Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Second-Generation Histone Deacetylase 6 Inhibitors Enhance the Immunosuppressive Effects of Foxp3+ T-Regulatory Cells

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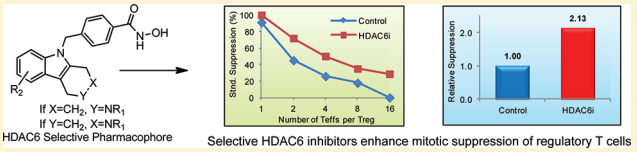
Supporting Information

ABSTRACT: Second-generation Tubastatin A analogues were synthesized and evaluated for their ability to inhibit selectively histone deacetylase 6 (HDAC6). Substitutions to the carboline cap group were well-tolerated with substitution at the 2-position of both β- and γ-carbolines being optimal for HDAC6 activity and selectivity. Some compounds in this series were determined to have subnanomolar activity at HDAC6 with more than 7000 fold selectivity for HDAC6 versus HDAC1. Selected compounds were then evaluated for their ability to augment the immunosuppressive effect of Foxp3+ regulatory T cells. All compounds tested were found to enhance the ability of regulatory T cells to inhibit the mitotic division of effector T cells both in vitro and in vivo, suggesting that further investigation into the use of these compounds for the treatment of autoimmune disorders is warranted.



Selective HDAC6 inhibitors enhance mitotic suppression of regulatory T cells

INTRODUCTION

Histone deacetylases (HDACs), along with histone acetyltransferases (HATs), are responsible for regulating the expression of an extensive array of genes by catalyzing the reversible deacetylation of ε-amino groups located on the N-terminal lysine residues of nuclear histones.[1] In addition to histones, HDACs act on a large number of other substrates including various transcription factors, chaperone proteins, and redox regulators.[2,3] As a result, HDAC inhibitors (HDACi) have been proven to be therapeutic for the treatment of diseases ranging from cancer to neurodegeneration, but one of the major obstacles facing the development of these drugs for clinical uses beyond oncology stems from the toxicity associated with inhibition of the class I isoforms. Whereas some toxicity may be tolerable and even necessary when designing drugs for oncologic purposes, it may well prevent these drugs from ever reaching clinical trials for the vast majority of other disease states.[4,5] Therefore, elimination of these side effects is desirable, and one way to accomplish this is to develop inhibitors that are selective for the particular isoform of interest. In our case, HDAC6 has emerged as a promising drug target because recent research has indicated that selective inhibition does not exhibit the cytotoxic profile associated with inhibition of the class I isoforms.[6]

Consistent with the broad expression of HDACs, HDACi were recently shown to enhance the suppressive effects of Foxp3+ regulatory T cells (Tregs).[7,8] The pharmacological enhancement of Treg suppression is a potential therapeutic approach to slow or reverse the pathogenesis of autoimmune disorders and prevent allograft rejection, inflammatory bowel disease, and rheumatoid arthritis.[7−9] It is well known that HDACs are involved in regulating Treg immunosuppression, although the particular isoform or isoforms responsible for this activity remain under investigation.[7,8] Use of HDACi results in increased acetylation of Foxp3, an important transcription factor responsible for the proper maturation of Tregs and the normal functioning of the immune system.[7] Foxp3 is part of a multiprotein complex that reduces Treg expression of certain cytokines such as IL-2, and HDACi use enhances Treg expression of CTLA-4, a protein with an important role in immunosuppression.[8] As a result, in murine models of allograft survival, HDACi in combination with low-dose rapamycin therapy resulted in donor-specific allograft tolerance, in stark contrast with recipients treated with rapamycin alone.[7]

Previously, our lab reported on the synthesis of a first-generation, potent, and selective HDAC6i, Tubastatin A, and showed that Tubastatin A enhanced the suppressive functions of murine Foxp3+ Tregs in an HDAC6-specific manner.[10,11] We now report a second generation of these compounds, a series of substituted β- and γ-carbolines, further optimized for activity, selectivity, and physiochemical properties. Usually, HDACi consist of a cap group that interacts with the surface of the protein, a linker that occupies a hydrophobic channel leading to the active site, and a metal chelator that interacts with the zinc ion at the bottom of the catalytic pocket.[12] Having determined that a benzyl linker was optimal for potent, selective HDAC6 inhibition and keeping with the standard zinc-binding group, a hydroxamic acid, we sought to explore modifications to the cap group of these HDACi.

Received: June 14, 2011
Published: December 13, 2011

   dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

We synthesized compounds with varying substitutions to the carboline cap groups and evaluated their HDAC inhibitory activity using purified recombinant human HDAC proteins isolated from a baculovirus expression system. Selected compounds were then tested for their ability to enhance the function of murine Foxp3+ Tregs in vitro and in vivo.

CHEMISTRY

The three series of Tubastatin A derivatives employed in this study were prepared from inexpensive, commercially available starting materials according to the synthetic routes outlined in Schemes 1−3. The first series was synthesized using Fischer indole synthesis to generate 6-, 7-, 8-, and 9-substituted γ-carboline cap groups **1a−h** from the respective phenylhydrazine and 1-methyl-4-piperidone.[13] Alkylation at the 5-position with 4-bromomethyl-benzoic acid methyl ester returned intermediate esters **2a−h**, which were subsequently converted to hydroxamic acids **3a−h** using hydroxylamine hydrochloride and sodium methoxide (Scheme 1).[10]

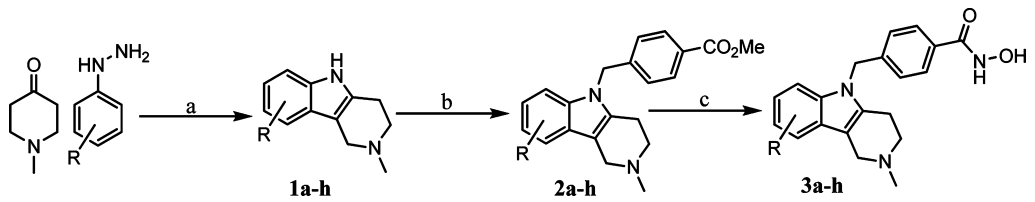
The second series of compounds consisting of 2-substituted γ-carbolines was prepared from 1-benzylpiperidin-4-one and phenylhydrazine. Fischer indole synthesis was employed to generate the 2-benzyl substituted γ-carboline, which was deprotected via catalytic hydrogenation to yield common intermediate 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4**.[13,14] Orthogonal alkylation at the 2-position, followed by the 5-position with the appropriate alkyl/benzyl halide and 4-bromomethyl-benzoic acid methyl ester, respectively, yielded intermediate esters **5a−l**.[10,14] The penultimate esters were then converted to hydroxamic acids **6a−l** using hydroxylamine hydrochloride in the presence of sodium methoxide in the same manner as the compounds described in the first series (Scheme 2).[10]

The third series of compounds consisting of 2-substituted β-carbolines was synthesized from commercially available 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole. Boc protection at the 2-position followed by alkylation at the 9-position with 4-bromomethyl-benzoic acid methyl ester yielded common intermediate **7**.[10,15] Deprotection and alkylation at the 2-position with the appropriate alkyl/benzyl halide yielded intermediate esters **8a−c**, which were subsequently converted to hydroxamic acids **9a−c** again using hydroxylamine hydrochloride and sodium methoxide (Scheme 3).[10,14] With the desired compounds in hand, we now sought to assay the activity of each at HDAC1 and HDAC6 as well as determine the ability of selected compounds to enhance Treg-mediated mitotic suppression.

RESULTS AND DISCUSSION

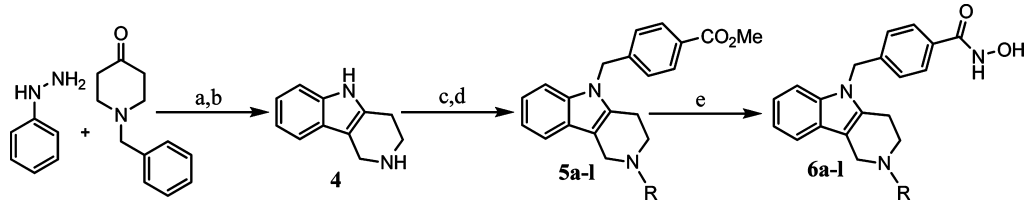
HDAC Inhibition. We investigated substitutions to the aromatic portion of the γ-carboline cap group in an effort to increase both potency and selectivity in comparison with our lead, Tubastatin A. The results of the HDAC inhibition assays for the compounds in the first series are displayed in Table 1.

Scheme 1. Preparation of 6-, 7-, 8-, and 9-Substituted γ-Carbolines



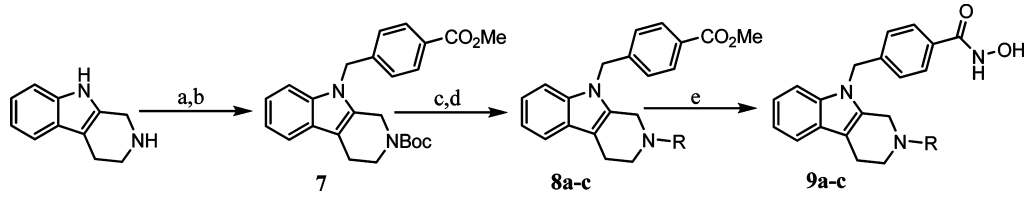
Reagents and conditions: (a) H_2SO_4, 1,4-dioxane, 2 h, 60 °C; (b) 4-bromomethyl-benzoic acid methyl ester, KO^{t}Bu, DMF, 80 °C, 2 h; (c) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, 0 °C to rt, 16 h.

Scheme 2. Synthesis of 2-Substituted γ-Carbolines



Reagents and conditions: (a) H_2SO_4, 1,4-dioxane, 60 °C, 2 h; (b) H_2, 10% Pd/C, 70% EtOH/H_2O, 70 °C, 24 h; (c) alkyl/benzyl halide, Et_3N, MeCN, 60 °C, 2 h; (d) 4-bromomethyl-benzoic acid methyl ester, KO^{t}Bu, DMF, 80 °C, 2 h; (e) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, 0 °C to rt, 16 h.

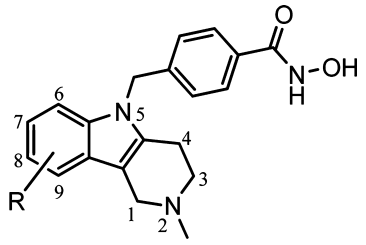
Scheme 3. Synthesis of 2-Substituted β-Carbolines



Reagents and conditions: (a) Boc anhydride, THF, reflux, 8 h; (b) 4-bromomethyl-benzoic acid methyl ester, KO^{t}Bu, DMF, 80 °C, 2 h; (c) 10% TFA/DCM, 30 °C, 2 h; (d) alkyl/benzyl halide, Et_3N, MeCN, 60 °C, 2 h; (e) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, 0 °C to rt, 16 h.

dx.doi.org/10.1021/jm200773h | *J. Med. Chem.* 2012, 55, 639−651

Table 1. IC_{50} and HDAC6 Selectivity for 6-, 7-, 8-, and 9-Substituted γ-Carbolines



compound ID	R	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (nM)	selectivity HDAC1/HDAC6 (fold)
Tubastatin A		16.4 ± 2.6	15 ± 1	1093
3a	6-Me	22.1 ± 6.2	34.7 ± 5.5	636
3b	7-Me	4.05 ± 0.94	11.5 ± 0.0	352
3c	8-Me	2.07 ± 0.27	4.90 ± 0.05	422
3d	7,9-diMe	10.3 ± 1.5	38.4 ± 0.1	268
3e	8-^{t}Bu	14.1 ± 3.5	22.5 ± 4.9	626
3f	8-OMe	1.88 ± 0.66	3.06 ± 0.49	614
3g	8-Cl	1.02 ± 0.13	1.02 ± 0.13	1000
3h	8-Br	0.427 ± 0.133	0.704 ± 0.197	606

Introduction of a methyl group to the 6- or 7-position, compounds **3a** and **3b**, respectively, as well as two methyl groups simultaneously to the 7- and 9-positions, **3d**, did little to increase the potency at HDAC6 but did increase the potency at HDAC1, resulting in a decrease in selectivity. The introduction of a methyl group to the 8-position, **3c**, increased potency at both isoforms, and thus we continued to look for substitutions at this position that would result in the same increase in potency at HDAC6 but not HDAC1. The introduction of a bulky *tert*-butyl group at the 8-position, **3e**, reduced potency at HDAC6, whereas incorporation of a methoxy group at this position, **3f**, had effects similar to that of the methyl group. In general, alkyl substitutions to the 6-, 7-, and 9-positions of the γ-carboline cap resulted in an increase in potency at HDAC1 with little effect on potency at HDAC6. Substitutions at the 8-position did increase potency at HDAC6 with the 8-chloro compound **3g** exhibiting an HDAC6 IC_{50} of ∼1 nM and retaining the selectivity of our lead, Tubastatin A. However, introduction of an 8-bromo substituent, **3h**, while increasing potency at both HDAC1 and HDAC6, significantly reduced the selectivity for HDAC6.

In light of these results, it was apparent that substitutions to the 6-, 7-, 8-, and 9-positions of the cap group were not beneficial to increasing the selectivity of these compounds, nor were they increasing the potency at our desired isoform. Therefore, we investigated substitutions to the other side of the cap group, at the 2-position specifically. HDAC IC_{50}s and HDAC6 selectivity data for compounds **6a**−**l** are given in Table 2. The desmethyl derivative of Tubastatin A, **6b**, was synthesized to evaluate the necessity of the methyl group at the 2-position. We found that removal of the methyl group resulted in a compound that was twice as potent and selective as Tubastatin A. Introduction of an ethyl group instead of a methyl group at this position, **6c**, increased both potency and selectivity from 15 to 3 nM and from ∼1000 to nearly 3500 fold, respectively. Introduction of a bulkier *iso*-propyl group, **6d**, increased the potency at and selectivity for HDAC6 compared with Tubastatin A; however, the increase was not as dramatic as with the ethyl group, suggesting that smaller or linear substituents might be more desirable. Incorporation of an allylic group at the 2-position, **6e**, dramatically improved the HDAC6 IC_{50} to <1 nM and increased the selectivity for HDAC6 versus HDAC1 to nearly 6000 fold.

We hypothesized that there could be a benefit to introducing aromatic functionalities at the 2-position for π-stacking purposes. Therefore, we introduced a benzyl substituent, **6f**, and observed a dramatic increase in selectivity to over 7000 fold versus HDAC1 as well as a moderate increase in potency with an HDAC6 IC_{50} of ∼1 nM. The introduction of methoxy substituents to the benzyl group, **6g** and **6h**, resulted in a decrease in potency and selectivity compared with the benzyl group alone. In an attempt to increase aqueous solubility in addition to improving potency and selectivity at HDAC6, we replaced the phenyl substituent of **6f** with a 4-pyridyl group, **6i**, which resulted in subnanomolar potency at HDAC6 as well as approximately 5000 fold selectivity over HDAC1.

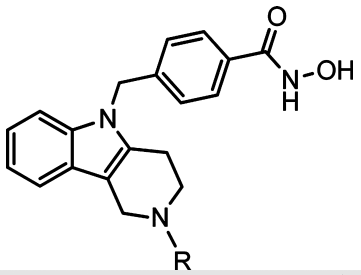
Once we had determined that the 2-position was optimal for substitution, we decided to investigate the breadth of substituents that would not only maintain the excellent potency/selectivity profile that we had elucidated but also enhance the drug-like properties of these molecules. Because amides are known to be very polar and could function to improve aqueous solubility, we decided to incorporate an acetamide group tethered with an ethyl linker to mimic the ethyl substituent of **6c**. This resulted in **6j** with an IC_{50} of 0.5 nM at HDAC6 and ∼3500 fold selectivity over HDAC1. Because this compound exhibited a good activity profile, we wanted to investigate the optimal length of the linker between the acetamide substituent and the nitrogen at the 2-position. We found that a one carbon linker was optimal, **6k**, with an HDAC6 IC_{50} of 0.8 nM and a selectivity of nearly 5000 fold compared with HDAC1. This compound was also found to have a lower cLogP ($cLogP_{6k}$ = 1.38) compared with our lead compound, Tubastatin A (cLogP = 2.38), indicating the potential for improved aqueous solubility.

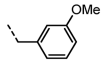
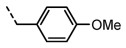
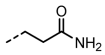
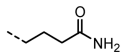
For the investigation of the position of the nitrogen atom in the tricyclic ring system, a small series of β-carbolines was also synthesized and the results of the HDAC inhibition assays are displayed in Table 3. The β-carboline regioisomer of Tubastatin A, **9b**, was very potent at HDAC6 with an IC_{50} of ∼1 nM and a selectivity of ∼3500 fold over HDAC1. The desmethyl derivative **9a** was equipotent at HDAC6 with an IC_{50} of ∼2 nM and a selectivity of ∼4000 fold over HDAC1. To determine if increased HDAC6 selectivity could be obtained by incorporating a benzyl substituent at the 2-position, as was the case with **6f**, we synthesized compound **9c**, and it was found to exhibit an improved HDAC6 IC_{50} of <1 nM. Whereas **9c** was not quite as selective as **6f**, it still maintained ∼5000 fold selectivity for HDAC6 compared with HDAC1.

Treg Suppression of T Cell Proliferation. After having identified a series of potent and selective Tubastatin A analogues with improved IC_{50}s against HDAC6 and selectivity versus HDAC1, we sought to evaluate the ability of these compounds to enhance the suppressive function of Tregs in vitro using Tregs and effector T cells (Teffs) isolated from C57BL/6 mice. CFSE-labeled Teffs were incubated in the presence and absence of Tregs, with or without the addition of selected HDAC6 inhibitors at multiple working concentrations. Flow cytometry was used to measure CFSE fluorescence, and CFSE−dilution plots were generated with the percentage of

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

Table 2. IC_{50} and HDAC6 Selectivity for 2-Substituted γ-Carbolines



compound ID	R	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (nM)	selectivity HDAC1/HDAC6 (fold)
Tubastatin A	Me	16.4 ± 2.6	15 ± 1	1093
6a		8.62 ± 0.06	2.25 ± 0.39	3831
6b	H	16.4 ± 0.1	6.56 ± 0.48	2500
6c	Et	10.2 ± 0.7	2.99 ± 0.67	3411
6d		15.7 ± 3.2	8.96 ± 1.62	1752
6e		5.82 ± 0.09	0.972 ± 0.125	5987
6f	Benzyl	10.3 ± 1.4	1.44 ± 0.07	7152
6g		5.19 ± 0.01	2.96 ± 0.85	1753
6h		4.64 ± 0.14	3.05 ± 0.25	1521
6i		2.74 ± 0.31	0.582 ± 0.022	4707
6j		1.61 ± 0.45	0.459 ± 0.134	3507
6k		3.98 ± 0.33	0.799 ± 0.205	4981
6l		7.45 ± 0.07	4.06 ± 0.68	1834

cells determined to be undergoing mitotic division displayed in the top left of each plot (Figure 1 and Supplementary Figure 1 of the Supporting Information). As the ratio of Tregs/Teffs increased, the number of cells undergoing mitosis decreased, and this effect was augmented by the addition of HDAC6i to the culture media.

Raw mitotic division data obtained from the CFSE−dilution plots was standardized with respect to a control for each compound at each tested concentration. The standardized cell division data were then converted to standardized suppression data and plotted against the ratio of Teffs/Tregs (Figure 2 and Supplementary Figure 2 of the Supporting Information).

The area under the standardized suppression curve (AUC) for each compound at each tested concentration, along with the AUC for the respective control, was calculated, and the AUC ratio between the compound at a particular concentration and the respective control was determined (Figure 3). All of the compounds tested enhanced Treg suppression of Teff proliferation, with Tubastatin A and **6i** exhibiting the most pronounced effect. Both Tubastatin A and **6i** more than doubled the immunosuppressive function of Tregs in vitro, an effect similar to that observed using pan-HDACi.[8]

To validate further these results, we undertook 7-day homeostatic proliferation assays to assess the in vivo effects of our HDAC6i (Figure 4). We have previously demonstrated the utility of HDAC6 targeting in these assays.[11] The principle of the assay is such that conventional T cells adoptively transferred to an immunodeficient (RAG−/−) host undergo rapid proliferation within secondary lymphoid tissues. This homeostatic expansion is decreased by the actions of Foxp3+ Tregs, and HDAC6−/− Tregs are more effective than wild-type Tregs in suppressing the proliferation of the transferred cells.[11] To ensure that the effects of our HDAC6i were not attributable to inhibition of HDAC6 in proliferating Teffs, we used wild-type CD4+CD25+ Tregs and CFSE-labeled HDAC6−/− CD4+CD25− Teffs. We found that mice treated with HDAC6i had significantly lower numbers of Teffs in their peripheral lymph nodes (p = 0.03) due to enhancement of the Treg suppressive function. Both **3h** and **6i** tended to be more effective than Tubastatin A, although the differences were not statistically significant.

Functionalization of the carboline cap group was well-tolerated with respect to both inhibition of the recombinant HDAC6 protein and the ability to enhance Treg induced mitotic suppression of Teffs both in vitro and in vivo. Substitution at the 2-position of both the β- and γ-carboline was found to be optimal with some compounds exhibiting subnanomolar potency at HDAC6 as well as greater than 7000

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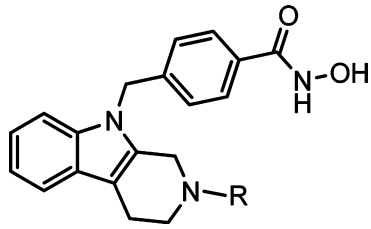
Table 3. IC_{50} Values and HDAC6 Selectivity for 2-Substituted β-Carbolines



compound ID	R	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (nM)	selectivity HDAC1/HDAC6 (fold)
9a	H	9.70 ± 0.03	2.49 ± 0.60	3895
9b	Me	5.18 ± 0.12	1.40 ± 0.30	3700
9c	benzyl	4.32 ± 0.70	0.872 ± 0.195	4954

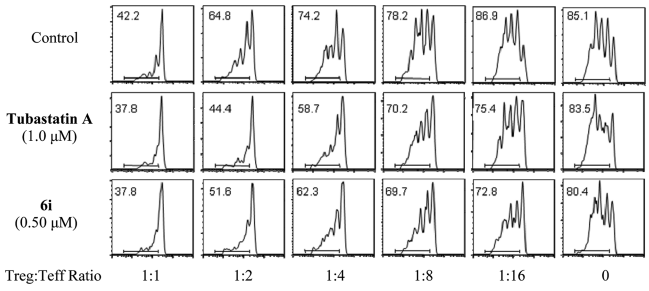


Figure 1. CFSE−dilution plots for Tubastatin A and **6i** at the highest working concentration. HDAC6 inhibitors were evaluated for their ability to enhance regulatory T cell suppression of effector T cell proliferation. The percentage of cells undergoing division is displayed in the top left of each plot.

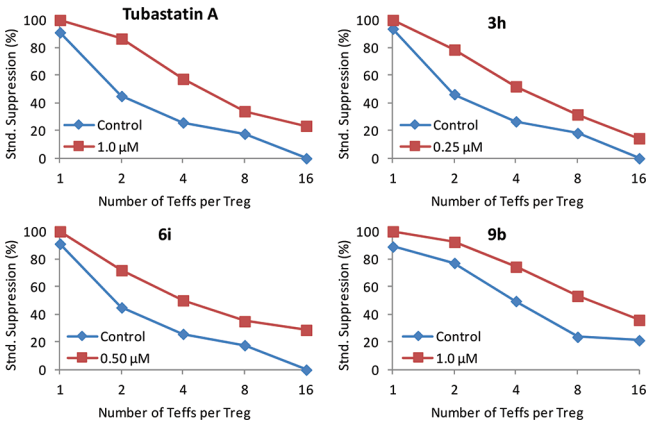


Figure 2. Standardized suppression curves for selected compounds at their highest working concentration. Standardized suppression curves were generated by applying min−max normalization to the raw cell division data, which was then converted to percent mitotic suppression (% suppression = 100 − % dividing cells) and plotted against the ratio of Teffs/Tregs.

fold selectivity versus HDAC1 in the HDAC inhibition assays. The wide range of tolerated functional groups allows for the selection of substituents to enhance the drug-like properties of these compounds. In addition, it is known that placing bulky substituents at the 2-position of the γ-carboline eliminates undesirable off-target activity sometimes observed with other carbolines such as Dimebolin.[16]

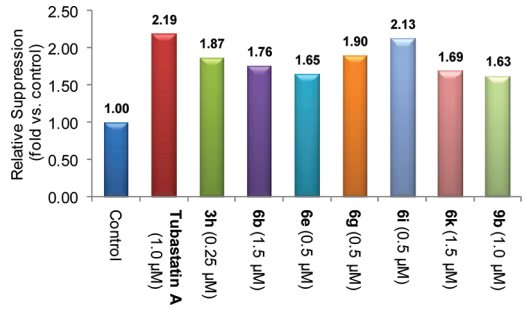


Figure 3. AUC suppression ratio for each compound at highest working concentration. Relative suppression ratios were determined by calculating the area under the standardized suppression curves for each compound with its respective control using GraphPad Prism 5 (relative suppression = $AUC_{compound}/AUC_{control}$).

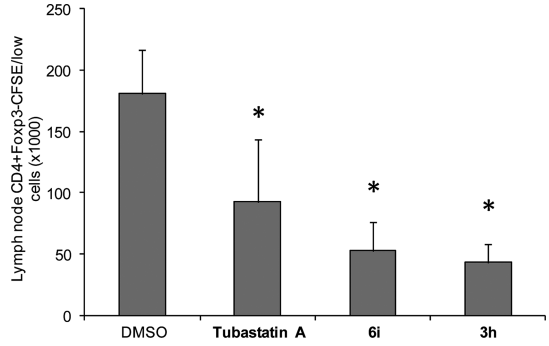


Figure 4. In vivo assessment of HDAC6i activity using a 7-day homeostatic proliferation assay with HDAC6−/− CFSE+/low conventional T cells in the presence of HDAC6+ Tregs (2:1 ratio) and daily injections of DMSO, Tubastatin A, or second-generation HDAC6i (1 mg/kg). Numbers of CD4+ Foxp3− CFSE+/low conventional T cells in peripheral lymph nodes of adoptively transferred RAG−/− mice (3/group) are shown (mean ± SEM, *p < 0.03 vs DMSO).

CONCLUSIONS

Pan-HDACi and selective HDAC6i were previously shown to promote the acetylation and function of Foxp3, a key transcription factor responsible for control of Treg-dependent T cell immune responses.[7,11] Furthermore, HDAC inhibition enhances the expression of CTLA-4 in Foxp3+ Tregs and directly correlates with their ability to suppress Teff proliferation.[8] Whereas it is known that broad spectrum HDACi can enhance the suppressive effect of Tregs in vitro, whether one or more particular isoforms are responsible for this action remains to be determined. Here we have developed a series of second generation HDAC6 selective compounds and demonstrated their ability to enhance Treg suppression of Teff proliferation both in vitro and in vivo. Because of the prevalence of rheumatoid arthritis, which the Center for Disease Control estimates affects 1 to 2% of the population worldwide, and other autoimmune diseases, as well as the need for new therapies to improve the prognosis after organ transplantation, we believe that further investigation into the immunosuppressive effects of these HDAC6 selective agents is warranted. The development of isoform-selective HDAC inhibitors continues to be a widely undertaken endeavor, and we believe that their discovery will provide invaluable research tools for identifying the specific functions of each HDAC isoform.

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

EXPERIMENTAL SECTION

General Information. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker spectrometer with TMS as an internal standard. The following standard abbreviations indicating multiplicity were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, and br = broad. HRMS experiments were carried out using a Shimadzu IT-TOF instrument with MeCN and H_2O spiked with 0.1% formic acid as the mobile phase. Reaction progress was monitored by TLC using precoated silica gel plates (Merck silica gel 60 F_{254}, 250 μm thickness). Automated column chromatography was performed using the CombiFlash Rf apparatus available from Teledyne ISCO and prepacked 25 g cartridges loaded with Merck silica gel (40−60 mesh) along with the following conditions: Gradient: 100% DCM, 5 min; 0−10% MeOH/DCM, 20 min, 10% MeOH/DCM, 5 min; flow rate = 30 mL/min with wavelength monitoring at 254 and 280 nm. Preparatory HPLC was carried out using a Shimadzu preparative liquid chromatograph with the following specifications: Column: ACE 5 AQ (150 × 21.2 mm) with 5 μm particle size. Gradient: 8−100% MeOH/H_2O, 30 min; 100% MeOH, 5 min; 100−8% MeOH/H_2O, 4 min; 8% MeOH/H_2O, 1 min; flow rate = 17 mL/min with wavelength monitoring at 254 and 280 nm. Both solvents were spiked with 0.05% TFA. Analytical HPLC was carried out using an Agilent 1100 series instrument with the following specifications: Column: Luna 5 μ C18(2) 100A (150 × 4.60 mm) with 5 μm particle size. Flow rate = 1.4 mL/min with wavelength monitoring at 254 nm. Gradient: 10−100% MeOH/H_2O, 18 min; 100% MeOH, 3 min; 100−10% MeOH/H_2O, 3 min; 10% MeOH/H_2O, 5 min. Both solvents were spiked with 0.05% TFA. The purity of all tested compounds was ≥95%, as determined by analytical HPLC.

General Procedure A. The appropriate phenylhydrazine and 1-methylpiperidin-4-one (1 mol equiv) were dissolved in 1,4-dioxane (35 mL). The reaction was placed in an ice bath and concentrated H_2SO_4 (5 mL) was added dropwise. The reaction was then removed from the ice bath and heated to 60 °C for 2 h. When the reaction was complete as evidenced by TLC, it was cooled to room temperature, then placed on ice and the pH was adjusted to approximately 13 by the addition of saturated $NaHCO_3$ (100 mL) and solid NaOH. The organic products were extracted with EtOAc (3 × 30 mL), washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated in vacuo. The resulting product was purified via automated column chromatography.

General Procedure B. The appropriate γ-carboline was dissolved in anhydrous DMF (3 mL) and added to a suspension of potassium *tert*-butoxide (1 mol equiv) in anhydrous DMF (2 mL) under argon at room temperature. The reaction mixture was heated to 80 °C for 15 min, after which 4-bromomethyl-benzoic acid methyl ester (1 mol equiv) dissolved in anhydrous DMF (2 mL) was added at 80 °C. The reaction was stirred at 80 °C for 2 h, after which the reaction was cooled to room temperature and poured into cold water (15 mL). The organic products were extracted with EtOAc (3 × 20 mL), washed with water (3 × 15 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. The resulting product was purified via automated column chromatography.

General Procedure C. The appropriate ester and hydroxylamine hydrochloride (6 mol equiv) were dissolved in MeOH (5 mL) and cooled to 0 °C in an ice bath. (DMF can be added to improve the solubility of the ester as this is important.) A 25% sodium methoxide solution in MeOH (8 mol equiv) was added, upon which a precipitate formed. The reaction was allowed to warm to room temperature and then stirred for 16 h. Upon completion as evidenced by TLC, the reaction was quenched by the addition of a 10% TFA/DCM solution (~5 mL) and then filtered to remove residual NaCl. The filter cake was washed with additional MeOH (5 mL), and the combined filtrate and wash were concentrated in vacuo. The crude product was then dissolved in DMF and purified by preparatory HPLC.

General Procedure D. The γ-carboline was placed under argon in a two-necked round-bottomed flask fitted with a condenser. Anhydrous MeCN (5 mL) was added, followed by the addition of Et_3N (2 mol equiv) at room temperature. The reaction was heated to 60 °C; then, the appropriate alkyl/benzyl halide (1 mol equiv) was added in anhydrous MeCN (2 mL). The reaction was stirred for 2 h at 60 °C and then poured in a 1:1 mixture of EtOAc/H_2O (20 mL). The organic layer was isolated and the aqueous layer was further extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. The desired 2-substituted γ-carboline was isolated by automated column chromatography and then dissolved in anhydrous DMF (3 mL) and added to a suspension of potassium *tert*-butoxide (1 mol equiv) in anhydrous DMF (2 mL) under argon at room temperature. The reaction mixture was heated to 80 °C for 15 min, after which 4-bromomethyl-benzoic acid methyl ester (1 mol equiv) dissolved in anhydrous DMF (2 mL) was added at 80 °C. The reaction was stirred at 80 °C for 2 h, after which the reaction was cooled to room temperature and poured into cold water (15 mL). The organic products were extracted with EtOAc (3 × 30 mL), and the combined extracts were washed with water (3 × 15 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. The resulting product was purified via automated column chromatography.

General Procedure E. The β-carboline was placed under argon in a two-necked round-bottomed flask fitted with a condenser. Anhydrous MeCN (5 mL) was added, followed by the addition of Et_3N (2 mol equiv) at room temperature. The reaction was heated to 60 °C; then, the appropriate alkyl/benzyl halide (1 mol equiv) was added in anhydrous MeCN (2 mL). The reaction was stirred for 2 h at 60 °C and then poured into a 1:1 mixture of EtOAc/H_2O (20 mL). The organic layer was isolated, and the aqueous layer was further extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. The resulting product was purified via automated column chromatography.

*2,6-Dimethyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1a**).* The title compound was synthesized from *o*-tolylhydrazine (2.00 g, 16.4 mmol) according to general procedure A (0.40 g, 12%). ^{1}H NMR (400 MHz, MeOD): δ 7.18 (d, J = 7.6 Hz, 1H), 6.88 (m, 2H), 3.65 (s, 2H), 2.91 (t, J = 5.5 Hz, 2H), 2.83 (t, J = 5.7 Hz, 2H), 2.52 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 135.9, 131.2, 125.3, 121.1, 119.8, 118.6, 114.4, 106.8, 63.8, 53.0, 34.0, 22.9, 15.7. ESI-HRMS: calc. for $C_{13}H_{16}N_2$: $[M+H]^+$ = 201.1386 m/z, found: $[M+H]^+$ = 201.1389 m/z.

*2,7-Dimethyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1b**).* The title compound was synthesized from *m*-tolylhydrazine hydrochloride (2.00 g, 12.6 mmol) according to general procedure A (1.68 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 7.32 (d, J = 7.9 Hz, 1H), 6.91 (d, J = 7.9 Hz, 1H), 6.83 (s, 1H), 3.71 (s, 2H), 2.77 (m, 2H), 2.64 (s, 3H), 2.61 (s, 3H), 2.39 (s, 2H). ^{13}C NMR (100 MHz, MeOD): δ 136.8, 131.3, 130.5, 123.9, 120.6, 116.9, 111.0, 107.7, 52.6, 51.9, 45.8, 23.4, 21.7. ESI-HRMS: calc. for $C_{13}H_{16}N_2$: $[M+H]^+$ = 201.1386 m/z, found: $[M+H]^+$ = 201.1381 m/z.

*2,8-Dimethyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1c**).* The title compound was synthesized from *p*-tolylhydrazine hydrochloride (2.00 g, 12.6 mmol) according to general procedure A (2.18 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (s, 1H), 7.17 (m, 1H), 6.95 (s, 1H), 3.66 (br, 2H), 2.84 (m, 4H), 2.57 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 134.9, 131.5, 127.3, 126.0, 121.8, 116.4, 110.0, 106.0, 52.1, 51.4, 44.3, 22.9, 20.2. ESI-HRMS: calc. for $C_{13}H_{16}N_2$: $[M+H]^+$ = 201.1386 m/z, found: $[M+H]^+$ = 201.1386 m/z.

*2,7,9-Trimethyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1d**).* The title compound was synthesized from (3,5-dimethylphenyl)-hydrazine hydrochloride (2.00 g, 11.5 mmol) according to general procedure A (1.08 g, 44%). ^{1}H NMR (400 MHz, MeOD): δ 6.87 (s, 1H), 6.54 (s, 1H), 3.88 (s, 2H), 2.85 (t, J = 5.4 Hz, 2H), 2.78 (t, J = 5.3 Hz, 2H), 2.51 (s, 3H), 2.50 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 135.4, 128.5, 128.4, 126.6, 121.4, 119.6, 106.6, 105.2, 51.9, 50.0, 42.8, 21.3, 18.7, 17.1. ESI-HRMS: calc. for $C_{14}H_{18}N_2$: $[M+H]^+$ = 215.1543 m/z, found: $[M+H]^+$ = 215.1534 m/z.

*8-tert-Butyl-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1e**).* The title compound was synthesized from (4-*tert*-butylphenyl)-hydrazine hydrochloride (2.00 g, 10.0 mmol) according to general

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

procedure A (1.53 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (s, 1H), 7.20 (m, 2H), 3.75 (s, 2H), 2.83 (m, 4H), 2.60 (s, 3H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 139.6, 133.2, 129.9, 124.0, 117.0, 110.9, 108.3, 105.0, 50.6, 49.9, 42.8, 32.4, 29.6, 21.4. ESI-HRMS: calc. for $C_{16}H_{22}N_2$: $[M+H]^+$ = 243.1856 m/z, found: $[M+H]^+$ = 243.1855 m/z.

*8-Methoxy-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1f**).* The title compound was synthesized from (4-methoxyphenyl)-hydrazine hydrochloride (2.00 g, 11.5 mmol) according to general procedure A (1.71 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (d, J = 8.7 Hz, 1H), 6.77 (s, 1H), 6.66 (dd, J = 2.5 Hz, 8.7 Hz, 1H), 3.75 (s, 3H), 3.55 (s, 2H), 2.72 (m, 4H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.8, 132.8, 131.3, 126.4, 111.2, 110.6, 108.4, 100.0, 55.9, 52.5, 51.8, 45.8, 23.9. ESI-HRMS: calc. for $C_{13}H_{16}N_2O$: $[M+H]^+$ = 217.1335 m/z, found: $[M+H]^+$ = 217.1340 m/z.

*8-Chloro-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1g**).* The title compound was synthesized from (4-chlorophenyl)-hydrazine hydrochloride (2.00 g, 11.2 mmol) according to general procedure A (1.08 g, 44%). ^{1}H NMR (400 MHz, MeOD): δ 7.33 (s, 1H), 7.23 (d, J = 8.6 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 3.67 (s, 2H), 2.92 (m, 4H), 2.61 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 133.4, 131.9, 125.2, 122.5, 118.8, 114.6, 109.9, 104.9, 50.4, 49.5, 42.7, 21.3. ESI-HRMS: calc. for $C_{12}H_{13}ClN_2$: $[M+H]^+$ = 221.0840 m/z, found: $[M+H]^+$ = 221.0838 m/z.

*8-Bromo-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (**1h**).* The title compound was synthesized from (4-bromophenyl)-hydrazine (3.00 g, 16.0 mmol) according to general procedure A (2.82 g, 94%). ^{1}H NMR (400 MHz, MeOD): δ 7.47 (d, J = 1.6 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 7.12 (dd, J = 1.8 Hz, 6.7 Hz, 1H), 3.62 (s, 2H), 2.90 (t, J = 5.3 Hz, 2H), 2.84 (t, J = 5.2 Hz, 2H), 2.52 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 134.8, 132.9, 127.1, 122.6, 118.9, 111.5, 111.0, 106.0, 51.6, 50.6, 43.9, 22.4. ESI-HRMS: calc. for $C_{12}H_{13}BrN_2$: $[M+H]^+$ = 265.0335 m/z, found: $[M+H]^+$ = 265.0348 m/z.

*Methyl 4-((2,6-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2a**).* The title compound was synthesized from 2,6-dimethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1a** (0.40 g, 2.0 mmol) according to general procedure B (0.07 g, 9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.3 Hz, 2H), 7.03 (m, 2H), 6.93 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 7.2 Hz, 1H), 5.54 (s, 2H), 3.89 (s, 3H), 3.84 (s, 2H), 2.95 (t, J = 5.9 Hz, 2H), 2.78 (t, J = 5.7 Hz, 2H), 2.64 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 144.7, 135.4, 133.3, 130.2, 129.6, 129.0, 126.5, 125.1, 124.6, 120.5, 119.5, 115.8, 63.7, 52.1, 51.3, 47.9, 45.2, 22.2, 19.5. ESI-HRMS: calc. for $C_{22}H_{24}N_2O_2$: $[M+H]^+$ = 349.1911 m/z, found: $[M+H]^+$ = 349.1898 m/z.

*Methyl-4-((2,7-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2b**).* The title compound was synthesized from 2,7-dimethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1b** (0.35 g, 1.7 mmol) according to general procedure B (0.36 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 7.9 Hz, 1H), 7.07 (d, J = 8.2 Hz, 2H), 6.96 (m, 2H), 5.27 (s, 2H), 3.90 (s, 3H), 3.73 (s, 2H), 2.83 (t, J = 5.6 Hz, 2H), 2.76 (br, 2H), 2.58 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 143.2, 134.2, 132.3, 131.0, 130.1, 129.3, 126.1, 123.7, 121.0, 117.5, 109.2, 108.2, 52.3, 52.1, 51.7, 46.1, 45.4, 22.6, 21.9. ESI-HRMS: calc. for $C_{22}H_{24}N_2O_2$: $[M+H]^+$ = 349.1911 m/z, found: $[M+H]^+$ = 349.1904 m/z.

*Methyl 4-((2,8-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2c**).* The title compound was synthesized from 2,8-dimethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1c** (0.30 g, 1.5 mmol) according to general procedure B (0.35 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, J = 8.1 Hz, 2H), 7.26 (m, 2H), 7.12 (d, J = 8.1 Hz, 1H), 7.08 (d, J = 8.2 Hz, 2H), 5.26 (s, 2H), 4.09 (s, 2H), 3.91 (s, 3H), 3.57 (t, J = 5.9 Hz, 2H), 3.18 (t, J = 6.3 Hz, 2H), 3.01 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.5, 141.9, 135.6, 130.3, 130.0, 129.8, 129.7, 125.9, 125.0, 124.4, 117.6, 109.4, 101.5, 52.2, 50.7, 50.6, 46.5, 41.6, 21.4, 19.2. ESI-HRMS: calc. for $C_{22}H_{24}N_2O_2$: $[M+H]^+$ = 349.1911 m/z, found: $[M+H]^+$ = 349.1910 m/z.

*Methyl 4-((2,7,9-Trimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2d**).* The title compound was synthesized from 2,7,9-trimethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1d** (0.30 g, 1.4 mmol) according to general procedure B (0.40 g, 78%).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 6.92 (s, 1H), 6.75 (s, 1H), 5.24 (s, 2H), 4.26 (s, 2H), 3.91 (s, 3H), 3.57 (t, J = 5.8 Hz, 2H), 3.19 (t, J = 6.4 Hz, 2H), 3.02 (s, 3H), 2.43 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.6, 148.9, 135.7, 131.4, 129.7, 129.6, 128.8, 128.5, 127.5, 124.4, 123.0, 107.4, 107.2, 65.9, 52.4, 50.0, 41.2, 30.5, 21.6, 20.0, 19.9. ESI-HRMS: calc. for $C_{23}H_{26}N_2O_2$: $[M+H]^+$ = 363.2067 m/z, found: $[M+H]^+$ = 363.2065 m/z.

*Methyl 4-((8-tert-Butyl-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**2e**).* The title compound was synthesized from 8-*tert*-butyl-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido-[4,3-*b*]indole **1e** (0.50 g, 2.1 mmol) according to general procedure B (0.64 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.2 Hz, 2H), 7.45 (s, 1H), 7.20 (m, 1H), 7.09 (m, 3H), 5.38 (s, 2H), 3.92 (s, 3H), 3.78 (s, 2H), 2.85 (m, 2H), 2.78 (m, 2H), 2.61 (br, 3H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 166.7, 144.0, 141.9, 135.1, 132.7, 129.5, 128.9, 126.1, 125.4, 119.1, 113.1, 108.5, 107.5, 52.0, 51.3, 51.2, 45.5, 44.2, 34.0, 31.1, 22.0. ESI-HRMS: calc. for $C_{25}H_{30}N_2O_2$: $[M+H]^+$ = 391.2380 m/z, found: $[M+H]^+$ = 391.2397 m/z.

*Methyl 4-((8-Methoxy-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**2f**).* The title compound was synthesized from 8-methoxy-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido-[4,3-*b*]indole **1f** (0.50 g, 2.3 mmol) according to general procedure B (0.40 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.0 Hz, 2H), 7.04 (m, 3H), 6.93 (d, J = 8.7 Hz, 1H), 6.77 (dd, J = 2.4 Hz, 8.6 Hz, 1H), 5.27 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.71 (s, 2H), 2.83 (m, 2H), 2.77 (m, 2H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 154.0, 143.2, 134.0, 131.9, 130.1, 129.3, 126.2, 126.1, 110.7, 109.7, 108.5, 100.3, 55.9, 52.5, 52.1, 51.9, 46.3, 45.8, 23.0. ESI-HRMS: calc. for $C_{22}H_{24}N_2O_3$: $[M+H]^+$ = 365.1860 m/z, found: $[M+H]^+$ = 365.1859 m/z.

*Methyl 4-((8-Chloro-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2g**).* The title compound was synthesized from 8-chloro-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1g** (0.15 g, 0.7 mmol) according to general procedure B (0.18 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, J = 8.1 Hz, 2H), 7.35 (s, 1H), 7.05 (m, 2H), 7.02 (d, J = 8.0, 2H), 5.27 (s, 2H), 3.89 (s, 3H), 3.67 (s, 2H), 2.82 (br, 2H), 2.62 (br, 2H), 2.57 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 166.8, 144.0, 142.0, 135.2, 132.7, 129.5, 128.9, 126.1, 125.3, 119.1, 113.0, 108.4, 107.4, 51.1, 45.5, 44.1, 34.0, 30.9, 22.0. ESI-HRMS: calc. for $C_{21}H_{21}ClN_2O_2$: $[M+H]^+$ = 369.1364 m/z, found: $[M+H]^+$ = 369.1373 m/z.

*Methyl 4-((8-Bromo-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**2h**).* The title compound was synthesized from 8-bromo-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **1h** (0.50 g, 1.9 mmol) according to general procedure B (0.37 g, 47%). ^{1}H NMR (400 MHz, MeOD): δ 7.87 (d, J = 8.4 Hz, 2H), 7.55 (s, 1H), 7.14 (br, 2H), 7.04 (d, J = 8.4 Hz, 2H), 5.33 (s, 2H), 3.84 (s, 3H), 3.74 (s, 2H), 2.93 (t, J = 5.9 Hz, 2H), 2.82 (t, J = 5.5 Hz, 2H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 166.3, 142.9, 135.2, 134.0, 129.2, 128.7, 126.7, 125.7, 123.3, 119.5, 111.9, 110.3, 106.3, 51.3, 50.8, 50.4, 45.2, 43.5, 21.4. ESI-HRMS: calc. for $C_{21}H_{21}BrN_2O_2$: $[M+H]^+$ = 413.0859 m/z, found: $[M+H]^+$ = 413.0859 m/z.

*4-((2,6-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)-N-hydroxybenzamide·TFA (**3a**).* The title compound was synthesized from methyl 4-((2,6-dimethyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2a** (0.07 g, 0.2 mmol) according to general procedure C and the TFA salt was isolated as a white solid (12 mg, 18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1H), 10.22 (br, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 7.8 Hz, 1H), 6.93 (m, 4H), 5.62 (m, 2H), 4.67 (br, 1H), 4.35 (br, 1H), 3.78 (br, 1H), 3.60 (br, 1H), 3.01 (br, 5H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 143.2, 135.5, 132.3, 132.2, 127.9, 125.8, 125.5, 125.4, 121.2, 120.4, 116.3, 103.1, 51.0, 50.4, 47.8, 42.2, 20.0, 19.4. ESI-HRMS: calc. for $C_{21}H_{23}N_3O_2$: $[M+H]^+$ = 350.1863 m/z, found: $[M+H]^+$ = 350.1863 m/z.

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639–651

*4-((2,7-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)-N-hydroxybenzamide·TFA (**3b**).* The title compound was synthesized from methyl-4-((2,7-dimethyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2b** (0.10 g, 0.3 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (27 mg, 27%). ^{1}H NMR (400 MHz, MeOD): δ 7.68 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.1 Hz, 1H), 7.19 (s, 1H), 7.11 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 8.2 Hz, 1H), 5.44 (m, 2H), 4.74 (d, J = 15.0 Hz, 1H), 4.39 (d, J = 12.4 Hz, 1H), 3.84 (br, 1H), 3.58 (br, 1H), 3.14 (s, 2H), 3.12 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 166.2, 141.7, 137.7, 132.4, 131.4, 129.5, 127.2, 126.1, 122.5, 121.6, 117.1, 109.4, 102.0, 51.4, 51.8, 45.5, 41.6, 20.5, 19.7. ESI-HRMS: calc. for $C_{21}H_{23}N_3O_2$: $[M+H]^+$ = 350.1863 m/z, found: $[M+H]^+$ = 350.1868 m/z.

*4-((2,8-Dimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)-N-hydroxybenzamide·TFA (**3c**).* The title compound was synthesized from methyl 4-((2,8-dimethyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2c** (0.30 g, 0.7 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (39 mg, 15%). ^{1}H NMR (400 MHz, MeOD): δ 7.70 (d, J = 8.2 Hz, 2H), 7.31 (s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 7.8 Hz, 2H), 5.44 (s, 2H), 4.55 (br, 2H), 3.71 (br, 2H), 3.12 (m, 5H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 141.6, 135.4, 132.3, 131.6, 128.8, 127.6, 126.9, 125.0, 123.8, 117.9, 110.3, 102.2, 51.0, 50.5, 46.0, 42.3, 21.5, 20.0. ESI-HRMS: calc. for $C_{21}H_{23}N_3O_2$: $[M+H]^+$ = 350.1863 m/z, found: $[M+H]^+$ = 350.1862 m/z.

*N-Hydroxy-4-((2,7,9-trimethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA (**3d**).* The title compound was synthesized from methyl 4-((2,7,9-trimethyl-3,4-dihydro-1*H*-pyrido-[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2d** (0.40 g, 1.1 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (28 mg, 23%). ^{1}H NMR (400 MHz, MeOD): δ 7.64 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.05 (s, 1H), 6.68 (s, 1H), 5.37 (s, 2H), 4.29 (s, 2H), 3.67 (t, J = 6.0 Hz, 2H), 3.23 (t, J = 6.3 Hz, 2H), 3.12 (s, 3H), 2.41 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (100 MHz,): δ 164.7, 140.2, 136.2, 130.7, 129.7, 127.4, 127.3, 125.6, 124.5, 121.4, 120.5, 105.6, 100.9, 50.9, 49.4, 43.9, 40.1, 18.8, 18.1, 17.0. ESI-HRMS: calc. for $C_{22}H_{25}N_3O_2$: $[M+H]^+$ = 364.2020 m/z, found: $[M+H]^+$ = 364.2016 m/z.

*4-((8-tert-Butyl-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**3e**).* The title compound was synthesized from methyl 4-((8-*tert*-butyl-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2e** (0.20 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (55 mg, 27%). ^{1}H NMR (400 MHz, MeOD): δ 7.66 (d, J = 8.2 Hz, 2H), 7.52 (s, 1H), 7.31 (m, 2H), 7.14 (d, J = 8.0 Hz, 2H), 5.46 (br, 2H), 4.59 (br, 2H), 3.72 (br, 2H), 3.13 (br, 5H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 142.6, 141.6, 135.2, 132.3, 131.4, 127.7, 126.9, 124.6, 120.4, 114.0, 110.1, 102.7, 51.0, 50.7, 46.1, 42.2, 34.8, 32.2, 20.0. ESI-HRMS: calc. for $C_{24}H_{29}N_3O_2$: $[M+H]^+$ = 392.2333 m/z, found: $[M+H]^+$ = 392.2337 m/z.

*N-Hydroxy-4-((8-methoxy-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA (**3f**).* The title compound was synthesized from methyl 4-((8-methoxy-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2f** (0.09 g, 0.2 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (11 mg, 13%). ^{1}H NMR (400 MHz, MeOD): δ 7.58 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 7.8 Hz, 1H), 7.01 (d, J = 8.2 Hz, 2H), 6.91 (s, 1H), 6.74 (d, J = 8.6 Hz, 1H), 5.36 (m, 2H), 4.44 (br, 2H), 3.69 (s, 3H), 3.28 (br, 2H), 3.01 (br, 5H). ^{13}C NMR (100 MHz, MeOD): δ 168.4, 155.1, 146.3, 130.4, 129.5, 129.1, 128.3, 128.0, 127.7, 111.4, 111.3, 109.2, 100.7, 63.3, 54.7, 50.6, 39.6, 29.1, 19.2. ESI-HRMS: calc. for $C_{21}H_{23}N_3O_3$: $[M+H]^+$ = 366.1812 m/z, found: $[M+H]^+$ = 366.1803 m/z.

*4-((8-Chloro-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**3g**).* The title compound was synthesized from methyl 4-((8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2g** (0.10 g, 0.3 mmol) according to general procedure C, and the TFA salt was isolated as a white solid (16 mg, 15%). ^{1}H NMR (400 MHz, MeOD): δ 7.68 (d, J = 8.0 Hz, 2H), 7.51 (s, 1H), 7.38 (d, J = 8.6 Hz, 1H), 7.17 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 7.8 Hz, 2H), 5.48 (s, 2H), 4.55 (br, 2H), 3.71 (br, 2H), 3.18 (br, 2H), 3.12 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 166.1, 141.1, 135.7, 132.2, 131.5, 127.3, 126.2, 125.7, 125.6, 122.3, 117.0, 110.9, 102.0, 51.2, 50.7, 45.8, 41.6, 19.7. ESI-HRMS: calc. for $C_{20}H_{20}ClN_3O_2$: $[M+H]^+$ = 370.1317 m/z, found: $[M+H]^+$ = 370.1323 m/z.

*4-((8-Bromo-2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**3h**).* The title compound was synthesized from methyl 4-((8-bromo-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **2h** (0.25 g, 0.6 mmol) according to general procedure C, and the TFA salt was isolated as an off-white solid (75 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.18 (s, 1H), 10.23 (s, 1H), 9.03 (s, 1H), 7.75 (s, 1H), 7.68 (d, J = 7.6 Hz, 2H), 7.47 (d, J = 8.8 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.10 (d, J = 7.7 Hz, 2H), 5.48 (s, 2H), 4.63 (br, 1H), 4.32 (br, 1H), 3.73 (br, 1H), 3.54 (br, 1H), 3.09 (s, 2H), 2.99 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 140.7, 135.4, 133.1, 132.0, 127.3, 126.5, 126.2, 124.4, 120.4, 112.4, 112.3, 102.2, 50.4, 49.9, 45.8, 41.9, 19.7. ESI-HRMS: calc. for $C_{20}H_{20}BrN_3O_2$: $[M+H]^+$ = 414.0812 m/z, found: $[M+H]^+$ = 414.0817 m/z.

*2,3,4,5-Tetrahydro-1H-pyrido[4,3-b]indole (**4**).* 1-Benzyl-4-piperidone (5.0 g, 26.4 mmol) and phenylhydrazine (2.86 g, 26.4 mmol) were dissolved in 1,4-dioxane (35 mL). The reaction was placed in an ice bath, and to it was added conc. H_2SO_4 (5 mL). The reaction was then removed from the ice bath and heated to 60 °C for 2 h. When the reaction was complete, as evidenced by TLC, it was cooled to room temperature, and the pH was adjusted to ∼13 by the addition of saturated $NaHCO_3$ (100 mL) and then by solid NaOH. The organic products were extracted with EtOAc (3 × 30 mL), washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo to yield 2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (6.13 g, 93%) as a beige solid. Further purification was not required, and the compound was used directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.48 (d, J = 7.0 Hz, 2H), 7.38 (m, 4H), 7.23 (d, J = 7.4 Hz, 1H), 7.13 (m, 2H), 3.87 (s, 2H), 3.79 (s, 2H), 2.91 (t, J = 5.8 Hz, 2H), 2.76 (t, J = 5.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.6, 136.1, 132.2, 129.2, 128.4, 127.2, 126.2, 121.1, 119.2, 117.5, 110.7, 108.7, 62.4, 50.2, 49.8, 23.7. ESI-HRMS: calc. for $C_{18}H_{18}N_2$: $[M+H]^+$ = 263.1543 m/z, found: $[M+H]^+$ = 263.1533 m/z.

2-Benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (1.0 g, 3.8 mmol) and 10% Pd/C (150 mg) were suspended in a 70% EtOH/H_2O solution (20 mL) and placed under hydrogen at atmospheric pressure. The reaction was heated to 70 °C and stirred for 24 h, after which the reaction was filtered through filter paper before cooling to remove Pd/C. The filter cake was washed with 70% EtOH/H_2O (3 × 50 mL) and the combined washes and filtrate were concentrated in vacuo. The crude product was recrystallized from a 70% EtOH/H_2O solution, and the precipitate was isolated by filtration, washed with cold MeOH, and dried in vacuo. The title compound was isolated (0.61 g, 93%) as a light-brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 7.28 (q, J = 7.7 Hz, 2H), 6.98 (m, 1H), 6.89 (m, 1H), 3.85 (s, 2H), 3.01 (t, J = 5.6 Hz, 2H), 2.66 (t, J = 5.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 135.4, 133.4, 125.7, 120.1, 118.2, 117.1, 110.7, 108.4, 43.1, 41.8, 24.2. ESI-HRMS: calc. for$C_{11}H_{12}N_2$: $[M+H]^+$ = 173.1073 m/z, found: $[M+H]^+$ = 173.1065 m/z.

*tert-Butyl 5-(4-(Methoxycarbonyl)benzyl)-3,4-dihydro-1H-pyrido-[4,3-b]indole-2(5H)-carboxylate (**5a**).* Boc anhydride (0.70 g, 3.2 mmol) was placed in a round-bottomed flask fitted with a condenser under argon, and to it was added 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) suspended in THF (20 mL), upon which the reaction turned from cloudy white to yellow. The reaction was heated at reflux with stirring for 8 h after which the reaction was allowed to cool to room temperature and concentrated in vacuo. The reaction mixture was then poured into a 1:1 mixture of EtOAc/H_2O (30 mL). The organic layer was isolated, and the aqueous layer was further extracted with EtOAc (2 × 10 mL). The organic layers were combined, washed with water (10 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639–651

chromatography (SiO_2, 0−100% EtOAc/hexane) afforded *tert*-butyl 3,4-dihydro-1*H*-pyrido[4,3-*b*]indole-2(5*H*)-carboxylate (0.66 g, 83%) as a dark yellow oil. ^{1}H NMR (400 MHz, MeOD): δ 7.36 (d, J = 7.7 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.06 (m, 1H), 6.99 (m, 1H), 4.60 (s, 2H), 3.79 (t, J = 5.6 Hz, 2H), 2.81 (t, J = 5.7 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 155.6, 136.4, 131.9, 125.4, 120.6, 118.4, 116.6, 110.4, 105.7, 79.9, 60.1, 40.6, 27.3, 22.9. ESI-HRMS: calc. for $C_{16}H_{20}N_2O_2$: $[M+H]^+$ = 273.1598 m/z, found: $[M+H]^+$ = 273.1592 m/z.

tert-Butyl 3,4-dihydro-1*H*-pyrido[4,3-*b*]indole-2(5*H*)-carboxylate (0.44 g, 1.6 mmol) was dissolved in anhydrous DMF (3 mL) and added to a suspension of potassium *tert*-butoxide (0.18 g, 1.6 mmol) in anhydrous DMF (2 mL) under argon at room temperature. The reaction mixture was heated to 80 °C for 15 min, after which 4-bromomethyl-benzoic acid methyl ester (0.37 g, 1.6 mmol) dissolved in anhydrous DMF (2 mL) was added at 80 °C. The reaction was stirred at 80 °C for 2 h, after which the reaction was cooled to room temperature and poured in cold water (15 mL). The organic products were extracted with EtOAc (3 × 30 mL), washed with water (3 × 15 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 0−80% EtOAc/hexane) afforded the title compound (0.50 g, 74%) as a dark orange oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 6.7 Hz, 1H), 7.16 (m, 3H), 7.07 (d, J = 8.1 Hz, 2H), 5.31 (s, 2H), 4.71 (s, 2H), 3.90 (s, 3H), 3.83 (s, 2H), 2.72 (s, 2H), 1.52 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 154.7, 142.5, 136.3, 133.3, 129.8, 129.1, 125.7, 125.1, 121.2, 119.2, 117.5, 108.8, 107.3, 79.5, 51.7, 45.8, 41.1, 40.3, 28.1, 22.1. ESI-HRMS: calc. for $C_{25}H_{28}N_2O_4$: $[M+H]^+$ = 421.2122 m/z, found: $[M+H]^+$ = 421.2104 m/z.

*Methyl 4-((3,4-Dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-benzoate (**5b**).* *tert*-Butyl 5-(4-(methoxycarbonyl)benzyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indole-2(5*H*)-carboxylate **5a** (0.24 g, 0.57 mmol) was dissolved in DCM (4 mL), and to it was added TFA (0.5 mL), upon which the reaction turned from light orange to dark orange. The reaction was allowed to stir at room temperature until completion, as evidenced by TLC, after which volatiles were removed in vacuo. The residue was resuspended in EtOAc (30 mL) and washed with saturated $NaHCO_3$ (2 × 15 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Further purification was not required, and the title product was obtained (0.16 g, 85%) as a dark-orange oil. ^{1}H NMR (400 MHz, MeOD): δ 7.88 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 7.6 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.07 (m, 4H), 5.34 (s, 2H), 4.15 (s, 2H), 3.84 (s, 3H), 3.28 (t, J = 5.8 Hz, 2H), 2.79 (t, J = 5.8 Hz, 2H).^{13}C NMR (100 MHz, MeOD): δ 166.7, 143.7, 136.7, 132.5, 129.5, 128.9, 126.1, 125.5, 121.3, 119.2, 117.3, 109.0, 106.2, 51.2, 45.3, 42.2, 41.1, 21.1. ESI-HRMS: calc. for $C_{20}H_{20}N_2O_2$: $[M+H]^+$ = 321.1598 m/z, found: $[M+H]^+$ = 321.1591 m/z.

*Methyl 4-((2-Ethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)benzoate (**5c**).* 2,3,4,5-Tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and acetaldehyde (0.24 mL, 4.4 mmol) were placed in a round-bottomed flask and dissolved in anhydrous MeOH at room temperature. Sodium cyanoborohydride (0.42 g, 6.7 mmol) was added in small portions over a 15 min period, and the reaction was then stirred at room temperature for 3 h. The reaction was then quenched by the addition of 2 N HCl (20 mL) and stirred for 15 min. The pH was adjusted to 12 with 1 N NaOH, and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. The isolated product was then directly subjected to general procedure B to afford the title compound (0.30 g, 29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (d, J = 6.8 Hz, 2H), 7.38 (m, 2H), 7.13 (d, J = 8.2 Hz, 2H), 7.01 (m, 2H), 5.41 (s, 2H), 3.80 (s, 3H), 3.59 (s, 2H), 2.74 (br, 2H), 2.69 (br, 2H), 2.57 (q, J = 7.2 Hz, 2H), 1.10 (t, J = 7.12 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.0, 144.1, 136.4, 134.2, 129.6, 129.2, 128.9, 126.7, 120.7, 118.9, 117.5, 109.5, 108.1, 63.0, 52.1, 51.3, 48.6, 45.1, 22.6, 12.6. ESI-HRMS: calc. for $C_{22}H_{24}N_2O_2$: $[M+H]^+$ = 349.1911 m/z, found: $[M+H]^+$ = 349.1918 m/z.

*Methyl 4-((2-Isopropyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**5d**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 2-bromopropane (0.27 mL, 2.9 mmol) according to general procedure D (0.37 g, 35%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 (d, J = 8.3 Hz, 2H), 7.39 (m, 2H), 7.14 (d, J = 8.3 Hz, 2H), 7.02 (m, 2H), 5.41 (s, 2H), 3.81 (s, 3H), 3.68 (s, 2H), 2.94 (m, 1H), 2.79 (t, J = 5.5 Hz, 2H), 2.66 (br, 2H), 1.08 (d, J = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.4, 144.6, 136.7, 134.7, 130.0, 129.3, 127.1, 126.0, 121.0, 119.2, 117.8, 109.8, 108.9, 53.9, 52.5, 49.0, 45.8, 44.5, 23.6, 18.7. ESI-HRMS: calc. for $C_{23}H_{26}N_2O_2$: $[M+H]^+$ = 363.2067 m/z, found: $[M+H]^+$ = 363.2072 m/z.

*Methyl 4-((2-Allyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)benzoate (**5e**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 3-bromoprop-1-ene (0.25 mL, 2.9 mmol) according to general procedure D (0.24 g, 23%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, J = 8.2 Hz, 2H), 7.49 (dd, J = 3.3 Hz, 3.0 Hz, 1H), 7.15 (m, 5H), 6.03 (m, 1H), 5.29 (m, 4H), 3.90 (s, 3H), 3.79 (s, 2H), 3.32 (d, J = 6.8 Hz, 2H), 2.90 (t, J = 5.7 Hz, 2H), 2.77 (t, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 142.7, 136.4, 135.2, 133.2, 129.7, 129.0, 125.8, 125.6, 120.8, 118.9, 117.6, 117.4, 108.7, 108.4, 60.8, 51.7, 49.7, 49.3, 45.9, 22.5. ESI-HRMS: calc. for $C_{23}H_{24}N_2O_2$: $[M+H]^+$ = 361.1911 m/z, found: $[M+H]^+$ = 361.1917 m/z.

*Methyl 4-((2-Benzyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)-methyl)benzoate (**5f**).* The title compound was synthesized by subjecting the intermediate obtained during the synthesis of compound **4**, 2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (0.50 g, 1.9 mmol), to general procedure B (0.38 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 6.7 Hz, 2H), 7.30 (m, 8H), 7.13 (m, 3H), 5.30 (s, 2H), 3.91 (s, 3H), 3.84 (s, 2H), 3.82 (s, 2H), 2.91 (t, J = 5.8 Hz, 2H), 2.75 (t, J = 5.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 143.1, 138.5, 136.7, 133.6, 130.1, 129.3, 129.1, 128.3, 127.1, 126.2, 126.0, 121.1, 119.3, 117.8, 109.0, 108.8, 62.3, 52.1, 50.0, 49.9, 46.3, 22.8. ESI-HRMS: calc. for $C_{27}H_{26}N_2O_2$: $[M+H]^+$ = 411.2067 m/z, found: $[M+H]^+$ = 411.2074 m/z.

*Methyl 4-((2-(3-Methoxybenzyl)-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**5g**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 1-(bromomethyl)-3-methoxybenzene (0.41 mL, 2.9 mmol) according to general procedure D (0.23 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 8.2 Hz, 2H), 7.44 (m, 3H), 7.18 (m, 5H), 7.06 (d, J = 7.6 Hz, 2H), 5.34 (br, 2H), 4.54 (m, 4H), 3.96 (s, 3H), 3.90 (s, 3H), 3.81 (s, 2H), 3.39 (br, 1H), 3.23 (br, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.5, 161.1, 141.7, 137.2, 133.2, 132.0, 130.9, 130.6, 130.4, 129.8, 129.5, 125.9, 124.7, 123.0, 120.7, 117.2, 116.6, 109.6, 101.6, 58.3, 55.3, 52.2, 49.1, 48.1, 46.6, 19.1. ESI-HRMS: calc. for $C_{28}H_{28}N_2O_3$: $[M+H]^+$ = 441.2173 m/z, found: $[M+H]^+$ = 441.2163 m/z.

*Methyl 4-((2-(4-Methoxybenzyl)-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**5h**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 1-(chloromethyl)-4-methoxybenzene (0.39 mL, 2.9 mmol) according to general procedure D (0.64 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 8.3 Hz, 2H), 7.46 (dd, J = 2.1 Hz, 4.7 Hz, 1H), 7.36 (d, J = 8.6 Hz, 2H), 7.16 (m, 5H), 6.92 (d, J = 8.6 Hz, 2H), 5.29 (s, 2H), 3.92 (s, 3H), 3.84 (s, 3H), 3.80 (s, 2H), 3.78 (s, 2H), 2.90 (t, J = 5.7 Hz, 2H), 2.75 (t, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 158.8, 143.1, 136.7, 133.7, 130.6, 130.3, 130.1, 129.3, 126.2, 126.0, 121.1, 119.3, 117.8, 113.7, 109.0, 108.9, 61.7, 55.3, 52.1, 49.9, 49.7, 46.2, 22.8. ESI-HRMS: calc. for $C_{28}H_{28}N_2O_3$: $[M+H]^+$ = 441.2173 m/z, found: $[M+H]^+$ = 441.2174 m/z.

*Methyl 4-((2-(Pyridin-4-ylmethyl)-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**5i**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 4-(bromomethyl)pyridine hydrobromide (0.73 g, 2.9 mmol) according to general procedure D (0.89 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (d, J = 5.1 Hz, 2H), 7.94 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 6.8 Hz, 1H), 7.34 (d, J = 5.2 Hz, 2H), 7.11 (m, 5H), 5.21 (s, 2H), 3.85 (s, 3H), 3.76 (d, J = 9.1 Hz, 4H), 2.84 (t, J = 5.2 Hz, 2H), 2.71 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 149.5, 147.7, 142.7, 136.4, 133.1, 129.7, 129.0, 125.8, 125.5, 123.4, 120.9,

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

119.1, 117.4, 108.8, 108.1, 60.6, 51.7, 49.9, 49.6, 45.8, 22.5. ESI-HRMS: calc. for $C_{26}H_{25}N_3O_2$: $[M+H]^+$ = 412.2020 *m/z*, found: $[M+H]^+$ = 412.2014 *m/z*.

*Methyl 4-((2-(3-Amino-3-oxopropyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzoate (**5j**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.35 g, 2.3 mmol) and 3-bromopropanamide (0.40 g, 2.3 mmol) according to general procedure D (0.23 g, 29%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (br, 1H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.36 (m, 1H), 7.04 (m, 3H), 6.96 (d, *J* = 8.2 Hz, 2H), 5.76 (br, 1H), 5.17 (s, 2H), 3.78 (s, 3H), 3.71 (s, 2H), 2.81 (m, 4H), 2.65 (br, 2H), 2.43 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 166.7, 142.9, 136.8, 133.3, 130.1, 129.4, 126.1, 125.7, 121.5, 119.6, 117.7, 109.2, 108.0, 53.2, 52.1, 49.9, 49.3, 46.3, 32.8, 22.7. ESI-HRMS: calc. for $C_{23}H_{25}N_3O_3$: $[M+H]^+$ = 392.1969 *m/z*, found: $[M+H]^+$ = 392.1973 *m/z*.

*Methyl 4-((2-(2-Amino-2-oxoethyl)-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**5k**).* The title compound was synthesized from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) and 2-bromoacetamide (0.40 g, 2.9 mmol) according to general procedure D (0.55 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 (d, *J* = 8.1 Hz, 2H), 7.37 (m, 2H), 7.28 (br, 1H), 7.16 (m, 3H), 7.01 (m, 2H), 5.43 (s, 2H), 3.81 (s, 3H), 3.71 (s, 2H), 3.13 (s, 2H), 2.84 (br, 2H), 2.76 (br, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.1, 166.0, 144.1, 136.3, 133.9, 129.6, 128.6, 126.8, 125.4, 120.8, 119.0, 117.5, 109.6, 107.6, 60.7, 52.2, 50.3, 49.4, 45.5, 22.3. ESI-HRMS: calc. for $C_{22}H_{23}N_3O_3$: $[M+H]^+$ = 378.1812 *m/z*, found: $[M+H]^+$ = 378.1824 *m/z*.

*Methyl 4-((2-(4-Amino-4-oxobutyl)-3,4-dihydro-1H-pyrido[4,3-b]-indol-5(2H)-yl)methyl)benzoate (**5l**).* 2,3,4,5-Tetrahydro-1*H*-pyrido-[4,3-*b*]indole **4** (0.50 g, 2.9 mmol) was placed under argon in a two-necked round-bottomed flask fitted with a condenser. Anhydrous MeCN (5 mL) was added, followed by the addition of Et_3N (2 mol equiv) at room temperature. The reaction was heated to 60 °C; then, methyl 4-bromobutanoate (1 mol equiv) was added in anhydrous MeCN (2 mL). The reaction was stirred for 2 h at 60 °C and then poured into a 1:1 mixture of EtOAc/H_2O (20 mL). The organic layer was isolated, and the aqueous layer was further extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Methyl 4-(3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-2(5*H*)-yl)butanoate was isolated by automated column chromatography (0.66 g, 84%). ^{1}H NMR (400 MHz, MeOD): δ 8.80 (br, 1H), 7.46 (br, 1H), 7.14 (br, 3H), 3.77 (s, 2H), 3.71 (s, 3H), 2.83 (br, 2H), 2.69 (t, *J* = 7.2 Hz, 2H), 2.59 (br, 2H), 2.46 (t, *J* = 7.3 Hz, 2H), 2.03 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ 174.2, 136.3, 132.4, 126.1, 120.9, 119.0, 117.3, 110.9, 107.8, 57.2, 51.6, 50.8, 40.5, 32.1, 23.4, 22.7. ESI-HRMS: calc. for $C_{16}H_{20}N_2O_2$: $[M+H]^+$ = 273.1598 *m/z*, found: $[M+H]^+$ = 273.1607 *m/z*.

Methyl 4-(3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-2(5*H*)-yl)butanoate (0.66 g, 2.4 mmol) was dissolved in MeOH (15 mL) and cooled to 0 °C. A 25% aqueous NH_3 solution (15 mL) was added and the reaction was stirred at 0 °C for 2 h after which the reaction was allowed to come to room temperature and then refluxed for an additional 12 h. After TLC indicated that the reaction was complete, it was concentrated in vacuo and then poured into a 1:1 mixture of EtOAc/H_2O (60 mL). The organic fraction was isolated, and the aqueous layer was further extracted with EtOAc (2 × 15 mL). Combined organic fractions were washed with water (15 mL) and brine (10 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Further purification was not required, and the isolated product, 4-(3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-2(5*H*)-yl)butanamide (0.34 g, 54%), was subjected to the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.75 (s, 1H), 7.29 (m, 3H), 6.96 (m, 2H), 6.73 (br, 1H), 3.56 (s, 2H), 2.76 (br, 4H), 2.53 (t, *J* = 7.06 Hz, 2H), 2.14 (t, *J* = 7.3 Hz, 2H), 1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.8, 136.4, 133.3, 126.1, 120.5, 118.6, 117.4, 111.2, 107.6, 57.4, 50.9, 49.7, 33.5, 24.0, 23.4. ESI-HRMS: calc for $C_{15}H_{19}N_3O$: $[M+H]^+$ = 258.1601 *m/z*, found: $[M+H]^+$ = 258.1598 *m/z*.

The title compound was synthesized from 4-(3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-2(5*H*)-yl)butanamide (0.34 g, 1.3 mmol) according to general procedure B (0.21 g, 39%). ^{1}H NMR (400 MHz, MeOD): δ 7.83 (d, *J* = 8.2 Hz, 2H), 7.41 (d, *J* = 6.9 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.04 (m, 4H), 5.21 (s, 2H), 3.80 (s, 3H), 3.71 (s, 2H), 2.80 (br, 2H), 2.68 (br, 2H), 2.61 (t, *J* = 7.1 Hz, 2H), 2.28 (t, *J* = 7.3 Hz, 2H), 1.96 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ 177.0, 166.7, 143.8, 136.9, 133.3, 129.5, 128.9, 126.1, 125.8, 121.0, 119.1, 117.3, 109.0, 107.5, 56.9, 51.2, 50.2, 49.3, 45.4, 32.9, 22.7, 22.0. ESI-HRMS: calc. for $C_{24}H_{27}N_3O_3$: $[M+H]^+$ = 406.2125 *m/z*, found: $[M+H]^+$ = 406.2133 *m/z*.

*tert-Butyl 5-(4-(Hydroxycarbamoyl)benzyl)-3,4-dihydro-1H-pyrido[4,3-b]indole-2(5H)-carboxylate (**6a**).* The title compound was synthesized from *tert*-butyl 5-(4-(methoxycarbonyl)benzyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indole-2(5*H*)-carboxylate **5a** (0.11 g, 0.3 mmol) according to general procedure C and isolated as an orange solid (7 mg, 7%). ^{1}H NMR (400 MHz, MeOD): δ 7.65 (d, *J* = 8.3 Hz, 2H), 7.46 (d, *J* = 7.4 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 1H), 7.09 (m, 4H), 5.41 (s, 2H), 4.66 (s, 2H), 3.81 (br, 2H), 2.75 (br, 2H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 166.4, 155.5, 142.3, 136.9, 133.3, 131.1, 127.1, 126.0, 125.3, 121.1, 119.1, 117.1, 109.0, 106.8, 80.0, 45.3, 41.6, 40.8, 27.3, 22.0. ESI-HRMS: calc. for $C_{24}H_{27}N_3O_4$: $[M+H]^+$ = 422.2074 *m/z*, found: $[M+H]^+$ = 422.2073 *m/z*.

*4-((3,4-Dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**6b**).* The title compound was synthesized from methyl 4-((3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)-benzoate **5b** (0.38 g, 1.2 mmol) according to general procedure C, and the TFA salt was isolated as a yellow solid (152 mg, 38%). ^{1}H NMR (400 MHz, MeOD): δ 7.68 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.13 (m, 3H), 5.49 (s, 2H), 4.51 (s, 2H), 3.65 (t, *J* = 5.9 Hz, 2H), 3.09 (t, *J* = 5.5 Hz, 2H).^{13}C NMR (100 MHz, MeOD): δ 161.2, 141.6, 137.0, 131.4, 130.8, 127.2, 126.2, 124.9, 122.1, 119.8, 117.3, 109.4, 102.2, 46.9, 41.5, 40.8, 19.2. ESI-HRMS: calc. for $C_{19}H_{19}N_3O_2$: $[M+H]^+$ = 322.1550 *m/z*, found: $[M+H]^+$ = 322.1549 *m/z*.

*4-((2-Ethyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**6c**).* The title compound was synthesized from methyl 4-((2-ethyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5c** (0.30 g, 0.8 mmol) according to general procedure C, and the TFA salt was isolated as a yellow-orange solid (26 mg, 9%). ^{1}H NMR (400 MHz, MeOD): δ 7.67 (d, *J* = 8.3 Hz, 2H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.21 (t, *J* = 7.1 Hz, 1H), 7.14 (m, 3H), 5.48 (q, *J* = 14.0 Hz, 2H), 4.80 (d, *J* = 14.1 Hz, 1H), 4.39 (d, *J* = 14.2 Hz, 1H), 3.92 (br, 1H), 3.56 (br, 1H), 3.45 (q, *J* = 7.4 Hz, 2H), 3.16 (br, 2H), 1.49 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 141.1, 136.6, 132.0, 131.6, 127.3, 126.6, 124.6, 121.9, 119.7, 118.0, 110.2, 102.3, 50.4, 48.8, 47.8, 45.7, 19.8, 9.5. ESI-HRMS: calc. for $C_{21}H_{23}N_3O_2$: $[M+H]^+$ = 350.1863 *m/z*, found: $[M+H]^+$ = 350.1871 *m/z*.

*N-Hydroxy-4-((2-isopropyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA (**6d**).* The title compound was synthesized from methyl 4-((2-isopropyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5d** (0.21 g, 0.6 mmol) according to general procedure C, and the TFA salt was isolated as an orange solid (35 mg, 17%). ^{1}H NMR (400 MHz, MeOD): δ 7.67 (d, *J* = 8.3 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 7.21 (t, *J* = 7.1 Hz, 1H), 7.15 (m, 3H), 5.48 (q, *J* = 16.3 Hz, 2H), 4.59 (dd, *J* = 13.8 Hz, 29.5 Hz, 2H), 3.86 (m, 1H), 3.81 (m, 1H), 3.57 (m, 1H), 3.17 (br, 2H), 1.51 (d, *J* = 3.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 141.1, 136.6, 132.0, 131.7, 127.3, 126.7, 124.7, 122.0, 119.7, 118.0, 110.2, 102.6, 57.1, 46.2, 45.7, 44.5, 20.3, 17.1, 16.4. ESI-HRMS: calc. for $C_{22}H_{25}N_3O_2$: $[M+H]^+$ = 364.2020 *m/z*, found: $[M+H]^+$ = 364.2029 *m/z*.

*4-((2-Allyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**6e**).* The title compound was synthesized from methyl 4-((2-allyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)-methyl)benzoate **5e** (0.21 g, 0.6 mmol) according to general procedure C, and the TFA salt was isolated as a yellow solid (49 mg, 25%). ^{1}H NMR (400 MHz, MeOD): δ 7.68 (d, *J* = 8.1 Hz, 2H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.15 (m, 3H), 6.11 (m, 1H), 5.70 (m, 2H), 5.49 (d, *J* = 11.3 Hz, 2H), 4.74 (br, 1H), 4.44 (br, 1H), 4.03 (d, *J* = 6.8 Hz, 2H), 3.91 (br,

1H), 3.56 (br, 1H), 3.16 (s, 2H). ^{13}C NMR (100 MHz, MeOD): δ 165.8, 141.1, 136.9, 131.1, 130.2, 126.8, 125.9, 125.8, 125.3, 124.3, 121.9, 119.6, 117.0, 109.2, 101.6, 57.5, 48.9, 48.5, 45.3, 19.3. ESI-HRMS: calc. for $C_{22}H_{23}N_3O_2$: $[M+H]^+$ = 362.1863 m/z, found: $[M+H]^+$ = 362.1860 m/z.

4-((2-Benzyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA ***(6f)***. The title compound was synthesized from methyl 4-((2-benzyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5f** (0.18 g, 0.4 mmol) according to general procedure C, and the TFA salt was isolated as an off-white solid (35 mg, 19%). ^{1}H NMR (400 MHz, MeOD): δ 7.66 (d, J = 8.4 Hz, 2H), 7.54 (m, 5H), 7.46 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.19 (m, 1H), 7.12 (m, 3H), 5.47 (s, 2H), 4.57 (m, 4H), 3.75 (br, 2H), 3.14 (s, 2H). ^{13}C NMR (100 MHz, MeOD): δ 147.5, 137.2, 131.4, 130.8, 130.7, 130.0, 129.4, 129.1, 127.2, 126.2, 124.7, 122.2, 119.9, 117.2, 109.5, 101.9, 59.2, 49.3, 48.9, 45.6, 19.7. ESI-HRMS: calc. for $C_{26}H_{25}N_3O_2$: $[M+H]^+$ = 412.2020 m/z, found: $[M+H]^+$ = 412.2034 m/z.

N-Hydroxy-4-((2-(3-methoxybenzyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA ***(6g)***. The title compound was synthesized from methyl 4-((2-(3-methoxybenzyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5g** (0.23 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as a beige solid (112 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.17 (s, 1H), 10.33 (s, 1H), 9.02 (s, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.47 (m, 3H), 7.15 (m, 5H), 7.07 (m, 2H), 5.46 (dd, J = 17.3 Hz, 25.4, 2H), 4.51 (s, 2H), 4.41 (br, 1H), 3.80 (s, 3H), 3.49 (br, 1H), 3.17 (m, 3H), 3.06 (br, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 159.5, 141.1, 136.6, 132.0, 131.7, 131.3, 130.1, 127.3, 126.7, 124.5, 123.3, 122.0, 119.8, 117.8, 116.6, 115.3, 110.2, 101.9, 58.2, 55.3, 48.7, 48.4, 45.7, 19.7. ESI-HRMS: calc. for $C_{27}H_{27}N_3O_3$: $[M+H]^+$ = 442.2125 m/z, found: $[M+H]^+$ = 442.2104 m/z.

N-Hydroxy-4-((2-(4-methoxybenzyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA ***(6h)***. The title compound was synthesized from methyl 4-((2-(4-methoxybenzyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5h** (0.23 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as a beige solid (123 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1H), 10.13 (s, 1H), 9.05 (br, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.49 (t, J = 8.9 Hz, 4H), 7.11 (m, 6H), 5.46 (dd, J = 16.8 Hz, 28.1 Hz, 2H), 4.50 (br, 1H), 4.47 (s, 3H), 4.37 (br, 1H), 3.78 (s, 2H), 3.59 (m, 1H), 3.46 (m, 1H), 3.17 (m, 1H), 3.02 (m, 1H). ^{13}C NMR (100 MHz,): δ 163.8, 160.3, 141.0, 136.6, 132.8, 132.0, 131.7, 127.3, 126.7, 124.5, 122.0, 121.7, 119.8, 117.8, 114.4, 110.2, 101.9, 57.9, 55.3, 48.5, 48.0, 45.7, 19.7. ESI-HRMS: calc. for $C_{27}H_{27}N_3O_3$: $[M+H]^+$ = 442.2125 m/z, found: $[M+H]^+$ = 442.2140 m/z.

N-Hydroxy-4-((2-(pyridin-4-ylmethyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide·TFA ***(6i)***. The title compound was synthesized from methyl 4-((2-(pyridin-4-ylmethyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5i** (0.82 g, 2.0 mmol) according to general procedure C, and the TFA salt was isolated as a light-yellow solid (327 mg, 40%). ^{1}H NMR (400 MHz, MeOD): δ 8.81 (d, J = 5.8 Hz, 2H), 7.85 (d, J = 6.1 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.1 Hz, 1H), 7.12 (m, 3H), 5.46 (s, 2H), 4.71 (s, 2H), 4.59 (s, 2H), 3.80 (t, J = 5.8 Hz, 2H), 3.17 (t, J = 5.5 Hz, 2H). ^{13}C NMR (100 MHz, MeOD): δ 166.2, 147.9, 142.3, 141.5, 137.2, 131.4, 130.6, 127.2, 126.7, 126.2, 124.7, 122.2, 119.9, 117.3, 109.5, 101.8, 57.5, 50.1, 49.6, 45.6, 19.7. ESI-HRMS: calc. for $C_{25}H_{24}N_4O_2$: $[M+H]^+$ = 413.1972 m/z, found: $[M+H]^+$ = 413.1979 m/z.

4-((2-(3-Amino-3-oxopropyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA ***(6j)***. The title compound was synthesized from methyl 4-((2-(3-amino-3-oxopropyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5j** (0.13 g, 0.35 mmol) according to general procedure C, and the TFA salt was isolated as a light-yellow solid (111 mg, 43%). ^{1}H NMR (400 MHz, MeOD): δ 7.67 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 7.7 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.17 (m, 4H), 5.47 (br, 2H), 4.80 (br, 1H), 4.49 (br, 1H), 3.92 (br, 1H), 3.65 (m, 3H), 3.17 (br, 2H), 2.89 (t, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, MeOD): δ 172.7, 166.2, 141.5, 137.3, 131.4, 130.5, 127.2, 126.2, 124.7, 122.2, 119.9, 117.4, 109.5, 101.8, 51.7, 50.0, 49.3, 45.6, 28.8, 19.5. ESI-HRMS: calc. for $C_{22}H_{24}N_4O_3$: $[M+H]^+$ = 393.1921 m/z, found: $[M+H]^+$ = 393.1920 m/z.

4-((2-(2-Amino-2-oxoethyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA ***(6k)***. The title compound was synthesized from methyl 4-((2-(2-amino-2-oxoethyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5k** (0.50 g, 1.3 mmol) according to general procedure C, and the TFA salt was isolated as a light-yellow solid (75 mg, 15%). ^{1}H NMR (400 MHz, MeOD): δ 7.69 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 7.7 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.21 (t, J = 7.1 Hz, 1H), 7.15 (m, 3H), 5.49 (s, 2H), 4.66 (br, 2H), 4.19 (s, 2H), 3.79 (br, 2H), 3.18 (br, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.6, 163.9, 141.5, 136.9, 132.3, 131.8, 127.7, 126.9, 125.0, 122.3, 120.1, 118.3, 110.5, 102.2, 55.5, 50.3, 49.7, 46.0, 19.8. ESI-HRMS: calc. for $C_{21}H_{22}N_4O_3$: $[M+H]^+$ = 379.1765 m/z, found: $[M+H]^+$ = 379.1778 m/z.

4-((2-(4-Amino-4-oxobutyl)-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)-N-hydroxybenzamide·TFA ***(6l)***. The title compound was synthesized from methyl 4-((2-(4-amino-4-oxobutyl)-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzoate **5l** (0.21 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as a light-yellow solid (42 mg, 20%). ^{1}H NMR (400 MHz, MeOD): δ 7.68 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.21 (t, J = 7.2 Hz, 1H), 7.14 (m, 3H), 5.48 (s, 2H), 4.82 (br, 1H), 4.44 (br, 1H), 3.94 (br, 1H), 3.59 (br, 1H), 3.42 (t, J = 7.4 Hz, 2H), 3.17 (br, 2H), 2.51 (t, J = 6.6 Hz, 2H), 2.16 (m, 2H). ^{13}C NMR (100 MHz, MeOD): δ 176.0, 166.2, 141.5, 137.3, 131.4, 130.6, 127.2, 126.2, 124.8, 122.2, 119.9, 117.4, 109.5, 102.1, 55.7, 49.8, 49.3, 45.6, 31.7, 19.8, 19.7. ESI-HRMS: calc. for $C_{23}H_{26}N_4O_3$: $[M+H]^+$ = 407.2078 m/z, found: $[M+H]^+$ = 407.2086 m/z.

tert-Butyl 9-(4-(Methoxycarbonyl)benzyl)-3,4-dihydro-1H-pyrido-[3,4-b]indole-2(9H)-carboxylate ***(7)***. Boc anhydride (7.60 g, 34.8 mmol) was placed in a round-bottomed flask fitted with a condenser under argon, and to it was added 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (5.00 g, 29.0 mmol) suspended in THF (100 mL), upon which the reaction turned from cloudy white to yellow. The reaction was then heated to reflux and stirred for 8 h, after which the reaction was allowed to cool to room temperature and concentrated in vacuo. The reaction mixture was poured into a 1:1 mixture of EtOAc/H_2O (100 mL). The organic layer was isolated, and the aqueous layer was further extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 0−100% EtOAc/hexane) afforded *tert*-butyl 3,4-dihydro-1*H*-pyrido[3,4-*b*]indole-2(9*H*)-carboxylate (7.32 g, 93%) as a dark-yellow oil.

tert-Butyl 3,4-dihydro-1*H*-pyrido[3,4-*b*]indole-2(9*H*)-carboxylate (5.00 g, 18.4 mmol) was dissolved in anhydrous DMF (30 mL) and added to a suspension of potassium *tert*-butoxide (2.06 g, 18.4 mmol) in anhydrous DMF (20 mL) under argon at room temperature. The reaction mixture was heated to 80 °C for 15 min, after which 4-bromomethyl-benzoic acid methyl ester (4.21 g, 18.4 mmol) dissolved in anhydrous DMF (20 mL) was added. The reaction was stirred at 80 °C for 2 h, after which the reaction was cooled to room temperature and poured into cold water (75 mL). The organic products were extracted with EtOAc (3 × 30 mL), washed with water (3 × 20 mL) and brine (15 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 0−80% EtOAc/hexane) afforded the title compound (3.78 g, 49%) as a dark-orange oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 6.8 Hz, 1H), 7.18 (m, 5H), 5.27 (s, 2H), 4.54 (br, 2H), 3.91 (s, 3H), 3.79 (br, 2H), 2.88 (br, 2H), 1.52 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 154.7, 142.2, 136.5, 131.5, 129.8, 129.2, 126.5, 125.9, 121.3, 119.2, 117.9, 108.7, 108.3, 79.8, 51.7, 46.1, 42.1, 40.5, 28.1, 21.1. ESI-HRMS: calc. for $C_{25}H_{28}N_2O_4$: $[M+H]^+$ = 421.2122 m/z, found: $[M+H]^+$ = 421.2124 m/z.

Methyl 4-((3,4-Dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)methyl)-benzoate ***(8a)***. *tert*-Butyl 9-(4-(methoxycarbonyl)benzyl)-3,4-dihydro-1*H*-pyrido[3,4-*b*]indole-2(9*H*)-carboxylate 7 (3.00 g, 7.1 mmol) was dissolved in DCM (24 mL), and to it was added TFA (3 mL),

dx.doi.org/10.1021/jm200773h | J. Med. Chem. 2012, 55, 639−651

upon which the reaction turned from light orange to dark orange. The reaction was allowed to stir for 2 h at room temperature, after which volatiles were removed in vacuo. The residue was resuspended in EtOAc (50 mL) and washed with saturated $NaHCO_3$ (2 × 20 mL) and brine (15 mL), dried with Na_2SO_4, filtered, and concentrated in vacuo. Further purification was not required, and the title product was obtained (1.75 g, 76%) as a dark-orange oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 7.8 Hz, 2H), 7.57 (d, J = 7.1 Hz, 1H), 7.24 (m, 1H), 7.19 (m, 2H), 7.10 (d, J = 8.1 Hz, 2H), 5.29 (s, 2H), 4.53 (s, 2H), 3.91 (s, 3H), 3.78 (br, 2H), 2.87 (br, 2H). ^{13}C NMR (100 MHz, MeOD): δ 164.8, 141.9, 134.9, 130.5, 127.6, 127.1, 125.3, 124.1, 119.2, 117.0, 115.6, 106.9, 106.0, 49.3, 43.6, 40.9, 39.2, 19.1. ESI-HRMS: calc. for $C_{20}H_{20}N_2O_2$: $[M+H]^+$ = 321.1598 m/z, found: $[M+H]^+$ = 321.1610 m/z.

*Methyl 4-((2-Methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)-methyl)benzoate (**8b**).* The title compound was synthesized from methyl 4-((3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-9(2*H*)-yl)methyl)-benzoate **8a** (0.50 g, 1.6 mmol) and iodomethane (0.10 mL, 1.6 mmol) according to general procedure E (0.18 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, J = 8.2 Hz, 2H), 7.54 (m, 1H), 7.16 (m, 3H), 7.15 (d, J = 8.1 Hz, 2H), 5.25 (s, 2H), 3.89 (s, 3H), 3.53 (s, 2H), 2.90 (m, 2H), 2.80 (m, 2H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 142.8, 136.9, 132.4, 130.2, 129.4, 126.9, 126.1, 121.5, 119.5, 118.3, 109.1, 107.9, 52.7, 52.1, 51.3, 46.3, 45.5, 21.3. ESI-HRMS: calc. for $C_{21}H_{22}N_2O_2$: $[M+H]^+$ = 335.1727 m/z, found: $[M+H]^+$ = 335.1724 m/z.

*Methyl 4-((2-Benzyl-3,4-dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)-methyl)benzoate (**8c**).* The title compound was synthesized from methyl 4-((3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-9(2*H*)-yl)methyl)-benzoate **8a** (0.50 g, 1.6 mmol) and benzyl bromide (0.19 mL, 1.6 mmol) according to general procedure E (0.36 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.1 Hz, 2H), 7.56 (m, 1H), 7.33 (m, 4H), 7.17 (m, 4H), 7.05 (d, J = 8.0 Hz, 2H), 5.21 (s, 2H), 3.92 (s, 3H), 3.77 (s, 2H), 3.61 (s, 2H), 2.94 (br, 2H), 2.91 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 142.9, 138.4, 136.9, 133.2, 130.1, 129.4, 129.0, 128.4, 127.2, 127.1, 126.2, 121.3, 119.4, 118.2, 109.0, 108.5, 61.9, 52.1, 50.6, 49.3, 46.3, 21.3. ESI-HRMS: calc. for $C_{27}H_{26}N_2O_2$: $[M+H]^+$ = 411.2067 m/z, found: $[M+H]^+$ = 411.2071 m/z.

*4-((3,4-Dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**9a**).* The title compound was synthesized from methyl 4-((3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-9(2*H*)-yl)methyl)-benzoate **8a** (0.10 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as an off-white solid (57 mg, 57%). ^{1}H NMR (400 MHz, MeOD): δ 7.67 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 7.3 Hz, 1H), 7.46 (m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 5.47 (s, 2H), 4.39 (s, 2H), 3.62 (t, J = 5.7 Hz, 2H), 3.15 (t, J = 5.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.4, 143.8, 137.1, 130.0, 129.2, 128.2, 127.3, 126.2, 122.6, 120.1, 118.7, 110.4, 106.7, 52.6, 46.2, 41.8, 18.6. ESI-HRMS: calc. for $C_{19}H_{19}N_3O_2$: $[M+H]^+$ = 322.1550 m/z, found: $[M+H]^+$ = 322.1556 m/z.

*N-Hydroxy-4-((2-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)methyl)benzamide·TFA (**9b**).* The title compound was synthesized from methyl 4-((2-methyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-9(2*H*)-yl)methyl)benzoate **8b** (0.18 g, 0.5 mmol) according to general procedure C, and the TFA salt was isolated as an off-white solid (26 mg, 15%). ^{1}H NMR (400 MHz, MeOD): δ 7.70 (d, J = 6.6 Hz, 2H), 7.58 (d, J = 7.9 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.22 (t, J = 7.0 Hz, 1H), 7.13 (m, 3H), 5.46 (s, 2H), 4.66 (br, 1H), 4.35 (br, 1H),3.81 (br, 1H), 3.50 (br, 1H), 3.20 (t, J = 6.5 Hz, 2H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 166.3, 141.4, 137.5, 131.2, 127.3, 126.2, 126.1, 125.9, 122.5, 119.8, 118.1, 109.5, 106.0, 52.1, 49.8, 45.8, 41.9, 18.3. ESI-HRMS: calc. for $C_{20}H_{21}N_3O_2$: $[M+H]^+$ = 336.1707 m/z, found: $[M+H]^+$ = 336.1700 m/z.

*4-((2-Benzyl-3,4-dihydro-1H-pyrido[3,4-b]indol-9(2H)-yl)methyl)-N-hydroxybenzamide·TFA (**9c**).* The title compound was synthesized from methyl 4-((2-benzyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-9(2*H*)-yl)methyl)benzoate **8c** (0.36 g, 0.9 mmol) according to general procedure C, and the TFA salt was isolated as an off-white solid (152 mg, 42%). ^{1}H NMR (400 MHz, MeOD): δ 7.60 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.47 (m, 5H), 7.74 (d, J = 8.2 Hz, 1H), 7.21 (dt, J = 0.9 Hz, 7.2 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.03 (d, J = 8.2 Hz, 2H), 5.33 (s, 2H), 4.51 (s, 2H), 4.36 (br, 2H), 3.68 (br, 2H), 3.16 (t, J = 5.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ164.2, 141.2, 137.3, 132.4, 131.6, 130.2, 130.1, 129.3, 127.7, 127.4, 127.0, 125.9, 122.6, 120.1, 118.9, 110.5, 106.5, 58.4, 49.3, 47.5, 46.3, 18.4. ESI-HRMS: calc. for $C_{26}H_{25}N_3O_2$: $[M+H]^+$ = 412.2020 m/z, found: $[M+H]^+$ = 412.2034 m/z.

HDAC Inhibition Assays. HDAC inhibition assays were performed by the Reaction Biology Corporation (Malvern, PA) using human, full-length recombinant HDAC1 and 6 isolated from a baculovirus expression system in Sf9 cells. An acetylated, fluorogenic peptide derived from residues 379−382 of p53 ($RHKK_{Ac}$) was used as the substrate in the assays. The reaction buffer contained 50 mM Tris-HCl pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/mL BSA, and a final concentration of 1% DMSO. The enzyme was delivered into wells of the reaction plate, and compounds were delivered in 100% DMSO into the enzyme mixture by Acoustic Technology (Echo550; nanoliter range). The plates were spun down and preincubated for 5−10 min. The substrate was then delivered to all reaction wells to initiate the reaction, and the reaction was incubated for 2 h at 30 °C. After incubation, developer and Trichostatin A were added to quench the reaction and generate fluorescence. Then, kinetic measurements were taken for 1.5 h in 15 min intervals to ensure that development was complete. End-point readings were taken for analysis after the development reached a plateau. Dose−response curves were generated, and the IC_{50} for each compound was extrapolated from the generated plots. (Ten-dose IC_{50} curves were generated using a three-fold serial dilution pattern starting with concentrations of 30 μM.) All IC_{50} determinations were done in duplicate, and the values expressed in this Article are the average of both trials ± the standard deviation.

Treg Mitotic Suppression Assays. Spleens and lymph nodes from C57BL/6 mice were harvested, and a single-cell suspension of lymphocytes was prepared. We used magnetic beads (Miltenyi Biotec, Auburn, CA) for cell separation into effector T-cells ($CD4^+CD25^-$, Teffs), regulatory T-cells ($CD4^+CD25^+$, Tregs), and antigen-presenting cells ($Thy1.2^-$, APC). Teffs were CFSE-labeled and then incubated in the presence of irradiated APC and varying concentrations of each compound at different Treg/Teff ratios.[17] CD3ε mAb (1 μg/mL) was added to stimulate cell division, and after 3 to 4 days of incubation, the percent of dividing cells was determined by CFSE dilution.[8] To eliminate false positives due to intrinsic compound toxicity, we defined working concentrations of each compound as those in which the difference between the number of Teffs undergoing mitosis in the presence and absence of drug and with no Tregs present was <10%. CFSE−dilution plots were generated for each experiment, and the percentage of cells undergoing mitosis is displayed in the top left of each plot (Figure 1 and Supplementary Figure 1 of the Supporting Information). Raw data regarding the percentage of cell divisions were standardized for each compound and its respective control at each tested concentration by applying min−max normalization in GraphPad Prism 5 (La Jolla, CA). Standardized cell division data were converted to percent mitotic suppression (% mitotic suppression = 100 − % mitotic division) and then plotted against the ratio of Teffs/Tregs (Figure 2 and Supplementary Figure 2 of the Supporting Information). The area under the standardized suppression curves was calculated using GraphPad Prism 5, and the AUC ratios for each compound versus the control were calculated at multiple working concentrations (Figure 3). Compounds exhibiting AUC ratios greater than 1.25 were considered to be significant.[8]

Homeostatic Proliferation Assays. We isolated HDAC6−/− CD4+CD25− Teffs from 3 HDAC6−/− mice and WT CD4+CD25+ Tregs from 19 WT C57BL/6 mice. HDAC6−/− cells were labeled with CFSE and injected into 12 RAG1−/− mice along with Tregs; each mouse received 4 × 10^5 Tregs and 8 × 10^5 Teffs intravenously. Mice were divided into four groups (n = 3/group) and treated daily for 7 days with DMSO control, Tubastatin A, **3h** or **6i** (1 mg/kg, i.p.). On day 8, peripheral lymph nodes were collected, and the total number of viable cells was calculated separately for each mouse. After cell staining with CD4 and Foxp3 monoclonal antibodies, the absolute

number of lymph node CD4+ Foxp3− CFSE+/low cells in each mouse was determined by flow cytometry. Data from each group of mice were expressed as mean ± SEM, and differences between groups were compared using the Mann−Whitney U test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional HDAC isoform inhibition data, additional CFSE-dilution plots, and additional standardized suppression curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

Kyle Butler was supported by an American Chemical Society Division of Medicinal Chemistry predoctoral fellowship.

■ ABBREVIATIONS USED

HDAC, histone/protein deacetylase; HAT, histone/protein acetyltransferase; HDACi, histone deacetylase inhibitors; Foxp3, forkhead box P3; Tregs, T-regulatory cells; CTLA-4, cytotoxic T-lymphocyte antigen 4; Teffs, T-effector cells; CFSE, carboxyfluorescein succinimidyl ester; AUC, area under the curve

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