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SYNTHESIS AND BIOLOGICAL EVALUATION OF SMALL MOLECULES DERIVATIVES OF THE NATURAL HISTONE DEACETYLASE INHIBITOR FR235222

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Abstract

Discovery of new more selective and potent Histone deacetylase inhibitors is one of the most promising therapeutical approach for tumor growth arrest and proliferation control. Natural cyclotetrapeptide FR235222 (**1**) from *Acremonium* sp. was identified as potent immunosuppressant and HDAC inhibitor with low-nanomolar range activity against HDACs Class I and IIa. The strong potency elicited by FR235222, even thought the cyclotetrapeptide scaffold is a good cupping group (CAP), is due to its natural Zinc-Binding group (ZBG), based on the α-hydroxyketone moiety. Herein we report the synthesis and the biological evaluation of amides as molecular simplification of natural cyclotetrapeptide (**1**), in order to evaluate their antiproliferative and HDAC inhibition activities. Linear derivatives show interesting results in terms of antiproliferative effects.

Key words: Histone deacetilase inhibitors, Peptides, Antitumor Agents, Epigenetic

Introduction

Specific histone modifications are used as 'active' or 'inactive' marks of euchromatin and heterochromatin, respectively. The *N*-ε-acetylation of lysine residues is a major histone modification involved in transcription, chromatin structure, and DNA repair. Acetylation neutralizes lysine's positive charge and may consequently weaken the electrostatic interaction between histones and negatively charged DNA. For this reason, histone acetylation is often associated with ''open'' chromatin conformation. Acetylation is highly dynamic and is regulated by the competing activities of two enzymatic families, the histone lysine acetyltransferases (HATs) and the histone deacetylases (HDACs) [1]. HDACs, based on their subcellular localization, can be subdivided in four classes: Class I (HDAC1, 2, 3, 8) in the nucleus; b) Class II, further divided into: Class IIa (HDAC4, 5, 7, 9) in the nucleus and cytoplasm; and Class IIb (HDAC6, 10) mainly in the cytoplasm; Class IV (HDAC11); Class III, also called Sirtuins (SIRTs). The common feature of classes I, II and IV enzymes resides in their Zn^{2+}-dependent nature, where a catalytic site in a pocket at the bottom accommodates on a hand the acetyl-lysine residue, as natural substrate, on the other hand chelating compounds, as HDAC inhibitors (HDACis) [2].

Many HDAC inhibitors have been designed and studied, showing to be potent inducers of growth arrest, differentiation and apoptotic cell death of transformed cells *in vitro* and *in vivo*.

In fact, a generally accepted pharmacophoric model for the HDACis, have common structural characteristics: zinc-binding group (ZBG), the pharmacophoric portion, responsible for the crucial Zn^{2+} ion-chelation event in the catalytic pocket; capping group (CAP) interacting with surface recognition domain; a straight-chain alkyl, vinyl or aryl linker connecting the two parts, inserted in a tube-like channel of the enzyme active site (FIGURE 1) A kink function connecting unit (CU) which relates the linker to the CAP.

The aberrant overexpression, function, and recruitment of class I and II HDACs, associated with malignancies including T-cell lymphomas [3-5] as well as the increased expression of class I (HDAC1, 2, and 3) is considered essential for cancer cell proliferation and survival. Two HDAC inhibitors have been approved by U.S. regulatory authority (FDA): vorinostat (SAHA; Zolinza®) and romidepsin (depsipeptide; FK-228; Istodax®) (figure2), both for the treatment of *cutaneous T cell lymphoma* (CTCL), a rare form of non-Hodgkin's lymphoma which affects the skin. [6]

Recently, the immunosuppressant fungal metabolite FR235222 (**1,** fig 2**)**, emerged as a potent natural inhibitor of mammalian HDACs belonging to the class of reversible zinc chelator cyclic tetrapeptide HDAC inhibitors, some of which have been already shown to act as effective anticancer agents. [7] Strong potency and activity is elicited by the presence of rare amino acids, such as 2-amino-9-hydroxy-8-oxodecanoic acid (Ahoda), capable of tightly tethering the Zn^{2+} ion, showing more chemical and metabolic stability despite of hydroxamic acids.

FR235222 represents an attractive basis for new drug designs and we also proposed a 3D model for the interaction of this natural ligand with its biological target together with its total synthesis with the aim to elucidate the precise interactions of this natural compound with the biological target and its correct conformation of binding. [8]

The big relevance of FR235222 resides in its nanomolar activity as HDACi despite of the relatively low affinity of the zinc-binding group constituted from a terminal α-hydroxyketone instead of the most common, but metabolically instable, hydroxamic acid.

Considering our great interest for the FR235222 pharmacological properties, [9-15] we already undertook the synthesis of a focused array of analogues of the natural compound. [16] This study was important to demonstrate that it is possible synthesize simplified analogues that preserve the inhibitory activity starting from commercially available amino acids. [17-19] On the other hand, the main structural feature to be maintained is the Ahoda (2-amino-9-hydroxy-8-oxadecanoic acid) side chain, which acts as pharmacophore in the active binding pocket. Moreover, we demonstrated that compounds bearing the naturally occurring (2*S*,9*R*)-Ahoda fragment are in general more active than the stereoisomers 2*S*,9*S*.

With these achievements in mind, our approach was then focused on the synthesis of new analogues where the new ZBG is kept, whilst the CAP-group is further modified through progressive molecular simplifications. After the evaluation of the modifications in the cyclopeptide ring and in the stereochemical arrangement of the Ahoda fragment and taking into account the methabolic problems related to the peptide skeleton, we undertook the synthesis of FR235222 simplified analogues in order

to improve its pharmacokinetic profile.
Linear amides were chosen as primary scaffolds, all of them containing terminal hydroxyketone as zinc-binding group (Figure 3).
Two types of amides were designed according to the CU inserted: the amide function was used in the two possible bonding positions and the nitrogen atom was linked with the CAP first and then reversed on the linker portion. The CAP groups were chosen according to the simple HDACis SAHA-like reported in literature. [20-22]

Materials and Methods

Compounds **7a-g** were prepared following standard processes and completely characterized through ^{1}H, ^{13}C NMR, and high resolution mass spectrometry. All reagents and solvents were purchased from Sigma-Aldrich (St. Louis,MO, USA) and used as received. All microwave reactions were carried out in CEM Explorer® apparatus under monomode irradiation. Hydrogenations were run into Parr® apparatus. All reactions were performed under dry nitrogen atmosphere. Flash column chromatography was performed with silica gel 60, 0.040 −0.063 mm (230−400 mesh)
In vitro activity of linear amides analogues: 50 pmol of an acetylated peptide of histone H4 (Ac-S-G-R-G-K-G-G-K-G-L-G-K-G-G-A-K(ac)-MCA, generous gift of Dr. M. Yoshida) was incubated in a total volume of 10 µL with 0,5 µg of a HeLa nuclear extract (BioVision, Mountain View, CA, USA) in 20 mM Tris, 150 mM NaCl, pH 8 for 1h at 37 °C in presence or absence of inhibitors. The reaction was stopped by heating the samples for 5 min at 95 °C. 1 mL of each sample was spotted onto an H4 proteinchip (Ciphergen, Fremont, CA, USA) and allowed to dry. Spots were then washed twice with 5 µL of 5 mM HEPES. Finally 2 x 0.8 µL of 20% CHCA (in 50% acetonitrile and 0.5% TFA) were spotted onto each spot. Peptide masses were acquired by SELDI-TOF (Ciphergen, Fremont, CA, USA) mass spectrometry and IC_{50} values were calculated using the peak areas in the following way: % of deacetylation was calculated as the percent of deacetylated peptide with respect to the sum of deacetylated and acetylated peptides. Inhibitory activities were then calculated as percent inhibition of the deacetylation reaction. The cytotoxic effects of the most promising compound (1-100 µM) in DMSO solution were evaluated on H460 and HCT-116 cell lines by MTT assay. The assay is based on the observation that viable cells have the ability to metabolize a water-soluble tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma), into an insoluble formazan salt. Reduction of cell viability by more than 30% is considered a cytotoxic effect, otherwise the IC_{50} value is considered not available (NA). The concentration of each compound which inhibit cell viability of 50% (IC_{50}) was determined using Prism 5.0 (GraphPad Software Inc.). IC_{50} values were presented as means ± SEM of three independent experiments carried out by triplicate.

Chemistry

Hydroformilation [23] of butenylamides **4a-g**, previously prepared by standard coupling of acyl chlorides and butenylamine hydrochloride (synthetic details not reported), to corresponding aldehydes **5a-g**, and subsequent Horner-Wadsworth-Emmons (HWE) homologation furnished α,β-unsaturated ketones **6a-g**. Final Pd-catalyzed hydrogenation and TBAF-mediated (tetrabutyl-ammonium fluoride) TBS (tert-butyl-dimethylsilyl) deprotection furnished hydroxyketones **7a-g** in good yields (scheme 1).

Results and Discussion

Antiproliferative activies of synthesized amides **7a-g** were evaluated after 48h treatment of H460 (human pleural effusion adenocarcinoma) and HCT-116 (human colon carcinoma) and H4 acetylation level were tested as shown in table 1. Although most of the amides **7a-g** did not present any representative antiproliferative activity for the tested concentrations from 1 to 20 µL, compound **7e** showed a certain activity (mM range) in HCT-116 and H460 cells (table 1, entry 6).

Conclusion

New linear amides peptidomimetics with the α-hydroxyketone functionality as Zn-binding group were designed in order to find small molecule alternative to large peptides as FR235222. This preliminary work provides bases for lead optimization, identifying compound **7e** as a new candidate for further modifications.

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Figure 1. Pharmacophoric model of HDAC inhibitors.

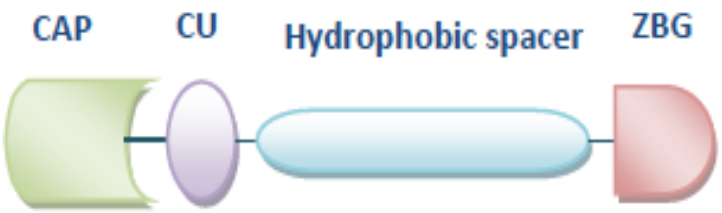


Figure 2. Structure of HDAC inhibitors.

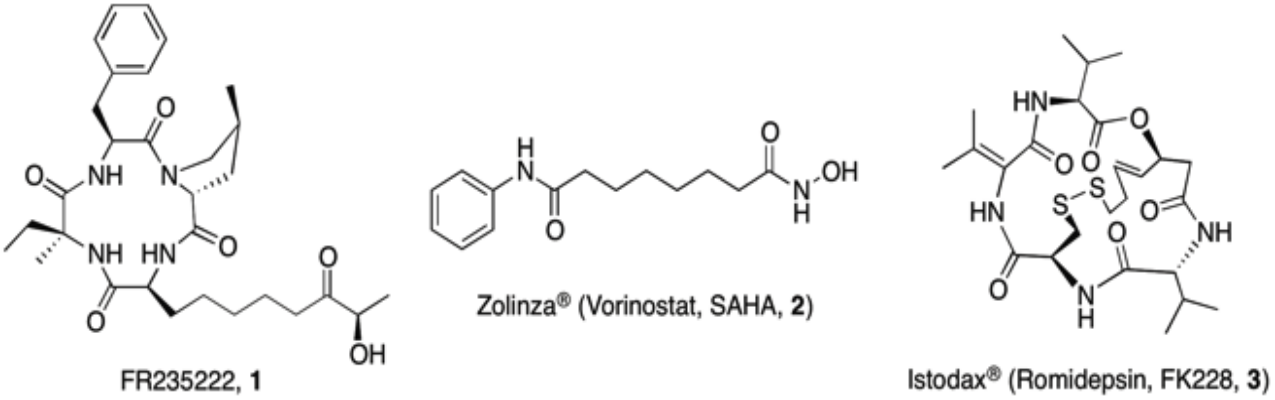
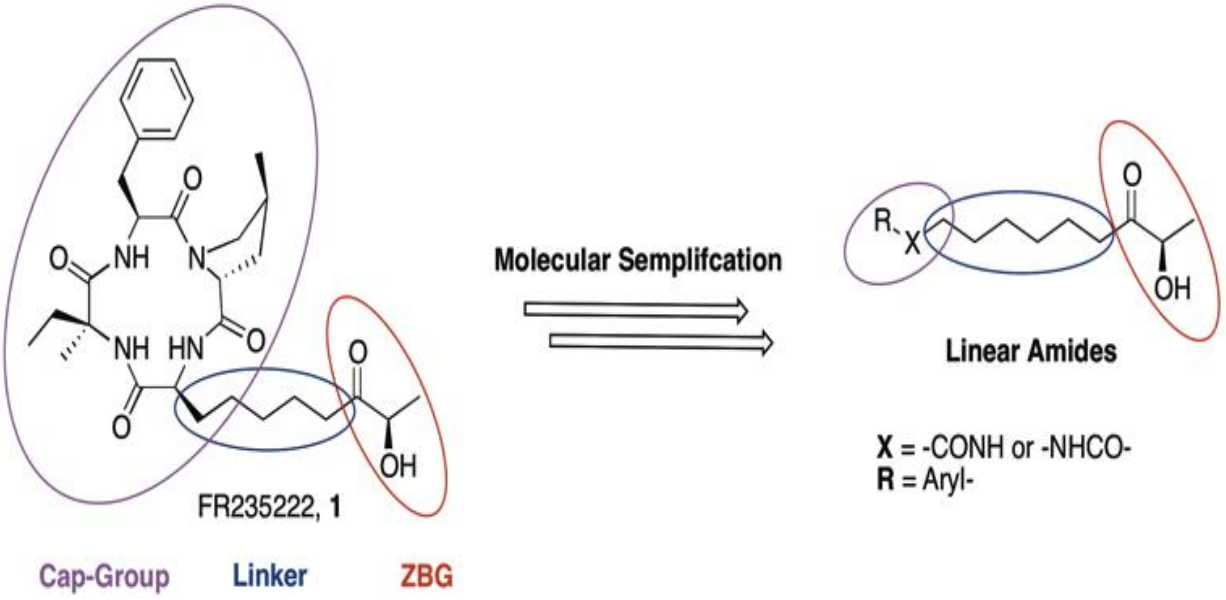
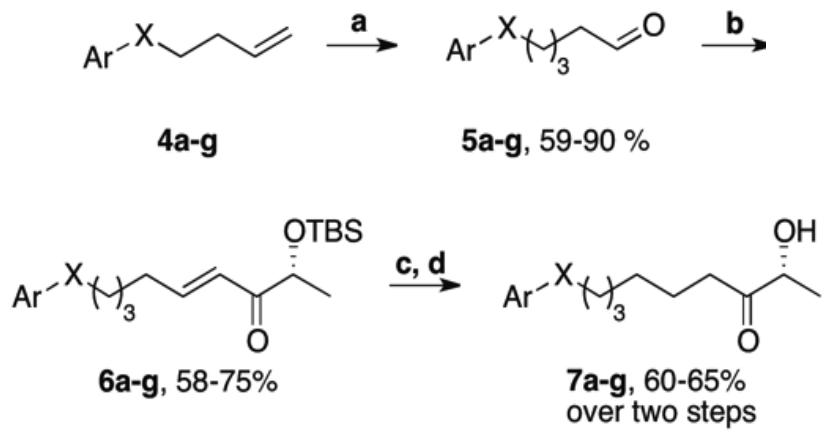


Figure 3. Molecular semplification of FR235222 into linear amides.

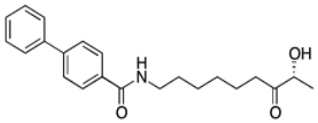
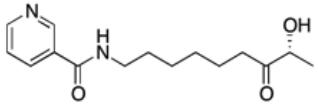
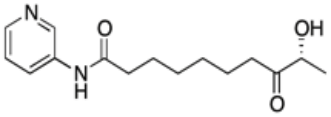
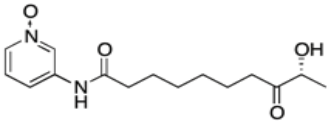
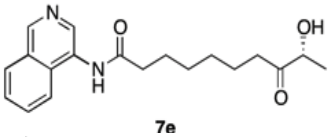
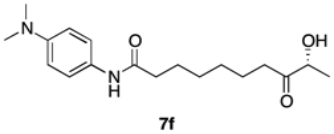
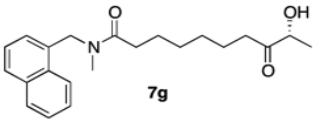


Scheme 1: Synthetic approach for linear amides.



Reagents and conditions: a) $(PPh_3)_3$ Rh(CO)H, Xantphos, CO/H_2 (60 psi), Toluen, [bmim][BF_4], MW, 110 °C, 5 min, **b)** *(R)*-dimethyl-[2-(*tert*-butyldimethylsilyloxy)-1-oxopropyl] phosphonate, DIPEA, LiCl, CH_3CN, r.t., 12h; **c)** Pd/C, H_2, MeOH, r.t.; **d)** TBAF, THF, r.t., 20 min.

Table 1. Antiproliferative activities of synthesized amides **7a-g**.

Compound	**H4 acetylation** (at 5 mM)	**H460 cells (IC_{50} μM)**	**HCT 116 cells (IC_{50} μM)**
 7a	No acetylation	>20	>20
 7b	No acetylation	>20	>20
	No acetylation	>20	>20
	No acetylation	>20	>20
 7e	No acetylation	12.2 ±1.1	7.8 ±1.1
 7f	No acetylation	>20	>20
 7g	No acetylation	>20	>20