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Review Article

Druggability, molecular targets, and nanocarrier delivery of natural triterpenoid celastrol against chronic diseases

Chi Lui Chan[a,1], Yilu Sun[a,1], Xiaowen Mao[b], Hansen Chen[c], Jia Zhao[a,d,e,*] and Jianhui Rong[a,*]

[a]School of Chinese Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 3 Sassoon Road, Pokfulam, Hong Kong, China
[b]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China
[c]Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, United States
[d]Department of Chinese Medicine, The University of Hong Kong Shenzhen Hospital, Shenzhen, China
[e]Zhu Nansun's Workstation and Yu Jin's Workstation, School of Chinese Medicine, The University of Hong Kong, 3 Sassoon Road, Pokfulam, Hong Kong, China

[1]These authors contributed equally to this work.
***Correspondence:** zhaojia7@hku.hk, Tel.: (852)-3917-6508, Fax: (852)-2872-5476 (J. Zhao); jrong@hku.hk, Tel.: (852)-3917-6537, Fax: (852)-2872-5476 (J. Rong)

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ABSTRACT

Celastrol is an active compound from the root of *Tripterygium wilfordii* Hook F that shows great potential in the treatment of inflammation, cancer, neurodegeneration, diabetes, and obesity. However, the clinical application of celastrol has been hindered by its low bioavailability and severe systemic toxicity. The aim of this review was to discuss the druggability, molecular targets, and nanocarrier delivery of the natural triterpenoid, celastrol, against chronic diseases. We sequentially investigated the physicochemical properties of celastrol using online tools (pkCSM and SwissADME), reviewed the recent studies on the molecular mechanisms underlying the therapeutic effects of celastrol, and examined the potential of nanoparticle-mediated delivery systems for safe and effective delivery of celastrol. The cancer-related targets and pathways involved were further predicted through network pharmacologic analysis. This review provides molecular insights into the pharmacologic activities and molecular mechanisms underlying celastrol, as well as useful information for the selection of nanocarrier drug delivery system for the clinical delivery of celastrol against various chronic diseases.

Keywords: Celastrol, chronic diseases, nanoparticle, network pharmacology, mechanism

1. INTRODUCTION

Thunder God Vine (*Tripterygium wilfordii* Hook F. [TwHF]) of the Celastraceae family possesses anti-inflammatory, neuroprotective, anti-cancer, anti-autoimmune, and antioxidant properties and thereby holds promise to treat challenging diseases, such as systemic lupus erythematosus and rheumatoid arthritis (RA) [1, 2]. Previous studies have isolated >100 bioactive compounds, including terpenoids (diterpenoids and triterpenoids), flavonoids, and alkaloids from Thunder God Vine [3, 4]. Terpenoids, including triptolide, mainly contribute to the Thunder God Vine pharmacologic activities and appear to target multiple signalling pathways, including the PI3K/AKT and Notch pathways [5].

Celastrol is an important bioactive triterpenoid from TwHF and exhibits broad pharamacologic activities against inflammation, cancer, diabetes, and neurodegeneration [6, 7]. Celastrol regulates various signalling pathways at the molecular level, such as the PI3K/AKT/mTOR and Janus-activated kinase-1/2 (JNK-1/2) pathways, and thereby affects the expression of different proteins, like heat shock protein 90 (HSP90) and interleukin-1β (IL-1β) [8]. However, the application of celastrol in clinical settings is largely restricted by low water solubility, low bioavailability, dosage-dependent toxicity on different organs, and side effects [9, 10]. Specifically, a concentration of 2.5 μg/g/d celastrol effectively reduces inflammation but causes liver and thymic lesion when the concentration reaches 7.5 μg/g/d [11]. Nevertheless,

celastrol delivery using nanotechnology appears to be safe and effective [12].

2. PHYSIOCHEMICAL PROPERTIES OF CELASTROL

Celastrol (molecular formula, $C_{29}H_{38}O_4$; and molecular weight, 450.6 g/mol) is a structurally unique pentacyclic triterpene with the presence of quinone methides [13]. Celastrol exists as a crystalline powder pale brown-to-orange in color, a melting point of 219–230°C and a boiling point of approximately 647.5°C in 760 mmHg [13, 14]. Celastrol is highly hydrophobic and poorly soluble in polar solvents. For example, the solubility of celastrol in water is approximately 13.25 µg/mL at 37°C, while highly soluble in non-polar solvents, such as dimethylsulfoxide (DMSO) and ethanol [15]. The quinone methide in the structure allows celastrol to form covalent adducts with a cysteine residue in the target protein via an electrophilic reaction that represents a key mechanism by which celastrol exhibits its biological function [16]. Indeed, celastrol is a potential therapy for various diseases, including inflammation [13], cardiovascular diseases [17], neurologic diseases [18], diabetes [19], and cancer [20].

3. METHOD OF CELASTROL EXTRACTION

Celastrol is present in several plants, such as TwHF, and *Celastrus orbiculatus* and *C. reglii* in the Celastraceae family [13, 21]. Two common methods have been developed to isolate celastrol, as follows: 1) column and thin-layer chromatography, in which the dried stem bark is subjected to $CHCl_3$ extraction, water washed, and separated by silica gel column chromatography using $CHCl_3$-acetone and hexane-EtOAc as a solvent [22]; and 2) counter-current chromatography, a technology that possesses a coil planet centrifuge holding four identical multilayer coil columns for large-scale isolation of celastrol [21]. Crude celastrol is prepared by extraction of the powdered raw roots with 95% ethanol under reduced air pressure and separation via silica gel flash chromatography. For large-scale purification of crude celastrol, counter-current chromatography flows the solvent (light petroleum–ethyl acetate–tetrachloromethane–methanol–water) at a 1:1:8:6:1 ratio through the column from head-to-tail while rotating to yield celastrol with a purity of 99.5% [21].

4. ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION, AND TOXICITY (ADMET) PROPERTIES OF CELASTROL

The pharmacokinetics of celastrol, especially ADMET properties, were analysed by two different online ADME tools (pkCSM [https://biosig.lab.uq.edu.au/pkcsm/] and SwissADME [http://www.swissadme.ch/]) (**Table 1**).

The absorption index showed that celastrol exhibits relatively low water solubility and skin permeability, moderate Caco-2 permeability, and a high percentage of intestinal absorption (100%). The good Caco-2 permeability and intestinal absorption scores supported oral administration [23]. P-glycoprotein is known as an efflux transporter protein and has important role in drug absorption regulation and multidrug resistance in cancer cells [24]. Interestingly, celastrol is neither the substrate nor the inhibitor of P-glycoprotein, suggesting good intracellular retention. The volume of distribution at steady state (VDss) indicates the preferred distribution of the drug in tissue or blood plasma. Celastrol has a very low VDss value and zero fraction is unbound, suggesting that celastrol is preferably distributed in plasma and efficiently binds to the plasma proteins. Celastrol may readily penetrate the central nervous system (CNS) given the blood-brain barrier (BBB) permeability (0.078) and CNS permeability (–1.278) [23]. Celastrol does not act as a CYP isoenzyme inhibitor but is likely metabolised by cytochrome P450 3A4 isoforms. Total clearance refers to the clearance in the liver, biliary metabolism, and kidney excretion, which is related to bioavailability [23]. The total clearance of celastrol is very low, confirming the notion of low bioavailability. To predict the previously described potential side effects and clearance [23], celastrol was screened by renal organic cation transporter 2 (OCT2) substrate analysis and proven to be negative. Thus, OCT2 did not boost the renal transport and clearance of celastrol.

Drug safety is a priority in the development of new drugs. The AMES test uses bacteria that do not synthesize histidine or tryptophan to predict the mutagenicity and carcinogenicity of synthetic and natural chemical compounds due to DNA mutations [25]. Celastrol had negative AMES toxicity assay results and it is therefore unlikely that celastrol will cause mutations and have a carcinogenic effect in the human body. However, celastrol has a low maximum tolerated dose (0.192) and low bioavailability score (0.85), indicating the possibility of strong toxicity at higher concentrations. Celastrol does not appear to be an inhibitor of hERG I and II, therefore excluding cardiotoxicity and induction of fatal ventricular arrhythmias, although hepatotoxicity is still possible. Acute and chronic toxicity were assessed based on LD50 and LOAEL tests. The toxicologic assays revealed that celastrol has a *Tetrahymena pyriformis* toxicity value >–0.5 log ug/L and a fathead minnow toxicity value <–0.3, suggesting that celastrol is a toxic compound and might exhibit high acute toxicity [26]. The PAINS and Brenk tests indicated that celastrol is not a promiscuous or metabolically unstable compound [27]. Taken together, these results provide important information for the development of celastrol as a clinical drug.

5. MEDICINAL PROPERTIES OF CELASTROL

5.1 Anti-inflammatory effect and autoimmune diseases

Inflammation is activated as a defensive immune mechanism by pathogens, allergens, and cellular injury [28].

Table 1 | Summary of the predicted ADMET properties of celastrol.

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	−3.311	Numeric (log mol/L)
	Caco2 permeability	0.464	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	100	Numeric (% Absorbed)
	Skin permeability	−2.735	Numeric (log Kp)
	P-glycoprotein substrate	No	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
	Lipophilicity	(iLOGP) 3.37 (XLOGP3) 5.94 (WLOGP) 6.70 (MLOGP) 4.42 (SILICOS-IT) 5.39	Numeric (Log Po/w)
Distribution	VDss (human)	−1.329	Numeric (log L/kg)
	Fraction unbound (human)	0	Numeric (Fu)
	BBB permeability	0.078	Numeric (log BB)
	CNS permeability	−1.278	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Categorical (Yes/No)
	CYP1A2 inhibitor	No	Categorical (Yes/No)
	CYP2C19 inhibitor	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	Categorical (Yes/No)
Excretion	Total clearance	−0.094	Numeric (log mL/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.192	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral rat acute toxicity (LD50)	2.362	Numeric (mol/kg)
	Oral rat chronic toxicity (LOAEL)	1.873	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	Yes	Categorical (Yes/No)
	Skin sensitisation	No	Categorical (Yes/No)
	T. pyriformis toxicity	0.285	Numeric (log ug/L)
	Minnow toxicity	−0.642	Numeric (log mM)
	Bioavailability score	0.85	Numeric (% Absorbed)
	PAINS (Pan-assay interference compounds)	0	Numeric (Alert)
	Brenk	0	Numeric (Alert)

Abnormal inflammatory responses often lead to the onset of autoimmune and chronic diseases, such as rheumatoid arthritis (RA), multiple sclerosis, inflammatory bowel disease, psoriasis, atopic dermatitis, and ankylosing spondylitis. Earlier studies evaluated the anti-inflammatory effects of celastrol and investigated the underlying mechanisms.

Inflammatory responses cause damage to synovial tissues and joints in patients with RA, a chronic autoimmune disease [29]. Inflammatory stimuli activate the nuclear factor-kB (NF-κB) pathway in T cells, macrophages, osteoclasts, and fibroblast-like synoviocytes (FLSs) to release IL-17, matrix metalloproteinases (MMPs), and reactive oxygen species (ROS), which lead to bone erosion and cartilage destruction [30]. Celastrol has been proven to suppress the levels of pro-inflammatory cytokine expression, including IL-1β, IL-6, tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) in a collagen-induced arthritis (CIA) mouse model [28, 31, 32]. Celastrol also inhibits related molecule expression, including Bax, cleaved caspase-3, collagen I, collagen III, and α-SMA, which are related to apoptosis and tissue repair [33]. Interestingly, celastrol effectively reduces oxidative stress-associated inflammation in RA via multiple mechanisms: 1) boosting superoxide dismutase activity and reducing NADPH oxidase (NOX) activity, superoxide anion, and malondialdehyde [32]; 2) inhibiting the PI3K/AKT/mTOR signalling pathway and autophagy [31]; 3) downregulating the level of hypoxia-inducible factor expression [34]. In a Sprague-Dawley (SD) rat model of complete Freund's adjuvant-induced arthritis, celastrol not only inhibited activation of the NF-κB signalling pathway and NLRP3 inflammasome to downregulate cytokine IL-1β, IL-18, and intracellular ROS secretion [28, 35] but also induced autophagy in synovial fibroblasts via the Ca^{2+}/calmodulin-dependent kinase kinase-β–AMP-activated protein kinase (AMPK)–mTOR pathway and suppressed endoplasmic reticulum (ER) Ca^{2+} ATPase pump activity [36]. It is possible that celastrol alleviated RA symptoms by controlling the number of FLSs [34].

CNS inflammation is implicated in patients with multiple sclerosis, an autoimmune disease, while neuronal degeneration is mainly caused by ROS and reactive nitrogen species (RNS) [37]. Celastrol attenuates CNS inflammation by regulating extracellular signal-regulated kinase (ERK) and p38 pathways and suppressing SGK1 in T helper 17 (Th17)/T regulatory (Treg) cells, reducing the secretion of several cytokines, including IL-6, IL-8, IL-12p40, TNF-α, and Th17/Th22 in monocyte-derived dendritic cells, and upregulating brain-derived neurotrophic factor (BDNF) for effective neuroprotection in experimental autoimmune encephalomyelitis (EAE) mice [38, 39].

Chronic inflammation is the pathologic hallmark of ulcerative colitis with an accumulation of $CD8^+$ $IL\text{-}17^+$ T cells and overproduction of IL-17 and TNF, which leads to tissue damage in the colon [40]. Celastrol was shown to directly regulate the Treg:Th1 and Treg:Th17 ratios and cooperate with gut microbiota to reduce inflammatory cytokines (IFN-γ and IL-17A) in a BALB/c mouse model [41]. Celastrol inhibited activation of the NF-κB signalling pathway, HSP-90, and the NLPR3 inflammasome in a rat model of acute colitis, thereby downregulating pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) [42].

Chronic inflammation has been implicated in psoriasis with scaly red skin plaques on the skin surface [43]. Various cytokines (IL-6, IL-17, IL-22, TNF, and IFN) mediate the interaction between keratinocytes and immune cells and induce epidermal cell proliferation [44]. In 2D and 3D human models of psoriasis involving human $CD4^+$ T cells, epidermal keratinocytes, and the epidermis, celastrol effectively attenuated the Th17:Treg balance, chronic inflammation, and keratinocyte proliferation by inhibiting the differentiation of Th17 and Th22 and promoting Treg cells, which led to a reduction in IL-17A, IL-22, INF-γ, and TNF-α, downregulation of DEFB4A, PI3, S100A7A, SLPI, CXCL1, CCL5, CCL20, CXCL-8, IL-19, IL-23A, and IL-36γ and upregulation of CD25, IL-10, and TGF-β [45]. It is possible that celastrol exhibits such broad anti-inflammatory activity by interfering with transcription key factor retinoid-related orphan receptor gamma t (RORγt) and inhibiting STAT3 phosphorylation [28, 45]. Moreover, in a mouse model of imiquimod-induced psoriasis, celastrol inhibited store-operated calcium entry (SOCE) by breaking the functional coupling of STIM1 with Orial1 and subsequently decreasing SOCE signals [46].

Skin inflammation causes itch and eczematous lesions in atopic dermatitis due to activation of immunoglobulin E and the Th 2 cell-mediated immune response [47]. Celastrol decreased the levels of cytokines (IL-4, IL-5, and IL-13) and reduced secretion of thymic stromal lymphopoietin in C57BL/6 and NC/Nga mouse models [48].

In addition, human parvovirus B19 infection stimulates the production of cytokines, like TNF-α and IFN-γ, and causes persistent anemia, erythema, and arthropathy [49, 50]. Celastrol dramatically decreased the levels of inflammatory cytokines (IL-6, TNF-α, IL-1β, and IL-18) and thereby decreased the migration and phagocytosis of macrophages in acute monocytic leukemia cell lines [51].

Taken together, these studies basically confirmed the anti-inflammatory effects of celastrol and revealed that celastrol likely targets multiple signalling pathways (NF-kB, STAT3, and MAPKs) to downregulate inflammatory chemokines and cytokines while upregulating anti-inflammatory mediators, as summarized in **Figure 1**.

5.2 Anti-cancer activity and mechanisms

Cancers are characterized by uncontrolled proliferation of cells, which is likely due to the accumulation of mutations [52]. Among the therapies that are available, many active compounds from medicinal herbs are promising interventions for cancers [53]. Celastrol is a key drug candidate for treating various cancers due to

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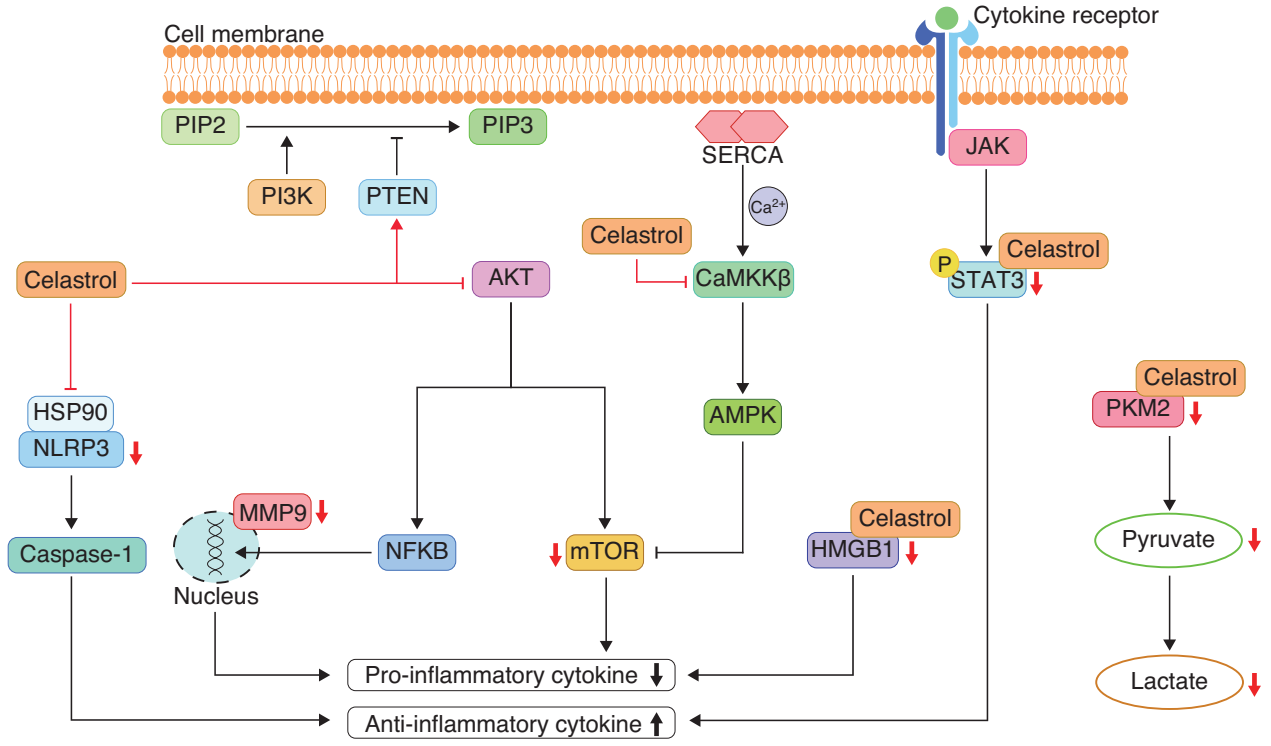

Figure 1 | Summary of the mechanisms underlying anti-inflammatory activities of celastrol.
↑ Refers to upregulation; ↓ Refers to inhibition or downregulation.

its properties in the regulation of cancer-associated signalling pathways and cytokine expression [54].

Colorectal cancer (CRC) involves the accumulation of cancer stem cells and the formation of tumours at the base of colon crypts, while the progression of CRC is driven by activation of WNT, MAPK, PI3K, TGFβ, and p53 signalling [55]. Celastrol was evaluated in the xenograft CRC tumour mouse model and in an *in vitro* cell model using HCT-116 and SW480 cell lines. Celastrol induced cancer cell apoptosis and autophagy by reducing the expression of nerve growth factor-induced gene B (Nur77) and increasing the expression of autophagy-related protein 7 (ATG7) [56]. In other studies using HCT116 and SW620 cell lines, celastrol inhibited the spread, attachment, and migration of CRC cells by inhibiting the TGF-β1/Smad signalling pathway, nitric oxide synthase (NOS) activity, and MMP9, TNF-α, and IL-1β production [57, 58]. Celastrol was reported to regulate the activity of immune cells and suppressed inflammatory response by inhibiting histone deacetylase in the C57BL/6J - $APC^{min/+}$ mouse model of spontaneous CRC [59]. Celastrol skews macrophage polarization from the pro-inflammatory M1 type to the anti-inflammatory M2 type to modulate the activity of immune cells, primarily by targeting the ERK/MAPK signalling pathway, which leads to the production of cytokines and chemokines (IL-24, TGF-β, IL-32, CD155, IL-17, and CXCL14) [59]. C57BL/6 mice with a p27Kip1 gene deletion in germline cells and an Smad4 gene deletion in T cells were treated with celastrol to determine the exact molecular mechanism. Celastrol appeared to inhibit the activity of IκB kinase to downregulate the expression of NFκB and inducible iNOS, inhibited the activity of Hsp90 to block the binding of transcriptional factors to the iNOS promoter, and promoted the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) to downregulate the expression of proinflammatory cytokines [60].

Breast cancer is caused by the accumulation of mutations in glands, dysfunction of the oestrogen receptor pathway, and uncontrolled cell proliferation [61]. Previous studies have revealed that celastrol might exhibit anti-cancer activity through the following mechanisms: 1) inhibiting Hsp90 ATPase activity to disrupt the Hsp90-Cdc37 complex, inhibiting the phosphorylation of mTOR to facilitate its ubiquitination, and subsequent degradation in human breast cancer cell lines (MCF7 and MDA-MB231) [62]; 2) suppressing the mitogen-activated protein kinase (MEK)/ERK signalling pathway to downregulate amphiregulin expression and inhibit the amphiregulin-induced epidermal growth factor receptor signalling pathway in MCF7, T47D, ZR75–1, HCC1143, and HCC1806 cells [63]; 3) reducing the expression of Hakai, upregulating E-cadherin expression, and downregulating N-cadherin expression in MDA-MB-231 and MDA

MB-468 cells [64]; 4) inhibiting the MEK/ERK-dependent pathway and downregulating the expression of IL-1β, IL-8, MMP-1, and MMP-9 in T47D, SK-BR-3, HCC1143, HCC1806, ZR75-1, MDA-MB-231, MDA-MB-157, MCF7, and Hs578T cells [65]; and 5) inhibiting activation of the NF-κB pathway as a key mechanism to downregulate IL-6 expression and reduce the migration and invasion of MDA-MB-468 and MDA-MB-231 cells [66].

Lung cancer is classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC arises from pulmonary neuroendocrine cells [67]. Due to the complexity of lung composition, some cells are altered to escape from immune surveillance and progress into cancer [68]. The anti-cancer potential of celastrol has been mainly evaluated in different cell models. Indeed, celastrol regulates the methylation, phosphorylation, and acetylation of histone in A549, HeLa, LLC-1, and H1299 cell lines [69]. Celastrol reduces the proliferation, migration, and invasion of cancer cells and increases cancer cell apoptosis by promoting oxidative and ER stress in NSCLC cell lines (BEAS-2B lineage cells, PC-9, H460, and H520) [70]. Presumably, dosage-dependent suppression of the signal transducer and activator of transcription-3 (STAT3) signalling pathway is the underlying mechanism.

Gastric cancer also refers to stomach tumour, predominantly as gastric adenocarcinoma (GAC), while other variants include neuroendocrine tumours, and mesenchymal and lymphoproliferative disorders. Gastric cancer is highly linked to mutations in the germline mismatch repair genes, including breast cancer gene 1 (BRCA1), BRCA2, serine/threonine kinase 11 (STK11), tumour protein p53 (TP53), and myosin heavy chain (MYH) [71]. The anti-cancer activity has mainly been investigated using gastric cancer cell lines. Celastrol possibly suppresses the growth, viability, and invasiveness of cancer cells by suppressing peroxiredoxin-2, STAT, and HSP70e, and promoting ROS production in BGC-823 and SGC-7901 cells [72]. Celastrol has been shown to induce CIP2A degradation via the ubiquitin-proteasome pathway and increase cell apoptosis in HGC-27, BCG-823, AGS, and KATO-III cells [73]. Mechanistic studies revealed that celastrol suppresses activation of the PI3K/AKT pathway and downregulates forkhead box A1 (FOXA1) and claudin 4 (CLDN4) expression in BGC-823, SGC-7901, SNU-216, NCI-87, MKN-28, and AGS cells [74]. Downregulation of miR-21 may be a potential mechanism by which celastrol inhibits the PTEN/PI3K/AKT and NFκB signalling pathways [75].

The anti-cancer potential of celastrol has also been studied in several cancer cell models to determine the anti-cancer mechanisms, as follows: 1) inhibiting the ATF4-CHOP-DR5 and FoxO3a-Bim signalling pathways in human oesophageal squamous cell carcinoma cell line models (Eca109 and EC1) [76]; 2) arresting the cell cycle in the G2/M phase, activating caspase-3, -8, and -9 and promoting poly(ADPadenosine diphosphate-ribose) polymerase (PARP) cleavage in the human oral squamous cell carcinoma cell line (SAS) [77]; 3) enhancing the release of histone-linked DNA fragments, activating caspase-3 and -7, upregulating the expression of DNA damage-inducible transcript 3 (DDIT3) and activating transcription factor 3 (ATF3) in human pancreatic cancer cell lines (BxPC-3, Capan-1, Capan-2, Colo-357, Panc-1, Suit-2, and Suit-007) [78]; 4) reducing the expression of MMP-2 and MMP-9, which causes mitochondrial fragmentation and enhancing caspase-3 expression in cervical cancer HeLa cells [79]; 5) arresting the cell cycle in the G2/M phase and increasing intracellular ROS levels in human ovarian cancer cell lines (SKOV3 and A2780) [80]; and downregulating the expression of Pin1, cancer stem cell biomarkers (and CD44, Oct4, and Klf4), CDK2, CDK4, and Cyclin D1 to arrest the cancer cell cycle in the G2/M phase, decreasing Bcl-2 expression, increasing Bax expression, and inhibiting the AKT, JNK/P38, NF-κB and IL-6/STAT3 signalling pathways in SKOV3, A2780, and OVCAR3 cells [81]; 6) inhibiting PTEN/PI3K/Akt/mTOR signalling axis and upregulating the Bax:Bcl-2 ratio in human cholangiocarcinoma cell lines (HuCCT-1 and TFK-1) [82]; 7) increasing the intracellular ROS level, upregulating p53 expression, and promoting mitochondrial damage in human leukemic cancer cell lines (HL-60 and NB-4) and the nude mouse xenograft model via the cysteine metabolism/ROS/p53/Bax/caspase 9/caspase 3 pathway [83]; 8) inhibiting the activity of proteasome proteases (caspase-, chymotrypsin-, and trypsin-like subunits) and inducing cell cycle arrest at G0/G1 in myeloma cell lines (MM.1R, MM.1S, U266, and RPMI8226) and the CB17 severe combined immunodeficiency (SCID) mouse model [84]; and 10) activating the ROS/JNK signalling pathway and suppressing the activation of Akt and mTOR in human glioma cell lines (U87 and U251) and rat glioma C6 cells [85].

5.3 Neuroprotective activity and mechanisms

Neurodegeneration in the CNS and peripheral nervous system (PNS) leads to cognition, memory, and motor function deficits due to the progressive loss of neurons [86]. Alzheimer's and Parkinson's diseases are two common types of neurodegenerative diseases. Alzheimer's disease is characterized by cognition and memory impairment due to the formation of β-amyloid (Aβ)-containing extracellular plaques and tau-containing intracellular neuro-fibrillary tangles [87]. Parkinson's disease is characterized by a range of motor symptoms (akinesia, bradykinesia, tremor, rigidity, gait disturbances, impaired handwriting, grip force, and speech deficits) due to the aggregation of misfolding α-synuclein [88]. Celastrol has been shown exhibit neuroprotective effects on Alzheimer's and Parkinson's diseases [85, 89].

Celastrol was evaluated for the treatment of Parkinson's disease in dopaminergic neuronal cells and a mouse model of Parkinsonism. Neurotoxin rotenone was used to induce the cell model of Parkinsonism in the dopaminergic neuronal cell line, SH-SY5Y. Celastrol induced ERK-dependent mitophagy to protect the

neuronal cells from apoptosis by increasing the levels of mitochondrial serine/threonine-protein kinase PINK1, the ubiquitous cytoprotective protein, DJ-1, and decreasing the level of leucine-rich repeat kinase 2 (LRRK2) [90]. Another study showed that celastrol targets the PINK1-Parkin pathway to inhibit Parkin recruitment to mitochondria by disrupting the interaction between translocase of outer mitochondrial membrane subunit 20 (TOM20) and PINK1 [91]. Another study used neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) to induce dopaminergic neuronal damage in the substantia nigra pars compacta and striatum of C57BL/6 mice. Celastrol effectively reduced MPTP-induced oxidative stress, mitochondrial apoptosis, inflammation, excitotoxicity, and formation of inclusion bodies. Activation of Nrf2 signalling is a key mechanism by which celastrol targets the Nrf2-NLRP3-caspase-1 pathway to elicit a neuroprotective effect [85]. Celastrol also reduces miR-146a expression and activates the PI3K/Akt/mTOR pathway to reduce ROS production, restores the mitochondrial membrane potential, and decreases neuronal apoptosis [92].

Alzheimer's disease is often modelled with transgenic mouse and cell culture systems. In a study using mice expressing human APP695sw and presenilin-1 mutations in M146L and HEK293 cells as models, respectively, celastrol markedly reduced the production of Aβ peptide (Aβ1-38, Aβ1-40, and Aβ1-42) by reducing amyloid precursor protein β-cleavage and inhibiting NFκB-dependent BACE-1 expression [93]. In another study using HeLa, HEK293, and N2a cells and the homozygous human P301S Tau transgenic mouse model, celastrol ameliorated memory deficiency by promoting the degradation of phosphorylated Tau aggregates via transcription factor EB (TFEB)-mediated autophagy [89]. Inhibition of the mammalian target of rapamycin complex 1 (mTORC1) is an important mechanism by which celastrol mediates the dephosphorylation and activation of TFEB to induce the autophagy lysosomal pathway, increases the degradation of phosphorylated Tau, and ultimately improves memory deficiency.

Neuronal death has been implicated in cerebral ischemia-reperfusion (CIR) injury due to the restriction of blood supply to the brain, which induces rapid activation of innate and adaptive immune systems and cell death programs [94]. In a study using a male adult SD rat model by middle cerebral artery occlusion and a primary cortical neuron model by oxygen–glucose deprivation, celastrol effectively reduced neuroinflammation by increasing HSP70 expression and decreasing NF-κB p65 expression [95]. In another study using a rat model of transient global CIR, celastrol reduced oxidative stress and neuroinflammation by decreasing the production of inflammatory cytokines (IL-1β, IL-6, and TNF-α) and oxidative markers and inhibiting the HMGB1/NF-κB signalling pathway [96]. In a study using a C57BL/6 mouse model of CIR injury, celastrol decreased the expression of HIF-1α and PDK1 genes while enhancing glucose metabolism [97]. By regulating the PI3K/Akt signalling pathway, celastrol attenuates mitochondrial dysfunction, reduces ROS production, and increases mitochondrial biogenesis in hippocampal cells [98]. Celastrol inhibits immune cell activation and oxidative stress by decreasing ACOD1 expression in cortices of the brain [99, 100]. In a C57BL/6J mouse model, celastrol reduced ROS production and oxidative stress by binding to Nedd4 to prevent the binding of Nedd4 and Nrf2 in astrocytes [101]. Moreover, celastrol also inhibited the JNK signalling pathway and restored the expression of pro-survival Bcl-2 via reducing the expression of the non-coding RNA sequence, AK005401, and mitogen-activated protein kinase kinase 12 (MAP3K12).

In addition, celastrol was evaluated for neuroprotective activity in several other types of neurologic diseases. Celastrol was shown to preserve BBB integrity by inhibiting MMP-9 expression and neuroinflammatory damage in an SD rat model of subarachnoid hemorrhage. In addition, celastrol prevented necroptosis by downregulating the necroptosis-related protein, RIP3, and mixed lineage kinase domain-like protein (MLKL) [102]. In a study involving age-related macular degeneration using the human retinal pigment epithelial cell line, ARPE-19, celastrol alleviated cell autophagy by reducing ROS production and increasing the expression of Sirtuin3 mRNA and protein [103]. In a C57BL/6J mouse model of intracerebral hemorrhage, celastrol reduced oxidative stress and subsequent neuronal apoptosis by increasing the pre-reduced OPA1 to maintain mitochondrial cristae morphology and promote mitochondrial fusion [104]. Furthermore, in an SD rat model with spinal cord injury, celastrol attenuated microglial pyroptosis by reducing pro-inflammatory cytokines (IL-1β, IL-18, and TNF-α), iNOS, caspase-1, gasdermin D, and the NLRP3 inflammasome, while increasing the IL-10 level [105].

5.4 Anti-diabetes and anti-obesity activity and mechanisms

Obesity is a highly prevalent chronic inflammatory disease with excessive fat buildup and an elevated body mass index, which is a causative factor for type 2 diabetes mellitus, fatty liver disease, cancer, and cardiovascular and neurologic diseases [106]. In an SD rat model, celastrol enhanced insulin sensitivity by increasing the gut Bacteroidetes and Firmicutes populations and increasing energy consumption by activating the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) transcriptional axis [107]. Another study demonstrated that celastrol improves insulin sensitivity by enhancing GLUT4 translocation to the plasma membrane, enhances mitochondrion function by increasing PGC-1α deacetylation and stimulating AMPK/Sirtuin 1 (SIRT1) activities and reduces the inflammatory response by inhibiting macrophage polarization and NF-κB activity [108]. In a C57BL/6J mouse model of a high-fat diet (HFD)-induced obesity, celastrol promoted browning of inguinal white adipose tissue (iWAT) and upregulated

thermogenic factors (UCP1 and PGC-1α) to increase insulin sensitivity and the thermogenic effect, while increased energy consumption by activating the NLRP3 inflammasome and Toll-like receptor 3 (TLR3) signalling [109]. To elucidate the putative targets of celastrol on intracellular glucose utilization and mitochondrial oxidative metabolism, Abu Bakar et al. performed experiments using isolated quadriceps skeletal muscle in HFD-induced obese male C57BL6/J mice [110]. Abu Bakar et al. reported that celastrol exhibited remarkable anti-obesity effects by improving systemic glucose tolerance and insulin sensitivity. Celastrol strongly upregulates the expression of several glycolysis and the tricarboxylic acid cycle (TCA) rate-limiting enzymes. Moreover, celastrol effectively upregulates the pyruvate dehydrogenase complex (PDC) and succinate dehydrogenase (SDH), while downregulating pyruvate dehydrogenase kinase 4 (PDK4). Ultimately, celastrol facilitates establishment of a stable electrochemical gradient in the respiratory chain for ATP production and mitochondrial biogenesis.

Type 2 diabetes mellitus is characterized by a high blood glucose level, insulin resistance, and elevated glycated haemoglobin A1c level, whereas type 1 diabetes mellitus is an autoimmune disease with an inability to produce insulin [111, 112]. Several studies have demonstrated the anti-diabetes properties of celastrol in animal models and cell culture systems. To target the blood glucose level, celastrol suppresses glucose release from the liver by reducing expression of the catalytic subunit of glucose-6-phosphatase (G6PC), phosphoenolpyruvate carboxykinase (PEPCK), GLUT4, and IRS1 at the mRNA level in Wistar rats [113]. Celastrol also increases the phosphorylation of Akt, while suppressing 11β-HSD1 expression. Consequently, celastrol alleviates oxidative stress and promotes insulin signalling and mitochondrial biogenesis. To target insulin resistance, obesity and insulin resistance was modelled by introducing a G-to-T point mutation in the leptin receptor gene, generating C57BLKS/Lepr^{db} transgenic mice [114]. Celastrol effectively reduced food intake by regulating the leptin signalling pathway in C57BLKS/Lepr^{db} mice [115, 116]. To target inflammation, celastrol was shown to ameliorate metabolic syndrome and inflammation in sub-strain C57BL/6J mice against a HFD [117]. Mechanistic studies revealed that celastrol directly binds to adenylyl cyclase-associated protein 1 (CAP1) and prevents binding of CAP1 with resistin, leading to inhibition of the cAMP/PKA/NF-κB signalling pathway. Activation of the NLRP3 inflammasome was effectively prohibited, while the levels of IL-1β, thioredoxin-interacting protein (TXNIP), and chREBP degradation were reduced. Ultimately, pancreatic β cells apoptosis was reduced [115, 116]. Downregulation of p38MAPK and NF-κB p65 not only reduced secretion of inflammatory factors (IL-8 and PAI-1) but also preserved liver and kidney function against diabetic lesions [118]. The PI3K/AKT/mTOR/autophagy axis is an important target by which celastrol reduces NF-κB mRNA expression and ameliorates diabetic nephropathy [119]. Similarly, activation of the Nrf-2/HO-1 signalling pathway is a key mechanism by which celastrol protects liver integrity in a C57BL/6 mouse model of type 2 diabetes mellitus [120, 121]. The SIRT1-farnesoid X receptor (FXR) pathway is another important pathway for celastrol to regulate SIRT1 enzyme expression and suppress the WNT/β-catenin and EZH2 pathways against diabetic nephropathy in a sub-strain of C57BLKS/J db/m and db/db mice [122]. The molecular mechanisms were elucidated in different cell culture systems. Celastrol reduced the inflammatory response and apoptosis by inhibiting miR-345-5p, activating Gas6, and reducing the Bax:Bcl-2 ratio in H9c2 cardiomyocytes [123]. Celastrol was shown to directly bind to STAT3 and reduce STAT3 phosphorylation and matrix protein expression, which might alleviate cardiac deficit symptoms [124]. In human retinal endothelial cells, celastrol inhibited cell invasion and angiogenesis by reducing angiogenin-2, vascular endothelial growth factor receptor-2, VEFGA, and CD31 expression and improving HIF-1α and VEGF expression [19]. Moreover, celastrol enhanced miR223 and GLUT4 expression in human hepatocellular carcinoma HepG2 cells, implying insulin resistance reversal [125].

Collectively, celastrol was shown to be effective for treatment of inflammation, cancer, neurologic diseases, obesity, and diabetes. By analyzing target genes/proteins celastrol was shown to target common genes/proteins (NF-κB and PI3K/AKT) and genes/proteins for specific diseases (CAMKK β for inflammation, TGF-β1/Smad for cancer, PINK1/Parkin for neurologic diseases, and CREB/PGC-1α for obesity and diabetes) (**Figure 2**). Indeed, these results highlight the important role of inflammation in these diseases.

5.4.1 Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. GO and KEGG enrichment analyses were performed on cancer-related genes using the online tool, DAVID (https://david.ncifcrf.gov/home.jsp), to gain molecular insight into the anti-cancer activity of celastrol, as previously described [126]. Based on GO enrichment analysis, the 10 most significantly enriched ($P < 0.01$) GO biological processes (BPs), cellular component (CCs), molecular function (MF), and the KEGG pathway were selected. The most enriched GO-BP terms were related to the apoptotic process, whereas the most enriched GO-MF terms were related to protein kinase binding, transcription regulatory region sequence-specific DNA binding, and ubiquitin protein ligase binding (**Figure 3**). Based on the GO-CC enrichment results, the most involved cellular component was shown to be the cytosol and cytoplasm (**Figure 3C**). Moreover, the KEGG pathway analysis (**Figure 3D**) showed that the most regulated pathways were mainly related to cancer and p53 signalling pathways. These results highlight the potential therapeutic targets and related

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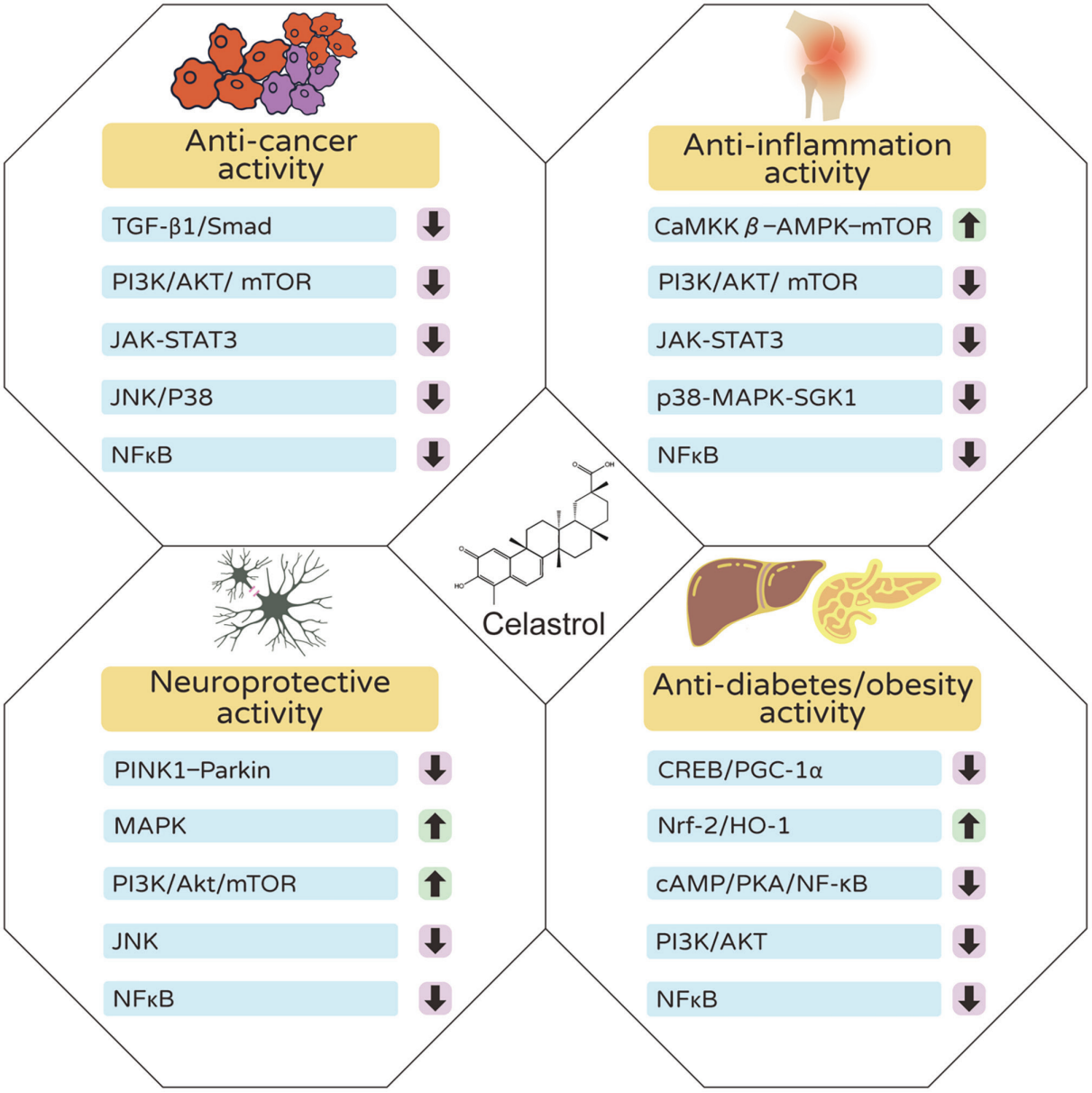

Figure 2 | Summary of celastrol properties on anti-inflammation, anti-cancer, neuroprotective, anti-diabetes, and anti-obesity activities.
Chemical structure of celastrol was generated via ChemDraw software.

signalling pathways as the key molecular mechanisms underlying the anti-cancer activity of celastrol.

5.4.2 Protein-protein interaction analysis. Network pharmacology analysis was performed to map the interaction network of 58 cancer-associated genes that are regulated by celastrol. GeneMANIA in Cytoscape software (https://cytoscape.org/) was applied to identify the protein-protein interactions. The most significant targets (TP53, CDK2, AKT1, CDKN1A, CCND1, and CDK4) were shown to be related to cell cycle arrest in the G2/M phase, cancer cell apoptosis, and the PTEN/PI3K/AKT and PI3K/Akt/mTOR signalling pathways (Figure 4).

6. THERAPEUTIC POTENTIAL OF CELASTROL-LOADED NANOPARTICLES

The effort to translate bioactive celastrol as a clinical drug is greatly limited by low water solubility, low bioavailability, and related toxicity in the gastrointestinal tract, reproductive system, liver, heart, and nervous

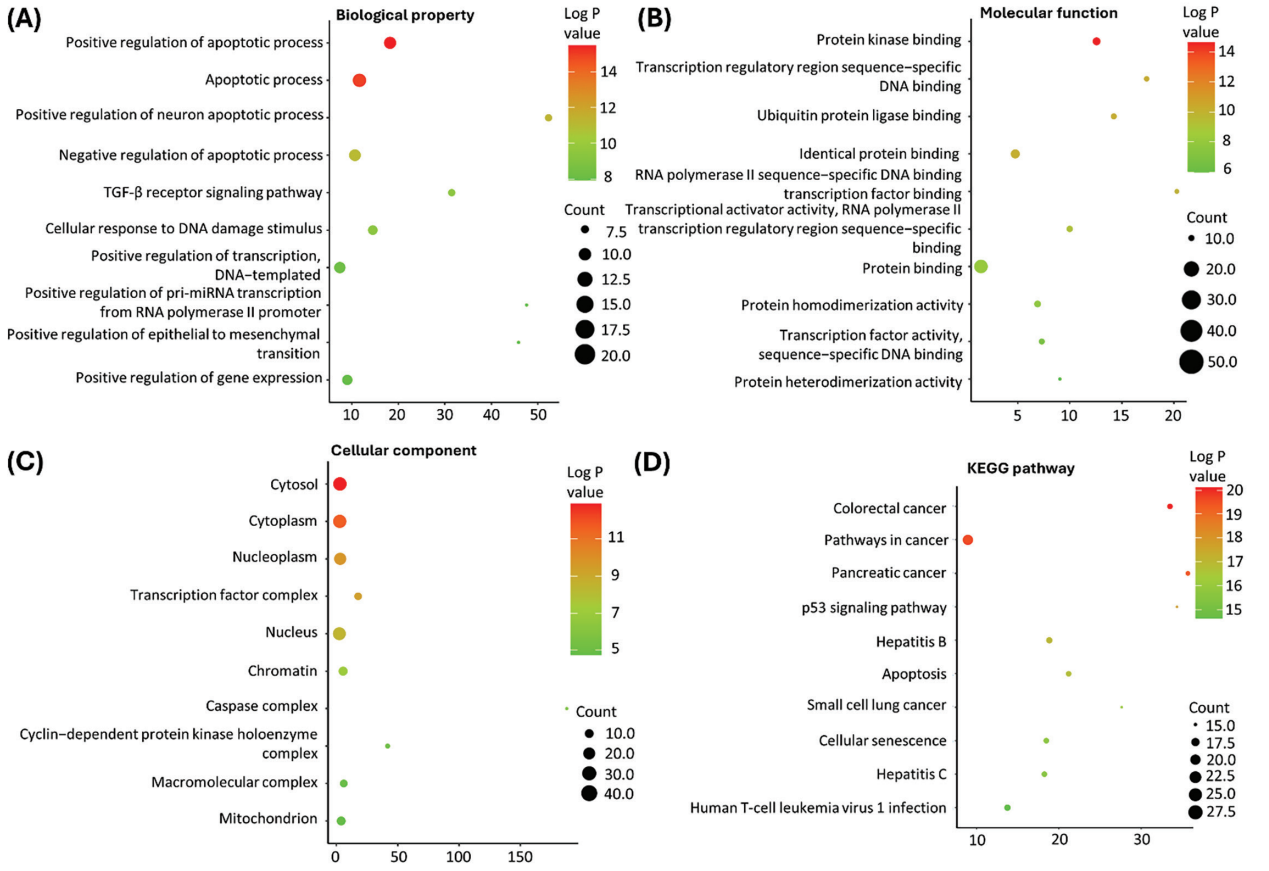

Figure 3 | GO terms enrichment and KEGG pathway analyses of the protein targets related to cancer.
(A) The most significantly enriched GO terms were specifically chosen based on relevance to "biological property." (B) The most significantly enriched GO terms were specifically chosen based on relevance to "molecular function." (C) The most significantly enriched GO terms were specifically chosen based on relevance to "cellular component." (D) Ten most significant enriched KEGG pathways.

system [127, 128]. Several potential strategies, including structural modifications, combined therapy, and nanocarrier delivery systems, were adopted to overcome these obstacles [129]. As an example, structural optimization generated less toxic celastrol analogues due to covalent targeting of peroxiredoxin 1 [20]. Importantly, different nanocarrier delivery systems have been recently developed to reduce the side effects, toxicity, drug resistance, and achieve organ-targeting drug delivery [130]. Thus, the following section will discuss the nanocarrier delivery systems for celastrol in the treatment of various diseases (**Figure 5**). Potential properties of celastrol loaded nanoparticles were summarized in **Table 2**.

6.1 Inflammation

Nanoparticle-mediated delivery systems of celastrol were used in the treatment of RA to reduce the side effects and non-targeted toxicity. For example, Liu et al. developed celastrol-loaded arginylglycylaspartic acid (RGD)-modified bovine serum albumin (BSA) nanoparticles with an encapsulation efficiency (EE%) of 75.8 ± 1.2% and a mean size of 118.0 ± 3.1 nm using the desolvation method [131]. Inflammatory neutrophils (INEs) were specifically targeted by the interaction of integrin and RGD in a CIA mouse model, thereby obstructing the recruitment of INEs and effectively alleviating articular cartilage damage. The surface modification of BSA nanoparticles with covalently linked polyethylene glycol helped evade immune recognition and metabolism, increase the celastrol circulation time, which reduced systemic toxicity and enhanced celastrol bioavailability [131, 132]. Another study encapsulated celastrol in polymeric micelle-based poly(ethylene glycol)-block-poly(propylene sulfide) [C-PEPS] with a loading content of 4.71% and a mean size of 135 nm by self-assembly [133]. The hydrophobic block, poly(propylene sulphide), which is oxidized by ROS and releases drugs by losing hydrophobic interactions, targets drug delivery to inflamed tissues [133, 134]. Similarly, self-assembled celastrol-loaded poly(lactic-co-glycolic acid) hybridized with triglycerol monostearate (TG-18) nanoparticles were shown to effectively reduce bone erosion and cartilage damage in CIA mice by selectively disassembling with the presence of an enzyme in RA

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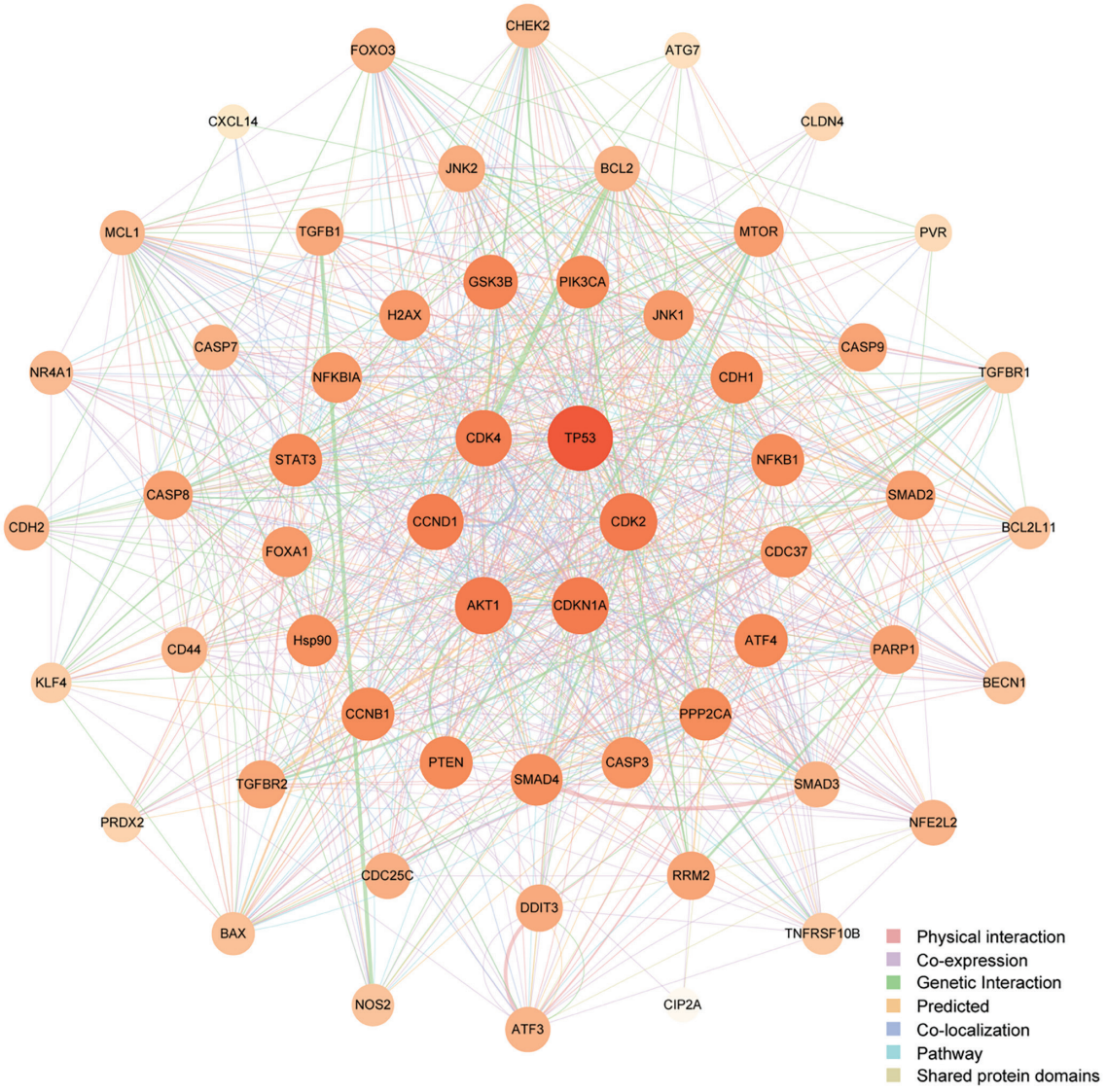

Figure 4 | The network pharmacologic analysis of anti-cancer targets of celastrol.

inflammatory environments [135]. Furthermore, Lin et al. loaded celastrol in folic acid-modified nanoparticles to target the overexpressed folate receptors on activated macrophages to achieve a macrophage-targeting effect and target ROS in inflammation sites in CIA rats [136].

While conventional inhalants effectively target the lungs and minimize systemic side effects in the treatment of asthma, utilization of nanomaterials has shown promise in prolonging the blood circulation time and enhancing pharmacokinetics [137]. Celastrol-loaded liquid crystalline nanoparticles (EE%, 99.1 ± 0.2%; particle size, 194.1 ± 9.7 nm) were designed to enhance bioavailability and evaluated using a human bronchial epithelial cell line (Bci-Ns1.1) [138]. Liquid crystalline nanoparticles can self-assemble reversed micellar hexagonal and cubic phases to increase the half-life for drug delivery [138]. Peng et al. developed ovalbumin-conjugated celastrol-loaded nanomicelles for allergic asthma using the thin-film hydration method with an EE% of 99.89 ± 0.85% and drug loading percentage of 4.76 ± 0.03% [139]. The small particle size (50.72 ± 0.98 nm) of ovalbumin-conjugated nanomicelles induce a strong humoral immune response and more rapidly penetrate tissue barriers, helping induce immune tolerance via re-exposure to allergen [139].

In the context of acute pancreatitis, celastrol loaded in PEG–ether-block-poly-(lactide-co-glycolide) [PEG-PLGA] neutrophil membrane-coated nanoparticles reduce ascites fluid weight and systemic toxicity in acute pancreatitis (AP) rats [140]. The PEG-PLGA nanoparticles (NP60 particle size, 61.4 ± 2.8 nm; NP150 particle

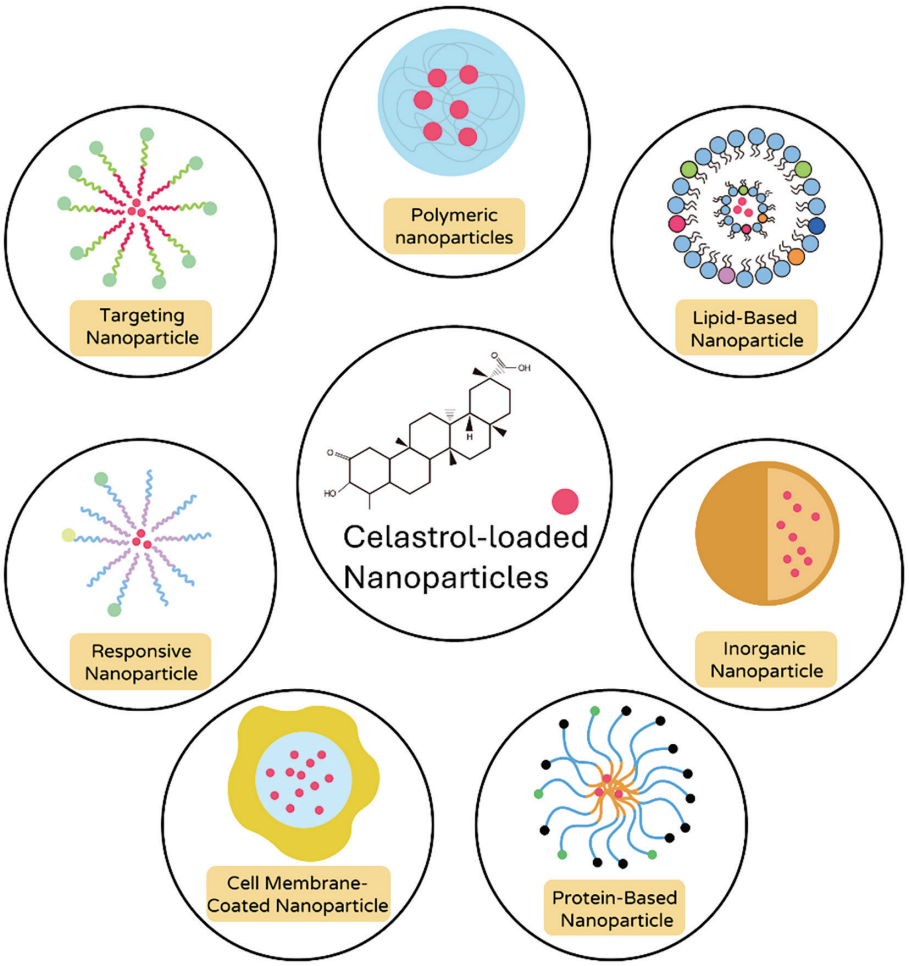

Figure 5 | Summary of different types of celastrol-loaded nanoparticles.

size, 156.8 ± 2.3 nm) were prepared by emulsion and solvent evaporation methods, along with the ability to target pancreatic tissue and improve uptake efficiency via selectively uptake by inflammatory cells [140]. For knee osteoarthritis (OA), celastrol loaded in hollow mesoporous silica nanoparticles with chitosan improves the water solubility, reduces the degradation of type II collagen, and exerts OA protective effects in OA rats. Chitosan is a pH-responsive amino-polysaccharide targeting severe inflammation sites with acidic microenvironments [141].

6.2 Cancer

At least 15 cancer nanomedicines were approved by the FDA and used in the clinic setting, indicating the enormous potential of nanomedicine in cancer treatment [142]. In the context of breast cancer, Liu et al. directly self-assembled celastrol into nanoparticles using the anti-solvent method and the particle size could be controlled from 52.5 ± 14.7 nm to 874.9 ± 315.8 nm depending on the solvent. The self-assembled celastrol nanoparticles efficiently accumulated in MCF-7 breast cancer cells and selectively increased the cytotoxicity on the cancer cells to decrease the systemic toxicity in tumour bearing mice [143]. Tian et al. self-assembled celastrol with erianin (EE%: celastrol, 96.45%; erianin, 82.61%; drug loading: celastrol, 63.15%; erianin, 37.85%; particle size, 142.9 ± 2.75 nm), another natural compound from *Dendrobium*, to provide a combination therapy effect and improve the bioavailability at the same time in mouse mammary carcinoma cells 4T1 [144]. Celastrol nanoparticles using triphenyl phosphonium-tocopherol polyethylene glycol succinate (TPGS) modified with PLGA and pH-sensitive chondroitin sulphate-folic acid was shown to have good biocompatibility, an increased uptake rate in 4T1 cells, and low toxicity on non-target cells. The nanoparticles were prepared by single emulsion solvent evaporation method, exhibiting an encapsulation efficiency of 75.4% ± 2.8%, drug loading of 36.1% ± 2.1%, and a size range of 70–100 nm. The binding of folic acid to chondroitin sulfate enables precise targeting of tumour cells with the acidic

Table 2 | Summary of potential properties of celastrol loaded in nanoparticles in different experimental settings.

Disease	Model	Encapsulate	Mechanism	Reference
Rheumatoid arthritis	8–12-week-old male C57BL/6 mice Administration: 2 mg/kg after the 28th day of immunization via injection of chicken type II collagen	Arginylglycylaspartic acid (RGD)-modified bovine serum albumin nanoparticles	– ↓ Side effects and non-targeted toxicity – ↓ Release of neutrophil extracellular traps – ↑ Bioavailability, apoptosis efficiency	[131]
	T cell line Jurkat and RAW264.7 cell line Administration: 10 mg/kg on days 30 and 40 after immunization by injected bovine collagen type II and Freund's adjuvant	Polymeric micelle based poly(ethylene glycol)-block-poly(propylene sulfide) [PEPS]	– ↓ TNFα, IL-6 and iNOS transcription – ↓ M1 macrophage activation – ↓ NF-κB signalling pathway, and Notch1 pathway – ↑ Water solubility	[133]
	Macrophage cell line RAW264.7 Administration: 12.5, 25, 50, 100, 200, 400, 800, and 1600 ng/mL, 2 days	Celastrol loaded poly(lactic-co-glycolic acid) nanoparticles	– No obvious systemic toxicity – ↓ Bone erosion and cartilage damage	[135]
	4-week-old male SD rats with collagen-induced arthritis by intradermally injected with 100 μg bovine type II collagen at the tail RAW264.7 cell	Celastrol loaded in folic acid-modified ROS-responsive nanoparticle	– Selectively targeted macrophage and regulate its proliferation – ↓ Inflammatory cytokines production, toxicity	[136]
Asthma	Human bronchial epithelial cell line: Bci-Ns1.1 Administration: 90 μL, 24 h	Liquid crystalline nanoparticles	– ↓ IL-1β level – ↓ NF-κB signalling pathway – ↑ Bioavailability	[138]
Allergic asthma	6–8-week-old female BALB/c mice Administration: 10 μg OVA and celastrol with 200 μg nanomicelles on days 28, 33, and 38	Ovalbumin-conjugated celastrol-loaded nanomicelles	– ↓ Inflammatory cell infiltration, expression level of IgE, IL-4, IL-5 and histamine level – ↑ IgG1/IgG2a expression level	[139]
Acute Pancreatitis	Male SD rat model with acute pancreatitis produced by sterile laparotomy after providing 1% sodium pentobarbital and infused 3% sodium taurocholate Administration: equivalent dose of 1 mg/kg of celastrol dissolved in 75 mg/kg of DiD solution, intravenously at 1 and 3 h	PEG–PLGA neutrophil membrane-coated nanoparticles	– ↓ Ascites fluid weight and systematic toxicity – Targeted pancreatic tissue and improved uptake efficiency via selective uptake by inflammatory cells	[140]
Knee osteoarthritis	6-week-old male SD rat model with left knee osteoarthritis induced by monosodium iodoacetate intra-articular injection Administration: equivalent dose of 10 μg/mL celastrol, 2 weeks	Celastrol loaded in hollow mesoporous silica nanoparticles utilizing chitosan	– ↓ Type II collagen degradation – ↑ IL-1β, IL-6, MMP-3, MMP-13 and TNF-α expression level – ↓ NF-κB signalling pathway	[141]
Broad cancer	Breast cancer cell line: MCF-7	Celastrol self-assemble nanoparticle	– ↑ Celastrol accumulation efficiency in cancer cell – ↑ Cancer cell selectively targeted cytotoxicity – ↓ Systemic toxicity	[143]
	Mouse melanoma cell: B16F10 Human lung carcinoma cell: A549 Hela, 4T1 and HepG2	Celastrol carrier poly(2-(N-oxide-N,N-dimethylamino) ethyl methacrylate)-block-poly(2-hydroxyethyl methacrylate)	– ↓ Cytotoxicity – ↑ Water solubilities	[161]
Melanoma	Murine melanoma cells line: B16F10 Administration: 27 μg/mL, 2 or 6 h 5–6-week-old male C57BL mice Administration: 2 and 4 mg/kg/2 days, 12 days, tail vein injection	Methoxyl poly(ethylene glycol)-b-poly(L-lysine)	– ↑ Efficiency against tumour – ↓ Systematic toxicity, liver degeneration	[147]

Table 2 | Continued

Disease	Model	Encapsulate	Mechanism	Reference
	Mouse melanoma and breast cancer cell line: B16F10 and 4T1 Human cancer cell lines: A375, A875, A2058, SKBR-3, SK-MEL-28, MDA-MB-231, and MCF-7 Female C57BL/6, female BALB/c, and nude mice	P-selectin targeting peptide and low molecular weight heparin nanoparticle	– ↑ ROS production – ↓ PI3K/Akt/mTOR signalling pathway – ↓ Side effects of toxicity on organ and blood – ↓ Mitochondrial potential	[148]
	Murine melanoma cell line: B16F10 Human melanoma cell line: A375 and M10 6–8-week-old female C57BL/6 mice	Celastrol nanoemulsion	– ↑ Water solubility and bioavailability – ↓ NF-κB signalling pathway, PD-L1 expression – Activated dendritic cells and T cells and affected PD-1/PD-L1 pathway	[149]
Breast cancer	Murine breast cancer cell line: 4T1 Administration: 2 mg/kg/d, intravenous injection, 2 weeks	pH-sensitive chondroitin sulfate-folic acid with triphenyl phosphonium-tocopherol polyethylene glycol succinate modified with polylactic acid glycol acid and pH-sensitive chondroitin sulfate-folic acid	– Good biocompatibility – Enable localized target location – ↓ Toxicity on non-target cell – ↑ apoptotic protein expression level – Induce damage and injury of mitochondria	[145]
	Female BALB/c mice 4T1 cells Administration: 2 mg/kg/3 times per day, 28 d	Celastrol loaded in nanoparticles combined by poly (lactic-co-glycolic acid) modified with β-cyclodextrin and polyethyleneimine grafted with benzimidazole	– Arresting the cell cycle and apoptotic mechanism – ↓ Systemic toxicity by targeting tumour cell – ↑ Cellular uptake of drug	[146]
Liver cancer	HepG2 cells	Polydopamine-modified self-assembly celastrol nanosuspension	– ↑ Endocytosis – ↑ Efficacy and bioavailability of the drug – ↑ Nucleat rupture	[150]
Ovarian cancer	Ovarian cancer cell line: (Epithelial) SKOV3, ES-2; (Normal) IOSE80	Zeolitic imidazolate framework-8 nanoparticles grafted with polyethylene glycol conjugated biotin	– ↑ Therapeutic effect, water solubility, stability and bioavailability – ↑ Intracellular ROS level and affected function of mitochondria – ↓ Drug damage on non-target organs by selectively releasing encapsulated drug on targeted tumour tissue – ↑ JNK and p38 signalling pathway – ↑ Expression of caspase-3 and Bax	[152]
Retinoblastoma	Human umbilical vein endothelial cell line: EA.hy 926 Human retinoblastoma cell line: SO-Rb 50 5–6-week-old female NOD-SCID mice model subcutaneously injected with SO-Rb 50 cells	Celastrol-loaded poly(ethylene glycol)-b-poly(ε-caprolactone) copolymers (PEG-b-PCL) nano micelles	– ↓ Tumour growth, new blood vessels formation – ↓ Hypoxia-inducible factors-1α/ vascular endothelial growth factor pathway	[12]
Osteosarcoma	Rat bone marrow stem cell line: rBMSCs Human osteosarcoma cell lines: HOS and 143b 4-week-old female BALB/c nude mice model	Celastrol-loaded layered double hydroxide-coated magnesium alloy	– ↓ Bone tumour cell migration and proliferation – ↑ p53, Bax, and Caspase 3 expression level – ↓ PI3K-Akt-mTOR signalling pathway – ↓ Expression of Bcl-2	[155]

Table 2 | Continued

Disease	Model	Encapsulate	Mechanism	Reference
Obesity	8-week-old C57 male mice RAW264.7, renal tubular, and renal basement membrane cells	Celastrol-loaded dextran sulfate with PVGLIG peptide linkage nanoparticle micelle	– ↓ Toxicity – ↓ Food intake of model – ↓ Insulin resistance – Restore homeostasis of blood glucose level	[156]
Non-alcoholic fatty liver disease	3–4-week-old male C57BL/6 N mice non-alcoholic fatty liver disease model with 60 kcal % high fat diet for 3 months Administration: 0.3 mg/kg/d, 16 days, i.p. HepG2 cells	Celastrol loaded in lactosylated bovine serum albumin	– ↓ Body fat and fat deposit in liver – ↓ Lipogenesis biomarkers and lipolysis mediator expression – ↓ Insulin resistance	[158]
Corneal allograft	8-week-old female SD and Wistar rats 8-week-old male C57BL/10ScNJNju and C57BL/10JNju (TLR4$^{-/-}$) mice Administration: 20 μL/three times per day, 40 days, topical instillation 2–2.5 kg New Zealand white rabbits Raw264.7 cells	Celastrol-loaded in triblock copolymer poly(ethylene glycol)-poly(ε-caprolactone)-g-polyethyleneimine	– ↓ IL-1α, IL-6, iNOS, IFN-γ, MCP-1, TNF-α and VEGF expression level – ↓ Macrophage and inflammatory mediator secretion	[160]
Acetaminophen-induced liver injury	6–8-week-old male BALB/c mice with established acute liver injury by intraperitoneal injection with acetaminophen Administration: 0.75 mg/kg, 24 h, tail vein injection RAW264.7 cell line	Celastrol-loaded erythrocyte membrane vesicles	– ↑ Bioavailability – ↓ Systemic toxicity and targeted liver inflammatory site – ↓ Polarization of M1 of macrophage – ↑ Polarization of M2	[159]

↑ refers to activated or enhanced by celastrol; ↓ refers to inhibited or downregulated by celastrol

tumour microenvironment triggering the degradation of chondroitin sulfate and thereby allowing the nanoparticles to target mitochondria and release celastrol effectively [145]. Another study loaded celastrol in PLGA nanoparticles modified with β-cyclodextrin and polyethyleneimine (PEI) grafted with benzimidazole and low molecular weight heparin (LMWH) by solvent displacement method with an EE% of 98.56 ± 0.07% and an average particle size of 108.37 ± 1.02 nm. The IC_{50} value in 4T1 cells showed a significant decrease attributed to the enhanced cellular uptake of the drug. The pH-sensitive β-cyclodextrin can respond to the acidic tumour microenvironment, facilitating drug release to target tumour cells. The PEI, with low molecular weight and low toxicity, promotes cellular uptake and lysosome escape. Additionally, LMWH helps prevent the first-pass effect and prolongs the circulation time of the nanoparticles in the bloodstream [146].

For melanoma treatment, Li et al. developed celastrol-loaded methoxyl poly(ethylene glycol)-b-poly(L-lysine) [mPEG-PLL] nanoparticles via the "*in situ* chemical conjugation-induced self-assembly" method, exhibiting a particle size of 103.1 ± 10.7 nm, a drug-loading content (DLC) of 9.95 wt%, and a drug loading-efficiency (DLE) of 53.9%. The nanoparticles showed increased efficiency against tumours, reduced systematic toxicity, and lower liver degeneration compared to free celastrol treatment in murine melanoma cells (B16F10) and tumour-bearing mice. The hydrophilic shielding layer composed of the mPEG segment stabilized the nanoparticle during blood circulation, while the PLL segment led to a celastrol self-cross-linked core by having electrostatic interaction with celastrol and its conjugate to celastrol via imine bonds, allowing it to disperse well in water and exhibit sustained release behavior [147]. Zhou et al. designed a self-assembled celastrol nanodrug using LMWH and P-selectin targeting peptide (PSN) to target tumour sites and reduce side effects with a mean particle size of 115.83 ± 6.93 nm. The nanoparticles targeted cancer more effectively due to the strong binding interaction to the tumour site vascular endothelial cells by LMWH and the targeting of highly expressed P-selectin in various cancer cells (B16F10 and 4T1 cells) by PSN. Additionally, the study demonstrated that the nanodrug demonstrated potent anti-metastasis efficacy in B16F10 lung metastasis mice [148]. Another study developed a celastrol nanoemulsion (EE%, 90 ± 2%; particle size, 91 nm) against melanoma using an ultrasonic emulsification method. The celastrol nanoemulsion increased the solubility and cellular uptake of celastrol and reduced the concentration of celastrol to efficiently induce immunogenic cell death and suppress

the expression of PD L1 in B16F10 tumour-bearing mice. Moreover, it was noted that the nanoemulsion exhibited no apparent organ toxicity [149].

In a study using HepG2 cells as a model, a polydopamine (PDA)-modified self-assembly celastrol nanosuspension was developed (drug loading, 60.33 ± 1.09%; particle size, 189.67 ± 2.08 nm) to enhance the efficacy and bioavailability of celastrol in liver cancer. The self-assembly celastrol was initially prepared through the precipitation method and subsequently modified with PDA using the oxidative polymerization technique. PDA was highlighted for high chemical reactivity and excellent biocompatibility. Furthermore, the system exhibited enhanced drug stability, efficacy, and bioavailability with a high drug-loading capacity [150]. Chen et al. developed liver-targeting celastrol-loaded liposomes (EE%, 90.5 ± 1.5%; particle size, 139.4 ± 2.7 nm) with galactose-modified 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-PEG (gala-PEG-DSPE), natural soybean phosphatidylcholine (SPC), and cholesterol using the film dispersion method. The inclusion of galactose in the formulation was aimed at leveraging its strong affinity for the asialoglycoprotein receptor (ASGPr)-rich hepatic parenchymal cytomembrane to achieve liver targeting. These liver-targeting celastrol-loaded liposomes had improved bioavailability, stability, and sustained release characteristics. The *in vivo* study confirmed that these liposomes significantly improved the therapeutic efficacy of celastrol in hepatocellular carcinoma (HCC) mice while concurrently reducing side effects [151].

Zhou et al. found that zeolitic imidazolate framework-8 (ZIF-8) nanoparticles grafted with PEG conjugated biotin (EE%, 60.52% ± 2.79%; drug loading, 31.6 ± 2.85; particle size, 234.5 nm) enhanced the therapeutic effect, water solubility, stability, and bioavailability of celastrol in epithelial ovarian cancer cell lines (SKOV3 and ES-2), a normal ovarian epithelial cell line, and an SKOV3 xenograft mouse model. The nanoparticles reduced drug damage to non-target organs by selectively releasing encapsulated drugs in targeted tumour tissue. The pH-sensitive nature of ZIF-8 nanoparticles allows for the controlled release of drugs in an acidic microenvironment, facilitating precise drug delivery to tumours. Moreover, the biotin-conjugated PEG enables specific targeting of tumours that overexpress the biotin receptor [152]. Niu et al. developed ROS-sensitive celastrol nanoparticles (EE%, 56%; DLC, 11.2 ± 0.7%; particle size, 155 ± 4 nm) with FA-modified thioketal-bonded PLGA-PEG using a solvent evaporation technique. The study demonstrated that celastrol was released faster in a high-concentration ROS environment and promoted cell apoptosis in an SKOV3 cell line [153].

Li et al. used the human umbilical vein endothelial cell line, EA.hy 926, the retinoblastoma cell line, SO-Rb 50, and the SO-Rb 50 xenograft mouse model to investigate the effects of celastrol-loaded PEG-b-poly(ε-caprolactone) copolymer (PEG-b-PCL) nanomicelles. These nanomicelles, with a DLC of 7.36% and a mean particle size of 48 nm, demonstrated the ability to inhibit tumour growth via inducing apoptosis and reducing the formation of new blood vessels through inhibiting hypoxia-inducible factors-1α/vascular endothelial growth factor pathway. Additionally, PEG-b-PCL nanomicelles were shown to improve the solubility of celastrol, provide enhanced control over drug release kinetics, and prolong the drug circulation time in the bloodstream [12, 154].

In a study focusing on osteosarcoma, rat bone marrow stem cells (rBMSCs) and human osteosarcoma cell lines (HOS and 143b) were used to examine the effects of celastrol-loaded layered double hydroxide-coated magnesium alloy on reducing the migration and proliferation of bone tumour cells. The magnesium alloy was biodegradable and exhibited anti-tumour properties. The layered double hydroxide coating served to enhance the corrosion resistance of the magnesium alloy by forming a protective barrier. Additionally, this coating enables control over drug delivery, thereby reducing systemic side effects [155].

6.3 Obesity and liver diseases

Chen et al. designed an MMP-2 enzyme sensitive celastrol-loaded dextran sulfate nanoparticle micelle with PVGLIG peptide linkage (EE%, 38.07%; DLE%, 3.46%; particle size, 201.3 nm) for obesity. The treatment reduced insulin resistance, restored homeostasis of blood glucose levels, reduced food intake in HFD-induced obesity mice, and exerted an anti-obesity effect. PVGLIG peptides are sensitive to MMP-2 enzyme, which are excessively secreted during inflammation. Dextran sulfate is a hydrophilic ligand for scavenger receptor class A and improved the accumulated concentration, which reduce the systemic toxicity induced by high-concentration celastrol [156]. Fan et al. developed celastrol-loaded BSA nanoparticles by high-pressure homogenization method with an EE% of 75%, a DLE% of 13.88%, and a particle size of 125.6 ± 2.2 nm. The nanoparticle improved the cellular uptake rate and capability of passing the intestinal barrier, thereby improving insulin sensitivity and liver function more significantly in HFD obese mice [157]. Furthermore, in a study focused on non-alcoholic fatty liver disease (NAFLD), celastrol loaded in lactosylated bovine serum albumin (EE%, 79.0%; DLE%, 13.62%; particle size, 158.6 ± 3.4 nm) has been shown to be precisely deposited in the livers of NAFLD mice, indicating the ability to target liver cells. Celastrol loaded in lactosylated bovine serum albumin also reduced body fat and fat deposits in the liver via inhibiting the expression of lipogenesis biomarkers and lipolysis mediators and enhancing insulin resistance. The cell surface of hepatocytes features the lectin asialoglycoprotein receptor, which exhibits a strong affinity for lactose. This characteristic enables the binding of lactosylated nanoparticles to hepatocytes through clathrin-mediated endocytosis, which targets liver cells to reduce systemic cytotoxicity and

improves the bioavailability of hydrophobic drugs, like celastrol [158].

Furthermore, for acetaminophen-induced liver injury, the application of celastrol-loaded erythrocyte membrane vesicles has been observed to provide extended bioavailability, lower systemic toxicity, and the ability to target liver inflammatory sites [159].

6.4 Allograft

Moreover, a study using SD and Wistar rats and Raw264.7 cells as models for corneal allografts demonstrated that celastrol-loaded in triblock copolymer PEG-poly(ε-caprolactone)-g-polyethyleneimine inhibit macrophage activity and reduce inflammatory mediator secretion by regulating the TLR4/MyD88/NF-κB signalling pathway. This positive nanoparticle can elongate the retention time on the ocular surface and enhance the corneal permeability of celastrol due to its PEGylated shells and small size [160].

7. CONCLUSION

This review discussed the druggability, molecular targets, and nanocarrier delivery for the therapeutic potential of celastrol in the treatment of chronic diseases. It becomes clear that celastrol not only targets several common signalling pathways (NF-kB, TP53, PTEN/PI3K/AKT, and PI3K/Akt/mTOR) in inflammation, cancer, neurodegeneration, diabetes, and obesity but also regulates disease-specific target proteins and signalling pathways. Nanocarrier drug delivery system may effectively overcome the key pharmacologic issues (low water solubility, low bioavailability, and high systemic toxicity) and warrant the clinical applications of celastrol in the treatment of various chronic diseases. Nevertheless, *in silico* prediction of druggability does not guarantee the clinical efficacy and safety of celastrol. Further work is needed to determine the accurate toxicologic values and optimize the nanocarrier drug delivery system for guiding the clinical applications of celastrol. Ultimately, it is important to translate celastrol as a safe and effective drug for various chronic diseases.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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