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Aurones as histone deacetylase inhibitors: Identification of key features

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ABSTRACT

In this study, a total of 22 flavonoids were tested for their HDAC inhibitory activity using fluorimetric and BRET-based assays. Four aurones were found to be active in both assays and showed IC_{50} values below 20 μM in the enzymatic assay. Molecular modelling revealed that the presence of hydroxyl groups was responsible for good compound orientation within the isoenzyme catalytic site and zinc chelation.

Histone acetylation is one of the most important epigenetic processes and largely influences gene expression. Increases in chromatin acetylation are associated with transcriptionally active genes whereas hypoacetylation causes transcriptionally silent genes. The acetylation degree of histones depends on the enzymatic activity balance between histone deacetylase (HDAC) and histone acetylase (HAT) enzymes. In eukaryotes, 18 HDAC isoforms, classified into 4 classes, have been identified. Classes I, II and IV are zinc-dependant enzymes. Class III contains the sirtuins (SIRT), which are NAD-dependent. Epigenetic perturbation due to a dysfunction in the acetylation process have been involved in the development of several diseases including cancer,[1] cardiovascular,[2] and neurodegenerative disorders.[3,4] HDAC inhibition has emerged as an interesting therapeutic strategy to restore the HDAC/HAT balance. Some HDAC inhibitors (HDACi) are currently under clinical studies, with others being approved by the FDA for cancer therapy.[5] The general HDACi pharmacophore consists of three distinct structural parts: the zinc-binding group that chelates a zinc residue within the active site, the recognition cap group responsible for HDAC subtype selectivity and a hydrophobic linker which links these 2 groups.[6,7] The first discovered HDACi are from natural sources. Some of them are very potent such as trichostatin A[8] and have been studied in the context of cancer therapy. Weak HDAC ligands have also been identified in food and are mostly associated with cancer chemoprevention. Some examples are butyrate, organosulfur and organoselenium compounds, as well as polyphenols.[9] Among flavonoids, a common group of plant phenolic compounds,[10] some isoflavones and flavones are known as in vitro HDAC inhibitors.[11–13] Considering this, the HDAC inhibitory activity of another flavonoid subgroup, the aurones, was investigated in the present study.

Aurones, (*Z*)-2-benzylidenebenzofuran-3-(2*H*)-ones are responsible for the bright yellow color of some popular ornamental flowers, and are biosynthesized from chalcones by the key enzyme aureusidin synthase.[14] Probably due to their scarce occurrence in nature, aurones have not been extensively studied for their biological activities although this is rapidly changing. Natural and synthetic aurones have been shown so far to possess a broad spectrum of bioactivity including anticancer,[15,16] antioxidant,[17]

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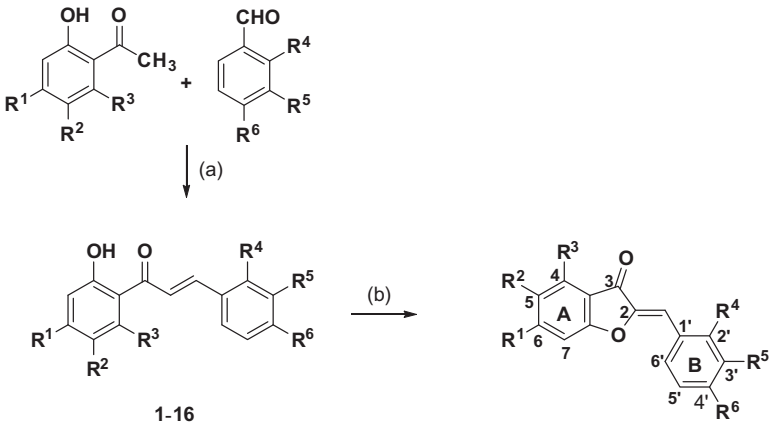
antiparasitic[18,19] and neuroprotective.[20] Some aurone analogues have been recently identified by Manjulatha et al. as SIRT1 inhibitors.[21] In the frame of our research involving the design, synthesis and activity evaluation of aurones, a series of 20 natural and synthetic analogues were tested for their zinc-dependent HDAC inhibitory activity.

The synthesis of the aurones under study was implemented via the methodology that was previously applied[17,18] and consisted of the oxidative cyclization of the corresponding chalcones using mercury(II) acetate as the oxidative agent. The required chalcones were prepared through the Claisen–Schmidt condensation reaction between appropriately substituted 2′-hydroxy-acetophenones and benzaldehydes carried out using 20% aqueous KOH in ethanol (Scheme 1).[17,18]

The synthesis and structure characterization of aurones **17**–**19**, **21**–**23**, and **25**–**29** were reported in detail in previously published work.[17,18] Aurones **20**, **24**, **30**, **31** and **32** are new compounds which were synthesized for the purpose of this study. The hydroxylated aurones **33**–**35** were prepared by deprotection of the corresponding methoxylated aurones **17**, **23** and **24**, respectively, using BBr_3 in CH_2Cl_2 as the deprotecting agent, whereas the natural product aureusidin (**36**) was synthesized by removing the methoxymethyl-protecting group from aurone **29**, as described previously (Scheme 2). Experimental details and analytical data for the new compounds are given in Supplementary data.

The 20 synthesized aurones as well as chalcones **2** and **3**, precursors of aurones **18** and **19**, respectively, were tested for their HDAC inhibitory activity at 100 μM by using a HeLa nuclear extract in a fluorimetric assay (Table S1). The IC_{50} of compounds **23**, **29**, **33**, **34**, **35** and **36** (Table 1) exhibiting an inhibition ⩾50% was determined. Among these, only the hydroxylated aurones **33**–**36** presented an IC_{50} lower than 20 μM and were further tested with various HDAC isoforms (HDAC1, HDAC2 and HDAC6). Phenolic groups on ring B appear to be critical for the activity as the alkoxylated analogues **17**, **23**, **24** and **29**, respectively, showed no or significantly lower activity. Aurones **34** and **35** possessing a catechol substitution pattern were the most active compounds on each HDAC isoform tested, showing no isoform selectivity, whereas aurone **33** which is lacking the catechol system on ring B was less active. The natural product aureusidin (**36**), which possesses two additional hydroxyl groups at the 4- and 6-positions of ring A, exhibited lower activity than aurone **34**, therefore implying that substitution on ring B is more important for the activity.

In order to confirm the HDACi properties of the compounds, histone H3 acetylation was measured in living cells by using a bioluminescent resonance energy transfer technology (BRET)-based assay.[22] An increase in BRET signal reflects an increase in histone H3 acetylation. Experimental details are given in Supplementary data. All BRET experiments were validated by using SAHA as a positive control (Fig. S1A and B). Compounds **23**, **30**, **33**, **34**, **35** and **36** increased BRET signal in a dose dependent manner (Fig. 1) demonstrating their HDACi activity in living cells. A dose dependent toxicity was observed for all of these compounds



17 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=OCH_3$

18 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=CH_3$

19 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=Cl$

20 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=COOH$

21 $R^1=R^2=R^3=R^5=R^6=H$, $R^4=OCH_3$

22 $R^1=R^2=R^3=R^4=R^6=H$, $R^5=OCH_3$

23 $R^1=R^2=R^3=R^4=H$, $R^5=R^6=OCH_3$

24 $R^1=R^2=R^3=R^6=H$, $R^4=R^5=OCH_3$

25 $R^1=R^3=OCH_3$, $R^2=R^4=R^5=R^6=H$

26 $R^1=R^3=R^6=OCH_3$, $R^2=R^4=R^5=H$

27 $R^1=R^3=R^4=OCH_3$, $R^2=R^5=R^6=H$

28 $R^1=R^3=OCH_3$, $R^4=Cl$, $R^2=R^5=R^6=H$

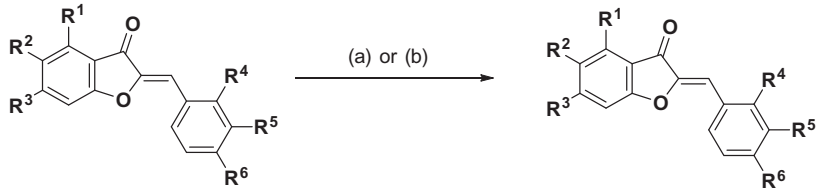
29 $R^1=R^3=R^5=R^6=OCH_2OCH_3$, $R^2=R^4=H$

30 $R^1=R^3=R^4=H$, $R^2=Br$, $R^5=R^6=OCH_3$

31 $R^1=R^3=R^6=H$, $R^2=Cl$, $R^4=R^5=OCH_3$

32 $R^1=R^3=R^4=R^5=H$, $R^2=Cl$, $R^6=COOH$

Scheme 1. General synthetic route to chalcones **1**–**16** and aurones **17**–**32**. Reagents and conditions: (a) 20% aq KOH, EtOH, rt. (b) $Hg(OAc)_2$, pyridine, 110 °C, 1 h.

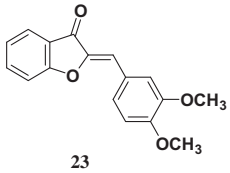
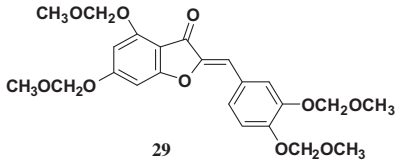
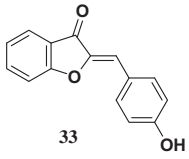
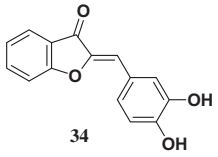
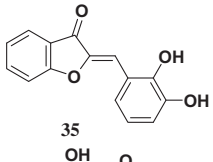
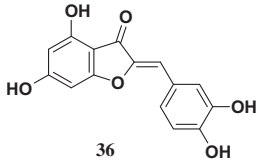
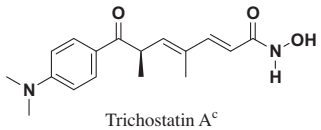


17 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=OCH_3$
23 $R^1=R^2=R^3=R^4=H$, $R^5=R^6=OCH_3$
24 $R^1=R^2=R^3=R^6=H$, $R^4=R^5=OCH_3$
29 $R^1=R^3=R^5=R^6=OCH_2OCH_3$, $R^2=R^4=H$

33 $R^1=R^2=R^3=R^4=R^5=H$, $R^6=OH$
34 $R^1=R^2=R^3=R^4=H$, $R^5=R^6=OH$
35 $R^1=R^2=R^3=R^6=H$, $R^4=R^5=OH$
36 $R^1=R^3=R^5=R^6=OH$, $R^2=R^4=H$

Scheme 2. Procedure for the synthesis of hydroxylated aurones **33**–**36**. Reagents and conditions: Route (a) (for aurones **33**–**35**): BBr_3, CH_2Cl_2, 0 °C; Route (b) (for aurone **36**): 10% aq HCl, MeOH, reflux 15 min.

Table 1
HDAC inhibition (IC_{50} in μM[a]) of selected aurones in a nuclear extract and towards various isoforms

Compounds	HeLa nuclear extract	Class I		Class IIb
		HDAC1	HDAC2	HDAC6
 **23**	71.0 ± 11.6	ND[b]	ND	ND
 **29**	40.0 ± 6.0	ND	ND	ND
 **33**	12.6 ± 3.4	129.2 ± 25.6	100.5 ± 13.0	135.9 ± 21.9
 **34**	8.2 ± 1.2	11.4 ± 0.7	5.1 ± 0.10	26.9 ± 6.9
 **35**	18.6 ± 2.5	21.0 ± 4.1	16.4 ± 0.3	26.9 ± 8.5
 **36**	18.5 ± 2.3	38.0 ± 7.7	38.9 ± 6.2	48.5 ± 5.7
 Trichostatin A[c]	0.0075 ± 0.0017	0.0074 ± 0.0027	0.0282 ± 0.0009	0.0082 ± 0.0024

[a] Results are the means ± SD of three independent experiments.
[b] ND: not determined.
[c] Positive control.

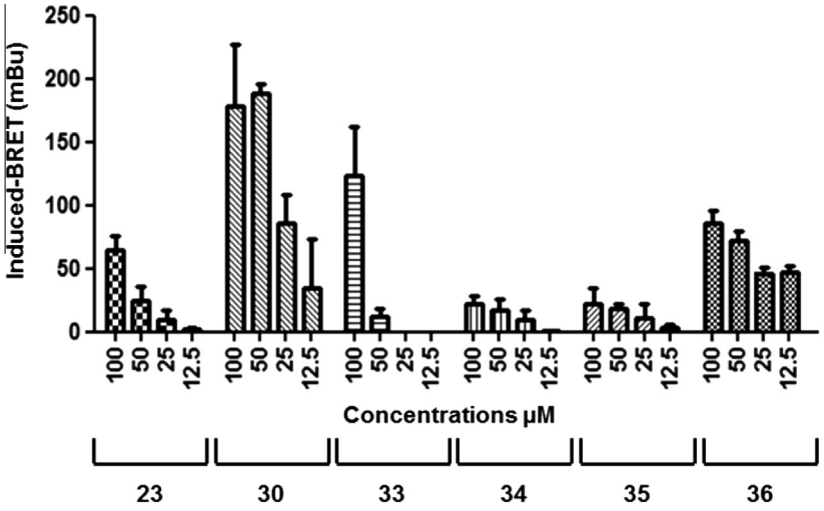


Figure 1. Validation of HDACi activity of compounds in living cells. MeT-5A cells were transfected with phRluc-C1-BrD and pEYFP-C1 histone H3 and treated for 16 h with increasing doses of the various compounds. BRET signals were measured as described in Experimental section. Results are the means ± SEM of three independent experiments.

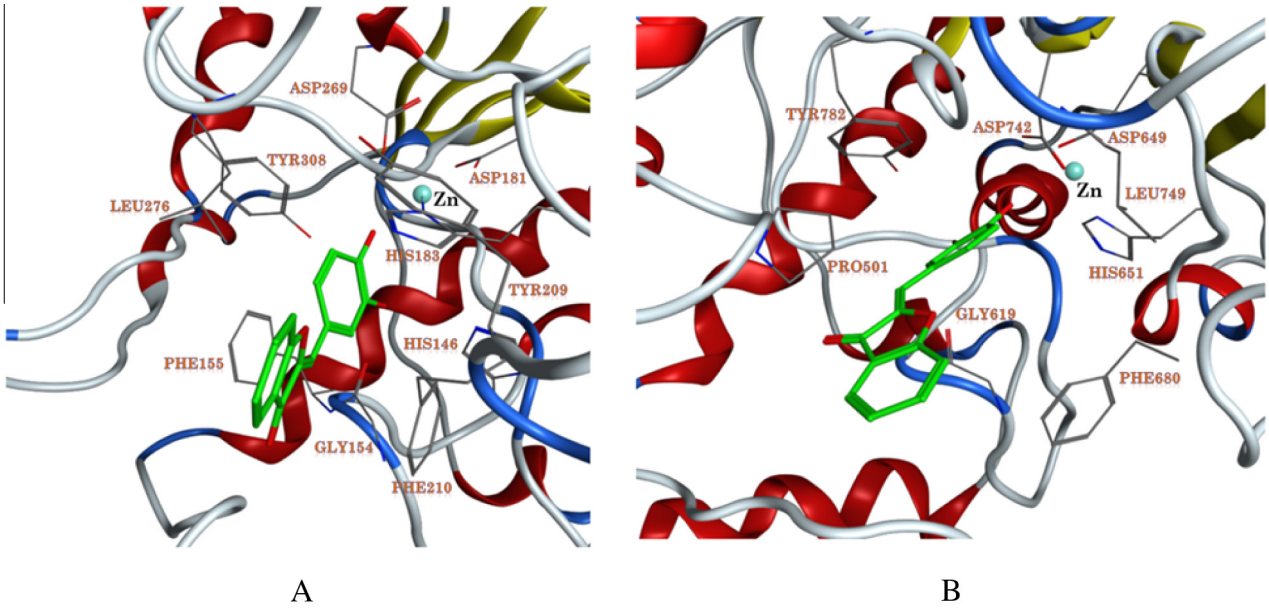


Figure 2. Compound **34** in complex with HDAC2 and HDAC6. (A) Compound **34** (green sticks) in complex with HDAC2. (B) Compound **34** (green sticks) in complex with HDAC6.

(Fig. S1C). It was not possible to confirm the HDACi activity of compound **29** due to strong toxicity (Table S1 and Fig. S2). Compound **30**, which is different from compound **23** by the presence of a bromine at the 5-position of ring A, showed an HDACi activity only in the cell-based assay. This could be explained by a cellular process leading to the active molecule or impacting its stability and then its intracellular concentration (Fig. 1). Each compound was also tested for the modulation of sirtuin 2 by thermal shift assay and for changes in DNA methylation based on specific PCR for RARβ. None of the compound showed any activity (data not shown).

In order to rationalize the results coming from the enzymatic assays, the four most active aurones (Table 1) were docked into the catalytic pocket of a representative isoform from class I (HDAC2)[23] and class II (HDAC6). The molecular docking protocol was first validated in both enzymes through the re-docking of co-crystallized SAHA within the HDAC2 protein structure.[24] Docking calculations revealed the capability of the docking tool to retrieve the correct binding mode within HDAC2. Poses with Root Mean Square Deviation (RMSD) values lower that 1 Å with respect to the ligand of reference were obtained (Fig. S3A). The same inhibitor was then docked into the catalytic pocket of HDAC6.[25] A unique binding mode, in line with data coming from the literature,[26] was retrieved (Fig. S3B). The docking of **33** in both HDAC2 and HDAC6 isoforms suggested that this compound, through the single *para*-hydroxy substitution, can occupy similar positions in the catalytic pocket through a monodentate chelation of the zinc ion. Other hydrophobic interactions stabilized the complexes (further details provided in Supplementary information, Fig. S4).

Regarding **34** and **35**, molecular docking results showed a conserved hydrophobic interaction network within the HDAC proteins but a different hydrophilic one. The aurone **34**–HDAC2

complex presented a monodentate zinc chelation via the 4′-OH group and polar interactions between the 3′-OH and the His146 side chain (Fig. 2A). Through the 4′- and 3′-OH groups, **34** interacted with the zinc ion and Gly619 of the HDAC6 catalytic pocket, respectively (Fig. 2B). Aurone **35** interacted via 2′-OH with the catalytic zinc ion of HDAC2 whereas the 3′-OH formed a hydrogen bond with the Gln265 side chain (Fig. S5A). In HDAC6, a monodentate zinc chelation via the 3′-OH group and polar contacts between the 2′-OH group and Asp742 were observed (Fig. S5B). Aureusidin (**36**) was the most substituted compound among the active ones (Table 1). In HDAC2, a convergent docking pose highlighted a hydrophilic network between 3′-OH and the catalytic metal, between 4′-OH and the His146 side chain, and between 6-OH and the Leu276 backbone (Fig. S6A). Whereas the docking algorithm identified only one possible position of **36** in the HDAC2 pocket, two statistically represented binding modes were observed in HDAC6. The first one was in line with the position adopted by compounds **33**–**35** (Fig. S6B). The second one, on the contrary, displayed a flip of the ligand within the pocket, with the hydroxy groups of ring A pointing toward the zinc binding domain (Fig. S6C). The four most active aurones are hydroxylated (*Z*)-2-benzylidenebenzofuran-3-(2*H*)-one compounds. Hydroxylation seems to play a key role on HDAC modulation via zinc chelation. Moreover, docking results of the inactive compounds (data not shown) confirmed their importance: methylation or apolar substitutions were responsible for the missing interactions with the catalytic zinc ion. From the enzymatic data (Table 1), a slight preferential inhibition of the aurone derivatives toward class I HDACs can be observed. As elucidated in the past, HDAC2 and HDAC6 catalytic sites present differences in terms of residues, shape and size (Fig. S7).[25,26] Thus, it can be hypothesized that this feature may be responsible for a hydrophobic sandwich effect, stabilizing the linker portion and the ring A of the compounds in the HDAC2 pocket. In fact, by docking, a higher number of hydrophobic contacts within HDAC2 active site were detected.

Based on the wide range of aurone bioactivity, a series of 20 analogues was tested for their HDAC inhibitory activity. Among these compounds, four exhibited an IC_{50} lower than 20 μM and were considered active. Unfortunately, they did not show any selectivity with the isoforms tested. The activity of the compounds was confirmed in living cells by using a BRET-based assay. The presence of hydroxyl groups on ring B was identified to play an important role for the activity. These results could lead to the development of more selective and potent HDAC inhibitors.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.019.

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