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Article

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# (*N*-Hydroxycarbonylbenylamino)quinolines as Selective Histone Deacetylase 6 Inhibitors Suppress Growth of Multiple Myeloma *in vitro* and *in Vivo*

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**ABSTRACT.** A series of bicyclic arylamino/heteroarylamino hydroxamic acids (**7-31**) have been examined as novel histone deacetylase 6 (HDAC6) inhibitors. One compound (**13**) exhibits remarkable inhibitory activity of HDAC6 with an $IC_{50}$ value of 0.29 nM, which is 4000-43000 times more selective over other HDAC isoforms. Compound **13** was shown to have antiproliferative activity against human multiple myeloma RPMI 8226, U266, and NCI-H929 cells with no effect on normal bone marrow cells. Compound **13**, as a single drug, suppresses the growth of tumors by a %TGI factor of 60.4% in human

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multiple myeloma RPMI 8226 xenograft models, and in combination with bortezomib, shows significant *in vivo* antitumor activity (%TGI = 86.2%). Compound **13** also demonstrates good human hepatocytic stability and high permeability, without any effect on mutagenicity and cytotoxicity. Thus compound **13** is a potent HDAC6 inhibitor that could be developed for the treatment of multiple myeloma in the future.

## Introduction

Targeting proteins related to epigenetic regulation has emerged as an attractive therapeutic strategy for various diseases. Among these epigenetic enzymes, histone deacetylase (HDAC) is the most studied protein. HDAC is able to remove the acetyl groups from *N*-terminal lysines of histones, which frees the genetic component and triggers the transcriptional process. In addition, it also deacetylates various non-histone protein substrates such as tubulin[1] and p53[2,3]. These crucial roles of HDAC make it a promising target for drugs to treat various diseases. The HDAC family has eighteen members which are grouped into four classes (I, II, III, and IV) according to their homologies. The development of HDAC inhibitors started with the discovery of pan-HDAC inhibitors and has brought various compounds into the clinic, for instance, vorinostat, romidepsin, belinostat, and panobinostat.[4-7] A survey of the results of clinical trials indicates that the use of pan-HDAC inhibitors results in some side effects, such as fatigue, diarrhea, nausea,[8] QTc-interval prolongation[9-11], and thrombocytopenia.[12] Consequently, in the past decade scientific attention was drawn to the discovery of selective inhibitors of HDAC isoforms, specifically

HDAC6 inhibitors. Compound **1** (tubacin) was discovered by high-throughput screening and was recognized as the first selective HDAC6 inhibitor,[13] which opened an avenue to the subsequent development of other HDAC6 inhibitors. For instance, Compound **2** (tubastatin A) shows remarkably selective HDAC6 inhibition and neuroprotective activity[14] and compound **3** (ACY-1215) currently in phase II clinical trials, is able to suppress the growth of multiple myeloma in combination therapy.[15] The correlation of HDAC6 with diverse diseases has encouraged us to develop potent selective HDAC6 inhibitors with effective biological activity.

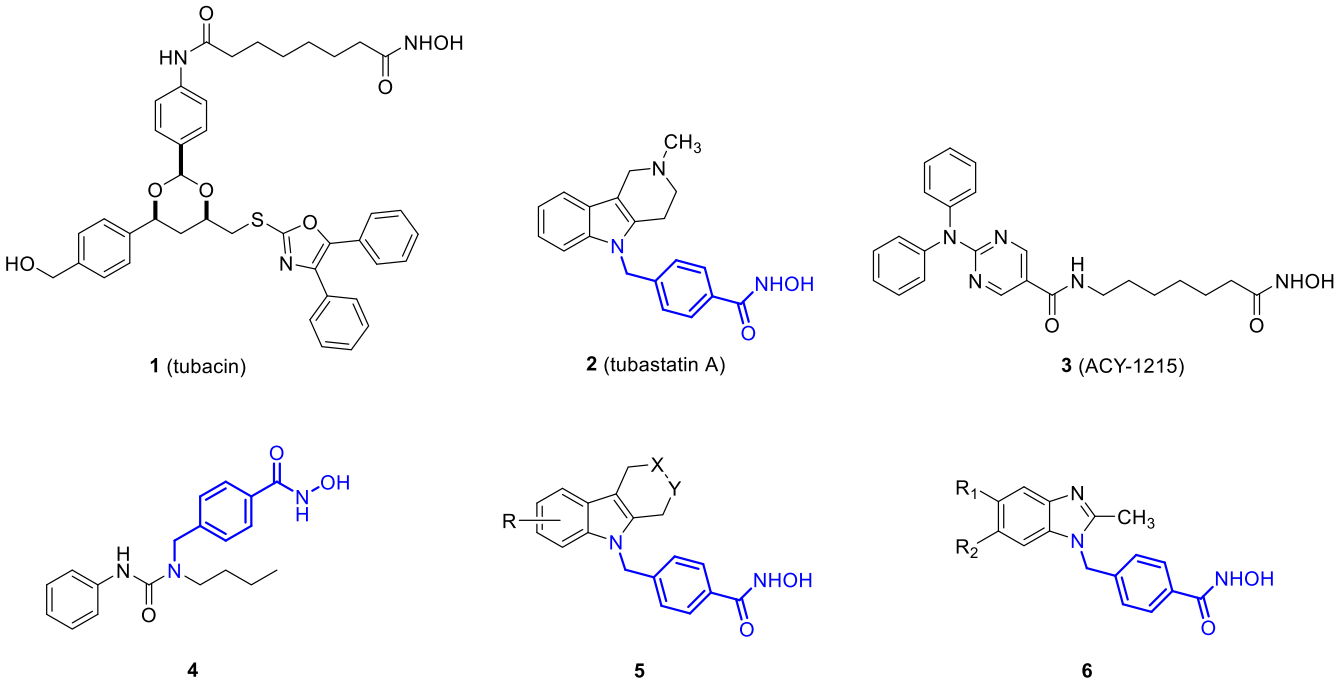



**Figure 1.** Structures of HDAC6 inhibitors.

In addition to the HDAC6 inhibitors cited above, some additional examples of HDAC6 inhibitors (**4-6**) are also shown in Figure 1.[16-18] HDAC6 is a zinc-dependent enzyme; therefore, the structure of HDAC6 inhibitors comprises three parts: zinc binding domain, linker, and surface recognition region.

Upon inspection of their structures, compounds **2**, **4**, **5**, and **6** can be seen to share a common moiety, the 4-(*N*-hydroxyaminocarbonyl)benzylamino group (-NHCH$_2$PhCONHOH, shown in blue in Figure 1). This moiety is considered to be a combination of a zinc ion binding domain and a linker section, and it is connected with various aromatic rings acting as a surface recognition region. This led to a series of bicyclic arylamino/heteroarylamino hydroxamic acids (**7**-**31**, Figure 2). The current study is also aimed at assays of their activity against both HDAC6 and the growth of multiple myeloma cells.

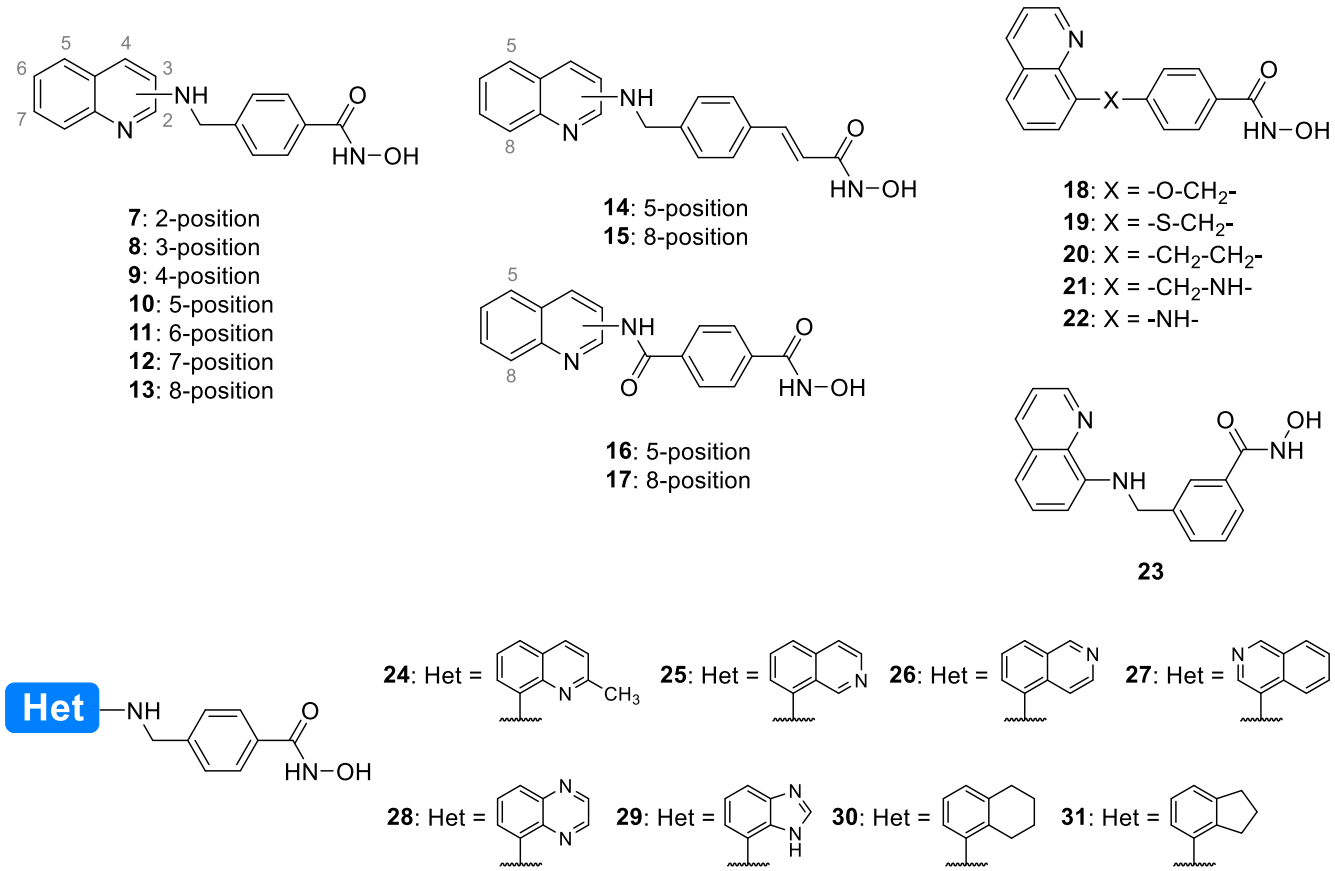


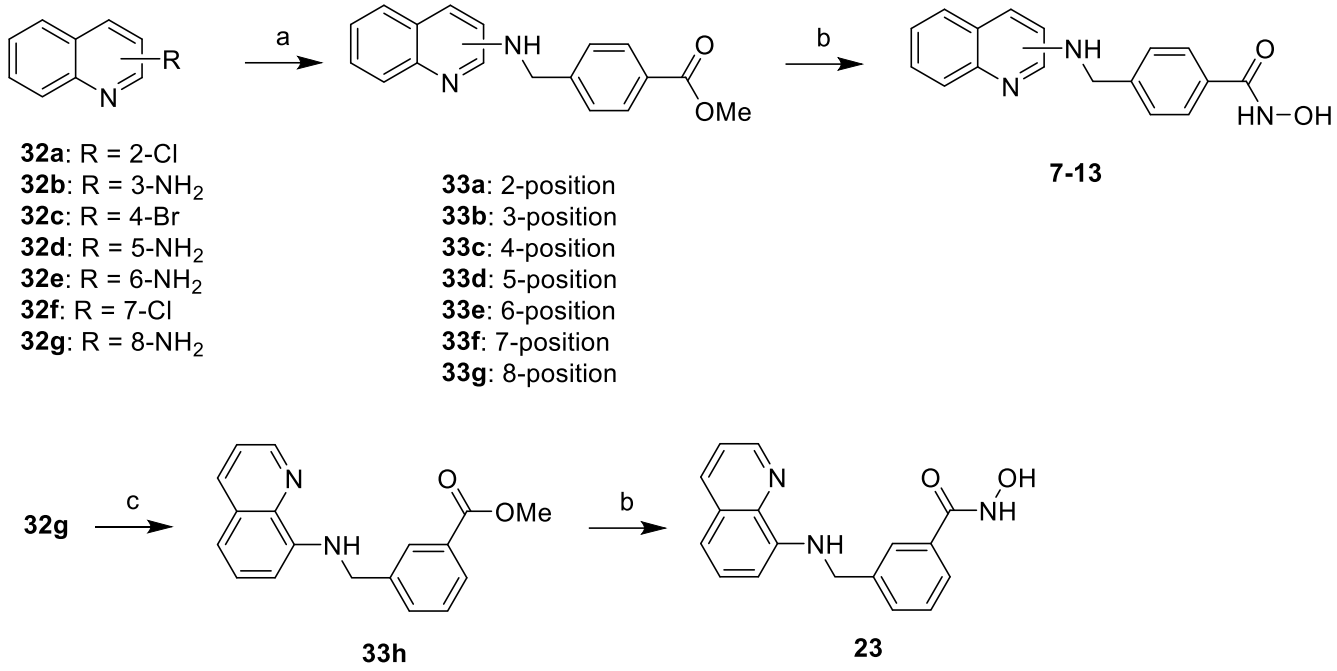

**Figure 2.** Synthetic bicyclic arylamino/heteroarylamino hydroxamic acids (**7**-**31**).

## Results and Discussion

## Chemistry

Scheme 1 shows the synthesis of 4-(*N*-hydroxyaminocarbonyl)benzylaminoquinolines (**7-13** and **23**). Amino-substituted quinolines (**32b**, **32d**, **32e**, and **32g**) reacted with methyl terephthalaldehydate in the presence of acetic acid followed by reduction with $NaBH(OAc)_3$ to generate the corresponding secondary amines (**33b**, **33d**, **33e**, and **33g**). To afford C2-, C4-, and C7-(4-(*N*-hydroxyaminocarbonyl)benzylamino)quinolines (**7**, **9**, and **12**), the synthetic route started from halogenated quinolines (**32a**, **32c**, and **32f**) *via* a Buchwald-Hartwig amination reaction with methyl 4-(aminomethyl)benzoate with the assistance of $Pd(OAc)_2$, which provided compounds **33a**, **33c**, and **33f**. Under basic conditions, the methyl ester groups of **33a**-**g** were converted into the corresponding hydroxamic acid by $NH_2OH$, which generated the anticipated *N*-hydroxy-4-(aminomethyl)benzamide (**7-13**). In addition, compound **23** was prepared from reaction of **32g** with methyl 3-formylbenzoate followed by conversion of ester (**33h**) into hydroxamic acid.

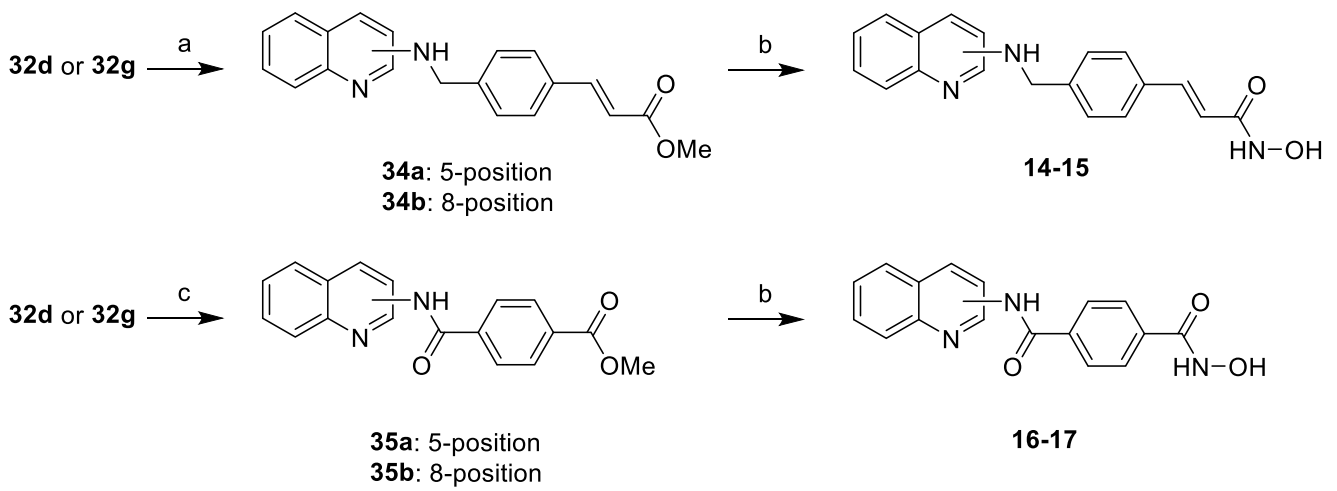
**Scheme 1.** Synthetic Approaches to Compounds **7-13** and **23**[a]




[a]Reagents and conditions: (a) for **33b**, **33d**, **33e**, and **33g**: methyl terephthalaldehydate, $NaBH(OAc)_3$, AcOH, rt; for **33a**: methyl 4-(aminomethyl)benzoate, $Pd(OAc)_2$, BINAP, $K_2CO_3$, toluene, 100 °C; for **33c** and **33f**: methyl 4-(aminomethyl)benzoate, $Pd(OAc)_2$, DPEphos, $K_3PO_4$, toluene, 100 °C; (b) $NH_2OH$, NaOH, MeOH, rt; (c) methyl 3-formylbenzoate, $NaBH(OAc)_3$, AcOH, rt.

To understand the effect on enzymatic activity of the linker between hydroxamic acid and the quinoline, compounds **14**-**17** were synthesized as shown in Scheme 2. The synthesis of compounds **14** and **15** possessing a -C=C- linker started from the treatment of 5-aminoquinoline (**32d**) and 8-aminoquinoline (**32g**) with methyl (*E*)-3-(4-formylphenyl)acrylate in the presence of $NaBH(OAc)_3$ and acetic acid. The preparation of **16** and **17** which have an amide linkage, was carried out by the reaction of **32f** and **32g** with methyl 4-(chloroformyl)benzoate and monomethyl terephthalate, respectively. All the resultant esters (**34a-b** and **35a-b**) were converted into the corresponding hydroxamic acid by treating $NH_2OH$ under basic conditions, which generates the designed compounds **14-17**.

**Scheme 2.** Synthetic Approaches to Compounds **14-17**[a]

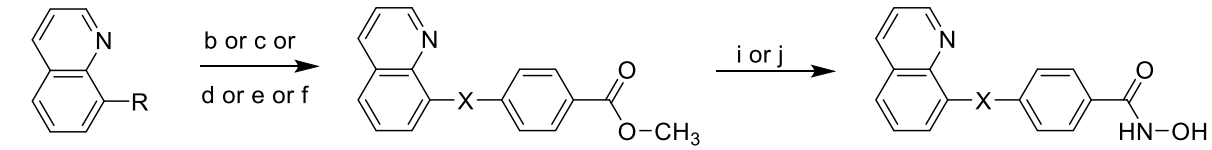



[a]Reagents and conditions: (a) (*E*)-methyl 3-(4-formylphenyl)acrylate, $NaBH(OAc)_3$, AcOH, rt; (b) $NH_2OH$, NaOH, MeOH, rt; (c) for **35a**: methyl 4-(chloroformyl)benzoate, TEA, DCM, 0 °C to rt; for **35b**: monomethyl terephthalate, EDC-HCl, DMAP, DCM, rt.

In addition to the secondary amine linker, several bridging groups such as ether, sulfide, and ethylenyl, were inserted between the quinoline and 4-(*N*-hydroxyaminocarbonyl)phenyl moieties. Their synthetic route was illustrated in Scheme 3. Access to compounds **18** and **19** possessing ether and sulfide linkage respectively, started from the $S_N2$ reaction of **36a** and **36b** with ethyl 4-(bromomethyl)benzoate. The resulting benzoate (**37a** and **37b**) was transformed to the hydroxamic acid (**18** and **19**) under conditions similar to those shown in Scheme 1. The Wittig olefination of **36c** followed by reduction of the resulting C=C bond gave compound **37c**, which possesses an ethylenyl group, and the subsequent reaction with $NH_2OH$ under basic conditions yielded **20**. To prepare compound **21** with a reverse secondary amino group, compound **36c** underwent reductive amination with methyl 4-aminobenzoate in the presence of $NaBH(OAc)_3$, followed by reaction with $NH_2OH$,

which leads to the designed compound **21**. Compound **22** with a shortened amino linkage was prepared by the Buchwald-Hartwig amination reaction of **36d** with methyl 4-aminobenzoate to afford the corresponding carboxylate **37e** which can be converted into compound **22**.

**Scheme 3.** Synthetic Approaches to Compounds **18-22**[a]




**36a**: R = -OH
**36b**: R = -SH
**36c**: R = -CHO
**36d**: R = -Br

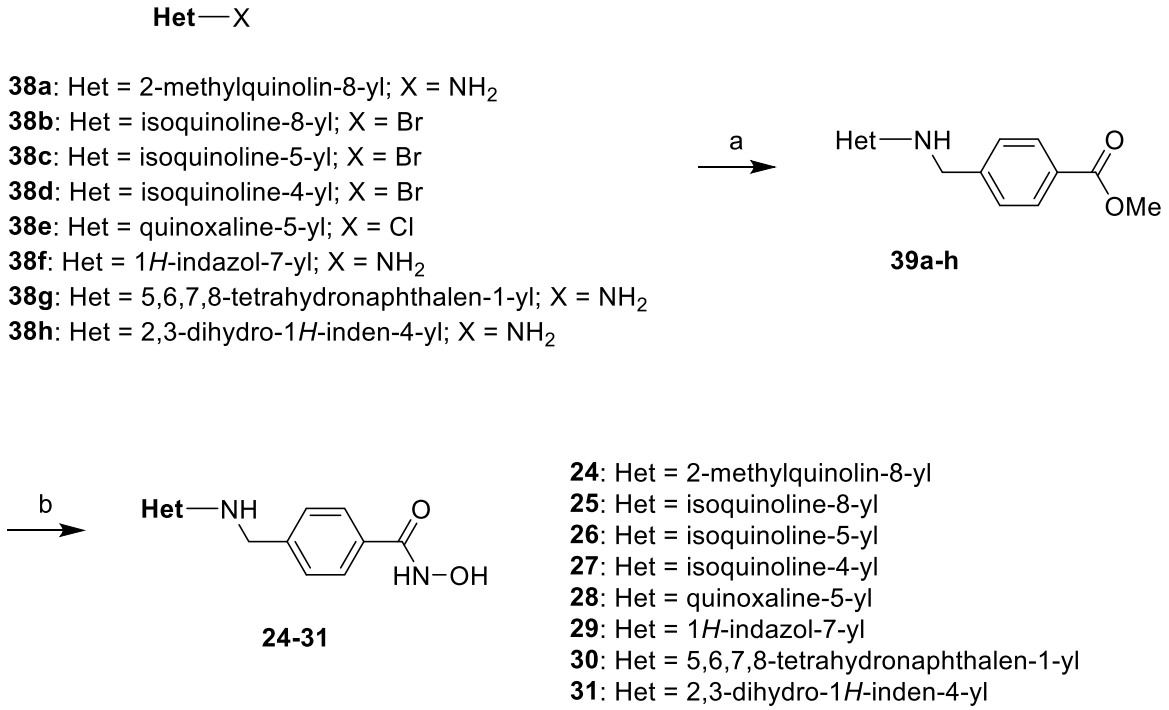
**37a**: -X- = -$OCH_2$-
**37b**: -X- = -$SCH_2$-
**37c**: -X- = -$CH_2CH_2$-
**37d**: -X- = -$CH_2NH$-
**37e**: -X- = -NH-

**18**: -X- = -$OCH_2$-
**19**: -X- = -$SCH_2$-
**20**: -X- = -$CH_2CH_2$-
**21**: -X- = -$CH_2NH$-
**22**: -X- = -NH-

[a]Reagents and conditions: (a) for **37a-b**: methyl 4-(bromomethyl)benzoate, $K_2CO_3$, acetone, reflux; for **37c**: i. triphenyl-(4-methoxycarbonylbenzyl)phosphonium chloride, *t*-BuOK, DCM, 0 °C to rt; ii. Pd/C, $H_2$, MeOH, rt; for **37d**: methyl 4-aminobenzoate, $NaBH(OAc)_3$, AcOH, rt; for **37e**: methyl 4-aminobenzoate, $Pd(OAc)_2$, DPEphos, $K_3PO_4$, toluene, 100 °C; (b) $NH_2OH$, NaOH, MeOH, rt.

The replacement of quinoline by various heterocycles or bicyclic rings is beneficial to an understanding of the influence of quinoline on the biological activity. The synthesis of bicyclic ring-containing molecules (**24-31**) is shown in Scheme 4. Halogen-containing bicyclic rings (**38b-e**) underwent Buchwald-Hartwig amination with methyl 4-(aminomethyl)benzoate, and the resulting benzoate was converted into the designed hydroxamic acid (**25-28**). Meanwhile, the reductive amination of amino-containing rings (**38a** and **38f-h**) with methyl terephthalaldehydate followed by reaction with $NH_2OH$ gave the designed compounds **24** and **29-31**.

**Scheme 4.** Synthetic Approaches to Compounds **24-31**[a]




[a]Reagents and conditions: (a) for **25-28**: methyl terephthalaldehydate, $NaBH(OAc)_3$, AcOH, rt; for **24** and **29-31**: methyl 4-(aminomethyl)benzoate, $Pd(OAc)_2$, DPEphos, $K_3PO_4$, toluene, 100 °C; (b) $NH_2OH$, NaOH, MeOH, rt.

## Biological Evaluation.

### A. HDAC6 Inhibitory Activity.

All synthesized adducts (**7-31**) and a reference compound, trichostatin A were examined for their HDAC6 inhibitory activity, and the results are shown in Table 1. The activities of compounds **7-13** indicate the effective substitution position where the 4-(*N*-hydroxycarbonyl)benzylamino group binds, on enzymatic activity. The result shows that the 4-(*N*-hydroxycarbonyl)benzylamino group favors the C5 or C8 positions of quinoline, while **10** and **13** have better HDAC6 inhibitory activity. This result reveals that quinoline-5-yl and quinoline-8-yl are suitable moieties as surface recognition area. The $IC_{50}$

values of **10** and **13** against HDAC6 are 0.795 and 0.291 nM, respectively. The shift from C5 or C8 position to C3, C6, or C7 position led to a slight decrease of HDAC inhibitory activity, but their $IC_{50}$ values remain in the single-digit nM range. The C2-*N*-hydroxyaminocarbonylbenzylamino group resulted in a distinct loss of enzymatic potency. The insertion of a -C=C- linkage between *N*-hydroxyaminocarbonyl and phenyl ring resulted in a decrease of activity if one compares **10** and **13** with **14** and **15**, respectively. The influence of the bridging groups linking the quinoline to the hydroxamate group and the position of the hydroxamate group on HDAC6 inhibition can be studied in compounds **18-22**. The replacement of nitrogen atom with oxygen (**18**) or sulfur (**19**) as well as reversal of alignment (**21**) slightly reduces activity, whereas replacement of the nitrogen with carbon (**20**) led to marked loss of HDAC6 inhibitory activity. The removal of a carbon atom (**22**) and *meta*-hydroxamate group (**23**) resulted in a dramatic decrease of enzymatic activity. The results from **24-31** show the effect of replacement of quinoline with a variety of heterocycles and bicyclic rings on biological activity. It reveals that most of nitrogen-containing [6,6]- or [6,5]-heterocycles (**25-29**) show HDAC6 inhibitory ability with $IC_{50}$ values in single-digit nM range, the single exception being the 2-methylquinoline (**24**). The bicyclic rings which incorporate saturated rings like 1,2,3,4-tetrahydronaphthalene (**30**) and 2,3-dihydro-1H-indene (**31**) decrease their ability to inhibit HDAC6. In summary, this study provides a detailed study of structure-activity relationship that assists in the further development of selective HDAC6 inhibitors.

**Table 1.** HDAC6 inhibitory activity ($IC_{50}$) of tested compounds (**7-31**)

| Cpd | HDAC6 $IC_{50}$ (nM)[a] |
|---|---|
| **7** | 4.24 |
| **8** | 403 |
| **9** | 21.5 |
| **10** | 0.795 |
| **11** | 4.78 |
| **12** | 3.25 |
| **13** | 0.291 |
| **14** | 25.3 |
| **15** | 41.7 |
| **16** | 525 |
| **17** | 265 |
| **18** | 2.83 |
| **19** | 9.48 |
| **20** | 41.7 |
| **21** | 7.40 |
| **22** | 129 |
| **23** | 1130 |
| **24** | 11.4 |
| **25** | 3.35 |
| **26** | 2.33 |
| **27** | 2.31 |
| **28** | 5.89 |
| **29** | 3.56 |
| **30** | 25.3 |
| **31** | 19.6 |
| Trichostatin A | 3.55 |

[a]These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in at least 10-dose $IC_{50}$ mode with 3-fold serial dilution starting at 10 μM. $IC_{50}$ values displayed the result of a single experiment.

Compounds **10** and **13**, which showed the most potent HDAC6 inhibition were further examined for their inhibitory ability to other HDAC isotypes (Table 2). Compounds **10** and **13** inhibit HDAC6 with $IC_{50}$ values of 0.795 and 0.291 nM, respectively. This result shows that **10** and **13** exhibit distinct selective HDAC6 inhibition over other isoforms. The values inside the bracket of Table 2 are selectivity ratio of tested compounds over HDAC6. The result indicates that the selectivity potency of **10** and **13** toward HDAC6 is dramatically better than that of compound **3**. Importantly, compound **13** is highly selective for HDAC6 and more active than with HDAC1, 2, 3, 4, 5, 7, 8, 9, 10, and 11 by factors of 32817, 42955, 26632, 15250, 10694, 2436, 4089, 5258, 33646 and 1292, respectively.

**Table 2.** Inhibitory activity ($IC_{50}$, nM) of test compounds **10**, **13**, and reference **3**.

| | $IC_{50}$ (nM)[a] | | |
|---|---|---|---|
| HDAC subtype | **10** (selectivity ratio)[c] | **13** (selectivity ratio) | **3**[b] (selectivity ratio)) |
| HDAC1 | 7050 (8868) | 9550 (32817) | 58 (12) |
| HDAC2 | 8610 (10830) | 12500 (42955) | 48 (10) |
| HDAC3 | 5480 (6893) | 7750 (26632) | 51 (11) |
| HDAC4 | 4817 (6059) | 4438 (15250) | 7000 (1500) |
| HDAC5 | 2251 (2831) | 3112 (10694) | 5000 (1100) |
| HDAC6 | 0.795 (-) | 0.291 (-) | 4.7 (-) |
| HDAC7 | 766 (964) | 709 (2436) | 1400 (300) |
| HDAC8 | 636 (800) | 1190 (4089) | 100 (21) |

| | | | |
|---|---|---|---|
| HDAC9 | 1915 (2409) | 1530 (5258) | >10000 (> 2100) |
| HDAC10 | 1256 (1580) | 9791 (33646) | NA (-) |
| HDAC11 | 1004 (1263) | 376 (1292) | >10000 (> 2100) |
| SIRT1 | >20000 (> 25157) | >20000 (> 68729) | >10000 (> 2100) |

[a]These assays were conducted by the Reaction Biology Corporation, Malvern, PA. All compounds were dissolved in DMSO and tested in at least 10-dose $IC_{50}$ mode with 3-fold serial dilution starting at 10 μM. $IC_{50}$ values displayed the result of a single experiment. [b]Data obtained from reference 15. [c]Selectivity ratio: selectivity ratio of HDAC subtypes over HDAC6

The comparison of the influence of compounds **10** and **13** on the acetylation of α-tubulin in RPMI 8226, U266, and NCI-H929 cell lines is shown in Figure 3. Treatment with **10** or **13** led to upregulation of acetylated α-tubulin in a dose-dependent manner, which corresponds to the functionality of HDAC6 inhibitors.

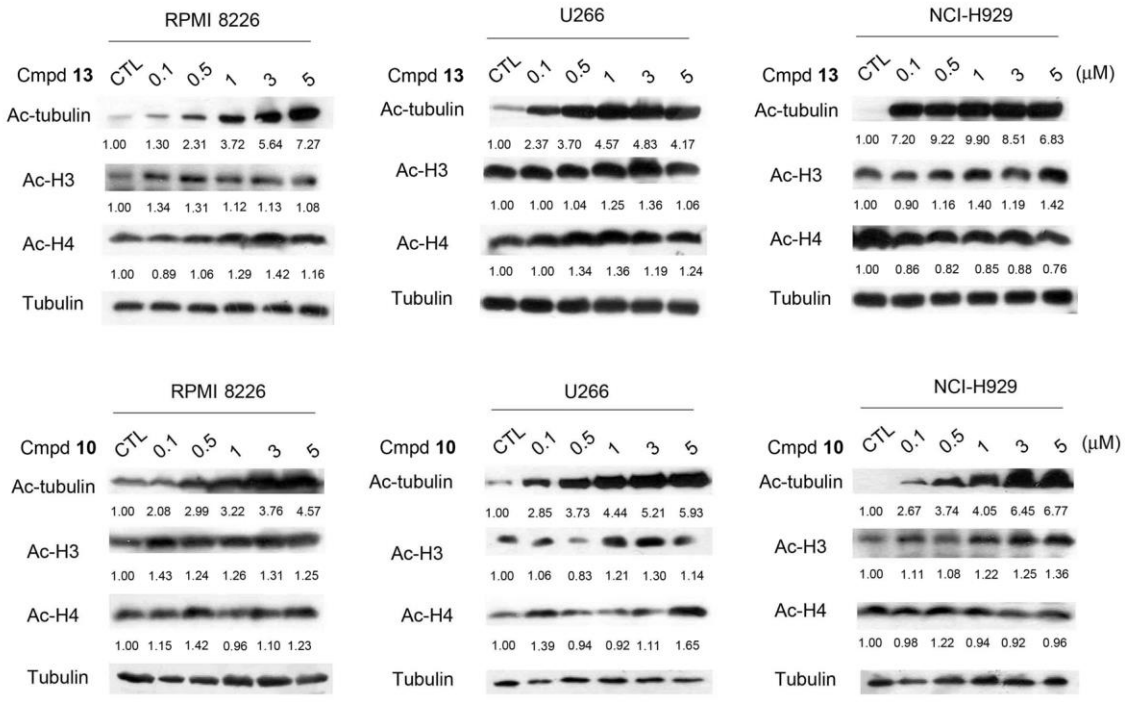



**Figure 3.** Compounds **10** and **13** increased acetyl-α-tubulin expression in human multiple myeloma cell lines.

**B. *In Vitro* Cell Growth Inhibition.**

A survey of the literature indicates that HDAC6 inhibitors are specifically able to inhibit the growth of multiple myeloma cells, and their combination with proteasome inhibitors trigger marked apoptosis of multiple myeloma cells.[19] Consequently, compounds **10** and **13** were selected to study their influence on the growth of three multiple myeloma cells, RPMI 8226, U266, and NCI-H929, using compound **3** as a reference compound (Table 3). It was found that U266 and NCI-H929 cells are more sensitive to compounds **10** and **13**, as compared to the reference compound **3**, and both **10** and **13** have antiproliferative activity for RPMI 8226 cells comparable to that of **3**. In addition, **10**, **13**, and **3** have no influence on normal bone marrow HS-5 cells. Further studies of the apoptotic effect of compound **13** and bortezomib, a proteasome inhibitor, were evaluated by flow cytometry. As shown in Figure 4, compound **13** (5 μM) alone or bortezomib (2.5 nM) alone caused mild apoptosis in U266 and NCI-H929 cells, but not in RPMI 8226 cells. However, the combination of the drugs exhibited significant apoptotic effect in all three multiple myelomas. We determine the apoptotic effect by Annexin V/PI stain in response to compound **13** and bortezomib treatment in human multiple myeloma cells. As shown as supporting information Figure S1, compound **13** (5 μM) or bortezomib (2.5 nM) alone for 24 h only caused mild apoptosis (upper right quadrant) in three multiple myeloma cells. However, the combination of the drugs exhibited significant apoptotic effect in all three multiple myelomas.

**Table 3.** Growth inhibition activity ($GI_{50}$, μM) of **10**, **13**, and reference **3** against multiple myeloma cell lines.

| | Multiple myeloma cells | | | Normal bone marrow cells |
|---|---|---|---|---|
| Cmpd | RPMI 8226 | U266 | NCI-H929 | HS-5 |
| **10** | 10.96±2.52 | 28.40±1.07 | 6.20±1.41 | >100 |
| **13** | 7.49±3.63 | 40.61±9.84 | 9.14±2.33 | >100 |
| **3** | 6.40±2.68 | >100 | >100 | >100 |

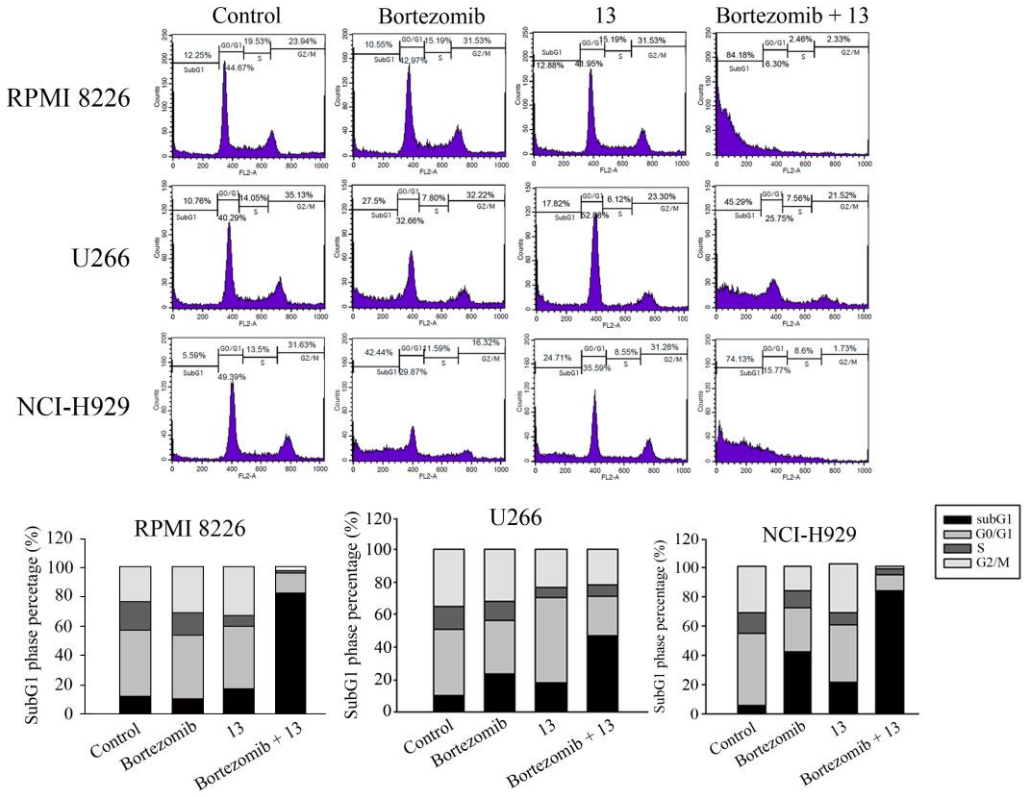



**Figure 4.** A combination of compound **13** with bortezomib induces significant apoptosis in three multiple myeloma cells (U266, NCI-H929 cells, and RPMI 8226 cells). Upper panel shows the result of flow cytometry and lower panel presents the distribution of cell cycle phases.

## C. Growth Inhibition of Human Multiple Myeloma Xenografts *in vivo*.

Figure 5 shows the *in vivo* activity of the mesylate of compound **13** in human multiple myeloma RPMI 8226 xenograft models, using bortezomib as a reference compound. Bortezomib and **13**·mesylate were used intravenously or intraperitoneally to treat human multiple myeloma RPMI 8226 xenograft mice, in the form either of a single-drug or a combination therapy (Figure 5). The use of **13**·mesylate alone at the concentration of 30 mg/kg led to the suppression of tumor growth by a tumor growth inhibition factor (%TGI) of 60.4% (Figure 5A), which is comparable to the response to 1 mg/kg of bortezomib (%TGI = 70.1%). Notably, treatment with bortezomib led to the death of a test animal. The combination treatment of bortezomib and **13**·mesylate in an *in vivo* study showed enhancement of tumor suppression by a %TGI of 86.2%. Unlike use of bortezomib alone, treatment with **13**·mesylate alone or combined with bortezomib has no influence on body weight of testing animals (Figure 5B). Investigation of the maximal tolerated dose of compound **13** is described in Figure 6. Compound **13** was administered intraperitoneally to healthy mice at doses of 50, 100, and 200 mg/kg, and body weights were recorded over 14 days. No significant adverse reaction with **13** was observed, suggesting that it was well tolerated and safe within the dosage range of up to 200 mg/kg.

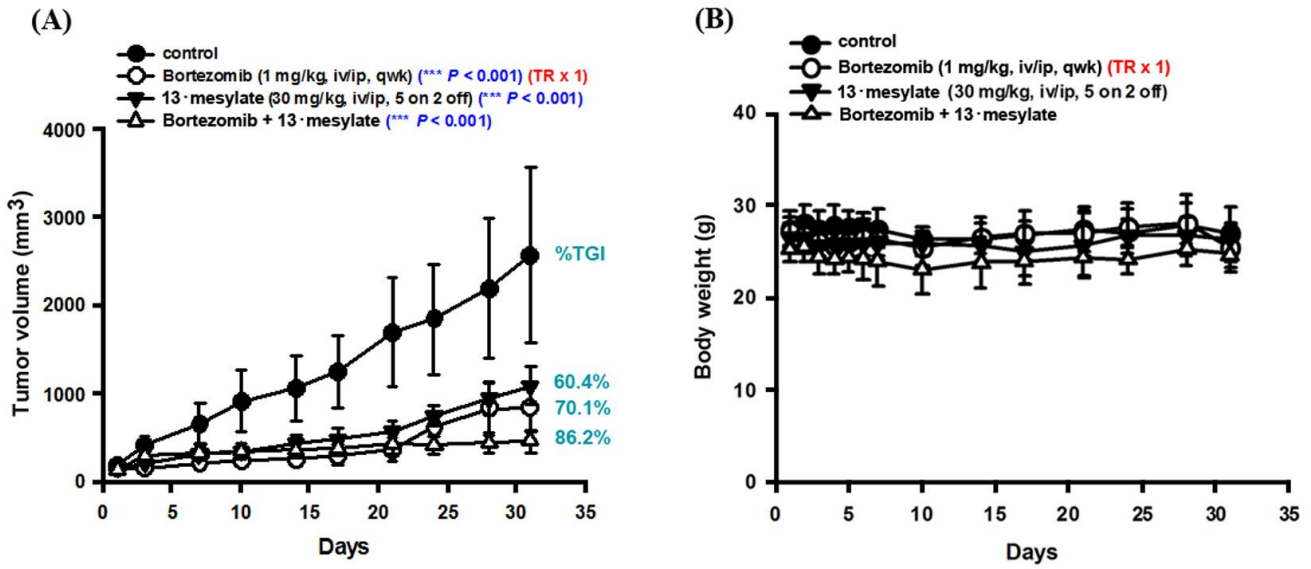



**Figure 5.** Anticancer activities of **13**·mesylate alone and combined with bortezomib in human multiple myeloma RPMI 8226 xenograft models. Tumor growth is tracked by the mean tumor volume ($mm^3$) ± S.E and calculated as % tumor growth inhibition (%TGI). Tumor volume was determined using caliper measurements and was calculated as the product of 1/2 x length x $width^2$. *, $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared with the control group. TR, treatment related death.

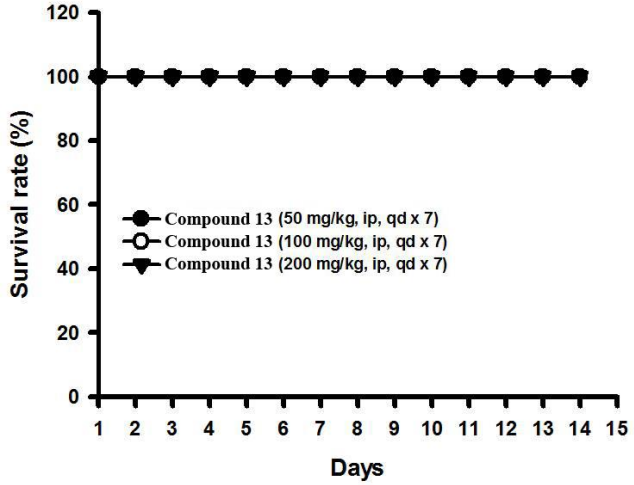



**Figure 6**. Maximum tolerance dose (MTD) evaluation of compound **13**. Thirty animals were randomized into three groups. The treated animals were administered compound **13** at doses of 50, 100, and 200 mg/kg. The animals were dosed intraperitoneally daily for 7 days and then monitored for one week. The animals were weighed daily.

## D. Kinase selectivity analysis

To know if compound **13** has off target effects, the kinase selectivity profiling against a comprehensive panel of 97 kinases was assessed using DiscoveRx's KinomeScan technology. Compound **13** was screened at a concentration of 1 μM. In this study, the potential of the test compound to disrupt the complex between a high affinity ATP-mimic probe immobilized on a solid support and the selected kinase was measured. The screening platform provides "Percent of Control" (POC) values which are indicative of a compound's affinity for kinases. Low POC values indicate stronger binding of the test compound with the test kinase. The results of the preliminary screening clearly indicates that there is no high affinity binding of compound **13** against the kinases tested (Supporting Information Table S1).

**E. *In vitro* hepatocyte stability**

A preliminary investigation of the ADME properties of compound **13** to ascertain its potential for further development was conducted. *In vitro* hepatocyte stability of compound **13** was determined using substrate depletion methodology for the determination of half-life ($t_{1/2}$) $_{and}$ $CL_{int}$ in cryopreserved hepatocytes from male SD rats, male beagle dogs and human hepatocytes. As shown in Table 4, compound **13** demonstrated good human hepatocytic stability. The stability order is human > dog > rat.

**Table 4**. Compound **13** stability, apparent half-life and *in vitro* clearance in rat, dog and human hepatocytes spiking concentration of 10 μM.

| Cryopreseved Hepatocytes | 60 min remaining (%) | Half life (min) | $CL_{int}$ (mL/min/kg) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Rat | 28.4 ± 1.5 | 55.83 | 150.85 |
| Dog | 70.6 ± 1.4 | 118.17 | 39.59 |
| Human | 86.4 ± 5.8 | 239.82 | 32.52 |

**F. Caco-2 cell permeability study**

An *in vitro* Caco-2 bi-directional permeability study was conducted to gain insights into membrane penetration, efflux from cells and possible interaction with the P-glycoprotein (P-gp) of compound **13**. Compound **13** (10 µM) and rhodamine 123 (10 µM) were added to either the apical or basolateral chambers of the *trans* well plate assembly and were incubated at 37 °C for a 2-hour period. Compounds with an efflux ratio greater than 2 are typically considered to be a substrate of the efflux transporter, especially the P-glycoprotein. Table 5 presents the data as apparent permeability ($P_{app}$) and efflux ratio. The Caco-2 cell permeability coefficients for apical to basolateral (*AP* → *BL*) movement and basolateral to apical (*BL* → *AP*) movement of compound **13** are 24.03 ± 2.87 x $10^{-6}$ cm/sec and 20.13 ± 3.13 x $10^{-6}$ cm/sec. This result shows that the ($P_{app,A\rightarrow B}$) of compound **13** (24.03 ± 2.87 x $10^{-6}$ cm/sec) was higher than the high ($P_{app,A\rightarrow B}$)cutoff (10 x $10^{-6}$ cm/sec). On the basis of permeability coefficient ($P_{app,A\rightarrow B}$), compound **13** could be considered to be highly permeable. The efflux ratio (($P_{app,B\rightarrow A}$)/($P_{app,A\rightarrow B}$)) of compound **13** is 0.84 indicating that it should not serve as a substrate of apical efflux transporters.

**Table 5.** Permeability, recovery and efflux ratio of compound **13** and P-gp control in both directions across Caco-2 cell Monolayers

| Compound | Direction | $P_{app}$ (x $10^{-6}$ cm/sec) | Recovery (%) | Efflux ratio |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **13** | A → B | 24.03 ± 2.87 | 72.21 ± 2.93 | 0.84 |
| | B → A | 20.13 ± 3.13 | 69.05 ± 2.38 | |
| Rhodamine 123 (P-gp control) | A → B | 1.16 ± 0.33 | 89.75 ± 3.90 | 6.50 |
| | B → A | 7.52 ± 0.48 | 93.86 ± 2.93 | |

**G. Antimutagenicity test**

An Ames test was performed to evaluate the mutagenicity of compound **13** against S. typhimurium TA 98 and TA 100 both with and without metabolic activation. Compound **13**, at concentrations of 3000, 300, 30 or 3 μg/plate was evaluated for possible activity to induce reversion of mutations at the histidine loci of two His-auxotrophic strains of *Salmonella typhimurium* (TA98 and TA100). In this assay, positive mutagenicity (induction of reversion) was evidenced by 3-fold increase in the reversion frequency of the treated group compared to the spontaneous reversion of the vehicle control group. Colonies numbering ≤50% of the vehicle control indicated cytotoxicity. Results clearly indicate that TA98 and TA100 strains failed to increase the number of revertant colonies when the bacteria was treated with compound **13** at various concentrations either in the presence or absence of metabolic activation enzymes (S9) (Supporting Information Table S2 and S3). The results of the antimutagenicity test revealed the absence of any significant mutagenicity and cytotoxicity.

**Conclusion**

This study resulted in the synthesis of a series of bicyclic arylamino/heteroarylamino hydroxamic acids (**7**-**31**) and testing of their HDAC6 inhibitory activity. Among them, compounds **10** and **13**, with a

quinoline moiety showed the most potent HDAC6 inhibition with $IC_{50}$ values in sub-nM range. The screening of **10** and **13** for HDAC isoforms inhibition revealed that **10** and **13** possess remarkable HDAC6 selectivity over other isoforms, and are between ten and several thousand times more potent than compound **3**, an HDAC6 inhibitor currently in clinical trials. The result of cellular assays demonstrated that **10** and **13** exhibit potent antiproliferative activity against multiple myeloma cells such as RPMI 8226, U266, and NCI-H929 cells. The mesylate of **13**, as a single drug or in combination with bortezomib is able to suppress the growth of tumors in human multiple myeloma xenograft models. Notably, combinatorial use of **13**·mesylate with bortezomib avoided the death of test animals. This study provides a series of (*N*-hydroxycarbonylbenzylamino)quinolines that show marked HDAC6 selectivity and could be further developed as efficient and safer therapeutic agents for the treatment of multiple myeloma.

## Experimental Section

(A) **Chemistry.** Nuclear magnetic resonance ($^1$HNMR) spectra were obtained with a Bruker DRX-500 spectrometer operating at 300 MHz, with chemical shift reported in parts per million (ppm, $\delta$) downfield from TMS, an internal standard. High-resolution mass spectra (HRMS) were measured with a JEOL (JMS-700) electron impact (EI) mass spectrometer. Elemental analyses were performed on a Heraeus varioIII-NCH. The purity of the final compounds was determined using an Agilent 1100 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) and was

found in all cases to be ≥ 95%. Flash column chromatography was carried out using silica gel (Merck Kieselgel 60, no. 9385, 230-400 mesh ASTM). All reactions conducted under an atmosphere of dry $N_2$.

**_N_-Hydroxy-4-((quinolin-2-ylamino)methyl)benzamide (7)**

NaOH powder (10 eq) was added to a mixture of hydroxylamine hydrochloride (10 eq) in MeOH at 0 $^oC$ and stirred for 30 min. The resulting mixture was filtered and the filtrate was added to a solution of compound **33a** (1 eq) in MeOH (0.1 M). Additional NaOH powder (1-4 eq) was added at 0 $^oC$ and the mixture was stirred at room temperature until the reaction was complete. The reaction mixture was then quenched with $H_2O$ and the pH value was adjusted to pH 7. The precipitate was filtered and washed with boiling MeOH to give compound **7** (yield 85%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.67 (d, $J$ = 5.7 Hz, 2H), 6.83 (d, $J$ = 9.0 Hz, 1H), 7.10-7.17 (m, 1H), 7.42-7.46 (m, 4H), 7.54-7.62 (m, 2H), 7.69 (d, $J$ = 8.1 Hz, 2H), 7.87 (d, $J$ = 9.0 Hz, 1H), 8.98 (s, 1H), 11.14 (s, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ [M + H]$^+$ calcd 294.1243, found 294.1208.

**_N_-Hydroxy-4-((quinolin-3-ylamino)methyl)benzamide (8)**

The title compound was obtained in 93% overall yield from compound **33b** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.43 (d, $J$ = 5.7 Hz, 2H), 6.93-6.98 (m, 2H), 7.27-7.38 (m, 2H), 7.67 (d, $J$ = 8.1 Hz, 2H), 7.54-7.58 (m, 1H), 7.69-7.78 (m, 3H), 8.54 (d, $J$ = 2.7 Hz, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ [M + H]$^+$ calcd 294.1243, found 294.1209.

**_N_-Hydroxy-4-((quinolin-4-ylamino)methyl)benzamide (9)**

The title compound was obtained in 83% overall yield from compound **33c** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.59 (d, $J$ = 5.1 Hz, 2H), 6.29 (d, $J$ = 5.4 Hz, 1H), 7.42-7.48 (m, 3H), 7.59-7.65 (m, 1H), 7.69 (d, $J$ = 8.1 Hz, 2H), 7.78 (d, $J$ = 8.4 Hz, 1H), 7.95 (s, 1H), 8.29 (d, $J$ = 5.4 Hz, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1209.

***N*-Hydroxy-4-((quinolin-5-ylamino)methyl)benzamide (10)**

The title compound was obtained in 91% overall yield from compound **33d** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.54 (d, $J$ = 5.7 Hz, 2H), 6.37 (d, $J$ = 7.5 Hz, 1H), 7.18 (d, $J$ = 8.4 Hz, 1H), 7.24 (t, $J$ = 6.0 Hz, 1H), 7.34-7.48 (m, 4H), 7.69 (d, $J$ = 8.4 Hz, 2H), 8.69 (d, $J$ = 8.4 Hz, 1H), 8.80 (dd, $J$ = 1.5, 4.2 Hz, 1H), 9.00 (s, 1H), 11.14 (s, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1236.

***N*-Hydroxy-4-((quinolin-6-ylamino)methyl)benzamide (11)**

The title compound was obtained in 93% overall yield from compound **33e** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.56 (d, $J$ = 5.7 Hz, 2H), 6.62 (d, $J$ = 2.4 Hz, 1H), 6.88 (t, $J$ = 5.7 Hz, 1H), 7.22-7.30 (m, 2H), 7.51 (d, $J$ = 8.1 Hz, 2H), 7.71 (d, $J$ = 9.0 Hz, 1H), 7.88-7.92 (m, 3H), 8.46 (dd, $J$ = 1.5, 4.2 Hz, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1242.

***N*-Hydroxy-4-((quinolin-7-ylamino)methyl)benzamide (12)**

The title compound was obtained in 90% overall yield from compound **33f** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.45 (d, $J$ = 5.7 Hz, 2H), 6.70 (d, $J$ = 2.1 Hz, 1H), 6.99-7.14 (m, 3H), 7.47 (d, $J$ = 8.1 Hz, 2H), 7.62 (d, $J$ = 8.7 Hz, 1H), 7.71 (d, $J$ = 8.4 Hz, 2H), 8.00 (dd, $J$ = 1.2, 8.0 Hz, 1H), 8.54-8.57 (m, 1H), 9.02 (s, 1H), 11.15 (s, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ $[M + H]^+$ calcd 293.1243, found 294.1222.

**_N_-Hydroxy-4-((quinolin-8-ylamino)methyl)benzamide (13) and 13·mesylate**

The title compound was obtained in 92% overall yield from compound **33g** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.57 (d, $J$ = 6.3 Hz, 2H), 6.51 (dd, $J$ = 1.2, 7.8 Hz, 1H), 7.04 (dd, $J$ = 1.2, 8.4 Hz, 1H), 7.21-7.28 (m, 2H), 7.45 (d, $J$ = 8.1 Hz, 2H), 7.50 (dd, $J$ = 4.2, 8.4 Hz, 1H), 7.68 (d, $J$ = 8.1 Hz, 2H), 8.20 (dd, $J$ = 1.8, 8.4 Hz, 1H), 8.76 (dd, $J$ = 1.8, 4.2 Hz, 1H), 8.96 (brs, 1H), 11.11 (brs, 1H). HRMS (ESI) for $C_{17}H_{18}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1217. For **13**·mesylate: a solution of MsOH in dioxane (0.2M) was added to a solution of compound **13** in dioxane (0.2M), and the resulting mixture was stirred at room temperature. The reaction mixture was filtered and washed with dioxane to afford the **13**·mesylate. $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 2.34 (s, 3H), 4.59 (s, 2H), 6.56 (dd, $J$ = 0.9, 7.8 Hz, 1H), 7.08 (dd, $J$ = 1.2, 8.1 Hz, 1H), 7.26-7.32 (m, 1H), 7.46 (d, $J$ = 8.4 Hz, 2H), 7.55 (dd, $J$ = 8.4, 8.4 Hz, 1H), 7.68 (d, $J$ = 8.1 Hz, 2H), 8.26 (dd, $J$ = 1.8, 8.4 Hz, 1H); Anal. Calcd for $C_{18}H_{19}N_3O_5S$: C, 55.52; H, 4.92; N, 10.79; S, 8.23. Found: C, 55.65; H, 4.81; N, 10.72; S, 7.91.

**(_E_)-_N_-Hydroxy-3-(4-((quinolin-5-ylamino)methyl)phenyl)acrylamide (14)**

The title compound was obtained in 96% overall yield from compound **34d** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.51 (d, $J$ = 6.0 Hz, 2H), 6.36-6.43 (m, 2H), 7.15-7.24 (m, 2H), 7.37-7.45 (m, 5H), 7.50 (d, $J$ = 8.1 Hz, 2H), 8.69 (d, $J$ = 8.4 Hz, 1H), 8.78-8.81 (m, 1H), 9.01 (s, 1H), 10.72 (s, 1H). HRMS (ESI) for $C_{19}H_{18}N_3O_2$ $[M + H]^+$ calcd 320.1399, found 320.1375.

**(*E*)-*N*-Hydroxy-3-(4-((quinolin-8-ylamino)methyl)phenyl)acrylamide (15)**

The title compound was obtained in 88% overall yield from compound **34g** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.55 (d, $J$ = 6.6 Hz, 2H), 6.40 (d, $J$ = 15.6 Hz, 1H), 6.53 (dd, $J$ = 0.9, 7.5 Hz, 1H), 7.02-7.06 (m, 1H), 7.19-7.29 (m, 2H), 7.38-7.53 (m, 6H), 8.20 (dd, $J$ = 1.8, 8.4 Hz, 1H), 8.76 (dd, $J$ = 1.5, 4.2 Hz, 1H), 8.99 (brs, 1H), 10.72 (brs, 1H). HRMS (ESI) for $C_{19}H_{18}N_3O_2$ $[M + H]^+$ calcd 320.1399, found 320.1382.

***N*$_1$-Hydroxy-*N*$_4$-(quinolin-5-yl)terephthalamide (16)**

The title compound was obtained in 93% overall yield from compound **35a** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 7.55 (dd, $J$ = 4.2 Hz, 1H),7.70-7.73 (m, 1H), 7.77-7.83 (m, 1H), 7.90-7.98 (m, 3H), 8.14 (d, $J$ = 8.4 Hz, 2H), 8.41 (d, $J$ = 8.1 Hz, 1H), 8.93 (dd, $J$ = 1.5, 4.2 Hz, 1H), 9.17 (s, 1H), 10.64 (s, 1H), 11.41 (s, 1H). HRMS (ESI) for $C_{17}H_{14}N_3O_3$ $[M + H]^+$ calcd 308.1035, found 308.1035.

***N*$^1$-Hydroxy-*N*$^4$-(quinolin-8-yl)terephthalamide (17)**

The title compound was obtained in 88% overall yield from compound **35b** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$): $\delta$ 7.61-7.77 (m, 3H), 7.95-8.16 (m, 4H), 8.45 (d, $J$ = 8.4 Hz, 1H), 8.69-8.75 (m, 1H), 8.97 (d, $J$ = 3.6 Hz, 1H), 10.68 (s, 1H). HRMS (ESI) for $C_{17}H_{14}N_3O_3$ $[M + H]^+$ calcd 308.1035, found 308.1035.

***N*-Hydroxy-4-((quinolin-8-yloxy)methyl)benzamide (18)**

The title compound was obtained in 90% overall yield from compound **37a** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 5.37 (s, 2H), 7.25-7.29 (m, 1H), 7.46-7.57 (m, 3H), 7.61 (d, $J$ = 8.1 Hz, 2H), 7.78 (d, $J$ = 8.1 Hz, 2H), 8.30-8.34 (m, 1H), 8.85-8.88 (m, 1H), 9.03 (brs, 1H), 11.22 (brs, 1H). HRMS (ESI) for $C_{17}H_{15}N_2O_3$ $[M + H]^+$ calcd 295.1083, found 295.1057.

***N*-Hydroxy-4-((quinolin-8-ylthio)methyl)benzamide (19)**

The title compound was obtained in 92% overall yield from compound **37b** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.38 (s, 2H), 7.47-7.61 (m, 5H), 7.68-7.73 (m, 3H), 8.35 (dd, $J$ = 1.2, 8.4 Hz, 1H), 8.88 (dd, $J$ = 1.8, 4.2 Hz, 1H), 9.00 (brs, 1H), 11.17 (brs, 1H). HRMS (ESI) for $C_{17}H_{15}N_2O_2S$ $[M + H]^+$ calcd 311.0854, found 311.0848.

***N*-Hydroxy-4-(2-(quinolin-8-yl)ethyl)benzamide (20)**

The title compound was obtained in 92% overall yield from compound **37c** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.03-3.09 (m, 2H), 3.47-3.53

(m, 2H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.45-7.58 (m, 3H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.81(dd, *J* = 1.5, 8.1 Hz, 1H), 8.31-8.35 (m, 1H), 8.95 (dd, *J* = 1.8, 4.2 Hz, 1H), 8.98 (s, 1H), 11.15 (s, 1H). HRMS (ESI) for $C_{18}H_{17}N_2O_2$ $[M + H]^+$ calcd 293.1290, found 293.1262.

***N*-Hydroxy-4-(quinolin-8-ylmethylamino)benzamide (21)**

The title compound was obtained in 87% overall yield from compound **37d** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.95 (d, *J* = 6.0 Hz, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 6.79-6.84 (m, 1H), 7.45-7.68 (m, 5H), 7.87 (d, *J* = 8.1 Hz, 1H), 8.37-8.41 (m, 1H), 8.64 (s, 1H), 8.96-8.99 (m, 1H), 10.71 (brs, 1H). HRMS (ESI) for $C_{17}H_{16}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1238.

***N*-Hydroxy-4-(quinolin-8-ylamino)benzamide (22)**

The title compound was obtained in 75% overall yield from compound **37e** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 7.33-7.38 (m, 2H), 7.57-7.63 (m, 2H), 7.70-7.78 (m, 4H), 8.62 (dd, *J* = 1.5, 8.4 Hz, 1H), 8.97 (dd, *J* = 1.5, 4.5 Hz, 1H). HRMS (ESI) for $C_{16}H_{14}N_3O_2$ $[M + H]^+$ calcd 280.1086, found 280.1081.

***N*-Hydroxy-3-((quinolin-8-ylamino)methyl)benzamide (23)**

The title compound was obtained in 92% overall yield from compound **33h** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.57 (d, *J* = 6.3 Hz, 2H), 6.54 (d, *J* = 7.2, 8.1 Hz, 1H), 7.04 (d, *J* = 7.5 Hz, 1H), 7.19-7.29 (m, 2H), 7.34-7.40 (m, 1H), 7.47-7.60 (m,

3H), 7.82 (s, 1H), 8.17-8.22 (m, 1H), 8.75-8.77 (m, 1H), 8.99 (s, 1H), 11.19 (s, 1H). HRMS (ESI) for $C_{17}H_{16}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1209.

***N*-Hydroxy-4-(((2-methylquinolin-8-yl)amino)methyl)benzamide (24)**

The title compound was obtained in 91% overall yield from compound **39a** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 2.67 (s, 3H), 4.57 (d, $J$ = 6.6 Hz, 2H), 6.47 (d, $J$ = 7.5 Hz, 1H), 6.97-7.05 (m, 2H), 7.17 (t, $J$ = 8.1 Hz, 1H), 7.38 (d, $J$ = 8.1 Hz, 1H), 7.44 (d, $J$ = 7.8 Hz, 2H), 7.68 (d, $J$ = 8.1 Hz, 2H), 8.08 (d, $J$ = 8.4 Hz, 1H), 8.96 (brs, 1H), 11.11 (brs, 1H). HRMS (ESI) for $C_{18}H_{18}N_3O_2$ $[M + H]^+$ calcd 308.1399, found 308.1399.

***N*-Hydroxy-4-((isoquinolin-8-ylamino)methyl)benzamide (25)**

The title compound was obtained in 90% overall yield from compound **39b** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.56 (d, $J$ = 5.7 Hz, 2H), 6.42 (d, $J$ = 7.8 Hz, 1H), 7.02 (d, $J$ = 8.1 Hz, 1H), 7.34-7.40 (m, 1H), 7.47 (d, $J$ = 8.1 Hz, 2H), 7.58-7.63 (m, 2H), 7.70 (d, $J$ = 8.1 Hz, 2H), 8.40 (d, $J$ = 5.7 Hz, 1H), 9.03 (brs, 1H), 9.64 (s, 1H), 11.14 (brs, 1H). HRMS (ESI) for $C_{17}H_{16}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1221.

***N*-Hydroxy-4-((isoquinolin-5-ylamino)methyl)benzamide (26)**

The title compound was obtained in 90% overall yield from compound **39c** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.55 (d, $J$ = 6.0 Hz, 2H), 6.51 (d, $J$ = 7.5 Hz, 1H), 7.20-7.24 (m, 2H), 7.31 (t, $J$ = 7.8 Hz, 1H), 7.44 (d, $J$ = 8.1 Hz, 1H), 7.68 (d, $J$ = 8.1

Hz, 1H), 8.11 (d, *J* = 6.0 Hz, 1H), 8.43 (d, *J* = 5.7 Hz, 1H), 8.97 (s, 1H), 9.11 (s, 1H), 11.11 (s, 1H). HRMS (ESI) for $C_{17}H_{16}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1210.

**_N_-Hydroxy-4-((isoquinolin-4-ylamino)methyl)benzamide (27)**

The title compound was obtained in 88% overall yield from compound **39d** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.58 (d, *J* = 6.0 Hz, 2H), 7.13 (d, *J* = 6.0 Hz, 1H), 7.46-7.54 (m, 3H), 7.59-7.65 (m, 1H), 7.67-7.74 (m, 3H), 7.88-7.96 (m, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.98 (s, 1H), 11.13 (s, 1H). HRMS (ESI) for $C_{17}H_{16}N_3O_2$ $[M + H]^+$ calcd 294.1243, found 294.1212.

**_N_-Hydroxy-4-((quinoxalin-5-ylamino)methyl)benzamide (28)**

The title compound was obtained in 78% overall yield from compound **39e** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.59 (d, *J* = 6.6 Hz, 2H), 6.56 (dd, *J* = 0.6, 7.8 Hz, 1H), 7.16 (dd, *J* = 0.9, 8.4 Hz, 1H), 7.43-7.52 (m, 4H), 7.68 (d, *J* = 8.4 Hz, 2H), 8.77 (d, *J* = 1.8 Hz, 1H), 8.89 (d, *J* = 1.8 Hz, 1H), 8.97 (brs, 1H), 11.12 (brs, 1H). HRMS (ESI) for $C_{16}H_{15}N_4O_2$ $[M + H]^+$ calcd 295.1195, found 295.1190.

**4-((1*H*-Indazol-7-ylamino)methyl)-*N*-hydroxybenzamide (29)**

The title compound was obtained in 88% overall yield from compound **39f** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 4.50 (d, *J* = 5.7 Hz, 2H), 6.14-6.18 (m, 1H), 6.27 (d, *J* = 7.2 Hz, 1H), 6.79-6.86 (m, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 8.4 Hz,

2H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.93 (s, 1H), 8.99 (brs, 1H), 11.14 (brs, 1H), 12.65 (brs, 1H). HRMS (ESI) for $C_{15}H_{15}N_4O_2$ $[M + H]^+$ calcd 283.1195, found 283.1187.

**_N_-Hydroxy-4-((5,6,7,8-tetrahydronaphthalen-1-ylamino)methyl)benzamide (30)**

The title compound was obtained in 93% overall yield from compound **39g** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 1.64-1.69 (m, 2H), 1.77-1.81 (m, 2H), 2.43-2.51 (m, 2H), 2.59-2.64 (m, 2H), 4.35 (d, *J* = 5.7 Hz, 2H), 5.56 (t, *J* = 6.0 Hz, 1H), 6.11 (d, *J* = 7.8 Hz, 1H), 6.27 (d, *J* = 7.5 Hz, 1H), 6.73 (t, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 2H), 8.97 (brs, 1H), 11.11 (brs, 1H). HRMS (ESI) for $C_{18}H_{21}N_2O_2$ $[M + H]^+$ calcd 297.1603, found 297.1575.

**4-((2,3-Dihydro-1*H*-inden-4-ylamino)methyl)-*N*-hydroxybenzamide (31)**

The title compound was obtained in 89% overall yield from compound **39h** in a manner similar to that described for the preparation of **7**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 1.94-2.04 (m, 2H), 2.68-2.74 (m, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 4.35 (d, *J* = 6.0 Hz, 2H), 5.72-5.77 (m, 1H), 6.11 (d, *J* = 7.8 Hz, 1H), 6.43 (d, *J* = 7.5 Hz, 1H), 6.76-6.82 (m, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 8.96 (brs, 1H), 11.11 (brs, 1H). HRMS (ESI) for $C_{17}H_{18}N_2O_2$ $[M + H]^+$ calcd 297.1447, found 297.1423.

**Methyl 4-((quinolin-2-ylamino)methyl)benzoate (33a)**

NaOH powder (1.5 eq) was added to a mixture of methyl 4-(aminomethyl)benzoate hydrochloride (1.5 eq) in MeOH at 0 $^o$C and stirred for 1 h. The resulting mixture was filtered and the filtrate was

evaporated *in vacuo*. The residue was extracted with EtOAc and $H_2O$, and then the organic layer was collected, dried, and dissolved in toluene (0.5 M). A mixture of $Pd(OAc)_2$ (0.06 eq), DPEphos (0.12 eq), and $K_3PO_4$ (3 eq) was added at room temperature under $N_2$ to compound **32a** (1 eq). The resulting mixture was heated to 100 ºC for 10 h then cooled to room temperature. The mixture was filtered and washed with EtOAc. The organic layer was collected and purified through column chromatography (EtOAc/*n*-hexane) to afford compound **33a** (yield 29%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H), 4.72 (d, $J$ = 6.0 Hz, 2H), 6.84 (d, $J$ = 9.0 Hz, 1H), 7.10-7.17 (m, 1H), 7.40-7.48 (m, 2H), 7.52 (d, $J$ = 8.4 Hz, 1H), 7.59-7.64 (m, 2H), 7.85-7.93 (m, 3H).

**Mthyl 4-((quinolin-3-ylamino)methyl)benzoate (33b)**

A solution of aminoquinoline (1 eq) and methyl 4-formylbenzoate in AcOH (1 M) was stirred at room temperature for 30 min. A solution of sodium triacetoxyborohydride (2 eq) in AcOH (1 M) was added dropwise to this solution, and the resulting solution was stirred at room temperature for 2 h. The reaction mixture was quenched by ice water and the pH of the residue was adjusted to pH 10 by addition of NaOH solution. The mixture was extracted with EtOAc and the organic layer was purified through column chromatography (EtOAc/*n*-hexane) to afford compound **33b** (yield 89%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H), 4.48 (d, $J$ = 6.0 Hz, 2H), 6.94 (d, $J$ = 2.7 Hz, 1H), 6.98-7.03 (m, 1H), 7.27-7.38 (m, 2H), 7.53-7.57 (m, 3H), 7.74-7.78 (m, 1H), 7.90-7.95 (m, 2H), 8.55 (d, $J$ = 2.7 Hz, 1H).

**Methyl 4-((quinolin-4-ylamino)methyl)benzoate (33c)**

The title compound was obtained in 88% overall yield from compound **32c** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.63 (d, $J$ = 6.0 Hz, 2H), 6.27 (d, $J$ = 5.4 Hz, 1H), 7.43-7.53 (m, 3H), 7.59-7.65 (m, 1H), 7.77-7.81 (m, 1H), 7.89-7.93 (m, 2H), 7.97 (t, $J$ = 6.0 Hz, 1H), 8.27-8.31 (m, 2H).

**Methyl 4-((quinolin-5-ylamino)methyl)benzoate (33d)**

The title compound was obtained in 93% overall yield from compound **32d** in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.58 (d, $J$ = 6.0 Hz, 2H), 6.34 (d, $J$ = 7.8 Hz, 1H), 7.19 (d, $J$ = 8.4 Hz, 1H), 7.28 (t, $J$ = 6.0 Hz, 1H), 7.34-7.40 (m, 1H), 7.43 (dd, $J$ = 1.2, 8.4 Hz, 1H), 7.53 (d, $J$ = 8.1 Hz, 2H), 7.91 (d, $J$ = 8.4 Hz, 2H), 8.69 (d, $J$ = 8.1 Hz, 1H), 8.80 (dd, $J$ = 1.5, 4.2 Hz, 1H).

**Methyl 4-((quinolin-6-ylamino)methyl)benzoate (33e)**

The title compound was obtained in 85% overall yield from compound **32e** in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H), 4.47 (d, $J$ = 6.0 Hz, 2H), 6.62 (d, $J$ = 2.4 Hz, 1H), 6.86-6.91 (m, 1H), 7.22-7.30 (m, 2H), 7.54 (d, $J$ = 9.0 Hz, 2H) , 7.72 (d, $J$ = 9.0 Hz, 1H), 7.86-7.94 (m, 1H), 8.46 (dd, $J$ = 1.5, 4.2 Hz, 1H).

**Methyl 4-((quinolin-7-ylamino)methyl)benzoate (33f)**

The title compound was obtained in 50% overall yield from compound **32f** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO- $d_6$) $\delta$ 3.82 (s, 3H), 4.50 (d, $J$ = 6.0

Hz, 2H), 6.68 (d, *J* = 2.4 Hz, 1H), 7.02-7.13 (m, 3H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 9.0 Hz, 1H), 7.93 (dt, *J* = 1.8, 8.4 Hz, 2H), 7.98-8.02 (m, 1H), 8.55 (dd, *J* = 1.8, 4.2 Hz, 1H).

**Methyl 4-((quinolin-8-ylamino)methyl)benzoate (33g)**

The title compound was obtained in 90% overall yield from compound **32g** in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.62 (d, *J* = 6.3 Hz, 2H), 6.46-6.50 (m, 1H), 7.04 (dd, *J* = 0.9, 8.1 Hz, 1H), 7.21-7.32 (m, 2H), 7.47-7.53 (m, 3H), 7.89 (d, *J* = 8.4 Hz, 2H), 8.17-8.21 (m, 1H), 8.76 (dd, *J* = 1.8, 4.2 Hz, 1H).

**Methyl 3-((quinolin-8-ylamino)methyl)benzoate (33h)**

The title compound was obtained in 38% overall yield from compound **32e** with methyl 3-formylbenzoate in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.80 (s, 3H), 4.60 (d, *J* = 6.3 Hz, 2H), 6.49-6.53 (m, 1H), 7.03 (dd, *J* = 1.2, 8.1 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.28-7.34 (m, 1H), 7.43-7.53 (m, 2H), 7.66-7.70 (m, 1H), 7.79-7.83 (m, 1H), 8.00-8.12 (m, 1H), 8.18-8.22 (m, 1H), 8.77 (dd, *J* = 1.8, 4.2 Hz, 1H).

**(*E*)-Methyl 3-(4-((quinolin-5-ylamino)methyl)phenyl)acrylate (34a)**

The title compound was obtained in 81% overall yield from compound **32d** with (*E*)-methyl 3-(4-formylphenyl)acrylate in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.70 (s, 3H), 4.52 (d, *J* = 6.0 Hz, 2H), 6.38 (d, *J* = 7.2 Hz, 1H), 6.57 (d, *J* = 16.2 Hz,

1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.23 (t, *J* = 6.0 Hz, 1H), 7.34-7.45 (m, 4H), 7.59-7.67 (m, 3H), 8.69 (d, *J* = 8.4 Hz, 1H), 8.80 (d, *J* = 1.5, 4.2 Hz, 1H).

**(*E*)-Methyl 3-(4-((quinolin-8-ylamino)methyl)phenyl)acrylate (34b)**

The title compound was obtained in 90% overall yield from compound **32g** with (*E*)-methyl 3-(4-formylphenyl)acrylate in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.70 (s, 3H), 4.53 (d, *J* = 5.7 Hz, 2H), 6.38 (d, *J* = 7.8 Hz, 1H), 6.58 (d, *J* = 16.2 Hz, 1H), 7.17-7.25 (m, 2H), 7.35-7.43 (m, 4H), 7.44-7.67 (m, 3H), 8.70 (d, *J* = 8.7 Hz, 1H), 8.79-8.81 (m, 1H).

**Methyl 4-(quinolin-5-ylcarbamoyl)benzoate (35a)**

Methyl 4-(chlorocarbonyl)benzoate (1 eq) was added slowly at 0 $^o$C to a solution of compound **32d** (1.3 eq) and triethylamine (2 eq) in DCM (0.5 M) and the solution was then stirred at room temperature under $N_2$ for 12 h. The resulting solution was diluted with EtOAc and washed with 2 N HCl. The organic layer was collected *in vacuo* to compound **35a** (yield 9%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.91 (s, 3H), 7.56 (dd, *J* = 1.2, 8.4 Hz, 1H), 7.70-7.73 (m, 1H), 7.77-7.83 (m, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.21 (d, *J* = 8.4 Hz, 2H), 8.41 (d, *J* = 8.7 Hz, 2H), 8.92-8.95 (m, 1H), 10.73 (s, 1H).

**Methyl 4-(quinolin-8-ylcarbamoyl)benzoate (35b)**

A solution of compound **32g** (1 eq), EDC·HCl (1.2 eq), 4-dimethylaminopyridine (1 eq) and monomethyl terephthalate (1.2 eq) in DCM (0.1 M) was stirred at room temperature under $N_2$ for 12 h. The reaction mixture was quenched with $H_2O$ and extracted with DCM. The organic layer was collected and purified through column chromatography (EtOAc/*n*-hexane) to afford compound **35b** (yield 86%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.90 (s, 3H), 7.61-7.69 (m, 2H), 7.73-7.77 (m, 1H), 8.14 (s, 4H), 8.44 (dd, $J$ = 1.5, 8.4 Hz, 1H), 8.70 (dd, $J$ = 1.5, 7.5 Hz, 1H), 8.96 (dd, $J$ = 1.8, 4.2 Hz, 1H), 10.70 (s, 1H).

**Methyl 4-((quinolin-8-yloxy)methyl)benzoate (37a)**

A solution of compound **38a** (1 eq), methyl 4-(chloromethyl)benzoate (1.1 eq) and potassium carbonate (2 eq) in acetone (10 mL) was heated at reflux for 12 h. The reaction mixture was filtered and the filtrate was purified through column chromatography (EtOAc/*n*-hexane) to provide the product (yield 89%). $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.85 (s, 3H), 5.42 (s, 2H), 7.25-7.29 (m, 1H), 7.46-7.59 (m, 3H), 7.69 (d, $J$ = 8.1 Hz, 2H), 7.99-8.03 (m, 2H), 8.30-8.34 (m, 1H), 8.86-8.89 (m, 1H).

**Methyl 4-((quinolin-8-ylthio)methyl)benzoate (37b)**

The title compound was obtained in 89% overall yield from compound **36b** in a manner similar to that described for the preparation of **37a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H), 4.43 (s, 2H), 7.46-7.52 (m, 1H), 7.55-7.64 (m, 4H), 7.69-7.73 (m, 1H), 7.89-7.92 (m, 2H), 8.35 (dd, $J$ = 1.8, 8.4 Hz, 1H), 8.88 (dd, $J$ = 1.8, 4.2 Hz, 1H).

**Methyl 4-(2-(quinolin-8-yl)ethyl)benzoate (37c)**

A solution of compound **36c** (1 eq), methyl triphenyl-(4-methoxycarbonylbenzyl)phosphonium chloride (1.1 eq), sodium hydroxide (1.1 eq), and THF was stirred at room temperature for 12 h. The reaction mixture was quenched with $H_2O$ and the organic layer was dried *in vacuo*. The resulting residue was dissolved in MeOH and then palladium on carbon (50 mg) was added slowly and the mixture was stirred at room temperature under $H_2$ for 2 h. The reaction mixture was filtered and the filtrate was purified through column chromatography (EtOAc/*n*-hexane) to provide compound **37c** (yield 55%). $^1$HNMR (300 MHz, $CDCl_3$) $\delta$ 3.16-3.22 (m, 2H), 3.58-3.64 (m, 2H), 3.91 (s, 3H), 7.29 (d, $J$ = 8.4 Hz, 1H), 7.38-7.44 (m, 3H), 7.66-7.72 (m, 1H), 7.94-8.00 (m, 2H), 8.15 (dd, $J$ = 1.8, 8.4 Hz, 1H), 8.97 (dd, $J$ = 1.8, 4.2 Hz, 1H).

**Methyl 4-(quinolin-8-ylmethylamino)benzoate (37d)**

The title compound was obtained in 82% overall yield from compound **36c** with methyl 4-aminobenzoate in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.71 (s, 3H), 4.98 (s, 2H), 6.61-6.65 (m, 2H), 7.14-7.18 (m, 1H), 7.25-7.67 (m, 5H), 7.88 (d, $J$ = 8.1 Hz, 1H), 8.37-8.41 (m, 1H), 8.97-8.99 (m, 1H).

**Methyl 4-(quinolin-8-ylamino)benzoate (37e)**

The title compound was obtained in 93% overall yield from compound **36d** with methyl 4-aminobenzoate in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz,

DMSO-$d_6$): $\delta$ 3.79 (s, 3H), 7.44-7.54 (m, 4H), 7.56-7.61 (m, 1H), 7.66-7.70 (m, 1H), 7.87 (d, $J$ = 9.0 Hz, 2H), 8.32-8.36 (m, 1H), 8.86-8.89 (m, 1H), 9.12 (s, 1H).

**Methyl 4-(((2-methylquinolin-8-yl)amino)methyl)benzoate (39a)**

The title compound was obtained in 79% overall yield from compound **38a** in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, $CDCl_3$) $\delta$ 2.71 (s, 3H), 3.91 (s, 3H), 6.36 (d, $J$ = 6.0 Hz, 2H), 6.52 (dd, $J$ = 0.6, 7.5 Hz, 1H), 6.72-6.77 (m, 1H), 7.01-7.05 (m, 1H), 7.20-7.28 (m, 2H), 7.50 (d, $J$ = 8.4 Hz, 1H), 7.94-8.03 (m, 3H).

**Methyl 4-((isoquinolin-8-ylamino)methyl)benzoate (39b)**

The title compound was obtained in 73% overall yield from compound **38b** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.35 (s, 3H), 4.60 (d, $J$ = 5.7 Hz, 2H), 6.39 (d, $J$ = 7.8 Hz, 1H), 7.28 (d, $J$ = 8.1 Hz, 1H), 7.36 (t, $J$ = 7.8 Hz, 1H), 7.54 (d, $J$ = 8.1 Hz, 2H), 7.58-7.66 (m, 1H), 7.89-7.92 (m, 2H), 8.40 (d, $J$ = 5.7 Hz, 1H), 9.64 (s, 1H).

**Methyl 4-((isoquinolin-5-ylamino)methyl)benzoate (39c)**

The title compound was obtained in 75% overall yield from compound **38c** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.59 (d, $J$ = 5.7 Hz, 2H), 6.48-6.51 (m, 1H), 7.21-7.34 (m, 3H), 7.52 (d, $J$ = 8.1 Hz, 2H), 7.90 (d, $J$ = 8.4 Hz, 2H), 8.12 (d, $J$ = 6.0 Hz, 1H), 8.44 (d, $J$ = 6.0 Hz, 1H), 9.12 (s, 1H).

**Methyl 4-((isoquinolin-4-ylamino)methyl)benzoate (39d)**

The title compound was obtained in 23% overall yield from compound **38d** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.62 (d, $J$ = 6.0 Hz, 2H), 7.13 (t, $J$ = 6.0 Hz, 1H), 7.52-7.57 (m, 3H), 7.58-7.64 (m, 1H), 7.68-7.74 (m, 1H), 7.90-7.96 (m, 3H), 8.29 (d, $J$ = 8.4 Hz, 1H), 8.51 (s, 1H).

**Methyl 4-((quinoxalin-5-ylamino)methyl)benzoate (39e)**

The title compound was obtained in 90% overall yield from compound **38e** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H), 4.63 (d, $J$ = 6.6 Hz, 2H), 6.53 (dd, $J$ = 0.9, 7.8 Hz, 1H), 7.16 (dd, $J$ = 1.2, 8.4 Hz, 1H), 7.45-7.54 (m, 4H), 7.87-7.91 (m, 2H), 8.77 (d, $J$ = 1.8 Hz, 1H), 8.90 (d, $J$ = 1.8 Hz, 1H).

**Methyl 4-((1*H*-indazol-7-ylamino)methyl)benzoate (39f)**

The title compound was obtained in 85% overall yield from compound **38f** in a manner similar to that described for the preparation of **33b**: $^1$HNMR (300 MHz, $CDCl_3$) $\delta$ 3.83 (s, 3H), 4.55 (d, $J$ = 5.7 Hz, 2H), 6.18 (t, $J$ = 5.7 Hz, 1H), 6.26 (d, $J$ = 7.2 Hz, 1H), 6.79-6.85 (m, 1H), 6.96 (d, $J$ = 8.1 Hz, 1H), 7.55 (d, $J$ = 8.1 Hz, 2H), 7.92-7.96 (m, 3H), 12.62 (brs, 1H).

**Methyl 4-((5,6,7,8-tetrahydronaphthalen-1-ylamino)methyl)benzoate (39g)**

The title compound was obtained in 87% overall yield from compound **38g** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, $CDCl_3$) $\delta$ 1.37-1.82 (m, 2H), 1.85-1.94 (m, 2H), 2.45-2.50 (m, 2H), 2.77 (t, $J$ = 6.0 Hz, 2H), 3.92 (s, 3H), 3.98 (brs, 1H), 4.45 (s, 2H), 6.36 (d, $J$ =

8.1 Hz, 1H), 6.54 (d, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.8 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 8.02 (d, *J* = 8.4 Hz, 2H).

**Methyl 4-((2,3-dihydro-1H-inden-4-ylamino)methyl)benzoate (39h)**

The title compound was obtained in 86% overall yield from compound **38h** in a manner similar to that described for the preparation of **33a**: $^1$HNMR (300 MHz, $CDCl_3$) $\delta$ 2.09-2.19 (m, 2H), 2.72-2.78 (m, 2H), 2.95 (t, *J* = 7.5 Hz, 2H), 3.92 (m, 4H), 4.47 (s, 2H), 6.35 (d, *J* = 7.8 Hz, 1H), 6.69 (d, *J* = 7.5 Hz, 1H), 7.00-7.05 (m, 1H), 7.46 (d, *J* = 8.1 Hz, 2H), 8.03 (d, *J* = 8.1 Hz, 2H).

(B) **Biology.**

(a) **HDAC enzymes inhibition Assays.** Enzyme inhibition assays were conducted by the Reaction Biology Corporation, Malvern, PA. Compounds were dissolved in DMSO and tested in at least 10-dose $IC_{50}$ mode with 3-fold serial dilution starting at 10 μM. HDAC Control Compound trichostatin A was tested in a 10-dose $IC_{50}$ with 3-fold serial dilution starting at 10 μM.

**(b) *In Vitro* Cell Growth Inhibitory Activity.**

***Culture medium and culture condition of Cell lines.*** RPMI 8226, MDA-MB-231 and MCF-7 human breast cancer cells were purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan), and the cells were cultured in RPMI 1640 medium supplemented with 20% (v/v) fetal bovine serum (both from Invitrogen™ Life Technologies, Carlsbad, CA, USA), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Biological Industries, Kibbutz Beit Haemek, Israel). All cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ in air.

***Cell Growth Inhibitory Assay*.** Cells were incubated for 48 h with the indicated concentrations of test compounds, then were fixed with 10% trichloroacetic acid, stained for 30 min with SRB (0.4% in 1% AcOH), and washed repeatedly with 1% AcOH, then protein-bound dye was dissolved in 10 mM Tris base solution and the optical density at 510 nm was measured.

***Cell cycle analysis***. Cells were incubated with drugs for 24 h, then cells were collected and washed with PBS and fixed in 70% EtOH at -20°C for at least 30 min. After washing with PBS, cells were treated with DNA extraction (0.2 M $Na_2HPO_4$, 0.1M citric buffer, pH 7.8) for 20 min and finally stained with propidium iodide (PI) solution (0.1% Triton X-100, RNAase A and PI) for 20 min. DNA contents were analyzed by flow cytometry using FACS Calibur (BD Biosciences).

***Western blot analysis*.** Cells were harvested using a lysis buffer (1 mM phenylmethylsulfonyl fluoride, 1 μg/mL of leupeptin, 5 mM NaF, 1 mM sodium orthovanadate, 10 μg/mL of aprotinin, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100 and 2.5 mM sodium pyrophosphate in 20 mM Tris-HCl buffer (pH 7.4)), and then centrifuged at 17,000 g for 30 min at 4 ºC. Cell lysates were collected and analyzed by immunoblotting with the specific antibodies. The signals were visualized using an enhanced chemiluminescence (ECL) reagent (Advansta Corp., Menlo Park, CA, USA) and exposure to photographic film.

***Antitumor Activity in vivo*.** RPMI 8226 cells ($1 \times 10^7$) were implanted subcutaneously into eight week old male nude mice. When the tumors reached an average volume of 200 $mm^3$, the mice were randomly divided into four groups (n = 8) and then were treated intravenouslt/intraperitoneally with the vehicle

(0.5% EtOH/0.5% Cremophor in 5% dextran, 0.2 mL/20 g mouse), bortezomib (1 mg/kg, i.v./i.p., qwk), **13**·mesylate (30 mg/kg, i.v./i.p., 5 on 2 off) or combination bortezomib with **13**·mesylate treatment. The length (L) and width (W) of the tumor were measured by caliper every 3 to 4 days, and the tumor volume was calculated as $L \times W^2/2$. The percentage of tumor growth inhibition (%TGI) as determined by the formula: $\{1-[(T_t/T_0)/(C_t/C_0)]/1-[C_0/C_t]\}*100$. $T_t$: tumor volume of treated at time t. $T_0$: tumor volume of treated at time 0. $C_t$: tumor volume of control at time t. $C_0$: tumor volume of control at time 0. Animal experiments were performed in accordance with relevant guidelines and regulations followed ethical standards, and protocols have been reviewed and approved by Animal Use and Management Committee of Taipei Medical University (IACUC no. LAC-2015-0163).

***Determination of the Maximum tolerated dose (MTD)***. The MTD was determined using male balb/c mice at 8-week age by administering compound **13** intraperitoneally at doses ranging from 50 to 200 mg/kg once a day for 1 week and then monitoring for 1 week. Each dose was tested on ten mice in the individual experiments. MTD was defined as the highest dose that could be given resulting in no drug-related moribund state or death, and body weight loss under 20%.

***Kinase assays.*** For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 min). The lysates were centrifuged (6,000 x g) and filtered (0.2 μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for

qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 % SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 ml. The assay plates were incubated at room temperature with shaking for 1 h and the affinity beads were washed with wash buffer (1x PBS, 0.05 % Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR. The compound(s) were screened at the concentration(s) specified, and results for primary screen binding interactions are reported as '%Ctrl', where lower numbers indicate stronger hits in the matrix.

%Ctrl Calculation

$$\left(\frac{test\ compound\ signal - positive\ control\ signal}{negative\ control\ signal - positive\ control\ signal}\right) X\ 100$$

test compound = compound submitted by Kim Forest
negative control = DMSO (100%Ctrl)
positive control = control compound (0%Ctrl)

***Hepatocyte stability.*** The metabolism of compound **13** was investigated in cryopreserved hepatocytes from Sprague-Dawley rat, beagle dog and human, Incubation of compound **13** (10 µM) and hepatocytes was conducted for 4 hours in 12- well plates containing approximately 0.5X $10^6$ hepatocytes/well (rat and dog) and 0.24X $10^6$ hepatocytes/well (human) at 37° C. Reactions were quenched with equal volume of MeCN. The test article depletion and metabolite identification were performed using LC-MS/MS, which consisted of and Agilent LC 1200 HPLC pump and autosampler system, and an AB SCIEX 5500 QTRAP mass spectrometer equipped with a turbo ion spray source, Data-dependent ion, multiple reaction monitoring, precursor ion, neutral loss, glucuronide neutral loss scans in positive ion electrospray mode were used to identify compound **13** and its metabolites.

***Caco-2 Bi-directional Permeability***. Caco-2 cells were seeded at a density of 8.4 x $10^4$ cells/$cm^2$ onto cell culture inserts with polycarbonate membrane for 21 days. For absorptive ($AP \rightarrow BL$) permeability, transport was initiated by adding 0.4 mL of drug solution (HBSS/MES, pH 6.5 containing 10 µM test compound) to the apical chamber (donor chamber) of inserts bathed with 0.6 mL of transport medium (HBSS/HEPES, pH 7.4) in the basolateral chamber (receiver chamber). For secretory ($BL \rightarrow AP$) permeability, transport was initiated by adding 0.6 mL of drug solution (HBSS/HEPES, pH 7.4 containing 10 µM test article) to the basolateral chamber (donor chamber) of wells with 0.4 mL of transport medium (HBSS/MES, pH 6.5) in the apical chamber (receiver chamber). Samples (100 µL) were withdrawn from the receiver chamber at 30, 60, 90 and 120 min and from the donor chamber at 0

and 120 min. The volume withdrawn was replaced with fresh transport medium. The concentrations of test compound were determined by LC-MS/MS.

***Antimutagenicity testing.*** Two histidine auxotrophic mutants (TA98 and TA100) of *Salmonella typhimurium* were used. Test strains were obtained from the frozen working stock vial and thawed at room temperature. A 0.2 mL aliquot was inoculated into 25 mL nutrient broth medium and then incubated at 35-37 °C with shaking (120 rpm) for 16-18 h. Test substances were dissolved in DMSO with 10-fold dilutions to obtain 4 stock concentrations of 30,000, 3,000, 300 and 30 μg/mL. Rat liver microsome enzyme homogenate (S9) mixture was prepared containing 8 mM $MgCl_2$, 33 mM KCl, 4 mM NADP, 5 mM glucose-6-phosphate, 100 mM $NaH_2PO_4$ (pH 7.4) and 4 % (v/v) Aroclor 1254-induced male rat liver microsome enzyme homogenate (S9). A 0.2 mL aliquot of stock test compound solution was combined with 0.1 mL of strain culture and with 0.5 mL of rat liver enzyme homogenate (S9) mixture or 0.5 mL PBS, then was incubated at 35-37 °C with shaking (120 rpm) for 20 min. Molten top agar (2 mL containing 0.05 mM histidine and 0.05 mM biotin) was added, then the mixture was poured onto the surface of a minimal glucose agar plate (30 mL of bottom agar per petri plate) to obtain final test substance concentrations of 3000, 300, 30 and 3 μg/plate. The plates were incubated at 37°C for 72 h, and then the numbers of $His^+$ revertant colonies were counted. Treatments resulting in a three-fold increase (≥3×) in revertant colonies compared to the vehicle control were considered mutagenic. Treatments that reduce the colony counts to ≤50% of the vehicle control were considered cytotoxic. Assays were performed in triplicate.

**Supporting Information Available:** The HPLC purity results and $^{1}$HNMR spectra of target compounds **7-31**. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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**ABBREVIATIONS USED**

HDAC6, histone deacetylase 6; DPEphos, bis[(2-diphenylphosphino)phenyl] ether; $NaBH(OAc)_3$, sodium triacetoxyborohydride; EDC·HCl, *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride.

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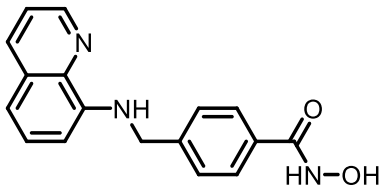


**13**

HDAC6 $IC_{50}$ = 0.291 nM