Pharmacological Research 155 (2020) 104694

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Pirfenidone is a cardioprotective drug: Mechanisms of action and preclinical evidence

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ARTICLE INFO

Chemical compounds studied in this article:
Pirfenidone (PubChem CID: 40632)

Keywords:
Pirfenidone
Cardiac protection
Fibrosis
Inflammation

ABSTRACT

Myocardial fibrosis is an endogenous response to different cardiac insults that may become maladaptive over time and contribute to the onset and progression of heart failure (HF). Fibrosis is a direct and indirect target of established HF therapies, namely inhibitors of the renin-angiotensin-aldosterone system, but its resilience to therapy warrants a search for novel, more targeted approaches to myocardial fibrosis.

Pirfenidone is a drug approved for idiopathic pulmonary fibrosis, a severe form of idiopathic interstitial pneumonias. Pirfenidone is a small synthetic molecule with high oral bioavailability, exerting an antifibrotic activity, but also anti-oxidant and anti-inflammatory effects. These effects have been attributed to the inhibition of several growth factors (in particular transforming growth factor-β, but also platelet-derived growth factor and beta fibroblast growth factor), matrix metalloproteinases, and pro-inflammatory mediators (such as interleukin-1β and tumour necrosis factor-α), and possibly also an improvement of mitochondrial function and modulation of lymphocyte activation. Given the activation of similar profibrotic pathways in lung and heart disease, the crucial role of fibrosis in several cardiac disorders, and the wide spectrum of activity of pirfenidone, this drug has been evaluated with interest as a potential treatment for cardiac disorders. In animal studies, pirfenidone has shown cardioprotective effects across different species and in a variety of models of cardiomyopathy. In the present review we summarize the pharmacological characteristics of pirfenidone and the data from animal studies supporting its cardioprotective effects.

1. Introduction

Myocardial fibrosis is an endogenous response to different cardiac insults that offers structural support when cardiomyocyte loss is occurring in the absence of appropriate cardiomyocyte replacement. Fibrosis, either "reparative" (as the development of a myocardial scar after myocardial infarction [MI], or "reactive" (as in non-ischemic dilated cardiomyopathy or in the remote myocardium after MI), is a constant feature of heart failure (HF), and is considered a crucial determinant in HF development and progression, as it promotes contractile dysfunction and arrhythmias [1]. Cardiac fibrosis can be targeted by established HF therapies, namely inhibitors of the renin-angiotensin-aldosterone system (RAAS), but its resilience to therapy "requires additional major efforts to control fibrotic remodeling" [1]. So far, specific anti-fibrotic medications have received little or no attention as a therapeutic option for human patients with cardiac disorders.

Pirfenidone is a drug approved for the treatment of idiopathic pulmonary fibrosis (IPF), which is the most common and lethal form of idiopathic interstitial pneumonias, characterized by the unregulated deposition of extracellular matrix (ECM) within the lungs causing progressive pulmonary insufficiency [2–6]. IPF is believed to develop

Abbreviations: DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; IPF, idiopathic pulmonary fibrosis; LA, left atrium; LV, left ventricle; MI, myocardial infarction; PDGF, platelet-derived growth factor; PIROUETTE, Efficacy and Safety of Pirfenidone in Patients With Heart Failure and Preserved Left Ventricular Ejection Fraction; RAAS, renin-angiotensin-aldosterone system; TAC, transverse aortic constriction; TGFβ, transforming growth factor-β

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https://doi.org/10.1016/j.phrs.2020.104694
Received 1 December 2019; Received in revised form 10 February 2020; Accepted 11 February 2020
Available online 12 February 2020
1043-6618/

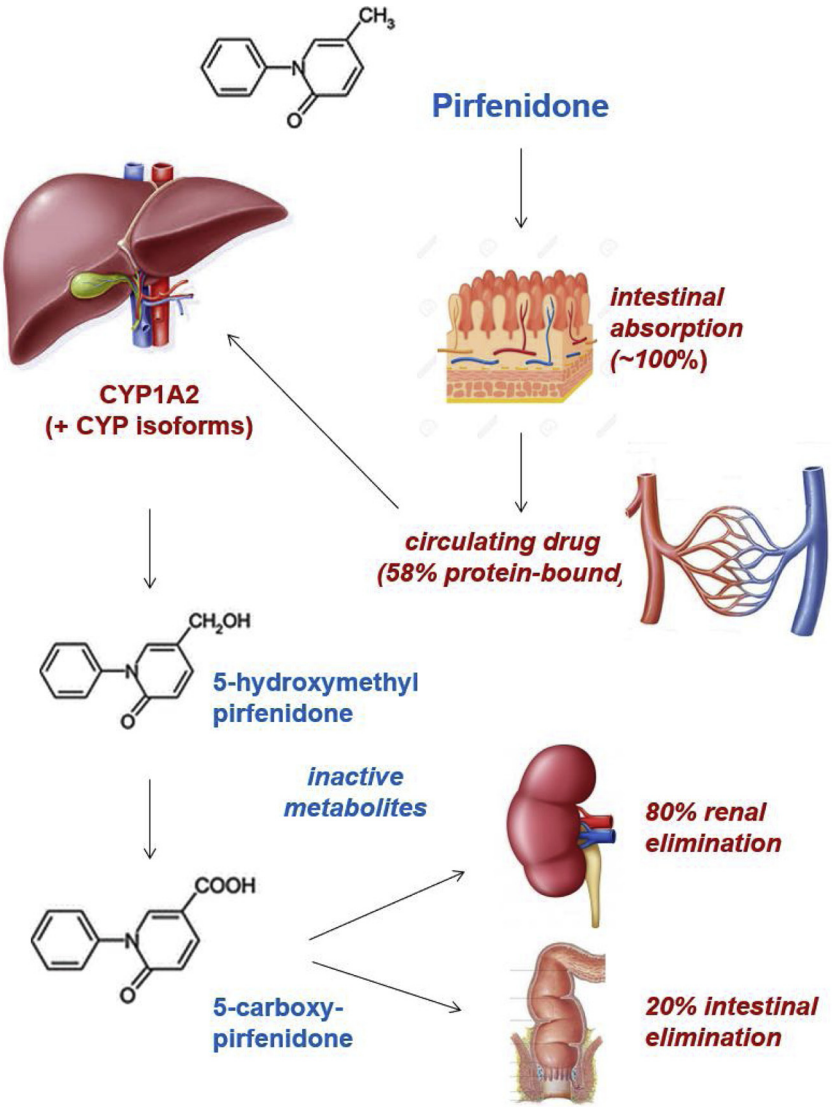

Fig. 1. Metabolism of pirfenidone.

when the alveolar epithelium of genetically susceptible individuals is exposed to a repetitive injury. The dysfunctional epithelium produces pro-fibrotic mediators such as transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), angiotensinogen and multiple chemokines, which recruit and activate mesenchymal cells. Fibroblasts differentiate into myofibroblasts, which produce ECM and promote apoptosis of epithelial cells [5,7]. Interestingly, the mechanisms of pulmonary fibrosis are partially overlapping with those of myocardial fibrosis [7], and pirfenidone proved effective in several animal models of cardiac disorders. The present review provides an overview of the pharmacology of pirfenidone and summarizes the evidence suggesting that pirfenidone warrants consideration as a novel therapeutic approach for human patients with at risk of developing HF or with established HF.

2. Pharmacokinetics and safety profile of pirfenidone

Pirfenidone (5-methyl-1-phenylpyridin-2(1H)-one) is a synthetic agent structurally similar to pyridine 2,4-dicarboxylate. This small molecule is very soluble in alcohol and chloroform; in aqueous solutions, the maximum concentration is about 10 mg/mL. Pirfenidone can move through cell membranes by passive diffusion [8].

2.1. Pharmacokinetics

Orally administered pirfenidone is very rapidly absorbed in the gastrointestinal tract, with a median time to maximal plasma concentration of 0.5 h [9]. Around 60 % of circulating pirfenidone binds to plasma proteins (primarily albumin). The drug reaches most tissues and crosses the brain-blood barrier, with a moderate degree of extravascular distribution. Concomitant intake of food reduces by around 20 % the rate and extent of absorption and tissue exposure to pirfenidone [8,9]. The bioavailability of pirfenidone has not been determined in humans. Drug half-life is 3 h [8–10]. Pirfenidone is metabolized in the liver by CYP1A2 and by other CYPs (CYP2C9, 2C19, 2D6, and 2E1). The principal site of metabolism is the 5-methyl position of the pyridone ring, resulting in stepwise formation of 5-hydroxymethyl pirfenidone and then a 5-carboxylic acid metabolite, which are not pharmacologically active at the concentrations measured in humans. Pirfenidone is mostly excreted as the metabolite 5-carboxy-pirfenidone (around 99.6 % of the drug recovered). The drug is excreted by 80 % through the urine (80 %), and by 20 % though intestinal elimination (Fig. 1). No parent drug or metabolites were detected 24 h after a single dose [8–13].

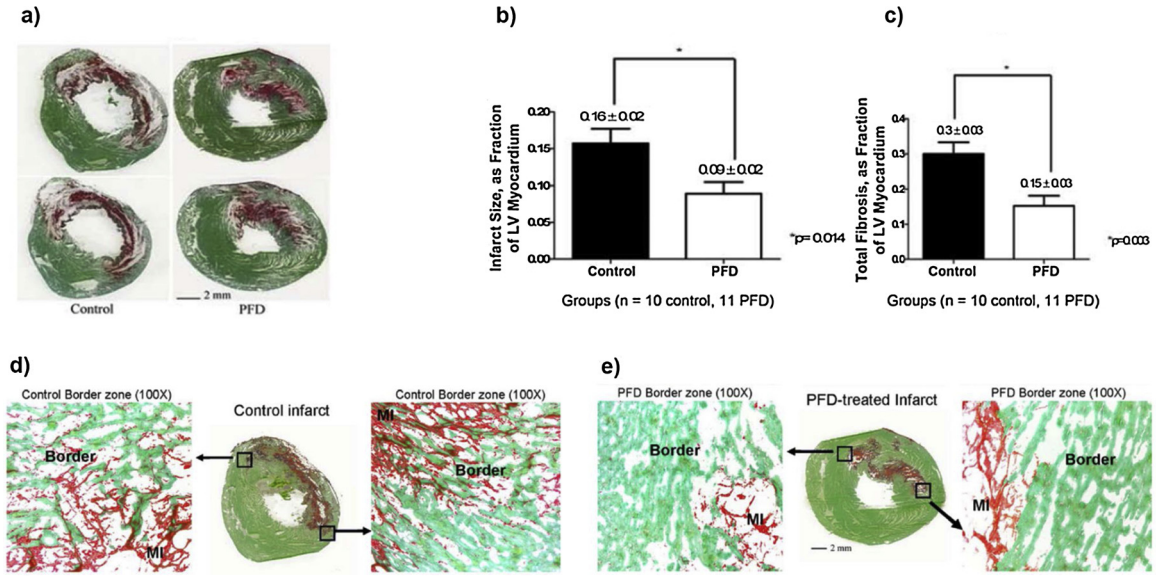

Fig. 2. Effects of pirfenidone on left ventricular fibrosis after myocardial infarction.
a) Representative myocardial sections stained with Sirius red and fast green. Control animals have larger dense infarct areas (*red stain*) than pirfenidone (PFD)-treated animals. b) Mean scar size was calculated as percentage of left ventricular (LV) myocardium. c) For total amount of LV fibrosis, controls had twice as much fibrosis as the PFD group. d, e) In the PFD-treated animal, fibrosis is less extended, and the border between the infarcted area and the healthy myocardium is much more clearly demarcated.
Modified with permission from: Nguyen et al. [38].

2.2. Safety profile and drug interactions

In a post-authorization safety study (PASSPORT), 73 % of patients with IPF experienced adverse drug reactions (ADRs), most often nausea (21 %) and fatigue (19 %) [14]. At least one dose adjustment was needed in 37 % of patients. On the other hand, only 29 % of patients experienced ADRs leading to treatment discontinuation, most frequently nausea (4%), weight decrease (3%) and skin rash (3%). Serious ADRs were recorded in 6% of patients, leading to a fatal outcome in less than 1%. In the overall population, 69 % of patients experienced ADRs of special interest, most often gastrointestinal disturbances (38 %) and skin rash or photosensitivity reactions (29 %). Older age, history of steroid use and female gender were factors associated with early discontinuation due to ADRs within the first 6 months. Creatinine clearance < 50 mL/min and mild-to-moderate liver dysfunction represent relative contraindications to pirfenidone therapy [14], although pirfenidone is regarded with interest as a potential therapy for renal fibrosis [15], and this drug exerts an antifibrotic action in liver cirrhosis [16].

Smoking induces CYP1A2 and thus decreases the exposure to pirfenidone [26]; patients should be instructed to stop smoking, and not to smoke while on therapy with pirfenidone. Exposure to pirfenidone may be increased by concomitant treatment with CYP1A2 inhibitors, including fluvoxamine, ciprofloxacin, amiodarone and propafenone. Conversely, rifampicin induces CYP1A2 expression and reduces systemic exposure to pirfenidone [17].

Adverse effects of pirfenidone have been attributed to the suboptimal pharmacokinetic profile of pirfenidone. These effects in humans improve with dose reduction [18] or administration of the drug with meals (which reduces peak plasma concentration) (13).

3. Mechanisms of antifibrotic activity of pirfenidone

Several targets of pirfenidone have been proposed, but the exact mechanisms whereby pirfenidone modulates these targets, and how this action translates into relief from tissue damage, remain largely speculative [19]. Several groups have reported that pirfenidone blunts TGF-β signalling, which is a crucial determinant of pulmonary and cardiac fibrosis [7]. For example, pirfenidone reduces TGF-β mRNA levels and the mature TGF-β protein [20,21], inhibits TGF-β-stimulated collagen synthesis in human lung fibroblasts [20], and blocks the proliferation and differentiation of fibroblasts into myofibroblasts by inhibiting several targets of TGF-β (Smad3, p38, Akt42) [20,22]. Pirfenidone treatment also inhibits other fibrogenic growth factors, most notably PDGF and basic fibroblast growth factor (bFGF) [20], and upregulates several MMPs [23].

Pirfenidone treatment modulates several inflammatory cytokines, including tumour necrosis factor α (TNF-α). This mediator promotes the recruitment of inflammatory cells, proliferation of fibroblasts, hyperplasia and apoptosis of epithelial cells in the airways [24]. Pirfenidone also downregulates IL-1β, which stimulates fibroblasts to produce PDGF, TGF-β and other fibrogenic mediators. Additionally, pirfenidone reduces the expression of type 2 cytokines IL-4 and IL-13. IL-4 stimulates the deposition of ECM, and IL-13 overexpression in mice promotes TGF-β expression and pulmonary fibrosis [25]. Pirfenidone might also inhibit the formation of the NLRP3 inflammasome, a protein complex responsible for the recognition of stress signals and involved in the onset and maintenance of inflammation [26]. Finally, pirfenidone seems to modulate the activity and proliferation of both T and B lymphocytes [27,28], although the mechanisms are still unclear. Lymphocyte modulation could explain many of the beneficial effects of pirfenidone, as T and B cells can produce a number of pro-inflammatory and pro-fibrotic mediators that are modulated by pirfenidone, including TNF-α and TGF-β [27,28].

In animal models of lung and liver fibrosis, pirfenidone reduces circulating levels of markers of oxidative stress, possibly by stabilizing membrane potential in the mitochondria, which could sustain energy production while reducing the production of reactive oxygen species and the activation of apoptosis [29]. Finally, a direct potentiation of L-type calcium channel conduction in rat cardiomyocytes acutely exposed to pirfenidone has been proposed, which might reduce the susceptibility to atrial fibrillation [30].

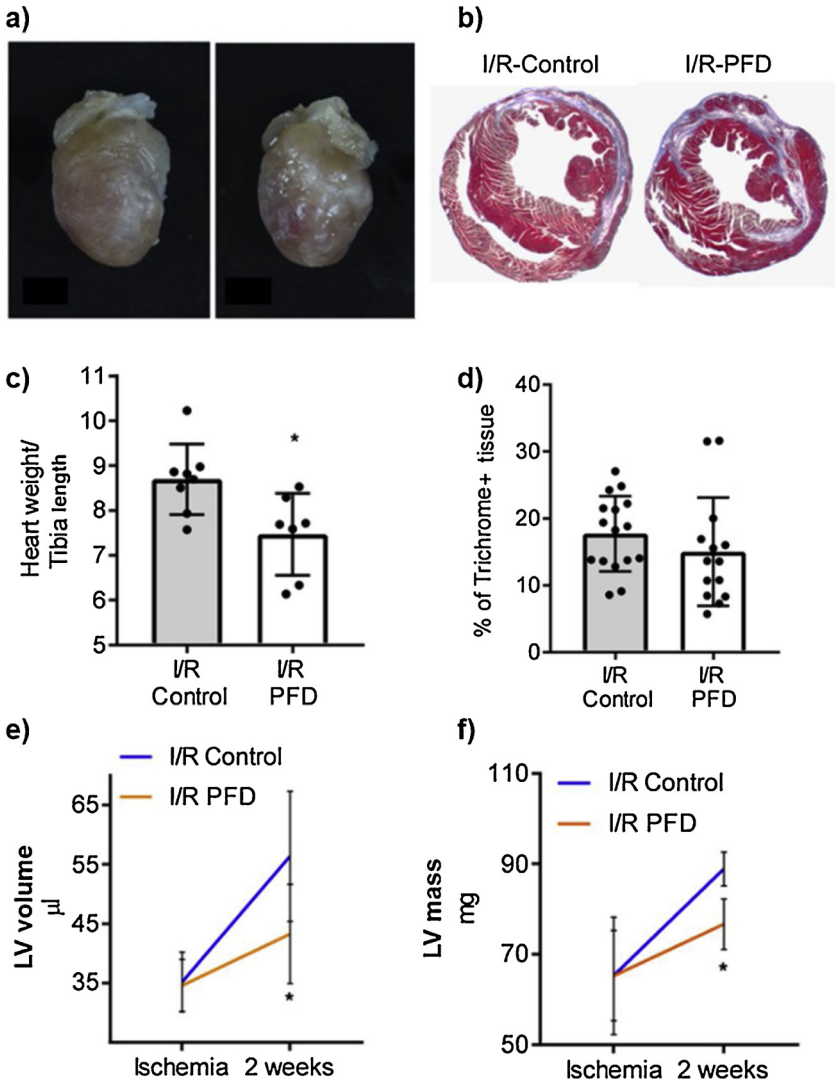

Fig. 3. Pirfenidone and left ventricular (LV) remodelling after myocardial infarction.
Wild-type mice underwent 90-minute closed-chest ischaemia and 2 weeks of reperfusion (I/R injury). a) Hearts from control (*left*) and pirfenidone (PFD)-treated animals (*right*) after 2 weeks from the I/R injury. b) Sections of hearts from control (*left*) and PFD-treated animals (*right*); trichrome staining. c) Weights of hearts from controls or PFD-treated animals. d) Percentage trichrome-positive staining. e) Echocardiographic LV end-diastolic volume. f) LV mass. *$p < 0.05$.
Modified with permission from: Adamo et al. [39].

4. Preclinical evidence of cardiac protection

4.1. Models of cardiomyopathy caused by increased afterload

In rats with hypertension induced by nephrectomy, pirfenidone treatment for 2 weeks reduced the hypertrophic response of ventricular myocytes, the development of myocardial fibrosis and diastolic dysfunction [31]. In another study, mice were divided into 3 groups, receiving saline infusion, angiotensin-II, or angiotensin-II plus pirfenidone. After 2 weeks, mice receiving angiotensin-II plus pirfenidone displayed a significantly lower hypertrophic response than mice on angiotensin-II alone, as well as less perivascular and interstitial fibrosis, lower expression of TGF-β, mineralocorticoid receptors, and natriuretic peptides. Pirfenidone did not inhibit blood pressure elevation by angiotensin-II [32]. Similarly, pirfenidone did not affect blood pressure levels in mice exposed to transverse aortic constriction (TAC), while reducing hypertrophy and inflammation in the left ventricle (LV) [26]. In mice with TAC-induced HF, pirfenidone relieved contractile dysfunction and LV fibrosis [33].

4.2. Pirfenidone in other cardiomyopathies

Pirfenidone has been evaluated also in animal models of very heterogeneous forms of cardiomyopathy. In rats with streptozotocin-induced diabetes, 4-week treatment with either pirfenidone or spironolactone reduced perivascular and interstitial collagen deposition in the LV and kidneys, and attenuated diastolic dysfunction, but did not normalize cardiac contractility or chronotropic responses to norepinephrine. Pirfenidone and spironolactone yielded similar effects [34]. In another study, rats received intraperitoneal injections of doxorubicin, alone or together with pirfenidone for 2 weeks, and were evaluated 25 days after the last drug dose. The pirfenidone group had a lower mortality rate to day 25, reduced heart and kidney weight, and less prominent histopathological changes in both sites [35]. Finally, Lee et al. assessed the effects of pirfenidone in 15 dogs divided equally into 3 groups: control, dogs with HF induced by high-frequency LV pacing (220 beats per minute for 3 weeks), and dogs with HF and receiving pirfenidone. In this case, no difference in LV function was noted, possibly also for the small group sizes, but treatment with pirfenidone resulted in a 50 % reduction in left atrial (LA) fibrosis, which was associated with a lower susceptibility to atrial fibrillation [36]. Finally,

mdx mice (a model of Duchenne muscular dystrophy) receiving pirfenidone for 7 months had lower levels of TGF-β mRNA in the heart and better LV contractility, despite no significant differences in diastolic stiffness or fibrosis [37].

4.3. Myocardial infarction

The expansion of cardiac fibrosis following MI is thought to affect viable tissue adjacent to the infarcted area, predisposing to impulse fragmentation and the development of ventricular arrhythmias. In a rat model, pirfenidone treatment was initiated 1 week after ischemia-reperfusion injury and continued for 4 weeks. Pirfenidone caused a reduction in scar size and myocardial fibrosis in the border zone, with better preserved LV systolic function (Fig. 2), and lower rates of ventricular tachycardia inducibility [38].

In rats subjected to ligation of the left anterior descending coronary artery, pirfenidone reduced cardiac fibrosis and scar size, and slowed down the progression toward HF [19]. Accordingly, pirfenidone attenuated LV remodelling and improved survival in mice with diphtheria toxin-mediated acute myocardial injury and closed-chest ischemia-reperfusion injury (Fig. 3) [39]. The Authors observed a reduced percentage of B lymphocytes in mice treated with pirfenidone, and depletion of B lymphocytes abolished the beneficial effects of pirfenidone. Stimulation with lipopolysaccharide and extracts of necrotic cells activated B lymphocytes, and pirfenidone blocked this activation. The Authors then postulated that the cardioprotective effects of pirfenidone depended at least partially on the modulation of myocardial B lymphocytes [39].

5. Ongoing clinical trials and future perspectives

At the time of writing (November 2019), clinical trials on pirfenidone are focusing on several disorders characterized by an excessive and dysregulated deposition of ECM, either in the lungs or in other organs (Supplemental Table 1). Despite the prominent role of fibrosis in the pathophysiology of several cardiac disorders, and the evidence that therapy with pirfenidone is not associated with an increased incidence of cardiovascular events [40], only a single study of pirfenidone for a cardiac condition has been started, the Efficacy and Safety of Pirfenidone in Patients With Heart Failure and Preserved Left Ventricular Ejection Fraction (PIROUETTE) trial (Supplemental Table 1). This is a randomized, double-blind, placebo-controlled, phase 2 study aiming to evaluate the efficacy and safety of pirfenidone in the treatment of patients with HF and preserved LV ejection fraction (HFpEF), based on the pathogenetic mechanisms shared by IPF and HFpEF [7].

At present, there are no disease-modifying therapies for HFpEF; therefore, starting a phase 2 clinical trial of pirfenidone in this patient population based on pathophysiological considerations may therefore be justified. In addition, findings from several animal models suggest that pirfenidone might effectively reduce the development of fibrosis and LV remodelling following myocardial damage (Supplemental Table 2). Nonetheless, it remains to be established whether pirfenidone confers an additive benefit over RAAS blockers, also considering that the mechanisms of action seem to be partially overlapping, since RAAS antagonists act firstly by reducing TGF-β signalling [41]. Finally, since pirfenidone has several shortcomings that seem to be related to its poor pharmacokinetic profile, it would be important to develop pirfenidone derivatives with improved pharmacokinetics, especially reduced penetration in the central nervous system and increased half-life.

Declaration of Competing Interest

L.A. is founder of i-Cordis, a start-up company focused on the development of pirfenidone derivatives for the treatment of heart failure; the other Authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.104694.

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