Bioorganic & Medicinal Chemistry Letters 19 (2009) 2840–2843

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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ARTICLE INFO

Article history:
Received 13 February 2009
Revised 18 March 2009
Accepted 21 March 2009
Available online 26 March 2009

Keywords:
HDAC inhibitors
Bis-indoles
Hydroxamic acids
Antitumor

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.[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).

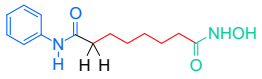

Figure 1. SAHA—vorinostat (Zolinza®).

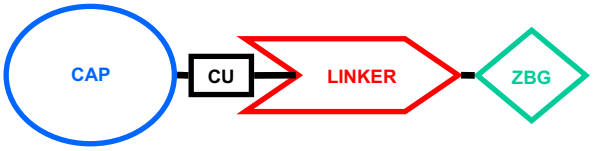

Figure 2. HDACIs common pharmacophore schematic segmentation.

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doi:10.1016/j.bmcl.2009.03.101

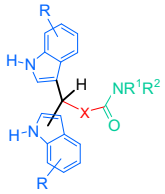


Figure 3. Bis-(indolyl)methane derivatives general structure.

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**).

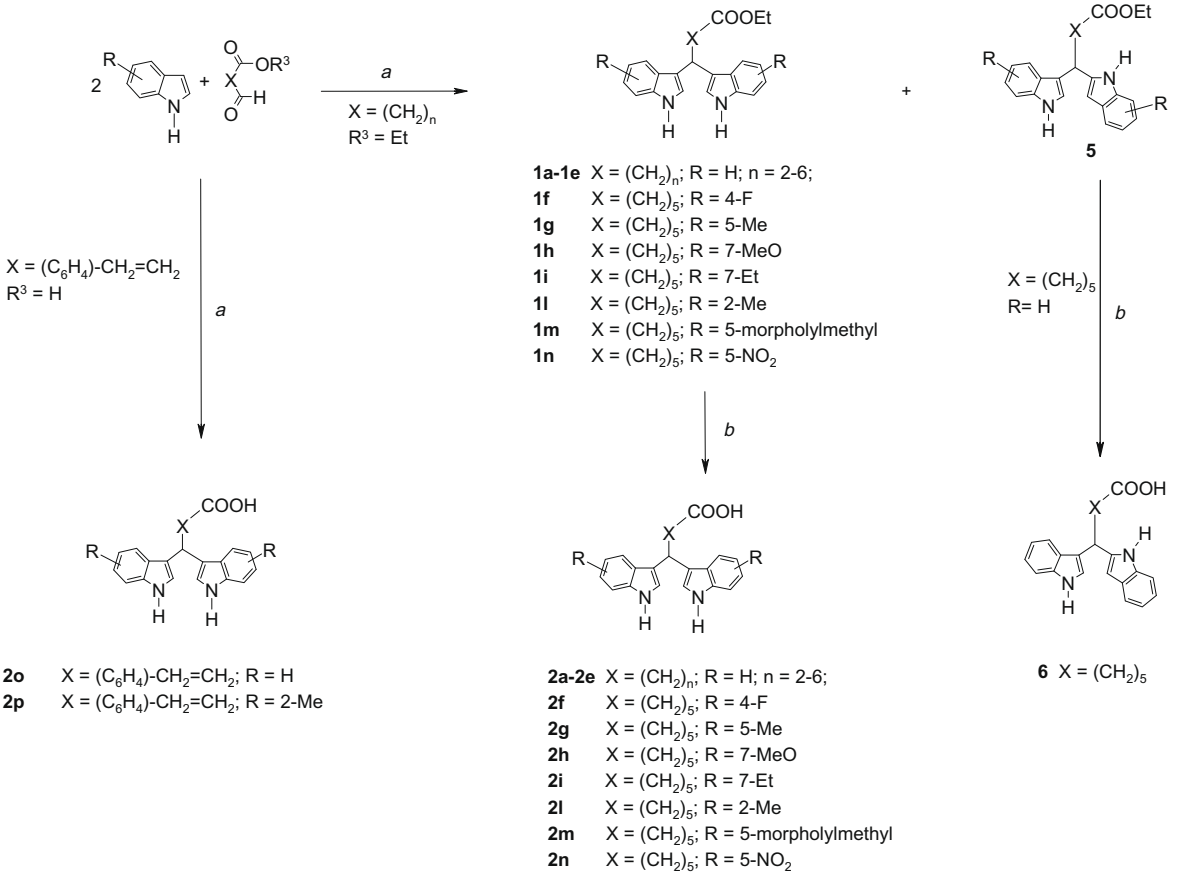
Finally, in order to evaluate an alternative ZBG, we prepared compound **3q** which is the *o*-aminobenzamide analogue of compound **4d**.

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.