Overview of Piperlongumine Analogues and their Therapeutic Potential

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Review article

Overview of piperlongumine analogues and their therapeutic potential

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ABSTRACT

Natural products have long been an important source for discovery of new drugs to treat human diseases. Piperlongumine (PL) is an amide alkaloid isolated from *Piper longum* L. (long piper) and other piper plants and has received widespread attention because of its diverse biological activities. A large number of PL derivatives have been designed, synthesized and assessed in many pharmacological functions, including antiplatelet aggregation, neuroprotective activities, anti-diabetic activities, anti-inflammatory activities, anti-senolytic activities, immune activities, and antitumor activities. Among them, the antitumor effects and application of PL and its derivatives are most extensively studied. We herein summarize the development of PL derivatives, the structure and activity relationships (SARs), and their therapeutic potential on the treatments of various diseases, especially against cancer. We also discussed the challenges and future directions associated with PL and its derivatives in these indications.

Contents

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1. Introduction

Natural products play critical roles in drug development and serve as vital sources for discovery and development of new drugs [1–4]. It has been suggested that approximately 35% of drugs on the market are originated from natural products [5]. Many anticancer drugs are developed from natural products, such as vinorelbine, docetaxel and topotecan [6]. Herbal medicine, an integral part of natural products, has been practiced for centuries in China and many other east Asian countries and is still widely used for the prevention and treatment of a variety of diseases. Traditionally used as mixtures of multiple components, herbal medicine has evolved over the years. Medicinal chemists have been able to extract and isolate active ingredients from natural products, determine their chemical structures, and design and synthesize large numbers of derivatives to gain in-depth understanding of structure-activity relationships (see Figs. 6 and 7).

Piperaceae, or the pepper family, is a large family of flowering plants with ~3600 species. *Piper longum* L is the most well-known species of the family and is widely distributed in tropical and subtropical regions [7]. *Piper longum* L. provides most peppercorns that are widely used as spices, including black pepper. In addition, the roots, fruits and seeds of *Piper longum* L. have been shown to have a variety of medicinal properties and can be used as herbal medicine to treat a number of diseases [8]. A variety of alkaloid components have been isolated from *Piper longum* L. including piperlongumine (PL), as shown in Fig. 1 [9–11]. PL has also been extracted from other piper plants such as *Piper tuberculatum* [12].

The chemical structure of PL, 5,6-Dihydro-1-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2-(1H)-pyridone, was first determined by Chatterjee and co-workers in the 1960s, who also reported its chemical synthesis [13]. PL exists as both *cis* and *trans* (or Z and E) isomers [14]. The trans PL is more stable than the cis isomer and showed higher tumor cytotoxic activity in a dose-dependent manner, whereas the cis PL failed to induce cytotoxicity even at high concentrations of 50 μM [15]. Therefore, the *trans* isomer has been more extensively studied. PL in this paper refers to the *trans* isomer if not otherwise noted (Fig. 2). Various PL derivatives have been synthesized and evaluated over the years. PL and/or these analogues have been found to possess diverse pharmacological activities, including antiplatelet aggregation, neuroprotective, anti-diabetic, anti-inflammatory activity, anti-senolytic activity, immune activity, and particularly promising antitumor activities in different animal models [16–19]. In this review, we aim

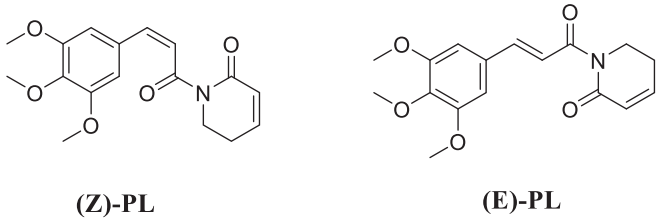

Fig. 2. The chemical structure of (Z)- and (E)-PL.

Piper longum L.

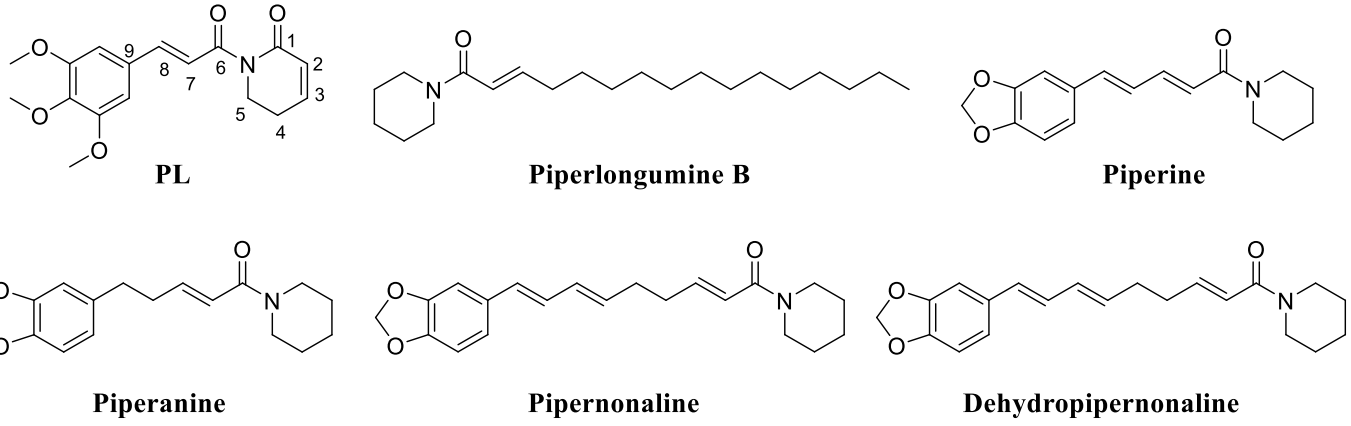

Fig. 1. Structures of representative alkaloids from *Piper longum* L.

to summarize the development of PL derivatives and their biological activities based on these therapeutic applications. It should be noted that the anti-cancer potential of these PL derivatives has received particular attention and has been discussed in several recent reviews [20,21], whereas the other therapeutic indications have received less attention.

2. Biological activities of PL and its derivatives

2.1. Antiplatelet aggregation of PL and its derivatives

Thrombotic disorders are a major cause of morbidity and death throughout the world. A primary approach to treat thrombotic diseases is to use antiplatelet agents [22,23]. PL has been shown to exert in vitro antiplatelet aggregation effect induced by agonists such as collagen, adenosine 5′-diphosphate (ADP), arachidonic acid (AA) and thrombin. This effect may result from the ability of PL to decrease thromboxane A (2) formation and inhibit cyclooxygenase activity [12].

Park et al. synthesized a serious of PL derivatives and investigated their effects on platelet aggregation inhibition. SAR studies showed that introduction of a methyl group to the C-2 position of PL enhanced the inhibition of thrombin and collagen-induced platelet aggregation, but reduced the inhibition of arachidonic acid-induced platelet aggregation. Among the analogues synthesized, compound **1** (Fig. 3) displayed the best inhibitory activity to platelet aggregation induced by arachidonic acid (100%), greater than PL (76.4%), at the concentration of 150 μM [24].

Wang and colleagues proposed that PL shared the same skeleton with thienopyridines (such as 2), a class of drugs wildly used in the clinic for treating platelet aggregation by selectively blocking adenine diphosphate (ADP)-induced platelet aggregation. The authors predicted that the trimethoxy-phenyl-acryloyl moiety from PL and thienopyridine contributed to their antiplatelet aggregation activities. Therefore, the authors designed and synthesized a series of PL derivatives by merging these two key structural components. Among them, compounds **3** and **4** (Fig. 3) significantly inhibited AA-induced platelet aggregation with IC_{50} values of 0.130 ± 0.023 mM and 0.108 ± 0.014 mM, respectively, which were significantly lower than PL (20.751 ± 1.450 mM). Compounds **3** and **4** also increased the inhibitory activity to platelet aggregation induced by ADP with IC_{50} values of 0.403 ± 0.80 mM and 3.145 ± 0.720 mM, respectively, lower than PL (6.710 ± 0.697 mM) [25].

2.2. Neuroprotective activity of PL and its derivatives

Millions of people around the world are affected by neurodegenerative disorders, with symptoms including cognitive impairment and depressive behaviors [26]. *Trans*-cinnamic acid derivatives such as PL have been reported to show anti-anxiety, anti-depression effects by targeting 5-hydroxytryptamine (5-HT), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and nuclear factor 2 (Nrf2) [14,27–29].

Since 3,4,5-trimethoxycinnamic acid (TMCA) has been found to alleviate opiate withdrawal syndrome and exhibit antidepressant-like activity in mice, Jung et al. developed a simple synthesis of *trans*-3,4,5-trimethoxycinnamamides as novel antinarcotic agents and evaluated their radical scavenging, neuroprotective and antinarcotic activities. Potent neuroprotective activities were observed for PL derivative **5** and **6** (Fig. 4) at doses ranging from 5 to 20 μM in primary cortical neuronal cells induced by glutamate. Meanwhile, PL derivative **7** exerted the most potent inhibitory activity on the morphine withdrawal syndrome in mice [30].

Cinepazide (**8**, Fig. 4), a PL-like drug, is a marketed drug for the treatment of cerebrovascular diseases and peripheral vascular diseases. Zhao et al. first demonstrated that Cinepazide protected PC12 neuronal cells by maintaining the mitochondrial function through stabilizing mitochondrial membrane potential, promoting oxygen–glucose deprivation (OGD)-induced suppression of mitochondrial respiratory complex activities and enhancing ATP (adenosine-triphosphate) production [31].

Elevated oxidative stress plays a key role in many neurodegenerative disorders. The Fang group synthesized a series of analogues at the C2-position and identified PL analogues **9** and **10** as promising neuroprotective agents by enhancing the antioxidant effects (Fig. 4). These compounds relieved cell injuries against hydrogen peroxide- and 6-hydroxydopamine-induced PC12 cell damage and apoptosis, and also prevented the accumulation of ROS, while displaying low cytotoxicity. This cytoprotection against neuronal cell oxidative damage appeared to be mediated by promotion of antioxidant/detoxifying genes (HO-1, Trx 1, TrxR1, NQO1, GCLC and GCLM), genes driven by transcription factor Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), a master regulator of the cellular stress responses. Silencing Nrf2 suppressed the cytoprotection of these molecules, confirming its involvement [32].

Li et al. designed and synthesized a number of PL analogues by combining the 3,4,5-trimethoxyphenyl group and the diketene skeleton, which is known to have antioxidant effect with low toxicity [33]. A total of 34 compounds were synthesized via a novel green synthesis method. Most compounds exerted less toxicity to PC12 cells than PL. Among the analogues, **11** was identified as a promising antioxidant agent, which significantly protected PC12 cells from H_2O_2-induced cell injury and improved the survival rate to 80%. Further studies suggested that **11** was a promising candidate for the treatment of cerebral ischemia-reperfusion injury (CIRI) without apparent cytotoxicity and attenuated brain injury in vivo.

2.3. Anti-diabetes activity of PL and its derivatives

Aldose reductase (ALR2) is a rate-limiting enzyme that regulates the polyol pathway from glucose to sorbitol [34]. During diabetes, the ability of ALR2 to convert excess glucose to sorbitol is significantly enhanced compared to euglycemic conditions. The accumulation of high concentrations of sorbitol in kidney, lens, retina and nerve, contributes to diabetic complications [35]. Rao et al. first discovered that PL could inhibit the recombinant human ALR2 with

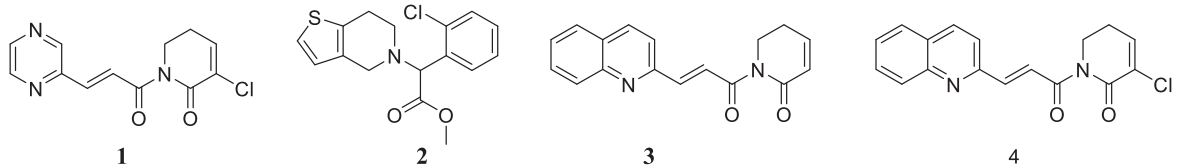

Fig. 3. Structure of synthetic PL derivatives as antiplatelet aggregation agents (**1**–**4**).

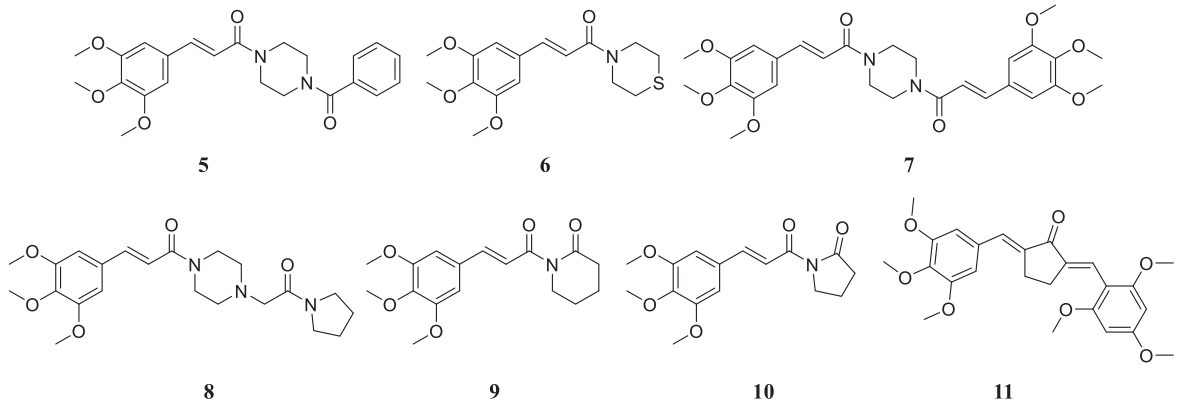

Fig. 4. Structure of synthetic PL derivatives as neuroprotective agents (**5**–**11**).

an IC_{50} of 160 μM. In order to improve the potency, they synthesized a series of PL derivatives by introducing various substituents around the aromatic/styryl and heterocyclic moieties [36]. Among them, analogues **12**, **13** and **14** (Fig. 5) had strong inhibitory activity on aldose reductase with IC_{50} of 8 μM, 4 μM and 4 μM, respectively. Compounds **12**–**14** also prevented the accumulation of sorbitol in human red blood cells, thus facilitating prevention and treatment of diabetes. Docking studies of ALR2 with **13** showed that **13** was docked into the active site of ALR2 and interacted with residues Trp-20, Tyr-48, His-110, Trp-111, Cys-298, Leu-300, and Leu-301.

2.4. Anti-inflammatory activity of PL and its derivatives

PL and its derivatives have received widespread attention due to its anti-inflammatory activities [37]. Fibroblast-like synoviocytes (FLS) from patients with Rheumatoid arthritis (RA) exhibit "tumor-like" properties of hyperproliferation, apoptosis and migration [38]. Xu et al. discovered that PL inhibited the proliferation, induced the apoptosis and reduced the migration and invasion of RA FLS by activating the p38, JNK, NF-κB and STAT3 pathways [39]. They also reported that PL exerted anti-inflammatory actions in human Osteoarthritis (OA) chondrocytes. It reversed IL-1β-inhibited cell viability in a dose-dependent manner. Mechanistic studies showed that the production of NO and PGE2 was significantly inhibited after the treatment of PL. PL also significantly suppressed the production of MMP-3 and MMP-13 and prevented NF-κB activation stimulated by IL-1β in human OA chondrocytes [40].

Ku et al. synthesized a number of PL analogues by combining carbazole with a cinnamoyl group (Fig. 6). Compound **15** was identified as a potent agent for inhibiting HMGB1 (high mobility group box-1 protein)-mediated inflammatory responses. Compound **15** inhibited hyperpermeability with the most potent inhibition of 70.2% at the dose of 10 μM, also suppressed HMGB1-mediated response with the inhibition of 58.9% on mice model. These were both considerably greater than PL [41].

Seo et al. synthesized PL and its derivatives through a direct reaction between the acid chloride of various amides/lactams and 3,4,5-trimethoxycinnamic acid [42]. In further studies, they evaluated the anti-inflammatory effects of PL and its derivatives (Fig. 6) in RAW-264.7 macrophages induced by lipopolysaccharide (LPS). The results showed that the inhibitory effect of PL ($IC_{50} = 3$ μM) was potent than that of compound **16** ($IC_{50} = 6$ μM); however, compound **16** with an α,β-unsaturated c-butyrolactam moiety exerted less cytotoxicity than PL.

More recently, Sun et al. synthesized several PL derivatives, including compound **17** (Fig. 6) which had increased electrophilicity by replacing the amide of PL with a carbonyl group [43]. Compound **17** exerted greater potential than PL in reducing the production of NO and PGE2 and down-regulating the COX-2 and iNOS expressions in RAW264.7 macrophage, and exerted cytotoxicity with an IC_{50} of 6 μM in RAW264.7 macrophage. Further structure-activity relationship studies showed that the two Michael acceptor groups of PL are indispensable structural moieties for their anti-inflammatory activity, whereas the phenyl ring and methoxy group have no significant effect on the anti-inflammatory activity.

Gu et al. synthesized a series of PL derivatives, among which compound **18** (Fig. 6) was identified as a novel anti-inflammatory agent by inhibiting the production of NO and the activity of NF-kB in LPS-stimulated HBZY-1 mesangial cells [44].

2.5. Senolytic activity of PL and its derivatives

Senolytics are a class of drugs that selectively induce apoptosis of senescent cells (SCs), cells that accumulate in many tissues with aging and at sites of pathology in multiple chronic diseases [45,46]. Persistent senescence seems to exert detrimental effects fostering

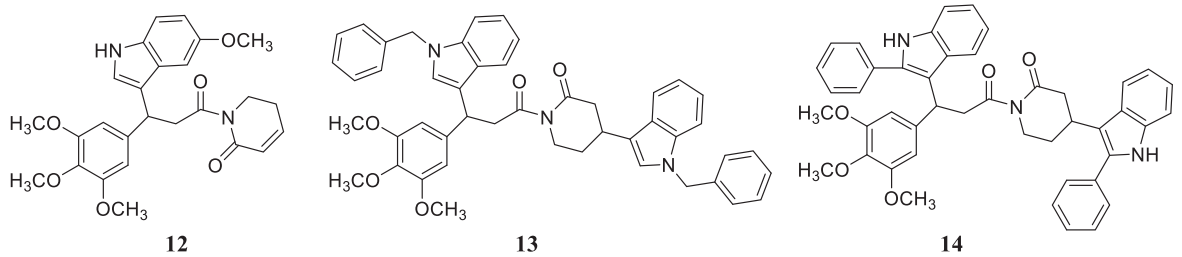

Fig. 5. Structure of synthetic PL derivatives as anti-diabetes agents (**12**–**14**).

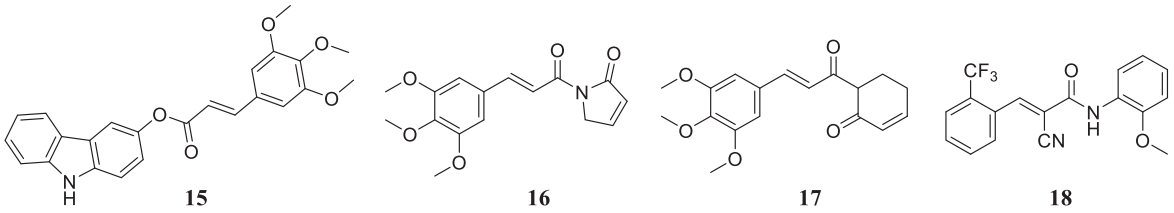

Fig. 6. Structure of synthetic PL derivatives as anti-inflammatory agents (**15**–**18**).

aging and age-related disorders including cancer [47]. PL could selectively kill various kinds of cancer cells by inhibiting oxidative stress response proteins that regulate the expression of a variety of antioxidant enzymes, which are important for cancer cell survival under elevated ROS levels [48]. The oxidative stress in SCs are higher than normal cells (NCs) [49]. Selective clearance of SCs has emerged as a potential therapeutic approach for age-related diseases.

Liu et al. first identified PL as a novel senolytic agent, which selectively killed SCs over normal or non-senescent cells [50]. They also established the structure-senolytic activity relationships of PL analogues through a series of structural modifications of PL. The Michael acceptor on the lactam ring was essential for the senolytic activity, which increased when the C2—C3 double bond was replaced by an exocyclic methylene at the C-2 position. In addition, the analogues contain an α-methylene (Fig. 7) were more potent than PL in inducing ROS production in WI-38 SCs. Among all the analogues, compound **19** showed potent senolytic activity by reducing the protein levels of oxidation resistance 1 (OXR1), which is an important oxidative stress response protein that regulates the expression of a variety of antioxidant enzymes.

2.6. Immune activity of PL and its derivatives

Jarvius et al. reported that PL displayed anti-tumor activities through ROS-independent mechanisms by inhibiting the ubiquitin-proteasome system [51], a key intracellular protein degradation system [52]. The protease is formed of a 20S catalytic core particle (CP) complexed with regulatory particles such as 19S for constitutive proteasome or 11S for inducible immunoproteasome [53]. Elodie et al. first demonstrated this new mechanism independent of ROS elevation, in which PL selectively inhibited human immunoproteasome with no noticeable inhibition of human constitutive proteasome. The Structure-activity relationships of PL demonstrated that the in vitro inhibitory efficiency against immunoproteasome and cellular toxicity could be further improved when the lactam ring of PL was replaced by a linear olefin (compound **20**, Fig. 8) [54].

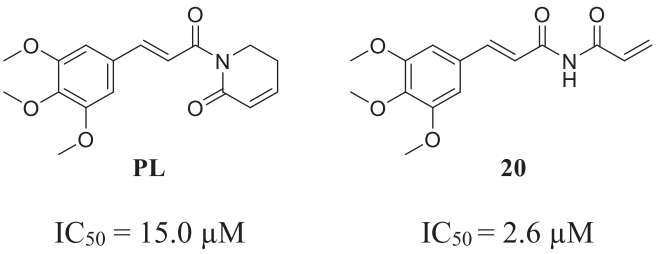

Fig. 8. Structure of synthetic PL and its derivative as senolytic agents (**20**).

2.7. Antitumor activity of PL and its derivatives

Among all the biological activities of PL, its antitumor potential has been the most investigated. While many anti-tumor drugs have significant side effects and limited selectivity towards tumor cells [55], PL has shown specific cytotoxicity to tumor cells and almost no cytotoxic on normal cells [56]. Therefore, PL and its derivatives have attracted considerable interesting for anti-cancer drug development.

2.7.1. Antitumor activity of PL

In the early 1990s, Duh et al. extracted and isolated PL from pepper leaves with chloroform and found that PL was cytotoxic to tumor cells [57]. Since then, PL has been examined against a variety of tumor cells. As summarized in Table 1, the tumor cells that PL was effective against include nasopharyngeal, leukemia, colon, breast, bladder, pancreatic, osteosarcoma, but not in normal cells. Its cytotoxicity against these tumor cells is generally in the low micromolar range.

2.7.2. The antitumor mechanisms of PL

Reactive oxygen species (ROS) play an important role in cell signal transduction, cell proliferation and differentiation. However, the excessive accumulation of ROS can induce oxidative damage to biomolecules [70,71], and this strategy has been explored for

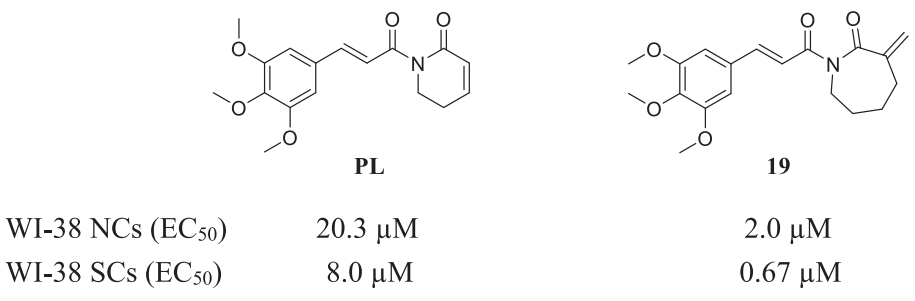

Fig. 7. Structure of synthetic PL and its derivative as senolytic agent (**19**).

Table 1
In vitro cytotoxic effects of PL against tumor cell lines.

Cancer types	Cell lines	IC_{50} (μM)	References
Human Nasopharyngeal	KB	5.6	[57,58]
Human Lung cancer	A-549	27.3	[59]
Mouse Leukemia lymphocytic	P-388	2.8	[57,58]
Human Leukemia promyelocytic	HL-60	5.3	[60]
Human Leukemia lymphocytic	CEM	4.4	[60]
Human Leukemia myeloid	K-562	5.7	[61]
Human Leukemia lymphocytic	JUKART	5.3	[61]
Human Leukemia lymphoblastic	MOLT-4	1.7	[61]
Human Colon cancer	HT-29	1.4	[57]
Human Colon cancer	HCT-8	2.2	[60]
Human Colon cancer	HCT-116	13.9	[62]
Human Breast cancer	MCF-7	~25	[63]
Human Breast cancer	MDA-MB-231	~20	[63]
Human Gliomas cancer	LN229/U87/8 MG	10–20	[64]
Human Prostate cancer	DU-145	3.4	[65]
Human Prostate cancer	PC-3	4.9	[65]
Human Pancreatic cancer	H660	0.4	[66]
Human Oral cancer	OC2	7.4	[67]
Human Oral cancer	OCSL	11.3	[67]
Human Gastric cancer	MKN45/AGS	20–25	[68]
Human Melanoma cancer	A375/A875	2.5–5	[69]

antitumor purposes [72,73]. PL has been reported to selectively induce apoptotic by ROS accumulation in cancer cells via different molecular mechanisms. Much effort has since been devoted to verifying the direct or indirect role of PL in regulating ROS levels. Jarvius et al. confirmed PL's effects in increasing ROS levels and promoting apoptosis of tumor cells and concluded that the antitumor activity of PL involving induction of ROS is at least partly, or even entirely, due to proteasome inhibition [51]. In another report, PL inhibited proteasome including suppression of FOXM1 (Forkhead box protein M1), increased the level of ROS in tumor cells, and promoted apoptosis of tumor cells [74]. Zou et al. revealed that the anti-cancer activity of PL involved induction of ROS by directly inhibiting thioredoxin reductase 1 (TrxR1) activity, which in turn induced ROS-mediated apoptosis in human gastric cancer [75]. Consistent with this, Karki et al. found that the ROS and apoptosis introduced by PL could be attenuated after cotreatment with glutathione (GSH), the most abundant antioxidant [76]. Hang et al. reported that the glutathione S-transferase π1 (GSTπ1), highly expressed in HNSCC tissues, could be suppressed by PL, resulting in ROS-mediated cell death [77]. Wang et al. demonstrated that PL could inhibit both glutathione and thioredoxin and thus induce ROS elevation, which enhanced sensitivity of colorectal cancer cells to radiation [78].

Han et al. reported that PL inhibited the growth and survival of human Burkitt lymphoma by inducing the inhibition of IBα degradation and downregulation of c-Myc and LMP1 and the Caspase-3-dependent apoptosis of Burkitt lymphoma cells in vitro. Further studies showed that PL inhibited malignant B proliferation derived from double-transgenic mouse model mCD40-LMP1/IMycEμ and induced apoptosis of neoplastic but not normal B cells [79–81]. It was also found that PL selectively inhibited B lymphoma, but had no damage to normal lymphocytes, and therefore PL can be used for the treatment of hematological malignancies.

PL could downregulate Bcl-2 and Mcl-1 and decrease the expression of STAT-3 and its phosphorylation at Y705 of melanoma cells [82]. Bharadwaj et al. identified PL as a direct STAT3 inhibitor by drug-repositioning screening and found that PL inhibited STAT3 nuclear translocation and phosphorylation and modulated expression of multiple STAT3-regulated genes in breast cancer [83]. Ginzburg et al. found that PL could affect the adhesion and invasion of prostate cancer cells [65]. Niu and colleagues described a novel molecular mechanism underlying PL anti-tumor action, where it bound the conserved Cys 528 of CRM1, and inhibited the interactions between CRM1 and tumor suppressor proteins [84].

Golovine et al. demonstrated for the first time that PL rapidly reduced the androgen receptor protein level of prostate cancer cells (PC) by proteasome-mediated ROS-dependent mechanism, therefore significantly inhibiting the proliferation of PC cells. The authors suggested PL could be used for the prevention and treatment of prostate malignant tumors [85].

In summary, PL could kill multiple cancer types via different pathways including inhibiting proteasome, raising intracellular levels of reactive oxygen species (ROS) in cancer cells, inducing DNA damage, or promoting tumor cell apoptosis (see Table 2).

2.7.3. Antitumor activity of PL derivatives

Adams et al. designed and synthesized nearly 80 PL derivatives by reduction of C7 and C8 positions of PL or substitution of amide ring or the C4 position of PL [106]. While some compounds showed weak potency against H1703 and HeLa, **21** and **22**, obtained by introduction of an alkynyl group at the C2 position, were superior to that of the corresponding amide (Fig. 9). The IC_{50} values of **21** for H1703 and HeLa were 0.7 μM and 1.3 μM respectively. The IC_{50} values of compound **22** for H1703 and Hella were 0.4 μM and 1.0 μM, respectively. The authors also showed that the C2–C3 double bond of PL was critical for ROS elevation and C7–C8 olefin was necessary for cytotoxic effects of PL.

Considering that ROS generation depends on electrophilicity of α, β-unsaturated imides of PL, Yan et al. introduced an α-substituent chlorine on the lactam ring (**23**) to increase its electrophilicity (Fig. 9). This structural alteration improved the cytotoxicity and lowered the IC_{50} value in A549 to 4.14 μM as compared to 10.17 μM for PL. Further, it was reported for the first time that **23** could strongly promote ROS generation by inhibiting thioredoxin reductase (TrxR). The accumulation of ROS led to S-phase arrest and mitochondria-mediated apoptotic in A549 cells [107].

Liao et al. combined PL with SAHA, an HDAC inhibitor, to design a new class of compounds with DNA damage and interference with GSH regulation. Some of these compounds (such as **24** and **25**) as shown in Fig. 9 possessed significant anti-tumor effects [108].

Similarly, Punganuru et al. designed and synthesized a series of PL derivatives by combining the structure of PL and tubulin polymerization inhibitors to introduce aromatic groups at the C7 position of PL [109]. Most of the derivatives had significant inhibitory effects on various tumor cells, among which **26** (Fig. 9) exerted the greatest anti-proliferative activity. Its IC_{50} values were 0.84 μM, 0.62 μM and 0.86 μM toward SKBR3, SF188 and T98G cancer cell lines, respectively, all lower than that of PL (4.04 μM, 3.9 μM, 4.92 μM). The mechanism of its cytotoxicity involved microtubule disruption, elevation of ROS and imparting biological activity to the mutant p53.

Meegan et al. found that PL could depolymerize microtubules in breast cancer MCF-7 cells, and therefore combined PL with tubulin aggregation inhibitor CA4 to design a series of new derivatives [110]. Compound **27** (Fig. 9) had the best cytotoxicity towards breast cancer MCF-7 cells and was relatively non-toxic to non-tumorigenic MCF-10a cells, representing a potential clinical candidate for breast cancer treatment.

Wu et al. synthesized 16 novel PL derivatives by introducing halogen or morpholine substituents at C2 and alkyl substituents at C7 of PL. Most of the 2-halogenated derivatives selectively killed four cancer cells when tested. Compound **28** (Fig. 9) exerted potent cytotoxicity with low IC_{50} values in HCT116 (9.85 μM), MDA-MB-231 (6.07 μM) and Hep3B (16.69 μM), which were about 3–4 better than those of PL (21.80 μM, 19.53, μM 69.46 μM, respectively). The results indicated that halogen substituents as electrophilic group at C2 played an important role in increasing cytotoxicity.

Table 2
Summary of the anticancer mechanism of PL.

Cancer types	Mechanism of action	References
TNBC[a]	Increases ROS level and decreases expression of Bcl-2	[86]
TNBC	Inhibition of PI3K/Aκt/mTOR to induce caspase-dependent apoptosis	[87]
Human ovarian cancer	Induces cell apoptosis, G2/M phase arrest and accumulation of ROS	[88]
B-ALL	Elevation of ROS	[89]
HGG	Induces ER stress by inhibiting PRDX4	[90]
GBM	Suppresses tumor invasion and metastasis	[91]
Prostate cancer	PL-mediated inhibition of NF-κB	[65]
Prostate and renal cancer	Promotes autophagy via inhibition of Aκt/mTOR signaling	[92]
Breast cancer	Induces apoptosis via inactivating Keap1 thereby activating the Nrf2/HO-1 pathway	[93]
Breast cancer	Synergizes with TRAIL to stimulate apoptosis	[94]
Hela cell lines	Inhibits the interactions between CRM1 and tumor suppressor proteins	[84]
Lung cancer	Induces apoptosis and autophagy via inhibition of PI3K/Aκt/mTOR pathway	[95]
NSCLC	Induces apoptotic and suppresses the DNA binding activity of NF-κB	[96]
PHEOs	Activates both apoptosis and necroptosis	[97]
OSCC	Regulates cell cycle and induce apoptosis and senescence	[67]
Gastric cancer	Induces G2/M cell cycle arrest and cell apoptosis both in vitro and in vivo	[98]
NEPC	Inhibits p-STAT3 signaling and promote apoptosis	[66]
CCA	Induces G2/M phase arrest and apoptosis through the ROS-JNK-ERK pathway	[99]
Bladder cancer	Inhibits epithelial mesenchymal transition and F-actin reorganization	[100]
Pancreatic cancer	Induction of ferroptosis	[101]
Leukemic cells	Targets the PI3K/Aκt/mTOR and p38 signaling	[102]
Melanoma cells	ROS mediated mitochondria disruption and JNKs pathway	[103]
BC	Induces autophagy via ROS-activated Erκ signaling pathway	[104]
Osteosarcoma cells	Induces apoptosis and G2/M phase arrest by regulating ROS/PI3K/Akt pathway	[105]

[a] Triple-negative breast cancer (TNBC); phosphatidylinositol 3-kinase (PI3K); mammalian target of rapamycin (mTOR); B-cell acute lymphoblastic leukemia (B-ALL); high-grade glioma (HGG); endoplasmic reticulum (ER); peroxiredoxin 4 (PRDX4); glioblastoma multiform (GBM); neuroendocrine prostate cancer (NEPC); Kelch-like ECH-associated protein-1 (Keap1); nuclear factorerythroid-2-related factor-2 (Nrf2); heme oxygenase-1 (HO-1); CRM1 (chromosome maintenance region 1); non-small cell lung cancer (NSCLC); tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); Nuclear Factor-kappa B (NF-κB); Pheochromocytomas(PHEOs); Oral squamous cell carcinoma (OSCC); Neuroendocrine prostate cancer (NEPC);Cholangiocarcinoma (CCA); Endoplasmic reticulum(ER); Biliary cancer (BC).

Compound **28** also significantly increased ROS levels in A549 cells and suppressed tumor growth by 48.58% at the dose of 2 mg/kg in lung cancer xenograft model [111]. However, despite the improved in vitro anti-tumor activity, the selectivity of **28** for tumor cells was only modest. To overcome this problem, the group designed and synthesized another set of 12 derivatives of PL with non-substituted benzyl rings or heterocycles [112]. Among them, **29** had the best activity of the series, with IC_{50} values of 4.12 μM, 1.67 μM, 1.67 μM, 0.71 μM, 1.17 μM, and 0.011 μM for cancel cells A549, HCT-116, MDA-MB-231, SK-Hep-1, U2OS, and Saos-2, respectively. Importantly, the IC_{50} of **29** for normal cell W138 was 84.6 μM, indicating high selectivity for tumor cells. These SAR studies provided valuable directions for further optimization of non-trimethoxylphenyl-containing PL derivatives.

Wang et al. modified the para and C2 positions on the PL phenyl ring to obtain a series of analogues, in which compound **30** enhanced the electrophilicity of PL (Fig. 9), inhibited glutathione S-transferase (GST) with an IC_{50} of 5.6 ± 0.7 μM, about 6-fold lower than PL (30.7 ± 1.2 μM). Compound **30** also reversed resistance to cisplatin in human lung cancer A549 cells [113].

Lad et al. designed and synthesized a class of PL derivatives containing a cyclosulfonamide on the PL core. Compounds **31** and **32** (Fig. 9) showed significant anticancer activities in HeLa cells, with GI_{50} values less than 0.1 μM [114].

Xu et al. merged the fragments of two natural products, PL and dicoumarol, into a new structure and synthesized a series of PL derivatives based on the merged structure. Among them, compound **33** (Fig. 9) exhibited apparent ROS elevation and excellent in vivo antitumor activity as inhibitor of NQO1, which suppressed tumor growth by 48.46% at the dose of 5 mg/kg [115].

PL is limited in its clinical applicability due to the poor aqueous solubility. In order to improve its aqueous solubility, Zou et al. synthesized a number of analogues by replacing the trimethoxyphenyl group of PL with N-containing heteroaromatic rings [116]. These modifications not only improved the water solubility of PL, but also enhanced cytotoxicity. Among them, the solubility of compound **34** (Fig. 9) was about 64-fold greater than PL in PBS aqueous solution (pH = 5). The IC_{50} of **34** against HCT-116 was 0.47 ± 0.04 μM, ~17-fold lower than that of PL (IC_{50} = 8.13 ± 0.51 μM). In addition, **34** enhanced ROS levels in colon cancer cells and suppressed tumor growth by 52% in an HCT-116 xenograft mouse model at 5 mg/kg. In a follow-up study, Zou et al. designed and synthesized a series of PL derivatives by replacing the trimethoxyphenyl of PL with ligustrazine moiety and introduced 2-Cl, -Br, and −I to the C2 position [117]. Similarly, in addition to overcoming the solubility problem of PL, compound **35**−**37** also demonstrated enhanced cytotoxicity (Fig. 9). For example, the aqueous solubility of **35** was 22.84 μg/mL and 74.62 μg/mL in water and PBS (pH 7.4), respectively, which was approximately 14 and 233 times better than PL in water (1.63 μg/mL) and PBS (0.32 μg/mL). The IC_{50} of **35** against K562 and HCT-116 were 0.25 ± 0.01 μM and 0.30 ± 0.03 μM, respectively, about 20−27-fold lower than PL (IC_{50} = 5.05 ± 0.02 μM, K562 and IC_{50} = 8.13 ± 0.51 μM, HCT116). At a dose of 5 mg/kg, **35** suppressed tumor growth by 71.4% in an HCT-116 xenograft mouse model, which was more potent than PL (60.6%).

Kim et al. synthesized a number of analogues by replacing the cyclic amide of PL with aliphatic amides. Compound **38** (CG-06, Fig. 9) was identified as a STAT3 inhibitor, and exerted strong cytotoxic by inhibiting STAT3 phosphorylation at tyrosine 705 in human prostate cancer DU-145 cells [118]. The GI_{50} value of **38** in these cells was 0.9 μM, considerably lower than that of PL (7.1 μM).

Oliveira et al. synthesized a novel platinum complex containing a PL demethylated derivative, *cis*-[PtCl(PIP-OH) $(PPh_3)_2$]PF_6 (**39**), which displayed enhanced cytotoxicity in several different cancer cells (Fig. 9). Specifically, **39** was more potent than PL in K-562 (16.9-fold), HSC-3 (14.1-fold), B16−F10 (9.6-fold), HL-60 (7.4-fold), SCC-9 (2.7-fold), MCF-7 (1.5-fold), HepG2 (1.4-fold), and HCT116

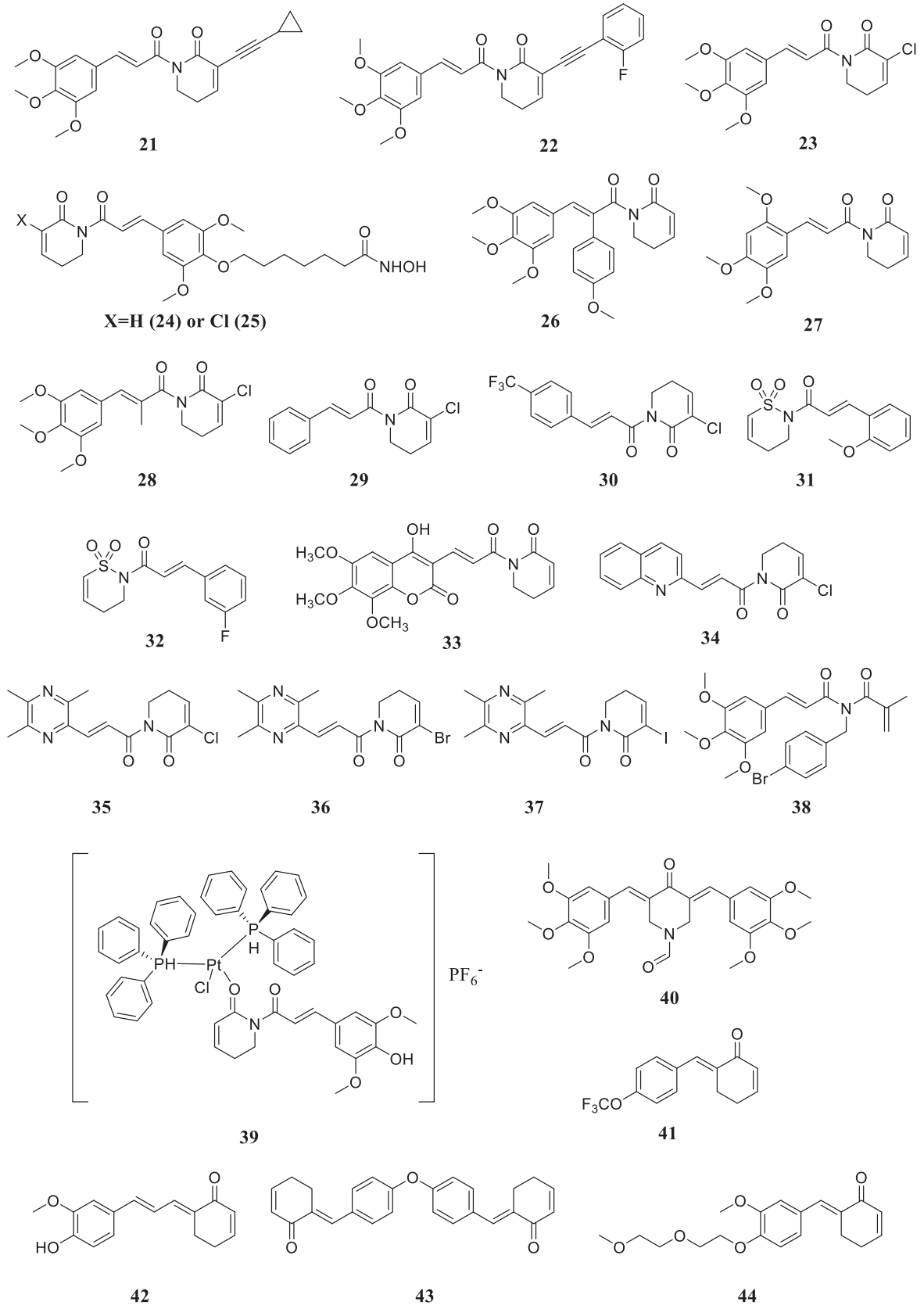

Fig. 9. The chemical structure of PL derivatives (**21**–**44**).

(1.1-fold). Further studies demonstrated that **39** induced apoptosis in human promyelocytic leukemia HL-60 cells via the ROS/ERK/p38 pathway [119].

Pyroptosis is a type of programmed cell death most studied in infectious and inflammatory diseases. Li et al. reported a PL analogue **40** (Fig. 9), which displayed greater antitumor activity with a lower IC_{50} value (0.6 μM) than PL (9.2 μM) towards human non-small-cell lung cancer (NSCLC) cell lines by elevating ROS and inducing pyroptosis [120].

Recently, our group has designed and synthesized a series of novel benzylidenecyclohexenone analogues based on the structure of PL, which were prepared in one step via an Aldol condensation reaction between cyclohexen-2-one and differently substituted benzaldehydes or arylformaldehydes. Among all the analogues synthesized, **41** (Fig. 9) behaved as a novel TrxR inhibitor with an IC_{50} of 0.12 μM, nearly 13 times better than PL (IC_{50} = 1.52 μM) in MDA-MB-231 cells. The antiproliferative activities of **41** against breast and lung cancer cells (IC_{50} = 0.44–1.04 μM) were about 4 to 8-fold stronger than PL (IC_{50} = 3.20–4.40 μM). Further, **41** displayed high anticancer activity by inducing ROS, apoptosis and autophagy in breast cancer cells. Finally, **41** suppressed tumor growth by 63.3% at the dose of 25 mg/kg, as compared to PL treated group (44.9%) at 10 mg/kg [121].

More recently, our group designed and synthesized another series of novel phenylallylidenecyclohexenone analogues based on the structures of PL and piperine in a similar one-step Aldol condensation. Compound **42** (Fig. 9) from the series displayed potent TrxR inhibitory activity with an IC_{50} of 0.18 μM, ~9-fold lower than PL (IC_{50} = 1.52 μM) in drug-resistant Bel-7402/5-FU human liver cancer cells. In these 5-FU resistant cancer cells, **42** displayed potent antiproliferative activities (IC_{50} = 0.8 μM), ~10-fold lower than PL (IC_{50} = 8.4 μM). Mechanistic studies showed that **42** exerted potent antiproliferation by inhibiting TrxR activity, increasing ROS levels, reducing mitochondrial transmembrane potential (MTP), inducing autophagy, activating the p38 signaling pathways and suppressing the Akt/mTOR signaling pathways in Bel-7402/5-FU cancer cells [122].

Our group also designed and synthesized a series of mono- and di-methylenecyclohexenone derivatives based on PL, such as **43** (Fig. 9) which was a potent TrxR inhibitors with an IC_{50} of 0.16 μM, ~10 times better than PL (IC_{50} = 1.52 μM) in MDA-MB-231 cells [123]. Compound **43** not only displayed potent antiproliferative activities against MDA-MB-231 cells (IC_{50} = 0.63 μM) compared to PL (IC_{50} = 1.72 μM), but also exerted strong inhibition potency toward solid tumors of breast cancer in vivo, which suppressed tumor growth by 67% at the dose of 25 mg/kg. What's more, **43** displayed prominent anti-metastasis effects by reversing the epithelial–mesenchymal transition induced by the transforming growth factor β1.

As mentioned above, the poor water solubility and moderate activity limited the application of PL. In order to improve the poor water solubility and moderate activity of PL, we synthesized another series of novel phenylmethylenecyclohexenone derivatives based on the structures of PL, prepared in a similar one-step Aldol condensation [124]. Compound **44** (Fig. 9) from the series which was a potent TrxR inhibitors with an IC_{50} of 0.15 μM, ~12 times better than PL (IC_{50} = 1.76 μM) in HGC27 cells. Compound **44** not only showed good water solubility and displayed potent antiproliferative activities against HGC27 cells (IC_{50} = 0.76 μM), ~10-fold lower than PL (IC_{50} = 7.53 μM), but also exhibited lower cytotoxicity in human normal gastric epithelial cells GES-1 compared with HGC27 cells. Mechanistic studies showed that **44** exerted potent antiproliferative by inhibiting TrxR activity, increasing ROS levels, reducing mitochondrial transmembrane potential (MTP), induced G2/M phase arrest, triggered cancer cell apoptosis and promoted DNA damage in HGC27 cells via activating the H2AX (S139 ph) and p53 signaling.

These results showed that PL derivatives could be promising candidates for the treatment of cancer, especially for the drug-resistant cancer cells and, as such, warrants further investigation.

2.8. Antitumor activity of PL in combination with other drugs

Although PL alone exerts moderate activity, studies found that PL could enhance the therapeutic effects when combined with other chemotherapeutics. PL increased the activity of a variety of anti-tumor agents, including 5-fluorouracil (5-FU) [125], diferuloylmethane [15], cisplatin [126], chloroquine (CQ) [127,128], doxorubicin (DOX) [129], paclitaxel [130,131] and gemcitabine [132,133].

Increased inhibitory effects were observed when tumor cell lines including leukemia (HL-60), colon (HCT-8), breast (MDA-MB-435), and brain (SF295) were incubated with 5-FU in the presence of PL. Table 3 shows the IC_{50} values for 5-FU in the absence and presence of PL. The distinct inhibitory effects of the combination treatments were apparent with the lower IC_{50} value 0.49 μM, as compared to 2.33 μM in SF-295 cells. In addition, the combination treatments not only enhanced the therapeutic effect, but also improved the immunological activity and reduced the toxicity to liver, spleen and kidney when compared with 5-FU alone treated group [125].

Jyothi et al. found that PL had good cytotoxicity against a variety of tumor cells including human neuroblastoma tumor (IMR-32), mouse macrophages (J774 and P388D1), mouse embryonal carcinoma (PCC4), and rat histiocytoma (BC-8). More interestingly, PL at the concentration of 15 μM significantly enhanced cytotoxicity when combined with the anti-inflammatory drug diferuloylmethane. The cytotoxicity was 3–4 times greater than that of PL treatment alone in IMR32 and J774 cells [15]. PL could selectively kill head and neck cancer (HNC) cells both in vitro and in vivo by elevating ROS levels. PL also increased the antitumor activity when combined with cisplatin under these conditions. Moreover, the combination of PL with autophagy inhibitor Chloroquine (CQ) showed high levels of cellular death and significant anti-tumor effects in a xenograft mouse mode [127,128].

Hansel et al. found that when combined with MTH1-inhibitor TH1579, PL increased ROS-induced damage to free deoxynucleotides (dNTPs) required for DNA replication and significant cellular death levels especially in ART NCI–H460 cells [134]. It was also found that the combination treatment of PL, Cotylenin A (CN-A; a plant growth regulator) and clinically approved sulfasalazine (SSZ) could exert potent antitumor activities against pancreatic cancer via increasing intracellular ROS levels [101].

Drug resistance is a significant obstacle to some cancer therapeutics. PL has been reported to have drug resistance reversing effects in human retinoblastoma cell lines and this effect might be mediated by the PI3K/AKT and PKCζ pathways [135]. PL was also used to reduce renal cell carcinoma (RCC) resistance to IFN-g treatment by suppressing NF-kB activation through elevation of the ROS levels [136]. It was reported that PL effectively reversed

Table 3
The *vitro* cytotoxicity of 5-FU in the combination treatments of PL on tumor cell lines.

Cell line	5-FU (IC_{50}, μM)	Combined PL (IC_{50}, μM)
leukemia (HL-60)	84.68 ± 0.66	38.13 ± 0.43
colon (HCT-8)	11.18 ± 0.71	4.70 ± 0.55
breast (MDA-MB-435)	9.19 ± 0.85	1.95 ± 0.27
brain (SF295)	2.33 ± 0.51	0.49 ± 0.11

resistance to doxorubicin in K562/A02 human leukemia cells by inhibiting the expression of P-glycoprotein, MDR1, survivin and *p*-Akt, and the transcriptional activities of NF-kappa B and twist [137]. PL has also been shown to effectively reverse resistance to cisplatin in human non-small cell lung cancer cells by inhibiting Akt phosphorylation via ROS regulation [126].

Anthracyclines such as doxorubicin (DOX) could be metabolized to the less active metabolite DOXol by the predominant enzyme carbonyl reductase 1 (CBR1), which results in cancer cell resistance [138]. The synergistic antiproliferative and proapoptotic effects and the significant anti-invasive properties could be observed when prostate cancer cells were co-treated with DOX and PL in DU-145. Further mechanistic studies showed that PL could interact with the catalytic domain of CBR1, which inhibited almost half the metabolism of DOX to the less active metabolite DOXol at the concentration of 20 μM, thus resulting in synergistic antiproliferative and anti-invasive effects on DU-145 prostate cancer cells [129].

2.9. Antitumor activity of PL in the application of nanomedicine

In addition to chemical modifications to improve the poor solubility and relatively high lipophilicity of PL, which limits its further applications, nanoparticle-based delivery systems have also been explored. Liu et al. prepared PL encapsulated polymeric micelles in a solid dispersion manner, which overcome the poor water solubility of PL and enhanced permeability and retention (EPR) effect and improved the anti-tumor effects [139]. Liu et al. later developed a nanocarrier system for co-delivery of paclitaxel (PTX) and PL via the organic solvent evaporation method. The nanoparticles with the concentration ratio of PTX/PL at 1:200 showed the best antitumor activity with reduced toxicity compared with free PTX [140].

Lee et al. synthesized core cross-linked ChitoPEG copolymers by using selenocystine (ChitoPEGse) as a ROS-sensitive moiety, and then PL was incorporated into the pH/ROS-sensitive core of the nanoparticles. The PL-incorporated ChitoPEGse nanoparticles exerted potent antitumor activity both in vitro carcinoma cells (A549 and CT26) and in vivo pulmonary metastasis CT26 cell model [141].

Finally, PL was used to optimized to biocompatible and bioadhesive nanoemulsions for intraductal, which is a new strategy for treatment of ductal carcinoma [142]. PL was stable in the nanoemulsion for 60 days, which revealed that this nanoemulsion may be a stronger candidate for intraductal delivery.

2.10. Other bioactivity of PL and its derivatives

In addition to the bioactivities described above, other therapeutic potential of PL and/or its derivatives have also been reported, such as cardiovascular protective effects [23], leishmanicidal effects [143], antiparasitic activity [144], anti-hypertension, antibacterial and insecticidal effects [145–149].

PL derivative **45** (Fig. 10) was shown to display lipid-lowering activity by inhibiting rat hepatic cholesterol ACAT (O-acyltransferase) in vivo. Interestingly, the metabolization of **45** in male rats was better than females, which may be due to the different CYP3A2 expression levels between male and female rats [150,151].

Wang et al. first reported a PL derivative **46** (Fig. 10), which was identified as a chronic hepatitis B virus (HBV) inhibitor by decreasing extracellular HBV DNA and intracellular DNA levels with IC_{50} values of 1.5 μM and 3 μM, respectively. Further studies showed that the capsid formation of core protein (Cp) could be interfered by **46** [142,152].

3. Discussion and conclusions

PL and its derivatives have shown a variety of biological activities, including antitumor, antiparasitic, antiplatelet aggregation, neuroprotective, anti-diabetic, anti-inflammatory activity, antisenolytic activity, immune activity. These diverse activities provide immense opportunities for interventions of various human diseases [14,21,132,144,153–156]. However, despite the great potential, PL itself has several limitations that limit its therapeutic application, including modest potency, poor water solubility and high lipophilicity [157]. In order to improve the overall properties, a significant number of PL derivatives have been designed, synthesized and evaluated in varying biological assays or models.

Among all bioactivities, the anti-tumor effects of PL and its derivatives have been the mostly extensively studied. Cancer is a great health burden to human health, and is the second leading cause of death worldwide [158]. Traditional anti-cancer drugs are often limited by undesirable side effects such as 5-fluorouracil, cisplatin, doxorubicin, and gemcitabine. Importantly, PL has no apparent cytotoxicity on normal cells but can selectively kill tumor cells through multiple mechanisms. However, PL's anti-tumor effect, the most studied biological activity, is only moderate. Therefore, considerable effort has been devoted to developing PL derivatives with improved biological activities, particularly anti-tumor potency.

Structure and activity relationship (SAR) studies on PL have demonstrated that the position and nature of the substituents on PL greatly affect its biological activity. The C2—C3 and C7—C8 double bonds and the α, β-unsaturated ketone moiety in PL, serving as Michael addition acceptors, can bind the highly reactive C-terminal selenocysteine (Sec) residue of TrxR to inhibit the enzyme activity, resulting in cytotoxicity to cancer cells [106,159]. The C2—C3 double bond, part of the unsaturated ketone system, has higher Michael addition activity and has been suggested to be the key active chemical pharmacophore. In contrast, the C7—C8 double bond has been reported to exert certain cytotoxicity but has less impact than the C2—C3 double bond on increasing ROS levels of tumor cells [60,160]. In addition, the double bonds, particularly the C2—C3 bond, have also been suggested to be responsible for ROS elevation by inhibiting the ubiquitin-proteasome system [161]. It has also been reported that α, β-unsaturated carbonyl groups could inactivate Kelch-like ECH-associated protein-1 (Keap1) through thiol modification, thereby inducing apoptosis via activating the Nrf2/

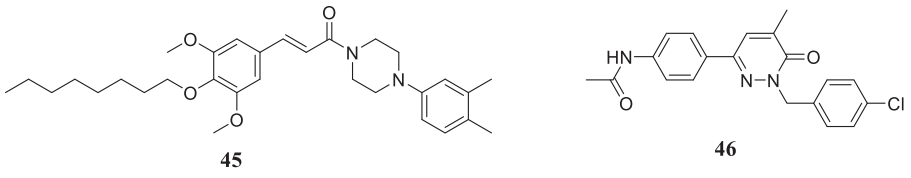

Fig. 10. Structure of synthetic PL derivatives for other activities (**45**—**46**).

HO-1 pathway [93]. Given the importance of C2—C3 and C7—C8 double bonds for anti-tumor activities, these two double bonds are mostly preserved in SAR studies. The commonly modified sites on PL are C2, C7, C8, and C9 positions (see Fig. 8).

When an electron withdrawing group, such as a halogen, is introduced at the C2 position, the antitumor activities are significantly improved. For example, compound **23** (Fig. 9) which was introduced an α-substituent chlorine on the lactam ring to increase its electrophilicity displayed improved cytotoxicity and lowered IC_{50} value than PL in A549 cells (4.14 μM vs. 10.17 μM). In addition, **21** and **22** (Fig. 9) were obtained by introduction of an electron withdrawing alkynyl groups at the C2 position. The IC_{50} values of **21** for H1703 and HeLa were 0.7 μM and 1.3 μM respectively and the IC_{50} values of compound **22** for H1703 and Hella were 0.4 μM and 1.0 μM, respectively, both of which were superior to that of the PL. The results indicated that halogen and alkynyl substituents as electrophilic group at C2 played an important role in increasing cytotoxicity. The antitumor activity is also increased when an aromatic group is introduced at the C7 or C8 position. For instance, **26** with aromatic groups at the C7 position exerted high antiproliferative activity with IC_{50} values lower than that of PL toward SKBR3, SF188 and T98G cancer cell lines [112]. These modifications presumably change the electronic properties of the C2—C3 or C7—C8 double bonds, thus increasing their reactivity as Michael donors.

It should be noted the SAR around the neuroprotective activity of PL and its derivatives is different from that of their antitumor activity at these positions. The C2—C3 double bond has been suggested to be responsible for ROS elevation by inhibiting the ubiquitin-proteasome system. Since cancer cells are more sensitive to elevation of ROS, which ultimately lead to ROS-mediated cell death, the C2—C3 double bond is crucial to the anti-tumor activities and is mostly preserved for the development of antitumor agents. However, elevated oxidative stress in general negatively affects neurodegenerative disorders. Neuroprotective agents should possess antioxidant effects and prevent accumulation of ROS, so the C2—C3 double bond, which is responsible for ROS elevation, should be removed or modified for the development of neuroprotective agents. This is consistent with that PL analogues **5**—**11** with no C2—C3 double bonds were identified as promising neuroprotective agents by enhancing the antioxidant effects.

The trimethoxy benzene ring at the C9 position can be replaced by an aryl (hetero) ring with different substituents to improve PL water solubility, without compromising the potency. For example, **35**, which was obtained by replacing the trimethoxyphenyl of PL with a ligustrazine moiety, not only had improved the cytotoxicity but also improved aqueous solubility [120]. Compound **44,** which had the trimethoxy benzene ring replaced with an aryl ring with an ether chain, displayed good water solubility and potent antiproliferative activities against HGC27 cells than PL. In addition, it has been observed that the trimethoxyphenyl moiety at the C9 position and the lactam ring of PL are easily metabolized, so these two groups have been structurally modified to improve stability as well [162]. These SAR studies helped establish the direction for the design and development of novel PL derivatives with high effectiveness and lower toxicity than PL.

Multidrug resistance (MDR) is a major challenge in cancer treatment. One of the mechanisms of PL in inhibiting tumor growth is by acting on TrxR. Overexpression of TrxR modulates drug-specific cytotoxic responses in cancer cells, suggesting that TrxR may be a promising target for the treatment of certain drug resistant cancers [163]. In addition, ROS plays an critical role in cell proliferation, metabolism and cancer drug resistance, and has been widely pursued as a promising target for cancer therapy [164,165]. It was reported that PL could be formulated into ROS-sensitive nanofibers to suppress the growth of cholangiocarcinoma cells [166]. The activity of PL and its derivatives in inhibiting TrxR and elevating ROS in cancers represent a novel strategy for the development of drug-resist cancer therapy. What's more, the multiple biological activities of PL and its derivatives may be advantageous to obtain improved antitumor activities. For example, PL has demonstrated immune activity and anti-inflammatory activities, which are associated with cancer progression and thus beneficial to exert antitumor activity [167—169].

Compared to the research on PL and its derivatives against cancer, there are relatively fewer studies on other biological activities. In studies of PL derivatives for other therapeutic indications than anti-tumor, the main structural modifications are on the aromatic ring of PL and removal of the conjugated double bond on the lactam ring of PL to form caprolactam to reduce toxicity. Recent studies on non-tumor activities have shown that PL derivatives not only exerted more potent biological activities than PL, but also significantly reduced their toxicity. Many of these studies provided encouraging results supporting the further development of these PL derivatives for applications in various of biological activities.

It is important to note that much of the research thus far has only characterized PL or its derivatives in enzymatic or cell-based assays, and only a small number of the compounds have been evaluated in animal models. The current compound evaluation strategies generally focus on characterization of the active compounds in in vitro systems to identify possible improvement on potency and/or affinity. Furthermore, the desired drug-like properties, including in vivo pharmacokinetics, pharmacodynamics, and relative bioavailability, also need to be explored in more depth in the future. Therefore, it is important to systematically evaluate these active PL derivatives in in vivo models and at a stage as early as possible.

Given the serious health threat of cancer, pharmaceutical companies and research institutions are increasingly focusing on the research and development of novel anti-tumor drugs [170]. Future investigations of PL and its derivatives could provide more encouraging results in the field of application of PL, especially in the development of novel clinical candidate for the treat of cancer. It is our belief that new PL derivatives targeting cancer related proteins will be identified, optimized and eventually advanced to the clinics to treat cancers.

Declaration of competing 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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