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Research paper

Synthesis, biological characterization and molecular modeling insights of spirochromanes as potent HDAC inhibitors

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ABSTRACT

In the last decades, inhibitors of histone deacetylases (HDAC) have become an important class of anti-cancer agents. In a previous study we described the synthesis of spiro[chromane-2,4′-piperidine] hydroxamic acid derivatives able to inhibit histone deacetylase enzymes. Herein, we present our exploration for new derivatives by replacing the piperidine moiety with various cycloamines. The goal was to obtain highly potent compounds with a good *in vitro* ADME profile. In addition, molecular modeling studies unravelled the binding mode of these inhibitors.

1. Introduction

Post-translational modifications on histones have been demonstrated to be essential regulators of the gene expression [1]. Among these, acetylation and deacetylation of the ε-amino groups of lysine residues represent crucial modifications of these proteins. The positively charged lysines present at the *N*-terminal tail of the histones allow tight binding of negatively charged DNA around them. This strong histone-DNA interaction blocks the binding sites of promoters and thus inhibits gene transcription. The neutralization of the charge through the acetylation of the amino groups of the lysines reduces the electrostatic interactions with the DNA leading to the nucleosome unwrapping [2]. This unfolding of the chromatin allows the transcriptional factors to access the gene promoter regions with the consequent gene expression. The balance between hyperacetylation and hypoacetylation is maintained by two counteracting enzyme families, namely histone acetyltransferases (HATs) and histone deacetylases (HDACs). Currently, 18

Abbreviations: HDAC, histone deacetylases; HAT, histone acetyltransferases; SQM, Semi-empirical Quantum Mechanics; CYP, cytochrome P450.

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different human HDACs have been identified and are grouped into two families: the classical HDACs and the silent information regulator (Sir)-related proteins (sirtuins). Classical HDACs are Zn^{2+} dependent metalloenzymes and based on their sequence homology to the yeast analogues can be further divided into 4 classes. Class I comprises HDACs 1, 2, 3, and 8, class IIa HDACs 4, 5, 7, 9, class IIb HDACs 6 and 10 and class IV HDAC11 [3–9]. Sirtuins, also known as class III HDACs, form a structurally and mechanistically distinct group and comprise Sirt1 to Sirt7. Class III HDACs are characterized by their dependency on NAD^+ as cofactor [10]. Since the acetylation status of histones is critical for the gene expression modulation and cell fate, there is no surprise that its dysregulation is involved in the development of several cancers. Recent studies revealed that HDACs are responsible for the deacetylation of several non-histones too, such as proteins relevant for tumorigenesis, for cancer cell proliferation, and for immune functions [11,12].

In the past two decades the inhibition of HDACs has emerged as an attractive therapy to reverse aberrant epigenetic changes associated with cancer, inflammation and neurodegenerative diseases [13–18]. Several compounds reached clinical investigations and they demonstrated to be particularly efficacious in the treatment of different hematological malignancies [19–21]. So far, the clinical studies have culminated in the market approval of four distinct chemical entities (see Fig. 1). The hydroxamic acid vorinostat, known also as SAHA or Zolinza® [22], and the cyclic depsipeptide romidepsin, known FK228 or Istodax® [23,24], have been approved for the treatment of the cutaneous T–cell lymphoma (CTCL). The disulfide romidepsin, which is converted in cells into the active (reduced) form [24,25], has also been approved for the treatment of peripheral T-cell lymphoma (PTCL) as well as the hydroxamate belinostat, known also as PXD101 or Beleodaq® [26,27]. Finally, on February 2015 the FDA approved oral panobinostat (known also as LBH589 or Farydak®) in combination with bortezomib and dexamethasone in patients with recurrent multiple myeloma [28]. Despite these successes, the known HDAC inhibitors are less efficacious in other tumors, in particular in solid malignancies. Therefore, there is still a need to develop HDAC inhibitors with a better clinical profile [19,20,29].

In previous studies we described a spiropiperidine hydroxamic acid scaffold with potent HDAC inhibitory activity [30]. Structure-activity relationship studies (SAR) showed that modifications on the spirochromane core moiety had a substantial influence on the biological activities. The shift of the *N*-hydroxyacrylamide moiety or the replacement of the chromane ring by spirobenzofuran generally furnished less active compounds. Furthermore, the exploration at the 4-oxo moiety demonstrated that the ketone group is critical for the target inhibition. In fact, modification of this group was detrimental for HDAC and antiproliferative activity. On the other hand, various *N*-substitutions on the piperidine group were well tolerated, and no major differences in the activities between these compounds were found. Considering that HDAC inhibitors are composed by a Zn^{2+} binding moiety, a spacer, which can be further divided into linker and connecting unit, and a cap group [31,32], this minor effect of the different modifications could be ascribed to an outside orientation of the *N*-substituents as the cap group [33]. Based on these observations we further explored this scaffold in order to find more active inhibitors maintaining their good ADME properties. For this purpose, various cycloamines were studied as replacement of the piperidine moiety. In the present study, we describe the synthesis of spirochromane azetidines, pyrrolidines, azepanes and piperidin-3yl derivatives with various *N*-substituents as well as their biological activity in detail. At the same time, molecular modeling investigations were performed on a number of spirochromane inhibitors. Hence, molecular docking combined with Semi-empirical Quantum Mechanics (SQM) optimization allowed us to elucidate the ligand-binding mode of these molecules and to understand their SAR at the atomic level.

2. Results and discussion

The synthesis of compounds **1a** to **1c** has been previously described [30,33].

The secondary amines **15**–**18** were prepared starting from commercially available 2-hydroxy-5-bromoacetophenone (**2**) as illustrated in Scheme 1. Cyclization of compound **2** with *N*-BOC protected cycloamine-ones in methanol in presence of pyrrolidine gave the spirocycles **3**–**6**, which were then coupled with methylacrylate in presence of palladium acetate [$Pd(OAc)_2$], tri(2-methylphenyl)phosphine ($P(o\text{-}tol)_3$), triethylamine (TEA) affording the spirocyclic acrylic methyl esters **7**–**10**. The methyl esters **7** and **9** were hydrolyzed with sodium hydroxide (NaOH) in a water/dioxane mixture, while intermediates **8** and **10** were treated with hydrogen chloride (HCl) in acetic acid (AcOH). The resulting carboxylic acids were coupled with NH_2OTHP (*O*-(tetrahydropyran-2-

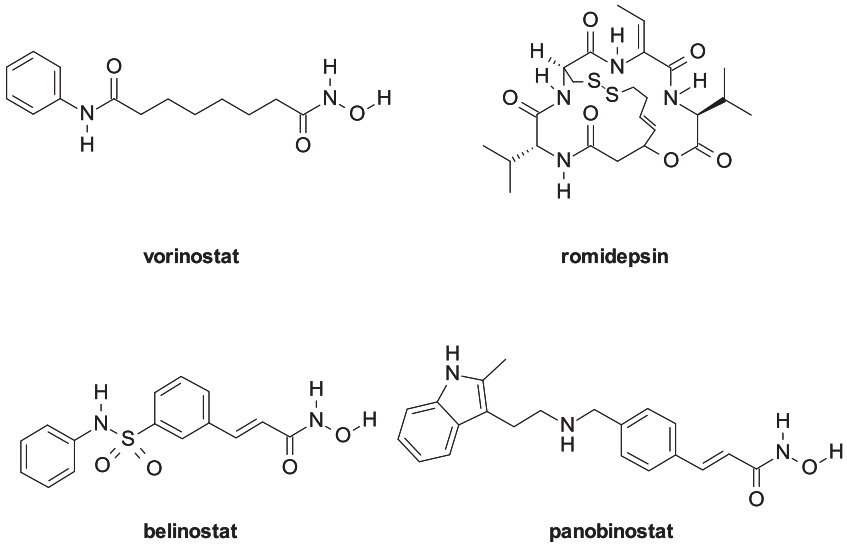

Fig. 1. FDA approved HDAC inhibitors.