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Brief Article

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Improved Synthesis and Structural Reassignment of MC1568: A Class IIa Selective HDAC Inhibitor

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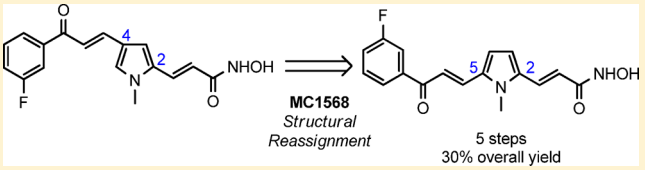
Supporting Information

ABSTRACT: An improved synthesis and structural reassignment of the class IIa selective histone deacetylase (HDAC) inhibitor MC1568 are described.



INTRODUCTION

The dysregulation of histone deacetylase (HDAC) enzymes is associated with a variety of disease states including cancer, cellular metabolism disorders, and inflammation.[1−3] It is therefore no surprise that the design and synthesis of HDAC inhibitors is of interest for the treatment of these conditions.[1,4,5] In humans, the HDAC family comprises 18 enzymes categorized into four distinct classes (classes I, IIa/IIb, III, and IV). Each class is defined according to structural similarities, gene expression patterns, cellular localization, tissue distribution, and number of active sites.[1,4−8]

It has been reported that HDAC5 represses important glucose transporters in the skeletal muscle.[9] Hence, selectively inhibiting the HDAC5 isoform may be beneficial for the regulation of skeletal muscle metabolism and offer a new pathway to treat conditions such as diabetes.[4,9]

To the best of our knowledge, rocilinostat is the only class II isoform selective HDAC inhibitor to successfully reach clinical trials.[10] The lack of potent class II HDAC inhibitors currently in clinical trials is most likely due to the fact that only a handful of class IIb (HDAC6) selective inhibitors have been reported and even fewer for the selective inhibition of class IIa enzymes.[11] In 2005 the group of Mai reported MC1568 (**1**), one of the only known selective inhibitors of class IIa HDACs to be documented in the literature (Figure 1).[12,13]

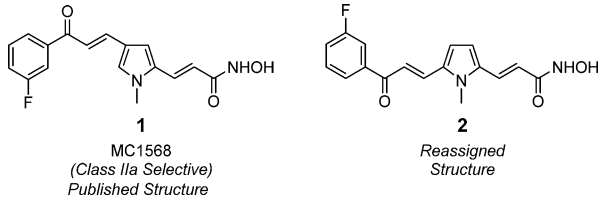


Figure 1. Published structure of class II selective HDAC inhibitor, MC1568 (**1**), and its actual structure (**2**).

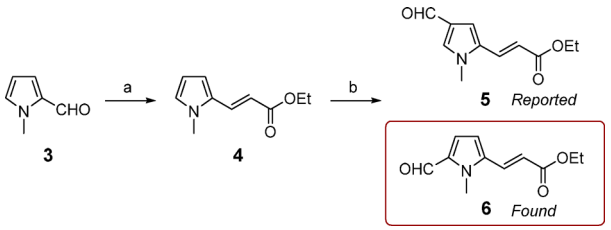
In this Brief Article we present the reassigned structure of MC1568 and a robust procedure for its synthesis.[12]

RESULTS AND DISCUSSION

Compound MC1568 was first reported among a series of similarly disubstituted pyrroles and has since been used as a biological tool.[12,14] The compound has also been the starting point for a number of structure−activity relationship and molecular modeling studies.[13,14]

During efforts to repeat the synthesis of MC1568 several peculiarities with the reported structure of formylated pyrrole **5** were noted (Scheme 1). Despite using a modified synthesis,[15] a

Scheme 1. Synthesis of Aldehyde 6[α]



[α]Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, ^tBuOK, THF, 21 °C, 24 h (74%); (b) $(COCl)_2$, DMF, microwave, 100 °C, 14 min (83%).

product spectroscopically identical to that reported by Mai was obtained.[12] However, a coupling constant of 4.5 Hz for the pyrrole protons (δ_H 6.65 and 6.90) was recorded in the ^1H NMR spectra, indicative of $^3J_{H3,H4}$ coupling. Typical $^4J_{H3,H5}$ coupling constant for pyrrole is approximately 1.5 Hz.[16]

Moreover, it was observed that the ^1H NMR resonance corresponding to the *N*-methyl group appeared to encounter significant anisotropic deshielding from the newly installed

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carbonyl (δ_H 3.68 in the pyrrole **4** to δ_H 4.03 in the formylated pyrrole **6**; $\Delta\delta_H$ = 0.35). A comparable trend was observed for a similar *N*-methylpyrrole system reported by Rapoport et al., in which both 4- and 5-substituted pyrroles were isolated (Figure 2).[17]

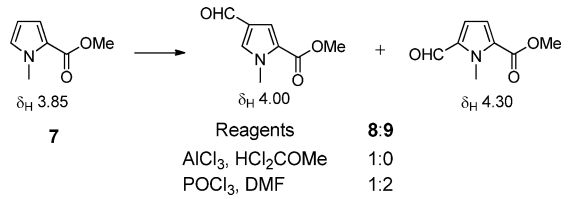


Figure 2. Synthesis of 2,4- and 2,5-substituted pyrroles as reported by Rapoport (δ_H refers to N-Me).[17]

Rapoport identified that when Friedel–Crafts conditions (dichloromethyl methyl ether (HCl_2COMe), $AlCl_3$) were employed, the aldehyde was introduced exclusively to the 4-position of the pyrrole ring (**8**), and little change (from δ_H 3.85 to δ_H 4.00, $\Delta\delta_H$ = 0.15) in the chemical shifts corresponding to the *N*-methyl protons was observed.[17] This trend has also been observed in other reports in which 2,4-substituted pyrroles were synthesized using similar Friedel–Crafts conditions.[18,19]

In contrast, Vilsmeier–Haack formylation of ester (**7**) gave a 1:2 mixture of 4- and 5-formylated pyrroles. The *N*-methyl protons of the 5-formylated pyrrole (**9**) are assigned to the singlet at δ_H 4.30 (from δ_H 3.85, $\Delta\delta_H$ = 0.45), due to the additional anisotropic deshielding of the aldehyde.[17]

The HSQC spectrum of aldehyde **6** synthesized in this study showed that the two pyrrole ^{1}H NMR resonances (δ_H 6.65 and 6.90) correlated to ^{13}C NMR resonances δ_C 111.0 and 124.2, respectively (Figure 3). The aldehyde proton (δ_H 9.58)

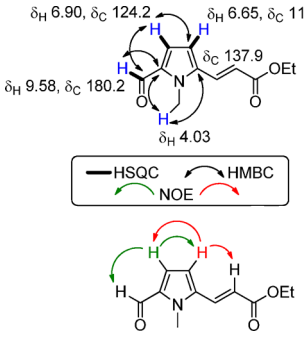


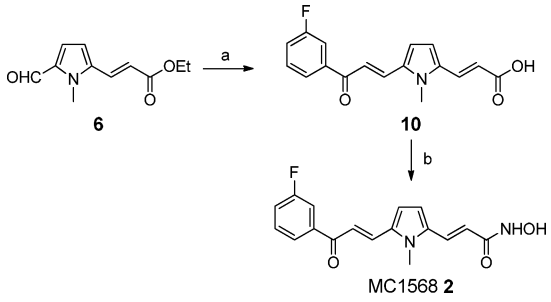
Figure 3. Key HSQC, HMBC, and NOE correlations of formylated pyrrole **6** in $CDCl_3$.

correlated to the ^{13}C NMR resonance δ_C 180.2. In the HMBC the *N*-methyl protons showed correlations to the quaternary carbons (δ_C 134.2 and 137.9) of the pyrrole. A HMBC correlation between the H-4 proton (δ_H 6.90) and the aldehyde carbon (δ_C 180.2) was also observed. If the reported 2,4-substitution pattern was present (aldehyde **5**), a HMBC correlation between both pyrrole protons (H-3 and H-5) and the aldehyde carbon would be expected. These results unequivocally confirm that formylation occurred at the 5-position of the pyrrole, not the 4-position as originally reported.[12]

The proposed structural reassignment of intermediate **6** was further supported by 1D selective NOE difference experiments (Figure 3). The remaining steps of the synthesis were then carried out to obtain MC1568 (**2**). In most cases modifications were made to the reported procedure (see Experimental Section).

Optimum results for the condensation of aldehyde **6** with 3′-fluoroacetophenone to give chalcone **10** employed $Ba(OH)_2$ in MeOH and microwave irradiation at 40 °C for 30 min. The chalcone was consistently isolated in high yield (96–98%, five repeats, Scheme 2).

Scheme 2. Synthesis of MC1568 (2)[α]



[α]Reagents and conditions: (a) 3′-fluoroacetophenone, $Ba(OH)_2$, MeOH, microwave, 40 °C, 30 min (96–98%); (b) (i) NH_2OTHP, EDCI·HCl, HOBt, Et_3N, CH_2Cl_2, 21 °C, 16 h (70%); (ii) *p*-TsOH·H_2O, MeOH, 21 °C, 30 min (70%).

For the installation of the hydroxamic acid moiety, coupling and deprotection of a protected hydroxylamine, *O*-(methyoxy-2-propyl)hydroxylamine was originally reported.[12] In our hands commercially available *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine (NH_2OTHP) was coupled to acid **10** using EDCI. Deprotection of the THP group was achieved by treatment with *p*-TsOH in MeOH at 21 °C to afford analytically pure (LCMS) MC1568 (**2**) in good yield (70%, Scheme 2).

As only a general procedure for the final two synthetic steps were originally reported, NMR spectra of MC1568 (**2**) were not provided.[12] Therefore, analysis of ^{1}H and ^{13}C NMR spectra, in conjunction with 1D NOE and 2D NMR experiments (HSQC and HMBC), was used to confirm the successful synthesis of MC1568 (**2**). Detailed synthesis and characterization are provided in the Experimental Section and Supporting Information.

In conclusion the structure of the class II selective inhibitor MC1568 has been reassigned from a 2,4- to a 2,5-disubstituted pyrrole and an improved synthesis developed with an overall yield of 30% achieved over five steps. Preliminary biological evaluation confirmed class II selectivity (see Supporting Information) and further supports the reassigned structure as the active isomer.

EXPERIMENTAL SECTION

General. General reagents and solvents for the synthesis of compounds were analytical grade (AR) and used as supplied. Anhydrous THF was obtained using a Pure Solv (Innovative Technologies) solvent drying system. Oxalyl chloride was distilled under N_2 before use. Melting points are uncorrected and were determined using a Bibby Stuart Scientific SMP3 melting point apparatus. NMR spectra were collected on a JEOL Eclipse JNM-Ex 270 MHz, 400 MHz FT-NMR, or Bruker Avance 500SB spectrometer as specified. Samples were dissolved (0.5 mL) in either deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6). High resolution mass spectra (HRMS) were recorded on an Aligent Technologies LC/MSD TOF mass spectrometer. Column chromatog-

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raphy was performed using Merck 230−400 silica gel. Petroleum spirits refer to the fraction boiling between 40 and 60 °C. Microwave mediated reactions were conducted using a CEM Discover S-class microwave reactor, operating at a frequency of 50/60 Hz and continuous irradiation power from 0 to 200 W. All reactions were conducted in a 10 mL microwave vial sealed with a Teflon snap cap. Purity of the described compound was determined by LCMS. Chromatographic analysis was performed on an Agilent HPLC/MS system using a Poroshell 120 EC-C18 column (3.0 mm × 50 mm, 2.7 μm). HPLC conditions were as follows; injection volume = 1 μL; solvent A = H_2O containing 0.1% formic acid; solvent B = MeCN containing 0.1% formic acid; compound was eluted with a gradient of 5−100% solvent B over 3.8 min; flow = 0.5 mL/min. All biologically active compounds were >98% pure as analyzed by HPLC

Ethyl 3-(1-Methyl-1*H*-pyrrol-2-yl)-2-propenoate (4). To a stirring solution of triethyl phosphonacetate (565 mg, 2.04 mmol) in THF (15 mL) at 0 °C was added potassium *tert*-butoxide (265 mg, 2.38 mmol). After 30 min, a suspension of *N*-methyl-2-pyrrolecarboxaldehyde **3** (186 mg, 1.70 mmol) in THF (5 mL) was added, and the mixture was stirred at 21 °C for 24 h. The mixture was diluted with H_2O (50 mL) and the aqueous phase extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with saturated $NaHCO_3$ (10 mL), brine (10 mL), dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (30% Et_2O in petroleum spirits) afforded the title compound (225 mg, 74%, R_f = 0.3) as a pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.30 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 3.68 (s, 3H, NCH_3), 4.22 (q, *J* = 7.0 Hz, 2H, CH_2CH_3), 6.13 (m, 2H, pyrrole H-4, CH═CHO), 6.64 (dd, *J* = 4.1, 1.6 Hz, 1H, pyrrole H-3), 6.72 (t, *J* = 2.2 Hz, 1H, pyrrole H-5), 7.58 (d, *J* = 15.7 Hz, 1H, CH═CHO). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 14.3, 33.7, 60.8, 109.7, 112.0, 112.7, 126.5, 129.4, 132.0, 167.9. HRMS (ESI, *m*/*z*): calculated for $C_{10}H_{13}NO_2$ $[M + H]^+$ 180.1019; found 180.1026.

Ethyl 3-(5-Formyl-1-methyl-1*H*-pyrrol-2-yl)-2-propenoate (6). In a 10 mL microwave vial, a solution of oxalyl chloride (213 mg, 1.67 mmol) in DMF (1 mL) was stirred at 0 °C for 45 min. A solution of ethyl 3-(1-methyl-1*H*-pyrrol-2-yl)-2-propenate **4** (100 mg, 0.56 mmol) in DMF (0.5 mL) was added, and the resulting mixture was heated using microwave irradiation at 100 °C for 14 min. After the mixture was cooled to 21 °C, H_2O (9 mL) was added, affording a precipitate, which was isolated by vacuum filtration. The title compound was obtained (95 mg, 83%) as a fine tan solid; mp 124−125 °C (lit. 102−104 °C).[20] ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.33 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 4.03 (s, 3H, NCH_3), 4.26 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 6.40 (d, *J* = 15.6 Hz, 1H, CH═CHO), 6.65 (d, *J* = 4.5 Hz, 1H, pyrrole H-3), 6.90 (d, *J* = 4.5 Hz, 1H, pyrrole H-4), 7.61 (d, *J* = 15.8 Hz, 1H, CH═CHO), 9.58 (s, 1H, CHO). ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.25 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 3.93 (s, 3H, NCH_3), 4.20 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 6.63 (d, *J* = 15.7 Hz, 1H, CH═CHO), 6.97 (d, *J* = 4.4 Hz, 1H, pyrrole H-3), 7.06 (d, *J* = 4.4 Hz, 1H, pyrrole H-4), 7.61 (d, *J* = 15.7 Hz, 1H, CH═CHO), 9.58 (s, 1H, CHO). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 14.4, 32.6, 60.9, 111.0, 120.8, 124.2, 130.5, 134.2, 137.9, 166.6, 180.2. HRMS (ESI, *m*/*z*): calculated for $C_{11}H_{13}NO_3$ $[M + H]^+$ 208.0968; found 208.0961.

3-(5-(3-(3-Fluorophenyl)-3-oxo-1-propen-1-yl)-1-methyl-1*H*-pyrrol-2-yl)-2-propenoic Acid (10). In a 35 mL microwave vial, a solution containing 3′-fluoroacetophenone (134 mg, 0.97 mmol) and barium hydroxide octahydrate (1.220 g, 3.86 mmol) in MeOH (5 mL) was stirred at 21 °C for 15 min. A solution of ethyl 3-(5-formyl-1-methyl-1*H*-pyrrol-2-yl)-2-propenate **6** (200 mg, 0.97 mmol) was added, and the mixture was heated using microwave irradiation at 40 °C for 30 min. The mixture was diluted with H_2O (20 mL) and adjusted to pH 7 using 1 M HCl. The orange precipitate was isolated by vacuum filtration, washing thoroughly with H_2O to afford the desired compound (283 mg, 98%) as an orange solid; mp 209−211 °C (lit. 210−212 °C).[12] ^{1}H NMR (270 MHz, DMSO-d_6): δ 3.75 (s, 3H, NCH_3), 6.36 (d, *J* = 13.5 Hz, 1H, CH═CHOOH), 6.86 (d, *J* = 5.4 Hz, 1H, pyrrole H-3), 7.24 (d, *J* = 5.4 Hz, 1H, pyrrole H-4), 7.53 (m, 3H, CH═CHOOH, ArH-2,5), 7.74 (m, 2H, COCH═CH, COCH═CH), 7.89 (d, *J* = 8.1 Hz, 1H, ArH-4), 7.97 (d, *J* = 8.1 Hz, 1H, ArH-6). ^{13}C NMR (125 MHz, DMSO-d_6): δ 30.5, 112.6, 114.1, 114.7 (d, $^2J_{C-F}$ = 22.7 Hz), 117.3, 118.1, 119.7 (d, $^2J_{C-F}$ = 21.6 Hz), 124.4 (d, $^4J_{C-F}$ = 2.4 Hz), 130.9 (d, $^3J_{C-F}$ = 8.1 Hz), 131.0, 131.8, 133.8, 134.4, 140.3 (d, $^3J_{C-F}$ = 6.4 Hz), 162.4 (d, $^1J_{C-F}$ = 243.5 Hz), 167.8, 186.9 (d, $^4J_{C-F}$ = 2.5 Hz). HRMS (ESI, *m*/*z*): calculated for $C_{17}H_{14}FNO_3$ $[M + H]^+$ 300.1031; found 300.1032.

3-(5-(3-(3-Fluorophenyl)-3-oxo-1-propen-1-yl)-1-methyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamide (2). To a stirring solution of 3-(5-(3-(3-fluorophenyl)-3-oxo-1-propen-1-yl)-1-methyl-1*H*-pyrrol-2-yl)-2-propenoic acid **10** (220 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) was added *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (103 mg, 0.88 mmol), EDCI·HCl (456 mg, 2.94 mmol), anhydrous HOBt (199 mg, 1.47 mmol), and Et_3N (526 mg, 5.20 mmol). After the mixture was stirred at 21 °C for 16 h, it was transferred into a separatory funnel and was washed with brine (2 × 10 mL), dried over $MgSO_4$, and concentrated to dryness. Purification by flash column chromatography (2% MeOH in 1:1 EtOAc/petroleum spirits) afforded the THP-protected hydroxamic acid (202 mg, 70%, R_f = 0.18) as a red oil that solidified upon standing. To a solution of this THP-protected hydroxamic acid (142 mg, 0.356 mmol) in MeOH (10 mL) was added *p*-TsOH·H_2O (20 mg, 0.107 mmol). After the mixture was stirred at 21 °C for 1 h, an orange precipitate formed that was isolated by gravity filtration and further purified by recrystallization (DMSO/H_2O) to afford the title compound (80 mg, 70%) as an orange solid; mp 216−218 °C (lit. 212−215 °C).[12] ^{1}H NMR (270 MHz, DMSO-d_6): δ 3.76 (s, 3H, NCH_3), 6.37 (d, *J* = 15.5 Hz, 1H, CH═CHONHOH), 6.72 (d, *J* = 4.0 Hz, 1H, pyrrole H-3), 7.25 (d, *J* = 4.0 Hz, 1H, pyrrole H-4), 7.53 (m, 3H, CH═CHONHOH, ArH-2,5), 7.75 (m, 2H, COCH═CH, COCH═CH), 7.90 (d, *J* = 10.2 Hz, 1H, ArH-4), 7.97 (d, *J* = 7.6 Hz, 1H, ArH-6), 9.03 (br s, 1H, NHOH), 10.72 (br s, 1H, NHOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.2, 111.6, 114.8, 115.3 (d, $^2J_{C-F}$ = 21.8 Hz), 117.9, 118.7, 120.2 (d, $^2J_{C-F}$ = 21.1 Hz), 124.9, 126.4, 131.5 (d, $^3J_{C-F}$ = 7.7 Hz), 132.5, 133.5, 135.7, 140.9 (d, $^3J_{C-F}$ = 6.3 Hz), 162.9 (d, $^1J_{C-F}$ = 243.9 Hz), 163.6, 187.4. HRMS (ESI, *m*/*z*): calculated for $C_{17}H_{15}FN_2O_3$ $[M + H]^+$ 315.1139; found 315.1138. HPLC: t_R = 3.50 min.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results and experimental protocols for cytotoxicity and inhibitory property testing and full ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

HDAC, histone deacetylase

■ REFERENCES

(1) Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. Histone Deacetylase Inhibitors: From Bench to Clinic. *J. Med. Chem.* **2008**, *51*, 1505−1529.

(2) Miller, T. A.; Witter, D. J.; Belvedere, S. Histone Deacetylase Inhibitors. *J. Med. Chem.* **2003**, *46*, 5097−5116.
(3) McKinsey, T. A. Isoform-Selective HDAC Inhibitors: Closing In on Translational Medicine for the Heart. *J. Mol. Cell. Cardiol.* **2011**, *51*, 491−496.
(4) Suzuki, T. Explorative Study on Isoform-Selective Histone Deacetylase Inhibitors. *Chem. Pharm. Bull.* **2009**, *57*, 897−906.
(5) Bertrand, P. Inside HDAC with HDAC Inhibitors. *Eur. J. Med. Chem.* **2010**, *45*, 2095−2116.
(6) Balasurbramanain, S.; Verner, E.; Buggy, J. J. Isoform-Specific Histone Deacetylase Inhibitors: The Next Step? *Cancer Lett.* **2009**, *280*, 211−221.
(7) Bieliauskas, A. V.; Pflum, M. K. H. Isoform-Selective Histone Deacetylase Inhibitors. *Chem. Soc. Rev.* **2008**, *37*, 1402−1413.
(8) Haberland, M.; Montgomery, R. L.; Olson, E. N. The Many Roles of Histone Deacetylases in Development and Physiology: Implications for Disease and Therapy. *Nat. Rev. Genet.* **2009**, *10*, 32−42.
(9) Christensen, D. P.; Dahllöf, M.; Lundh, M.; Rasmussen, D. N.; Nielsen, M. D.; Billestrup, N.; Grunnet, L. G.; Mandrup-Poulsen, T. Histone Deacetylase (HDAC) Inhibition as a Novel Treatment for Diabetes Mellitus. *Mol. Med.* **2011**, *17*, 378−390.
(10) Celgene Invests $15M in Acetylation To Support HDAC Inhibitor Development. *Genet. Eng. Biotechnol. News*. **2012**, February 9.
(11) For examples of class II selective HDAC inhibitors, see the following: (a) Lobera, M.; Madauss, K. P.; Pohlhaus, D. T.; Wright, Q. G.; Tracha, M.; Schmidt, D. R.; Baloglu, E.; Trump, R. P.; Head, M. S.; Hofmann, G. A.; Murray-Thompson, M.; Schwartz, B.; Chakravorty, S.; Wu, Z.; Mander, P. K.; Kruidenier, L.; Reid, R. A.; Burkhart, W.; Turunen, B. J.; Rong, J. X.; Wagner, C.; Moyer, M. B.; Wells, C.; Hong, X.; Moore, J. T.; Williams, J. D.; Soler, D.; Ghosh, S.; Nolan, M. A. Selective Class IIa Histone Deacetylase Inhibition via Nonchelating Zinc-Binding Group. *Nat. Chem. Biol.* **2013**, *9*, 319−325. (b) Kong, Y.; Jung, M.; Wang, K.; Grindrod, S.; Velena, A.; Lee, S. A.; Dakshanamurthy, A.; Yang, Y.; Miessau, M.; Zheng, C.; Dritschilo, A.; Brown, M. L. Histone Deacetylase Cytoplasmic Trapping by a Novel Fluorescent HDAC Inhibitor. *Mol. Cancer Ther.* **2011**, *10*, 1591−1599. (c) Tessier, P.; Smil, D. V.; Wanhhab, A.; Leit, S.; Rahil, J.; Li, Z.; Déziel, R.; Besterman, J. M. Diphenylmethylene Hydroxamic Acids as Selective Class IIa Histone Deacetylase Inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5684−5688.
(12) Mai, A.; Massa, S.; Pezzi, R.; Simeoni, S.; Rotili, D.; Nebbioso, A.; Scognamiglio, A.; Altucci, L.; Loidl, P.; Brosch, G. Class II (IIa)-Selective Histone Deacetylase Inhibitors. 1. Synthesis and Biological Evaluation of Novel (Aryloxopropenyl)pyrrolyl Hydroxyamides. *J. Med. Chem.* **2005**, *48*, 3344−3353.
(13) Rango, R.; Simeoni, S.; Rotili, D.; Caroli, A.; Botta, G.; Brosch, G.; Massa, S.; Mai, A. Class II-Selective Histone Deacetylase Inhibitors. Part 2: Alignment-Independent GRIND 3-D QSAR, Homology and Docking Studies. *Eur. J. Med. Chem.* **2008**, *43*, 621−631.
(14) For examples, see the following: (a) Kahali, B.; Gramling, S. J. B.; Marquez, S. B.; Thompson, K.; Lu, L.; Reisman, D. Identifying Targets for the Restoration and Reactivation of BRM. *Oncogene* **2013**, 1−12. (b) Palmisano, I.; Chiara, G. D.; D'Ambrosio, R. L.; Huichalaf, C.; Brambilla, P.; Corbetta, S.; Riba, M.; Piccirillo, R.; Valente, S.; Casari, G.; Mai, A.; Boneschi, F. M.; Gabellini, D.; Poli, G.; Schiaffino, M. V. Amino Acid Starvation Induces Reactivation of Silenced Transgenes and Latent HIV-1 Provirus via Down-Regulation of Histone Deacetylase 4 (HDAC4). *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2284−2293. (c) Munkacsi, A. B.; Chen, F. W.; Brinkman, M. A.; Higaki, K.; Gutiérrez, G. D.; Chaudharis, J.; Layer, J. V.; Tong, A.; Bard, M.; Boone, C.; Ioannou, Y. A.; Sturley, S. L. An "Exacerbate-Reverse" Strategy in Yeast Identifies Histone Deacetylase Inhibition as a Correction for Cholesterol and Sphingolipid Transport Defects in Human Niemann-Pick Type C Disease. *J. Biol. Chem.* **2011**, *286*, 23842−23851.
(15) Gupton, J. T.; Banner, E. J.; Sartin, M. D.; Coppock, M. B.; Hempel, J. E.; Kharlamova, A.; Fisher, D. C.; Giglio, B. C.; Smith, K. L.; Keough, M. J.; Smith, T. M.; Kanters, R. P. F.; Dominey, R. N.; Sikorski, J. A. The Application of Vinylogous Iminium Salt Derivatives and Microwave Accelerated Vilsmeier−Haack Reactions to Efficient Relay Synthesis of the Polycitone and Storniamide Natural Products. *Tetrahedron* **2008**, *64*, 5246−5253.
(16) Katrikzky, A. R; Akhmedov, N. G.; Doskocz, J.; Mohapatra, P. P.; Hall, C. D.; Güven, A. NMR Spectra, GIAO and Charge Density Calculations of Five-Membered Aromatic Heterocycles. *Magn. Reson. Chem.* **2007**, *45*, 532−543.
(17) Barker, P.; Gendler, P.; Rapoport, H. 2-(Trichloroacetyl)-pyrroles as Intermediates in the Preparation of 2,4-Disubstituted Pyrroles. *J. Org. Chem.* **1978**, *25*, 4849−4853.
(18) Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Rango, R.; Bottoni, P.; Scatena, R.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 2. Effect of Pyrrole-C_2 and/or -C_4 Substitutions on Biological Activity. *J. Med. Chem.* **2004**, *47*, 1098−1109.
(19) Joule, J. A.; Mills, K. *Heterocyclic Chemistry*, 4 th ed.; Blackwell Science Ltd.: Oxford, U.K., 2000; Chapter 13, pp 241−243.
(20) Mai, A.; Massa, S.; Pezzi, R.; Rotili, D.; Loidl, P.; Brosch, G. Discovery of (Aryloxopropenyl)pyrrolyl Hydroxyamides as Selective Inhibitors of Class IIa Histone Deacetylase Homologue HD1-A. *J. Med. Chem.* **2003**, *46*, 4826−4829.