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Fibrogenic disorders in human diseases: from inflammation to organ dysfunction

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Abstract

Fibrosis is an inadequate response to tissue stress with very few therapeutic options to prevent its progression to organ dysfunction. There is an urgent need to identify drugs with a therapeutic potential for fibrosis, either by designing and developing new compounds or by repurposing drugs already in clinical use which were developed for other indications. In this Perspective, we summarize some pathways and biological targets involved in fibrosis development and maintenance, focusing on common mechanisms between organs and diseases. We review the therapeutic agents under experimental development, clinical trials or in clinical use for the treatment of fibrotic disorders, evaluating the reasons for the discrepancies observed between preclinical and clinical results. We also discuss the improvement that we envision in the development of therapeutic molecules able to achieve improved potential for treatment, including indirect modulators, targeting approaches or drug combinations.

1. Introduction. Molecular and cellular players mediating fibrosis.

Fibrosis is a maladaptive response to tissue or organ injury, such as chronic inflammation or chemical and mechanical insults. These result in metabolic dysregulation, abnormal production of extracellular matrix (ECM), formation of stiff scar tissue and compromised organ function. Fibrosis can affect any organ of the body. In response to tissue injury, the repair process may result in two distinct phenomena, a *normal* regenerative process, limited in time, in which injured tissues and cells are replaced by cells of the same type, maintaining tissue homeostasis; and a *chronic* fibrotic process, non-controlled, in which connective tissue replaces normal tissues. During the process of normal remodeling, resolution of organ fibrosis includes regeneration of the normal cell and tissue functions and disappearance of myofibroblasts,[1] which is a process not observed in chronic fibrotic processes. The mechanisms leading to the development of fibrosis are not completely understood and depend on the underlying diseases and/or local tissue properties. An inflammatory response to an initial injury, whatever the injury, can be postulated in most but not all fibrotic processes. For example, inflammation is involved in liver fibrosis; metabolic disorders and inflammation are involved in heart and kidney diseases, whereas there is no real inflammation in idiopathic pulmonary fibrosis (IPF). The quality of the connective tissue, especially of the collagens, is also important in the maintenance of homeostasis in the different tissue compartments. Pathological fibrotic processes are associated with abnormal and excessive deposition of an altered ECM by activated (myo)fibroblasts, a consequence and a driver of fibrosis,[2,3] which finally results in the replacement of normal tissue with permanent scar tissue of increased stiffness.[4] Whereas there has been progress in understanding some of the mechanisms of fibrosis, there is still an urgent need to find new biological targets and therapeutics to control fibrosis development, progression and resolution. Indeed, there are very few treatment options for this progressive, often fatal condition, which may be responsible of up to 45% of deaths in the industrialized world.[5,6] Current therapeutics are mostly supportive rather than curative, but as ongoing research identifies the molecular pathways that initiate and propagate fibrotic processes, better antifibrotic therapeutic possibilities may become available.

Immune cells (T-cells and macrophages), fibroblasts and epithelial cells all contribute to the

development of tissue fibrosis. In fibrotic diseases involving inflammation, macrophages and other immune cells are recruited to inflamed/injured tissues to promote diseased tissue clearance, repair and healing, and are crucial in maintaining tissue homeostasis. Profibrotic M1-type macrophages can locally synthesize a variety of growth factors, pro-inflammatory cytokines, enzymes and ECM proteins, whereas M2-type macrophages produce anti-inflammatory compounds, together influencing fibrogenesis and its resolution. However, activation of resident and/or recruited tissue fibroblasts into activated (myo)fibroblasts is central to the development of fibrosis,[7] in conjunction with the transforming growth factor-β (TGF-β)-associated signaling pathways.[8,9] TGF-β is predominantly produced by circulating monocytes, tissue macrophages and cancer cells. Epithelial damage triggers the production of TGF-β and its associated molecules, which in turn activate the epithelial-to-mesenchymal transdifferentiation (EMT) program as well as fibroblasts to become ECM-secreting (myo)fibroblasts. TGF-β is synthesized as an inactive latent peptide, needing transconformational and/or proteolytic processing to become active. In addition to TGF-β, cytokines (such as interleukin (IL)-4, IL-6, IL-13), chemokines (C-C motif chemokine ligand (CCL2)), growth factors (such as platelet-derived growth factor, PDGF), cadherins, integrins (in particular the $\alpha_v\beta_6$ integrin) able to activate latent TGF-β via a RGD-binding site, also contribute to the fibrotic processes and hence are potential therapeutic targets. Other inducers of fibrosis include the cellular pathways associated with the components of the angiotensin and endothelin systems. Angiotensin and endothelin peptides exhibit profibrotic activity, enhancing the production and signaling of active TGF-β, ECM deposition and fibroblast proliferation and differentiation into collagen-producing myofibroblasts via autocrine amplifying loops. During the normal process of tissue remodeling, the final stages include not only reduced synthesis but also increased degradation of collagens. An imbalance between the levels and activities of proteases secreted by myofibroblasts and/or their inhibitors, may compromise this regenerative phase and drive a progressive fibrotic process, suggesting that protease inhibitors also are potential therapeutics for controlling fibrosis. Several proteolytic enzymes, such as the matrix metalloproteinases (MMPs) or enzymes of post-prolyl-cleaving specificities such as dipeptidyl peptidase IV (DPP IV/CD26) or fibroblast activation protein-α (FAP-α) are involved in fibrotic

processes. Resolution of organ fibrosis can also be envisioned as a biological process under the control of three critical components: 1) eradication of the cause of injury; 2) degradation and removal of the fibrotic ECM, mainly by increasing the activity of specific MMPs and/or decreasing the expression level of MMP inhibitors; and 3) elimination of fibrogenic myofibroblasts, through apoptosis, senescence, de-differentiation and reprogramming, according to not well-defined processes.[1]

As stated above, the accumulation of proliferating activated ECM-producing fibroblasts and myofibroblasts in response to an initial inflammatory stress or tissue injury is central to tissue fibrosis, across a range of pathologic states. In comparison with their resting counterparts, activated ECM-secreting (myo)fibroblasts express *de novo* specific molecules;[10] however, all the precise molecular mechanisms are not yet elucidated. Myofibroblasts are activated by a variety of stimuli, including mechanical stress as well as autocrine and paracrine inflammatory and non-inflammatory cell-derived factors, in particular from the TGF-β pathway. Myofibroblasts are present at very low number in normal tissues, but in increased number in healing wounds, fibrotic and cancerous tissues. The hallmarks of myofibroblasts consist in the expression of α-smooth muscle actin (α-SMA), comparable to smooth muscle cells, the production of ECM, including several collagens, and of ECM-modifying enzymes. The source of activated fibroblasts and myofibroblasts is believed to be multiple, potentially including tissue-resident fibroblasts, circulating bone-marrow-derived fibrocytes, vascular pericytes and epithelial/endothelial cells via EMT and endothelial-to-mesenchymal transdifferentiation (EndMT) mechanisms.[10-12] The increased number of (myo)fibroblasts could also originate from excessive proliferation or acquired resistance to physiological apoptosis of tissue-resident cells. Fibroblasts represent a heterogeneous population of cells[7,10-15] with diverse features between anatomic sites and even within a single tissue. Fibroblasts exhibit considerable functional diversity, but it is not clear whether this is due to intrinsic differentiation properties of these cells or if it is a response to environmental factors. The identification and characterization of distinct lineages of fibroblasts, based on functional roles or with intrinsic fibrogenic potential, suggest that some populations of fibroblasts are more prone than others to induce fibrosis. Recently, in the mouse skin,

at least two lineages of fibroblasts with different location, capacities for wound healing and the production of ECM proteins and to express the Wnt/β-catenin pathway could be distinguished.[16,17] DPP IV was shown to be a cell marker of this fibrogenic lineage and exposure to a DPP IV/FAP-α inhibitor during wound healing resulted in diminished cutaneous scarring. Fibroblast heterogeneity was stable following transplantation, suggesting tissue origin memory rather than tissue environmental differences. In summary, myofibroblasts producing an altered ECM[3] with increased stiffness[4] are found in fibrotic, metabolic, oncogenic and inflammatory diseases, and in implant-related fibrotic disorders. In this Perspective, we will review what is known about the mechanisms involved in the development and maintenance of fibrotic processes in an organ-specific manner in order to determine potential therapeutic targets which may be common to all organs. We will also discuss some of the drugs developed for therapeutic intervention presently available.

2. Organ-specific fibrosis and therapeutic targets.

As stated above, fibrosis, the excessive scarring of tissues, is a non-specific terminal pathway of many toxic, metabolic and inflammatory diseases and can develop in almost all organs exposed to chronic injury.[9] Fibrogenic mechanisms are initially aimed at repairing short-term tissue insults, however, when repetitive, they lead eventually to organ scaring and failure. While fibrosis represents a final common response to injury from ubiquitous processes, the resulting phenotype is tissue-specific and the course of organ failure can be highly variable, dependent on local tissue characteristics,[18] thus presenting opportunities for targeted therapeutic intervention.[19] However, in all organs, fibrosis has been linked to the activation of the TGF-β signaling pathway, which currently represents the main pathway under investigation for the development and evaluation of therapeutic approaches. Below we will discuss for the most commonly affected organs some novel therapeutic options to control fibrosis and organ dysfunction. The chemical structures of the molecules discussed in the following paragraphs are presented in Figures 2 to 16, and some selected clinical trials in Table 1.

2.1. Liver fibrosis

Liver fibrosis leading to cirrhosis results from persistent healing attempts to replace defective/dead hepatocytes in response to injury and is a common consequence of chronic liver disorders. Following liver injury, either toxic or metabolic, hepatic stellate cells and portal fibroblasts transform into myofibroblasts and with disease progression express the integrin $\alpha_v\beta_3$. Hepatic stellate cells are responsible for maintaining the ECM in the liver and activation of these cells leads to excess collagen formation and fibrosis. Controlling the functions of stellate cells may thus represent means of regulating the fibrotic response.[20] Kupffer cells are an important source of cytokines and are known to stimulate stellate cells to secrete hepatocyte growth factor (HGF). Presently, there is no effective therapy for liver fibrosis. However, as compared to other organs, the liver has some potential for regeneration. Proteolytic digestion of collagens with disappearance of myofibroblasts may resolve fibrosis upon cessation of liver injury.[21,22] Liver fibrosis has been mostly studied in nonalcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), either in animal models of the disease or human surgical specimens.[23] NASH covers a range of diseases that result from fat accumulation in the liver leading to liver inflammation, scarring, irreversible damage and liver failure. NASH is currently estimated to affect up to 20 to 30% of the general population in the western world. Patients with type-2 diabetes mellitus and morbid obesity are the most affected. Thus, fatty acid metabolic pathways have been evaluated as therapeutic targets. Peroxisome proliferator-activated receptor (PPAR)-γ agonists combined with cholesterol-lowering agents, the "statins", possibly associated with omega 3 fatty acids, antidiabetic agents or vitamin E, have been proposed;[24,25] but only very limited clinical trial data are presently available. Farnesoid X-activated receptor (FXR) agonists are under investigation as potential treatment for multiple metabolic and liver disorders. For example, single agonists to the FXR receptor[26] and the PPAR-γ receptor [27] or dual PPAR-γ/FXR agonists[28] are under clinical development for NASH. A liver-directed oral inhibitor of acetyl CoA carboxylase (ACC), an enzyme involved in the metabolism of fatty acids in the liver, has shown beneficial effects in animal models and in a clinical trial in overweight adult male subjects.[29] In NASH, the protein sterol regulatory element-binding protein 1 (SREBP-1), which regulates fatty acid biosynthesis in the liver, is increased, and could represent another therapeutic target.[30] The renin-angiotensin system

(RAS) has also been implicated in NASH. However, trials including angiotensin receptor type 1 (AT$_1$R) blockers produced mixed results, while control of obesity, of insulin resistance and of hypercholesterolemia may be more efficient to reverse fibrosis.[31] Fibroblast growth factor 21 (FGF21) is a hepatoprotective hormone identified as a substrate of FAP-α, a membrane-bound protease expressed at sites of tissue remodeling, inflammation and fibrosis. Cleavage by FAP-α inactivates FGF21, while FAP-α inhibition increases endogenous levels of active FGF21, making FAP-α an attractive target for liver diseases.[32,33] The lysyloxidase-like 2 (LOXL2) enzyme catalyzes the oxidation of ε-amines of lysine residues within collagen, generating reactive aldehydes that condense to form collagen cross-linkages. Dysregulation of this process can lead to fibrosis and LOX(L) inhibitors were shown to significantly reduce fibrosis in mouse models. Following on these experimental results, a phase I trial was completed in healthy volunteers.[34] Blocking chemokine pathways is also a promising approach and a dual C-C chemokine receptor (CCR)-2/5 antagonist is under a phase IIb study in adults with NASH and liver fibrosis (CENTAUR study) after promising results in experimental rodent models.[35] The clinically approved family of DPP IV inhibitors, the "gliptins", and several kinase inhibitors are evaluated in various clinical trials, either completed or ongoing, but no molecule has been presently approved for this indication. In animal models, another promising target, besides TGF-β, is the sphingosine axis,[36] expressed and active in many organs and tissues. Sphigosine-1-phosphate (S1P)/S1PR signaling has both pro- and anti-fibrotic effects depending on the context and site of action.[37] In the liver, S1P is involved in profibrotic processes, including the differentiation of resident hepatic stellate cells into activated myofibroblasts.[38]

2.2. Lung fibrosis

In the lung, fibrosis encompasses a variety of idiopathic disorders with distinct clinical phenotypes, characterized by progressive replacement of normal alveolar structures by dense connective tissue that prevents normal gas exchange. Idiopathic pulmonary fibrosis (IPF) is the most frequent, progressive and fatal fibrotic lung disease that eventually leads to respiratory failure and death. Presently, lung transplantation is the sole curative intervention for IPF. The contribution of

inflammation is not the main driver of IPF, but can orchestrate existing fibrotic responses.[39] Unfortunately, no animal model truly reproduces the pathogenesis of IPF. The mouse bleomycin model has been nevertheless extensively studied, but bleomycin is associated with a marked influx of inflammatory cells into the lung parenchyma, in contrast to IPF in humans. A recent review has described the therapeutic agents of interest in IPF.[40] We will not repeat the discussion of the molecules presented in this review with some exceptions for the compounds that we believe are of a broader interest in the development of therapies for fibrotic diseases other than IPF. Currently no approved treatment can cure this disease, nevertheless, pirfenidone (1, structure in Figure 2), a pleiotropic anti-inflammatory, anti-fibrotic and antioxidant molecules, and nintedanib (63, structure in Figure 9), a multikinase inhibitor, have been shown (ASCEND, CAPACITY, TOMORROW and IMPULSIS trials) to slow down disease progression and prevent acute exacerbations in patients with IPF, and have been recently approved for the treatment of IPF. In lung fibrosis, most mechanisms of disease induction converge toward the TGF-β pathway, making this pathway an obvious target for the development of therapy.[41] In addition, myofibroblasts are activated by the components of the endothelin axis, another potential therapeutic target.[42] TGF-β induces endothelin (ET)-1 expression, forming an autocrine amplifying loop. ET-1 is chemotactic and proliferative for fibroblasts, induces α-SMA expression, ECM accumulation and contraction, and the myofibroblast phenotype in human lung fibroblasts, mediated by the ET_A and ET_B receptors. ET receptor antagonists are approved for the treatment of pulmonary hypertension. The dual ET-1 receptor antagonist bosentan (40, structure in Figure 6) decreases collagen I and III synthesis by fibroblasts, suggesting that ET-1 receptors antagonists may have therapeutic potential in lung fibrosis. However, several phases II or III clinical trials in patients with established pulmonary fibrosis of the dual $ET_{A/B}$ antagonists 40 (Build-1, -2 and -3 trials) and macitentan (41, structure in Figure 6) (MUSIC trial) and the ET_A-selective ambrisentan (43, structure in Figure 6) (ARTEMIS trial) produced negative results. Acquisition of an apoptosis-resistant myofibroblast phenotype in the injured lung is mediated, at least in part, by the sustained activation of focal adhesion kinase (FAK) and protein kinase B (PKB/Akt).[43] A C-Jun N-terminal kinase (JNK)-1 inhibitor is currently being evaluated in a phase I/II trial. A reversible inhibitor of

PI3K/Akt/mammalian target of rapamycin (mTOR) and the approved immunosuppressive drug sirolimus/rapamycin (16, structure in Figure 3), also targeting mTOR, are being evaluated in double-blind placebo-controlled trials. A Rho-associated protein kinase (ROCK) II inhibitor is in phase II trial. Compounds with anti-inflammatory and anti-oxidative properties were shown to attenuate or even reverse fibrosis in several animal and clinical studies.[44] A multikinase inhibitor related to the natural antioxidant quercetin (110, structure in Figure 11) is presently being evaluated in a phase I trial. Basic fibroblast growth factor (bFGF) is mitogenic for human lung fibroblasts via the plasminogen activator inhibitor type 1 (PAI-1) cascade.[45] Inhibitors targeting among others platelet-derived growth factor receptor (PDGFR)α/β, and vascular endothelial growth factor receptors (VEGFRs) have been evaluated in several clinical trials with some positive effects.[46] The protease FAP-α is selectively expressed by activated myofibroblasts. FAP-α, in concert with MMPs, participates in collagen catabolism and clearance, scar resolution and restoration of lung homeostasis, displaying protective effects in murine experimental models.[47] Loss of this protease was associated with fibrosis exacerbation in FAP-α-deficient mice exposed to bleomycin. Fatty acid pathways have also been studied. Agonists to the S1P pathways are pro-fibrotic in human lung fibroblasts in a Smad-independent mechanism.[48] Lysophosphatidic acid (LPA) is a bioactive phospholipid acting on LPA receptors. Results in LPA1 receptor-knockout mice suggested that blocking LPA1 signaling could provide a potential novel approach for the treatment of IPF.[49] A selective LPA1 antagonist inhibited proliferation and contraction of normal human lung fibroblasts following LPA stimulation.[50] An antagonist to the LPA1 receptor has been tested in a phase II trial, but results are not yet published. An inhibitor of autotaxin (ATX), an enzyme involved in the synthesis of LPA, is evaluated in an ongoing phase II trial. As IPF is characterized by a paucity of inflammatory cells within the lung parenchyma, classical anti-inflammatory treatments such as glucorticosteroids or purine inhibitors have proven ineffective, except for acute exacerbation episodes. A small molecule antagonist of the $\alpha_v\beta_6$ integrin was evaluated in phase I trials.[51] Drugs modulating the immune system have mostly involved antagonizing antibodies, which we will not discuss in the present Perspective. Stem cell therapies and anti-senescence therapies are novel therapeutic approaches to repair damaged tissue.

They are too recent and only in early preclinical stage to draw conclusion, but they may be interesting targets to pursue.

2.3. Kidney fibrosis

Fibrosis in the kidney is the final common pathway following severe acute and chronic kidney diseases, independent of the type of initial injury. Inflammatory and non-inflammatory stresses can affect the structure and physiological function of the glomeruli (the main filtration barrier that determines global kidney function) and/or the tubulointerstitial compartment, leading to progressive renal failure requiring dialysis or renal transplantation.[52,53] Acute kidney injury is an increasing common clinical disorder, in particular in frail and hospitalized patients, due to multiple causes, such as ischemic injury and exposure to nephrotoxic substances.[18,54,55] Oxidative stress-mediated injury as well as toxins mainly affect the tubulointerstitial compartment resulting in an inflammatory response. This process is characterized by fibroblast proliferation near the site of the injury, the appearance of α-SMA-positive activated myofibroblasts depositing excessive ECM in the interstitial space, and the activation of an EMT program in tubular epithelial cells.[54,56-58] The crosstalk between tubular cells and myofibroblasts in driving fibrosis is not clear, but seems to involve the transcription factors Snail and Twist that regulate EMT.[56,59,60] The glomerular compartment can also be damaged by acute or chronic primary glomerulopathies as well as during systemic ongoing diseases such as diabetes. Defaults in the metabolism of fatty acids, driven by TGF-β1 and involving PPAR-γ pathways, have also been implied. PPAR-γ agonists activating the S1P axis are anti-fibrotic in the kidney.[61] Activation of PPAR-γ by synthetic agonists,[62] the "glitazones" rosiglitazone (117) or pioglizazone (118, structures in Figure 12), approved in the treatment of type-2 diabetes, inhibit TGF-β profibrotic effects. These molecules are presently under clinical evaluation for kidney fibrosis. Activation of ET-1 receptors has also been implicated in the pathophysiology of chronic kidney disease and particularly in diabetic nephropathy. Animal models have shown beneficial effects on proteinuria and kidney function of the blockade of the endothelin axis, using either dual $ET_{A/B}$ or ET_A-selective antagonists.[42] For example, an ET_A-selective antagonist is in phase III for diabetic nephropathy.[42] Central to the

therapy of kidney diseases are antagonists to the RAS, either angiotensin converting enzyme (ACE) inhibitors, AT_1R antagonists or antagonists to mineralocorticoids, which have been in clinical use for decades for hypertension and cardiovascular disorders. However, in glomerular fibrosis, RAS blockade only modestly slows down progression. The RAS is a potent inducer of TGF-β, suggesting that anti-RAS drugs should be associated with TGF-β antagonists.[63] Angiotensin (Ang) II activates the epidermal growth factor receptor (EGFR) pathway, another potential therapeutic target in renal fibrosis.[54,64] The bone morphogenic protein (BMP) receptor activin–like kinase 3 (Alk3) has anti-fibrotic properties in the tubular epithelium, inhibiting TGF-β1/Smad3 signaling, epithelial damage and fibrosis. The peptide AA123/THR123 BMP7 agonist could reverse fibrosis in mouse models of kidney injury and are presently in Phase I for acute kidney injury.[65,66] Combining AA123/THR123 and the approved ACE inhibitor, captopril (35, structure in Figure 5), exhibited additive therapeutic benefit in controlling fibrosis. Anti-TGF-β therapeutics clinically evaluated include neutralizing antibodies and antisense or silencing nucleotides. The small molecule antagonist of the TGF-β receptor 1 has been examined in several clinical trials for diabetic nephropathy and was proposed to be effective in slowing the decline of renal function. Tranilast (17, structure in Figure 3), a drug approved for bronchial asthma and hypertrophic scar, as well as new cynnamoylanthranilate analogs,[67] have been shown to inhibit the TGF-β and PDGF pathways and are presently under clinical evaluation for the treatment of diabetic nephropathy. Additional pathways such as integrin-linked kinases and Wnt/β-catenin, which have a central role in EMT regulation, have been suggested to be potential therapeutic targets for renal fibrosis. Activation of the Wnt/β-catenin, pathway, of which the RAS is a target in the kidney, enhances renal fibrotic processes.[68] Inhibition of the Ca^{2+}-activated K^+ channel (KCa3.1) inhibits TGF-β-induced upregulation of ECM-associated genes in renal fibroblasts.[54,57,69] Janus kinase/signal transducers and activators of transcription (JAK/STAT)-3/6 that governs lymphocyte functions has an important role in interstitial fibrosis development which is abolished by a JAK3 inhibitor.[70] An antagonist to the CCR2 chemokine is being evaluated in three phase II trials for diabetic nephropathy.[71] The kidney is the organ expressing the highest levels of the protease DPP IV which has been associated with cell survival and ECM remodeling, suggesting that

beside their glucose-lowering action, DPP IV inhibitors, the "gliptins",[72] may have potential renal protective effects, inhibiting EndMT.[73,74] Anti-cholesterol agents, the "statins", diminish EMT, TGF-β signaling and oxidative stress in glomerular cells.[75] An antioxidant NADPH oxidases (NOX1/4) inhibitor is in phase II trial for diabetic nephropathy. A non-selective phosphodiesterase (PDE) inhibitor is under one phase III and two phase IV clinical trials and the PDE-5-selective PF00489791 is in phase II all for diabetic nephropathy.[76] It has to be underlined that in the kidney the course of fibrosis progression is heterogeneous between patients, depending on the nature of the injury, continuous activation of the (myo)fibroblasts and patient-associated environmental and genetic factors.[77] Facilitating regeneration through the ability of resident progenitor cells to differentiate into new renal cells was shown in experimental models to enhance recovery from acute kidney injury.[78]

2.4. Cardiovascular system-associated fibrosis

Fibrillar collagen-1 is the main protein of the heart structural ECM network, providing a scaffold for and regulating the contraction of cardiomyocytes. This ECM scaffolding is maintained by interstitial fibroblasts. In heart diseases, such as ischemic cardiomyopathy, cardiomyocytes loss is observed but the heart structural integrity is maintained by activated myofibroblasts in a healing response, with formation of a scar tissue involving stiff crosslinked fibrillar collagen-1 and various ECM proteins. With disease progression, this initial reparative process results in an amplification loop of continuous fibrogenesis and general cardiomyocyte dysfunction. The mediators and pathways involved are the RAS, the ET-1 axis and TGF-β-dependent signaling, growth factors, macrophage-derived pro-inflammatory molecules, such as tumor necrosis factor (TNF)-α, oxidative stress, and proteases. Candidate proteases include MMP-2 and MMP-9,[79] as well as the serine proteases FAP-α[80] and DPP IV expressed by activated fibroblasts and smooth muscle cells.[81] The PREMIER clinical trial of a MMP inhibitor was not conclusive of a beneficial effect.[82] FAP-α is induced by TNF-α in activated myofibroblasts and can degrade type-1 collagen.[80,83] In vascular diseases, atherosclerotic plaque rupture is facilitated by the protease activity of FAP-α able to degrade type-1 collagen.[83] Antifibrotic therapeutics for heart diseases include anti-oxidants such as flavonoids, mitochondrial regulators such

as cyclosporin A (**20**, structure in Figure 3), β-adrenergic receptor antagonists and inhibitors of the angiotensin and endothelin pathways, such as the "sartan" family of AT_1R antagonists or ACE inhibitors (ACEI) and the "sentan" family of ETRs antagonists. Both activators and antagonists of the fatty acid pathways, such as the S1P/S1PR axis, must also be considered, as well as antagonists to inflammatory mediators, since most chronic heart diseases have an inflammatory component. Antagonizing the binding of stromal cell-derived factor 1 (SDF1, also known as C-X-C motif chemokine 12 (CXCL12)) to its CXCR4 receptor was as efficient in reducing cardiac fibrosis as inhibiting the binding of Ang II to AT_1R, independently of the level of blood pressure control.[84] Notch signaling has also been involved in cardiac and other organs fibrotic processes.[85] Notch activation was shown to be protective by reducing the effects of the profibrotic cytokine TGF-β on the differentiation of fibroblasts into myofibroblasts, the production of fibrillary type-1 collagen, as well as EMT and EndMT. These data suggest that activating Notch functions, for example with soluble Notch ligands or Notch pathway activators including the hormone relaxin, may be beneficial in preventing cardiac fibrosis.[86,87] Relaxin indeed affects collagen metabolism, inhibiting collagen synthesis and enhancing its breakdown by MMPs. Adenosine and its G-protein coupled receptors (GPCR), in particular the $A_{2B}AR$, are able to modulate fibrosis in the heart following myocardiac damage. In the early stages of the disease, Adenosine receptor (AR) agonists seem to be anti-fibrotic whereas antagonists seem to be of interest for a chronic treatment.[88]

2.5. Wound healing

Wound repair involves stages of inflammation, tissue regeneration and remodeling. In the healing skin, α-SMA-positive myofibroblasts contract and exert mechanical tension on the ECM causing it to be reorganized into a functional connective tissue with the formation of a normal temporary fibrogenic process, the closing of the wound and the reconstruction of a functional skin. As already stated, discrete skin fibroblast lineages have been described, at least in mice, depending on their embryonic origin and their expression of specific markers, in particular of the Wnt/β-catenin pathway, and their capacity to deposit ECM in response to fibrogenis stresses. The subset of fibroblasts

contributing mainly to ECM deposition are DPP IV- and likely also FAP-α-positive,[16,17] and express ADAM12,[89] suggesting potential therapeutic intervention targeting these proteases. Two deviations from the normal process of healing may happen in the skin: an over-reaction of the myofibroblasts resulting in scar tissue as observed for example in scleroderma or in cheloid formation, or on the contrary the absence of wound closing and the formation of ulcers. Scleroderma is an autoimmune chronic connective tissue disease that may be limited to the skin of the face and extremities, but it may also affect internal organs (systemic sclerosis) including lungs, heart, gastrointestinal tract and kidneys. The etiology is not well defined and no specific treatment is currently available. The prognosis is determined by the form of the disease and the extent of visceral involvement. The underlying mechanism involves abnormal growth of connective tissue, exaggerated deposition of collagen and ECM, and tissue fibrosis, which is believed to occur as a result of an initial insult by the immune system. Some biological pathways have been implicated, including the TGF-β and PDGF signaling pathways, as well as the endothelin axis. The purinergic P2X7 receptor, a nucleotide-gated ionotropic channel primarily involved in inflammatory response, may also play a role.[90] Therapeutic approaches have been based on the control of myofibroblasts differentiation and recruitment.[91] In this regard, the Wnt/β-catenin/tankyrase signaling is an important mediator of sustained fibroblast activation in fibrotic diseases, including systemic sclerosis.[92] Chronic non-healing wounds are characterized by unresolved inflammation, impaired fibroblast function and ECM deposition, and increased levels of proteolytic activity. To date, approved therapies for chronic cutaneous wounds include human skin substitutes and recombinant human PDGF. Blockade of the endothelin axis by the dual $ET_{A/B}$ antagonist **40** for digital ulcers is presently approved, following the demonstration in phase III and IV trials, of beneficial effects on the development of new ulcers. However, many patients affected with chronic ulcers remain unhealed, suggesting that the design of novel topical therapies is necessary.[93]

2.6. Allogeneic transplant-associated fibrosis

Solid organ, cell or engineered tissue transplantation are therapies required in the treatment of patients

with end-stage organ diseases. Graft-associated fibrosis is a predictor of dysfunction of solid organ transplants and implanted biomaterials.[5,57,94] While the development of potent immunosuppressive regimens has resulted in improved short-term allograft outcome, long-term survival and functioning of the grafts remain a challenge. Beside alloimmune injuries such as cell- and antibody-mediated rejection, late allograft loss is often due to a conjunction of non-immune factors, such as local oxidative stress due to ischemic injury, or the toxicity of immunosuppressive drugs used for maintenance therapy, leading to progressive non-specific inflammation, ECM deposition by tissue (myo)fibroblasts and fibrosis. Some degree of ischemia-reperfusion injury is unavoidable in the early phase of organ transplantation and influences both short-term and long-term allograft outcome. Carbon monoxide (CO) has attracted attention as a medical gas with anti-inflammatory and anti-apoptotic effects. CO decreases oxidative stress and mRNA expression of proinflammatory cytokines. In experimental models of kidney transplantation, CO inhibited inflammation, interstitial fibrosis, tubular apoptosis and injury.[95] By controlling leukocyte trafficking, S1PR agonists produce clinical immunosuppression useful for preventing transplant rejection and treating immune diseases. However, they also cause side effects due to the activation of different S1PRs, suggesting that receptor-specific agonists or antagonists may be preferable. S1PR modulators attenuated myocardial fibrosis following heart transplantation by reducing oxidative stress and apoptosis in a rat experimental model.[96] The complement pathway is part of the innate immune system. Inappropriate activation of this system is involved in organ dysfunction in transplantation, in particular in kidney transplantation. The complement cascade may be activated by ischemia/reperfusion injury and other non-specific inflammatory processes, representing a potential therapeutic target.[97] But to the best of our knowledge, no small molecule has presently been developed able to inhibit complement activation (apart from anti-C3a and C5a antibodies). DPP IV/CD26 is a co-stimulator of T-cells and a therapeutic target for type-2 diabetes. Following transplantation, type-2 diabetes is a common side effect of immunosuppressive anti-rejection therapies, which is improved by DPP IV inhibition.[98] In a murine model of lung allograft DPP IV inhibition promoted graft acceptance by reducing T-cell infiltration and modulating cytokine expression.[99] The secretion of TGF-β by activated fibroblasts

together with other cytokines and chemokines, the activation of the mammalian target of rapamycin (mTOR) pathway, the release of ET-1, prostacyclins, MMPs and the activation of the RAS by immunosuppressive drugs are some of the identified culprits of allograft fibrosis and dysfunction. However, no clinically evidence-based regimen has emerged so far. Another form of transplantation and regenerative medicine is represented by the direct transfer of cells, mostly by injecting them in the blood or following encapsulation into a polymeric device. Therapeutic mesenchymal stem cell therapy[100] has raised hopes for new treatments as these cells have high self-renewal capacity and can generate multiple cell lineages. They can be isolated from many tissues such as bone marrow, amniotic fluid, skin, heart, kidneys, liver and the adipose tissue. Amnion-derived fetal epithelial cells which are non-immunogenic and have anti-inflammatory and anti-fibrotic potential[101,102] are of potential interest. Autologous or allogeneic mesenchymal stem cells are presently under evaluation in many clinical trials (ClinicalTrials.gov) for the treatment of organ fibrosis, including after transplantation. As many adult tissues contain stem and progenitor cells able to proliferate, differentiate and maintain tissue homeostasis and repair, efforts have been made to enhance these cell populations using small molecules.[103]

2.7. Medical bio-device implants-associated fibrosis

Biomedical devices have important applications as orthopedic, dental and breast implants, pacemakers, vascular grafts, heart valves, intraocular lenses, drug delivery devices and biosensors. The fate of almost all medical implants is dictated by the biological response at the interface of host tissue and the implanted devices.[104] The engineering of safe biomaterials is fundamental since tissue-replacement scaffolds in regenerative medicine provide physical support and deliver biologically active molecules and cells, or mobilize endogenous cells to repair, maintain, replace or enhance the function of a specific tissue or organ.[105] Medical devices must be biocompatible, not activating the immune system,[106] and functional, displaying properties adapted to the aims of the replacement.[107] However, many bio-materials promote local inflammation, the adhesion, proliferation and activation of (myo)fibroblasts, resulting in abnormal tissue repair and fibrosis, ultimately hindering long-term

functioning of the devices. For example, we have shown (LJJ; unpublished results) that human fibroblasts grown in 3-dimensional collagen lattices in which a synthetic surgical implant was incorporated enwrap the implant in a thick ECM layer (**Figure 1**).

Figure 1. *Implant-induced fibrosis.* Three-dimensional culture in collagen gels of human fibroblasts with a surgical mesh. Left: at the initiation of the culture no ECM layer could be detected; right after two weeks of culture the fibroblasts adhered to the mesh and secreted a thick ECM layer (brown layer tightly surrounding the mesh).

The mechanisms behind implant-associated fibrosis are postulated to involve an initial, and probably repetitive, injury and tissue stress at the interface of the implant and the receiving tissue.[108] Manipulating the surface chemistry of biomaterials[104] is a way to modulate protein recognition and biomaterials-host contact and, subsequently local the inflammatory response. Several support systems may be envisioned, natural source-derived, polymer-derived or tissue-derived following sophisticated processing. To be accepted by the host tissue, a medical implant requires two main properties: first to resist inflammation and subsequent fibrosis development, and second to support vascularization, to bring nutriments, growth factors and oxygen for local physiological needs.[109,110] The development of a functional vascular system depends on the response of vascular cells to inflammation and oxidative stress.[111,112] Thus, controlling these processes will improve the viability of the bioengineered implants. These issues have been addressed by innovative approaches: the development of tissue-engineered blood vessels for arterial revascularization, and the production of non-immunogenic de-cellularized 3-D matrix organ scaffolds that can be re-cellularized with host-derived stem cells, as performed in experimental models and pioneering clinical studies in the respiratory system.[112,113] Modulating the local cell responses against engineered structures must also

involve controlling the release of pro-inflammatory cytokines/chemokines induced by biomaterials. Besides the modification of the physical and chemical properties of biomaterials to minimize inflammatory responses, novel therapies are being developed, based on cells with specific anti-inflammatory functions.[114] Presently, very few studies have aimed at developing anti-fibrotic strategies in the context of implanted biomaterials. However, there is an urgent need to develop strategies to prolong the physiological life of the implant and prevent implant failure. Up to now, most tissue-engineered products have been used in the clinic for the management of burns and severe wounds, as well as for cartilage and bone replacement. We believe that evaluating and comparing what has been developed in several organs as anti-fibrotic therapy for other situations may allow to define relevant strategies to prevent fibrosis associated to implants. Molecules able to favor medical device implantation are also under development, which includes **1**,[115] an approved drug for treating IPF.

2.8. Cancer-associated fibrosis

Tumors are heterogeneous populations of cells, the tumor cells themselves, inflammatory and immune cells, vascular cells and cancer-associated (myo)fibroblasts (CAFs) interacting with each other through direct interactions or mediated by secreted factors and their receptors, as well as enzymes, including many kinases, all influenced by inflammation-derived signaling processes.[116] The fibrotic tumor stroma has only recently emerged as a potential target for anti-cancer therapy. Many previous and recent reviews[117,118] have discussed the involvement of the stroma in cancer progression, including CAFs, thus we will only summarize the information relevant to our purpose provided in these reviews. CAFs express specific markers and display distinct properties and origin,[117-119] and are resistant to apoptosis. CAFs recruited to the stroma of tumors modulate oncogenic processes and cancer progression. They produce an altered cross-linked ECM[3] of increased stiffness,[4] which influences tumor immunity, vascularization and metastatic behavior, as well as the distribution of therapeutics. Collagen cross-linking is dependent on the action of LOX and LOXL enzymes. Inhibitors for these enzymes are under investigation. Collagen cross-linking might be also indirectly

targeted with the approved immunosuppressive drug tacrolimus/FK506 (**18**, structure in Figure 3) which inhibits FKBP65 a peptidyl prolyl isomerase that enhances lysine-hydroxylase-2 (LH2) activity.[120] Classically, CAFs are considered as pro-tumorigenic, however, when in a non-stimulated form, tumor-resident fibroblasts may also display anti-tumoral functions, promoting anti-tumor immunity similar to what is known about polarized pro- and anti-tumor (M1 and M2, respectively) populations of macrophages. From a therapeutic view, selectively promoting anti-tumor fibroblast populations would be more interesting, than eradicating all fibroblast populations. CAFs express and/or secrete TGF-β, CXCL12/SDF1 and its CXCR4 receptor, Notch, Wnt and HGF (the cMet ligand expressed on tumor cells and involved in cell scattering and invasion), several cytokines, chemokines, growth factors, proteins, and enzymes. CAFs also express fibroblast-specific protein-1 (FSP-1/S100A4), α-SMA, FAP-α and PDGFRβ. Altogether, this results in the activation of the EMT and the metastasis programs,[121] but also provides targets for therapy. In CAFs the transcriptional regulator heat shock factor 1 (HSF1) is activated and regulates cancer cell growth via TGF-β and CXCL12/SDF1 signaling in an autocrine loop.[122] CAFs have been involved in promoting cancer stem cell properties via insulin-like growth factor (IGF)1R-Akt signaling, suggesting the applicability of antagonists of IGFR, however, combination therapies will probably be necessary.[123] The enzyme FAP-α is expressed in the stroma directly surrounding epithelial cancers and in melanoma and sarcoma tumor cells. FAP-α inhibition is generally considered a potential therapeutic target for oncologic diseases. The proteolytic activity of FAP-α is pro-fibrogenic being involved in remodeling of the ECM, in particular increasing the levels of fibronectin and collagen fibers, an effect mediated by the $α_9β_1$ integrin which contributes to the recruitment of CAFs.[124-126] Thus, antagonists to integrins are also of potential therapeutic value in oncologic contexts. Tumor-associated S1P kinase signaling pathway promotes the differentiation of fibroblasts into myofibroblasts, then myofibroblasts-associated S1P kinase via a S1P receptor promotes tumor cell dissemination,[127] suggesting that the S1P axis is a potential target for controlling CAFs. The enzyme thrombin can directly stimulate ECM deposition, fibroblast proliferation and differentiation into myofibroblasts. Several thrombin inhibitors have been developed in the context of coagulopathies and may be of therapeutic interest.

In normal tissues, epithelial cells create an anti-inflammatory milieu which is lost in cancer and may be an initial signal for fibrotic responses. The combination of **1** and **63**, two drugs approved for the treatment of IPF has demonstrated a survival benefit in cancer.[128] A phase II clinical trial combining the approved AT_1R antagonist losartan (**37**, structure in Figure 5) and FOLFIRINOX regimen is underway in pancreatic cancer. Chemotherapy- and radiotherapy-induced fibrosis in cancer survivors is a common complication of therapeutic cancer regimens.[19] Targeting tumor stromal cells would be advantageous as these cells are genetically stable and therefore less prone to develop resistance mechanisms. Stromal cells also express specific markers, different of the markers expressed by tumor cells, thus, allowing more diversified combination and targeted therapies. In cancer, therapeutic attempts have been mainly, with a few exceptions, directed at blocking the effects in tumor cells of factors secreted by CAFs, or to develop drugs aimed at modulating the vascular and immune systems in the stroma. Very few preclinical and clinical attempts have been made (yet) aimed at directly targeting CAFs.

2.9. Common mechanisms and therapeutic strategies

In summary, during development and wound healing, physiological fibrogenesis maintains connective tissue integrity and structure through the synthesis of ECM. However, chronic organ stress results in fibrosis, overgrowth, hardening, and scarring of tissues, ultimately progressing to loss of organ function. Currently, there are very few approved anti-fibrotic therapeutic drugs (3.1.) and often these drugs are only slowing disease progression. Whilst the pathology of fibrosis and its functional significance are well described, its molecular regulation and therapeutic targets for preventing and treating fibrosis are less understood. Whereas fibrosis represents a final common pathway to injury, the course of organ failure can be highly variable, dependent on local tissue characteristics.[18] It seems however possible to generalize some findings between organs, as common key contributors to fibrotic diseases have been identified and are as outlined in Schema 1. The TGF-β1 signaling pathway (3.2.) is central to fibrosis,[129-131] and selectively antagonizing it is mandatory in the therapeutic arsenal. An inflammatory stress has been shown to be relevant in the initiation of fibrosis in defined organs, but

inflammation seems to be less involved in later stages. Thus, anti-inflammatory strategies (3.3.) must be initiated early in the course of the disease. Controlling the bioactive lipid S1P (3.4.) is also very relevant in anti-fibrotic therapies for all organs considered.[37,96] Depending on the context, targeting the kinases acting on sphigosine or developing agonists and/or antagonists for the S1PRs is to be considered. Targeting vasoactive peptides, in particular angiotensin and endothelin and their receptors (3.5.), also represent common mechanisms in the development and progression of fibrosis. In many organs, RAS blockade is currently the best available anti-fibrotic therapy.[82] Activation of AT_1R by Ang II mediates inflammation and fibrogenesis, whereas activation of the AT_2R has counter-regulatory anti-inflammatory and anti-proliferative effects, suggesting that compounds activating AT_2R have therapeutic interest.[132] The blockade of $ET_{A/B}$ receptors by itself was shown to be not sufficient to control fibrosis progression,[42] but combination therapies with ET-1 receptor(s) antagonists provided interesting results. Several enzymes, in particular some proteases and the lysyloxidases have been shown to be involved in fibrotic processes and inhibitors able to control their activity have been developed and evaluated (3.6.). Kinases and receptors involved in the differentiation of fibroblasts into activated (myo)fibroblasts have also been the targets of anti-fibrotic strategies (3.7.). Modulators of several metabolic pathways, in particular oxidative stress, PPAR-γ, FXR and the synthesis of fatty acids (3.8.) have also been developed and evaluated. Controlling the adenosine pathway, in particular the $A_{2B}AR$,[88] and the Notch1 pathway[85,86] are also relevant to fibrogenesis therapy. A few natural products have shown interesting properties and may be relevant for developing synthesis programs and optimization (3.9.). Finally, cell therapy options must be considered (3.10.) to replace organ transplantation in terminal diseases. Stem cell therapies offer opportunity to enhance tissue repair in chronic organ diseases.[101,102,133] but in this Perspective these therapeutic approaches will only be outlined and not discussed in detail. In conclusion, the recent development of treatment strategies offers the prospect of more efficacious therapies to prevent or even treat fibrosis (see Table 1 for the list of clinical trials). However, as these anti-fibrotic therapies may target widely expressed and important physiological pathways, it will be mandatory to develop tissue-selective approaches.
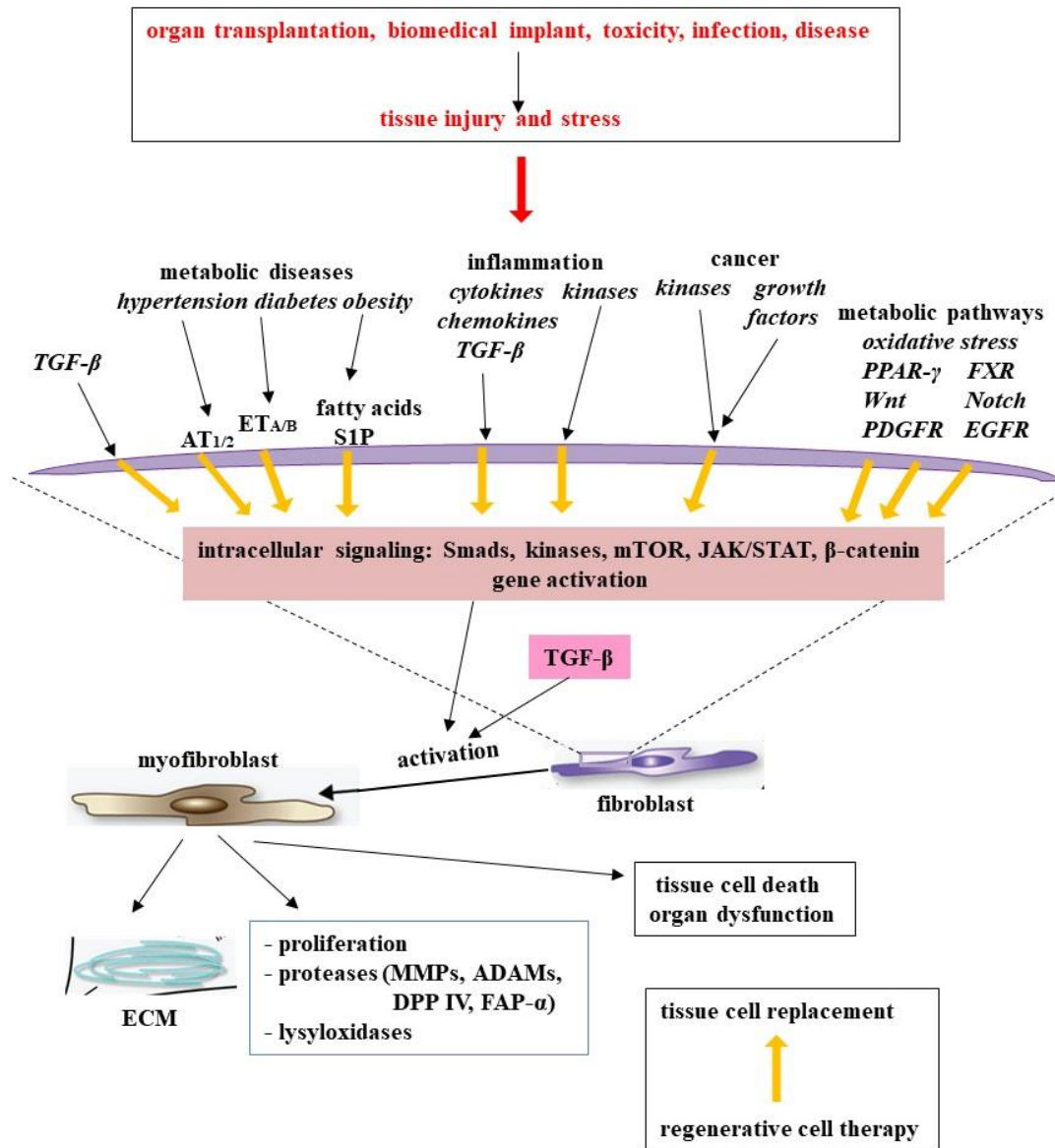
Schema 1. Schematic representation of cellular pathways mediating fibrosis.

3. Anti-fibrotic therapeutics.

A large amount of potential therapeutics against fibrotic diseases have been designed, prepared and evaluated in vitro, in animal experimental models (**Figures 2-10**) and for some of them in clinical trials (summarized in **Table 1**). Several excellent previous review papers have described in detail the molecules that have been designed, prepared and evaluated.[40,134-137] We will not repeat here in deep all this information, but only outline the most relevant characteristics of these previously described therapeutics. Therefore, in this chapter, we will discuss a selection of small molecules developed for the prevention and therapy of fibrosis and fibrosis progression, excluding genetic tools, antibodies

and analogs of proteins. We will also not discuss in detail cell-based therapies that were recently reviewed.[102] We have chosen to discuss the development of therapeutics according to the physiological pathways targeted, rather than the affected organ/tissue in an attempt to extract relevant information of more general interest for anti-fibrotic therapy in human diseases.

3.1. Clinically approved drugs for the prevention and treatment of fibrosis.

Presently, only very few drugs, in particular **1** (pirfenidone, trade names Esbriet, Pirespa, Etuary, structure in Figure 2) and **63** (nintedanib/BIBF1120, trade name Ofev, structure in Figure 9) have been specifically approved for clinical use as anti-fibrotic therapeutics. These two drugs have been shown in several clinical trials (the ASCEND, CAPACITY, TOMORROW and INPULSIS trials) to reduce the decline in lung function in patients with IPF and have been approved for this indication. **1** is an anti-fibrotic drug able to suppress fibroblast proliferation and to downregulate the production of growth factors, including TGF-β, and of procollagens I and II. It is also under evaluation for the treatment of other fibrotic processes, including renal, cardiac and liver fibrosis and abnormal wound healing.[138-141] **1** was evaluated in the CAPACITY-004 and -006 clinical trials, resulting in its approval by the FDA for IPF in 2014. **63** is an intracellular inhibitor of multiple receptor-associated tyrosine kinases, including PDGFR. **63** was evaluated in the INPULSIS-1 and -2 phase III clinical trials as an oral treatment for IPF, resulting in its approval by the FDA for this indication in 2014. In addition, several drugs designed, developed and clinically approved for other indications have been shown to be clinically interesting for the treatment of fibrotic conditions. **17** (tranilast, trade name Rizaben, structure in Figure 3) is a drug inhibiting IL-6 production, initially approved for allergic bronchial asthma. Therapeutic indication for hypertrophic scars was added later. In vitro it reduces collagen synthesis in fibroblasts.[142] and has been shown to also display modest anti-TGF-β activity, however, with disappointing results in the PRESTO study.[143] Roflumilast (**127,** trade names Daxas, Daliresp, structure in Figure 13), an orally active, selective, long-acting inhibitor of the enzyme PDE-4 with anti-inflammatory effects, was approved for the treatment of inflammatory conditions of the lungs.[144] **1** and **17** have adverse effects on liver function. It has to be noted that these therapeutics can slow

down the progression of the disease but cannot reverse it. Therapeutic anti-fibrotic strategies using approved drugs also include RAS antagonists, either ACEI, such as **35** (captopril, trade name Capoten) or AT_1 antagonists such as **37** (losartan, trade name Cozaar) (structures in Figure 5), as well as ET-1 receptor antagonists, such as bosentan (**40**, trade name Tracleer), macitentan (**41**, trade name OPSUMIT) or ambrisentan (**43**, trade names Letairis, Volibris) (structures in Figure 6).

3.2. Inhibitors of TGF-β signaling (Figure 2).

TGF-β1-4s are a family of multifunctional cytokines that bind to TGF-β receptors, composed of type 1 (TGF-βRI) and type 2 (TGF-βR2) receptor subunits. After the binding of TGF-β, TGF-βR2 kinase phosphorylates TGF-βR1 kinase, inducing a signaling cascade that recruits and activates the Smads proteins. Their translocation to the cell nucleus induces transcription of different effectors of downstream regulatory proteins, which results in differentiation, chemotaxis, proliferation and activation of target cells. TGF-β1 has been the most studied pro-fibrogenic factor. TGF-β1 is biosynthesized as an inactive latent peptide needing proteolytic activation by several proteases including MMPs, but also by transconformation by integrins, local pH, and reactive oxygen species. TGF-β1 key functions include regulation of inflammatory processes, ECM production, stem cell differentiation as well as T-cell regulation and differentiation. In the context of fibrosis, TGF-β signaling promotes the differentiation of quiescent fibroblasts into ECM-secreting myofibroblasts. TGF-β-dependent signaling via the Smad-3 pathway is responsible for many of its functions, therefore inhibiting TGF-β1 binding to its receptors and the associated Smad3 signaling pathway has been the target of many attempts in fibrosis. The profibrotic effects of TGF-β1-Smad 2/3 may also be antagonized by the activation of the BMP7-Smad1/5 axis, but to the best of our knowledge, no synthetic agonists for this axis have been described. Only small peptide analogs of BMP7 (AA123/THR123, Thrasos Therapeutics) have been prepared, presently in phase I clinical trials.[66] TGF-β antagonists have been examined in several clinical trials for diabetic nephropathy and were proposed to be effective in slowing fibrogenesis. GW 788388 (**2**), IN 1130 (**3**), LY 364947 (**4**), R 268712 (**5**), RepSox (**6**), SB 525334 (**7**) and ITD 1 (**8**) are potent and selective TGF-βRI inhibitors;

A 83-01 (**9**), and SB 431542 (**10**) inhibit TGF-βRI, anaplastic lymphoma kinase (ALK)-4 and ALK-7; D 4476 (**11**) inhibits TGF-βRI and casein kinase-1 (CK1); SD 208 (**12**) is a potent ATP-competitive TGF-βRI inhibitor. SIS3 (**13**), a selective Smad3 inhibitor, was shown to delay the progression of diabetic nephropathy in experimental models by reducing ECM proteins and antagonizing the effects of C5a receptor activation.[65,145] The new cynnamoylanthranilate analog FT011 (**14**) inhibits the TGF-β and PDGF pathways,[67] and is presently under clinical evaluation for the treatment of diabetic nephropathy. Hydronidone (**15**), a cyclo-oxygenase (COX) and TGF-β inhibitor, is in clinical trial for liver fibrosis, whereas PDE inhibitors, also able to decrease TGF-β, are in trials for diabetic nephropathy.[146.] Relaxin, an endogenous potent vasodilator hormone with pleiotropic effects, controls fibrosis by inhibiting TGF-β and Smads, regulating the MMP proteolytic balance and inhibiting local inflammatory response. Relaxin displayed anti-fibrotic effects in experimental models of cardiovascular diseases, but only when TGF-β1 levels were elevated.[147-150]
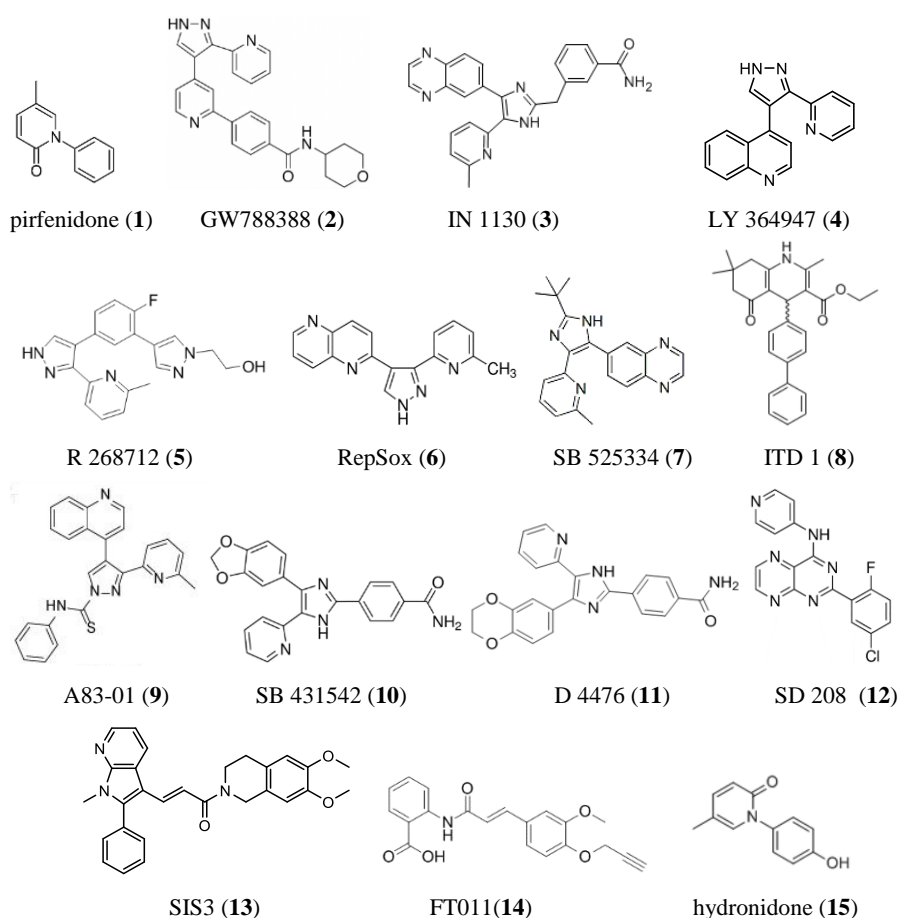

Figure 2. *Examples of inhibitors of the TGF-β signaling pathway.*

In summary, antagonizing TGF-β-associated pathways is necessary to control fibrosis, however, due to the pleiotropic effects of these pathways in normal physiological conditions, it will be necessary to develop disease-targeted and target-addressed therapeutics.

3.3. Inhibitors of inflammation and immunosuppressive drugs (Figure 3).

Although the trigger of fibrosis in different organs and clinical situations not always implies an inflammatory stress, the mechanisms leading to fibrosis frequently involve either initial or subsequent inflammation and the secretion of cytokines and chemokines by immune cells. In response to these stimuli, monocytes/macrophages infiltrate the interstitial space, perpetuating inflammation and inducing differentiation and proliferation of myofibroblasts. Then, it is generally thought that the fibrotic process progressively becomes independent of inflammation. Therefore, anti-inflammatory drugs targeting cytokine production and their cognate receptors may be relevant to be used early in the fibrogenic process. **16** and **17**, approved for bronchial asthma and hypertrophic scar, **18** and **20**, as well as **127**, an inhibitor of PDE-4 approved for chronic obstructive pulmonary disease, have also anti-inflammatory properties. The oral chemokine receptor antagonist cenicriviroc/TAK-652/TBR-652 (**19**), an inhibitor of CCR2/5 receptors, has been evaluated in the CENTAUR phase IIb study in NASH and liver fibrosis in adults at increased risk of progression to cirrhosis, but failed for this indication although it had a significant impact on fibrosis.[35] **18** and **20** are immunosuppressive drugs used in preventing transplant rejection. They inhibit the activation and effector function of T-cells, including the production of inflammatory cytokines.**20** forms a complex with cyclophilin to block the phosphatase activity of calcineurin. Thalidomide (**21**) and pomalidomide (**22**) are able to inhibit inflammation-induced angiogenesis. PBI-4050 (**23**) binds to GPR40 and GPR84 receptors, inhibiting collagen I production in epithelial cells and fibroblasts. Bardoxolone (**24**) inhibits the pro-inflammatory nuclear transcription factor NF-κB. Selective p38 mitogen-activated protein kinase (MAPK) inhibitors blocked the secretion of TNF-α and decreased cardiac fibrosis in mice,[151] and may represent a new treatment modality in humans. The RENEWAL study[152] examining the effect

of the TNF-α fusion protein antagonist etanercept in patients with heart failure was negative and the ATTACH trial was stopped prematurely as the high dose of the anti-TNF-α monoclonal antibody infliximab increased mortality in patients with moderate-to-severe chronic heart failure.[153] Statins are also anti-inflammatory and were shown to attenuate cardiac fibrosis in animal models and in a small clinical study, but the large-scale CORONA and GISSIF-HF trials displayed only a neutral effect.[154-157]

sirolimus (**16**) tranilast (**17**) tacrolimus (**18**)

cenicriviroc (**19**) cyclosporin A (**20**)

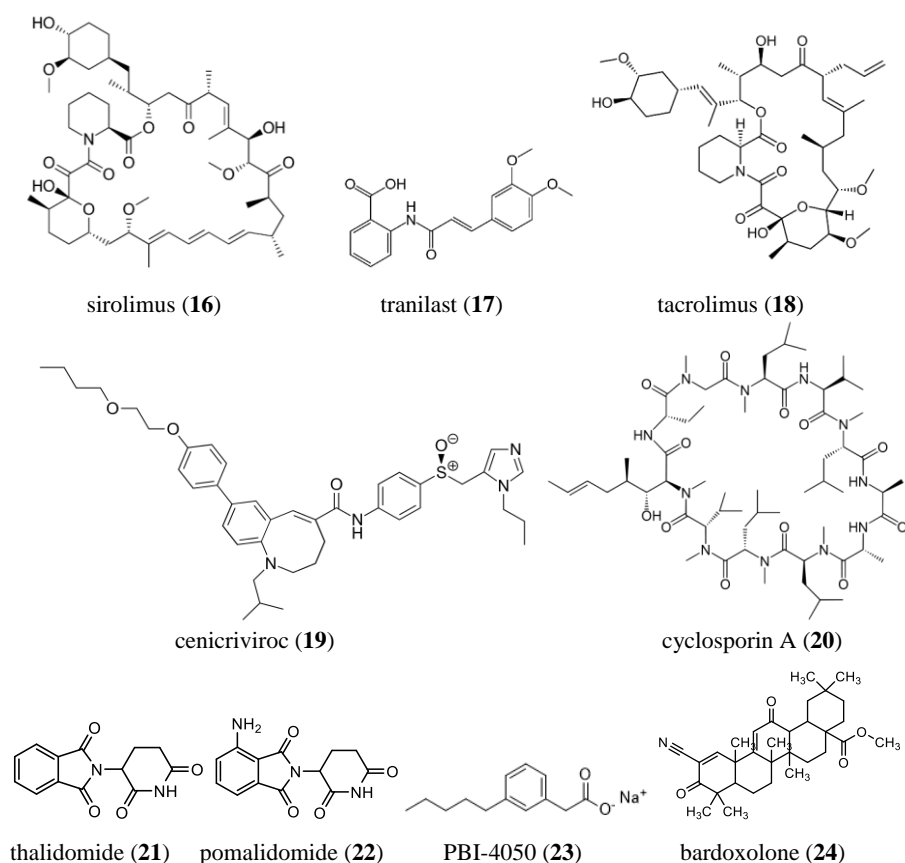thalidomide (**21**) pomalidomide (**22**) PBI-4050 (**23**) bardoxolone (**24**)

Figure 3. Examples of inhibitors of the immune/inflammatory stress.

In summary, inhibitors of inflammatory pathways are likely to be of therapeutic interest in defined organs in controlling the early phase, but not the late phase, of fibrosis development and progression.

3.4. Inhibitors of the sphingosine pathway (Figure 4).

The S1P/S1PR axis is expressed and active in many organs and tissues and involved in diverse cellular processes. Sphingosine (2-amino-4-octadecene-1,3-diol) is an unsaturated 18-carbon amino

alcohol cell membrane lipid. Sphingosine is phosphorylated in vivo by two kinases, sphingosine kinase-1 (SK1) and SK2, leading to the formation of S1P. S1P exhibits a broad spectrum of biological activities, including cell proliferation, survival, migration, cytoskeletal organization, morphogenesis and the differentiation of resident hepatic stellate cells into activated myofibroblasts. S1P is active intracellularly and can also bind extracellularly to five distinct GPCRs, $S1P_{1-5}R$, displaying both pro- and anti-fibrotic effects, depending on the context and site of action.[37] S1PR agonists and antagonists and SK inhibitors have been developed and evaluated, in particular in liver fibrosis,[38] and represent targets for the treatment of fibrosis. Side effects are observed due to the non-selective activation of different S1PRs, suggesting that receptor-specific agonists or antagonists may be preferable. The agonist FTY720/fingolimod (**25**) following its phosphorylation by SKs binds to $S1P_1R$, $S1P_3R$, $S1P_4R$ and $S1P_5R$. SEW2871 (**26**) is a selective $S1P_1R$ agonist not active on the $S1P_{2-5}R$. KRP203 (**27**) is a selective $S1P_1R$ agonist with potential immunosuppressive activity by decreasing the production by lymphocytes of inflammatory cytokines, such as interferon (IFN)-γ, IL-12 and TNF-α. VPC23019 (**28**) is a dual $S1P_{1/3}R$ antagonist. W146 (**29**) is a $S1P_1R$ antagonist with no agonist or antagonist activity on $S1P_2R$, $S1P_3R$, or $S1P_5R$. JTE-013 (**30**) is a potent (IC_{50} = 20 nM), selective $S1P_2R$ antagonist of the human and rat receptors modulating cell migration, contraction and cyclic AMP accumulation. CAY-10444/BML-241 (**31**) is a selective antagonist of $S1P_3R$, blocking calcium increase in cells. However, the selectivity of several S1P agonists and antagonists currently under development has been questioned.[158] For example, **30** also inhibited the effects of ET-1. PF543 (**32**) is a cell-permeable hydroxyl methyl pyrrolidine compound that reversibly inhibits SK1-catalyzed sphingosine phosphorylation in a sphingosine-competitive manner (K_d=5 nM), exhibiting no affinity toward S1PRs, with no effect on cell proliferation and survival. SKI-II (**33**) is a dual orally active SK1/2 inhibitor (IC_{50} = 35 and 20 μM for SK1 and SK2, respectively) which inhibits tumor growth in vivo. N,N-dimethylsphingosine (**34**), a natural metabolite of sphingosine inhibiting both SKs, induces apoptosis, decreases airway inflammation and is cardioprotective. Selectively silencing SKs or S1PRs with siRNAs has also been attempted.
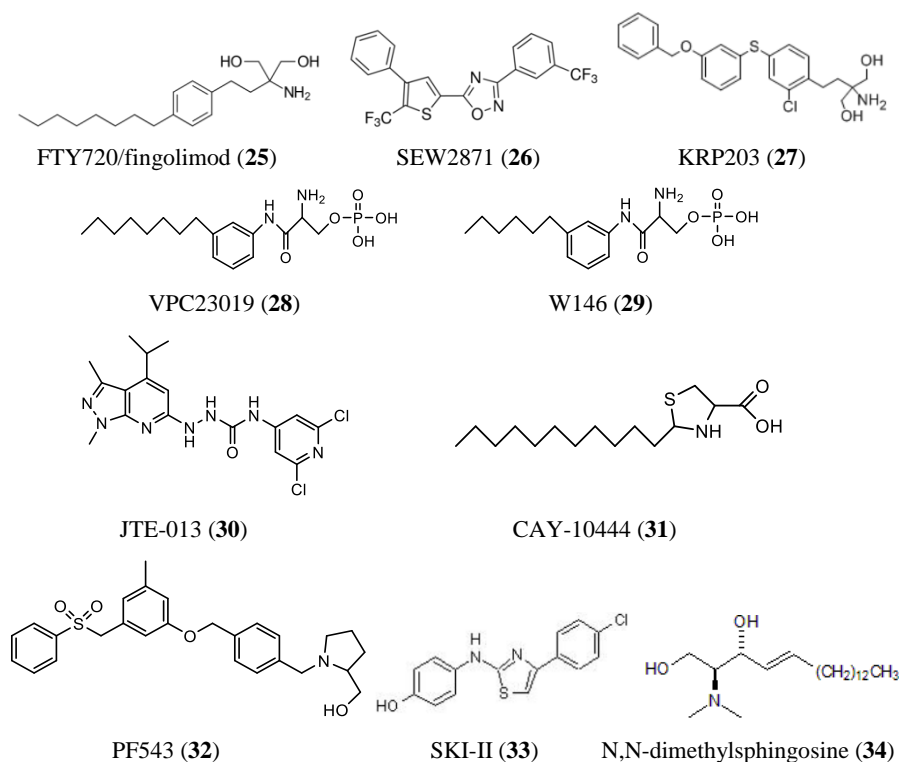
Figure 4. Examples of modulators of the sphingosine pathway.

In summary, S1PR-selective agonists and antagonists and SK inhibitors have the potential to be of therapeutic use in fibrosis. However, their target organs and their optimal molecular structures and timing of therapeutic efficacy in fibrogenesis need to be better defined.

3.5. Inhibitors of vasoactive peptides: angiotensin and endothelin (Figures 5 and 6).

Angiotensin (Ang) and endothelin (ET) peptides are pro-fibrotic, enhancing TGF-β1 production, fibroblast proliferation and activation and EMT/EndMT, effects mediated by autocrine amplifying loops. Antagonists of the Ang and ET pathways may represent interesting molecules for anti-fibrotic therapy, since several drugs are already approved for clinical use in the management of cardiovascular and hypertensive diseases.

Angiotensin pathway inhibitors (**Figure 5**). From the angiotensinogen precursor, the enzyme renin selectively releases an inactive decapeptide Ang I, further activated by ACE to the active octapeptide Ang II which acts on two GPCRs AT_1R and AT_2R. AT_1R activation is pro-fibrogenic, pro-

inflammatory and pro-oxidative.[31,159] whereas AT_2R activation counteracts AT_1R activation, being protective against fibrosis.[132] Ang(1-7) produced by the action of ACE2 and acting on the Mas receptor attenuates myofibroblasts activation,[160] hence functioning as a negative regulator of Ang II-mediated fibrosis. Ang II may be released locally by activated macrophages and fibroblasts, activating an inflammatory response, TGF-β production and signaling, and fibroblast proliferation and differentiation into ECM-producing myofibroblasts. Inhibition of the conversion of Ang I to Ang II with ACE inhibitors or blockade of AT_1R by antagonists demonstrated the role of the RAS in fibrosis. Inhibitors of ACE, including **35** or enalapril (**36**), as well as antagonists of the AT_1R, including **37** or valsartan (**38**), in clinical use for the treatment of cardiovascular disorders, have been evaluated in the context of fibrosis, showing some benefit. For example, **37** was shown in cancer models to reprogram CAFs, reduce their number and decrease TGF-β, connective tissue growth factor (CTGF) and ET-1.[161] Agonists of AT_2R have been developed. such as the AT_2R-selective non-peptidyl molecule C21 (**39**) (Ki 0.4 nM for AT_2R and 10 µM for AT_1R) are anti-fibrotic in cardiovascular and renal diseases.[132]
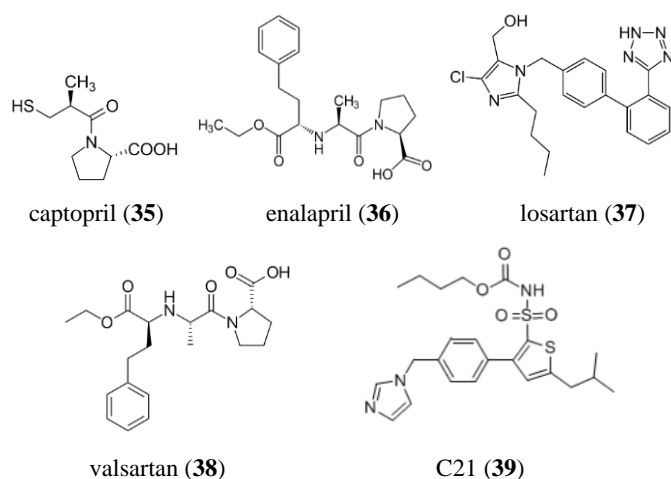

Figure 5. Examples of inhibitors of the angiotensin pathways.

Endothelin pathway inhibitors (**Figure 6**). Precursor endothelin polypeptides (ppET1-3) are proteolytically activated in two steps, first by the intracellular serine proteases subtilisin-like convertases/furins, releasing the 38 aa-long pro-ETs/big ETs, then subsequently by the more specific

membrane-bound endothelin converting enzyme-1 (ECE-1) to yield the 21aa active ET-1, ET-2 and ET-3 peptides. Following secretion, ET peptides act on two distinct high-affinity GPCRs, ET_A and ET_B, located on target cell membranes and signaling through the MAPK pathway. The ET-1 axis plays a fundamental role in the pathogenesis of fibrosis,[162,163] mediating the profibrotic effects of TGF-β. ET-1 is chemotactic for cells and induces fibroblast proliferation, ECM accumulation and contraction, mediated by the two ET-1 receptors. Most cells express both receptors, rendering difficult to define the culprit receptor in the context of fibrosis. A consensus exists that dual $ET_{A/B}$ antagonists are probably to be preferred.[42,163] Antagonists for the ET receptors, either dual $ET_{A/B}$ or ET_A-selective, are in clinical use for the treatment of pulmonary hypertension.[42] Receptors antagonists decrease collagen I and III synthesis by fibroblasts. Several phases II or III clinical trials were performed in patients with fibrosis-associated diseases. The dual $ET_{A/B}$ receptor antagonist **40** was evaluated in the BUILD-1 and -3 trials in lung fibrosis and in the RAPIDS-1 and -2 trials in skin fibrosis. The dual $ET_{A/B}$ receptor antagonists **41** and enrasentan (**42**) were evaluated in the MUSIC trial in lung fibrosis and in heart fibrosis, respectively. The ET_A-selective antagonist **43** was evaluated in the ARTEMIS trial for lung fibrosis. The ET_A-selective antagonists avosentan (**44**), sitaxentan (**45**) and darusentan (**46**) were evaluated in the ASCEND trials for kidney fibrosis and in the EARTH trial for heart fibrosis; whereas the ET_B-selective BQ-788 (**47**) was evaluated only in liver fibrotic diseases. All trials showed mixed outcomes, mostly being ineffective to reverse fibrosis, with the exception of **40**, which has received clinical approval for digital ulceration in systemic sclerosis. ET_A-selective antagonists demonstrated some reduction of proteinuria in kidney fibrosis, but they are associated with side effects when prescribed in combination with RAS blockade.[42,163] The dual ET_A/AT_1R antagonist, sparsentan/RE-021/retrophin/BMS456567 (**48**) is presently in a phase II trial for diabetic nephropathy.

bosentan (**40**) macitentan (**41**) enrasentan (**42**) ambrisentan (**43**)

avosentan (**44**) sitaxentan (**45**) darusentan (**46**)
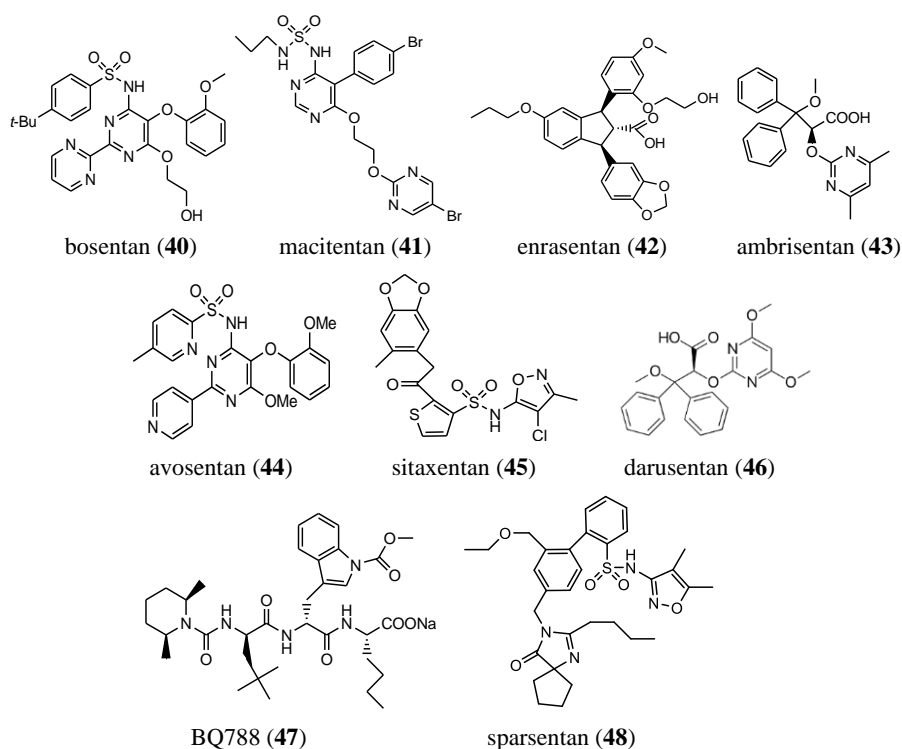
BQ788 (**47**) sparsentan (**48**)

Figure 6. *Examples of inhibitors of ET-1 receptors.*

In summary, blockers of the Ang and ET pathways may present interest in the treatment of fibrosis, mainly by their beneficial effects on associated pathologies, such as diabetes or hypertension. In the context of fibrosis, they are likely to be mandatory in combination regimens.

3.6. Enzyme inhibitors (Figures 7 and 8).

In fibrosis, two families of enzymes have crucial roles in ECM remodeling. Proteases involved in ECM degradation, the Zn-dependent proteases such as MMPs and ADAMs or the prolyl-specific serine-proteases of the DPP IV family, and enzymes involved in ECM stabilization, the lysyloxidases LOX and LOXL1-4.

Inhibitors of proteases (**Figure 7**). Inhibitors of the proteases. MMPs and ADAMs are implicated in a variety of physiological processes as well as in pathological conditions such as inflammation, cancer, fibrosis and tissue repair,[164] acting on cytokines, chemokines, adhesion and signaling molecules and structural proteins.[165] MMPs and ADAMs mediate ECM remodeling and release

fibrogenic factors including TGF-β or TNF-α, triggering inflammation and fibrosis.[166] The role of MMPs in fibrogenic diseases has been previously reviewed by several authors.[167] The first MMP inhibitors failed in clinical trials due to their low selectivity. Later on, MMP inhibitors with higher affinity and increased selectivity or MMP inhibitors targeting exosites mediating cell surface interactions and activation, were designed. Whereas MMP inhibitors, for instance **18** (structure in Figure 3) and batimastat/BB-94 (**49**) or marimastat (**50**), can control the perpetuation of fibrosis induced by MMP overproduction,[169] the PREMIER clinical trial of the MMP inhibitor PG-116800 (**51**) was not conclusive of a beneficial effect.[168] Specific inhibitors have been developed against the proteases able to process biologically active prolyl-containing peptides: DPP IV, FAP-α and POP/PREP.[72,124] DPP IV/CD26 is a co-stimulator of T-cells and a therapeutic target for type-2 diabetes. Indeed, DPP IV inhibitors, the "gliptins", are approved in the clinic for the treatment of type 2 diabetes,[72] a condition frequently associated with fibrosis. Diabetic nephropathy is associated with increased expression of DPP IV on endothelial and tubular epithelial cells. The proteolytic activity of FAP-α is pro-fibrogenic but the protein itself is a regulator of cell apoptosis, adhesion and migration. FAP-α protein and/or activity has been associated with fibrosis in many organs.[124] FAP-α inhibition increases endogenous levels of active FGF21, making FAP-α an attractive target for the treatment of liver diseases and NASH.[32,33] Synthetic POP inhibitors have been developed and evaluated mainly in the context of neurodegenerative diseases. However, as POP, and possibly also FAP-α, have been involved in the activation of the anti-fibrotic Ac-Ser-Asp-Lys-Pro peptide from thymosin-β4,[124] its inhibition in the context of fibrosis would be detrimental. The DPP IV inhibitor linagliptin (**52**) has incretin-independent anti-fibrotic effects in diabetic nephropathy, preventing renal fibrosis mediated by TGF-β. **52** has the advantage that it can be used in patients with renal dysfunction without dose-adjustment since it is not excreted by the kidney. New onset diabetes after transplantation is a common side effect of immunosuppressive therapies, which could be improved by the DPP IV inhibitor vildagliptin (**53**).[98] In a murine model of lung allograft, **53** promoted graft acceptance by reducing T-cell infiltration and modulating cytokine expression.[99] In DPP IV- and likely FAP-α-positive fibroblasts, able to deposit ECM in response to fibrogenic stresses, diprotin A/Ile-Pro-Ile

(**54**), a competitive substrate of DPP IV-like proteases could reduce scar formation.[16,17] The only FAP-α inhibitor which has been evaluated in clinical trials is the small molecule dual DPP IV/ FAP-α inhibitor PT-100/ValboroPro/talabostat (**55**). Clinical trials of **55** demonstrated positive response in a phase II trial of stage IV melanoma patients.[124] Dabigatran (**56**) is in clinical use to inhibit thrombin-induced fibroblast proliferation, presently under consideration for clinical trial.
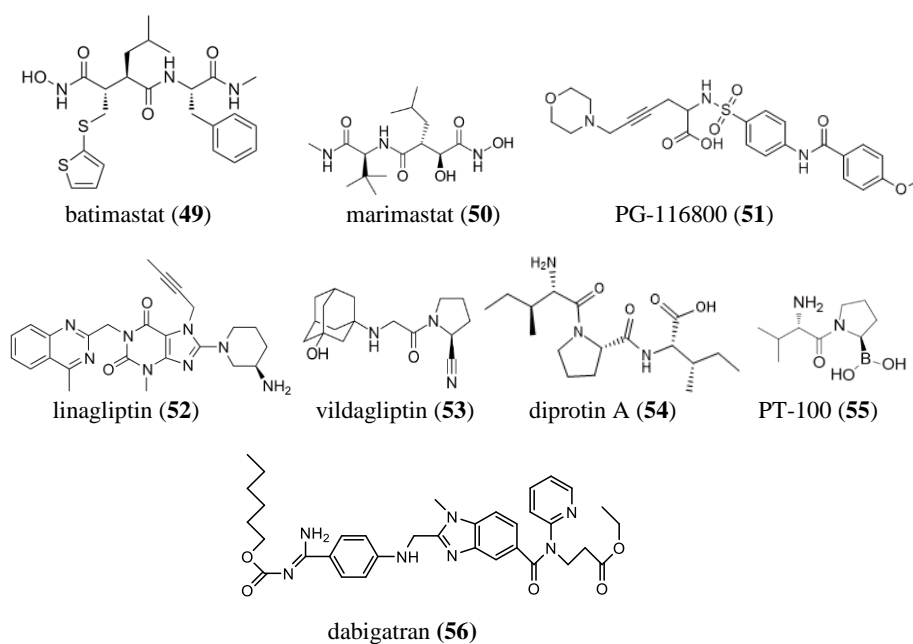

batimastat (**49**)   marimastat (**50**)   PG-116800 (**51**)

linagliptin (**52**)   vildagliptin (**53**)   diprotin A (**54**)   PT-100 (**55**)

dabigatran (**56**)

Figure 7. *Examples of protease inhibitors.*

Inhibitors of lysyloxidases (**Figure 8**). LOX and LOXL-1–4 enzymes are a family of lysine-tyrosylquinone-dependent copper amine oxidases which are upregulated by TGF-β1 before the appearance of fibrotic lesions. These enzymes catalyze the oxidation of ε-amines of lysine residues within collagen, generating reactive aldehydes that condense to form covalent collagen cross-linkages in the ECM.[170,171] Crosslinking by LOX and LOXLs not only stabilizes collagen fibers, rendering them more resistant to degradation, but also contribute to myofibroblast activation due to increased stiffness, causing progression of interstitial fibrosis.[172,173] Specific LOXL inhibitors could prevent fibrosis. β-Aminopropionitrile (**57**, BAPN), a small molecule inhibitor of the LOX family, improved the outcome of experimental liver and reversed cardiac fibrosis.[174-176] Based on this lead, LOX(L) inhibitors have been designed. PAT-1251 (**58**) was identified as a potent, highly selective, irreversible

inhibitor of LOXL2, significantly reducing fibrosis in mouse bleomycin-mediated lung injury models. It has completed a healthy volunteer phase I trial.[34] CCT365623 (**59**) successfully disrupted LOX signaling pathway by decreasing the EGFR pathway in cancer models.[177] Out of two series of potent chemical inhibitors for LOXL-2, either para-substituted benzylamines or 2-substituted pyridin-4-ylmethanamines, the most potent and reversible inhibitor was the 2-chloropyridin-4-yl)methanamine (**60**), selective for LOXL-2 compared to LOX.[34] Other selective inhibitors under development with wide application from fibrotic disease to cancer include the 3-fluoro-4-aryloxyallylamine inhibitors PXS-S1A (**61**) able to significantly reduce the activation of CAFs in cancer models,[178] as well as collagen accumulation and cross-linking in CCl4-induced liver fibrosis. Gilead Sciences is testing simtuzumab, a recombinant humanized monoclonal antibody against LOXL2 designed as an immunomodulator for the treatment of fibrosis. It is in multiple phase II trials for several organ fibrosis. Tipelukast/MN-001 (**62**) is an oral dual leukotriene receptor antagonist/LOXL-2 inhibitor, which also inhibits PDE-3 and PDE-4, as well as 5-lipoxygenase. It downregulates collagen type 1, MMP inhibitors, pro-inflammatory chemokines and is presently being evaluated in a phase II trial.
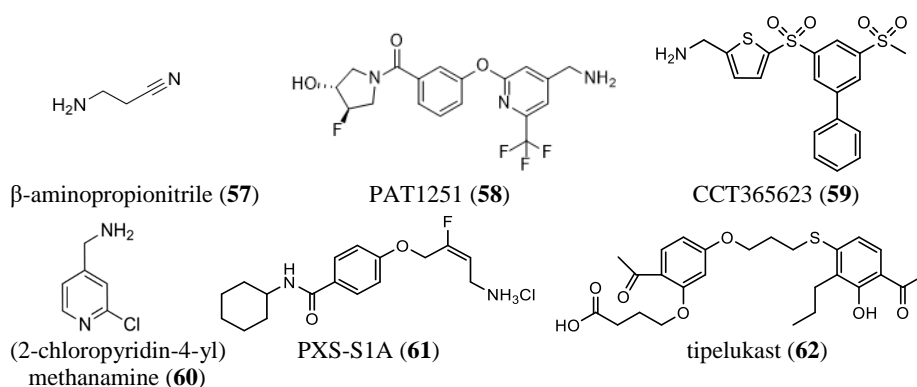

Figure 8. *Examples of LOX and LOXL inhibitors.*

In summary, inhibiting the activity of proteases and lysyloxidases in the early phase of fibrosis development is relevant. However, more information is necessary to ascertain their effect in progression and late phase of fibrosis. Combination therapies would probably be necessary.

3.7. Inhibitors of kinases and cellular signaling pathways (Figures 9 and 10).

Several kinases and cellular signaling pathways participate in the development and progression of fibrotic processes. These include the EGFR-MAPK, IGF1R-Akt, JAK3/STAT6, Wnt/β-catenin and Notch signaling pathways, CXCL12/ SDF1 and its CXCR4 receptor, integrins and cadherins. Thus, controlling activation of these pathways with antagonists and/or inhibitors has been attempted, but reaching selectivity for one pathway over the others is difficult. Moreover, many of these pathways are involved in tissue homeostasis in normal physiological conditions. It is impossible in this Perspective to describe all pathways and molecules; thus, we present a selection that we hope is representative of the attempts made.

Kinases inhibitors (**Figure 9**). EGFR (ErbB-1; HER1 in humans) belongs to a family of receptors for members of the EGF protein ligands. Overexpression of EGFR signaling is associated with the development of a wide variety of diseases. Upon activation by its growth factor ligands, EGFR dimerizes which stimulates its intrinsic intracellular protein-tyrosine kinase activity. The resulting autophosphorylation initiates signal transduction cascades, principally the MAPK-ERK, PI3K-Akt-mTOR and STAT/JNK pathways, leading to cell activation, proliferation and migration. Interruption of EGFR signaling can be achieved either by blocking EGFR binding sites on the extracellular domain of the receptor or by inhibiting intracellular tyrosine kinase activity. Therapeutics directed against EGFR include small molecule kinase inhibitors or antibodies, targeting the ligand binding site or the downstream signaling pathways. CAFs have also been shown to activate the insulin-like growth factor 1 receptor (IGF1R)-Akt signaling,[123] raising interest for antagonists of these receptors. However, due to the similarity of IGF-1R and the insulin receptor structures, especially in the ATP binding site and tyrosine kinase domain, side-effects can be expected. Selectivity of inhibitors for specific kinases has been generally difficult to achieve and several small molecule drugs display multikinase inhibition, suggesting that the design of targeted analogs will be necessary. **16** (structure in Figure 3), an immunosuppressive drug targeting mTOR, is being evaluated in a double-blind

placebo-controlled trial. **63**, clinically approved for IPF, is a tyrosine kinase inhibitor of VEGFR, FGFR and PDGFR. Dasatinib/BMS-483525 (**64**), a multikinase inhibitor, in association with the natural antioxidant **110** (structure in Figure 11) is presently being evaluated in a phase I trial. Suramin (**65**) is a polysulphonated naphthylurea with potential anti-tumor activity, able to block the binding of various growth factors, including IGF-I, EGF, PDGF and TGF-β to their receptors, thereby inhibiting cell proliferation and migration. Sunitinib (**66**), a multikinase inhibitor, was developed as a kidney-targeted therapeutic by conjugating the analog 17864 (**67**) to the kidney-specific enzyme lysozyme, but this did not result in anti-fibrotic effects.[179] Cabozantinib (**68**) is a small molecule inhibitor of the tyrosine kinases c-Met, VEGFR2, AXL and RET. Omipalisib/GSK2126458 (**69**) is a highly selective and potent inhibitor of p110α/β/δ/γ and mTORC1/2 with Ki in the low nM range, able to decrease mitogenic fibroblast responses through inhibition of the PI3K/Akt/mTOR pathway. It has been already tested in a completed phase I trial. KD025/Slx-2119 (**70**) is an orally available selective ROCK2 versus ROCK1 inhibitor, with IC_{50} and K_i of 100 nM and 40 nM, respectively, able to reduce the secretion of the IL-21 and IL-17 proinflammatory cytokines by leukocytes. It is presently evaluated in an ongoing phase II trial. Tanzisertib/CC930 (**71**), a potent, selective, and orally active anti-fibrotic inhibitor of the MAPK/JNK pathway (IC_{50} values 61 nM, 7 nM, 6 nM, 480 nM, and 3400 nM for JNK1, JNK2, JNK3, ERK1, and p38α, respectively) was evaluated in a phase II trial for the treatment of IPF. CC90001 (**72**), a second generation JNK inhibitor, selective for JNK1, has completed phase I and is presently evaluated in a phase II trial.[180] Several EGFR kinase inhibitors have been approved for clinical use, mostly in the context of cancer. Gefitinib/ZD1839 (**73**) was the first selective antagonist of EGFR, inhibiting the ATP-binding site of the enzyme and the anti-apoptotic Ras signal transduction cascade. Erlotinib (**74**), the second approved EGFR inhibitor, reversibly binds to the ATP site of the receptor. Afatinib (**75**), is an irreversible covalent inhibitor of EGFR and ErbB-2/HER2. Lapatinib (**76**), is an orally active dual HER2/EGFR inhibitor of the ATP-binding pocket of the kinase domains. Brigatinib/AP26113 (**77**), is a dual ALK/EGFR inhibitor, able to overcome resistance conferred by the EGFR C797S mutation when combined with an anti-EGFR antibody. Icotinib/BPI-2009H (**78**) is a highly selective, first generation EGFR tyrosine kinase

inhibitor, solely approved and marketed in China. Osimertinib/AZD9291 (**79**) is a third-generation irreversible and specific inhibitor of T790M or L858R mutated EGFR or of EGFR with exon 19 deletion. Imatinib (**80**, Glivec) and sorafenib/BAY43-9006 (**81**) are active on PDGFR and VEGFR. Synthesizing selective inhibitors of IGF-1R is difficult, but includes ChEBI:75252 (**82**), BMS-754807 (**83**) and NVP-AEW541 (**84**). The tyrphostins AG538 (**85**) and AG1024 (**86**) are in early pre-clinical testing. They do not appear to be ATP-competitive and show some selectivity towards IGF-1R. K252a (**87**), a staurosporine analog, is a cell permeable inhibitor of CaM kinase and phosphorylase kinase (IC_{50} = 1.8 and 1.7 nM, respectively). Monoclonal antibodies, such as figitumumab, are probably the most specific and promising therapeutic compounds currently undergoing trials.
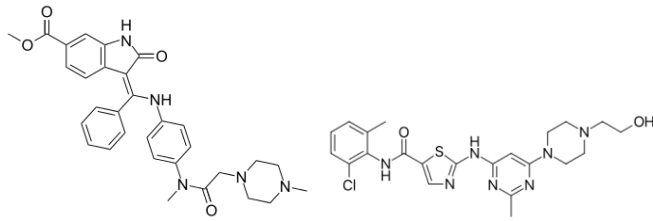
nintedanib (**63**)

dasatinib (**64**)
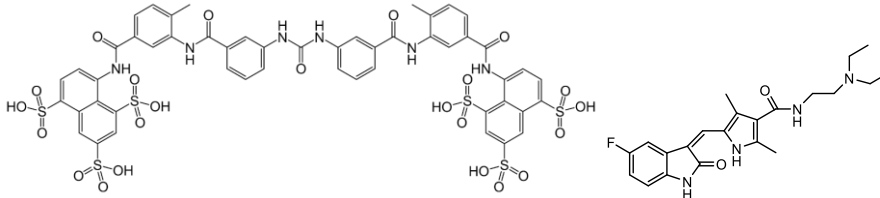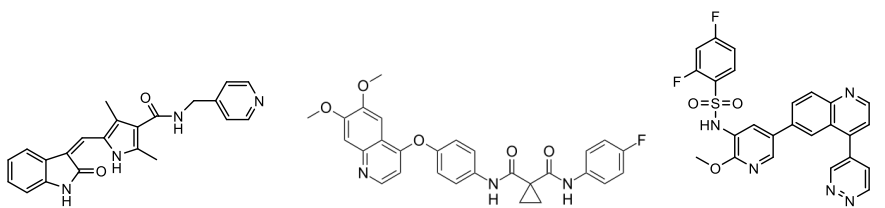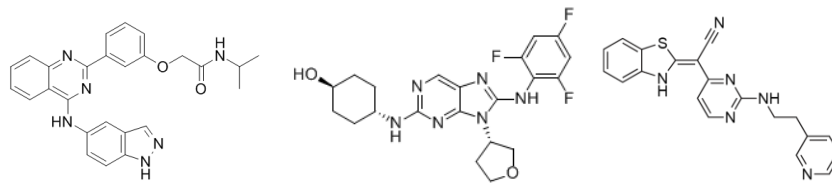
suramin (**65**)

sunitinib (**66**)

sunitinib analog 17864 (**67**)

cabozantinib (**68**)

GSK2126458 (**69**)

KD025/SLX-2119 (**70**)

tanzisertib (**71**)

CC90001 (**72**)
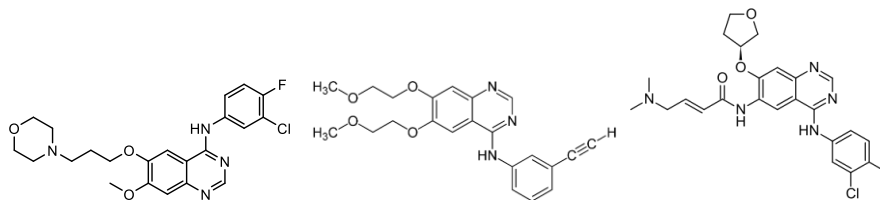
gefitinib (**73**)

erlotinib (**74**)

afatinib (**75**)
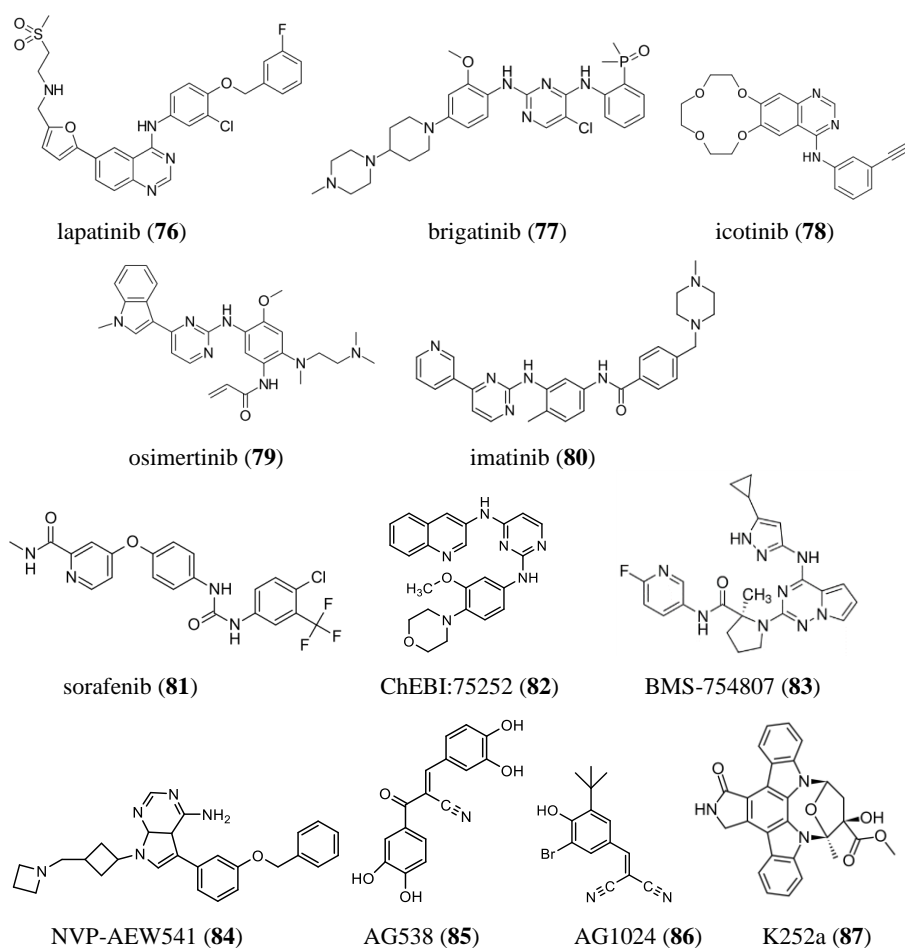
Figure 9. Examples of kinase inhibitors.

Inhibitors of cellular signaling pathways (**Figure 10**). Activation of fibroblasts depends on the profibrotic cytokines IL-4 and IL-13, resulting in activation of JAK3 and phosphorylation of STAT6, which translocates to the nucleus and promotes responsive gene transcription, production of ECM proteins (fibronectin and collagen I) and fibrosis. These effects can be antagonized by JAK3 inhibition.[70] Tofacitinib/CP-690550 (**88**) is a selective JAK3 inhibitor with an IC_{50} of 1 nM, that governs lymphocyte survival, proliferation, differentiation, cytokine and chemokine production and apoptosis. Treatment with **88** was shown to significantly reduce myofibroblast transformation and fibrosis development in a murine model of kidney fibrosis.[70] ZM 39923 (**89**) is a dual JAK1/3 inhibitor. WHI-P154 (**90**) is a JAK3 inhibitor with an IC_{50} of only 1.8 μM, but selective versus JAK1 or JAK2, preventing STAT3, but not STAT5 phosphorylation, and also inhibiting EGFR, VEGFR and MAPK. Cerdulatinib/PRT-062070 (**91**) is an orally active non-specific kinase inhibitor with an

IC_{50} of 12 nM, 6 nM or 8 nM for JAK1, JAK2 or JAK3, respectively. The Wnt/β-catenin-TCF signaling pathways are a group of highly conserved cell surface receptors and *signal transduction* pathways, mediating sustained fibroblast activation in fibrotic diseases, including systemic sclerosis. The binding of a Wnt-protein ligand to a receptor of the Frizzled family activates downstream signaling to the Dishevelled protein, which leads to gene transcription, cytoskeleton reorganization, intracellular calcium regulation and cell proliferation and migration. The Wnt-dependent pathways are necessary in embryo development, tissue regeneration and cancer progression, making interferences with these pathways prone to side-effects. PKF118-310 (**92**) disrupts the TCF4/β-catenin complex and inhibits the expression of TCF4-responsive genes and of survivin. ICG-001/PRI-724 (**93**) antagonizes Wnt/β-catenin/TCF-mediated transcription by specifically binding to CREB-binding protein (CBP) with an IC_{50} of 3 μM, it is presently in phase II clinical trials.[92] XAV-939 (**94**) selectively inhibits Wnt/β-catenin-mediated transcription through tankyrase1/2 inhibition with an IC_{50} of 11 nM, it does not affect NF-κB or TGF-β.[92] Lectin antagonism has also been examined. TD139 (**95**) is a high-affinity inhibitor of galectin-3 carbohydrate binding domain with a K_d of 14 nM able to decrease TGF-β1–induced β-catenin phosphorylation and translocation to the nucleus, reducing fibrosis. In a completed phase Ib/IIa clinical trial for IPF, it was shown that inhaled **95** is effective, safe and well-tolerated. The Notch signaling pathway is a highly conserved cell signaling system. The single-pass transmembrane receptor Notch is activated by direct cell-cell contact. Binding of protein ligands to the extracellular domain induce sequential proteolytic cleavage, in particular involving γ-secretase, releasing the intracellular domain, which migrates to the cell nucleus to modify gene expression. We have previously reviewed in detail Notch inhibition and the associated therapeutics.[85] Notch antagonism is mainly based on the use of γ-secretase inhibitors, such as MK-0752 (**96**, IC_{50}=5 nM, LY411575 (**97**, IC_{50}=0.39 nM), RO4929097 (**98**, IC_{50}=5nM), avagacestat/BMS-708163 (**99**, IC_{50}=58 nM), semagacestat/LY450139 (**100**, IC_{50}=14 nM) and LY-900009 (**101**, IC_{50}=27 nM). Inflammatory signaling has also been targeted. The CXCR4 and CCR2 receptors are inhibited, respectively, by plerixafor/AMD3100 (**102**), recently approved for hematopoietic stem cell mobilization, and CCX140B (**103**). Antagonists have been developed for the

c-Met/hepatocytes growth factor receptor/scatter factor (HGFR/SF). HGFR/SF tyrosine kinase is essential for wound healing and angiogenesis and the cMet ligand expressed on tumor cells is involved in tissue invasion and metastasis. SU11274 (**104**) is a selective c-Met inhibitor with an IC_{50} of 10 nM inactive on PDGFRβ, EGFR and Tie2. PHA665752 (**105**) is a potent, selective ATP-competitive c-Met inhibitor with an IC_{50} of 9 nM. Antagonists to the prostacyclin receptor, such as the PGI2 analog treprostinil (**106**), to the purinergic P2X2/3 receptor, such as gefaxinant/AF219/MK7264 (**107**) or to Hedghog, such as vismodegrib (**108**), are in clinical trials. Controlling the integrins and cadherins-associated pathways is also of interest in fibrotic therapies[181] as the integrin $α^3β^1$ and the cadherin-11 adhesion molecule are mediators of tissue fibrosis and the integrin $α_vβ_6$ activates latent TGF-β. Naphthyridine derivatives, such as GSK3008348 (**109**) have been developed for the treatment of fibrotic diseases as antagonists of the integrin $α_vβ_6$.

CP-690550/tofacitinib (**88**)   ZM 39923 (**89**)   WHI-P154 (**90**)

cerdulatinib (**91**)   PKF118-310 (**92**)   ICG-001/PRI-724 (**93**)   XAV939 (**94**)

TD139 (**95**)   MK-0752 (**96**)   LY411575 (**97**)

RO4929097 (**98**)   avagacestat (**99**)   semagacestat (**100**)   LY-900009 (**101**)
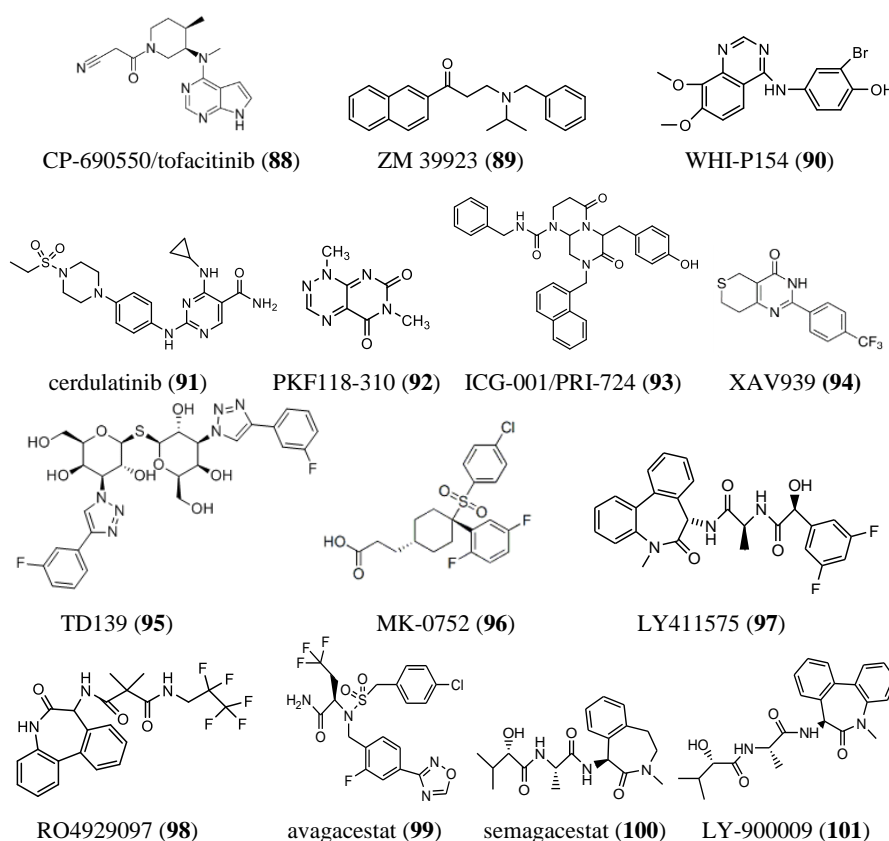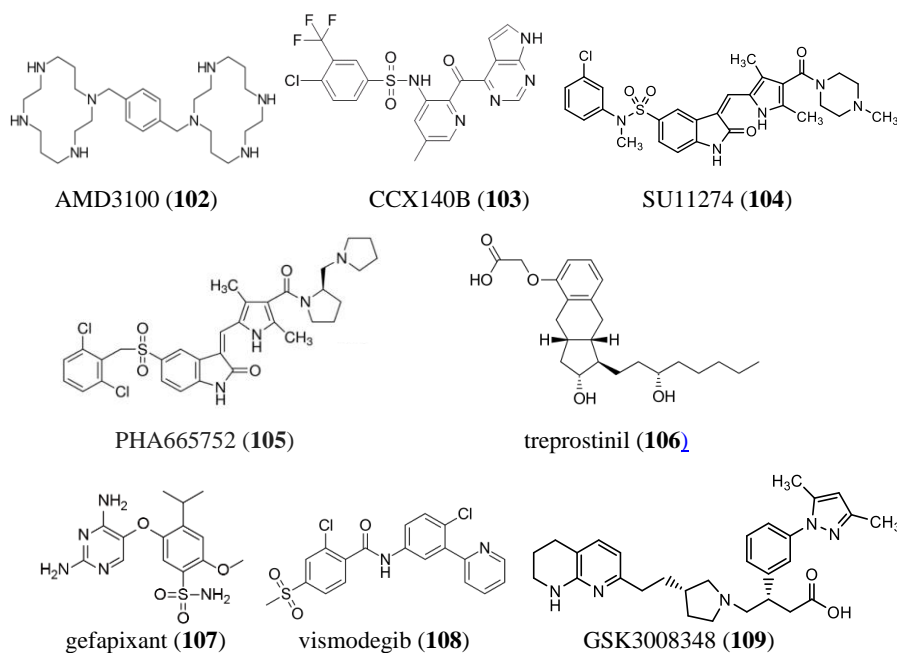
Figure 10. *Examples of inhibitors of cell signaling pathways*.

In summary, inhibitors of kinases and antagonists to cellular pathways are likely to play an indirect role by modifying the functions of activated myofibroblasts as well as fibrosis-associated cells other than fibroblasts. More information from the outcome of clinical trials is needed to define the most relevant pathway(s).

3.8. Inhibitors of metabolic pathways (Figures 11 to 14).

Several metabolic pathways, including oxidative stress, FXR and PPAR receptors and fatty acid synthesis have been targeted with small molecules in the aim to achieve treatment of fibrosis and able to modulate the level of activity of these pathways.

Inhibitors of oxidative stress (**Figure 11**). In humans, oxidative stress is involved in the development of many diseases, including fibrosis. Oxidative stress reflects an imbalance between the production of reactive oxygen species (ROS) and detoxification mechanisms. Oxidative stress disrupts normal mechanisms of cellular signaling and damages all components of the cell, including proteins, lipids and nucleic acids. Oxidative stress plays a role in the inflammatory cascade in ischemic-reperfusion injury, an important problem in solid organ transplantation procedures. But some ROS also act as

cellular messengers in redox signaling, and the immune system uses the lethal effects of oxidants in its mechanism of killing pathogens. ROS under normal conditions in humans are produced by the mitochondria during oxidative phosphorylation as well as by various oxidases. Some organic compounds, such as quinones cycling with their conjugate semiquinones and hydroquinones, in addition to metal redox catalysts can produce ROS. Cellular antioxidant enzymes encompass superoxide dismutase, catalase, glutathione peroxidase, glutathione-S transferases and various aldehyde dehydrogenases. Several physiological pathways, such as TGF-β signaling and the RAS, as well as some pathologic conditions, such as type-2 diabetes, have been associated with oxidative stress. Therefore, drugs developed to control these pathways are of interest in controlling oxidative stress. In addition, small molecules have been developed as more specific anti-oxidative therapeutics. The natural anti-inflammatory and anti-fibrotic compounds **110** and curcumin (**111)** are antioxidant, protecting DNA and regulating the immune system. Melatonin/N-acetyl-5-methoxytryptamine (**112**), a regulator of circadian rhythm, S-nitroso-N-acetylcysteine (**113**), a nitric oxide donor and antioxidant, regulates proteolytic balance, collagen deposition and TGF-β activation.[182] α-Lipoic acid (**114**) is a mitochondrial fatty acid organosulfur compound essential for aerobic metabolism. As a dietary supplement it is an antioxidant, with protective effects in inflammatory diseases. The approved antioxidant and free-radical scavenger compounds edaravone (**115**) can reduce cardiac fibrosis by decreasing TGF-β1/Smad2/3 signaling, collagen I synthesis and AT_1R signaling, while up-regulating AT_2R. They also decrease the recruitment of macrophages and myofibroblasts to the myocardium,[183,184] attenuating or even reversing fibrosis as shown in animal and clinical studies. The antioxidant NOX1/4 inhibitor GKT137831 (**116**) is in phase II for diabetic nephropathy.
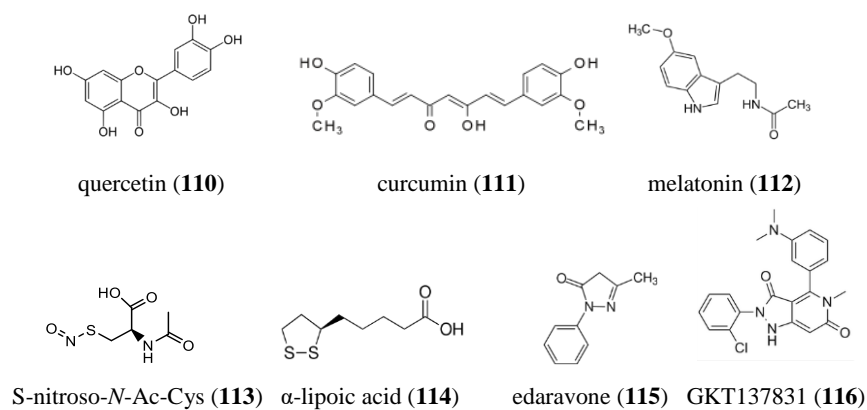
quercetin (**110**)   curcumin (**111**)   melatonin (**112**)

S-nitroso-*N*-Ac-Cys (**113**)   α-lipoic acid (**114**)   edaravone (**115**)   GKT137831 (**116**)

Figure 11. Examples of inhibitors of oxidative stress.

PPAR and FXR agonists (**Figure 12**). FXR is a nuclear receptor that, when activated, translocates to the cell nucleus, where it forms a heterodimer with retinoid X receptor (RXR) and binds to hormone response elements on DNA. This regulates gene expression, in particular cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in bile acid synthesis and hepatic triglycerides. FXR agonists are under investigation in early clinical and preclinical trials as potential therapeutics for NASH. Several FXR agonists are presently in phase I or II development for NASH, such as **117** and **118**. The synthetically modified bile acid obeticholic acid/ocaliva (**119**) is a potent agonist of FXR used to treat liver diseases. The FXR agonists EDP-305 (structure not disclosed) and tropifexor/LJN452 (**120**) were shown to perturb FXR-dependent gene expression and reduce hepatocyte ballooning and liver fibrosis in animal models. **120** was successfully tested in the phase II FLINT trial, showing a reduction of fibrosis and scaring. oral antidiabetic agents belonging to the thiazolidinedione class or GS-9674 (**121**). FXR can interact with PPAR-γ coactivator 1-alpha. The PPAR subfamily of nuclear receptors can form heterodimers which regulate transcription of various genes. The nuclear PPAR-γ/glitazone regulates fatty acid storage and glucose metabolism and is implicated in the pathology of numerous diseases. Many naturally occurring agents directly bind with and activate PPAR-γ, including various polyunsaturated fatty acids like arachidonic acid. PPAR-γ agonists decrease the inflammatory response. Many insulin-sensitizing drugs, the "thiazolidinediones", used in the treatment of diabetes activate PPAR-γ. Compounds that more weakly activate PPAR-γ as partial

agonists, such as the medium-chain triglyceride decanoic acid, are currently under study. **118** is also a PPAR-γ agonist, acting on adipocytes, hepatocytes and muscle cells to inhibit TGF-β profibrotic effects. Its combination with anti-cholesterol agents, the "statins", and omega 3 fatty acids or vitamin E has been proposed [24,25] but only limited clinical trial data are presently available. **117** is another antidiabetic drug of the thiazolidinedione class that works as an insulin-sensitizer by binding to PPAR-γ in fat cells. Elafibranor/GFT505 (**122**), a dual PPAR-α/δ agonist,[28] developed for the treatment of metabolic disorders, and in particular NASH, produced mixed results in phase II trials.

rosiglitazone (**117**)   pioglitazone (**118**)   obeticholic acid (**119**)

tropifexor (**120**)   GS-9674 (**121**)   elafibranor (**122**)
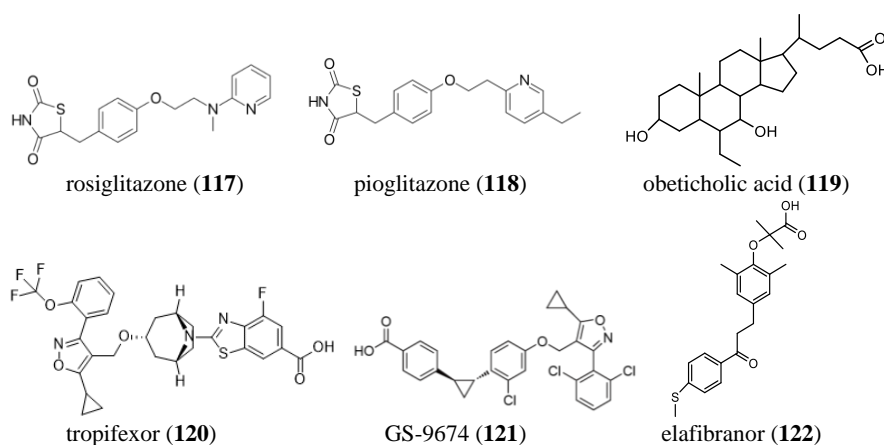
Figure 12. Examples of FXR and PPAR-γ modulators.

Fatty acid synthesis and phosphodiesterases (**Figure 13**). Lysophospholipids (LPs), which include S1P and lysophosphatidic acid (LPA), are bioactive phospholipids that transduce signals through their specific cell-surface GPCRs, $S1P_{1-5}R$ and $LPA_{1-6}R$, respectively. LPs and their receptors have been implicated in both physiological and pathological processes, including fibrosis. S1P has been discussed separately in paragraph **3.4**. Targeting the biosynthesis of fatty acids has been attempted, mostly in the liver, using either inhibitors of ACC and of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase or antagonists to the LPARs-MAPK signaling pathways, involved in the production of pro-inflammatory cytokines. NDI-010976/ND630 (**123**) is a potent liver-directed inhibitor of ACC in NASH. Pyrazole- and triazole-derived carbamates, such as RO6842262 (**124**), are selective antagonists for LPA_1R (IC_{50} 25 nM) versus LPA_3R. They inhibit the proliferation and contraction of normal human lung fibroblasts following LPA stimulation, suggesting a potential novel

approach for the treatment of IPF.[50] The clinically-approved cholesterol lowering agents, the "statins" family, diminish EMT, TGF-β signaling and oxidative stress in glomerular cells.[75,154-156] GLPG1690 (**125**), an ATX inhibitor, the major enzyme generating the LPA, is presently in a phase II clinical trial. BMS-986020/AM152 (**126**) is a LPAR antagonist presently tested in a completed phase II trial. Inhibitors of PDEs have also attracted interest as potential anti-fibrotic therapeutics. As already stated, **127**, an orally active, selective, long-acting inhibitor of the enzyme PDE-4 with anti-inflammatory effects, was approved for the treatment of inflammatory conditions of the lungs.[144] Pentoxifylline (**128**), a methylxanthine non-specific PDE inhibitor developed for vascular diseases, displaying also anti-inflammatory and anti-oxidative properties, is under one phase III and two phase IV clinical trials. The cyclic GMP-specific PDE5A-selective inhibitors sildenafil (**129**) and PF00489791 (**130**), in phase II for diabetic nephropathy and the PDE4-selective inhibitor **15**, approved for chronic obstructive pulmonary disease, are less potent therapeutics for fibrotic disorders. In the first proof-of-concept human study, **129** was shown to reduce TGF-β in IPF;[146] however, fibrosis parameters were not measured.

NDI-010976/ND630 (**123**)   RO6842262 (**124**)

GLPG1690 (**125**)   BMS-986020/AM152 (**126**)   roflumilast (**127**)

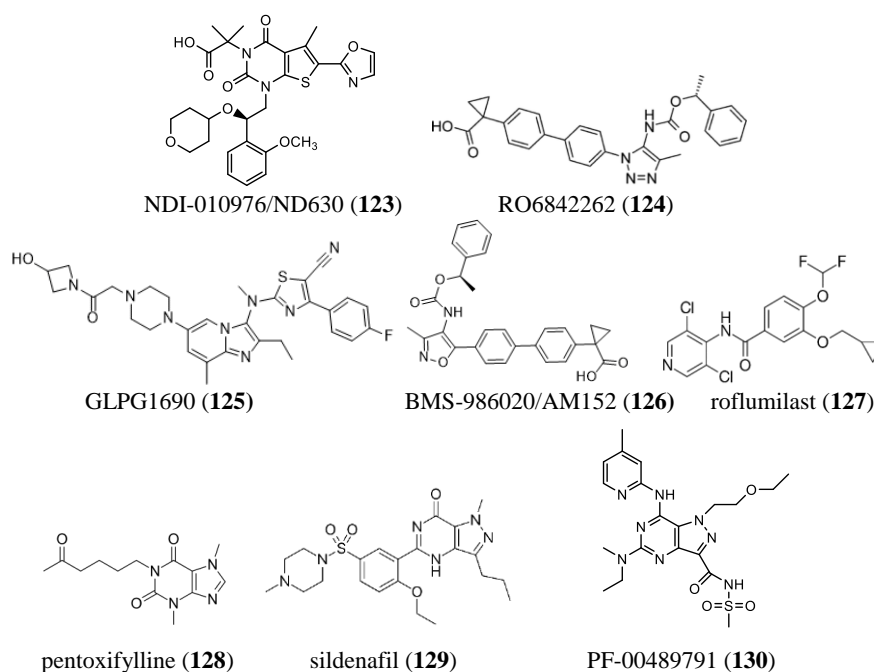pentoxifylline (**128**)   sildenafil (**129**)   PF-00489791 (**130**)

Figure 13. *Inhibitors of fatty acid synthesis and phosphodiesterases.*

Others (**Figure 14**). Therapeutic attempts have also included targeting histone H4 acetylation,

inhibition of the Ca^{2+}-activated K^+ channel (KCa3.1), the mineralocorticoid/aldosterone receptors or the vitamin D-dependent pathways. TGF-β1- or PDGF-mediated profibrotic and inflammatory responses in lung fibroblasts of patients with IPF were attenuated by the bromodomain 4 (Brd4) inhibitor JQ1 (**131**).[185] Inhibition of KCa3.1 channel by TRAM34 (**132**) suppresses TGF-β-induced upregulation of ECM-associated genes in renal fibroblasts.[69] Inhibitors of the mineralocorticoid/aldosterone receptor, such as spironolactone (**133**) tested in the RALES trial, finerenone/BAY-94-8862 (**134**) in a phase III trial, esaxerenone/CS-3150 (**135**) or PF-03882845 (**136**), can suppress fibrosis development in the heart and the kidney.[186] Stimulation of the adenosine $A_{2B}R$ has anti-fibrotic properties, mediated by antagonizing ET-1 effects on fibroblasts.[187] Adenosine receptor antagonists include caffeine, theophylline or theobromine and the pharmaceutical drug regadenoson (**137**).

JQ1 (**131**) TRAM34 (**132**) spironolactone (**133**) finerenone (**134**)

esaxerenone (**135**) PF-03882845 (**136**) regadenoson (**137**)
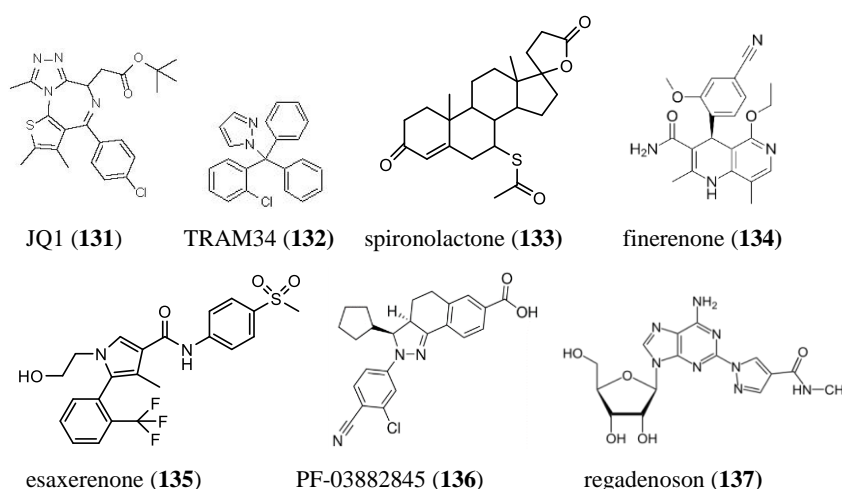
Figure 14. *Examples of various pathways modulators of potential interest in fibrosis treatment.*

In summary, modulators of metabolic pathways and FXR and PPAR-γ agonists display anti-fibrotic properties and anti-inflammatory properties. They may be of therapeutic interest in combination therapy regimens, but they are likely to act in the early phase of fibrosis.

3.9. Natural products (*Figure 15*).

Several natural products with antioxidant properties as well as immunomodulatory and anti-inflammatory activities, such as **110**, **111** or **112** (structures in Figure 11), have been evaluated as

anti-fibrotic therapeutics. Schisandrin B (**138**) reduces ROS formation, inhibits the apoptotic mitochondrial pathway and is a regulator of TGF-β signaling.[188] Astragaloside IV (**139**), a glycoside of cycloartane-type triperpene, protects against fibrosis development in several organs by attenuating ECM deposition.[189] Echinacoside (**140**) was demonstrated to inhibit the TGF-β signaling pathway.[190]

schisandrin B (**138**)   astragaloside IV (**139**)   echinacoside (**140**)

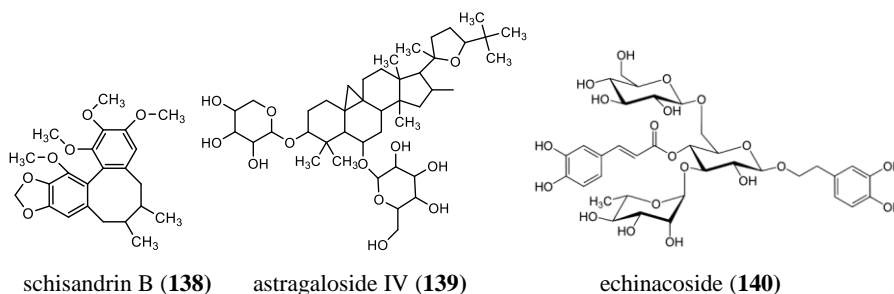**Figure 15**. *Examples of natural products of potential interest in fibrosis treatment.*

In summary, natural products display anti-fibrosis properties and may be of therapeutic interest as potential leads for the development of anti-fibrotic therapeutics.

3.10. Regenerative cell therapeutics for fibrosis treatment (Figure 16).

Healing from tissue injury depends on the regeneration of damaged tissue cells through de-differentiation of surviving cells and/or the ability of resident progenitor cells to proliferate and differentiate into new organ-specific cells. Since, for end-stage fibrotic diseases, the only treatment is organ transplantation and as donor organs are in very short supply, facilitating tissue regeneration is a key component to treating both acute and chronic organ diseases. Besides fetal-derived tissues,[101] many adult tissues contain stem and progenitor cells able to proliferate and differentiate, and to maintain tissue homeostasis and repair. In the kidney, mesenchymal stromal cells (MSCs, also called mesenchymal progenitor/stem cells) have been shown to have beneficial paracrine-mediated effects, both on the initial acute recovery from ischemia-reperfusion injury and in preventing the development of further chronic kidney disease.[78] This demonstrates that effective treatment may be possible, either through adoptive cell therapy or through stimulation of paracrine responses from resident MSCs after injury.[100] Direct transfer of autologous or allogeneic MSCs, mostly by injecting them in the blood or

following encapsulation into a polymeric device, is presently under evaluation in a large amount of clinical trials, mainly, but not only, for the treatment of acute liver injury and chronic fibrosis. Thus, efforts have been made to enhance these regenerative cell populations using small molecules. **102** (structure in Figure 10), a CXCR4 antagonist, SB497115/eltrombopag (**141**), a thrombopoietin mimetic, FT1050/16,16-dimethyl prostaglandin E2 (**142**), a PGE2 analog, and Isx-9 (**143**), a GPR68 agonist,[103] are under evaluation to enhance proliferation and differentiation of stem and progenitor cells.

SB497115 (**141**)   FT1050 (**142**)   Isx-9 (**143**)
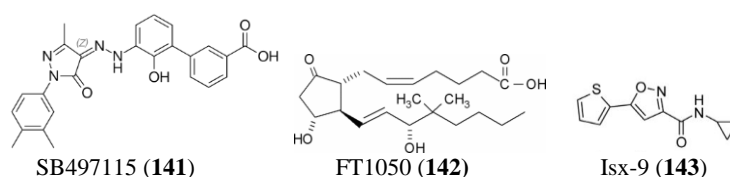
Figure 16. *Examples of small molecules of potential interest in regenerative cell therapy for fibrosis*.

In summary, recent progress in regenerative medicine has opened new therapeutic perspectives in the treatment of fibrosis, in particular the possibility to induce the repair of diseased tissues rather than reverse progression.

4. Lessons from clinical trials for the treatment of fibrosis.

Several hundreds of molecules able to interfere with the fibrotic process at each of its phases of progression have been designed, prepared and evaluated in preclinical models. The most promising of them have been evaluated in human clinical trials for possible therapy of fibrosis. Again, hundreds of trials, either phase I, II or III or IV trials, have been performed, much too many to discuss all of them in this Perspective. In this part, we will focus the discussion on clinical trials, either terminated or ongoing, which have the potential to provide therapeutics for the prevention and treatment of fibrosis. We will concentrate on small molecule drugs, excluding antibodies, fusion proteins, modified proteins or protein fragments, or genetic tools. Toward this aim, we have compiled the US clinical trials registry (ClinicalTrials.gov), published reviews and original manuscripts describing and

discussing the outcomes of these trials.[5,6,42,63,82,124,135,136,163] The information obtained is summarized

in **Table 1**.

Table 1. *Selected clinical trials for small molecule modulators of fibrotic pathways.*

drug / target	*trial code*	*organ*	*phase/status/outcome*
1 / TGF-β	NCT00287729 /CAPACITY	lung	III, positive results
	NCT01366209	lung	approved
	NCT00001959	kidney GS	II, completed
	NCT00063583	kidney DN	I/II, completed
15 / COX	NCT02499562	liver	II, no information
16 / mTOR	NCT01462006	lung	II, worsening, unpublished
19 / CCR2/5	NCT02217475 /CENTAUR	liver	II, completed
	NCT03028740 /AURORA	liver	III, ongoing
21 / inflammation	NCT00162760	lung	II, completed, unpublished
22 / inflammation	NCT01135199	lung	II, withdrawn
23 / CTGF	NCT02538536	lung	II, open, completed, no results
24 / NFκB	NCT01351675	kidney	III, terminated for safety concerns
37 / AT$_1$R	NCT00298714	liver	IV, completed, improvement
	NCT00879879	lung	pilot, stabilized lung function
	NCT01150461	heart	II, completed, lower progression
	NCT01051219 /FELINE	liver	III, no information
40 / ET$_{A/B}$	NCT00070590 /Build2	lung	II, completed, no improvement
	NCT00631475 /Build3	lung	open label, failed
	NCT00319696 /RAPIDS-1	digital ulcers	III, completed
	NCT01395732 /RAPIDS-2	digital ulcers	IV, completed, approved
41 / ET$_{A/B}$	NCT00903331	lung	II, completed, no improvement

	/MUSIC		
43 / ET$_A$	NCT00879229 /ARTEMIS-PH	lung	III, fail
	NCT01051960	Ssc	IV, terminated, unknown
	NCT00768300 /ARTEMIS-IPF	lung	III, ineffective, halted
44 / ET$_A$	NCT00120328/ ASCEND	kidney	III, lower proteinuria, halted
46 / ET$_A$	EARTH	heart	ineffective
48 / AT$_1$R/ET$_A$	NCT01613118	kidney	II, active
58/ LOXL2	NCT02852551	several organs	I, safety in volunteers, completed
62 / PDE3/LO /LTR	NCT02503657	lung	II, ongoing
63 / PDGFR/VEGFR	NCT00514683 /INPULSIS	lung	II, positive results
	NCT01335464 /INPULSIS	lung	III, completed, approved
	NCT 01335477	lung	III, completed, approved
	NCT02597933 /SENCSIS	Ssc	III, ongoing
64 / PDGFR	NCT00764309	lung (Ssc)	II, completed, ineffective
69 / PI3K/mTOR	NCT01725139	lung	I, completed, no published data
70 / ROCK2	NCT02688647	lung	II, open label, ongoing
71 / JNK	NCT01203943	lung	II, discontinued for side effects
80 / PDGFR	NCT00131274	lung	II/III, failed
	NCT006677092	lung	II, completed
81 / PDGFR/VEGFR	NCT01425216	keloids	II, terminated; approved
95 / galectin-3	NCT02257177	lung	I/II, completed, no information
103 / CCR2	NCT01028963	kidney	II, completed
	NCT01440257	kidney	II, completed
	NCT01447145	kidney	II, completed
109 / integrin αvβ6	NCT02612051	lung	II, safety and tolerability
106 / prostacyclin R	NCT00703339	lung/PH, IPF	II, completed, early termination
107 / purinergic R	NCT02502097	lung /cough	II, completed, unpublished
116 / NOX1/4	NCT02010242	kidney DN	II, completed
119 / FXR	NCT01265498/ FLINT	liver NASH	II, completed

	NCT02548351 /REGENERATE	liver NASH	III, completed
120 / FXR	NCT02855164	liver	II, recruiting
122 / PPARα/δ	NCT01694849.	liver NASH	II, resolution no fibrosis worsening
123 / ACC	NCT02876796	liver	I, safety, overweight volunteers
125 / ATX	NCT02738801	lung	II, partial report, stabilization
126 / LPAR	NCT02588625	Ssc	II, completed, no results
	NCT01766817	lung	II, withdrawn
128 / PDEs	NCT00285298	kidney DN	III, completed
	NCT01382303	kidney DN	IV, ongoing
	NCT01377285	kidney DN	IV, ongoing
130 / PDE5	NCT01200394	kidney DN	II, ongoing

Drug combinations

1+63 /TGF-β +PDGF/VEGF	NCT02598193	lung	IV, tolerability, completed
	NCT02579603	lung	IV, tolerability, completed
1+108 / Hedghog+TGF-β	NCT02648048	lung	I, completed, no results
37+FOLFIRINOX / AT$_1$+therapy regimen	NCT01821729	cancer	II, ongoing
44+37 / ET$_A$+AT$_1$R	ASCEND follow-up	kidney	tolerability, no information
110+64 / anti-ox+PDGF	NCT02874989	lung	I, open label, ongoing

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ACC: acetyl-CoA carboxylase; AT$_1$R: angiotensin receptor 1; ATX: autotaxin; CCR: C-C chemokine receptor; COX: cyclooxygenase; CTGF: connective tissue growth factor; DN: diabetic nephropathy; ET$_A$: endothelin receptor A; ET$_{A/B}$: endothelin receptors A and B; GS: glomerulosclerosis; IPF: idiopathic pulmonary fibrosis; LO: lipoxygenase; LOX-L: lysyloxidase-like; LPAR: lysophosphatidic acid receptor; LTR: leukotriene receptor; PDE: phosphodiesterase; PH; pulmonary hypertension; PPAR: peroxisome proliferator activated receptor; ROCK: Rho-associated protein kinase; Ssc: systemic scleroderma.

In summary, most completed and published clinical trials were rather disappointing, showing only limited efficacy to reverse established fibrotic diseases. The majority of recent and previous trials were performed using drug monotherapy and were aimed at the treatment of IPF, many with not yet published results. Trials of combination therapies and new anti-fibrotic agents are underway.[51,137]

Two very recent clinical reviews have analyzed clinical trials performed for IPF.[135,136] both reviews concluded that compounds that have shown efficacy in preclinical studies failed to demonstrate positive effects when translated into humans, due to the limitations of animal models. TGF-β is central to fibrosis development and small molecules able to decrease TGF-β production and inhibitors of the kinase activity of the TGF-β receptors or the intracellular signaling proteins Smads have been evaluated in clinical trials. However, they were associated with adverse cardiovascular and hepatic side-effects.[191,192] Only **1**, which decreases TGF-β production, and **63**, a multikinase inhibitor, have been approved for the treatment of IPF in humans; **1** is under trials for fibrosis therapy in other organs. The endothelin and angiotensin systems are also central to fibrosis development, promoting the myofibroblast phenotype and the production of TGF-β. Drugs able to control the endothelin and angiotensin pathways are in clinical use since many years for cardiovascular and hypertensive diseases. Clinical trials have evaluated ET_A-selective, ET_B-selective or dual $ET_{A/B}$ antagonists in disorders of the lung, liver, heart, kidney and the skin.[42,154] The primary objective of reducing mortality/morbidity was generally not achieved, with however, a positive trend. In the context of digital skin ulcer, even if **40** was not able to shorten the time to healing, it was effective in preventing the formation of new ulcers, suggesting that dual $ET_{A/B}$ antagonists may be interesting tools to *prevent* fibrosis in medical situations. Ang II interaction with AT_1R stimulates fibroblast proliferation and collagen synthesis. To the best of our knowledge, only very few dedicated clinical trials have been performed to test *per se* antagonism of the RAS to treat fibrosis. Most of the trials evaluated anti-fibrotic drugs in patients already on RAS inhibition for cardiovascular or diabetic disorders. Ang II receptor blockers, ACEI or mineralo-corticoid receptor antagonists demonstrated however, some potential to improve fibrotic processes by controlling the underlying diseases. Inflammatory mediators are involved in the initiation and progression of fibrosis, in some clinical situation like kidney fibrosis, but not in IPF, for example. Clinical trials attempting to decrease cytokine and chemokine secretion using kinase inhibitors or antagonists to their cognate receptors were performed. To date, mainly fusion proteins or blocking antibodies were used; the RENEWAL study[152] examined the effect of the TNF-α fusion protein antagonist etanercept and in the ATTACH trial the anti-TNF-

α monoclonal antibody infliximab was tested. These trials were halted for inefficacy regarding the primary outcome or for side-effects. The cholesterol-lowering agents statins are also anti-inflammatory. Two large-scale clinical trials, the CORONA and GISSIF-HF trials[155-157] demonstrated only a neutral effect of statins. PPAR agonists have anti-inflammatory properties, however, their cardiac safety profile is controversial.[193] Overall, there is yet a lack of effective fibrosis inhibitors in patients.

Conclusion and Perspective

Fibrosis proceeds in three steps: an initial phase of injury, an inflammatory phase in most clinical situations and a remodeling phase. Then, in normal situations, the third phase is followed by a fourth phase of resolution, which does not happen in the context of progressive fibrosis. Anti-fibrotic treatments under development and (pre-)clinical evaluation include small molecules, antibodies, genetic tools, peptides, protein analogs and receptor decoys. Progression to fibrosis is mediated by the activation of TGF-β-associated signaling pathways and the appearance of myofibroblasts producing an altered ECM. Thus, blockade of the TGF-β pathways is an obvious target for the treatment of fibrotic diseases. However, due to the ubiquitous and pleiotropic physiological functions of TGF-β, depending on the local microenvironment and the organ, a challenge to be addressed will be to achieve its targeted inhibition. Therefore, despite its critical importance in fibrosis, systemic and non-selective inhibition of TGF-β may not be the best approach to treat fibrosis. The same consideration applies for inhibition of other important signaling pathways, such as the Wnt or Notch pathways. Thus, one major challenge in anti-fibrotic therapy is selectivity and specificity of the drugs aimed at controlling fibrosis.[194] Functionalized therapeutics with tissue- and/or cell-selective molecules must be designed. For example, in a recent approach,[85.195] we used experimental rodent models of acute inflammatory and profibrotic kidney diseases to selectively target Notch1 signaling activation. We developed γ-secretase inhibitor-based prodrug strategies for enzymatic activities specifically expressed in injured kidneys. Using these functionalized prodrugs, we could demonstrate a nephroprotective effect without systemic toxicity. Drug-delivering nanoparticles displaying at their

surface reconnaissance molecules for overexpressed targets in fibrosis-associated cells may represent another approach to solve some of the problems of off-target side-effects, as shown in a rat model of pulmonary fibrosis.[196]

Drugs able to modulate physiological pathways other than the TGF-β, such as oxidative stress, lipids, enzymes, kinases or peptide receptors, have been evaluated. Most clinical trials were disappointing, although the molecules had shown efficacy in preclinical animal models of the diseases. The majority of the therapeutics evaluated in preclinical models were selected to prevent the development of the fibrotic process. Novel compounds are often tested in patients with diseases that did not respond to established therapies, meaning that many compounds are tested at the later refractory stages of disease. Animal models do not recapitulate the complex nature of human fibrotic diseases, since in these simplified models, treatment is initiated in the early phase of the disease. In humans, fibrosis takes decades to develop and has often reached a no-return point when therapeutic intervention is attempted, requiring long-term treatment to diminish its progression. The type of fibrosis, its tissue origin, its stage of progression, the exact cells involved and the level of heterogeneity between organs and diseases must be also considered. But this is generally not realistic in the medical context. In addition, young and healthy animals were studied, while patients with fibrosis are generally at a more advanced age, and may have other co-morbidities, suggesting that drug combination regimens must be considered to achieve optimal therapeutic efficacy with limited toxicity. It remains to be demonstrated however, which adjuvant drug(s) may be more appropriate and which combined-schedule therapy should be proposed to patients according to their level of risk. In order to improve clinical translation, it is important in the future to design more clinically-relevant research models, and to perform clinical trials that include larger cohorts of suitable patients.

Presently, end-stage chronic fibrotic diseases of the lung, kidney, liver, heart and skin require organ/tissue replacement by either autologous or allogeneic transplantation, or the implantation of artificial medical devices. In these processes, the time of injury is known but unavoidable, either due to ischemia/reperfusion injury or due to the presence of a foreign body and an accompanying inflammation. Therefore, anti-fibrotic therapies should ideally be delivered at the time of

transplantation/implantation. Recent progress in regenerative medicine to induce the repair of diseased tissues, rather than reverse progression, has open new therapeutic perspectives in the treatment of fibrosis.

In conclusion, to control and possibly reverse fibrosis progression, treatment approaches require the control of key pathways that influence cell functions and the development of a permissive environment, mainly composed of activated (myo)fibroblasts. In order to achieve clinically meaningful results in human trials, the strategy to treat fibrosis should include the three following challenges: 1) a targeted/addressed delivery of therapeutic drugs to the organ or to the tissue to avoid systemic side-effects; 2) an upfront combination therapy with molecules targeting different pathways; 3) a treatment administered very early in the course of the disease, since organ dysfunction is unlikely to regress significantly once present. We believe that selectively blocking the TGF-β signaling pathways will be necessary. Blocking the RAS was shown to improve fibrosis therapy. Thus, combining RAS blockade with the approved anti-fibrotic drugs **1** or **2** may be an option. Therapeutics able to modulate the endothelin pathway must also be considered in combination therapeutic regimens. Controlling inflammation and oxidative stress is probably not of interest in the late phase of the fibrotic processes. As several kinases, the PPAR, FXR and fatty acid pathways such as S1P, mediate a variety of pro-fibrotic effects in many tissues and organs, kinases and receptor-specific agonists or antagonists may be of interest.

Authors' biographies

Lucienne Juillerat-Jeanneret obtained her PhD from the University of Geneva, Switzerland. After post-doctoral experiences at the University of Geneva and the University Hospital of Lausanne (CHUV-UNIL), she joined the University Institute of Pathology of Lausanne as a tenured senior lecturer and a teacher at the University of Lausanne (UNIL) and the Swiss Federal Institute of Technology of Lausanne (EPFL). Her main research interests are focused at the interface between biomedicine, chemistry and biomaterials, to design and develop innovative devices or modified drugs to deliver therapeutics, as well as therapeutics repurposing. She is also involved in the development

of novel approaches for diagnosis and tissue engineering. The strategies investigated include nanotherapeutics, and the design and evaluation of targeted chemotherapeutics for the treatment of cancer and degenerative diseases.

John-David Aubert graduated from the Faculty of Biology and Medicine of the University of Lausanne (UNIL), Switzerland, and specialized in internal and respiratory medicine, first in Lausanne University Hospital then in UBC Research Laboratory in Vancouver (Prof JC Hogg) as a post-doctoral assistant. Back in Lausanne in 1993 he was involved in the creation of the new lung transplantation program, being presently its medical director and associate professor. He is also leading the pulmonary hypertension clinic and has been president of the Swiss Society for Pulmonary Hypertension. He has been appointed president of the Research Committee of the Swiss Lung League for 2016-2018. His main research interests focus on chronic allograft dysfunction after lung transplantation and clinical aspects of pulmonary hypertension.

Josip Mikulic graduated from the Faculty of Biology and Medicine of the University of Lausanne (UNIL), Switzerland, obtaining his Master in Molecular Life Science. He received his PhD from the University of Lausanne, in the field of mucosal immunology. He currently works as a research assistant at the Transplantation Center of the University Hospital of Lausanne (CHUV-UNIL) in the Transplantation Immunopathology Laboratory. His research is focusing on the identification of T and B cells signatures associated with graft rejection and outcome after kidney transplantation.

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Conflicts of interests. The authors declare no conflict of interest.

List of abbreviations

ACC: acetyl-CoA carboxylase; ACE(I): angiotensin converting enzyme (inhibitor); ADAM: a-disintegrin-and-metalloproteinase; ALK: anaplastic lymphoma kinase; AR: adenosine receptor; Ang: angiotensin; $AT_{1/2}R$: angiotensin receptor 1/2; ATX: autotaxin; BMP: bone morphogenic protein; CAF: cancer-associated fibroblast; CC/CXC (R/L): C-C/C-X-C motif chemokine (receptor/ligand); COX: cyclooxygenase; CTGF: connective tissue growth factor; DPP IV: dipeptidyl peptidase IV; ECM: extracellular matrix; EGF(R): epidermal growth factor (receptor); EMT/EndMT: epithelial/endothelial-to-mesenchymal transdifferentiation; ERK: extracellular regulated kinase; ET: endothelin; $ET_{A/B}$: endothelin receptor A/B; FAP-α: fibroblast activation protein-α; FGF: fibroblast growth factor; FXR: farnesoid X receptor; GPCR: G-protein coupled receptor; HGF: hepatocyte growth factor; IL: interleukin; IGF: insulin (like) growth factor; interleukin; JAK/STAT: Janus kinase/signal transducers and activators of transcription; JNK: C-Jun NH_2-terminal kinase; LOX(L): lysyl(like)oxidase; LPA(R): lysophosphatidic acid (receptor); MMP: matrix metalloproteinase; MSC: mesenchymal stem cells; mTOR: mammalian target of rapamycin; NAFLD/NASH: nonalcoholic fatty liver disease/nonalcoholic steatohepatitis; NFκB: nuclear factor κB; NOX: NADPH oxidase; IPF: idiopathic pulmonary fibrosis; PDGF®: platelet-derived growth factor (receptor); PDE: phosphodiesterase; POP/PREP: prolyl-oligopeptidase; PPAR: peroxisome proliferator activated receptor; RAS: renin angiotensin (aldosterone) system; ROCK: Rho-associated protein kinase; ROS: reactive oxygen species; S1P(R): sphingosine-1-phosphate (receptor); SK: sphingosine kinase; α-SMA: α-smooth muscle actin; Ssc: systemic scleroderma/sclerosis; TGF-β(R): transforming growth factor-beta (receptor); TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

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