# Purine-derived phenylhydroxamate compounds exhibit antineoplastic activity in hematological malignancies

Jorge Antonio Elias Godoy Carlos[1], Larissa Costa de Almeida[1], Keli Lima[1], Mauricio Temotheo Tavares[2], Karoline de Barros Waitman[2], Leticia Veras Costa-Lotufo[1], Roberto Parise-Filho[2], João Agostinho Machado-Neto[1]

1Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Brazil.
2Department of Pharmacy, Faculty of Pharmaceutical Science, University of São Paulo.

## Background

Acute leukemias are a group of malignant diseases characterized by exacerbated levels of myeloid or lymphoid progenitor cells showing high rates of recurrence and mortality. High levels of histone deacetylases (HDAC) have been observed in patients with solid neoplasms and leukemias being associated with poor prognosis.

## Objectives

The present study aimed to evaluate the cellular effects of three novel potential HDAC inhibitors in acute leukemia cellular models.

## Methods

A panel containing myeloid (OCI-AML3, Kasumi 1, HL60, THP1, MOLM13, MV4-11, U937, NB4, NB4-R2, K562, KU812, SET2, and HEL) and lymphoid (Jurkat, CEM, Namalwa, NALM6, Daudi, Raji, SUP-B15, REH, U266, MM1.S, MM1.R, Karpas 422, and MEC-1) neoplasm cells and normal leukocytes (from four healthy donors) were used. Three novel purine-derived phenylhydroxamate with potential HDAC inhibitor activity (82, 83, and 84) were used. Vorinostat was used as a reference drug. Cell viability was assessed by MTT assay, apoptosis and cell cycle by annexin V/propidium iodide labeling and flow cytometry (FC), and clonogenicity by autonomous colony formation. Protein analyses were performed by Western Blot. IC50 values were obtained by non-linear regression. Statistical analysis was performed by ANOVA and Bonferroni post-test. A p<0.05 was considered significant.

## Results

**All compounds reduced cell viability. In both neoplasic cells tested, compound 82 was more potent than the others exhibiting less concentration necessary to reduce cell viability by 50% (IC50 ranged from 0.09 to 2.04 μM). THP1, MOLM13, NB4-R2, and HEL leukemia cells were more sensitive to these compounds. In healthy hematopoietic cells, our data showed that vorinostat and compound 82 did not reduce cell viability at the same extension as observed for leukemia cells, indicating a selective antineoplastic activity.**

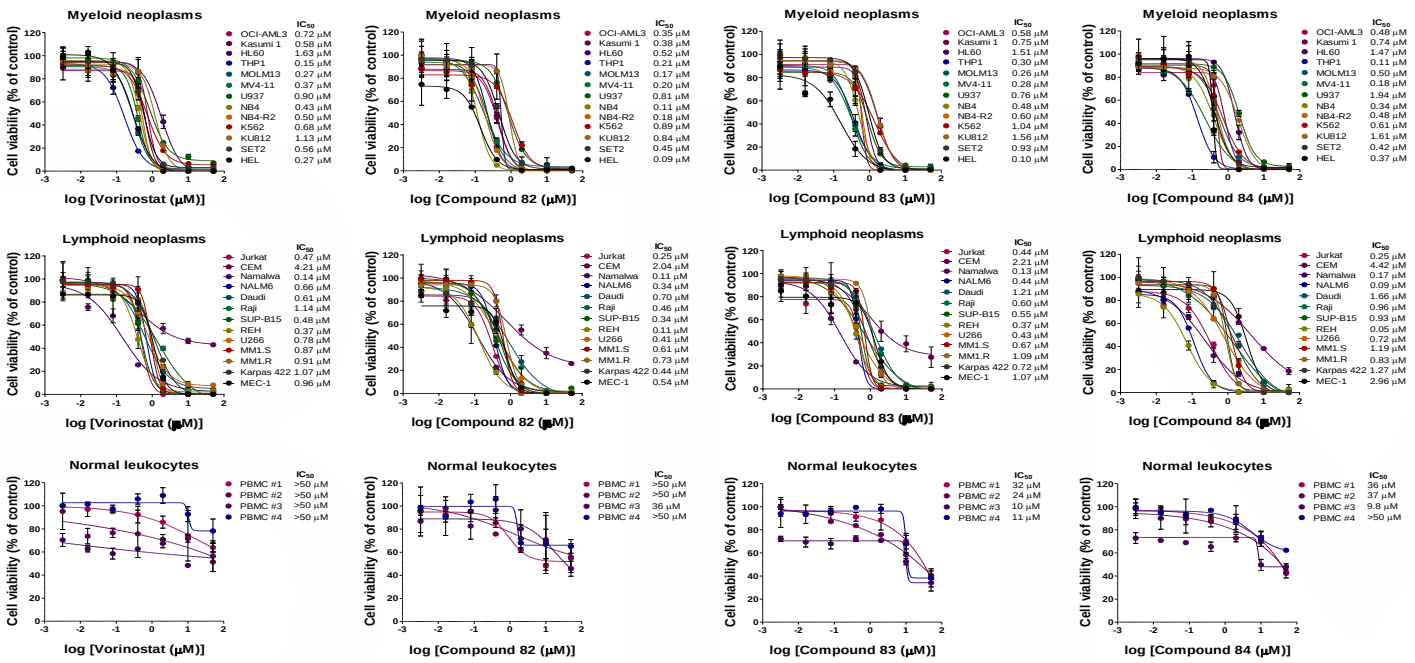


Figure 1. New potential HDAC's inhibitors reduces cell viability in leukemia cells. OCI-AML3, Kasumi 1, HL60, THP1, MOLM13, MV4-11, U937, NB4, NB4-R2, K562, KU812, SET2, HEL, Jurkat, CEM, Namalwa, NALM6, Daudi, Raji, SUP-B15, REH, U266, MM1.S, MM1.R, Karpas422 and peripheral blood mononuclear cells (PBMC) were exposed to increased concentrations (0.0032, 0.016, 0.08, 0.4, 2, 10 and 50μM) of the drugs (SAHA, 82, 83 e 84) previously selected by 72 hours. Normal leukocytes wasn't sensibilized in the same manner as soon in leukemia cells. Cell viability was analyzed by MTT assay. IC50 values were obtained to each compound.

**Hel, MOLM-13, NB4-R2 and THP1 cells were more sensible to HDAC inhibitors. Compound 82 was more potent than the others showing IC50 values between 1 to 2.8 in 24 hours, 0.7 to 1.3nM in 48 hours and 0.5 to 0.8μM in 72 hours.**

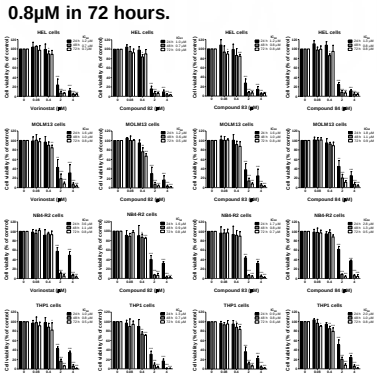

Figure 2. New potential HDAC's inhibitor induce cell death. HEL, MOLM13, NB4-R2 and THP1 cells were exposed to increased concentrations (0.08, 0.4, 2 and 4μM) of the drugs (SAHA, 82, 83 e 84) previously selected by 72 hours. IC50 values were obtained to each compound ($p$<0.05).

**All compounds induced apoptosis in THP1, MOLM13, NB4-R2, and HEL cells (all p<0.05) in dose-dependent manner .**

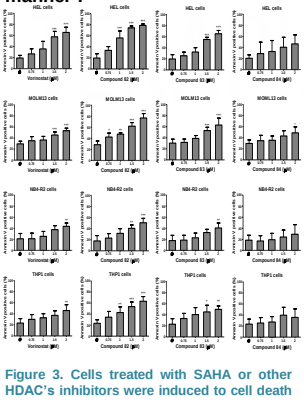

Figure 3. Cells treated with SAHA or other HDAC's inhibitors were induced to cell death by apoptosis. HEL, MOLM13, NB4-R2 and THP1 cells were exposed to increased concentrations (0.75, 1, 1.5 and 2μM) of the drugs (SAHA, 82, 83 e 84) previously selected by MTT assay ($p$<0.05).

**Compound 82 increased cell populations in SubG1 phase of cell cycle in THP1, MOLM13, NB4-R2, and HEL cells (all p<0.05).**

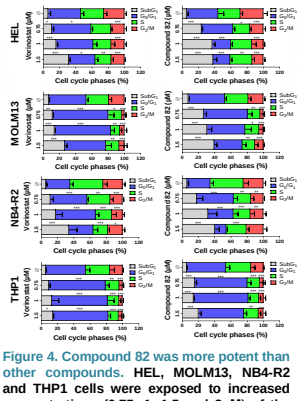


Figure 4. Compound 82 was more potent than other compounds. HEL, MOLM13, NB4-R2 and THP1 cells were exposed to increased concentrations (0.75, 1, 1.5 and 2μM) of the drugs (SAHA, 82, 83 e 84) previously selected. Compound 82 induced more subpopulations in SubG1 cell cycle phase ($p$<0.05).

**All compounds induced apoptosis in THP1, MOLM13, NB4-R2, and HEL cells (all p<0.05) in dose-dependent manner.**

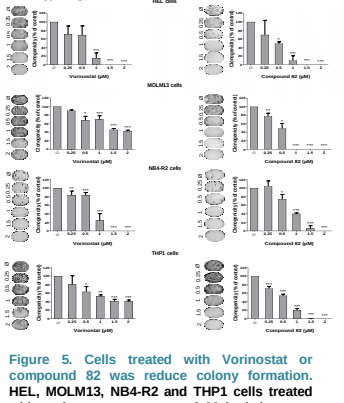

Figure 5. Cells treated with Vorinostat or compound 82 was reduce colony formation. HEL, MOLM13, NB4-R2 and THP1 cells treated with vorinostat or compound 82 had decrease capacity in forming new colonies ($p$<0.05).

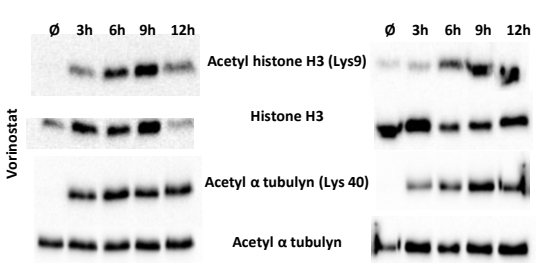


Figure 6. Compound 82 modulate protein expression in HEL cells. HEL cells were treated with vorinostat or compound 82, both molecules induced acetylation in histone and α tubulyn.

## Conclusion

Our results indicate that the three purine-derived phenylhydroxamate tested presented antileukemic effects with fewer effects in normal leukocytes. Since the higher expression of HDACs is associated with poor prognosis and higher levels of relapse in leukemia, these enzymes are interesting targets for the development of new pharmacological inhibitors. Supported by CNPq, CAPES, and FAPESP.

## Contact

**Laboratory of Cell Biology and Antineoplasics**
Institute of Biomedical Science – Department of Pharmacology
Ph.D. Candidate: Jorge Antonio E. G Carlos – jorgegdy@icb.usp.br
Supervisor: Ph.D. João Agostinho Macho Neto – jamachadoneto@gmail.com
University of São Paulo