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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5339–5344
Bioorganic & Medicinal Chemistry Letters

Synthesis of rigid trichostatin A analogs as HDAC inhibitors

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Received 8 June 2006; revised 24 July 2006; accepted 25 July 2006
Available online 14 August 2006

Abstract—New inhibitors of histone deacetylase (HDAC) have been synthesized and evaluated for their activity toward non small lung cancer cell line H661. Their design is based on indanone (or tetralone) systems leading to trichostatin A (TSA) analogs with limited conformational mobility. Molecular modelization at the AM1 level revealed that the conformations of indane-based analogs and TSA bound to HDAC like protein are similar. The synthesis of these new analogs was achieved by alkylation of an appropriate indanone (or tetralone) to introduce the side chain bearing a terminal ester group, the latter being a precursor of hydroxamic acid and aminobenzamide derivatives. Hydroxamic acids with the TSA side chain were found to be the most active compounds and the presence of the dimethylamino group on the phenyl ring turned out to be essential to achieve low micromolar activities against H661 cancer cells.

The eukaryotic DNA is structured in chromatin with repetitive units called nucleosomes that not only provide structural support for packing the nucleosomal DNA but also are critical sites that control gene activation and repression. At the center of the periodically assembled units (about every 200 nucleotide base pairs) sealed by histone H1 are the highly conserved histone proteins H2A, H2B, H3, and H4 that form the octameric core around which the DNA is folded. The assembly of DNA in nucleosomal units is a fluid structure that responds to the needs of the cells by remodeling regions that are actively involved in gene transcription and DNA replication. Regulation of histone acetylation, methylation, and phosphorylation control chromatin remodeling[1]. Modification of the Σ-amino group of specific lysines within histones by acetylation and deacetylation plays a crucial role in the transcriptional process and deregulation can lead to cancer.[2,3] This epigenetic modification is regulated by two groups of enzymes HAT (histone acetyl transferase) and HDAC (histone deacetylase) with opposite activities.[4,5] HDACs play prominent roles in the transcriptional inactivation of tumor suppressor genes. Many studies show that inhibition of HDAC elicits anticancer effects in several tumors by inhibition of cell growth and inducing cell differentiation.[6] In addition, recent data indicate that a variety of proteins, including p53 a tumor suppressor gene[7] and α-tubulin involved in microtubule stability,[8,9] are also substrates for HDACs. It is now clear that many HDACs target non-histone proteins and have functions beyond the alteration of chromatin structures.[10] Therefore, inhibiting HDAC is an attractive target for anticancer therapy.[11–13]

As a result of these findings, several programs for the development of HDAC inhibitors (HDACI) as anticancer drugs have been initiated.[14] Several natural compounds have been isolated, like trichostatin A **1**[15] (Fig. 1),

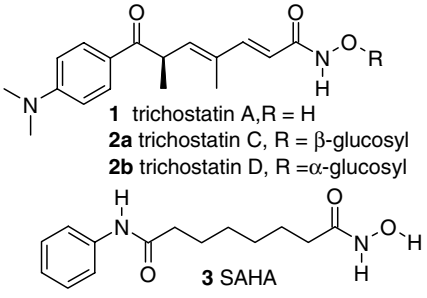

Figure 1. Natural (**1**,**2**) and synthetic (**3**) HDACI.

Keywords: Histones deacetylases; Trichostatin A; Analogs; Anticancer agents; Hydroxamic acids; Benzamides.
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doi:10.1016/j.bmcl.2006.07.080

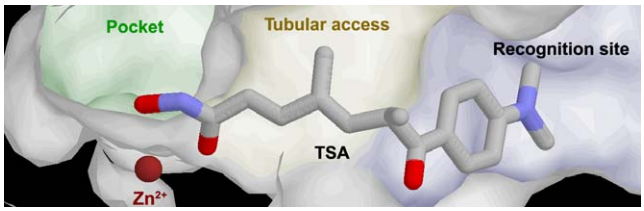


Figure 2. TSA in HDLP protein (pdb 1C3R[15]).

a reversible HDACI,[16] or synthesized, like SAHA **3**, actually in clinical trials (phase III).[17]

Structural requirements for HDAC inhibition have been rationalized:[18–20] a functional group able to chelate the zinc atom at the bottom of the active site, a recognition moiety interacting with the external surface of the protein, and a spacer linking these two moieties and fitting the tubular geometry of the site (Fig. 2). This led to the synthesis of a large number of more or less complex compounds for SAR studies.[21–26]

HDACIs were classified in several groups: second generation hybrid polar compounds (HPCs) as SAHA **3**, benzamides, arylketones, benzo[*de*]isoquinolines, and 1,3-dioxanes. Another group is the cyclic hydroxamic acid-containing compounds (CHAPs), where the recognition part is a cyclic tetrapeptide.

TSA **1** itself has been synthesized in either racemic[27] or enantiopure[28] form, and enantioselective preparation of trichostatin D **2**[29] has also been described. Biological studies on TSA showed some toxicity and biological instability limiting its clinical development: the hydroxamic acid is hydrolyzed first to amide and then to carboxylic acid, two inactive derivatives, and the amino group is demethylated, giving metabolites rapidly eliminated in biological systems.[30] Furthermore, it should be considered that TSA may be sensitive to enolization and thus, racemization leading to inactive compounds.

Taking into account these observations, it seemed worthwhile to explore the synthesis of rigid analogs of TSA designed by linking the α position of the ketone to the phenyl ring, leading to indanone ($n = 1$) or tetralone ($n = 2$) derivatives (Fig. 3). Furthermore, other modifications may be considered such ring substitution or replacement of the hydroxamic acid by another Zn chelating function.

For such analogs enolization will be impossible but the flexibility of the phenyl ring with respect to the remaining part of the molecule will also be suppressed. At this stage, conformational analysis of the indanone analog

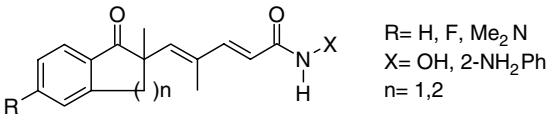


Figure 3. TSA analogs.

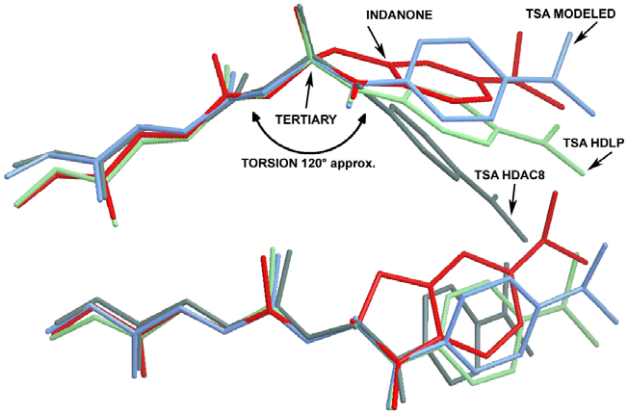


Figure 4. Modeled conformations of TSA and of an indanone analog, and crystallographic structures of TSA bound to HDLP (histone deacetylase like protein) and HDAC8.

was carried out at the AM1 level[31] (Fig. 4) and it was found that the more stable conformer is rather close to the computed and X-ray structures found for TSA in the active site of HDAC8 or HDLP.[18–20]

The synthesis of these new analogs was implemented by alkylation of an appropriate indanone (or tetralone, Fig. 5) to introduce the unsaturated side chain bearing a terminal ester group, the latter being a precursor of hydroxamic acid and aminobenzamide derivatives.

Indanone **4a** is commercially available, tetralone **4b** was prepared from α-tetralone by methylation[32] (79% overall yield), and fluoroindanone **4c** was prepared from fluorobenzene and 2-bromo-2-methylpropionylbromide

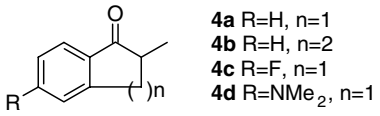
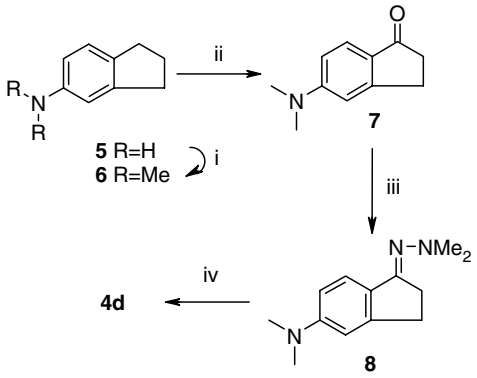
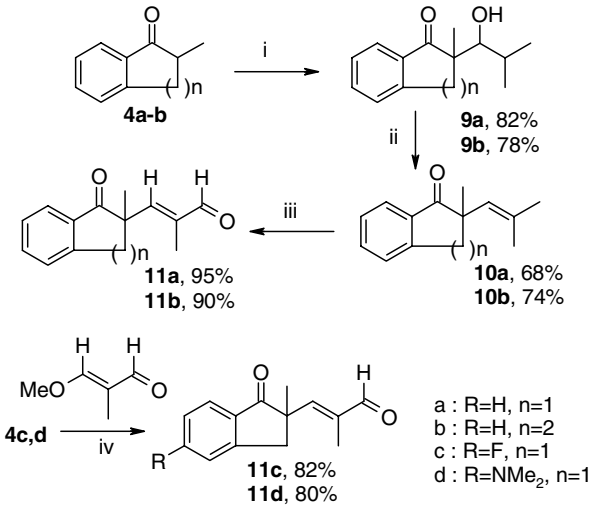


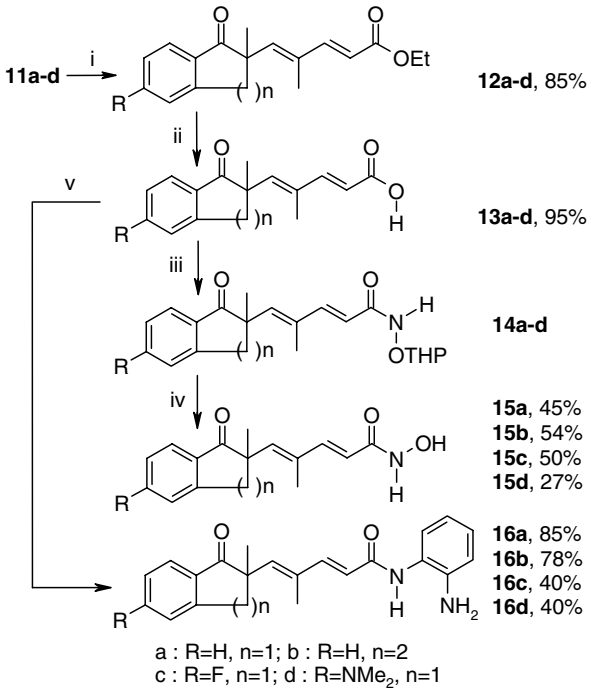
Figure 5. Indanones and tetralone.



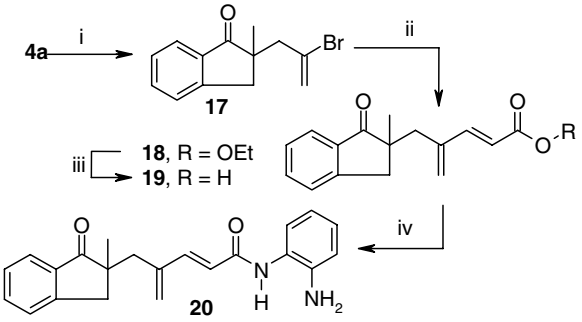
Scheme 1. Reagents and conditions: (i) CH_2O, $NaBH_3CN$, CH_3CN, 98%; (ii) DDQ, THF, 90%; (iii) H_2N–NMe_2, pTsOH, Toluene, Dean–Stark; (iv) LDA, MeI, −40°C; then H_3O^+, 78%.



Scheme 2. Reagents and conditions: (i) LDA, THF, −78 °C, isobutyraldehyde; (ii) $SOCl_2$, pyridine; (iii) SeO_2, EtOH; (iv) a—LDA, THF, −78 °C; b—H^+.



Scheme 3. Reagents and conditions: (i) $Ph_3P{=}CHCOOEt$, toluene, reflux, 12 h; (ii) LiOH, MeOH; (iii) H_2N–OTHP, TBTU, DMF, NEt_3; (iv) CSA, CH_2Cl_2, MeOH; (v) H_2N–Ph–NH_2, EDC, THF.



Scheme 4. Reagents and conditions: (i) LDA, THF, −78 °C, 85%; (ii) LiCl, Bu_4NCl, K_2CO_3, DMF, 80 °C, 65%; (iii) LiOH, MeOH, 60%; (iv) H_2N–Ph–NH_2, EDC, THF, 40%.

($AlCl_3$, CS_2, quantitative yield).[33] Attempts to directly convert fluoroindanone to dimethylamino-2-methyl-indanone **4d** by nucleophilic aromatic substitution (HMPA, 170 °C[34] or Me_2NH, NaH, DMSO, 120 °C[35]) gave poor yields (<10%).

Another strategy was used to prepare this material from aminoindane **5** (Scheme 1). The latter was dimethylated by reductive formylation (CH_2O, $NaBH_3CN$, CH_3CN) to give **6** which was then oxidized to **7** (DDQ, THF).[36] Monomethylation of **7** (LDA, THF, CH_3I) via its *N*,*N*-dimethylhydrazone **8** finally afforded **4d** (78% overall yield from **7**).

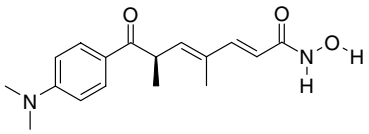
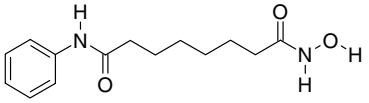
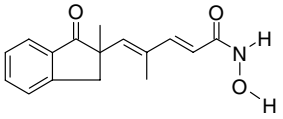
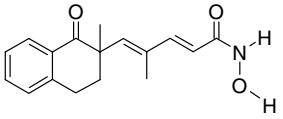
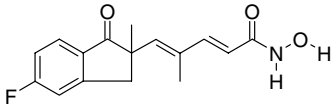
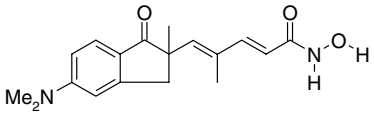
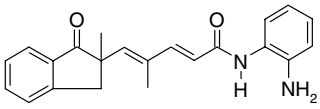
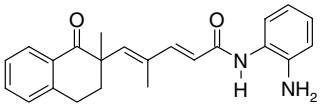
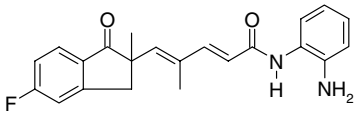
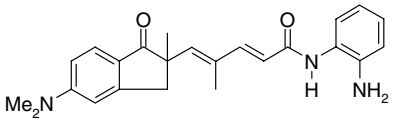
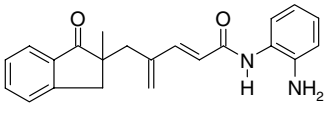
Introduction of the butadienoate chain was first carried out from **4a,b** via aldolization with isobutyraldehyde (LDA, THF, −78 °C, Scheme 2). Aldols **9a**,**b** were dehydrated ($SOCl_2$, pyridine) to give alkenes **10a**,**b** which were oxidized to afford **11a**,**b** as *E* isomers.[37] A better route was designed based on aldolization with 3-ethoxy-2-methyl-propenal as described by Wood,[38] the intermediate aldol being hydrolyzed in situ under acidic conditions to afford 3-substituted 2-methyl-pronenal derivatives. This methodology was first applied to **4b** and gave **11b** in better yield (80%) as compared to the first method (3 steps, 40% overall). Wood's procedure was then applied to fluoro and amino compounds **4c**,**d** to give the corresponding aldehydes **11c**,**d** in good yield.

Wittig homologation (Ph_3P = CHCOOEt, toluene, 12 h) of aldehydes **11a**–**d** gave the *E*,*E*-unsaturated esters **12a**–**d** (Scheme 3). After hydrolysis (LiOH, MeOH) to the corresponding carboxylic acids **13a**–**d**, hydroxamic acids **15a**–**d** were obtained by treatment of **13a**–**d** with the known hydroxylamine O–THP[39] ether (TBTU, DMF, NEt_3) giving **14a**–**d**, followed by acid catalyzed removal of the THP ether (CSA, CH_2Cl_2, MeOH). Aminobenzamides **16a**–**d** were obtained by coupling **13a**–**d** with 1,2-diaminobenzene (EDC, THF, 64–85%).

A different butadienoate derivative was also prepared with the aim of attempting double bond isomerization to afford an alternative route toward **12a**–**d** (Scheme 4). Alkylation of indanone **4a** with 2,3-dibromopropene afforded bromide **17** which was then submitted to Heck coupling with ethyl acrylate. The *E* dienoate **18** was hydrolyzed to acid **19**, converted as previously described to benzamide **20**. Compound **19** could not be converted to the corresponding hydroxamic acid and Pd catalyzed isomerization of **18** was not successful.

All the hydroxamic and benzamide derivatives were evaluated for their antiproliferative activity against non small cell lung cancer H661 cells. IC_{50}s were determined from two triplicate experiments by extrapolation

Table 1. Antiproliferative activities for TSA **1**, SAHA **2**, and TSA analogs

Entry	Compound		Inhibition IC_{50}, μM[a,b,c]	Cell lines
1	**1**		<0.3[a] 0.1 0.0013	H661 H1299[26] HCT116[26]
2	**3**		>5[a] 7.24 1.93	H661 H1299[26] HCT116[26]
3	**15a**		1.65[b]	H661
4	**15b**		3[b]	H661
5	**15c**		1.5[b]	H661
6	**15d**		<0.5[b] 0.2[c]	H661
7	**16a**		3[a]	H661
8	**16b**		5[a]	H661
9	**16c**		7[a]	H661
10	**16d**		2.5[a]	H661
11	**20**		>10	H661

[a] Extrapolated IC_{50} from XTT tests at concentrations in range 1–10 μM.
[b] 1–5 μM.
[c] 0.025–1 μM.

to 50% proliferation (Table 1 and supplementary material, Fig. 8). Values obtained were compared to previously reported data for TSA or SAHA.

Benzamides were found to be less active than the corresponding hydroxamic acids, which is in agreement with literature data. The tetralone analogs were found to be

half as potent than the corresponding indanones (Table 1, entries 3–8, compounds **15a**,**b**–**16a**,**b**). Compound **20** appeared to be rather unstable and was not active (Table 1, entry 11).

Analogs not substituted on the phenyl ring or bearing a fluoro substituent gave IC_{50}s in the 1–5 μM range (Table 1, entries 5 and 9). On the other hand, the presence of a dimethylamino group (Table 1, entries 6 and 10) appears to be critical to achieve submicromolar activity.

This can be rationalized by analysis of the crystallized form of HDLP with TSA which shows an interaction between this amino group and Tyr91 (Tyr100 in HDAC8) at the entry of the pocket, either with the carbonyl group of Tyr91 or by π–π stacking with the phenyl ring (Fig. 6).

Then, compounds showing the best antiproliferative activities were used for determination of acetylation of histone H4 and tubulin using Western blot (Fig. 7). The concentration of each compound was adjusted close to the corresponding IC_{50} value (**15c**: 2.5 μM, **15b**: 5 μM, **15d**: 0.3 μM, TSA **1**: 0.3 μM). At these concentrations, **15b**–**d** have good activities on acetylation of histone H4. In particular, **15d** shows a better response than TSA at the same concentration. Evaluation on tubulin acetylation was also determined for compounds **15b**–**d**, with activities similar to those of TSA at the given concentrations.

In conclusion, this work has demonstrated that conformationally constrained analogs of TSA, incorporating an indanone nucleus retain interesting antiproliferative and HDAC inhibitory activities. The dimethylamino group derivative compares favorably to TSA, although being a racemic compound. Work is in progress in our laboratory to prepare new substituted indanones as well as enantiomerically pure compounds.

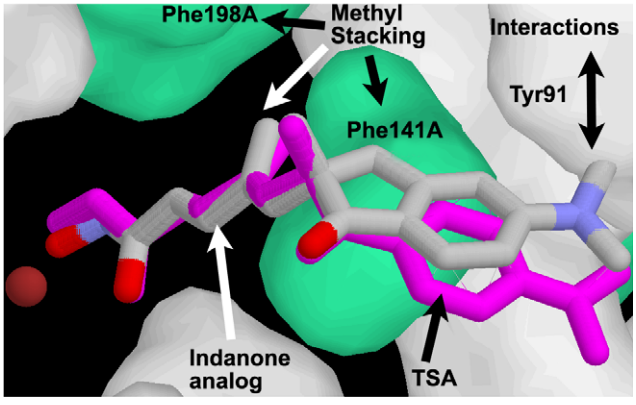


Figure 6. TSA (pink) and an indanone analog (CPK) manually docked in the active site of HDLP with Zn^{2+} (brown). Only Phe (green) and Tyr (white) are shown with stacking of the vinylic methyl between Phe141 and Phe198, and dimethylamino close to Tyr91 (produced from 1C3R[18] with MDL Chime plug in).

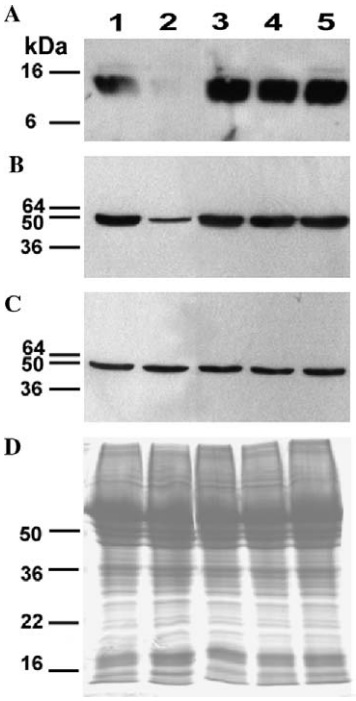


Figure 7. Western blots of: (A) histone H4 acetylation, (B) tubulin acetylation, (C) α-tubulin for H661 cell treatment during 5 h 30′ with: 1—**1** (TSA) 0.3 μM, 2—control untreated cells, 3—**15c** 2.5 μM, 4—**15b** 5 μM, 5—**15d** 0.3 μM, (D) total protein extract of H661 cells stained by Coomassie blue.

Acknowledgments

We thank MENRT, CNRS, and La Ligue Contre le Cancer, Comité de Charente-Maritime for financial support, and Stéphanie Sissoko for additional biological evaluations.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.07.080.

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