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Mechanistic insights into dimethyl cardamonin-mediated pharmacological effects: A double control of the AMPK-HMGB1 signaling axis

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ABSTRACT

Dimethyl cardamonin (DMC) has been isolated from diverse plants, notably from *Cleistocalyx operculatus*. We have reviewed the pharmacological properties of this natural product which displays anti-inflammatory, anti-hyperglycemic and anti-cancer properties. The pharmacological activities essentially derive from the capacity of DMC to interact with the protein targets HMGB1 and AMPK. Upon binding to HMGB1, DMC inhibits the nucleocytoplasmic transfer of the protein and its extracellular secretion, thereby blocking its alarmin function. DMC also binds to the AMP site of AMPK to activate phospho-AMPK and then to trigger downstream signals leading to the anti-inflammatory and anti-hyperglycemic effects. AMPK activation by DMC reinforces inhibition of HMGB1, to further reduce the release of the alarmin protein, likely contributing to the anticancer effects. The characterization of a tight control of DMC over the AMPK-HMGB1 axis not only helps to explain the known activities of DMC but also suggests opportunities to use this chalcone to treat other pathological conditions such as the acute respiratory distress syndrome (which affects patients with COVID-19). DMC structural analogues are also evoked.

1. Introduction

Dimethyl cardamonin, also known as 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC, Fig. 1) is a natural product found in diverse plant species. This chalcone derivative has been isolated mainly from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr (Fig. 1), also known as *C. nervosum* Kosterm., *Syzygium nervosum* and *Syzygium operculatum* (Roxb.) Nied (Myrtaceae) [1]. It is a tree 10–15 m tall, originating from tropical Asia. This plant is a rich source of bioactive compounds including acetophenones, chalcones, flavones and triterpenoids [2–6]. DMC has been isolated also from totally distinct plants, such as *Cyclosorus parasiticus* [7], *Myrica serrata* [8], *Campomanesia reitziana* [9], *Eugenia aquea* [10], *Luma gayana* (Barn.) Burret [11] and the shrub *Psorothamnus polydenius* (*S. Watson*) Rydb., also known as *Dalea polyadenia* (or the smoke bush), which grows in the desert of the Colorado plateau and other desertic regions in North America [12].

DMC is a small molecule ($C_{18}H_{18}O_4$), sparingly soluble in water which can be easily isolated from plants or obtained by chemical synthesis and derivatized [13–15]. DMC has revealed different types of pharmacological activities, including potent anti-inflammatory effects and significant antitumor activities both in vitro and in vivo. Here we have reviewed the pharmacological properties of DMC, to help defining the molecular basis of these effects. A common molecular basis has been identified. This is the first time that a central implication of the AMPK-HMGB1 axis (Fig. 2) in the mode of action of DMC is reported. Our observation raises novel opportunities for the use of DMC.

2. Antioxidative activity

DMC is an antioxidant compound. In fact, an essential oil of *C. operculatus* presents antioxidative properties and suppresses the expression of pro-inflammatory cytokines such as TNFα and IL-1β, and thus exerts an anti-inflammatory effect [16]. This essential oil can be used topically to treat burn wounds [17]. DMC effectively protects cells from oxidative injuries. DMC has shown hepatoprotective effects against hydrogen peroxide-induced liver injury by alleviating oxidative stress and apoptosis process in human hepatocytes L02 cells [18]. The gastroprotective activity of DMC and DMC-containing extracts is

Abbreviations: AMPK, AMP-dependent protein kinase; DMC, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone; HMGB1, high-mobility group box; ROS, reactive oxygen species.

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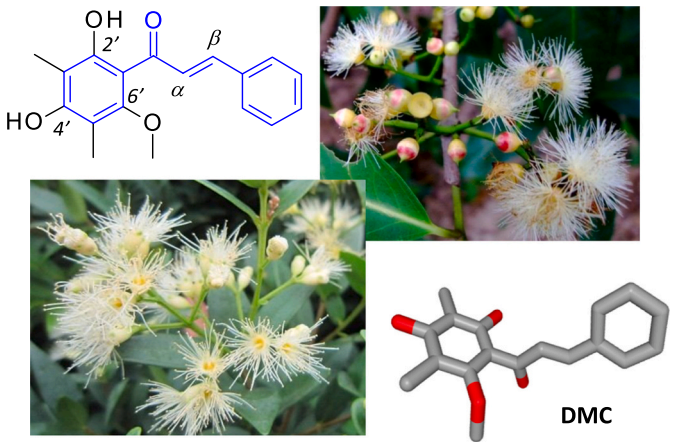

Fig. 1. Structure and conformation of 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC). The chalcone motif is shown in blue. DMC is a small molecule ($C_{18}H_{18}O_4$; Mw: 598.3 Da), with a solvent accessible surface aera (SASA) of 575.7 Å^2 (hydrophobic SASA: 238.8 Å^2 and hydrophilic SASA 100.6 Å^2) and with a limited aqueous solubility (log S: −4.5 and log P (octanol/water): 3.9). The drug properties were calculated with the BOSS 4.9 software. Illustrations of the flower buds of *Cleistocalyx operculatus*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

knowledgeable [9,19].

3. Anti-inflammatory activities

Recently, DMC has revealed potent anti-inflammatory activity in a conventional model of lipopolysaccharide (LPS)-induced endotoxic shock in macrophage cells and in mice. The pharmacological effects were attributed to a drug-induced activation of the Nrf2/HO-1 pathway (Fig. 2), in line with the antioxidative action mentioned above. But in parallel, DMC was also shown to induce intracellular reactive oxygen species (ROS) production, thereby enhancing the phosphorylation levels of proteins p38 and c-Jun NH_2-terminal kinase (JNK) in macrophage-like RAW264.7 cells [20]. In these cells, DMC potently attenuates LPS-induced inflammatory response via inhibition of Nuclear Factor kappa B (NFκB) activation and a potent inhibition of cytokines expression, such as tumor necrosis factor-α (TNFα), interleukin (IL)-1β and IL-6 [21,22]. But depending on the experimental conditions and cell types, inhibition or activation of nuclear factor erythroid 2-related factor 2 (Nrf2) have been reported with DMC. Activation of Nrf2 was observed using macrophage-like cells in culture, whereas Nrf2 inhibition was seen with cancer cells [20,23]. Interestingly, a marked inhibition of Nrf2 expression was observed upon treatment of cancer cells with DMC during the first 8 h of treatment, followed by an increased expression to recover a quasi-normal expression after 24 h, probably because of an autoregulatory feedback mechanism [23].

In a mouse model of LPS-induced endotoxin shock, the anti-inflammatory activity of DMC was characterized by a suppression of the secretion of TNFα, IL-6 and IL-1β in the blood serum, likely due to the drug-induced blockade of NFκB activation [21]. Importantly, the drug was also found to suppress LPS-stimulated secretion and nucleocytoplasmic translocation of the protein high-mobility group box 1 (HMGB1), which is a prototypical alarmin protein (or damage-associated molecular pattern, DAMP) [22]. DMC dose-dependently inhibited the phosphorylation of HMGB1 and its interaction with the enzyme Protein Kinase C (PKC). The drug efficiently blocked the pro-inflammatory activity of HMGB1 [24]. This key property is further discussed below, as HMGB1 is considered a direct molecular target of DMC.

4. Anticancer activities

DMC can inhibit the proliferation of diverse cancer cell lines and in most cases, the drug was found to affect cell cycle dynamic and to trigger apoptotic cell death via the mitochondrial pathway, with caspases activation. Induction of autophagy by DMC, associated with proliferation arrest, has also been reported. The effects observed in vitro are collated in Table 1. Comparison are not easy because the methods and experimental conditions used can vary from one study to another.

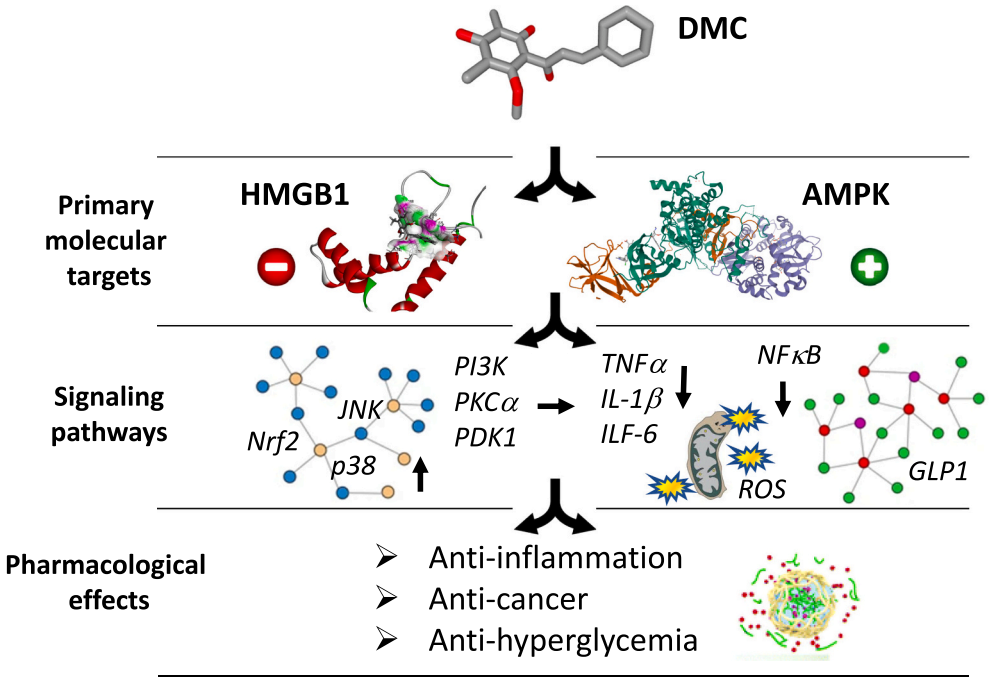

Fig. 2. Illustration of the mechanism of action of DMC. The drug bind to the two indicated protein targets to negatively regulates HMGB1 and positively regulates AMPK. These interactions trigger different pathways, through the indicated signaling molecules, leading to the indicated pharmacological effects.

Table 1
Activities of DMC against cancer cell lines in vitro.

Cancer cell or tumor types	Active drug concentrations	Observed effects	References
Lung cancer cells (A549)	IC_{50}: 19.6 μM (@48 h)	Cell growth inhibition and induction of apoptosis, with caspases activation.	[10]
Hepatocellular carcinoma cells, including multi-drug resistant cells (BEL-7402 and BEL-7402/5-FU)	IC_{50}: 34.7 and 40.6 μM, with sensitive (BEL-7402) and resistant (BEL-7402/5-FU) cells (@48 h)	Inhibition of cell growth and reversal of resistance to cytotoxic drugs (5-FU and doxorubicin). Induction of apoptotic cell death.	[26]
	10 μM DMC enhanced the sensitivity of the cancer cells to the drug 5-FU by 3.8 times.	DMC reduced drug efflux to reverse drug resistance by suppressing the Nrf2/ARE signaling pathway.	[23]
	IC_{50}: 44.2 μM (@48 h)	Cell growth inhibition, production of ROS, induction of G1 cell cycle arrest (through downregulation of cyclin D1 and CDK4) and activation of the mitochondria-dependent apoptotic pathway (with upregulation of p53 and inhibition of NFκB nuclear translocation).	[99]
Hepatocellular carcinoma cells (SMMC-7721)	Cellular effects measured upon treatment with 20 μM DMC (@48 h).	Drug-induced inhibition of cell proliferation and telomerase activity, and proteins expression (c-myc and hTERT). Induction of apoptosis	[100]
Pancreatic cancer cells (PANC-1 and MIA PACA2)	IC_{50}: 10–12 μM (@48 h)	Cell growth inhibition and induction of apoptosis, with caspases activation. Down-regulation of Bcl-2, up-regulation of Bax. Release of mitochondrial cytochrome *c*.	[101]
Human leukemia cells (K562)	IC_{50}: 14.2 μM (@48 h)	Cell growth inhibition and induction of apoptosis. Down-regulation of Bcl-2. Inhibition of colony formation.	[102]
Colon cancer cells (HCT116, LOVO)	IC_{50}: about 25 μg/mL with HCT116 (@48 h)	Time- and concentration-dependent antiproliferative activity, and induction of autophagy (up-regulation of LC3-II protein marker).	[103]
Colon cancer cells (HT29, HCT116)	IC_{50}: 12 μg/mL (@48 h)	Inhibition of cell growth and cell migration.	[104,105]
Liver cancer cells (SMMC-7721)	IC_{50}: 32.3 μM (@48 h)	Cell growth inhibition and induction of apoptosis, associated with the production of reactive oxygen species and caspases activation.	[106]
Breast cancer cells (MDA453)	IC_{50}: 24.5 μg/mL (@72 h)	Cell growth inhibition and induction of apoptosis, associated with caspases activation. Inhibition of the phosphorylation of erbB2, MAPK and AKT. Up-regulation of Bim.	[107]
Multiple cell lines: liver cancer cells (SMMC-7721), pancreas cancer cells (8898), cervical cancer cells (HeLa), lung cancer cells (SPC-A-1, 95-D), gall bladder carcinoma cells (GBC-SD).	IC_{50}: 31 to 85 μM (@48 h)	More efficient cell growth inhibition with SMMC-7721 liver and 8898 pancreatic cancer cells. Induction of hypodiploid cells (sub-$G_{0/1}$ population) suggesting apoptosis.	[108]
Multiple cell lines: breast cancer cells (MCF-7, MDA-MB-231), lung cancer cells (A549), hepatocarcinoma cells (HepG2), pancreatic cancer cells (SW 1990), leukemia cells (ALL-SIL)	IC_{50}: 2.8 to 9.7 μM (@72 h)	Cell growth inhibition and apoptosis. HepG2 cells were more sensitive than the other cell lines.	[7]

However, it seems that pancreatic cancer cells are more sensitive to DMC than colon and breast cancer cells for examples but overall, there is no marked cell type selectivity. DMC is not a potent cytotoxic agent, but it triggers cancer cell death and importantly it sensitizes cancer cells to chemotherapeutic drugs like doxorubicin and 5-fluorouracil (5-FU), both in vitro and in vivo. For example, a significant reduction of tumor growth was observed when combining DMC and the topoisomerase 2 inhibitor doxorubicin in a drug-resistant KB-A1 tumor xenograft model in vivo, without apparent toxic effect, whereas the two drugs individually were inactive. DMC dose-dependently facilitated the penetration and accumulation of doxorubicin in the tumor and decreased the gene expression of *MDR-1* which encodes for the classical efflux pump P-glycoprotein [25]. A DMC-induced reversal of the multidrug resistance phenotype has been observed also using multidrug-resistant human hepatocellular carcinoma cells (BEL-7402/5-FU) in vitro and in vivo [26,27]. In this case DMC was found to decrease both the mRNA and protein expressions of MRP1. Here again, DMC significantly enhanced the tumor accumulation of the cytotoxic drug 5-FU, leading to a prominent antitumor effect, with a reduction of the tumor volume by 72%, whereas 5-FU or DMC alone were very weakly active [27].

In 2005, another in vivo study reported the anticancer activity of DMC in a mouse xenograft model of human liver cancer SMMC-7721. When the drug was given intraperitoneally at 50, 100 and 150 mg/kg, the growth of the tumor was markedly reduced, in a dose-dependent manner, and the percentage of hypodiploid cells increased accordingly, to reach about 30% of the cancer cell population. The drug was

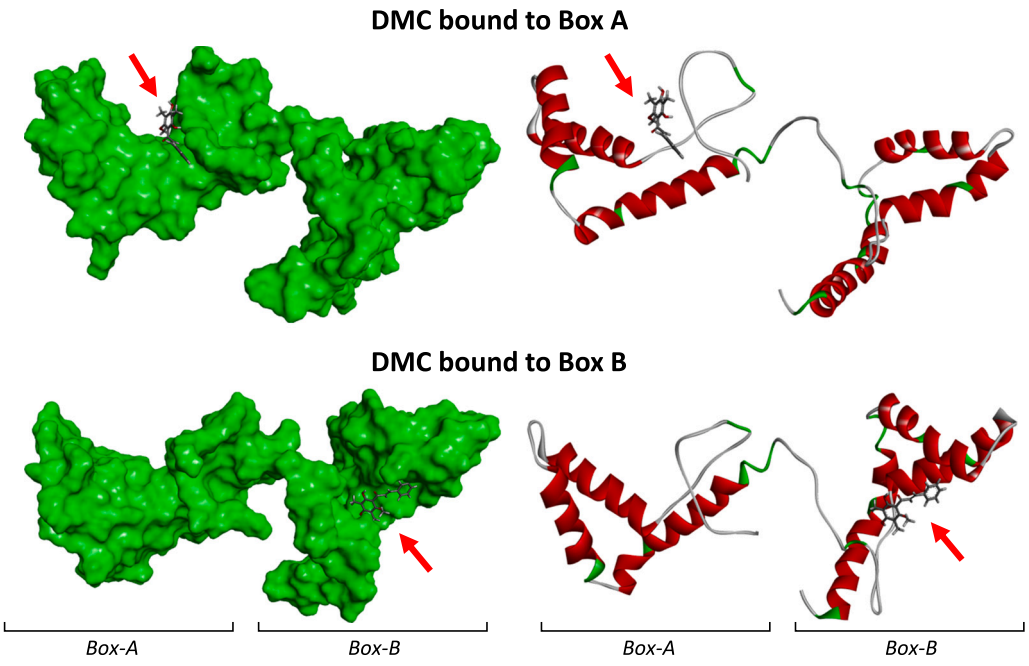

Fig. 3. Molecular models of the interaction between DMC and HMGB1. The drug is bound to Box-A or Box-B of HMGB1, in close interaction with the α-helices of each box. The protein surface is shown in green (left) or as a ribbon model in red (right) to illustrate the three helices of each box. The 3D structure of HMGB1 was retrieved from the Protein Data Bank (www.rcsb.org) under the PDB code 1HME. Molecular docking experiments were performed with the GOLD software (Cambridge Crystallographic Data Centre, Cambridge, UK). The drug-HMG structures have been optimized using a classical Monte Carlo conformational searching procedure as described in the BOSS software [96]. The ligand is defined as flexible during the docking procedure. Up to 30 poses that are energetically reasonable were kept while searching for the correct binding mode of the ligand. The decision to keep a trial pose is based on ranked poses, using the PLP fitness scoring function (which is the default in GOLD version 5.3 used here). In addition, an empirical potential energy of interaction ΔE for the ranked complexes is evaluated using the simple expression ΔE(interaction) = E(complex) - (E(protein) + E(ligand)). For that purpose, the Spectroscopic Empirical Potential Energy function SPASIBA and the corresponding parameters were used [97,98]. Molecular graphics and analysis were performed using the Discovery Studio 2020 Client software, Dassault Systemes Biovia Corp.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found to be well tolerated, with an LD_{50} of 3800 mg/kg (lethal dose 50%) [28,29]. Clearly, DMC appears to be relatively safe and functions as a potent anticancer agent and a chemosensitizer, enhancing the efficacy of chemotherapeutic drugs and their capacity to trigger apoptosis in vivo [23]. DMC acts directly on proliferating tumor cells but also on the tumor vasculature. The drug was found to inhibit the growth of human vascular endothelial HDMEC cells in vitro and to reduce the tumor vessel density in vivo. The anti-angiogenic activity of DMC was linked to the inhibition of the KDR tyrosine kinase (kinase insert domain receptor) [30]. Here again, a potent antitumor activity with a complete and dose-dependent suppression of tumor growth was observed upon treatment of Bel7402 hepatocarcinoma xenografted mice with DMC at doses ranging from 5 to 20 mg/kg ip [30].

5. Protection from cell damages

In parallel to its cell growth inhibitory function observed with cancer cells, DMC can also exhibit cell protection activity against chemical- or drug-induced damages. It is not uncommon for a chalcone derivative to observe pro-apoptotic and anti-apoptotic effects, depending on the conditions and cell types. Recently, the same property was described with the prenyl-flavonoid icaritin [31]. Similar natural products such as naringenin, quercetin and rutin can exert cytoprotective effects to reduce the oxidative stress induced by exogenous toxic chemicals, and function as anticancer agents [32–35]. DMC has been found to inhibit pancreatic MIN6 β-cell apoptosis, decreasing the endogenous production of reactive oxygen species (ROS) and consequently inhibiting apoptosis [36]. A cytoprotective activity of DMC was evidenced with rat neuronal PC12 cells and with L02 human hepatocytes treated with hydrogen peroxide. The drug scavenged ROS formation and reduced lipid peroxidation [37,38]. Similarly, DMC protects cells from acute liver injury induced by carbon tetrachloride, decreasing the oxidative damages [32]. A dysregulation of the ROS equilibrium, one way or another, causes major cellular malfunctions. Both the antioxidative and anti-inflammatory properties of DMC contribute to the cell protective functions [19]. The cytoprotective effects of chalcones is well recognized. This property can be exploited to derive DMC-containing skin care products, antiaging or hepatoprotective products [19,39].

6. Other pharmacological effects: diabetes prevention

Diverse other pharmacological effects have been reported with DMC, including mild spasmolytic [40,41] and gastro-protective effects linked to its antioxidant and anti-inflammatory properties [4]. Importantly, DMC presents anti-hyperglycemic effects. The drug has the capacity to increase insulin secretion by simulating the effect of glucagon-like peptide 1 (GLP-1) and enhancing the expression of the receptor GLP-1R [42]. As such, DMC restricts the glucotoxicity and its used in the management of hyperglycemia has been proposed [43]. At 1 mg/20 g body weight by oral administration, DMC was found to lower blood

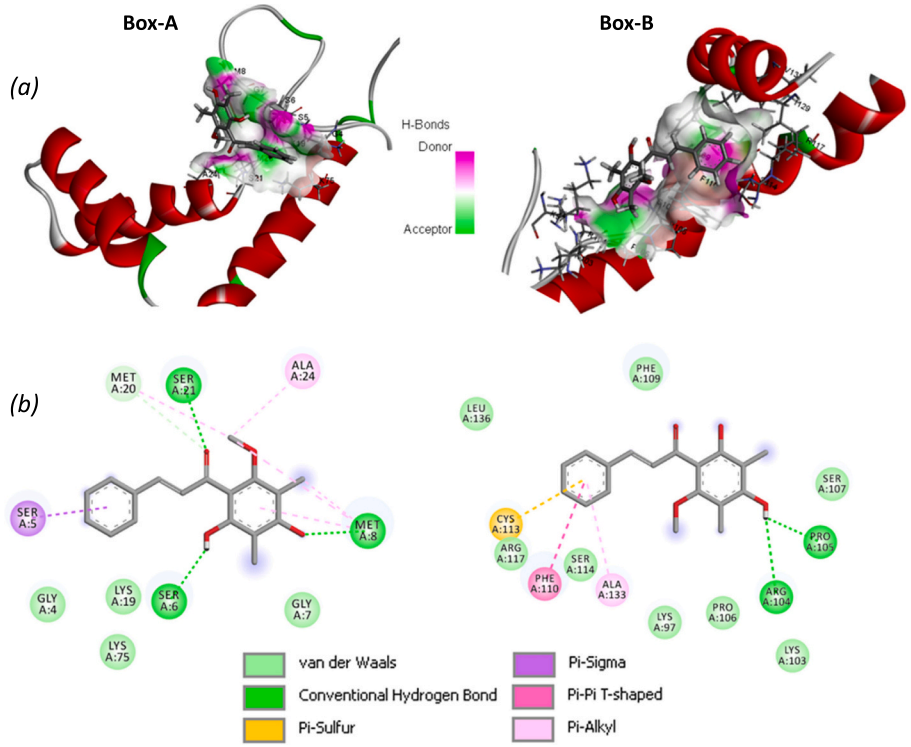

Fig. 4. (a) Detailed view of DMC bound to Box-A or Box-B of HMGB1, with the drug facing the H-bond donor and acceptor sites. (b) Binding map contacts for DMC bound to the HMGB1 boxes A and B. In each case, the color code is indicated.

glucose level in alloxan-induced diabetic mice [44]. The drug can also inhibit pancreatic α-amylase (but the IC_{50} is relatively high, 43 μM), and suppresses glucose transport in intestine [43]. But the drug presents a paradoxical effect on lipid accumulation: DMC promoted lipid production at a low dose but suppressed the differentiation from preadipocytes to adipocytes at high concentrations [45]. In fact, the effect of DMC on adipogenesis and anti-glucotoxic effects in pancreatic β-cells derives from its capacity to activate the AMPK pathway (see below) and consequently to increase glucose uptake and fatty acid oxidation [46]. As an AMPK activator, DMC could be useful to prevent the progression of hyperglycemia, and thus it may serve as a lead compound for a new class of drugs active against obesity and type 2 diabetes. Similar effects have been reported with structurally close chalcone derivatives such as 2-bromo- and 2-iodo-4′-methoxychalcones [47].

7. Molecular targets of DMC

7.1. Binding to and inhibition of HMGB1

The secretion of protein HMGB1 is usually a marker of tissue damages and inflammation. HMGB1 is a ubiquitous protein which plays diverse functions both in the nucleus of cell (as a guardian of the genome and DNA chaperone) and in the cytoplasm. HMGB1 is a major damage-associated molecular pattern molecule (DAMP) which interacts with other proteins such as the receptor for advanced glycation end products (RAGE) and Toll-like Receptors (TLR-2 and -4). It is considered an interesting target for the treatment of different inflammatory diseases, neurodegenerative disorders and cancers [48–50]. Inhibitors of HMGB1 are actively searched.

It has been reported that DMC can inhibit HMGB1 secretion and block the pro-inflammatory activity of the protein in a model of LPS-stimulated hepatic inflammation. Not only the drug dose-dependently inhibited the mRNA expression of *HMGB1* and the extracellular protein release from activated liver macrophages but it also reduced the phosphorylation of downstream signaling proteins such as PI3K, PKCα and PDK1 [24]. The authors showed that the drug blocked the nucleo-cytoplasmic translocation of HMGB1 through the suppression of HMGB1 phosphorylation. A direct interaction of DMC with HMGB1 was proposed based on molecular modeling using a small protein motif (Box-B only) of HMGB1. The docking analysis suggested that DMC binds to Box-B via H-bonding interactions with specific amino acid residues (notably Arg-91, Ser-94, Lys-90) and multiple hydrophobic contacts [24]. Subsequently, the same authors demonstrated that DMC had anti-inflammatory effects through reducing HMGB1 expression (and expression of both early cytokines TNF-α, IL-1β, and IL-6) via interfering with the PI3K-PDK1-PKCα signaling pathway [22]. Therefore, the initial direct interaction of DMC with HMGB1 seems to be a key factor that triggers the blockade of a signaling cascade leading to inflammation and cancer cell proliferation. There is no formal demonstration, as yet, that the blockade of the nuclear export of HMGB1 by DMC contributes to its anticancer effects. But it is known that inhibiting the cytoplasmic location of HMGB1 can reverse resistance to the anticancer drugs like cisplatin and inhibit tumor growth [51,52]. Inhibition of HMGB1 expression is a key point to inhibit tumor growth and induce apoptosis [53].

We have investigated further the potential binding of DMC to HMGB1 by means of molecular modeling, based on the crystallographic structure of the HMGB1 protein deleted from its C-terminal acidic tail but containing the two HMG boxes A and B in tandem (PDB code: 1HME). The two boxes contain each about 75 amino acids and have three α-helices defining two arms. Boxes A and B are separated by a flexible linker. We found that each box offers a suitable binding site for DMC but nevertheless, the drug appears to form a more stable complex with Box-A ($\Delta E = -40.3$ kcal/mol) than with Box-B ($\Delta E = -31.8$ kcal/mol). Molecular models of the drug-protein complexes are shown in Fig. 3. There is a significant difference of potential energy of interaction (ΔE) but also a difference of free energy of hydration ($\Delta G = -17.9$ kcal/mol for DMC bound to Box-A vs. −13.5 kcal/mol for DMC bound to Box-B). However, it seems clear that the chalcone derivative can bind to both boxes, establishing a range of molecular contacts with several key amino

acid residues of HMGB1 protein via H-bonding, van der Waals contacts and stacking interactions. Each box provides a cavity for DMC binding and the drug-HMG complexes are stabilized by hydrophobic and H-bonding interactions (Fig. 4). Thus, our own in silico analysis of the interaction of DMC with HMGB1 corroborates the initial observations made with a simpler model of HMGB1 Box-B [24]. At present, the direct binding of DMC to HMGB1 is based only on molecular models; it will necessitate an experimental validation. But the interaction is fully plausible and coherent with the observed anti-inflammatory and anti-cancer activities.

Parenthetically, in our modeling analysis we found that DMC occupies the same binding pocket that the well-characterized drug inflachromene which has been shown to bind to HMGB1 and to down-regulate its proinflammatory functions and to reduce neuronal damages in vivo [54]. As a HMGB1 regulatory agent, this drug is considered as an interesting lead for the design of molecules effective against sepsis [55] and to prevent the loss of pancreatic islet grafts in patients suffering from severe type 1 diabetes mellitus [56]. Therefore, with a similar mode of action, the use of DMC in these indications should be evaluated as well.

7.2. Binding to and activation of AMP-dependent protein kinase (AMPK)

The serine/threonine protein kinase AMPK (AMP-dependent protein kinase) is a central regulator implicated in multiple metabolic pathways. Notably, AMPK is a master regulator of cellular energy metabolism and it is one of the primary targets of the antidiabetic drug metformin, which also exhibits antitumor properties [57,58]. AMPK is a three-subunit protein kinase with two alternative kinase domain-containing subunits (α_{1-2}), two alternative carbohydrate-binding subunits (β_{1-2}), and three alternative AMP/ADP/ATP-binding subunits (γ_{1-3}). The protein functions as a central signaling hub that phosphorylates and regulates numerous targets [59]. Via the binding of AMP, AMPK has the capacity to adjust its enzymatic activity in order to sense the energy status of the cell and to maintain the balance between ATP production and consumption in eukaryotic cells. AMP binding induces a conformational switch that regulates the enzyme activity [60]. Novel activators of AMPK are actively searched [61,62].

Like metformin, DMC is capable of activating AMPK. DMC was found to bind directly to AMPK and to function as an agonist, thereby stimulating fatty acid oxidation in myotubes and inhibiting adipogenesis. In contrast to metformin which indirectly activates AMPK by increasing the AMP/ATP ratio through the inhibition of mitochondrial respiratory chain complex 1, DMC appears to activate AMPK directly by binding to the protein subunits and inducing the phosphorylation of the enzyme [46]. A direct interaction between DMC and the enzyme was evidenced by surface plasmon resonance, providing a KD value of 1.4×10^{-7} M, about 3.7-fold lower than that measured with AMP (Ka: 3.9×10^{-6} M). The affinity of DMC for the AMPK is thus superior to that of AMP and the two entities apparently bind the same site on the enzyme [46].

Cardamonin has been shown to increase phosphorylation of AMPK in cancer cells [63]. The capacity of DMC to regulate AMPK activity likely contributes to its anti-inflammatory and anticancer properties. For example, the chalcone phloretin exhibits anticancer effects via AMPK activation [64]. Other anticancer and/or anti-inflammatory chalcone derivatives have been shown to activate AMPK, such as isoliquiritigenin [65], 2-bromo-4′-methoxychalcone [47], the prenylated chalcone xanthohumol [66], the kava chalcone flavokawain B [67] and other chalcones [68–70]. An interesting case is that of licochalcone A which displays antitumor and anti-inflammatory properties, like DMC [71]. There are numerous natural products known to activate AMPK but they generally function in an indirect manner and therefore they may cause off-target effects [72]. Only a few compounds are known to interact directly with AMPK, such as the drug salicylate, the synthetic allosteric activator A-769662 and a few others [73–75].

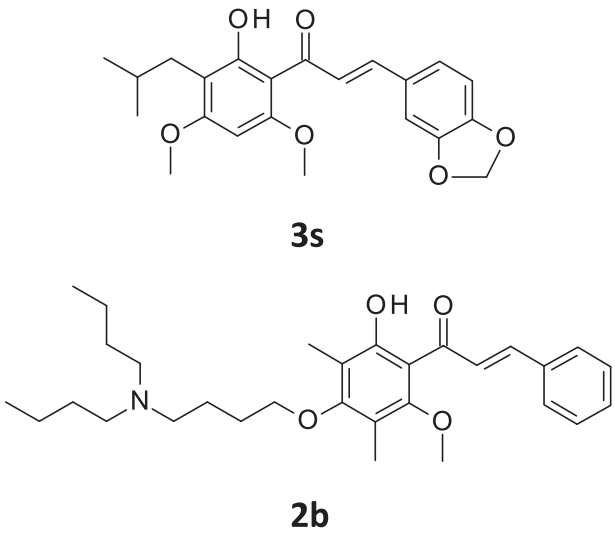

Fig. 5. Structure of two synthetic DMC derivatives. Compound 3 s exhibits neuroprotective property [81]. Compound 2b is a potent anticancer agent [82].

7.3. Binding to other proteins

Recently, an interaction of DMC with caspase-3 has been proposed based on a molecular modeling analysis. Different potential drug-protein H-bond interactions were identified, as well as π-stacking interaction between the distal phenyl ring of DMC and a methionine residue of the protein [5]. The proposed molecular arrangement is reminiscent to that mentioned above with HMGB1. At this point, we can also mention the DMC derivative designated HNS10 which has been proposed as a ligand of the estrogen receptor ERα on the basis of a molecular modeling study, to combat estrogen receptor-positive breast cancer [76].

8. Structural analogues

The interesting pharmacological properties of DMC has encouraged the design of derivatives, to define the structure-activity relationships and to obtain more potent compounds, not only anticancer agents but also anti-infective agents. The chalcone scaffold offers large possibilities to modify the structure or introduce substituents, to generate active compounds. We will not refer here to the many chalcones synthesized in recent years; there are comprehensive reviews for that [77–80]. Nevertheless, it is worth to mention a few direct analogues of DMC such as the compounds **3s** incorporating a prenyl moiety (Fig. 5) which has revealed a marked neuroprotective activity against oxygen-glucose deprivation/reoxygenation-induced apoptosis in SH-SY5Y cell lines, by decreasing the expression of cleaved caspases-3 and -9, and proteins Bax and p53 [81]. There are also synthetic or semi-synthetic derivatives of DMC with improved anticancer properties. For examples, derivatives **2b** (Fig. 5) incorporating a non-cyclic tertiary amine group into the 4′-OH has shown superior activity to DMC against HeLa cells, including Hela/Tax cells resistant to Taxol. This dibutyl-amino DMC derivative is more water-soluble than DMC and showed a considerable selectivity toward cancer cells over normal cells and a marked synergistic activity with Taxol [82]. MDC represents an interesting scaffold for the design of anticancer derivatives.

9. Discussion

The pharmacological effects and mechanism of action of dimethyl cardamonin (DMC) have been much less investigated than those of its close analogue cardamonin, which is also an anti-inflammatory and anticancer agent. Cardamonin essentially targets the signaling

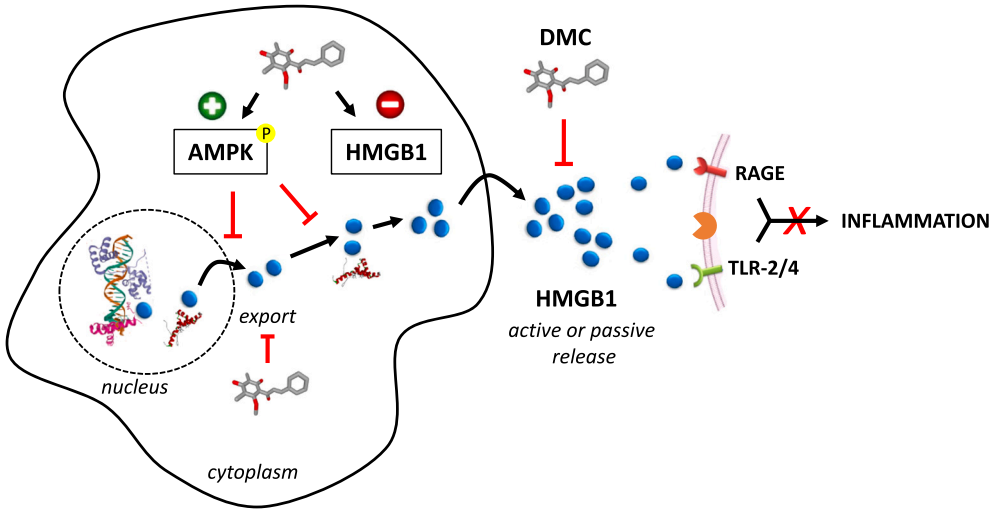

Fig. 6. Illustration of the mode of action of DMC via the AMPK-HMGB1 axis. The drug binds to HMGB1, inhibiting its transport from the nucleus to the cytoplasm of cells and blocking its extracellular secretion. DMC also binds to APMK, to activate its phosphorylation and activated AMPK represses HMGB1. The direct and indirect effects of DMC block the binding of HMGB1 to its receptors, such as TLR-2/4 and RAGE, and subsequently induce an anti-inflammatory action.

molecules mTOR, NFκB, Akt, STAT3, Wnt/β-catenin and COX-2 [83]. There is no indication that cardamonin binds to or interfere with HMGB1 or AMPK, as it is the case with DMC. The two related products may thus exploit different signaling routes to exert their pharmacological actions. Nevertheless, cardamonin displays also marked anticancer effects in vitro and like DMC, it enhances the activity the chemotherapeutic drugs like 5-FU and cisplatin [84,85]. But the in vivo activity of cardamonin is limited, probably because the drug is rapidly metabolized and eliminated [86], and apparently much less pronounced than that of DMC. This is a characteristic trait of DMC, with a relatively modest potency in vitro but a pronounced activity in vivo (at least when the drug is injected intraperitoneally). There is no data available on the metabolization of DMC in vivo. The compound is likely hydroxylated and glucuronidated, as it is the case for similar compounds such as cardamonin and flavokawains [87,88]. The pharmacokinetic properties of DMC are not known.

Broadly speaking, the mechanism of action of DMC seems to rely, on the one hand on a regulation of the ROS equilibrium in cells and on the other hand on the direct targeting of two main proteins HMGB1 and AMPK. The modulation of the ROS equilibrium is a general, non-specific effects encountered with many chalcones, which frequently display cell-protective functions. More interestingly, DMC selectively interferes with the correct functioning of AMPK and HMGB1, activating the former, inhibiting the later (Fig. 6). These observations thus define the principal signal axis responsible for the pharmacological activities of DMC. Binding of the drug to AMPK favors its phosphorylation and thus activates its functions. DMC binding to HMGB1 blocks its pro-inflammatory activity, probably by inhibiting the nucleocytoplasmic transfer of the protein and its secretion. Once released to the extracellular space, HMGB1 acts as a proinflammatory cytokine that triggers inflammatory reaction. The anti-inflammatory activity likely comes from the blockade of the export of HMGB1. In fact, AMPK and HMGB1 are two central effectors in cells, implicated in multiple metabolic processes. It is not surprising thus that the modulation of this major signaling axis by DMC results in diverse pharmacological effects, from inflammation, to cancer and anti-hyperglycemia.

Interestingly, there is close relationship between AMPK activation and inhibition of HMGB1 release. Different studies have revealed that activation of AMPK with specific drugs, like the allosteric AMPK activator A769662, reduces the release of HMGB1 [89]. The knockdown of HMGB1 with specific siRNA was found to be associated with enhanced activation of AMPK in macrophages incubated with the pharmacological AMPK activator AICAR [90]. Metformin has been shown to improve survival in a mouse model of lethal endotoxemia by inhibiting HMGB1 release and AMPK activation was implicated as one of the mechanisms contributing to this inhibition of HMGB1 secretion [91]. Certain anti-inflammatory natural products, like the triterpenoid mogroside IIIE, retinoic acid or 13-ethylberberine, can activate AMPK to reduce the release of HMGB1 [92–94]. Therefore, activation of AMPK by DMC can reinforce the blockade of HMGB1 release.

Our analysis reveals for the first time the primary role of the AMPK-HMGB1 axis in the mechanism of action of DMC. It shows that DMC exerts complementary direct and indirect effects of HMGB1 release. This observation provides key elements to explain the mechanism of action of the drug and it also suggests novel opportunities for the use of this natural product. Indeed, a crosstalk between AMPK and HMGB1 has been evidenced in an experimental model of acute respiratory distress syndrome (ARDS). It has been shown that the restoration of AMPK activity with metformin or the specific neutralization of HMGB1 in bronchoalveolar lavage fluid leads to a decrease of lung inflammation during ARDS [95]. Therefore, we can hypothesize that DMC which both activates AMPK and neutralizes HMGB1 can be useful to combat inflammation in patients with ARDS, notably in the current period of the COVID-19 pandemic.

CRediT authorship contribution statement

Gérard Vergoten: Investigation; Visualization; Software. **Christian Bailly**: Conceptualization; Visualization; Writing - original draft; Writing - review & editing.

Declaration of competing interest

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