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Exploring bis-(indolyl)methane moiety as an alternative and innovative CAP group in the design of histone deacetylase (HDAC) inhibitors

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ABSTRACT

In order to gather further knowledge about the structural requirements on histone deacetylase inhibitors (HDACi), starting from the schematic model of the common pharmacophore that characterizes this class of molecules (surface recognition CAP group—connection unit—linker region—Zinc Binding Group), we designed and synthesized a series of hydroxamic acids containing a bis-(indolyl)methane moiety. HDAC inhibition profile and antiproliferative activity were evaluated.

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Inhibition of histone deacetylases is emerging as a promising new strategy in human cancer therapy.[1] Histones are nuclear core proteins accountable for the regulation of transcription and cell cycle progression. These activities are dependent on the level of acetylation and deacetylation of specific lysine ε-amino groups of the proteins backbone. These processes are controlled by two families of enzymes: histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively. The 18 known human HDACs members are classified into four categories. Class I (HDAC 1, 2, 3, 8), class II (HDAC 4, 5, 6, 7, 9, 10), and class IV (HDAC 11) are Zn-dependent enzyme, while class III (sirtuins) are NAD^+-dependent enzymes.[2]

The great potential of these epigenetic modulators was recognized early on, but most of the early research aiming at finding more potent HDAC inhibitors did not focus on the role of each isoform, giving rise to non selective inhibitors.

Over the past few years, a lot of efforts have been done in the field of HDACi and more than a hundred patents claiming new chemical series have emerged. A number of molecules targeting HDACs are under clinical investigation as anticancer and the first one (SAHA—vorinostat; Zolinza®; Fig. 1) has been approved by the FDA for the treatment of cutaneous T-cell lymphoma.[3]

Furthermore, few HDACi are also currently investigated as single agent therapy or in combination with other active ingredients. Most of them are pan-inhibitors, with only few exceptions of isoform-specificity. However, the exact mechanisms by which these inhibitors lead to the observed biological effect are still not known. The mode of action may differ from one inhibitor to another because of the chemical structure (leading to a particular modulation of the various HDACs isoforms) or because of the pharmacokinetic profile. Although, the requirement of isoform specific inhibition is not yet unambiguously established, research in this area is mainly oriented toward isoform-specific HDACi.[4]

According to the usual schematic segmentation of the common pharmacophore (Fig. 2), HDACi are characterized by a surface recognition zone (CAP group, blue), a connection unit (or kink atom, black), a linker region (usually hydrophobic, red) and a zinc binding group (ZBG, green).

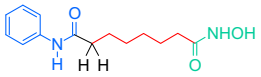

Figure 1. SAHA—vorinostat (Zolinza®).

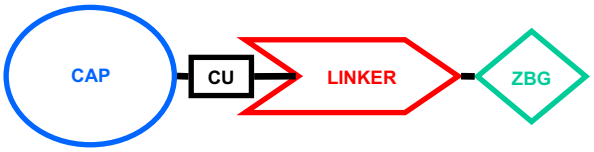

Figure 2. HDACIs common pharmacophore schematic segmentation.

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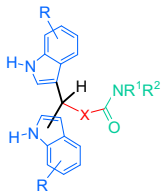


Figure 3. Bis-(indolyl)methane derivatives general structure.

Our project was oriented toward the exploration of a novel CAP group, the bis-(indolyl)methane moiety, as a substitution of SAHA and SAHA-like scaffolds.

The bis-(indolyl)methane derivatives show interesting biological activities.[5] Besides, bis-(indolyl)methane is a product obtained under spontaneous dehydratation and condensation of the well-known natural antitumoral agent, indole-3-carbinol.[6]

Geminal bis-(indolyl) moiety containing compounds were designed and synthesized in order to gather information regarding the steric and electronic requirements of HDACi. The compounds thus obtained enabled to elaborate a SAR around the above mentioned four regions identified in Figure 2 (Fig. 3).

We synthesized 2,2′-bis-(indolyl)methane derivatives **4a**–**e**—where R = H and X = $(CH_2)_n$ with *n* ranging from 2 to 6—to explore the influence of methylene chain length on biological activity. Experimental data showed that the optimal linker length consisted of five methylenes (**4d**; **ST2741**).

Based on these preliminary results, we synthesized pentyl derivatives with various substituents on the indole ring (**4f**–**n**). The hydrochloride salt of 5-morpholylmethyl derivative **4m** was prepared to enhance the solubility property.

We also modified the nature of the chain, replacing the aliphatic chain by an unsaturated variant (cinnamic system, **4o** and **4p**).

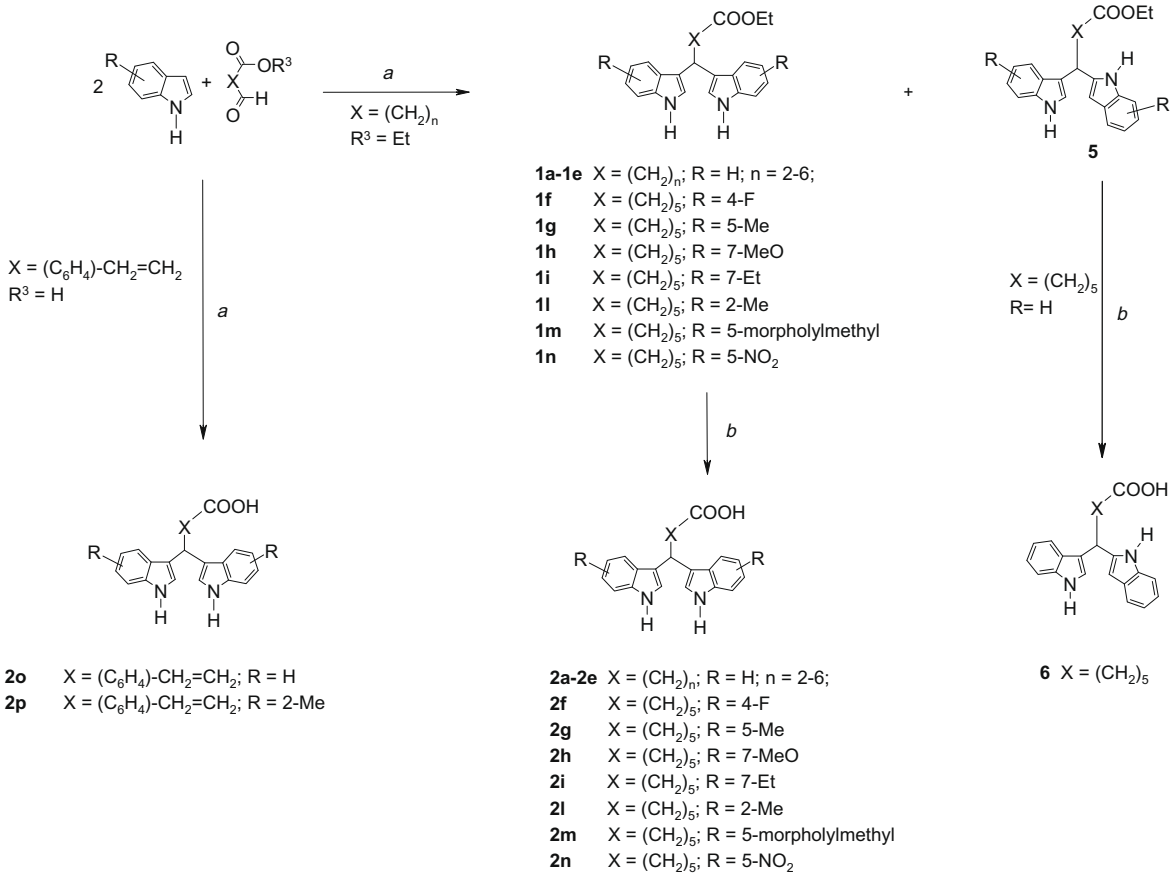
Finally, in order to evaluate an alternative ZBG, we prepared compound **3q** which is the *o*-aminobenzamide analogue of compound **4d**.

Condensation of two equivalents of (un)substituted indole starting material with one equivalent of ω-oxoaliphatic esters [Scheme 1, step *a*, X = $(CH_2)_n$] or *para*-formyl *trans*-cinnamic acid [Scheme 1, step *a*, X = Ph-CH_2=CH_2] using dysprosium triflate[7] as Lewis acid, led to the formation of the desired bis-indolyl system in excellent yields.

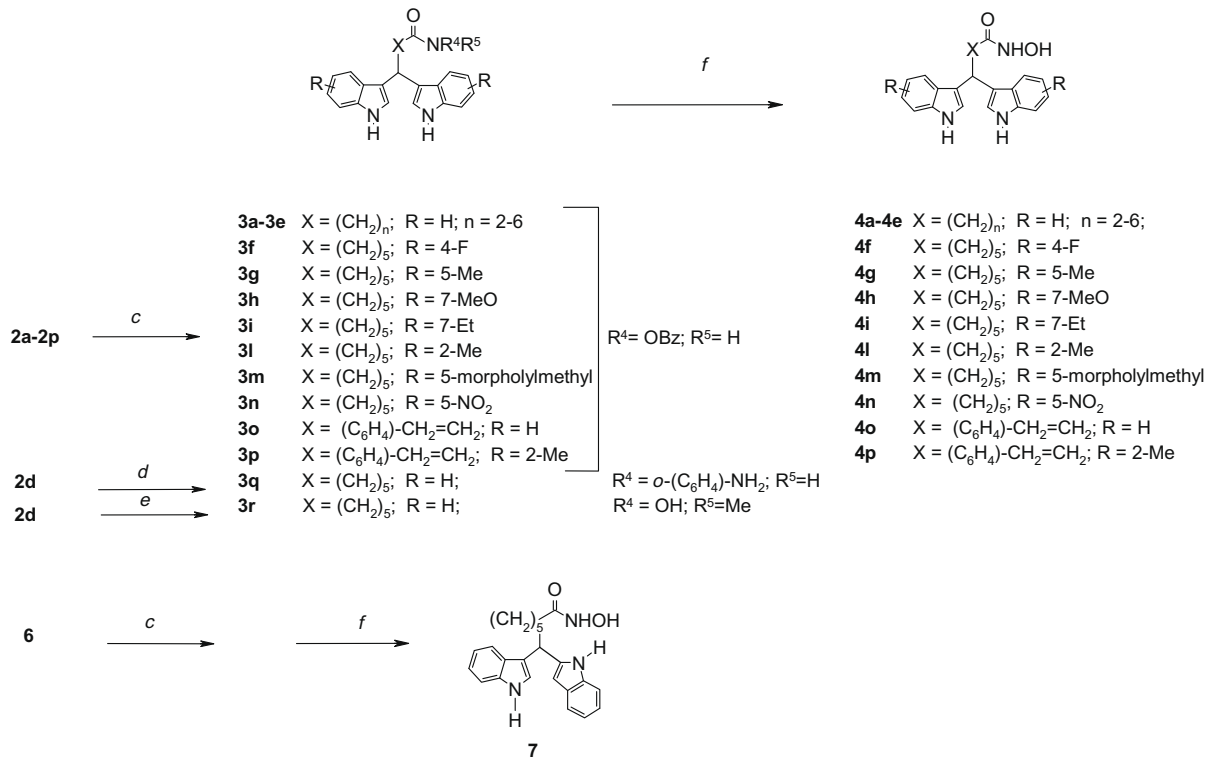
Besides $Dy(OTf)_3$, used as a Lewis acid stoichiometrically, on gram scale-up, we also used catalytic amount of I_2[8] or of trichloro-1,3,5-triazine (TCT) in CH_3CN at rt.[9] An alkaline hydrolysis (Scheme 1, step *b*) was necessary to obtain the carboxylic acids derivatives from the esters **1a–n**. Sometimes, asymmetric 2,3′ bis-indole derivatives (**5**) were isolated as side products. When R = H and X = $(CH_2)_5$ this by-product was used, according to step *b* (Scheme 1), to give intermediate **6** which was converted into the corresponding hydroxamate derivative (**7**) in a two step procedure (see Scheme 2).

The carboxylic acid intermediates **2a–p** were condensed (Scheme 2, step *c*) with *O*-benzyl-hydroxylamine hydrochloride or with *o*-phenylenediamine to give the corresponding protected hydroxamic acids intermediates **3a**–**p** or *o*-aminobenzamide derivative **3q**, which were easily purified by silica gel chromatography.

Subsequent condensation was performed using typical peptide synthesis condensing agents (i.e., PyBOP or HATU) or, for gram



Scheme 1. Bis-(indolyl)methane derivatives synthesis: condensation and hydrolysis steps. Reagents and conditions: (a) $Dy(OTf)_3$, $MeOH/H_2O$ (3/1), rt–50 °C; (b) NaOH, THF/$MeOH/H_2O$ (3:3:1), rt.



Scheme 2. Bis-(indolyl)methane derivatives synthesis coupling and deprotection steps. Reagents and conditions: (*c*) PyBOP, NMM, O-Bz-hydroxylamine·HCl, DCM, rt–70 °C (**3a**: 68%; **3b**: 60%; **3c**: 62%; **3d**: 70%; **3e**: 57%; **3f**: 70%; **3g**: 69%; **3h**: 52%; **3i**: 69%; **3l**: 49%; **3m**: 64%; **3n**: 68%; **3o**: 51%; **3p**: 57%); (*d*) PyBOP, *o*-$(NH_2)_2$-C_6H_4, TEA, DCM, rt; (*e*) ClCOOEt, TEA, CH_3NHOH·HCl, THF, 0 °C–rt (**3r**: 60%). (*f*) Pd/C, H_2, MeOH, rt (**4a**: 71%; **4b**: 97%; **4c**: 54%; **4d**: 78%; **4e**: 82%; **4f**: 35%; **4g**: 36%; **4h**: 40%; **4i**: 67%; **4l**: 41%; **4m**: 75%; **4n**: 51%; **4o**: 68%; **4p**: 80%; **7**: 8%).

scale-up, using the cheaper ClCOOEt (ethyl chloroformate). Finally, benzyl removal under reductive conditions (Scheme 2, step *f*) afforded the desired hydroxamic acids **4a**–**p**.

In order to establish a SAR and investigate the chemical stability of the inhibitors, we also focused our attention on N-atoms synthesizing various N-methylated derivatives. Direct coupling of intermediate **2d** with *N*-methylhydroxylamine hydrochloride led to compound **3r** (Scheme 2, step *e*).

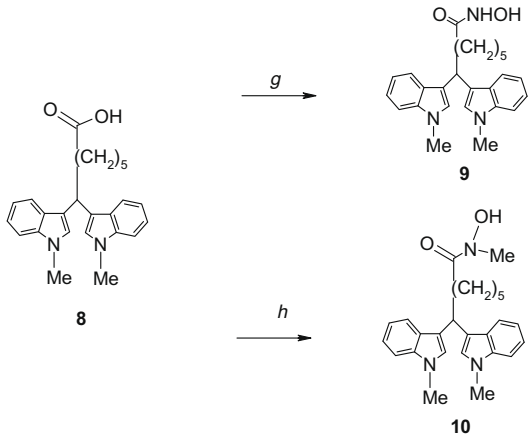
Starting from 1-methylindole, we obtained bis-indolyl carboxylic acid derivative **8**, according to step *a* and *b* of previous Scheme 1. This intermediate was subjected to direct coupling with hydroxylamine hydrochloride and with *N*-methylhydroxylamine hydrochloride to give compounds **9** and **10**, respectively (Scheme 3).

To evaluate the effect of the monoindolyl group as CAP, we synthesized an analogue of **4c** starting from the commercially available 6-(1*H*-indol-3-yl)hexanoic acid **11** to obtain compound **12** (Scheme 4).

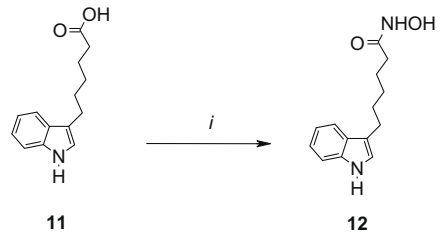
For all synthesized compounds, we first evaluated the in vitro anti-proliferative activity, using H460 and HCT116 tumor cell lines (Table 1) with SAHA[10] as a reference compound.

Biological data suggested product **4d** as possessing an optimal linker length as a hit compound among the un-substituted derivatives.

Then, the following compounds that were representative of the various structural modifications, although not always showing a good anti-proliferative activity, were selected for further biological investigation: 7-methoxy- (**4h**; ST3043) and the 5-nitro-derivative



Scheme 3. N-methylated derivatives synthesis. Reagents and conditions: (*g*) ClCOOEt, TEA, NH_2OH·HCl, THF, 0°–rt (**9**: 53%); (*h*) (i) ClCOOEt, TEA, NH_2OBz·HCl, THF, 0°–rt (67%), (ii) CH_3I, NaH, THF, rt, (iii) Pd/C, EtOH, H_2 (**10**: 52% 2 steps).



Scheme 4. Monoindol-3-yl derivate synthesis. Reagents and conditions: (i) HATU, DIPEA, NH_2OH·HCl, DMF, rt, 2 h (60%).

Table 1
In vitro cytotoxic activity of novel bis-indolyl derivatives[a]

Compound		X	R	R^1	R^2	H460 IC_{50}[b] (μM)	HCT116 IC_{50}[b] (μM)
SAHA						3.4	1.2
4a	3-3′	$(CH_2)_2$	H	OH	H	>20	>20
4b	3-3′	$(CH_2)_3$	H	OH	H	>20	>20
4c	3-3′	$(CH_2)_4$	H	OH	H	5.2	5.8
4d	3-3′	$(CH_2)_5$	H	OH	H	1.2	0.6
4e	3-3′	$(CH_2)_6$	H	OH	H	2.7	3.5
4f	3-3′	$(CH_2)_5$	4-F	OH	H	0.7	0.4
4g	3-3′	$(CH_2)_5$	5-Me	OH	H	0.51	0.27
4h	3-3′	$(CH_2)_5$	7-MeO	OH	H	0.55	0.41
4i	3-3′	$(CH_2)_5$	7-CH_3CH_2	OH	H	4.8	3.5
4l	3-3′	$(CH_2)_5$	2-Me	OH	H	4.3	3.2
4m	3-3′	$(CH_2)_5$	5-Morpholylmethyl	OH	H	1.1	0.96
4n	3-3′	$(CH_2)_5$	5-NO_2	OH	H	3.2	1.2
4o	3-3′	(C_6H_4)–CH_2=CH_2	H	OH	H	2.6	2.6
4p	3-3′	(C_6H_4)–CH_2=CH_2	2-Me	OH	H	>20	>20
4q	3-3′	$(CH_2)_5$	H	*o*-(C_6H_4)–NH_2	H	>20	16.5
10	3-3′	$(CH_2)_5$	1-Me	OH	Me	>20	>20
9	3-3′	$(CH_2)_5$	1-Me	OH	H	8.8	6.6
3r	3-3′	$(CH_2)_5$	H	OH	Me	>20	>20
7	3-2′	$(CH_2)_5$	H	OH	H	0.82	0.84
12			Monoindol-3yl			22	15

[a] Growth inhibition was measured by SRB (sulphorodamine B) assay after 24 h of treatment and 48 h of recovery.
[b] Values are means of three experiments.

Table 2
Compounds **4d**, **4h**, **4n**, **4o** and **4q** HDAC isoform selectivity profile[a,11]

Compounds	IC_{50}(nM)										
	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HDAC9	HDAC10	HDAC11
4d	730	2000	350	1300	590	6	530	240	640	870	430
4h	83	440	23	220	311	2.6	420	180	340	240	/
4n	140	590	18	220	140	20	220	600	150	250	/
4o	4800	3900	440	4800	4900	4900	4200	730	3300	4700	410
4q	21,000	1100	260	150,000	28,000	/	/	74,000	50,000	73,000	5800

[a] Assay condition was started with a 50 μM solution, 10 doses with 1:3 dilution. Trichostatin A (TSA) was used as reference compound.

(**4n**; ST5732), the cinnamic derivative (**4o**; ST2887) and the *o*-aminobenzamide derivative (**4q**; ST3071).

For these selected molecules, we evaluated the in vitro inhibitory activity against the 11 HDACs isoforms. In Table 2, we show IC_{50} (μM) data obtained using human HDAC enzymes and a fluorogenic peptide (p53 379–382 RHKKAc residues) at 50 μM, as the substrate.[11]

Compound **4d** together with the derivatives **4h** and **4n** showed an interesting HDACi profile ranging from low to high nM. These data were consistent with their anti-proliferative activity. **4h** was found particularly promising with a selective inhibition activity, against the isoforms HDAC1 and HDAC3 besides HDAC6, in the low nM range.

In summary, we have demonstrated that the bis-(indolyl)methane moiety can be used as a valid CAP in the design of HDAC inhibitors.

A Structure–Activity-Relationship (SAR) analysis has delineated the importance of the linker length in HDAC inhibition (the most potent derivatives present a penta-methylene linker) and the relevance of the bis-(indolyl)methane system (3-indolyl derivative **12** is less potent than bis-(indolyl) analogue **4d**).

The *o*-aminobenzamide derivative (**4q**), although less active than all the other examples of HDACi having as ZBG an *o*-aminobenzamide moiety, showed an unusual selectivity towards the HDAC3 isoform. However, the data confirm the importance of the hydroxamic acid as the ZBG of choice and show that the nature of the substituent on the indole ring, when not too hydrophilic, only slightly influences the in vitro activity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.101.

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10. SAHA was in-house synthesized.
11. The substrate, a fluorogenic moiety bound to specific p53 fragment—residues 379–392: Arg-His-Lys-Lys(Ac)—which comprises an ε-acetylated lysine side chain, was incubated with the 11 single HDAC purified enzymes. Upon deacetylation of the substrate, the fluorophore was released given rise to fluorescence emission. The latter was detected by a fluorimeter, and the IC_{50} values of the compounds were determined by analyzing dose-response inhibition curves. Trichostatin A (TSA) was used as reference compound.Screening was performed by Reaction Biology Corp.