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Review

Overview of the therapeutic potential of piplartine (piperlongumine)

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ABSTRACT

Piplartine (piperlongumine, 5,6-dihydro-1-[(2*E*)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone) is a biologically active alkaloid/amide from peppers, as from long pepper (*Piper longum* L. – Piperaceae). Long pepper is one of the most widely used in Ayurvedic medicine, which is used to treat many diseases, including tumors. The purpose of the current paper is to address to the chemical structure establishment and to systematically survey the published articles and highlight recent advances in the knowledge of the therapeutic potential of piplartine, establishing new goals for future research. The reported pharmacological activities of piplartine include cytotoxic, genotoxic, antitumor, antiangiogenic, antimetastatic, antiplatelet aggregation, antinociceptive, anxiolytic, antidepressant, anti-atherosclerotic, antidiabetic, antibacterial, antifungal, leishmanicidal, trypanocidal, and schistosomicidal activities. Among the multiple pharmacological effects of piplartine, its anticancer property is the most promising. Therefore, the preclinical anticancer potential of piplartine has been extensively investigated, which recently resulted in one patent. This compound is selectively cytotoxic against cancer cells by induction of oxidative stress, induces genotoxicity, as an alternative strategy to killing tumor cells, has excellent oral bioavailability in mice, inhibits tumor growth in mice, and presents only weak systemic toxicity. In summary, we conclude that piplartine is effective for use in cancer therapy and its safety using chronic toxicological studies should be addressed to support the viability of clinical trials.

Contents

Abbreviations: 5-FU, 5-fluorouracil; AHNAK, neuroblast differentiation-associated protein; ALT, alanine amino transaminase; ANXA5, Annexin A5; AST, aspartate amino transferase; Bcl-2, B-cell leukemia/lymphoma 2; Bim, Bcl-2 interacting mediator of cell death; CBR1, carbonyl reductase 1; CBR1, carbonyl reductase 1; CD31, cluster of differentiation 31 also known as platelet endothelial cell adhesion molecule (PECAM-1); Cdc-2, cyclin-dependent kinases; CDIP, cell death involved p53-target; Cdk2, cyclin-dependent kinase 2; CDKN1A, cyclin-dependent kinase inhibitor 1A; CNS, central nervous system; ERK1/2, extracellular signal-regulated kinases; GLO1, glyoxalase I; GSTM3, glutathione S-transferase M3; GSTO1, glutathione S-transferase omega 1; GSTP1, glutathione S-transferase pi 1; GSTp1, glutathione S-transferase pi 1; GSTZ1, glutathione S-transferase zeta 1; HDRA, histoculture drug response assay; HIF-2, hypoxia inducible factor-2; ^{1}H NMR, proton nuclear magnetic resonance; IR, infrared; MMTV-PyVT, transgenic mouse model of spontaneous breast cancer; NF-κB, nuclear factor-kappa B; MS, mass spectrometry; Noxa, (latin for damage) phorbol-12-myristate-13-acetate-induced protein 1; p120ctn, p120 catenin; p53, transformation related protein 53; PARP, poly-(ADP-ribose) polymerase; PDGF-BB, platelet-derived growth factor BB; PMBC, peripheral blood mononuclear cells; PLEKHM1, pleckstrin homology domain-containing family M member 1; PRDX1, peroxiredoxin 1; PUMA, p53 upregulated modulator of apoptosis also known as BCL2 binding component 3 (BBC3); Raf, receptor tyrosine kinase; Rf, retardation factor; ROS, reactive oxygen species; RPS5, ribosomal protein S5; SILAC, stable isotope labeling with amino acids in cell culture; Survivin, baculoviral IAP repeat containing 5 (BIRC5); TLC, Thin layer chromatography; Twist, bHLH transcription factor; UV–vis, ultraviolet–visible; VEGF, vascular endothelial growth factor; VIM, vimentin; XIAP, X-linked inhibitor of apoptosis; $\Delta\Psi m$, mitochondrial membrane.

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1. Introduction

Piplartine (piperlongumine, 5,6-dihydro-1-[(2*E*)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone) is a biologically active component from *Piper* species (Piperaceae), which have high economical and medicinal importance. Economically the *Piper* seeds are important due to the worldwide use as "pepper" in the spice markets. Interestingly, they have been used in traditional medicine, including the Indian Ayurvedic system of medicine and the folk medicine of Latin America. In particular, piplartine is the major alkaloid from long pepper (*Piper longum* L.) (Chatterjee and Dutta, 1963; Prabhu and Mulchandani, 1985; Zhang et al., 2012). Its dried unripe fruits are used as a tonic. The infusion of *P. longum* root is used to induce expulsion of the placenta after birth. Other traditional uses of *P. longum* include treating tumors, diseases of the spleen, malaria, viral hepatitis, bronchitis, cough, asthma, respiratory infections, stomachache, and gonorrhea (Chopra et al., 1956; Kumar et al., 2011).

In addition, piplartine is also found in other important medicinal plants, such as *Piper tuberculatum* J. (de Jacq.) (Braz-Filho et al., 1981; Navickiene et al., 2003), *Piper arborescens* Roxb. (Duh et al., 1990a, 1990b), *Piper chaba* Hunter (Mishra and Tewari, 1964), *Piper sylvaticum* Roxb. (Banerji et al., 1980), *Piper cenocladum* C.DC (Dodson et al., 2000), *Piper alatabaccum* Trel & Yuncker (Facundo et al., 2005), and *Piper puberulum* Benth (Wu et al., 1997). Table 1 summarizes the traditional uses of plant species that are natural sources of piplartine.

The reported pharmacological activities of piplartine include cytotoxic (Duh et al., 1990a, 1990b; Bezerra et al., 2005, 2007, 2008a; Tsai et al., 2005; Kong et al., 2008; Lin et al., 2007; Jyothi et al., 2009; Raj et al., 2011; Bokesch et al., 2011; Golovine et al., 2013; Adams et al., 2012), genotoxic (Bezerra et al., 2008b, 2009), antitumor (Bezerra et al., 2006, 2008a; Raj et al., 2011), antiangiogenic (Raj et al., 2011), antimetastatic (Raj et al., 2011), antiplatelet aggregation (Tsai et al., 2005; Park et al., 2007, 2008; Iwashita et al., 2007; Fontenele et al., 2009; Lee et al., 2010), antinociceptive (Rodrigues et al., 2009), anxiolytic (Felipe et al., 2007), antidepressant (Felipe et al., 2007), anti-atherosclerotic (Son et al., 2012), antidiabetic (Rao et al., 2012), antibacterial (Naika et al., 2010), antifungal (Navickiene et al., 2000; Silva et al., 2002), leishmanicidal (Bodiwala et al., 2007), trypanocidal (Cotinguiba et al., 2009), and schistosomicidal (Moraes et al., 2011, 2012a, 2012b) activities. Particularly, its anticancer potential has been extensively investigated by different research groups. As a consequence, a patent that provides methods for the treatment of cancer using piplartine and/or its analogs has been published (Lee and Mandinova, 2009).

Piplartine was one of the selected molecules for further studies from a total of 5166 samples screened, in the period between 2000 and 2007, in our screening program for the discovery and development of potential anticancer compounds carried out at the Laboratório Nacional de Oncologia Experimental – Universidade Federal do Ceará, LOE-UFC, Fortaleza, Ceará, Brazil (Costa-Lotufo et al., 2010). Therefore, the purpose of the current paper is to address the chemical structure establishment and to systematically survey the published articles and highlight recent advances in the knowledge of the therapeutic potential of piplartine, establishing new goals for future research. Since its anticancer property is the most promising and has been extensively investigated, herein we focused on this effect. This literature review includes journals, patents, internet databases, and books. Up to now, 60 research papers and one patent describing piplartine structure and activity were published.

2. Chemical structure establishment of piplartine

Atal and Banga, a pair of Indian chemists working with *P. longum*, first isolated in 1961 a new amide alkaloid to which they gave

Table 1
Reported traditional uses of plant species that are natural sources of piplartine.

Plant species	Vernacular name	Traditional use
Piper longum L.	Long pepper	In Indian Ayurvedic medicine this plant is used to treat tumors, bronchitis, asthma, cough, respiratory infections, cholera, malaria, viral hepatitis, gonorrhea, constipation, diarrhea, stomachache, paralysis of the tongue, diseases of the spleen, and as a contraceptive Chopra et al. (1956), Chaudhary et al. (2001), Kumar et al. (2011).
Piper tuberculatum J. (de Jacq.)	Pimenta-d'arda	In Northeastern Brazil, *P. tuberculatum* has been used as a sedative and antidote to snake poison Felipe et al. (2007). This plant is used in Peruvian traditional medicine as an anti-inflammatory and disinfectant of wounds in humans and domestic animals Palacios et al. (2009).
Piper arborescens Roxb.	Boyo–boyo	In Malaysia, *P. arborescens* is used for treatment of rheumatism Chai et al. (1989).
Piper chaba Hunter	Dee plee pepper	In Bangladesh, *P. chaba* stem is used to reduce several types of pains and diarrhea. The roots are traditionally used to treat asthma, bronchitis, and constipation. The fruit is used for asthma, bronchitis, fever, inflammation, pain, and hemorrhoids Kirtikar and Basu (1980), Yusuf et al. (1994).
Piper sylvaticum Roxb.	Rari	In India, *P. sylvaticum* leaves are used as a vegetable (Srivastava, 2009). The roots are used as an antidote to snake poison (Parmar et al., 1997).
Piper cenocladum C.DC	Ant pipers	–
Piper alatabaccum Trel & Yuncker	João brandinho	In the Amazon Rainforest, *P. alatabaccum* roots are used as a local anesthetic Ribeiro et al. (1999).
Piper puberulum Benth	Hairy pepper	In traditional Chinese medicine, *P. puberulum* is used to treat painful swelling from knocks and falls, hernia, menstrual pain, and pain in the stomach duct and abdomen Zhou et al. (2011).

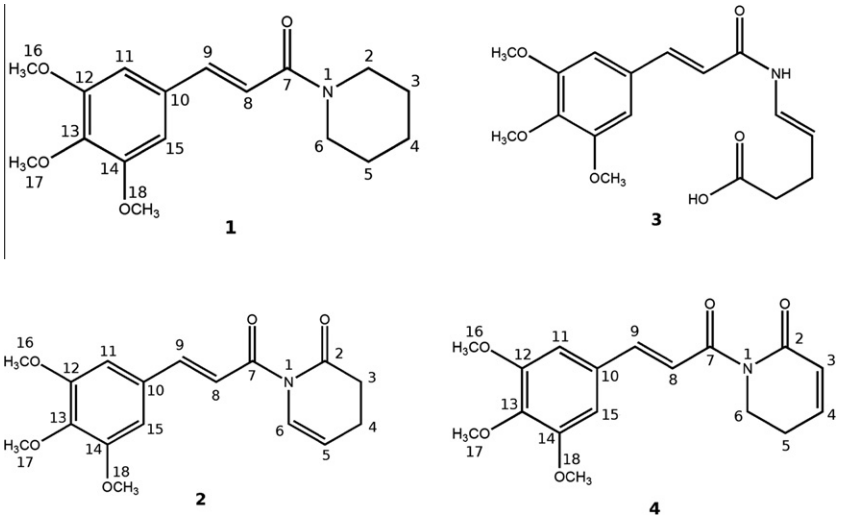

Fig. 1. Proposed chemical structures. Correct structure of piplartine (**4**, piperlongumine, 5,6-dihydro-1-[(2*E*)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone).

the name piplartine, and subsequently characterized its structure in 1963 (Atal and Banga, 1961, 1962, 1963). Also in 1963, another Indian group composed by Chatterjee and Dutta isolated an amide alkaloid from *P. longum* with physical characteristics similar to piplartine, but for which a different structure was proposed (Chatterjee and Dutta, 1963, 1967; Chatterjee, 1964). The former group suggested structure (**1**) for piplartine based mainly on the mass spectral investigation and elemental analysis of a hydrolysis product, determined to be 3,4,5-trimethoxycinammic acid, and the similarity of the retardation factor (Rf), on thin layer chromatography (TLC), of both piplartine and the condensation adduct of 3,4,5-trimethoxycinammoyl chloride and piperidine (Atal and Banga, 1963). Meanwhile, the latter group suggested structure (**2**) for piperlongumine based on a more extensive study of the compound itself and of its hydrolysis products, including a novel compound which they named piperlongumic acid (**3**) (Chatterjee and Dutta, 1963, 1967). The chemical structures are shown in Fig. 1. The attribution was made based on analyses of mass spectrometry (MS), infrared (IR), ultraviolet–visible (UV–vis) and proton nuclear magnetic resonance (^{1}H NMR) spectra of piperlongumine and piperlongumic acid, coupled with the investigation of hydrogenation and ozonolysis products and the synthesis of piperlongumic acid, among other chemical and physical identification techniques. During the study of piperlongumine, Atal reported in personal communication to the authors that he was convinced that piplartine and piperlongumine were in fact the same compound (Chatterjee and Dutta, 1967).

Soon after, in 1968, a paper by Joshi, Kamat and Saksena was published criticizing the 1967 study and pointing out several oversights that lead to the incorrect identification of the structure of piplartine/piperlongumine, including a >100% yield of a proposed product during a key identification reaction and an erroneous interpretation of the ^{1}H NMR data. A more careful ^{1}H NMR spectrum analysis, emphasizing previous integration and assignment mistakes, afforded the correct structure (**4**), where the double bond is conjugated to the pyridinone carbonyl. The authors suggested the compound should retain the first name given, piplartine (Joshi et al., 1968). Further studies in the 1980s allowed comparison of the X-ray crystallographic data of piplartine obtained from an improved synthetic route (Boll et al., 1984), and of piplartine isolated from *P. longum* (Boll et al., 1984; Banerjee and Chaudhuri, 1986), definitively establishing the equivalence of piplartine and piperlongumine and the accuracy of structure (**4**). It is unclear why, despite "piplartine" being the most used term in the literature, Aldrich Co. commercially adopted the name "piperlongumine", running against the historical order and usage frequency. Thus, piplartine and piperlongumine are synonyms which refer to 5,6-dihydro-1-[(2*E*)-1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)-pyridinone, (**4**). The structure is shown in Fig. 1.

3. Anticancer effects of piplartine

3.1. Cytotoxicity studies

The direct cytotoxic activity of piplartine against tumor cell lines has been described in many studies. Table 2 summarizes all results found. Piplartine is able to kill cancer cells of several histotypes, including hematological, colon, melanocyte, lung, breast, central nervous system (CNS), pancreatic, nasopharyngeal, osseous, bladder, renal, and prostate. Its cytotoxicity was observed in the micromolar range in tumor cells, but not in normal cells. This compound showed selective cytotoxicity over cancer cells and presents only a weak activity in normal cells (Duh et al., 1990a, 1990b; Bezerra et al., 2005, 2007, 2008a; Kong et al., 2008; Lin et al., 2007; Jyothi et al., 2009; Lee and Mandinova, 2009; Raj et al., 2011; Bokesch et al., 2011; Golovine et al., 2013; Adams et al., 2012).

In addition, piplartine was also able to inhibit cancer cell growth in patient-derived samples. The cytotoxic activity of piplartine was compared to a variety of known anticancer agents in a histoculture drug response assay (HDRA). Piplartine showed superior cytotoxicity against patient-derived osteosarcoma, breast, and colon cancer when compared to several chemotherapeutic agents, including adriamycin, cyclophosphamide, mitomycin C, and methotrexate (Lee and Mandinova, 2009).

For reducing the mechanisms of resistance in clinical cancer chemotherapy, anticancer drugs are often used in combination. Interestingly, piplartine increased the cytotoxicity of 5-fluorouracil (5-FU) in several cell lines, as observed by the lower IC_{50} value and increase in the maximum response achieved. For example, when 5-FU was used in combination with 5 μM of piplartine, the IC_{50} value diminished from 2.33 to 0.49 μM in SF-295 cells (Bezerra et al., 2008a). In a similar work, Jyothi et al. (2009) investigated the combinatorial treatment of piplartine with diferuloylmethane (curcumin), an anti-inflammatory and anticancer agent. Diferuloylmethane combination enhanced piplartine induced cytotoxicity. These findings showed that piplartine not only possesses a cytotoxic effect, but can also increase the antitumor activity of chemotherapeutic drugs.

Table 2
In vitro cytotoxic effects of piplartine against normal and tumor cell lines.

Cell lines	Histotype	Origin	IC_{50} (μM)	References
P-388	Leukemia lymphocytic	Mouse	2.8	Duh et al. (1990a, 1990b)
HL-60	Leukemia promyelocytic	Human	5.3	Bezerra et al. (2005)
CEM	Leukemia lymphocytic	Human	4.4	Bezerra et al. (2005)
K-562	Leukemia myeloid	Human	5.7	Bezerra et al. (2007)
JUKART	Leukemia lymphocytic	Human	5.3	Bezerra et al. (2007)
MOLT-4	Leukemia lymphoblastic	Human	1.7	Bezerra et al. (2007)
HT-29	Colon carcinoma	Human	1.4	Duh et al. (1990a)
HCT-8	Colon carcinoma	Human	2.2	Bezerra et al. (2005)
HCT116	Colon carcinoma	Human	~7	Raj et al. (2011)
SW620	Colon carcinoma	Human	~7	Raj et al. (2011)
DLD-1	Colon adenocarcinoma	Human	~7	Raj et al. (2011)
B16	Melanoma	Mouse	5.3	Bezerra et al. (2005)
MDA-MB-435	Melanoma	Human	~7	Bezerra et al. (2008a)
A-549	Lung carcinoma	Human	1.9	Duh et al. (1990a)
H1975	Lung carcinoma	Human	~7	Raj et al. (2011)
BT-474	Breast carcinoma	Human	~7	Raj et al. (2011)
MDA-MB-231	Breast carcinoma	Human	~7	Raj et al. (2011)
SF-295	Glioblastoma	Human	~7	Bezerra et al. (2008a)
IMR32	Neuroblastoma	Human	>25	Jyothi et al. (2009)
Panc-1	Pancreatic carcinoma	Human	~7	Raj et al. (2011)
Mia PaCa-2	Pancreatic carcinoma	Human	~7	Raj et al. (2011)
U2OS	Osteosarcoma	Human	~7	Raj et al. (2011)
SaoS-2	Osteosarcoma	Human	~7	Raj et al. (2011)
J774	Macrophages	Mouse	>25	Jyothi et al. (2009)
P388D1	Macrophages	Mouse	~5	Jyothi et al. (2009)
EJ	Bladder carcinoma	Human	~7	Raj et al. (2011)
PC-3	Prostate carcinoma	Human	~10	Kong et al. (2008)
LNCaP	Prostate carcinoma	Human	>30	Kong et al. (2008)
786-0	Renal carcinoma	Human	61.4	Bokesch et al. (2011)
BC-8	Histiocytoma	Rat	~5	Jyothi et al. (2009)
PCC4	Embryonal carcinoma	Mouse	~5	Jyothi et al. (2009)
KB	Nasopharyngeal	Human	5.6	Duh et al. (1990a, 1990b)
V79	Normal lung fibroblast	Hamster	~60	Bezerra et al. (2008b)
PBMC	Normal lymphocytes	Human	>31.5	Bezerra et al. (2007)
PAE	Normal aortic endothelial	Human	>15	Raj et al. (2011)
76N	Normal breast epithelial	Human	>15	Raj et al. (2011)
HKC	Normal keratinocytes	Human	>15	Raj et al. (2011)
HDF	Normal skin fibroblasts	Human	>15	Raj et al. (2011)
184B5	Immortalized breast epithelial	Human	>15	Raj et al. (2011)
MCF 10A	Immortalized breast epithelial	Human	>15	Raj et al. (2011)
Melan-a	Immortalized melanocyte	Mouse	~10	Lin et al. (2007)

The differences between *E*-piplartine (*trans*-piplartine) and *Z*-piplartine (*cis*-piplartine) in cytotoxicity against tumor cell lines were also examined. For the *trans*- and *cis*-piplartine, only *trans*-piplartine showed cytotoxicity. *Cis*-piplartine failed to induce cytotoxicity even at higher concentrations (50 μM). *Trans*-piplartine (*E*-piplartine) is the main form that this amide is found in nature (Jyothi et al., 2009). Additionally, by comparing the cytotoxicity of selected molecules that differ in structural features, the presence of two α,β-unsaturated carbonyl moieties seem to be responsible for its cytotoxic activity (Duh et al., 1990a, 1990b; Bezerra et al., 2005).

The cytotoxicity of piplartine was also assessed in alternative models. This compound showed high toxicity to brine shrimp nauplii (LD_{50} = 32.3 ± 3.4 μg/ml). In addition, it induced a dose-dependent inhibition on egg development during all phases examined, first and third cleavage and blastulae, which completely inhibited sea urchin mitosis at concentrations lower than 4 μg/ml. Moreover, piplartine was not hemolytic even at the highest tested concentration (200 μg/ml), suggesting that the cytotoxicity of piplartine is not related to membrane damage (Bezerra et al., 2005).

3.2. Mechanism of action studies

Understanding the molecular mechanism of action of drugs is essential to predict the potential therapeutic and side effects of these substances. For this purpose, the molecular and cellular mechanism by which piplartine induces cytotoxicity has been under investigation. Fig. 2 summarizes all results found.

The first work was performed by Bezerra et al. (2007), where a series of studies evaluated the human leukemia cells, HL-60 and K-562, treated with piplartine for 24 h. These experiments suggested that piplartine can suppress leukemia growth and reduce cell survival, triggering cell death by both caspase-dependent apoptosis and/or necrosis. However, only weak cytotoxicity was observed in normal lymphocytes (PMBC). In a subsequent study, Bezerra et al. (2008b) demonstrated the effects of piplartine in the cell cycle progress employing a permanent cell line derived from Chinese hamster lung fibroblasts (V79 cell line). Piplartine induced G_2/M cell cycle arrest followed by mitochondrial-dependent apoptosis, as observed by chromatin condensation, internucleosomal DNA fragmentation, and loss of mitochondrial membrane potential. Consequently, several other studies were also designed to identify the mechanism of action of piplartine. Kong et al. (2008) examined the anti-proliferative effects of piplartine on two human prostate cancer cell lines, PC-3 (androgen-independent prostate carcinoma) and LNCaP (androgen-dependent prostate carcinoma). Piplartine preferentially inhibited the proliferation of the androgen-independent cells more than the androgen-dependent cells. Cell cycle arrest at the G_2/M phase was also observed in PC-3 cells treated with piplartine. Additionally, a dose-dependent decrease of cdc-2 expression was observed in piplartine-treated PC-3 cells, which correlated with the G_2/M

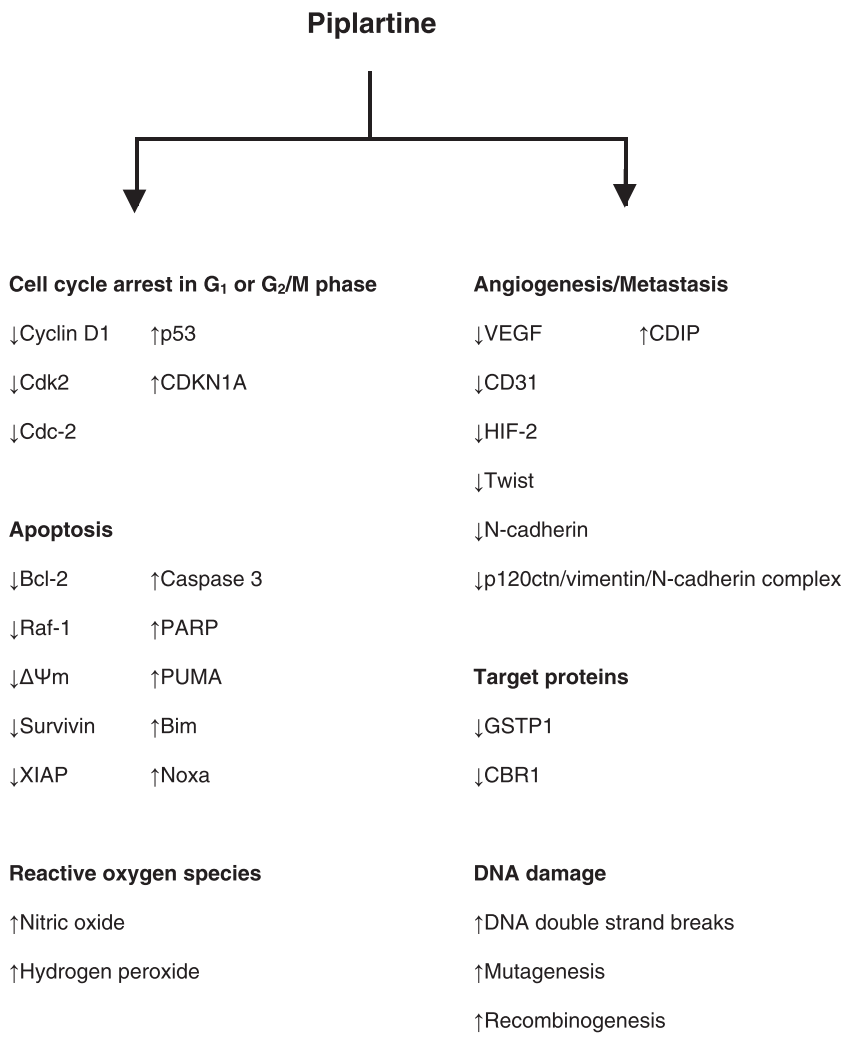

Fig. 2. Summary of the anticancer mechanism of piplartine. Cdk2: cyclin-dependent kinase 2; Cdc-2: cyclin-dependent kinase; p53: transformation related protein 53; CDKN1A: cyclin-dependent kinase inhibitor 1A; Bcl-2: B-cell leukemia/lymphoma 2; Raf: receptor tyrosine kinase; $\Delta\Psi m$: mitochondrial membrane potential; Survivin: baculoviral IAP repeat containing 5 (BIRC5); XIAP: X-linked inhibitor of apoptosis; PARP: poly-(ADP-ribose) polymerase; PUMA: p53 upregulated modulator of apoptosis, also known as BCL2 binding component 3 (BBC3); Bim: Bcl-2 interacting mediator of cell death; Noxa: (latin for damage) phorbol-12-myristate-13-acetate-induced protein 1; VEGF, vascular endothelial growth factor; CD31: cluster of differentiation 31, also known as platelet endothelial cell adhesion molecule (PECAM-1); HIF-2: hypoxia inducible factor-2; Twist: bHLH transcription factor; p120ctn: p120 catenin; CDIP: cell death involved p53-target; GSTP1: glutathione S-transferase pi 1; CBR1: carbonyl reductase 1.

phase cell cycle arrest, but the expression of cyclin B1, another target molecule critical for G_2/M transition, was not altered. Changes in the levels of bax were undetectable, but bcl-2, an inhibitor of the intrinsic apoptosis pathway, decreased significantly, suggesting that piplartine induced apoptosis through down-regulation of bcl-2. The activation of caspase-3 and poly(ADPribose)polymerase, effector proteins in apoptosis, was also found. Moreover, Golovine et al. (2013) demonstrated that piplartine induces rapid androgen receptor depletion in LNCaP cells through a proteasome-mediated reactive oxygen species (ROS) – dependent pathway, which coincides with reduced functional activity of the androgen receptor signaling.

Piplartine effects on cell death, cell cycle and signal transduction pathways were also evaluated in the mouse embryonal carcinoma cell line, PCC4 (Jyothi et al., 2009). In contrast to previous works, piplartine induced a significant increase of cells in the G_1 phase of the cell cycle. The effect of piplartine on receptor tyrosine kinase (Raf-1), extracellular signal-regulated kinases (ERK1/2), cell cycle dependent kinase (cdk2), and the major cell cycle regulator (cyclin D1) were also examined. Although piplartine failed to inhibit ERK1/2 signaling, it inhibited the Raf-1 activation. The decrease of the kinase levels of cdk2 and protein levels of cyclin D1, two G_1 cell cycle regulators, were also found. Moreover, Hsp70, a heat shock protein, over expressed by BC-8, showed a reduction in piplartine-induced cell death, suggesting that Hsp70 protects cells from cytotoxicity caused by piplartine. Additionally, Bokesch et al. (2011) tested the ability of this compound to modulate hypoxia inducible factor-2 (HIF-2) transcription activities in the renal carcinoma cell line, 786-0. Piplartine showed HIF-2 inhibitory activity with an EC_{50} value of 4.8 μM.

In an issue of Nature, Raj et al. (2011) showed that piplartine treatment markedly induced cell death in cancer, but not normal cells. The cell death was also associated to the induction of expression of p53 and the induction of p53 acetylation. Surprisingly, piplartine treatment killed both wild-type p53 and mutant p53 tumor cells. Additionally, piplartine treatment was able to repress the expression of several pro-survival proteins, including BCL2, survivin, and XIAP. Moreover, after piplartine treatment, several pro-apoptotic genes, including Bim, PUMA, and Noxa, were up-regulated in the cancer cells, while survival genes showed significantly reduced mRNA levels. In piplartine-treated cells, activation of the CDIP (cell death involved p53-target) gene was also found. However, no significant changes in these transcripts were observed in normal cells.

Piplartine suppresses tumor progression and migration. The inhibition of the expression of Twist and N-cadherin was also observed in tumor cells treated with piplartine in culture. Piplartine treatment also was able to disrupt the p120-ctnvimentin/N-cadherin complex, which plays a critical role in tumor progression and invasion/metastasis (Lee and Mandinova, 2009).

Raj et al. (2011) identified the targets in which piplartine induces cytotoxicity through a combination of stable isotope labeling with amino acids in cell culture (SILAC) and quantitative proteomics. Using this method, the authors identified 12 potential targets for piplartine that were conserved across two cell lines, EJ (human bladder carcinoma, mutant p53) and U2OS (human osteosarcoma, wt-p53). The target proteins were: glutathione S-transferase pi 1, GSTp1; carbonyl reductase 1, CBR1; glutathione S-transferase zeta 1, GSTZ1; glutathione S-transferase M3, GSTM3; neuroblast differentiation-associated protein, AHNAK; pleckstrin homology domain-containing family M member 1, PLEKHM1; glyoxalase I, GLO1; glutathione S-transferase omega 1, GSTO1; Annexin A5, ANXA5; ribosomal protein S5, RPS5; vimentin, VIM; peroxiredoxin 1, PRDX1. Interestingly, seven of the potential targets are involved in the cellular response to oxidative stress. GSTp1 and CBR1 were the highest-confidence hits. In addition, it was found that piplartine is able to interact directly with purified recombinant GSTp1, inhibit glutathione S-transferase activity, decrease the reduced glutathione levels and increase the oxidized glutathione levels in tumor cells. Hydrogen peroxide and nitric oxide were found among the reactive oxygen species (ROS) induced by piplartine in tumor cells. Moreover, the combination of piplartine with N-acetyl-L-cysteine, an antioxidant agent, prevents ROS production and cell death (Raj et al., 2011; Parkinson and Hergenrother, 2011.).

In contrast to the results in cancer cells, piplartine did not cause an increase in ROS levels in normal cells. To better understand the selectivity of piplartine for cancer cells, basal expression levels of GSTpi and CBR1 were determined in non-transformed, oncogenically transformed, and cancer cells. The expression of GSTpi and CBR1 is enhanced in oncogenically transformed cells and cancer cells. Therefore, Raj et al. (2011) conclude that piplartine induces cell death selectively in tumor cells, presumably by its effects on oxidative stress response enzymes such as GSTp1 and CRB1, which are unregulated in cancer cells. These enzymes are novel targets of cancer therapy. Therefore, piplartine represents a novel class of chemotherapy drugs.

More recently, Adams et al. (2012) synthesized 80 structural analogs of piplartine to identify modifications that retain, enhance, or ablate its potency. Structure/activity relationships suggest that the electrophilicity of the C2–C3 olefin is critical for ROS elevation, glutathione depletion, and cellular toxicity. In addition, analogs lacking a reactive C7–C8 olefin can elevate ROS to levels observed with piperlongumine but show markedly reduced cell death, suggesting that ROS-independent mechanisms, including cellular cross-linking events, may also contribute to piperlongumine's induction of apoptosis. Moreover, irreversible protein glutathionylation was identified as a process associated with cellular toxicity.

3.3. Studies in tumor-bearing mice

Preclinical efficacy testing for antitumor agents can be performed in a series of models including genetically engineered animals, orthotopic, xenograft, and combination therapy models. Pharmacology and toxicology models in tumor-bearing animals are useful to direct the clinical trials of a new drug. Several studies in tumor-bearing mice treated with piplartine are found. Table 3 summarizes these studies.

The *in vivo* antitumor activity of piplartine was evaluated for the first time in *Swiss* mice transplanted with sarcoma 180 tumor. Piplartine inhibited sarcoma 180 tumor growth in mice after the administration of 7 doses of 50 or 100 mg/kg, showing an *in vivo* antitumor effect (Bezerra et al., 2006). Besides this, when the tumor-bearing animals were treated with piplartine plus 5-FU, the tumor inhibition rate increased additively (Bezerra et al., 2008a). Additionally, no significant changes in body weights or organ weights were seen in any animals treated with piplartine. Renal (urea levels) and liver (enzymatic activity of transaminases: AST and ALT) parameters remained unchanged in sarcoma 180-transplanted mice treated with piplartine. In histopathological analyses, piplartine had no effect on the spleen or liver of treated animals, but had the kidney as its toxicological target (Bezerra et al., 2006, 2008a). However, the kidney damage observed in piplartine-treated animals could also be considered reversible. Additionally, leukocytopenia was observed with 5-FU treatment alone, which was prevented by the association with piplartine, indicating a protective effect of piplartine against delayed hematopoietic depression induced by 5-FU. The combination of piplartine and 5-FU did not induce substantial changes in biochemical, hematological and histopathological parameters. It is possible that this combination augments the antitumor activity without augmenting side effects, and, moreover, it could even attenuate 5-FU toxicity (Bezerra et al., 2008a).

In xenograft models, the antitumor effect of piplartine was tested against EJ (human bladder carcinoma), MDAMB436 (human breast carcinoma), and A549 (human lung carcinoma). In addition, C57BL/6 mice inoculated with melanoma B16-F10 were also treated with piplartine. The animals were treated for 21 days at the dose of 1.5 mg/kg/day. As expected, marked antitumor effects were observed in tumor-bearing mice treated with piplartine. The inhibition rate was near the positive control used. Piplartine treatment also enhanced the expression of CDKN1A, PUMA, and caspase 3 in EJ-cell tumors, which represents the pro-apoptotic effect of piplartine in tumor-bearing mice. Moreover, piplartine treatment inhibited the formation of blood vessels in xenograft-tumor mice, as observed by the reduction of the expression of VEGF, suggesting also an antiangiogenic activity (Raj et al., 2011). The angiogenesis is required by solid tumors to sustain growth. Thus, reduction of angiogenesis provides an alternative treatment for many tumors.

The effect of piplartine treatment in a transgenic mouse model of spontaneous breast cancer, MMTV-PyVT, was also studied. A reduction of the tumor development was observed when the animals were treated with piplartine by intraperitoneal route

Table 3
In vivo antitumor effects of piplartine against mouse and human tumor cell lines.

Tumor	Histotype	Origin	Dose (mg/kg/day)	*n*	Days of treatment	Route	Inhibition rate (%)	References
Sarcoma 180	Sarcoma	Mouse	50/100	10	7	i.p.	28–52	Bezerra et al. (2006, 2008)
EJ	Bladder carcinoma	Human	1.5	14	21	i.p.	~80	Raj et al. (2011)
MDAMB436	Breast carcinoma	Human	1.5	14	21	i.p.	~50	Raj et al. (2011)
A549	Lung carcinoma	Human	1.5	14	21	i.p.	~50	Raj et al. (2011)
B16-F10	Melanoma	Mouse	1.5	14	21	i.p.	~50	Raj et al. (2011)
MMTV-PyVT	Breast adenocarcinoma	Mouse	2.4	12	13	i.p.	~75	Raj et al. (2011)

for 13 consecutive days. Once again, an inhibition of the formation of blood vessels was found in piplartine-treated MMTV-PyVT, as observed by the reduction of the expression of CD31 (also known as platelet endothelial cell adhesion molecule – PECAM-1). In the MMTV-PyVT model, it is also possible to assess the antimetastatic potential. Metastasis of the primary tumor produces secondary tumors and disseminated cancer. Moreover, the development of spontaneous metastasis to the lungs was not found, showing that piplartine also has antimetastatic activity (Raj et al., 2011).

3.4. Toxicological analysis

Preclinical toxicology studies are fundamental for assessing the security of new therapeutic drugs. These tests include acute and chronic toxicity and genotoxic studies. Several toxicology studies of piplartine are found in literature.

As genotoxic compounds are potentially mutagenic or carcinogenic, the genotoxic potential of piplartine has been investigated in many models. The genotoxic activity could be biologically relevant as an alternative strategy to the killing of tumor cells by anticancer agents, but generally also classified the genotoxic antineoplastic agents as secondary carcinogens. The genotoxicity of piplartine was evaluated through neutral and alkaline comet assays, *in vitro* micronucleus analysis, and in chromosome aberration assays in the V79 cell line; its mutagenic and recombinogenic potential was evaluated in the simple eukaryote *Saccharomyces cerevisiae,* mouse bone-marrow micronucleus tests, and prokaryote *Salmonella*/microsome assay.

Piplartine treatment induced DNA strand breaks in V79 cells, as detected by neutral and alkaline comet assays. In addition, it was found that DNA double-strand break, as detected by neutral comet assay, was significantly higher in V79 cells following exposure to this substance (Bezerra et al., 2008b). The literature has also reported that piplartine is able to induce significant chromatid and chromosomal aberrations at different concentrations as assessed using a chromosome aberration test in V79 cells. Additionally, the literature has shown that piplartine significantly increased the incidence of micronuclei in the same cells (Bezerra et al., 2009). This agent also induced dose-dependent genotoxicity in *S. cerevisiae* cultures in the exponential growth phase as observed by the increase in the frequency of point, frameshift, and forward mutations; however, it was not mutagenic in the stationary phase (Bezerra et al., 2008b). In mouse bone-marrow micronucleus assay, piplartine at 50 mg/kg does not induce micronucleus formation, but 100 mg/kg of this compound increased the levels of micronucleated immature polychromatic erythrocytes. In contrast, piplartine did not induce a significant mutagenic response in bacteria either in the presence, or absence, of metabolic activation, suggesting that piplartine is able to induce genotoxicity in eukaryotic but not in prokaryotic model systems (Bezerra et al., 2009; Morandim-Gianetti et al., 2011). Probably this effect is due to many metabolic and physiological peculiarities between the eukaryote and prokaryote systems.

Toxicological aspects of piplartine were also target of investigation in healthy mice. Hematological, biochemical, histopathological and morphological analyses of the piplartine-treated animals were also performed on healthy *Swiss* mice after 7 days of treatment at a dose of 50 mg/kg. Neither the enzymatic activity of transaminases nor the urea levels were significantly modified when compared with the control group; hematological parameters also remained unchanged. The histopathological analysis showed that the kidneys of treated animals were only slightly and reversibly affected (Bezerra et al., 2008a).

Recently, acute toxicity studies were performed in rats and mice treated with piplartine at doses of 100, 300, 1000, 2000, and 3000 mg/kg by oral route. However, no obvious clinical indications were observed. Additionally, CD-1 mice were intraperitoneally treated with piplartine at a dose of 2.4 mg/kg for 6 days, and whole blood samples as well as vital organs (kidney, liver, lung and spleen) were collected for hematological and histopathological analyses, respectively. Piplartine-treated CD-1 mice remained healthy throughout the treatment time, and no significant differences between the vehicle-treated and piplartine-treated animals were observed (Raj et al., 2011). These results show that piplartine has only weak systemic toxicity.

3.5. Pharmacokinetic studies

Pharmacokinetic data are very important to better understand the *in vivo* pharmacological and toxicological effects of new compounds. However, up to now, little information about the absorption and metabolism of piplartine is available.

In concentration–time profiles, piplartine showed kinetic absorption of a monocompartimental model in mice and rats. Piplartine showed excellent oral bioavailability in mice. After intravenous injection, $T_{1/2}$ was 0.95 h and 1.60 h at the doses of 5 mg/kg and 10 mg/kg, respectively, where C_{max} was 905.62 μg/l and 1658.61 μg/l at the same doses. After oral administration, $T_{1/2}$ was 1.42 h and 0.84 h at the doses of 5 mg/kg and 10 mg/kg, respectively, where C_{max} was 884.31 μg/l and 201.42 μg/l at the same doses. The bioavailability of piplartine following oral administration at 5 mg/kg and 10 mg/kg were 76.39% and 50.08%, respectively (Raj et al., 2011). By intraperitoneal administration, a single dose of 50 mg/kg of piplartine in rats showed a plasma concentration of 1511.9 ng/ml, 418.2 ng/ml and 41.9 ng/ml for 30 min, 3 h and 24 h, respectively (Bezerra et al., 2011).

Biomimetic oxidation of piplartine catalyzed by iron(III) and manganese(III) porphyrins were also performed to mimic its metabolism by cytochrome P450 enzymes. It was observed that the oxidation of piplartine can take place both at the lactam ring and at the trimethoxyphenyl moiety of the molecule (Schaab et al., 2010).

4. Other biological effects of piplartine

Beyond the anticancer effect, piplartine has been reported to show multiple *in vitro* and *in vivo* pharmacological activities. These activities include antiplatelet aggregation, anxiolytic, antidepressant, antinociceptive, anti-atherosclerotic, antidiabetic, antibacterial, antifungal, leishmanicidal, trypanocidal, and schistosomicidal properties.

4.1. Antiplatelet aggregation effect

The results of piplartine studies as a crude drug for the treatment of the disorder of peripherally poor circulation are also promising. Piplartine showed an inhibitory effect on washed rabbit platelet aggregation induced by collagen, arachidonic acid, platelet-activating factor, and thrombin (Tsai et al., 2005; Park et al., 2007). A structure–activity relationship study indicates that the trimethoxybenzene or the dihydropyridone moiety in piplartine may contribute to its *in vitro* antiplatelet effect (Park et al., 2008). The mechanism underlying the piplartine antiplatelet action could be related to the action as a prostanoid thromboxane A_2 receptor antagonist and/or inhibition of cyclooxgenase activity, like the non-steroidal anti-inflammatory drugs (Iwashita et al., 2007; Fontenele et al., 2009). Next, the antinociceptive effect of piplartine was also examined in an acetic acid-induced abdominal constriction model. It was noteworthy to see that piplartine in-

Table 4
Summary of the reported pharmacological effects of piplartine.

Effect	Models	References
Cytotoxic and antitumor activities	Selective cytotoxicity over cancer cells presents only a weak activity in normal cells	Duh et al. (1990a, 1990b), Bezerra et al. (2005, 2006, 2007, 2008a), Tsai et al. (2005), Kong et al. (2008), Lin et al. (2007), Jyothi et al. (2009), Raj et al. (2011), Bokesch et al. (2011), Golovine et al. (2013), Adams et al. (2012)
	Cell cycle arrest in G_1 or G_2/M phase Induction of apoptotic cell death Induction of oxidative stress by inhibition of GSTp1 and CRB1 *In vivo* antitumor effects against Sarcoma 180 (murine tumor), B16-F10 (murine melanoma), EJ (human bladder carcinoma), MDAMB436 (human breast carcinoma), A549 (human lung carcinoma), and MMTV-PyVT (transgenic mouse model of spontaneous breast cancer) Enhancement of 5-fluorouracil activity in tumor cells in culture and in animal models	
Genotoxicity	Genotoxicity in Chinese hamster fibroblast lung-cultured cells (V79) using comet assay Mutagenecity in yeast *Saccharomyces cerevisiae* Negative results in *Salmonella typhimurium* TA 97a, TA 100, and TA 102 with and without metabolic activation (Ames test) Positive results in the *in vitro* micronuclei and chromosomal aberration assays using V79 cells Clastogenecity in mice using the bone-marrow micronucleus test	Bezerra et al. (2008b, 2009), Morandim-Gianetti et al. (2011)
Antiangiogenic effect	*In vivo* inhibition of the formation of blood vessels in tumors observed by the reduction of the expression of VEGF and CD31	Raj et al. (2011)
Antimetastatic effect	*In vitro* inhibition of the expression of Twist and N-cadherin, and disruption of the p120-ctnvimentin/N-cadherin complex *In vivo* inhibition of the spontaneous metastasis to lungs in a transgenic mouse model	Raj et al. (2011)
Antiplatelet aggregation action	*In vitro* studies using collagen-, arachidonic acid-, platelet-activating factor-, and thrombin-induced platelet aggregation *In vivo* study using collagen-induced platelet aggregation	Tsai et al. (2005), Park et al. (2007, 2008), Iwashita et al. (2007), Fontenele et al. (2009), Lee et al. (2010)
Antinociceptive effect	Acetic acid-induced abdominal constriction model in mice	Rodrigues et al. (2009)
Anxiolytic and antidepressant action	Elevated plus maze, open field, and forced swimming tests in mice	Felipe et al. (2007)
Anti-atherosclerotic effect	*In vivo* inhibition of atherosclerosis plaque formation *In vitro* inhibition of platelet-derived growth factor receptor pathways	Son et al. (2012)
Antidiabetic effect	*In vitro* inhibition of the recombinant human aldose reductase	Rao et al. (2012)
Antibacterial	*In vitro* antibacterial activity against clinical strains of *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus*	Naika et al. (2010)
Antifungal	*In vitro* antifungal activity against *Cladosporium sphaerospermun* and *Cladosporium cladosporioides*	Navickiene et al. (2000), Silva et al. (2002)
Leishmanidal	*In vitro* antileishmanial activity against *Leishmania donovani* *In vivo* antileishmanial activity in a hamster model of visceral leishmaniasis	Bodiwala et al. (2007)
Trypanocidal	*In vitro* trypanocidal activity against *Trypanosoma cruzi*	Cotinguiba et al. (2009)
Schistosomicidal	*In vitro* schistosomicidal activity against *Schistosoma mansoni*	Moraes et al. (2011, 2012a, 2012b)

duced a significant antinociceptive action in this visceral pain model in mice (Rodrigues et al., 2009).

In a proteomic study on antiplatelet activity of piplartine, 33 proteins decreased and 24 proteins increased after collagen induction was recovered with piplartine treatment. Among the 24 increased proteins after collagen treatment, 5 FLNA proteins, actinins, tubulins, progesterone-induced blocking factor, glutathione peroxidase, G-protein, and acid phosphatase were identified in the platelet cells. The decreased amount of PLS-1 protein (a coagulation factor XIII), three caldesmons, WDR1 protein, coronin B, adenylyl cyclase-associated protein 1, fibrinogen, biliverdinreductase A, lactate dehydrogenase, thiol-specific reductase, and peroxiredoxin 3 were identified in the platelet cells induced by collagen addition (Lee et al., 2010).

An antithrombogenic effect of piplartine was assessed in a pulmonary thrombosis model in mice. Piplartine (50 mg/kg) was orally administered 90 min before the administration of a mixture of collagen and epinephrine by intravenous route. Interestingly, the protection rate of piplartine on the thrombogenic effect of the mixtures was higher than that of aspirin (Lee et al., 2010). Taken altogether, these results strongly suggest that piplartine may be used as a potent medicine for treating diseases with disorders of platelet cell aggregation.

4.2. Anti-atherosclerotic activity

Atherosclerosis is a progressive disease characterized by the accumulation of lipids, fibrous materials, and mineral in the arteries (Riccioni et al., 2003). Piplartine was able to suppress *in vivo* atherosclerosis plaque formation (Son et al., 2012). Local treatment with piplartine significantly reduced atherosclerotic plaque formation as well as proliferation and nuclear factor-kappa B (NF-κB) activation in an *in vivo* setting. Piplartine also inhibits the migration and platelet-derived growth factor BB (PDGF-BB)-induced proliferation in aortic vascular smooth muscle cells. It inhibited PDGF-BB-induced PDGF receptor beta activation and suppressed downstream signaling molecules such as phospholipase Cγ1, extracellular signal-regulated kinases 1 and 2 and Akt. Moreover, piplartine significantly attenuated activation of NF-κB-a downstream transcriptional regulator in PDGF receptor signaling, in response to PDGF-BB stimulation.

4.3. Antidiabetic studies

In vitro antidiabetic potential of piplartine has been also assessed. Piplartine was found to inhibit recombinant human aldose reductase (Rao et al., 2012). Aldose reductase is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. Sorbitol accumulation in some tissues is involved in diabetic complications. Therefore, the use of inhibitors of aldose reductase is useful to reduce diabetic complications (Narayanan, 1993). A series of analogs prepared by the Michael addition of piplartine with indole derivatives displayed potent aldose reductase inhibitory activity. Piplartine and its analogs were also capable of preventing sorbitol accumulation in human red blood cells (Rao et al., 2012).

4.4. Anxiolytic and antidepressant effects

Felipe et al. (2007) performed a CNS pharmacological screening in piplartine-treated mice. The anxiolytic, sedative, muscle relaxant, anticonvulsant, and antidepressant effects were investigated. Piplartine presented significant anxiolytic and antidepressant activities as observed in the elevated plus maze, open field, and forced swimming tests in mice. In the elevated plus maze test, the increased number of entrances and the time of permanence in the open arms induced by piplartine were completely blocked by the previous administration of flumazenil, which suggests the involvement of benzodiazepine type receptors. However, the exact mechanism of action is not yet understood.

4.5. Antimicrobial studies

The antimicrobial effects of piplartine were also evaluated. An erroneous study was performed by Lokhande et al. (2007), where piplartine and piperlongumine were considered different compounds, but in fact they are the same compound as previously discussed. As the identification of the tested compounds was not appropriate, the results have no scientific value. In another work, piplartine demonstrated promising antibacterial activity against clinical strains of *Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus* (Naika et al., 2010).

In vitro antifungal activity was also found for piplartine as determined by direct bioautography against *Cladosporium sphaerospermun* and *Cladosporium cladosporioides* (Navickiene et al., 2000; Silva et al., 2002). The fungicidal activity of piplartine against six phytopathogenic fungi – *Pyricularia oryzae*, *Rhizoctonia solani*, *Botrytis cineria*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis* – was also tested using a whole plant method *in vivo*. On the other hand, no activity was observed from treatments with piplartine (Lee et al., 2001). Additionally, suppression of mycotoxin production by piplartine has been found in many *Aspergillus* species (Lee et al., 2002, 2007). The mode of action of piplartine against mycotoxin production or as an antimicrobial agent is not known.

Piplartine has shown potential activity against species that cause neglected tropical diseases. For instance, piplartine treatment displayed significant *in vitro* antileishmanial activity against *Leishmania donovani*, and it reduced the parasitic burden and spleen weight *in vivo* using a hamster model of visceral leishmaniasis at a dose of 30 mg/kg (Bodiwala et al., 2007). Similarly, piplartine also showed *in vitro* trypanocidal activity against epimastigote forms of *Trypanosoma cruzi* (Cotinguiba et al., 2009) and *in vitro* schistosomicidal activity against larval- and adult-stage of *Schistosoma mansoni* (Moraes et al., 2011, 2012a). Moreover, piplartine combined with the antimicrobial peptide dermaseptin showed synergistic effect against *S. mansoni* schistosomula and adult worms (Moraes et al., 2012b). However, no larvicidal activity against *Aedes aegypti* mosquito larvae was observed for piplartine (Yang et al., 2002). The mechanism of the piplartine-induced antiparasitary effect remains to be determined.

5. Conclusion

In the present study, an attempt was made to address the anticancer properties of piplartine. Piplartine is an active herbal component present in important ethnopharmacological plants used in the Indian Ayurvedic system of medicine and the folk medicine of Latin America. *In vitro* and *in vivo* preclinical research has shown multiple biological activities for piplartine, such as cytotoxic, genotoxic, antitumor, antiangiogenic, antimetastatic, antiplatelet aggregation, anxiolytic, antidepressant, antinociceptive, anti-atherosclerotic, antidiabetic, antibacterial, antifungal, leishmanicidal, trypanocidal, and schistosomicidal properties. Table 4 summarizes the reported pharmacological effects of piplartine; among them, its anticancer effect is the most promising. Its molecular pathway includes induction of oxidative stress selectively in cancer cells by inhibition of GSTp1 and CRB1, which are novel targets of cancer therapy. As an alternative strategy to killing tumor cells, piplartine can be used to induce genotoxicity. In addition, piplartine has excellent oral bioavailability and inhibits tumor growth in mice. Furthermore, this compound demonstrated weak systemic toxicity that makes it suited as a novel anticancer agent. We conclude that piplartine is effective for use in cancer therapy and its safety using chronic toxicological studies should be addressed to support the viability of clinical trials.

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