REVIEW

Therapeutic potential of agents targeting cannabinoid type 2 receptors in organ fibrosis

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Abstract

The endocannabinoid system has garnered attention as a potential therapeutic target in a range of pathological disorders. Cannabinoid receptors type 2 (CB2) are a class of G protein-coupled receptors responsible for transmitting intracellular signals triggered by both endogenous and exogenous cannabinoids, including those derived from plants (phytocannabinoids) or manufactured synthetically (synthetic cannabinoids). Recent recognition of the role of CB2 receptors in fibrosis has fueled interest in therapeutic targeting of CB2 receptors in fibrosis. Fibrosis is characterized by the alteration of the typical cellular composition within the tissue parenchyma, resulting from exposure to diverse etiological factors. The pivotal function of CB2 agonists has been widely recognized in the regulation of inflammation, fibrogenesis, and various other biological pathologies. The modulation of CB2 receptors, whether by enhancing their expression or activating their function, has the potential to provide benefits in numerous conditions, particularly by avoiding any associated adverse effects on the central nervous system. The sufficient activation of CB2 receptors resulted in the complete suppression of gene expression related to transforming growth factor β1 and its subsequent fibrogenic response. Multiple reports have also indicated the diverse functions that CB2 agonists possess in mitigating chronic inflammation and subsequent fibrosis development in various types of tissues. While currently in the preclinical stage, the advancement of CB2 compounds has garnered significant attention within the realm of drug discovery. This review presents a comprehensive synthesis of various independent experimental studies elucidating the pivotal role of identified natural and synthetic CB2 agonists in the pathophysiology of organ fibrosis, specifically in the cardiac, hepatic, and renal systems.

1 | INTRODUCTION

Organ fibrosis is correlated with perturbed cellular homeostasis and aberrant architectural organization of the organ. This phenomenon occurs as a reaction to an etiological agent, typically a chronic inflammatory response that continuously causes damage to the endothelium or epithelium tissue of the organ. Consequently, the original functional tissue is gradually replaced by connective tissue. During the process of replacement, as observed in numerous instances, the regeneration of the endothelium tissue tends to be inadequate owing to the varying regenerative and proliferative capacities exhibited by different organs. Therefore, this process results in a vigorous accumulation of extracellular matrix (ECM) proteins by the activated mesenchymal cells. As a consequence, this leads to the alteration of the impacted organ, resulting in the development of scar tissue, increased thickness, and rigidity, ultimately leading to compromised functionality.[1] This pathological anomaly has the potential to impact various organs, encompassing the integumentary system, cardiovascular system, respiratory system, hepatic system, and renal system. While wound healing serves as a vital adaptive process necessary for addressing cellular damage and restoring injured tissue, an inadequate response fails to effectively restore tissue homeostasis, leading to the development of fibrosis.[2] Inflammation serves as the initial stage in the process of cellular repair and clearance. Nevertheless, chronic inflammation plays a pivotal role in the initiation of fibrosis by facilitating the secretion of fibrogenic cytokines and instigating an abnormal buildup of collagen fibers.[3] Hence, anti-inflammatory drugs are extensively employed as a cornerstone in the therapeutic approach toward fibrosis, owing to the compelling body of evidence elucidating the intricate connection between inflammation and this pathological fibrosis.[4]

The endocannabinoid system (ECS) is comprised of endocannabinoids, which are signaling molecules, and their corresponding cannabinoid receptors. Additionally, enzymes play a crucial role in regulating the overall turnover of these compounds within the ECS. 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (anandamide) are the two main endogenous endocannabinoids that are the most well studies. The production of those ligands is stimulated upon request in response to an external injury. They are fatty in nature and synthesized from phospholipids. There are two enzymes responsible for their degradation. Fatty acid amide hydrolase hydrolyzes AEA and monoglyceride lipase degrades 2-AG.[5] The neuromodulatory effect of the ECS has sparked significant interest as a potential therapeutic target in various pathological diseases. The effect exerted by endogenous endocannabinoids is mediated through their interaction with cannabinoid receptors type 1 (CB1) and cannabinoid receptors type 2 (CB2) receptors, which are G-protein-coupled receptors. The CB1 receptors exhibit a higher degree of prevalence within the anatomical components of the central nervous system (CNS), including the basal ganglia and hippocampus. These receptors are found in the axon terminal of cholecystokinin and glutamatergic neurons. In comparison, the expression of CB2 receptors within the CNS is significantly lower when compared to the expression of CB1 receptors.[6,7]

The CB2 receptors are predominantly located on microglia cells and cerebral vasculature. It has been observed that their expression is said to be stimulated in response to nerve injury.[8] Studies have additionally elucidated a prevailing expression of CB2 receptors in various anatomical locations, including cardiomyocytes,[9] the gastrointestinal tract,[10] the liver,[11] bone,[12] and the reproductive system[13,14] with high expression in immune cells including leukocytes, lymphocytes and other cell populations.[15] The indication of therapeutic potential in various pathological conditions has been observed through the effects mediated by the CB2 receptor in several models. In response to the inflammatory trigger, there has been a notable upregulation of CB2 receptors, while the introduction of CB2 agonists has resulted in a reduction in the secretion of pro-inflammatory mediators from fibroblast cells within the inflamed dermal tissue.[16]

Due to their heightened expression on various immune cells such as macrophages, natural killer cells, T and B cells, eosinophils, and leukocytes, CB2 receptors exhibit a distinct affinity toward these cellular components[17]; a significant anti-inflammatory effect has been attributed to them.[18] The direct effect of CB2 receptors on fibroblast cells is illustrated in Figure 1. CB2 receptor activation has been observed to confer anti-inflammatory properties by inhibiting the progression of the inflammatory cascade and the secretion of inflammatory cytokines, including interleukin-2 (IL-2), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin-8, and interleukin-6 (IL-6).[19] This effect can be mediated by either synthetic or natural cannabinoids. CB2 receptors are predominantly expressed in response to an inflammatory stimulus owing to their extensive presence on circulating immune cells and cells derived from macrophages. Simultaneously, it has been reported that the activation of CB2 receptors exhibits a deficiency in the undesirable psychoactive effects that impose restrictions on their medical application.

These effects are primarily mediated by CB1 receptors. The restricted expression of CB2 receptors in the brain that is limited to microglia and brain endothelial cells supports the fact that CB2 receptors mediate no psychotropic effects which are largely mediated through CB1 receptors.[20–22] Therefore, the advancement of therapies based on cannabinoids that eliminate the undesired effects would be appropriate by specifically targeting the CB2 receptors.[23] Due to the intricate interplay between inflammation and fibrosis, the advantageous characteristic of cannabinoids in this context has been extrapolated and harnessed as an antifibrotic approach in fibrosis of various organs.[24] The cannabis plant and its derivatives have
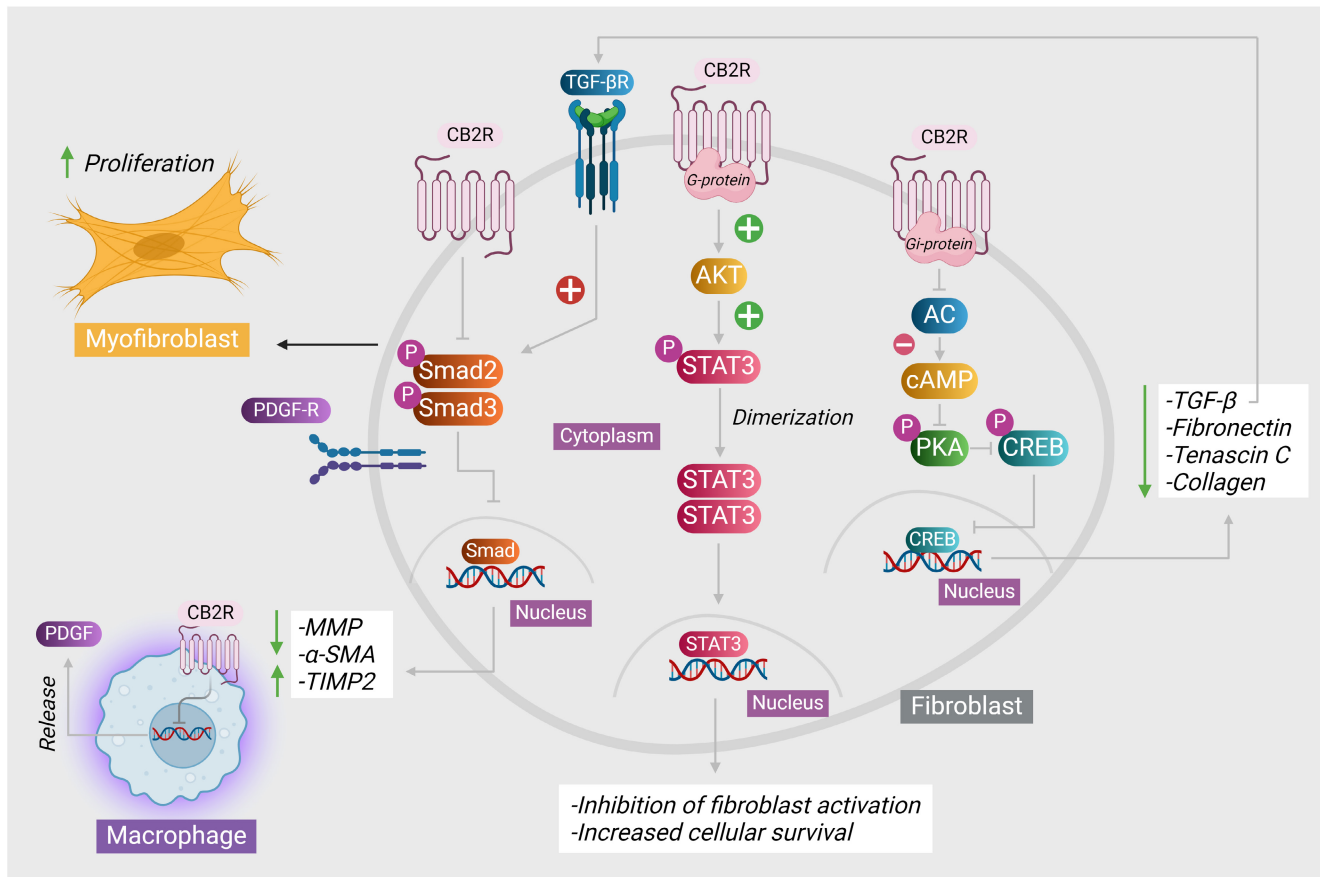
FIGURE 1 The protective effects of CB2 receptors on fibroblasts. Upon the activation of CB2 receptors following the binding of CB2 agonists, several intracellular pathways can be initiated. As CB2 receptor is a GPCR bound to G_i subunit this leads to the inhibition of AC that in turn leads to a decrease in the intracellular concentration of cyclic adenosine monophosphate (cAMP). Decreased cAMP inhibited the activation and phosphorylation of PKA which in turn inhibits the phosphorylation and nuclear translocation of CREB transcription factor which ultimately inhibits the gene expression of fibrogenic proteins including tumor growth factor-beta (TGF-β), fibronectin, collagen, and tenascin C. The decreased production of TGF-β results in decreased activation of TGF-βR that positively acts on transducing Smad2 and Smad3 signaling and producing TIMP2 and α-SMA, and decreased matrix metalloproteinase release. CB2 receptor also directly inhibit the action of Smad2 and Smad3 transcription factors. Besides, CB2 receptor activation increases activation of AKT, which in turn phosphorylates STAT3 transcription factor. Upon phosphorylation, STAT3 induces transcription of protective genes enhancing cellular survival. A side illustration of CB2 receptor expression on macrophages and the ultimate effect of inhibited PDGF production following CB2 receptor activation. AC, adenylyl cyclase; AKT, protein kinase B; cAMP, adenosine 3′;5′-cyclic monophosphate; CREB, cAMP response element-binding protein; PDGF, platelet-derived growth factor; PKA, protein kinase A; Smad, suppressor of mothers against decapentaplegic; STAT3, signal transducer and activator of transcription 3.

demonstrated notable involvement in the management of various afflictions, including pain, epilepsy, and other neurodegenerative disorders[25] as well as fibrosis.[26] This review comprehensively aims to elucidate the role of CB2 receptor agonists, encompassing both phytocannabinoids and synthetic ligands, in mitigating the advancement of fibrosis across diverse models of fibrosis in various organs.

2 | CB2 AGONISTS IN CARDIAC FIBROSIS

Myocardial fibrosis refers to a form of cardiac remodeling characterized by the pathological alteration of the heart, involving cellular and molecular abnormalities, functional decline, and phenotypic modifications. The pathogenesis of cardiac fibrosis encompasses the

occurrence of necrotic and apoptotic cellular demise, heightened inflammatory response, and oxidative insult caused by prolonged stimulation of receptors on cardiomyocytes or pressure-induced strain on the heart tissue due to uncontrolled blood pressure.[27] The ECS has been observed to exert regulatory influence on various physiological processes within the cardiovascular system. In this context, novel methodologies grounded in the ECS are currently under scrutiny for their potential therapeutic applications in the realm of cardiovascular ailments. Studies have shown the potential role of CB2 receptor activation in protecting heart from various injuries including inflammatory and oxidative ones taking advantage of expressed CB2 receptors in cardiomyocytes, endothelial cells, and smooth muscle cells, alongside immune cells within the cardiac region.[28]

Numerous investigations have elucidated that the stimulation of CB2 receptors induces a range of outcomes, including the suppression of inflammatory responses and the inhibition of fibrotic processes. In a model of myocardial fibrosis, the activation of CB2 receptors by AM1241 demonstrated a mitigating effect on the fibrosis that was induced after myocardial infarction. The i.p. injection of AM1241 in mice was observed to stimulate the activation of the CB2 receptor in the cardiac region. This activation led to the subsequent initiation of the Akt/nuclear factor erythroid 2-related factor 2 (Nrf2)/HO-1 signaling pathway, specifically in the cardiomyocytes of the heart. It was demonstrated to expedite the translocation of Nrf2 to the cellular nucleus, where it functioned to enhance the transcription of molecules involved in antioxidant defense. The potential mechanism underlying the prevention of fibrosis by inhibiting the activation of TGF-β1/Smad3 pathway and subsequent accumulation of collagen is believed to rely on the dependence of Akt/Nrf2/HO-1-dependent signaling cascade.[29] The protective mechanism of CB2 receptors is demonstrated in Figure 2. The efficacy of AM1241 in mitigating the induced fibrosis following myocardial infarction induced by left anterior descending ligation was further validated. Masson's trichrome staining revealed a notable decrease in the fibrotic region within the group that received the CB2 agonist AM1241, in comparison to the group that received phosphate buffer saline. This phenomenon was observed in parallel with a reduction in the synthesis and secretion of fibrotic cytokines TGF-β1 and platelet-derived growth factor (PDGF) by mesenchymal stem cells derived from adipose tissues.[30]

Additionally, the administration of AM1241 has exhibited a mitigating effect on atrial fibrosis through the restoration of TGF-β, matrix metalloproteinase (MMP), collagen I, and collagen III expression levels to a state that is relatively comparable to normal levels subsequent to the infusion of angiotensin II in mice.[31] The obtained consistent results provided additional confirmation regarding the involvement of AM1241 in the observed phenomenon. The administration of AM1241 to mice with myocardial infarction has shown a notable improvement in reducing fibrosis development in an experimental model of myocardial infarction. The administration of AM1241 has additionally facilitated cellular proliferation subsequent to myocardial infarction by augmenting the population of progenitor cells within the cardiac tissue. The findings unveiled an increased expression of the fluorescence-stained c-kit+ and Runx1+ proteins, both of which serve as markers for progenitor cells, in MI mice treated with AM1241.[32] Table 1 summarizes the cardioprotective mechanisms of various CB2 agonists in different fibrosis models.

Similarly, it has been demonstrated that oral administration of the lipid-based formulation (VCE-004) of EHP-101, a synthetic cannabidiol aminoquinone, has antifibrotic effects on the heart. The observed phenomenon demonstrated the inhibition of interstitial and perivascular collagen deposition in the heart interstitium, induced by angiotensin-II. Additionally, it prevented the transformation of fibroblasts into profibrotic-activated myofibroblasts. Furthermore, the population of T cells (CD3+ cells) and macrophages (F4/80+ cells) exhibited a notable reduction in the left ventricle of the heart upon administration of EHP-101, mirroring the effects observed in the positive control, losartan. In order to investigate the potential underlying mechanism behind this antifibrotic effect, a transcriptomic analysis was conducted to examine the genes that were either upregulated or downregulated in response to EHP-101. The analysis has unveiled a reduction in the transcriptional activity of genes associated with epithelial–mesenchymal transition, as well as E2F targets that govern the activity of transcription factors and influence the modulation of fibrosis. A subset of the E2F target genes encompasses cyclin-dependent kinase 1 (Cdk1), DNA topoisomerase II alpha (Top2a), and marker of proliferation Ki67 (Mki67). The aforementioned study has examined the antifibrotic function of AJULEMIC ACID, a synthetic CB2 receptor ligand,[38] and showed efficacy in decreasing the extent of induced fibrosis and accumulated collagen in the heart, with a lower potency compared to EHP-101.[33]

In further substantiation of the antifibrotic impact of CB2 agonism, it has been documented that both JWH-133 and AM1241 exhibited protective properties against quetiapine-induced cardiotoxicity and mitigated the initiation of cardiac fibrosis. Following administration of JWH-133 and AM1241, the progression of inflammatory cells and cytokines infiltrating the myocardium cells was observed to be impeded. Additionally, the buildup of fibrotic fibers on the myocardium cells was found to be suppressed. The observed effects were counteracted upon pretreatment with AM630, a specific antagonist of the CB2 receptor. Furthermore, it was observed that this pretreatment even intensified the toxic effect induced by quetiapine.[35]

Furthermore, it has been demonstrated that the cannabinoid naturally synthesized within the body exhibits advantageous impacts on cardiac fibrosis, which arises as a complication of long-term diabetes induction. The fibrotic region, which exhibited positive staining, displayed a notable reduction in streptozotocin-induced diabetic mice that were administered 2-arachidonoylglycerol. The mice treated with 2-arachidonoylglycerol exhibited consistently lower collagen content in comparison to the mice with untreated diabetes. The administration of 2-arachidonoylglycerol resulted in a suppression of protein expression of TGF-β1, p-Smad2/3, and Smad2/3, which play crucial roles as regulators in the process of fibrosis.[36]

Moreover, the vulnerability of mice lacking CB2 receptors to develop cardiac fibrosis was assessed in an ischemia/reperfusion model. The echocardiography examination revealed a notable degree of cardiac remodeling in the heart, characterized by an augmented expansion in the dimensions of the left ventricle and a heightened average of the cardiac myocyte-stained sections. Mice lacking the CB2 gene (CB2$^{-/-}$) exhibited an increased susceptibility to the formation of cardiac fibrosis, characterized by a higher occurrence of fiber disarray and accumulation of collagenous fibrils in the myocardium, in comparison to mice with the normal CB2 gene (wild-type mice). The molecular analysis revealed an increase in the stimulated messenger RNA (mRNA) expression of fibrogenic markers such as TGF-β1, collagen 1, and collagen 3. In contrast, the mRNA levels of these fibrogenic markers in the wild-type mice that underwent ischemia/reperfusion surgery were comparable to those of mice that
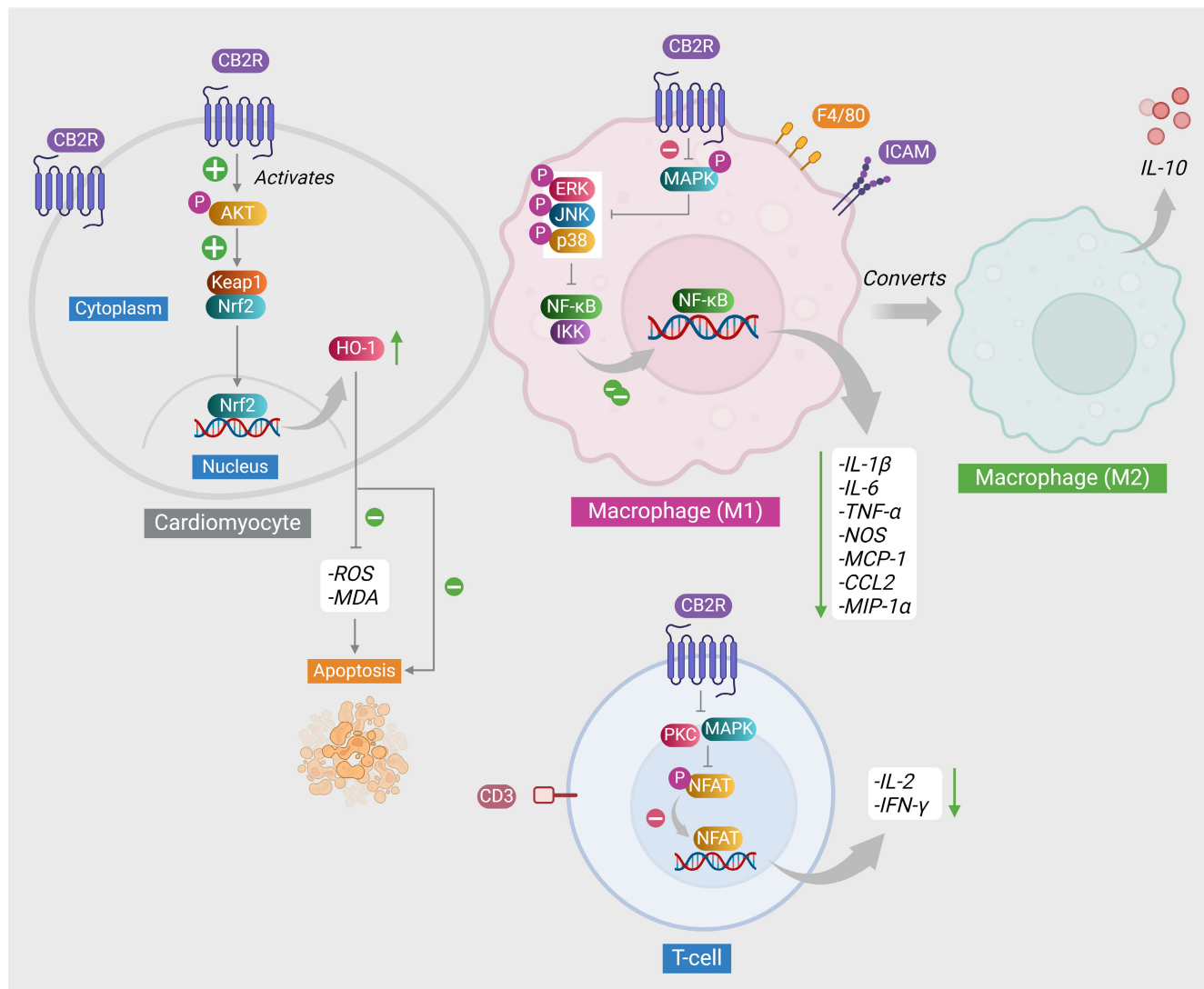
FIGURE 2 CB2 receptors' mechanisms of action in cardiomyocytes and inflammatory cells. As illustrated, the activation of CB2 receptors in cardiomyocytes phosphorylates AKT which degrades Keap1 leading to the dissociation of Nrf2 and its translocation to the nucleus where it increases the transcription of HO-1 gene. This decreases the generated ROS, and peroxidized lipids and inhibits apoptosis. In the macrophage, CB2 activation suppresses the activation of mitogen-activated protein kinases (MAPK) and its downstream mediators. Consequently, NF-κB is inhibited and there will be no dissociation from its inhibitor complex with IKK, and the release of inflammatory mediators is inhibited. With an unknown mechanism activated CB2 receptor causes the polarization of M1 macrophages into the anti-inflammatory phenotype M2 macrophages which contribute to the anti-inflammatory response through the release of anti-inflammatory cytokine IL-10. In T cells, activated CB2 receptor deactivates MAPK pathway, inhibiting PKC. As a result, NFAT phosphorylation, activation, and nuclear translocation are inhibited, resulting in a reduced release of IL-2 and INF-γ. AKT, protein kinase B; CCL2, chemokine (C-C motif) ligand 2; ERK, extracellular signal-regulated kinase; HO-1, heme oxygenase 1; IKK, inhibitor of nuclear factor-κB (IκB) kinase; IL-10, interleukin-10; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-2, interleukin-2; INF-γ, interferon gamma; JNK, c-Jun N-terminal kinase; keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MIP-1α, macrophage inflammatory protein-1 alpha; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; NOS, nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha.

underwent sham surgery. Furthermore, the investigation revealed that fibroblasts obtained from CB2$^{-/-}$ mice hearts exhibited heightened vulnerability to oxidative stress and the initiation of apoptosis upon exposure to hydrogen peroxide. This was accompanied by a significant increase in the release of TNF-α and a positive detection of alpha smooth muscle actin (α-SMA). The introduction of JWH133 into cellular systems has exhibited a safeguarding effect against the

initiation of apoptosis, mitigated irregular cardiac remodeling, and enhanced the secretion and activity of MMP-2 in cells of the wild type, while no discernible impact was observed in the knocked-out cells. This observation underscores the selectivity of JWH133 toward CB2 receptors.[34]

Moreover, the model utilizing CB2$^{-/-}$ was subsequently employed to elucidate the impact of CB2 receptor deficiency on the

TABLE 1 The cardioprotective role of different cannabinoids.

Compound	Dose, duration, and route of administration	Mechanisms of protection	Reference
AM1241	20mg/kg, i.p. for 7days to C57BL/6 male mice	↑Akt/Nrf2/HO-1	[29]
AM1241	20mg/kg, i.p. for 28days to mice	↓ROS, MDA, ↑p-Akt ↑p-Erk, ↑p-Stat3, ↓TGF-β1, PDGF	[30]
AM1241	20mg/kg, i.p. injected for 7days to C57BL/6 mice	↓MDA, TNF-α, IL-6, ↑c-kit⁺, Runx1⁺ ↑Nrf2, HO-1	[32]
AM1241	20mg/kg, i.p. for 21days to C57BL/6 mice	↓TGF-β, collagen I, collagen III, MMP	[31]
EHP101	5, 10, and 20mg/kg, p.o. for 2weeks to C57-BL/6 mice	↓NFAT, ↓ERK1+2, ↓IL1β, ↓IL6, ↓Col1A2, ↓CCL2, ↓CD3⁺, F4/80⁺ cells, Cdk1, Top2a, Ki67	[33]
JWH133	3mg/kg, i.p., single injection to CB2⁻/⁻ mice 0.5μM to fibroblasts	↓TNF-α, ↓α-SMA ↑MMP-2	[34]
AM1241, JWH133	AM 1241 (5μM) and JWH-133 (1μM) 30min prior to quetiapine-to-myocardial HL-1 cells	↓p-MLKL, ↓Inflammation ↓fibrosis	[35]
2-Arachidonoylglycerol	10μg/kg for 4weeks to C57BL/6 mice	↓TGF-β1 ↓p-Smad2/3	[36]
HU910	10mg/kg, i.p. for 14days to C57BL/6J mice	↓CD45⁺ cells, ↓ MIP1α, IL1α, and TLR4, ↓E-selectin, VCAM-1, ICAM-1, ↓COX1,2, ↓3-NT, and MDA	[37]

enhancement of fibrosis. The ventricles of the CB2⁻/⁻ group exhibited a greater accumulation of fibrotic tissue in comparison to the wild-type group. This was accompanied by a pronounced activation of B-cells subsequent to the induction of infarction.[39] Similarly, the hearts of mice lacking CB2 receptors exhibited maladaptation to pressure overload. Following transverse aortic constriction (TAC) surgery, there was an observable presence of confluent and well-defined patchy regions exhibiting heightened cell density, suggesting the substitution of healthy cardiomyocytes with fibrotic tissue. Mice lacking the CB2 receptor (Cnr2⁻/⁻) exhibited collagenous regions that were more densely stained, displaying a non-compacted pattern. These mice also showed reduced production of the arachidonic acid, endocannabinoid 2-AG, and anandamide, in comparison to their Cnr2⁺/⁺ counterparts. The absence of the CB2 receptor resulted in a compromised antioxidant defense mechanism in the cardiac tissue, accompanied by reduced levels of Hmox1 protein expression. This reduction serves as an indicator of impaired differentiation of M2 macrophages, indicating a disrupted polarization of macrophages.

Simultaneously, an intriguing observation was made regarding the upregulation of pro-inflammatory cytokines, which enhanced the inflammatory response in mice lacking the Cnr2 gene (Cnr2⁻/⁻) following TAC surgery, in comparison to mice with intact Cnr2 gene (Cnr2⁺/⁺ mice).[40] In accordance with the established pattern, the absence of CB2 receptors in myocardial ischemia and reperfusion (I/R) model resulted in a significant increase in replacement fibrosis, which served as a compensatory mechanism for the loss of cardiomyocytes. Notably, the microinfarcted regions in Cnr2⁻/⁻ mice exhibited augmented collagen deposition. Furthermore, it is noteworthy to mention that Cnr2⁻/⁻ mice exhibited only a partial regression of fibrosis 60days post-I/R injury, in contrast to the complete regression observed in mice of the wild type. The justification for this observation stems from the prevalence of irreversible collagen isoforms and the absence of the lysyl oxidase enzyme, which is responsible

for facilitating the cross-linking of collagen fibers in Cnr2⁻/⁻ mice.[41] This exemplifies the intricate and interconnected relationship between endocannabinoids and CB2 receptors, which play a crucial role in mediating cardioprotection and preserving the integrity of cardiomyocytes. The implicated mechanisms involve the expression of CB2 receptors and the subsequent anti-inflammatory effect when CB2 receptors are activated in the heart. The data provided highlights the crucial significance of the endocannabinoid–CB2 receptor axis in the cardiac response to diverse stimuli.

3 | CB2 AGONISTS IN LIVER FIBROSIS

Chronic liver diseases, which can be triggered by excessive alcohol consumption, viral hepatitis, or non-alcoholic fatty liver disease (NAFLD), ultimately lead to the development of liver fibrosis and cirrhosis. These conditions pose a significant risk to patients, as they can result in severe complications such as heightened portal pressure, encephalopathy, and in the most severe instances, hepatocellular carcinoma. The ECS has been discovered to play a significant role in regulating the development and progression of liver diseases, as well as providing protective effects. The upregulation of CB2 receptors has been observed in Kupffer cells and hepatic myofibroblasts as a protective response to liver injury. This upregulation is believed to have an anti-inflammatory and antifibrogenic effect, which helps to mitigate the progression of fibrosis.[42]

Previous studies have reported on the involvement of endocannabinoids in the pathogenesis of liver fibrosis. The study conducted by Siegmund and Schwabe in 2008 demonstrated that a lack of CB2 receptors was associated with a higher likelihood of experiencing enhanced fibrogenesis and increased proliferation of myofibroblasts. The activation of CB2 receptors has been documented to exhibit inhibitory properties on the proliferation of hepatic myofibroblasts.

TABLE 2 The hepatoprotective role of different cannabinoids.

Compound	Dose, duration, and route of administration	Mechanisms of protection	Reference
Δ9-Tetrahydrocannabinolic Acid	20 or 40 mg/kg, i.p. for 2 weeks to C57BL/6 mice	↓α-SMA, tenascin C, ↓ALT, ↓Col1a2, Col3a1, Acta2, TGFβ, ↓Il1b, and Il6 ↓CD3, F4/80$^+$ cells	[45]
JWH133	3 mg/kg, i.p. for 3 weeks to wild-type and CB2$^{-/-}$ C57BL/6 mice 5 μM to hepatic stellate cell	↓ IL-6, IL-1β, TNF-α, and iNOS, ↓ p-P38, NF-κB/p65 ↓ Collagen I	[47]
JWH133	10 mg/kg, i.p. for 3 days to C57BL/6 mice	↓F4/80$^+$ cells, ↓PKA, ↓Ubiquitin-specific peptidase 4	[48]
JWH133	1 mg/kg, s.c. for 9 days to Wistar rats	↓α-SMA, Collagen I ↑MMP-2, ↑caspase-3 ↓CD68$^+$ cells	[24]
HU910	10 mg/kg, i.p. for 14 days to C57BL/6J mice	↓CD45$^+$ cells, ↓IL1β, IL6, MIP1α, TNFα ↓E-selectin, VCAM-1, ICAM-1, ↓ALT and ALP	[37]
Cannabigerol	2.46, 24.6 mg/kg, i.p. 3 times a week for 2 weeks to C57BL/6 mice	↓α-SMA, collagen ↓CD45$^+$ cells	[46]
β-Caryophyllene	5 mg/kg, p.o. for 2 weeks to Wistar rats	↓collagen ↑CB2 expression	[49]
AM1241	3 and 6 mg/kg, i.p. for 3 weeks to Wistar rats	↓NF-κβ p65, ↑GSH, SOD ↓MDA, ↓TNF-α, IL-1β and IL-6 ↓Vimentin, ↑E-cadherin	[50]
AM1241	3 mg/kg, i.p. to C57BL/6J mice	↓PDGF, ↓Col-III	[51]
AM1241	1 mg/kg, i.p. for 5 weeks to rats	↓PDGF, ↓α-SMA, collagen, ↑MMP ↓TIMP	[52]
JWH-015	1 μmol/L to hepatic myofibroblasts and stellate cells	↓forskolin-stimulated cAMP, ↓PDGF ↑COX-2	[44]

Furthermore, it has been observed to promote apoptosis, inducing a cytotoxic effect on both hepatic myofibroblasts and hepatic stellate cells, thereby reducing their viability.[43] The activation of CB2 receptors has been documented to exhibit inhibitory properties on the proliferation of hepatic myofibroblasts. Furthermore, it has been observed to promote apoptosis, inducing a cytotoxic effect on both hepatic myofibroblasts and hepatic stellate cells, thereby reducing their viability.[44] The signaling pathway associated with CB2 has been recognized as an antifibrogenic mechanism, as evidenced by multiple scientific investigations. δ9-Tetrahydrocannabinolic acid (Δ9-THCA), which serves as the precursor to Δ9-tetrahydrocannabinol (Δ9-THC), the primary and most prevalent compound found in Cannabis sativa, has been the subject of scientific inquiry due to its potential antifibrogenic and anti-inflammatory properties. The administration of Δ9-THCA has effectively impeded the initiation of hepatic fibrosis caused by CCl4, as evidenced by a notable reduction in the fibrotic region, decreased levels of α-SMA, and diminished expression of tenascin C. The treatment with Δ9-THCA resulted in a significant reduction in the gene expression of fibrotic markers Col1a2, Col3a1, and TGFβ. Furthermore, a reduction in the infiltration of T lymphocytes and macrophages in the liver resulted in a decrease in the induced hepatic inflammation. Comparable phenomena were documented in a 23-week experimental setup involving the induction of liver fibrosis through a high-fat diet.[45]

In a comparable pattern, the administration of cannabigerol has exhibited a safeguarding effect against the initiation of hepatic

fibrosis and inflammation. This has been substantiated by observing a reduction in the presence of α-SMA (alpha-smooth muscle actin) in liver tissue sections, as well as by impeding the infiltration of immune cells, specifically leukocytes and macrophages, into the liver.[46] However, the utilization of the CB2 agonist HU910 has demonstrated a significant reduction in liver fibrosis. This reduction is achieved by mitigating the infiltration of leukocytes and the secretion of proinflammatory cytokines, as well as vascular inflammation markers. Additionally, HU910 restores the hepatic microcirculation, transforming it into a state of intact and healthy vasculature in mice with bile duct ligation.[37] Mechanisms implicated in antifibrotic effect of CB2 agonists are collectively illustrated in Table 2.

In order to gain more understanding of the functionality of CB2 receptors, cellular entities were subjected to transfection with CB2 small interfering RNA (siRNA), resulting in the suppression of CB2 receptor activity and subsequent reduction in receptor expression levels when compared to the group transfected with negative control siRNA. The observed downregulation of CB2 receptors was found to be correlated with an augmented secretion of pro-inflammatory cytokines from Kupffer cells upon stimulation by lipopolysaccharide (LPS). Furthermore, the process of phosphorylation of p38 MAPK and the subsequent nuclear translocation of NF-κB/p65 exhibited heightened levels in CB2 knocked-out cells as compared to the control cells. Using an alternative methodology, the identical investigation assessed the impact of CB2 receptor activation on the activation of Kupffer cells induced by LPS. The administration of

JWH133 effectively suppressed the release of IL-6, IL-1β, TNF-α, and inducible nitric oxide synthase from activated Kupffer cells in response to LPS stimulation. The expression levels of p-P38 MAPK and activated NF-κB/p65 were observed to be significantly reduced in cells transfected with negative control siRNA, but not in cells with CB2 knockout, following treatment with JWH133. The antifibrotic effects were further examined in mice with CCl4-induced liver fibrosis, encompassing both CB2$^{-/-}$ and wild-type specimens. JWH133 has exhibited a mitigating effect on the artificially induced pathological fibrotic lesion, as evidenced by a reduction in the presence of stained α-SMA and collagen I in the wild-type mice. However, this effect was not observed in the CB2$^{-/-}$ CCl4-challenged mice.[47]

Moreover, the administration of JWH133 resulted in a notable decrease in the accumulation of collagen fibers around the veins and portal areas, accompanied by a reduction in the distortion of the liver's structure. This was further supported by the preservation of the liver's functional tissue, as confirmed through Sirius red staining.[24] The observed deletion of CB2 receptors was additionally demonstrated to intensify hepatic injury and fibrosis upon exposure to CCl4. In the fibrotic model induced in mice lacking the CB2 receptor gene (CB2$^{-/-}$), a notable augmentation in cellular necrosis and infiltration of inflammatory cells was observed. Additionally, collagen fibers, which are found in various regions of the liver tissue, were observed to be more widely distributed in the CB2$^{-/-}$ model group compared to the control group. This increased distribution of collagen fibers was accompanied by an upregulation of α-SMA and TGF-β1 expression. Simultaneously, the observed protein expression levels of A20 and phosphorylated NF-κB p65, along with the mRNA levels of IL-6 and TNF-α, exhibited significant upregulation in the CB2$^{-/-}$ model mice in comparison to the wild-type mice that were exposed to CCl4.[53]

Furthermore, additional investigations have demonstrated that the progression of fibrosis was augmented in mice lacking the CB2 receptor. In an experimental setup involving bile duct ligation, the fibrogenic response of both CB2-deficient and wild-type mice was assessed. In individuals lacking CB2, there was an increased presence of type 1 T helper cells (Th1) or Th2 markers, as well as Th17 markers such as Rora, Rorct, and Il23r, along with heightened levels of IL-17 in the liver, as compared to their counterparts with a normal CB2 gene. The observed increase in the immune response was correlated with an augmented fibrotic response, as evidenced by elevated levels of collagen I and α-SMA mRNA expression. Interestingly, wild-type mice demonstrated a remarkable expression of the CB2 receptors, as evidenced by the augmented mRNA expression of Cnr2, the enzymes Dagla and Daglb responsible for synthesizing 2-AG, and a diminished expression of the enzyme Mgll responsible for degrading 2-AG. Conversely, the mice lacking the CB2 receptor gene showed no discernible impact on the expression of these components. Furthermore, the administration of IL-17 has resulted in a notable augmentation of collagen I, asma, and Tgfb mRNA expression in a population of CB2-deficient mice. In order to gain a deeper understanding of the impact of CB2 receptor activation, Th17 lymphocytes undergoing differentiation in a controlled

culture were subjected to treatment with JWH-133. The findings indicated a notable decrease in the quantity of IL-17+ cells and the production of IL-17, accompanied by a reduction in the intensity of stained phospho-STAT5. The treatment of macrophages with JWH-133 resulted in the inhibition of the activation of the pro-inflammatory M1 phenotype and the downregulation of the expression of Il1b, Il6, and chemokine ligand 2 genes.[54] Mice lacking the CB2 gene (CB2$^{-/-}$) have also exhibited an increased vulnerability to hepatic steatosis and fibrogenesis following prolonged exposure to ethanol. A notable augmentation in inflammation, activation of hepatic stellate cells, and accumulation of collagen were observed in comparison to both CB1$^{-/-}$ and wild-type mice.[55]

The presence of endogenous cannabinoids has also been observed, with a notable increase in the concentration of anandamide, up to 10 to 20 times higher, in fibrotic mice when compared to control mice. The increased abundance of anandamide functions as a defensive mechanism, providing protection against artificially induced fibrosis.[56] In rats subjected to bile duct ligation, there was a notable downregulation in the gene expression of CB2 receptors. This downregulation was found to be linked with an increased occurrence of hepatic fibrosis. However, the administration of β-caryophyllene, a naturally occurring cannabinoid agonist, has been observed to reduce the accumulation of collagen in the central vein and portal tract of liver of the rats.[49] Furthermore, the ameliorative effects of curcumin on hepatic fibrosis were observed to be facilitated through the modulation of CB2 receptor expression and the subsequent restoration of gene expression to normal levels after exposure to CCL4.[57] The antifibrotic protective effects of a curcumin derivative known as C66 were observed in a study. These effects were found to be associated with the upregulation of CB2 receptors, as evidenced by both mRNA and protein analysis.[58]

Subsequent investigations delved into the examination of the impact of CB2 receptor activation on the reversal of fibrosis initiation or progression. The administration of AM1241 exhibited a protective effect against the release of inflammatory cytokines induced by thioacetamide. The levels of TNF-α, IL-1β, and IL-6 were constrained by the presence of AM1241, which additionally exhibited an antioxidant impact by augmenting the activity of superoxide dismutase (SOD) and the concentration of reduced glutathione (GSH). The findings additionally showcased the antifibrotic impact of AM1241, which effectively reinstated the expression of E-cadherin while concurrently reducing the expression of vimentin in hepatocytes. AM1241 exhibited a downregulatory effect on the genetic expression of fibrotic markers, specifically toll-like receptor 4, TGF-β1, α-SMA, and microRNA-155.

Additionally, the administration of AM1241 has demonstrated improvements in the histopathological changes observed in rats treated with thioacetamide. The rats that received AM1241 treatment exhibited limited necrotic changes, portal inflammation, fibroplasia, cytoplasmic vacuolation, and pseudolobulation of hepatic parenchyma. The histological examination revealed that the rats treated with AM1241 exhibited mild focal degeneration and portal inflammation, which were notably less severe compared to the rats

treated with thioacetamide. The latter group displayed an almost normal histological appearance. The fibrotic extent was assessed using the Ishak scoring system. The group treated with AM1241 exhibited the lowest fibrosis score, suggesting a minimal presence of fibroplasia and indicating a restricted proliferation of fibrotic tissue.[50] AM1241 exerts its influence on hepatic fibrosis by modulating the expression of PDGF and impeding its activity, thereby leading to a decline in the synthesis of ECM.[51] Similar results were reported previously, confirming the antifibrotic role of AM1241 in chronically CCL$_4$-treated rats.[52] In genetic analysis, we examined the connection between allelic variants (Q63R and PNPLA3 variant) in the CB2 receptor gene. Our findings unveiled a significant correlation between the genetic variation and the severity of NAFLD in a group of children who were diagnosed with NAFLD through liver biopsy.[59] The data demonstrated the activation of CB2 receptors and their effects on reducing inflammation and fibrosis, thereby mitigating the impact of fibrogenic stimuli.

4 | CB2 AGONISTS IN LUNG FIBROSIS

The respiratory passages exhibit a susceptibility to inflammatory responses triggered by various environmental irritants and stimulants. The primary objective in managing respiratory disease is to mitigate the inflammatory response and its potential for becoming chronic, which could potentially heighten the susceptibility to developing pulmonary fibrosis. Given the correlation observed between pathological states and the ECS, numerous therapeutic strategies rely on the manipulation of constituents of this system, particularly by exerting influence on its receptor entities.[60]

The immunomodulatory impact of cannabinoids is ascribed to their interaction with immune cells and the subsequent release of cytokines from these inflammatory cells. Human eosinophils have been found to express a discernible quantity of CB2 receptors, suggesting a notable level of responsivity of these cells to cannabinoid agonists. CB2 receptors are additionally involved in regulating the functioning of dendritic cells through the inhibition of their movement, which is accompanied by a significant decrease in the production of MMP.[61]

Pulmonary fibrosis is classified as an interstitial lung disease characterized by the concurrent presence of inflammation and fibrosis within the pulmonary tissue. Given that CB2 receptors are predominantly found on immune cells within the lung, their stimulation leads to the initiation of programmed cell death known as apoptosis. This process effectively hinders the growth and reproduction of cells while also reducing the production and release of pro-inflammatory signaling molecules called cytokines. Consequently, the activation of CB2 receptors is recognized for its ability to exhibit anti-inflammatory, immunosuppressive, and anti-fibrotic effects.[62] In a model of allergic asthma induced by ovalbumin, the administration of cannabidiol has demonstrated the ability to counteract the heightened presence of collagen fibers within the alveolar septa. In this study, AM630 (a selective CB2 antagonist) was administered

in combination with cannabidiol. This concurrent administration conferred reversed reduction in collagen deposition in airways. Therefore, it can be considered that CBD antifibrotic effect is possibly attributed to CB2 receptor activation.[63]

In a comparable manner, the compound WIN 55212-2, which serves as an analog for endocannabinoids, has exhibited the ability to inhibit the manifestation of various fibrotic indicators. Additionally, it has shown the capacity to augment the expression and stimulation of M2 macrophages, thereby adopting a defensive strategy against inflammation. This defensive mechanism is further supported by an elevation in the release of anti-inflammatory cytokines. Interleukin-10 (IL-10) is a cytokine that plays a crucial role.[64] A recent investigation has successfully identified a newly discovered synthetic CB2 agonist, known as YX-2102, through the utilization of organocatalytic methodologies. This compound has subsequently been subjected to systematic analysis in order to evaluate its potential therapeutic applications. The administration of YX-2102 has demonstrated an improvement in the extent of lung fibrosis induced by bleomycin, resulting in reduced lung coefficients. The application of the Szapiel score revealed a reduction in fibrotic lesions and a decrease in the accumulation of ECM proteins following the administration of the treatment.[65]

The additional findings highlighted the observed protective properties exhibited by CB2 agonists. The administration of JWH 133 has demonstrated a mitigating effect on the pathological alterations that were observed in conjunction with the fibrotic condition induced by nicotine. After the administration of JWH 133, an observation was made of a typical structure characterized by a slender septum, the absence of excessive blood flow, and reduced collagen accumulation compared to the other experimental groups.[66] The investigation also encompassed the examination of the protective effect of JWH 133 in a model of lung fibrosis induced by bleomycin. The co-administration of JWH 133 and bleomycin resulted in a notable reduction in induced fibrosis. The computed tomography images revealed a notable decrease in pulmonary density within the upper, central, and lower regions of the lung in mice treated with JWH 133. Moreover, the administration of JWH 133 has exhibited regulatory effects on the progressive fibrotic mechanism, resulting in a reduction of intralobular opacity and fibrosis stranding that is typically induced by bleomycin.[67]

Additional compounds were examined within the context of a bleomycin-induced lung fibrosis model. In the study conducted by Parlar et al. it was observed that AM1241 exhibited a comparable impact on bleomycin-induced pulmonary fibrosis,[68] which was evidenced by a reduction in the accumulation of collagen fibers and the content of hydroxyproline, as indicated by staining techniques. Additionally, AM1241 demonstrated concurrent anti-inflammatory and antioxidant properties, further contributing to its therapeutic potential in combating pulmonary fibrosis. The protective mechanisms of AM1241 and other CB2 agonists are shown in Table 3.

The evaluation of the anti-fibrotic effect of EHP-101 was conducted in a study involving bleomycin-induced fibrosis. The researchers assessed the impact of EHP-101 on collagen deposition by

Compound	Dose, duration, and route of administration	Mechanisms of protection	Reference
Cannabidiol	5 or 10 mg/kg i.p. for 8 days to Balb/c mice	↓Collagen, ↓IL-4, IL-5, IL-13	[63]
WIN 55212–2	0.2–1 mg/kg, i.p. for 28 days to C57BL/6 mice	↓TGF-β, α-SMA, PDGFRa, fibronectin, and collagen I, ↓TNF-α and IL-6, ↑CD206, CD163 ↑IL-10 ↓CCR7⁺ cells (M1)	[64]
YX-2102	25 mg/kg, i.p. for 21 days to Sprague–Dawley rats	↓fibronectin and α-SMA, ↑E-cadherin ↓myeloperoxidase, CD68, ↓IL-6, MCP-1, IL-1β, ↑CD206	[65]
JWH 133	1 mg/kg, i.p. for 14 days to Swiss mice	↓Hyperemia ↓hemorrhage ↓leucocytes infiltration ↓hyperplasia ↓collagen	[66]
JWH133	2.5 mg/kg, i.p. for 21 days to C57BL/6 mice	↓Hydroxyproline ↓collagen I, ↓α-SMA, ↓TGF-β/Smad, ↓PDGF	[67]
AM1241	1, 3 mg/kg, i.p. for 28 days to Wistar rats.	↓IL-6 and TNF-α ↓MDA, ↑GSH	[68]
EHP-101	5, 10, and 25 mg/kg, p.o. for 4 weeks to BALB/c mice	↑vessels perimeter ↓myofibroblast ↓collagen	[69]
Pirfenidone	300 mg/kg, p.o. for 15 days to C57BL/6 mice	↓collagen, ↓TNF-α, IL-1β, and IL-6	[70]
Lenabasum	0.1, 0.3, 1, 3, 10, and 30 μM to monocyte-derived macrophages	↓IL-8, TNF-α, and IL-13	[71]
Ajulemic acid	1 mg or 5 mg/kg, p.o. for 21 days to DBA/2 mice	↓HO-proline, ↓TGF-β1, ↑PPAR-γ, ↓pSMAD2/3, ↓CTGF, ↓α-SMA	[72]

analyzing the Ascroft score and conducting picrosirius red staining. The results revealed a significant reduction in collagen deposition in mice treated with EHP-101 compared to those in the bleomycin-only group.[69] The antifibrotic effects of pirfenidone have been investigated in order to elucidate their reliance on the activation of CB2 receptors. The findings demonstrated a notable elevation in the protein abundance of CB2 receptors in mice that were co-administered with Pirfenidone, as compared to the group of mice treated solely with bleomycin. The lung sections obtained from mice subjected to pirfenidone treatment exhibited a notable reduction in collagen accumulation and diminished fibrosis progression upon staining with Masson's trichrome.[70]

Synthetic compounds are subjected to evaluation and subsequent reporting regarding their therapeutic potential in the context of targeting and mitigating lung fibrosis. Lenabasum, alternatively referred to as ajulemic acid, is a synthetic compound that acts as a selective CB2 agonist. It is an analog of THC and has demonstrated notable anti-inflammatory and antifibrotic properties. Treating macrophages derived from pediatric individuals with cardiac fibrosis resulted in a diminished polarization of macrophages, characterized by a decreased proportion of CD80⁺ M1 macrophages,

alongside an enhanced phagocytic activity.[71] Additionally, it has been observed that Ajulemic acid exhibits a protective effect against the development of lung fibrosis in mice induced by bleomycin. The utilization of ajulemic acid treatment in a preventative manner exhibited a mitigating effect on the inflammation that was induced in the sub-pleural regions. Furthermore, it provided protection against the extension of damage to the parenchymal tissue and impairment of the alveolar units. This was evidenced by a reduction in collagen deposition as observed in the histological analysis of the lung sections.[72]

5 | CB2 AGONISTS IN RENAL FIBROSIS

The maintenance of body homeostasis is achieved by the kidneys, which play a crucial role in the clearance of toxins, regulation of body volume, and preservation of hemodynamic balance. Hence, it is imperative to comprehend the fundamental pathways that undergo modifications leading to diverse manifestations of renal impairment and can be harnessed to develop efficacious therapeutic strategies for managing renal disorders. Cannabinoid receptors can be found

within the typical kidney, exhibiting a notably abundant presence of CB2 receptors in glomeruli, podocytes, and proximal tubule cells.

Studies conducted on animal models have demonstrated that the activation of CB2 receptors has a positive impact on conditions such as type 1 diabetes mellitus, renal fibrosis, and drug-induced tubular injury. The presence of endocannabinoids in the renal tissue suggests significant biological importance and serves as evidence for the direct impact of the ECS on the kidney. This impact is observed through the regulation of blood flow and the reabsorption of sodium in the kidney tubules.[73]

Given that kidney fibrosis is widely recognized as the primary consequence of chronic kidney diseases, we conducted a unilateral ureteral obstruction experiment to assess the nephroprotective effects of SMM-295 against the development of induced renal fibrosis. The administration of SMM-295 exhibited a protective effect against tubulointerstitial fibrosis, thereby resulting in a notable reduction in DNA damage by approximately 50% when compared to the mice treated with the vehicle.[74]

In a different investigation, the compound HU-910 was assessed for its potential protective properties in the context of renal fibrosis following bile duct ligation, which is an extraneous complication. The findings revealed a noteworthy reduction in kidney injury, characterized by diminished tubular dilatation, cellular necrosis, infiltration of leukocytes, and collagenous tissue. The administration of HU-910 also resulted in the restoration of microvascular flow, as well as the inhibition of vascular inflammation and endothelial activation.[75] Various compounds that activate the CB2 receptor have demonstrated the ability to induce an antifibrotic response in diverse models of experimentally induced renal fibrosis. The (+)-enantiomer of the naturally occurring (−)-cannabidiol, along with its derivative (+)-CBD hydroxypentylester ((+)-CBD-HPE), have been shown to exhibit pharmacological properties in reducing the severity of nephropathy, a diabetic complication, in mice induced with streptozotocin.

Functional assays demonstrated an enhanced CB2 receptor binding affinity of (+)-CBD-HPE with agonist function compared to the original cannabidiol. The administration of (+)-CBD-HPE resulted in a 1.95-fold decrease in the presence of interstitial fibrotic lesions. Various staining techniques revealed a notable improvement in fibrosis within the renal glomeruli and interstitium after administering (+)-CBD-HPE treatment to mice, as compared to mice treated with a control substance.[76]

In another approach, celastrol, a natural compound, demonstrated an antifibrotic effect through the upregulation of CB2 receptors and the reversal of the induced downregulation of CB2 receptors that is a result of the unilateral ureteral obstruction and therefore blunting the development of fibrosis. In vitro results also exhibited similar results with reversed effect on the TGF-β1-induced downregulation of CB2 receptors. Same study demonstrated that the administration of a CB2R antagonist SR144528 augmented the unilateral ureteral obstruction-induced fibrosis and prevented celastrol-mediated protective effect, indicating that the protective effect of celastrol is dependent on increasing the expression of CB2 receptor.[77] Additionally, treatment with AM1241 helped reducing the accumulation of collagen and the expression of TGF-β in the kidney tissue of high-fat diet-fed rats.[78] The anti-fibrotic effect of CB2 agonists in kidney is presented in Table 4.

6 | CB2 AGONISTS IN SKIN FIBROSIS

Fibrosis is a prevalent pathological dermal condition characterized by the disrupted metabolic processes of the connective tissue. The process of fibroblast activation and subsequent aberrant synthesis of ECM proteins is commonly referred to as cutaneous fibrosis or fibrosing connective tissue disorder. Skin fibrotic disorders are recognized to be facilitated by an immune response that triggers the generation

TABLE 4 The protective role of different cannabinoids in the kidneys.

Compound	Dose, duration, and route of administration	Mechanisms of protection	Reference
SMM-295	12 mg/kg, i.p. for 7 days to mice	↓α-SMA, ↓γ-H2AX ↓fibronectin	[74]
HU-910	10 mg/kg, i.p. for 14 days C57BL/6J male mice	↓KIM-1, OPN and NGAL, ↓Col1a1, Col3a1, Tgfβ mRNA ↓CD68 and CD11b ↓ICAM-1, SOD1, ↑Catalase, SOD2, ↓4-HNE, MDA, 3-NT	[75]
(+)-CBD-HPE	10 mg/kg, i.p. for 7 days to C57BL6J mice	↓CD3+, ↓ECM proteins	[76]
Celastrol	1 mg/kg, i.p. for 7 days to BALB/C mice	↓fibronectin, ↑CB2, collagen I, α-SMA ↓F4/80+, Ly-6G+, CD3+, ↓Smad3	[77]
AM1241	3 mg/kg, i.p. for 6 weeks to Sprague–Dawley rats	↓ collagen, ↓TGF-β ↓VEGF	[78]

of autoantibodies, resulting in damage to endothelial cells and an inflammatory process that leads to impaired functionality of dermal fibroblasts and an excessive accumulation of collagen, thereby contributing to the development of fibrosis. Endocannabinoid receptors are distributed within the suprabasal layers of the epidermis, as well as in hair follicles and other occasional regions of the basal layer, such as nerve fibers, hair follicles, and sebaceous glands, specifically within the keratinocytes.[79]

Various physiological functions of the ECS and the activation of CB2 receptors have been documented in various dermatological conditions. Cannabinoids demonstrate anti-inflammatory and immunosuppressive effects, which are the primary underlying mechanisms observed in the majority of skin pathologies.[80] Additionally, there have been reports indicating that the activation of CB2 receptors leads to a decrease in mast cell degranulation, which serves as an additional origin of profibrotic factors.[81] The antifibrotic effect of CB2 agonists is additionally ascribed to the downregulation of adhesion molecules, decreased activation of endothelial cells, and the induction of apoptosis in activated fibroblasts.[80,82]

Bleomycin has been observed to induce an augmentation in the vascular wall's thickness, which is a morphometric characteristic commonly observed in individuals with scleroderma. This condition is characterized by an excessive deposition of collagen surrounding the blood vessels. The observed histological alterations have been demonstrated to exhibit recovery upon administration of EHP-101. EHP-101 effectively restored the expression levels of activated myofibroblasts and tenascin in the treated mice, thereby bringing them back to a state comparable to that of the mice challenged with bleomycin. The characterization involved examining the impact of bleomycin induction and the ameliorating effects of EHP-101 on gene expression at a transcriptomic level. The expression of genes involved in inflammatory and fibrotic pathways, such as the epithelial–mesenchymal transition, was downregulated upon administration of EHP-101. Conversely, the same genes were upregulated in response to bleomycin treatment.[69]

The VCE-004.3 derivative, which is derived from cannabidiol, has been found to possess antifibrotic properties. It has the capacity to decrease the accumulation of collagen, suppress the release of inflammatory cytokines, and inhibit the degranulation of mast cells.[83] Its efficacy was further proven based on an in vitro and in vivo analysis. The in vitro findings demonstrated that VCE-004.8 exerted an inhibitory impact on the transcriptional process by targeting the collagen gene promoter. Consequently, this hindered collagen synthesis and impeded gene transcription in response to TGFβ1 stimulation. This is in addition to its efficacy in the murine model of experimentally induced skin scleroderma. VCE-004.8 exhibited a mitigating effect on the consequent fibrosis of the skin by reducing the thickness of the dermal layer, while also preserving the content of subcutaneous adipose tissue. Additionally, it hindered the triggered release of granules from mast cells.[84] Table 5 demonstrates the protective role of CB2 receptor activation in alleviating skin fibrosis.

Similarly, the effect of JWH-133 on the progression of dermal fibrosis in mice subjected to hypochlorous acid (HOCl) challenges was examined. The experimental findings demonstrated that the administration of JWH-133 to the mice resulted in a notable decrease in the thickness of the skin and the accumulation of collagen. JWH-133 exhibited protective effects against dermal fibrosis through the inhibition of fibroblast proliferation, as observed both in vitro and in vivo. The study revealed that mice lacking the CB2 gene (CB2$^{-/-}$) exhibit a higher susceptibility to developing augmented fibrosis compared to mice with intact CB2 genes (CB2$^{+/+}$). The histopathological analysis revealed an observed increase in dermal thickness accompanied by heightened skin fibrosis in CB2$^{-/-}$ individuals following exposure to HOCl.[85]

Recently, it has been demonstrated that the activation of CB2 receptors plays a role in the differentiation of human fibroblasts and the synthesis of collagen. The administration of JWH-133 to human fibroblasts stimulated by TGF-β resulted in a notable decrease in the synthesis of α-SMA and collagen production.[86,87] Gp1a, a highly selective CB2 agonist, has been discovered to exhibit efficacy in the reduction of inflammation and promotion of wound healing. This effect is observed specifically in incised skin wound healing, achieved by diminishing the infiltration of M1 macrophages.[92] Previous studies have reported that Gp1a has the potential to improve the simultaneous development of fibrogenesis that arises from skin wounds. The fibrotic markers, which are produced by cells that release fibrotic factors, consist of fibroblasts (positive for pro-collagen I), myofibroblasts (positive for pro-collagen I and α-smooth muscle actin), and fibrocytes (positive for CD45 and pro-collagen I). In the treated mice, these markers were observed at a reduced level compared to the mice treated with the vehicle. Additionally, the ratio of fibroblast-to-myofibroblast differentiation was low in the treated mice.[88]

In another study, the involvement of GP1a was further elucidated in a simulated scenario of cutaneous wound healing. The observations revealed a reduction in the amount of collagen deposited and thinner fibers when compared to mice treated with the control substance. In contrast, the vehicle-treated mice displayed a higher presence of polymorphonuclear cells and an accumulation of fibroblasts with a spindle-like shape in the injured wounded area.[89] Furthermore, the compound WIN55,212-2 exhibited a suppressive impact on the increase in dermal thickness caused by bleomycin, while maintaining the presence of subcutaneous adipose tissue in the perivascular spaces and reducing the infiltration of inflammatory cells in the subcutaneous layer. Additionally, it was observed that the administration of WIN55,212-2 resulted in a suppression of PDGF and connective tissue growth factor expression. Furthermore, there was a decrease in the phosphorylation of p-SMAD2/3 in WIN55,212-2-treated mice compared to mice challenged with bleomycin.[90]

In an in vitro study, the effectiveness of WIN55,212-2 was observed by its ability to hinder the process of profibrotic fibroblast transdifferentiation. Fibroblasts obtained from patients with diffuse cutaneous systemic sclerosis exhibited a significantly elevated

TABLE 5 The protective role of different cannabinoids in skin fibrosis.

Compound	Dose, duration, and route of administration	Mechanisms of protection	Reference
JWH-133	1,1.5, 2, 2.5, 3, 4 mg/kg, i.p. for 6 weeks to BALB/c Mice	↓collagen ↓dermal thickness	[85]
JWH-133	2.5 mg/kg, i.p. for 4 weeks to C57BL mice	↓dermal thickness	[86]
JWH-133	3 μM for 18 hours to human fibroblasts	↓collagen, ↓α-SMA	[87]
EHP-101	5, 10, and 25 mg/kg, p.o. for 3 weeks to BALB/c mice	↓collagen, ↓Tenascin, ↓VCAM, ↓Vimentin	[69]
VCE-004.3	20 mg/kg, i.p. for 6 weeks to BALB/C mice	↓collagen, ↓F4/80$^+$ ↓SMAD2/SMAD3 ↓ERK1/2, ↓CD3$^+$, ↓ macrophages	[83]
VCE-004.8	10, 20 mg/kg, i.p. for 3 weeks to BALB/c mice	↓Smad2/Smad3 ↓F4/80$^+$, ↓Col3A1, Col1A2, IL-1β and IL-13	[84]
GP1a	3 mg/kg, i.p. BALB/c mic	↓MCP-1, SDF-1, TNF-α and TGF-β1 ↓L-6, IL-1β, and VEGF, ↓collagen	[88]
GP1a	3 mg/kg/day, i.p. BALB/c mice	↓collagen I ↓TGF-β1, P-Smad3	[89]
WIN55,212-2	1 mg/kg/day, s.c. for 21 days to DBA/2 J mice	↓hydroxyproline ↓α-SMA, ↓PDGF and ↓TGFβ	[90]
WIN55,212-2	1 and 10 M for 24 days to dermal fibroblasts	↓ collagen I ↓TGF- β and CTGF ↓p-ERK-1/2	[91]

protein expression of α-SMA in comparison to fibroblasts derived from individuals without the condition. The fibroblasts exhibited a decrease in protein expression subsequent to the administration of WIN55,212-2 treatment.[91] The antifibrotic effects of CB2 receptors were further investigated and confirmed by utilizing CB2 receptor knockout mice. In this investigation, the mice lacking CB2 receptors displayed an increased vulnerability to the initiation of dermal fibrosis compared to the mice with intact CB2 receptors. The pharmacological suppression of CB2 receptors in CB2$^{+/+}$ mice using AM-630 led to a significant increase in the development of fibrosis and thickening of the skin. On the contrary, the administration of JWH-133 resulted in the activation of CB2 receptors, leading to a notable improvement in the measured dermal thickness.[86]

7 | CONCLUSIONS

This review provides an outline showcasing the antifibrogenic effect exhibited by the CB2 agonists. The pharmacological manipulation of these therapeutic properties holds potential for their application in the treatment of fibrosis and preservation of organ histology. The observed elevated rates of mortality in relation to fibrosis indicate a pressing requirement for the exploration and identification of

pharmacotherapeutic interventions possessing antifibrogenic properties, to address this incapacitating ailment.

The existing evidence supports the utilization of CB2 ligands to stimulate CB2 receptors or alternative methods to enhance the expression of these receptors in order to protect against organ fibrosis. Convincing number of studies are currently being conducted on discovering antifibrogenic therapies. The therapeutic potential of the ECS in the treatment of fibrosis has been observed to be quite intriguing. The significance of CB2 agonism arises from its ability to induce anti-inflammatory responses, thereby aiding in the mitigation of fibrosis progression.

The present review emphasizes the significant importance of the potential of activated CB2 receptors to suppress the production of pro-inflammatory cytokines and impede the activity of diverse transcriptional factors that contribute to the amplification of the inflammatory process. The antifibrotic effect of CB2 agonists is hypothesized to arise from their ability to impede the differentiation process of fibroblasts, thereby preventing their transition into cells that secrete fibrotic proteins. The established correlation between inflammation and fibrosis has been extensively documented in scientific literature.

Nevertheless, to uncover additional molecular mechanisms that contribute to the antifibrotic effect, further comprehensive

investigations are necessary. The extensive data presented in this study regarding the biological activities of CB2 agonists hold great promise for their potential clinical applications. However, it is also advised to carry out pharmacokinetic studies to unravel the kinetic properties of these diverse ligands, thereby ensuring the secure and precise extrapolation of these compounds in forthcoming clinical investigations.

8 | NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology. org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[93,94] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[95]

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
This is a review and the majority of the articles referred to are cited appropriately in the manuscript.

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