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Synthesis and biological evaluation of the pirfenidone derivatives as antifibrotic agents

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ABSTRACT

A total of 24 pirfenidone derivatives were designed, synthesized and evaluated for their inhibitory activity against the human lung fibroblast cell line MRC-5. These compounds showed the remarkable proliferation inhibition against MRC-5 compared to pirfenidone as the positive control. The possible mechanism of this kind of derivatives as antifibrotic agents was explored. The molecular docking and p38 binding affinity assays demonstrated that the antifibrotic potential of the pirfenidone derivatives was possibly through the inhibition of p38 MAPK signaling pathway. The data from this study suggested that p38 might be a potential therapeutic target for the new generation antifibrotics. All the pirfenidone derivatives are reported here for the first time.

Idiopathic pulmonary fibrosis (IPF) is a typical chronic, progressive fibrosing interstitial pneumonia, primarily occurring in older adults, and limited to the lungs. It is characterized by progressive worsening of dyspnea and lung function with a poor prognosis.[1] With a median survival of 3 to 5 years and a 5-year survival rate of 20%, IPF is more mortality than most of the malignant cancers.[2] The pathogenesis of the IPF is complex and usually involved the interaction among various pathways driven by proinflammatory or profibrogenetic mediators.[3] However, the precise mechanisms are still unknown.

More recently, pirfenidone was approved as the first antifibrotic therapy for IPF in Europe and Asia.[4] To date, pirfenidone is the only orally administered drug that has orphan designation for the treatment of mild to moderate IPF. Its mechanism has been studied extensively and it is generally thought to be a multiple-targets drug against inflammatory, antioxidative stress and antiproliferative processes. For example, pirfenidone reduced inflammatory cytokines such as TNF-α[5,6] and downregulated the transcription of key profibrotic growth factors including TGF-β.[7,8] Beneficial effects have been shown for pirfenidone in the fibrotic disease, including pulmonary, liver, renal, and cardiac muscle fibrosis.[9]

However, some side effects including gastrointestinal upset, fatigue and photosensitivity have been observed in clinical practices for pirfenidone.[10] It was speculated that these adverse symptoms might due to the requirement for high customary doses in order to counteract the reduced bioactivity by the fast metabolism in human body.[11] Thus, considerable efforts have been devoted towards the modification of the pirfenidone scaffold in order to increase the half-time and/or the antifibrotic activity. For example, Chen et al. substituted the 5-methyl group of pirfenidone by trifluoromethyl group or chlorine, and two series of novel (5-substituent)-2(1*H*)-pyridone compounds were designed with the purpose of overcoming the drawbacks of fast metabolism while maintaining its multi-targeting property. The compounds with 4-methoxy phenyl at the 1-position of pyridone scaffold of pirfenidone showed more antifibrotic activity.[12] Liu and co-workers modified 1-(substituted aryl)-5-trifluoromethyl-2(1*H*) pyridones with carbohydrate to prepare antifibrotic compounds with higher water solubility.[13] Wu et al. described a N_1-substituted phenylhydroquinolinone scaffold which can be used for developing novel antifibrotic agents.[14] More recent work by Kossen and co-workers demonstrated 6-oxo-7-phenyl-6,7-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine derivatives for treating inflammatory and fibrotic disorders.[15]

In order to assess the impact of different side chain on antifibrotic potency in vitro, we introduced a side chain with 4-alkoxyl phenyl group including terminal amine to the 1-position of pyridone scaffold (Fig. 1, **6a**–**9f**). Firstly, we focused on the length of linker between scaffold and amine. Secondly, different amines were selected according to their structure to determine the size of side chain and hydrophobic influence on the biological activity. The potential mechanism of pirfenidone derivatives as antifibrotic agents was also investigated.

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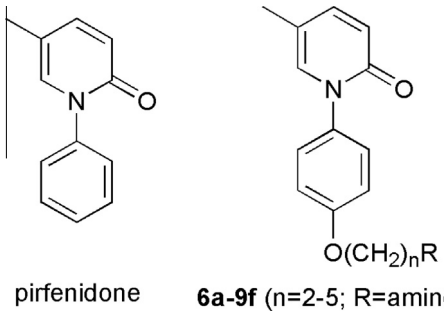
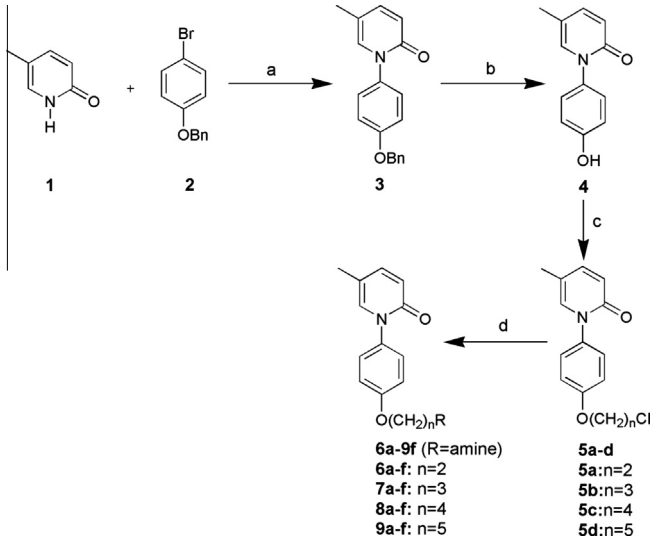


Figure 1. The structure of pirfenidone and compounds **6a**–**9f**.



Scheme 1. Reagents and conditions: (a) CuI, K_2CO_3, DMF, reflux, 74%; (b) 5% Pd/C, THF, H_2, rt, 78%; (c) $Cl(CH_2)_nBr$, acetone, K_2CO_3, reflux, 90–95%; (d) NaI, K_2CO_3, DMF, amine, 70 °C, 40–90%.

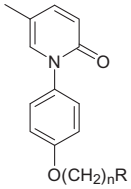
Scheme 1 shows the flow for the general preparation of these pirfenidone derivatives. The starting material **1** was obtained as reported.[16] Another starting material **2** was generated by protection of 4-bromophenol with benzyl bromide.[17] Using cuprous iodide as catalyst, **3** was synthesized by the Ullmman coupling reaction of **1** with **2** in dry DMF. The key intermediate **4** was successfully obtained by benzyl deprotection of **3** in THF with 5% Pd/C under H_2 atmosphere. After the reaction between intermediate **4** and alkyl dihalides at reflux temperature, compounds **5a**–**d** were achieved respectively. Finally, target compounds were obtained by amination reaction of **5a**–**d** with alkyl or heterocyclic amine.

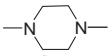
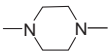
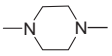
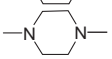
All target compounds were tested for their cell proliferation inhibitory activity against MRC-5 cell, an ideal in vitro cell model for screening antifibrotic drugs,[18] by MTT assay using pirfenidone as the positive control. DMSO was used as solvent. The results are summarized in Table 1.

As shown in Table 1, all the synthesized compounds exhibited marked proliferation inhibition against MRC-5 compared to pirfenidone as the positive control. For instance, **8a**–**f** showed IC_{50} from 1.77 to 3.29 mM, and **9a**–**f** showed IC_{50} from 1.47 to 4.35 mM, while the corresponding IC_{50} value for pirfenidone was 14.44 mM. Compound **6e** exhibited the most potent inhibitory with approximately 10-fold lower IC_{50} compared to pirfenidone.

In this Letter, we investigated the effect of the modification of substituent at the 1-position of pyridone scaffold on antifibrotic activity. At first, we focused on the length of linker between scaffold and amine. A series of compounds with two methylenes, three methylenes, four methylenes and five methylenes spacer were designed and synthesized. As the IC_{50} values of these new compounds were on one order of magnitude, the length of linker from two to five methylenes demonstrated no significant impact on antifibrotic activity. Furthermore, different amines were selected according to their structure to determine the size of side chain and hydrophobic influence on the biological activity. Replacement with different heterocyclic amine including pyrrolidin-1-yl group, piperidin-1-yl group, (4-methyl-piperazin)-1-yl and morpholin-4-yl group was carried out. A view on inhibitory data of compounds **6c**–**9f** showed that both the carbon number of heterocyclic rings and the hydrophobicity of heterocyclic amine had little effect on antifibrotic activity. For example, **7c** with a pyrrolidin-1-yl group, **7d** with a piperidin-1-yl group, **7e** with a

Table 1
The structures of pirfenidone derivatives and their inhibitory activities against MRC-5 cells



Compound	*n*	R	IC_{50} (mM)
6a	2	$-N(CH_3)_2$	6.88
6b	2	$-N(C_2H_5)_2$	6.20
6c	2		3.78
6d	2		2.48
6e	2		1.36
6f	2		7.64
7a	3	$-N(CH_3)_2$	5.22
7b	3	$-N(C_2H_5)_2$	2.10
7c	3		3.00
7d	3		2.55
7e	3		4.36
7f	3		5.28
8a	4	$-N(CH_3)_2$	1.80
8b	4	$-N(C_2H_5)_2$	2.16
8c	4		1.77
8d	4		3.29
8e	4		2.33
8f	4		3.03
9a	5	$-N(CH_3)_2$	2.98
9b	5	$-N(C_2H_5)_2$	4.35
9c	5		1.47
9d	5		3.67
9e	5		3.41
9f	5		2.59
Pirfenidone			14.44

(4-methyl-piperazin)-1-yl group and **7f** with a morpholin-4-yl group had the IC_{50} values of 3.00, 2.55, 4.36 and 5.28 mM, respectively.

As we known, fibrogenesis is strongly associated with inflammation. TNF-α and TGF-β are two major molecules for tissue fibrosis, which play central mediation roles in the induction of fibrosis. Further p38, a member (subfamily) of the stress-activated protein kinase family, is also considered critical to the inflammatory response and tissue remodeling.[19] Therefore we hypothesized that the antifibrotic potential of the synthesized compounds might be mediated through the inhibition of p38 MAPK signaling pathway. To confirm this hypothesis, a molecular docking study of **7d** with p38, TNF-α and TGF-β was performed using the Discovery Studio 2.1/Flexible Docking protocol on a model structure (PDB ID:3HP2, 1QSC and 3FAA). As show in Figure 2a, the carbonyl group of **7d** formed two hydrogen bonds with p38 Met109 and Gly110. In contrast, **7d** could not form hydrogen bonds with TNF-α or TGF-β (Figs. 2b and 2c). In addition, the binding free energy between **7d** and three targets, which calculated by the Discovery Studio 2.1/Calculate Binding Energy protocol, showed that ΔG_{p38} is significantly lower than $\Delta G_{TNF\text{-}\alpha}$ and $\Delta G_{TGF\text{-}\beta}$ ($\Delta G_{p38} = -33.60$ kcal/mol, $cG_{TNF\text{-}\alpha} = -5.25$ kcal/mol, $\Delta G_{TGF\text{-}\beta} = -5.86$ kcal/mol). The results indicated that **7d** was more likely to be the ligand of p38 compared to TNF-α and TGF-β.

To further verify our hypothesis, pirfenidone and several synthesized derivatives were selected to test their binding activity with p38 in vitro using ADP-Glo™ Kinase Assay (Promega Corporation). This assay is a luminescent ADP detection assay that provides a universal, homogeneous, high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction.[20] The results are shown in Table 2.

As indicated in Table 2, pirfenidone showed IC_{50} values of 165.4 μM against p38, while all tested derivatives showed superior inhibitory activity with an IC_{50} of 4.07–54.31 μM. The most potent compound **7d** displayed about 40-fold stronger potency than pirfenidone. **7d** and **8d** showed increased potency than **6d** and **9d**. In addition, **7d** showed more potent inhibitory activity than **7a**. These data suggested that three or four methylenes between scaffold and amine, and heterocyclic amines might be beneficial for inhibitory activity against p38. The molecular docking study and p38 binding affinity suggested that the antifibrotic potential of the pirfenidone derivatives is probably mediated through the inhibition of p38 MAPK signaling pathway.

In summary, a total of 24 pirfenidone derivatives were designed and synthesized. Inhibitory activities of these compounds against MRC-5 were evaluated. The binding affinities studies suggested the possible mechanism of the compounds as antifibrotic agents was via p38 MAPK pathway. The study indicated that p38 might be a potential target to develop new-generation antifibrotics.

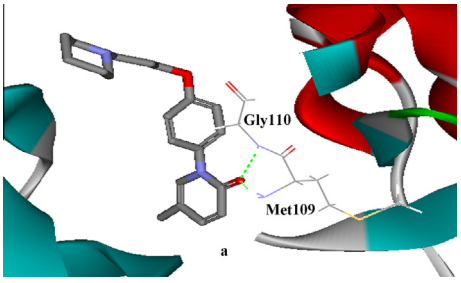


Figure 2a. The docking results of **7d** with p38.

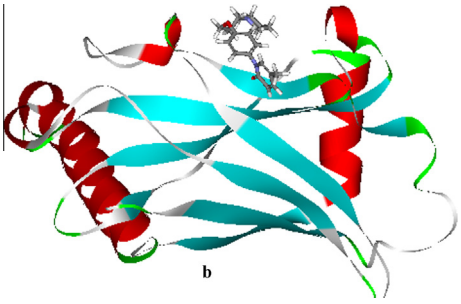


Figure 2b. The docking results of **7d** with TNF-α.

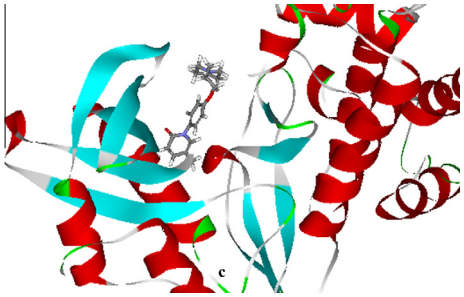


Figure 2c. The docking results of **7d** with TGF-β.

Table 2
Inhibitory activities against p38 of selected compounds

Compound	*n*	R	p38 IC_{50} (μM)
6d	2		54.31
7a	3	$-N(CH_3)_2$	14.17
7d	3		4.07
8d	4		5.21
9d	5		16.19
pirfenidone			165.40

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.038.

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