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Pharmacological overview of hederagenin and its derivatives

Xing Huang,† Qing-Kun Shen, Hong-Yan Guo, Xiaoting Li* and Zhe-Shan Quan *

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Hederagenin is a pentacyclic triterpenoid isolated from plants and widely distributed in a variety of medicinal plants. By integrating and analyzing external related literature reports, the latest research progress on the pharmacological effects and structural modification of hederagenin was reviewed. Hederagenin has a wide range of pharmacological activities, including antitumor, anti-inflammatory, antidepressant, anti-neurodegenerative, antihyperlipidemic, antidiabetic, anti-leishmaniasis, and antiviral activities. Among them, it shows high potential in the field of anti-tumor treatment. This paper also reviews the structural modifications of hederagenin, including carboxyl group modifications and two hydroxyl group modifications. Future research on hederagenin will focus on prolonging its half-life, improving its bioavailability and structural modification to enhance its pharmacological activity, accelerating the preclinical research stage of hederagenin for it to enter the clinical research stage as soon as possible.

1. Introduction

Natural products and metabolites found in animals, plants, insects, marine organisms, and microorganisms are important for the development of new drugs. Many endogenous chemical components in humans and animals are collectively referred to as natural products. Their abundance, novel properties, and diverse structures offer exciting possibilities for researchers to develop new molecular entities for disease treatment.[1–3] Natural products have achieved great success in drug development. For example, in 2015, the Nobel Prize in Physiology or Medicine was awarded to Professor Youyou Tu for her contribution to the study of the drug artemisinin and its breakthrough in the treatment of a devastating parasitic infection.[4]

Hederagenin was first discovered in the seeds of England ivy (*Hedera helix* L., a species of flowering plant of the family Araliaceae, native to most of Europe and western Asia) in 1849 and is a triterpenoid saponin.[5] Hederagenin is also found in other species of plants, from various sources (Table 1).

Structurally, hederagenin has hydroxyl groups at positions C-3 and C-23 in ring A, and double bonds at positions C-12 and C-13 in ring C, and a carboxylic acid group is present at the C-28 position in ring E (Fig. 1).

Hederagenin has received increasing attention over the past decade due to its favorable therapeutic effects (Fig. 2).

Key Laboratory of Natural Medicines of the Changbai Mountain, Affiliated Ministry of Education, College of Pharmacy, Yanbian University, Yanji, Jilin, 133002, China. E-mail: sypulixiaoting@163.com, zsquan@ybu.edu.cn
† These authors contributed equally.

In this review, we briefly summarize the biological and pharmacological data of hederagenin and highlight the therapeutic potential and value of hederagenin as a promising drug development platform. We hope to improve the existing knowledge and development capabilities of hederagenin and encourage the development of more potent drug candidates from its derivatives for potential clinical applications.

2. Pharmacological activities

Hederagenin is known to have different biological activities such as: anti-inflammatory,[35] anti-fungal,[36] anti-bacterial,[37] anti-diabetic,[38] anti-depressant,[34] anti-neurodegenerative,[39] anti-tumor,[40] leishmanicidal,[41] and antiatherosclerosis effects[42] (Fig. 1).

2.1. Anti-tumor activity

There have been some breakthroughs in the research on the anti-tumor effect of hederagenin. Hederagenin has been shown to have broad potential antitumor effects *in vitro* and *in vivo*, and has been evaluated in a variety of cancers, including colon, liver, head and neck, breast and cervical cancers. Next, a brief overview of the research progress of hederagenin in anti-tumor pharmacology will be introduced, which will provide a basis for future research and development in the field of anti-tumor treatment.

Cheng *et al.*[43] confirmed that hederagenin inhibited breast cancer cells (MCF-7 and MDA-MB-231 cells) in a concentration- and time-dependent manner. The results showed that the experimental group significantly reduced the

Table 1 Lists the source and distribution of some plants containing hederagenin

Plant	Family	Medicinal parts	Ref.
Hedera helix L.	Araliaceae	Leaves	6–8
Paeonia lactiflora	Paeoniaceae	Roots	9
Astrantia major	Saniculoideae	Leaves	10
Patrinia scabiosofolia I	Caprifoliaceae	Roots	11
Campsis grandiflora	Bignoniaceae	Leaves	12
Cussonia bancoensis	Cussonia	Stem bark	13
Klainedoxa gabonensis	Irvingiaceae	Stem bark	14
Kalopanax pictus	Araliaceae	Stem bark	15
Clematis chinensis Osbeck	Clematis	Roots and rhizomes	16
Clematis mandshurica Rupr			
Clematis hexapetala Pall			
Clematis mandshurica Ruprecht	Ranunculaceae	Roots and rhizomes	17
Akebia quinata	Lardizabalaceae	Stems	5
Saponaria officinalis L.	Caryophyllaceae	Rhizomes	18
Salvia sclareoides	Salvia	Aerial parts	19
Dipsacus asper	Dipsacaceae	Roots	20
Cyclocarya paliurus	Juglandaceae	Dried plant	21
Cyclocarya paliurus	Juglandaceae	Leaves	22
Akebia quinata	Lardizabalaceae	Fruits	23
Cornus florida	Comaceae	Bark	24
Gardenia jovis-tonanti	Rubiacea	Roots	25
Crossopteryx febrifuga	Rubiaceae	Bark	26
Lonicera macranthoides Hand.-Mazz.	Caprifoliaceae	Dried buds or first blooms	27
Trapa acornis Nakano	Trapaceae	Husk	28
Callicarpa integerrima Champ.	Verbenaceae	Stems and leaves	29
Pulsatilla dahurica	Ranunculaceae	Rhizome	30
Genianthus laurifolius (Roxb.) Hook. f.	Genianthus	Aerial parts	31
Gypsophila oldhamiana	Caryophyllaceae	Roots	32
Chenopodium quinoa Willd	Chenopodiaceae	Flowers, fruits, seeds	33
Fructus Akebiae	Lardizabalaceae	Fruits	34
Sapindus mukorossi Gaertn	Sapindaceae	Fruit husks	35

content of mitochondrial APAF-1 and cytochrome c protein in breast cancer cells, and up-regulated the activities of caspase-3 and caspase-9. Therefore, hederagenin may induce breast cancer cell apoptosis by regulating the mitochondrial apoptotic pathway.

Hederagenin showed excellent cytotoxicity against A549 and BT20 cells with an IC_{50} of 26.23 and 11.8 μM, respectively. Hederagenin induces apoptosis in A549 cells by blocking mitochondrial potential, and increases cell membrane permeability and inhibits NF-κB activation.[22]

Hederagenin inhibits the viability of colon cancer cells LoVo in a dose- and time-dependent manner with IC_{50} values of 1.39 μM (24 h) and 1.17 μM (48 h). Hederagenin also induced nuclear changes characteristic of apoptosis and increased reactive oxygen species levels in LoVo cells. Furthermore, in LoVo cells, hederagenin up-regulated Bax, caspase-3 and caspase-9, and down-regulated MMP, Bcl-2, Bcl-xL, survivin, procaspase-9, procasp-3, and poly (ADP-ribose) polymerase.[44]

Hederagenin selectively induces cell death in cisplatin-sensitive and cisplatin-resistant HNC cells by promoting changes in $\Delta\Psi m$ and inducing apoptosis. Hederagenin inhibits the Nrf2-antioxidant response element (ARE) pathway and activates p53 in HNC cells, thereby enhancing reactive oxygen species (ROS) production and promoting glutathione depletion. Hederagenin activates the intrinsic apoptotic pathway through cleaved PARP, cleaved caspase-3 and Bax. Selective inhibition of hederagenin was demonstrated in a cisplatin-resistant HNC xenograft model. Taken together, hederagenin induces cell death in resistant HNC cells *via* the Nrf2-ARE antioxidant pathway.[45]

Hederagenin showed the best anticancer activity against HeLa cells with an IC_{50} of 17.42 μg mL^{-1}. Hederagenin may induce apoptosis in HeLa cells through the mitochondrial

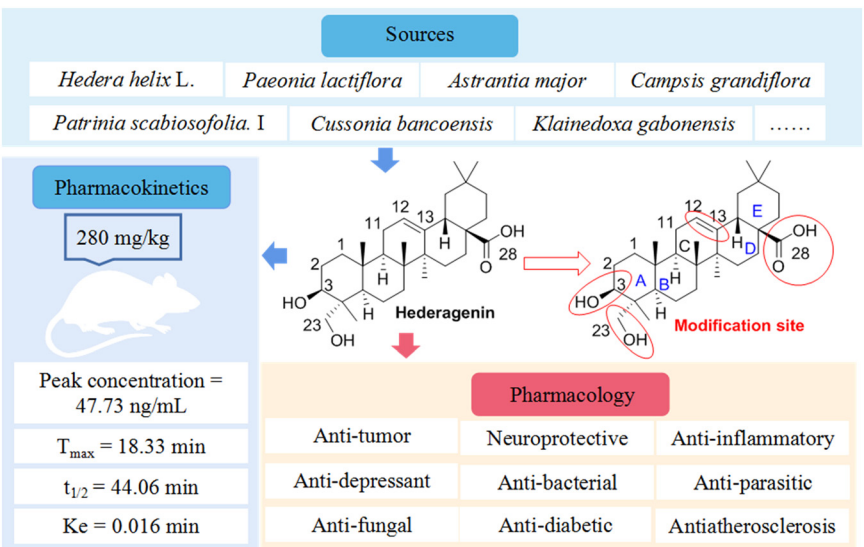


Fig. 1 Overview of sources, pharmacokinetics, pharmacology, and modification site of hederagenin.

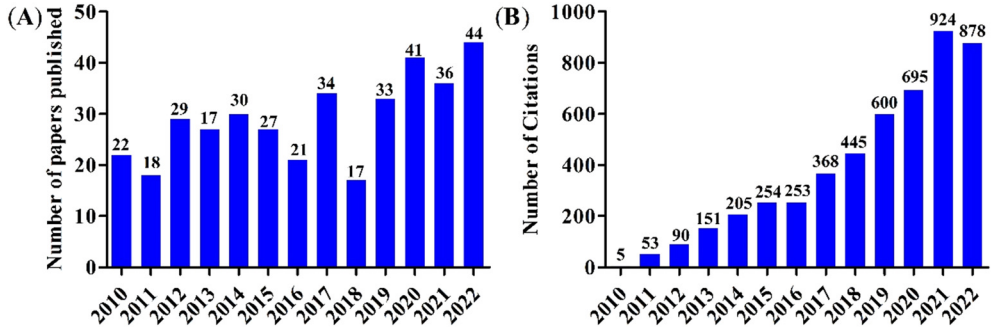


Fig. 2 (A) Number of papers published between 2010 and 2022 containing the keyword "hederagenin", searched according to Web of Science. (B) Citations between 2010 and 2022 using the keyword "hederagenin", searched according to Web of Science.

pathway. By inducing apoptosis and up-regulating MMP, ROS generation and apoptosis-related proteins (up-regulating Bax, cytochrome c, caspase-3 and caspase-9, down-regulating Bcl-2) in a dose-dependent manner, the proliferation ability of HeLa cells was significantly reduced.[46]

Hederagenin decreased the survival rate of lung cancer cells (A549 and H1299 cells) in a dose-dependent manner. Furthermore, hederagenin significantly inhibited colony formation in A549 and H1299 cells at 10 and 20 μM. Hederagenin treatment significantly reduced the protein levels of Aurora A, Aurora B and *p*-Aurora A and the activity of Aurora family kinases in A549 cells.[47]

Bai *et al.*[7] investigated the immunomodulatory and antitumor effects of hederagenin in immunofluorescence in H22 tumor-bearing mice by RT-PCR and western blotting. The results showed that the tumor weight was significantly reduced in the hederagenin-treated group. Hederagenin can repair the damaged thymus and spleen of H22 tumor-bearing mice, and can increase the proliferation ability of spleen lymphocytes. Compared with the control group, hederagenin up-regulated the expression of Bax and down-regulated the expression of Bcl-2 (Table 2).

2.2. Anti-inflammatory activity

Rats were cecal ligated and punctured to induce ALI, and then treated with hederagenin by gavage. Administration of hederagenin increased survival, improved lung injury, and decreased the lung wet/dry ratio and inflammatory cell accumulation in bronchoalveolar lavage fluid (BALF) in ALI rats. Hederagenin exerts protection against sepsis-induced ALI by reducing inflammatory response and macrophage M1 polarization. Hederagenin may activate NLRP3 inflammasome through NF-κB pathway regulation.[48]

Hederagenin treatment of RAW 264.7 cells inhibited lipopolysaccharide (LPS)-stimulated protein expression levels of iNOS, COX-2, and NF-κB as well as NO, PGE2, TNF-α, IL-1β, and IL-6. The results of the carrageenan-induced hind paw edema test in mice showed that hederagenin has an anti-edema effect. In addition, hederagenin inhibited carrageenan-induced skin thickness increase, inflammatory cell infiltration, and mast cell degranulation.[17]

2.3. Anti-bacterial activity

Hederagenin inhibits hemolytic activity, interferes with oligomerization of PLY in a concentration-dependent manner, does not affect the growth of *S. pneumoniae* D39 *in vitro*, and inhibits PLY-mediated cell damage. Therefore, hederagenin has great potential in the treatment of pneumococcal disease.[49]

Liu *et al.* found that hederagenin exhibited significant antibacterial activity against *Staphylococcus aureus* KCTC 503 with an MIC of 16 μg mL^{-1}.[50] Hederagenin showed moderate

Table 2 Anti-tumor activity of hederagenin both *in vitro* and *in vivo*

Cancer	Cell experiment	Potential molecular mechanisms	Ref.
Breast cancer	MCF-7 cells and MDA-MB-231 cells[a]	Inducing apoptosis, mitochondrial Apaf-1↓, cytochrome c proteins↓, caspase-3 and −9↑	43
Lung cancer Breast cancer	IC_{50} (A549 cells) = 26.3 μM, IC_{50} (BT20 cells) = 11.8 μM	Inducing apoptosis, cell membrane permeability↑, mitochondrial potential↓, NF-κB activation↓	22
Colon cancer	IC_{50} (LoVo cells) = 1.39 μM (24 h), 1.17 μM (48 h)	Bax↑, caspase-3↑, caspase-9↑, MMP↓, Bcl-2↓, Bcl-xL↓, survivin↓, procaspase-9↓, procasp-3↓, poly (ADP-ribose) polymerase↓	44
Neck cancer	HNC cells[a]	Activated p53, ROS↑, cleaved PARP, cleaved caspase-3 and Bax, activate apoptosis pathway	45
Cervical cancer	IC_{50} (HeLa cells) = 17.42 μg mL^{-1}	Inducing apoptosis, MMP↑, ROS↑, Bax↑, cytochrome c↑, caspase-3 and −9↑, Bcl-2↓	46
Lung cancer	A549 and H1299 cells[a]	Aurora A↓, Aurora B↓, *p*-Aurora A↓, Aurora family kinase activity↓	47

[a] Hederagenin inhibited the growth of tumor cells, but did not give IC_{50}. ↓Reduce, inhibition or down-regulated. ↑Increase or up-regulated.

activity against *Enterococcus faecalis* with an MIC value of 128 μg mL^{-1}.[26] Hederagenin has significant antibacterial activity against *Streptococcus mutans* ATCC 25175 (Gram-pos.) at a concentration of 9.7 μg mL^{-1}, but almost no antibacterial activity against *Fusobacterium nucleatum* ATCC 10953 (Gram-neg.).[51]

2.4. Neuroprotective effect

Hederagenin protects PC12 cells from CORT-induced damage. In addition, hederagenin prevents the decrease of mitochondrial membrane potential, reduces the production of intracellular ROS, and reduces CORT-induced apoptosis. The protective effects of hederagenin were reversed by the specific phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002 and the AKT (also known as protein kinase B) inhibitor MK2206, suggesting that the effects of hederagenin are mediated by the PI3K/AKT pathway.[52]

Cerebral ischemia/reperfusion (CI/R) injury is a major challenge due to the lack of effective neuroprotective drugs. Hederagenin treatment attenuated CI/R-induced apoptosis and inflammatory cytokine expression in the infarcted area. Hederagenin treatment also decreased the activation of the MLK3 signaling pathway, thereby enhancing CI/R injury through MAPK and NF-κB pathways.[53]

2.5. Anti-depressant activity

Hederagenin has antidepressant effects by inhibiting the reuptake of extracellular monoamines, including serotonin (5-HT), norepinephrine (NE), and dopamine (DA), and enhancing central monoamine signaling.[54]

In uptake assays using rat synaptosomes and transfected cells, hederagenin was found to inhibit 5-HT, NE, and DA in a dose- and time-dependent manner. Furthermore, hederagenin increased the extracellular concentrations of 5-HT, NE, and DA in the frontal cortex of freely moving rats. Therefore, hederagenin was shown to be a novel triple inhibitor of monoamine transporters.[34]

Hederagenin showed significant increases in norepinephrine and serotonin levels in the behavioral despair test and in a rat model of unpredictable chronic mild stress. And it showed a trend of increasing serotonin 1A (5-HT1A) receptor mRNA expression and significantly decreasing serotonin transporter (5-HTT) mRNA expression. Thus, the antidepressant-like effects of hederagenin in the behavioral despair test and in the UCMS rat model may be related to the mRNA expression of monoamine neurotransmitters and 5-HTT.[55]

2.6. Other pharmacological activities

Wu *et al.*[39] confirmed that hederagenin can improve the movement disorders in Parkinson's disease (PD) mouse models and has neuroprotective effects. In addition, hederagenin is regarded as a new autophagy enhancer. Choi *et al.*[56] found that the analgesic properties of hederagenin had a significant inhibitory effect on rheumatoid arthritis.

After treatment with hederagenin, the cardiac dysfunction in diabetic mice was alleviated, and the degree of cardiac hypertrophy and fibrosis was reduced. Hederagenin may achieve cardioprotective effects by reducing inflammation-related activation.[57]

The expression of Bax and p53 decreased and the expression of Bcl-2 increased after hederagenin treatment. Hederagenin treatment attenuated the ethanol-induced increase in activated p38 MAPK and increased the levels of phosphorylated AKT and ERK. Hederagenin attenuates ethanol-induced liver injury through anti-inflammatory and anti-apoptotic activities.[58]

In vitro cell models confirm that hederagenin reduces levels of mutated proteins in neurodegenerative diseases, including huntingtin 74 (HTT74), P301L tau, and A53T α-synuclein (A53T α-syn).[59]

Hederagenin can correct the imbalance of endothelial function by inhibiting the release of a large amount of iNOS and increasing the content of eNOS, inhibiting the IKKβ/NF-κB signaling pathway and reducing the release of IL-6, IFN-γ, TNF-α, and other inflammatory factors. The experimental results show that hederagenin can inhibit or improve the pathological changes related to atherosclerosis and has a good preventive effect on atherosclerosis.[42]

Hederagenin has strong anti-*Toxoplasma* activity and low cytotoxicity, and the inhibition rate of mouse celiac tachyzoites is 64.8%, which is higher than 56.8% of spiramycin. Hederagenin can effectively reduce the liver damage caused by *Toxoplasma gondii* and prolong the survival time of mice to a certain extent.[60]

Xie *et al.*[61] found that hederagenin up-regulated the mRNA and protein levels of TFEB, increasing the distribution of TFEB in the nucleus and the expression of its target genes. Hederagenin also promoted the expression of LC3BII/LC3BI and LAMP2, and promoted the degradation of autophagy and Aβ. Hederagenin reduced Aβ deposition and Aβ-induced paralysis in the nematode head region. Hederagenin improves cognitive dysfunction and pathological changes in APP/PS1 mice by promoting autophagy and activating TFEB. Hederagenin has strong targeting effects on PPARα, and these effects are reversed by MK-886, a selective PPARα antagonist. Studies have shown that hederagenin alleviates the pathology of Alzheimer's disease by inducing autophagy, and the mechanism is related to the PPARα/TFEB pathway.

Acute kidney injury (AKI) is a serious kidney disease caused by ischemia and toxicity.[62] lncRNA-A330074k22Rik was initially found in the kidney tissue of cisplatin induced AKI, and is an important participant in the pathogenesis of AKI. Xie *et al.*[63] found that hederagenin had a protective effect on cisplatin induced AKI and LPS-induced inflammatory damage of renal tubular epithelial cells. Mechanically, inhibition of LncRNA A330074k22Rik by hederagenin significantly inhibited the Axin2/β-catenin pathway, thereby significantly down-regulating the inflammatory response of AKI *in vivo* and *in vitro*. In conclusion, hederagenin inhibits the Axin2/β-catenin pathway

by down-regulating lncRNA-A330074k22Rik and improves cisplatin induced acute kidney injury in mice.

Hederagenin is a white crystalline powder, odorless, with a bitter taste. It is highly insoluble in water and slightly soluble in methanol and ethanol.[38] At an oral dose of 280 mg kg^{-1}, hederagenin could be detected in rat plasma within 5 min and reached a peak concentration of 47.73 ng mL^{-1}. The T_{max} was 18.33 min, indicating that hederagenin could be rapidly absorbed in the gastrointestinal tract. Likewise, hederagenin was rapidly eliminated with a $t_{1/2}$ of 44.06 min, a clearance of 128.36 L min kg, and an elimination constant (K_e) of 0.016 min.[64]

Therefore, hederagenin is rapidly metabolized in the body and has a short elimination half-life. The poor water solubility and short half-life of hederagenin may imply its low *in vivo* bioavailability, which may limit its clinical application.

It is well known that hemolysin-induced toxicity in most animals, including humans, is a major challenge for the clinical development of most saponins or sapogenins as pharmaceutical formulations, especially when administered by injection. This class of compounds produces massive foam when mixed with water, and their amphiphilic nature allows them to act as surfactants to interact with red blood cell membranes, disrupting them.[65,66]

As an aglycon, hederagenin exhibits moderate intrinsic hemolytic activity with an HD100 value (100% hemolytic dose) of approximately 2000 μg mL^{-1}.[67] Hederagenin has some hemolytic activity, possibly due to its ability to penetrate red blood cell membranes.[40]

3. Structural modification of hederagenin

Hederagenin is a promising natural bioactive substance with various pharmacological activities for potential treatment of various diseases. Hederagenin's low efficacy, poor water solubility, and low bioavailability hinder its further clinical development. In addition, hederagenin's hemolytic activity also limits its development as an injectable drug. Among them, the structure–activity relationship study of hemolytic activity showed that the polar group (carboxyl group) at the C-28 position can significantly enhance hemolysis,[67] so it is necessary to modify the C-28 position of hederagenin. In addition to the C-28 position, the C-3, C-12, C-13 and C-23

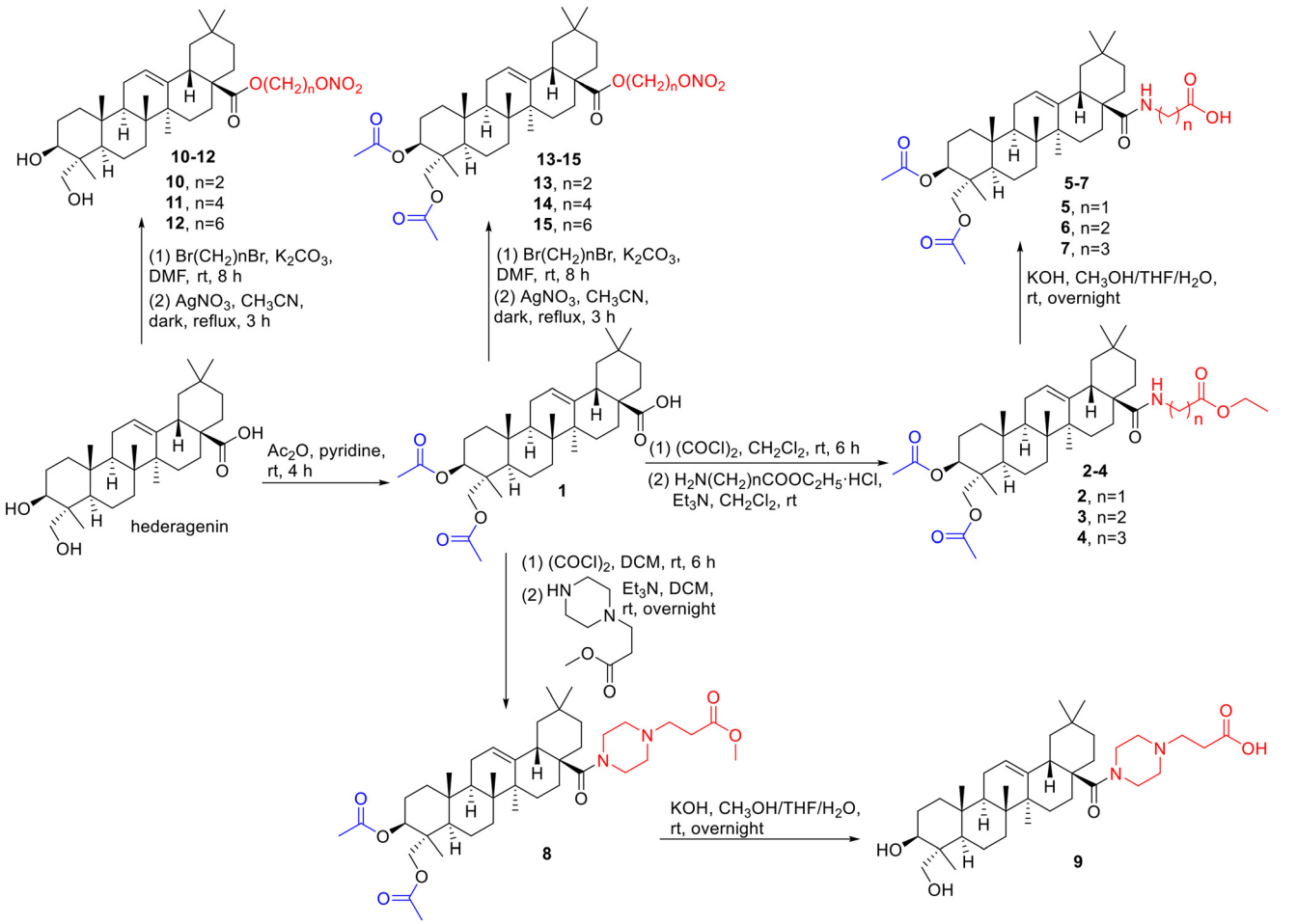


Fig. 3 Modification route of C-28 derivatives of hederagenin (2–15).

positions of hederagenin are also possible sites for structural modification, which enables the synthesis of new compounds with potentially higher potency and selectivity. Given the shortcomings of the hederagenin backbone and the necessity of its modification, we review the synthesis and activity studies of hederagenin-based derivatives reported by some researchers.

3.1. Anti-tumor activity of C-28 derivatives of hederagenin

Chen *et al.*[68] synthesized six novel derivatives (**2–7**) from hederagenin (Fig. 3). The cytotoxic effects of the new derivatives against five cancer cell lines (A549, MDA-MB-231, KB, KB-VIN, and MCF-7 cells) and the hemolytic toxicity against rabbit erythrocytes were evaluated. The modified compound **3** showed cytotoxicity against all five cancer cell lines (IC_{50} = 2.8–8.6 μM), respectively, and no hemolytic toxicity (HD_{50} > 500 μM) (Table 3). Therefore, compound **3** was identified as a potential antitumor drug candidate.

Tong *et al.*[69] used a piperazine ring linked to the C28–COOH of hederagenin to obtain derivatives **8** and **9**, and evaluated the cytotoxicity and hemolytic toxicity of seven tumor cell lines (Fig. 3). Compound **8** showed strong cytotoxicity against five cancer cells (IC_{50} = 4.68–10.74 μM), and no hemolytic toxicity (HD_{50} > 500 μM) (Table 3). The pharmacological mechanism demonstrated that compound **8** induced MDA-MB-231 cells to arrest in the G1 cell cycle.

Chen *et al.*[70] synthesized a series of helexin derivatives (**10–15**) (Fig. 3). The cytotoxicity of the new derivatives against five cancer cell lines (A549, MDA-MB-231, KB, KB-VIN, and MCF-7 cells) and the levels of nitrate/nitrite produced in H1975 cells treated with the new derivatives were determined. Among these derivatives, compound **14** significantly inhibited the proliferation of five tumor cell lines (IC_{50} = 4.6–5.2 μM) (Table 3). At the same time, compound **13** exhibited significant EGFR-LTC kinase inhibitory effects (IC_{50} = 0.01 μM) on H1975 (IC_{50} = 8.1 μM) and H1975-LTC (IC_{50} = 7.6 μM) tumor cell lines, and the inhibitory effect was stronger than hederagenin (IC_{50} > 20 μM). Meanwhile, compound **14** also produced the most nitrite (7.4 μM). In conclusion, compound **14** was identified as an effective lead compound for the treatment of non-small cell lung cancer.

3.2. Anti-tumor activity of hederagenin-pyrazine derivatives

Fang *et al.*[71] designed and synthesized 26 novel hederagenin-pyrazine derivatives (**16–41**) and screened them for *in vitro* cytotoxicity against five tumor cell lines (Fig. 4). Among them, the antitumor activity of compound **24** against A549 (IC_{50} = 3.45 μM) was comparable to the positive drug cisplatin (IC_{50} = 3.85 μM) and exceeded that of hederagenin (IC_{50} > 50 μM). Compound **24** induced early apoptosis of A549 cells and induced cell arrest in the S phase in a concentration-dependent manner.

3.3. Anti-tumor activity of C-28 amide derivatives of hederagenin

Rodriguez-Hernandez *et al.*[72] synthesized a series of hederagenin C-28 amide derivatives (**42–57**) with or without acetyl groups at positions 3 and 23 in ring A and screened them for cytotoxicity with six cancer cell lines (FaDu, A2780, HT29, MCF-7, SW1736, and NIH 3T3) (Fig. 5). Acetylated derivatives (**48–57**; EC_{50} in the range 0.4–9.0 μM) were more active than hydroxylated derivatives (**42–47**; EC_{50} in the 1.2–22.5 μM range). Hydroxylated derivative **44** with a pyrrolidinyl substituent was the strongest against HT29 human cells (EC_{50}= 1.2 μM). However, its acetylated derivative **50** showed the strongest antitumor activity with EC_{50} values of 0.4, 1.6, 1.8, 2.5, and 1.7 μM against A2780, FaDu, HT29, MCF-7, and SW1736 cells, respectively (Table 4).

3.4. Anti-tumor activity of C-28 ester and amide derivatives of hederagenin

Rodriguez-Hernandez *et al.*[73] designed and synthesized a series of novel C-28 ester and amide derivatives of hederagenin (**58–86**), and selected six human cancer cell lines for their antitumor activity (Fig. 6). Most compounds showed moderate to high levels of cytotoxic activity. The most active compounds were compound **83** with ethylpyrimidinyl and compound **84** with ethylpyrrolidinyl. The EC_{50} of compound **83** was 3.7, 1.8, 1.3, 6.5, 2.7, and 3.6 μM for 518A2, A2780, HT29, MCF-7, A549 and 8505C cell lines, respectively. Meanwhile, the EC_{50} values of compound **84** for 518A2, A2780, HT29, MCF-7, A549, and 8505C cell lines were 2.0, 1.1, 1.2, 3.7, 1.9, and 1.8 μM, respectively (Table 5). In addition, the acridine orange/propidium iodide assay (AO/PI)

Table 3 The *in vitro* cytotoxicities and haemolytic activities of hederagenin (**He**) derivatives (**3**, **8**, and **14**)

Comp.	IC_{50}[a] (μM)					HD_{50}[b] (μM)
	A549	MDA-MB-231	KB	KB-VIN	MCF-7	
He	10.25	36.61	14.63	27.90	39.39	>200
3	2.8	3.7	3.6	5.1	8.6	>500
8	4.68	5.54	5.08	5.25	10.74	>500
14	4.6	5.2	5.1	4.8	5.0	—[c]

[a] Concentration inhibiting 50% of cell growth for 48 h (compound **8**) or 72 h (compound **3** and **14**) exposure period of tested samples. Data represent mean values ± standard deviation for three independent experiments. [b] HD_{50} is the concentration inducing 50% of erythrocyte hemolysis. [c] No relevant data.

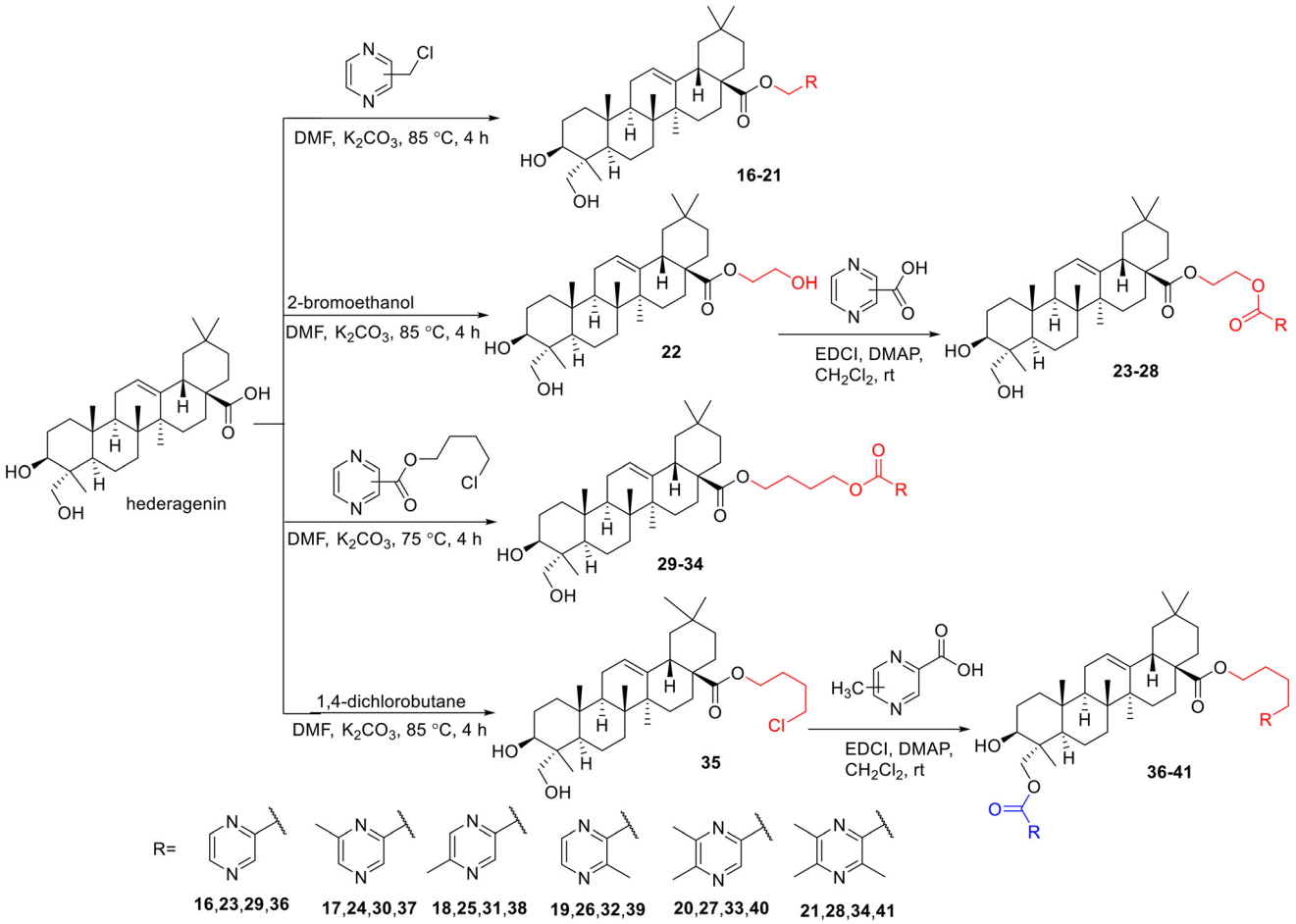


Fig. 4 Synthesis of hederagenin-pyrazine derivatives (**16**–**41**).

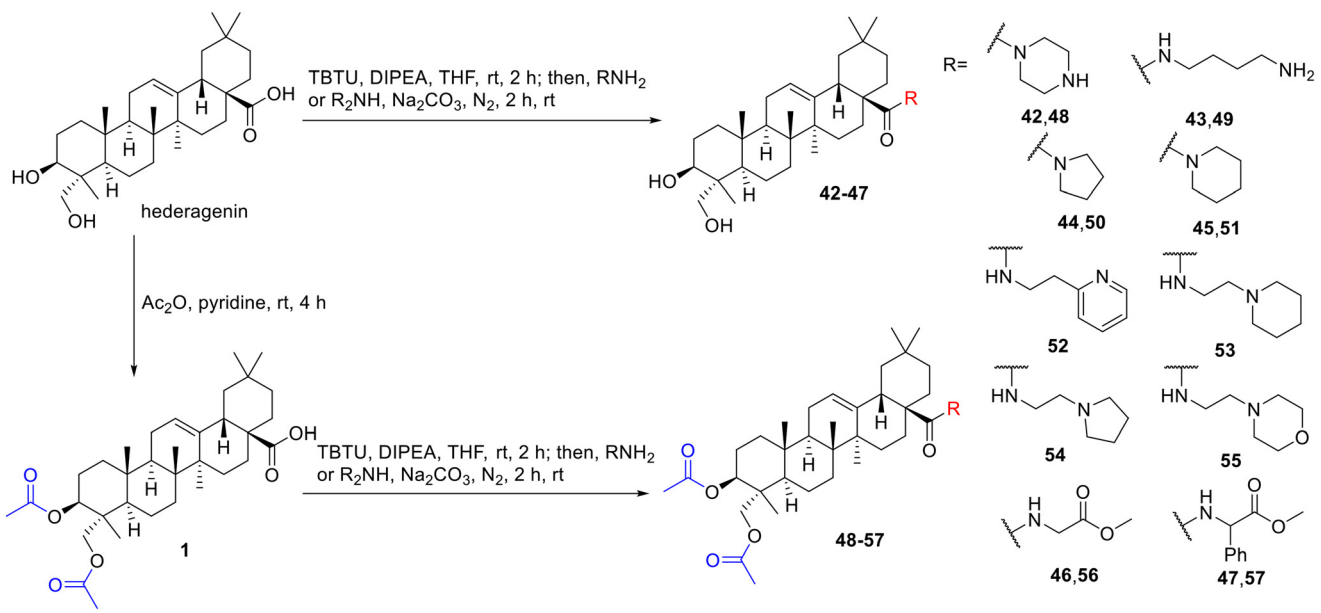


Fig. 5 Synthesis of C-28 amide derivatives of hederagenin (**42**–**57**).

Table 4 Cytotoxicity of hederagenin derivatives (**44** and **50**)

Comp.	EC_{50}[a] (μM)					
	FaDu	A2780	HT29	MCF-7	SW1736	NIH 3T3
He	>30	>30	>30	>30	>30	>30
44	2.8	3.0	1.2	n.d.	3.6	2.2
50	1.6	0.4	1.8	2.5	1.7	9.6

[a] EC_{50} values in μM from the SRB assay after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%. n.d. not detected/not determined.

Table 5 *In vitro* cytotoxicity of hederagenin derivatives (**83** and **84**)

Comp.	EC_{50}[a] (μM)					
	518A2	A2780	HT29	MCF-7	A549	8505C
He	34.9	19.9	50.0	25.7	29.0	38.0
83	3.7	1.8	1.3	6.5	2.7	3.6
84	2.0	1.1	1.2	3.7	1.9	1.8

[a] EC_{50} values in μM from the SRB assay after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%.

demonstrated that compound **84** exerted its antitumor effect mainly through apoptosis.

3.5. Anti-tumor activity of hederagenin-linked aryl-1*H*-1,2,3-triazoles

Rodriguez-Hernandez *et al.*[74] carried out structural modification on the C-28 position of hederagenin, and synthesized hederagenin derivatives **87–116** by connecting aryl-1*H*-1,2,3-triazoles with ester bonds and amide bonds, respectively (Fig. 7). These derivatives showed higher cytotoxicity than hederagenin against all six human tumor cell lines. The ester derivatives **89**, **93**, and **100** with *m*-Br, *m*-Cl, and *m*-NO_2 substituents were the most active compounds with EC_{50} values ranging from 3.2–4.0 μM, 3.1–4.0 μM, 3.2–4.1 μM, respectively (Table 6), and the activity is at least 8-fold that of hederagenin. In addition, compound **96** was the most cytotoxic to cellular HT29 with EC_{50}= 1.6 μM and a selectivity index of 5.4.

3.6. Anti-leishmanicidal activity of hederagenin derivatives

Leishmaniasis is caused by more than 20 protozoan parasites belonging to the family Kinetoplastids and the genus *Leishmania*. *Leishmania* are often zoonotic in nature, carried by rodents and canids, which are their primary hosts. Humans are infected by sand fly bites during their blood meal when parasites invade local phagocytic host cells.[75] *Leishmania infantum* is the main cause of visceral leishmaniasis in southern Europe. Thus, canine

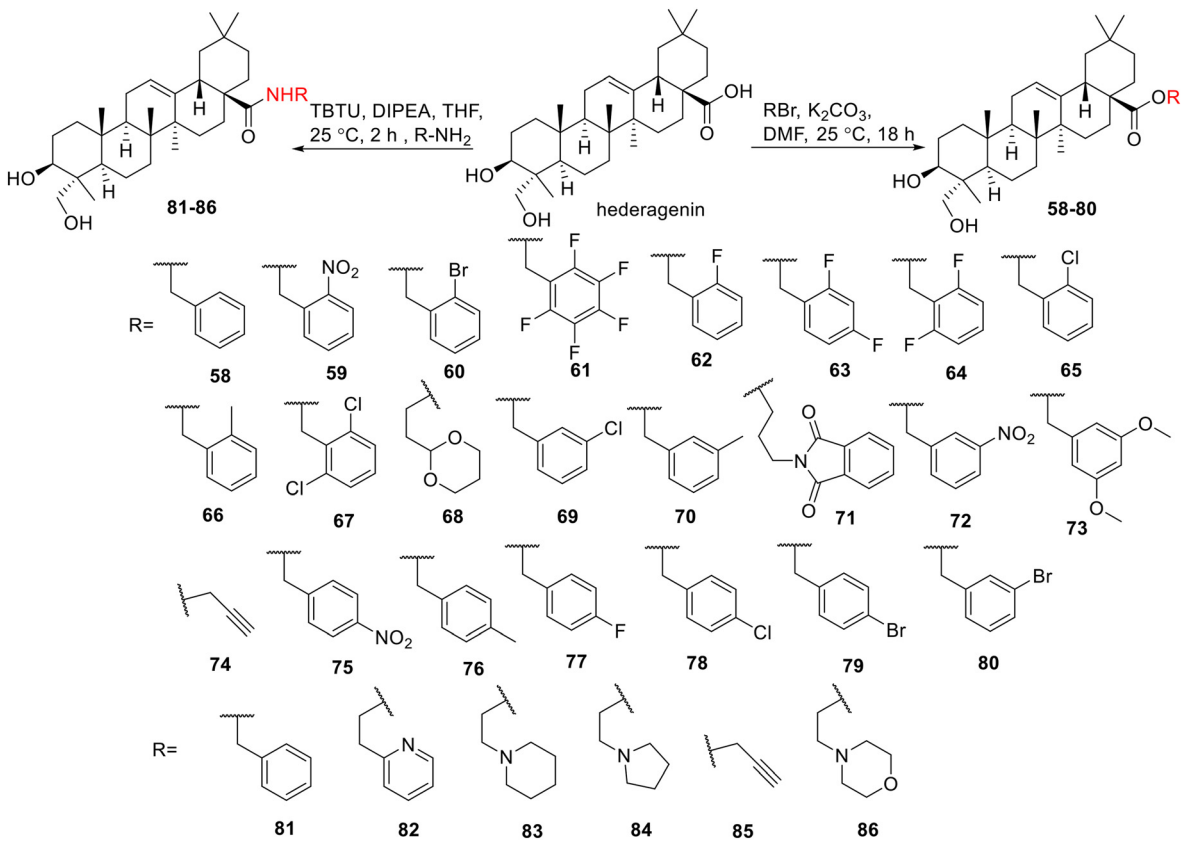
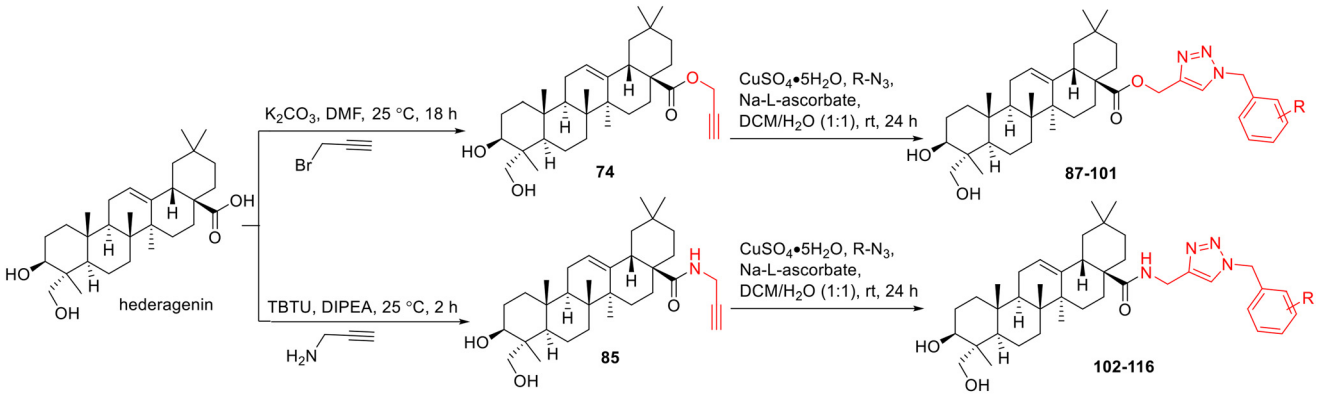


Fig. 6 Synthesis of C-28 ester and amide derivatives of hederagenin (**58–86**).



Compound	R	Compound	R	Compound	R
87,**102**	H	**92**,**107**	*p*-Cl	**97**,**112**	2,6-F
88,**103**	*o*-Br	**93**,**108**	*m*-Cl	**98**,**113**	*p*-F
89,**104**	*m*-Br	**94**,**109**	*o*-Cl	**99**,**114**	*p*-NO_2
90,**105**	*p*-Br	**95**,**110**	2,4-F	**100**,**115**	*m*-NO_2
91,**106**	2,6-Cl	**96**,**111**	*o*-F	**101**,**116**	*o*-NO_2

Fig. 7 Synthesis of hederagenin-linked aryl-1*H*-1,2,3-triazoles (**87**–**116**).

Table 6 Cytotoxicity for hederagenin (**He**) and analogs **4**, **8**, **11**, and **15**

	EC_{50}[a] (μM)						
Comp.	518A2	A2780	A549	HT29	MCF7	8505C	NIH 3T3
He	>30	>30	>30	>30	>30	>30	>30
85	4.0	3.7	3.7	3.7	3.2	3.7	3.4
93	4.0	3.8	3.8	3.5	3.1	3.7	3.1
96	11.3	9.1	16.9	1.6	6.2	12.8	8.7
100	4.1	3.6	3.7	3.5	3.2	3.6	3.1

[a] EC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from at least three independent experiments performed each in triplicate; confidence interval CI = 95%.

leishmaniasis, caused by *L. infantum*, is a major global zoonotic disease that is potentially fatal to humans and dogs and constitutes a major source of infection in humans.[76]

Rodriguez-Hernandez *et al.*[77] synthesized a series of hederagenin derivatives (**22**, **46**–**47**, **52**–**55**, **58**–**60**, **62**–**67**, **69**–**70**, **72**–**80**, **85**, and **87**–**116**) and evaluated their anti-*L. infantum* activity. Intracellular amastigotes of macrophages (DH82) parasitized by *Lactobacillus infantis* (BH46) were also studied, and their metabolic activity was also screened using cells of the BGM and HepG2 lines. Several hederagenin derivatives (**72**, **79**, **95**, **101**, and **116**) exhibited micromolar activity, were less cytotoxic to BGM and HepG2, and also inhibited *L. infantum* growth *in vitro*. Derivatives **72**, **79**, **95**, and **116** (IC_{50} of 9.7, 12.0, 11.0, and 2.0 μM, respectively) prevented the proliferation of the amastigotic form in *L. infantum* over the positive control drug antimony potassium tartrate trihydrate (IC_{50} = 80 μM) (Table 7). Derivatives **72**, **79**, **95**, and **116** have the ability to inhibit the growth of *L. infantum* in addition to good selectivity indices and low toxicity.

Table 7 Antileishmanial activity *in vitro* of hederagenin (**He**) and its derivatives (**72**, **79**, **95**, and **116**) and positive drug control against the intracellular amastigotes of *L. infantum* parasite of macrophages

Comp.	IC_{50}[a] (μM)	Toxicity DH82 macrophages LD_{50}[b] (μM)	SI[c] intracellular amastigote forms
He	61.6	<1000	10
72	9.7	63.0	6.4
79	12.0	83.0	6.9
95	11.0	66.0	6.0
116	2.0	45.0	22.5
Control[d]	80.0	8.0	0.1

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth. [b] LD_{50} = minimal lethal dose for 50% of human adherent macrophages at concentrations that weakly inhibit intracellular amastigote growth. [c] SI = selective index corresponding to the ratio between LD_{50} and IC_{50}. [d] Potassium antimonyl tartrate trihydrate used as the positive control.

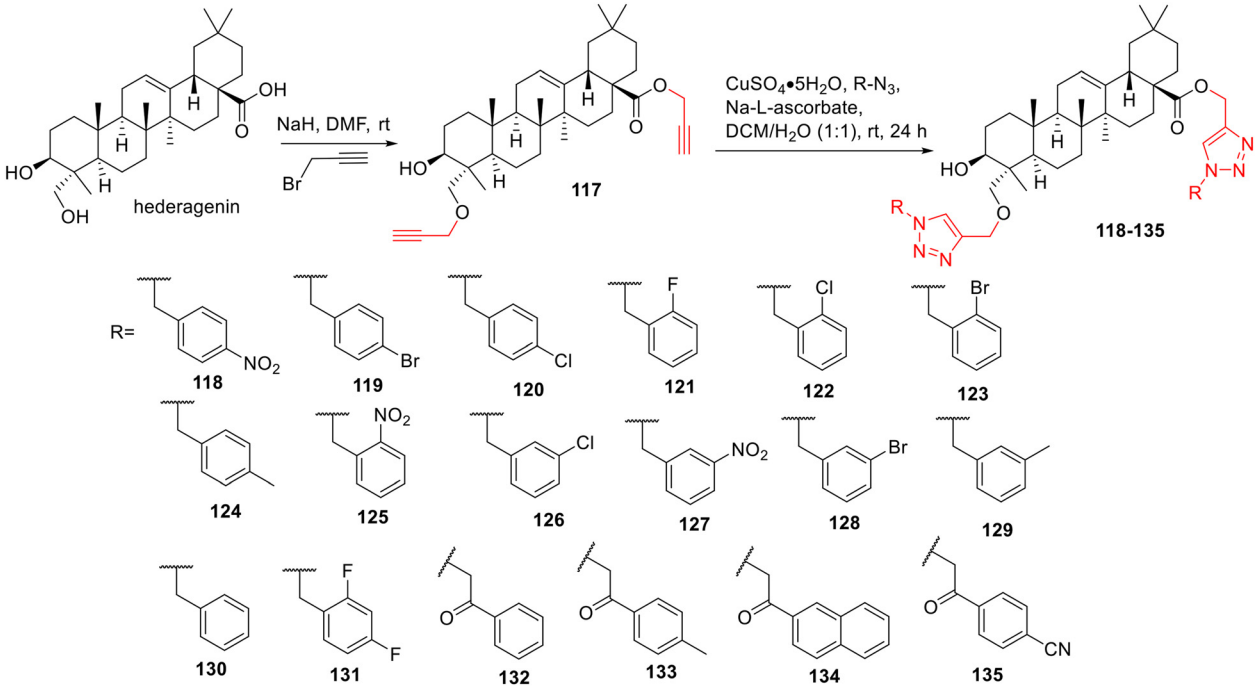


Fig. 8 Synthesis of hederagenin-bistriazolyl derivatives (**118–135**).

Rodriguez-Hernandez *et al.*[77] also reported a series of novel bistriazolyl derivatives (**118–135**) that simultaneously modified the C-23 and C-28 positions of hederagenin (Fig. 8). The *in vitro* cytotoxicity of all new compounds against five cancer cell lines and *in vitro* effects on *L. infantum* growth were investigated. Derivative **135** showed potent cytotoxicity against FaDu, A2780, HT29, A375, and SW1736 cell lines with EC_{50} values of 12.1, 11.2, 7.4, 10.6, and 11.9 μM, respectively. Derivatives **117**, **118**, **121**, and **133** were very effective in preventing the proliferation of the amastigotic form in *L. infantum* with IC_{50} values of 28.8, 25.9, 5.6, and 7.4 μM, respectively (Table 8).

3.7. Anti-tumor activity of the C-12 modification of hederagenin

Liu *et al.*[78] designed and synthesized a series of hederagenin derivatives (**136–141**) (Fig. 9). The *in vitro* antiproliferative activity against HepG2 and normal cell line L929 was determined by the MTT assay. Derivatives **136** and **138–141** showed better effects than the positive control 5-fluorouracil. Among them, compound **139** showed the strongest antitumor activity against HepG2 cells with an IC_{50} value of 1.88 μM. Meanwhile, it showed low cytotoxicity to normal cell line L929 with an IC_{50} value of 21.23 μM.

3.8. A-cyano ketone derivatives of hederagenin

Subba Rao *et al.*[79] synthesized a new derivative **146** using isoxazole to synthesize A-cyano ketone, and tested the anti-inflammatory and cytotoxic activity of compound **146** (Fig. 10). Compound **146** inhibits IFN-γ-induced nitric oxide production with an EC_{50} of 1.6 μM. The EC_{50} values of compound **146** for A549, A431, HL60, MCF-7, T47D, and HT1080 were 7.936, 1.697, 0.548,

Table 8 Antileishmanial activity *in vitro* of hederagenin (**He**) and its derivatives (**117**, **118**, **121**, and **133**) and positive drug control against the intracellular amastigotes of *L. infantum* parasite of macrophages

Comp.	Amastigotes IC_{50}[a] (μM)	Toxicity DH82 canine macrophages CC_{50}[b] (μM)	SI[c] intracellular amastigote forms
He	61.6	>1000	>10
117	28.8	259	9
118	25.9	>1000	>38
121	5.6	>1000	>178
133	7.4	>1000	>135
Control[d]	80.0	4.7	0.1

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth. [b] CC_{50} = cytotoxic concentration for 50% of canine macrophages DH82. [c] SI = selective index corresponding to the ratio between CC_{50} and IC_{50}. [d] Potassium antimonyl tartrate trihydrate used as the positive control.

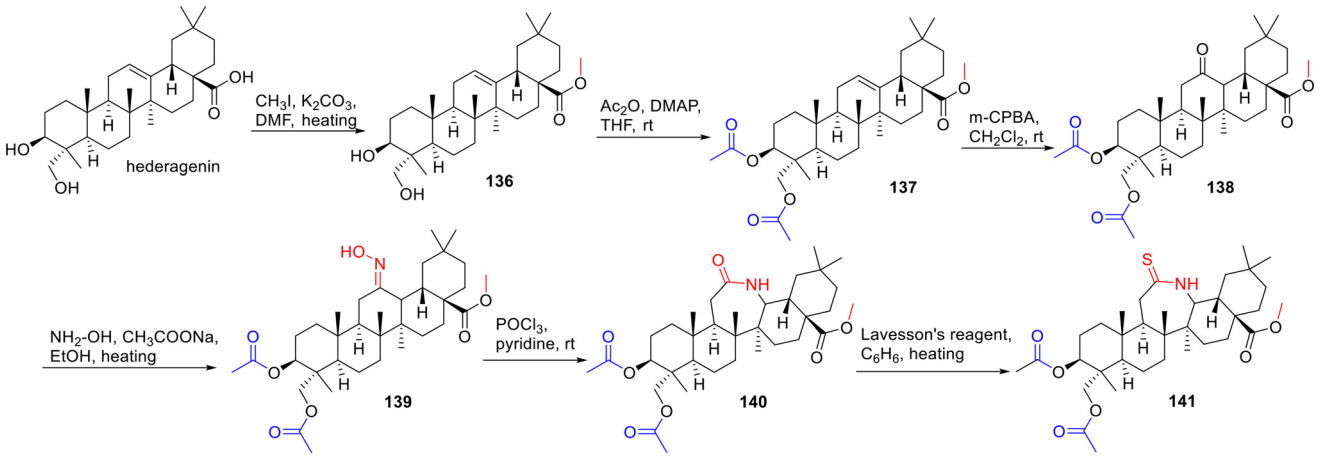


Fig. 9 Modification route of the C-12 position of hederagenin.

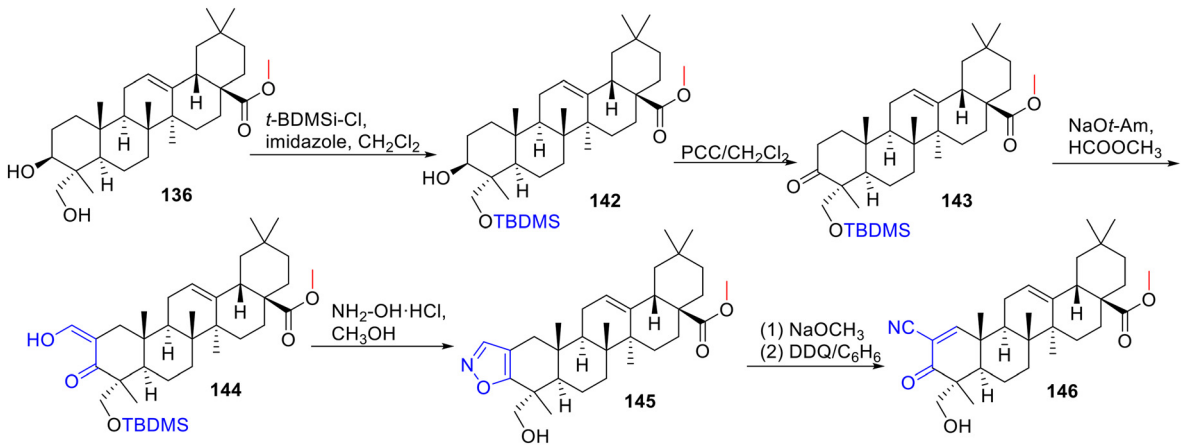


Fig. 10 Synthesis of A-cyano ketone derivatives (**146**).

2.249, 3.907, and 3.029 μM, respectively. In conclusion, compound **146** has certain anti-inflammatory and anti-tumor activities.

3.9. Nitrogen-containing heterocyclic derivatives of hederagenin

Multidrug resistance (MDR) is a complex phenomenon in which tumor cells acquire cross-resistance to multiple chemotherapeutic drugs with different structures or biological functions after being exposed to a single drug. The development of multidrug resistance is one of the major obstacles to inhibiting the growth and survival of cancer cells, and multidrug resistance is the cause of chemotherapy failure in more than 90% of patients with metastatic tumors.[80–83] Therefore, overcoming multidrug resistance is the basic premise for the development of new antitumor drugs.

Huang and Wang *et al.*[84,85] synthesized a series of isoxazole, pyrazole and thiazole ring derivatives (**152–158**, **160–161**, **166–167**, and **170**) (Fig. 11). The cytotoxicity and tumor MDR reversal activity of synthetic hederagenin derivatives in KBV cells were evaluated by the MTT assay. The results of *in vitro* cell experiments showed that when the A ring was a fused isoxazole and the compound was modified with a nitrogen-containing heterocycle, the tumor multidrug resistance reversal activity was enhanced. Compound **155** enhanced the sensitivity of KBV cells to paclitaxel, vincristine, mitoxantrone and cisplatin with IC_{50} values of 3.19, 0.65, 125.30, and 4.54 nM, respectively. Compound **155** inhibits P-gp mediated efflux by activating the activity of P-gp ATPase, thereby reversing tumor multidrug resistance. Compound **155** also induced apoptosis in KBV cells in the G2/M phase. In *in vivo* experiments, compound **155** enhanced the efficacy of paclitaxel on KBV cancer cell-derived xenograft tumors in nude mice, with a tumor inhibition rate of 56.24%. The pharmacological results showed that compound **155** is a promising P-gp modulator, which can effectively restore the sensitivity of drug-resistant tumors to paclitaxel.

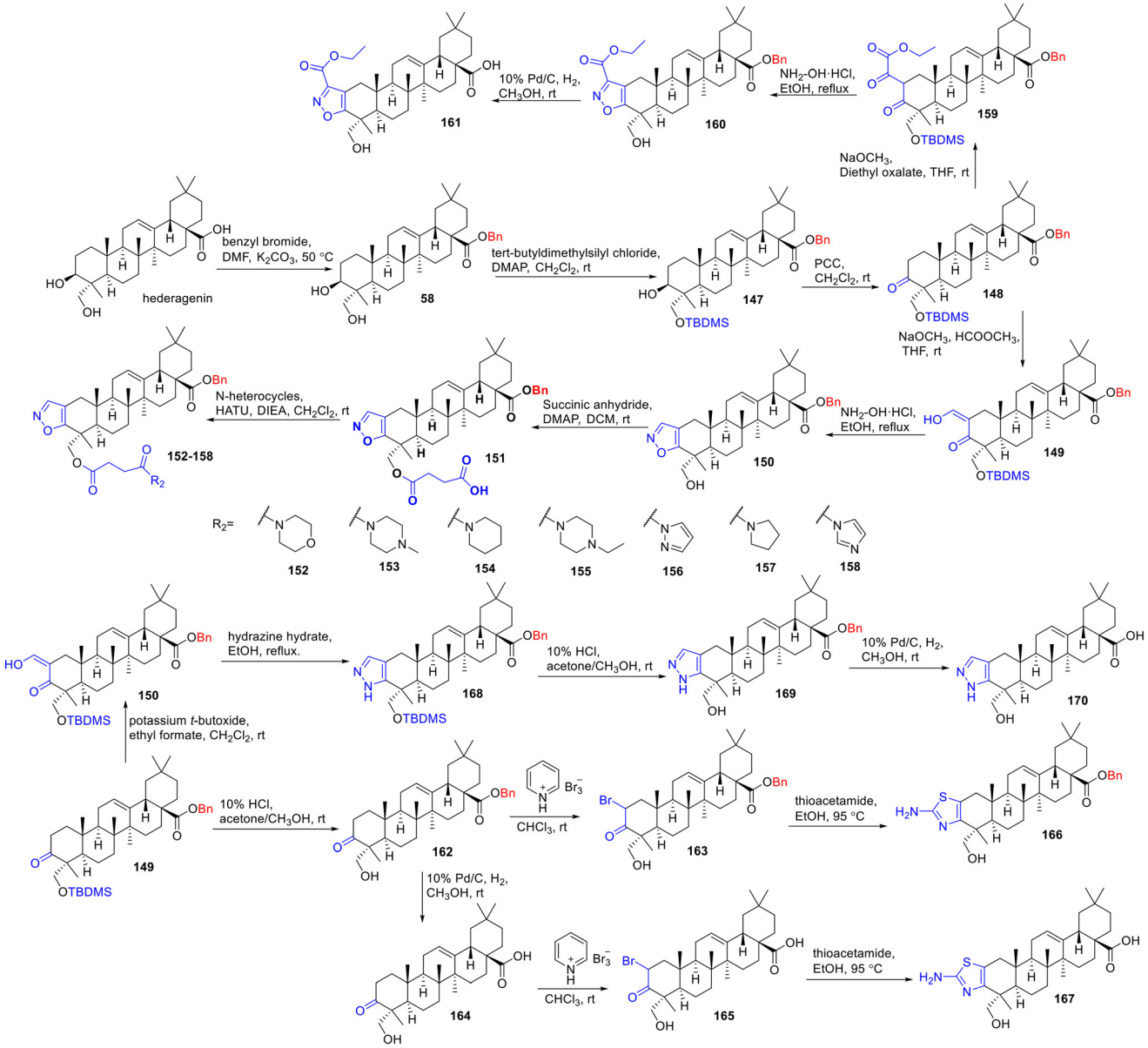


Fig. 11 Synthesis of hederagenin derivatives (**152–158**, **160–161**, **166–167**, and **170**) of nitrogen-containing heterocycles.

3.10. Heterocycle-free derivatives of hederagenin

Liu and Wang *et al.*[85,86] synthesized a series of hederagenin derivatives (**171–177**, **180–181**, **182–185**, **188–189**, and **194–203**) and performed an *in vitro* cytotoxicity screen on human cancer cell lines (Fig. 12). Compounds resulting from the introduction of a polyamine substituent at the C-23 position of hederagenin showed good antiproliferative activity. Among them, derivative **176** showed higher activity than hederagenin, with IC_{50} values of 4.75, 8.05, 4.22, 6.30, 7.0, 5.15, and 4.40 μM for KB, KBV, HeLa, A549, MKN45, BGC-823, and AGS cells, respectively. Furthermore, derivative **176** down-regulated the expression of Bcl-2 protein, up-regulated the expression of Bax protein and increased the ratio of Bax/Bcl-2, indicating that it disrupted mitochondrial potential and induced apoptosis in MKN45 cells.

3.11. Synthesis and modification of compound 204

Huang and Liu *et al.*[84,86] designed and synthesized a series of pyrazine-containing hederagenin derivatives (**207–215** and **217–226**) (Fig. 13). Liu *et al.*[86] found that compounds **207–215** did not show significant inhibitory activity against MKN45 and KB cells. At 10 μM concentration of these derivatives, the cell viability was greater than 50%.

Huang *et al.*[84] assessed the MDR-reversal activity of derivatives **217–226** in KBV cells. Cell experiments demonstrated that more than half of the compounds had MDR-reversing activity. Among them, derivative **221** showed the strongest antitumor reversal activity. When derivative **221** (10 μM) was used in combination with paclitaxel (100 nM), the survival rate of KBV cells reached 18.60%, which exceeded the survival rate of compound

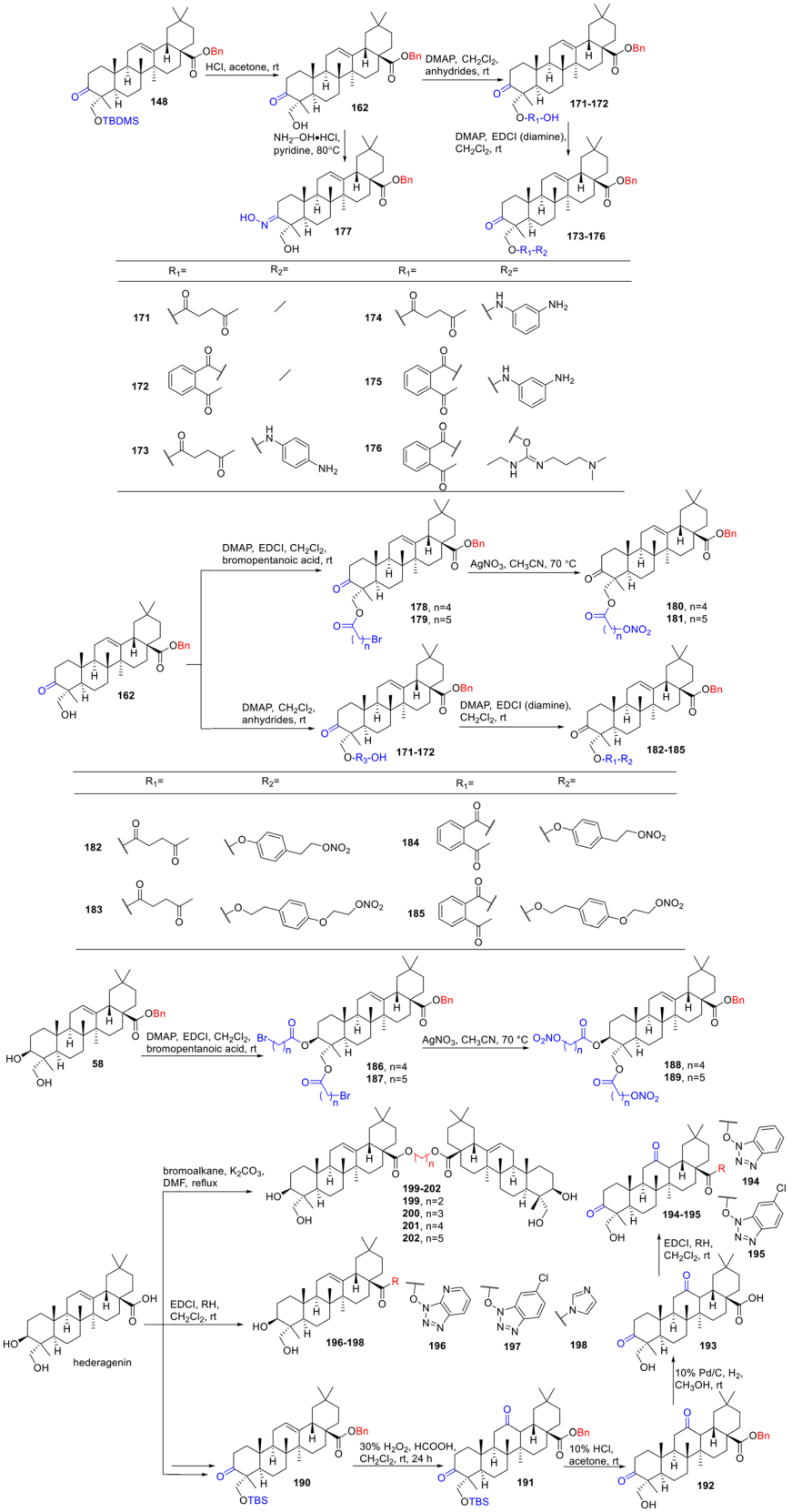


Fig. 12 Synthesis of heterocycle-free derivatives of hederagenin (**171–177**, **180–181**, **182–185**, **188–189**, and **194–203**).

204 (25.34%), and also exceeded the survival rate of hederagenin (149.47%).

Yu *et al.*[87] reported the *in vitro* anti-inflammatory activity of hederagenin derivative **216**. *In vivo* evaluation of anti-inflammatory activity showed that compound **216** reduced inflammation in a mouse model of sepsis with acute liver injury caused by LPS. Compound **216** also inhibited the expression of STING, p-IRF3, p-TBK1, p-p65, and p-IκB

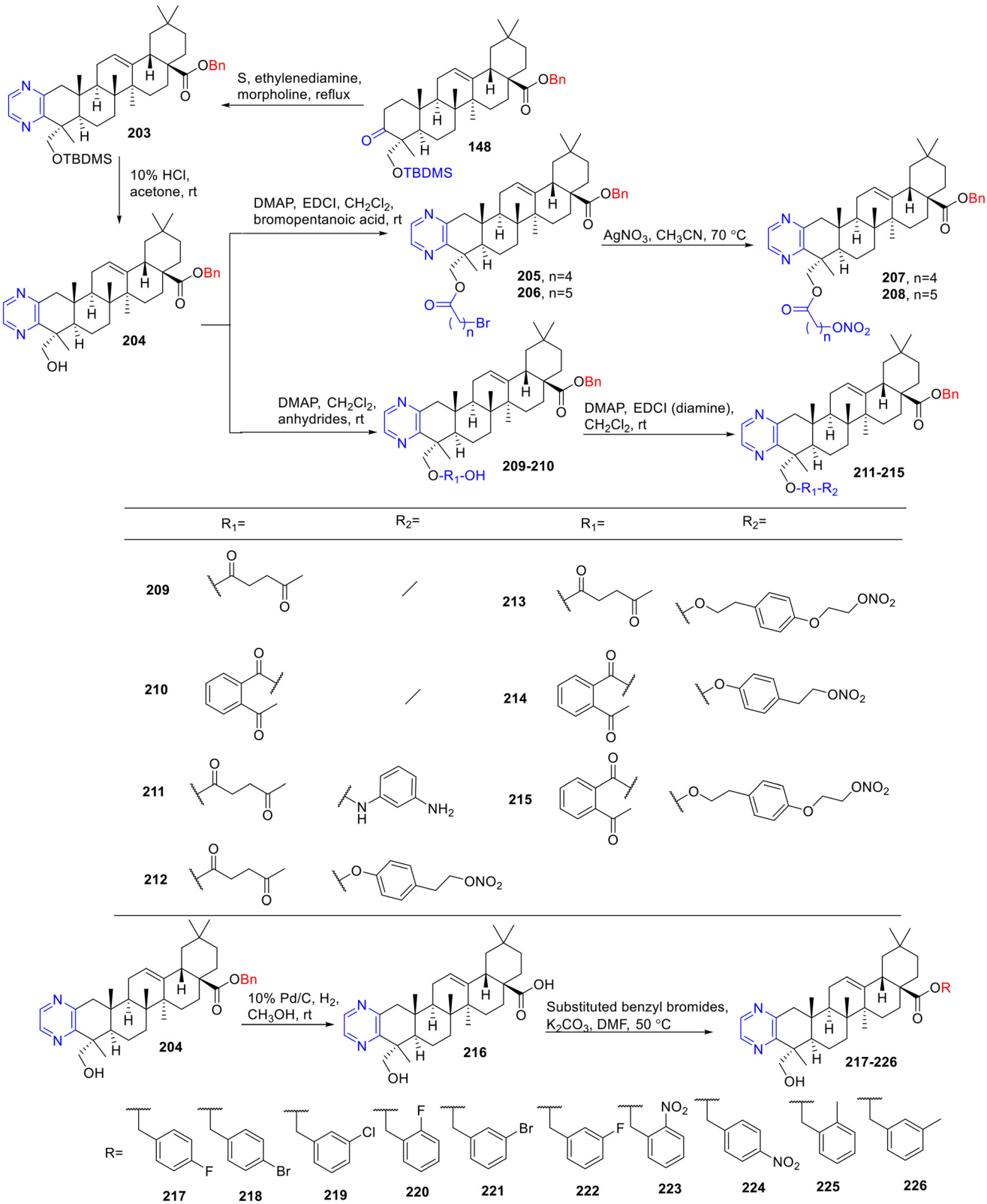


Fig. 13 Synthesis of hederagenin derivatives (**207–215** and **217–226**).

proteins in cgas-STING related signaling pathways. These results suggest that compound **216** reduces abnormal activation of the IRF3/NF-κB signaling pathway and protects sepsis mice from liver damage by reducing STING expression.

Yu *et al.*[88] identified several hederagenin derivatives with potential anti-inflammatory activity. Among them, compound **216** showed the strongest attenuating effect on inflammation in mice with acute liver injury caused by sepsis caused by LPS. Compound **216** also inhibits the role of STING, p-IRF3, p-TBK1, p-p65, and p-IκB proteins in CGAS-STING-related signaling. Thus, compound **216** reduces inflammation by inhibiting STING expression and thereby reducing the activation of STING and NF-κB signaling.

Derivative **204** is a hederagenin derivative that binds to paclitaxel at 10 μM with an IC_{50} value of 2.4 nM against drug-

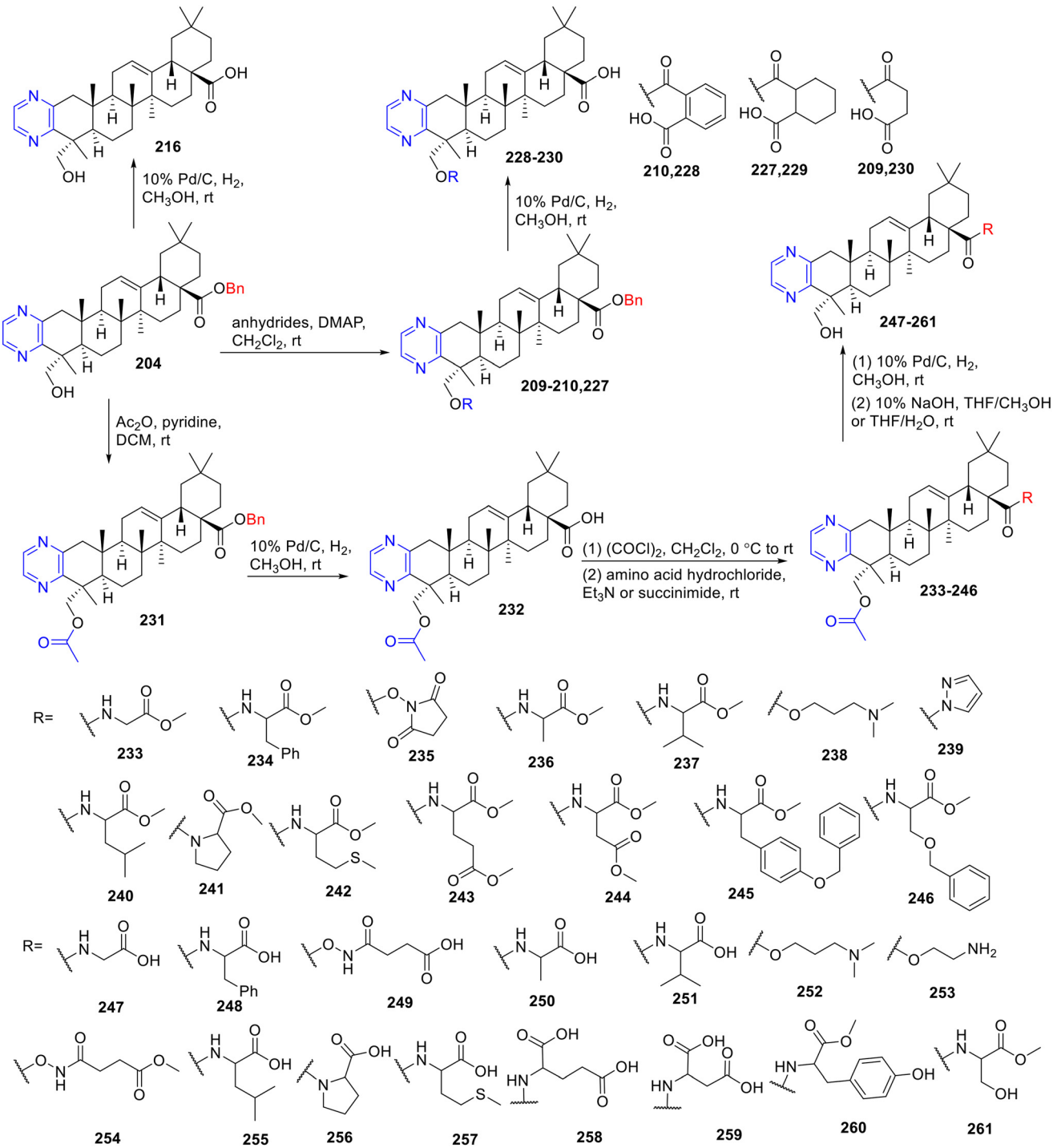


Fig. 14 Synthesis of hederagenin derivatives (**216**, **228–230** and **247–261**).

resistant KBV cells. Derivative **204** can activate P-gp ATPase, resulting in the inability of drug-resistant cells to eliminate the drug from the body. Therefore, derivative **204** can enhance the antitumor activity of paclitaxel in KBV cells, and the drug reversal effect is stronger than that of verapamil (IC_{50} = 4.9 nM). In addition, *in vivo* experiments showed that under the combined use of paclitaxel (30 mg kg^{-1}) and derivative **204** (10 mg kg^{-1}), the body weight of xenograft nude mice decreased slightly, and the tumor weight decreased to 41.88%. The results showed that derivative **204** reversed multidrug resistance by stimulating the ATPase activity of P-gp and then competing with chemotherapeutic drugs for binding to P-gp, but was less soluble due to the benzyl group at C-28.[89]

In order to improve the drug resistance reversal activity of **204**, Wang *et al.*[85] synthesized a series of novel hederagenin

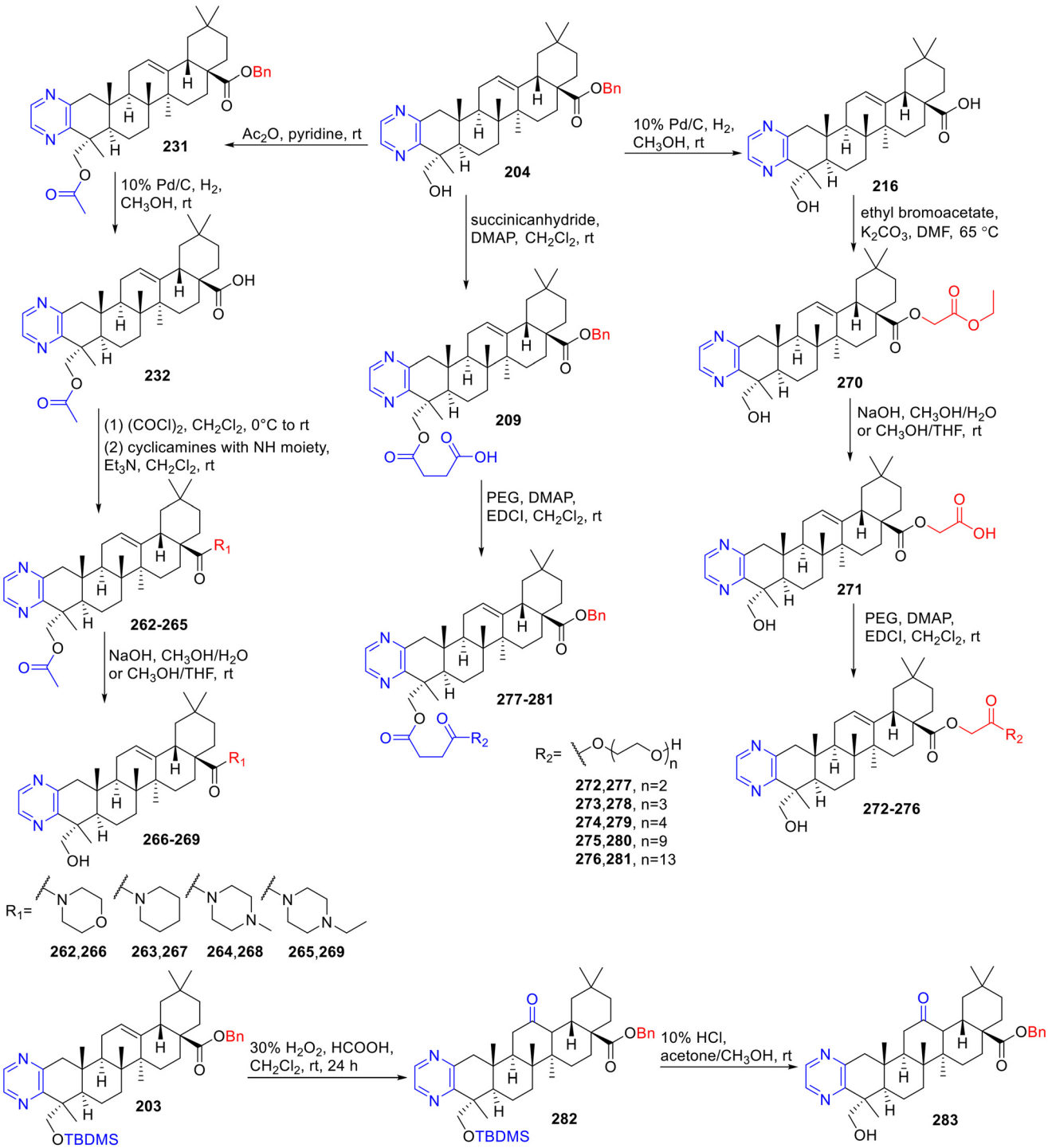


Fig. 15 Synthesis of hederagenin derivatives (**266–269**, **272–281**, and **283**).

derivatives (**216**, **228–230**, and **247–261**) through esterification, amidation and hydrolysis reactions (Fig. 14). All **204** analogs were screened for drug resistance reversal activity and cytotoxic activity in KBV cells. Compound **252** at 5 μM significantly enhanced the cytotoxicity of paclitaxel on resistant KBV cells and sensitized cells to paclitaxel, thereby preventing cells from entering the G2/M phase and inducing apoptosis. Compound **252** may block P-gps drug efflux by stimulating P-gp ATPase activity. *In vivo* experiments demonstrated that compound **252** enhanced the efficacy of paclitaxel on KBV cancer cell-derived xenograft tumors.

In order to improve the water solubility and tumor multidrug resistance reversal activity of **204**, Wang *et al.*[90] designed and synthesized a new series of hederagenin derivatives (**266–269**, **272–281**, and **283**) (Fig. 15). These derivatives significantly reversed the multidrug resistance phenotype of KBV cells to paclitaxel at a concentration of 10 μM. The water solubility of PEGylated derivatives **279–281** increased 18–657 fold compared to **204**, while maintaining tumor multidrug resistance reversal activity. Therefore, pegylation is an effective method to improve water solubility while maintaining tumor multidrug resistance reversal activity.

Compound **279**, the most active compound *in vitro*, showed good chemical stability to esterases within 24 h and enhanced the sensitivity of KBV cells to paclitaxel and vincristine with IC_{50} values of 4.58 and 0.79 nM, respectively. Compound **279** also increased the sensitivity of MCF-7 T cells to paclitaxel and vincristine with IC_{50} values of 0.89 and 0.04 nM, respectively. The combination of compound **279** and paclitaxel significantly increased the apoptosis rate of KBV cells. Compound **279** treatment increased the accumulation of rhodamine 123 and Flutax1 in KBV and MCF-7 T cells at 5 and 10 μM concentrations, suggesting that compound **279** played a role in reversing tumor resistance by effectively inhibiting the efflux function of P-gp.[90]

3.12. Selective oxidation of hederagenin by *Streptomyces griseus* ATCC 13273

S. griseus ATCC 13273 is a useful biocatalyst that exhibits efficient site-selective oxidation capacity (especially at C-29) on triterpene substrates. After treatment of hederagenin, derivatives **284** and **285** were obtained (Fig. 16). Compound **285** has inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages with an IC_{50} value of 0.078 μM.[91]

3.13. Hederagenin glycosides as α-glucosidase inhibitors

α-Glucosidase is a membrane-bound enzyme of the small intestinal epithelium that breaks down glucose from disaccharides and oligosaccharides by hydrolysis. α-Glucosidase inhibitors can inhibit the breakdown of oligosaccharides and disaccharides in carbohydrates, slow the absorption of monosaccharides, and reduce postprandial insulin and glucose peaks.[92,93]

Liu *et al.*[94] efficiently synthesized four hederagenin glycosides **303–306** by glycosylation and evaluated the activity of compounds **303–306** against α-glucosidase type IV (Fig. 17). Among them, compound **306** containing α-L-rhamnopyranosyl showed the best activity with an IC_{50} value of 47.9 μM.

3.14. Synthesis of anemoclemosides A, anemoclemosides B, and pulsatilla saponin D

Anemoclemosides A (**310**) and B (**312**) are structural isomers of hederagenin glycosides δ- and α-hederin (Fig. 18), which were first isolated and characterized from the leaves of common English ivy (*Hedera helix*, Araliaceae).[95] Bouillon *et al.*[96] synthesized the saponins anemoclemoside A (**310**) and B (**312**) in 6 and 18 steps with an overall yield of 46% and 18%, respectively.

Pulsatilla saponin D (**316**) belongs to oleanolic acid type saponins (Fig. 18), isolated from *Pulsatilla japonica*.[97,98] In mice with Lewis lung cancer, it showed very potent antitumor activity *in vivo*, surpassing even paclitaxel and doxorubicin.[99] Kim *et al.*[100] designed and synthesized pulsatilla saponin D (**316**). The *in vitro* anti-tumor activity of totally synthesized pulsatilla saponin D (**316**) against A549 had a similar IC_{50} value (IC_{50} = 5.8 μM) to that of isolated pulsatilla saponin D (**316**) (IC_{50} = 6.3 μM).

3.15. Antileishmanial activity of semi-synthetic saponins

Anderson *et al.*[101] described the study of a library of 9 natural and 128 semisynthetic hederagenin saponins and investigated the antileishmanial activity of these saponins. The synthesis of compounds **309**, **317–331** was reported in detail by Anderson *et al.* (Fig. 19). And most of the compounds have strong anti-leishmanial activity. Among

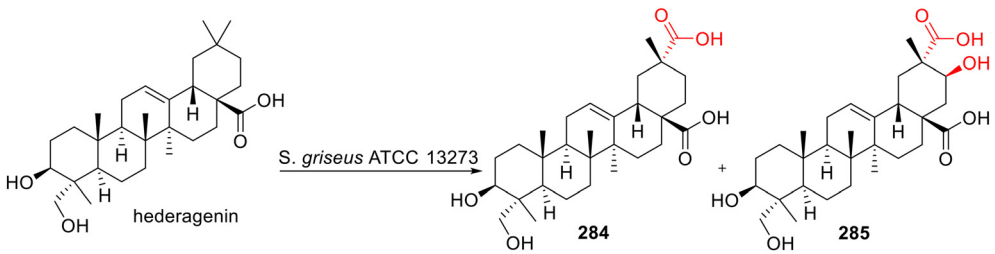


Fig. 16 The selective oxidation route of hederagenin.

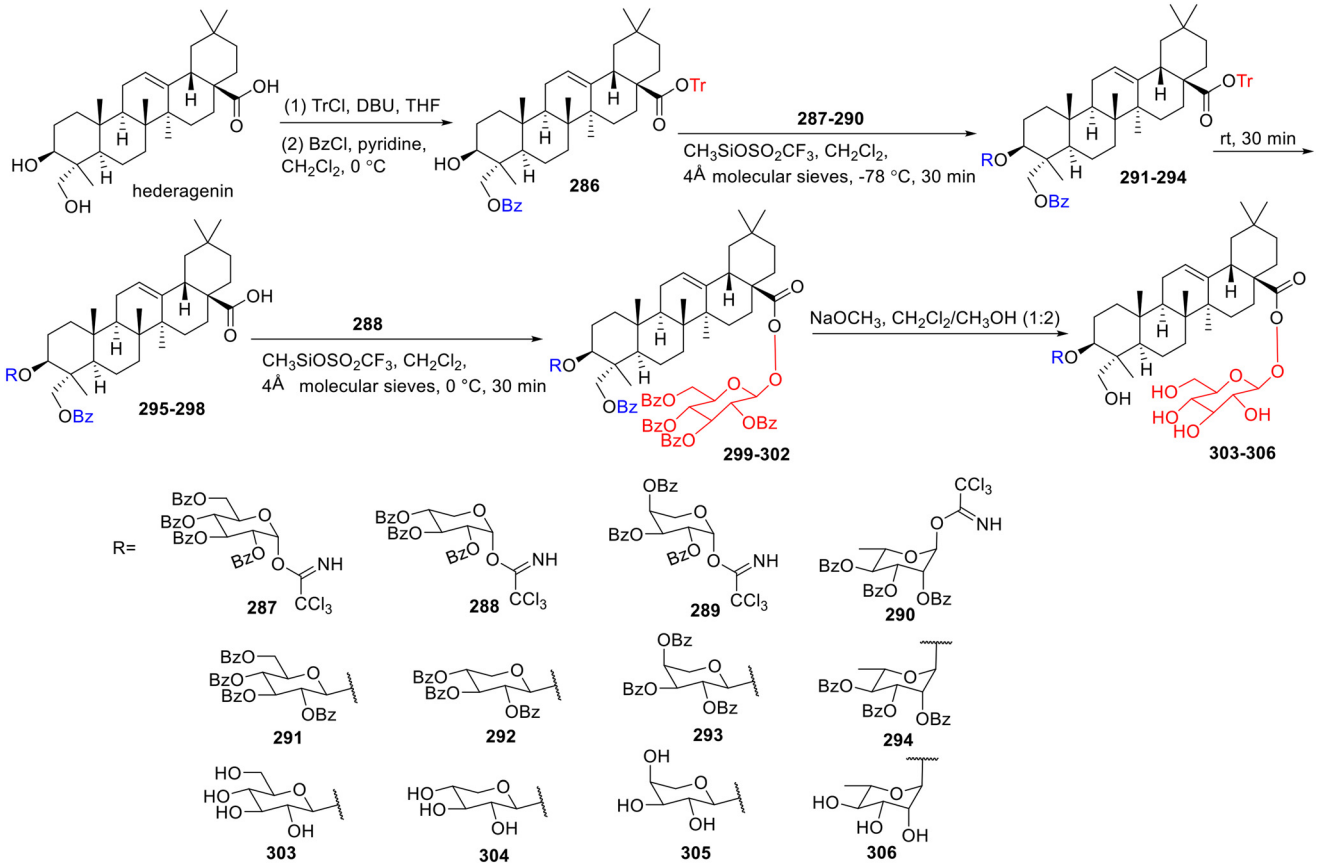


Fig. 17 Synthetic route of hederagenin glycosides **303**–**306**.

them, compound **320** had the strongest activity against *Leishmania* amastigotes with an ED_{50} of 0.9 μM (Table 9).

3.16. Summary on the structure–activity relationship of hederagenin analogues

Hederagenin structural modification sites are mainly concentrated in C-28, C-3, C-12, C-13, and C-23, and modification of these sites may lead to derivatives with improved activity. This chapter reviews the recent progress in the structural modification of hederagenin, aiming to explore the biological activities of various hederagenin analogs. The synthesis of various hederagenin analogs and their anticancer effects on various malignant cells and results against *L. infantum* established meaningful SARs, as shown in Fig. 20 and Table 10.

1) C-28 is an important modification site, and modification at this site is the most common. C-28 linked to pyridine (**24**) *via* an ester bond could enhance antitumor activity, and linked substituted benzyl could enhance anti-*L. infantum* activity (**72**, **79**).

2) C-28 linked chain amine (**3**, **83**, **84**) or cyclic imine (**8**) through an amide bond can improve antitumor activity. Furthermore, the C-3 and C-23 acetylated derivatives (**50**) had stronger antitumor activity than the unacetylated derivatives (**44**).

3) C-28 linking aryl-1*H*-1,2,3-triazole *via* an ester bond and amide bond can improve antitumor (**96**) and anti-*L. infantum* activity (**116**). However, C-3 and C-23 acetylated derivatives were not seen.

4) Simultaneous attachment of the C-28 carboxyl group and C-3 hydroxyl group to aryl-1*H*-1,2,3-triazole can improve the anti-*L. infantum* activity (**121**).

5) The modification of C-12 and C-13 is less reported, because the double bond of C-12 and C-13 is the key to cytotoxicity. In addition, when C-12 was a ketone, the antitumor activity was reduced. However, acetylation of C-3 and C-23 hydroxyls, and C-12 to oxime (**139**), amide (**140**) or sulfonamide (**141**) enhanced antitumor activity.

Tumor MDR reversal activity is a research hotspot of hederagenin derivatives. At present, based on the drug resistance reversal activity of hederagenin derivatives, reasonable structure–activity relationships (SARs) can be inferred:

1) The activity was improved when the saturated nitrogen-containing heterocycle was substituted by C-28; however, the open-chain nitrogen-containing group was superior to the saturated nitrogen-containing heterocycle.

2) When the hydroxyl group at C-3 and the double bond at C-12 are converted into ketones, the activity decreases; when ring-A is a fused pyrazine and the double bond at C-12 is converted into ketones, the activity increases.

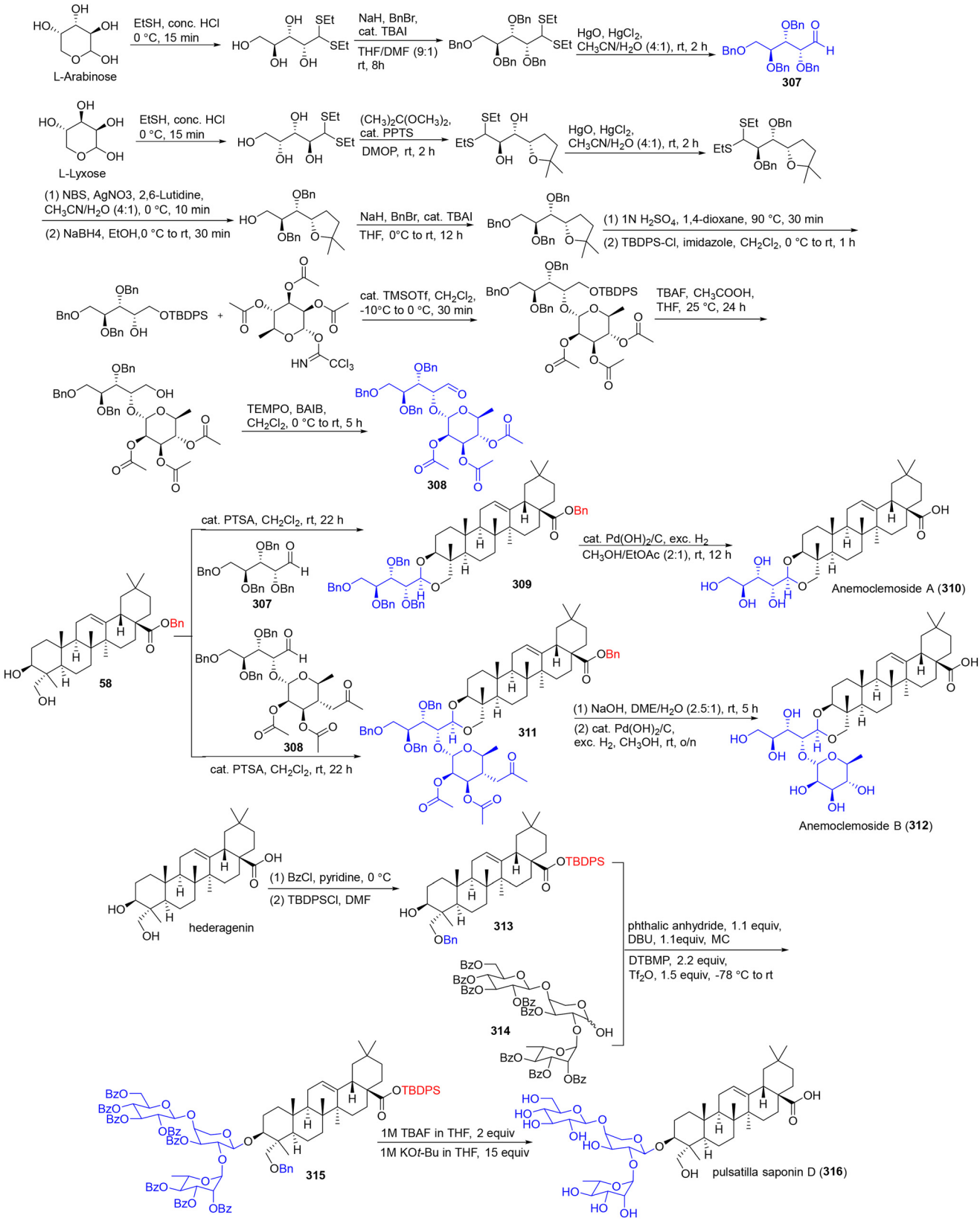


Fig. 18 Synthetic route of anemoclemoside A (**310**), anemoclemoside B (**312**) and pulsatilla saponin D (**316**).

3) When the A-ring is a fused isoxazole (**155**), the activity increases. When the A-ring is a fused isoxazole, the C-23 hydroxyl group is esterified with succinic anhydride and the terminal carboxyl group is exposed, resulting in increased

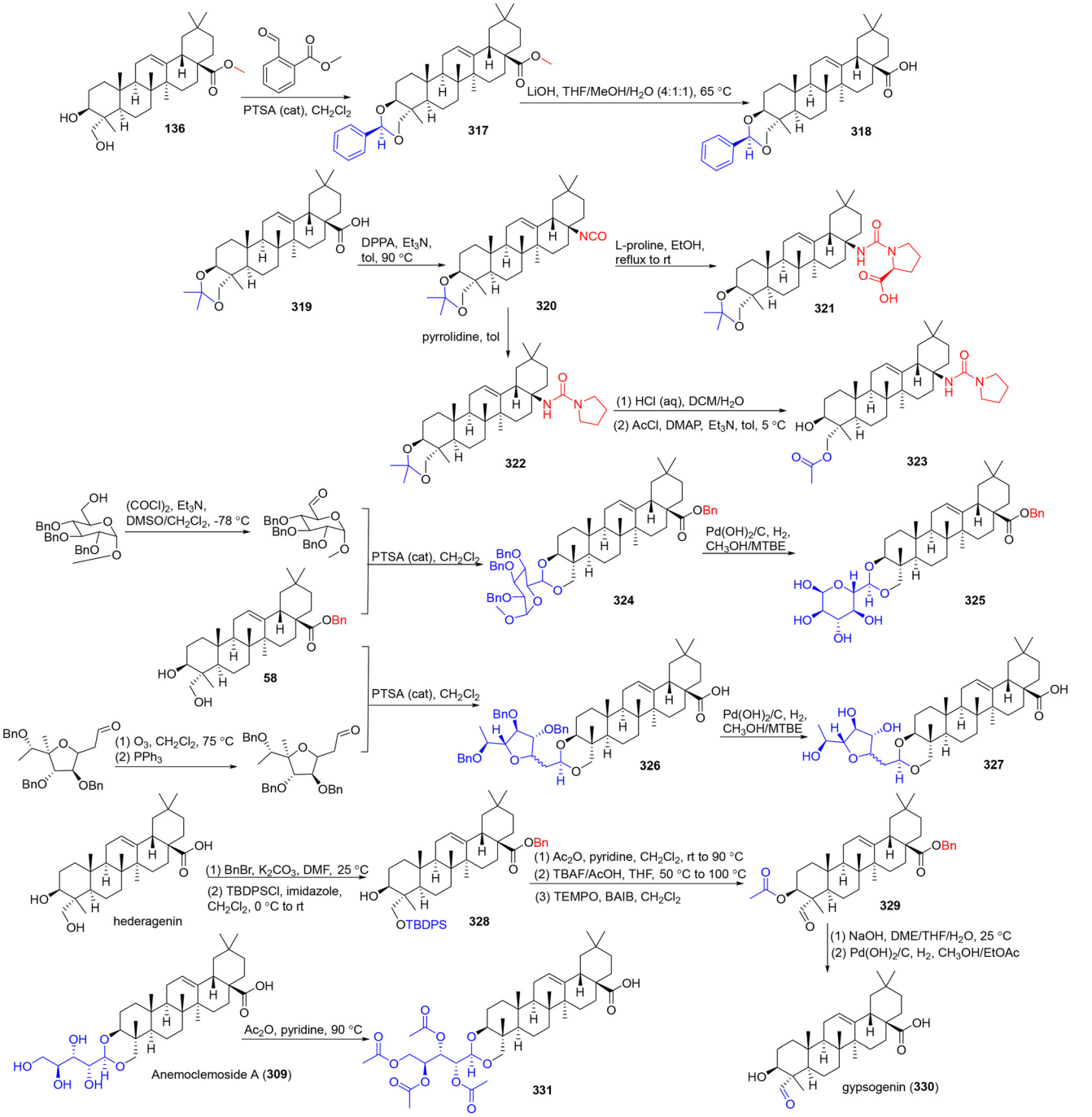


Fig. 19 Synthesis of semi-synthetic saponins.

cytotoxicity and synergistic antitumor activity. In conclusion, the activity was enhanced when ring A was a fused unsaturated nitrogen-containing heterocycle, and was further enhanced when the C-28 carboxyl group was substituted with a benzyl group.

4) The tumor MDR reversal activity is reduced when the A ring is an ethyl ester substituted isoxazole, and when the C-28 carboxyl group is exposed, the activity is reduced.

The A-ring fused pyrazine of hederagenin obtained derivative **204**, which showed good antitumor activity against drug-resistant KBV cells. It can be said that the resistance reversal activity of the ring-A fused pyrazine-based derivatives

Table 9 Activity of compounds (**309**, **317**–**323**, **325**, **327**, and **330**–**331**) against *Leishmania* amastigotes

Comp.	ED_{50} (μM)	Comp.	ED_{50} (μM)	Comp.	ED_{50} (μM)
309	4.5	**320**	0.9	**325**	6.0
317	2.9	**321**	81.1	**327**	10.3
318	10.0	**322**	92	**330**	9.6
319	83.6	**323**	10.4	**331**	7.2

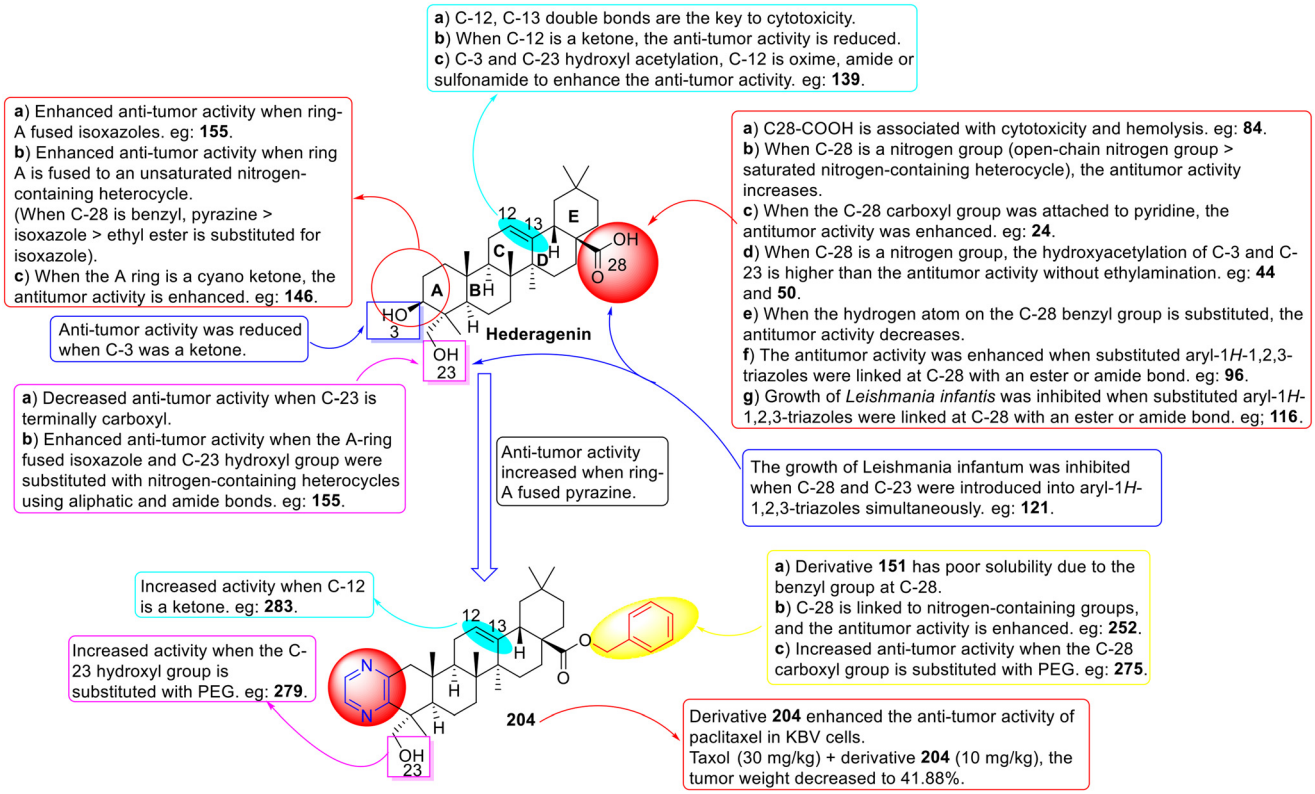


Fig. 20 Graphical representation of the general structure–bioactivity relationship of hederagenin derivatives.

is improved. However, the solubility of derivative **204** is poor due to the presence of a benzyl group at C-28. Therefore, further modification based on derivative **204** is also a research hotspot. At present, based on the drug resistance reversal activity of compound **204** analogs, reasonable structure–activity relationships (SARs) can be inferred:

1) When ring A is a fused pyrazine and the C-28 carboxyl and C-23 hydroxyl groups are replaced by PEG (**277**), the activity is further enhanced.

2) When the hydroxyl group at C-3 and the double bond at C-12 are converted to ketones, the activity decreases.

3) When the carboxyl group at C-28 is converted to a terminal amino group, the activity increases. When both carboxyl and hydroxyl groups were introduced into the C-28 site, the activity decreased. The activity was increased when the nitrogen group was substituted at C-28.

In conclusion, the existing synthetic studies provide useful and efficient modification strategies for the further development of hederagenin derivatives with enhanced biological activity and pharmaceutical properties.

4. Conclusion

Natural products are a valuable source of molecular diversity for drug discovery, and drug development has made a huge contribution. Hederagenin is a pentacyclic oleanane-type triterpenoid found in the fruit of *Sapindus saponaria*. Its fruit accumulates a large amount of saponins in the peel, the main aglycone of which is hederagenin.

The anticancer activity of hederagenin has aroused great interest among researchers over the past decade. Numerous studies have shown that hederagenin can achieve anti-tumor effects through a variety of pathways, including inhibiting tumor cell division and proliferation, inducing tumor cell differentiation and apoptosis, and blocking the tumor cell cycle. Therefore, hederagenin has great anticancer potential. However, its pharmacological activity and clinical potential are affected by its low water solubility, low bioavailability, and short half-life. Therefore, in order to avoid these problems, various derivatives have been designed and synthesized in order to obtain highly efficient compounds. Multiple potential targets and signaling pathways related to hederagenin and some of its derivatives have been identified, providing new candidate compounds for more effective anticancer drugs. However, there are still some questions and new directions for future development in the advancement of hederagenin analogs into viable treatments:

(i) Although hederagenins have received considerable attention in the past decade, their exact molecular mechanisms in the treatment of cancer and other diseases remain to be elucidated. The current findings suggest that hederagenin is difficult to apply in clinic. We hope that follow-up studies will map the complete signaling network associated with hederagenin to facilitate future research on new drugs for potential clinical indications.

Table 10 The pharmacological activity of hederagenin derivatives

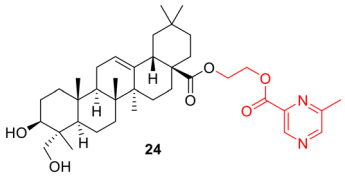
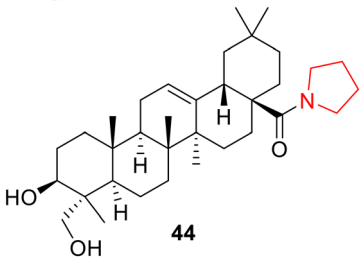
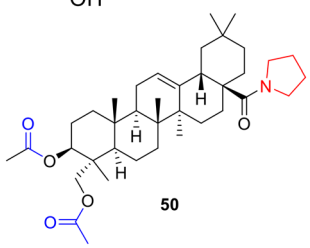
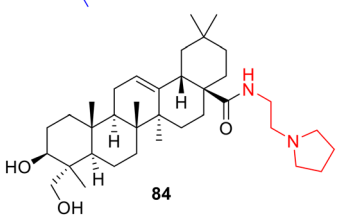
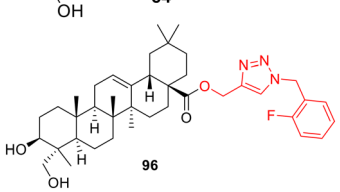
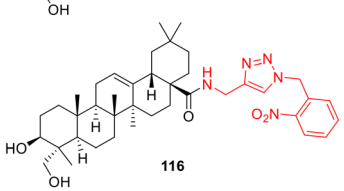
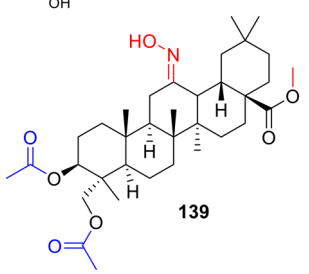
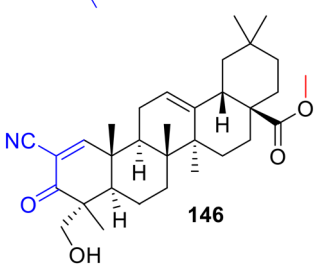
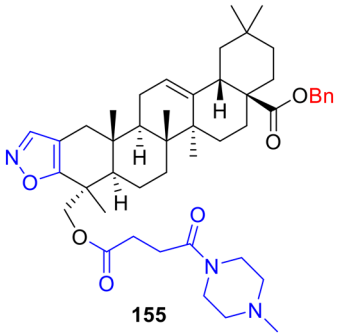
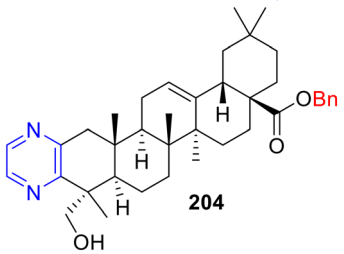
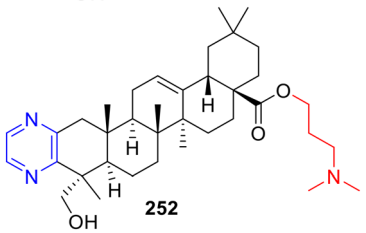
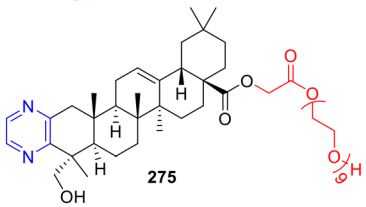
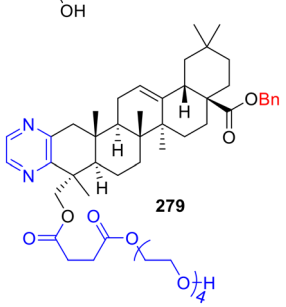
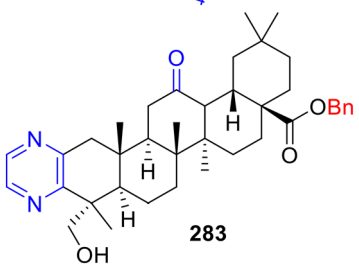
Comp.	Activity	Effect	Ref.
 **24**	Antitumor	Compound **24** against A549 (IC_{50} = 3.45 μM) was comparable to cisplatin (IC_{50} = 3.85 μM) and exceeded that of hederagenin (IC_{50} > 50 μM)	71
 **44**	Antitumor	Compound **44** against HT29 (ED_{50} = 1.2 μM) exceeded that of hederagenin (IC_{50} > 30 μM)	72
 **50**	Antitumor	Compound **50** against A2780 (ED_{50} = 0.4 μM) exceeded that of hederagenin (IC_{50} > 30 μM)	72
 **84**	Antitumor	Compound **84** against A2780 (ED_{50} = 1.1 μM) exceeded that of hederagenin (IC_{50} = 19.9 μM)	73
 **96**	Antitumor	Compound **96** against HT29 (ED_{50} = 1.6 μM) exceeded that of hederagenin (IC_{50} > 30 μM)	74
 **116**	Anti-*L. infantum*	Compound **116** inhibited *L. infantum* growth (IC_{50} = 2.0 μM) and exceeded that of hederagenin (IC_{50} = 61.6 μM) and tartrate trihydrate (IC_{50} = 80 μM)	77
 **139**	Antitumor	Compound **139** against HepG2 (IC_{50} = 1.88 μM) exceeded that of hederagenin (IC_{50} = 35.87 μM) and 5-fluorouracil (IC_{50} = 16.42 μM)	78
 **146**	Anti-inflammatory Antitumor	Compound **146** inhibits IFN-γ-induced nitric oxide production with an EC_{50} of 1.6 μM. Compound **146** against HL60 (IC_{50} = 0.548 μM)	79

Table 10 *(continued)*

Comp.	Activity	Effect	Ref.
 155	Antitumor	Compound **155** enhanced the efficacy of paclitaxel on KBV cancer cell-derived xenograft tumors in nude mice, with a tumor inhibition rate of 56.24%; the inhibitory rate exceeded that of paclitaxel by 22.63%	84
 204	Antitumor	Derivative **204** binds to paclitaxel at 10 μM with an IC_{50} value of 2.4 nM against drug-resistant KBV cells. With combined use of paclitaxel (30 mg kg^{-1}) and derivative **204** (10 mg kg^{-1}), the body weight of xenograft nude mice decreased slightly, and the tumor weight decreased to 41.88%	89
 252	Antitumor	Derivative **252** (5 μM) binds to paclitaxel at 100 nM with a KBV cell survival rate of 10%, and less than hederagenin has a 100% survival rate	85
 275	Antitumor	Derivative **275** (10 μM) binds to paclitaxel at 100 nM with a KBV cell survival rate of 25.16%, and less than hederagenin has a 107.17% survival rate	90
 279	Antitumor	Derivative **279** (10 μM) binds to paclitaxel at 100 nM with a KBV cell survival rate of 11.97%, and less than hederagenin has a 107.17% survival rate	90
 283	Antitumor	Derivative **283** (10 μM) binds to paclitaxel at 100 nM with a KBV cell survival rate of 20.77%, and less than hederagenin has a 107.17% survival rate	90

(ii) The pharmacological studies of hederagenin and its derivatives mainly focus on antitumor and antiparasitic activities, and there are few reports on other fields of activity. Moreover, the pharmacological activities of these compounds are mainly concentrated in *in vitro* experiments, and relatively few in *in vivo* experiments. Furthermore, the activities of many synthetic derivatives have not been explored in depth, which is a pity.

(iii) The pharmacological study of hederagenin and its derivatives initially revealed the development value of hederagenin and its derivatives. In the field of anti-tumor therapy, compounds **44**, **50**, **84**, **96**, and **139** (Fig. 20) are representative of their anti-tumor activity. Compound **204** is a representative compound in overcoming tumor MDR reversal activity. Compound **204** combined with paclitaxel showed a good anti-tumor effect on drug-resistant KBV cells, and it was still effective in *in vivo* experiments, but its water solubility was poor, which limited its application. Compounds **252** and **279** were synthesized based on the further modification of compound **204**. These two derivatives were more active than compound **204**, providing a new strategy for the development of hederagenin derivatives.

(iv) In the field of antiparasitic drugs, mainly *L. infantum*, hederagenin derivatives showed strong activity. Among them, compounds **116** and **121** are the most representative, with IC_{50} values of 2.0 and 5.6 μM, respectively. However, other parasites have not been reported; hederagenin has anti-*T. gondii* activity, but no anti-*T. gondii* activity of its derivatives has been reported. It is hoped that follow-up studies will explore the activity of hederagenin derivatives in other parasite fields.

(v) In most of the above studies, the reported modifications are mostly in the C-3 hydroxyl group, C-23 hydroxyl group, C-28 carboxyl group and A ring of hederagenin, and the modification of C-12 and C-13 is less reported. Therefore, future work should be devoted to the modification of C-12 and C-13 and the simultaneous modification of multiple sites.

(vi) Many natural products are biologically active while providing opportunities for drug discovery in different therapeutic areas. Hederagenin is known to have antitumor, anti-inflammatory, antibacterial, antidepressant, antifungal, anti-neurodegenerative, *L. infantum* and antidiabetic biological activities. However, the research of hederagenin derivatives has focused on the field of anti-tumor and anti-*L. infantum* activities, and the anti-inflammatory activities of the derivatives are rarely reported (compounds **6** and **285** have certain anti-inflammatory activities), and other activities have not been reported at all. We hope that researchers will be able to explore the anti-inflammatory and other pharmacological activities of hederagenin derivatives.

(vii) Novel drug delivery systems are effective strategies to improve the water solubility, absorption, distribution, metabolism, excretion (ADME), and toxicity of many drugs.[102,103] For example, hederagenin-loaded magnetic nanoparticles (HMP) can significantly enhance antitumor activity, and HMP can be effectively used as a drug delivery carrier to enhance the applicability of hederagenin.[104] The immunomodulatory and anti-inflammatory effects of hederagenin-coated maghemite (γ-Fe_2O_3) nanoparticles (HM) were evaluated in an atopic dermatitis model. HM dose-dependent treatment inhibited inflammation-induced expression of IL-2 and TNF-α in HaCaT and Jurkat cells.[105] Therefore, encapsulating hederagenin with nanocarriers can effectively deliver drugs and improve drug efficacy. In addition, novel drug delivery formulations of nanosuspensions, micelles, nanoparticles and nanogels can be used to improve the efficacy, water solubility and targeting of hederagenin.

(viii) The development of hederagenin-based drug combinations may be a useful strategy, such as combining hederagenin with other drugs for stronger activity, thereby overcoming the limitation of insufficient hederagenin activity.

We believe that hederagenin provides a natural product platform for drug development for the treatment of cancer and parasitic diseases. So far, this platform provides a good basis for developing new derivatives that are more potent and water-soluble than the natural product hederagenin. Hederagenin derivatives may be potential drugs for clinical treatment of human diseases.

List of abbreviations

MAPK	Mitogen-activated protein kinase
LPS	Lipopolysaccharide
TNF-α	Tumor necrosis factor alpha
Bcl-2	B-cell lymphoma 2
Bax	Bcl-2-associated X protein
NF-κB	Nuclear factor κB
p53	Tumor protein p53
PI3K	Phosphoinositide 3-kinase
IL-1β	Interleukin-1β
IL-6	Interleukin-6
COX-2	Cyclooxygenase-2
AKT	Also known as PKB (protein kinase B)
SAR	Structure activity relationship
PGE2	Prostaglandin E2
Nrf2	NF-E2-related factor
APAF-1	Apoptotic protease activating factor-1
MMP	Matrix metallopeptidase
ROS	Reactive oxygen species
ALI	Acute lung injury
BALF	Bronchoalveolar lavage fluid
iNOS	Inducible nitric oxide synthase
MIC	Minimal inhibitory concentration
CORT	Corticosterone
CI/R	Cerebral ischemia/reperfusion
MLK3	Mixed lineage kinase 3
PD	Idiopathic Parkinson's disease, short name Parkinson's disease
IFN-γ	Interferon-γ

HTT74	Huntingtin 74
A53T α-syn	A53T α-synuclein
AKI	Acute kidney injury
Nrf2	Transcription factor nuclear factor (erythroid-derived 2)-like 2
ARE	Antioxidant response element
PARP	Poly(ADP-ribose)polymerase
NLRP3	The NOD-like receptor (NLR) family pyrin domain-containing protein 3

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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