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Discovery and development of natural product oridonin-inspired anticancer agents

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Abstract

Natural products have historically been, and continue to be, an invaluable source for the discovery of various therapeutic agents. Oridonin, a natural diterpenoid widely applied in traditional Chinese medicines, exhibits a broad range of biological effects including anticancer and anti-inflammatory activities. To further improve its potency, aqueous solubility and bioavailability, the oridonin template serves as an exciting platform for drug discovery to yield better candidates with unique targets and enhanced drug properties. A number of oridonin derivatives (e.g. HAO472) have been designed and synthesized, and have contributed to substantial progress in the identification of new agents and relevant molecular mechanistic studies toward the treatment of human cancers and other diseases. This review summarizes the recent advances in medicinal chemistry on the explorations of novel oridonin analogues as potential anticancer therapeutics, and provides a detailed discussion of future directions for the development and progression of this class of molecules into the clinic.

Graphical Abstract

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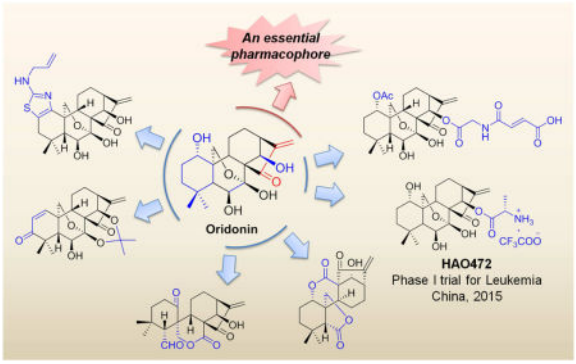
Conflict of interest

The authors confirm that this article content has no conflicts of interest.



Keywords

Natural product; Oridonin; Diterpenoids; Anticancer agents; Drug discovery; Chemical biology

1. Introduction

Natural products extracted from microbes, plants, and animals have remarkable structural diversity and biological characteristics, providing researchers with exciting possibilities to develop novel molecular entities for human therapeutics [1–5]. Of the 112 first-in-class drugs approved by Food and Drug Administration (FDA) between 1999 and 2013, 31 (28%) are developed based on natural pharmacophores (Fig. 1A) [6]. Notably, the 2015 Nobel Prize in Physiology or Medicine highlighted the significant importance of natural products (e.g. Artemisinin) in the treatment of devastating parasitic infections [7, 8].

In the kingdom of natural products, diterpenoids have emerged as one of the most important families given their distinct biological activities and drug-like properties as demonstrated by the success of taxane-type diterpenoids in preclinical studies and clinical treatments [9, 10]. Besides the taxanes, the kaurane-type diterpenoid, oridonin (**1,** Fig. 1B), has attracted an increasing amount of attention in recent years, due to its impressive pharmacological activities and a safety profile necessary for developing new therapeutics (Figs. 1C and 1D).

Oridonin, initially isolated from various *Isodon* species which are commonly used as a home remedy herb in China and Japan, was proven to possess considerable anticancer effects [11–13]. Despite a promising safety and efficacy profile for cancer treatment, the relatively moderate potency, limited aqueous solubility and bioavailability, as well as imprecise mechanisms of action have greatly hindered its further preclinical development and clinical applications. To overcome such disadvantages and yield better drug candidates with enhanced activity, a number of oridonin derivatives have been designed and synthesized. Substantial progress has been achieved in the identification of new agents and relevant molecular mechanistic studies towards the treatment of human cancers and other diseases over the past decade. As one of the major milestones achieved (Fig. 2), *L*-alanine-(14-oridonin) ester trifluoroacetate (**2**, HAO472) [14] was recently advanced into a Phase I human clinical trial (CTR20150246; www.chinadrugtrials.org.cn) in China by Hengrui Medicine Co. Ltd, for the treatment of acute myelogenous leukemia. Herein, we seek to

briefly overview the biological and pharmacological investigations of oridonin, and summarize the recent medicinal chemistry advances of novel oridonin analogues, aiming to appreciate the therapeutic potential and value of the oridonin template serving as an exciting platform for drug discovery.

2. The natural product oridonin and its mechanisms of action

Isodon rubescens (a.k.a. "*Rabdosia rubescens*", traditional Chinese medicine name "*Donglingcao*"), the primary natural source of oridonin, has been used for the treatment of inflammation and cancer in Asian countries for hundreds of years [15]. To date, *Donglingcao* remains a commonly available over-the-counter (OTC) herbal medicine for the treatment of inflammation in China [16, 17]. Mechanistic studies reveal that the herbal extract can suppress breast cancer *in vitro* and *in vivo* by regulating the MAPK and the Akt signaling pathways [18], and control aortitis inflammation through its anti-TNF-α effect, as seen in a United Kingdom patient study [19].

Oridonin is one of the major efficacious components of the herbal extract with an elucidated chemical structure that has attracted considerable interest [20–22]. Accumulating evidence suggests that oridonin triggers autophagy, inhibits angiogenesis, arrests cell cycle progression and promotes apoptosis through several major molecular mechanisms by modulating the relevant signaling pathways involved in the regulation of intracellular reactive oxygen species (ROS), Bcl-2/Bax, NF-κB, p53/p21, MAPK, PI3K, microRNA and fatty acid synthase pathways. These pathway modulations may be levied for the treatment of broad-spectrum cancers, as demonstrated both *in vitro* and *in vivo* (Table 1). These studies support the idea that cross-talk amongst these targets and signaling pathways are critically associated with the pharmacological effects of oridonin for human cancer treatment [23–26].

It has been increasingly recognized that the therapy for a challenging disease with resistance may benefit more from a polypharmacological approach, which modulates a network of disease related targets, rather than by "switching" a single target on or off [37, 38]. Intriguingly, oridonin is a multifunctional compound, capable of inhibiting cell proliferation and inducing apoptosis of cancerous cells in a polypharmacological manner. Cheng *et al.* demonstrated that oridonin induces L929 cell apoptosis by regulation of ROS-mediated signaling pathways, and simultaneously induces autophagy to block apoptosis by regulating p38/NF-κB signaling [39]. The anti-inflammatory and cytoprotective effects of oridonin have also been demonstrated using *in vitro* and *in vivo* models focused on the Nrf2 and NF-κB signaling pathways [40–42].

Oridonin not only inhibits cancer cell proliferation and induces apoptosis, but also suppresses inflammation and stimulates cytoprotection (Fig. 3). These seemingly paradoxical results may be attributed to the partial and dose-dependent regulation of signaling pathways in various cell types. For instance, transcription factor Nrf2, which is controlled by its repressor protein Keap1 and chemical activators (e.g. oridonin), defends normal cells from oxidative stress and injury [40, 43–45]. In contrast to these physiological or inflammatory conditions, some pathological factors (e.g. oncogenes) can directly increase Nrf2 transcription and cause resistance to chemotherapy in the tumor microenvironment

[46–48]. Given such evidence, oridonin-induced activation of the Nrf2 signaling pathway is beneficial in cytoprotection and anti-inflammation models, whereas the anticancer mechanism of oridonin is not primarily attributed to this target. In addition, the aforementioned self-contradictory results also depend upon different treatment dosages and concentrations of oridonin in the biological models. In the arsenic-induced cytotoxicity model, low concentrations of oridonin (1.4 μM) significantly protect human urothelial UROtsa cells from arsenic-induced damage by activation of Nrf2, whereas oridonin at higher concentrations (> 14 μM) is a pro-apoptotic agent [40].

3. Advances in oridonin-inspired medicinal chemistry

Despite oridonin's attractive safety and biological efficacy profiles, its relatively moderate potency, limited aqueous solubility and oral bioavailability diminish its therapeutic potential and further clinical applications [49]. Due to its unique scaffold, interesting pharmacological profile, and rich natural abundance for commercialization, the oridonin template may serve as an exciting platform for drug discovery. Based on its structural characteristics, we have clustered most of the reported analogues into four major categories: 1) derivatization on C-1 and/or C-14 hydroxyl functional groups; 2) modifications of the A ring system; 3) modifications on the α,β-unsaturated ketone; and 4) transformation and derivatization of the skeletal structure. These medicinal chemistry efforts for drug discovery of new molecules are discussed in detail below.

3.1 Derivatization on C-1 and/or C-14 hydroxyl functional groups

To improve aqueous solubility and anticancer activity, Xu *et al.* prepared a series of oridonin derivatives **3–11** with modifications at the C-1 and C-14 hydroxyl groups by introducing various side chains containing hydrophilic moieties (Fig. 4) [50]. Most of these compounds exhibit improved cytotoxicity (EC_{50} = 0.93–21.70 μM for B16 murine melanoma cells) and aqueous solubility (> 50 mg/mL) in comparison with oridonin (EC_{50} = 26.15 μM for B16 murine melanoma cells [50]; aqueous solubility 1.29 mg/mL [51]). Compounds **3**–**5** bearing an acetate at the C-1 position display an inhibitory activity more potent than the corresponding compounds **6**–**9** bearing a propylsulfonyl or carbonyl group at the same position. The introduction of fumaryl amino acids at the C-14 hydroxyl (**4**, **7**, and **11**) results in a significant improvement of antiproliferative effects, while the saturated acid substituted analogues (**5**, **8**, and **10**) exhibit only moderately enhanced activity. Therefore, compound **4** bearing both a C-1 acetate and a C-14 fumaryl glycine ester was identified as the most potent analogue of this series *in vitro* (EC_{50} = 0.93 μM for B16 murine melanoma cells), which is 28-fold more potent than oridonin. *In vivo*, compound **4** demonstrates a significant reduction (69.9%) of tumor growth in the melanoma B16 xenograft model at a dosage of 20.0 mg/kg (ip), while oridonin displays relatively lower efficacy with a 45.9% reduction in tumor growth in the same study [50].

Based upon the initial structure-activity relationship (SAR) study mentioned above, a follow-up drug discovery effort on the modifications of the C-1/C-14 hydroxyl groups was conducted. The introduction of a saturated, long-chain terminal acid in the C-14 position resulted in compound **12** with an enhanced antiproliferative effect (EC_{50} = 2.06 μM) against

BEL-7402 human hepatocarcinoma cell line in comparison with oridonin (EC_{50} = 29.80 μM). In addition, **12** exerts a more efficacious antitumor effect (tumor growth inhibition 63.7%) than oridonin (42.7%) at the dose of 20.0 mg/kg (ip) in mice bearing H22 liver tumor xenografts [52]. Similarly, the introduction of the reported antitumor functionalities, such as the furoxan nitric oxide (NO) donor [53, 54] or nitrogen mustard [55, 56] to the C-14 position provides another direction for activity optimization. Compound **13** produces a high concentration of NO and exhibits a more potent antiproliferative effect (EC_{50} = 0.86 μM, BEL-7402 human hepatocarcinoma cells; EC_{50} = 1.82 μM, K562 human leukemic cells) than oridonin (EC_{50} = 7.48 μM and 4.76 μM, respectively) [57]. The oridonin skeleton and the DNA alkylating agent nitrogen mustard moieties were hybridized with a significant enhancement of efficacy and selectivity against both drug-sensitive and drug-resistant cancer cell lines. The representative compound **14** was selected for initial mechanistic studies, which indicated that the antiproliferative activity was attributed to the induction of cell cycle arrest and apoptosis in cancer cells [58].

In addition, a similar effort around the C-14 position to improve oridonin's drug-like properties was made by Huang's and Guo's research teams, respectively [59, 60]. Considering that the concentration of glutathione (GSH) in tumor cells is much higher than that in blood plasma, the oridonin derivative **15** was designed to be specifically activated by GSH through the cleavage of the disulfide bond at C-14 position for a controlled release of the potential anticancer fragment **16** in neoplastic tissues [59]. In an effort to improve the drug-like properties of lipophilic drugs, polyethylene glycol (PEG) can be applied as a highly hydrophilic carrier as drug delivery system [61, 62]. Therefore, Xue *et al.* employed the self-assembled monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) as a drug carrier to prepare oridonin-loaded micelles with improved aqueous solubility [63]. Inspired by these advances, Guo *et al.* designed and synthesized C-14 position PEGylated oridonin prodrugs **17–20**. The study suggests that the molecular weight of PEGs is the key factor contributing to solubility and oridonin-releasing features. The lowest molecular weight PEGylated prodrug **17** exhibits the highest aqueous solubility (74.4 mg/mL) and the most rapid release profile ($t_{1/2}$ = ~5 h, pH = 7.4) *in vitro*, while the highest molecular weight PEGylated prodrug **20** exhibits the lowest aqueous solubility (3.5 mg/mL) and the slowest release profile ($t_{1/2}$ = ~12 h, pH = 7.4). Therefore, the conjugates **18** and **19** with moderate molecular weight PEGs were selected for further *in vivo* pharmacokinetic evaluation. In comparison with oridonin, they display about a 2.1-fold increased area under the plasma concentration time curve (AUC), prolonged (10~20-fold) elimination rate ($t_{1/2}$) and mean residence time (MRT), as well as approximately 54% reduced blood clearance values (CL). The findings support that PEGylation at C-14 position represents a promising strategy to improve the solubility and pharmacokinetic properties of oridonin [60].

Recently, HAO472 (structure depicted in Fig. 2) was designed to bear an alanine ester trifluoroacetate at C-14 position for significantly improving its aqueous solubility (i.e. 165 mg/mL). Additionally, HAO472 can be metabolized through the cleavage of its C-14 ester bond to release the parent compound oridonin *in vivo*, thereby acting like a prodrug. HAO472 was claimed to retain the anticancer activities of oridonin (data not disclosed) with less toxicity (no-observed-adverse-effect level, NOAEL = 40 mg/kg) and markedly

improved drug-like properties [14]. Moreover, HAO472 was found to inhibit the proliferation and activation of T cells by down-regulating the NF-κB signaling pathway in the treatment of inflammatory bowel disease, which is an established colorectal cancer risk factor [64]. HAO472 has been advanced into Phase I human clinical trials in China for the treatment of acute myelogenous leukemia (80–320 mg/d, iv, CTR20150246).

3.2 Modifications on A-ring system

Over the past several years, our group explored new scaffold constructions around the A-ring system of oridonin [51, 65–67]. The aromatic thiazole heterocycle represents an important anticancer pharmacophore that exists in bioactive natural products and FDA approved drugs, such as epothilone B and ixabepilone [68–70]. A series of thiazole fused oridonin analogues **21**–**30** with an additional nitrogen-containing side chain have been designed and synthesized to improve both potency and aqueous solubility. Most of these nitrogen-enriched novel analogues exhibit significantly enhanced antiproliferative activity against the human breast cancer MCF-7 and MDA-MB-231 cell lines with low micromolar to submicromolar EC_{50} values (Table 2). Compound **28** displays the most potent antiproliferative activity against the triple-negative breast cancer MDA-MB-231 cell line with an EC_{50} value of 0.2 μM, indicating approximately 147-fold potency increase relative to oridonin. Apoptosis is an important biological process of cell death that is critical for elimination of unwanted, damaged or infected cells and is associated with diverse biological events including cell development, differentiation and proliferation [71–74]. Pilot mechanistic studies indicate that **28** induces apoptosis at low concentrations in a dose-dependent manner through the regulation of NF-κB, Bcl-2/Bax and PARP signaling pathways. The aqueous solubility of compound **28** has been significantly improved with a saturated concentration of 42.4 mg/mL, which is approximately 32-fold higher than that of oridonin (1.29 mg/mL). Moreover, compound **28** significantly suppresses MDA-MB-231 xenograft tumor growth *in vivo* at a dosage of 5 mg/kg (ip, tumor growth inhibition > 66%), while oridonin at the same dosage displays no significant efficacy in the same animal model [51].

The pyran ring is also a critical moiety in various bioactive natural products and their derivatives (e.g. Eribulin mesylate and Bryostatin-1) [75, 76]. More diversified analogues were developed using a similar strategy by incorporating the pyran ring system into the oridonin template [65]. A lanthanide Lewis acid-catalyzed, cross inverse-electron-demand hetero-Diels-Alder (IEDHDA) reaction (Table 3) was investigated starting from the key intermediate **31** to install the substituted dihydropyran framework at C1-C2 position of the oridonin A-ring in a controlled manner. It was found that the optimized reaction conditions using 10% mol $Yb(fod)_3$ as the catalyst at 32 °C result in predominated *endo* isomers **32**–**36** and a small amount of *exo* isomers **37**–**41** with the approximate diastereomeric ratio 90:10, which are separable with open column chromatography. Most of these novel pyran-fused oridonin analogs display potent growth inhibitory effect on cancer cells. Interestingly, the representative compound **33** exhibits the highest potency for anticancer activity against adriamycin-resistant breast cancer MCF-7/ADR cells with low micromolar to submicromolar EC_{50} values, indicating a potential use for the treatment of chemoresistant breast cancer [65].

It is well known that the α,β-unsaturated carbonyl moiety (enone) is a Michael acceptor with an electrophilic center which is susceptible to attack by nucleophilic reagents, such as the sulfhydryl group of cysteine residues in the core domain of target proteins, leading to β-position adducts and the corresponding biological response [77]. Therefore, the enone system is generally viewed as an important pharmacophore in natural anticancer agents and FDA approved irreversible kinase inhibitors exemplified by the triterpenoid derivative CDDO-Me [78] and the synthetic small molecule drug Ibrutinib [79]. The enone system in oridonin D-ring also appears to be one of the most critical pharmacophores for its biological effects [23]. In accordance with the general SAR analysis of enone systems, the construction of an additional enone pharmacophore in the A-ring is likely to be favorable for biological activity and sensitivity enhancement against drug resistance. To this end, oridonin A-ring based derivatives of the enone functionality were explored as novel dienone analogues effective for highly aggressive breast cancer by inducing apoptosis [66]. Dienone analogues **42**–**46** (Fig. 5) exhibit significantly improved antiproliferative effects relative to oridonin against MCF-7, MDA-MB-231 as well as drug-resistant MCF/ADR breast cancer cells with low micromolar to submicromolar potency. Compound **45** shows growth inhibition against triple-negative breast cancer MDA-MB-231 cells with an EC_{50} value of 5.6 μM, and induces apoptosis of MDA-MB-231 cells in a dose-dependent manner (1.0–10.0 μM) by regulation of Bcl-2/Bax, NF-κB and PARP signaling pathways. Moreover, in comparison with the natural product oridonin, dienone analogue **45** displays lower toxicity to normal mammary epithelial cells, and is more efficacious regarding antitumor activity (ip, growth inhibition > 55% at 5.0 mg/kg) with no significant loss of body weight in the MDA-MB-231 xenograft tumor model [66].

The 1,2,3-triazole scaffold, a typical pharmacophore, is generally formed between azides and terminal alkynes via the click reaction, and can readily bind to biological targets through hydrogen bonds and dipole interactions [80–82]. Therefore, we developed an efficient synthesis of novel nitrogen-enriched oridonin derivatives such as **47**–**49** with azide functionalities at the C-1, C-2 or C-3 position in a highly regio- and stereospecific manner, and subsequently utilized the click reaction to generate relevant oridonin-based 1,2,3-triazoles as potential anticancer agents. The representative triazole-substituted oridonin analogue **50** exhibits a 61-fold increase in potency of antiproliferative activity (EC_{50} = 0.48 μM) against triple-negative breast cancer MDA-MB-231 cell line relative to oridonin (EC_{50} = 29.4 μM) [67]. Taken together, our work on the efficient construction of oridonin A-ring derivatives opens new avenues to the development of novel natural product-like anticancer agents and valuable chemical tools in biomedical research existing outside the repertoire of compounds found in nature. The selected molecules of this class generated through A-ring modifications are currently in preclinical development by our research group.

3.3 Modifications on the α,β-unsaturated ketone

As mentioned above, the presence of the enone system as a core pharmacophore in the D-ring appears to be critical for its biological effects, and removal of this enone system may abrogate its anticancer activity [66]. Nan *et al.* have modified the enone system in the D ring to synthesize cyclopentanedione derivative **51**, reduction product **52**, and nitrogen-containing heterocycle fused D-ring oridonin analogues such as **53** and **54** (Fig. 6).

Interestingly, none of these compounds exhibit significant antiproliferative effects relative to oridonin [83], indicating that the enone system in the D-ring appears to be an essential pharmacophore for the anticancer activities.

The phytochemical study of secondary metabolites produced by oridonin offers three dimeric oridonin metabolites **55**–**57** involving the conversion of the enone system in the D-ring (Fig. 7). The metabolite **55** containing the enone group displays significant antiproliferative activity against broad-spectrum tumor cell lines with EC_{50} values ranging from 0.54 to 1.85 μM, while **56** and **57** without the enone moiety were found to be inactive against the cancer cells (EC_{50} > 100 μM) [84]. These studies further support the notion that the enone functionality in the D-ring is an essential pharmacophore for oridonin-based diterpenoid anticancer activity.

3.4 Transformation and derivatization of the skeletal structure

Oridonin is a typical, widespread and commercially available kaurane-type diterpenoid found abundantly in natural resources. Interestingly, some other types of diterpenoids such as spirolactone-type and enmein-type diterpenoids also possess considerable antitumor activity [85–88]. Unfortunately, the limited resources of these natural products restrict their pharmacological research and drug development. To this end, the naturally abundant oridonin may serve as a valuable starting material to explore the efficient and concise synthetic approaches for scaffold transformation to various spirolactone-type diterpenoids, enmein-type diterpenoids and other relevant derivatives.

3.4.1 Spirolactone-type diterpenoid derivatives—The carbon bond between the C-6 and C-7 positions in oridonin can be oxidized and cleaved by lead tetraacetate in the presence of sodium carbonate to yield the spirolactone-type diterpenoid **59** almost quantitatively (Fig. 8) [89]. Diterpenoid **59** exhibits efficacious anticancer effects against a broad spectrum of cancer cell lines such as human cervical cancer Hela cells and human lung carcinoma A549 cells with EC_{50} values of 4.63 μM and 4.58 μM, respectively, which are approximately 6~8-fold more potent than oridonin. It also displays moderately improved activity in suppressing MGC-803 xenograft tumor growth (ip, growth inhibition = 31.4% at 10 mg/kg) relative to oridonin (ip, growth inhibition = 26.3% at 10 mg/kg) [89]. To further explore the benefit of novel skeletons, another series of spirolactone-type diterpenoid derivatives **60**–**71** with the C-14 hydroxyl group acylated with various acyl groups and with C-1 as a ketone or an acetate have been investigated. Most of these compounds display significant antiproliferative effects (Table 4). The preliminary SAR studies suggest that the replacement of C-1 position carbonyl with an acetyl ester is generally tolerable, except in compound **60** which demonstrates decreased activity. The introduction of chemically diversified esters at the C-14 position results in a significant enhancement of anticancer activity. Compounds **69** and **70** bearing an acetate at the C-1 position and chlorine-substituted aromatic acid esters at the C-14 position proved to be the most potent molecules of this series against cancer cell proliferation by, at least in part, cell cycle G2/M phase arrest and apoptosis at low concentrations (0.6–1.0 μM) [89, 90].

3.4.2 Enmein-type diterpenoid derivatives—On the basis of oxidation at the C-6 and C-7 positions, the kaurane-type substrate **1** bearing a free hydroxyl group at the C-1 position can also be oxidized by sodium periodate in water to generate an unstable spirolactone-type intermediate, subsequently followed by hemiacetal formation between the hydroxyl group at C-1 and the aldehyde at C-6 to form the enmein-type diterpenoid **71** (Fig. 9) [89]. Compound **71** exhibits a weaker antiproliferative effect than the kaurane-type diterpenoid **1** or the spirolactone-type diterpenoid **59** (Table 5). The treatment of **71** with Jones reagent provides the corresponding lactone **72** with moderately improved activity. Based upon the enmein-type skeleton, the further substitution of the C-14 position hydroxyl with various esters leads to a significant enhancement of antiproliferative activity. Generally, the derivatives bearing C-14 position aromatic acid esters possess greater potency than those bearing alkyl acid esters. The substituents of the aromatic ring appear not to substantially impact the activity of corresponding compounds (Table 5). The typical enmein-type diterpenoid **76** was selected for pilot mechanism studies using human hepatoma BEL-7402 cells. The results reveal that drug-induced cell cycle G_2/M phase arrest and apoptosis are regulated via the oxidative stress induced mitochondria-related apoptotic pathway [91]. Moreover, administration of salts of water-soluble compound **83** at a dose of 40 mg/kg was found to exhibit more efficacious anti-gastric cancer effect (ip, growth inhibition = 64.8%) than oridonin (ip, growth inhibition = 37.3%) in mice [92]. In summary, these synthetic spirolactone-type and enmein-type diterpenoids may provide new platforms for drug discovery toward human cancer treatment.

3.4.3 Other kaurane-type natural diterpenoids—In addition to its application in the synthesis of spirolactone-type and enmein-type diterpenoids, oridonin was also utilized in the construction of a kaurane-type diterpenoid library. Rubescensin S (**87**; Fig. 10) is a natural kaurane-type diterpenoid with cytotoxic activities against the human leukemia K562 cell line (EC_{50} =18.48 μM) [93]. Inspired by the biogenetic structural transformation, Nan *et al.* reported a facile and mild synthetic approach through D ring-opening at the C15-C16 bond, together with installation of a lactone functionality at the C-6 and C-15 positions in a quantitative yield as depicted in Fig. 10. Subsequently, the acid **85** was effectively converted to rubescensin S via a Weinreb amide intermediate in modest yield of 32% [94].

Semiaquilegin A is a natural kaurane-type diterpenoid ester isolated from the roots of *Semiaquilegia adoxoides* (DC.) Makino., which exhibits antiproliferative activity against a broad spectrum of cancer cell lines with EC_{50} values ranging from 4.36 μM to 10.88 μM [95]. As depicted in Fig. 11, hydrolysis of semiaquilegin A afforded the crucial kaurane-type diterpenoid **88**. Tu *et al.* has succeeded in the development of a formal synthesis of **88** from oridonin via a 19-step synthetic route. This approach, involving elimination of the C-1 hydroxyl (Fig. 11, synthesis of **90**) and opening of the oxygen bridge between the C-7 and C-20 positions (Fig. 11, synthesis of **92**), provided more chemically diversified kaurane-type diterpenoids of potential biological importance [96].

3.5 Summary on structure-activity relationship of oridonin analogues

Taken together, this review captures the recent advances in medicinal chemistry efforts to explore a variety of oridonin analogues as potential anticancer agents. The accumulated

results on synthesis of various structural analogs and their anticancer effects against various malignant cancer cells have established meaningful SARs which are depicted in Fig. 12. Briefly, modifications on the A-ring and C-14 position may significantly improve the anticancer efficacy and aqueous solubility of oridonin. The oxidative rearrangements around C-6 and C-7 positions afford chemically diversified spirolactone-type and enmein-type diterpenoids, instead of kaurane-type oridonin template. The enone system in the D-ring seems to be an essential pharmacophore for the anticancer activity. While modifications on the A-ring, the C-14 position or the carbon bond between C-6 and C-7 appear to be tolerable, the reconstruction of the enone system in D-ring may result in a significant activity loss. Modifications of the oxygen bridge between the C-7 and C-20, and the C-6 and C-7 position hydroxyls are relatively less explored, and more efficient methodologies are imperative to establish useful SAR for these two structural regions. In summary, the available SAR studies provide useful and efficient modification strategies to further develop oridonin derivatives with enhanced biological activities and drug-like properties.

4. Conclusions and future perspectives

Natural products continue to serve as an invaluable source of molecular diversity for drug discovery. In the past decade, oridonin, a commonly utilized natural product in traditional Chinese and Japanese herbal medicine, has attracted considerable interest due to its biological potential as a cytoprotective, anti-inflammatory and anticancer agent. Its anticancer effect receives considerable attention since a multitude of studies demonstrate oridonin is capable of regulating cell cycle, autophagy, and inducing apoptosis in various malignant cancer cells through a multifunctional approach [97]. Despite its attractive pharmacological profile of safety and efficacy, oridonin has not been widely adopted into clinical practice due to its relatively moderate potency, imprecise mechanisms of action, limited aqueous solubility and oral bioavailability. Hence, a variety of potential targets and signaling pathways have been identified to be associated with oridonin and its derivatives, and a number of oridonin derivatives with diverse modifications have been designed and synthesized in the search of more effective and drug-like analogues for cancer chemotherapy. Excitingly, compound **2** was the first human clinical trial oridonin-like drug candidate for the treatment of leukemia in 2015. Nevertheless, to advance oridonin analogues into viable therapies, there remains to be several issues and new directions for future development.

1. Although oridonin has already received tremendous attention in the past decade, its precise molecular mechanisms involved in the treatment of cancer and other diseases remain to be further elucidated. It is known that the polypharmacology and network pharmacological approach have been widely recognized in drug discovery to enhance efficacy and overcome drug resistance of singular-target drugs [98–100]. Such approaches have been successfully applied in the field of natural product research based upon the systematic elucidation and prediction of mechanisms, as well as functional phenotypic measurements *in vivo* and *in vitro* [101, 102]. These relevant advances inspire researchers to identify new and unexplored targets of oridonin and its derivatives (e.g. death receptor 5 (DR5) [103];

Krüppel-like factor 5 (KLF5) [104]; X-box binding protein 1 (XBP1) [105]) for cancer therapy, and map the integral signaling networks associated with oridonin-like compounds to facilitate the future Investigational New Drug (IND)-enabling studies towards potential clinical indications.

2. The proof of concept for developing oridonin derivatives as a novel therapy for cancer, inflammation and other diseases has been initially validated by the application of oridonin-containing herbal medicines and the investigation of clinical oridonin prodrug HAO472. Additionally, new analogs **28** and **45** (Fig. 12) exhibit potent anticancer activities against the aggressive triple negative breast cancer with low micromolar to nanomolar EC_{50} values *in vitro* and demonstrated efficacy *in vivo* at a low dosage of 5 mg/kg. These newly discovered molecules are superior to oridonin, and are currently under preclinical development. With the established SAR information of explored oridonin derivatives (Fig. 12) and useful chemical probes [106], fragment-based drug design (FBDD) [107–112] by taking advantage of privileged fragments, especially the critical pharmacophores in marketable and clinical trial natural drugs, may provide an additional strategy to develop more effective and drug-like oridonin derivatives with higher potency, better oral bioavailability and safety performance.

3. The *in vivo* pharmacokinetic safety concerns of oridonin derivatives should also be cautiously considered in the early stage of drug discovery. Oridonin itself displays high first-pass effects and low bioavailability under oral administration (F = 4.3%) or intraperitoneal injection (F = 12.6%) [49]. The introduction of the hydrophilic groups as prodrug fragments at the C-14 position (e.g. HAO472, **18** and **19**) and the further development of intravenous drug delivery system are useful strategies for improving the absorption and distribution properties of oridonin-like compounds. In addition, modifications of oridonin skeleton by introducing hydrophilic functionalities (e.g. **28**) were an effective approach to enhance the aqueous solubility and avoid the excessive first-pass metabolism. Therefore, more extensive investigations involving ADME and safety profiles of oridonin derivatives are imperative to provide comprehensive information for the further drug design and development. Computational approaches, such as the publicly available program pkCSM [113], may assist an earlier elimination of the risks of compounds with potential poor pharmacokinetic and safety issues.

4. Development of novel drug delivery systems is an available strategy to improve aqueous solubility, ADME and toxicity of many drugs [114, 115]. Consequently, such strategies may also be useful for oridonin and its derivatives to improve drug-likeness. The preparation of oridonin or its active analogs in the form of a nanosuspension can result in enhanced aqueous solubility and efficiency against human cancer cell lines *in vitro* and *in vivo* [116–118]. The exploration of novel drug delivery

formulations containing nanosuspension, nanogels, nanoparticles, micelles and self-microemulsifying systems would provide aqueous soluble, tumor targeting and potent oridonin-like agents for potential clinical applications [63, 119–123].

5. In most of the aforementioned literature, the synthetic oridonin derivatives with enhanced activity were primarily tested against various cancers. In fact, many natural products are multifunctional agents simultaneously providing opportunities for drug discovery in different therapeutic areas. For instance, the natural triterpenoid derivative CDDO-Me has profound anti-oxidative, anti-inflammatory and cytoprotective effects in low nanomolar range (0.4–100 nM), as well as significant antiproliferative and pro-apoptotic effects in relative higher concentrations (1–5 μM) [124, 125]. Based upon the multifunctional effects of CDDO-Me, researchers explored different indications in clinical trials, including the treatments of chronic kidney disease (Phase II, NCT02316821), pulmonary arterial hypertension (Phase III, NCT02657356), and advanced malignant melanoma (Phase II, NCT00535314). Hence, additional attention should also be paid to explore other beneficial effects, including cytoprotection [40], anti-mycobacterial [126, 127], anti-fibrosis [128, 129] and anti-inflammatory effects [130], produced by oridonin and its derivatives. Interestingly, recent findings support that oridonin and its analogues may be considered a promising therapeutic option for Alzheimer’s disease (AD) and other neurodegenerative diseases [41, 42].

It is the opinion of the authors that oridonin offers an important natural product platform for drug development for treatment of cancer, inflammation and other diseases. As of now, this platform has successfully provided an excellent base from which to develop a variety of new agents that are more potent and drug-like than the parent natural product oridonin. Oridonin derivatives may be viable therapeutics for the treatment of human diseases in clinic.

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Abbreviations used

FDA	Food and Drug Administration
OTC	Over-The-Counter
MAPK	Mitogen-activated protein kinase
TNF-α	tumor necrosis factor alpha

ROS	reactive oxygen species
Bcl-2	B-cell lymphoma 2
Bax	Bcl-2-associated X protein
NF-κB	nuclear factor κ light chain enhancer of activated B cells
p53	tumor protein p53
p21	p21Cip1 protein
PI3K	phosphoinositide 3-kinase
MTT	thiazolyl blue tetrazolium bromide
CCK-8	cell counting kit-8
EST	median survival time
SIRT1	NAD-dependent protein deacetylase sirtuin-1
FADD	Fas-associated protein with death domain
Nrf2	nuclear erythroid 2-related factor 2
Keap1	kelch like ECH associated protein 1
ARE	antioxidant responsive element
EC_{50}	half maximal effective concentration
ip	intraperitoneal
SAR	Structure-activity relationship
NO	nitric oxide
GSH	glutathione
PEG	polyethylene glycol
MPEG-PCL	poly(ethylene glycol)-poly(ε-caprolactone)
AUC	area under the plasma concentration time curve
MRT	mean residence time
CL	clearance values
NOAEL	No-observed-adverse-effect level
iv	intravenous
PARP	poly (ADP-ribose) polymerase
IED HAD	inverse-electron-demand hetero-Diels-Alder

IND	Investigational New Drug
ADME	absorption, distribution, metabolism and excretion
FBDD	fragment-based drug design
AD	Alzheimer's disease

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Highlights

- Oridonin displays significant anticancer activities via multi-signaling pathways.
- Recent advances in medicinal chemistry of oridonin-like compounds are presented.
- The article summarizes the SAR and mechanism studies of relevant drug candidates.
- The milestones and future direction of oridonin-based drug discovery are discussed.

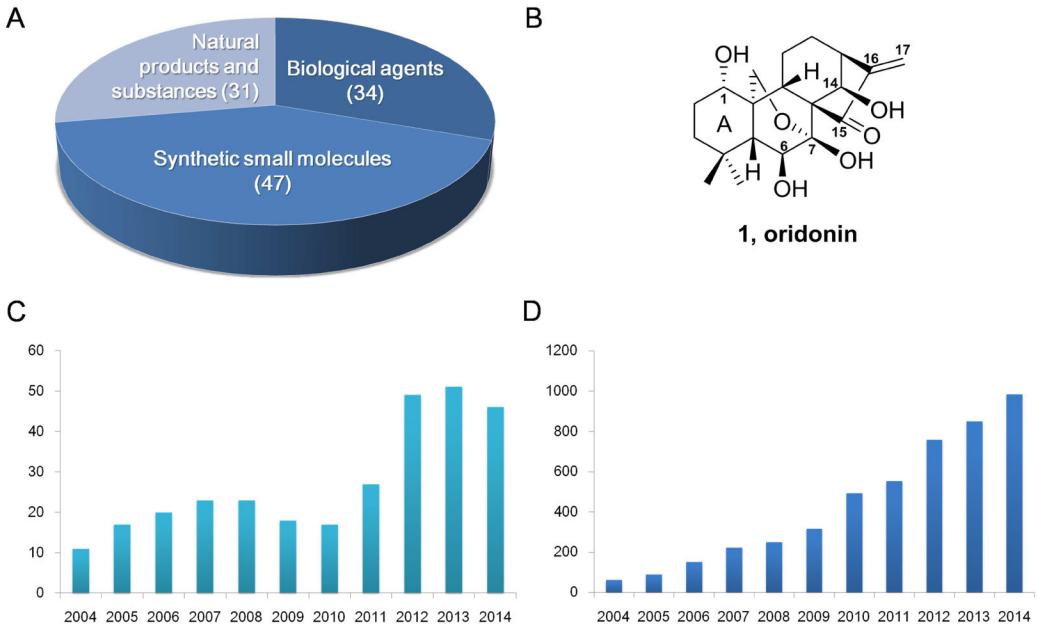


Fig. 1.

A) Of the 112 FDA-approved first-in-class drugs from 1999 to 2013, 31 drugs (28%) originated from natural products and substances versus 47 drugs (42%) which are synthetic small molecules, and 34 drugs which are biological agents. **B**) The structure of the natural diterpenoid oridonin. **C**) Number of papers published between 2004 and 2014 containing the keyword “oridonin” according to the Web of Science search. **D**) Citations between 2004 and 2014 according to the Web of Science search using the keyword “oridonin”.

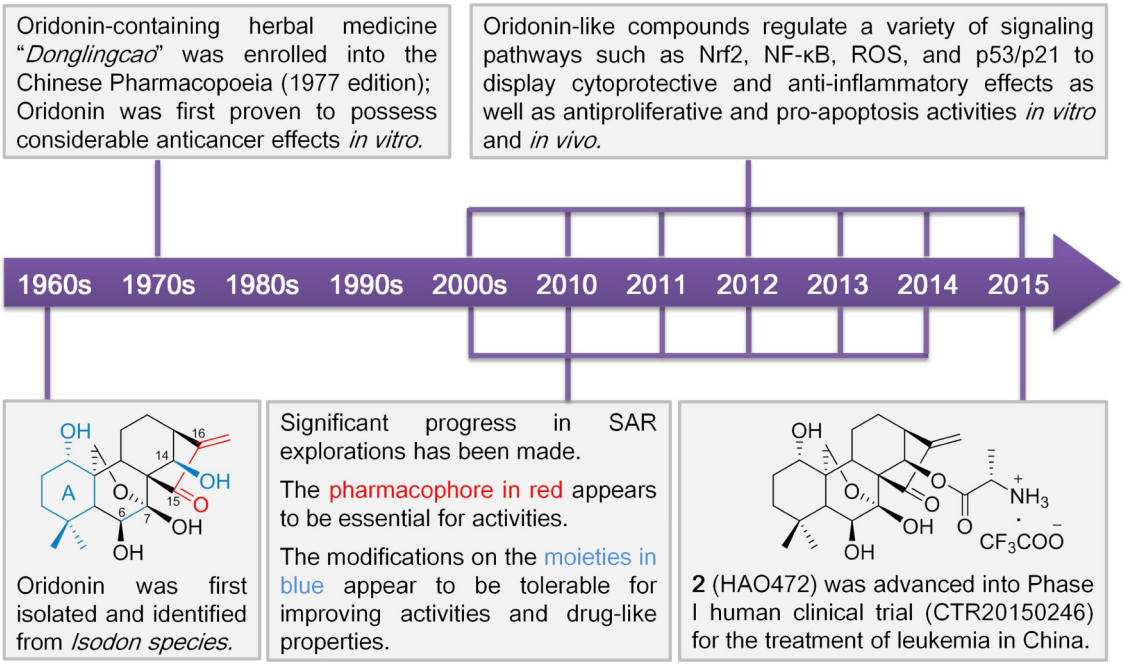


Fig. 2.
Major milestones achieved in oridonin-inspired drug discovery and development.

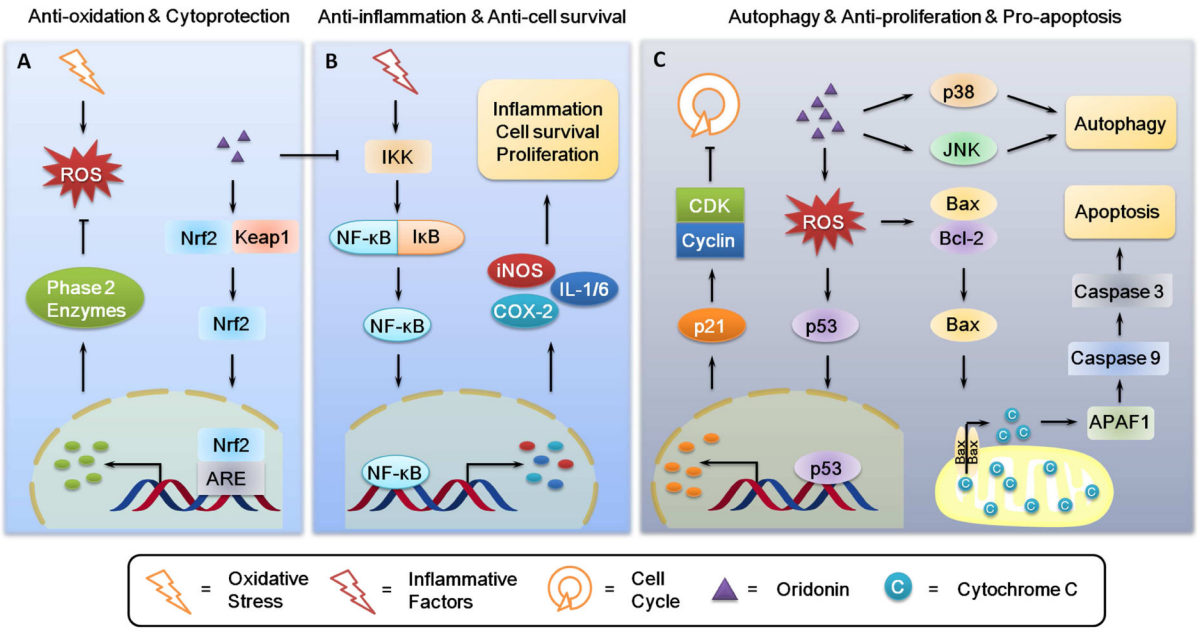


Fig. 3.

Oridonin regulates multi-signaling pathways to display corresponding multifunctional effects. **A**) and **B**). At low concentrations, oridonin regulates Keap1-Nrf2-ARE and NF-κB pathways to exert cytoprotective and anti-inflammatory effects. **C**). Elevated concentrations of oridonin inhibit proliferation and induce apoptosis by regulating ROS-mediated and other signaling pathways. These paradoxical activities may be correlated with partial signaling pathway modulation in different cell conditions and depend upon the dosage of treatment.

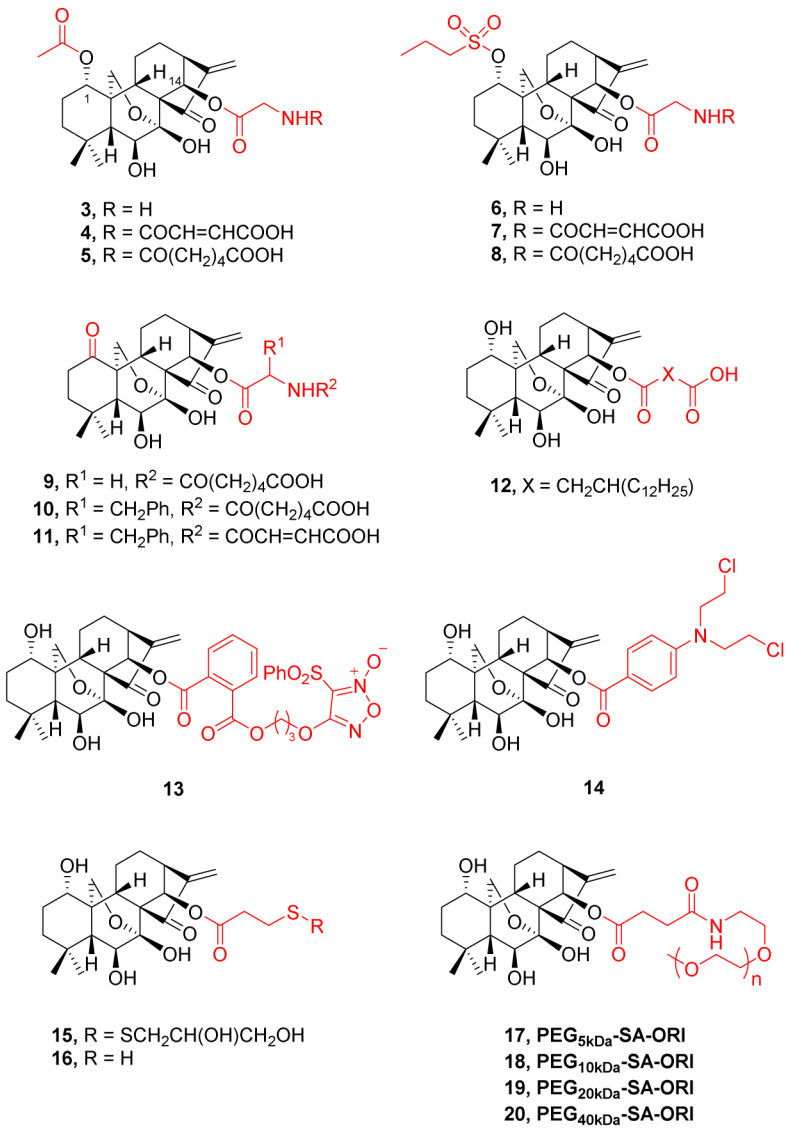


Fig. 4.

The structures of C-1 and/or C-14 position modified oridonin derivatives.

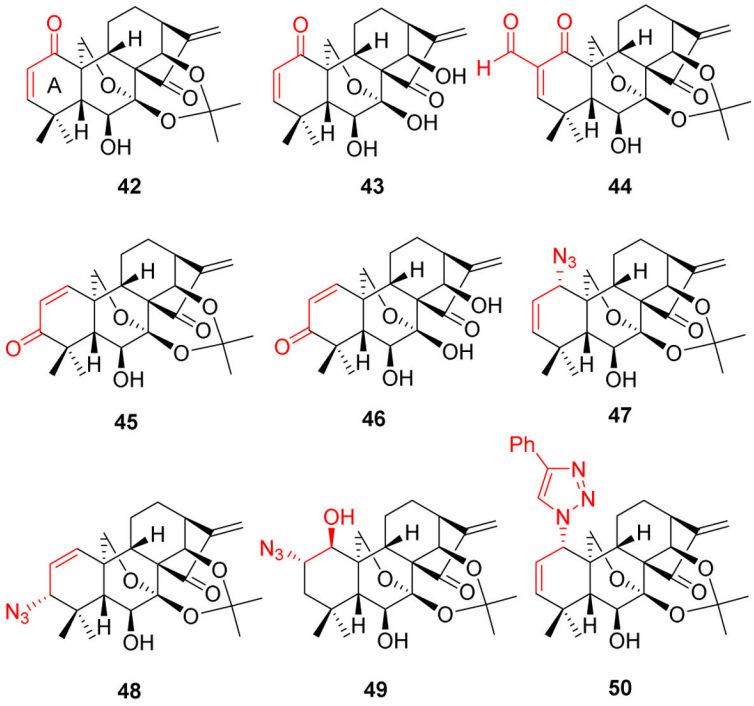


Fig. 5.
Diversified modifications on A-ring of oridonin template.

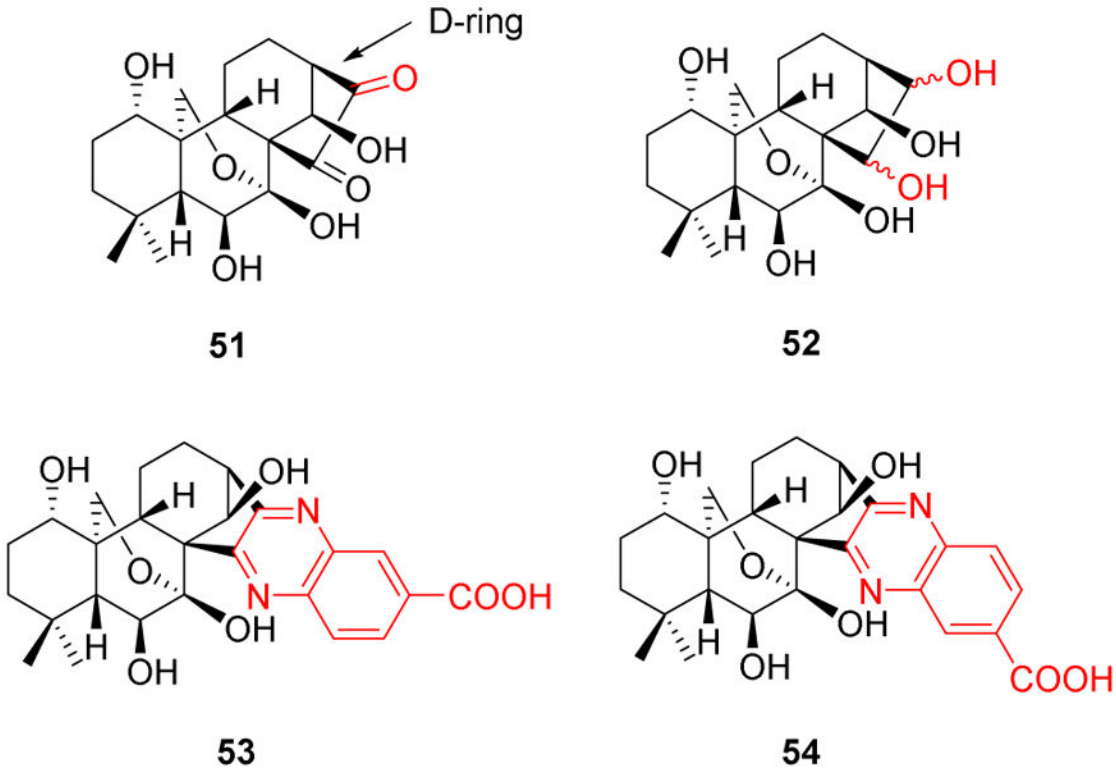


Fig. 6.
Modifications on D-ring of oridonin.

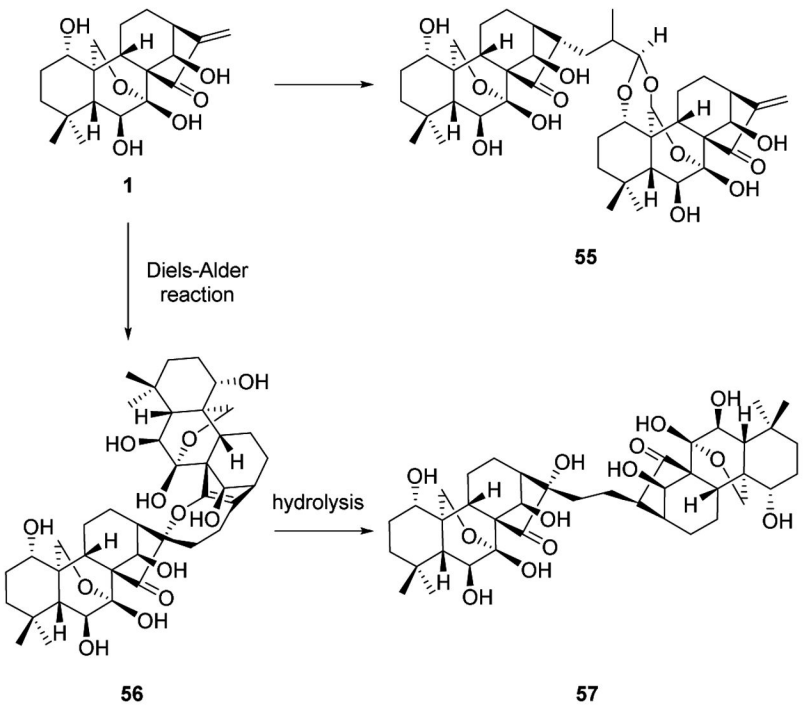


Fig. 7.
Biosynthetic pathway of oridonin secondary metabolites.

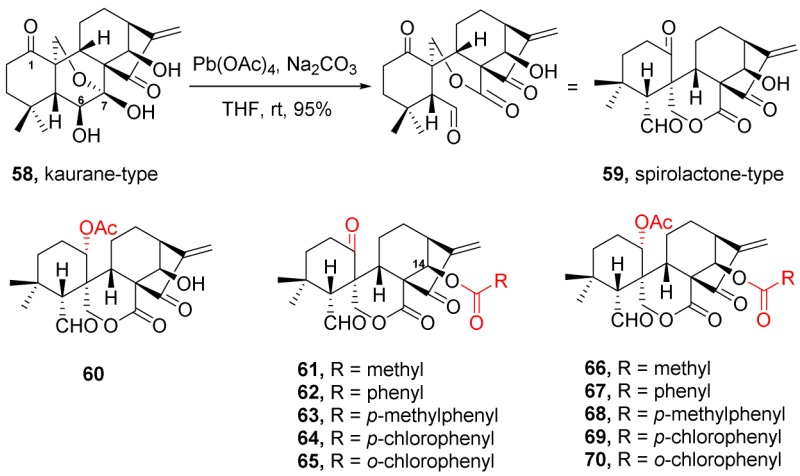


Fig. 8.
Spirolactone-type diterpenoid derivatives generated from oridonin template.

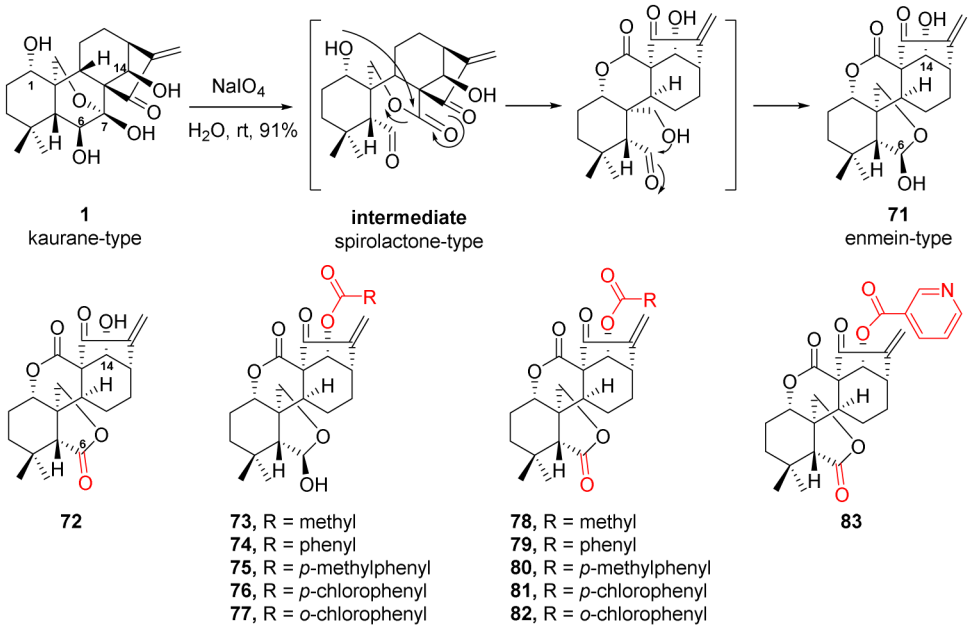


Fig. 9.

Enmein-type diterpenoid derivatives generated from oridonin.

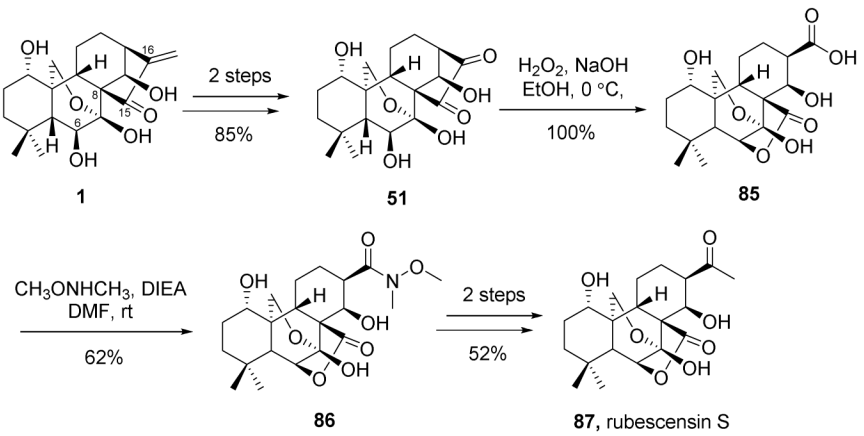


Fig. 10.

Synthesis of the kaurane-type diterpenoid rubescensin S starting from oridonin.

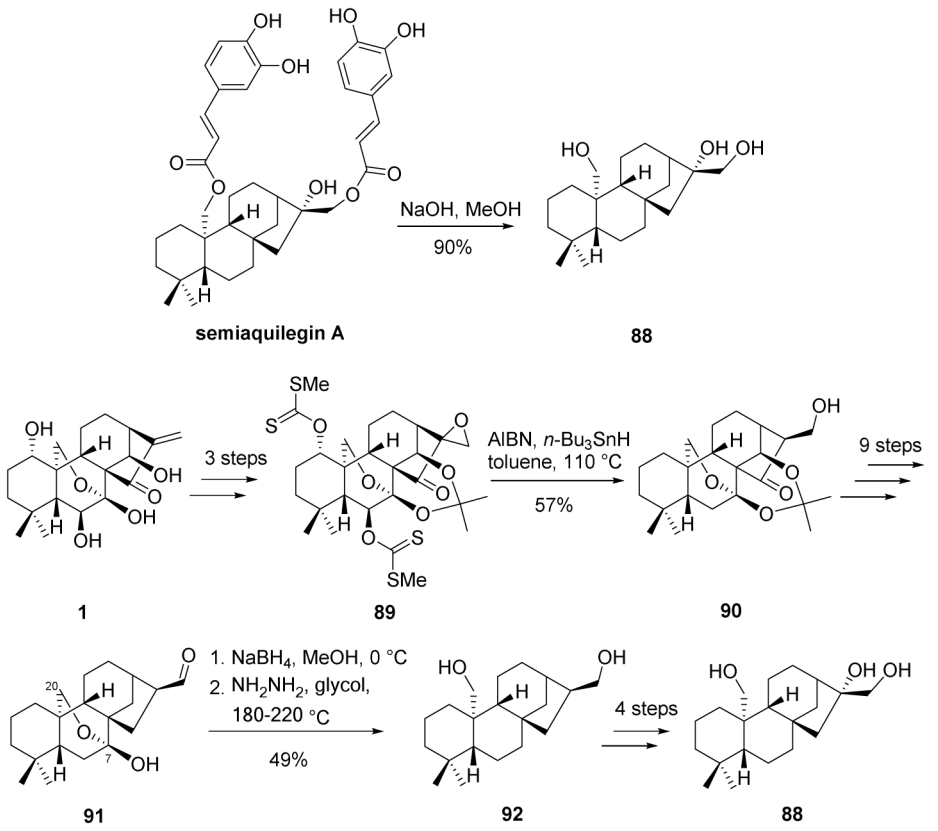


Fig. 11.

Synthesis of kaurane-type pharmacophore in the structure of semiaquilegin A starting from oridonin.

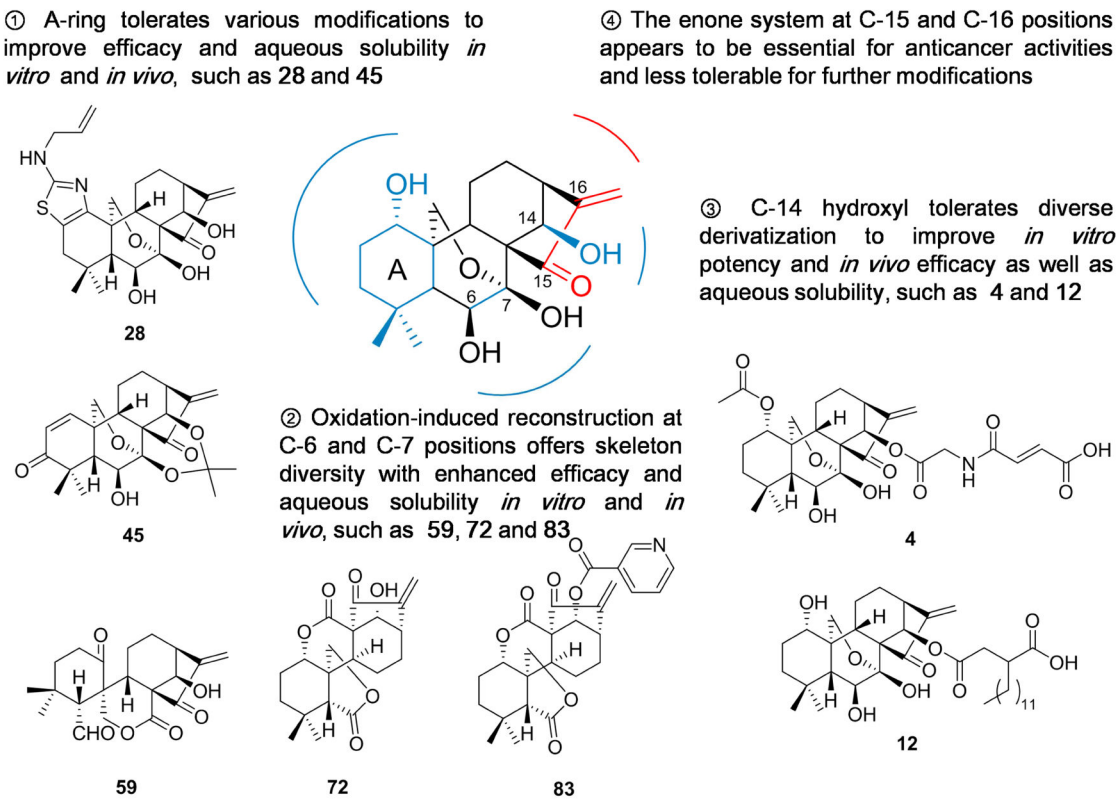


Fig. 12.

Graphical depiction of the general structure-anticancer activity relationship of oridonin derivatives based upon the available *in vitro* and *in vivo* biological results.

Table 1

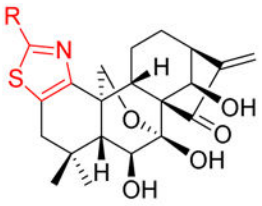
Summary of *in vitro* and *in vivo* studies with oridonin against various cancers.

Diseases	*In vitro* studies	*In vivo* studies	Potential molecular mechanisms	Ref.
Acute myeloid leukemia	CCK-8 assay (48 h) indicated: EC_{50} = 2.5 μM for AML-Eto9a cells, EC_{50} = 1.33 μM for Kasumi-1 cells	Prolong EST from 83.5 days to 112.5 days in AML-Eto9a mice (20 mg/kg), and inhibit tumor growth in t(8;21) leukemia murine models (7.5 and 15 mg/kg)	Generate ROS, down-regulate Bcl-2, activate caspase-3, and degrade AML1-ETO fusion protein	[27, 28]
Chronic myeloid leukemia	MTT assay (24 h) indicated: EC_{50} = 14.6 μM for K562 cells, EC_{50} <40 μM for K562/ADR cells	Inhibit tumor growth in K562 xenograft nude mice (10 and 15 mg/kg)	Down-regulate miR-17 and miR-20a, up-regulate BIM-S, and degrade c-Myc	[29, 30]
T-cell leukemia	Trypan Blue assay (48 h) indicated: EC_{50} = 1.2 – 2.7 μM for MT-1, MT-2, U266, RPMI8226 and Jurkat cells	-[a]	Inhibit NF-κB signaling	[31]
Fibrosarcoma	-	-	Generate ROS, and up-regulate Bax	[32]
Laryngeal cancer	MTT assay (24 h) indicated EC_{50} = 37.1 μM for HEp-2 cells	-	Generate ROS, up-regulate Bax, FADD, caspase-8 and caspase-3, down-regulate Bcl-2 and SIRT1	[33]
Liver fibrosis	Alamar Blue assay (48 h) indicated: EC_{50} = 7 μM for LX-2 and HSC-T6 cells	-	Up-regulate p53/p21 signaling	[34]
Prostate cancer	CCK-8 assay (48 h) indicated: EC_{50} = 25 μM for PC-3 and LNCAP cells	-	Up-regulate p21 signaling	[35]
Colorectal cancer	MTT assay (24 h) indicated: EC_{50} = 20.8 – 37.0 μM for SW480 and SW620 cells	-	Inhibit fatty acid synthase	[36]

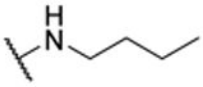
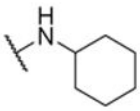
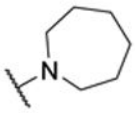
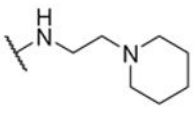
[a]Not available.

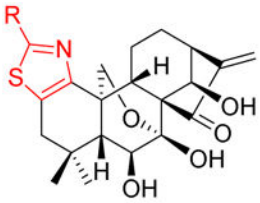
Table 2

The structures and effects of nitrogen-enriched oridonin analogues with thiazole-fused A-ring on proliferation of human breast cancer cell lines.

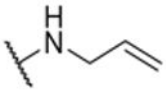
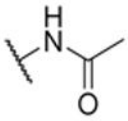
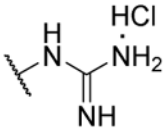


21 ~ 30

Compd	R	EC_{50} (μM)	
		MCF-7	MDA-MB-231
1	-	6.6	29.4
21		1.0	3.2
22		1.3	2.1
23		0.8	0.3
24		0.6	1.1
25		0.9	0.8
26		1.2	6.8
27		1.0	1.8



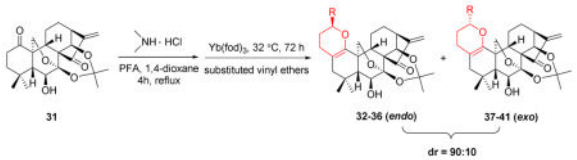
21 ~ 30

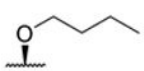
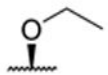
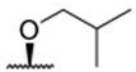
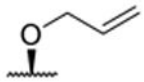
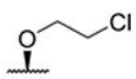
Compd	R	EC_{50} (μM)	
		MCF-7	MDA-MB-231
28		0.2	0.2
29		2.0	6.8
30		3.4	> 10

Eur J Med Chem. Author manuscript; available in PMC 2017 October 21.

Table 3

The design and synthesis of dihydropyran-fused oridonin derivatives as potential anticancer agents.



Compd	R	EC_{50} (μM)			
		MCF-7	MDA-MB-231	MDA-MB-468	MCF-7/ADR
1	-	4.4	28.0	5.3	34.8
Adriamycin	-	0.7	2.3	0.7	> 10
32		2.4	3.7	5.8	6.8
33		0.44	0.54	0.52	1.6
34		5.8	8.2	7.0	10.3
35		2.1	3.3	4.4	3.2
36		6.8	7.2	7.3	8.7

Compd	R	EC_{50} (μM)			
		MCF-7	MDA-MB-231	MDA-MB-468	MCF-7/ADR
37		2.4	2.2	3.0	3.8
38		3.5	6.1	5.4	4.7
39		2.2	1.8	2.6	4.4
40		4.3	7.1	4.9	4.3
41		2.3	3.3	3.0	3.1

Table 4

The antiproliferative effects of spirolactone-type diterpenoid derivatives against various human cancer cell lines.

Compd	EC_{50} (μM)			
	K562	MGC-803	CaEs-17	Bel-7402
1	4.76	5.69	11.03	7.48
59	2.18	-[a]	5.85	5.03
60	9.56	-	72.55	75.44
61	1.39	1.66	1.35	1.73
62	1.48	1.92	1.71	1.85
63	1.27	2.24	1.05	1.54
64	1.38	3.12	2.55	3.84
65	1.22	2.66	2.01	2.89
66	1.74	3.47	2.80	3.96
67	1.40	2.18	1.99	3.05
68	1.31	1.90	1.83	2.09
69	0.87	1.73	1.25	1.51
70	0.39	1.28	0.60	1.39

[a]Not available.

Table 5

The antiproliferative effects of enmein-type diterpenoid derivatives against human cancer cell lines.

Compd	EC_{50} (μM)			
	K562	MGC-803	CaEs-17	Bel-7402
1	4.76	5.69	11.03	7.48
71	8.11	14.21	30.84	32.96
72	2.64	-[a]	23.67	15.98
73	1.11	1.99	1.59	1.97
74	0.21	0.93	0.54	1.42
75	0.14	0.61	0.45	1.01
76	0.24	1.22	0.28	0.87
77	0.22	0.80	0.60	0.85
78	0.35	1.27	0.69	1.37
79	0.33	0.46	0.81	0.79
80	0.52	1.54	1.78	1.58
81	0.14	0.34	0.34	0.89
82	0.26	0.82	0.28	0.72
83	1.74	1.16	3.54	0.71

[a]Not available.