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A comprehensive review of cycloastragenol: Biological activity, mechanism of action and structural modifications

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ABSTRACT

Cycloastragenol (CAG) is a sapogenin of Astragaloside IV (AG-IV), isolated from the dried roots of *legumes Astragalus mongolica* or *Astragalus membranaceus*. Cycloastragenol has a steroidal skeleton of tetracyclic triterpene and possess diverse pharmacological activities such as anti-aging, anti-inflammatory, anti-fibrosis, pro-wound healing, liver protection and endothelial protection. In addition, cycloastragenol is the only telomerase activator reported in natural products, which is closely related to MAPK and PI3K/Akt signaling pathways. This review provides a theoretical basis for the development of cycloastragenol into the candidate for the treatment of multi-factorial diseases in clinic, focusing on the extensively biological activities as well as the mechanism of action and structural modification and aiming to attract researchers to conduct in-depth studies on cycloastragenol. Meanwhile, the pharmacokinetics and toxicology studies of CAG are also described for further druggability exploration to provides valuable reference.

1. Introduction

Natural products have extensive pharmacological and biological activities that can be widely used in disease treatment. More than half of FDA-approved drugs are natural products and their derivatives [1]. Natural products provide a crucial scaffold for drug development in the case of the subsequent high-throughput screening of synthetic chemical libraries for targets [2].

Cycloastragenol (CAG) with steroid-skeletal characteristics is a cycloartan saponin of Astragaloside IV (AG-IV) which is one of the main active components of *Astragalus membranaceus* (Fig. 1) [3]. The most abundant studies demonstrated that CAG has a wide variety of pharmacological activities, including anti-inflammatory, anti-fibrosis, anti-oxidative stress, antibacterial, antiviral, liver protection, endothelial protection and immune regulation, mainly through the regulating of the ERK/JNK, Wnt/β-catenin, AKT1-mTOR-RPS6KB1 and JAK/STAT3 signaling pathways respectively (see Table 1).

On the basis of current literature research, this review focuses on current advances in pharmacological mechanism of action. In addition, the structure modifications and pharmacokinetics profile of CAG were also discussed. Due to diverse biological functions, CAG would be considered as promising therapeutic agents against multiple diseases.

2. The biology of CAG

2.1. Telomerase activation and its mechanism

Cycloastragenol has been reported as the only telomerase activator in the extract of traditional Chinese medicine. Telomerase activity plays an important role in prolonging the lifespan of somatic cells [4]. However, compared with newborns, the telomerase activity of adult animals significantly decreased [5]. Recently, telomerase dysfunction has been detected in multiple diseases, such as rare multi-system diseases [6], cardiovascular disease [7–9], autoimmune diseases [10,11], respiratory diseases [12,13] and neurodegenerative diseases [14–16]. Therefore, it is very promising to conduct in-depth research on CAG.

The reported telomerase activation by CAG mainly through regulating MAPK and Akt signaling pathways. The activated Akt signaling pathways could promote the phosphorylation of the catalytic subunit telomerase reverse transcriptase (TERT) through post-transcriptional modification [17]. Additionally, Epidermal growth factor (EGF)

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Abbreviations

AAA	Abdominal aortic aneurysm
AHR	Airway hyperresponsiveness
Akt	Protein kinase B
ALP	Alkaline phosphatase
AMI	Acute myocardial infarction
AMPK	5′AMP-activated Protein Kinase
AG-IV	Astragaloside IV
Aβ	Amyloid beta-peptide
Bax	Bcl2-associated x
Bcl2	B-cell lymphoma-2
CAG	Cycloastragenol
CREB	cAMP-Response Element Binding Protein
COX-2	Cyclooxygenase-2
DIPEA	*N, N*-diisopropylethylamine
EGF	Epidermal Growth Factor
EpSCs	Epidermal stem cells
ERK	Extracellular Regulated Protein Kinases
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
FXR	Farnesoid X receptor
hMSC	Human bone marrow mesenchymal stem cells
IL-1β	Interleukin-1β
IGF	Insulin-like Growth Factor
IPF	Idiopathic pulmonary fibrosis
JAK	Janus kinase
JNK	Jun N-terminal Kinase
KLB	β-Klotho
LRP1	Low density lipoprotein receptor related protein 1
MAPK	Mitogen-activated Protein Kinase
MEK	ERK kinase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NLRP3	NLR family pyrin domain containing 3
NF-κB	Nuclear factor kappa B
PARP	Poly ADP-ribose polymerase
PDK	3-Phosphoinositide-dependent protein kinase
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol-4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5- trisphosphate
RAGE	Advanced Glycosylation End-product Specific Receptor
RhoA	Recombinant ras homolog gene family, member A
ROS	Reactive Oxygen Species
SAEC	small airway epithelial cells
SIRT1	Sirtuin 1
Src	Steroid receptor coactivator
STAT3	Signal transducer and activator of transcription 3
TERT	Telomerase reverse transcriptase
TNF-α	Tumor necrosis factor-α
TXNIP	Thioredoxin interacting protein
VEGF	Vascular Endothelial Growth Factor
VSMC	Vascular smooth muscle cell

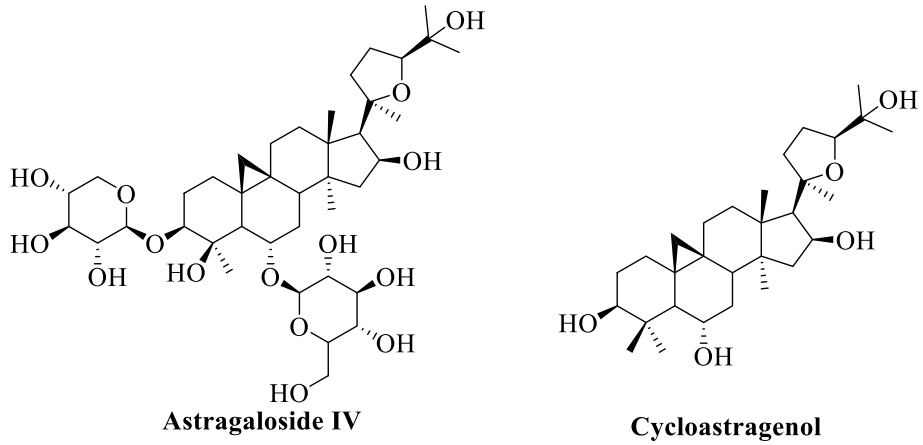


Fig. 1. The structure of Astragaloside IV (AST) and Cycloastragenol (CAG).

directly acts on the hTERT promoter through the Ras/MEK/ERK pathway and Ets factor [18]. Notably, CAG boosted the phosphorylation of cell signal-regulated kinase (ERK) in HEK293 cells and HEK-neonatal keratinocytes via activating the Src/MEK/ERK signaling pathway [19]. However, its detailed mechanism of action regarding telomerase activation needs to be deeply studied.

2.2. Anti-aging effect

Aging is the main fuse of many life-threatening diseases [20]. It was forcefully indicated that CAG has potential for the treatment of age-related dysfunction [21]. As reported, the average telomere length measured in human blood lymphocytes is usually considered as a biomarker for aging, survival and mortality [22]. Inhibition of telomerase activity promotes cell senescence and ultimately cell death, while telomerase activity decreases with age [23,24]. Therefore, CAG may exhibit anti-aging effect by acting on telomerase, and CAG has been popular among anti-aging health products.

Recently, Xu et al. has demonstrated that CAG could significantly upregulate the expression of β-Klotho (KLB) and alleviate the age-related dysfunction in primary bovine granulosa cells by activating the ROS-FGFR1-TERT-β-Klotho signaling pathway [25]. Klotho gene can regulate telomere length and telomerase activity to postpone aging, which was first discovered in transgenic mice lines in 1997 [26]. Overexpression of the Klotho gene could prolong life span, while, mutation of klotho would induce symptoms similar to premature aging syndrome [27,28]. CAG and TERT plasmid have shown an ability to improve expression of KLB, reduce ROS level and inhibit cell apoptosis *in vitro* cultured bovine granulosa.

Table 1
Cycloastragenol pharmacological effects and mechanisms.

Author, year	Research object	Experimental method	Biological efficacy	Mechanism of action
Xu L et al., 2021	Bovine granulosa cells, oocytes and early embryos	Treat with d-gal, TERT plasmid injection	Age-related dysfunction	FGFR1/ β-Klotho signal pathway
Deng G et al., 2019	Mouse model	Imiquimod-induced	Psoriasis-like skin inflammation	NLPR3 inflammasome
Zhao Y et al., 2015	Endothelial cells	Palmitate stimulates	Endothelial dysfunction	TXNIP/NLRP3 inflammasome
Cao Y et al., 2019	Human epidermal stem cells	CAG cocultivation of EpSCs	Diabetic wound healing	Wnt/β-catenin signal pathway
Ip FC et al., 2014	PC12 cells, primary neuronal cells, mouse model	Compulsory swimming-induced	Depression	CREB
Huang MY et al., 2021	Endothelial cell line	Immortalized	Alzheimer's disease	LRP1, RAGE
Li M et al., 2020	Mouse model	45 min reperfusion after middle cerebral artery occlusion	Ischemic brain injury	SIRT1
Wang J et al., 2018	Rat model	Isoproterenol-induced	Heart failure	AKT1-mTOR-RPS6KB1
Gao XM et al., 2017	Rat model	Permanent left anterior descending branch ligation	Acute myocardial infarction	RhoA/AKT signal pathway
Le Saux CJ et al., 2013	Mouse model	Bleomycin-induced	Idiopathic pulmonary fibrosis	TERT
Wan Y et al., 2018	Mouse model	ISO-induced	Cardiac fibrosis	NLPR3 inflammasome
Ryer EJ et al., 2015	Mouse model; Rat vascular smooth muscle cells	Elastase-induced and angiotensin II-induced; 100 ng/mL-1TNF-α stimulated	Abdominal aortic aneurysm	ERK/JNK signal pathway
Hwang ST et al., 2019	SNU-1 and SNU-16 cells	Compared with normal GES-1 cells	Stomach cancer	JAK/STAT3 signal pathway
Gu M et al., 2017	Mouse model	HF-fed diet induces obesity	Non-alcoholic fatty liver disease	FXR receptor
Yu Y et al., 2020	Mouse model,	Telomerase overexpression	Osteoporosis	IGF/Akt signal pathway

2.3. Anti-inflammation effect

Inflammation is an adaptive mechanism through the human immune system in response to microbial invasion or harmful stimulus [29]. The anti-inflammatory activity of CAG has always been a research hotspot. A common hereditary chronic inflammatory skin disease, psoriasis, mainly includes histological features such as spinous epidermal hypertrophy, hyperkeratosis and incomplete keratinization [30]. In 2019, Deng et al. reported that 50 mg/kg CAG could significantly improve psoriasis-like skin inflammation *in vivo* imiquimod-induced mice and downregulate the levels of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6 *in vitro* primary BMDMs [31]. Moreover, the enzyme activity of LDH was decreased as well as the expression of caspase-1 and cleaved-gasdermin D was inhibited in serum of mice treated with 50 mg/kg CAG, which suggested that the potential target of CAG is NLRP3 inflammasomes. Further studies have shown that NLRP3 inflammasomes play a significant role in inflammatory disease [32–34]. It has been clearly demonstrated that CAG can inhibit NLRP3 inflammasomes, restoring the loss of mitochondrial membrane via inhibiting the activation of caspase-1 and caspase-3, further splicing pro-IL-1β and pro-IL-18 into IL-1β and IL-18, ultimately causing endoplasmic-reticulum-stress-induced apoptosis [35] (Fig. 2).

In addition, CAG also regulated endothelial homeostasis by inhibiting NLRP3 inflammation via targeting AMPK. CAG could effectively inhibit the phosphorylation of endoplasmic reticulum stress inducer IRE1α (67%) and the production of ROS in endothelial cells, indicating its therapeutic potential on endothelial dysfunction in the early onset of cardiovascular diseases. Moreover, it was also observed that phosphorylation of AMPK was increased in endothelial cells treated with 10 μM CAG [36]. The novelty of their research lies in the further discovery of the upstream target of CAG to inhibit the NLRP3 inflammasome.

2.4. Pro-wound healing effect

Wound healing in diabetic patients is impaired and delayed, exposes the open wound to infection and trauma, emerging as a global public health issue that concerns physical and mental health [37]. Interestingly, CAG has shown potential to treat diabetic wound healing.

Wnt/β-catenin signaling plays a crucial role in stem cells, and regulates expression of telomerase, which was considered to be primarily responsible for diabetic inflammation, wound proliferation, wound remodeling and stem cell control (Fig. 2) [38,39]. Significantly, the expression of Wnt/β-catenin and TERT were activated in epidermal stem cells (EPSCs) treated with CAG. Compared with normal cells, the cell viability of proliferation and migration effectively increased in co-culture with 0.3 μM CAG at days 3, 5 and 7 [40]. Their studies provided a new perspective of diabetic wounds' therapeutic, but current research and the relevant mechanisms at the cellular level remain inadequate.

2.5. Treatment of neurodegenerative diseases

2.5.1. Anti-depressant effect

Depression is a common mental illness and has developed into a significant human blight, which is the second leading cause of death in people aged from 15 to 29 at present [41]. In 2014, Fanny et al. demonstrated for the first time that CAG exhibits antidepressant-like properties in mice by intraperitoneal injection of 100 mg/kg CAG [42]. Furthermore, CREB phosphorylation at Ser 133 was induced in PC12 cells and primary neurons treated with 0.3 μM CAG. In addition, CAG was able to upregulate the expression of CREB regulatory gene Bcl2 and TERT, suggesting its therapeutic potential for depression (Fig. 3). They performed a variety of *in vitro* assays for cell signaling changes and *in vivo* behavioral tests of depression in mice, however, their toxicity experiments are insufficient at high dose of 100 mg/kg.

2.5.2. Anti-Alzheimer

Alzheimer is an age-related neurodegenerative disease, characterized by cognitive decline as well as behavioral, emotional and mental disorders, whose hallmark is overproduction of amyloid beta-peptide (Aβ) in the brain [43,44]. Significantly, CAG has been approved to inhibit $A\beta_{1\text{-}42}$-induced blood-brain barrier (BBB) disruption and enhance soluble Aβ efflux *in vitro* [45].

It was observed that CAG could dose-dependently (10, 50, 75 μM) reduce the early apoptosis rate of the immortalized endothelial cell line (bEnd.3) treated with 20 μM oligomer $A\beta_{1\text{-}42}$, along with increasing the expression of tight junction scaffold protein. Importantly, they detected the toxicity of CAG on bEnd.3 cells before biological test and suggested that the safety concentration was no more than 75 μM. Furthermore, it was known that CAG promoted water-soluble Aβ to flow out of BBB via upregulating expression of density lipoprotein receptor-related protein 1

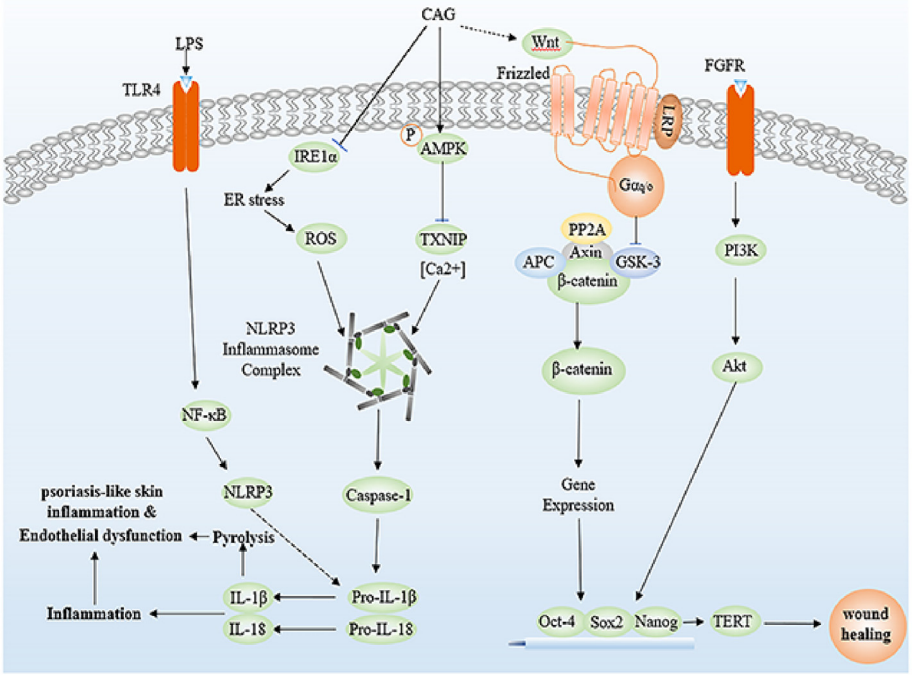


Fig. 2. Potential signaling pathways for CAG in the treatment of psoriasis-like skin inflammation, endothelial dysfunction and wound healing.

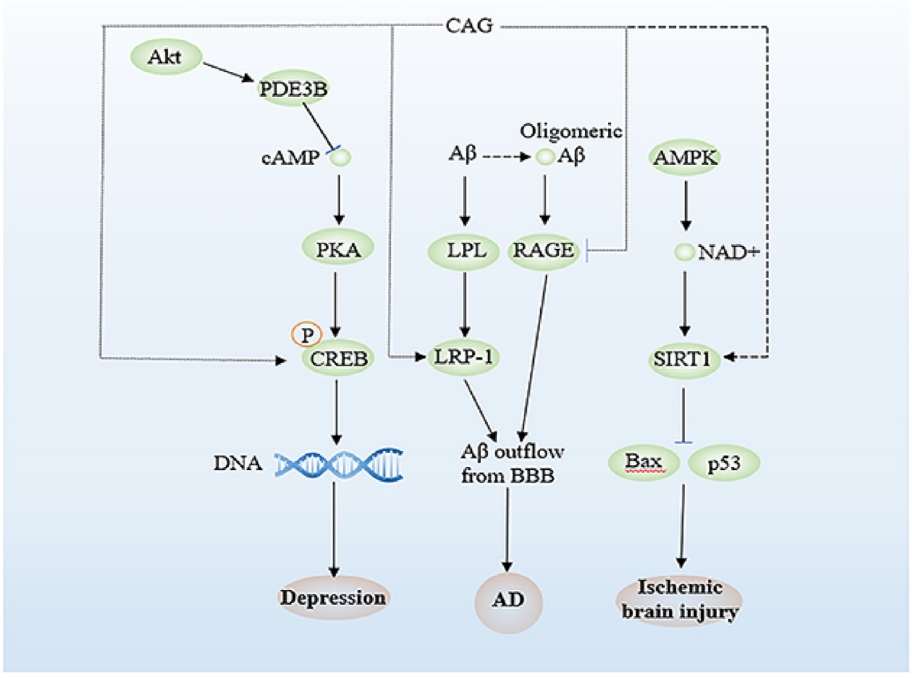


Fig. 3. Potential signaling pathways for cycloastragenol in the treatment of depression, Alzheimer's and ischemic brain injury.

(LRP1) and P-GP as well as downregulating utterance of advanced glycation end-product receptor (RAGE), which in turn improved Alzheimer (Fig. 3).

2.5.3. *Anti-ischemic stroke*

Stroke is a major cause of adult disability and death, among which ischemic stroke accounts for more than 79% in China. Silent information regulator 1 (SIRT) is attributed to many neurodegenerative diseases due to its neuroprotective properties, which has been the promising target for ischemic stroke [46,47].

CAG exhibited significant neuroprotective effects on ischemic brain injury in MACO mice at the dose of 20 mg/kg by suppressing apoptotic signaling pathway and inhibiting neuroinflammation via indirectly upregulating SIRT1 [48]. Concomitant with the upregulation of SIRT1 expression, CAG downregulated MMP-9 activity and the tight junction protein degradation, thereby attenuating BBB disruption (Fig. 3). Furthermore, CAG could dose-dependently (5, 10, 20 mg/kg) decrease the neurological deficit scores and steadily reduced the brain infarct volume from 52.3 mm^3 to 13.7 mm^3, ameliorating functional deficits and neuronal cell loss. It is undeniable that their research provides a new

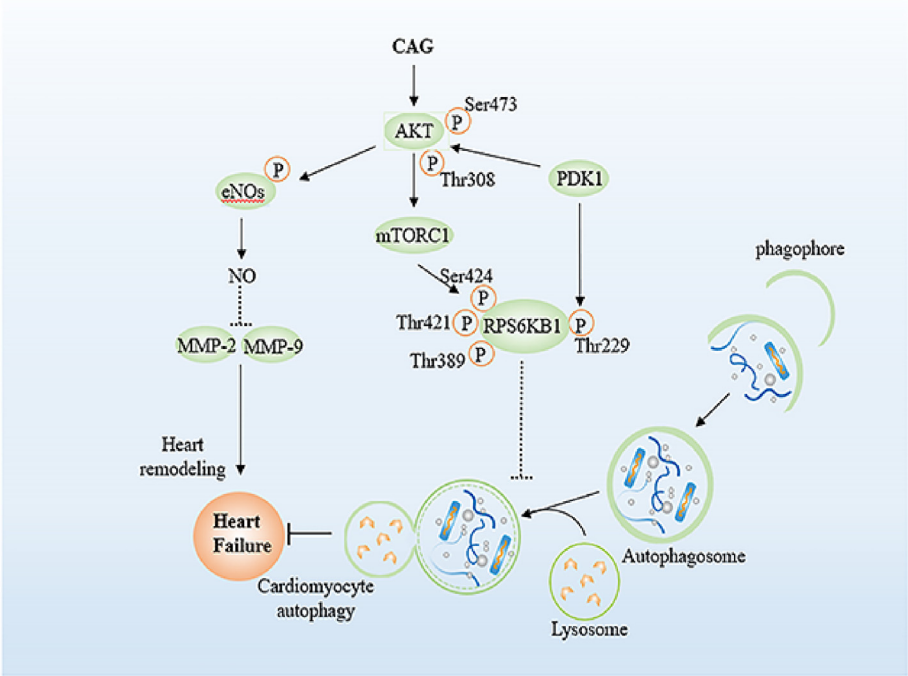


Fig. 4. Potential signaling pathways of CAG in the treatment of heart failure.

therapeutic direction for ischemic stroke, but clinical trials of CAG for this brain disorder are indispensable.

2.6. Treatment of cardiovascular diseases

2.6.1. Anti-heart failure

The incidence of heart failure is multiplying among older adults. The progression of heart failure is characterized by cardiac remodeling, and an important pathophysiological factor is dysfunctional cardiomyocytes autophagy [49]. CAG could significantly ameliorate disordered cardiac parameters (LVESP, dp/dt_{min}, dp/dt_{max}, LVEDP and LVWI), ejection fraction (EF, %) and cardiac histological changes (such as cardiac desmoplasia), along with simultaneously downregulating the serum levels of neuroendocrine factors (NE, ALD, BNP, ET-1, ANG II, CAMK2 and CK) in a dose-dependent manner (10, 30,90 mg/kg). Especially, the protective effect of 90 mg/kg CAG in isoproterenol-induced heart failure was comparable to the positive control metoprolol. [50]. Notably, the rate of LC3-I-to-LC3-II-conversion and the expression of p62 were remarkably decreased *in vivo* ISO-induced rat and *in vitro* primary neonatal rat cardiomyocytes treated with CAG, indicating that autophagy was involved in the CAG-mediated cardiac protection.

Furthermore, *in vitro* assay illustrated the inhibition of Akt1 Ser473 and Thr308 phosphorylation along with the reduction of p-RPS6 and p-RPS6KB1 expression, representing the potential role of AKT1-RPS6KB1 signaling pathway on CAG-induced myocardial autophagy (Fig. 4) This study firstly revealed the protective effects of CAG on heart failure.

2.6.2. Anti-cardiac fibrosis

Cardiac fibrosis is present in many patients with cardiovascular diseases, characterized by excessive deposition of extracellular matrix (ECM) proteins in the cardiac interstitium [51,52]. CAG could downregulate the mRNA expression of fibrosis-related genes (Col-1, Col-3 and TGF-β1) at the dose of 62.5 and 31.25 μg/ml, but only the dose of 62.5 μg/ml CAG inhibited ISO-induced cardiac fibrosis *in vivo* assay [53]. The data *in vivo* assay indicated that CAG significantly downregulated the mRNA expression of pivotal genes in NLRP3 inflammasome pathway (NLRP3, caspase-1, IL-18, IL-6) and decreased the infiltration of inflammatory cells at the dose of 62.5 μg/ml.

2.6.3. Anti-myocardial infarction

Acute myocardial infarction (AMI) is a significant cause of high mortality rates of cardiovascular disease [54]. Inflammatory factors play a vital role in the treatment in AMI, therefore, inhibiting inflammatory signals and infiltration is suggested as a feasible treatment [55]. It was observed that CAG efficiently suppressed the expression of inflammatory factors, such as TNF-α (from 59.7% to 21.9%) and IFN-γ (from 29.5% to 18.6%) at the dose of 10 μM, as well as increasing the production of anti-inflammatory factors, such as IL-4 (from 11.56% to 21.11%) and IL-10 (from 1.96% to 5.00%) in AMI patient PBMCs [56]. Simultaneously, the same results were observed in 20 mg/kg CAG-treated rat experiments.

Furthermore, CAG could significantly decreased expression of Akt, RhoA and apoptotic protein (caspase-9, caspase-3 and Bax) in hypoxic H9C2 cells. Although this study preliminarily explored the connection between CAG and RhoA/Akt signal pathways, there still need a specific elucidation on the mechanism in the future.

2.6.4. Anti-high cholesterol

Fat accumulation is tightly related to hyperlipidemia, obesity and atherosclerosis [57]. Simultaneously, 3T3-L1 preadipocytes have been accounted for the obesity experimental model [58]. Formerly, CAG has been used as biologically active supplements for hypercholesterolemia and obesity [59].

CAG dose-dependently stimulated intracellular calcium mobilization ($EC_{50} = 21.9$ μM) and reduced cytoplasmic lipid droplet ($IC_{50} = 13.0$ μM) in 3T3-L1 preadipocytes. Reassuringly, the cytotoxicity assay showed that CAG did not induce injury of HepG2 cells up to 60 μM. Additionally, CAG was gainful in combination with atorvastatin, showing better ability to alleviate weight and improve cardiac remodeling and myocardial dysfunction [59].

2.6.5. Anti-abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) comprises oxidative stress, extracellular matrix (ECM) degradation as well as vascular smooth muscle cell (VSMC) apoptosis and its chief inducement is chronic inflammation [60]. CAG at the dose of 125 mg/kg/d effectively reduced aortic dilatation, elastin degradation and AAA incidence in both elastase-induced AAA

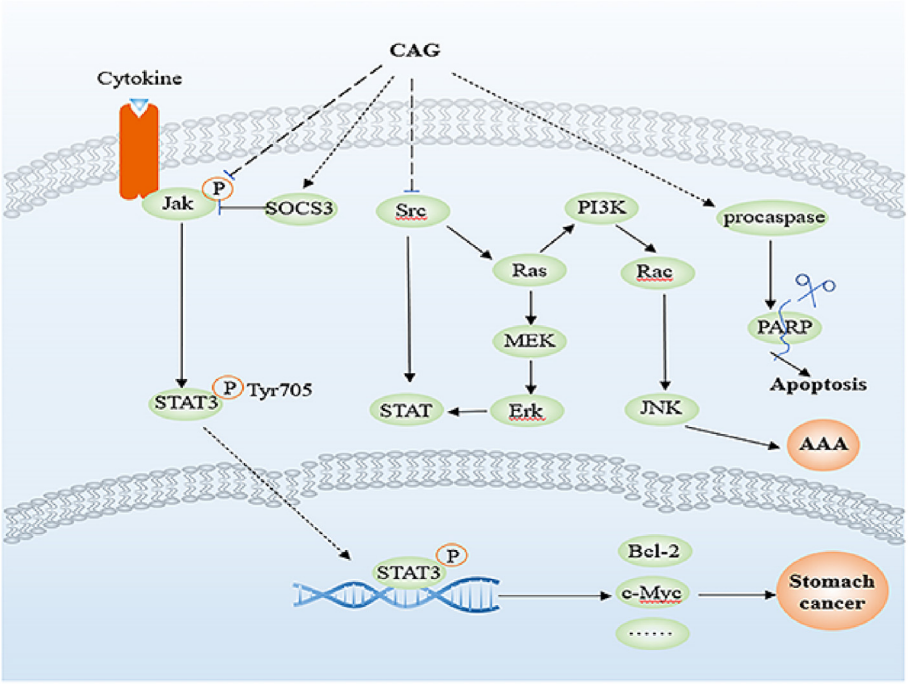


Fig. 5. Potential signaling pathways of CAG in the treatment of antibacterial and anti-tumor.

mice and angiotensin II-induced ApoE-/- mice [61]. Apart from preventing AAA and protecting the abdominal aorta, CAG has also shown therapeutic effects on formed AAA. Simultaneously, 31.25 μg/mL CAG extenuated the inflammation, oxidation, phenotype conversion and apoptosis *in vitro* experiments of rat vascular smooth muscle cells stimulated by 100 ng/mL TNF-α. Furthermore, CAG downregulated p-ERK and p-JNK *in vivo* and *in vitro*, indicating the protective mechanism against AAA was potentially ERK/JNK signaling pathway (Fig. 5).

2.7. Treatment of respiratory diseases

2.7.1. Anti-pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic and interstitial lung disease and frequently occurs in middle-aged and older adults [62]. The hallmark of IPF is the collagen accumulation in the parenchyma, to a certain extent, which is associated with higher mortality compared with tumors.

CAG possessed an obviously anti-fibrosis pharmacological effect, indicating its therapeutic potential on IPF [63]. CAG significantly reduced the accumulation of collagen *in vivo* bleomycin-induced mice at the dose of 20 mg/kg/d, attenuating the development of the fibrotic phenotype in a dose dependent manner. Compared to untreated mice, telomerase activity was increased both *in vivo* bone marrow progenitor cells and lung tissue treated with 10 mg/kg/d and 20 mg/kg/d CAG. Furthermore, CAG decreased the senescence associated-βgal activity and p21 mRNA expression but unchanged the proportion of the infiltrating cells, indicating that CAG protected pulmonary fibrosis is associated with anti-aging not anti-inflammatory. With special emphasis, the effects on telomerase activity and cellular senescence of CAG are manifested only in specific lung cell (SAEC).

2.7.2. Anti-asthma

Asthma is a common chronic disease in the respiratory system, manifested by airway inflammation, airway hyperresponsiveness (AHR) and airway remodeling [64,65]. There is an urgent need to develop novel safe and effective therapies [66]. Recently, Fance et al. reported CAG could significantly attenuated OVA-induced AHR *in vivo* and inhibited the expression level of autophagy-related proteins (LC3B and Beclin1) at the dose of 125 mg/kg, which made it potentially gainful in the treatment of asthma [67]. Although CAG made hydrogen-bonding interaction with LEU232 and GLN43 in the ATG4-LC3B complex (PDB ID: 2Z0D) in the molecular docking, the highest binding energy (−8.0 kcal/mol) is not ideal. At present, whether CAG directly triggered autophagy-related targets is still pending verification.

2.8. Anti-HIV

Comparing with the modest effect on cells from healthy adults, CAG (TAT2) significantly facilitated proliferation of PBMC and $CD8^+$ T lymphocytes from HIV-1-infected donors at the dose of 1 μM via upregulating telomerase activity, as well as increasing the antiviral functions [68]. It is worth noting that CAG could upregulate the expression of RSK1 and phosphorylation of ERK at the concentration of 10 nM within 30–60 min, via activating MAPK/ERK pathway in $CD8^+$ T lymphocytes. Meaningfully, the upregulation of telomerase treated with CAG is reversible and in short term, which provides a basis for the safety of CAG.

2.9. Anti-gastric cancer

Gastric cancer ranks high among human common cancers diagnosed and STAT3 has been used as a hallmark for the prognosis of gastric cancer patients [69]. Compared with normal GES-1 cells, CAG exhibited higher cytotoxicity to SNU-1 and SNU-16 cells at the concentration of 25 μM and significantly inhibited the constitutive phosphorylation of STAT3 (Tyr705) and the STAT3-DNA binding [70]. In addition, CAG could concentration-dependently upregulate the expression of SOCS-1/3 protein and suppressed the phosphorylation of JAK and Src (Fig. 5). Simultaneously, CAG directly increased procaspase-3 activity and poly ADP-ribose polymerase (PARP) cleavage in tumor cells, inducing cell apoptosis, and not autophagy.

According to the report, the combined treatment with CAG and paclitaxel enhanced SNU-16 cells apoptosis, along with the reduction levels of Bcl-2, Bcl-xL, COX-2, VEGF and MMP-2/9, intensely alleviating severe side effects and chemotherapeutic resistance of paclitaxel.

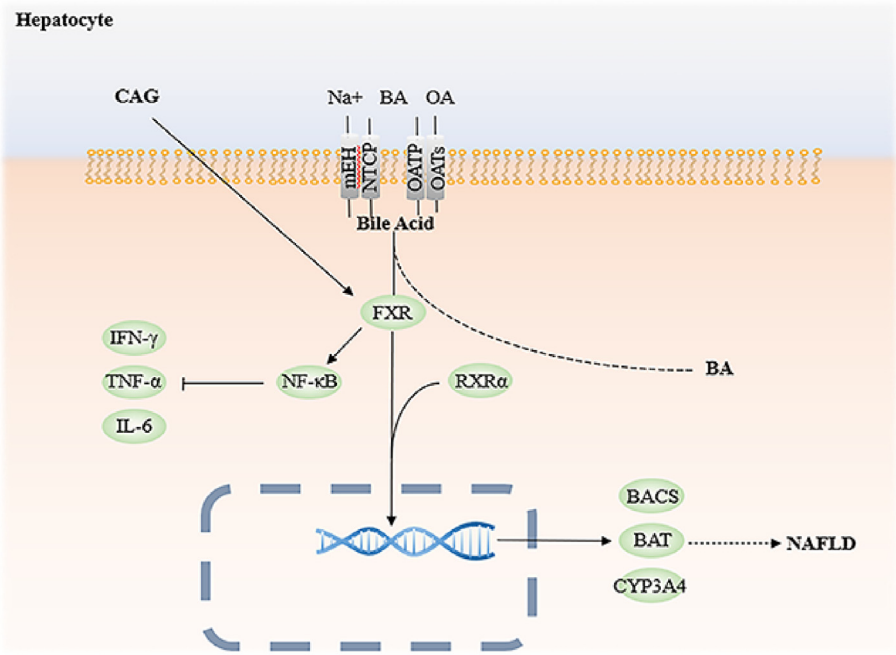


Fig. 6. Potential signaling pathways of CAG in the treatment of NAFLD.

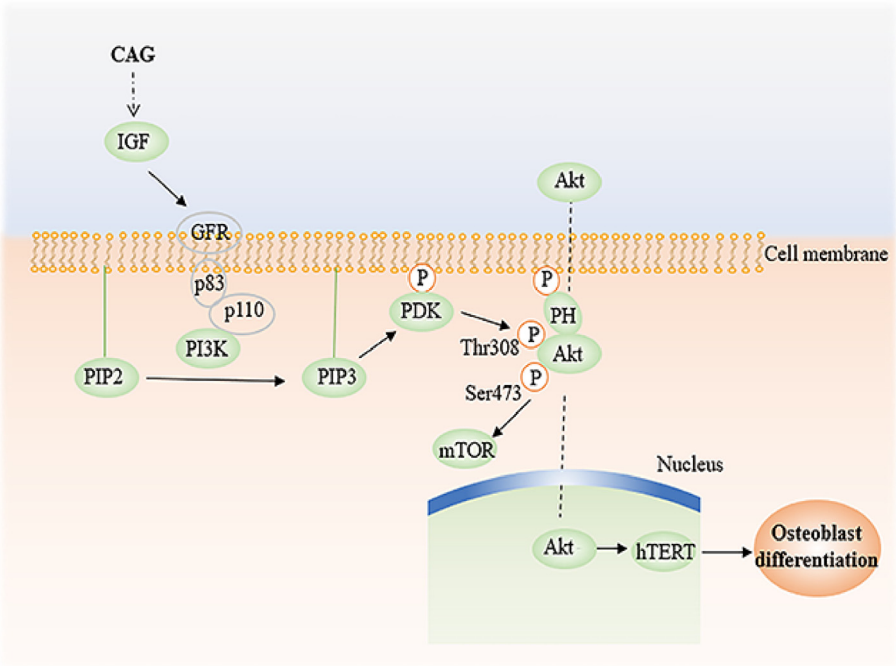
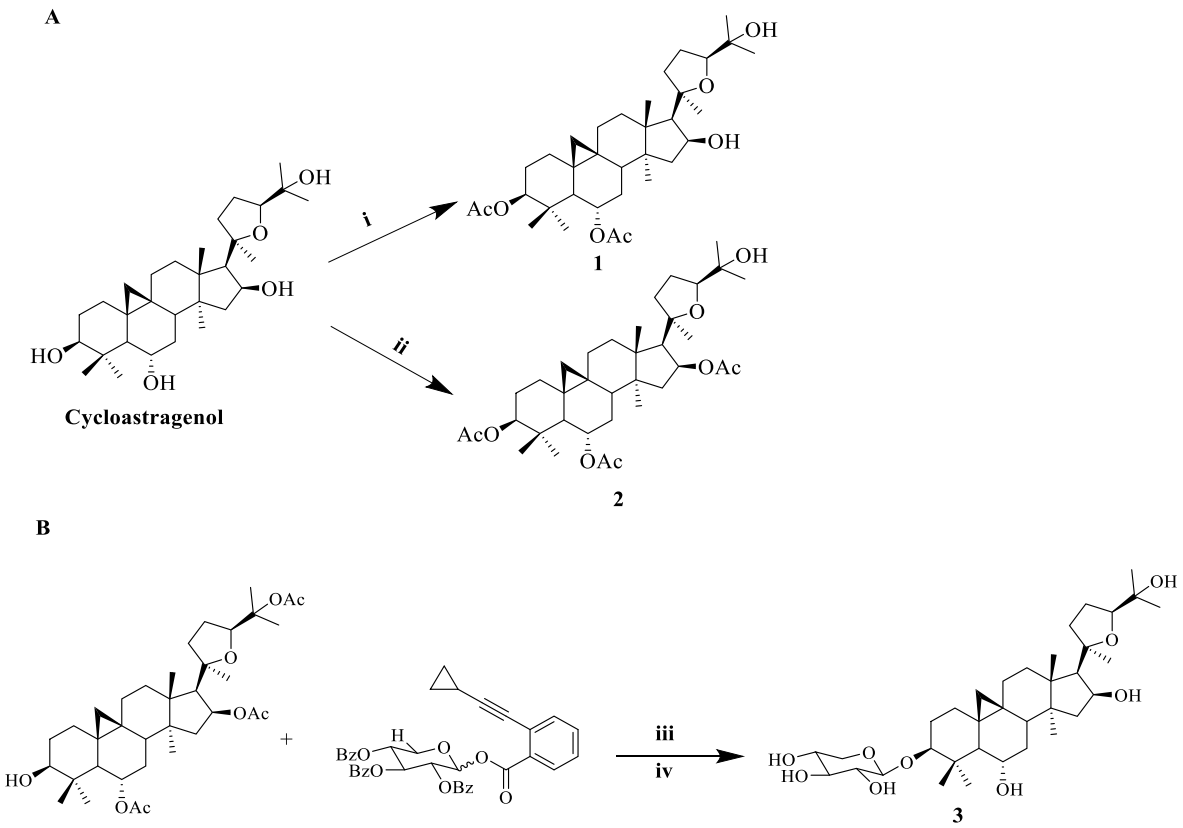


Fig. 7. Potential signaling pathways of CAG in the treatment of osteoporosis.

2.10. Treatment of metabolic diseases

Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disease induced by obesity, insulin resistance, dyslipidemia and metabolic disorders [71,72]. FXR, as a ligand-activated nuclear receptor transcription factor, not only maintains the homeostasis of bile acid and cholesterol, but also has the functions of regulating lipid metabolism and glucose homeostasis, improving liver inflammation as well as inhibiting liver fibrosis [73]. Therefore, FXR activator is provided with therapeutic effects on NAFLD.

CAG has been observed beneficially on NAFLD while screening FXR agonists from the natural compound library. Subsequently, CAG markedly regulated the expression of FXR target genes *in vivo* and *iv vitro* assay, which further suggesting that CAG could selectively activate FXR transcription (Fig. 6) [74]. Moreover, CAG significantly decreased the blood glucose, serum triglyceride levels and hepatic bile acid pool size of the high-fat (*HF*) diet-induced obesity (DIO) mice at the dose of 100 mg/kg. Simultaneously, CAG has been demonstrated to inhibit liver steatosis and inflammation both in DIO and L-amino acid diet (MCD) mice, indicating the potential treatment of CAG on NAFLD.



Scheme 1. CAG was prepared through different acetylation reactions and Yu glycosylation protocol to obtain derivatives **1**, **2** and **3**. Reagents and conditions: A (i) Ac_2O-Pyr (1:2), 5 °C, 10 h; (ii) Ac_2O, PPY, DIPEA, toluene, 105 °C, 2 h; B (iii) $Ph_3PAuNTf_2$, 4A MS, CH_2Cl_2, rt; (iv) NaOMe, MeOH; PPY: polypyrrole.

2.11. Treatment of osteoporosis

Osteoporosis is a systemic bone disease prone to fractures, causing bone density, bone quality reduction, bone microstructure destruction and bone fragility addition [75]. Studying the differentiation process and regulation of osteoblasts is of great significance to understand the osteoporosis. While alkaline phosphatase (ALP) expression begins osteoblast differentiation, the mineralization marks osteoblast maturation.

CAG dose-dependently increased ALP activity, mineralization and mRNA of runt-related genes (Runx2, OCN, OPN and Col l) *in vitro* MC3T3-E1 cells and DEX-treated MC3T3-E1 cells (Fig. 7) [76]. Similarly, in DEX-treated zebrafish juvenile vivo experiments, CAG (25, 50, 100 μM) significantly stimulated mineralization area and IOD, indicating the protection of CAG on bone damage. However, the viability of MC3T3-E1 cells was decreased with CAG at doses over 75 μM. The safe dose of CAG *in vivo* should be evaluated in the future.

Additionally, CAG effectively reduced bone loss and promoted osteoblast differentiation, further enhancing bone biomechanical properties as well as an increasing bone activator (*OA*) in D-galactose-induced and natural aging mice, which proves that CAG has the potential therapeutic effect on osteoporosis [77].

3. Pharmacokinetics of CAG

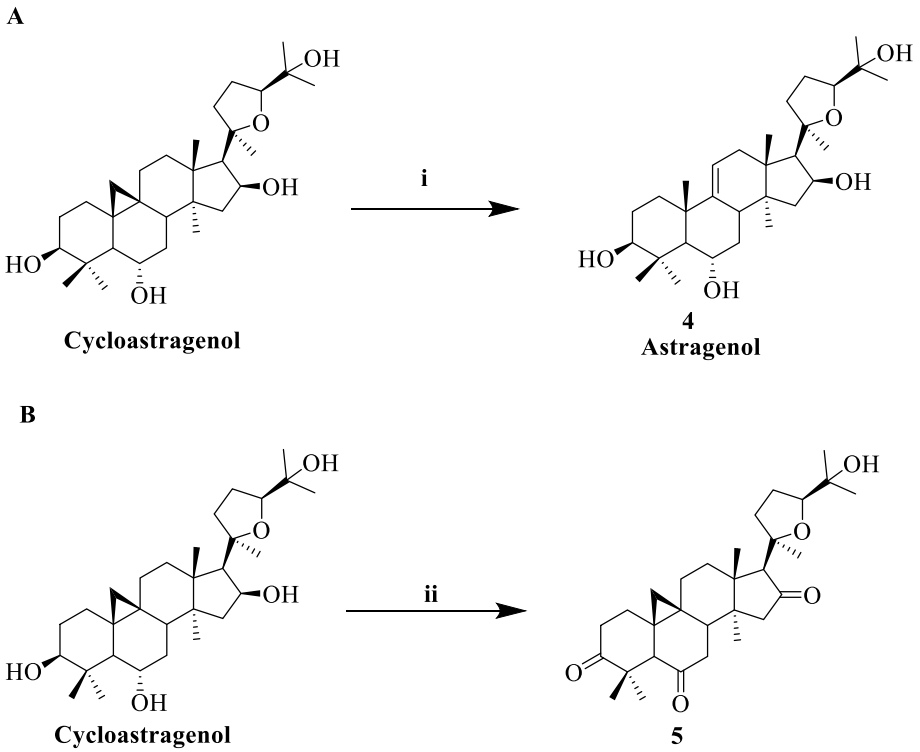
Pharmacokinetic studies are important on drug discovery and optimization, which have become an indispensable anchor for the development of new therapeutic agents [78]. In 2010, the pharmacokinetic study of CAG was reported by Zhu's group using the Caco-2 model and liver microsomes *in vitro* [79]. The study indicated that CAG rapidly passes through the Caco-2 cell monolayer by passive diffusion, occurring first-class intestinal metabolism. Besides that, only 17.4% and 8.2% of CAG remained after 30 min incubation in rat and human liver microsomes, indicating that CAG extensively underwent phase I metabolism.

Furthermore, Ma et al. developed a rapidly and sensitive LC-MS method to quantify pharmacokinetic parameters of CAG [80]. Refer to Zhang' group [81], Ma et al. grinded CAG with defined amount of PEG-400 sufficiently and suspended it in 0.5% carboxymethyl cellulose sodium (CMC-Na), then they performed experimental verification by oral administration. The results showed that the mean absorption time of CAG at 10 mg/kg was 5.70 ± 1.62 h and the oral bioavailability was 25.70%. All these remarkable results showed a base for CAG to develop into a new clinical agent.

4. Toxicology of CAG

Cycloastragenol is favored by researchers because of its broad and good pharmacological activities, therefore, the safety of cycloastragenol is particularly important. Cycloastragenol is a telomerase activator [82]. Fortunately, the experimental results did not show an increased risk of cancer and other adverse effects. A pathological analysis was performed that of female mice sacrificed after 4 months of treatment, and it showed the slightly decreased sarcomas and tracely increased lymphomas; however, comparing to the untreated mice, the increased incidence of cancer of CAG-treated mice were not significant [83]. Also, there was no treatment-related death or adverse events occurred in rats ingested at the highest dose of 150 mg/kg/d CAG for 91 consecutive days, and no induced-toxic or genotoxic expressed *in vitro* chromosome aberration assay or *in vivo* erythrocyte micronucleus assay [84].

Moreover, Calvin reported the health of humans taking a commercial age-management product, PattonProtocol-1, composed of CAG and other dietary supplements. Over five years since the program was launched, there were nearly 7000+ person exposure 50 mg/kg dose equivalence per year, and only few of them showed adverse reactions, which were not



Scheme 2. CAG was prepared by ring-opening reaction and oxidation reaction to produce derivatives **4** and **5**. Reagents and conditions: a (i) H_2SO_4, MeOH, 70 °C; b (ii) Cr_3O, pyridine, rt, 8 h.

attributed to CAG [85]. The food and administration (FDA) declared that CAG was been generally recognized as safe in November 2014.

5. The chemical structural modification of CAG

Over the past decades, based on the extensive good pharmacological activities and challenging compound skeleton, related-structural modifications of CAG were focused on acetylation, glycosylation, ring-opening reaction and oxidation reaction. Due to the rigidity and selectivity of its construction, there are few reports on chemical synthesis. Therefore, there are still great development prospects in the search for candidate molecules with good drug-like properties and excellent biological activity.

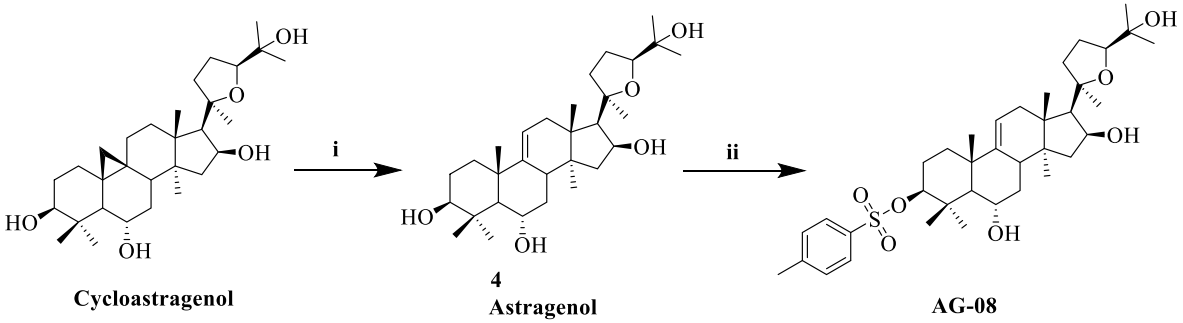
5.1. Acetylation and glycosylation reaction

Significantly, Liu et al. firstly determined the reactivity and reaction sequence of the four hydroxyl groups, solving the problem of the complexity of the polyhydroxy reaction. They demonstrated that 3,6-acetylated product **1** could be synthesized using acetic anhydride-pyridine as a solvent, then stirring for 10 h at 5 °C (see Scheme 1) [86]. However, under the combined effects of Ac_2O, PPY and *N, N*-diisopropylethylamine (DIPEA), CAG was fluently acetylated in toluene at 105 °C, delivering the 3,6,16-triacetate **2** [87]. In addition, via the Yu glycosylation protocol, cycloastragenol and glucosyl donor were in dichloromethane solution under $Ph_3PAuNTf_2$ or $Ph_3PAuOTf$ catalysis to obtain glycosylation products **3** in high yield [87].

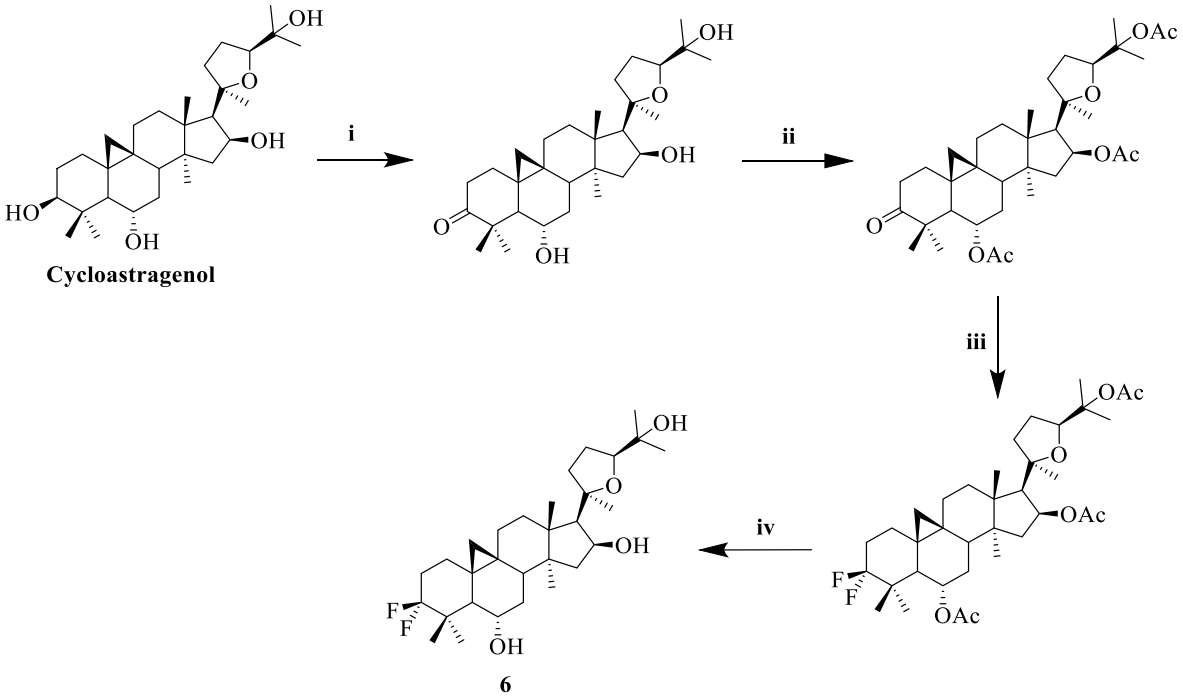
5.2. Ring-opening reaction and oxidation reaction

Additionally, the cycloastragenol derivative Astragenol **4** was obtained in good yields through a ring-opening reaction [86]. Subsequently, the study offered a novel method to synthesis 3,6,16-trione derivatives **5** by treating the cycloastragenol solution in pyridine with chromium oxide-pyridine solution and stirring the whole mixture at 25 °C for 8 h (see Scheme 2) [86].

Based on the synthesis of Astragenol **4,** Üner G et al. demonstrated the semi-synthesis of **AG-08** (see Scheme 3) [88]. First, Astragenol **4** was dissolved in pyridine, and p-TsCl (p-tosyl chloride, Acros Organics) reagent was added. Then the reaction was continued for 4 h at room temperature. Lastly, **AG-08** was purified from the dried reaction mixture on silica gel (34.7% yield).



Scheme 3. Semi-synthesis route of **AG-08** from cycloastagenol. Reagents and conditions: (i) H_2SO_4, MeOH, 70 °C; (ii) p-TsCl, pyridine.



Scheme 4. Synthetic route of difluoro-cycloastraganol derivatives. Reagents and conditions: (i) DMSO, SO_3-Pyridine, Et3N; (ii) Ac_2O, 4-(pyrrolidin-1-yl)-pyridine, toluene, Et_3N; (iii) DAST, toluene; (iv) DABALH, CH_2Cl_2; DMSO: dimethyl sulfoxide; DAST: diethyl amino sulfur trifluoride; DABALH: di-isobutyl aluminum hydride.

Interestingly, this study showed that the semi-synthetic compound **AG-08** was a new cytotoxic sapogenin derivative. The cell experiment data showed that **AG-08** induces noncanonical cell death with necrotic features, potentially becoming an interesting lead compound for anti-cancer drug research.

5.3. Halogenation reaction

Currently, Zhang et al. reported an effective method for synthesizing halogenated cycloastragenol derivatives [89]. The synthetic route of derivative **6** is shown in Scheme 4. Firstly, they used Parikh-Doering oxidation to oxidize cycloastragenol to a ketone derivative, which was the key step in the whole synthesis process. Subsequently, the remaining three hydroxyl groups were all protected with acetic anhydride. Then the critical intermediate difluoro-substituted derivative was generated by reaction with diethyl amino sulfur trifluoride. Finally, they used di-isobutyl aluminum hydride to remove the protective group to produce the derivative **6**.

Furthermore, Zhang et al. also conducted pharmacokinetics and biological pharmacodynamic research on halogenated cycloastragenol derivatives [89]. The study data indicated that the derivative **6** was stable in human and rat liver microsomes, better than cycloastragenol. Additionally, it showed better chronic heart failure efficacy than clinical standard treatment drug Enalapril and new drug LCZ696 (Sacubitril Valsartan Sodium) with good clinical prospects and greater social benefits.

6. Discussion and conclusion

In this review, the research progress of extensively biological efficacy and involved signaling pathways of cycloastragenol were comprehensively summarized. We found that the preventive and therapeutic effects of cycloastragenol on many diseases are usually the result of the joint action of multiple signaling pathways, which belongs to one of the characteristics of natural product drugs. And the structure modifications of cycloastragenol and preliminary study on pharmacokinetics were also mentioned. A number of evidences indicate that cycloastragenol has therapeutic potential on neurodegenerative, cardiovascular, respiratory, metabolic diseases et al. The combination therapy with cycloastragenol and other drugs could alleviate the side effects of drugs. Therefore, further multicenter clinical studies are expected for specific diseases.

At present, poor water solubility, faster metabolic conversion and lower oral bioavailability still restrict the clinical application of cycloastragenol. Therefore, it is urgent to attempt structural modification for improving absorption, distribution, metabolism and excretion (ADME) of cycloastragenol. In the perspective of future development, it is hoped that, cycloastragenol is valuable clinical choice for those specific diseases caused by inflammation, oxidative stress, cell senescence, or autophagy in the near future.

Author contribution

The manuscript was written through the contributions of all authors. All authors have given their approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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