



Cite this: *RSC Adv.*, 2024, 14, 9314

Design, synthesis and activity evaluation of arctigenin derivatives with HDAC inhibition activity†

Xinyue Jiang,^a Yuchao Yan,^a Huali Yang,^a Maosheng Cheng,^a Deqiang Dou^{*b} and Yang Liu^{†a}

Arctigenin, a natural product with diverse pharmacological activities, can inhibit cell proliferation and survival and has shown promising potential in cancer research. In this study, we designed a series of arctigenin derivatives with HDAC inhibitory activity based on the synergistic effects between HDAC inhibitors and arctigenin. Among them, compound **B7** exhibited significantly higher antiproliferative activity in the MV411 cell line compared to the positive control, tucidinostat. Additionally, enzymatic activity testing was performed with compound **B7**. Further mechanistic studies indicated that compound **B7** induced apoptosis through the Caspase-3 pathway in MV411 cells and enhanced histone acetylation levels in the MV411 cell line. These findings highlight the broad potential application of these arctigenin derivatives in cancer therapy.

Received 3rd January 2024
Accepted 11th March 2024

DOI: 10.1039/d4ra00050a

rsc.li/rsc-advances

Introduction

Arctigenin is a natural product primarily found in plants such as *Arctium lappa* (burdock). The molecular formula of arctigenin is $C_{21}H_{24}O_6$, and the compound has been extensively studied and shown to possess certain anticancer activities.^{1–3} The anticancer effects of arctigenin are attributed to multiple mechanisms. Some studies have indicated that arctigenin can inhibit the proliferation and growth of tumour cells,^{4–7} induce apoptosis (programmed cell death),⁸ and impede the invasion and metastasis of cancer cells.^{9,10} Regarding haematologic malignancies, arctigenin has also demonstrated inhibitory effects. For instance, *in vitro* experiments have shown that arctigenin can inhibit the proliferation and growth of leukaemia cells, including acute lymphoblastic leukaemia and acute myeloid leukaemia cells, and induce apoptosis in these cells.¹¹ Furthermore, arctigenin has shown inhibitory effects on the growth of lymphomas and multiple myeloma cell lines, among other haematologic malignancies.¹²

Histone deacetylases (HDACs) are a class of enzymes that play a crucial role in regulating gene expression and epigenetic modifications^{13,14} (Fig. 1). They are responsible for removing acetyl groups from lysine residues in histone proteins, leading

to chromatin condensation and transcriptional silencing.¹⁵ Aberrant upregulation or excessive activity of HDACs frequently occurs in various malignancies, and as a result, tumour suppressor genes are silenced and tumour cells proliferate.¹⁶ Consequently, over the past 20 years, HDACs have been widely targeted for cancer therapy.^{17,18} To date, five HDAC inhibitors, including vorinostat (SAHA),¹⁹ romidepsin,²⁰ belinostat,²¹ panobinostat,²² and tucidinostat,²³ have been approved for the treatment of haematologic malignancies, and many others are currently in various stages of development.¹⁷

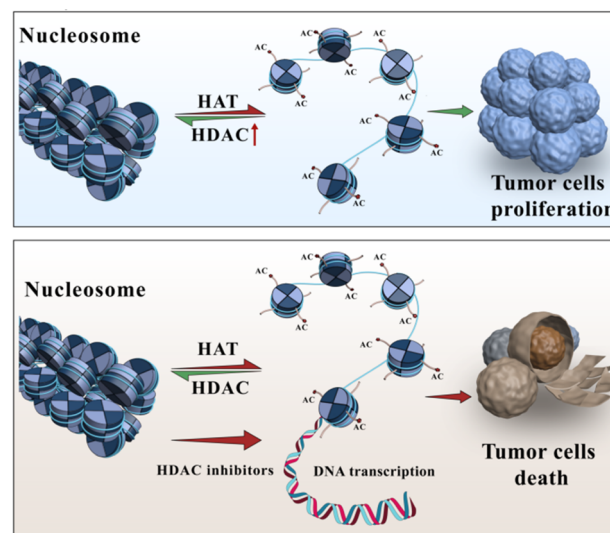


Fig. 1 HATs and HDACs jointly regulate the acetylation pattern of histones.

^aKey Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, 110016, P.R. China. E-mail: y.liu@syphu.edu.cn

^bDepartment of Chinese Medicine Chemistry, Liaoning University of Traditional Chinese Medicine, Dalian, 116000, P.R. China. E-mail: deqiangdou@126.com

† Electronic supplementary information (ESI) available: The ESI contains additional figures, compound characterization including ¹H and ¹³C NMR spectra, ultra-high resolution mass spectrometry, and western blot experimental images. See DOI: <https://doi.org/10.1039/d4ra00050a>



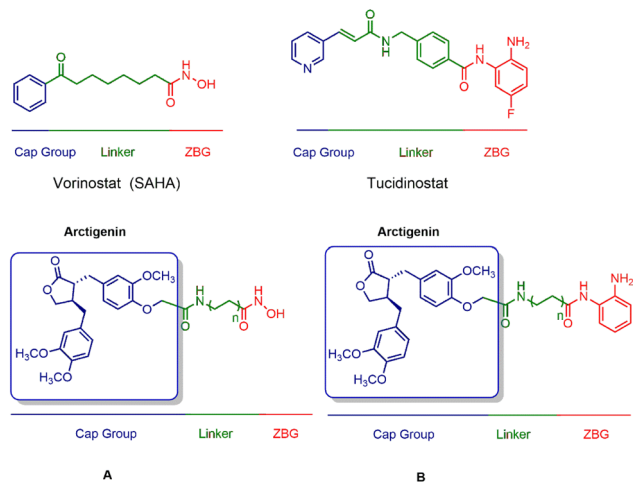


Fig. 2 Design strategy of target compounds.

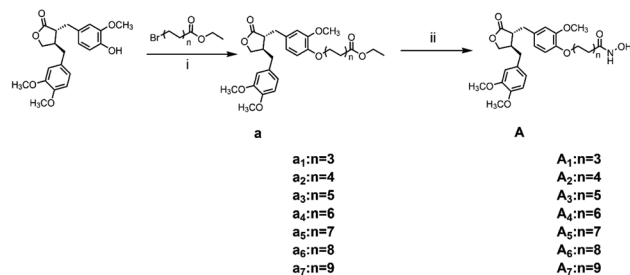
Combining histone deacetylase (HDAC) inhibitors with arctigenin, a natural product, is aimed at achieving anticancer activity, thereby potentially demonstrating synergistic anti-tumor effects. Importantly, by introducing HDAC inhibitory fragments, such as zinc ion-binding hydroxamic acid and *ortho*-benzamide moieties, the derivatives of arctigenin may demonstrate HDAC inhibitory activity, thereby enhancing the therapeutic efficacy of the natural product. Furthermore, by employing arctigenin as the cap group, it is anticipated to impact the selectivity towards different HDAC isoforms (Fig. 2).

Using arctigenin as our foundational compound in this study, we incorporated essential zinc-binding elements from the commercial drugs SAHA and tucidinostat. Consequently, we synthesized two series, A and B, that contained 18 distinct compounds. *In vitro* evaluation of their antitumour activity revealed that the optimized compound **B7** displayed superior antiproliferative effects against the MV411 cell line. Further mechanistic investigations demonstrated that **B7** induced cell apoptosis in MV411 cells in a dose-dependent manner mediated by Caspase-3. Caspase-3 plays a critical role in the process of programmed cell death or apoptosis. Additionally, compound **B7** significantly increased cellular acetylation levels. Overall, compound **B7** exhibited more potent antitumour activity than that of arctigenin and tucidinostat. The mechanism underlying the activity involves the induction of cell apoptosis and an increase in cellular acetylation levels. These findings provide valuable insights for the further development and potential clinical applications of this compound.

Results and discussion

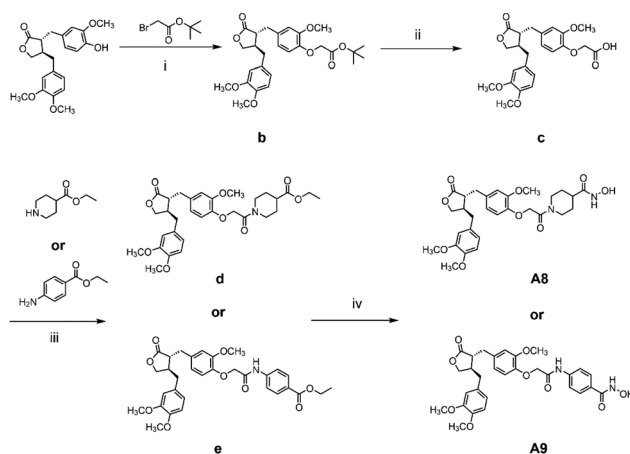
Chemistry

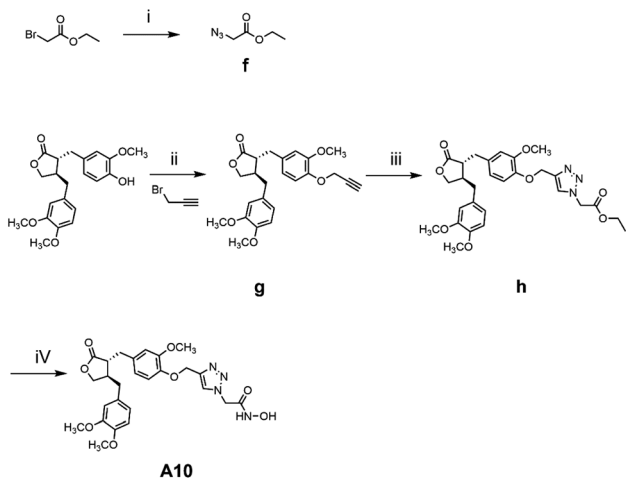
The general synthetic strategy for synthesizing a series of A–B target derivatives is outlined in Schemes 1–6. The synthesis route for the A-series derivatives begins with the natural product arctigenin. First, arctigenin undergoes a reaction with bromo-substituted alkyl esters of different carbon chain lengths. Afterwards, under basic conditions, the compound reacts with hydroxylamine hydrochloride to yield compounds **A1–A7**.

Scheme 1 Reagents and conditions: (i) K_2CO_3 , DMF, 60 °C, 6 h; (ii) hydroxylamine hydrochloride, KOH, MeOH, HCl, 0 °C – r.t., 1.5 h.

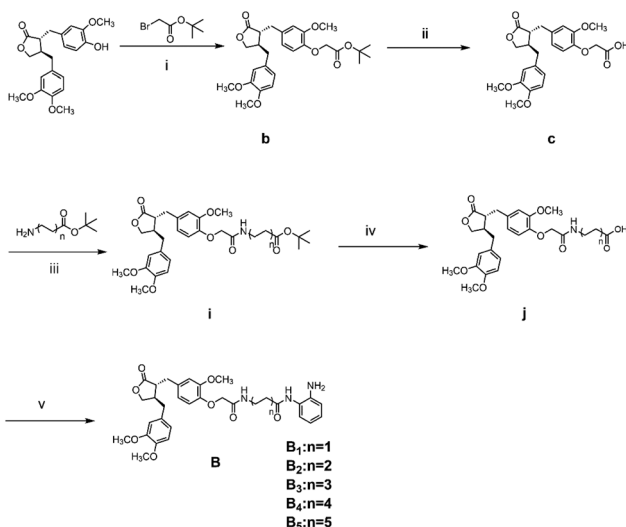
Arctigenin, when reacted with *tert*-butyl bromoacetate, produces compound **b**. Following this, the protective group is removed in acidic conditions (using TFA), resulting in compound **c**. When coupled with ethyl 4-piperidinecarboxylate and ethyl 4-aminobenzoate under the influence of EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and HOBT (1-hydroxy benzotriazole), this compound forms compounds **d** and **e**. In basic conditions, compounds **d** and **e** react with hydroxylamine hydrochloride to produce compounds **A8** and **A9**. Ethyl bromoacetate reacts with sodium azide in a reaction to form compound **f**. Compound **f**, when reacted with propargyl bromide, results in compound **g**. Subsequently, compound **g** undergoes a click reaction with compound **f** in the presence of copper(II) ions and sodium ascorbate, leading to compound **h**. Finally, under alkaline conditions, compound **h** reacts with hydroxylamine hydrochloride to produce compound **A10**.

The synthesis route for the B-series derivatives starts with the natural product arctigenin. Arctigenin initially undergoes a reaction with *tert*-butyl bromoacetate. Then, under acidic conditions provided by TFA (trifluoroacetic acid), the protective group is removed, resulting in compound **c**. This intermediate then reacts with amino acid *tert* butyl esters with different carbon chain lengths under the influence of EDCI and HOBT and then deprotects under acidic conditions with TFA to yield compounds **j1–j5**. Then, in the basic conditions provided by

Scheme 2 Reagents and conditions: (i) K_2CO_3 , DMF, 60 °C, 6 h; (ii) TFA, DCM, r.t., 2 h; (iii) EDCI, HOBT, Et_3N , DCM, r.t., 6 h; (iv) hydroxylamine hydrochloride, KOH, MeOH, HCl, 0 °C – r.t., 1.5 h.

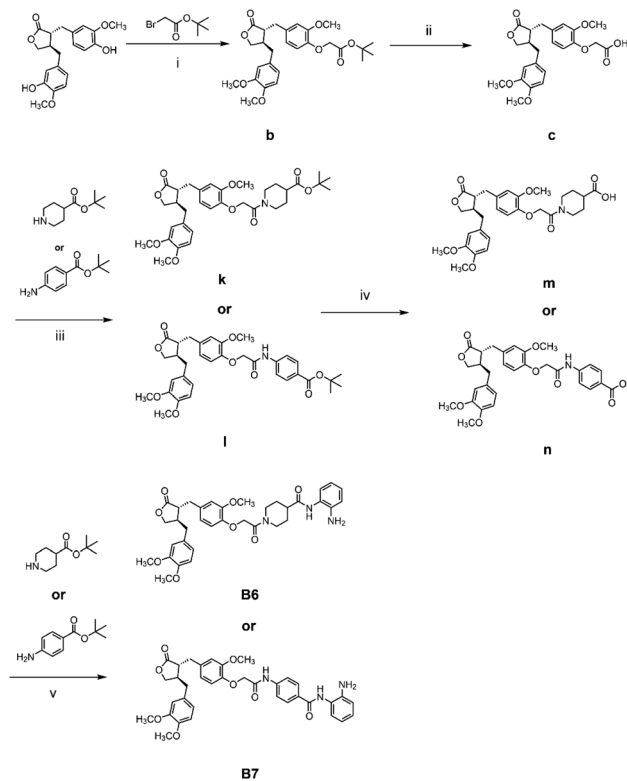


Scheme 3 Reagents and conditions: (i) DMF, NaN₃, 80 °C, 6 h; (ii) K₂CO₃, DMF, r.t., 6 h; (iii) CuSO₄·5H₂O, sodium ascorbate, H₂O : *t*-BuOH = 1 : 1 (V/V), 60 °C, 4 h; (iv) hydroxylamine hydrochloride, KOH, MeOH, HCl, 0 °C – r.t., 1.5 h.

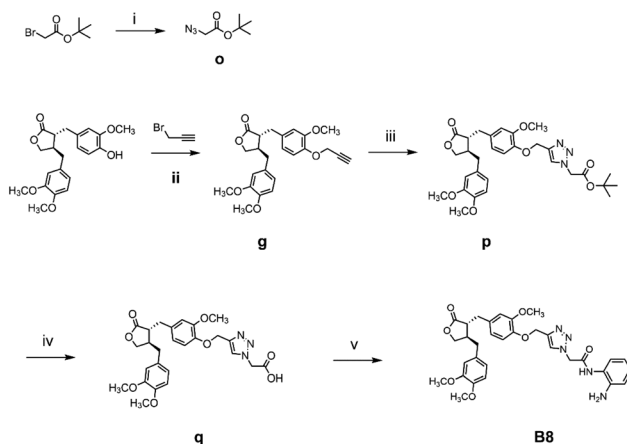


Scheme 4 Reagents and conditions: (i) K₂CO₃, DMF, 60 °C, 6 h; (ii) TFA, DCM, r.t., 2 h; (iii) EDCI, HOBT, Et₃N, DCM, r.t., 6 h; (iv) TFA, DCM, r.t., 2 h; (v) *o*-phenylenediamine, HATU, DIPEA, DMF, r.t., 6 h.

DIPEA (*N,N*-diisopropylethylamine), the compounds condense with *o*-phenylenediamine in the presence of the coupling agent HATU(*o*-(7-azabenzotriazol-1-yl)-*N,N,N',N'* tetramethyluronium hexafluorophosphate) to produce compounds **B1**–**B5**. Compound **c** undergoes condensation with ethyl 4-piperidinecarboxylate and ethyl 4-aminobenzoate in the presence of EDCI and HOBT, yielding compounds **k** and **l**. After deprotection under acidic conditions provided by TFA, compounds **m** and **n** react in a condensation with *o*-phenylenediamine through the coupling agent HATU to produce compounds **B6** and **B7**. *Tert*-butyl bromoacetate reacts with sodium azide to form compound **o**. Arctigenin reacts with propargyl bromide to yield compound **g**. This compound then undergoes a click reaction with compound **o** in the presence of copper(II) ions and sodium



Scheme 5 Reagents and conditions: (i) K₂CO₃, DMF, 60 °C, 6 h; (ii) TFA, DCM, r.t., 2 h; (iii) EDCI, HOBT, Et₃N, DCM, r.t., 6 h; (iv) TFA, DCM, r.t., 2 h; (v) *o*-phenylenediamine, HATU, DIPEA, DMF, r.t., 6 h.



Scheme 6 Reagents and conditions: (i) NaN₃, DMF, 80 °C, 6 h; (ii) K₂CO₃, DMF, 60 °C, 6 h; (iii) CuSO₄·5H₂O, sodium ascorbate, H₂O : *t*-BuOH = 1 : 1 (V/V), 60 °C, 4 h; (iv) TFA, DCM, r.t., 2 h; (v) *o*-phenylenediamine, HATU, DIPEA, DMF, r.t., 6 h.

ascorbate, resulting in compound **p**. After deprotection under acidic conditions, compound **p** condenses with *o*-phenylenediamine under the influence of HATU to produce compound **B8**.

In vitro antiproliferative activity evaluation

The antiproliferative activities of two series of target compounds, **A1** to **A10** and **B1** to **B8**, were screened in five



Table 1 *In vitro* antiproliferative activity of selected compounds

Compd.	Antiproliferative activity ^a (IC ₅₀ /μM)				
	K562	MV411	KU812	Kasumi-1	CCRF-CEM
A1	>5	>5	>5	>5	>5
A2	>5	>5	>5	>5	>5
A3	2.12 ± 0.12	0.85 ± 0.17	3.88 ± 1.06	>5	1.57 ± 0.13
A4	1.19 ± 0.25	1.66 ± 0.26	1.41 ± 0.75	>5	1.37 ± 0.11
A5	1.82 ± 0.52	1.18 ± 0.44	2.27 ± 0.68	>5	1.35 ± 0.21
A6	3.03 ± 0.63	1.93 ± 0.13	3.72 ± 0.81	>5	2.09 ± 0.17
A7	>5	>5	>5	>5	>5
A8	>5	>5	>5	>5	>5
A9	>5	>5	>5	>5	>5
A10	>5	>5	>5	>5	>5
B1	>5	>5	>5	>5	>5
B2	>5	>5	>5	>5	>5
B4	>5	>5	>5	>5	>5
B5	>5	>5	>5	>5	>5
B6	>5	>5	>5	>5	>5
B7	1.96 ± 0.19	0.75 ± 0.09	2.09 ± 0.31	0.86 ± 0.04	2.21 ± 0.26
B8	>5	>5	>5	>5	>5
Arctigenin	24.22 ± 3.12	4.27 ± 1.68	>30	>30	>30
SAHA	1.11 ± 0.05	1.00 ± 0.09	1.00 ± 0.11	0.47 ± 0.03	0.89 ± 0.03
Tucidinostat	2.68 ± 0.65	2.00 ± 0.15	4.26 ± 0.48	>5	2.25.23

^a IC₅₀ value (means ± SD, *n* = 3, independent experiments).

Table 2 HDAC isoform inhibitory activity of compound B7

Compd.	HDACs isoforms						
	IC ₅₀ (nM)						
	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC10
Compound B7	261.1	130.7	298.7	>10 000	>10 000	>10 000	1746
Tucidinostat	95.4	160.1	733.2	>10 000	>10 000	>10 000	78.6

human haematological malignancy cell lines (K562, MV411, KU812, Kasumi-1, CCRF-CEM) using a CCK8 assay. Arctigenin was used as the negative control, and tucidinostat was used as the positive control. Five optimized compounds were selected, and their IC₅₀ values are presented in Table 1.

Notably, compound **B7** exhibited higher antiproliferative activity than that of arctigenin and tucidinostat in the MV411 cell line, with an IC₅₀ value of 0.75 ± 0.09 μM. Additionally, the negative control arctigenin also showed good antiproliferative activity in the MV411 cell line, with an IC₅₀ value of 4.271 ± 1.68 μM. Therefore, the preliminary mechanism of MV411 cell antiproliferation was further explored using compound **B7**.

Due to the strong inhibitory effect of compound **B7** on MV411 cells, we further tested its enzyme activity against different subtypes of HDACs (refer to Table 2). The results showed that compound **B7** exhibited slightly better inhibition of HDAC2 than that of tucidinostat (with IC₅₀ values of 130.6 and 160.1 nM). The compound also displayed mild inhibition of HDAC10 (with an IC₅₀ of 1746.0 nM) but had no effect on HDAC4, HDAC5, and HDAC6 (IC₅₀ > 1000 nM). Overall, the enzyme activity tests indicated that compound **B7** selectively inhibits the activity of HDAC1, HDAC2, and HDAC3. The

improved inhibition of HDAC2 by compound **B7**, emphasizes the significant role of arctigenin in enhancing the compound's selective inhibitory activity. The inclusion of arctigenin's structural motifs is instrumental in this efficacy, highlighting its utility in the strategic design of enzyme inhibitors.

Analysis of apoptosis by annexin V-FITC/PI

To evaluate whether compound **B7** can induce apoptosis in cancer cells, we conducted an annexin V-FITC apoptosis assay in MV411 cells, as shown in Fig. 3. The results demonstrate that **B7** can induce apoptosis in MV411 cells. As the concentration of **B7** increased (0.25, 0.5 and 1.0 μM) after 48 hours of treatment, the overall proportion of apoptotic cells (Q1-UR + Q1-LR) increased in a dose-dependent manner (21.84%, 37.90%, and 77.00%, respectively, at concentrations of 0.1, 0.5 and 1.0 μM). Additionally, compared to arctigenin and tucidinostat, **B7** exhibits a stronger ability to induce apoptosis in the MV411 cell line.

Analysis of the cell cycle

In the cell cycle analysis, *in vitro* antiproliferation experiments demonstrated that compound **B7** can inhibit the proliferation

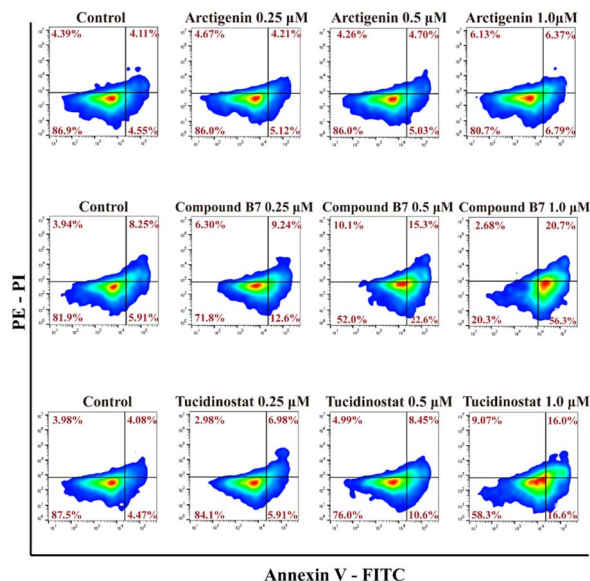


Fig. 3 MV411 cells were treated with different concentrations of compounds, including arctigenin, compound B7, and tucidinostat (at levels of 0, 0.25, 0.5 and 1.0 μM). Subsequently, apoptosis was detected using flow cytometry after annexin V/PI staining.

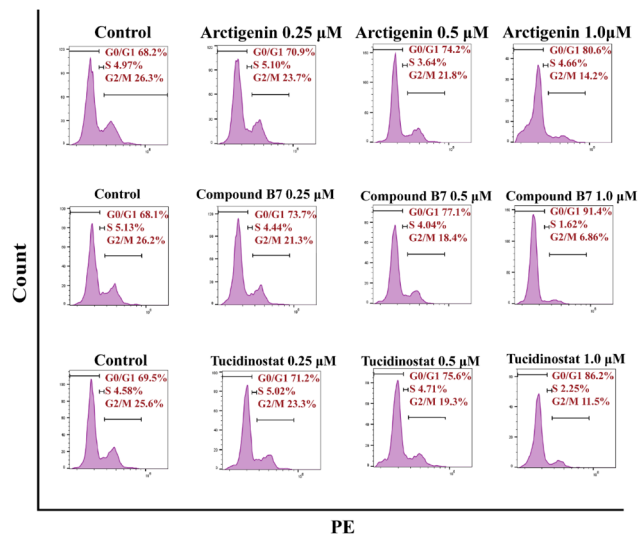


Fig. 4 MV411 cells were exposed to varying concentrations of several compounds, namely arctigenin, compound B7, and tucidinostat (0, 0.25, 0.5 and 1.0 μM). Following this, an analysis of the cell-cycle distribution was conducted utilizing flow cytometry. The panel showcases the distribution percentages of cells throughout different stages of the cell cycle.

of the MV411 cell line. To further investigate the mechanism of compound B7, flow cytometry (FCM) analysis was performed on the MV411 cell line using propidium iodide (PI) staining to study its effect on cell cycle progression. Under arctigenin and tucidinostat conditions, the proportion of cells arrested in the G0/G1 phase was increased with compound B7 compared to the control. Fig. 4 shows that as the concentration of compound B7 increased, the proportion of cells in the G0/G1 phase gradually

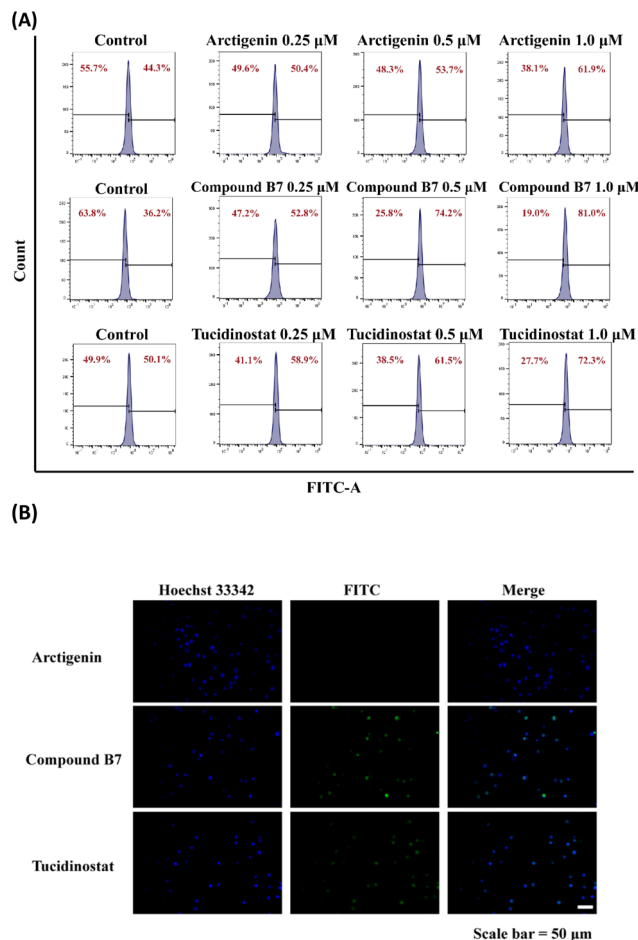


Fig. 5 (A) Flow cytometric analysis: flow cytometric analysis of MV411 cells treated with increasing concentrations of arctigenin, compound B7, and tucidinostat (0, 0.25, 0.5 and 1.0 μM) revealed a concentration-dependent increase in Caspase-3 activity. As shown in the figure, the percentage of cells with high Caspase-3 activity increased in a dose-dependent manner, with the highest proportion observed at 1.0 μM B7 treatment. (B) Fluorescence microscopy: fluorescence microscopy images of MV411 cells stained with the GreenNuc™ Caspase-3 assay kit further confirmed the induction of apoptosis by compound B7. The control group (0 μM B7) showed minimal Caspase-3 activity, indicated by weak fluorescence. In contrast, cells treated with increasing concentrations of compound B7 (1.0 μM) displayed intensified fluorescence signals, affecting Caspase-3 activity and, hence, increased apoptosis. The scale bar denotes 50 μm .

increased, reflecting that compound B7 induced concentration-dependent cell cycle arrest in the G0/G1 phase.

Caspase 3 activity assay

First, we used Beyotime Biotechnology's GreenNuc™ Caspase-3 activity assay kit to monitor the activity of Caspase-3 in real-time. Flow cytometric analysis results showed that under different concentrations of compound B7 treatment, Caspase-3 activity exhibited a clear concentration-dependent increase, with a more pronounced trend compared to the arctigenin and tucidinostat conditions. This result indicates that compound B7 enhances the activity of Caspase-3 in the MV411 cell line, thereby promoting cell apoptosis.



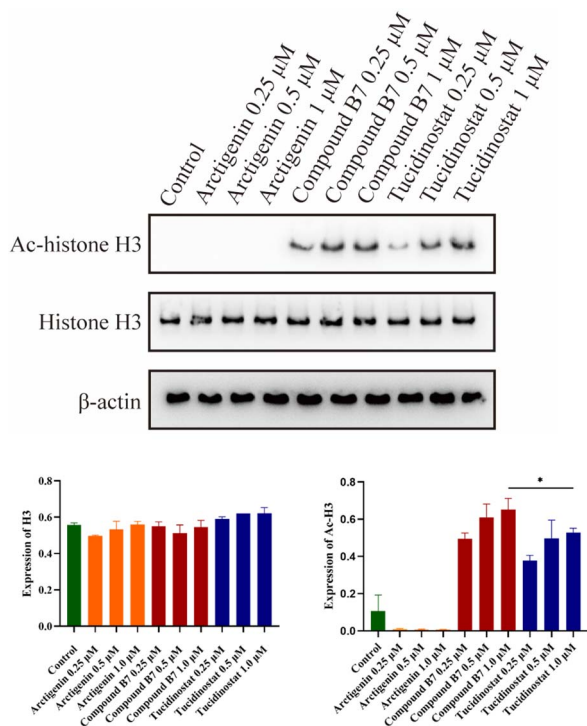


Fig. 6 MV411 cells were treated with arctigenin, compound B7, and tucidinostat at concentrations of 0, 0.25, 0.5 and 1.0 μM for 48 hours. The levels of H3 and acetylated H3 (Ac-H3) were determined using western blot analysis. β -Actin was used as the loading control.

Next, we observed the activity of Caspase-3 using fluorescence microscopy. Through live cell staining, we directly observed the fluorescence signals of Caspase-3. In the experiment, we similarly observed a concentration-dependent increase in Caspase-3 fluorescence signals under different concentrations of compound B7 treatment, with a more pronounced trend compared to the arctigenin and tucidinostat conditions. This further confirmed the results obtained from flow cytometry, demonstrating that compound B7 significantly increases caspase-3 activity in the MV411 cell line, leading to the induction of cell apoptosis.

In summary, the results obtained from both monitoring methods consistently indicate that compound B7 promotes caspase-mediated apoptosis in the MV411 cell line (Fig. 5).

Western blotting

Given the outstanding antiproliferative activity of compound B7 in MV411 cells, we conducted western blotting experiments to verify its intracellular mechanism of HDAC inhibition. The results showed that at different doses, compound B7 significantly increased the level of acetylated H3 in MV411 cells in a dose-dependent manner. This result indicates that compound B7 strongly inhibits the HDAC1/2/3 signalling pathway, and its HDAC inhibitory activity is significantly superior to that of arctigenin and slightly superior to that of tucidinostat. Thus, the HDAC inhibitory activity of compound B7 were confirmed (Fig. 6).

Conclusions

The objective of this study was to design and synthesize arctigenin derivatives that can inhibit HDAC, thoroughly evaluate their antiproliferative effects on the leukaemia cell line MV411, and investigate their underlying mechanisms. We successfully synthesized a series of compounds and meticulously assessed their potential as antitumour agents. Our findings revealed that compound B7 exhibited exceptional antiproliferative activity among all the designed derivatives, outperforming the standards arctigenin and tucidinostat. Further enzymatic assays demonstrated that compound B7 showed comparable levels of HDAC inhibitory activity to tucidinostat, the positive control. After thoroughly investigating the mechanism of action, we discovered that compound B7 induced cell apoptosis in MV411 cells through the Caspase-3 pathway, effectively inhibiting cell proliferation. Caspase-3 plays a crucial role in driving cell apoptosis, and its increased activity contributes to enhanced cell death.

Moreover, compound B7 significantly increased the levels of acetylation within MV411 cells, a key protein modification that regulates various cellular functions. This result provided further evidence for the potent HDAC inhibitory activity of compound B7, aligning with the characteristics of commercially available HDAC inhibitors.

In summary, through designing and synthesizing arctigenin derivatives with HDAC inhibitory properties, we discovered a novel antileukaemia compound, namely, compound B7. This compound exhibited significant antiproliferative effects and demonstrated its antitumour properties by inducing cell apoptosis and enhancing cellular acetylation levels. Furthermore, our research paves the way for further investigations on arctigenin derivatives in the field of cancer treatment, offering valuable insights that could aid in the discovery of additional potential anticancer drugs.

Experimental section

Chemistry

General information. ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE600 spectrometer (Bruker Company, Germany) using tetramethylsilane (TMS) as an internal standard and dimethyl sulfoxide (DMSO)- d_6 as the solvent. Chemical shifts are given in ppm (δ). Mass spectra were recorded on an FT-MS mass spectrometer. Silica gel thin-layer chromatography was performed on precoated GF-254 plates (Qingdao Haiyang Chemical, China), and TLC was performed on silica gel plates (Indicator F-254) and visualized by UV light (254 nm/365 nm). Commercially available reagents were used without further purification. All target compounds were >98% pure by HPLC analysis, performed on an Agilent 1260 Infinity II HPLC instrument using an Agilent XDB C18 column (3.5 μm , 4.6×150 mm).

General procedure for the synthesis of A1–A6 in Scheme 1. Arctigenin (50.0 mg, 0.13 mmol), bromo ester (0.156 mmol), and K_2CO_3 (35 mg, 0.26 mmol) were stirred in 15 mL DMF at 60 $^\circ\text{C}$ for 6 h. The mixture was extracted with EtOAc and



concentrated under reduced pressure, yielding compounds **a1**–**a6**. Hydroxylamine hydrochloride (90.0 mg, 1.3 mmol), anhydrous sodium sulfate, and potassium hydroxide (72.9 mg, 1.3 mmol) were dissolved in methanol, added at 0 °C, and stirred for 0.5 h until room temperature. The resulting mixture was then extracted and filtered, and the obtained filtrate was added dropwise to the methanol solution of compounds **a1**–**a7**. Potassium hydroxide (36.5 mg, 0.65 mmol) was added to the mixture, and after 1.5 hours, 20 mL of water was added; the aqueous solution was acidified to pH 4–5 with dilute hydrochloric acid (1 M) and extracted with dichloromethane (50 mL \times 3). The dichloromethane layer was concentrated and dried in a vacuum, and the residue was purified by flash chromatography (silica gel, 200–300 mesh, DCM/MeOH = 50 : 1, V/V) to afford compounds **A1**–**A7**.

4-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxypentanamide (A1). White solid, yield: 72.7%. m.p. 102.1–103.4 °C, ^1H NMR (600 MHz, DMSO- d_6) δ 10.41 (s, 1H), 8.70 (s, 1H), 6.85–6.81 (m, 2H), 6.77 (d, J = 1.7 Hz, 1H), 6.68 (dd, J = 8.2, 1.7 Hz, 1H), 6.65–6.58 (m, 2H), 4.09 (t, J = 8.0 Hz, 1H), 3.91–3.86 (m, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.34 (s, 3H), 2.85–2.80 (m, 1H), 2.77 (dd, J = 13.7, 6.9 Hz, 1H), 2.74–2.69 (m, 1H), 2.50–2.44 (m, 3H), 2.12 (t, J = 7.4 Hz, 2H), 1.90 (dd, J = 13.9, 7.1 Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.90, 169.14, 149.28, 149.11, 147.76, 147.11, 131.67, 131.27, 121.77, 120.84, 113.83, 113.59, 112.80, 112.25, 71.18, 68.11, 55.93, 55.91, 55.80, 46.06, 41.26, 37.36, 34.12, 29.25, 25.42. FT-MS: calcd for $\text{C}_{25}\text{H}_{32}\text{NO}_8$, $[\text{M} + \text{H}]^+$, 474.2128, found 474.2122. HPLC purity: 98.24% (t_{R} = 7.06 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

5-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxypentanamide (A2). White solid, yield: 57.3%. m.p. 104.3–104.5 °C, ^1H NMR (600 MHz, DMSO- d_6) δ 10.36 (s, 1H), 8.70 (d, J = 0.8 Hz, 1H), 6.85–6.83 (m, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 1.6 Hz, 1H), 6.68 (dd, J = 8.2, 1.6 Hz, 1H), 6.64 (d, J = 1.5 Hz, 1H), 6.60 (dd, J = 8.1, 1.4 Hz, 1H), 4.09 (t, J = 8.0 Hz, 1H), 3.91–3.89 (m, 1H), 3.89–3.86 (m, 2H), 3.71 (s, 3H), 3.70 (d, J = 1.9 Hz, 3H), 3.70 (s, 3H), 3.34 (s, 1H), 2.84 (dd, J = 13.7, 5.4 Hz, 1H), 2.77 (dd, J = 13.7, 6.9 Hz, 1H), 2.74–2.69 (m, 1H), 2.48 (t, J = 8.2 Hz, 1H), 2.43 (dd, J = 18.2, 10.1 Hz, 1H), 2.01 (t, J = 7.0 Hz, 2H), 1.66 (dd, J = 12.0, 6.3 Hz, 2H), 1.65–1.60 (m, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.90, 169.42, 149.20, 149.11, 147.76, 147.22, 131.67, 131.06, 121.75, 120.85, 113.79, 113.35, 112.80, 112.25, 71.18, 68.24, 55.91, 55.80, 55.39, 46.07, 41.25, 37.37, 34.11, 32.41, 28.80, 22.35. FT-MS: calcd for $\text{C}_{26}\text{H}_{34}\text{NO}_8$, $[\text{M} + \text{H}]^+$, 488.2277, found 488.2278. HPLC purity: 99.27% (t_{R} = 5.81 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

6-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxyhexanamide (A3). Yellow oil, yield: 60.5%. ^1H NMR (600 MHz, DMSO- d_6) δ 10.34 (s, 1H), 8.67 (s, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.81 (t, J = 8.7 Hz, 1H), 6.77 (d, J = 1.2 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.63 (d, J = 14.0 Hz, 1H), 6.60 (d, J = 8.0 Hz, 1H), 4.09 (t, J = 7.9 Hz, 1H), 3.90–3.88 (m, 1H), 3.87 (d, J = 6.1 Hz, 2H), 3.71 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.34 (s, 1H), 2.84 (dd, J = 13.7, 5.3 Hz, 1H), 2.77 (dd, J = 13.6, 7.0 Hz, 1H), 2.73–2.68 (m, 1H), 2.48 (d, J = 8.0 Hz, 1H), 2.44

(dd, J = 12.7, 5.4 Hz, 1H), 1.96 (t, J = 7.3 Hz, 2H), 1.68 (dd, J = 13.9, 6.9 Hz, 2H), 1.53 (dd, J = 15.0, 7.5 Hz, 2H), 1.37 (dd, J = 16.9, 9.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.90, 169.49, 149.22, 149.11, 147.76, 147.26, 131.67, 131.05, 121.76, 120.84, 113.79, 113.38, 112.80, 112.25, 71.18, 68.52, 55.91, 55.79, 55.39, 46.07, 41.27, 37.37, 34.13, 32.70, 29.07, 25.68, 25.39. FT-MS: calcd for $\text{C}_{27}\text{H}_{36}\text{NO}_8$, $[\text{M} + \text{H}]^+$, 502.2433, found 502.2435. HPLC purity: 99.87% (t_{R} = 2.71 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

7-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxyheptanamide (A4). Yellow oil, yield: 42.8%. ^1H NMR (600 MHz, DMSO- d_6) δ 10.33 (s, 1H), 8.66 (s, 1H), 6.85–6.83 (m, 1H), 6.80 (d, J = 17.5 Hz, 1H), 6.75 (d, J = 26.9 Hz, 1H), 6.68 (d, J = 7.9 Hz, 1H), 6.63 (d, J = 13.7 Hz, 1H), 6.58 (t, J = 14.4 Hz, 1H), 4.09 (t, J = 7.7 Hz, 1H), 3.89 (d, J = 3.1 Hz, 1H), 3.87 (s, 2H), 3.73 (s, 9H), 3.34 (s, 1H), 2.84 (dd, J = 13.6, 5.0 Hz, 1H), 2.77 (dd, J = 13.5, 7.0 Hz, 1H), 2.71 (d, J = 5.9 Hz, 1H), 2.49–2.44 (m, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.69–1.64 (m, 2H), 1.52–1.47 (m, 2H), 1.37 (d, J = 6.3 Hz, 2H), 1.28 (d, J = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.90, 169.56, 149.25, 149.11, 147.76, 147.27, 131.67, 131.05, 121.76, 120.84, 113.82, 113.43, 112.80, 112.25, 71.18, 68.57, 55.93, 55.91, 55.79, 46.07, 41.28, 37.37, 34.14, 32.69, 29.20, 28.83, 25.76, 25.56. FT-MS: calcd for $\text{C}_{28}\text{H}_{38}\text{NO}_8$, $[\text{M} + \text{H}]^+$, 516.2594, found 516.2591. HPLC purity: 98.01% (t_{R} = 6.15 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

8-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxyheptanamide (A5). Yellow oil, yield: 49.2%. ^1H NMR (600 MHz, DMSO- d_6) δ 10.32 (s, 1H), 8.65 (s, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.77 (d, J = 1.3 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 1.2 Hz, 1H), 6.59 (d, J = 8.1 Hz, 1H), 4.08 (t, J = 7.9 Hz, 1H), 3.88 (t, J = 6.5 Hz, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.33 (s, 1H), 2.84 (dd, J = 13.7, 5.2 Hz, 1H), 2.77 (dd, J = 13.7, 7.0 Hz, 1H), 2.71 (dd, J = 13.2, 7.2 Hz, 1H), 2.47 (d, J = 6.3 Hz, 1H), 2.44 (dd, J = 11.2, 6.0 Hz, 1H), 1.93 (t, J = 7.3 Hz, 2H), 1.66 (dd, J = 13.8, 6.4 Hz, 2H), 1.48 (dt, J = 14.5, 7.3 Hz, 2H), 1.39–1.35 (m, 2H), 1.31–1.28 (m, 2H), 1.24 (d, J = 7.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.90, 169.57, 149.25, 149.11, 147.76, 147.27, 131.67, 131.03, 121.76, 120.84, 113.81, 113.43, 112.80, 112.25, 71.18, 68.60, 55.93, 55.91, 55.79, 46.06, 41.28, 37.37, 34.15, 32.71, 29.25, 29.01, 28.95, 25.92, 25.54. FT-MS: calcd for $\text{C}_{29}\text{H}_{40}\text{NO}_8$, $[\text{M} + \text{H}]^+$, 530.2754, found 530.2748. HPLC purity: 98.04% (t_{R} = 6.82 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

9-4-(((3R,4R)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-N-hydroxyheptanamide (A6). Yellow oil, yield: 50.3%. ^1H NMR (600 MHz, DMSO- d_6) δ 10.32 (s, 1H), 8.65 (s, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.77 (d, J = 1.3 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 1.2 Hz, 1H), 6.59 (d, J = 8.1 Hz, 1H), 4.08 (t, J = 7.9 Hz, 1H), 3.89 (d, J = 4.0 Hz, 1H), 3.88 (d, J = 6.3 Hz, 2H), 3.71 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.33 (s, 1H), 2.84 (dd, J = 13.7, 5.2 Hz, 1H), 2.77 (dd, J = 13.7, 7.0 Hz, 1H), 2.73–2.69 (m, 1H), 2.48 (s, 2H), 2.47–2.39 (m, 2H), 1.93 (t, J = 7.3 Hz, 2H), 1.67 (dd, J = 14.0, 7.0 Hz, 2H), 1.48 (dd, J = 14.4, 7.2 Hz, 2H), 1.38–1.34 (m, 2H), 1.30–1.27 (m, 2H), 1.24 (d, J = 7.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6)



δ 177.81, 168.47, 148.16, 148.02, 146.67, 146.18, 130.58, 129.94, 120.67, 119.75, 112.72, 112.35, 111.71, 111.16, 70.09, 67.53, 55.21, 54.84, 54.82, 54.70, 44.97, 40.20, 36.27, 33.07, 31.63, 28.98, 28.19, 28.09, 24.92, 24.48. FT-MS: calcd for $C_{30}H_{42}NO_8$, $[M + H]^+$, 544.2911, found 544.2904. HPLC purity: 99.45% (t_R = 1.94 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min $^{-1}$ in 15 min.

10-(4-(((3*R*,4*R*)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)-*N*-hydroxyheptanamide (A7). Yellow oil, yield: 32.1%. 1H NMR (600 MHz, DMSO- d_6) δ 10.32 (s, 1H), 8.68 (d, J = 37.2 Hz, 1H), 6.86–6.82 (m, 1H), 6.82–6.78 (m, 1H), 6.78–6.70 (m, 1H), 6.70–6.65 (m, 1H), 6.62 (d, J = 20.0 Hz, 1H), 6.57 (t, J = 15.4 Hz, 1H), 4.09 (dd, J = 16.2, 8.6 Hz, 1H), 3.89 (d, J = 5.0 Hz, 1H), 3.88 (d, J = 6.1 Hz, 2H), 3.71 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.55–3.46 (m, 1H), 3.33 (s, 1H), 2.84 (dt, J = 24.4, 12.3 Hz, 1H), 2.76 (dd, J = 13.6, 7.1 Hz, 1H), 2.74–2.66 (m, 1H), 2.48 (s, 1H), 2.46 (d, J = 7.2 Hz, 1H), 2.43 (dd, J = 18.4, 5.5 Hz, 1H), 1.93 (t, J = 7.3 Hz, 2H), 1.66 (dd, J = 14.0, 6.8 Hz, 2H), 1.49–1.45 (m, 2H), 1.37 (d, J = 6.3 Hz, 2H), 1.24 (s, 6H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 177.82, 168.50, 148.19, 148.04, 146.69, 146.20, 130.60, 129.96, 120.69, 119.76, 112.75, 112.37, 111.72, 111.18, 70.11, 67.54, 54.86, 54.84, 54.72, 44.99, 40.23, 36.30, 34.61, 33.10, 31.66, 28.31, 28.22, 28.16, 28.10, 24.95, 24.53. FT-MS: calcd for $C_{31}H_{44}NO_8$, $[M + H]^+$, 558.3062, found 558.3061. HPLC purity: 98.53% (t_R = 3.44 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min $^{-1}$ in 15 min.

General procedure for the synthesis of A8, A9 in Scheme 2. Arctigenin (50.0 mg, 0.13 mmol), *t*-butyl bromoacetate (0.156 mmol), and K_2CO_3 (35.0 mg, 0.26 mmol) were stirred in 15 mL DMF at 60 °C for 6 h. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **b**. Compound **b** was dissolved in a solution of DCM : TFA = 1 : 1 (V/V) and stirred for 2 h at room temperature, and then the mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **c**. Compound **c** was dissolved with ethyl 4-piperidinecarboxylate or ethyl 4-aminobenzoate, EDCI (30.6 mg, 0.16 mmol), and HOBt (21.6 mg, 0.16 mmol) in dry DMF and stirred for 6 h at room temperature. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compounds **d** or **e**; hydroxylamine hydrochloride (90.0 mg, 1.3 mmol), anhydrous sodium sulfate and potassium hydroxide (72.9 mg, 1.3 mmol) were added at 0 °C and stirred for 0.5 h at room temperature. The resulting mixture was then extracted and filtered, and the obtained filtrate was added dropwise to the methanol solution of compounds **d** or **e**. Potassium hydroxide (0.65 mmol) was added to the mixture, and after 1.5 hours, 20 mL of water was added; the aqueous solution was acidified to pH 4–5 with dilute hydrochloric acid (1 M) and extracted with dichloromethane (50 mL \times 3). The dichloromethane layer was concentrated and dried in a vacuum, and the residue was purified by flash chromatography (silica gel, 200–300 mesh, DCM/MeOH = 50 : 1, V/V) to afford compounds **A8** or **A9**.

1-(2-(4-(((3*R*,4*R*)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetyl)-*N*-hydroxypiperidine-4-carboxamide (A8). White solid, yield: 42.1%. m.p. 105.0–105.6 °C. 1H NMR (600 MHz, DMSO- d_6) δ 10.47 (s,

1H), 8.74 (s, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.78 (d, J = 8.2 Hz, 2H), 6.72–6.62 (m, 2H), 6.60 (d, J = 8.0 Hz, 1H), 4.72 (d, J = 4.4 Hz, 2H), 4.30 (d, J = 11.9 Hz, 1H), 4.10 (t, J = 8.0 Hz, 1H), 3.73 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.37 (s, 2H), 3.02 (t, J = 11.1 Hz, 1H), 2.83 (dd, J = 13.7, 5.3 Hz, 1H), 2.77 (dd, J = 13.7, 6.8 Hz, 1H), 2.72 (dd, J = 8.4, 6.2 Hz, 1H), 2.61 (t, J = 12.1 Hz, 1H), 2.49–2.42 (m, 2H), 2.25 (t, J = 10.9 Hz, 1H), 2.12 (s, 1H), 1.63 (s, 3H), 1.44–1.37 (m, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 207.76, 177.79, 170.23, 165.06, 148.09, 148.01, 146.66, 145.50, 130.66, 130.56, 120.48, 119.78, 112.80, 111.73, 111.15, 70.09, 67.89, 66.37, 54.90, 54.81, 54.71, 44.95, 36.26, 33.00, 31.49, 30.07, 28.97, 28.04, 27.40. FT-MS: calcd for $C_{29}H_{37}N_2O_9$, $[M + H]^+$ 557.2502, found 557.2493. HPLC purity: 98.39% (t_R = 6.90 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min $^{-1}$ in 15 min.

1-(2-(4-(((3*R*,4*R*)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)-*N*-hydroxybenzamide (A9). Yellow solid, yield: 28.3%. m.p. 118.1–118.3 °C. 1H NMR (600 MHz, DMSO- d_6) δ 9.23 (s, 1H), 7.17 (d, J = 6.8 Hz, 1H), 6.90–6.86 (m, 1H), 6.83 (s, 1H), 6.82 (s, 1H), 6.78 (d, J = 1.8 Hz, 1H), 6.78 (s, 1H), 6.76 (s, 1H), 6.71 (dd, J = 8.0, 1.1 Hz, 1H), 6.66 (d, J = 1.7 Hz, 1H), 6.61–6.59 (m, 2H), 6.55–6.51 (m, 1H), 4.71 (s, 2H), 4.10 (s, 1H), 3.88 (s, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.07 (d, J = 9.4 Hz, 2H), 2.7–2.77 (m, 2H), 2.48 (d, J = 5.8 Hz, 1H), 2.46 (s, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 177.81, 172.24, 164.99, 148.08, 148.02, 146.68, 145.53, 141.30, 130.62, 130.58, 125.11, 124.71, 122.74, 120.51, 119.79, 115.48, 115.23, 112.83, 112.76, 111.75, 111.17, 70.10, 66.39, 54.92, 54.83, 54.73, 44.97, 42.30, 36.28, 33.02. FT-MS: calcd for $C_{30}H_{33}N_2O_9$, $[M + H]^+$, 565.2171, found 565.2180. HPLC purity: 99.32% (t_R = 4.61 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min $^{-1}$ in 15 min.

General procedure for the synthesis of A10 in Scheme 3. The *t*-butyl bromoacetate was dissolved in DMF along with NaN $_3$ and reacted for 6 hours at 80 °C. The resulting mixture was extracted with ethyl acetate (20 mL \times 3) and concentrated under reduced pressure to obtain compound **f**. Arctigenin (1.0 equiv.), bromopropyne (1.5 equiv.), and K_2CO_3 (2.0 equiv.) were dissolved in DMF and reacted for 6 hours at 60 °C. The mixture was then extracted with ethyl acetate (20 mL \times 3) and concentrated under reduced pressure to obtain compound **n**. Compound **g**, compound **f**, $CuSO_4 \cdot 5H_2O$ (41.5 mg, 0.26 mmol), and sodium ascorbate (25.8 mg, 0.13 mmol) were mixed in H_2O : *t*-BuOH = 1 : 1 (V/V) and stirred at 60 °C for four hours. The mixture was poured into water and extracted with DCM (20 mL \times 3). The combined organic layers were concentrated to obtain compound **h**. Hydroxylamine hydrochloride (90.0 mg, 1.3 mmol), anhydrous sodium sulfate and potassium hydroxide (72.9 mg, 1.3 mmol) were added at 0 °C and stirred for 0.5 h at room temperature. The mixture was then extracted and filtered, and the obtained filtrate was added dropwise to the methanol solution of compound **h**. Potassium hydroxide (0.65 mmol) was added to the mixture, and after 1.5 hours, 20 mL of water was added. The aqueous solution was acidified to pH 4–5 with dilute hydrochloric acid (1 M) and extracted with DCM (50 mL \times 3). The dichloromethane layer was concentrated and dried under vacuum, and the residue was purified by flash column

chromatography (silica gel, 200–300 mesh, DCM/MeOH = 50 : 1, V/V) to obtain compound **A10**.

2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-hydroxyacetamide (**A10**). White solid, yield: 38.3%. m.p. 98.3–99.1 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.15 (s, 1H), 7.05–6.83 (m, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.78 (d, *J* = 1.5 Hz, 1H), 6.72–6.69 (m, 1H), 6.65 (d, *J* = 1.6 Hz, 1H), 6.60 (dd, *J* = 8.1, 1.6 Hz, 1H), 5.06 (s, 2H), 4.95 (d, *J* = 46.5 Hz, 2H), 4.10 (t, *J* = 8.0 Hz, 1H), 3.89 (t, *J* = 8.4 Hz, 1H), 3.70 (s, 6H), 3.69 (s, 3H), 2.83 (dd, *J* = 18.1, 12.9 Hz, 1H), 2.81–2.72 (m, 2H), 2.55–2.52 (m, 1H), 2.49–2.43 (m, 2H), 1.23 (s, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.91, 162.66, 149.22, 149.10, 147.76, 146.67, 142.97, 132.01, 131.65, 131.58, 129.14, 126.36, 121.63, 120.86, 113.71, 112.81, 112.26, 71.19, 65.50, 62.03, 55.92, 55.81, 50.51, 46.06, 37.38, 34.14. FT-MS: calcd for C₂₆H₃₁N₄O₈, [M + H]⁺, 527.2130, found 527.2136. HPLC purity: 98.69% (*t*_R = 7.21 min, 10% MeCN/90% H₂O/0.1% TFA), 1.0 mL min^{−1} in 15 min.

General procedure for the synthesis of B1–B5 in Scheme 4.

Arctigenin (50.0 mg, 0.13 mmol), *t*-butyl bromoacetate (0.02 mL, 0.156 mmol), and K₂CO₃ (35 mg, 0.26 mmol) were stirred in 15 mL DMF at 60 °C for 6 h. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **e**. Compound **e** was dissolved in a solution of DCM : TFA = 1 : 1 (V/V) and stirred for 2 h at room temperature, and then the mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **c**. Compound **c** was dissolved with amino acid *t*-butyl esters of different carbon chain lengths (0.16 mmol), EDCI (30.6 mg, 0.16 mmol), and HOBt (21.6 mg, 0.16 mmol) in dry DMF and stirred for 6 h at room temperature. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compounds **d1–d5**. Compound **i** was dissolved in a solution of DCM : TFA = 1 : 1 (V/V) and stirred for 2 h at room temperature, and then the mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compounds **j1–j5**. *o*-Phenylenediamine (17.3 mg, 0.26 mmol), DIPEA (2.8 mL, 0.26 mmol), and HATU (60.8 mg, 0.26 mmol) were added to a solution of acid compound **j1–j5** anhydrous DMF. The mixture was stirred at room temperature for 6 h. Water was added, and the mixture was extracted with ethyl acetate. The organic layers were combined, concentrated, and purified with flash column chromatography (DCM/MeOH = 50 : 1, V/V) to afford compounds **B1–B5**.

N-(2-Aminophenyl)-2-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)acetamide (**B1**). White solid, yield: 53.1%. m.p. 104.2–104.9 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.21 (s, 1H), 8.17–8.15 (m, 1H), 7.12 (d, *J* = 7.4 Hz, 1H), 6.91 (t, *J* = 7.0 Hz, 2H), 6.82 (d, *J* = 8.1 Hz, 2H), 6.7–6.66 (m, 2H), 6.65 (d, *J* = 1.7 Hz, 1H), 6.59 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.53 (dd, *J* = 10.9, 4.1 Hz, 1H), 4.90 (s, 2H), 4.51 (s, 2H), 4.10 (t, *J* = 8.0 Hz, 1H), 4.00 (d, *J* = 5.5 Hz, 2H), 3.88 (t, *J* = 8.5 Hz, 1H), 3.75 (s, 3H), 3.70 (d, *J* = 1.0 Hz, 6H), 3.33 (s, 1H), 2.85 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.75 (ddd, *J* = 20.9, 13.2, 6.8 Hz, 2H), 2.51 (d, *J* = 8.0 Hz, 1H), 2.49–2.46 (m, 1H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.88, 168.83, 167.85, 149.48, 149.10, 147.76, 146.39, 143.01, 132.67, 131.64, 126.71, 126.36, 123.02,

121.75, 120.87, 116.40, 115.99, 115.09, 113.95, 112.81, 112.25, 71.19, 68.93, 56.03, 55.91, 55.81, 46.02, 42.65, 41.31, 37.36, 34.15. FT-MS: calcd for C₃₁H₃₆N₃O₈, [M + H]⁺, 578.2495, found 578.2496. HPLC purity: 99.22% (*t*_R = 7.07 min, 10% MeCN/90% H₂O/0.1% TFA), 1.0 mL min^{−1} in 15 min.

N-(2-Aminophenyl)-3-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)propanamide (**B2**). White solid, yield: 44.1%. m.p. 105.1–106.3 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.27 (s, 1H), 8.04 (t, *J* = 5.6 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 6.90–6.84 (m, 2H), 6.83–6.80 (m, 2H), 6.70 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.68–6.64 (m, 2H), 6.59 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.54–6.49 (m, 1H), 4.97 (d, *J* = 67.1 Hz, 2H), 4.42 (s, 2H), 4.10 (dd, *J* = 8.6, 7.4 Hz, 1H), 3.88 (t, *J* = 8.5 Hz, 1H), 3.74 (s, 3H), 3.70 (d, *J* = 2.4 Hz, 6H), 3.43 (q, *J* = 6.6 Hz, 2H), 2.84 (dd, *J* = 13.7, 5.3 Hz, 1H), 2.74 (ddd, *J* = 20.9, 13.1, 6.9 Hz, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.47 (dd, *J* = 15.7, 5.5 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.87, 169.88, 168.32, 149.50, 149.09, 147.75, 146.40, 142.41, 132.67, 131.63, 126.19, 125.84, 123.71, 121.74, 120.86, 116.40, 116.19, 115.15, 113.96, 112.82, 112.25, 71.18, 69.06, 56.01, 55.91, 55.82, 46.01, 41.32, 37.34, 35.98, 35.50, 34.17. FT-MS: calcd for C₃₂H₃₇N₃NaO₈, [M + H]⁺, 614.2480, found 614.2472. HPLC purity: 99.89% (*t*_R = 6.49 min, 10% MeCN/90% H₂O/0.1% TFA), 1.0 mL min^{−1} in 15 min.

N-(2-Aminophenyl)-3-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)propanamide (**B3**). White solid, yield: 34.1%. m.p. 106.2–106.8 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.09 (s, 1H), 7.91 (t, *J* = 5.5 Hz, 1H), 7.16 (d, *J* = 7.0 Hz, 1H), 6.88 (dd, *J* = 11.1, 3.9 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 2H), 6.73–6.67 (m, 2H), 6.65 (d, *J* = 1.5 Hz, 1H), 6.60–6.57 (m, 1H), 6.53 (t, *J* = 7.0 Hz, 1H), 4.86 (s, 2H), 4.41 (s, 2H), 4.09 (t, *J* = 7.7 Hz, 1H), 3.88 (t, *J* = 8.2 Hz, 1H), 3.75 (s, 3H), 3.70 (d, *J* = 1.4 Hz, 6H), 3.33 (s, 2H), 3.15 (dd, *J* = 12.7, 6.5 Hz, 2H), 2.79–2.70 (m, 2H), 2.46 (dd, *J* = 13.2, 5.3 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.57 (dd, *J* = 14.9, 7.5 Hz, 2H), 1.50–1.45 (m, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.87, 171.46, 168.20, 149.53, 149.10, 147.76, 146.47, 142.24, 132.69, 131.64, 126.14, 125.70, 124.05, 121.74, 120.85, 116.67, 116.37, 115.22, 113.94, 112.82, 112.25, 71.18, 69.22, 55.98, 55.91, 55.81, 46.02, 41.35, 38.52, 37.35, 35.83, 34.21, 29.18, 23.14. FT-MS: calcd for C₃₂H₃₈N₃O₈, [M + H]⁺, 606.2819, found 606.2809. HPLC purity: 99.42% (*t*_R = 6.89 min, 10% MeCN/90% H₂O/0.1% TFA), 1.0 mL min^{−1} in 15 min.

N-(2-Aminophenyl)-5-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)pentanamide (**B4**). Yellow oil, yield: 29.3%. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.09 (s, 1H), 7.91 (t, *J* = 5.5 Hz, 1H), 7.16 (d, *J* = 7.0 Hz, 1H), 6.88 (dd, *J* = 11.1, 3.9 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 2H), 6.73–6.67 (m, 2H), 6.65 (d, *J* = 1.5 Hz, 1H), 6.60–6.57 (m, 1H), 6.53 (t, *J* = 7.0 Hz, 1H), 4.86 (s, 2H), 4.41 (s, 2H), 4.09 (t, *J* = 7.7 Hz, 1H), 3.88 (t, *J* = 8.2 Hz, 1H), 3.75 (s, 3H), 3.70 (d, *J* = 1.4 Hz, 6H), 3.33 (s, 2H), 3.15 (dd, *J* = 12.7, 6.5 Hz, 2H), 2.79–2.70 (m, 2H), 2.46 (dd, *J* = 13.1, 5.3 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.60–1.55 (m, 2H), 1.50–1.46 (m, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.86, 171.20, 168.38, 149.55, 149.10, 147.76, 146.47, 142.48, 131.64, 126.22, 125.89, 123.88, 121.74, 120.86, 116.53, 116.24, 115.26, 113.97, 112.83, 112.26, 71.18, 69.24, 55.98, 55.91, 55.82, 46.02, 41.34, 38.37, 37.34, 34.19, 33.59, 29.49, 25.86.



FT-MS: calcd for $C_{34}H_{41}N_3O_8$, $[M + H]^+$, 620.2969, found 620.2966. HPLC purity: 98.44% (t_R = 6.86 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

N-(2-Aminophenyl)-5-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)pentanamide (**B5**). Yellow oil, yield: 56.2%. 1H NMR (600 MHz, $DMSO-d_6$) δ 9.13 (s, 1H), 7.89 (d, J = 5.8 Hz, 1H), 7.16 (dd, J = 7.8, 1.0 Hz, 1H), 6.90–6.80 (m, 4H), 6.73–6.67 (m, 2H), 6.65 (d, J = 1.8 Hz, 1H), 6.59 (dd, J = 8.1, 1.8 Hz, 1H), 6.55–6.50 (m, 1H), 4.83 (s, 2H), 4.40 (s, 2H), 4.09 (d, J = 1.3 Hz, 1H), 3.88 (s, 1H), 3.75 (s, 3H), 3.70 (d, J = 2.2 Hz, 6H), 3.12 (dd, J = 13.1, 6.7 Hz, 2H), 2.86 (dd, J = 13.6, 5.2 Hz, 1H), 2.79–2.70 (m, 2H), 2.51 (d, J = 1.7 Hz, 1H), 2.47 (dd, J = 13.6, 5.6 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 1.61–1.56 (m, 2H), 1.48–1.43 (m, 2H), 1.29 (d, J = 7.0 Hz, 2H), 0.85 (ddd, J = 16.5, 9.7, 5.2 Hz, 3H). ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 178.87, 171.56, 168.16, 149.50, 149.09, 147.76, 146.45, 142.31, 132.67, 131.63, 126.10, 125.71, 124.06, 121.74, 120.85, 116.60, 116.34, 115.16, 113.93, 112.81, 112.24, 71.18, 69.18, 55.99, 55.91, 55.81, 46.02, 41.35, 38.65, 37.34, 36.17, 34.20, 29.35, 26.50, 25.49. FT-MS: calcd for $C_{35}H_{43}N_3O_8$, $[M + H]^+$, 634.3133, found 634.3122. HPLC purity: 98.67% (t_R = 7.01 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

General procedure for the synthesis of B6 and B7 in Scheme 5. Arctigenin (50.0 mg, 0.13 mmol), *t*-butyl bromoacetate (0.156 mmol), and K_2CO_3 (35.0 mg, 0.26 mmol) were stirred in 15 mL DMF at 60 °C for 6 h. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **b**. Compound **b** was dissolved in a solution of DCM : TFA = 1 : 1 (V/V) and stirred for 2 h at room temperature, and then the mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compound **c**. Compound **c** was dissolved with *t*-butyl piperidine-4-carboxylate or *t*-butyl 4-aminobenzoate, EDCI, and HOBT in dry DMF and stirred for 6 h at room temperature. The mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compounds **k** or **l**. Dissolved compound **k** or **l** in a solution of DCM : TFA = 1 : 1 (V/V) was stirred for 2 h at room temperature, and then the mixture was extracted with EtOAc and concentrated under reduced pressure, yielding compounds **m** or **n**. *o*-Phenylenediamine (17.3 mg, 0.26 mmol), DIPEA (2.8 mL, 0.26 mmol), and HATU (60.8 mg, 0.26 mmol) were added to a solution of acid compound **m** or **n**, anhydrous DMF. The mixture was stirred at room temperature for 6 h. Water was added, and the mixture was extracted with ethyl acetate. The organic layers were combined, concentrated, and purified with flash column chromatography (DCM/MeOH = 50 : 1, V/V) to afford compounds **B6** or **B7**.

4-(2-(4-(((3*R*,4*R*)-4-(3,4-Dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)-*N*-hydroxybenzamide (**B6**). White solid, yield: 52.0%. m.p. 109.4–110.2 °C. 1H NMR (600 MHz, $DMSO-d_6$) δ 9.26 (s, 1H), 7.18 (d, J = 7.0 Hz, 1H), 6.90–6.86 (m, 1H), 6.82 (d, J = 8.1 Hz, 2H), 6.80 (d, J = 8.3 Hz, 2H), 6.78 (s, 2H), 6.76 (s, 1H), 6.71 (d, J = 7.9 Hz, 1H), 4.71 (s, 2H), 4.10 (s, 2H), 3.87 (d, J = 8.4 Hz, 2H), 3.73 (s, 3H), 3.72 (s, 3H), 3.70 (s, 6H), 3.08 (s, 2H), 2.84–2.81 (m, 2H), 2.78–2.75 (m, 2H), 2.49–2.46 (m, 2H), 1.23 (s, 4H). ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 178.89, 173.33, 166.20, 166.07, 149.16, 149.10, 147.76, 146.60, 142.37, 131.65, 126.17, 125.77, 123.83, 121.59,

120.87, 116.55, 116.30, 113.90, 113.84, 112.82, 112.25, 71.18, 70.25, 67.47, 55.99, 55.91, 55.80, 46.05, 45.47, 44.46, 43.37, 42.35, 41.27, 37.36, 34.10. FT-MS: calcd for $C_{35}H_{41}N_3NaO_8$, $[M + H]^+$, 631.2933, found 654.2780. HPLC purity: 98.87% (t_R = 7.45 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

N-(2-Aminophenyl)-4-(2-(4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)acetamido)benzamide (**B7**). Yellow solid, yield: 45.3%. m.p. 118.1–118.3 °C. 1H NMR (600 MHz, $DMSO-d_6$) δ 10.29 (s, 1H), 9.59 (s, 1H), 7.97 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.17 (t, J = 10.8 Hz, 1H), 7.00–6.94 (m, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 6.80 (t, J = 6.2 Hz, 1H), 6.78 (dd, J = 8.0, 1.2 Hz, 1H), 6.71 (dd, J = 8.2, 1.7 Hz, 1H), 6.65 (d, J = 1.8 Hz, 1H), 6.60 (td, J = 8.6, 1.5 Hz, 2H), 5.05–4.73 (m, 2H), 4.68 (s, 2H), 4.11 (dd, J = 8.5, 7.4 Hz, 1H), 3.89 (t, J = 8.4 Hz, 1H), 3.78 (s, 3H), 3.69 (s, 3H), 3.69 (s, 3H), 2.94–2.84 (m, 1H), 2.76 (ddd, J = 20.9, 13.1, 6.9 Hz, 2H), 2.50–2.42 (m, 3H). ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 178.87, 167.67, 149.48, 149.10, 147.76, 146.49, 143.62, 141.63, 135.34, 132.66, 131.64, 129.90, 129.20, 127.14, 126.87, 123.91, 121.76, 120.86, 119.05, 117.78, 116.74, 116.61, 115.03, 114.91, 114.02, 112.81, 112.23, 71.19, 69.11, 56.03, 55.90, 55.80, 46.02, 41.36, 37.36, 34.18. FT-MS: calcd for $C_{33}H_{38}N_3NaO_8$, $[M + H]^+$, 640.2653, found 640.2653. HPLC purity: 99.87% (t_R = 6.33 min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

General procedure for the synthesis of B8 in Scheme 6. *t*-Butyl bromoacetate (0.04 mL, 0.26 mmol) was dissolved in DMF along with NaN_3 (16.9 mg, 0.26 mmol), and the mixture was heated at 80 °C for six hours. The resulting mixture was extracted with EtOAc (20 mL \times 3) and concentrated under reduced pressure to obtain compound **o**. Arctigenin (50.0 mg, 0.13 mmol), propargyl bromide (0.01 mL, 0.16 mmol), and K_2CO_3 (35 mg, 0.26 mmol) were dissolved in DMF, and the reaction mixture was heated at 60 °C for six hours. The mixture was then extracted with EtOAc (20 mL \times 3) and concentrated under reduced pressure to obtain compound **g**. Compound **g**, compound **o**, $CuSO_4 \cdot 5H_2O$ (41.5 mg, 0.26 mmol), and sodium ascorbate (25.8 mg, 0.13 mmol) were mixed in H_2O : *t*-BuOH = 1 : 1 (V/V) and stirred at 60 °C for four hours. The mixture was poured into water and extracted with DCM (20 mL \times 3). The combined organic layers were concentrated to obtain compound **p**. Compound **p** was dissolved in a solution of DCM : TFA = 1 : 1 (V/V) and stirred at room temperature for two hours. The mixture was then extracted with ethyl acetate and concentrated under reduced pressure to obtain compound **q**. Compound **q** was mixed with *o*-phenylenediamine (17.3 mg, 0.26 mmol), DIPEA (2.8 mL, 0.26 mmol), and HATU (60.8 mg, 0.26 mmol) in anhydrous DMF. The mixture was stirred for 6 h at room temperature. Water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were concentrated and purified by flash column chromatography (DCM/MeOH = 50 : 1, V/V) to obtain compound **B8**.

N-(2-Aminophenyl)-2-(4-(((4-(((3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-oxotetrahydrofuran-3-yl)methyl)-2-methoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide) (**B8**). White solid, yield: 33.9%. m.p. 130.0–130.3 °C. 1H NMR (600 MHz, $DMSO-d_6$) δ 9.80 (s, 1H), 8.23 (s, 1H), 7.20 (dd, J = 7.8, 1.1 Hz, 1H), 7.06 (d, J = 8.3 Hz,



1H), 6.93–6.89 (m, 1H), 6.83–6.81 (m, 1H), 6.79 (d, $J = 1.7$ Hz, 1H), 6.74–6.69 (m, 2H), 6.65 (d, $J = 1.6$ Hz, 1H), 6.60 (dd, $J = 8.1$, 1.6 Hz, 1H), 6.55–6.50 (m, 1H), 5.39 (s, 2H), 5.09 (s, 2H), 5.07 (s, 2H), 4.10 (t, $J = 8.0$ Hz, 1H), 3.88 (t, $J = 8.4$ Hz, 1H), 3.70 (s, 9H), 2.85 (dd, $J = 13.7$, 5.3 Hz, 1H), 2.79 (dd, $J = 13.7$, 6.9 Hz, 1H), 2.74–2.70 (m, 1H), 2.49 (d, $J = 8.3$ Hz, 2H), 2.47–2.45 (m, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.92, 164.84, 149.25, 149.11, 147.76, 146.69, 143.00, 142.71, 131.66, 131.60, 126.75, 126.71, 125.94, 122.66, 121.64, 120.87, 116.35, 116.06, 113.76, 113.71, 112.82, 112.26, 71.20, 70.25, 62.09, 55.92, 55.81, 52.42, 46.07, 41.25, 37.38, 34.15. FT-MS: calcd for $\text{C}_{32}\text{H}_{35}\text{N}_5\text{NaO}_7$, $[\text{M} + \text{H}]^+$, 624.2423, found 624.2428. HPLC purity: 99.56% ($t_{\text{R}} = 7.58$ min, 10% MeCN/90% H_2O /0.1% TFA), 1.0 mL min^{-1} in 15 min.

Pharmacology

Cell cytotoxicity assay. In this study, the *in vitro* cytotoxicity of test compounds against five different tumor cell lines was evaluated using the CCK-8 assay. Initially, cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and incubated under stable conditions at 37 °C with 5% CO_2 for 24 hours. Subsequently, various concentrations of compounds, including **A1–10**, **B1–B8**, arctigenin, and tucidinostat, were added to each well. The cells were then incubated for 48 hours in the presence of the compounds. After that, CCK-8 solution (100 μL , with a concentration of 0.5 mg mL^{-1}) was added to each well, followed by an additional 4 hour incubation period. Finally, the absorbance of the samples was measured at 450 nm. By calculating the inhibition rate using the Logit method, the IC_{50} values of the compounds were determined, representing the concentration at which the compounds inhibit cell proliferation by half.

Cell enzyme activity assay. The activity of HDAC in cells was detected using Bio-Vision's HDAC 1, 2, 3, 4, 5, 6, and 10 Activity Assay Kit and followed its protocols. Transfer 50 μL compounds were transferred into 384 plates, each column containing 2 replicates. The 384 assay plate was centrifuged at 1000 rpm, 2.5 μL HDAC was added into each assay well and incubated for 15 min, 37 °C. 2.5 μL substrate was added into each assay well and incubated for 60/180 min, 37 °C. 5 μL Developer mixture was added into each assay well and incubated for 10 min. FI signal (em: 340 nm; ex: 450 nm) was read using BMG.

Annexin V/PI staining assay. In the presented study, the level of cell apoptosis was assessed through the application of the Annexin V/PI staining procedure. An initial seeding of the cells was done in a 96-well plate at a concentration of 5×10^5 cells in each well. The cells were then incubated at 37 °C under a 5% CO_2 environment for a duration of 24 hours. Post incubation, the cells were exposed to varying quantities (0, 0.25, 0.5 and 1.0 μM) of the compound **B7** and incubated for an additional 48 hours. Upon completion of the incubation period, the cells were harvested and subjected to a wash with 500 μL of the annexin-binding buffer. The cells were then marked with 5 μL of Annexin V-FITC and 5 μL of Propidium Iodide (PI) and kept for 15 minutes at 37 °C for staining. In the final step of the process, a flow cytometer (Beckman Coulter cytoFLEX, based in Brea, California, USA) was used for the analysis of the samples.

Cell cycle analysis. Cell cycle analysis was conducted using the PI staining method in this study. Initially, MV411 cells were seeded at a density of 5×10^5 cells per well in a 6-well plate and incubated for 24 hours. Subsequently, the cells were treated with different concentrations (0, 0.25, 0.5 and 1.0 μM) of compound **B7** and further incubated for 48 hours. The cells were then collected and washed with ice-cold PBS buffer, followed by fixation with 70% ethanol at 4 °C for 12 hours and another wash with PBS. After that, 100 μL of RNase A was added, and the cells were incubated at 37 °C for 30 minutes. Finally, the cells were stained with 400 μL of PI at 4 °C for 30 minutes and analyzed for absorbance at 488 nm wavelength using a flow cytometer (Beckman Coulter cytoFLEX, USA).

GreenNuc living cell caspase 3 activity assay. At per hole 1×10^6 cells of MV411 cells were cultured in a six-well plate and treated with different concentrations of **B7** compounds (0, 0.25, 0.5 and 1.0 μM) for 48 hours. The treated cells were incubated in GreenNuc™ Caspase-3 substrate and then analyzed by flow cytometry. MV411 cells were seeded uniformly at a concentration of 5000 cells per well in 6-well plates. After 6 h incubation, arctigenin, compound **B7**, and tucidinostat were respectively added to each well. 48 hours later, the cells were collected in Eppendorf tubes and centrifuged at 1000 rpm for 5 minutes. Following the manufacturer's instructions, the cells were washed twice with PBS and subjected to Caspase-3 staining. After a 40 minute incubation at room temperature, the cells were washed twice with PBS and stained with the live-cell dye Hoechst 33342 for 5 minutes at room temperature, followed by a PBS wash. The stained cells were resuspended in PBS and subsequently observed and photographed under a fluorescence microscope.

Western blotting assay. Cell extracts were obtained using a chilled lysis buffer supplemented with protein inhibitors, then centrifuged at 12 000 g at 4 °C for a duration of 15 minutes. Protein levels in the clear supernatants were determined with the BCA protein assay kit. Following this, the protein samples underwent 12% SDS-PAGE separation, were moved onto a PVDF sheet, and then blocked using 5% non-fat milk in TBS with 0.10% Tween 20 for an hour at ambient temperature. The sheets were later treated with primary antibodies within 5% non-fat milk for 6 hours at 4 °C, then with secondary antibodies for an additional hour at ambient conditions. Antibody interactions were displayed using an ECL detection solution.

Statistical analysis. Values are presented as mean \pm SD. The distinction between two groups was evaluated using the Student's *t*-test. For contrasting multiple groups, ANOVA was applied, succeeded by the Student-Newman-Keuls method. A *P* value of less than 0.05 indicated statistical relevance.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its ESI.†

Author contributions

Compound design and synthesis: Xinyue Jiang, Yuchao Yan; *in vitro* activity testing: Xinyue Jiang, Huali Yang; writing: Xinyue



Jiang; review and editing: Yang Liu; arctigenin supply and subject cooperation: Deqiang Dou; funding and supervision: Yang Liu, Maosheng Cheng.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This project was financially supported by National Natural Science Foundation of China (No. 22177079). Dr Y. Liu wishes to express his thanks for the support by the Program for Liaoning Innovative Talents in University (LR2017043). Dr D. Dou wishes to express his gratitude for the support provided by the Key Scientific Research Projects of Intercollegiate Cooperation in Colleges and Universities of Liaoning Province (2020JYT005). The figure in Entry for the Table of Contents was made by Figdraw.

Notes and references

- 1 Q. C. Li, Y. Liang, Y. Tian and G. R. Hu, *J. BUON*, 2016, **21**, 87–94.
- 2 N. Kudou, A. Taniguchi, K. Sugimoto, Y. Matsuya, M. Kawasaki, N. Toyooka, C. Miyoshi, S. Awale, D. F. Dibwe, H. Esumi, S. Kadota and Y. Tezuka, *Eur. J. Med. Chem.*, 2013, **60**, 76–88.
- 3 Y. Tezuka, K. Yamamoto, S. Awale, F. Lia, S. Yomoda and S. Kadota, *Nat. Prod. Commun.*, 2013, **8**, 463–466.
- 4 S. Su, X. Cheng and M. Wink, *J. Pharm. Pharmacol.*, 2015, **67**, 1316–1323.
- 5 S. Susanti, H. Iwasaki, M. Inafuku, N. Taira and H. Oku, *Phytomedicine*, 2013, **21**, 39–46.
- 6 S. Yang, J. Ma, J. Xiao, X. Lv, X. Li, H. Yang, Y. Liu, S. Feng and Y. Zhang, *Anat. Rec.*, 2012, **295**, 1260–1266.
- 7 K. Kang, H. J. Lee, J. H. Yoo, E. H. Jho, C. Y. Kim, M. Kim and C. W. Nho, *DNA Cell Biol.*, 2011, **30**, 623–629.
- 8 C. J. Hsieh, P. L. Kuo, Y. C. Hsu, Y. F. Huang, E. M. Tsai and Y. L. Hsu, *Free Radical Biol. Med.*, 2014, **67**, 159–170.
- 9 J. H. Yoo, H. J. Lee, K. Kang, E. H. Jho, C. Y. Kim, D. Baturen, J. Tunsag and C. W. Nho, *Food Chem. Toxicol.*, 2010, **48**, 2247–2252.
- 10 Y. Gu, C. Scheuer, D. Feng, M. D. Menger and M. W. Laschke, *Anticancer Drugs*, 2013, **24**, 781–791.
- 11 S. Kuehn, S. Schroecksnadel, V. Temml, J. M. Gostner, H. Schennach, D. Schuster, S. Schwaiger, J. M. Rollinger, D. Fuchs and H. Stuppner, *Phytomedicine*, 2013, **20**, 1190–1195.
- 12 J. J. Tong, J. Liu, N. R. Bertos and X. J. Yang, *Nucleic Acids Res.*, 2002, **30**, 1114–1123.
- 13 T. Eckschlager, J. Plch, M. Stiborova and J. Hrabeta, *Int. J. Mol. Sci.*, 2017, **18**, E1414.
- 14 D. Li, Z. Zhang, Y. Li, X. Wang, H. Zhong, H. Yang, Y. Xi, H. Liu, A. Shen and Y. Hu, *J. Med. Chem.*, 2023, **66**, 7016–7037.
- 15 M. Brindisi, A. P. Saraswati, S. Brogi, S. Gemma, S. Butini and G. Campiani, *J. Med. Chem.*, 2020, **63**, 23–39.
- 16 J. Roche and P. Bertrand, *Eur. J. Med. Chem.*, 2016, **121**, 451–483.
- 17 Y. Huang, N. Liu, Z. Pan, Z. Li and C. Sheng, *J. Med. Chem.*, 2023, **66**, 1239–1253.
- 18 T. C. S. Ho, A. H. Y. Chan and A. Ganesan, *J. Med. Chem.*, 2020, **63**, 12460–12484.
- 19 P. A. Marks and R. Breslow, *Nat. Biotechnol.*, 2007, **25**, 84–90.
- 20 L. P. Yang, *BioDrugs*, 2011, **25**, 393–395.
- 21 R. M. Poole, *Drugs*, 2014, **74**, 1543–1554.
- 22 K. P. Garnock-Jones, *Drugs*, 2015, **75**, 695–704.
- 23 R. Parveen, D. Harihar and B. P. Chatterji, *Cancer*, 2023, **129**, 3372–3380.

