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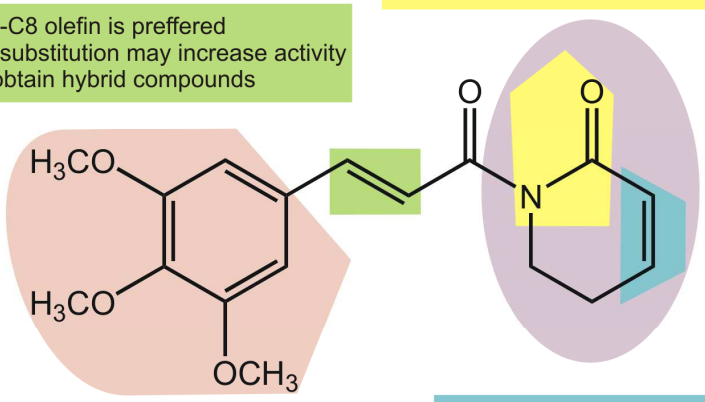
1. Nitrogen atom may be replaced by carbon
2. Carbonyl moiety may be replaced by sulfonamide

1. Presence of C7-C8 olefin is preffered
2. C7 alkyl or aryl substitution may increase activity and is used to obtain hybrid compounds



1. Can be replaced by other heterocycles

1. Other aromatics are acceptable
2. Strongly electron withdrawing groups increase activity
3. Possible linker for hybrid compounds/oligomers

1. Presence and electrophilicity determines drug cytotoxicity and ROS-generating activity
2. C2 substitution can provide increased activity (e.g. 2-chloro)

Piperlongumine (piplartine) as a lead compound for anticancer agents – synthesis and properties of analogues

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Abstract

Piperlongumine, also known as piplartine, is an amide alkaloid of *Piper longum* L. (long piper), a medical plant known from Ayurvedic medicine. Although was discovered well over fifty years ago, its pharmacological properties have been uncovered in the past decade. In particular, piperlongumine has been most extensively studied as a potential anticancer agent. Piperlongumine has exhibited cytotoxicity against a broad spectrum of human cancer cell lines, as well as demostrated antitumor activity in rodents. Piperlongumine has also been found to be a proapoptotic, anti-invasive, antiangiogenic agent and synergize with modern chemotherapeutic agents. Because of its clinical potential, several studies were undertaken to obtain piperlongumine analogues, which have exhibited more potent activity or more appropriate drug-like parameters. In this review, the synthesis of piperlongumine analogues and piperlongumine-based hybrid compounds, as well as their anticancer properties and the molecular basis for their activity are explored. General structure-activity relationship conclusions are drawn and directions for the future research are indicated.

Keywords: piperlongumine, piplartine, natural product compounds, SAR, Ayurveda, chemotherapy

Piperlongumine as anticancer agent

Piperlongumine (**Fig. 1**), also known as piplartine, is an amide alkaloid constituent of the *Piper longum* L. (long piper), a medical plant known from Ayurvedic medicine. This amide alkaloid was for the first time isolated in 1961 [1,2], while its molecular structure was determined in 1984 [1,3]. Despite being discovered several decades ago, the pharmacological properties of piperlongumine have not been intensively investigated. However, over the past years, diverse biological activities of this molecule have been discovered and described including antiplatelet, antinociceptive, anxiolytic, antidepresant, neuroprotective, antiartheriosclerotic, antimicrobial and, especially, anticancer properties [1]. Piperlongumine has been considered as a potential anticancer agent since it was evaluated during a screening program of 5166 molecules in searching for antineoplastic drugs carried out at the Laboratório Nacional de Oncologia Experimental – Universida de Federal do Ceará, between 2000 and 2007 [4].

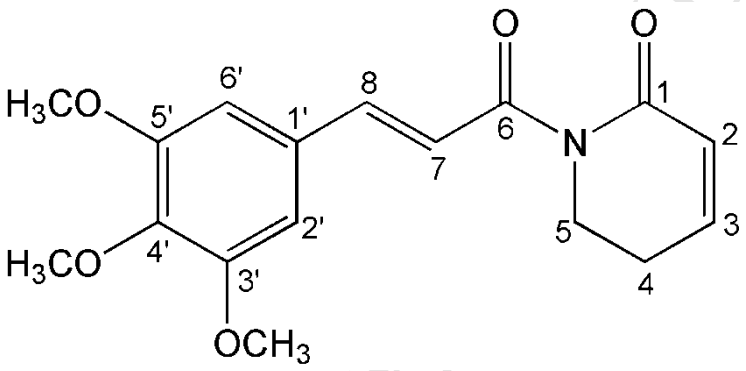

Fig. 1 Structure of piperlongumine

The antineoplastic activities of piperlogumine have been investigated in several cancer models [5-8], demonstrating cytotoxic and cytostatic properties against a number of cancer cell lines, including: colon, lung, breast, pancreatic, renal and prostate [7-9]. Moreover, antitumor activity has been noted in a variety of animal models [5,6,9]. Piperlongumine has also been found to trigger cell death by both caspase-dependent apoptosis and necrosis [7,9-13]. The molecular proapoptotic effects of piperlongumine activity includes downregulation of Bcl2, and activation of caspase-3, poly(ADPribose)polymerase (PARP) [11] and c-Jun NH2-terminal kinase [12,13]. Interestingly, piperlongumine was found to restore normal function of a mutant p53 protein in human colon cancer cell lines [14]. Piperlongumine has also been found to induce cell death by autophagy [10,11,15].

The anticancer properties of piperlongumine are generally related to its inhibition of glutathione S-transferase π (GSTπ) and carbonyl reductase 1 (CBR1), followed by a subsequent disruption of redox homeostasis and the generation of reactive oxygen species

(ROS) in cancer cells [8,9,16]. Additionally, ROS-independent molecular targets (e.g. nuclear export or PI3K/Akt/mTOR inhibition) have also been identified [10,17].

In addition to the above described anti-cancer properties, piperlongumine also has been noted to have anti-invasive properties. Piperlongumine inhibited the migration of highly metastatic human glioma LN229 and U87 MG cells in a scratch-wound experimental model [18]. It was also found to inhibit the expression of Twist and N-cadherin, proteins essential in cancer metastasis, as well as disrupted the p120-ctn/vimentin/N-cadherin complex, abrogating the ability of cells to metastasize [19]. In highly metastatic prostate cancer cells, piperlongumine decreased cells invasiveness and expression of IL-6, IL-8, MMP-9, and ICAM-1, a cell-to-matrix adhesion protein [20]. Antiangiogenic properties of piperlongumine have also been observed, mediated by the decreasing of VEGF protein level [9,21].

Piperlongumine has demonstrated interesting properties when combined with other chemotherapeutics, increasing the activity of 5-fluorouracil [22], cisplatin [23] doxorubicin [24], paclitaxel [25] and curcumin [26]. Piperlongumine, at a non-cytotoxic concentration, enhanced the radio-sensitivity of MDA-MB-231 cells. This may be related to its regulation of the expression apoptosis-related proteins and an increase of intracellular ROS levels, thus increasing radiation-induced apoptosis [27].

The pharmacological activity of piperlongumine is characterized by high selectivity – it does not affect healthy cells in concentrations which are highly toxic against cancer cells [9]. Moreover, *in vivo* toxicological examinations of piperlongumine indicate a good safety profile [9,22]. Another advantage is its high absorption from gastro-intestinal track. In mice, the molecule demonstrated 50-76% bioavailability following oral administration [9].

The numerous reports of piperlongumine activity against a variety of molecular targets essential in different stages of cancer development and progression, as well as low toxicity and beneficial pharmacokinetic properties make this molecule a strong potential candidate for use in future anticancer treatments. However, due to a lack of nanomolar potency and low water solubility its applicability is limited, however, medicinal chemists may be able to help to obtain a compound characterized by both better anticancer activity and appropriate drug-like physico-chemical parameters. This article describes recently published research concerning the synthesis of piperlongumine analogues and their anticancer structure-activity relationship.

Structure activity relationship of piperlongumine and its analogues

- **Electrophillicity and activity**

The piperlongumine structure contains two Michael acceptors – 2,3- and 7,8-unsaturated bonds. Their influence on the compounds activity was investigated in several studies. Adams et al. obtained two saturated derivatives - **1** and **2**. Both showed decreased cytotoxic properties against H1703 and HeLa cell lines. Derivative **1** failed to increase cellular ROS level, while derivative **2** showed lower pharmacologic activity than piperlongumine [28]. A decrease in cytotoxic and ROS-generating properties of both **1** and **2** had been found by other research groups [29]. These compounds were also characterized by a lower inhibitory activity against GST, while the 2,3- and the 7,8-saturated compounds (**3**) failed to inhibit the enzyme at all [30].

Not only the presence, but also electophilicity of olefins, has importance in the drugs activity [28,30]. Wang et al. showed correlation (R=0.85) between the molecules electrophilicity and its inhibitory activity toward GST [30]. Thus, steric blockade (**4**) and cyclization (**5,6**) diminished the reactivity of these electrophilic centers and decreased the compounds activity [28]. An increase of electrophillicity was mainly achieved by the substitution of a lactam ring, by a chlorine atom (**7**), or replacement of a nitrogen of the lactam by a carbon atom (**8**) [29]. However, the most significant results were obtained by introducing a trifluoromethyl group to the aromatic ring. Consequently, the most active of the molecules with respect to inhibition of GST activity were compounds with trifluoromethyl group and 2-chlorine (**9**) [30].

Olefins are significant due to their role in the binding of piperlongumine with GST. It was suggested that piperlongumine by 7,8-olefin interacts with the sulhydryl groups of the active site of GST, while by 2,3-olefin interacts with glutathione [28,30]. Harshbarger et al. examined the X-ray crystal structure of GSTP1 co-crystallized with piperlongumine and glutathione, and found that not piperlongumine, but the product of its hydrolysis conjugated with glutathione was bounded with the enzyme. The authors indicated that piperlongumine first undergoes conjugation by 2,3-olefin with intracellular thiols which makes the molecule unstable. Thus, hydrolysis occurs and the product of this reaction conjugate by 7,8-olefin with glutathione. This complex in turn is the real inhibitor of the enzyme. These results suggest that piperlongumine is, from the perspective of GST inhibition, a prodrug and its 2,3-olefin plays a role in molecule activation, while the 7,8-olefin is important in the formation of a complex with glutathione, which is able to inhibit the enzyme [31]. Further research is needed

to determine whether hydrolysis and conjugation with glutathione also influences the anticancer activity of its derivatives, as well as plays a role in the molecular mechanisms involving other targets.

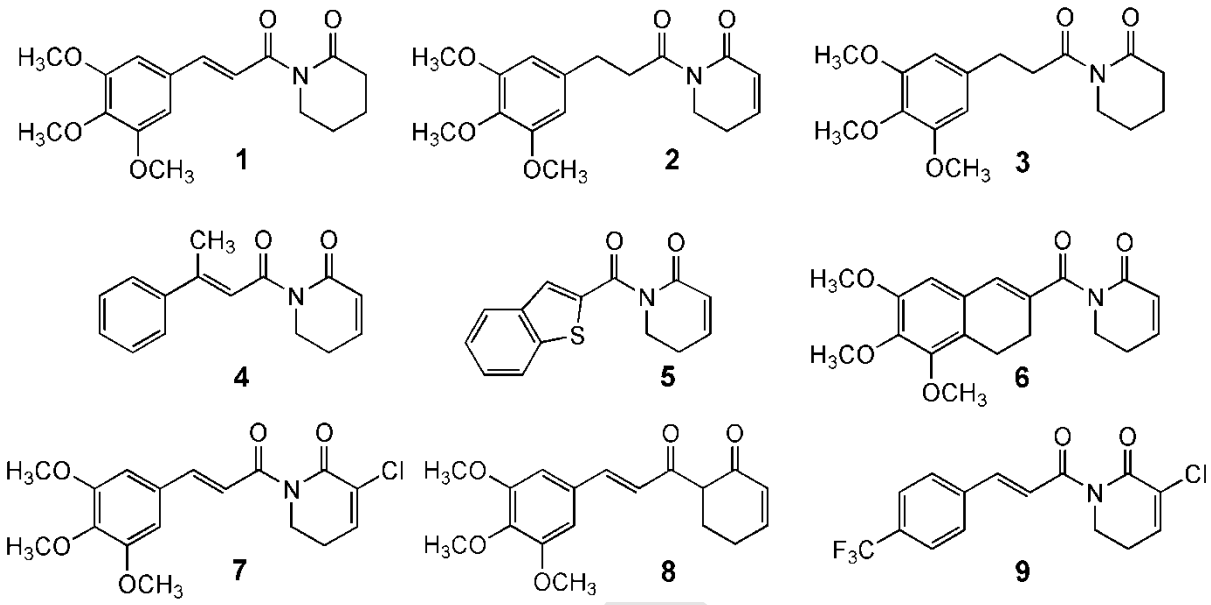

Fig.2 Piperlongumine modifications affecting olefins electophilicity

- **Aromatic ring and activity**

Piperlongumine structures lacking an aromatic ring (**10**) exert decreased inhibition of GST [30] and decreased cytotoxicity against cancer cells [29]. A similar result was obtained by the cyclization of the linkage between aromatic and 7,8-olefin (**5,6**) [28]. Han et al. found that the substitution of a phenyl ring by aromatic heterocycles, i.e. pyridine or furan, did not significantly alter anticancer properties [32]. However, Zou et al. described the synthesis of nine piperlongumine pyridine, pyrazine and quinolone-based analogues, which showed increased anti-proliferative activity against colon cancer cell lines. The most active ones i.e. quinoline-2-yl derivative (**11**) and its 2-chloro analogue (**12**) achieved less than 1 μM IC_{50} values. Derivative **12** also induced ROS generation and inhibited tumor growth in mice similarly to piperlongumine. All of the obtained derivatives also exerted better water solubility [33].

Removal of methoxy groups, as described by Adams et al., did not result in substantial changes in drug cellular toxicity or ROS elevation [28]. Meegan et al. synthetized piperlongumine derivatives with aromatic rings differently substituted by mono-, di- and tri-methoxy groups. The most cytotoxic was 2',4',5'-trimethoxy derivate (**13**), however, its activity was lower than piperlongumine and comparable to derivate with an unsubstituted aromatic ring. Compound **13** exerted slightly selectivity (MCF-7 vs. MCF-10a) and its

activity was reversible by antioxidants [34]. These results suggest that a methoxy substitution of phenyl is not optimal for a drug activity, however, other modifications could provide an increase in anticancer properties. A step in this direction were studies investigating the influence of an electron withdrawing effect of aromatic ring on activity. Chlorine substitution in the 2' position (**14**) slightly decreased activity [28]. Both 4'-bromide (**15**) and 2',6'-dichloride (**16**) derivatives also showed lower activity than piperlongumine, while a 4'-fluorine-3'-methoxy (**17**) analogue exhibited increased activity [32]. As described previously, trifluoromethyl group (especially in 4' position) significantly increases the drug's action [30]. The 4'-nitro substituted compound (**18**) was also found to slightly increase drug activity [32]. To-date, studies have not fully determined the influence of electron withdrawing groups on derivatives properties. However, it seems that strong withdrawing groups (-CF_3, -NO_2) are preferred over weak withdrawing groups (e.g. halides).

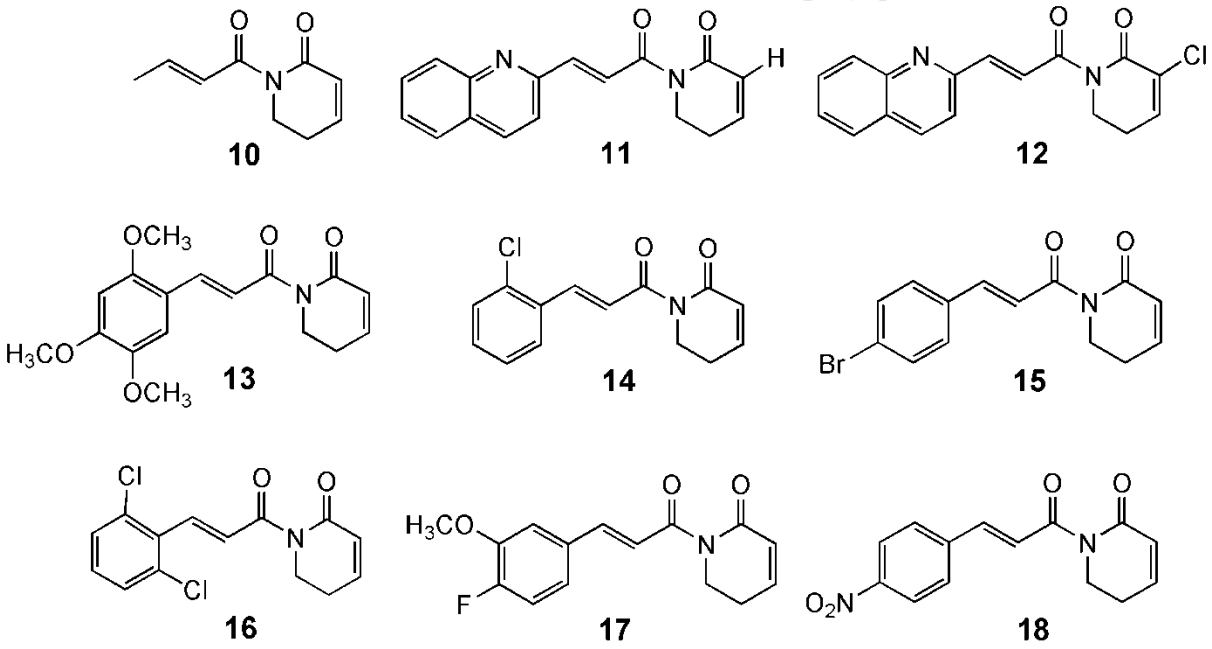

Fig. 3 Piperlongumine analogues modified in aromatic ring

- **Lactam ring modifications**

As described previously, the 2,3-olefin of lactam and its electrophilicity play a critical role in piperlongumine cytotoxicity and elevation of ROS [28]. Thus, modification in this part of molecule may be expected to have high influence on a derivatives activity. Interestingly, replacement of dihydropyridinone by azepine or by tetrahydroazepineone had minimal effect on cytotoxicity [28].

Substitution of the C2 and C3 atoms was found to significantly affect piperlongumine activity. 2-Methyl (**19**), 3-methyl (**20**) or 2-phenyl (**21**) substitutions completely impaired cytotoxic properties, while substitution by more complex groups, containing multiple bonds,

such as 2-(2-fluorinephenyl)prop-1-yne (**22**) or 2-cyclopropyloprop-1-yne (**23**), significantly increased activity [28]. A 2-morpholine substitution decreased activity of piperlongumine, as well as that of a series of its 7-alkyl derivatives [35]. Introduction of electron-withdrawing chlorine into the 2 position, significantly increased the GST-inhibitory properties of the compounds, but only those substituted in the aromatic ring by a trifluoromethyl group [30]. However, a 2-chlorine (**7**) substitution was also found to increase cytotoxicity and ROS-generating ability, inhibit thioredoxin reductase, intensify apoptosis, and arrest the cell cycle. Apoptosis induced by 2-chloropiperlongumine was induced via a mitochondrial pathway, while the cell cycle was arrested at S phase [29]. 2-Chlorine substitution also increased the activity of 7-alkyl derivatives (described below) [35].

Lad et al. described the synthesis and biological evaluation of a series of piperlongumine sulfonamide analogues, in which the lactam carbonyl moiety was replaced by a sulfone moiety (**24**). Moreover, the tested compounds possessed various substituents in their aromatic ring. Several of the compounds showed potent activity in sulforhodamine B assay. Interestingly, the most active ones i.e. 2'-methoxy, 3',5'-dimethoxy, as well as 3'-fluorine substituted, exhibited GI_{50} values of less than 0,1 μM [36]. Thus, it seems that introduction of a sulfonamide instead of carbonyl moiety leads to significant changes in drugs properties and may be an interesting direction for the development of novel derivatives.

Other C4 and C5 substituted derivatives were not extensively studied and showed comparable or lower cytotoxicity than piperlongumine [28].

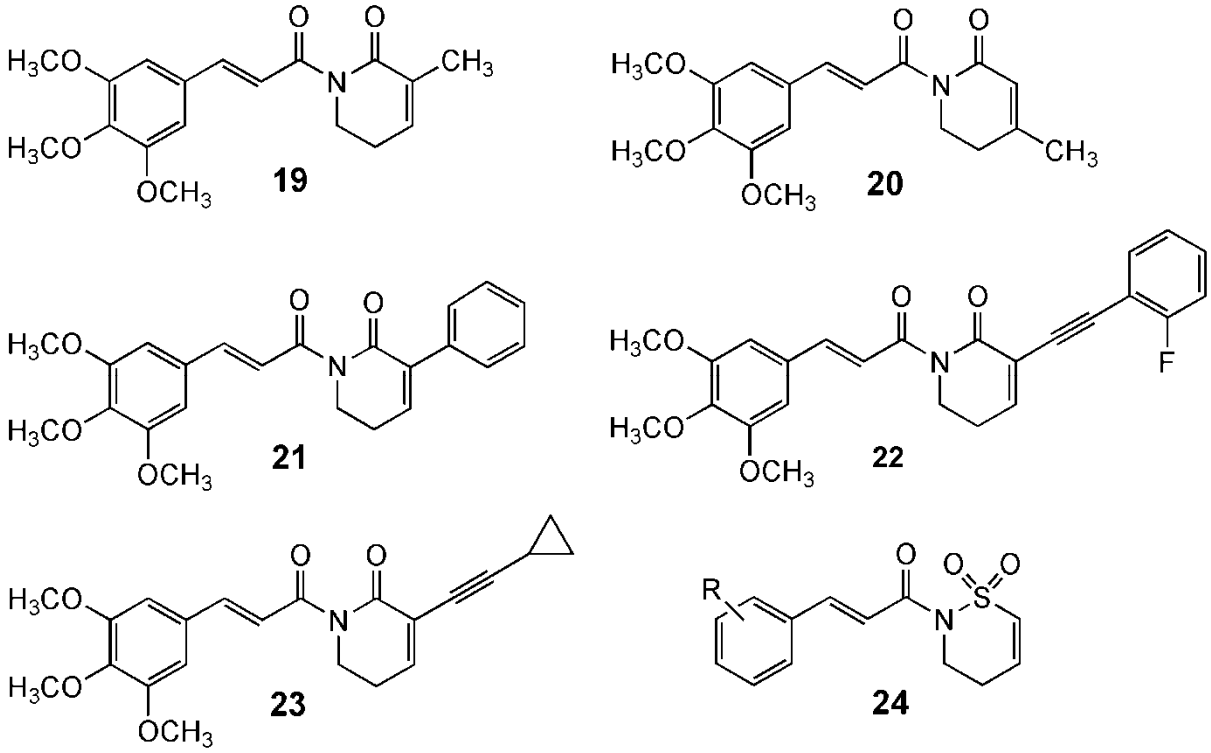

Fig. 4 Modification of piperlongumine lactam ring

- **7- substitution**

7,8-olefin plays an essential role in piperlongumine activity. In a previous study, this part of the structure was modified to form three derivates: removed, replaced by cyclopropane ring and by a triple bound. While the first two derivatives have significantly decreased activity, introduction of a triple bound greatly improved drug cytotoxicity [37]. The 7 position has also been used to introduce novel functional groups.

Punganuru et al. [39] obtained a series of 7-aryl piperlongumine derivatives to employ as microtubule destabilizing agents. The idea of such a substitution had been derived from the structure of combretastatin-A (**25**), a well-known microtubule targeting drug, binding to the site of colchicine, a drug with potent cytotoxic and antiangiogenic properties. 7-Phenyl was 4''-substituted with methyl (**26**), methoxy (**27**), methylthio (**28**), ethoxy (**29)** groups and fluorine (**30**) or was unsubstituted (**31**). Among them, the most cytotoxic derviates were compound **27** followed by compound **29**, which achieved IC_{50} values of less than 1 µM. Compound **27** exerted ROS-generating properties and induced protein glutathionylation, and, similarly to piperlongumine [38] had the ability to restore the function of a mutant p53 protein. Finally, compound **27** was found to potently destabilize microtubules, arrest the cell cycle at G2/M phase and induce apoptosis [39]. Further research are required to determine the

significance of a 7-aryl substitution, and methoxy groups in the activity of abovementioned compounds.

Wu et al. obtained a series of 7-alkyl derivatives of piperlongumine. This approach did not significant alter cytotoxic properties, however, 7-alkyl derivatives, especially the 7-methyl (**32**) of 2-chloropiperlongumine, demonstrated increased cytotoxic properties. Compound **32** was an ROS inductor and suppressed tumor growth more than piperlongumine (48.58% vs. 38.31%) [35].

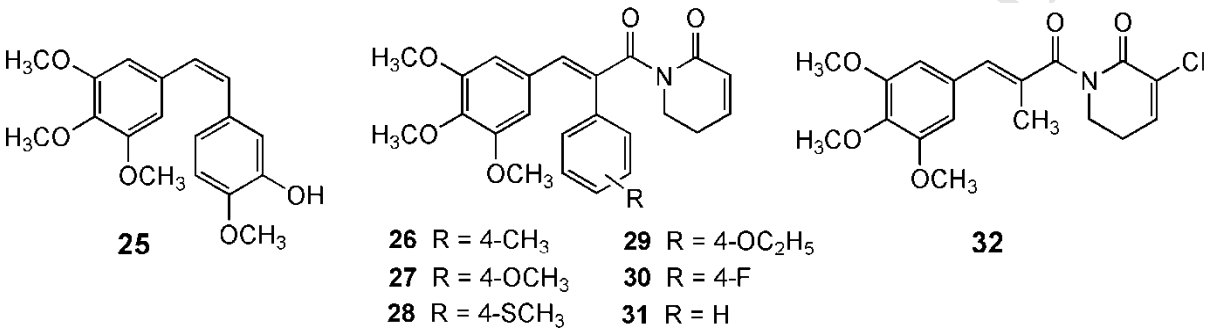

Fig. 5 Combretastatin-A and 7-substituted derivatives of piperlongumine

- **Oligomerization and conjugation**

An aromatic ring was used to obtain piperlongumine oligomers and hybrid compounds containing structural elements characteristic for anticancer molecules, which is an effective approach to obtain compounds endowed with the properties of both agents.

Adams et al. synthetized 4-(dimethylamino)ethyl monomer (**33**), as well as an analogical dimer (**34**) and trimer (**35**) linked by aminoethyl bridges. Such an approach provided for a meaningful increase in cytotoxicity and intracellular ROS generation: the dimer was 10 times more potent than the monomer, while the trimer was 2-fold more potent the than dimer [28]. Compound **34** was found to exert potent action against renal carcinoma cell lines at a nanomolar concentration, while antioxidant treatment reversed cellular toxicity. Compound **34** also decreased the growth of PNX0010 (patient derived renal cell carcinoma) xenograft tumors more effectively than piperlongumine [40].

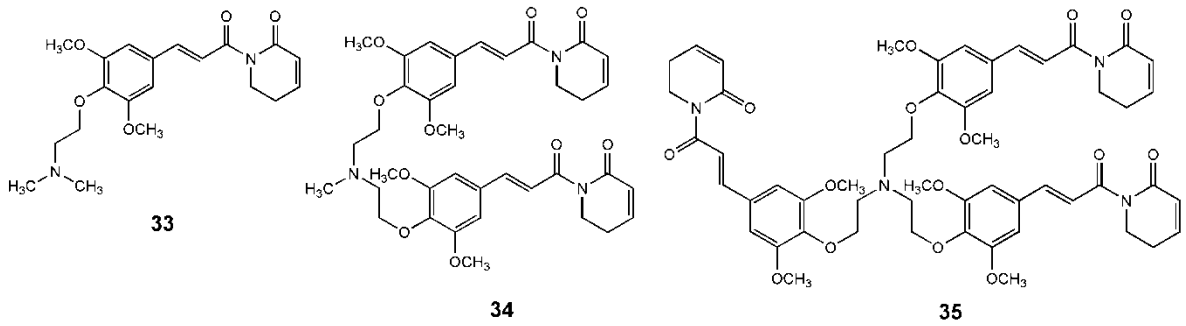

Fig. 6 Piperlongumine monomer, dimer and trimer

Liao et al. attempted to increase cytotoxicity by co-treatment of AML (Acute Myeloid Leukemia) cells with a combination of piperlongumine with the histone deacetylases (HDAC) inhibitor – vorinostat (**36**). Synergistic interaction between drugs led to a conception to obtained hybrid of these agents. The hybrid (**37**) showed significant cytotoxicity against leukemic cell lines, potently inducing apoptosis and DNA damage. Compound **37** also exhibited characteristic piperlongumine properties: antioxidant treatment protected cells against cytotoxicity and compound was conjugating with glutathione, as well as maintaining HDAC inhibitory properties. Introduction of chlorine into the C2 position increased pharmacologic activity however decreased selectivity. As other derivatives with an eliminated 7,8-olefin, the hybrid lacking this Michael acceptor was less active [41].

By a replacement of the phenyl ring with a 6, 7 and 8 substituted 4-hydroxycoumarin, a fragment of a dicoumarol structure (**38**), a series of cytotoxic agents were obtained. Dicumarol is a phytoconstitutent, having chemosensitizing and cytotoxic properties. As such, a combination of pharmacophores was expected to yield compounds with increased anticancer properties. Methoxy, methyl, halides derivatives were moderate cytotoxic agents. The most active was the 7-fluorine compound (**39**), which was found to elevate ROS and induce apoptosis in A549 cells, as well as had improved physicochemical properties. When administered to human lung cancer-bearing xenografts, compound **39** inhibited tumor growth, similarly to piperlongumine (48.46% and 41.23%, respectively) [42].

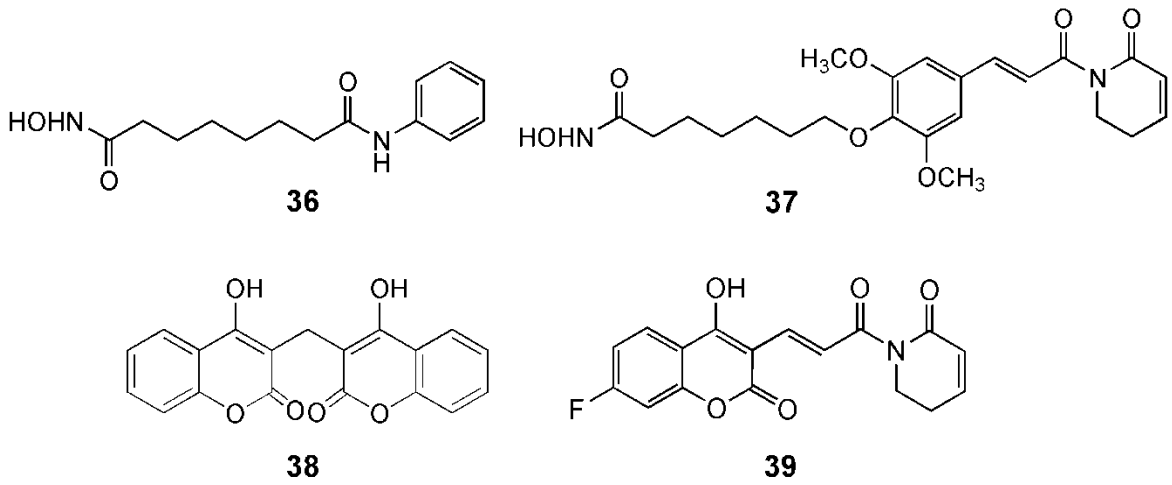

Fig.7 Piperlongumine conjugates

- **Piperlongumine as metal binding agent**

Use of metal complexes in cancer treatment is an old conception which recently is under intensive investigation. Many molecules were considered as ions chelating agents of different metals ions, and some complexes are currently studied in clinical trials. Pioneering research with piperlongumine as chelating agent were conducted by D'Sousa Costa et al. (2017) who used piperlongumine as a ligand in complex ruthenium chelates. Two obtained complexes were from 2 to 12 fold more active than metal-free piperlongumine in cytotoxicity assay against broad spectrum of cancer cell lines also in 3D model. These compounds, in the most sensitive HTC116 colon cancer cell line, were inducing caspase-3 and mitochondria involved intrinsic apoptosis, DNA fragmentation and ROS generation [43]. Fang et al. (2018) described synthesis of piperlongumine analogue with pyrrolidinone instead of dihydropyridinone ring, which was characterized as copper (Cu^{2+}) ions chelator. It acted as Cu^{2+} ionophore, which significantly increased intracellular concentrations of copper, especially Cu^{1+} in HepG2 cell, what caused redox imbalance, apoptosis and necrosis [44].

Synthesis

One of the first methods of piperlongumine synthesis was published by Boll et al., who used 3,4,5-trimethoxycinnamic anhydride and 5,6-dihydropyridin-2(1H)-one reacting in toluene and pyridine (5.66%) [3]. Currently, piperlongumine and its analogs are synthesized in similar ways via amide bond formation between carboxylic acid derivatives and appropriately (un)substituted (α,β-unsaturated) δ-lactams [32]. A common method involves the utilization of *n*-butyllithium (n-BuLi) in tetrahydrofuran (THF, at -78ºC) to deprotonate lactam and then coupling it with pivaloyl or oxalyl chloride-activated acids forming a mixed

anhydride [34,36,41,46]. Another method employs acid chloride for N-acylation of an unsaturated cyclic amide. This reaction may be carried out for example in THF with the addition of sodium hydride, [29,30] or THF with the addition of triethylamine [42]. If saturated lactams (eg. piperidin-2-one, pyrrolidin-2-one) are used in the reaction of N-acylation by acid chloride, the reagents may be refluxed in toluene for several hours or mixed in dichloromethane with the addition of trimethylamine [28,45]. To obtain a C2-C3 unsaturated bond, the compound needs to be subjected to further reactions including, for example, the formation of a selene derivative by means of phenylselenyl chloride, followed by oxidation with hydrogen peroxide [45]. Han et al. employed a late state olefination of the Horner-Wadsworth-Emmons type reagent (corresponding N-phosphonoacetylo derivative of 5,6-dihydropyridin-2(1H)-one, e.g. diethyl [2-oxo-2-(6-oxo-3,6-dihydropyridin-1(2H)-yl)ethyl]phosphonate) reacting with an appropriate aldehyde in THF in the presence of sodium hydride at 0ºC to room temperature. The synthesis of the reagent is a multistep process employing commercially available δ-valerolactam (piperidin-2-one) as a starting substrate. This approach is beneficial in the synthesis of analogs modified in a phenyl ring as it eliminates the need for the synthesis of substituted cinnamates and enables the obtainment of compounds possessing other aryl components as pyridine or furan instead of phenyl (**Fig. 8**) [32].

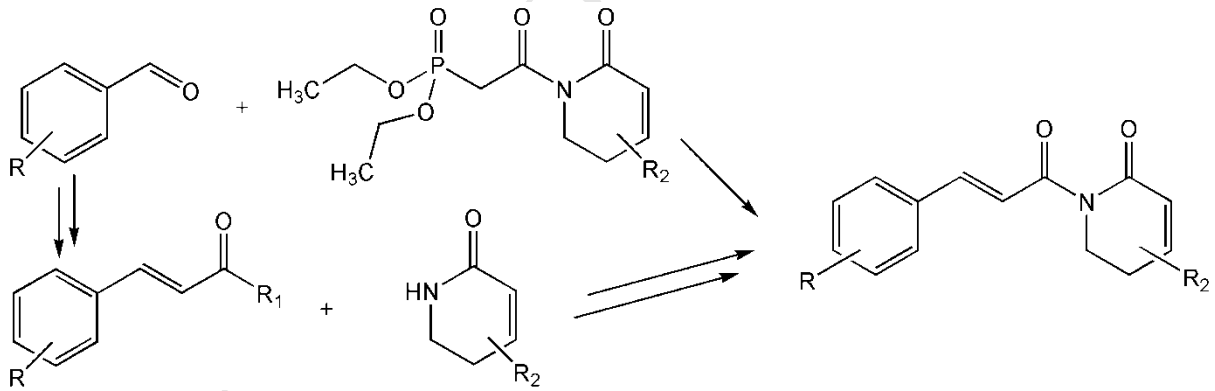

Fig. 8 General methods in the synthesis of piperlongumine analogs.

Several studies have emphasized that in the synthetic route of piperlongumine synthesis, the major challenge is the preparation of an appropriately substituted 5,6-dihydropyridin-2(1H)-one. The compound is frequently synthesized in the reaction of a metathesis of an appropriate amide possessing an olefinic bond e.g. N-(but-3-en-1-yl)prop-2-enamide. A Grubbs second generation catalyst is often used in this procedure [32,34,39] (**Fig. 9a**). Similarly, in order to obtain piperlongumine derived cyclic sulfonamides, a

Grubbs second generation catalyst is used to form a cyclic N-(but-3-en-1-yl)ethenesulfonamide [36] (**Fig. 9b**). In the synthesis of 2-chloro substituted piperlongumine derivatives, 3-chloro-5,6-dihydropyridin-2(1H)-one is used. The substrate obtained from piperidin-2-one in the reaction with phosphorus pentachloride gives 3,3-dichloropiperidin-2-one, which subsequently undergoes dehydrohalogenation [29].

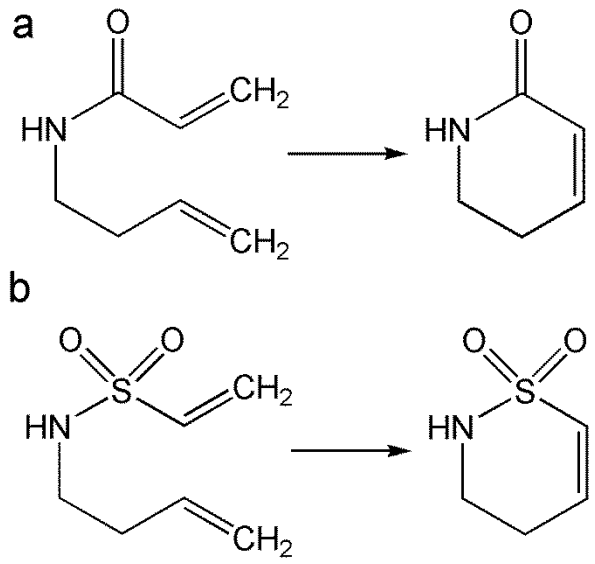

Fig. 9 Final step of obtaining of 5,6-dihydropyridin-2(1H)-one (a) and its sulfonamide analog (b).

A mixed anhydride approach (acids activated by means of pivaloyl or oxalyl chloride) enables the obtainment of not only simple analogs, but also other substances, including hybrid compounds combining the structure of piperlongumine with a well-known HDAC inhibitor - vorinostat. In a reported series, a seven carbon linker connected to a hydroxamic acid moiety was introduced at the 4' position of piperlongumine. [41]. Piperlongumine analogs substituted at the 2 position with alkyl, aryl or alkynyl group can be synthesized by a selective iodination of the parent compounds at the 2 position and palladium-catalyzed cross-coupled. While oligomeric piperlongumine analogs, linked with oxygen and diethyleneamine group at the 4' position, are obtained from 1-[3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-5,6-dihydropyridin-2(1H)-one in the reactions with 2-(dimethylamino)ethanol, N-methyldiethanolamine, or triethanolamine, carried out in toluene and THF, with the addition of triphenylphosphine and (*E*)-diisopropyl diazene-1,2-dicarboxylate [28].

Conclusions

The synthesis of piperlongumine analogues fits into the practice of obtaining derivatives of natural compounds exhibiting desirable pharmacological properties. This widely applied concept has previously resulted in the development of many drugs, used in the treatment of various diseases [47].

Based on the literature to-date, several general structure-activity relationships have been identified. It appears that the presence of a 2,3-olefin is required, while a 7,8-olefin is favorable for compound activity. In all studies, a 2-chloro substitution, probably by increasing 2,3-olefin electophilicity, was found to enhance activity. However, despite chloro substitution, the 2- position has not been widely studied, this may be an attractive point to introduce other functional groups. Another requirement for activity is an aromatic ring, however, the original benzyl may be successfully replaced by other aromatics, as well as substituted by other methoxy groups. Groups with especially strong electron withdrawing properties are preferred. The lactam ring may also be subjected to far reaching modifications. Very limited data is available concerning a 4-substitution, while 5- and 8- substitutions need to be studied. This may also be an interesting direction for future research.

Very interesting results have been achieved through the synthesis of piperlongumine hybrids with other anti-cancer molecules. This approach has brought a significant potentiation of activity. However, further studies of the toxicity and pharmacokinetic issues of this hybrid are required.

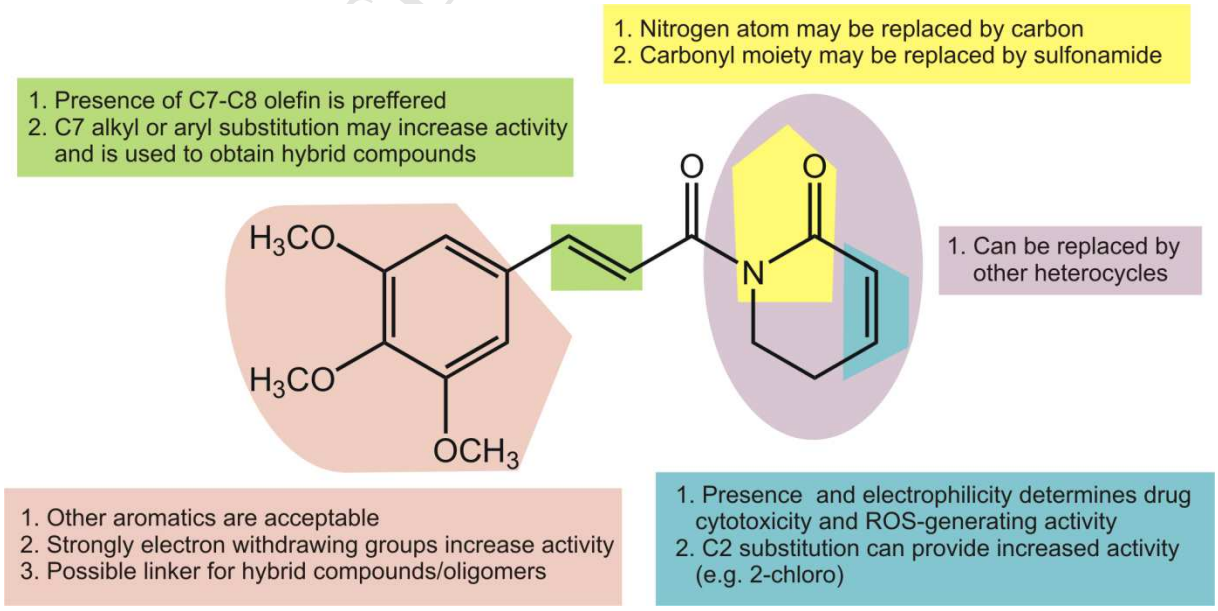

Fig. 10 Main concepts of piperlongumine modifications and structure-activity relationship

A summary of concepts regarding piperlongumine modification are presented in **fig. 10**. Recently synthetized piperlongumine analogues have exhibited a satisfactory activity both *in vitro* and *in vivo*. However, there is a lack of information concerning physico-chemical properties of these compounds, especially water solubility, safety profile and pharmacokinetics - ADME parameters. Future studies that investigate these issues - as they are equally important to pharmacological activity in the drug development process- are necessary. Piperlongumine has been found to exert a broad spectrum of anticancer properties. However, studies concerning the synthesis of piperlongumine analogues, focused mainly on its cytotoxic and, in some cases, prooxidant and antitumor properties of the obtained derivatives. Future studies should also explore if more cytotoxic piperlongumine analogues possesses as good proapoptotic, anti-invasive, and anti-angiogenic effects as piperlongumine. Also, more attention should be paid to molecular targets of piperlongumine, since these are attractive targets for anticancer and resistance-reversing agents [48].

It should also be noted that the use of modern drug delivery systems were applied in only a single piperlongumine studies [21,25]. However, these systems may significantly improve not only compound efficacy, but also could be a solution for its poor water solubility.

In recent years, great progress has been made in the optimization of piperlongumine activity, however, despite an increase in cytotoxicity, more attention must be devoted to the optimization of piperlongumine physico-chemical parameters, pharmacokinetics, and safety.

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- Piperlongumine is a promising antitumor agent
- This article describes synthesis and anticancer properties of piperlongumine analogues
- Some of them have showed increased anticancer properties and more appropriate drug-like parameters
- General structure-activity relationship conclusions are drawn and directions for future research are indicated