

Supplemental material

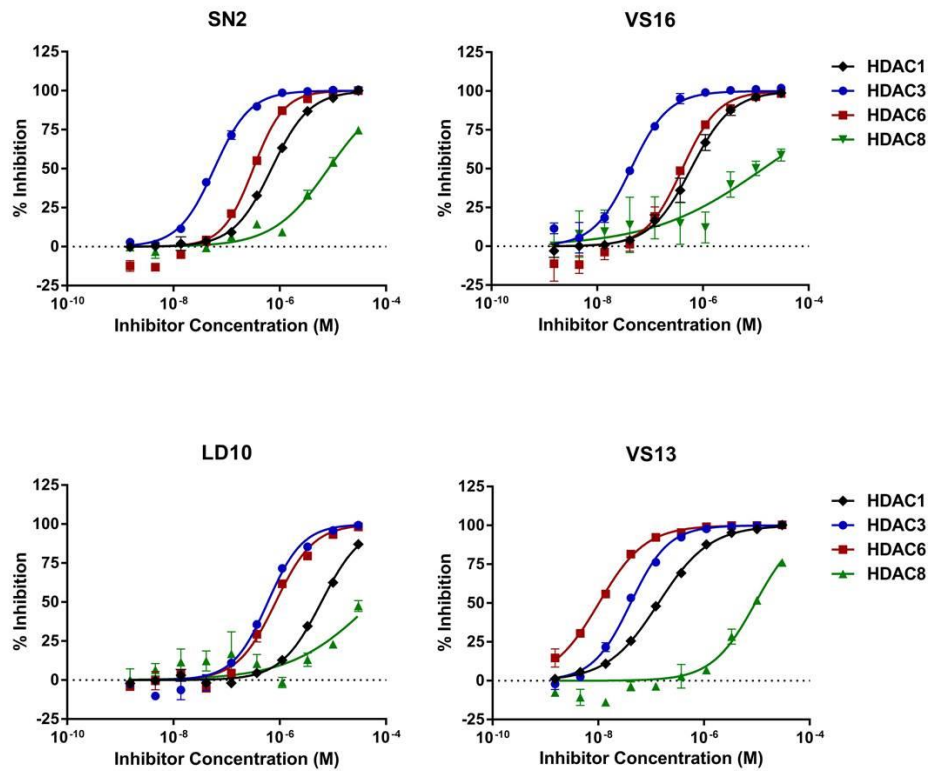
# Identification of Histone Deacetylase Inhibitors with (Aryliden)aminoxy Scaffold active in Uveal Melanoma Cell Lines

Susanna Nencetti<sup>a,\*</sup>, Doretta Cuffaro<sup>a</sup>, Elisa Nuti<sup>a</sup>, Lidia Ciccone<sup>a</sup>, Armando Rossello<sup>a,c</sup>, Marina Fabbi<sup>d,\*</sup>, Flavio Ballante<sup>e,f</sup>, Gabriella Ortore<sup>a</sup>, Grazia Carbotti<sup>d</sup>, Francesco Campelli<sup>d</sup>, Irene Banti<sup>a</sup>, Rosaria Gangemi<sup>d</sup>, Garland R. Marshall<sup>c</sup>, Elisabetta Orlandini<sup>b,c</sup>

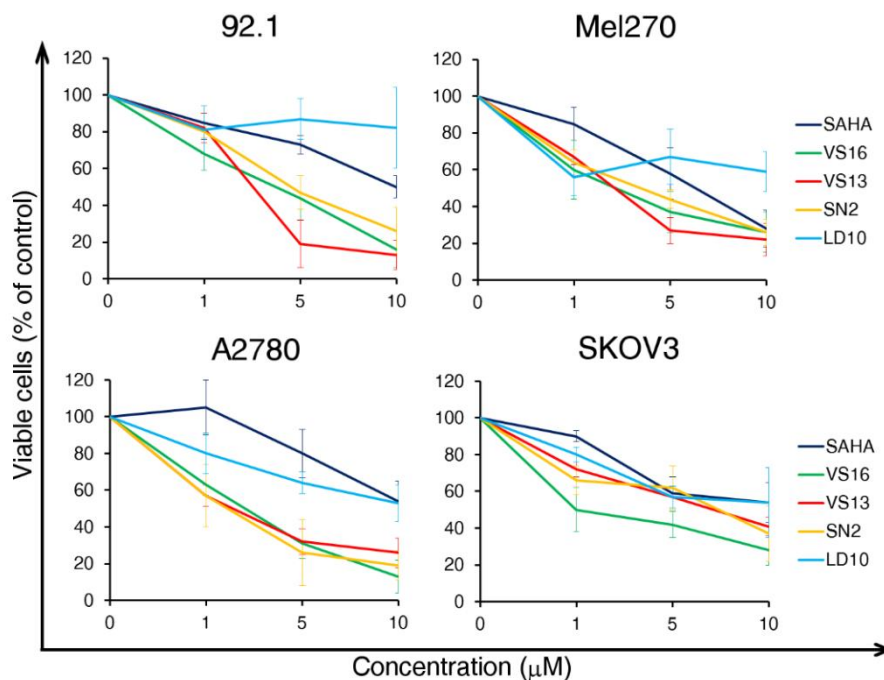
*<sup>a</sup>Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126, Pisa, Italy. <sup>b</sup>Dipartimento di Scienze della Terra, Università di Pisa, Via Santa Maria 53-55, 56100, Pisa, Italy. <sup>c</sup>Research Center “E. Piaggio”, Università di Pisa, Pisa, Italy. <sup>d</sup>IRCCS Ospedale Policlinico San Martino, Genova, Italy. <sup>e</sup>Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA. <sup>f</sup>Present address: Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, BMC Box 596, SE-751 24 Uppsala, Sweden.*

Corresponding author:

*E-mail address:* susanna.nencetti@unipi.it

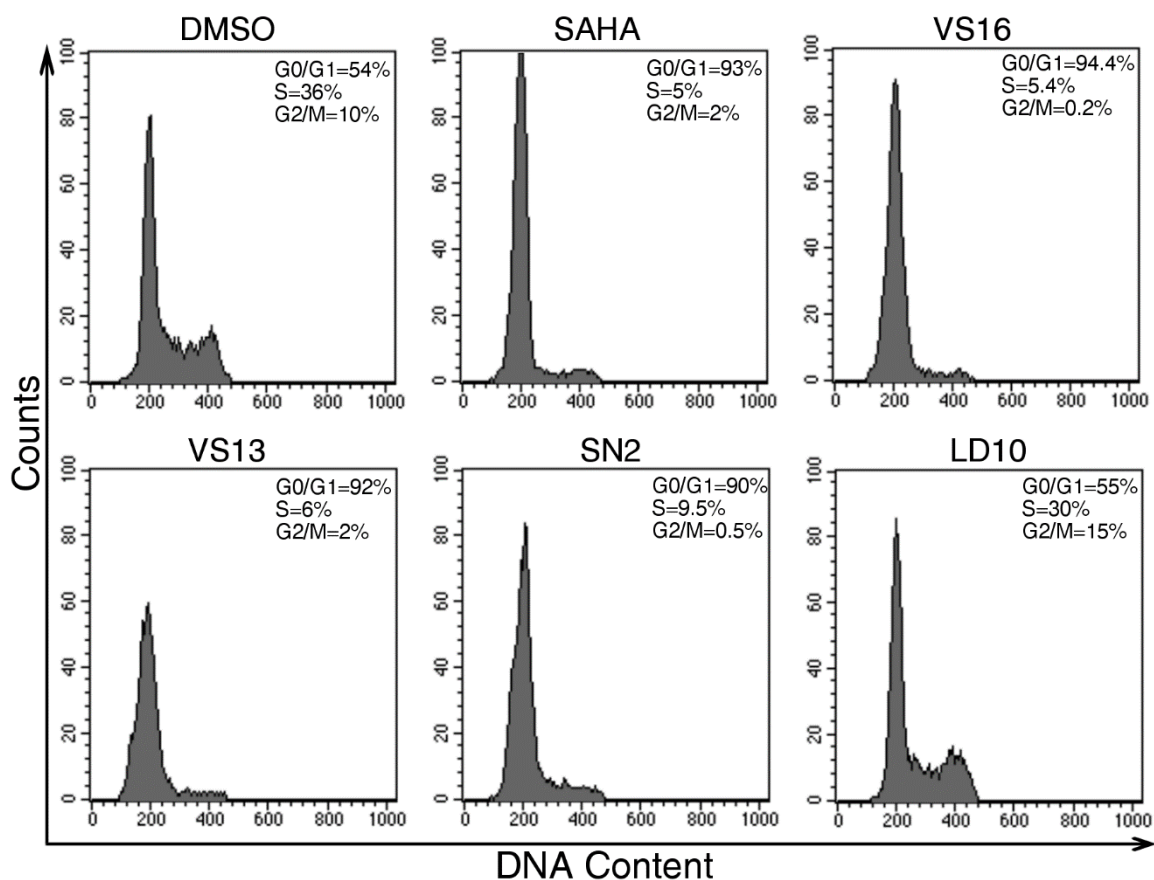


**Figure S1 HDAC isoform selectivity assays.** Dose-response curves for compounds SN2, VS16, LD10 and VS13. Data are present as mean  $\pm$  SEM. Compounds were tested in duplicate in a 10-point dose curve with 3-fold serial dilution starting from 30 $\mu$ M.

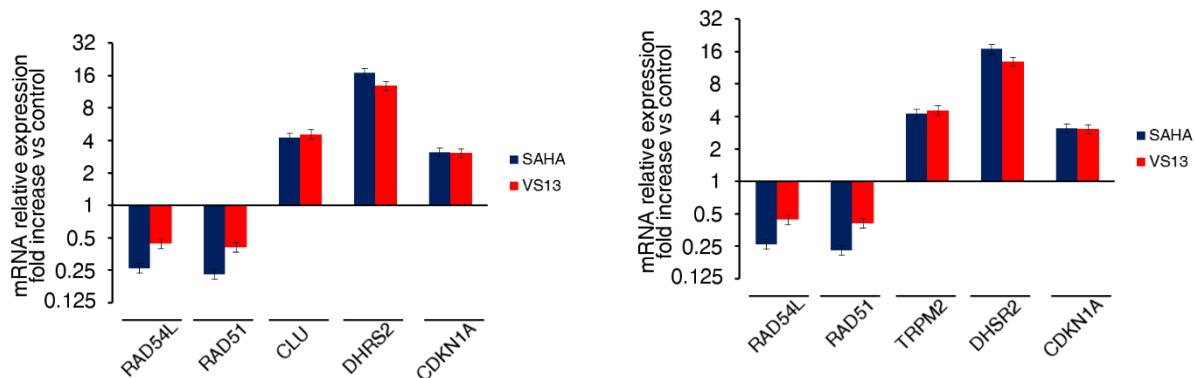


**Figure S2 MTT assay.** In vitro cytotoxicity at the 72 hour time point of the different compounds in human uveal melanoma cell lines 92.1 and Mel270 and ovarian cancer cell lines A2780 and SKOV3, as assessed by the MTT cell viability assay. Data are expressed as percent of control with the DMSO

solvent, which was used at the same amount present in the highest compound concentration. Error bars represent SD of quadruplicates. One representative experiment is shown.



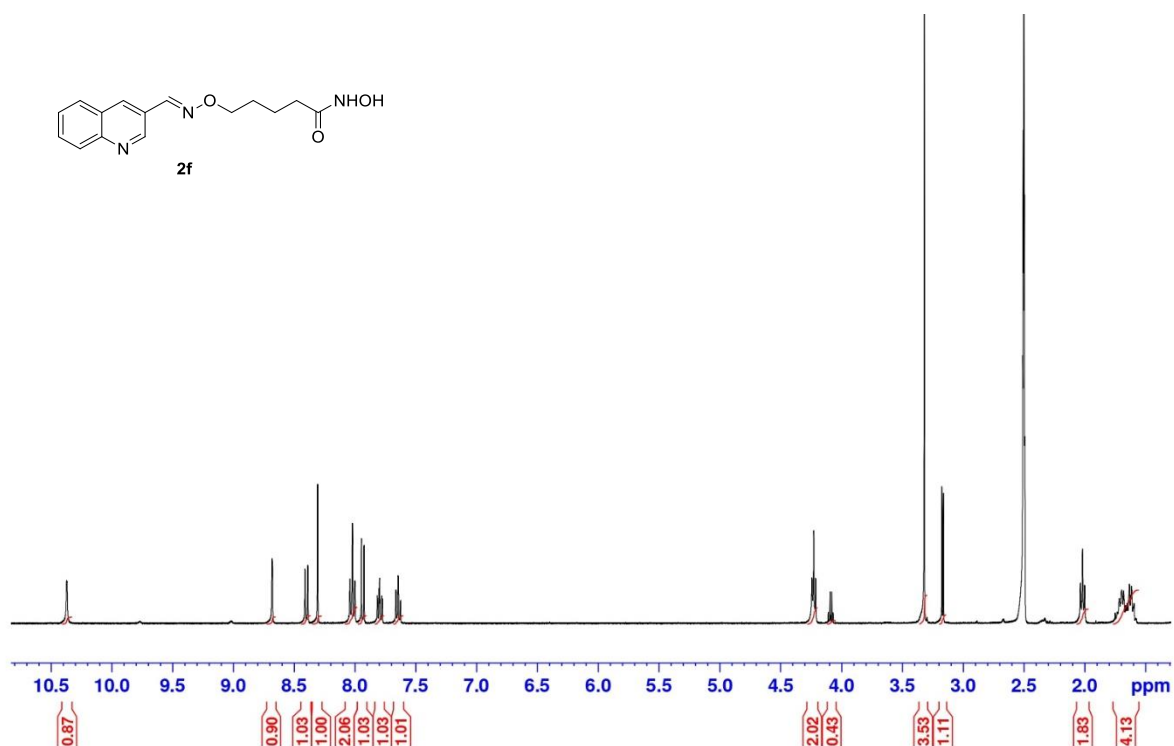
**Figure S3 Cell cycle analysis.** Flow-cytometric analysis of the DNA content of uveal melanoma 92.1 cells after treatment with 10  $\mu$ M of the indicated compounds for 48 hours. The cells were treated and cell cycle distribution was analyzed by flow cytometry after fixation and staining with PI. The percentage of cells in each category is indicated. One representative experiment is shown.



**Figure S4 qRT-PCR.** Comparison of the modulation of expression of five different genes induced by SAHA and VS13 in uveal melanoma cells 92.1. Cells were treated for 48 hours with 10  $\mu$ M compound or the corresponding amount of DMSO. Data, normalized to GAPDH housekeeping gene, are expressed as fold change relative to the DMSO control. Error bars represent SD of triplicates. One representative experiment is shown

NMR characterization of compound **2f** (**VS13**):

$^1\text{H}$  NMR (400 MHz  $\text{DMSO-}d_6$ ):



$^{13}\text{C}$ -NMR (100 MHz  $\text{DMSO-}d_6$ ):

