2-Oxabicyclo[2.2.2]octane bioisostere of Vorinostat: synthesis and biological activity

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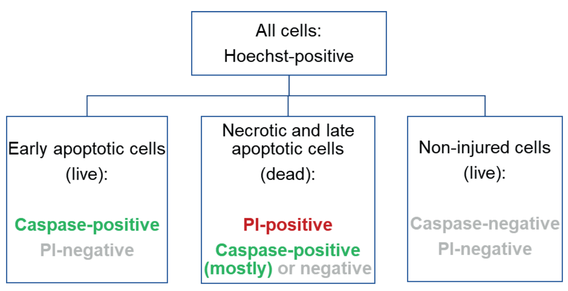
Introduction and Aim

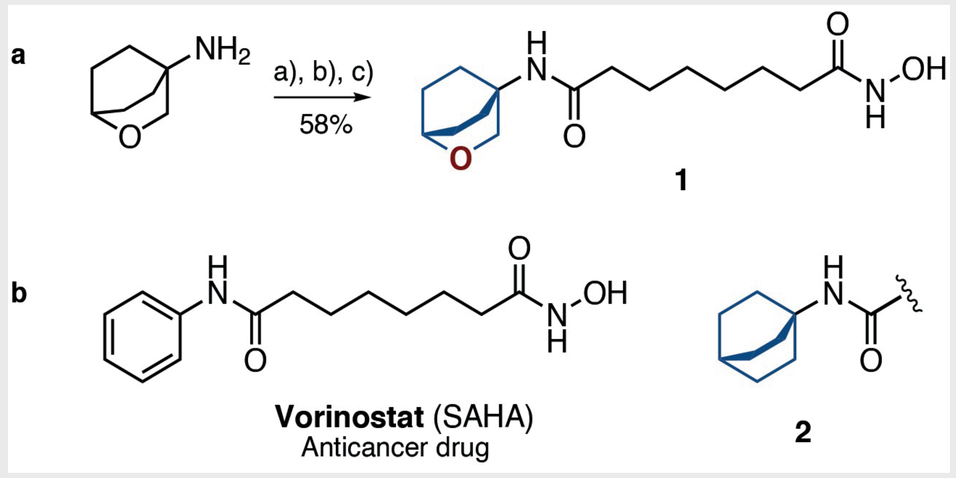
Vorinostat (suberoylanilide hydroxamic acid; SAHA) is the first histone deacetylase inhibitor (HDACi) approved for cancer treatment. It facilitates chromatin decondensation that enhances transcription levels and is crucial for the expression of genes needed to induce cell differentiation.
Although this is a potent drug for the treatment of different types of cancer, it is a high-clearance drug with limited aqueous solubility and oral bioavailability.
In this study, we incorporated the new bioisostere, 2-oxabicyclo[2.2.2]octane scaffold, into the Vorinostat structure instead of the monosubstituted phenyl ring.

Methods

N^1-(2-oxabicyclo[2.2.2]octan-4-yl)-N^8-hydroxyoctanediamide **(analog 1)** was synthesized from amine (Scheme 1), in three steps. For comparison, **(analog 2)** with the bicyclo[2.2.2]octane skeleton was also obtained. Kinetic solubility (2% final DMSO) and $LogD_{7.4}$ of all three compounds were assessed by the shake-flask method. Metabolic stability was tested in human liver microsomes (HLM). The bioactivity of Vorinostat and its analogs, 1 and 2, was tested on HepG2 cells (hepatocellular carcinoma) using high-content imaging.



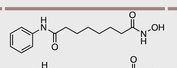
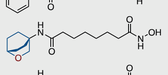
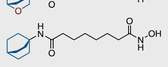
- HepG2 cells to 384-well plate, 4000 cells in 30uL total volume per well;
- Test compounds treatment 50 uM, 48h;
- Staining for 30 min.
- Dyes: Hoechst 33342 (nuclei); CellEvent Caspase-3/7 Green Detection Reagent (cells with activated caspases); Propidium Iodide (PI) for dead cells with permeabilized membrane



Scheme 1. Replacement of the phenyl ring with saturated bioisosteres in anticancer drug Vorinostat (SAHA). a Synthesis of compound 1 – a saturated analog of Vorinostat. Reaction conditions: a) $Cl(O)C(CH_2)_6CO_2Me$, NEt_3, CH_2Cl_2, rt, 2h. b) NaOH, MeOH, reflux, 30min. c) NH_2OH•HCl, DMF, CDI, rt, 30min. b Structure of Vorinostat (SAHA), and its saturated analog 2.

Results

Table 1. ADME properties of Vorinostat and analogs

Compound	Structure	Solubility*, PBS pH 7.4, μM	LogD, pH 7.4	CLint, ul/min/mg, HLM	$t_{1/2}$, min, HLM	k_{el}, min^{-1}, HLM
Vorinostat		≥200	1.0±0.01	8	200	0.003
Analog 1		182±8	0.1±0.04	8	211	0.003
Analog 2		196±3	1.6±0.01	12	139	0.005

***kinetic solubility, 2% final DMSO**

Vorinostat treatment resulted in 7.2% and 12.2% of early apoptotic cells (caspase-positive, propidium iodide-negative) upon incubation at concentrations 5 μM and 50μM, respectively. Analogs 1 and 2 demonstrated similar efficacy only at 50 μM. The nuclei area was slightly increased after treatment with Vorinostat at 1 uM and 5 uM which might be morphological evidence for its histone deacetylase inhibition activity. Analog 1 revealed a similar effect at 50 uM. Analog 2 at 50 uM induced chromatin decondensation similar to the Vorinostat effect at the same concentration, which might indicate the similarity in their effects on transcription.

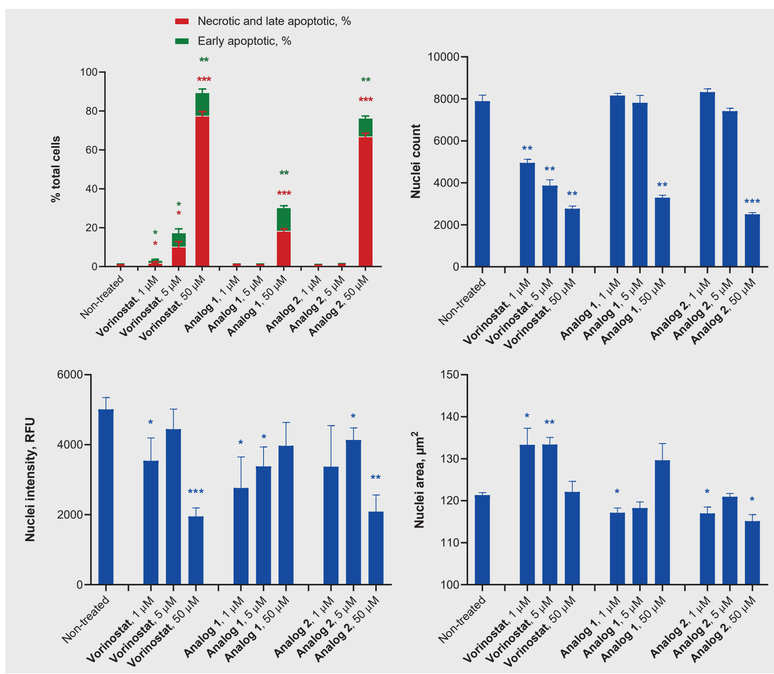


Figure 2. Biological effects induced in HepG2 cells after treatment with Vorinostat and its analogs. The data are represented as mean (n=3 ± SD); * indicates $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ compared with the non-treated group.

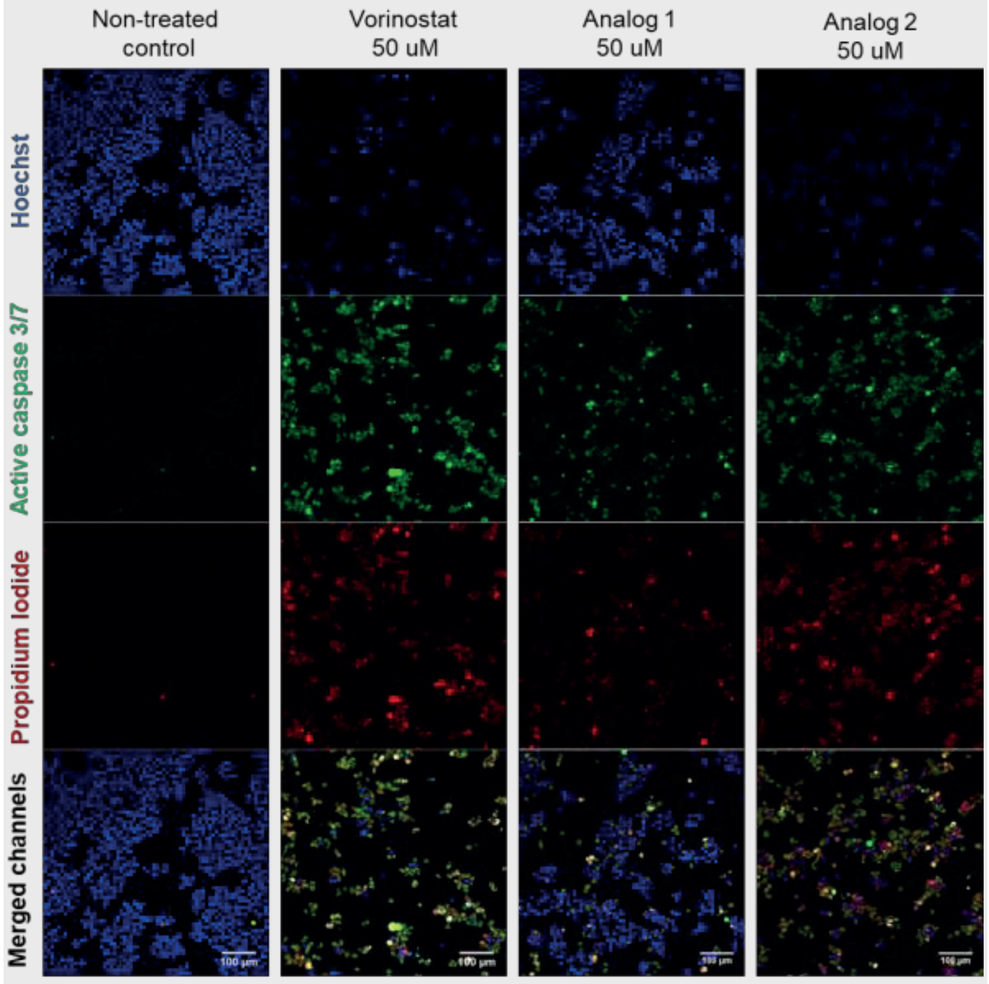


Figure 1. Confocal fluorescent microscopy images of HepG2 cells treated with Vorinostat and analogs. Hoechst 33342 was used for nuclei staining, CellEvent Caspase 3/7 detection reagent was used to assess early apoptotic cells and propidium iodide for staining dead cells with permeabilized membrane.

Conclusions

These primary data suggested that Vorinostat and both its analogs, 1 and 2, could have similar cytotoxic and cytostatic activities in cells. These results also show that 2-oxabicyclo[2.2.2]octane core could indeed mimic the phenyl ring in bioactive compounds. Thus, 2-oxabicyclo[2.2.2]octane can be added to the set of available saturated bioisosteres of (hetero)aromatic rings for use in drug discovery projects.

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