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Dual-acting histone deacetylase-topoisomerase I inhibitors

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ABSTRACT

Current chemotherapy regimens are comprised mostly of single-target drugs which are often plagued by toxic side effects and resistance development. A pharmacological strategy for circumventing these drawbacks could involve designing multivalent ligands that can modulate multiple targets while avoiding the toxicity of a single-targeted agent. Two attractive targets, histone deacetylase (HDAC) and topoisomerase I (Topo I), are cellular modulators that can broadly arrest cancer proliferation through a range of downstream effects. Both are clinically validated targets with multiple inhibitors in therapeutic use. We describe herein the design and synthesis of dual-acting histone deacetylase–topoisomerase I inhibitors. We also show that these dual-acting agents retain activity against HDAC and Topo I, and potently arrest cancer proliferation.

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Current chemotherapeutic options for the treatment of cancer are often plagued by debilitating side effects and off-target toxicities. While other pharmacological options, such as gene or immunotherapies, are attaining increasing viability for researchers, most clinical options still center on traditional small molecule chemotherapy. There is considerable interest in designing novel small molecule agents that retain efficacy, while increasing the specificity toward the target of choice, thereby reducing side effects. While single-target drugs remain a popular design endpoint, there has been a recent surge of interest toward multivalent ligand design. It is thought that these drugs could possess a greater therapeutic advantage, by modulating multiple targets and avoiding the side effects of any single agent. Additionally, multivalent ligands are not expected to face the inherent pharmacokinetic and pharmacodynamics disadvantages of administering two or more separate drugs, a common liability that may complicate the outcome of traditional combination therapy.[1] The benefit of drugs with multiple targets relative to the conventional combination therapies has only begun to be elucidated, and these therapies are becoming increasingly common across a variety of pharmacological applications.[1–5]

Cancer offers a unique opportunity for the design of a multifunctional drug due to the multiple pathways contributing to the disease state. One promising pathway for tumor growth inhibition is that of epigenetic and protein acetylation state modulation by histone deacetylases (HDACs). HDACs function within a pathway that was originally discovered to alter the acetylation of histone proteins, leading to a more condensed nucleosome and decreased transcription.[6,7] The counterpart enzyme, histone acetyltransferase (HAT), has the opposite effects; acetylating histones and upregulating transcription.[8] The proposed cancer-promoting mechanism of HDAC involves transcriptional silencing of tumor suppressors via deacetylation of nucleosomes containing tumor suppressor genes.[9,10] However, recent evidence has shown HDAC involvement in the deacetylation of important non-histone regulatory proteins such as p53,[11] E2F,[12] and tubulin.[13] HDACs inhibitors (HDACi) have been shown to cause growth arrest, differentiation, and apoptosis in cancer cells.[14–16] Two HDACi, **SAHA** (Vorinostat) (Fig. 1) and **FK-228** (Romidepsin), have been approved by the FDA for the treatment of cutaneous T-cell lymphoma,[17,18] thus opening the door for HDACi as viable therapeutic agents.[19,20] For these reasons, HDACs remain an attractive target for small molecule inhibition.

Another proven anticancer target is topoisomerase I (Topo I). The Topo I enzyme relieves the torsional strain on DNA during DNA replication by cutting one strand of the DNA double helix and passing one strand over the other.[21,22] Due to the inherent need for rapid replication in cancer, inhibitors of topoisomerases result in DNA strand breaks, cell cycle arrest, and apoptosis.[23–27] Many small molecule inhibitors of Topo I have proven clinically

Abbreviations: HDAC, histone deacetylase; HAT, histone acetyltransferase; HDACi, histone deacetylase inhibitors; **SAHA**, suberoylanilide hydroxamic acid; TSA, trichostatin A; Topo I, topoisomerase I class of enzymes.

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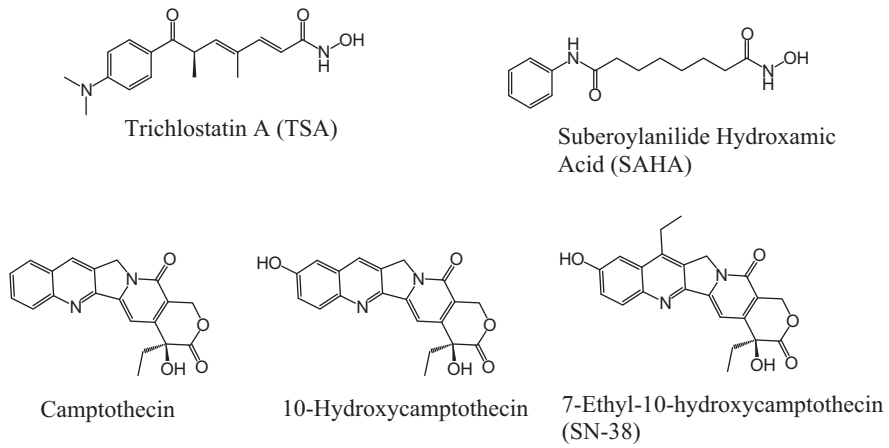

Figure 1. Representative HDAC and Topo I inhibitors.

effective and are currently FDA-approved for cancer chemotherapy.[25] Since both HDAC and Topo I enzymes are localized to the nucleus, the opportunity for dual inhibition from a single agent is a promising possibility. Creating a dual-acting HDAC–Topo I inhibitor could prove beneficial for many reasons. First, HDACi have been shown to act synergistically with Topo I inhibitors, resulting in enhanced apoptosis in cancer.[28] Also, since both enzymes are nuclear-localized, dual-acting agents may have better therapeutic indices.

Using fused-frameworks design approach,[1] we have previously, described dual-acting agents derived from an anthracycline, a topoisomerase II (Topo II) inhibitor and **SAHA** analogs, prototypical HDACi. A subset of these dual-acting HDAC–Topo II inhibitors inhibited Topo II and HDAC activities more potently compared to parent anthracycline and **SAHA**, respectively.[29] Furthermore, a lead compound from this series was equipotent to daunorubin against selected breast, lung and prostate cancer cell lines. As a follow-up to our work on dual-acting HDAC–Topo II inhibitors, we have designed and synthesized dual-acting HDAC–Topo I inhibitors derived from the camptothecin ring system and the linker region of **SAHA**-like HDACi. We show here that an alternative designed multiple ligand approach, merged-frameworks strategy,[1b] proved successful in the design of HDAC–Topo I inhibitors. We present evidence here that these compounds retain inhibitory activities against both target enzymes and inhibit the proliferation of selected cancer cell lines.

The camptothecin family of Topo I inhibitors are potent anticancer drugs that form a ternary complex at the interface of the cleavage complex, inhibiting dissociation of Topo I from DNA. We chose 10-hydroxycamptothecin and 7-ethyl-10-hydroxycamptothecin (**SN-38**) (Fig. 1) as the Topo I inhibiting templates for the design of the proposed dual-acting HDAC–Topo I inhibitors due to their promising activity against a range of tumor types and the presence of a functionalizable phenolic group at their C-10 position. Also, both templates have demonstrated more potency and less toxicity than camptothecin.[30–32] From structure–activity relationship (SAR) studies on camptothecins, substitution at the 10-hydroxy group has been found to be tolerable,[33] so we used this position as the point of attachment for the HDACi moiety. We have already reported the suitability of 1,2,3-triazole ring as a surface recognition cap group-linking moiety in **SAHA**-like HDAC inhibitors.[34] These studies showed cap group-dependent preference for five to six methylene linkers. In the designed dual-acting compounds, the linker region of **SAHA**-like HDACi is coupled through a triazole moiety to the camptothecin template, which in turn is anticipated to act as an aromatic surface recognition cap group essential for HDAC

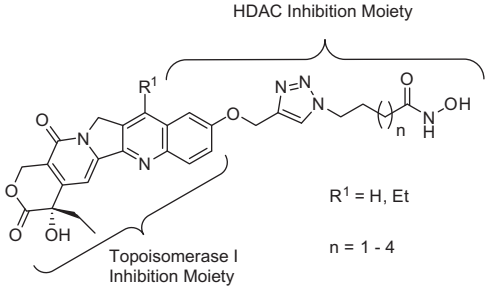

Figure 2. Designed dual-acting HDAC–Topo I inhibitors.

inhibition while also retaining its Topo I inhibition activity (Fig. 2). We introduced variations into the linker region to test the linker length-dependent potency of the resulting dual-acting agents. Additionally, incorporation of the triazole ring into compound design helped to simplify synthesis and SAR studies.

The reaction route to all the designed compounds is shown in Scheme 1. The phenolic OH-group of 7-ethyl-10-hydroxycamptothecin **1a** and 10-hydroxycamptothecin **1b** was alkylated with propargyl bromide to yield the corresponding alkyne intermediates **2a** and **2b**, respectively. Cu-catalyzed Huisgen cycloaddition[35] with known azido intermediates **3a–e**[34,36] afforded trityl-protected compounds **4a–h**. Subsequent TFA deprotection of **4a–h** yielded the desired compounds **5a–h** in good yields with minimal purification required.

Building on our previous observations about the linker length-dependent potency of aryltriazolyl HDACi,[34,36] we first synthesized and evaluated the anti-HDAC activity of 7-ethylcamptothecin-derived compounds **5a–e** against HeLa cell nuclear extract HDACs as described previously with a slight modification.[34] Briefly, camptothecin has a fluorescence emission (excitation $\lambda = 370$ nm, emission $\lambda = 434$ nm) close to the wavelength (460 nm) of the fluorescence generated by the HDAC enzyme cleavage of its fluorogenic substrate. To circumvent this potential interference, controls containing the same concentration of the test compound without the enzyme were used, and the background fluorescence of these controls were subtracted from the experimental fluorescence readings. Compound **5a**, an analog with a three methylene linker separating the triazole ring and the hydroxamate moiety, has no measurable anti-HDAC activity at concentrations as high as 10 μM (Table 1). The inactivity of **5a** may be due to the fact that its linker region is too short to effectively position its hydroxamate