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Exploring medicinal potential and drug delivery solutions of Celastrol from the Chinese "Thunder of God Vine"

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ABSTRACT

Tripterygium wilfordii (TRWI), known as 'Thunder of God Vine' or 'Lei Gong Teng' in traditional Chinese medicine (TCM), is a perennial vine that has been used for centuries for its potent therapeutic properties. This plant, which belongs to the Celastraceae family, has been documented in various TCM texts, where it has been attributed with a wide range of benefits, including anti-inflammatory, antirheumatic, and anti-autoimmune activities. Central to the medicinal potential of TRWI is celastrol, a triterpenoid with extensive pharmacological activities. Research on celastrol has revealed its effects on combating inflammation, oxidative stress, cancer proliferation, and neurological disorders. However, celastrol's high toxicity, low water solubility, and limited stability pose challenges for its clinical application. In this review, we explore the chemical structure of celastrol, emphasizing its key pharmacological activities and the structure-activity relationships (SARs) that influence its efficacy and toxicity. Various studies have demonstrated that modifications at specific sites, such as the C-29 carboxylic group, C-6, and C-3, can enhance celastrol's therapeutic potential while reducing adverse effects. Moreover, recent advances in drug delivery systems offer promising avenues to overcome the inherent limitations of celastrol. These include direct modifications such as PEGylation and indirect modifications through encapsulation in dendritic polymers, phytosomes, liposomes, and exosomes. Each method seeks to improve celastrol bioavailability, water solubility, and target capabilities, thus enhancing its clinical viability. The objective of this review is to synthesize current knowledge about celastrol's therapeutic potential and discuss the future of its development in drug delivery and pharmaceutical applications. These findings could open the door to new treatment methods that combine traditional remedies with modern pharmacology, helping us unlock the complete potential of celastrol in clinical use.

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1. Introduction

Tripterygium wilfordii Hook F. (NCBI: txid458696) (TRWI), commonly known as 'Thunder of God Vine' or 'Lei Gong Teng' in Chinese, has long been valued in traditional Chinese medicine (TCM) [1]. This perennial vine of the genus *Tripterygium* of the family Celastraceae has been a staple of traditional Chinese medicine practices for centuries. Its therapeutic properties, deeply rooted in ancient wisdom, span a wide spectrum of applications. TRWI is traditionally recognized for its anti-inflammatory and anti-autoimmune benefits and its efficacy in the treatment of rheumatism, cancer, and skin disorders.

The first documented use of TRWI dates to the South Yunnan Materia Medica in the Ming Dynasty [2]. It has been noted for its ability to "dispel pathogenic wind", "remove dampness", and "promote blood circulation". Over the centuries, TRWI has been used to address various health issues, due to its unique chemical composition, including diterpenoids, triterpenoids, alkaloids, and glycosides. Contemporary research has explored its pharmacological properties in depth, revealing celastrol, a powerful triterpenoid with a diverse array of health benefits.

In recent years, researchers have focused on bridging the traditional and modern aspects of TRWI, exploring its applications in contemporary pharmacology. Exploring the therapeutic potential of TRWI and celastrol offers a unique opportunity to connect ancient healing methods with modern medical advancements. By investigating its mechanisms, safety profiles, and potential drug delivery systems, scientists aim to unlock TRWI's potential for clinical use in diverse disease conditions. This convergence of traditional medicine and modern research highlights the enduring importance of TRWI and celastrol in the current medical field.

2. Background

Chemical investigations have revealed that the complex components of TRWI include diterpenoids, triterpenoids, alkaloids, and some glycosides. Of these, celastrol (Cel, **1**, Figure 1), also known as tripterine, is a water-insoluble triterpenoid quinone methide triterpene and the main active principle of *Tripterygium wilfordii* Hook F.

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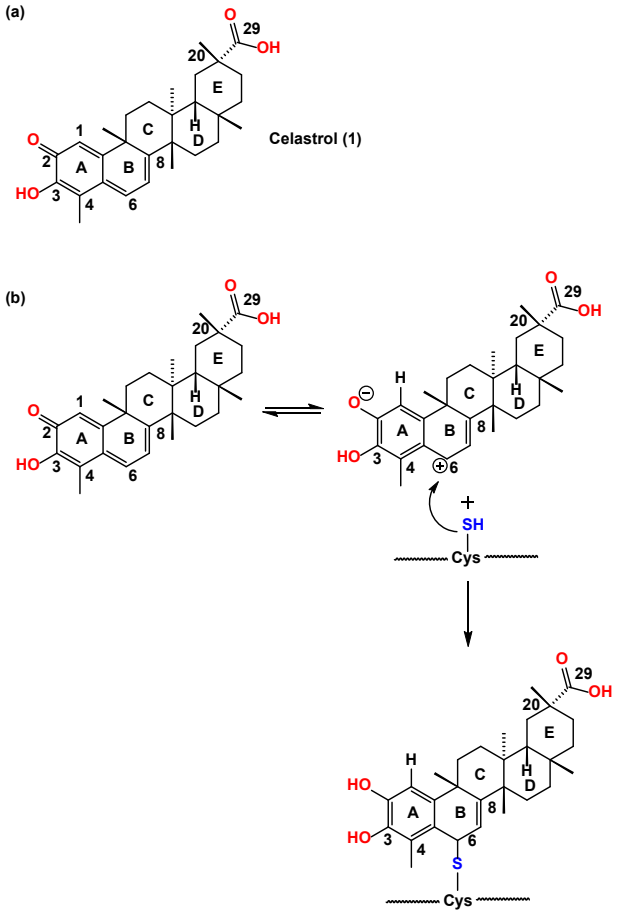

Figure 1. (a) Molecular structure of compound **1** and (b) Michael adducts interact with the biological target.

The natural product has a wide range of promising pharmacological activities relevant to inflammation [3], antioxidant [4], anti-allergy [5], anti-osteoarthritis [6], cardiac protection [7], neuronal degeneration [8], infectious disease [9] and obesity [10]. It has also been reported to inhibit cancer cell growth [11], induce apoptosis [12,13], and suppress invasion and metastasis of tumor cells [14]. Recently, extensive research has indicated that Cel manifests its pharmacological functions through several molecular targets [15], such as SERCA, Hsp90-Cdc37 [16], NF-kB/IKKb [17], the VEGFR1, VEGFR2 and PI3K/Akt signaling pathway. Previous studies suggested that the quinone methide moiety of celastrol was crucial for its cytotoxic activity in cancer cell lines [18]. Celastrol has electrophilic sites within the A and B rings, where nucleophilic groups of amino acid residues react to form covalent Michael adducts (Figure 1).

Intracellular calcium (Ca^{2+}) acts as a second messenger, playing a critical role in regulating a variety of cellular processes such as gene transcription, cell morphology, motility, proliferation, mitochondrial function, apoptosis, and immune responses [19]. Two decades ago, researchers recognized the significant role Ca^{2+} plays in various diseases, including cardiovascular disease, stroke, diabetes, immune responses (like inflammation), and cancer [20]. Ca^{2+} has a crucial role in autoimmunity and inherited immunological disorders. Recent studies indicate that intracellular Ca^{2+} signaling is involved in the development of autoimmune diseases such as lupus erythematosus and rheumatoid arthritis (RA) [21]. Celastrol has shown antiarthritic properties in rheumatoid arthritis (RA), but its role in Ca^{2+} mobilization in the treatment of RA is not yet fully understood. Research indicates that celastrol can induce endoplasmic reticulum (ER) stress through the mobilization of intracellular calcium (Ca^{2+}), suggesting a potential pathway for its therapeutic effects. However, more studies are needed to elucidate the exact mechanisms by which celastrol influences Ca^{2+} dynamics in RA [22].

Celastrol was used as a molecular tool to examine the profile of inflammatory gene expression regulated by Ca^{2+}. In a previous study, Wong *et al.* found that treatment with celastrol led to cytosolic Ca^{2+} mobilization in rheumatoid arthritis synovial fibroblasts (RASF) derived from patients [23]. In their study [24], Wong *et al.* investigated the regulatory role of celastrol-induced Ca^{2+} signaling in synovial fibroblasts of rheumatoid arthritis patients and adjuvant-induced arthritis (AIA) in rats. They employed computational docking, Ca^{2+} dynamics, and functional assays to examine the activity of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump (SERCA). The sarco-endoplasmic reticulum (SR) Ca^{2+} transport ATPase (SERCA) is the sole pump responsible for transporting calcium ions from the cytoplasm into the SR. The study reported that celastrol inhibited the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), triggering autophagy-dependent cytotoxicity in rheumatoid arthritis synovial fibroblasts via the Ca^{2+}/calmodulin-dependent kinase kinase-β-AMP-activated protein kinase-mTOR pathway. This action also led to suppression of arthritis symptoms in adjuvant-induced arthritis (AIA) rats.

2.1. Medicinal chemistry of celastrol

Despite the beneficial therapeutic properties currently understood, it has some undesirable pharmacological properties, such as high toxicity, poor water solubility, and stability, which hinder its future clinical application [25].

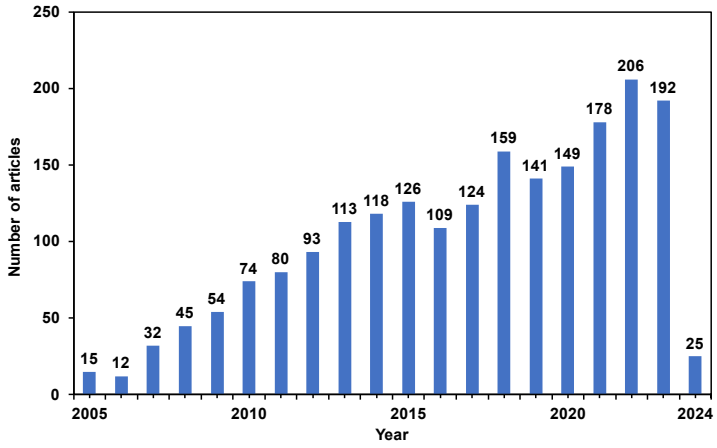

Figure 2. Number of articles published between 2005 and 2024 containing the keyword "celastrol" according to the Science Finder database. This bar graph shows a noticeable increase in publications over the years, indicating a growing interest in celastrol-related research. The growing trend reflects the expanding exploration of celastrol's pharmacological properties and its potential applications in various therapeutic areas.

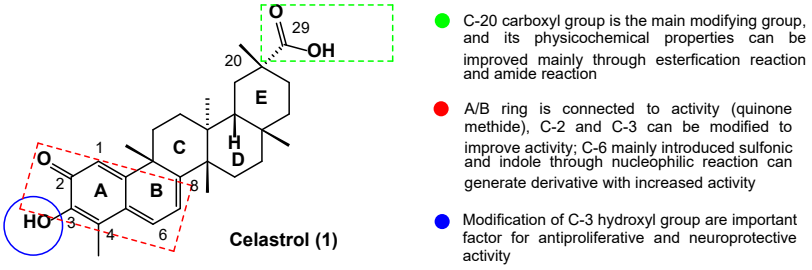

Figure 3. The structure-activity relationship of celastrol. Based on the analysis of the structure of celastrol, it was found that the quinone methyl of the A/B rings, the hydroxyl at the C-3 position, and the carboxyl group at the 29-position were the main pharmacodynamic groups. Among them, the C-29 carboxylic group is the prime modification site of celastrol (green site), which can play a key role in its chaperone activity (I); Modification of C-6 with C–S or C–C bonds could generate derivatives with increased antiproliferactive activities (red site) (II); the C-3 hydroxyl group plays a key role in its antiproliferative activity and neuroprotective activity (blue site) (III).

Reviews related to its structural modifications, structure-activity relationships (SAR), pharmacology, and toxicology have emerged recently (Figure 2).

In recent years, great effort has been devoted to the study of celastrol structure-activity relationships (SARs) (Figure 3). Many studies [26,27] on the chemical modification of celastrol have been carried out at home and abroad in the last decade, and the preliminary SARs are as follows (Figure 3).

(i) The C-29 carboxylic group stands out as the primary site for modification in celastrol, potentially influencing its chaperone activity significantly. Typically, structural modifications aim to introduce various groups through acylation and esterification, with the goal of developing potential drug candidates exhibiting enhanced pharmacological profiles and minimal toxicity [28]. The addition of halogen elements, a benzene ring, and a hydroxyl group significantly improved the anticancer effectiveness of the compounds. However, the efficacy of six-ring variants decreased markedly [25]. In particular, alterations to the carboxylic acid group of celastrol did not substantially affect its ability to disrupt Hsp90/CDC37 inhibition. However, derivatives featuring aromatic phenyl substituents exhibited potentially reduced toxicity to normal cells compared to those with nonaromatic alkyl groups. Furthermore, the conjugation of celastrol with other mechanistically distinct anticancer agents, such as ferulic acid and its derivatives, or biotins through the C-29 carboxylic group, has the potential to produce hybrids with synergistic effects or targeted delivery capabilities (Figure 4) [29-31]. We previously designed, synthesized, and evaluated the eleven celastrol derivatives for their *in vitro* cytotoxic activities against human cancer cell lines and normal cells [32].

(ii) Introducing C–S or C–C bonds at the C-6 position has the potential to produce derivatives with enhanced antiproliferative properties. Sulfonation and sulfidation at C-6 may increase cytotoxicity, whereas carbonation significantly decreases cytotoxic effects. Furthermore, subsequent acetylation at C-2 and C-3 is shown to be more effective than propionylation in terms of cytotoxicity. While the anticancer efficacy of 6-substituted indole (Figure 5a) exhibited a decrease, the addition of an exocyclic C–C bond at the C-6 position led to the deactivation of the Michael acceptor system within the ring A (see Figure 3, red square). This observation suggests that the presence of the Michael acceptor is not always essential for potent cytotoxic activity [33].

(iii) The presence of the C-3 hydroxyl group is critical for both the antiproliferative and neuroprotective properties of the compound. The specific type and dimensions of the carbamate substituents attached to this hydroxyl group significantly influenced its cytotoxic efficacy in vitro. Although modifying the C-3 hydroxyl group with size-constrained groups had minimal impact on its antiproliferative potential, incorporating hydrophilic moieties like piperazine proved beneficial in improving solubility (Figure 5b [34,35]. Furthermore, derivatives obtained from the coupling of tripterine with anticancer drugs (lipoic acid or ligustrazine) can show cytoprotective effects against various stress-induced nerve injuries [8].

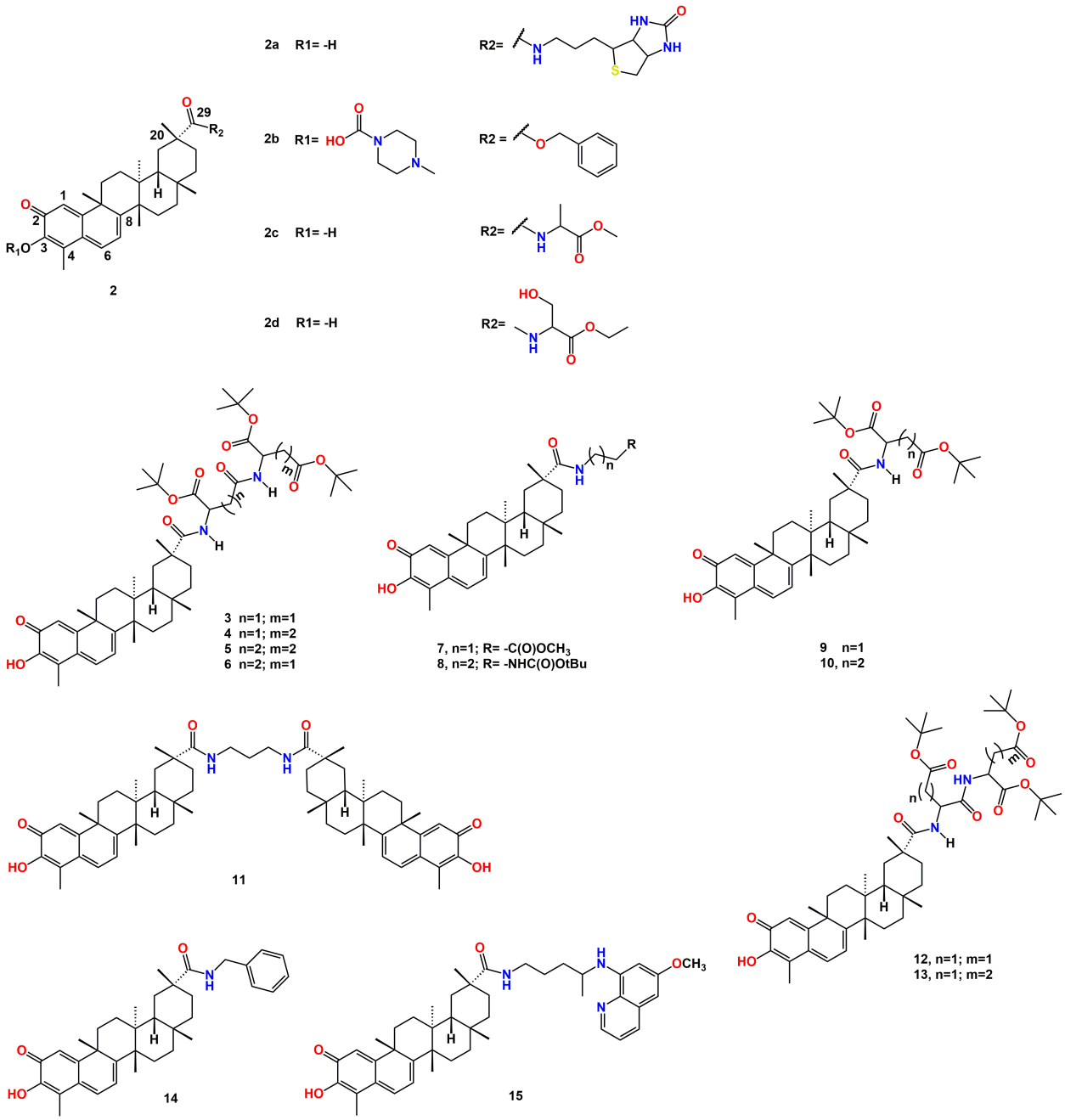

Figure 4. Chemical structures of celastrol derivatives **2**–**15**, which have been modified at the C-29 carboxyl group. This figure illustrates the various modifications made to the chemical structure of celastrol to explore its impact on the pharmacological properties (I) [28-32,36].

(iv) Modification of ring A: During the lead compound screening for potential treatments targeting neurodegenerative diseases, Westerheide and colleagues identified celastrol as a stimulator of the human heat shock response [37]. To elucidate the structural specificity of celastrol, they conducted additional experiments to evaluate the efficacy of various derivatives (at concentrations of 3 and 8 mM) in activating the heat shock promoter reporter, in particular the results indicated that **1** and dihydrocelastrol diacetate **23** are all active at 3 mM (Figure 5b). To identify celastrol derivatives possessing robust neuroprotective properties while exhibiting reduced toxicity, Sun and collaborators synthesized a range of derivatives modified at the C-2, C-3 (Figure 5c) [8].

2.2. Triazole derivatives and click chemistry of celastrol

Triterpenoids, natural compounds renowned for their diverse biological activities, exhibit considerable promise in therapeutic applications. Despite their advantageous low toxicity and abundant availability from natural sources, their clinical utility remains restricted due to higher IC_{50} values and inferior pharmacological profiles compared to established therapeutics. Motivated by this limitation, numerous researchers have embarked on endeavors to engineer novel terpenic derivatives with enhanced pharmacological properties, aiming to unlock their full potential for clinical use [38].

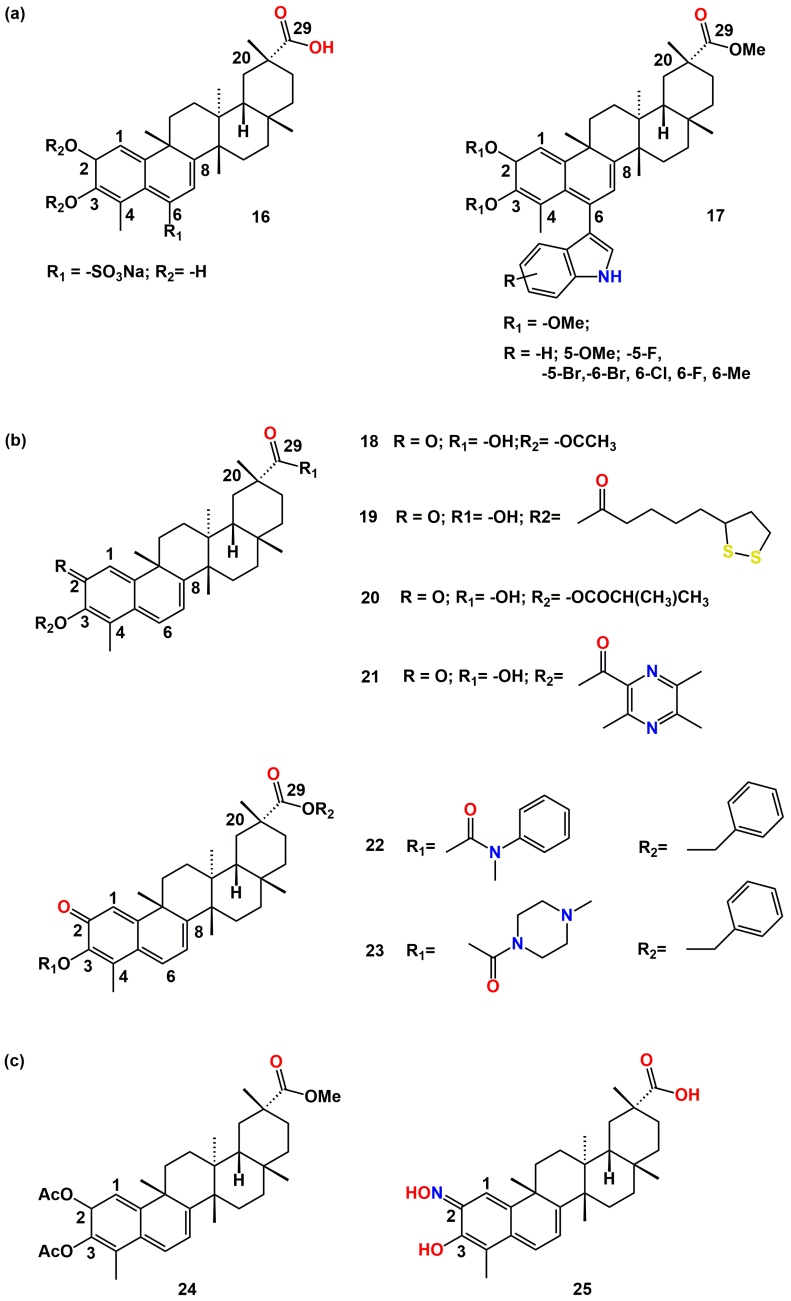

Figure 5. (a) Celastrol derivatives **16**,**17** chemically modified by the C-6 group (II) [33,35]; (b) Celastrol derivatives chemically **18**–**23** modified by the C-3-hydroxyl group [8,30]; (c) Celastrol derivatives chemically **24**, **25** modified at ring A [37].

In recent years, significant exploration has been conducted in improving the activity and ADME-Tox properties of terpenes by linking them to modifying molecules through click reactions. This synthetic strategy has shown promising advancements, leading to notable improvements in the parent compounds.

The prominent Cu(I) (copper)-catalyzed azide–alkyne cycloaddition (CuAAC, Click Chemistry, Figures 6a and 6b), discovered independently by Meldal *et al*. [39] and Sharpless *et al*. [40], is the most common and promising approaches that are used to produce the most efficiently in the synthesis of various 1,2,3-triazoles [41] (Figure 6a and 6b). The Nobel Prize in Chemistry 2022 was awarded jointly to Carolyn R. Bertozzi, Morten Meldal, and K. Barry Sharpless for the development of click chemistry and bioorthogonal chemistry [42]. The 1,2,3-triazole core can form π-π interaction with aromatic rings such as the phenyl ring of amino acids in targets. Their unique structural characteristics, including their polarity, dipole moment, rigidity, and ability to function as both hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), allow them to mimic a variety of functional groups while offering significant stability against hydrolytic, oxidative, and reductive conditions. These compounds generally withstand hydrolysis in both acidic and basic environments, as well as remain stable in oxidative-reductive conditions, indicating high resistance to metabolic degradation and substantial aromatic stabilization. In particular, 1,2,3-triazoles are among the most prevalent amide/guanidine isosteres. Their structural attributes allow them to closely resemble amide-binding groups, facilitating effective interaction with various targets.

The click chemistry strategy was also adopted for the structure modification of Cel C-3 OH (Figure 7a). Zhang *et al.* synthesized by modifying carboxylic acid at the 20th position with amino acid, amine, and triazole derivatives [43].

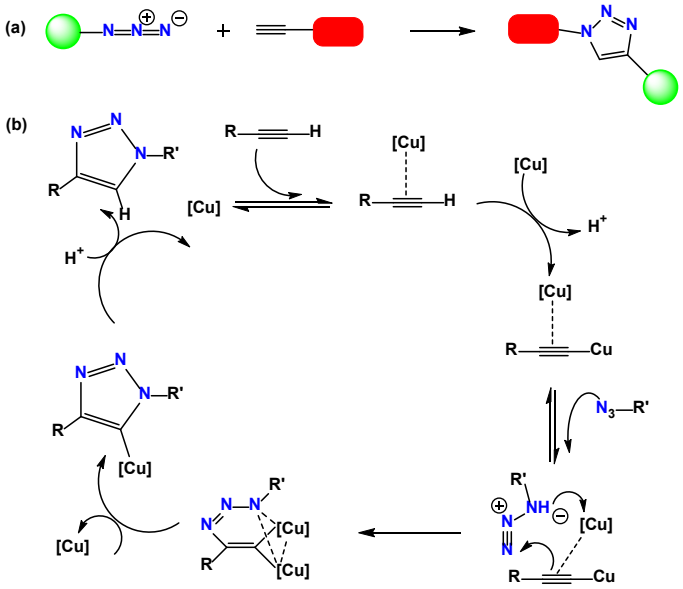

Figure 6. (a) Schematic representation of click chemistry and (b) mechanism of the copper-catalyzed azide-alkyne cycloaddition (CuAAC). The diagram shows the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) process, demonstrating the formation of 1,2,3-triazoles from azide and alkyne reactants.

Recently, the click chemistry modification strategy was successfully applied to the structure modification of the C-28 carboxylic group of C-20 carboxylic group of compound **1**, and the obtained celastrol-1,2,3-triazole derivatives that exhibited potent anticancer activities (Figure 7b) [44].

3. Drug delivery of celastrol

Celastrol, a natural compound derived from the thunder god vine, has garnered significant attention because of its potential to treat a variety of diseases, such as cancer, obesity, and neurodegenerative disorders. However, despite its promising therapeutic effects, celastrol's clinical application is hampered by several notable side effects and limitations. These include its low bioavailability, which indicates that only a small fraction of the drug reaches the systemic circulation; weak water solubility, making it difficult to dissolve in biological fluids; cytotoxicity, which could harm healthy cells; and hepatotoxicity, which poses risks to liver health [46].

Given these limitations, researchers have been exploring innovative approaches to improve the safety and effectiveness of celastrol in medical treatment. One of the key strategies involves the development of specialized drug delivery systems that can improve the transportation of celastrol to targeted areas within the body. By ensuring precise delivery, these systems aim to maximize the therapeutic benefits of the drug while minimizing its adverse effects [47].

Current celastrol drug delivery methods can be divided into two categories: Direct and indirect modification.

3.1. Direct modification

Direct modification delivery methods involve direct chemical modification of the celastrol molecule itself to improve its bioavailability, water solubility, targeting, and reduced toxicity. For example, by attaching durable, highly water-soluble, and biocompatible polyethylene glycol (PEG) to celastrol in an appropriate manner, its water solubility can be improved. When fatty acid chains are attached to celastrol, you can increase its lipophilicity and membrane penetration capabilities, which in turn increases its bioavailability and therapeutic effectiveness. The introduction of an ester group into the molecule can protect the active part through an esterification reaction. Upon entering the body, the enzymatic action cleaves the ester group, releasing the active celastrol.

Such modifications can significantly improve the physicochemical characteristics of the molecules, thereby addressing common obstacles in drug delivery, such as rapid degradation, recognition by the immune system, and limited cellular uptake.

Pengjin Ge *et al.* have proposed a collaborative design strategy using dendrimer polymers for targeted celastrol delivery in both in vitro SW620 colon cancer cell models and in vivo SW620 tumor-bearing nude mice, demonstrating good specificity, efficiency and biosafety. Successful design can also be applied to the treatment of liver cancer, not limited to colon and liver cancer [48]. Yanan Tan *et al*. utilized lipophilic cationic CTPP (a type of TPP cation) to modify the liposome carrier CSOSA, preparing targeted mitochondrial nanoparticles loaded with celastrol, resulting in CTPP-CSOSA/celastrol micelles. *In vivo* experiments demonstrated that micelles can facilitate rapid drug release into the mitochondria, reducing drug leakage into the cytoplasm and lysosome, and significantly increasing the tumor inhibition rate of celastrol from 54.89 to 80.17% [49].

3.2. Indirect modification

Indirect modification drug delivery systems work by encapsulating or wrapping the celastrol molecule rather than directly altering its chemical structure. In this system, the therapeutic agent is encapsulated or embedded within the carrier structure, thereby preventing degradation, improving solubility, controlling release, and enhancing bioavailability.

Dendritic polymers are highly defined artificial polymer macromolecules characterized by a combination of numerous functional groups and a compact molecular structure. B. Niu and colleagues conjugated **1** with PEGylated EpCAM aptamer or antibody dendritic macromolecules, obtaining two types of nanoconjugates [50]. The results showed that, compared to the antibody counterpart, the PEGylated aptamer nanoconjugate demonstrated enhanced accumulation and specific retention at the tumor site, as well as stronger intratumoral penetration ability by reducing the macrophage reservoir effect in solid tumors. When **1** was delivered to a mouse model of colorectal heterograft tumor through PEGylated aptamer dendritic polymers, the therapeutic efficacy increased by 20% compared

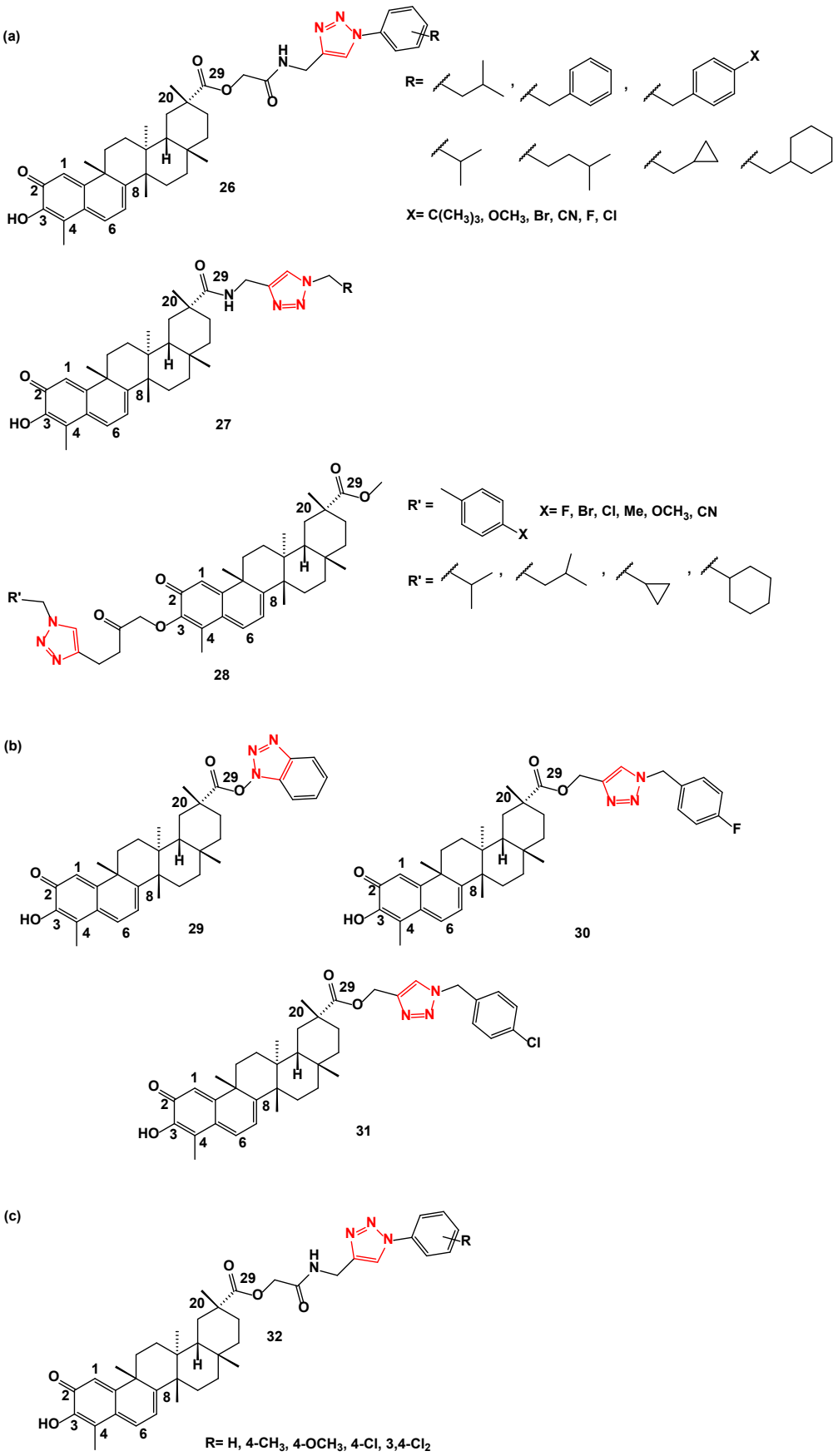

Figure 7. (a) 1,2,3-Triazole derivatives **26** – **28** obtained by click chemistry [44] and (b,c) Some representative in the literature of celastrol triazole **29–32** [36,43,45].

Table 1. Different drug delivery for celastrol.

Drug delivery	Type	Advantages	Disadvantages
Direct	Glucolipid [56]	- Improved water solubility: Glucolipids combine sugar and lipid groups, enhancing the solubility of lipophilic drugs. - Biocompatibility: Typically derived from natural sources, glucolipids are generally biocompatible. - Targeted delivery: Their amphiphilic nature allows for better cellular uptake and potentially targeted drug delivery.	- Stability: Glucolipids can be prone to degradation under certain conditions. - Limited versatility: They may not be suitable for all drug types.
	Nucleic acid aptamer [47]	- High specificity: Aptamers can be designed to bind specific molecular targets, leading to precise drug delivery. - Small size: This can facilitate better tissue penetration and cellular uptake. - Biodegradability: Aptamers are biodegradable, reducing toxicity concerns.	- Susceptibility to degradation: Aptamers can be degraded by nucleases in the body. - Cost: Custom synthesis of aptamers can be costly and time-consuming.
	Nucleic acid aptamer-dendrimer [48]	- Enhanced stability: Dendrimers provide structural stability to aptamers, improving resistance to degradation. - Controlled release: Dendrimers can enable a controlled release of the drug at the target site. - Increased loading capacity: Dendrimers offer high loading capacity due to their branched structure.	- Complexity: The combination of dendrimers and aptamers can lead to more complex synthesis and manufacturing processes. - Potential toxicity: Some dendrimers may be toxic or trigger immune responses.
Indirect	Polymer [57,58]	- Versatility: Polymers can be customized for various drug types and release profiles. - Controlled release: Polymers can offer tailored drug release rates. - Stability: Polymers generally provide good stability and protection for the drug.	- Biocompatibility: Some polymers can cause adverse reactions or inflammation. - Complexity: The variety of polymers can require more extensive testing for biocompatibility.
	Dendrimer [48,59]	- High loading capacity: Dendrimers' branched structure allows for a high drug loading capacity. - Precise delivery: Their defined structure can offer precise drug delivery and controlled release.	- Potential toxicity: Certain dendrimers can be toxic, depending on their structure and terminal groups. - Complex synthesis: Dendrimer production can be complex and expensive.
	Albumin [60,61]	- Biocompatibility: Albumin is a natural protein, reducing the risk of immune reactions. - Prolonged circulation: Albumin-based carriers can extend drug circulation time. - Reduced toxicity: Albumin generally has a lower toxicity profile.	- Limited customization: Albumin's structure might limit the customization options for drug delivery. - Stability concerns: Under certain conditions, albumin can denature, impacting drug stability.
	Phytosomes [51,62,63]	- Enhanced bioavailability: Phytosomes can improve the absorption and bioavailability of phytochemicals. - Biocompatibility: Phytosomes are generally biocompatible and derived from plant-based materials. - Improved solubility: They can increase the solubility of certain drugs.	- Limited application: Phytosomes may be more effective for specific compounds, limiting their broader use. - Stability: They might degrade under certain environmental conditions.
	Liposome [53,54,64]	- Versatility: Liposomes can encapsulate a wide range of drugs, including hydrophilic and hydrophobic. - Biocompatibility: Liposomes are biocompatible and biodegradable. - Controlled release: Liposomes can be designed for targeted and controlled drug release.	- Stability issues: Liposomes can be prone to leakage and instability under certain conditions. - Cost: Liposome production and stabilization can be costly.
	Exosomes [55,65]	- Natural origin: Exosomes are derived from cells, which reduces immune reactions. - Targeted delivery: Exosomes can inherently target specific cells or tissues. - Low toxicity: Exosomes generally exhibit low toxicity due to their natural origin.	- Scalability: The production and isolation of exosomes on a large scale can be challenging. - Heterogeneity: Exosomes from different sources may vary in composition and properties, affecting consistency.

to antibody modification. Furthermore, 2 mg/kg celastrol delivered by PEGylated aptamer dendritic polymer exhibited significant anticancer efficiency (nearly 92%) with no apparent side effects [50].

Phytosomes are a class of lipid-based nanoparticles designed to enhance the solubility, absorption, and bioavailability of poorly soluble compounds such as celastrol. Freag *et al*., for the first time, focused on developing self-assembled phytosome nanocarriers for celastrol to enhance its solubility and oral bioavailability [51]. Comparative *in vitro* release studies showed that phytosomes significantly enhanced the release of celastrol. Pharmacokinetic studies in rabbits demonstrated that the oral bioavailability of celastrol in self-assembled phytosome nanocarriers improved significantly compared to crude celastrol, with a four-fold increase in AUC(0-8) and a five-fold increase in C(max). Ultimately, the results confirmed the potential of phytosome nanocarriers to improve oral administration of celastrol [51].

Albumin is an attractive carrier for the delivery of poorly water-soluble drugs. Fan N *et al*. developed a biocompatible albumin-based nanoparticle carrier system for the controlled release of celastrol in diet-induced obese mice [52]. Celastrol was loaded into bovine serum albumin (BSA) nanoparticles, forming celastrol-BSA-NPs through high-pressure homogenization. Celastrol-BSA-NPs exhibited better bioavailability and in vivo efficacy in the treatment of diet-induced obesity. Importantly, this albumin-based nanoparticle system could potentially be a general biocompatible drug carrier system used for the controlled release of hydrophobic compounds, such as celastrol, for the treatment of obesity and nonalcoholic fatty liver disease [52].

Liposomes are a type of vesicular nanocarrier, and vesicular nanocarrier delivery systems can transport both hydrophilic and hydrophobic drugs with low toxicity, prolong the drug's half-life, and control drug release [53]. Liposome technology was the first therapeutic carrier approved by the US Food and Drug Administration (FDA) for the delivery of anticancer drugs. Xinyan Chen and colleagues developed galactosylated liposomes for the delivery of celastrol (C-GPL) using galactose-modified 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-

polyethylene glycol. C-GPL increased the water solubility of celastrol and exhibited a high encapsulation rate, good serum stability, and slow drug release characteristics. *In vitro* studies showed that C-GPL enhanced the cellular uptake of celastrol through receptor-mediated endocytosis, thus increasing celastrol's cytotoxicity and cancer cell apoptosis. The improved therapeutic effects of C-GPL can be attributed to inhibition of AKT activation, induction of cell apoptosis, and delay of cell proliferation. Importantly, C-GPL exhibited low toxicity to normal tissues and did not cause significant weight loss in mice [54].

In addition to liposomes, vesicular nanocarriers also include exosomes. Farrukh Aqil and colleagues investigated the effects of celastrol-loaded exosomes on two nonsmall cell lung cancer (NSCLC) cell lines [55]. Compared to free celastrol, celastrol-loaded exosomes exhibited enhanced antitumor efficacy in lung cancer xenografts. The data demonstrated the chemotherapeutic potential of celastrol in lung cancer, and the exosome formulation enhanced its efficacy while reducing dose-related toxicity [55].

4. Future directions and clinical trials

Future directions for celastrol research are promising, with several key areas poised for exploration and innovation. One of the primary focuses will be on improving the drug's pharmacokinetic profile to enhance its clinical viability. This includes addressing challenges such as low water solubility, toxicity, and stability, which have historically hindered the celastrol's therapeutic use.

Additionally, the integration of celastrol into combinatorial therapies with other drugs has potential for synergistic effects, offering more effective treatment options in antimalarial therapy [9,28]. In particular, celastrol exhibits strong IC_{50} activity, with values ranging from 0.50 to 0.82 μM against drug-sensitive and drug-resistant asexual blood stage Plasmodium falciparum (Pf). It also shows IC_{50} levels of 1.16 and 0.28 μM against immature and late-stage Pf NF54 gametocytes, respectively. When combined with the known antimalarial drugs artemisone or methylene blue, celastrol's effects against asexual blood stage Pf are additive, suggesting the potential for synergistic treatment strategies [9].

As research progresses, understanding the molecular mechanisms underlying celastrol's action will be vital, providing insights that could lead to new therapeutic targets and strategies. Ultimately, these future directions could significantly impact the field of drug development, bringing celastrol-based treatments closer to clinical application and offering hope for patients with challenging medical conditions.

4.1. Completed and ongoing clinical trials

The exploration of celastrol in clinical trials involves testing its safety, efficacy, dosing, and overall therapeutic potential in humans. Despite its promise, celastrol's transition from preclinical studies to human trials has faced challenges, mainly due to its potential side effects and toxicity. The clinical trials with celastrol aim to assess whether these risks can be mitigated through innovative drug delivery systems, dosage adjustments, or other methods.

There are ongoing clinical trials investigating celastrol for various applications [66,67]. Some studies focus on its role in the management of obesity, where celastrol is believed to influence satiety and reduce body weight [10]. Other trials examined its anticancer properties, looking at its ability to inhibit tumor growth and promote cancer cell apoptosis [68] and diabetes [69]. Clinical trials are also exploring its effects on autoimmune diseases, such as Crohn's disease [70] and rheumatoid arthritis [65,71,72], to determine whether celastrol can reduce inflammation and alleviate symptoms.

Clinical trials with celastrol must address several key challenges: (i) Toxicity and side effects: Celastrol's hepatotoxicity, cytotoxicity, and overall safety profile require careful monitoring and control to ensure patient safety; (ii) bioavailability and solubility: Celastrol's low bioavailability and poor water solubility can limit its therapeutic efficacy, necessitating novel delivery systems and (iii) Dosing and administration: Determining the optimal dose and administration method is critical to minimize adverse effects while maximizing therapeutic results.

To overcome these challenges, clinical trials often explore advanced drug delivery systems such as liposomes, nanoparticles, and other targeted delivery methods. These systems aim to improve celastrol's bioavailability, stability, and targeted delivery, thus reducing side effects and enhancing therapeutic outcomes. Clinical trials with celastrol hold promise, but require a cautious approach to ensure safety and efficacy. As research progresses, these trials will provide valuable insights into how celastrol can be used effectively in clinical settings, potentially offering new treatment options for a variety of diseases.

5. Conclusions

Celastrol, a key compound derived from *Tripterygium wilfordii* Hook F., emerges as a promising natural product with a diverse range of pharmacological properties. Its potential therapeutic applications extend across various health domains, including anti-inflammatory, anticancer, neuroprotective, and cardioprotective effects. However, despite its promise, several challenges remain, particularly regarding celastrol's high toxicity, low water solubility, and limited stability, which can hinder its widespread clinical use.

To fully harness celastrol's therapeutic potential, ongoing research must address these challenges through innovative drug delivery systems and chemical modifications that improve bioavailability and reduce toxicity. Current studies have demonstrated promising approaches, such as direct modifications to the celastrol molecule, including PEGylation and fatty acid conjugation, as well as indirect modifications through encapsulation in nanocarriers, liposomes, and dendritic polymers. These developments are crucial for advancing celastrol from a promising natural compound to a practical and effective therapeutic agent in clinical settings.

Given its broad therapeutic scope, celastrol represents a compelling target for future drug discovery and development. Further investigation is required to refine its pharmacokinetic profile and explore its applications in the treatment of various diseases. This exploration has significant potential to develop novel treatments that incorporate traditional wisdom with modern pharmacology, ultimately contributing to improved patient outcomes and advancing the field of medicine.

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Abbreviations

SERCA = Sarco-Endoplasmic Reticulum Calcium ATPase; Hsp90-Cdc37 = Heat shock protein 90 - (cell division cycle 37); NF-kB/IKKb = Nuclear factor kappa-light-chain-enhancer of activated B cells - inhibitor of the nuclear factor kappa-B kinase subunit beta; VEGFR1 = Vascular endothelial growth factor receptor 1; PI3K/Akt = Phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt).

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Conceptualization: Paolo Coghi; Methodology: Zimo Ren; Software: Zimo Ren; Validation: Zimo Ren; Formal Analysis: Zimo Ren; Resources: Zimo Ren; Data Curation: Zimo Ren; Writing - Original Draft: Zimo Ren; Writing - Review and Editing: Zimo Ren, Paolo Coghi; Visualization: Paolo Coghi; Funding acquisition: Paolo Coghi; Supervision: Paolo Coghi; Project Administration: Paolo Coghi.

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