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Graphical Abstract

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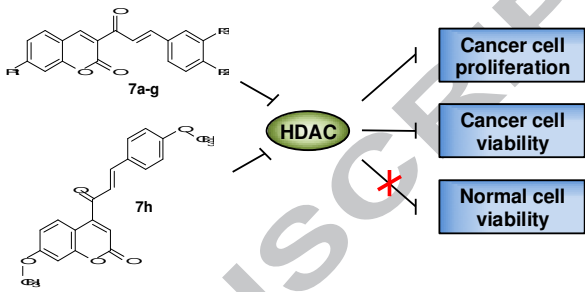

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Novel inhibitors of human histone deacetylases: design, synthesis and bioactivity of 3-alkenoylcoumarines

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ABSTRACT

Histone deacetylases (HDACs) are well-established, promising targets for anticancer therapy due to their critical role in cancer development. Accordingly, an increasing number of HDAC inhibitors displaying cytotoxic effects against cancer cells have been reported. Among them, a large panel of chemical structures was described including coumarin-containing molecules. In this study, we described synthesis and biological activity of new coumarin-based derivatives as HDAC inhibitors. Among eight derivatives, three compounds showed HDAC inhibitory activities and antitumor activities against leukemia cell lines without affecting the viability of peripheral blood mononuclear cells from healthy donors.

Histone deacetylases (HDACs) are enzymes that catalyze removal of acetyl groups from lysine residues. Beyond their originally identified histone substrates, HDACs target non-histone proteins including α-tubulin, heat shock protein 90 or p53 [1]. The HDAC family comprises 18 members subdivided into four classes based on sequence similarity and catalytic activity [2]. HDACs play a critical role in epigenetic gene regulation and therefore control multiple cellular processes [1, 3].

Since expression and/or activity of HDACs are deregulated in various cancer subtypes, they became an interesting target for anticancer therapy [4, 5]. Accordingly, numerous compounds from natural sources as well as synthetic derivatives were identified and further developed as HDAC inhibitors (HDACi) and some of them are already undergoing clinical trials for anticancer therapy [6-8]. Among HDACi, chalcone-based compounds (1,3-diaryl-2-propen-1-ones) are a group of aromatic natural or synthetic unsaturated ketones with anti-inflammatory and anticancer activities [9, 10]. Ease of preparation, oral administration and safety also support the feasibility of chalcone-based compounds as therapeutic agents [11-13].

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Additionally, their simple and efficient synthesis makes them attractive for industrial production [14].

Curcumin is a promising molecule that also modulates the acetylation status of proteins [15]. *In vitro* studies demonstrated that it possesses potent cytotoxic and chemotherapeutic properties in different models [16-24]. Whilst curcumin itself has limited efficacy due to its low bioavailability and stability in physiological media [25], analogs including *N*-methylpiperidone were generated [26]. Natural products bearing 2*H*-1-benzopyran-2-one (coumarin) possess cytotoxic antitumor potential [27, 28]. Coumarin-based compounds were previously described as Cdc25 phosphatase and HDAC inhibitors [27, 29-32].
So far only two molecules, FK228 (Romidepsin) and suberoylanilide hydroxamic acid (SAHA, Vorinostat), gained Food and Drug Administration approval for cutaneous T-cell lymphoma [8]. In this context, development of novel HDACi with good anticancer properties and low toxicity remains a challenge. Here we designed novel coumarin-containing analogs **7a-h** and we assessed their HDACi potential and effects on cell proliferation and viability in K-562 and U-937 leukemia cell lines compared to peripheral blood mononuclear cells (PBMCs) of healthy donors.

The new series of coumarin-based analogues (**7a-h**) bearing an α,β-(mono- or bis)-unsaturated ketone at the C3 or C4 position (**Figure 1**) were prepared carrying out aldolic condensation between 3-acetylcoumarins (**5a-b**) or 4-acetyl coumarin (**5c**), previously synthesized by us according to the literature [33-35]

and the appropriate aldehydes (**6a-h**) following an adapted procedure of Cechinel-Filho *et al.* [36]. All the chemical-physical data, elemental analyses, ^{1}H NMR and ^{13}C NMR of the compounds as well as all the conditions for their biological evaluation are described in the supporting information.

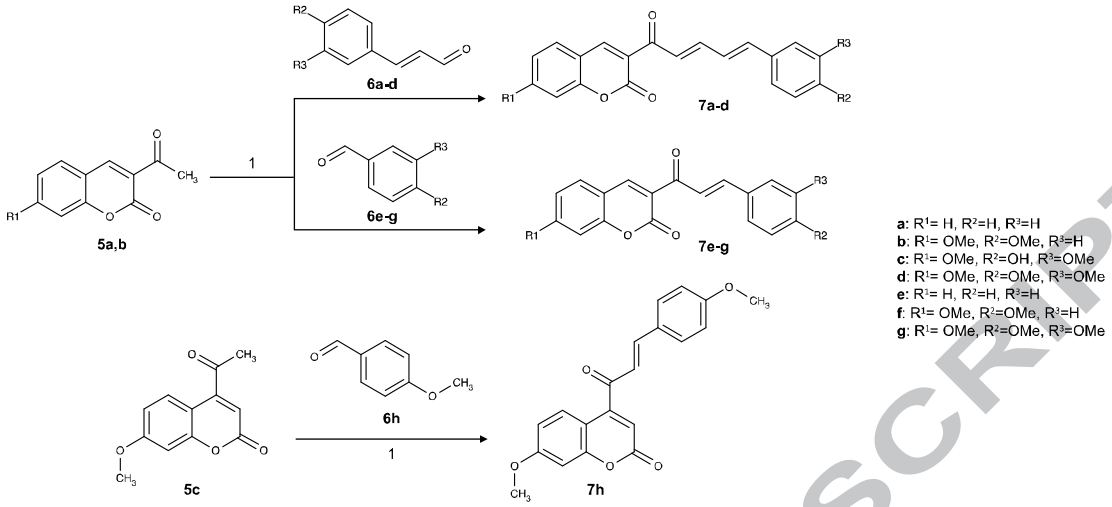

Figure 1: Synthesis of coumarin-based compounds **7a-h**.
Reagent and conditions (1): pyrrolidine, ethanol, 80°C, 1-3 h.

Coumarin-based compounds were tested for their total HDAC inhibitory potential on K-562 nuclear extracts [37, 38]. **7b**, **7d** and **7h** showed a 20 to 50% of inhibition of total HDAC activity at 100 µM (**Table 1**). In opposition to **7b**, compound **7d,** with a methoxy group at R^3, presented increased levels of inhibition. Noteworthy, compound **7c** with a hydroxyl group instead of the methoxy group in R^2 was inactive against HDACs further demonstrating the importance of the methoxy group in this position. Compound **7d** was tested against seven HDAC isoenzymes representing classes I, IIb and IV and acted as a pan-HDACi (**Table 2**) with IC_{50}s between 12 and 85 µM.
Interestingly, **7d** inhibited HDAC3 with an IC_{50} at 12 µM and may serve as a lead for targeting this nuclear isoenzyme.

Table 1: Effect of compounds **7a-h** on *in vitro* total HDAC activity. Values represent the mean of the percentage of inhibition measured in two independent experiments. Inactive means inhibition < 10% at 100 µM.

Compound	Effect on HDAC activity
7a	Inactive
7b	30% inhibition at 100 µM
7c	Inactive
7d	50% inhibition at 100 µM
7e	Inactive
7f	Inactive
7g	Inactive
7h	20% inhibition at 100 µM

Table 2: Effect of **7d** on *in vitro* activity of HDAC isoenzymes.
Values represent the mean of the percentage of inhibition measured in two independent experiments. Inactive means inhibition < 10% at 100 µM.

HDAC		IC_{50} (µM)
Class	**Isoenzyme**	
I	HDAC1	59
	HDAC2	33
	HDAC3	12
	HDAC8	28
IIb	HDAC6	32
	HDAC10	85
IV	HDAC11	74

Compounds **7b** and **7d** showed moderate effects on proliferation and viability of chronic myeloid leukemia K-562 and histiocytic lymphoma U-937 cell lines (**Figure 2A**). Compound **7h** strongly inhibited proliferation in both cell lines. In U-937 cells, loss of proliferation was accompanied by a marked decrease of cell viability (**Figure 2B**). We noticed that compound **7d** precipitated at 100 µM in cell culture medium that could explain why this compound was less effective at this concentration compared to 50 µM. Thus, we observed a close effect between compounds **7b** and **7d** on leukemia cancer cell lines. Indeed, these two compounds are structurally identical except a methoxy group present in compound **7d**. Interestingly, among compounds with one unsaturation (**7e-g**), compound **7h**, the more active compound on cell viability and proliferation, was the only structure inhibiting HDAC activities and corresponds to the C4 regioisomer of compound **7f**, which was inactive. These differential activities could result from 3D structure variations depending on the position of the keto function. To assess for differential toxicity, PBMCs from healthy donors [39] were treated with compounds **7b**, **7d** and **7h** under the same conditions. Results showed no effect on PBMC viability (**Figure 2C**).

Since among newly synthetized compounds, **7d** was the most active compound on HDAC activity, we further tested whether this hybrid compound possessed an HDAC inhibitory potential superior to the two parent compounds, namely the 3-acetylcoumarin **5b** and the aldehyde **6d**. First, **5b** and **6d** were tested on *in vitro* total HDAC activity. Results demonstrated that 100 µM compound **5b** inhibited only 20% of total HDAC activity, whereas at the same concentration compound **6d** enhanced total HDAC activity by 80%. Compounds **5b** and **6d** were further tested on cancer as well as on PBMCs from healthy donors. Results demonstrated that both compounds slightly decreased proliferation only in U-937 cells after 72 hours of treatment without affecting viability of both cancer K-562 and U-937 cell lines and healthy PBMCs (**Figure 3**). All together these results clearly demonstrated that the newly synthetized hybrid compound **7d** possesses an inhibitor potential superior to both parent compounds **5b** and **6d**.

In this study, we have described three new coumarin-based derivatives, **7b**, **7d** and **7h,** endowed with HDAC inhibitory and antitumor properties. Regarding differences in their chemical structures and biological effects, these compounds can support a new design of molecules to increase their reactivity against HDAC activity and cancer cells.

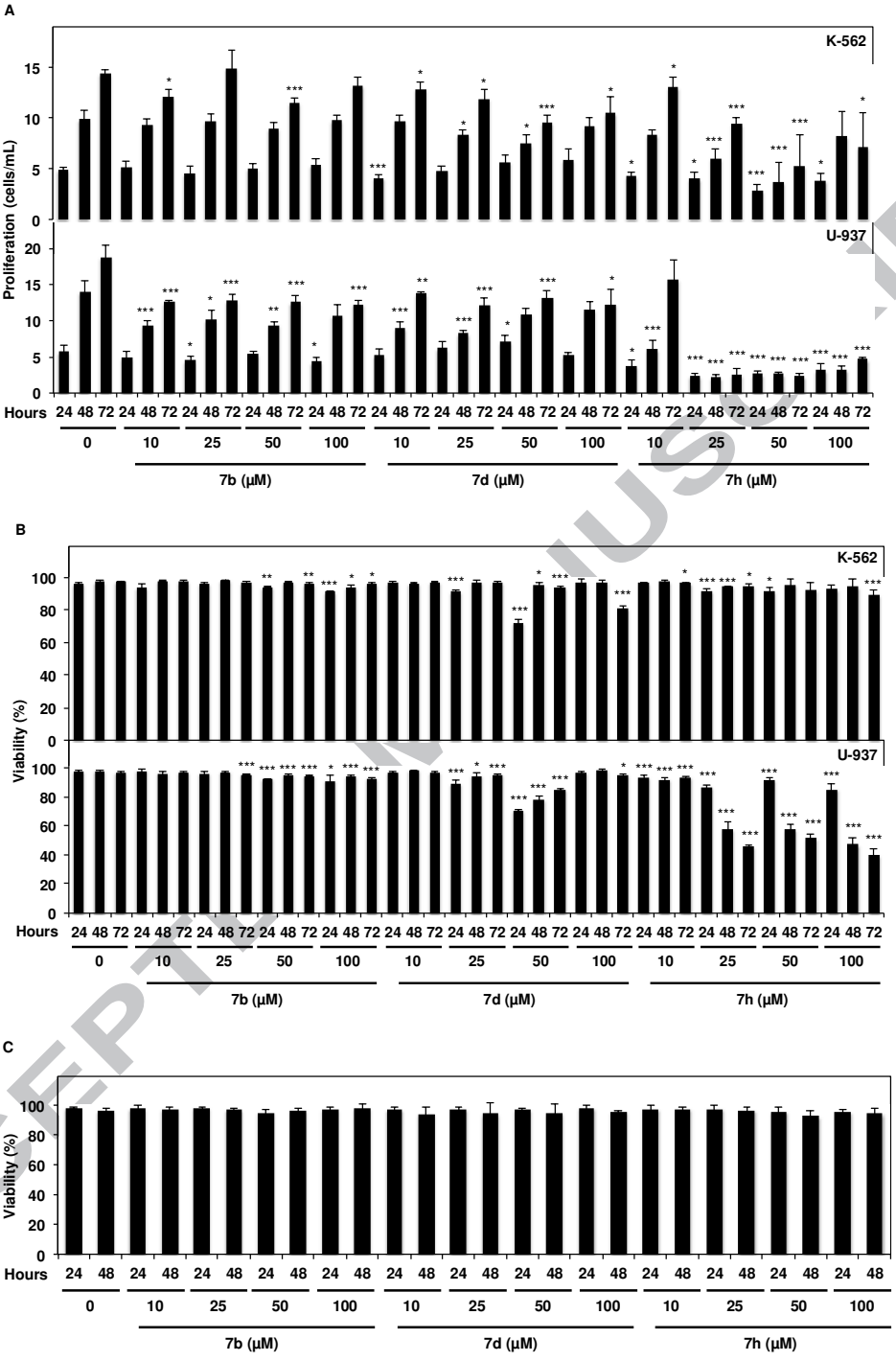

Figure 2: Effect of **7b**, **7d**, **7h** on cell proliferation and viability.
K-562 and U-937 cells were treated with the indicated concentration of compound. (A) Cell viability and (B) proliferation were assessed after 24, 48 and 72h. (C) PBMCs from healthy donors were incubated with **7b**, **7d**, **7h** and then cell viability was evaluated after 24 and 48h of treatment. Data are the mean ± SD of three independent cultures. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ versus control.

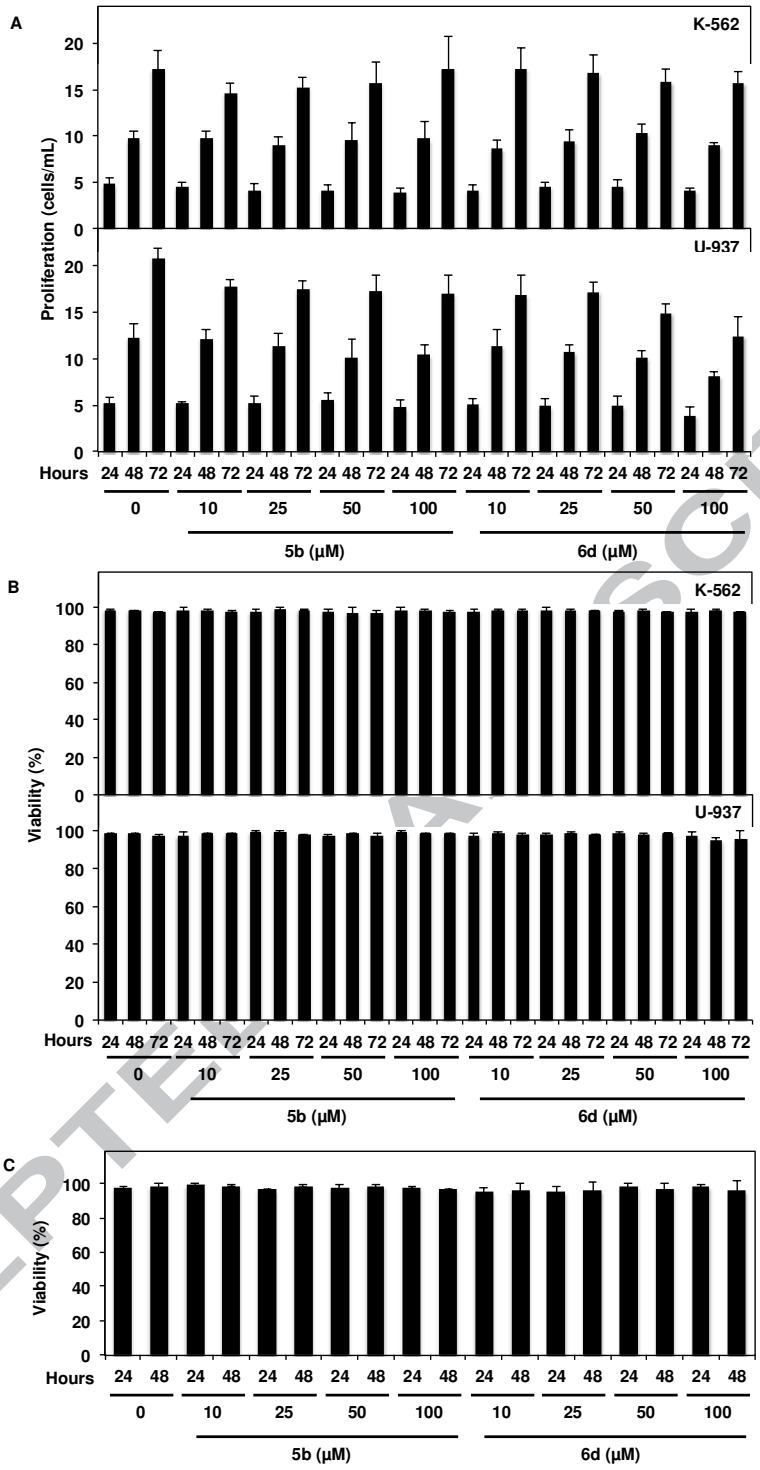

Figure 3: Effect of **5b** and **6d** on cell proliferation and viability.
K-562 and U-937 cells were treated with the indicated concentration of compound. (A) Cell viability and (B) proliferation were assessed after 24, 48 and 72h. (C) The viability of PBMCs from healthy donors was evaluated after 24 and 48h of treatment with compounds **5b** and **6d**. Data are the mean ± SD of three independent cultures. * $p < 0.05$, *** $p < 0.005$ versus control.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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