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(2-Amino-phenyl)-amides of ω-substituted alkanoic acids as new histone deacetylase inhibitors

Arkadii Vaisburg,[a,*] Naomy Bernstein,[a] Sylvie Frechette,[a] Martin Allan,[a] Elie Abou-Khalil,[a] Silvana Leit,[a] Oscar Moradei,[a] Giliane Bouchain,[a] James Wang,[a] Soon Hyung Woo,[a] Marielle Fournel,[b] Pu T. Yan,[b] Marie-Claude Trachy-Bourget,[b] Ann Kalita,[b] Carole Beaulieu,[b] Zuomei Li,[b] A. Robert MacLeod,[b] Jeffrey M. Besterman[b] and Daniel Delorme[a,*,†]

[a]*Department of Medicinal Chemistry, MethylGene Inc., 7220 Frederick-Banting, Montreal, Quebec, Canada H4S 2A1*
[b]*Department of Molecular Biology, MethylGene Inc., 7220 Frederick-Banting, Montreal, Quebec, Canada H4S 2A1*

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Abstract—A variety of ω-substituted alkanoic acid (2-amino-phenyl)-amides were designed and synthesized. These compounds were shown to inhibit recombinant human histone deacetylases (HDACs) with IC_{50} values in the low micromolar range and induce hyperacetylation of histones in whole cells. They induced expression of p21WAF1/Cip1 and caused cell-cycle arrest in human cancer cells. Compounds in this class showed efficacy in human tumor xenograft models.

Identification of potent HDAC inhibitors possessing adequate 'drug-like' properties represents a considerable effort in the development of therapeutics for the treatment of cancers. In eukaryotic cells, histone acetylation/deacetylation is co-regulated by enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1] Acetylation of histones is involved in many cellular functions including chromatin remodeling and functional regulation of gene transcription.[1] HDACs modulate the deacetylation of ε-amino groups of lysines located near the N-termini of core histone proteins.[2] Deregulation of HDAC activity is implicated in malignant diseases.[3] Small molecules hydroxamic acids, including the natural product trichostatin A (**1**, TSA)[4] or its analogues,[5] suberoylanilide hydroxamic acid (**2**, SAHA),[6] scriptaid (**3**)[7] or its analogues,[8] oxamflatin (**4**)[9] or the 2-aminoanilide MS-275 (**5**),[10] are potent HDAC inhibitors (Fig. 1).

In our efforts to identify novel HDAC inhibitors we initially designed and synthesized arylsulfonamido-*N*-hydroxycinnamides **6** and arylsulfonamido-2-amino-phenyl cinnamides **7**.[11] The next class of compounds was SAHA-TSA-like straight-chain hydroxamates **8**.[12] Here we describe the synthesis and biological activity of novel 2-aminophenylamides of ω-substituted alkanoic acids **9**–**10**, structurally relevant to compounds **8** and having the same optimized chain length (Fig. 2). As HDACs are viewed as an important new family of cancer targets, efforts to identify non-hydroxamate small molecule HDAC inhibitors are underway in many research groups working in this area. For instance, a

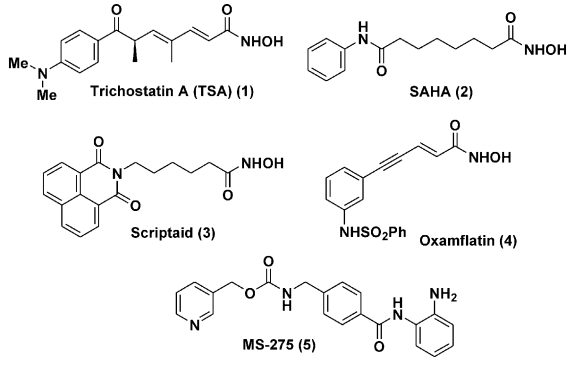

Figure 1.

* Corresponding authors. Tel.: +1-514-337-3333; fax: +1-514-337-0550; e-mail: vaisburga@methylgene.com
† Present address: PhageTech Inc., 7170 Frederick-Banting, 2nd Floor, Montreal, Quebec H4S 2A1, Canada.

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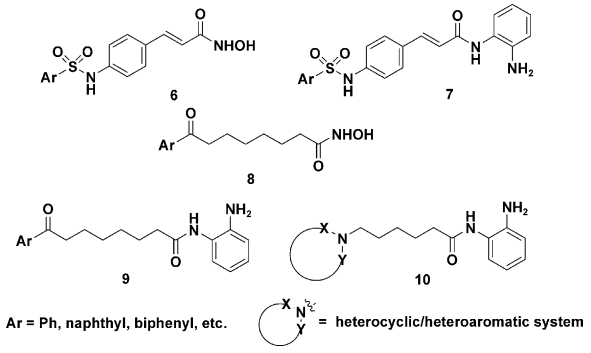
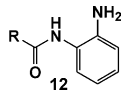


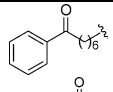
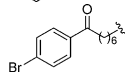
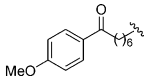
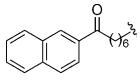
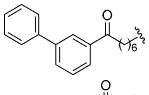
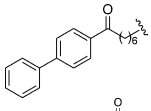
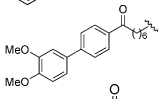
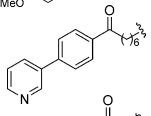
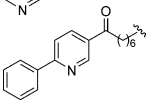
Figure 2.

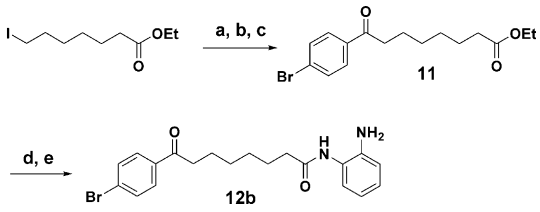
recent publication from Abbott Laboratories[13] describes the synthesis and biological activity of a series of long-chain trifluoromethyl ketones.

The first series of target compounds, phenyl, biphenyl and naphthyl anilides **12a–g** (Table 1) was synthesized starting from ethyl 7-iodoheptanoate as outlined in Scheme 1 (exemplified by anilide **12b**).[12] Formation of the copper-zinc organometallic intermediate, followed by reaction with *p*-bromobenzoyl chloride yielded compound **11**. This aryl ketoester was saponified and then coupled to *o*-phenylenediamine using standard peptide

Table 1. 8-Oxo-8-aryl-octanoic acid (2-amino-phenyl)-amides



No.	R	HDAC-1 IC_{50} (μM)	MTT HCT116 IC_{50} (μM)	H4-Ac EC_{50} (μM)
12a		9	>25	25
12b		6	20	15
12c		5	21	5
12d		2	9	25
12e		1	12	25
12f		2	7	10
12g		4	4	3
12h		10	19	20
12i		3	5	5

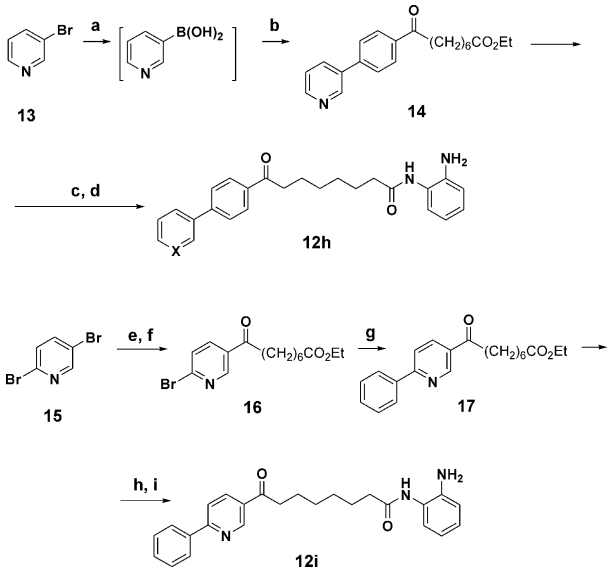


Scheme 1. Reagents and conditions: (a) $Zn/BrCH_2CH_2Br/Me_3SiCl$; (b) LiCl/CuCN/THF; (c) *p*-BrC_6H_4COCl/THF, 80% three steps; (d) $NaOH/H_2O$/THF/MeOH, 94%; (e) HOBt/EDC/DMF then *o*-phenylene diamine, 30%.

coupling chemistry (HOBt/EDC) to afford the final product **12b** in moderate yield (Scheme 1).

Compounds **12a** and **12c–g** (see Table 1) were prepared similarly to the compound **12b**, using the corresponding arylcarbonyl chlorides as intermediates. Two pyridine-based anilides (Scheme 2) were prepared from bromopyridines. Starting from 3-bromopyridine (**13**), the corresponding boronic acid was generated in situ and then reacted with ketoester **11** to form ketoester **14**. Saponification followed by HOBt/EDC coupling produced anilide **12h**. Its isomer, compound **12i**, was synthesized from 2,5-dibromopyridine (**15**) which was converted to the trimethyltin intermediate and subsequently coupled to the monomethyl ester of suberoyl chloride under Stille conditions. The bromopyridyl ketoester **16** underwent Suzuki coupling with phenylboronic acid to yield the intermediate **17**, which after saponification and treatment with *o*-phenylenediamine afforded compound **12i**.

A series of heterocyclic derivatives **20a–g** (Table 2) were prepared via condensation reactions (Scheme 3). *o*-Phthalaldehyde (**18**) was readily converted to oxo-



Scheme 2. Reagents and conditions: (a) *n*-BuLi, Et_2O, hexane then $B(OMe)_3$; (b) **11**, $Pd(Ph_3)_4$, K_2CO_3, toluene, H_2O, 25%; (c) NaOH, H_2O, THF, MeOH, 75%; (d) HOBt, EDC, DMF then *o*-phenylene diamine, 6%; (e) *n*-BuLi then Me_3SnCl; (f) $ClCO(CH_2)_6CO_2Me$, $PdCl_2(PPh_3)_2$, 39% two steps; (g) $PhB(OH)_2$, $Pd(Ph_3)_4$, K_2CO_3, toluene, EtOH, 88%; (h) NaOH, H_2O, THF, MeOH, 90%; (i) HOBt, EDC, DMF then *o*-phenylene diamine, 33%.

isoindole **19** using known procedure[14] and then to the final product **20a**. Compounds **20b–e** were prepared starting from the appropriate phthalic anhydrides reacting with 6-aminohexanoic acid, followed by peptide coupling with *o*-phenylenediamine (Scheme 3). In the case of **20e**, where the desired anhydride was not commercially available, it was prepared from 4-hydroxyphthalic acid (**21**) simply by heating under vacuum. Mannich reaction was employed to convert the hydroxy-substituted phthalimide **23** to piperidinylmethyl-intermediate **24** which led to compound **20f**.

Compounds **28e–28g** (see Table 3) were prepared similarly to the compound **20e**, from the appropriately substituted phthalic or naphthalic anhydrides. Compounds **20g**, **28h**, and **28i** (see Tables 2 and 3) were prepared similarly to compound **20f**.

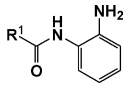
Scheme 4 outlines the synthesis of several quinazolinones **28a**, **28c**, **28d**, and benzotriazinone **28b**, all stemming from the same starting material. Reaction of isatoic anhydride (**25**) with 6-aminohexanoic acid followed by cyclization in the presence of either formic acid or sodium nitrite/HCl resulted in quinazolinone (**26**: X = CH) or benzotriazinone (**27**: X = N), respectively.[15] Each was then coupled to yield anilides **28a** and **28b**. Reaction of isatoic anhydride (**25**) with 6-aminohexanoic acid followed by consecutive treatment of the resultant reaction mixture with methyl chloroformate and then a base afforded quinazolinedione **30**.[16] This material upon alkylation with methyl iodide followed by reaction with an alkali led to **29**. Both acids **30** and **29** were coupled in the usual manner to form compounds **28c** and **28d**.

Naphthalimides **28e–g** (Table 3) were derived from the corresponding naphthalic anhydrides using chemistry depicted in the Scheme 3.

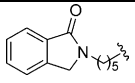
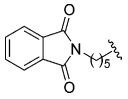
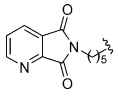
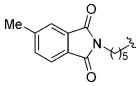
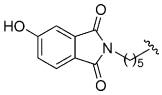
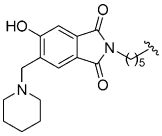
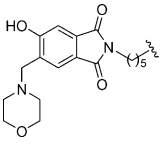
All compounds were initially screened for the ability to inhibit recombinant human HDAC-1.[17] We chose this isozyme since it is widely implicated in transcriptional repression and chromatin remodeling. The first compound in this series **12a** showed modest activity against HDAC-1 with the IC_{50} of 9 μM (Table 1). Introduction of substituents on the phenyl ring (compounds **12b** and **12c**) slightly improved their activity while increasing the size of the entire aryl fragment, compounds **12d–12f**, resulted in a 5-fold gain in potency (IC_{50} 1–2 μM, Table 1). Neither a moderate increase of the size of the aryl substituent nor introduction of a basic site (compounds **12g** and **12h**) proved to be beneficial. However, the isomer of **12h**, compound **12i**, showed potency similar to the one of compounds **12e** and **12f** (Table 1).

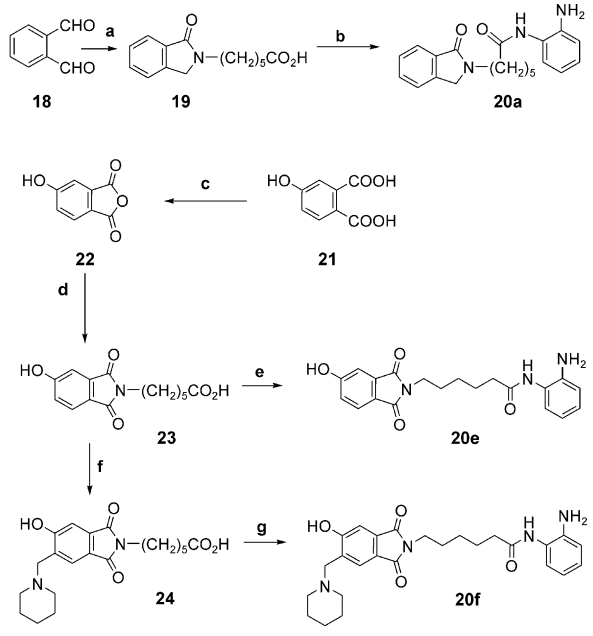
To explore more comprehensively the SAR of this series we hypothesized that restrictions in the flexibility of the chain between the carbonyl groups may improve potency. The first two compounds of this type were the bi-cyclic compounds of the isoindole series **20a** and **20b**, having HDAC-1 potency of 4 and 2 μM, respectively

Table 2. 6-Heteroaryl-hexanoic acid (2-amino-phenyl)-amides



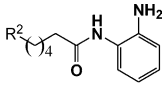
20

No.	R^1	HDAC-1 IC_{50} (μM)	MTT HCT116 IC_{50} (μM)	H4-Ac EC_{50} (μM)
20a		4	15	99
20b		2	24	5
20c		5	>47	>25
20d		3	6	3
20e		3	11	10
20f		3	31	—
20g		4	12	—

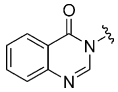
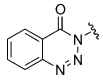
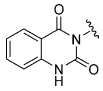
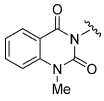
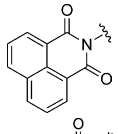
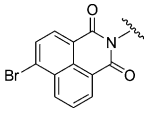
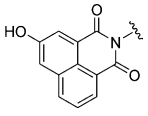
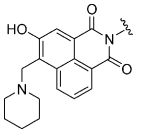
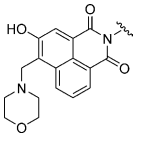


Scheme 3. Reagents and conditions: (a) $H_2N(CH_2)_5COOH$, AcOH, reflux 97%; (b) HOBt, EDC, DMF then *o*-phenylene diamine 27%; (c) Δ, 220 °C, 2 h; (d) $H_2N(CH_2)_5COOH$, AcOH, reflux 98%; (e) HOBt, EDC, DMF then *o*-phenylene diamine 18%; (f) paraform, piperidine, dioxane, DMF 37%; (g) HOBt, EDC, DMF then *o*-phenylene diamine 44%.

Table 3. 6-Heteroaryl-hexanoic acid (2-amino-phenyl)-amides

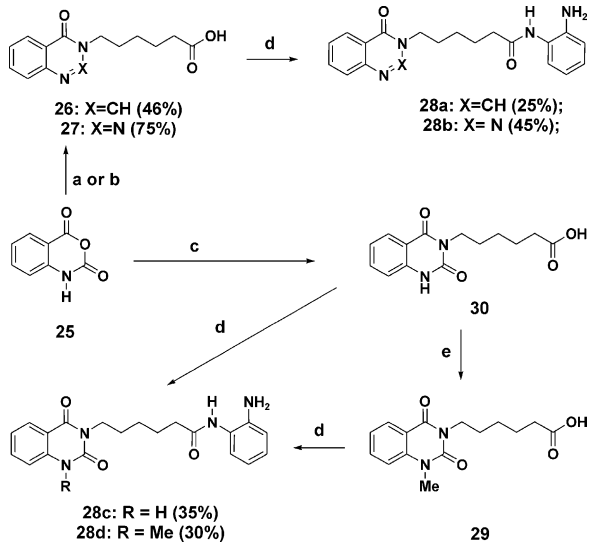


28

No.	R^2	HDAC-1 IC_{50} (μM)	MTT HCT116 IC_{50} (μM)	H4-Ac EC_{50} (μM)
28a		12	10	5
28b		8	10	10
28c		4	5	10
28d		4	5	—
28e		3	2	3
28f		1	6	10
28g		1	0.9	5
28h		2	1	1
28i		0.5	1	2
	MS-275	1	0.5	1

(Table 2). Introduction of a variety of substituents on the phenyl ring (compounds **20d–g**) or replacing phenyl with pyridine (compound **20c**) did not improve the potency (Table 2), prompting us to prepare compounds of quinazolinone type **28a–d** and later benzoisoquinolinediones **28e–i** (Table 3). The last four turned out to be quite potent, in fact, as potent as MS-275 (**5**) which is currently undergoing phase I clinical trials.[18]

We evaluated the in vitro antiproliferative activities of the compounds using the 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium] bromide (MTT) assay against various human cancer cell lines, in particular HCT116 (human colon cancer, Tables 1–3). The compounds showed a range of antiproliferative activities from 1 to 50 μM.



Scheme 4. Reagents and conditions: (a) $H_2N(CH_2)_5COOH$, Et_3N, H_2O then HCOOH, reflux; (b) $H_2N(CH_2)_5COOH$, Et_3N then $NaNO_2$, HCl; (c) $H_2N(CH_2)_5COOH$, Et_3N, MeOCOCl then NaOH 45%; (d) HOBt, EDC, DMF then *o*-phenylene diamine; (e) MeI, K_2CO_3 then NaOH 90%.

To confirm the ability of the amino anilides to inhibit HDAC in whole cells, all compounds were evaluated for the ability to induce histone acetylation in T24 human bladder cancer cells. The anilides (**12f**, **12i**, **28e**, **28g**, and **28i**) caused dose-dependent histone hyperacetylation in these cells (Tables 1–3, Fig. 3). Similarly to other known HDAC inhibitors the compounds **12f**, **12i**, **28e**, **28g** and **28i** also induced expression of p21WAF1/Cip1 protein in T24 human cancer cell lines (Fig. 4), while compounds **12f** and **28g** caused G2/M cell cycle arrest in HCT116 (Fig. 5).

HDAC small molecule inhibitors with attractive profiles from in vitro screening (**12f**, **12i**, **28e**, **28g**, and **28i**) were evaluated in vivo in several different human tumor xenograft models: A549 (non-small cell lung), PANC-1 (pancreatic), DU145 (prostate) and HCT116, Colo205 and SW48 (colon). Compounds were administered daily by either intraperitoneal (ip) injection or orally (po). Compounds **12f**, **12i**, **28e**, **28g**, and **28i**

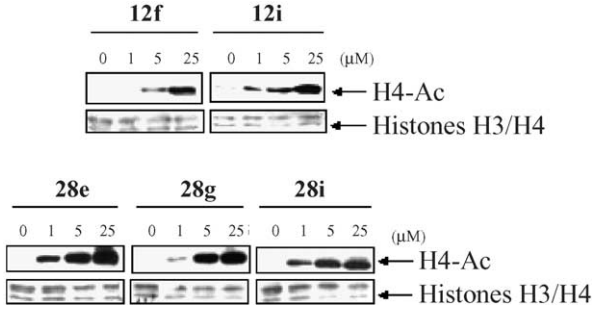


Figure 3. Human bladder carcinoma T24 cells were treated with compounds at 0, 1, 5, and 25 μM for 16 h. Cells were harvested and histones were acid-extracted, analyzed by SDS-PAGE and immunoblotting with antibodies specific for acetylated H4 histones. Histones were stained by Coomassie Blue to reveal their amount loaded on the gels.

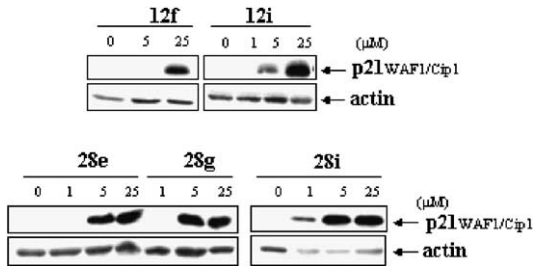


Figure 4. Induction of p21WAF1/Cip1 protein expression in human bladder carcinoma T24 cells treated with HDAC inhibitors. Human T24 cells were treated with compounds at indicated concentrations for 16 h before whole cell lysates were harvested and analyzed by Western Blot using antibodies against human p21WAF1/Cip1 protein. The expression level of actin was analyzed to reveal protein loading in each lane.

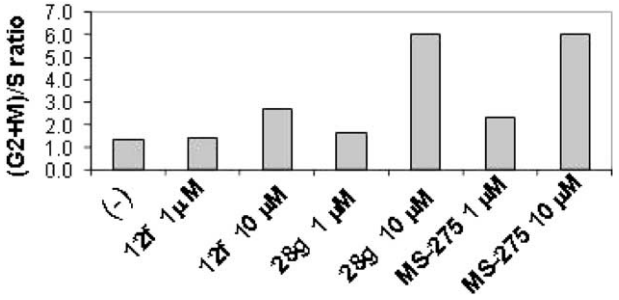


Figure 5. HDAC inhibitors caused G2/M arrest of human cancer cells. Human HCT116 cancer cells were treated with HDAC inhibitors or MS-275 in 1% DMSO for 16 h before harvesting by trypsinization. Propidium iodide stained DNA contents in fixed cells were analyzed by flow cytometry. The (G2+M)/S ratio was calculated using the percentage of cells among total gated cells in G2/M phase, divided by the percentage of cells in S phase. Percentages of cells in G1 phase were not affected by HDAC inhibitors.

showed measurable antitumor activity in vivo. Compounds **12f** and **28g** displayed the greatest antitumor activity with % TGIs (tumor growth inhibitions relative to vehicle controls) of 53 and 28%, respectively, when dosed at 40 mg/kg per day for 3 weeks. This activity was obtained without any associated body weight loss as a measure of gross toxicity. However the activity was lower than that of MS-275 (**5**) which was used as a positive control in these experiments. These results may be attributed to relatively short half-life and poor bioavailability of the listed entities (data not shown).

In summary, the (2-amino-phenyl)-amides of ω-substituted alkanoic acids were shown to inhibit recombinant human HDACs with IC_{50} values in the low micromolar range, induce hyperacetylation of histones, increase expression of p21WAF1/Cip1 and cause cell-cycle arrest in human cancer cells in a dose dependent manner. Although the title compounds are not yet completely optimized, the results indicate a significant step towards the development of small molecule HDAC inhibitors with acceptable pharmaceutical characteristics and potent antitumor activity.

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