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Bifunctional conjugates with potent inhibitory activity towards cyclooxygenase and histone deacetylase

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ABSTRACT

We herein disclose a series of compounds with potent inhibitory activities towards histone deacetylases (HDAC) and cyclooxygenases (COX). These compounds potently inhibited the growth of cancer cell lines consistent with their anti-COX and anti-HDAC activities. While compound **2b** showed comparable level of COX-2 selectivity as celecoxib, compound **11b** outperformed indomethacin in terms of selectivity towards COX-2 relative to COX-1. An important observation with our lead compounds (**2b**, **8**, **11b**, and **17b**) is their enhanced cytotoxicity towards androgen dependent prostate cancer cell line (LNCaP) relative to androgen independent prostate cancer cell line (DU-145). Interestingly, compounds **2b** and **17b** arrested the cell cycle progression of LNCaP in the S-phase, while compound **8** showed a G0/G1 arrest, similar to SAHA. Relative to SAHA, these compounds displayed tumor-selective cytotoxicity as they have low anti-proliferative activity towards healthy cells (VERO); an attribute that makes them attractive candidates for drug development.

1. Introduction

Aberrant epigenetic regulation and inflammation play significant roles in tumor development and progression. Posttranslational acetylation and deacetylation of histones, both epigenetic events regulated by histone acetyl transferases (HAT) and histone deacetylases (HDAC), respectively, control the expression and/or silencing of tumor suppressor genes.[1] While these two epigenetic regulators exist in equilibrium in non-transformed cells, HDAC activity predominates in most malignant tumors, effectively leading to silencing of tumor suppressor genes and uncontrolled proliferation of cancer cells.[2] Eighteen isoforms of HDAC are known, eleven of which depend on zinc for their catalytic activities and are group into: class I (HDACs 1–3 and 8); class II (subdivided into class II A (HDACs 4, 5, 7 and 9) and class IIB (HDACs 6 and 10)); and class IV (HDAC 11).[3] Class III HDACs, also known as sirtuins, are non-zinc dependent and require NAD^+ for their catalytic activity.[3b] The expression profiles of HDAC isoforms in different tumors vary with each isoform playing unique roles in driving tumorigenesis.[4] The therapeutic potential of HDAC inhibition has been validated by the US food and drugs administration's (FDA) approval of HDAC inhibitors (HDACi), vorinostat, romidepsin, belinostat and panabinostat (Fig. 1) for the treatment of cutaneous T-cell lymphoma, peripheral T-cell lymphoma and multiple myeloma.[5] Cardiotoxicity, short half-life, and inactivity towards solid tumors are few of many challenges faced by HDACi in the clinic.[3a,6]

Among the several drivers of inflammation in tumors, the inducible isoform of cyclooxygenases (COX), COX-2, plays a crucial role by ensuring a continuous supply of prostaglandin E2 (PGE_2) to the tumors.[7] The other COX isoform, COX-1, is constitutively expressed in the body where it performs housekeeping functions.[8] In contrast to COX-1, COX-2 expression is short-lived[9] and is upregulated in most tumors to meet up with the requirement for PGE_2 in the rapidly proliferating cells.[7b] Both COX isoforms facilitate the conversion of arachidonic acid to prostaglandin H2, which is in turn transformed to prostaglandins, by specific synthases, as required by the cells.[7b,10] Several COX inhibitors (Fig. 2), also

Abbreviations: HDAC, histone deacetylase; HDACi, histone deacetylase inhibitors; COX, cyclooxygenases; NSAIDs, non-steroidal anti-inflammatory drugs; AR, androgen receptor; ZBG, zinc binding group; PGE_2, prostaglandin E_2.

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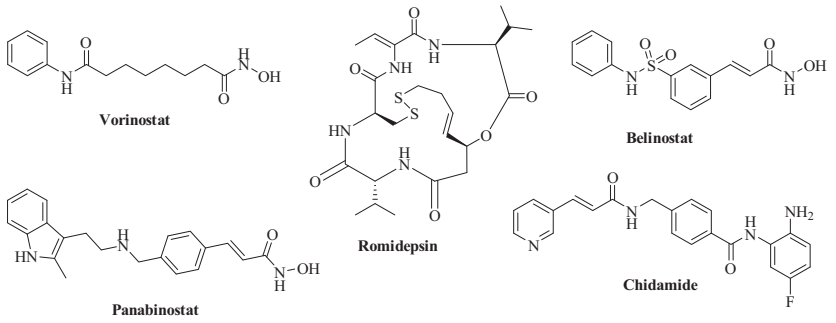

Fig. 1. HDACi in use in the clinic.

known as non-steroidal anti-inflammatory drugs (NSAIDs), have been approved by the FDA for managing inflammation associated with pains and fever.

Due to high expression of COX-2 in most tumors, it has been suggested that NSAIDs could someday find applications in the prevention and/or cure of some cancers, especially colon and prostate cancer.[11] Several mechanisms of cytotoxicity of NSAIDs towards cancer cells have been reported; most are believed to be independent of COX-2 inhibition. In androgen dependent prostate cancer cell line (LNCaP), celecoxib exerts its cytotoxic effect via induction of c-jun[12] and EP2 signaling leading to suppression of androgen receptor (AR).[13] Induction of apoptosis,[14] Wnt/beta-catenin pathway suppression,[15] cell cycle arrest[16] and inhibition of angiogenesis[17] are some of the other mechanisms through which NSAIDs exert their anticancer activity. In addition to being a possible therapeutic target, COX-2 upregulation in tumors has been exploited for tumor imaging through the use of contrast agents containing COX-2-selective NSAIDs.[18]

Recently, there has been enormous interest in the development of dual-acting compounds comprising of an HDACi and another cytotoxic component.[19] In such compounds, one of the "warheads" is usually the surface recognition group (cap) of the HDACi (see pharmacophoric model in Fig. 4a). While dual-acting compounds comprising NSAIDs and other agents exist,[20] none contain HDACi and NSAIDs combined as a single component. Moreover, results from *in vitro* studies suggest that enhanced cytotoxic effect could be achieved by combining NSAIDs and HDACi in cancer cell lines.[21] In this study, we designed and synthesized bifunctional compounds with HDAC and COX-2 inhibitory activities. These compounds are capable of harnessing the cytotoxic effects of HDAC inhibition, COX-2 inhibition, and perturbation of other non-COX dependent pathways. Our design has indomethacin or celecoxib as the cap, methylenes as linkers, and hydroxamate as the zinc binding group (ZBG) (Fig. 4b–e). These compounds potently inhibited the HDAC isoforms tested and retained COX-2 inhibitory activity comparable to both celecoxib and indomethacin. The potent HDAC and COX-2 inhibitory activities of these conjugates are reflected in their growth inhibitory activities in MCF-7 (breast cancer), A549 (non-small cell lung cancer), HCT-116 (colon cancer), DU-145 (androgen independent prostate cancer) and LNCaP (androgen dependent prostate cancer) cell lines. They are also less toxic towards healthy cell (VERO) compared to vorinostat.

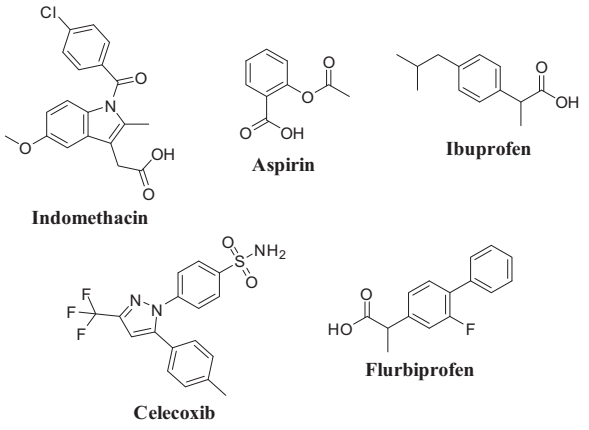

Fig. 2. Representative examples of US FDA approved NSAIDs.

2. Results and discussion

2.1. Design rationale

The residues presented at the outer rim of HDAC enzymes form rugged landscapes designed to flexibly accommodate a diverse class of substrates. This may explain the tolerance of the HDAC outer rim for incorporation of various surface recognition groups into the design of structurally dissimilar HDACi. Taking this into consideration, we hypothesized that incorporation of celecoxib (a COX-2 selective inhibitor) and indomethacin (a non-selective inhibitor of COX isoforms) into the surface recognition cap group of an HDACi may result in dual-acting agents that inhibit both HDAC and COX-2. Such agents are likely to show enhanced tumor cell cytotoxicity and superior therapeutic index compared to the individual HDACi and COX-2 inhibitors.

To determine which site to modify on celecoxib, we analyzed the orientation of celecoxib in the COX-2 active site. We found that the sulfonamide (SO_2NH_2) and trifluoromethyl (CF_3) moieties of celecoxib are projected towards different solvent exposed regions of the enzyme (Fig. 3a and b). Based on this analysis, the sulfonamide and trifluoromethyl moieties could be suitable points for the attachment of HDAC-inhibiting pharmacophores. Modifications at these two ends should minimally perturb the binding of celecoxib-based conjugates to the COX-2 active site, as shown in previous studies.[20b,22] Because of the relaxed specificity for hydrophobic groups at the HDAC outer rims, the celecoxib aromatic moiety of the resulting dual-acting agents is expected to be accommodated as a surface recognition group when bound to HDAC enzymes. To test this deduction, we designed and synthesized celecoxib-HDACi conjugates in which: (i) HDACi template is attached to the sulfonamide (series 1, Fig. 4), (ii), the "CF_3" is replaced by HDACi template (series 2, Fig. 4) and (iii) the sulfonamide is replaced with a methyl sulfone (SO_2Me) and "CF_3" is replaced by HDACi template (series 3, Fig. 4).

Similarly for indomethacin, the carboxylic acid moiety is projected towards the solvent exposed region of COX-2 (Fig. 3c). Modification of this moiety is known to convert indomethacin from a non-selective COX inhibitor to a COX-2 selective inhibitor.[23] This