Agrimonolide from *Agrimonia pilosa* Ledeb.

Subjects: Pharmacology & Pharmacy

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Agrimonolide (AM), which is a derivative of isocoumarins, is found mainly in the herb *Agrimonia pilosa* Ledeb. This compound is highly lipophilic and readily crosses the blood–brain barrier. Interest has grown in the use of AM as a multitarget natural treatment for various diseases, such as cancer, inflammation, hepatic injury, myocardial damage, and diabetes mellitus. The potential mechanisms of these pharmacological effects have been clarified at cellular and molecular levels. AM shows no cytotoxicity over a range of concentrations in different types of cells, providing evidence for its good safety profile in vitro. These findings indicate that AM is a promising medicinal agent.

agrimonolide | Agrimonia pilosa Ledeb. | pharmacological effect | safety

1. Introduction

Agrimonolide (AM), also known as agrimolide, is a bioactive compound that naturally occurs in the plants *Agrimonia pilosa* Ledeb. [1] and *Spiraea formosana* Hayata [2]. AM was first isolated from the fresh root of *A. pilosa* in 1958 by a Japanese scholar [1]. In 2004, AM was subsequently found in the fresh stems of *S. formosana* [2].

Structurally, AM is a derivative of isocoumarins and its molecular formula is $C_{18}H_{18}O_5$. The chemical name of AM is 3,4-dihydro-6,8-dihydroxy-3-[2-(4-methoxyphenyl) ethyl]-1*H*-2-benzopyran-1-one. This racemate compound possesses one methoxyl and two phenolic hydroxyls, one of which is chelated to a lactonic C = O. The chemical structure of AM is shown in **Figure 1**.

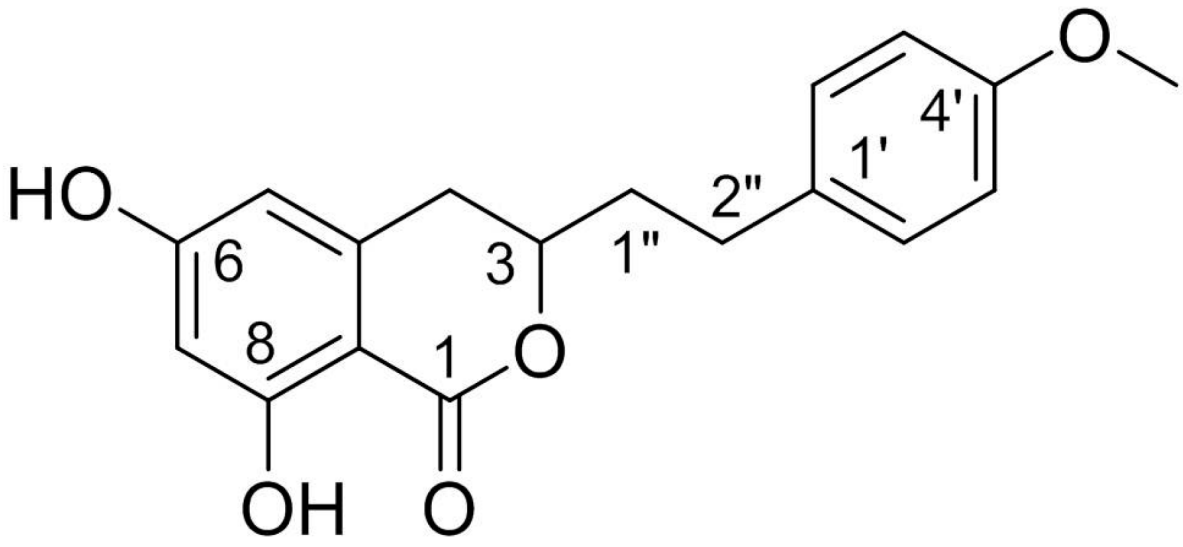

Figure 1. Chemical structure of AM.

Investigation of the bioactivities of AM has demonstrated that this compound has a wide range of potential pharmacological activities [3][4][5][6][7][8][9][10][11][12]. According to the current literature, AM acts as an α_{1A} adrenergic receptor antagonist [3]. AM also exerts anti-inflammatory [4], antitumor [5][6], antioxidant [7], hepatoprotective [8], and myocardial protective effects [9][10]. Furthermore, AM effectively increases insulin-mediated glycogen levels in hepatocytes, suggesting that it could be a promising natural product for the prevention and treatment of diabetes mellitus [11][12].

In recent years, there have been several reviews on the botany, phytochemistry, ethnopharmacology, pharmacology, and traditional uses of *A. pilosa* [13][14][15][16], from which AM is isolated. However, there are few systematic summaries of the knowledge about this compound. In particular, important information regarding AM, such as aspects of its in vivo pharmacokinetics, biodistribution, bioavailability, and the application of drug delivery systems, is lacking. These obstacles limit the use and development of AM.

2. Sources

2.1. Derivation from Plants

As mentioned above, AM has been found in the plants of *A*. *pilosa* and *S*. *formosana*, which belong to the Rosaceae family. *A*. *pilosa* is a perennial herb with an erect stem that is 30–120 cm in height, and it grows along roadsides or in grassy areas at diverse altitudes. This plant is distributed in China, central Europe, the former Soviet Union, Mongolia, North Korea, Japan, and northern Vietnam [17]. *A*. *pilosa* is used in traditional Chinese medicine for mainly treating hemoptysis, metrorrhagia, hematemesis, and bloody dysentery [18]. *S*. *formosana* is a shrub endemic to Taiwan that grows in alpine woodlands at an altitude of 2100–2950 m [19]. The tender leaves, fruits, and roots of this plant have traditionally been used as diuretics, antidotes, and analgesics to treat inflammation, cough, headache, and toothache [20][21].

Conventional approaches are usually used to extract and separate AM from the above-mentioned plants. **Table 1** summarizes the extraction and separation methods of AM [1][2][4][8][11][22][23]. The extraction and isolation process of AM from *S. formosana* can be summarized as follows [2]. Briefly, the fresh stems of *S. formosana* are extracted with hot ethanol, and the water suspension of the ethanol extract is subjected to a liquid-liquid partition to obtain chloroform, n-butanol, and water subfractions. AM is then separated from the chloroform subfraction using a combination of silica gel column chromatography. Finally, 5.6 mg of AM is obtained from 8.6 kg of *S. formosana*, equivalent to the content of 0.65 mg/kg. This result indicates that the content of AM in *S. formosana* is low.

Table 1. Extraction and separation methods of AM.

Parts	Methods of Extraction and Isolation	Yield	Content	Ref.
Fresh stems	8.6 kg of *S. formosana* is extracted with hot ethanol, and the water suspension of the ethanol extract is subjected to a liquid-liquid partition to obtain chloroform, n-butanol, and water subfractions, respectively. The chloroform subfraction is then fractionated by silica gel column chromatography.	5.6 mg	0.65 mg/kg	[2]
Fresh roots	10 kg of *A. pilosa* is extracted with methanol, and the extract is shaken with diethyl ether. The soluble part is boiled several times with petroleum ether, and the residue is heated and extracted repeatedly with benzene. Finally, the precipitated crystals are recrystallized from benzene and then from methanol.	3000–4000 mg	300–400 mg/kg	[1]
Dried plant	50 kg of *A. pilosa* is extracted with 60% ethanol, and the 30% ethanol elution part of macroporous resin is separated by silica gel column chromatography, recrystallization, ODS column chromatography, Sephadex LH-20 gel column chromatography and preparative high-performance liquid chromatography.	202 mg	4.04 mg/kg	[22]
Dried aerial parts	13 kg of *A. pilosa* is extracted with methanol and the extract is suspended in water. The suspension is partitioned between hexane, ethyl acetate, and n-butanol. The ethyl acetate fraction is then fractionated by repeated silica gel column chromatography.	43.7 mg	3.36 mg/kg	[11]
NA	Ethyl acetate fraction of methanol extract of *A. pilosa* is chromatographed repeatedly with silica gel columns and purified by	6.5 mg	NA	[4]

Parts	Methods of Extraction and Isolation	Yield	Content	Ref.
	preparative thin layer chromatography.			
Dried roots	290 g of *A. pilosa* is extracted with hot water and the filtrated aqueous solution is partitioned with ethyl acetate and n-butanol, successively. The ethyl acetate soluble fraction is chromatographed by silica gel column repeatedly.	44 mg	151.7 mg/kg	[8]
Dried plant	500 g of *A. pilosa* is extracted with 70% ethanol. The extract is then eluted with different concentrations of ethanol on the macroporous resin. The 50% ethanol eluted fractions is collected and used for subsequent high-speed counter-current chromatography separation.	385.2 mg	770.4 mg/kg	[23]

ow toxicity solvents, such as absolute methanol and 60–70% ethanol solutions, are usually used to extract AM. Conventional separation techniques, such as liquid–liquid extraction, silica gel column chromatography, recrystallization, macroporous resin, and preparative high-performance liquid chromatography, have been used to separate AM. AM yields vary from 3–4 mg/kg to 300–400 mg/kg [1][8][11][22]. These classic separation methods are complicated, inefficient, and time consuming. However, in recent years, high-speed counter-current chromatography (HSCCC) coupled with ultraviolet detection or evaporative light-scattering detection has been considered an efficient protocol for separating AM from *A. pilosa* [23]. The AM yield obtained using this method is higher than that using conventional methods, producing an AM content of 770 mg/kg. Therefore, HSCCC is a powerful technique for separating AM from *A. pilosa*.

In summary, there are substantial differences in AM yields extracted from *A. pilosa* owing to differences in the origin of plants, the extraction and separation methods used, extraction parts, and other potential factors. Notably, *A. pilosa* has a much higher content of AM than *S. formosana*, which explains why *A. pilosa* is the major source of AM.

2.2. Obtaining AM by Chemical Synthesis

The low accumulation of AM in plants, its cumbersome extraction and separation processes, and the overexploitation of natural resources are generally considered the main driving factors for its high production cost. These factors are also the main causes of supply shortages of AM. These obstacles have made chemical synthesis an appealing alternative method for obtaining AM. Although AM has a variety of pharmacological activities, there have been relatively few advances in its chemical synthesis.

A few attempts have been made to synthesize AM, with variable success. In 1976, Yamato et al. synthesized the racemate of AM in five steps for the first time, and confirmed its structure using nuclear magnetic resonance imaging [24]. They started with compound 1 and obtained compound 2 by protecting two phenolic hydroxyl groups

with a benzyl group. An overall yield of 2.6% AM was obtained through a series of reactions, including Stobbe condensation, ester hydrolysis, benzyl decarboxylation, bromine addition, and reduction [24]. The chemical synthesis route is shown in **Figure 2**A. Unfortunately, there were no subsequent reports of the chemical synthesis process of AM for many years. In 2018, a Chinese invention patent was published that contained a novel chemical synthesis method for AM [25]. The authors improved on the synthesis route of Yamato et al., and constructed the 3,4-dihydroisocoumarin core structure using microwave-assisted intramolecular esterification for the first time. The synthetic process began with 4-chlororesorcinol as the material, and the goal product was obtained in seven steps. The chemical synthesis route is shown in **Figure 2**B. This synthetic route increased the overall yield of AM to 20.7% [25][26], which is nearly eight times higher than that of Yamato et al. This exciting result suggests that microwave-assisted synthesis is a promising approach for the chemical construction of AM.

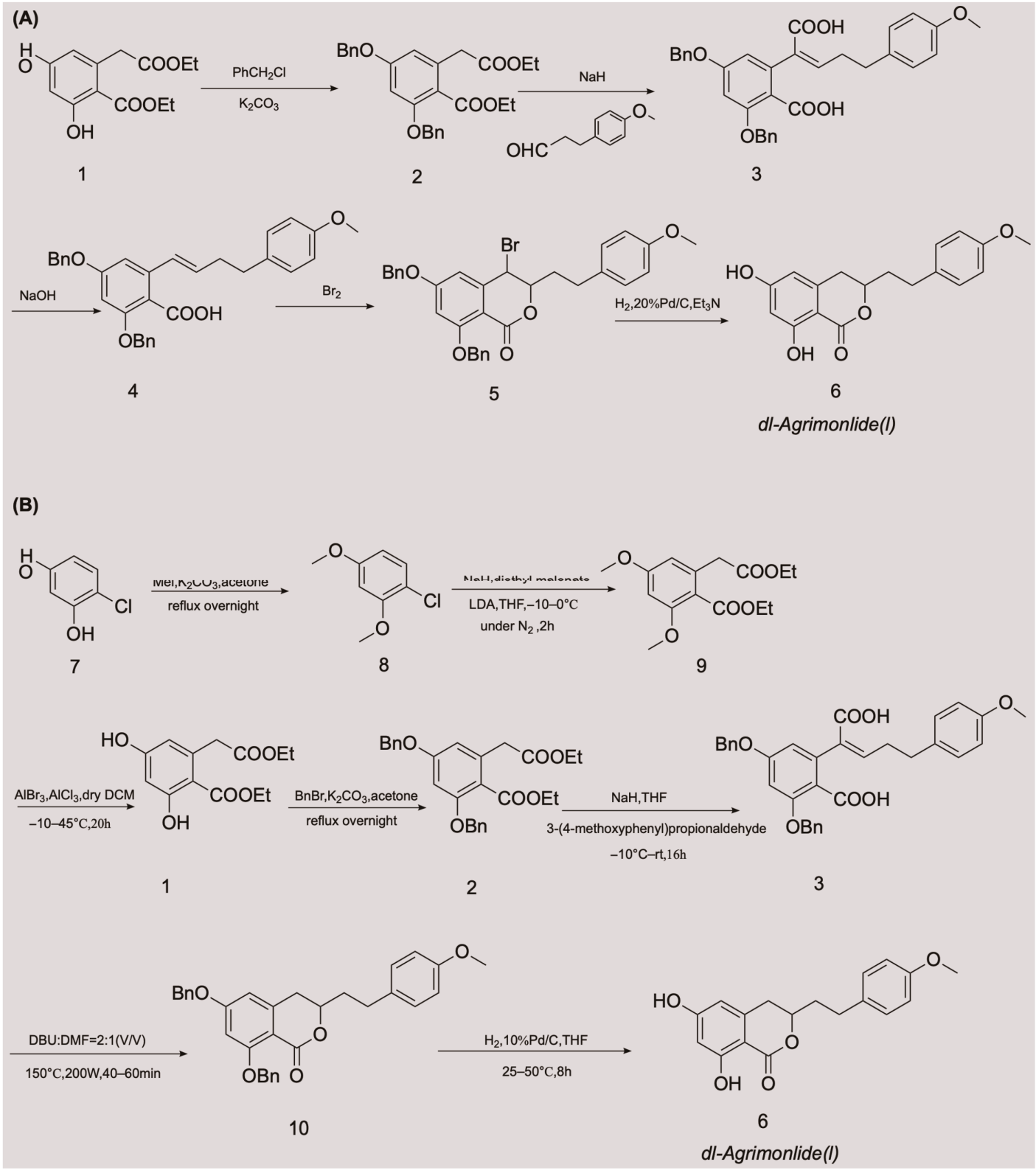

Figure 2. Chemical synthesis routes of AM [24][25][26].

3. Properties

3.1. Physicochemical Properties

Physicochemical properties, such as solubility in water and organic solvents, the acid dissociation constant, the oil/water partition coefficient, and chemical stability, are key factors that affect the pharmacokinetics, biopharmaceutics, and quality of drugs. The physicochemical properties of AM are summarized in detail in **Table 2** [27][28][29].

Table 2. Physicochemical properties of AM.

Physicochemical Properties	Property Value	Ref.
color/form	white powder	[28]
molecular weight	314.3 g/moL	[28]
partition coefficient	3.649	[27]
distribution coefficient	2.949	[27]
acid dissociation constant	8.10 ± 0.40	[28]
density	1.293 g/cm3	[28]
melting point	175.5–176.5 °C	[29]
boiling point	581.1 °C at 760 mmHg	[28]
refractive index	1.611	[28]
flash point	215.5 °C	[28]
vapour pressure	4.2E–14 mmHg at 25 °C	[28]

AM is a white powder with a molecular weight of 314.3 g/moL [28]. AM is soluble in chloroform, dichloromethane, ethyl acetate, dimethyl sulfoxide, acetone, and other solutions [30], but is only minimally soluble in petroleum ether. This solubility suggests that AM is lipophilic. The partition coefficient and distribution coefficient are important

parameters that describe the lipophilicity of a compound, which can be used to help predict the in vivo permeability. The reported partition coefficient and distribution coefficient values of AM are 3.649 and 2.949, respectively [27], indicating that AM has low solubility and moderate permeability. Such compounds usually have good intestinal tract permeability because there is a good balance between dissolution and passive diffusion penetration. According to the biopharmaceutics classification system, drug substances are categorized into four classes on the basis of their solubility parameter and permeability to biomembranes for evaluating the desired results of a formulation on oral bioavailability [31]. A low water solubility and poor oral bioavailability limit the biological effects of many natural products in vivo. The existing data suggest that AM belongs to biopharmaceutics classification system Class II and is likely to exhibit dissolution rate-limited absorption. However, this possibility requires further confirmation by determining the solubility of AM in water.

Regarding chemical stability, contact should be avoided between AM and strong oxidizing agents, reducing agents, strong acids, and alkalis. In the soluble form of AM, it should be sealed and stored below −20 °C to maintain its stability for several months. In the solid form of AM, it is stable at temperatures of 2–8 °C when kept in a dry place [30].

3.2. Predicted Absorption, Distribution, Metabolism, Excretion, and Toxicity Properties

Pharmacokinetic behaviors of drugs in vivo include absorption, distribution, metabolism, excretion, and toxicity (ADMET). The ADMET properties account for 50% of drug research and development (R&D) failures. Computer-aided design is an effective and alternative method of biological experimental evaluation, and helps to improve the R&D success rate. Computational approaches have increasingly been used to predict ADMET properties of compounds, especially in evaluating the ADMET properties of herbal medicines [32]. The predicted ADMET profiles of AM regarding its absorption, solubility, permeability across the blood-brain barrier (BBB), interactions with cytochrome P450 2D6, hepatotoxicity, and plasma protein binding (PPB) are shown in **Table 3** [27].

Table 3. Predicted ADMET properties of AM [27].

ADMET Properties	Prediction Value	Level
ADMET absorption	/	0
ADMET BBB	–0.241	2
ADMET solubility	–4.092	2
ADMET hepatotoxicity	0.655	1

ADMET Properties	Prediction Value	Level
ADMET CYP2D6	0.356	0
ADMET PPB	/	2
drug-likeness	0.842	good

ADMET absorption levels: 0, 1, 2, and 3 represent good, moderate, low, or very low absorption, respectively. ADMET BBB levels: 0, 1, 2, 3, 4, and 5 represent very high, high, medium, low, undefined, and molecules with one or more unknown AlogP98 types, respectively. ADMET solubility levels: 0, 1, 2, 3, 4, 5, and 6 represent extremely low, very low but possible, low, good, optimal, too soluble, and molecules with one or more unknown AlogP98 types, respectively. ADMET hepatotoxicity: 0 and 1 represent nontoxic and toxic effects, respectively. ADMET CYP2D6: 0 and 1 represent non-inhibitor and inhibitor, respectively. ADMET PPB levels: 0, 1, and 2 represent binding <90%, binding ≥90% and binding ≥95%, respectively.

AM appears to show a good absorption capacity in vivo with a predicted absorption level of 0. However, AM is predicted to have a low aqueous solubility, with a solubility level of 2, which contradicts the prediction result for in vivo absorption and needs to be further confirmed by in vivo testing. Regarding the prediction of BBB penetration, AM exhibits a moderate BBB penetration capability, with a level of 2. This indicates that AM may enter the brain tissue through the BBB and could be used to treat brain diseases. Furthermore, the ADMET predictor shows that AM exhibits potential hepatotoxicity, with a level of 1. Preliminary explorations and in-depth investigations are required to determine the specific mechanism of hepatotoxicity and whether it is dose dependent. In addition, AM is predicted to be a non-inhibitor of the cytochrome P450 2D enzyme and may be metabolized and excreted successfully. Therefore, drug-drug interactions are less likely when AM and the cytochrome P450 2D6 substrates are used simultaneously. Moreover, the PPB level is predicted to be 2, indicating that the binding rate of AM with plasma protein is ≥95%. The high degree of PPB limits the partitioning of AM from the blood into the tissues, where it could be metabolized. This limited partitioning may result in a delayed onset of action and longer half-life period, thereby reducing the elimination of AM. AM has been predicted to have a good drug-likeness, with a drug-likeness weight of 0.842. Generally, AM demonstrates promising ADMET profiles. However, to fully confirm the ADMET properties of AM, real-world tests are required to validate these properties, and more animal and human studies are required.

4. Pharmacological Effects

AM possesses a wide range of pharmacological activities, such as antitumor activity, antioxidation and hepatoprotection, antidiabetic activity, anti-inflammatory activity, myocardial protection, and α1A adrenergic receptor antagonist activity. The mechanisms of action of these effects are shown in **Table 4**.

Table 4. Mechanisms of pharmacological effects of AM.

Pharmacological Effects	Levels	Models	Concentrations or Doses of AM	Mechanisms	Ref.
anti-gastric cancer	in vitro	AGS cells	40 μM, IC_{50} = 25.9 μM	decrease the expression of Bcl-2; increase the expression of Bax; increase the level of phospho-ERK/ERK protein and the expression of phosphor-p38 protein; increase the activity of caspase-3; down-regulate the levels of the inactive pro-caspase-3, -8, and -9 proteins	[6]
anti-ovarian cancer	in vitro	A2780 and SKOV-3 cells	40 μM	increase the cleavage of caspase-3 and -9; increase the levels of ROS, total iron and ferrous ion, and down-regulate the levels of SLC7A11 and GPX4, thus inducing ferroptosis; direct inhibit tumor cell migration and invasion; inhibit the protein levels of SCD1	[5]
	in vivo	SKOV-3 xenograft model	50 mg/kg	down-regulate the expressions of Ki-67 and SCD1;	[5]

Pharmacological Effects	Levels	Models	Concentrations or Doses of AM	Mechanisms	Ref.
		(BALB/c mice)		lower the expressions of SCD1 mRNA and protein	
	in vitro	PANC-1 cell	1 μM; 5 μM	promote the expression of PDX-1	[22]
anti-diabetic	in vitro	/	IC_{50} = 37.4 μM	inhibit α-glucosidase	[11]
	in vitro	Insulin-resistance HepG2 cell	20 μM	elevate the activity of GK, and increase the content of glycogen; lower the activities of PEPCK and G6Pase, and constrain the gluconeogensis	[12]
anti-oxidative and hepatoprotective	in vitro	HepG2 cell; rat primary hepatocytes	EC_{50} = 88.2 μM; EC_{50} = 37.7 μM	scavenge the free radical	[8]
	in vitro	HepG2 cell	200 μM	scavenge the free radical; activate Nrf2-driven pathways; activate ERK, JNK, and MAPK phosphorylation; inhibit p38 phosphorylation; elevate the activity of antioxidative enzymes	[7]

Pharmacological Effects	Levels	Models	Concentrations or Doses of AM	Mechanisms	Ref.
anti-inflammatory	in vitro	RAW 264.7 cells	80 μM	reduce the levels of IL-1β, IL-6, and TNF-α; attenuate the expression of iNOS and COX-2; inhibit the activation of JNK and p38 MAPKs; decrease the activation of JAK-STAT and NF-κB	[4]
myocardial protective	in vitro	H9c2 cell	15 μM	regulate the gene expression involved in mitochondrial function; decrease the levels of cleaved Caspase 3 and Bax; boost the level of Bcl2; prevent the rate of apoptosis and shield H9c2 cells from hypoxia-induced apoptosis; reduce ROS production and preserve the normal shape of mitochondria; regulate the functional proteins to enhance the mitochondrial activity	[10]
	in vivo	CLP rat model	5 mg/kg	attenuate myocardial injury by Akt signaling;	[9]

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Pharmacological Effects	Levels	Models	Concentrations or Doses of AM	Mechanisms	Ref.
				suppress cardiac injury indicators, oxidative stress, and inflammation; restrain the activation of Akt, Erk, mTOR and the apoptosis of cardiomyocytes	
blocking α_{1A} adrenergic receptor	in vitro	rat prostate cell membrane	/	/	[3]

5. Safety

To the best of our knowledge, the available data on the safety of AM are limited. Safety studies of AM have mainly focused on in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tests of different types of cells, and AM shows no cytotoxicity over a range of concentrations [4][5][6][7][8][12]. Various concentrations of AM (25–200 μM) do not cause a substantial change in viability, or in the size and shape of HepG2 cells. Even at a high concentration of 200 μM, no cell shrinkage or shedding of adhesion molecules occurs [7]. In a hepatoprotective activity study, the survival rate of HepG2 cells was not altered in the presence (1–100 μM) or absence of AM [8]. A similar result was also found for HepG2 cells treated with AM at a concentration of 20 μM [12]. In a study of cytotoxic effects on RAW 264.7 cells, AM concentrations of 20–80 μM did not cause any changes in MTT-based cell viability. Annexin V/propidium iodide staining has been further used to characterize the cytotoxic effect of AM. After treatment with AM, the proportion of early apoptotic cells substantially decreases. These results indicate that AM does not show signs of cytotoxicity in RAW 264.7 cells, and that it does not inhibit the early events leading to apoptosis [4]. In another MTT test, AM concentrations of 10–40 μM showed no cytotoxicity on the growth of HepG2, HT-29, or MCF-7 cells [6]. The above-mentioned cytotoxicity tests provide preliminarily support for the safety of AM in vitro.

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