

# The Potential Application of Pentacyclic Triterpenoids in the Prevention and Treatment of Retinal Diseases

## Authors

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## ABSTRACT

Retinal diseases are a leading cause of impaired vision and blindness but some lack effective treatments. New therapies are required urgently to better manage retinal diseases. Natural pentacyclic triterpenoids and their derivatives have a wide range of activities, including antioxidative, anti-inflammatory, cytoprotective, neuroprotective, and antiangiogenic properties. Pentacyclic triterpenoids have great potential in preventing and/or treating retinal pathologies. The pharmacological effects of pentacyclic triterpenoids are often mediated through the modulation of signalling pathways, including nuclear factor erythroid-2 related factor 2, high-mobility group box protein 1, 11 $\beta$ -hydroxysteroid dehydrogenase type 1, and Src homology region 2 domain-containing phosphatase-1. This review summarizes recent *in vitro* and *in vivo* evidence for the pharmacological potential of pentacyclic triterpenoids in the prevention and treatment of retinal diseases. The present literature supports the further development of pentacyclic triterpenoids. Future research should now attempt to improve the efficacy and pharmacokinetic behaviour of the agents, possibly by the use of medicinal chemistry and targeted drug delivery strategies.

## Background

The retina is a light-sensitive layer at the rear of the eye that converts light into neuronal impulses to obtain vision. Retinal cells such as the RPE, photoreceptor cells, horizontal cells, bipolar cells, amacrine cells, ganglion cells, and Müller cells have been identified and their major circuitry and connections in the tissue have been summarised in detail recently [1]. The retina is one of the most metabolically active tissues in the body and requires appropriate levels of nutrients and oxygen for normal function [2]. Blood flow disruption and excessive oxygen consumption are

leading causes of impaired energy production in the retina and may also promote inflammation, neovascularisation, and even retinal cell death. Other factors like hypertension, high blood sugar level, and inheritance also contribute to disease progression [3, 4]. Common retinal diseases include AMD, DR, retinal detachment, and retinitis pigmentosa [5–7]. Patients suffering from these diseases have blurred or distorted vision, and their lives can be seriously affected [7]. However, effective treatments are few and current interventions are limited to intravitreal injections of anti-VEGF antibody or corticosteroids, surgery, laser treatment, and/or nutrient supplementations [8, 9]. Because most retinal dis-

## ABBREVIATIONS

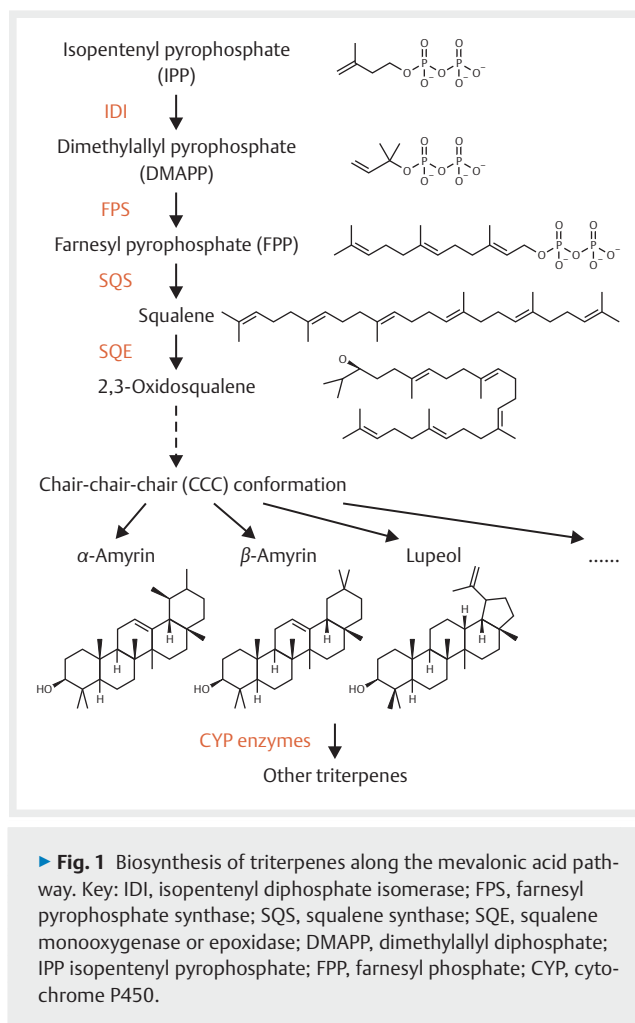
11 $\beta$ -HSD1	11 $\beta$ -hydroxysteroid dehydrogenase type 1
AKBA	acetyl-11-keto- $\beta$ -boswellic acid
AMD	age-related macular degeneration
CBX	carbenoxolone
CDDO	2-cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid
DMAPP	dimethylallylpyrophosphate
DR	diabetic retinopathy
FPP	farnesyl pyrophosphate
GCLC	glutamate-cysteine ligase catalytic subunit
GCLM	glutamate-cysteine ligase regulatory subunit
GL	glycyrrhizin
HMGB1	high-mobility group box protein 1
HO-1	heme oxygenase-1
HRMECs	human retinal microvascular endothelial cells
Hsp70	heat shock protein 70
HUVECs	human umbilical vein endothelial cells
I/R	ischaemia-reperfusion
ICAM-1	intercellular adhesion molecule-1
IPP	isopentenyl pyrophosphate
Keap1	Kelch-like ECH-associated protein 1
LPS	lipopolysaccharide
MVA	mevalonic acid
NQO1	NAD(P)H quinone oxidoreductase 1
Nrf2	nuclear factor erythroid-2 related factor 2
OA	oleanolic acid
OIR	oxygen-induced retinopathy
ONL	outer nuclear layer
PTs	pentacyclic triterpenoids
RGC	rat ganglion cell
ROS	reactive oxygen species
RPE	retinal pigment epithelium
SAR	structure-activity relationship
SHP-1	Src homology region 2 domain-containing phosphatase-1
UA	ursolic acid
VEGF	vascular endothelial growth factor

eases are irreversible, new therapies are urgently needed to prevent the initiation and progression of disease.

Natural compounds are the subject of intensive research interest for their potential in managing a range of diseases. This review focuses on recent advances in the potential application of natural compounds, particularly PTs and their derivatives, in the prevention and treatment of retinal disease.

### Biosynthesis of triterpenoids

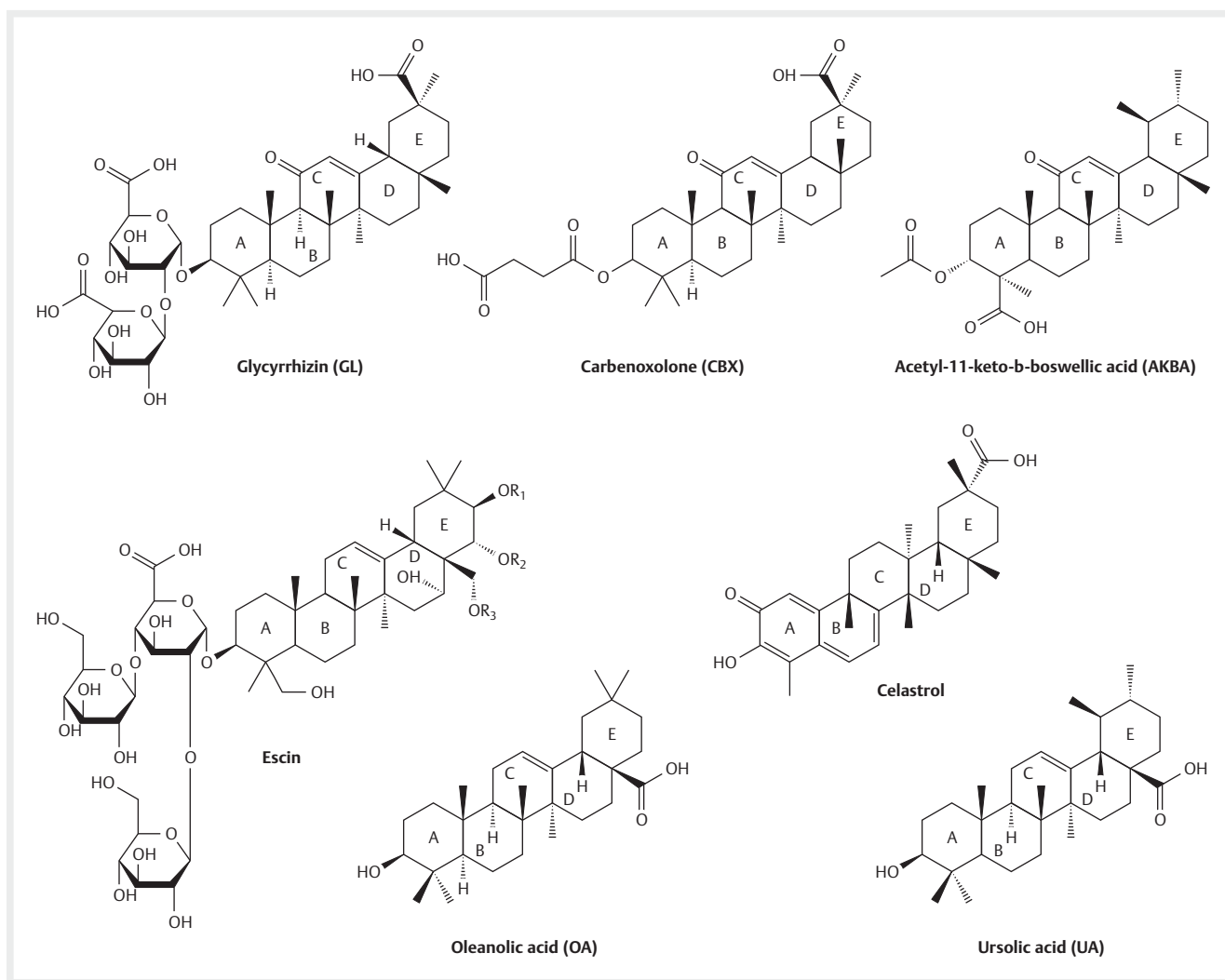
The terpenoids are a large and diverse group of natural products. The biosynthesis of terpenoids begins with condensation of the five-carbon isoprenoid subunits IPP and its isomer DMAPP via the MVA pathway [10–12]. Condensation with additional isoprenoid subunits produces the sesquiterpenoids (C15), diterpenoids (C20), sesterpenoids (C25), triterpenoids (C30), and tetraterpe-



noids (C40) [13]. As the number of component isoprenoid subunits increases, the potential for structural complexity increases.

In the peroxisome, head-to-tail condensation of DMAPP with two units of IPP generates C15 FPP [11, 12]. Head-to-head condensation of two molecules of FPP then generates the linear C30 triterpene precursor squalene [10–12], which undergoes epoxidation to 2,3-oxidosqualene [11, 12]. In its chair-boat-chair conformation, 2,3-oxidosqualene is cyclised to sterols such as lanosterol (in fungi and animals) or cycloartenol (in plants) [11, 12], while the chair-chair-chair conformation leads to a wide array of triterpenes such as taraxasterol, lupeol, and the  $\alpha/\beta$  amyrins [11, 12], which are transformed further to diverse triterpenes by CYP enzymes [11] (► Fig. 1).

The terms triterpene and triterpenoid are often used to describe the same C30-terpene compound, although triterpene refers more specifically to naturally occurring agents [13]. There are a number of triterpenes [14], with many being present as the aglycones (free hydroxyl or carboxylate moieties), while others are glycosylated (e.g., the saponins) or exhibit other types of conjugation [14]. Triterpenoid is a broader term, which covers natural degradation products, natural and synthetic derivatives, and hydrogenation products [13].



► **Fig. 2** Structures of common pentacyclic triterpenoids.

PTs have received considerable attention due to their potent biological and pharmacological properties [15]. Different PTs exhibit antitumour, antiviral, antimicrobial, antiparasitic, antidiabetic, and anti-inflammatory actions, and also mediate cardio-, hepato- and gastro-protection [14, 15]. Important PTs that have found clinical application include GL, celastrol, CBX, AKBA, escin, UA, and OA (► **Fig. 2**). These molecules have attracted attention for their potential in treating a range of human pathologies including retinal diseases.

## Methodology

We searched the databases of Pubmed, Google Scholar, and Clinicaltrials using the key words of “pentacyclic triterpenoid”, “pentacyclic triterpene”, “retina”, “oxidative”, “glycyrrhizin”, “carbenoxolone”, “AKBA”, “celastrol”, “escin”, “oleanolic acid”, “CDDO”, “pharmacokinetics”, “structure activity relationship”, and “docking”. We focused on articles discussing the biosynthesis of triterpenes and pharmacological actions (with reference to *in*

*vivo*, *in vitro*, and clinical reports) and the signaling pathways by which they mediate these actions. Their applications in the treatment of retinal diseases and the side effects of triterpenoids are also covered. In this review, 159 references from the years 1998 to 2020 are included.

## Naturally occurring pentacyclic triterpenoids

### Glycyrrhizin

GL (- (3 $\beta$ ,20 $\beta$ )-20-carboxy-11-oxo-30-norolean-12-en-3-yl-2-O- $\beta$ -D-glucopyranuronosyl- $\alpha$ -D-glucopyranosiduronic acid), also known as glycyrrhizic acid or glycyrrhizinic acid, is a triterpene glycoside from the root of the medicinal herb *Glycyrrhiza glabra* (licorice) [16]. GL has clinical potential in treating chronic hepatitis [16]. There is also evidence that GL has anti-inflammatory, antiviral, and antimicrobial actions [17]. Around 30 clinical trials have evaluated GL, principally for the treatment of liver disease and cancer [18]. According to clinical data, the pharmacokinetics of GL were linear to a maximum oral dose of 200 mg, administered 6 times per week [19], and accumulation occurred with on-

going administration [19]. The threshold GL plasma concentration for efficacy in the treatment of chronic hepatitis is around 5 mg/mL [20].

The protective actions of GL in the retina include maintenance of the structure and metabolic activities of retinal cells *in vitro* and *in vivo* in disease models. For example, He et al. [21] reported that GL significantly decreased the production of ROS induced by sodium iodate and prevented apoptosis in human ARPE-19 cells. GL was also found to promote the activation of Nrf2 and HO-1 (HMOX1) expression by increasing the phosphorylation and activation of the pro-survival Akt cascade. These findings are in broad agreement with another study that also reported that GL protected the retina by attenuating ROS production, increasing the poly [ADP-ribose] polymerase 1 DNA-repair enzyme and decreasing cell death mediated by caspase-3 [22].

In mice, GL (10 mg/kg by i.p. injection) administered prior to and following I/R injury protected the retina from neuronal and vascular damage [23]. In addition, Song et al. [24] reported that GL suppressed ocular hypertension induced by triamcinolone acetonide, improved electrophysiological parameters, and compensated for triamcinolone acetonide-induced changes in ocular metabolism.

GL also inhibits HMGB1, which is a ubiquitous nuclear protein that is released from damaged cells and induces proinflammatory responses [25]. The intravitreal injection of HMGB1 upregulated the proinflammatory ICAM-1 in the rat retina, which was attenuated after oral administration of GL [26]. Accordingly, GL has the potential to inhibit proinflammatory processes mediated by HMGB1.

Detailed studies by Mohammad's group [27] and others have established a role for HMGB1 in pathogenic mechanisms that are activated in DR. HMGB1 is proangiogenic [28] and has been shown to increase retinal proliferation in patients with DR [29]. By suppressing the increase in HMGB-1 expression and the activation of NF- $\kappa$ B in DR, GL attenuated pro-angiogenic signalling [30]. GL also prevented the diabetes-induced loss of brain-derived neurotrophic factor in rats [31], decreased excitotoxicity produced by high glutamate concentrations in the central nervous system [32], and restored retinal occludin [27]. In addition, GL prevented the activation of Toll-like receptor 4 and TNF- $\alpha$  in primary retinal endothelial cells that were cultured in high-glucose medium. This modulated the decrease in phosphorylated-Akt under the culture conditions and decreased caspase-3 cleavage to promote cell survival [23].

There have been no reports of side effects from the retinal application of GL, but several studies have noted that GL modulates the pharmacokinetics of coadministered drugs or chemicals, such as midazolam [33], paeoniflorin [34], ribavirin [35], puerarin [36], glibenclamide [37], omeprazole [38, 39], aconitine [40], talinolol [41], and even other PTs like asiatic acid [42] and celastrol [43], following systematic administration. These interactions may be due to altered functions of CYPs and P-glycoprotein.

### Carbenoxolone

CBX (-(3 $\beta$ )-3-[(3-carboxypropanoyl)oxy]-11-oxoolean-12-en-30-oic acid) is a derivative of GL and is also found in licorice root. CBX has been used in the treatment of ulcers of the stomach and

digestive tract [18], but this has decreased because of adverse effects, such as electrolyte disturbance and hypertension [24].

CBX is a nonselective inhibitor of the enzyme 11 $\beta$ -HSD1 [24] that regulates the biosynthesis of ligands for glucocorticoid and mineralocorticoid receptors [44]. Na et al. [45] reported that CBX prevented dry eye syndrome in the rat by inhibiting the expression and activity of 11 $\beta$ -HSD1 (► Table 1).

Pan et al. [46] found that CBX was a partially reversible inhibitor of gap junction channels, which are specialised membrane domains between adjacent cells that regulate the transfer of cytoplasmic components [47]. CBX is now used as an experimental reagent in *in vitro* and *in vivo* retinal models to decrease membrane potential, study the role of connexins in gap junctions [48], and investigate a range of retinal processes [49–52]. As an irreversible inhibitor of voltage-dependent calcium channels, CBX has been used to evaluate the role of these channels in the retina [53–55]. However, the clinical usage of CBX is limited by its toxicity that leads to retinal opacity and swelling [46] and retinal thinning [56]. CBX also decreases the responses of photoreceptors to light [57, 58] and photoreceptor-to-horizontal cell synaptic transmission [59].

### Acetyl-11-keto- $\beta$ -boswellic acid

Boswellic acids are PTs present in the resin of *Boswellia* species [60]. Boswellic acids have reported anti-inflammatory [60, 61], antimicrobial [60, 61], antiparasitic [60], anticancer [62], anti-arthritis [61], and immunomodulatory [61] actions. Although more than 12 different boswellic acids have been identified in resin extracts, 11-keto- $\beta$ -boswellic acid and AKBA [(3R,4R,4aR,6aR,6bS,8aR,11R,12S,12aR,14aR,14bS)-3-acetyloxy-4,6a,6b,8a,11,12,14b-heptamethyl-14-oxo-1,2,3,4a,5,6,7,8,9,10,11,12,12a,14a-tetradecahydronicene-4-carboxylic acid] appear to have the greatest pharmacological significance [60]. Three clinical trials have evaluated boswellic acids in relapsing remitting multiple sclerosis, osteoarthritis of the knee, and in pain, stiffness, and impaired function in joints; another trial of the efficacy of boswellic acids in the treatment of renal stones is scheduled, but recruitment has not yet started.

SHP-1 (also known as tyrosine-protein phosphatase non-receptor type 6) regulates growth, mitosis, differentiation, and oncogenic transformation in a range of cell types, including retinal cells. Indeed, SHP-1-deficient mice exhibit progressive retinal degeneration [63], while the activation of SHP-1 in retinal pericytes promotes apoptosis in DR [64].

AKBA has been reported to increase SHP-1 expression and activity in normoxic mouse retina explants [65], which modulates signalling by STAT3. This prevents the activation of hypoxia-inducible factor-1 $\alpha$  and VEGF in the oxygen-induced mouse model of retinopathy (OIR) [65]. AKBA decreased neovascularisation in the OIR mouse retina by suppressing STAT3 phosphorylation and VEGF expression. AKBA also inhibited cell proliferation and tube formation in this model. Similarly, AKBA prevented the increase in activated p-STAT3 in VEGF-treated HRMECs [66]. The antiangiogenic actions of AKBA are of potential value in studying the role of neovascularisation in the pathogenesis of retinal disease [66].

The fed/fasted state has a major impact on the pharmacokinetics of AKBA. An approximate fourfold increase in the  $C_{max}$  in

► **Table 1** Pharmacological actions of natural pentacyclic triterpenoids and mechanisms of improved retinal function.

PT	In vitro model	In vivo model	Disease model	Pharmacological activity	Pharmacological/pathological improvements	Molecular mechanisms*	Reference
Glycyrrhizin		Rabbits	Ocular hypertension	Reduced ocular hypertension	↓ Ocular hypertension, improved electrophysiology, modulated ocular metabolism induced by triamcinolone acetate		[24]
	ARPE-19 cells	Mice	Sodium iodate induced damage	Cytoprotection	Mice: ↓ apoptosis, ↓ retinal thinning, ↓ drusen number, ↑ amplitude of "a-wave" in photoreceptors and "b-wave" in bipolaris	ARPE-19 cells: ↑ p-Akt, ↑ Nrf2	[21]
	Rat RGC-5 cells		Advanced glycation end product-induced VEGF-A production	Antiangiogenic		↓ HMGB1, ↓ JNK2/3 activation	[28]
		Rats	Diabetes	Neuroprotection		↓ Brain-derived neurotrophic factor	[31]
		Rats	Diabetes	Antiangiogenic, anti-inflammatory		↓ CXCR4, ↓ HIF-1 $\alpha$ , ↓ Egr-1, ↓ CXCL12	[30]
		Rats	Diabetes	Anti-inflammatory		↓ HMGB-1, ↓ NF- $\kappa$ B activation	[27]
		Rats	Diabetes	Anti-inflammatory		↓ HMGB1, ↓ Etk1/2	[32]
	Primary human retinal endothelial cells	Mice	Cells: high glucose Mice: ischaemia/reperfusion	Anti-inflammatory, neuroprotection		Cells: ↓ HMGB1, ↓ TLR4, ↓ TNF- $\alpha$ , ↓ cleaved caspase-3; ↑ p-Akt	[23]
		Rats	Diabetes			↓ ROS, ↓ Nox2, ↓ p47phox, ↓ p22phox, ↓ cleaved caspase-3	[22]
	Carbenoxolone		Rats	Dry eye syndrome	Cytoprotective	Improved ocular surface, tear formation, corneal thickness, ↓ apoptosis in conjunctival epithelium	↓ 11 $\beta$ -HSD1, ↓ TNF- $\alpha$ , ↓ IL-6, ↓ Bax/Bcl-2
AKBA		Mice	Hypoxia	Cytoprotective		↑ SHP-1, ↓ p-STAT3, ↓ HIF-1 $\alpha$ , ↓ VEGF, ↓ phospho-VEGFR-2	[65]
	HRMECs	Mice	OIR	Antiangiogenic	HRMECs: ↓ cell proliferation, ↓ migration, ↓ tube formation Mice: ↓ neovascularisation	HRMECs: ↓ p-STAT3 Mice: ↑ SHP-1, ↓ p-STAT3, ↓ VEGF, p-VEGFR-2	[66]

continued

▶ **Table 1** Continued

PT	In vitro model	In vivo model	Disease model	Pharmacological activity	Pharmacological/pathological improvements	Molecular mechanisms*	Reference
Celastrol	ARPE-19 cells, RAW 264.7 cells	Mice	Retinal degeneration induced by bright light	Anti-inflammatory, antioxidant	Mice: ↓ ONL loss, ↑ scotopic a-wave and b-wave amplitudes, ↓ photoreceptor apoptosis, ↓ retinal inflammation, ↓ leukostasis, ↓ reactive gliosis, ↓ microglial activation	ARPE-19: ↓ ROS, ↓ IL1β, ↓ CCL2 RAW 264.7: ↓ TNF-α, IL1β, ↓ CCL2 Mice: ↓ IL1β, ↓ CCL2, ↓ TNF-α	[72]
	ARPE-19 cells		Inflammation	Anti-inflammatory		↓ IL-6, ↓ NF-κB p65 phosphorylation	[75]
		Rats	Optic nerve crush	Cytoprotective	↑ RGC survival, ↓ body weight,	↓ TNF-α	[74]
		Rats	Hypertension-induced degeneration	Cytoprotective	↑ RGC survival		[73]
Escin	ARPE-19 cells, primary murine RPE cells		H <sub>2</sub> O <sub>2</sub> -induced cytotoxicity	Antioxidative, cytoprotective	↓ Murine RPE cell damage, ↓ cell apoptosis	↓ ROS, ↑ Nrf2 phosphorylation, ↑ Akt phosphorylation	[80]
	ARPE-19 cells, HUVECs		BRB breakdown	Cytoprotective	HUVECs: improved tight junction	HUVECs: triamcinolone acetonide and escin: ↑ glucocorticoid receptor expression	[81]
		Rats	Ischaemia, BRB leakage	Cytoprotective	Escin: ↓ retinal thickness, triamcinolone acetonide and escin: ↓ BRB permeability		[82]
Ursolic acid	RPE cells		Broad light irritation	Cytoprotective		↓ NF-κB activation, ↑ ROS	[96]
	RPE cells		UV-induced oxidative stress	Cytoprotective	↓ Apoptosis	↓ p53, ↓ NF-κB activation	[97]
Oleanolic acid (OA)	HUVEC cells	Mice	Angiogenesis	Antiangiogenic	HUVECs and mice: ↓ angiogenesis	HUVEC cells: ↓ VEGFR-2, ↓ Erk1/2	[159]
Asiatic acid		Rat	Increased intraocular pressure, glaucoma	Cytoprotective	Rat: ↑ RGC survival, ↓ retinal thinning, ↓ apoptosis, ↑ photopic negative response amplitudes in COHT	↑ Bcl-2; ↓ Bax, ↓ caspase-3	[99]
Madecassic Acid	Human retinal microvascular endothelial cells		Hypoxia-induced oxidative stress	Antioxidative	↓ ER stress	↓ Cleaved caspase-3, -9 and -12, ↓ Bax, ↓ ROS, ↓ GRP78, ↓ CHOP, ↓ IRE1α, ↓ ATF6, ↓ ATF4, ↓ p-Erk, ↓ p-ef2α; ↑ Bcl-2	[100]
Corosolic acid	ARPE-19 cells	Eggs, rats	Angiogenesis	Antiangiogenic	Eggs: ↓ vascular area, ↓ number of junctions, ↓ vessels length and lacunarity		[101]

\* Signalling pathways and molecular mediators that are modulated by PTs and that improve retinal function



healthy volunteers, produced by a single oral dose of *Boswellia* extract (AKBA 20–30 mg), was attributable to increased absorbance following a high-fat meal [ $C_{\max}$ : 6 ng/mL (fasted) vs. 28.8 ng/mL (fed)] [67, 68]. After repeated oral administration of a *Boswellia* extract (800 mg, three times daily for 4 weeks), the steady-state concentration was 0.04  $\mu$ M (~ 20.5 ng/mL) [69]. Low absorption and/or extensive metabolism appear to contribute to the poor bioavailability of AKBA [70].

### Celastrol

Celastrol (3-hydroxy-9 $\beta$ ,13 $\alpha$ -dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oic acid) is a major constituent of the medicinal plant *Tripterygium wilfordii* Hook F. Its reported pharmacological activities are broad and include anti-inflammatory, cardioprotective, and neuroprotective actions, as well as anticancer, anti-obesity, and antidiabetic effects [71]. The anti-inflammatory actions of celastrol in the retina are attributed to its capacity to modulate multiple inflammatory mediators, including the cytokines IL-1 $\beta$ , CCL2, and TNF- $\alpha$ , Hsp70, and cyclooxygenase-2 [71–75]. Bian et al. demonstrated the efficacy of celastrol against light-induced retinal inflammation at low concentrations [72]. Pretreatment of ARPE-19 cells with 0.1–1.5  $\mu$ M celastrol inhibited the phosphorylation and activation of the NF- $\kappa$ B p65 subunit on Ser536 and decreased IL-6 secretion following the application of LPS.

Celastrol protected rat RGCs from damage due to ocular hypertension [73]. The intraperitoneal injection of celastrol (1 mg/kg for 14 days) promoted RGC survival in the rat optic nerve crush model [74]. Celastrol also preserved the outer nuclear layer structure and thickness in the mouse retina after damage by bright light, attenuated light-induced photoreceptor apoptosis, and increased the amplitudes of scotopic a- and b-waves [72].

Most studies have reported that the intraperitoneal administration of celastrol *in vivo* decreases body weight in experimental animals [74, 76, 77]. Thus, to retain the pharmacological benefits of celastrol, alternate delivery routes have been evaluated. Intravitreal administration of celastrol (1 mg/kg) was effective, but less so than daily intraperitoneal administration, and multiple applications may be required for optimal benefit. Detailed studies are now warranted to evaluate this possibility.

### Escin

Escin [(2S,3S,4S,5R,6R)-6-[[[(3S,4S,4aR,6aR,6bS,8R,8aR,9R,10R,12aS,14aR,14bR)-9-acetoxy-8-hydroxy-4,8a-bis(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-[2-methyl-1-oxobut-2-enoxy]-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-4-hydroxy-3,5-bis[[[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-tetrahydropyran-2-yl]oxy]-2-tetrahydropyran-2-carboxylic acid]] is a mixture of triterpenoid saponins from the horse chestnut tree, *Aesculus hippocastanum*. Earlier studies reported that escin has anti-inflammatory, anti-oedematous, and anticancer properties [78, 79], and has potential application in the treatment of chronic venous insufficiency, haemorrhoids, and postoperative oedema [78, 79]. It has been reported that escin functions by activating Akt-Nrf2 signalling [80].

In the ARPE-19 model, the combination of escin and triamcinolone acetonide prevented the disruption of the brain-retinal bar-

rier due to VEGF treatment, and increased the expression of occludin and the ZO-1 protein that maintains tight junctions [81]. Similar effects were also produced by the combination *in vivo*. Thus, escin and triamcinolone acetonide decreased retinal leakage in the rat, which was associated with loss of the integrity of the brain-retinal barrier following ischaemic injury [82].

Clinical studies by Wu et al. [83] reported that the  $C_{\max}$  of escin Ia and escin Ib were  $0.77 \pm 0.64$  ng/mL and  $0.38 \pm 0.26$  ng/mL, respectively, in healthy volunteers after oral administration of 60 mg escin saponin tablets (which contained escin Ia 18.6 mg and escin Ib 11.4 mg); both compounds reached  $T_{\max}$  at around 2 h.

### Oleanolic acid

OA [3-hydroxyolean-12-en-28-oic acid; (4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydronicene-4a-carboxylic acid] is one of the best-known PTs and is found in the bark, leaves, and fruits of over 1600 plant species as both a free acid and a glycosylated saponin [84]. OA is most abundant in members of the *Oleaceae* family, such as the principal commercial source olive (*Olea europaea*), *Lantana camara*, and *Ligustrum lucidum* [84–88].

OA has been used clinically in China as a hepatoprotective adjuvant agent for decades [84, 85, 87] and has antitumour, antidiabetic, antimicrobial, antiparasitic, and antihypertensive actions, as well as antioxidant and anti-inflammatory properties [87]. Studies in healthy Chinese volunteers reported that plasma concentrations of  $12.1 \pm 6.8$  ng/mL were attained after a single oral dose of 40 mg [89]. The highest oral dose that was used in rats was 50 mg/kg and produced a  $C_{\max}$  of  $132 \pm 122$  ng/mL [90].

OA also suppresses VEGF-induced activation of VEGF-receptor 2 and its downstream protein Erk1/2 in HUVECs. However, the antiangiogenic actions of OA in the mouse retina *in vivo* requires higher doses (up to 125 mg/kg), which may be due to its short half-life and low oral bioavailability (only ~ 0.7%), most likely due to poor absorption [91].

### Ursolic acid

UA (3 $\beta$ -hydroxy-urs-12-ene-28-oic acid; (1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picen-4a-carboxylic acid] is a secondary plant metabolite that is structurally similar to OA. UA is present in the bark, leaves, peel, and wax layers of many edible fruits [92]. UA reportedly has diverse pharmacological properties, including anticancer [92, 93], antimicrobial [92], antiviral [92], anti-inflammatory [93], and antidiabetic activities [93]. Clinical trials of UA have provided some evidence that it may have value in preventing muscle atrophy and sarcopenia (NCT02401113; study completed). However, the utility of UA in the treatment of metabolic syndrome (NCT02337933; study completed but results not reported) and primary sclerosing cholangitis (NCT03216876; study withdrawn due to lack of feasibility) is unclear at present. UA is rapidly absorbed ( $t_{\max} \leq 1$  h) [94, 95], but the bioavailability is low. A  $C_{\max}$  of 295 ng/mL after oral administration of a UA extract (80 mg/kg) was reported in Wistar rats [94], and a  $C_{\max}$  of 68.3 ng/mL was achieved in SD rats after administration of authentic UA (10 mg/kg) [95].

The photoprotective activity of UA in RPE cells has been assessed. UA was found to mitigate damage elicited by UV light by inhibiting the NF- $\kappa$ B pathway [96, 97], but also produced an increase in ROS [96]. However, the bioavailability of UA is low and both medicinal chemistry and formulation strategies have been undertaken to improve its activity. Alvarado et al. [98] designed and tested UA-loaded poly(dl-lactide-co-glycolide) acid nanoparticles that exhibited potent anti-inflammatory activity in the rabbit eye without producing toxicity. This approach could be further adapted in optimising the clinical application of PTs, especially those with poor pharmacokinetic properties, including poor oral bioavailability.

### Other pentacyclic triterpenoids

There are several other PTs with pharmacological potential in the treatment of retinal injury (► **Table 1**). The intravitreal injection of asiatic acid [(1*S*,2*R*,4*aS*,6*aR*,6*aS*,6*bR*,8*aR*,9*R*,10*R*,11*R*,12*aR*,14*bS*)-10,11-dihydroxy-9-(hydroxymethyl)-1,2,6*a*,6*b*,9,12*a*-hexamethyl-2,3,4,5,6,6*a*,7,8,8*a*,10,11,12,13,14*b*-tetradecahydro-1*H*-picene-4*a*-carboxylic acid] in rats with elevated intraocular pressure and chronic ocular hypertension improved RGC survival and prevented retinal dysfunction, such as retinal thinning. Asiatic acid prevented retinal apoptosis in chronic ocular hypertension by modulating the ratio of Bcl-2 and Bax [99].

Madecassic acid [(1*S*,2*R*,4*aS*,6*aR*,6*aS*,6*bR*,8*R*,8*aR*,9*R*,10*R*,11-*R*,12*aR*,14*bS*)-8,10,11-trihydroxy-9-(hydroxymethyl)-1,2,6*a*,6*b*,9,12*a*-hexamethyl-2,3,4,5,6,6*a*,7,8,8*a*,10,11,12,13,14*b*-tetradecahydro-1*H*-picene-4*a*-carboxylic acid] protected HRMECs from hypoxia-induced apoptosis by preventing the decline in the Bax:Bcl-2 ratio and attenuating caspase-3 and caspase-9 cleavage. Madecassic acid also decreased ROS production and lipid peroxidation, and modulated endoplasmic reticulum stress in hypoxic HRMECs [100].

Corosolic acid [(1*S*,2*R*,4*aS*,6*aR*,6*aS*,6*bR*,8*aR*,10*R*,11*R*,12*aR*,14*bS*)-10,11-dihydroxy-1,2,6*a*,6*b*,9,9,12*a*-heptamethyl-2,3,4,5,6,6*a*,7,8,8*a*,10,11,12,13,14*b*-tetradecahydro-1*H*-picene-4*a*-carboxylic acid] elicited antiangiogenic effects in a chorioallantoic membrane assay, characterised by a decrease in the vascular area and density and the number of gap junctions. Although, intravitreal administration of corosolic acid in Wistar rats was safe and well-tolerated pharmacological activity was low [101]. As with certain other PTs, the clinical use of corosolic acid may be enhanced by improved formulation.

In addition to the PTs mentioned, the activity of crude plant extracts that contain triterpenes has been assessed *in vivo*. Such extracts have been tested in rat models of DR and vasculopathy and have been found to exhibit antioxidant and antiproliferative activities. Because ROS-mediated cell death contributes to decreased retinal viability in ocular disease, PT analogues have significant therapeutic potential.

Taken together, naturally occurring PTs are potentially valuable in retinal disease. However, the pharmacokinetic profiles of PTs are suboptimal in the clinical setting. It would now be useful to improve the pharmacokinetic properties of these agents. This might be achieved using novel delivery modalities, such as nanoformulation.

## Chemically modified pentacyclic triterpenoid derivatives

As mentioned, naturally occurring PTs like OA exhibit favourable pharmacological activity in a number of retinal pathologies. Chemical modifications have been adopted in initial medicinal chemistry strategies to attempt to overcome their pharmacokinetic drawbacks and mitigate adverse effects. Accordingly, a series of semisynthetic PT derivatives based on OA have been developed for this purpose; their structures are shown in ► **Fig. 2**.

### 2-Cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid and dh404

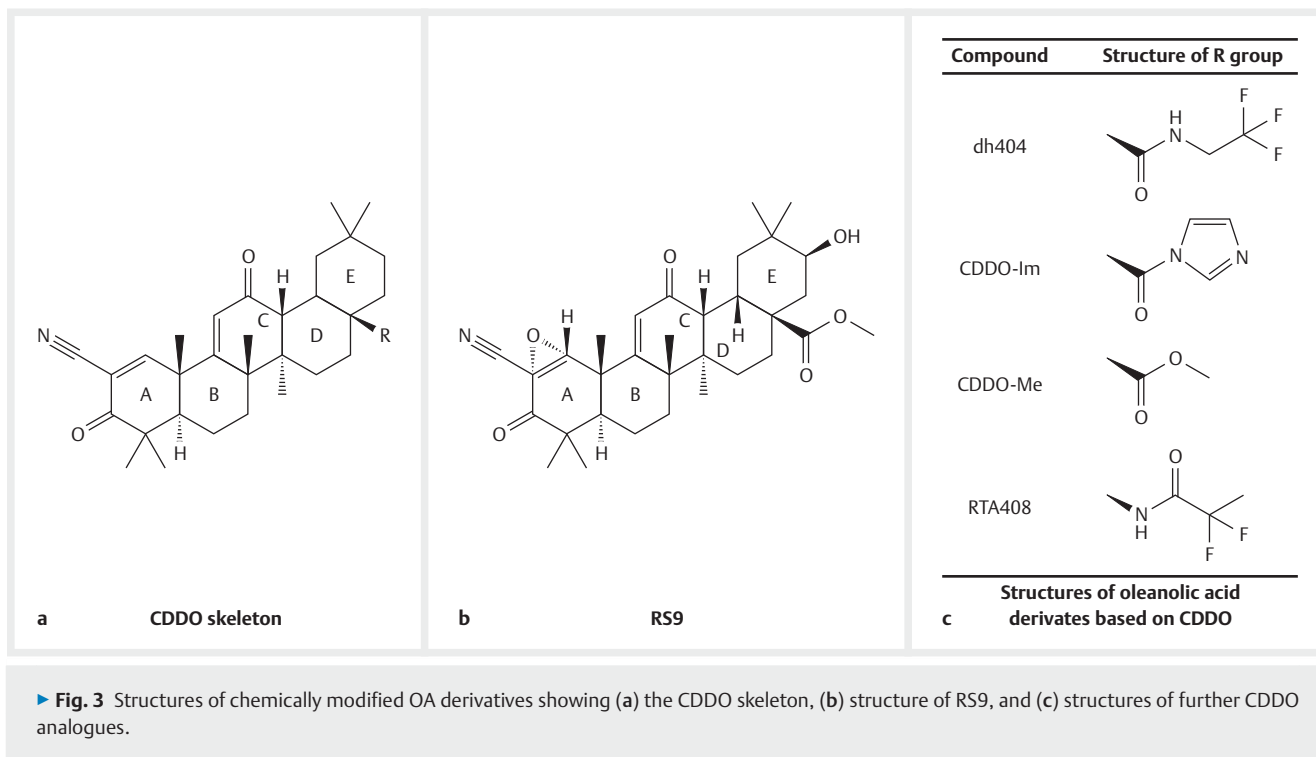
CDDO [2-cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid, also known as bardoxolone or RTA 401; (4*aS*,6*aR*,6*bS*,8*aR*,12*aS*,14*aR*,14*bS*)-11-cyano-2,2,6*a*,6*b*,9,9,12*a*-heptamethyl-10,14-dioxo-1,3,4,5,6,7,8,8*a*,14*a*,14*b*-decahydropicene-4*a*-carboxylic acid), where R=COOH in ► **Fig. 3a**] is a potent semisynthetic derivative of OA. Further structural modifications have been introduced to produce a series of CDDO analogues, including dh404 [R=CONHCH<sub>2</sub>CF<sub>3</sub> and where the unsaturated bond in the C-ring of CDDO is reduced; (4*aS*,6*aR*,6*bR*,8*aR*,12*aR*,14*aR*)-11-cyano-2,2,6*a*,6*b*,9,9,12*a*-heptamethyl-10,14-dioxo-*N*-(2,2,2-trifluoroethyl)-1,3,4,5,6,6*a*,6*b*,7,8,8*a*,9,10,12*a*,12*b*,13,14,14*a*,14*b*-octadecahydropicene-4*a*(2*H*)-carboxamide (► **Fig. 3c**)], CDDO methyl ester (CDDO-Me), RTA408, and CDDO-imidazolidine (CDDO-Im) (► **Fig. 3c**). A phase I clinical study of CDDO pharmacokinetics in patients with solid tumours reported that blood concentrations of at least 1  $\mu$ M (the effective preclinical concentration) can be attained using a continuous intravenous infusion of a dose of 38.4 mg/m<sup>2</sup>/h dose over a 5-day period every 28 days [102].

The CDDO analogues are potent activators of Nrf2, which is the master regulator of the Nrf2-inducible gene battery of antioxidant genes in response to oxidative stress [103]. Under normal conditions, Nrf2 is present in the cytoplasm bound to Keap1 and Cullin 3 [104–106]. In a normoxic environment, this complex may be rapidly degraded by ubiquitination [104–106]. However, in oxidative stress, Nrf2 is translocated to the nucleus and activates the transcription of genes that enhance the antioxidant response [104–106], including NQO1, GCLC, GCLM, sulfiredoxin 1, thioredoxin reductase 1, HO-1, and glutathione S-transferases [105–110]. Nrf2 and its downstream genes are a major component of the antioxidant defence against the pathogenesis of retinal injuries like AMD, DR, choroidal neovascularisation, I/R injury, posterior uveitis, and glaucoma [111–117]. Indeed, Nrf2 knockout animals exhibit increased retinal degeneration and retinopathy [111–117]. The capacity of CDDO analogues to activate the Nrf2-inducible gene battery affords protection to the retina.

Deliyanti et al. [118] reported the antioxidant and anti-inflammatory activities of dh404. In the OIR mouse model, dh404 activated the major Nrf2-responsive genes NQO1, glutathione synthase, HO-1, and GCLM. dh404 also alleviated inflammation by decreasing TNF- $\alpha$ , CCL2, and ICAM-1 expression and also prevented vascular leakage by restoring VEGF *in vitro* and *in vivo* (► **Table 2**).

Similar findings were made in a rat model of diabetes [119]. Thus, dh404 activated the Nrf2-responsive genes HO-1 and NQO1 in the retina, attenuated gliosis in Müller cells by decreasing glial fibrillary acidic protein, and suppressed the proinflammatory





tory TNF- $\alpha$ , IL-6, ICAM-1, and monocyte chemotactic protein 1. dh404 also prevented vascular leakage from the diabetic rat retina by inhibiting the increase in albumin and VEGF, and decreased angiopoietin 2. These actions of dh404 could be optimised by further structural modifications and utilising improved delivery approaches.

### 2-Cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid-Imidazolide

As mentioned, the Nrf2-inducible gene battery is important in maintaining the survival of ocular tissues after exposure to pro-oxidant stresses. Like dh404, CDDO-Im [(2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide; (4*aR*,6*aR*,6*aS*,6*bR*,8*aS*,12*aS*,14*bS*)-8*a*-(imidazole-1-carbonyl)-4,4,6*a*,6*b*,11,11,14*b*-heptamethyl-3,13-dioxo-4*a*,5,6,6*a*,7,8,9,10,12,12*a*-decahydropicene-2-carbonitrile] attenuated ROS production in murine photoreceptor 661 W cells and minimised I/R injury in mice by upregulating the major Nrf2-inducible genes NQO1, GCLC, GCLM, and HO-1 [120]. Similar findings were reported by Himori et al.; CDDO-Im protected mouse eyes *in vivo* and RGC cells *in vitro* against oxidative stress [121]. Two further CDDO derivatives – CDDO-trifluoroethyl-amide (CDDO-TFEA) and CDDO-ethyl-amide (CDDO-EA) – potentially activated NQO1 activity in 661 W cells in a concentration-dependent fashion within the nanomolar range [122]. CDDO-TFEA decreased light-induced retinal damage by preventing thinning of the ONL and increasing retinal NQO1 and GCLC expression in BALB/c mice [122].

### 2-Cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid-Methyl ester

CDDO-Me [also known as RTA402, bardoxolone methyl, or CDDO-methyl ester, methyl (4*aS*,6*aR*,6*bS*,8*aR*,12*aS*,14*aR*,14*bS*)-11-cyano-

no-2,2,6*a*,6*b*,9,9,12*a*-heptamethyl-10,14-dioxo-1,3,4,5,6,7,8,8*a*,14*a*,14*b*-decahydropicene-4*a*-carboxylate] activates Nrf2 and inhibits NF- $\kappa$ B. CDDO-Me has been evaluated in ~ 30 clinical trials for potential application in a range of pathological conditions, including renal diseases, diabetes, and pulmonary hypertension (<https://clinicaltrials.gov>). In a phase I clinical trial of CDDO-Me in cancer patients, Hong et al. reported that the maximum tolerated dose is 900 mg/d when orally administered once daily for 21 days over a 28-day cycle [123]. The  $C_{max}$  was  $25 \pm 13$  ng/mL and the  $C_{min}$  was  $8.8 \pm 4.3$  ng/mL. CDDO-Me also has low bioavailability, but this was improved by using an amorphous spray dried dispersion dosage form [124]. This highlights the potential pharmacokinetic advantages offered by novel delivery approaches.

CDDO-Me preserved the integrity of the blood-brain barrier, protected endothelial cells, and upregulated tight junction proteins [125]. CDDO-Me is highly potent in its protective actions against oxidative stress. Thus, CDDO-Me protected the mouse retina against I/R injury by abrogating superoxide levels and inhibiting retinal vascular degeneration, as well as by activating Nrf2 target genes such as NQO1, GCLM, GCLC, and HO-1 [126].

### RTA408

RTA408 [also known as omaveloxolone, *N*-[(4*aS*,6*aR*,6*bS*,8*aR*,12*aS*,14*aR*,14*bS*)-11-cyano-2,2,6*a*,6*b*,9,9,12*a*-heptamethyl-10,14-dioxo-1,3,4,5,6,7,8,8*a*,14*a*,14*b*-decahydropicene-4*a*-yl]-2,2-difluoropropanamide] is the only OA analogue to date that has been evaluated in clinical trials of corneal endothelial cell loss, ocular pain, and ocular inflammation following cataract surgery (NCT02128113, NCT02065375). RTA408 is protective in human foetal RPE cells at low concentrations ( $\leq 100$  nM) and inhibits H<sub>2</sub>O<sub>2</sub>-induced apoptosis and necrosis by modulating the Bcl-2: Bax ratio and inhibiting H<sub>2</sub>O<sub>2</sub>-induced protein glutathionylation

► **Table 2** Pharmacological actions of chemically modified pentacyclic triterpenoids and mechanisms of improved retinal function.

PT	In vitro model	In vivo model	Disease model	Effects	Pharmacological effects	Molecular mechanism	Reference
dh404	Primary astrocytes and rat primary Müller cells	Mice	OIR	Antioxidative, anti-vascularisation	Phase II OIR: ↓ Müller cell damage, ↓ vascular leakage, ↓ leukostasis, ↓ Iba1 <sup>+</sup> -positive microglia	Phase I OIR: ↑ retinal VEGF In astrocytes: ↓ ROS, ↓ TNF- $\alpha$ , ↓ iNOS Phase II OIR: ↓ VEGF, ↓ erythropoietin, ↓ p-Erk, ↓ ROS, ↓ TNF- $\alpha$	[118]
	Rat primary Müller cells	Rats	Diabetes	Antioxidative		Retina ↓ ROS, ↓ VEGF, ↓ angiopoietin-2, ↓ TNF- $\alpha$ , ↓ IL-6 Müller cells: ↓ GFAP, ↓ VEGF, ↓ IL-6, ↓ TNF- $\alpha$	[119]
CDDO-lm	RGCs	Mice	Axonal damage	Antioxidative		Mice: ↑ antioxidant defence	[121]
	ARPE-19, 661 W cells		t-BHP oxidative stress in ARPE-19 and 661 W cells	Antioxidative, cytoprotective	ARPE-19 and 661 W: ↑ cell survival	ARPE-19: ↑ antioxidant defence	[122]
	661 W cells	Mice	Ischaemia-reperfusion (I/R)	Neuroprotective	Mice: ↓ neuronal cell loss	↑ Antioxidant defence	[120]
CDDO-TFEA	ARPE-19, 661 W cells	Mice	t-BHP oxidative stress in cells, light induced retinal damage in mice	Antioxidative, cytoprotective	661 W: ↑ cell survival, Mice: ↑ ONL thickness	ARPE-19: ↑ antioxidant defence	[122]
	ARPE-19 cells		t-BHP oxidative stress	Antioxidative, cytoprotective		ARPE-19: ↑ antioxidant defence	[122]
RTA408	Human RPE cells	Wild-type mice	Ischaemia-reperfusion	Neuroprotective	↓ Superoxide, ↓ retinal vascular injury	↑ Antioxidant defence	[126]
			H <sub>2</sub> O <sub>2</sub> -induced cytotoxicity	Cytoprotective	↓ Apoptosis and necrosis, ↑ survival	↑ Bcl-2, ↑ Nrf2; ↓ Bax, ↓ ROS	[127]
RS9		Rhodopsin Pro347Leu rabbits	Retinitis pigmentosa	Inhibit ONL thinning	↓ ONL thinning, cell loss	↑ Antioxidant defence, ↓ IL-6	[134]
	ARPE-19 cells	Rats, rabbits	t-BHP in ARPE-19, BRB hyperpermeability in rabbits	Antioxidant, prevent neovascularisation, and BRB permeability	Rats: ↓ neovascularisation Rabbits: ↓ BRB hyperpermeability	↑ Antioxidant defence	[130]
	HRMEC cells	Mice, monkey	Microvascular endothelial barrier dysfunction, choroidal neovascularisation	Suppress ocular angiogenesis and hypermeability	HRMECs: ↓ migration, restore endothelial barrier Monkey: ↓ vascular leakage	HRMECs: ↑ Nrf2, ↑ PDGFR- $\beta$ , ↓ VEGF	[133]
	ARPE-19 cells	Zebrafish	Non-exudative model (ARPE-19), light-induced retinal degeneration (zebrafish)	Cytoprotective	ARPE-19: ↑ survival Zebrafish: ↓ ONL thinning, ↑ LC3-positive autophagosome	ARPE-19: ↑ antioxidant defence, ↑ LC3-III/LC3-I, ↑ SQSTM1 zebrafish: ↑ LC3-II, ↑ SQSTM1	[132]
	661 W cells	Mice	Light irradiation	Cytoprotective	661 W: ↑ survival Mice: ↓ retinal degeneration ↓ ONL thinning	↑ Antioxidant defence	[131]

continued

▶ <b>Table 2</b> Continued							
PT	In vitro model	In vivo model	Disease model	Effects	Pharmacological effects	Molecular mechanism	Reference
Betulinic acid derivative H7	RPE cells		Hypoxia-induced oxidative stress	Antioxidative	↑ Cell survival, ↓ apoptosis and necrosis	↓ ROS, ↑ Akt, Erk1/2, JNK	[135]
Betulinic acid derivatives H5, H7	Müller cells		Excitotoxicity-induced oxidative stress	Antioxidative	↑ Cell survival, ↓ necrosis	↓ ROS, ↑ Akt, Erk1/2, JNK	[136]

\* Signalling pathways and molecular mediators that are modulated by PTs and that improve retinal function. CDDO: 2-cyano-3,12-dioxo-oleana-1,9-dien-28-oic acid; Nrf2: nuclear factor erythroid-2 related factor 2; OIR: oxygen-induced retinopathy; ONL: outer nuclear layer; PTs: pentacyclic triterpenoids; RGC: rat ganglion cell; RPE: retinal pigment epithelium; ROS: reactive oxygen species; TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; VEGF: vascular endothelial growth factor

[127]. As an Nrf2 activator, RTA408 promotes cell survival by increasing the expression of Nrf2, HO-1, NQO1, superoxide dismutase-2, catalase, glutaredoxin-1, and thioredoxin-1 [127]. Clinical studies on patients with Friedreich' ataxia [128] and solid tumours [129] have shown similar pharmacokinetic profiles (AUC,  $C_{max}$ ,  $t_{1/2}$ ) at low oral doses (20 mg/d); the pharmacokinetics were linear over the dose range 2.5 mg/d and 300 mg/d [128].

## RS9

Nakagami et al. [130] used CDDO as a lead compound to prepare a series of derivatives using microbial transformation. One of the products – termed RS9 (methyl (1aR,3aR,5aS,5bR,7aR,9S,11aS,11bR,13bS,13cR)-1a-cyano-9-hydroxy-3,3,5a,5b,10,10,13b-heptamethyl-2,12-dioxo-1a,3,3a,4,5,5a,5b,6,7,8,9,10,11,11a,11b,12,13b,13c-octadecahydroniceno[1,2-b]oxirene-7a(2H)-carboxylate) – carries an epoxide moiety in the A-ring, an esterified carboxylate substituent at the D/E-ring junction, and a hydroxyl group in the E-ring. RS9 was more potent than CDDO-Me in inhibiting t-BHP-induced RPE cell death, mediated via Nrf2 activation and leading to increased expression of NQO1, HO-1, and GCLM. This further supports the potential value of medicinal chemistry in improving the efficacy of PT analogues. Multiple doses of RS9 [130] increased NQO1 and HO-1 expression in the retina of neonatal rats. Other studies corroborated these findings in murine photoreceptor and ARPE-19 cells [131, 132]. Indeed, when formulated with PLA-0020, RS9 protected the retina from light-induced ONL thinning in zebrafish and NaIO<sub>3</sub>-mediated oxidative damage in ARPE-19 cells [132]. RS9 also suppressed neovascularisation in the OIR rat model and inhibited blood-brain barrier hyperpermeability produced in rabbits by administration of glycated albumin. In contrast, CDDO-Me was relatively ineffective. The potency of RS9 was corroborated in another study [133]. Thus, RS9 increased the expression of HO-1 and NQO1 mRNAs at a low dose (1 and 3 mg/kg), whereas CDDO-Me only activated HO-1 expression at much higher doses (10 mg/kg). Again, RS9 improved the endothelial cell barrier *in vitro* and *in vivo* assays.

RS9 was also reported to be effective in certain genetic diseases of the retina. For example, Nakagami [134] showed that RS9 significantly inhibited ONL in rhodopsin Pro347Leu transgenic rabbits by activating the Nrf2-targeted genes NQO1 and HO-1 (▶ **Table 2**). This finding suggested that activation of the Nrf2-Keap1 signaling pathway could delay the pathogenesis of RP that is due to rhodopsin gene mutations. Considered together, RS9 is an example of the value of medicinal chemistry and drug delivery strategies to maximise efficacy and pharmacokinetics without increasing toxicity. Extension of these strategies is now warranted to produce superior OA analogues.

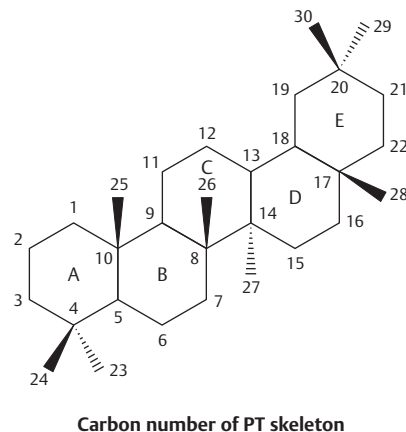
Apart from the OA analogues, very few other PTs have been subjected to structural modifications by medicinal chemistry or chemical biology approaches. Betulinic acid is structurally similar to OA but has a substituted cyclopentane E-ring in place of the cyclohexane system. Several betulinic acid derivatives with greater aqueous solubility showed improved cytoprotective activity and safety in RPE and Müller cells. Antioxidant activities were mediated by attenuating the activation of Akt, Erk1/2, and JNK pathways [135, 136].

## Structure-activity relationships of pentacyclic triterpenoids

PTs share similar structures, and investigations into their SAR could provide clues for drug design and enhancement of pharmaceutical actions. However, to our knowledge, there have been no SAR studies to date that have been based specifically on the retina. Rather, the focus of most SAR studies has been on anti-inflammatory and anticancer aspects of the molecules and their interactions with certain enzymes. It has been suggested that the activities of PTs are dependent on multiple structural and physicochemical factors, including hydrogen bond formation, hydrophobic character, and steric and electronic properties of chemical substituents. Further structural modifications to explore the pharmacological potential of PTs would now be justified [137].

Based on reports regarding the role of PTs in other diseases, it is feasible that hydrogen bonding capacity may play a role in their pharmacological actions. A notable feature of anti-inflammatory activity appears to be the presence of a hydrogen bond donor (such as a free hydroxyl group) at C3 and a hydrogen bond acceptor and/or dipolar contact at C16 (► Fig. 4) [138]. OA, UA and their derivatives show anti-inflammatory effects by inhibiting COX-2 via a hydrogen bond [139, 140]. Hydroxyl or substituted hydroxyl groups (containing, for example, acyl or amido substituents) at C2, C3, C15, C16, or C22 [141–145] and carboxyl or substituted carboxyl groups at C17 and C24 (such as alkyl, aryl, ethers, esters, amides, or nitrogen-containing heterocyclic moieties) have been reported to produce PTs with enhanced activity [142, 143]. Hydrogen bond-forming groups at C3 or C28 of UA analogues are important for their cytotoxicity as well as their inhibitory effects on NF- $\kappa$ B and mitochondrial transmembrane potential [146, 147]. At these two positions, their toxicity can be increased by the replacement of amino groups, while their inhibitory effect can be abolished by the change of electron-withdrawing groups such as  $\text{COC}_6\text{H}_5\text{Cl}$  or  $-\text{Cl}$  [146, 147]. Furthermore, heterocyclic groups such as indole [143, 148], thiazolidinedione [149], L-tyrosine [149], piperazine [140], 4-phenyl-1H-1,2,4-triazol-5(4H)-one [140], oxadiazole [140], and triazol [140] could enhance the biological activities of OA and UA as the nitrogen in the heterocycle can serve as a hydrogen bond acceptor or donor [150]. In addition, glycosylations at C3, C21, and C22 are also critical, since this can increase hydrogen bonding. Sugar chains are preferred at the C21, C22, and C3 positions, as the hydroxyl of sugar can serve as a hydrogen bond donor and acceptor [144]. The distance between the PT skeleton and aglycon also influences the activity. A shorter distance between the PT moiety and the sugar group leads to enhanced cellular effects [151], while the long sugar chain at C28 could result in reduced potency [144, 145]. C28 has also emerged as a potentially important position in these molecules that could be exploited in drug design because SAR analyses on betulinic acid analogues have indicated that bulky and electron-donating substituents promote activity [152, 153].

In addition, PTs with the structure of  $\alpha$ ,  $\beta$ -unsaturated carbonyl moieties are Michael acceptors, which are prone to react with nucleophile bioactive molecules. This accounts for the promising pharmaceutical activities [154–156]. For example, CDDO was reported to interact with sulfhydryl of cysteine residues in Keap1



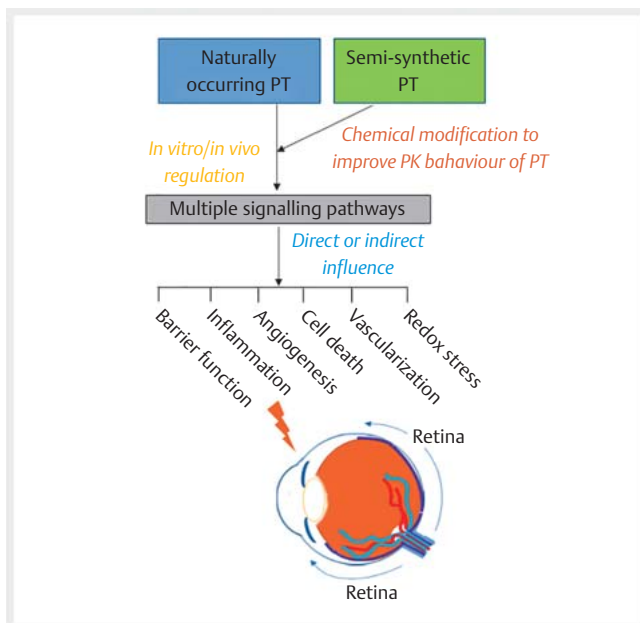
► Fig. 4 Carbon numbering in the pentacyclic triterpenoid skeleton; A–E indicates ring designations.

[157] and form covalent interaction with Cys151 [158], which is essential to detect increased oxidative stress [158].

## Conclusions

In summary, the PTs and their derivatives have considerable potential in the treatment of retinal pathologies because they have cytoprotective, antioxidant, neuroprotective, and anti-inflammatory actions (► Tables 1 and 2 and Fig. 5). Important considerations in future studies to assess PTs include:

1. That retinal cell damage *in vitro* or retinal degeneration *in vivo* may be caused by a range of injurious stimuli, including light, chemicals, ischaemia, and physical injury. Retinal damage and disease development are complex processes that involve multiple contributory mechanisms and that can be exacerbated by coincident diseases, such as diabetes and hypertension. Diagnostic markers should be monitored to guide the appropriate therapeutic use of PTs. For instance, inflammatory and metabolic indicators may be useful endpoints to monitor retinal degeneration and responses to PTs.
2. Information is increasing that retinal damage and retinal diseases are related to altered cellular signalling pathways. PTs differentially modulate such pathways, e.g., OA derivatives activate the Nrf2 gene battery, which promotes the antioxidant defence in cells, and GL inhibits HMGB1, which may attenuate inflammatory mechanisms. Cytoprotection afforded by PTs could enhance insight into the pathogenesis of retinal diseases and improved targeting of specific pathways that may produce new therapies.
3. Most PTs have been found to modulate several signalling mechanisms so that side effects during treatment are likely. By adapting advances in drug delivery, including targeted drug carriers, such unwanted actions of PTs may be minimised. Additionally, chemical modification of PTs should now be undertaken to improve the properties of PTs for use in retinal treatments. For example, CDDO analogues are more potent and exhibit greater retinal protection than the parent com-



► **Fig. 5** The graphical summary of the pharmacological actions of pentacyclic triterpenoids in retinal diseases.

compound OA. However, precautions are necessary because relatively minor chemical modifications can significantly alter the specific activities of analogues. For example, GL is an HMGB1 inhibitor with retina protective actions, while the structurally similar CBX is a 11 $\beta$ -HSD1 inhibitor and may elicit retinal toxicity.

4. The reported synergism between PTs and glucocorticoids suggests that combination treatments should now be evaluated in a systematic fashion. This may also enable effective PTs to be used at lower doses when used in combination with other agents.
5. Chemical modification of PTs should be rationally pursued with the aim of improving the aqueous solubility and the pharmacokinetic properties of active agents.

### Contributors' Statement

Drafted the manuscript: Z. Cheng, Y. Li, X. Zhu, Y. Ali. Critical revision of the manuscript: K. Wang, W. Shu, T. Zhang, L. Zhu, M. Murray, F. Zhou.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

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