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.

Inhibition of histone deacetylases is emerging as a promising new strategy in human cancer therapy. 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 enzymes, 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 HDMC 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. HDACi’s common pharmacophore schematic segmentation.
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. Besides, bis-(indolyl)methane is a product obtained under spontaneous dehydration and condensation of the well-known natural antitumoral agent, indole-3-carbinol.

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 \( X = (\text{CH}_2)_n \) 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-morpholymethyl 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 o-oxoaliphatic esters [Scheme 1, step a, \( X = (\text{CH}_2)_n \)] or para-formyl trans-cinnamic acid [Scheme 1, step a, \( X = \text{Ph-CH} = \text{CH}_2 \)] using dysprosium triflate as a 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 I₂ or of trichloro-1,3,5-triazine (TCT) in CH₃CN at rt. 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 \( X = (\text{CH}_2)_5 \) 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 scale-up, we also used catalytic amount of I₂ or of trichloro-1,3,5-triazine (TCT) in CH₃CN at rt. 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 \( X = (\text{CH}_2)_5 \) 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).

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scale-up, using the cheaper CICOOEt (ethyl chloroformate). Finally, benzyl removal under reductive conditions (Scheme 2, step e) 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 f).

Starting from 1-methylindole, we obtained bis-indolyl carboxylic acid derivative 8, according to step a and b of previous Scheme 2.

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-(1H-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 SAHA10 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 2. Bis-(indolyl)methylene 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: 60%; 3h: 52%; 3i: 69%; 3j: 49%; 3m: 64%; 3n: 68%; 3o: 51%; 3p: 57%); (d) PyBOP, o-(NH2)2-C6H4, TEA, DCM, rt; (e) CICOOEt, TEA, CH3NOH.HCl, THF, 0 °C–rt (3r: 60%). (f) Pd/C, H2, MeOH, rt; (4a: 71%; 4b: 57%; 4c: 54%; 4d: 78%; 4e: 82%; 4f: 35%; 4g: 36%; 4h: 67%; 4i: 41%; 4m: 75%; 4n: 51%; 4o: 68%; 4p: 80%; 7: 8%).

Scheme 3. N-methylated derivatives synthesis. Reagents and conditions: (g) CICOOEt, TEA, NH2OH.HCl, THF, 0 °C–rt (9; 53%); (h) (i) CICOOEt, TEA, NH2OBz.HCl, THF, 0 °C–rt (67%); (ii) CH3I, NaH, THF, rt; (iii) Pd/C, EtOH, H2 (10: 52% 2 steps).

Scheme 4. Monoindol-3-yl derive synthesis. Reagents and conditions: (i) HATU, DIPEA, NH2OBz.HCl, DMF, rt, 2 h (60%).
Supplementary data

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

References and notes

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 IC50 values of the compounds were determined by analyzing dose-response inhibition curves. Trichostatin A (TSA) was used as reference compound.