Letter

Fluorinated Analogues of the HDAC Inhibitor Vorinostat (Zolinza®): Validation of a Chiral Hybrid Bioisostere (BITE).

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00287 • Publication Date (Web): 19 Jul 2019

Fluorinated Analogues of the HDAC Inhibitor Vorinostat (Zolinza®): Validation of a Chiral Hybrid Bioisostere (BITE).

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KEYWORDS fluorine, bioisosteres, histone deacetylases, inhibitors, oncology.

ABSTRACT: A chiral, hybrid bioisostere of the CF_3 and Et groups (BITE) has been installed in a series of vorinostat (Zolinza®) analogues, and their HDAC inhibitory behavior studied relative to their non-fluorinated counterparts. Several of these compounds containing the 1,2-difluoroethylene unit showed greater *in vitro* potency than the clinically approved drug itself against HDAC1. This trend was found to be general with the BITE-modified HDAC inhibitors performing significantly better than the ethyl derivatives. Installed by the direct, catalytic *vicinal* difluorination of terminal alkenes using an I(I)/I(III) manifold, this underexplored chiral bioisostere shows potential in drug discovery.

Small molecule therapeutics have a long and venerable history in clinical oncology, where persistently high cancer mortality rates continue to provide a powerful impetus for drug design and discovery.[1] Therapeutic approaches based on non-selective DNA targeting cytotoxic agents have been complemented by a strategic shift towards more targeted molecular therapeutics.[2-4] This paradigm change is exemplified by the success of low molecular weight histone deacetylase inhibitors (HDACi).[5] Modulating histone deacetylase (HDAC), and by extension histone acetyltransferase (HAT), activity has a direct impact on the degree of histone acetylation: This subsequently alters the structure of chromatin and, allows gene transcription to be targeted.[6] Specifically, hypoacetylation, results in a compact chromatin structure (*heterochromatin*) that supresses the expression of tumour-repressor genes.[7] In contrast, hyperacetylated regions promote the expression of tumour-repressor genes because of an expanded, open chromatin structure (*euchromatin*), which is transcriptionally active. As targets for drug discovery, 18 human HDACs have been identified to date, and these can be sub-divided into four groups: class I HDACs (HDACs 1, 2, 3 and 8), class II HDACs (further subdivided into class IIa HDACs 4, 5, 7, and 9, and the class IIb HDACs 6 and 10), class III (sirtuin family) and class IV (HDAC11).[8] Enzymes from class I, II and IV share a catalytic mechanism that is zinc ion (Zn^{2+}) dependent, whereas HDACs from class III require the co-factor nicotinamide adenine dinucleotide (NAD) for enzyme activity.[9,10] Since the overexpression of HDACs is commonly associated with various cancer types, drugs that target HDACs have been intensively pursued.[11-14] HDACs 1-3 and 6, are particularly noteworthy for cancer drug development due to their distinguished mechanisms of action.[15,16] Currently, four HDACi drugs are FDA-approved for the treatment of multiple myeloma and T-cell lymphoma: vorinostat (Zolinza®, Figure 1),[17] belinostat (Beleodaq®), romidepsin (Istodax®), and panobinostat (Farydak®).

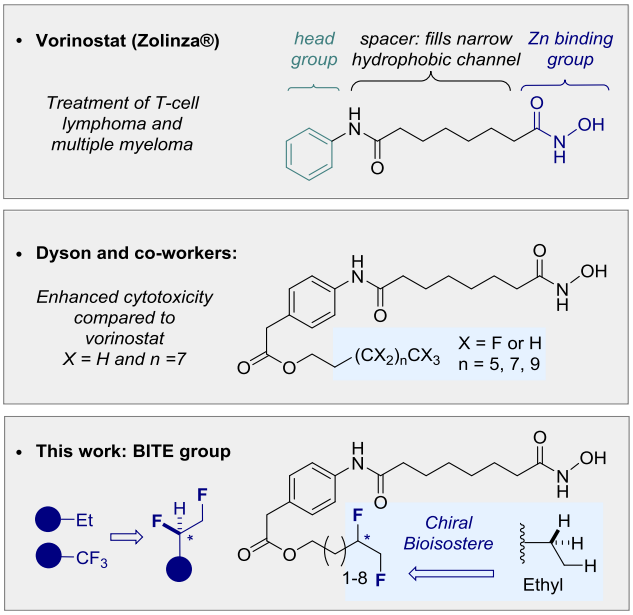


Figure 1. Top: Vorinostat for the treatment of cutaneous T-cell lymphoma. Centre: Vorinostat analogues with a pendant ester. Bottom: Target compounds of this study incorporating the BITE group.

With the exception of romidepsin (Istodax®) these drugs contain a common hydroxamic acid zinc-binding group (ZBG) that is essential for Zn^{2+} chelation in the enzyme active site. In the case of vorinostat, a lipophilic chain connects the hydrox-

amic acid to an aromatic head group that is occupies the entrance to this pocket. This ring lends itself to structural modifications, and is a convenient platform from which to conduct SAR studies.[18,19]

An elegant study by Dyson and co-workers reported a series of analogs containing perfluorinated alkyl chains attached to the aromatic head group (Figure 1, center).[20] This structural motif was introduced to induce thermoresponsiveness as a means to obtain more selective HDACi through activation by localised heating. Although only vorinostat itself was found to be responsive towards heat stimuli, the authors reported that several derivatives showed enhanced selectivity towards cancer cells over healthy cells. Interestingly, the best performing derivative was a non-fluorinated reference compound bearing a decyl ester functionality (Figure 1, center). Motivated by this study and our interest in the physicochemical properties of the *vicinal* difluoroethyl group, a study to explore the effect of the chiral hybrid, bioisostere of the trifluoromethyl and ethyl groups (BITE group) on HDAC inhibition was initiated (Figure 1, lower).[21-23]

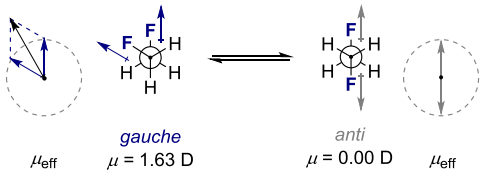


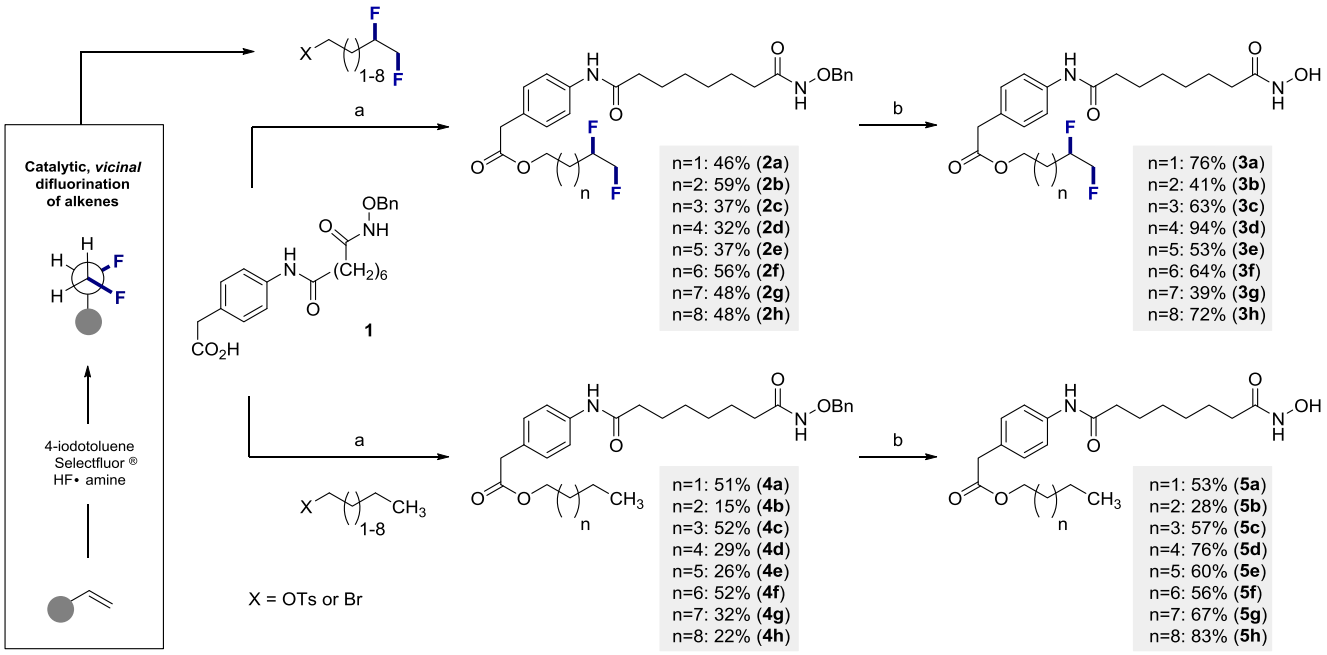
Figure 2. The *gauche* effect and dipole moment in 1,2-difluoroethylene.

The conformation of the 1,2-difluoroethylene motif is characterised by the *syn-clinal* arrangement of the C-F bonds due to reinforcing hyperconjugative interactions ($\sigma_{C\text{-}H} \rightarrow \sigma_{C\text{-}F}^*$): this is commonly known as the *gauche* effect.[24-30] In addition to restricting rotation about the C(sp^3)-C(sp^3) bond, the preferred *gauche* alignment (φ_{FCCF} = 60°) of the polarised carbon-fluorine bonds ($C^{\delta+}$-$F^{\delta-}$) give rise to a large dipole moment that directly influences the compounds physicochemical and pharmacological properties: Pertinent examples include lipophilicity, solubility, protein binding and rate of metabolism.[22,31] Moreover, the presence of a chiral centre in this motif renders it a useful addition to the medicinal chemistry toolkit. Although the 1,2-difluoroethyl group has a unique shape, it is comparable in size to the trifluoromethyl and ethyl groups,[23] and thus may be considered as a hybrid, chiral bioisostere (BITE). Regrettably, exploring this motif in the context of drug discovery has been hampered by preparative difficulties. Whilst an array of strategies have been developed that rely on oxidation / displacement sequences,[32-35] the direct *vicinal* difluorination of alkenes has only recently been achieved.[36-40] As validated in our recent study of fluorinated fingolimod (Gilenya®) analogues,[22] I(I)/(III) catalysis enables the direct installation of this group without substrate pre-functionalization. By extension, the preparation of fluorinated vorinostat analogues containing the BITE group is disclosed, and their HDAC inhibitory activity compared to their non-fluorinated congeners.

Preparation of the two test series (BITE *versus* Et) from the common precursor **1** is described in Scheme 1. Building block **1** was synthesised from 4-aminophenylacetic acid following the procedure described by Dyson and co-workers.[20] The fluorinated alkyl chains were prepared from the corresponding terminal alkenes *via* direct, catalytic *vicinal* difluorination. For this purpose *p*-iodotoluene was employed as an inexpensive organo catalyst, Selectfluor® as the terminal oxidant and an amine:HF (1:5) solution mixture, comprised of Et_3N•3HF and Olah's reagent, as fluoride source and Brønsted acid activator [Scheme 1 (right), please also see the Supporting Information for full details]. The corresponding non-fluorinated hydrocarbon chains were obtained from commercial sources, or prepared from readily available starting materials (Supporting Information). Non-fluorinated compounds **4e**, **4g**, **5e** and **5g** were previously reported by Dyson and co-workers.[20] Installation of the aliphatic chains was achieved via facile displacement (K_2CO_3, DMF, 70 °C to afford ethyl esters **2a-h** and **4a-h** (Scheme 1). Finally, the benzyl protecting group in intermediates **2a-h** and **4a-h** was cleaved using boron trichloride based on a previously reported procedure[41] to obtain the free hydroxamic acid.

The fluorinated and non-fluorinated target compounds (**3a-h** and **5a-h**) were initially tested for their *in vitro* inhibitory activity towards HDAC1 and HDAC6 (Figure 3, A and B) using a previously published biochemical assay.[42] In all cases, vorinostat (Zolinza®) was used as a reference compound. The IC_{50} values and standard deviations are summarized in Table S1. Compounds **5g** and **5h** did not reach 100% inhibition against HDAC1 and are therefore reported as % inhibition at 1 µM (see Table S1 in Supporting Information). In the majority of cases, the fluorinated compounds containing the BITE group ($CHFCH_2F$) outperformed their non-fluorinated (Et) equivalents. This distinction was particularly evident for the longer alkyl esters (i.e. heptyl and above). Notably, all the BITE containing compounds exceeded the activities of their ethyl bearing analogues towards HDAC1. Solely the non-fluorinated butyl and pentyl esters **5a** and **5b** had a slightly higher potency than their BITE fluorinated derivatives, with regards to HDAC6 inhibition. Furthermore, the potencies of compounds **5e** and **5g** were both lower than that of vorinostat, which is consistent with previously reported data.[20]

Several of the target compounds performed better than vorinostat (HDAC1 IC_{50}: 102 nM and HDAC6 IC_{50}: 47 nM) against both HDAC1 (**3a-d**) and HDAC6 (**3a-f** and **5a-c**). Furthermore, an apparent relationship was observed between the length of the alkyl ester and inhibitory activity: Potency gradually decreased with increasing alkyl chain length. This effect is much more prominent in the non-fluorinated compound series, particularly in the inhibition of HDAC1. Most of the target compounds possess a slightly higher selectivity index (SI: 3-7, Table S1) to that found in vorinostat (SI: 2) and therefore a slight preference for the class IIb enzyme HDAC6. This partiality becomes more evident with increasing size of the alkyl esters and particularly reduced potencies towards HDAC1. Subsequently, the compounds that displayed more promising potency against HDAC1 and HDAC6 (**3a-c** and **5a-c**) were screened against HDAC2 and HDAC3 (Figure 3, C and D), due to their particular interest as cancer targets.[43] The results did not however show any specific trend and the majority of compounds showed a comparable inhibitory activity to that of vorinostat. The only exception proved to be the non-fluorinated hexyl ester **5c**, whose potency was significantly lower than the remaining compounds, particularly towards HDAC2.



Scheme 1. Synthesis of fluorinated and non-fluorinated target compounds. *Reagents and conditions:* (a) K_2CO_3, DMF, RT; (b) BCl_3 in DCM (1 M), THF, 0 °C to ambient temperature.

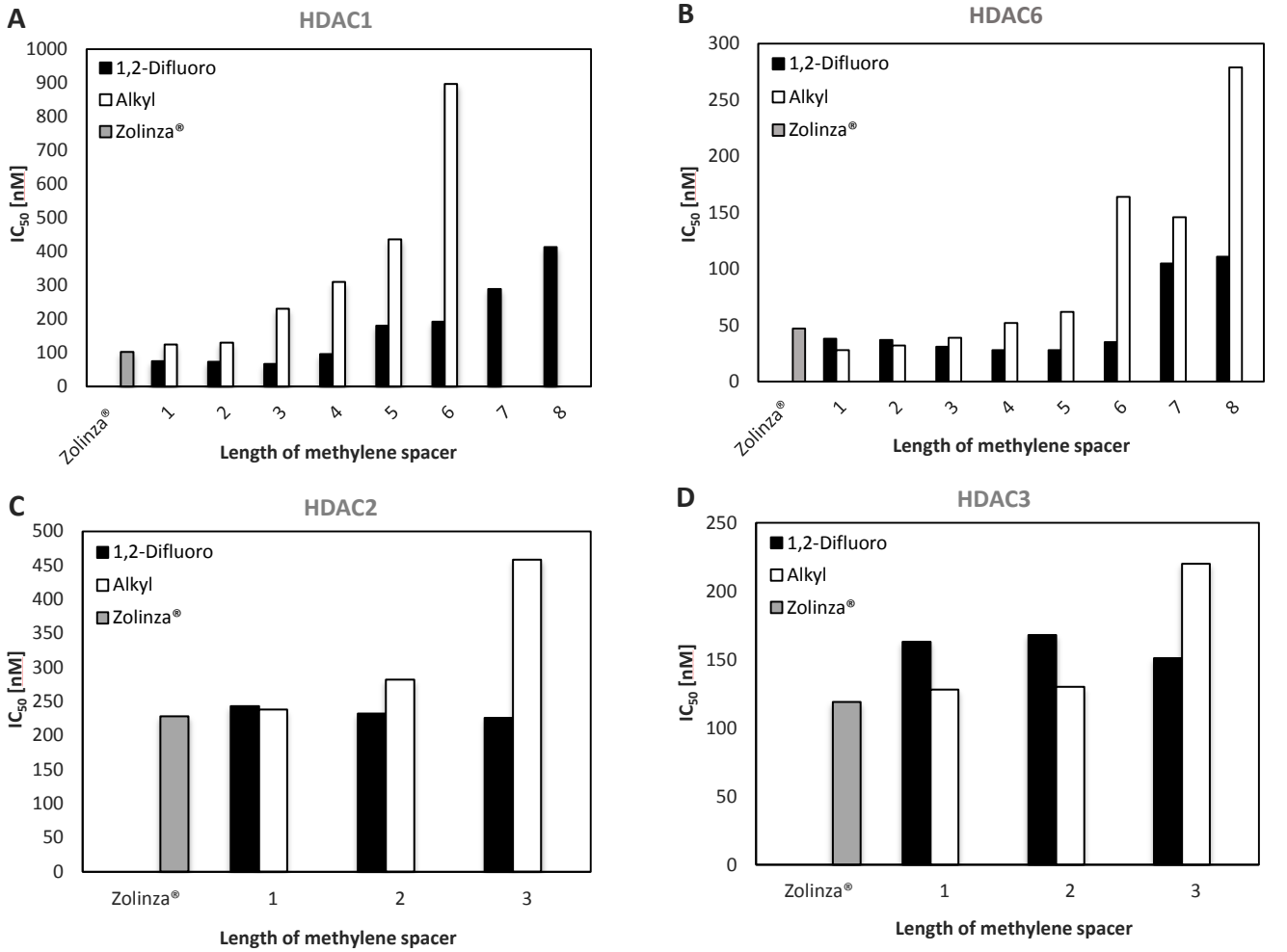


Figure 3. *In vitro* inhibitory activity of the two compound series as a function of methylene spacer length is shown towards: HDAC1 (A), HDAC6 (B), HDAC2 (C) and HDAC3 (D) (for more details see Table S1 in ESI). The drug vorinostat (Zolinza®) was used as a reference compound (grey).

In summary, a novel series of vorinostat analogues bearing a chiral, hybrid, bioisostere of the trifluoromethyl and ethyl groups (BITE group) has been prepared. This was achieved by the direct *vicinal* difluorination of alkenes via I(I)/I(III) catalysis. Evaluation as potential as HDAC inhibitors was conducted simultaneously with the non-fluorinated (Et) equivalents using vorinostat as the control. Several of these new, fluorinated compounds exceed the inhibition activities of the FDA approved drug vorinostat (eight towards HDAC6 and four against HDAC1). In the majority of cases, the BITE fluorinated compounds significantly outperformed their non-fluorinated analogues. In addition, a clear correlation between the inhibitory potency and the size of the alkyl ester was observed, where the inhibitory activity is gradually reduced with increasing alkyl chain length in both compound series. Interestingly, this effect is more pronounced in the non-fluorinated series. Furthermore, most of the compounds in this study showed an increased activity towards HDAC6 in comparison to HDAC1-3. A slightly increased preference for HDAC6 over HDAC1 was observed for a majority of compounds (SI: 3-7) when compared to that of vorinostat (SI: 2). Enabled by advances in catalysis, it is envisaged that the under-explored 1,2-difluoroethylene unit (BITE) will find further application as a useful fluorine-containing-bioisostere[44,45] for small molecule drug discovery. Since the BITE group is chiral, exploring the behavior of each enantiomer will be the subject of future research in our laboratory.

ASSOCIATED CONTENT

Supporting Information

Supporting information, including experimental, analytical data and assay protocols is provided as a PDF file.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

We acknowledge generous financial support from the WWU Münster, the DFG (SFB 858), and the European Research Council for an ERC Consolidator Grant (RG, 818949 RECON ERC-2018-CoG).

ACKNOWLEDGMENT

We thank the analytical departments of the Institute for Organic Chemistry at the WWU Münster for technical support.

ABBREVIATIONS

HATs, histone acetyltransferases; HDACs, histone deacetylases; HDACi, histone deacetylase inhibitors; BITE, bioisostere of the trifluoromethyl and ethyl groups; Et, ethyl; DNA, deoxyribonucleic acid; FDA, U.S. Food and Drug Administration; DMF, dimethylformamide; DCM, dichloromethane; THF, tetrahydrofuran; SI, selectivity index.

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