Dual Inhibitors of Inosine Monophosphate Dehydrogenase and Histone Deacetylases for Cancer Treatment

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Mycophenolic acid (MPA), an inhibitor of IMP-dehydrogenase (IMPDH), is used worldwide in transplantation. Recently, numerous studies showed its importance in cancer treatment. Consequently, MPA entered clinical trials in advanced multiple myeloma patients. Suberoylanilide hydroxamic acid (SAHA), a potent differentiation agent acting through inhibition of histone deacetylases (HDACs), was recently approved for treatment of cutaneous T cell lymphoma. We report herein the synthesis of dual inhibitors of IMPDH and HDACs. We found that mycophenolic hydroxamic acid (**9**, MAHA) inhibits both IMPDH (K_i = 30 nM) and HDAC (IC_{50} = 5.0 μM). A modification of SAHA with groups known to interact with IMPDH afforded a SAHA analogue **14**, which inhibits IMPDH (K_i = 1.7 μM) and HDAC (IC_{50} = 0.06 μM). Both MAHA (IC_{50} = 4.8 μM) and SAHA analogue **14** (IC_{50} = 7.7 μM) were more potent than parent compounds as antiproliferation agents. They were also significantly more potent as differentiation inducers.

Introduction

Chronic myelogenous leukemia (CML[a]) is a malignant cancer of the bone marrow that causes rapid growth of blood forming cells (myeloid precursors) in the bone marrow, peripheral blood, and body tissues. The hallmark of CML is the Philadelphia chromosome translocation, which fuses the *bcr* and *c-abl* genes, resulting in expression of a constitutively active tyrosine kinase. There is often little hope for patients with CML upon the onset of blast crisis (BC). Currently there are only two agents that are in clinical use for CML-BC, 4-[(4-methylpiperazin-1-yl) methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino] phenyl]benzamide (imatinib, tyrosine kinase inhibitor) and tiazofurin (TR, inosine monophosphate dehydrogenase, IMPDH inhibitor). TR is a prodrug that requires metabolic activation into its active form, tiazofurin adenine dinucleotide (TAD, Figure 1), a mimic of nicotinamide adenine dinucleotide (NAD). Patients develop resistance to both agents after several treatment cycles, and therefore, this condition is exceptionally difficult to repress. While imatinib is a phenomenal success, the drug does not affect a small population of leukemic stem cells (less than 1% of all tumor cells)[1–4] and resistance develops.

As the cells emerge from the progenitor stem cell, they are destroyed by imatinib, which needs to be taken every day for a lifetime. Little is known about the differences between normal and cancer stem cells that would allow for designing drugs that specifically target the malignant cancer stem cells. However, cancer stem cells, especially leukemic stem cells, can be selectively disarmed by induction of apoptosis and/or differentiation.[1] Parthenolide,[5] a natural product from feverfew

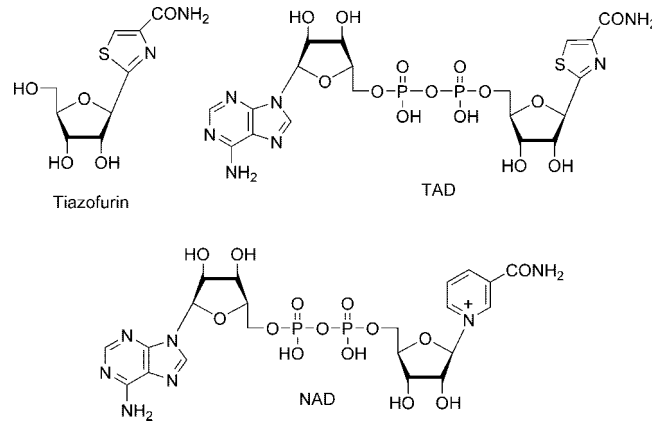

Figure 1

(plant), induces apoptosis in leukemic stem cells but not in normal stem cells. Recently it was found[6] that induction of differentiation severely limited the cancer stem cell's ability to form new tumors.[1,7,8] The mechanisms of apoptosis and differentiation have recently been studied extensively in CML cells, and these processes are now well characterized when triggered by Bcr-Abl inhibitors.[9] TR proved to be the most effective inducer of erythroid differentiation in CML leukemic (K562) cells among numerous compounds studied.[10,11] It showed good anticancer activity in both acute myelogenous leukemia (AML) and CML patients and regardless of its general toxicity was approved as an orphan drug for treatment of patients in CML blast crisis.[11]

Inhibitors of IMPDH, in general, show a significant ability to trigger differentiation and/or apoptosis.[12–14] Mycophenolic acid (MPA, Figure 2), one of the most potent inhibitors of human IMPDH (K_i = 10 nM), binds at the NAD binding domain of the enzyme and is used in clinic as an immunosuppressant. However, its potential anticancer activities are of current interest. Thus, MPA assisted differentiation of human prostate cancer PC-3 cells and DU145 cells has been reported by Huberman et al.[15–17] and it *was found to block tumor-induced angiogenesis in vivo*.[18]

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[a]Abbreviations: MPA, mycophenolic acid; IMPDH, inosine monophosphate dehydrogenase; SAHA, suberoylanilide hydroxamic acid; MAHA, mycophenolic hydroxamic acid; HDACs, histone deacetylases; CML, chronic myelogenous leukemia; BC, blast crisis; TR, tiazofurin; TAD, tiazofurin adenine dinucleotide; MAD, mycophenolic adenine dinucleotide; NAD, nicotinamide adenine dinucleotide.

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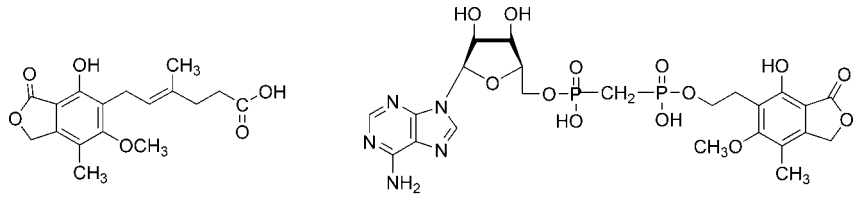


Figure 2

In CML, synergy between MPA and imatinib was reported in Bcr-Abl-expressing cell lines.[13] The authors suggested that these results "lay the foundation for clinical trials in which IMPDH inhibitors are added to imanitib in patients who have suboptimal molecular responses to single agent therapy". Meanwhile, because of significant apoptotic properties, MPA entered phase I clinical trial in advanced multiple myeloma patients.[19,20]

Our group has reported novel IMPDH inhibitors, mycophenolic adenine dinucleotides (MAD analogues), that mimic NAD cofactor. Among them, C2-MAD (Figure 2) was found to be more potent than MPA in both antileukemic activity and the ability to induce differentiation.[21] At concentrations as low as 1.5 μM, it converted 65% of CML leukemic cells (K562 cells) into mature, nonproliferating cells able to produce hemoglobin.[21] C2-MAD showed superior anticancer activity and lower toxicity than TR in mouse model of CML.[22]

All the above inhibitors (e.g., TR, MPA, and MAD analogues) mimic NAD cofactor (or part of it) and inhibit NAD-dependent IMPDH, a key enzyme in de novo synthesis of purine nucleotides. They induce differentiation due to depletion of guanine nucleotides. Such G-restriction affects DNA and RNA metabolism as well as the activity of GTP-binding proteins involved in transduction pathways.[23] In recent years IMPDH has emerged as a major therapeutic target[24] and its mechanism of action has been recently reviewed.[25]

Histone deacetylases (HDACs), enzymes that catalyze the removal of acetyl groups from lysine residues of histones represent new class of anticancer targets.[26] HDACs inhibitors alter gene transcription and exert antitumor effects through growth arrest, apoptosis, differentiation, and inhibition of tumor angiogenesis. *Thus, differentiation triggered by HDAC inhibitors is based on a fundamentally different mechanism*[27–30] than that caused by inhibition of IMPDH.

To date, four classes of human HDACs with 18 members have been identified. The class I, II, and IV HDACs require zinc for catalytic deacetylation, while class III HDACs consists of seven sirtuins[31–33] that are NAD-dependent and do not share homologies with the zinc-dependent histone deacetylases. Chemically, the zinc-dependent HDACs inhibitors can be divided into several classes such as short-chain fatty acids (e.g., compound **1**), hydroxamic acids **2**–**6**, and benzamides **7** and **8** (Figure 3). Three structural regions can be distinguished in these molecules; a cap (usually aromatic) region, a linker, and a metal binding group. For the metal binding group, hydroxamic acids usually exhibit higher potency than the corresponding benzamides.

The most representative member of HDACs inhibitors is suberoylanilide hydroxamic acid (SAHA), which was recently approved for treatment of cutaneous T cell lymphoma.[34] SAHA was studied in combination with imatinib[35,36] and was found to promote apoptosis in imatinib-resistant leukemic cells (Bcr/Abl positive). Recently, numerous studies showed synergism not only between imatinib and SAHA but also for combinations of new generation anti-CML drugs with SAHA.[9,37,38]

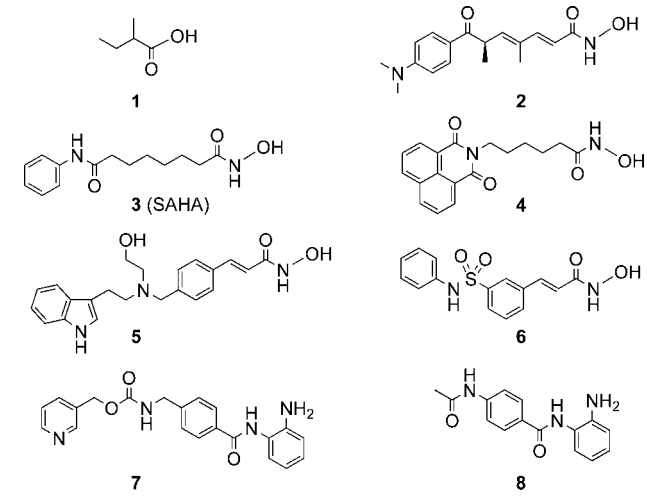


Figure 3

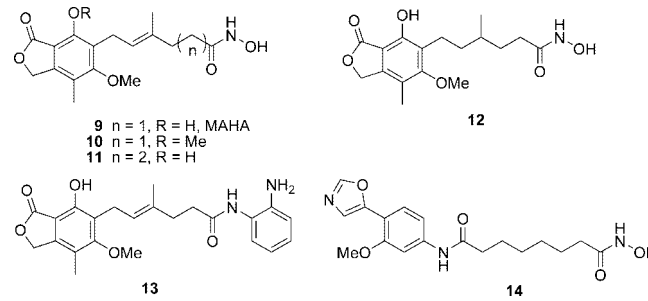


Figure 4

MPA's structure consists of an aromatic moiety and a linker; however, it does not contain a zinc binding group. Thus, we replaced the carboxylic group of MPA with a hydroxamic acid moiety and synthesized hydroxamic acid analogue **9** (MAHA, Figure 4). We also modified SAHA by addition of groups known to interact with IMPDH and prepared SAHA analogue **14**. Both compounds **9** and **14** were found to act as novel dual inhibitors of IMPDH and HDAC. In this paper we focused our efforts on synthesis and evaluation of dual IMPDH/HDAC inhibitors against CML; however, a similar anticancer activity of these novel inhibitors is expected (and was confirmed) for other cancers cell lines where SAHA proved to be highly effective.

Results and Discussion

1. Inhibition of IMPDH, HDAC, and Proliferation of K562 Cell. A conversion of the carboxylic group of MPA into hydroxamic acid moiety afforded MAHA (**9**) which, like other HADCs inhibitors, contain an aromatic cap (5-methoxy-4-methylphtalan-1-one), a linker (3-methylpent-2-enyl group), and a zinc binding group (hydroxamic acid). MAHA did not lose inhibitory activity against human IMPDH (K_i = 30 nM, type 2 isoform, Table 1) and was found to have a similar potency

Table 1. Inhibition of IMPDH, HDAC, and Proliferation of K562 Cells by SAHA, MPA, TR, and Novel Compounds[a]

inhibitor	IMPDH K_i (μM) type I	IMPDH K_i (μM) type II	HDAC IC_{50} nuclear extract (μM)	K562 IC_{50} (μM)	% of differ. K562 cells (μM)
SAHA	NA	NA	0.41	0.75	47.4 (at 0.50)
MPA	0.04	0.01	NA	7.7	46.4 (at 0.25)
MAHA (**9**)	0.07	0.03	5.0	4.8	75.0 (at 0.25)
10	NA	NA	14.0	42.3	ND
11	0.40	1.10	10.4	10.8	ND
12	2.07	0.54	20.1	44.7	ND
13	2.83	0.65	>100	60	ND
14	5.0	1.7	0.06	0.29	87.8 (at 0.005)
TR (TAD)	0.1	0.1	NA	3.0[b]	80.0 (at 5.30)[b]

[a] NA, not active. ND, not determined. [b] These data were reported previously by us.[21]

against IMPDH as MPA (K_i = 10 nM, type 2). It also inhibited a nuclear extract of HDACs enzymes (IC_{50} = 5.0 μM) as well as proliferation of K562 cells at a similar concentration (IC_{50} = 4.8 μM). MAHA's antiproliferative activity was slightly higher than that of MPA (IC_{50} = 7.7 μM).

At enzymatic level MAHA showed more potent inhibition of IMPDH than HDACs. Thus, the potential effect of HDACs inhibition on proliferation and differentiation of K562 cells may be negligible. In order to estimate the importance of HDACs inhibition by MAHA, we synthesized MAHA analogue **10** in which the 7-OH group is replaced by an OMe group. It is well-known that the 7-phenol group is crucial for MPA inhibitory activity. All attempts to protect this group or to replace it with groups such as −F, $-NH_2$, −CN resulted in inactive derivatives.[39] Indeed, 7-OMe analogue of MPA did not show any activity against human isoforms of IMPDH. However, replacement of the carboxylic group of the 7-OMe-MPA analogue with hydroxamic acid afforded 7-OMe-MAHA (**10**), which was only 3-fold less active against HDACs nuclear extract than MAHA and weakly inhibited K562 cell proliferation with IC_{50} = 42.3 μM. These results indicate that antileukemic activity of MAHA in K562 cells is related to its ability to inhibit IMPDH rather HDAC enzymes. Nevertheless, the inhibitory activity of compound **10** against K562 cells growth is only 14-fold less potent than tiazofurin. It is likely that this effect is related to HDACs inhibition.

In order to find more about structure–activity relationship (SAR) of these novel inhibitors, we synthesized compounds **11**–**13**, in which the linker of MAHA was extended by a one carbon (**11**), the double bond of the linker was reduced (**12**), and the hydroxamic group of MAHA was replaced by the ortho aminobenzamide group (**13**; see structures of HDACs inhibitors, Figure 2). None of these modifications produced a better inhibitor than MAHA. Interestingly, compound **11** is 1–2 orders of magnitude less potent an inhibitor of IMPDH [K_i = 0.4 μM (type 1), K_i = 1.1 μM (type 2)] than MAHA or MPA and only twice less potent (IC_{50} = 10.4 μM) than MAHA (IC_{50} = 5.0 μM) as HDACs inhibitor. It was found to inhibit proliferation of K562 cells (IC_{50} = 10.8 μM) at a concentration similar to that of MPA (IC_{50} = 7.7 μM) and MAHA (IC_{50} = 4.8 μM). These results suggest that at least for compound **11** the two components, IMPDH and HDACs inhibition, contribute to the antileukemic activity. On the other hand, the above structure–activity relationship appears to correlate again with the potency against IMPDH rather than HDACs inhibitory activity. Compounds **12** and **13** show similar inhibitory activity against IMPDH and similar antiproliferative potency against K562 cells despite the HDAC component for compound **12**, which is at least 5-fold higher than that of **13**. The same trend is valid for

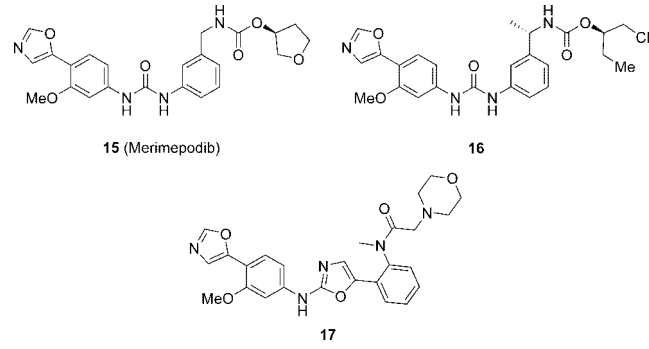


Figure 5

TR (TAD) when compared against compounds **11**–**13**. Clearly, more compounds with equally adjusted activity against IMPDH/HDACs are needed for a better understanding of their antileukemic activity.

Finally, we became interested in a chemical modification of SAHA that would result in inhibition of IMPDH without losing SAHA's ability to inhibit HDAC enzymes. In a series of IMPDH inhibitors developed by Vertex and Bristol-Myers-Squibb such as (*S*)-tetrahydrofuran-3-yl 3-(3-(3-methoxy-4-(oxazol-5-yl)phenyl)ureido)benzylcarbamate (**15**, merimepodib, Figure 5) and related inhibitors **16** and **17**, the phenyloxazole moiety with an ortho methoxy group was introduced and found to bind at the nicotinamide subdomain of NAD-binding pocket of IMPDH.[40–44] Thus, we modified SAHA by addition of oxazole group and methoxy group in ortho configuration, resulting in SAHA derivative **14**.

We found that compound **14** is more potent than SAHA as inhibitor of HDAC enzymes (IC_{50} = 0.06 μM) and proliferation of K562 cells (IC_{50} = 0.29 μM). It showed inhibition of IMPDH at low micromolar level. Again, compound **14** is 7-fold more potent than SAHA as an HDAC inhibitor; however, in spite of having both components for the activity, it is only 2.6-fold more potent than SAHA in the antiproliferative assay.

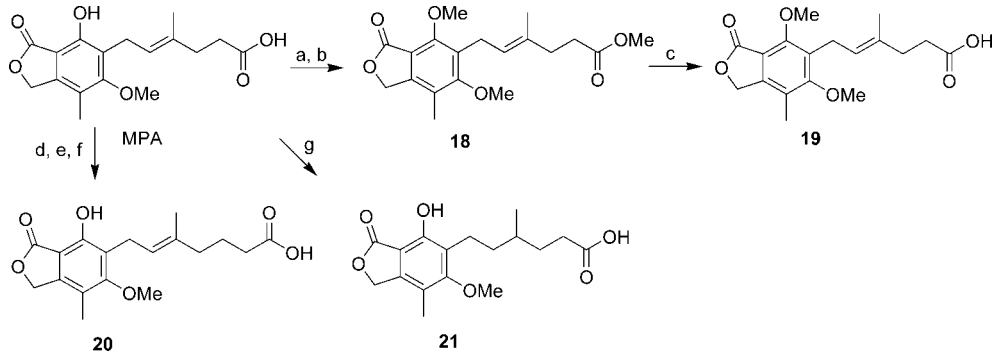
We expected that our double IMPDH/HDAC inhibitors would show an improved ability to differentiate leukemic cells such as K562 cells. Indeed, MAHA at concentration of 0.25 μM converted significantly larger number of K562 cells (75%) than SAHA (47%) at 2-fold higher concentration (0.5 μM). Interestingly, our modified SAHA analogue **14** was found to be one of the most potent (if not the most potent) K562 differentiation inducers (88% at 5 nM concentration) ever reported. In fact, compound **14** is a much more powerful differentiation inducer (3 orders of magnitude) than TR, a clinically used nucleoside with well established differentiation ability. Since compound **14** is much more active in cells than against isolated target enzymes, its differentiation activity could be due to additional interaction with cellular targets other than IMPDH and HDACs.

It was recently found that differentiation severely limits cancer stem cells' ability to form new tumors.[6] Thus, we are currently in the process of preparation of the second generation of dual IMPDH/HDAC inhibitors for their evaluation against cancer stem cells.

2. Chemical Synthesis. Chemical synthesis of MAHA (**9**) and its analogues **10**–**13** (Figure 4) requires preparation of the corresponding carboxylic acids and their subsequent conversion to the protected hydroxamates. Triphenylmethyl (trityl) was chosen as a hydroxylamine protecting group, and its facile removal under acidic conditions would afford the desired hydroxamic acids. As depicted in Scheme 1, carboxylic acids.

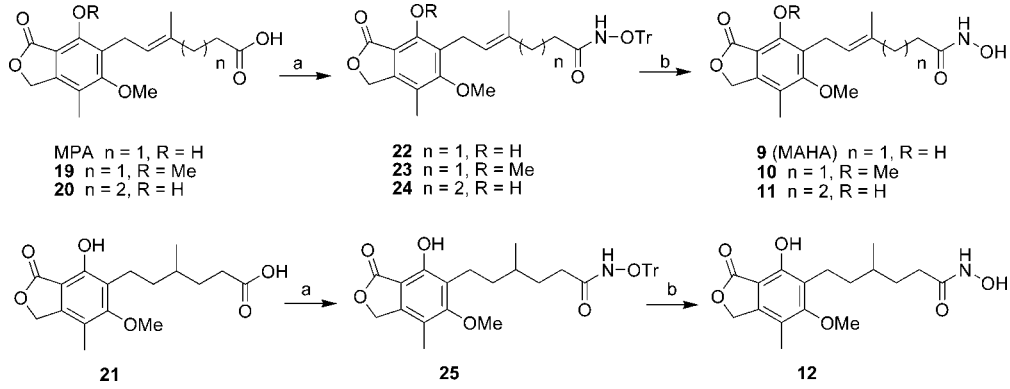
19–**21** were prepared from mycophenolic aicd (MPA). Methylation of the phenolic hydroxyl group in mycophenolic

Scheme 1[a]



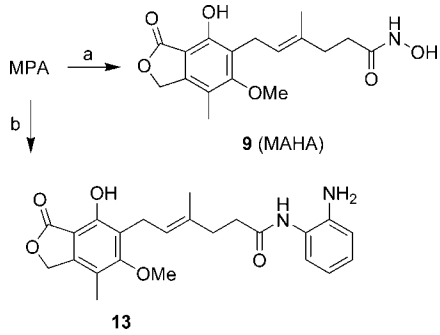
[a] Reaction conditions: (a) cat. H_2SO_4, MeOH; (b) MeI, Cs_2CO_3, CH_3CN, reflux; (c) NaOH, THF/H_2O and then H+; (d) $(COCl)_2$, cat. DMF, dichloroethane; (e) $TMSCHN_2$, CH_3CN, 0 °C to room temp; (f) AgOBz, THF/H_2O; (g) H_2, Pd/C, MeOH.

Scheme 2[a]



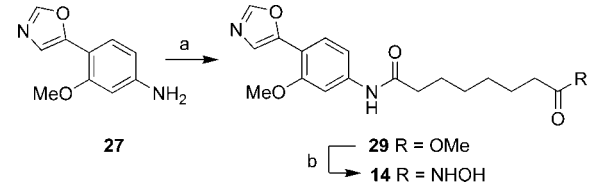
[a] Reaction conditions: (a) $TrONH_2$, EDC, HOBt, CH_2Cl_2; (b) TFA, Et_3SiH, CH_2Cl_2.

Scheme 3[a]



[a] Reaction conditions: (a) $(COCl)_2$, cat. DMF, CH_3CN and then $NH_2OH \cdot H_2O$; (b) **26**, BOP, Et_3N, DMF.

Scheme 4[a]



[a] Reaction conditions: (a) **28**, EDC, HOBt, CH_2Cl_2; (b) $NH_2OH \cdot H_2O$, MeOH.

acid methyl ester followed by hydrolysis of the resulting methyl ester **18** provided acid **19**.[45] Arndt−Eistert homologation[46] of MPA furnished acid **20**, which contains a side chain one carbon longer than that of MPA. Saturated acid **21**[47] was readily obtained by hydrogenation of MPA.

With these carboxylic acids available, coupling with trityl protected hydroxylamine gave hydroxamates **22–25** (Scheme 2). As expected, the removal of trityl protecting was accomplished under mildly acidic conditions, and the desired hydroxamic acids **9**−**12** were obtained.

Even though the method procedures described above produced hydroxamic acids smoothly, a more efficient one-pot procedure was used for the preparation of MAHA (**9**). It involved in situ formation of acyl chloride and subsequent treatment of hydroxylamine aqueous solution (Scheme 3). Since the product was readily precipitated from aqueous solution, this method is amenable to scale up of MAHA.

Benzamide derivative **13** was readily prepared by coupling of MPA and 1,2-phenylenediamine (**26**), which was mediated by benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP). The preparation of SAHA analogue **14** is depicted in Scheme 4. A coupling of known 3-methoxy-4-(5-oxazolyl)phenylamine (**27**)[48] and suberic acid monomethyl ester (**28**) afforded methyl ester **29**, which was converted into hydroxamic acid **14** under treatment with hydroxylamine aqueous solution.

In summary, we demonstrated that chemical modification of clinically used drugs such as MPA (IMPDH inhibitor) and SAHA (HDACs inhibitor) resulted in new molecules that show inhibitory activity of both IMPDH and HDAC enzymes. New inhibitors, MAHA and SAHA analogue **14**, were found to be more potent antiproliferative agents than parent drugs. Because of a dual mechanism of action, our novel inhibitors may be of therapeutic interest in cancer treatment. Since the inhibitory activity of our best compounds, MAHA and **14**, is not yet

perfectly balanced, we are working now on other SAHA−MPA hybrid combinations and evaluation of their biological potential.

Experimental Section

General Methods. All commercial reagents (Sigma-Aldrich, Acros) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, while two packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with Ultra Pure silica gel (Silicycle) with the indicated solvent system. Analytical HPLC was performed on a Varian Microsorb column (C18, 5 μm, 4.6 mm × 250 mm) with a flow rate of 0.5 mL/min with an isocratic or linear gradient of water with 0.1% (v/v) TFA and MeOH with 0.1% (v/v) TFA. Melting points were determined on a Mel-Temp apparatus and were uncorrected. ^{1}H nuclear magnetic resonance spectra were recorded on a Varian 300 or 600 MHz with Me_4Si, DDS, or signals from residual solvent as the internal standard. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), and dd (double–doublet). Values given for coupling constants are first-order. High-resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface.

Chemical Syntheses. Carboxylic Acid 20. To a solution of mycophenolic acid (811 mg, 2.53 mmol) in anhydrous CH_2Cl_2 (25 mL) were added oxalyl chloride (0.44 mL, 5.12 mmol) and three drops of DMF. The solution was stirred at room temperature for 2 h and concentrated. The residue was dissolved in anhydrous CH_3CN (25 mL) at 0 °C, and a solution of $TMSCHN_2$ (2.0 M in Et_2O, 5.1 mL, 10.2 mmol) was added. After being stirred at 0 °C for 30 min, the mixture was allowed to warm to room temperature and stirred for 48 h. After concentration, the resulting residue was dissolved in THF (12 mL) and H_2O (12 mL), and silver benzoate (120 mg, 0.52 mmol) was added. The mixture was stirred at room temperature overnight, diluted with EtOAc (150 mL), and subsequently washed with 0.5 N HCl (30 mL), H_2O (30 mL), and brine (2 × 60 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was chromatographed on a silica gel column (0%–4% MeOH/CH_2Cl_2) to give carboxylic acid **20** as a pale solid (673 mg, 80%), mp 124.2–126.0 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.24–5.18 (m, 3H), 3.77 (s, 3H), 3.39 (d, J = 6.6 Hz, 2H), 2.28 (t, J = 7.5 Hz, 2H), 2.15 (s, 3H), 2.02 (t, J = 7.5 Hz, 2H), 1.78 (s, 3H), 1.73 (t, J = 7.5 Hz, 2H). HRMS calcd for $C_{18}H_{23}O_6$ 335.1489 $(M + H)^+$, found 335.1489.

Protected Hydroxamates 22–25. Hydroxamate 22. A solution of mycophenolic acid (107 mg, 0.33 mmol), *O*-tritylhydroxylamine (102 mg, 0.37 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (104 mg, 0.53 mmol), and 1-hydroxybenzotriazole (HOBt) (51 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 20 h. After concentration, the residue was diluted with EtOAc (20 mL) and washed with water (3 × 10 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by flash column chromatography (20–40% EtOAc/hexanes) to give protected hydroxamate **22** as a white solid (118 mg, 62%), mp 187.0–188.5 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37–7.24 (m, 15H), 5.34–5.16 (m, 3H), 3.80 (s, 3H), 3.42 (d, J = 6.0 Hz, 2H), 2.34 (brs, 2H), 2.17 (s, 3H), 1.82 (brs, 2H), 1.27 (s, 3H). HRMS calcd for $C_{36}H_{35}NO_6Na$ 600.2362 $(M + Na)^+$, found 600.2363.

Hydroxamate 23. In a similar manner carboxylic acid **19**[45] (248 mg, 0.74 mmol) was coupled with *O*-tritylhydroxylamine to give **23** as a white solid (190 mg, 43%), mp 169.3–170.5 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48–7.25 (m, 15H), 5.12 (s, 2H), 4.98 (brs, 1H), 3.99 (s, 3H), 3.72 (s, 3H), 3.32 (d, J = 6.6 Hz, 2H), 2.15 (s, 3H), 1.90 (brs, 2H), 1.70–1.50 (m, 5H). HRMS calcd for $C_{37}H_{37}NO_6Na$ 614.2518 $(M + Na)^+$, found 614.2515.

Hydroxamate 24. In a similar manner carboxylic acid **20** (145 mg, 0.43 mmol) was coupled with *O*-tritylhydroxylamine to give **24** as a pale solid (69.3 mg, 27%), mp 132.8–134.3 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.70 (s, 1H), 7.66 (s, 1H), 7.52–7.24 (m, 15H), 5.19 (s, 2H), 5.10 (brs, 1H), 3.73 (s, 3H), 3.35 (d, J = 6.6 Hz, 2H), 2.14 (s, 3H), 1.86–1.66 (m, 6H), 1.53 (brs, 2H), 1.33 (brs, 1H). HRMS calcd for $C_{37}H_{37}NO_6Na$ 614.2513 $(M + Na)^+$, found 614.2513.

Hydroxamate 25. In a similar manner carboxylic acid **21**[47] (294 mg, 0.91 mmol) was coupled with *O*-tritylhydroxylamine to give **25** as a white solid (315 mg, 60%), mp 141.2–142.8 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.52–7.26 (m, 15H), 5.19 (s, 2H), 3.75 (s, 3H), 2.64–2.48 (m, 2H), 2.14 (s, 3H), 1.67–1.10 (m, 7H), 0.90-.072 (m, 3H). HRMS calcd for $C_{36}H_{37}NO_6Na$ 602.2513 $(M + Na)^+$, found 602.2518.

Hydroxamic Acids 9–12. Hydroxamic Acid 1 (MAHA) from Hydroxamate 22. To a solution of protected hydroxamate **22** (103 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (3 mL) was added TFA (0.15 mL). The resulting yellow solution was treated with Et_3SiH till it was colorless. After being stirred for an additional 10 min, the reaction mixture was concentrated and the residue was triturated with hot hexanes (2 × 10 mL). The syruplike residue was dissolved in CH_2Cl_2 (0.3 mL), and hexanes (10 mL) were added. After the organic solvents were discarded, the syrup formed was collected and dried under high vacuum to give hydroxamic acid **9** as a white solid (52.7 mg, 87%).

Hydroxamic Acid 9 (MAHA) Directly from Mycophenolic Acid. To a suspension of mycophenolic acid (552 mg, 1.72 mmol) in anhydrous CH_3CN were added oxalyl chloride (0.18 mL, 2.10 mmol) and then five drops of DMF. The reaction mixture turned clear, was stirred at room temperature for 1.5 h, and then cooled to 0 °C. A solution of 50% aqueous hydroxylamine (0.51 mL, 8.3 mmol) was added slowly, and the mixture was stirred at 0 °C for an additional 2 h. After addition of water (5 mL), the reaction mixture was warmed to room temperature and CH_3CN was removed in vacuo. A syrup that formed on the wall of the reaction flask was washed with water (5 mL) and then dissolved in CH_3CN (10 mL) and water (5 mL). A syrup was obtained after the removal of CH_3CN, and it was redissolved in CH_2Cl_2 (100 mL) and water (50 mL). After CH_2Cl_2 and some of the water were removed in vacuo, the solid formed was filtered, washed with water, and dried under high vacuum to give MAHA as a white solid (357 mg, 62%), mp 136.0–136.9 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.36–5.08 (m, 3H), 3.75 (s, 3H), 3.74 (d, J = 6.3 Hz, 2H), 2.29 (brs, 4H), 2.13 (s, 3H), 1.77 (s, 3H). HRMS calcd for $C_{17}H_{22}NO_6$ 336.1447 $(M + H)^+$, found 336.1451.

Hydroxamic Acid 10. In a similar manner protected hydroxamate **23** (164 mg, 0.28 mmol) was treated with TFA to give **10** as a pale solid (50.6 mg, 52%), mp 142.0–144.0 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 5.26–5.14 (m, 3H), 3.98 (s, 3H), 3.78 (s, 3H), 3.40 (d, J = 6.9 Hz, 2H), 2.27 (t, J = 7.2 Hz, 2H), 2.22–2.12 (m, 5H), 1.82 (s, 3H). HRMS calcd for $C_{18}H_{24}NO_6$ 350.1603 $(M + H)^+$, found 350.1607.

Hydroxamic Acid 11. In a similar manner protected hydroxamate **24** (65.2 mg, 0.11 mmol) was treated with TFA to give **11** as a pale syrup (3.1 mg, 8%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.24–5.18 (m, 3H), 3.80 (s, 3H), 3.40 (d, J = 6.6 Hz, 2H), 2.16 (s, 3H), 2.06 (t, J = 6.6 Hz, 2H), 2.01 (t, J = 6.9 Hz, 2H), 1.82–1.72 (m, 5H). HRMS calcd for $C_{18}H_{23}NO_6Na$ 372.1423 $(M + Na)^+$, found 372.1432.

Hydroxamic Acid 12. In a similar manner protected hydroxamate **25** (275 mg, 0.47 mmol) was treated with TFA to give **12** as a pale syrup (19.1 mg, 12%). ^{1}H NMR (CD_3OD, 600 MHz) δ 5.23 (s, 2H), 3.78 (s, 3H), 2.73–2.61 (m, 2H), 2.18–2.04 (m, 5H), 1.78–1.68 (m, 1H), 1.60–1.44 (m, 3H), 1.43–1.34 (m, 1H), 0.99 (d, J = 6.0 Hz, 3H). HRMS calcd for $C_{17}H_{24}NO_6$ 338.1598 $(M + H)^+$, found 338.1598.

Benzamide 13. A solution of mycophenolic acid (217 mg, 0.68 mmol), BOP (457 mg, 1.03 mmol), Et_3N (0.43 mL, 3.08), and 1,2-phenylenediamine (**26**, 184 mg, 1.70 mmol) in dry DMF (5 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with toluene (60 mL) and washed with water (2 × 10 mL). The organic layer was concentrated and the residue was

purified on a Chromatotron (2 mm, 0–4% MeOH/CH_2Cl_2) to give benzamide **13** as a tan solid (51.4 mg, 18%), mp 187.6–189.4 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 6.97 (dd, J = 7.2, 1.5 Hz, 1H), 6.88 (dd, J = 8.0, 1.0 Hz, 1H), 6.78 (dd, J = 7.8, 1.5 Hz, 1H), 6.63–6.55 (m, 1H), 5.34 (td, J = 7.0, 1.2 Hz, 1H), 5.21 (s, 2H), 3.74 (s, 3H), 3.41 (d, J = 6.9 Hz, 2H), 2.54–2.45 (m, 2H), 2.42–2.34 (m, 2H), 2.11 (s, 3H), 1.88 (s, 3H). HRMS calcd for $C_{23}H_{27}N_2O_5$ 411.1919 $(M + H)^+$, found 411.1931.

Monomethyl Ester 29. A solution of suberic acid monomethyl ester (**28**, 411 mg, 2.18 mmol), EDC (650 mg, 3.28 mmol), HOBt (295 mg, 2.18 mmol), and aniline **27** (458 mg, 2.41 mmol) in anhydrous CH_2Cl_2 (15 mL) was stirred at room temperature for 48 h. After concentration, the residue was diluted with EtOAc (100 mL) and washed with 0.1 N HCl (50 mL), water (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography (0–4% MeOH/CH_2Cl_2) to give amide **29** as a yellow solid (571 mg, 73%), mp 115.3–116.6 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88 (s, 1H), 7.71 (brs, 2H), 7.67 (d, J = 8.4 Hz, 1H), 7.49 (s, 1H), 6.91 (dd, J = 8.4, 1.8 Hz, 1H), 3.96 (s, 3H), 3.67 (s, 3H), 2.38 (t, J = 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 1.75 (q, J = 7.2 Hz, 2H), 1.65 (q, J = 7.2 Hz, 2H), 1.44–1.30 (m, 4H). HRMS calcd for $C_{19}H_{25}N_2O_5$ 361.1757 $(M + H)^+$, found 361.1787.

Hydroxamic Acid 14. To a solution of monomethyl ester **29** (253 mg, 0.70 mmol) in MeOH (6.6 mL) was added 50% aqueous hydroxylamine solution (2.2 mL), and additional MeOH (2.0 mL) was then added to clear the mixture. The reaction mixture was stirred at room temperature for 4 days, during which time hydroxylamine solution/MeOH was added to drive the reaction to completion. The solid formed was filtered, washed with MeOH/water, air-dried, and dried under high vacuum to give hydroxamic acid **14** as a pale solid (167 mg, 66%), mp 186.5–187.2 °C. ^{1}H NMR (DMSO, 600 MHz) δ 10.31 (s, 1H), 10.04 (s, 1H), 8.64 (s, 1H), 8.35 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.42 (s, 1H), 7.27 (d, J = 8.4 Hz, 1H), 3.89 (s, 3H), 2.30 (t, J = 7.2 Hz, 2H), 1.93 (t, J = 7.2 Hz, 2H), 1.62–1.54 (m, 2H), 1.52–1.44 (m, 2H), 1.34–1.20 (m, 4H). HRMS calcd for $C_{18}H_{24}N_3O_5$ 362.1710 $(M + H)^+$, found 362.1725.

Biological Evaluation. Enzyme Assays. IMPDH inhibition assays were performed as previously described.[49] Briefly, assays were set up in duplicate using two different concentrations of IMPDH type 1 (87 and 155 nM) and type 2 (33 and 66 nM) and varying concentrations of inhibitor. IMPDH and inhibitors were added to 100 μL of reaction buffer (50 mM Tris, pH 8.0, 100 mM KCL, 1 mM DTT, 100 μM IMP, 100 μM NAD) at 25 °C and mixed gently, and the production of NADH was monitored by following changes in absorbance at 340 nm on a Molecular Devices M5e multimode plate reader. Steady-state velocities were used to determine IC_{50} and K_i^{App} values by fitting the velocities vs inhibitor concentration to the sigmoidal concentration–response curve (variable slope) and the Morrison equation respectively using GraphPad Prism.[50]

HDAC inhibition assays were performed using the HDAC Activity/Inhibitor Screening Assay Kit (Cayman Chemical) per the manufacturer's instructions. Inhibitors were suspended in either DMSO or methanol. End point readings were used to determine IC_{50} values by fitting the fractional fluorescence vs inhibitor concentration to the sigmoidal concentration–response curve (variable slope) using GraphPad Prism. Curves were corrected for autofluorescence by making a standard concentration curve, substituting compound for solvent using the background well conditions.

Inhibition of Proliferation of K562 Cells. About 2000 cells/well of logarithmically growing human myelogenous leukemia K562 cells were plated into 96-well plates and incubated at 37 °C for 24 h. Compounds at final concentrations up to 100 μM were added in duplicate wells in 0.15% DMSO (final concentration), mixed, and incubated for 72 h. At the end of the incubation period, 20 μL of MTS reagent was added, mixed, and further incubated for 3 h and then absorbance read at 490 nm in a plate reader. Control cells exhibited three doublings (doubling time was 24 h).

Differentiation of K562 Cells. Logarithmically growing K562 cells (1.0×10^3 cells/0.1 mL) were plated in triplicate into 96-well plates in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum and antibiotics and incubated at 37 °C in an atmosphere of air and 5% CO_2. Twenty-four hours later, compounds were added and further incubated for 5 days. For examination of the effect of compounds on cytotoxicity, 20 μL of MTS reagent was added and further incubated for 3 h and the absorbance was read at 490 nm. For examination of the effect on cellular differentiation, 25 μL of 5-day incubated cell suspension was taken and mixed with 25 μL of differentiation reagent (0.2% benzidine in acetic acid and 0.15% H_2O_2) and about 500 cells were counted to determine the percent of differentiated cells.

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Supporting Information Available: HPLC profiles of compounds **9–14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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