Evaluation of a large library of (thiazol-2-yl)hydrazones and analogues as histone acetyltransferase inhibitors: enzyme and cellular studies

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · APRIL 2014

Impact Factor: 3.45

12 AUTHORS, INCLUDING:

Simone Carradori
Università degli Studi G. d'Annunzio Chieti …
92 PUBLICATIONS **931** CITATIONS
SEE PROFILE

Dante Rotili
Sapienza University of Rome
95 PUBLICATIONS **1,961** CITATIONS
SEE PROFILE

Marco Miceli
Second University of Naples
26 PUBLICATIONS **567** CITATIONS
SEE PROFILE

Lucia Altucci
Second University of Naples
194 PUBLICATIONS **6,685** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 80 (2014) 569–578

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Evaluation of a large library of (thiazol-2-yl)hydrazones and analogues as histone acetyltransferase inhibitors: Enzyme and cellular studies

Simone Carradori [a,1], Dante Rotili [a,1], Celeste De Monte [a], Alessia Lenoci [a], Melissa D'Ascenzio [a], Veronica Rodriguez [a], Patrizia Filetici [b], Marco Miceli [c], Angela Nebbioso [c], Lucia Altucci [c,d], Daniela Secci [a,*], Antonello Mai [a,e,*]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
[b] Institute of Molecular Biology and Pathology (IBPM), CNR National Research Council of Italy, c/o Sapienza Università di Roma, 00185 Rome, Italy
[c] Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, vico L. De Crecchio 7, 80138 Naples, Italy
[d] CNR-IGB, Institute of Genetics and Biophysics, via P. Castellino, 80100 Naples, Italy
[e] Pasteur Institute, Cenci Bolognetti Foundation, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form
7 April 2014
Accepted 12 April 2014
Available online 15 April 2014

Keywords:
Apoptosis
Epigenetics
HAT inhibitor
Hydrazone
Thiazole

ABSTRACT

Recently we described some (thiazol-2-yl)hydrazones as antiprotozoal, antifungal and anti-MAO agents as well as Gcn5 HAT inhibitors. Among these last compounds, CPTH2 and CPTH6 showed HAT inhibition in cells and broad anticancer properties. With the aim to identify HAT inhibitors more potent than the two prototypes, we synthesized several new (thiazol-2-yl)hydrazones including some related thiazolidines and pyrimidin-4(3*H*)-ones, and we tested the whole library existing in our lab against human p300 and PCAF HAT enzymes. Some compounds (**1x**, **1c'**, **1d'**, **1i'** and **2m**) were more efficient than CPTH2 and CPTH6 in inhibiting the p300 HAT enzyme. When tested in human leukemia U937 and colon carcinoma HCT116 cells (100 μM, 30 h), **1x**, **1i'** and **2m** gave higher (U937 cells) or similar (HCT116 cells) apoptosis than CPTH6, and were more potent than CPTH6 in inducing cytodifferentiation (U937 cells).

1. Introduction

N^{ε}-Lysine acetylation was the first discovered post-translational modification (PTM) of canonical and variant histones, and it is considered a direct regulator of chromatin structure and function. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the enzymes involved in this equilibrium, acting as concerted gene repressors or activators [1]. Histones can be also remodelled by other PTMs including methylation, phosphorylation, sumoylation, poly-ADP-ribosylation, and ubiquitilation. These changes alter dynamically and reversibly the chromatin structure recruiting specific effector proteins and thereby influencing gene expression, DNA replication and repair, and chromosome condensation and segregation [2]. Recent studies also investigated lysine acetylation levels of non-histone proteins, and highlighted the numerous roles that HATs and HDACs play as multifunctional factors in a variety of other cellular processes and vital physiological functions [3]. Nuclear histone lysine acetyltransferases (type A HATs or KATs) are grouped into five distinct evolutionary conserved major families (based on structural homology in the primary sequence and biochemical mechanism of acetyl transfer): general control non-derepressible 5 (Gcn5)-related *N*-acetyltransferases (GNATs) (Gcn5p, PCAF, Elp3, Hat1, Hpa2, and Nut1), MYST (Esa1, Morf, Ybp2, Sas2, Sas3, Tip60, and Hbo1), p300 (adenoviral E1A-associated protein of 300 kDa)/CBP (CREB-binding protein), general transcription factors HATs, and nuclear hormone receptor-related HATs [4].

In view of the increasing evidence that associates HAT function with cancer development and progression, these enzymes are appealing as drug targets for the development of small molecule inhibitors [5]. It may seem paradoxical to seek inhibitors of both HATs and HDACs when these enzymes have opposing catalytic reactions. However, the biology of HATs and HDACs is complex and it is unlikely that a simple "on-off" acetylation model will apply to gene transcription and cancer development [6]. The correlation between epigenetic aberrations and cancer underscores the importance of epigenetic mechanisms which may be the result of some pleiotropic effects. This evidence may indicate that: (i) a disequilibrium based on both genetic and epigenetic disregulations

* Corresponding authors. Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.
E-mail addresses: daniela.secci@uniroma1.it, antonello.mai@uniroma1.it (A. Mai).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.ejmech.2014.04.042
0223-5234/

brings to tumorigenesis; (ii) the deregulation of selective epi-enzymes might be crucial for cancer thus emphasizing the relevance of tissue-selective disregulations. In addition, multiple epi-enzyme-containing complexes might be altered in tumorigenesis, thus repressing or activating both HDAC- or HAT-dependent targets into chromatin [7].

More in detail, mutations in several HAT genes have been observed in solid tumors [8]; furthermore, biallelic mutations of *EP300* have been identified in epithelial tumors [9]. In hematological malignancies, and less commonly in solid tumors, chromosomal translocations involving HAT genes such as *EP300*, *CREBBP*, *MYST3* and *MYST4* (hematological malignancies) or *MYST4* and *NCOA1* (solid tumors) have been reported [10]. The translocations involved in leukemogenesis seem to be due to aberrant acetylation caused by mistargeting of HATs. The HAT *NCOA3* gene is frequently amplified and overexpressed in human breast cancer and behaves as a classical oncogene [11].

A large number of HDAC inhibitors have already entered into the clinical arena, alone or in combination with other therapeutics, and two of them, vorinostat and romidepsin, have been approved by US FDA for the treatment of refractory cutaneous T-cell lymphoma [12]. Differently, only a few HAT inhibitors (HATi) have been described so far, and a limited number of them showed efficacy in preclinical cancer models, mainly because they suffer from absence of a recognized pharmacophore structure for chemical optimization, lack of specificity, and/or low cell-permeability (Fig. 1) [13]. Thus, the urgency to find new selective HATi as pharmacological tools has emerged as a priority especially in the clinic. Overall, the understanding of structure–activity relationship of newly developed molecules as well as the knowledge of their biological mechanisms of action applied to the medical field will pave the way for novel epigenetic approaches to fight human cancer [4].

The chemistry of thiazole-containing compounds is particularly interesting because of its capability to easily furnish valuable chemotherapeutics such as anticancer, antibacterial, antifungal, and antiprotozoal agents as well as monoamine oxidase inhibitors [14]. Recently, we reported some (thiazol-2-yl)hydrazones screened by a chemo-genomic approach in *S. cerevisiae* yeast cultures, demonstrating that some of them were endowed with selective inhibitory activity against the Gcn5 HAT subfamily by means of a fluorescent read-out, and showed a moderate inhibitory activity against U87-glioblastoma, and BE-neuroblastoma [15]. Successively, some of the most potent derivatives (CPTH2, now also provided by commercial suppliers as selective Gcn5 inhibitor, and CPTH6, Fig. 2) were used as pharmacological tools to unravel the superoxide-generating system in leukocytes [16] and as new therapeutic options for the treatment of a panel of leukemia cell lines, confirming the predominant inhibition of Gcn5 HAT activity (inhibition of H3/H4 histones and α-tubulin acetylation, concentration- and time-dependent inhibition of cell viability paralleled by accumulation of cells in the *G*0/*G*1 phase, depletion from the S/*G*2M phases, induction of apoptosis *via* mitochondrial pathway, and involvement of Bcl-2 and Bcl-xL proteins) [17]. Solid tumor cell lines from several origins were shown to be differently sensitive to CPTH6 treatment in terms of cell viability, and a correlation between the inhibitory efficacy on H3/H4 histones acetylation and cytotoxicity was found [17]. In addition, differentiating effect on leukemia and neuroblastoma cell lines was also induced by CPTH6 exposure [17]. We also found that CPTH6 had an effect on autophagy developed through the Atg-7-mediated elongation of autophagosomal membranes and the blockage of autophagic cargo degradation indicating a putative role of α-tubulin acetylation in CPTH6-induced alteration in autophagy [18].

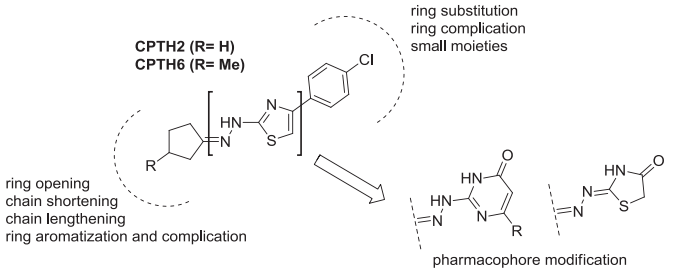


Fig. 2. Structural modifications applied on the reported lead compounds CPTH2 and CPTH6.

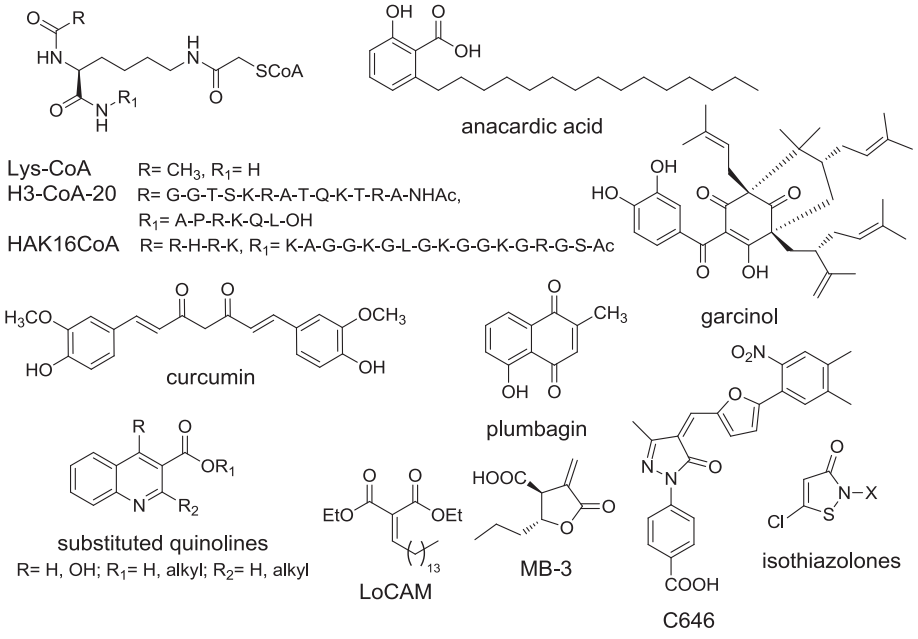


Fig. 1. Known HAT inhibitors.

In view of the above findings and our interest in designing new HAT inhibitors [19], we focused our attention on the (thiazol-2-yl) hydrazone scaffold and, having available a large in-house library of such compounds mostly assayed against other biological targets, [**14**c–f, h–j, **15**], we decided to test them against human HAT p300 and PCAF enzymes (Fig. 2) using a radiochemical assay and, for selected derivatives, against human U937 leukemia and HCT116 cells to determine their effects in cell cycle perturbation, apoptosis induction and cytodifferentiation.

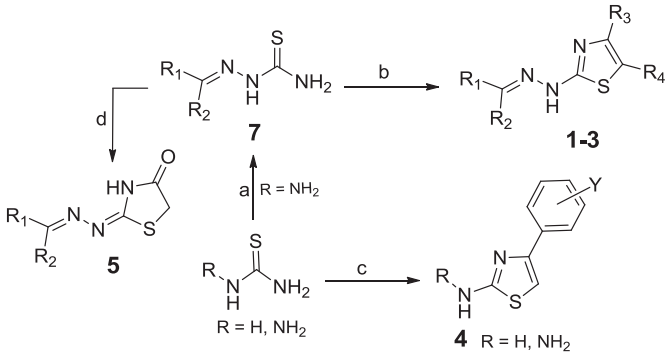
In this library, first we kept constant the 4-(4-chlorophenyl)-2-thiazolylhydrazone moiety of CPTH2 and CPTH6 replacing the cyclopentane portion with shorter and larger residues, either (cyclo)aliphatic or (hetero)aromatic, through processes of ring opening/chain shortening and chain lengthening/ring complication (**1a**–**j'**) (Fig. 3, section A).

Successively, we studied the influence of different substitutions at the C4-phenyl ring of the thiazole, or its replacement with smaller (methyl, ethoxycarbonyl) or larger (2-naphthyl, 3-chromenyl) groups, leaving the *iso*-propyl, (substituted)cyclopentyl, (substituted)cyclohexyl, or cycloheptyl hydrazone function at the thiazole C2 position (**2a**–**i'**) (Fig. 3, section B). Also the effect of methyl substitution at the C5 position of the thiazole ring has been explored (**3a**–**c**) (Fig. 3, section C). Lastly, some compounds have been designed and prepared by either deleting the hydrazone part (**4a**–**c**) (Fig. 3, section D) or replacing the thiazole nucleus with a thiazolidinone (**5a,b**) (Fig. 3, section E) or a 4-oxopyrimidin-2-yl nucleus (**6a**–**l**) (Fig. 3, section F).

2. Results and discussion

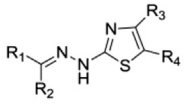
2.1. Chemistry

(Thiazol-2-yl)hydrazone derivatives (**1**–**3**) were synthesized in high yields according to an improved MW-assisted protocol developed in our laboratory as outlined in Scheme 1. Carbonylic compounds reacted directly with thiosemicarbazide in ethanol with catalytic amounts of acetic acid under MW irradiation (103 °C, 5 min, 300 W), and the obtained thiosemicarbazones **7a**–**j'** were subsequently converted into (thiazol-2-yl)hydrazones by Hantzsch reaction with α-bromo-substituted acetophenones/ketones/ketoesters in methanol under MW irradiation (90 °C, 10 min, 300 W). The 2-hydrazino- and 2-aminothiazoles **4a**–**c** [14a,b] were prepared by reaction of thiosemicarbazide or thiourea with the appropriate α-bromo-acetophenone in ethanol/acetic acid (Scheme 1). Thiazolidine compounds (**5**) were obtained by the same versatile thiosemicarbazone intermediates **7** by cyclization with ethyl bromoacetate and sodium acetate in methanol (Scheme 1).



Scheme 1. Synthesis of compounds **1**–**5**. *Reagents and conditions*: a) (for $R = NH_2$) R_1COR_2, CH_3COOH, EtOH, MW, 103 °C, 5 min, 300 W; b) $BrCH(R_4)COR_3$, MeOH, MW, 90 °C, 10 min, 300 W; c) $BrCH_2COPh(Y)$, CH_3COOH, EtOH, 12 h, rt; d) $BrCH_2COOEt$, CH_3COONa, MeOH, 2–6 h, rt.

The synthetic route followed for the preparation of 6 is depicted in Scheme 2. As reported previously [20], substituted ethyl



Section A

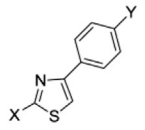
	R_1	R_2	R_3	R_4
1a	Me	Me	4-Cl-Ph	H
1b	Me	Et	4-Cl-Ph	H
1c	Et	Et	4-Cl-Ph	H
1d	Me	*n*-propyl	4-Cl-Ph	H
1e	Me	*i*-butyl	4-Cl-Ph	H
1f	Me	3-butenyl	4-Cl-Ph	H
1g	Me	*n*-butyl	4-Cl-Ph	H
1h	Me	*n*-pentyl	4-Cl-Ph	H
1i	Me	*n*-hexyl	4-Cl-Ph	H
1j	2-Me-cyclopentyl		4-Cl-Ph	H
1k	cyclohexyl		4-Cl-Ph	H
1l	2-Me-cyclohexyl		4-Cl-Ph	H
1m	3-Me-cyclohexyl		4-Cl-Ph	H
1n	(*R*)-(+)-3-Me-cyclohexyl		4-Cl-Ph	H
1o	4-Me-cyclohexyl		4-Cl-Ph	H
1p	4,4-Me_2-2-cyclohexenyl		4-Cl-Ph	H
1q	cycloheptyl		4-Cl-Ph	H
1r	cyclooctyl		4-Cl-Ph	H
1s	H	2-furyl	4-Cl-Ph	H
1t	H	2-thienyl	4-Cl-Ph	H
1u	H	3-pyridyl	4-Cl-Ph	H
1v	H	4-pyridyl	4-Cl-Ph	H
1w	H	1-naphthyl	4-Cl-Ph	H
1x	H	3(1*H*)-indolyl	4-Cl-Ph	H
1y	H	benzo[*d*][1,3]dioxol-5-yl	4-Cl-Ph	H
1z	Me	Ph	4-Cl-Ph	H
1a'	Me	2-furyl	4-Cl-Ph	H
1b'	Me	2-thienyl	4-Cl-Ph	H
1c'	Me	2-thiazolyl	4-Cl-Ph	H
1d'	Me	2-pyridyl	4-Cl-Ph	H
1e'	Me	3-pyridyl	4-Cl-Ph	H
1f'	Me	4-pyridyl	4-Cl-Ph	H
1g'	Me	cyclohexyl	4-Cl-Ph	H
1h'	Me	2-naphthyl	4-Cl-Ph	H
1i'	Me	2(2*H*)-chromenon-3-yl	4-Cl-Ph	H
1j'	2-carboxy-9(9*H*)-fluorenyl		4-Cl-Ph	H

Section B

	R_1	R_2	R_3	R_4
2a	Me	Me	Me	H
2b	cyclopentyl		Me	H
2c	Me	Me	COOEt	H
2d	cyclopentyl		COOEt	H
2e	3-Me-cyclopentyl		COOEt	H
2f	Me	Me	Ph	H
2g	cyclopentyl		Ph	H
2h	Me	Me	3-NO_2-Ph	H
2i	cyclopentyl		3-NO_2-Ph	H
2j	Me	Me	3-OCH_3-Ph	H
2k	cyclopentyl		3-OCH_3-Ph	H
2l	Me	Me	4-F-Ph	H
2m	cyclopentyl		4-F-Ph	H
2n	3-Me-cyclopentyl		4-F-Ph	H
2o	Me	Me	4-Br-Ph	H
2p	Me	Me	4-OCH_3-Ph	H
2q	cyclopentyl		4-OCH_3-Ph	H
2r	2-Me-cyclopentyl		4-OCH_3-Ph	H
2s	3-Me-cyclopentyl		4-OCH_3-Ph	H
2t	cyclohexyl		4-OCH_3-Ph	H
2u	2-Me-cyclohexyl		4-OCH_3-Ph	H
2v	3-Me-cyclohexyl		4-OCH_3-Ph	H
2w	3-Me-cyclohexyl*		4-OCH_3-Ph	H
2x	4-Me-cyclohexyl		4-OCH_3-Ph	H
2y	cycloheptyl		4-OCH_3-Ph	H
2z	3-Me-cyclopentyl		4-CN-Ph	H
2a'	Me	Me	2,4-Cl_2-Ph	H
2b'	cyclopentyl		2,4-Cl_2-Ph	H
2c'	3-Me-cyclopentyl		2,4-F_2-Ph	H
2d'	Me	Me	4-Ph-Ph	H
2e'	Me	Me	2-naphthyl	H
2f'	cyclopentyl		2-naphthyl	H
2g'	3-Me-cyclopentyl		2-naphthyl	H
2h'	Me	Me	2(2*H*)-chromenon-3-yl	H
2i'	3-Me-cyclopentyl		2(2*H*)-chromenon-3-yl	H

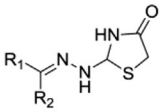
Section C

	R_1	R_2	R_3	R_4
3a	Me	Me	Ph	Me
3b	cyclopentyl		Ph	Me
3c	3-Me-cyclopentyl		Ph	Me



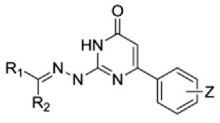
Section D

	X	Y
4a	-NHNH$_2$	H
4b	-NH$_2$	H
4c	-NH$_2$	Cl



Section E

	R_1	R_2
5a	Me	Me
5b	cyclopentyl	



Section F

	R_1	R_2	Z
6a	Me	Me	H
6b	Me	Me	3-NO_2
6c	Me	Me	4-Cl
6d	Me	Me	4-Br
6e	cyclopentyl		H
6f	cyclopentyl		3-NO_2
6g	cyclopentyl		4-Cl
6h	cyclopentyl		4-Br
6i	3-Me-cyclopentyl		H
6j	3-Me-cyclopentyl		3-NO_2
6k	3-Me-cyclopentyl		4-Cl
6l	3-Me-cyclopentyl		4-Br

Fig. 3. Library of (thiazol-2-yl)hydrazones and analogues described in this paper.

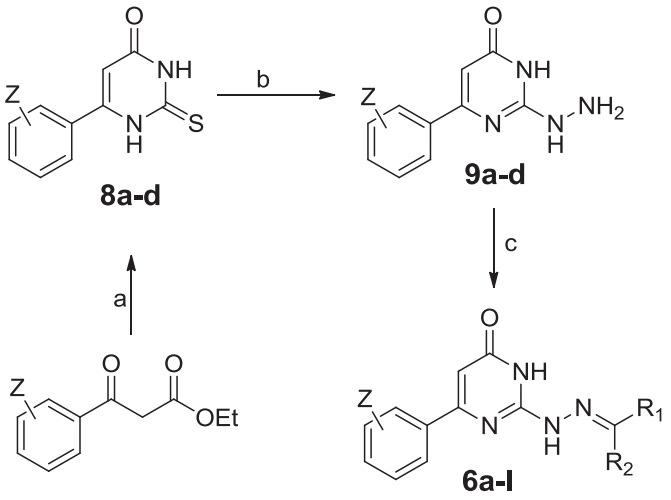
benzoylacetates and thiourea were added to an ethanolic solution of sodium, the resulting mixture was stirred at reflux and subsequent ring closure afforded the intermediates (8), which were then converted, in the presence of hydrazine monohydrate, to the corresponding (un)substituted 2-hydrazinyl-6-phenylpyrimidin-4(3*H*)-ones (9). The final compounds 6 were obtained by condensation between the intermediates (9) and the appropriate commercially available ketones in a refluxing solution of dry ethanol with glacial acetic acid as a catalyst.

2.2. Enzyme assays

Compounds **1**–**6** were tested at 100 μM against human p300 and PCAF (catalytic domains) using histone H3 as a substrate and [acetyl-^{3}H]-acetyl coenzyme A as an acetyl donor (HotSpot HAT activity assays). In these conditions, compounds **1**–**6** displayed very poor (if any) PCAF inhibiting activity (Tables S1–S5 in Supplementary Material). The percentages of p300 inhibitory activities for **1**–**6** tested at 100 μM are summarized in Fig. 4. Two compounds, **1x** and **1i'**, showed the highest p300 inhibition (>60%), and six further compounds (*i.e.*, **1c'**, **1d'**, **2i**, **2o**, **2i'** and **6k**) exhibited higher potency (>40%) than the two prototypes CPTH2 and CPTH6.

In the **1** series (section A) carrying the 4-chlorophenyl substituent at the C4-thiazole position, the replacement at the hydrazone function of the (3-methyl)cyclopentyl moiety of CPTH2 and -6 with smaller or larger, straight or branched, saturated or unsaturated (cyclo)alkyl portions (compounds **1a**–**r**) did not improve the p300 inhibitory activity of the derivatives. The introduction of (hetero) aryl substituents at this level was in general also ineffective, with some important exceptions. In particular, the insertion of a thiazol-2-yl or pyrid-2-yl ring at the hydrazone function yielded compounds (**1c'** and **1d'**) more potent than CPTHs in inhibiting p300. Among the bicyclic rings, the heteroaromatic (1*H*)-indol-3-yl and 2(2*H*)-chromenon-3-yl furnished the most potent derivatives (**1x** and **1i'**), while 2-naphthyl, benzo[*d*][1,3]dioxol-5-yl and 2-carboxy-9(9*H*)-fluorenyl substituents led to less potent analogues.

When the 4-chlorophenyl ring at the thiazole C4 was replaced with other (substituted)phenyl or (hetero)aromatic rings (compounds **2**, section B), an increase of p300 inhibitory activity respect to CPTHs was obtained with the introduction of a 3-nitrophenyl (**2i**), 4-bromophenyl (**2o**), or 2(2*H*)-chromenon-3-yl (**2i'**) portion



Scheme 2. Synthesis of compounds **6**. *Reagents and conditions*: a) thiourea, Na, dry EtOH, reflux; b) hydrazine monohydrate, reflux; c) R_1COR_2, dry EtOH, cat. CH_3COOH.

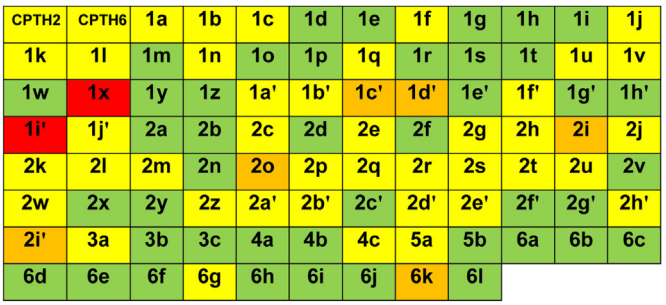


Fig. 4. Human p300 inhibitory activity of compounds **1**–**6**. Color code for inhibition: red, 60–80%; orange, 40–60%; yellow, 20–40%; green, <20% inhibition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at C4. The insertion of an additional methyl substituent at the thiazole-C5 position (compounds **3**, section C) did not improve the potency of the (thiazol-2-yl)hydrazones, as well as the removal of the hydrazine moiety (compounds **4**, section D) or the replacement of the thiazole with the thiazolidine ring (compounds **5**, section E). Among the 2-hydrazonopyrimidin-4(3*H*)-ones **6** (section F), compound **6k**, bearing the same substituents of CPTH6, displayed improved p300 inhibitory activity respect to the prototype.

Selected **1**, **2**, and **6** derivatives were also tested at 200 and 400 μM against human p300 (catalytic domain) to assess their percentages of inhibition. Data in Table 1 indicate that **1x**, **1d'** and **1i'** displayed the highest p300 inhibitory potency, with IC_{50} values of 77.6 ± 21.4 (**1x**), 99.3 ± 20.8 (**1d'**) and 78.3 ± 6.6 (**1i'**) μM, highlighting the crucial role of the 4-chlorophenyl substituent at the C4 position of the thiazole ring. Nevertheless, the high inhibition registered with **2h** and **2m** suggested the introduction of a 3-nitro- or 4-fluorophenyl at C4 as a valid alternative moiety. As regards to the hydrazone function, together with the *iso*-propyl and cyclopentyl residues also heterocycles such as 3(1*H*)-indolyl (**1x**), 2-pyridyl (**1d'**) and 2(2*H*)-chromenon-3-yl (**1i'**) furnished high inhibition.

2.3. Cellular studies

Selected compounds **1x**, **1c'**, **1d'**, **1i'**, **2h**, **2i**, **2m**, **2i'**, **6g** and **6k** were tested in human leukemia U937 and in human colon

Table 1
Human p300 inhibitory activities for the most active derivatives.[a]

Compound	% Inhibition		
	100 μM	200 μM	400 μM
1a	31 ± 1.7	45 ± 0.2	60 ± 2.2
1x	61 ± 3.4	79 ± 1.5	97 ± 0.4
1c'	46 ± 4.5	55 ± 1.4	67 ± 4.0
1d'	51 ± 1.6	69 ± 1.8	87 ± 3.4
1i'	62 ± 0.9	86 ± 5.6	97 ± 1.3
2g	37 ± 0.2	56 ± 2.2	54 ± 1.2
2h	33 ± 2.4	54 ± 1.6	76 ± 0.3
2i	44 ± 1.9	51 ± 1.7	60 ± 2.7
2k	31 ± 1.6	51 ± 1.2	62 ± 2.5
2m	34 ± 0.9	62 ± 2.4	91 ± 1.5
2o	42 ± 2.1	53 ± 2.3	63 ± 1.2
2q	33 ± 0.6	47 ± 2.0	57 ± 2.6
2h'	32 ± 0.3	46 ± 1.1	56 ± 2.1
2i'	40 ± 0.02	52 ± 1.8	69 ± 1.0
6g	31 ± 0.6	46 ± 1.8	54 ± 2.5
6k	41 ± 0.7	55 ± 2.9	64 ± 2.4
CPTH2	34 ± 4.4	47 ± 2.5	61 ± 2.0

[a] The assays were performed in duplicate.

carcinoma HCT116 cells at 100 μM for 30 h, to determine their effects on cell cycle progression and induction of apoptosis. In U937 cells, granulocytic cytodifferentiation at 30 h has been also evaluated. CPTH6, already known for its anticancer properties [17,18], was used as a reference drug.

In U937 cells, the treatment with **1d'** and **2m** among the tested derivatives, together with CPTH6, gave an arrest of cell cycle in the G1 phase, with lower percentages of cells in S phase respect to the control (Fig. 5A). For compounds **1x** and **1i'**, it was no possible to determine the changes in cell cycle due to the intense apoptosis. The pre-G1 peak in cell cycle was taken as an index of apoptosis induction. As shown in Fig. 5B, in U937 cells **1x**, **1i'** and **2m** elicited huge apoptosis (from 44 to 60%), they being 1.5/2-fold more potent than CPTH6 (% apoptosis: 29).

To confirm that the observed apoptotic effects of **1x**, **1i'** and **2m** in U937 cells were not due to off-target effects, such compounds were used in combination with CPTH6 (all at 100 μM for 30 h) in U937 cells, and the percentages of apoptosis were determined (Fig. 5C). Neither synergic nor additive effects were observed, thus confirming an on-target phenotype with these compounds.

On the basis of the relevance of histone acetylation changes on regulation of cell differentiation [25], we also investigated whether the selected compounds were able to induce granulocytic differentiation in U937 cells. The superficial antigen CD11c was taken as a marker of differentiation. After treatment of U937 cells with **1x, 1d', 1i'** and **2m** (100 μM, 30 h), a significant increase of the CD11c-positive cells was observed when compared with the control, after subtraction of the dead cells (propidium iodide (PI) positive cells) (Fig. 5D).

When tested in HCT116 cells, **2m**, **2i'** and, to a lower extent, **2h** gave a block in G1 phase of the cell cycle (Fig. 6A). As apoptotic inducers, **1x**, **1i'**, **2h** and **2m** were slightly less potent than CPTH6 displaying nearly 10% of apoptosis (CPTH6: 11.5%) (Fig. 6B).

The reversible acetylation of a broad range of histone and non-histone proteins is an epigenetic regulator in eukaryotic gene activation/deactivation. Aberrant HAT activity and mutation in several HAT genes are strictly related to tumorigenesis. Few HAT inhibitors have been characterized so far, and the development of new pharmacological agents is required.

By the use of thiazole chemistry, we prepared some (thiazol-2-yl)hydrazones as antimicrobial or anti-MAO agents [**14**c–f, h–j]. Other analogues were identified as Gcn5 HAT inhibitors in yeast, including CPTH2 and CPTH6 after recognized as valuable anti-tumor agents [15–18]. Since all these derivatives carried the same pharmacophoric core – the thiazole ring – linked to structurally diverse substituents at the thiazole C4 position and/or at the hydrazone moiety, we decided to increase our in-house thiazole library through the synthesis of further derivatives **2b,f,h,l,n,o,z,c',d'** including some thiazolidine (**5a,b**) and pyrimidin-4(3*H*)-one (**6a**–**l**) analogues, and to screen the whole library against human p300 and PCAF HAT enzymes, with the aim to identify compounds more potent than CPTH2 and CPTH6. From this screening, **1x**, **1c'**, **1d'**, **1i'** and **2m** were more efficient than the two prototypes in enzyme assays, highlighting the possibility to replace the (3-methyl)cyclopentyl group at the hydrazone moiety of CPTH2 and CPTH6 with a 3(1*H*)-indolyl or 2-pyridyl or 2(2*H*)-chromenon-3-yl moiety, and to insert at the thiazole-C4 position a 4-fluorophenyl instead of the 4-chlorophenyl ring typical of the two prototypes. When tested in human leukemia U937 cells at 100 μM for 30 h, **1x**, **1i'** and **2m** induced higher apoptosis and much more differentiation than CPTH6, used as reference drug. In colon carcinoma HCT116 cells, treated at 100 μM for 30 h, **1x**, **1i'**, **2h** and **2m** gave similar percentage of apoptosis as CPTH6. Further studies will be performed on such thiazole derivatives to assess their anticancer utility.

3. Conclusion

With the aim to identify HAT inhibitors more potent than our two promising prototypes (CPTH2 and CPTH6), we synthesized

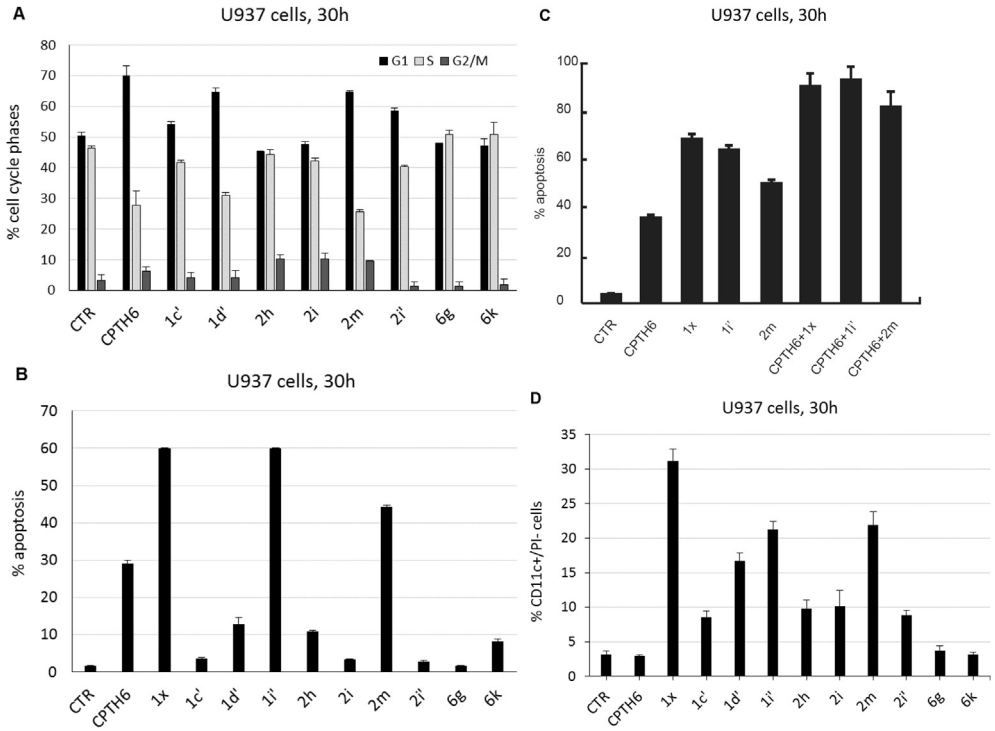


Fig. 5. Effects of selected derivatives **1x**, **1c'**, **1d'**, **1i'**, **2h**, **2i**, **2m**, **2i'**, **6g** and **6k** on cell cycle progression (A), apoptosis induction (B) and cytodifferentiation (D) in human leukemia U937 cells when tested at 100 μM for 30 h (C), percentages of apoptosis using **1x**, **1i'** and **2m** in combination with CPTH6 in U937 cells treatment at 100 μM for 30 h.

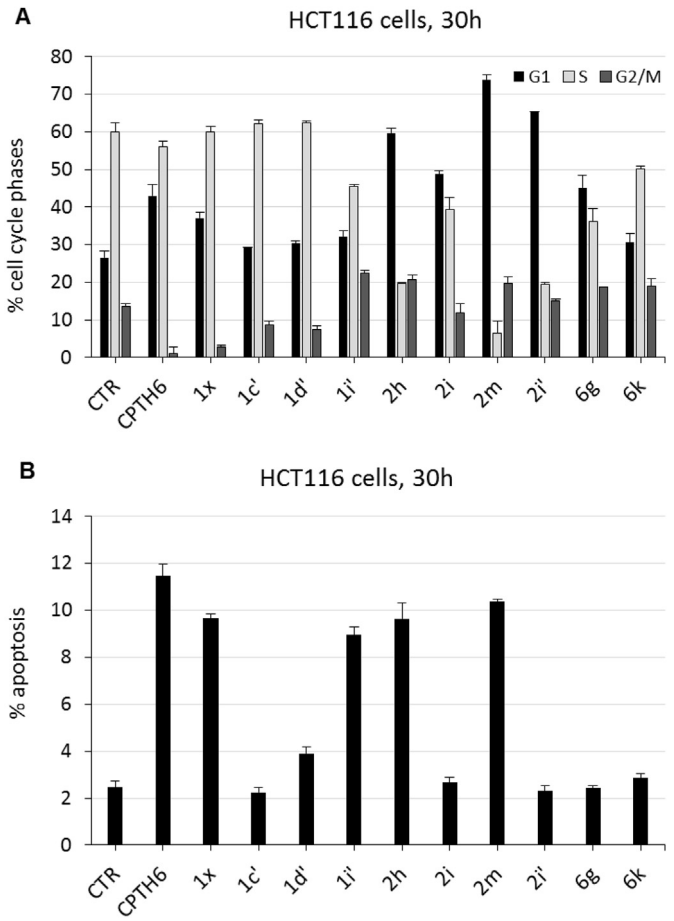


Fig. 6. Effects of selected derivatives **1x**, **1c'**, **1d'**, **1i'**, **2h**, **2i**, **2m**, **2i'**, **6g** and **6k** on cell cycle progression (A) and apoptosis induction (B) in human colon carcinoma HCT116 cells when tested at 100 μM for 30 h.

several new (thiazol-2-yl)hydrazones including some related thiazolidines and pyrimidin-4(3*H*)-ones to extrapolate broader structure–activity relationships within this scaffold. Moreover, we tested them, along with our in-house library [15], against human p300 and PCAF HAT enzymes *in vitro* with a radiochemical assay. Compounds **1x**, **1c'**, **1d'**, **1i'** and **2m** were potent p300 inhibitors in the micromolar range. In U937 and HCT116 cells at 100 μM, **1x**, **1i'** and **2m** determined remarkable apoptosis and cytodifferentiation.

4. Experimental section

4.1. Chemistry

Starting materials and reagents were obtained from commercial suppliers (Aldrich, Milan (Italy) and Lancaster Synthesis GmbH, Milan (Italy)) and were used without further purification. Microwave-assisted reactions were performed in a Biotage Initiator™ 2.0 (Uppsala, Sweden). Melting points (mp) were determined by the capillary method on an FP62 apparatus (Mettler-Toledo) and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC 400 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. Chemical shifts are expressed as δ units (ppm) relative to TMS. Coupling constants *J* are expressed in hertz (Hz). Elemental analyses for C, H, and N were determined with a Perkin–Elmer 240 B microanalyzer and the analytical results were ≥95% purity for all compounds. All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F_{254} Merck) with spots visualized by UV light. Preparative flash column chromatography was carried out on silica gel (230–400 mesh, G60 Merck). Organic solutions were dried over anhydrous sodium sulfate. Concentration and evaporation of the solvent after reaction or extraction was carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure of ca. 20 Torr.

4.1.1. General procedure for the synthesis of thiosemicarbazone intermediates (*7*)

Carbonylic compound (3.4 mmol) and the corresponding catalyst (glacial acetic acid) were added to a suspension of thiosemicarbazide (0.30 g, 3.4 mmol) in 2 mL of absolute ethanol in a 5 mL vessel suitable for microwave reactor (2.45 GHz high-frequency microwaves, power range 0–300 W). The vessel was sealed, the mixture pre-stirred for 30 s and then heated by microwave irradiation for 5 min at fixed temperatures (103 °C). If not set, the irradiation power reaches its maximum at the beginning of reaction and it subsequently decreases to lower and constant values. The vial internal temperature was controlled by an equipped IR sensor. After cooling in a stream of pressurized air the reaction mixture was filtered and the obtained solid washed with petroleum ether, *n*-hexane, and diethyl ether. The crude mixture was purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane).

4.2. Characterization data for new compounds

4.2.1. General procedure for the synthesis of (thiazol-2-yl) hydrazone derivatives ***1***–***3***

The appropriate thiosemicarbazone (**7**) (1.5 mmol) was added to a solution of the proper α-bromo-acetophenone in 2 mL of methanol. The mixture was pre-stirred in a sealed vessel for 1 min and then heated up by microwave irradiation for 10 min at fixed temperatures (90 °C). The reaction mixture was cooled down with pressurized air, filtered, and the obtained solid washed with n-hexane and diethyl ether. The crude mixture was purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane) to give all compounds in high yields.

*4.2.1.1. 1-Cyclopentylidene-2-(4-methylthiazol-2-yl)hydrazine (**2b**).* White powder, yield 79%, mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 1.90–1.94 (m, 2H, CH_2, cyclopentyl), 1.95–2.00 (m, 2H, CH_2, cyclopentyl), 2.35 (s, 3H, CH_3), 2.50–2.54 (m, 2H, CH_2, cyclopentyl), 2.61–2.64 (m, 2H, CH_2, cyclopentyl), 6.15 (s, 1H, C_5H-thiazole), 12.50 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_9H_{13}N_3S$: C, 55.35%; H, 6.71%; N, 21.52%. Found C, 55.60%; H, 6.44%; N, 21.87%.

*4.2.1.2. 1-(4-Phenylthiazol-2-yl)-2-(propan-2-ylidene)hydrazine (**2f**).* White solid, yield 77%, mp 220–223 °C; ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 6.71 (s, 1H, C_5H-thiazole), 7.49–7.51 (m, 3H, Ar), 7.73–7.75 (m, 2H, Ar), 12.47 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_{12}H_{13}N_3S$: C, 62.31; H, 5.66; N, 18.17. Found: C, 62.03; H, 5.89; N, 17.91.

*4.2.1.3. 1-(4-(3-Nitrophenyl)thiazol-2-yl)-2-(propan-2-ylidene)hydrazine (**2h**).* Light yellow solid, yield 78%, mp 215–216 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 6.97 (s, 1H, C_5H-thiazole), 7.62–7.66 (m, 1H, Ar), 8.12–8.14 (m, 1H, Ar), 8.19–8.22 (m, 1H, Ar), 8.56–8.57 (m, 1H, Ar), 12.42 (bs, 1H, NH, D_2O exch). *Anal.* Calcd for $C_{12}H_{12}N_4O_2S$: C, 52.16; H, 4.38; N, 20.28. Found: C, 52.30; H, 4.22; N, 20.16.

*4.2.1.4. 1-(4-(4-Fluorophenyl)thiazol-2-yl)-2-(propan-2-ylidene)hydrazine (**2l**).* Light yellow solid, yield 72%, mp 185–187 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 6.65 (s, 1H, C_5H-

thiazole), 7.21–7.23 (d, J = 7.0 Hz, 2H, Ar), 7.72–7.74 (d, J = 7.0 Hz, 2H, Ar), 12.64 (br s, 1H, NH, D_2O exch.). *Anal.* Calcd. for $C_{12}H_{12}FN_3S$: C, 57.81; H, 4.85; N, 16.85. Found: C, 57.65; H, 4.79; N, 16.64.

*4.2.1.5. 1-(4-(4-Fluorophenyl)thiazol-2-yl)-2-(3-methylcyclopentylidene)hydrazine (**2n**).* Yellow powder, yield 81%, mp 225–227 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 3H, CH_3), 1.38–1.40 (m, 1H, cyclopentyl), 1.50–1.51 (m, 1H, cyclopentyl), 2.01–2.03 (m, 1H, cyclopentyl), 2.17–2.19 (m, 1H, cyclopentyl), 2.23–2.25 (m, 1H, cyclopentyl), 2.57–2.58 (m, 1H, cyclopentyl), 2.71–2.73 (m, 1H, cyclopentyl), 6.63 (s, 1H, C_5H-thiazole), 7.18–7.20 (d, J = 7.7 Hz, 2H, Ar), 7.70–7.72 (d, J = 7.7 Hz, 1H, Ar), 12.23 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_{15}H_{16}FN_3S$: C, 62.26%; H, 5.57%; N, 14.52%. Found C, 62.41%; H, 5.33%; N, 14.87%.

*4.2.1.6. 1-(4-(4-Bromophenyl)thiazol-2-yl)-2-(propan-2-ylidene)hydrazine (**2o**).* Light brown solid, yield 73%, mp 240–243 °C; ^{1}H NMR (DMSO-d_6) δ 1.94 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 7.32 (s, 1H, C_5H-thiazole), 7.60–7.63 (d, J = 10.0 Hz, 2H, Ar), 7.79–7.81 (d, J = 10.0 Hz, 2H, Ar), 10.78 (br s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{12}H_{12}BrN_3S$: C 46.46; H, 3.90; N, 13.55. Found: C 46.59; H, 4.06; N, 13.32.

*4.2.1.7. 1-(4-(4-cyanophenyl)thiazol-2-yl)-2-(3-methylcyclopentylidene)hydrazine (**2z**).* Yellow powder, yield 89%; mp 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 3H, CH_3), 1.54–1.55 (m, 1H, cyclopentyl), 1.56–1.58 (m, 1H, cyclopentyl), 1.59–1.61 (m, 1H, cyclopentyl), 2.36–2.38 (m, 1H, cyclopentyl), 2.52–2.54 (m, 1H, cyclopentyl), 2.66–2.68 (m, 1H, cyclopentyl), 2.83–2.85 (m, 1H, cyclopentyl), 6.87 (s, 1H, C_5H-thiazole), 7.77–7.78 (d, J = 8.2 Hz, 2H, Ar), 7.84–7.87 (d, J = 8.2 Hz, 2H, Ar), 12.15 (bs, 1H, (*E*)-NH, D_2O exch.), 14.21 (bs, 1H, (*Z*)-NH, D_2O exch.). *Anal.* Calcd for $C_{16}H_{16}N_4S$: C, 64.84%; H, 5.44%; N, 18.90%. Found C, 65.08%; H, 5.26%; N, 19.11%.

*4.2.1.8. 1-(4-(2,4-Difluorophenyl)thiazol-2-yl)-2-(3-methylcyclopentylidene)hydrazine (**2c'**).* Light yellow powder, yield 83%, mp 131–134 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 3H, CH_3), 1.42–1.45 (m, 1H, cyclopentyl), 1.47–1.51 (m, 1H, cyclopentyl), 2.43–2.46 (m, 1H, cyclopentyl), 2.61–2.64 (m, 1H, cyclopentyl), 2.67–2.69 (m, 1H, cyclopentyl), 2.78–2.80 (m, 1H, cyclopentyl), 2.84–2.2.86 (m, 1H, cyclopentyl), 6.91 (s, 1H, C_5H-thiazole), 6.94–6.99 (m, 1H, Ar) 7.07–7.11 (m, 1H, Ar), 7.88–7.91 (m, 1H, Ar), 12.36 (bs, 1H, (*E*)-NH, D_2O exch.), 14.40 (bs, 1H, (*Z*)-NH, D_2O exch.). *Anal.* Calcd for $C_{15}H_{15}F_2N_3S$: C, 58.62%; H, 4.92%; N, 13.67%. Found C, 58.40%; H, 5.17%; N, 13.92%.

*4.2.1.9. 1-(4-(Biphenyl)thiazol-2-yl)-2-(propan-2-ylidene)hydrazine (**2d'**).* Brown solid, yield 69%, mp 241–243 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 6.73 (s, 1H, C_5H-thiazole), 7.25–7.52 (m, 9H, Ar), 12.40 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_{18}H_{17}N_3S$: C, 70.33; H, 5.57; N, 13.67. Found: C, 70.64; H, 5.84; N, 13.90.

4.3. General procedure for the synthesis of derivatives **4**

Thiourea or thiosemicarbazide (1.5 mmol) was added to a solution of the correspondent α-bromo-acetophenone in 2 mL of methanol. The mixture was pre-stirred in a sealed vessel for 1 min and then heated up by microwave irradiation for 10 min at fixed temperatures (90 °C). The reaction mixture was cooled down with pressurized air, filtered, and the obtained solid washed with n-hexane and diethyl ether. The crude mixture was purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane) to give all compounds in high yields.

4.4. General procedure for the synthesis of thiazolidine derivatives (**5**)

Equimolar amounts of the prepared thiosemicarbazone (**7**, 5 mmol) and ethyl bromoacetate (5 mmol), both suspended in 50 mL of methanol, were reacted at room temperature under magnetic stirring for 2–6 h in the presence of 5 mmol of CH_3COONa. The obtained solution was poured into ice and extracted with $CHCl_3$ (3 × 50 mL), dried over anhydrous sodium sulphate, and evaporated. The crude product has been purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane).

4.5. 2-(2-(Propan-2-ylidene)hydrazono)thiazolidin-4-one (**5a**)

White powder, yield 89%, mp 170–174 °C; ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 3.70 (s, 2H, CH_2, thiazolidinone), 11.91 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_6H_9N_3OS$: C, 42.09%; H, 5.30%; N, 24.54%. Found C, 41.82%; H, 5.12%; N, 24.79%.

4.6. 2-(2-Cyclopentylidenehydrazono)thiazolidin-4-one (**5b**)

White powder, yield 90%, mp 207–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79–1.82 (m, 4H, cyclopentyl), 2.50–2.53 (m, 4H, cyclopentyl), 3.79 (s, 2H, CH_2-thiazolidinone), 11.77 (bs, 1H, NH, D_2O exch.). *Anal.* Calcd for $C_8H_{11}N_3OS$: C, 48.71%; H, 5.62%; N, 21.30%. Found C, 48.99%; H, 5.34%; N, 21.67%.

4.7. General procedure for the preparation of 6-(substituted) phenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-ones (**8**)

4.7.1. Example: 6-(4-bromophenyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (**8d**)

Sodium metal (1.3 g, 56.1 mg-formula) was dissolved in 30 mL of absolute ethanol, then thiourea (2.9 g, 38.2 mmol) and 4-bromobenzoylacetate (6.9 g, 25.5 mmol) were added in sequence to the clear solution. The mixture was heated at reflux for 12 h. The resulting mixture was cooled at room temperature, the solvent was distilled *in vacuo* at 40–50 °C, and the residue was dissolved in a little amount of water to obtain a basic solution which was extracted with diethyl ether (2 × 50 mL). The basic aqueous solution was acidified while cooling with ice bath to pH = 2 to give a precipitate that was filtered and washed with water. The resulting compound was a white solid used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.11 (s, 1H, CH-pyrimidine ring), 7.64–7.66 (d, 2H, Ar), 7.69–7.71 (d, 2H, Ar), 12.56 (s, 2H, NH).

6-phenyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**8a**). See ref. [20a].
6-(3-nitrophenyl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**8b**). White powder, yield 65%, mp > 250 °C.
6-(4-chlorophenyl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**8c**). See ref. [20b].
6-(4-bromophenyl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**8d**). White powder, yield 86%, mp > 250 °C.

5. General procedure for the preparation of 2-hydrazinyl-6-(substituted)phenylpyrimidin-4(3*H*)-ones (9)

5.1. Example: 6-(4-chlorophenyl)-2-hydrazinylpyrimidin-4(3H)-one (**9c**)

A mixture of 6-(4-chlorophenyl)-3,4-dihydro-2-thiopyrimidin-4(3*H*)-one **8b** (500 mg, 2.1 mmol) and hydrazine monohydrate (7.15 mL, 7.36 g, 0.15 mol) was stirred at reflux for 4 h. Afterwards,

hydrazine was evaporated under reduced pressure. The residue was then triturated with water for 30 min. The obtained precipitate was filtered off and subsequently washed with water to give a white powder used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.11 (s, 1H, CH-pyrimidine ring), 7.49–7.51 (d, 2H, Ar), 7.98–8.01 (d, 2H, Ar), 8.57 (s, 1H, NH).

2-hydrazinyl-6-phenylpyrimidin-4(3*H*)-one (9a). White powder, yield 51%, mp 221–223 °C

2-hydrazinyl-6-(3-nitrophenyl)pyrimidin-4(3*H*)-one (**9b**). White powder, yield 62%, mp 227–229 °C

6-(4-chlorophenyl)-2-hydrazinylpyrimidin-4(3*H*)-one (**9c**). White powder, yield 59%, mp 243–245 °C

6-(4-bromophenyl)-2-hydrazinylpyrimidin-4(3*H*)-one (**9d**). White powder, yield 56%, mp > 250 °C

6. General procedure for the preparation of 2-(2-alkylidenehydrazinyl)-6-(substituted)phenylpyrimidin-4(3*H*)-ones (6). Example: 2-(2-cyclopentylidenehydrazinyl)-6-(3-nitrophenyl)pyrimidin-4(3*H*)-one (6f)

A mixture of 2-hydrazinyl-6-(3-nitrophenyl)pyrimidin-4(3*H*)-one **9b** (80 mg, 0.32 mmol), cyclopentanone (0.14 mL, 0.14 g, 1.6 mmol) and a catalytic amount of glacial acetic acid in dry ethanol (2 mL) was heated at reflux for 2 h. After cooling at room temperature, from the reaction mixture a white solid separated, which was collected by filtration, washed with ethanol and petroleum ether to provide the desired compound as a white solid finally purified by recrystallization from ethanol.

*6.1. 6-Phenyl-2-(2-(propan-2-ylidene)hydrazinyl)pyrimidin-4(3H)-one (**6a**)*

White powder, yield 78%, mp 232–234 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.97 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 6.24 (s, 1H, CH-pyrimidine), 7.45–7.47 (m, 3H, Ar), 7.99–8.01 (m, 2H, Ar), 10.49 (s, 2H, NH). *Anal.* Calcd for $C_{13}H_{14}N_4O$: C, 64.45%; H, 5.82%; N, 23.13%. Found C, 64.78%; H, 5.96%; N, 22.81%.

*6.2. 6-(3-Nitrophenyl)-2-(2-(propan-2-ylidene)hydrazinyl) pyrimidin-4(3H)-one (**6b**)*

White powder, yield 75%, mp 217–219 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.97 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 6.32 (s, 1H, CH-pyrimidine), 7.29–7.33 (m, 1H, Ar), 7.47–7.53 (m, 1H, Ar), 7.82–7.87 (m, 2H, Ar), 10.47–10.54 (s, 2H, NH). *Anal.* Calcd for $C_{13}H_{13}N_5O_3$: C, 54.35%; H, 4.56%; N, 24.38%. Found C, 54.11%; H, 4.39%; N, 24.61%.

*6.3. 6-(4-Chlorophenyl)-2-(2-(propan-2-ylidene)hydrazinyl) pyrimidin-4(3H)-one (**6c**)*

White powder, yield 81%, mp 237–239 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.96 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 6.26 (s, 1H, CH-pyrimidine), 7.51–7.54 (d, 2H, Ar), 8.02–8.05 (d, 2H, Ar), 10.50–10.62 (s, 2H, NH). *Anal.* Calcd for $C_{13}H_{13}ClN_4O$: C, 56.42%; H, 4.74%; N, 20.25%. Found C, 56.74%; H, 4.89%; N, 20.02%.

*6.4. 6-(4-Bromophenyl)-2-(2-(propan-2-ylidene)hydrazinyl) pyrimidin-4(3H)-one (**6d**)*

White powder, yield 69%, mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.96 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 6.26 (s, 1H, CH-pyrimidine), 7.66 (d, 2H, Ar), 7.96 (d, 2H, Ar), 10.53 (s, 2H, NH). *Anal.* Calcd for $C_{13}H_{13}BrN_4O$: C, 48.62%; H, 4.08%; N, 17.44%. Found C, 48.36%; H, 3.89%; N, 17.76%.

*6.5. 2-(2-Cyclopentylidenehydrazinyl)-6-phenylpyrimidin-4(3H)-one (**6e**)*

White powder, yield 80%, mp 231–233 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.70–1.72 (m, 2H, cyclopentyl ring), 1.78–1.80 (m, 2H, cyclopentyl ring), 2.42 (m, 4H, cyclopentyl ring), 6.23 (s, 1H, CH-pyrimidine), 7.45–7.47 (m, 3H, Ar), 7.99–8.01 (m, 2H, Ar), 10.39 (s, 2H, NH). *Anal.* Calcd for $C_{15}H_{16}N_4O$: C, 67.15%; H, 6.01%; N, 20.88%. Found C, 67.44%; H, 6.19%; N, 20.61%.

*6.6. 2-(2-Cyclopentylidenehydrazinyl)-6-(3-nitrophenyl) pyrimidin-4(3H)-one (**6f**)*

White powder, yield 67%, mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.69–1.81 (m, 4H, cyclopentyl ring), 2.41 (m, 4H, cyclopentyl ring), 6.31 (s, 1H, s, CH-pyrimidine), 7.29–7.32 (m, 1H, Ar), 7.47–7.53 (m, 1H, Ar), 7.82–7.87 (m, 2H, Ar), 10.38–10.49 (s, 2H, NH). *Anal.* Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50%; H, 4.83%; N, 22.35%. Found C, 57.23%; H, 4.79%; N, 22.68%.

*6.7. 6-(4-Chlorophenyl)-2-(2-cyclopentylidenehydrazinyl) pyrimidin-4(3H)-one (**6g**)*

White powder, yield 72%, mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.69–1.74 (m, 2H, cyclopentyl ring), 1.76–1.81 (m, 2H, cyclopentyl ring), 2.39–2.43 (m, 4H, cyclopentyl ring), 6.26 (s, 1H, CH-pyrimidine), 7.52 (d, 2H, Ar), 8.03 (d, 2H, Ar), 10.41 (s, 2H, NH). *Anal.* Calcd for $C_{15}H_{15}ClN_4O$: C, 59.51%; H, 4.99%; N, 18.51%. Found C, 59.76%; H, 5.12%; N, 18.24%.

*6.8. 6-(4-Bromophenyl)-2-(2-cyclopentylidenehydrazinyl) pyrimidin-4(3H)-one (**6h**)*

White powder, yield 88%, mp 237–240 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.67–1.81 (m, 4H, cyclopentyl ring), 2.40–2.41 (m, 4H, cyclopentyl ring), 6.26 (s, 1H, CH-pyrimidine), 7.66 (m, 2H, Ar), 7.96 (m, 2H, Ar), 10.44 (s, 2H, NH). *Anal.* Calcd for $C_{15}H_{15}BrN_4O$: C, 51.89%; H, 4.35%; N, 16.14%. Found C, 52.17%; H, 4.46%; N, 15.97%.

*6.9. 2-(2-(3-Methylcyclopentylidene)hydrazinyl)-6-phenylpyrimidin-4(3H)-one (**6i**)*

White powder, yield 73%, mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.03 (m, 3H, CH_3), 1.24–1.29 (m, 1H, cyclopentyl ring), 1.93–2.14 (m, 3H, cyclopentyl ring), 2.28–2.38 (m, 1H, cyclopentyl ring), 2.51–2.69 (m, 2H, cyclopentyl ring), 6.22 (s,1H, CH-pyrimidine), 7.46 (m, 3H, Ar), 8.00 (m, 2H, Ar), 10.39 (s, 2H, NH). *Anal.* Calcd for $C_{16}H_{18}N_4O$: C, 68.06%; H, 6.43%; N, 19.84%. Found C, 68.29%; H, 6.54%; N, 19.62%.

*6.10. 2-(2-(3-Methylcyclopentylidene)hydrazinyl)-6-(3-nitrophenyl)pyrimidin-4(3H)-one (**6j**)*

White powder, yield 69%, mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.02–1.06 (m, 3H, CH_3), 1.27–1.41 (m, 1H, cyclopentyl ring), 1.91–2.07 (m, 3H, cyclopentyl ring), 2.31–2.59 (m, 3H, cyclopentyl ring), 6.31 (s, 1H, CH-pyrimidine), 7.29–7.32 (m, 1H, Ar), 7.47–7.51 (m, 1H, Ar), 7.81–7.86 (m, 2H, Ar), 10.35–10.47 (s, 2H, NH). *Anal.* Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71%; H, 5.23%; N, 21.39%. Found C, 58.47%; H, 5.11%; N, 21.64%.

6.11. 6-(4-chlorophenyl)-2-(2-(3-methylcyclopentylidene) hydrazinyl)pyrimidin-4(3H)-one (**6k**)

White powder, yield 79%, mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (m, 3H, CH_3), 1.47–1.52 (m, 1H, cyclopentyl ring), 1.91 (m, 1H, cyclopentyl ring), 2.01–2.19 (m, 2H, cyclopentyl ring), 2.36–2.40 (m, 1H, cyclopentyl ring), 2.57–2.70 (m, 2H, cyclopentyl ring), 6.26 (s, 1H, CH-pyrimidine), 7.52 (d, 2H, Ar), 8.03 (d, 2H, Ar), 10.34–10.41 (s, 2H, NH). *Anal.* Calcd for $C_{16}H_{17}ClN_4O$: C, 60.66%; H, 5.41%; N, 17.69%. Found C, 60.94%; H, 5.57%; N, 17.33%.

6.12. 6-(4-bromophenyl)-2-(2-(3-methylcyclopentylidene) hydrazinyl)pyrimidin-4(3H)-one (**6l**)

White powder, yield 70%, mp 215–217 °C; ^{1}H NMR (DMSO-d_6) δ ppm 1.04 (d, 3H, CH_3), 1.35–1.40 (m, 1H, cyclopentyl ring), 1.96–2.06 (m, 3H, cyclopentyl ring), 2.30–2.37 (m, 1H, cyclopentyl ring), 2.53–2.58 (m, 2H, cyclopentyl ring), 6.26 (s, 1H, CH-pyrimidine), 7.66 (d, 2H, Ar), 7.96 (d, 2H, Ar), 10.36–10.48 (s, 2H, NH). *Anal.* Calcd for $C_{16}H_{17}BrN_4O$: C, 53.20%; H, 4.74%; N, 15.51%. Found C, 53.58%; H, 4.87%; N, 15.13%.

7. KAT assays

The effect of tested derivatives on the catalytic activity of p300 and PCAF was determined in a HotSpot HAT activity assay by Reaction Biology Corporation (Malvern, PA, USA) according to the company's standard operating procedure. In brief, the recombinant catalytic domains of PCAF (aa 492–658) or p300 (aa 1284–1673) were incubated with histone H3 as substrate (5 μM) and [Acetyl-^{3}H]-Acetyl Coenzyme A (3.08 μM) as an acetyl donor in reaction buffer (50 mM Tris–HCl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF, 1% DMSO) for 1 h at 30 °C in the presence or absence of a dose titration of the compounds. Histone H3 acetylation was assessed by liquid scintillation. Data at fixed doses were performed in duplicate. IC_{50} values were analyzed using Excel and GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA).

8. Biology

8.1. Cell culture

The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI-1640 medium (SIGMA) supplemented with 10% heat-inactivated fetal bovine serum (HYCLONE), penicillin G (100 units/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), amphotericin B (250 mg/mL). At the time indicated inductions were performed with ligands.

8.2. Cell cycle analysis

Cells were plated (2×10^5 cells/mL) and collected after stimulation. Then they were centrifuged and suspended in a solution containing 1× PBS, sodium citrate 0.1%, NP40 0.1% and propidium iodide 50 mg/mL. After 30 min of incubation at room temperature in the dark, cell cycle was evaluated by flow cytometry (FACSCalibur, Becton Dickinson Inc.) and analyzed with the program Mod Fit V3 (Verity).

8.3. FACS analysis of apoptosis

Apoptosis was measured as pre-G1 analyzed by FACS with Cell Quest software (Becton Dickinson) as previously reported [20].

8.4. Differentiation

The cells after centrifugation were resuspended in 10 μL of conjugate phycoerythrine CD11c (CD11c-PE, PharMingen). Control samples were incubated with 10 μL of mouse IgG1 PE conjugated; after incubation for 1 h at 4 °C in the dark, the cells were washed in PBS and resuspended in 500 μL PBS containing propidium iodide (0.25 μg/mL). The samples were then analyzed according to standard flow cytometry (FACSCalibur, BD).

Acknowledgements

This work was supported by FIRB RBFR10ZJQT, Sapienza Ateneo Project 2012, IIT-Sapienza Project, AIRC n° 11812, FP7 Project BLUEPRINT/282510 and COST/TD0905.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.042.

References

[1] a) X.-J. Yang, E. Seto, Oncogene 26 (2007) 5310–5318;
b) B.R. Selvi, T.K. Kundu, Biotechnology Journal 4 (2009) 375–390.
[2] A. Ganesan, L. Nolan, S.J. Crabb, G. Packham, Current Cancer Drug Targets 9 (2009) 963–981.
[3] a) K. Sadoul, C. Boyault, M. Pabion, S. Khochbin, Biochimie 90 (2008) 306–312;
b) S. Carradori, D. Secci, A. Mai, Expert Opinion on Therapeutic Patents 21 (2011) 1651–1656.
[4] K.K. Lee, J.L. Workman, Nature Reviews. Molecular Cell Biology 8 (2007) 284–295.
[5] A.G. Muntean, J.L. Hess, The American Journal of Pathology 175 (2009) 1353–1361.
[6] a) S. Sharma, T.K. Kelly, P.A. Jones, Carcinogenesis 31 (2010) 27–36;
b) M. Tovota, H. Suzuki, E. Yamamoto, H. Yamano, K. Imai, Y. Shinomura, Epigenomics 1 (2009) 291–299.
[7] K.L. Sommer, N.H. Myrtue, H.L. Lotte, European Journal of Pharmacology 625 (2009) 131–142.
[8] N.G. Iyer, H. Ozdag, C. Caldas, Oncogene 23 (2004) 4225–4231.
[9] a) M. Muraoka, M. Konishi, R. Kikuchi-Yanoshita, K. Tanaka, N. Shitara, J.M. Chong, T. Iwama, M. Miyaki, Oncogene 12 (1996) 1565–1569;
b) S.A. Gayther, S.J. Batley, L. Linger, A. Bannister, K. Thorpe, S.F. Chin, Y. Daigo, P. Russell, A. Wilson, H.M. Sowter, J.D. Delhanty, B.A. Ponder, T. Kouzarides, C. Caldas, Nature Genetics 24 (2000) 300–303.
[10] a) K. Shigeno, H. Yoshida, L. Pan, J.M. Luo, S. Fujisawa, K. Naito, S. Nakamura, K. Shinjo, A. Takeshita, R. Ohno, K. Ohnishi, Cancer Letters 213 (2004) 11–20;
b) K. Ida, I. Kitabayashi, T. Taki, M. Taniwaki, K. Noro, M. Yamamoto, M. Ohki, Y. Hayashi, Blood 90 (1997) 4699–4704;
c) N. Kakazu, T. Abe, M. Ohki, Leukemia 15 (2001) 89–94;
d) M. Chaffanet, L. Gressin, C. Preudhomme, V. Soenen-Cornu, D. Birnbaum, M.J. Pebusque, Genes, Chromosomes & Cancer 28 (2000) 138–144;
e) O.M. Sobulo, J. Borrow, R. Tomek, S. Reshmi, A. Harden, B. Schlegelberger, D. Housman, N.A. Doggett, J.D. Rowley, N.J. Zeleznik-Le, Proceedings of the National Academy of Sciences of the United States of America 94 (1997) 8732–8737;
f) J.L. Vizmanos, M.J. Larrayoz, I. Lahortiga, F. Floristan, C. Alvarez, M.D. Odero, F.J. Novo, M.J. Calasanz, Genes, Chromosomes & Cancer 36 (2003) 402–405.
[11] a) S.F. Chin, Y. Wang, N.P. Thorne, A.E. Teschendorff, S.E. Pinder, M. Vias, A. Naderi, I. Roberts, N.L. Barbosa-Morais, M.J. Garcia, N.G. Iyer, T. Kranjac, J.F. Robertson, S. Aparicio, S. Tavaré, I. Ellis, J.D. Brenton, C. Caldas, Oncogene 26 (2007) 1959–1970;
b) K. Chin, S. DeVries, J. Fridlyand, P.T. Spellman, R. Roydasgupta, W.L. Kuo, A. Lapuk, R.M. Neve, Z. Qian, T. Ryder, F. Chen, H. Feiler, T. Tokuyasu, C. Kingsley, S. Dairkee, Z. Meng, K. Chew, D. Pinkel, A. Jain, B.M. Ljung, L. Esserman, D.G. Albertson, F.M. Waldman, J.W. Gray, Cancer Cell 10 (2006) 529–541;
c) J. Fridlyand, A.M. Snijders, B. Ylstra, H. Li, A. Olshen, R. Segraves, S. Dairkee, T. Tokuyasu, B.M. Ljung, A.N. Jain, J. McLennan, J. Ziegler, K. Chin, S. Devries, H. Feiler, J.W. Gray, F. Waldman, D. Pinkel, D.G. Albertson, BMC Cancer 6 (2006) 96;
d) L.W. Loo, D.I. Grove, E.M. Williams, C.L. Neal, L.A. Cousens, E.L. Schubert, I.N. Holcomb, H.F. Massa, J. Glogovac, C.I. Li, K.E. Malone, J.R. Daling, J.J. Delrow, B.J. Trask, L. Hsu, P.L. Porter, Cancer Research 64 (2004) 8541–8549.
[12] a) A. Mai, Epigenomics 2 (2010) 307–324;
b) J. Tang, H. Yan, S. Zhuang, Clinical Science 124 (2013) 651–662;
c) P.A. Marks, R. Breslow, Nature Biotechnology 25 (2007) 84–90;

d) M. Kim, L.A. Thompson, S.D. Wenger, C.L. O'Bryant, The Annals of Pharmacotherapy 46 (2012) 1340–1348.

[13] a) F. Manzo, F.P. Tambaro, A. Mai, L. Altucci, Expert Opinion on Therapeutic Patents 19 (2009) 761–774;
b) S.D. Furdas, S. Kannan, W. Sippl, M. Jung, Archives of Pharmaceutical Chemistry in Life Sciences 345 (2012) 7–21;
c) A. Lenoci, S. Tomassi, M. Conte, R. Benedetti, V. Rodriguez, S. Carradori, D. Secci, S. Castellano, G. Sbardella, P. Filetici, E. Novellino, L. Altucci, D. Rotili, A. Mai, Chemmedchem 9 (2014) 542–548.

[14] a) A.R. Ali, E.R. El-Bendary, M.A. Ghaly, I.A. Shehata, European Journal of Medicinal Chemistry 69 (2013) 908–919;
b) M. Rudrapal, R.S. Satyanandam, T.S. Swaroopini, T.N. Lakshmi, S.K. Jaha, S. Zaheera, Medicinal Chemistry Research 22 (2013) 2840–2846;
c) F. Chimenti, B. Bizzarri, E. Maccioni, D. Secci, A. Bolasco, R. Fioravanti, P. Chimenti, A. Granese, S. Carradori, D. Rivanera, D. Lilli, A. Zicari, S. Distinto, Bioorganic & Medicinal Chemistry Letters 17 (2007) 4635–4640;
d) S. Carradori, D. Secci, A. Bolasco, D. Rivanera, E. Mari, A. Zicari, L.V. Lotti, B. Bizzarri, European Journal of Medicinal Chemistry 65 (2013) 102–111;
e) F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, S. Carradori, A. Granese, D. Rivanera, N. Frishberg, C. Bordòn, L. Jones-Brando, Journal of Medicinal Chemistry 52 (2009) 4574–4577;
f) F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, M. D'Ascenzio, M.M. Scaltrito, F. Sisto, Journal of Heterocyclic Chemistry 47 (2010) 1269–1274;
g) G. Mazzone, R. Pignatello, A. Panico, S. Mazzone, G. Puglisi, G. Pennisi, G. Raciti, P. Mazzone, M. Matera, Die Pharmazie 47 (1992) 902–910;
h) F. Chimenti, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, E. Maccioni, M.C. Cardia, M. Yáñez, F. Orallo, S. Alcaro, F. Ortuso, R. Cirilli, R. Ferretti, S. Distinto, J. Kirchmair, T. Langer, Bioorganic & Medicinal Chemistry 18 (2010) 5063–5070;
i) F. Chimenti, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, M. Yáñez, F. Orallo, F. Ortuso, S. Alcaro, Bioorganic & Medicinal Chemistry 18 (2010) 5715–5723;
j) F. Chimenti, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, M. D'Ascenzio, M. Yáñez, F. Orallo, Medicinal Chemistry Communications 1 (2010) 61–72.

[15] a) F. Chimenti, B. Bizzarri, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, R. Fioravanti, A. Granese, S. Carradori, F. Tosi, P. Ballario, S. Vernarecci, P. Filetici, Journal of Medicinal Chemistry 52 (2009) 530–536;
b) D. Secci, S. Carradori, B. Bizzarri, A. Bolasco, P. Ballario, Z. Patramani, P. Fragapane, S. Vernarecci, C. Canzonetta, P. Filetici, Bioorganic & Medicinal Chemistry 22 (2014) 1680–1689.

[16] H. Kikuchi, F. Kuribayashi, N. Kiwaki, Y. Takami, T. Nakayama, Immunol 186 (2011) 3015–3022.

[17] D. Trisciuoglio, Y. Ragazzoni, A. Pelosi, M. Desideri, S. Carradori, C. Gabellini, G. Maresca, R. Nescatelli, D. Secci, A. Bolasco, B. Bizzarri, C. Cavaliere, I. D'Agnano, P. Filetici, L. Ricci-Vitiani, M.G. Rizzo, D. Del Bufalo, Clinical Cancer Research 18 (2012) 475–486.

[18] Y. Ragazzoni, M. Desideri, C. Gabellini, T. De Luca, S. Carradori, D. Secci, R. Nescatelli, A. Candiloro, M. Condello, S. Meschini, D. Del Bufalo, D. Trisciuoglio, Cell Death & Disease 4 (2013) e524.

[19] a) P. Ornaghi, D. Rotili, G. Sbardella, A. Mai, P. Filetici, Biochemical Pharmacology 70 (2005) 911–917;
b) A. Mai, D. Rotili, D. Tarantino, P. Ornaghi, F. Tosi, C. Vicidomini, G. Sbardella, A. Nebbioso, M. Miceli, L. Altucci, P. Filetici, Journal of Medicinal Chemistry 49 (2006) 6897–6907;
c) A. Mai, D. Rotili, D. Tarantino, A. Nebbioso, S. Castellano, G. Sbardella, M. Tini, L. Altucci, Bioorganic & Medicinal Chemistry Letters 19 (2009) 1132–1135;
d) R. Ruotolo, F. Tosi, S. Vernarecci, P. Ballario, A. Mai, P. Filetici, S. Ottonello, Genomics 96 (2010) 272–280;
e) A. Mai, D. Cheng, M.T. Bedford, S. Valente, A. Nebbioso, A. Perrone, G. Brosch, G. Sbardella, F. De Bellis, M. Miceli, L. Altucci, Journal of Medicinal Chemistry 51 (2008) 2279–2290;
f) G. Sbardella, S. Castellano, C. Vicidomini, D. Rotili, A. Nebbioso, M. Miceli, L. Altucci, A. Mai, Bioorganic & Medicinal Chemistry Letters 18 (2008) 2788–2792;
g) C. Milite, S. Castellano, R. Benedetti, A. Tosco, C. Ciliberti, C. Vicidomini, L. Boully, G. Franci, L. Altucci, A. Mai, G. Sbardella, Bioorganic & Medicinal Chemistry 19 (2011) 3690–3701;
h) C. Colussi, J. Rosati, S. Straino, F. Spallotta, R. Berni, D. Stilli, S. Rossi, E. Musso, E. Macchi, A. Mai, G. Sbardella, S. Castellano, C. Chimenti, A. Frustaci, A. Nebbioso, L. Altucci, M.C. Capogrossi, C. Gaetano, Proceedings of the National Academy Sciences of the United States of America 108 (2011) 2795–2800;
i) C. Colussi, A. Scopece, S. Vitale, F. Spallotta, S. Mattiussi, J. Rosati, B. Illi, A. Mai, S. Castellano, G. Sbardella, A. Farsetti, M.C. Capogrossi, C. Gaetano, Arteriosclerosis, Thrombosis, and Vascular Biology 32 (2012) 2435–2443;
j) W. Wei, C.M. Coelho, X. Li, R. Marek, S. Yan, S. Anderson, D. Meyers, C. Mukherjee, G. Sbardella, S. Castellano, C. Milite, D. Rotili, A. Mai, P.A. Cole, P. Sah, M.S. Kobor, T.W. Bredy, The Journal of Neuroscience: the Official Journal of the Society for Neuroscience 32 (2012) 11930–11941.

[20] a) A. Mai, S. Massa, D. Rotili, S. Simeoni, R. Ragno, G. Botta, A. Nebbioso, M. Miceli, L. Altucci, G. Brosch, Journal of Medicinal Chemistry 49 (2006) 6046–6056;
b) A. Mai, D. Rotili, S. Massa, G. Brosch, G. Simonetti, C. Passariello, A.T. Palamara, Bioorganic & Medicinal Chemistry Letters 17 (2007) 1221–1225.