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Synthesis and biological evaluation of selenogefitinib for reducing bleomycin-induced pulmonary fibrosis

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ABSTRACT

Selenium has demonstrated effectiveness in the reduction of oxidative stress and inflammation *in vitro* and *in vivo*, both of which are key indicators of the pathogenesis of pulmonary fibrosis. Gefitinib, an FDA-approved EGFR inhibitor, effectively reverses the deterioration of bleomycin-induced pulmonary fibrosis. Based on this, we proposed introducing a selenium atom into the structure of gefitinib, resulting in the generation of selenogefitinib. Compared to gefitinib, selenogefitinib was significantly less hepatotoxic and cytotoxic in cells. The results of the H&E staining of lung tissue validated that Selenogefitinib effectively protected the structure of the alveolar tissue and mitigated the infiltration of inflammatory cells in bleomycin-induced pulmonary fibrosis models. The reduction in the deposition of collagen fibers in lung tissue determined by Masson staining and hydroxyproline (HYP) content also corroborated the efficacy of selenogefitinib in the treatment of pulmonary fibrosis. Furthermore, Selenogefitinib decreased the levels of pro-inflammatory markers IL-4, IL-6, and TNF-α more significantly than gefitinib, which indicated that it exhibited a higher anti-inflammatory activity. In addition, the presence of selenium manifested a greater reduction in oxidative stress based on the decrease in the levels of MDA in mice blood. These results suggested that Selenogefitinib may be a potential candidate for the treatment of IPF.

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease resulting from a variety of pathophysiological processes, including excessive inflammation, oxidative stress, and abnormal epithelial-mesenchymal transition (EMT).[1,2] The sequela of IPF mainly constitute dyspnea, dry cough, and general fatigue due to impaired gas exchange caused by scarring of the lung tissue.[3,4] It was previously reported that IPF was associated with a numerical expansion of fibroblasts and myofibroblasts, leading to the excessive production and deposition of extracellular matrix (ECM) and the formation of fibroblastic foci.[5,6] If not effectively managed, lung function will continue to be impaired after being diagnosed as pulmonary fibrosis, with approximately 50% of patients dying within 2–5 years.[7] In 2014, Nintedanib (Fig. 1) and Pirfenidone were approved by the Food and Drug Administration for the treatment of IPF, both of which were shown to slow down the progression of the disease.[8,9] As an TGF-β inhibitor, pirfenidone inhibits the progression of pulmonary fibrosis by downregulating the levels of vascular endothelial growth factor (VEGF) and procollagen I and II.[10,11] VEGF, a powerful angiogenic factor, mediates fibrogenic responses in myofibroblasts.[12] Nintedanib is a multi-target protein tyrosine kinase inhibitor that can block the autophosphorylation of VEGFR, FGFR, and PDGF, thereby slowing down the progression of IPF.[13–15] Although these new therapies have manifested a better prognosis, IPF remains an incurable disease, which is partly the result of its complex and poorly understood etiology.[16–18] A first-in-class selective autotaxin inhibitor, GLPG1690.[19], demonstrated superior efficacy compared to Pirfenidone and similar efficacy to Nintedanib in reducing the Ashcroft fibrotic score and collagen content in a mouse bleomycin-induced pulmonary fibrosis model in preclinical studies. It is now currently being evaluated in phase III clinical trials (NCT 02738801).

EGFR activation has been shown to stimulate fibroblast proliferation in animal models. Therefore, inhibition of EGFR could be advantageous in diminishing the progression of growth factor-α-induced pulmonary fibrosis. The EGFR inhibitor Gefitinib has been shown to significantly slow down the progression of IPF and partially reverses the fibrotic

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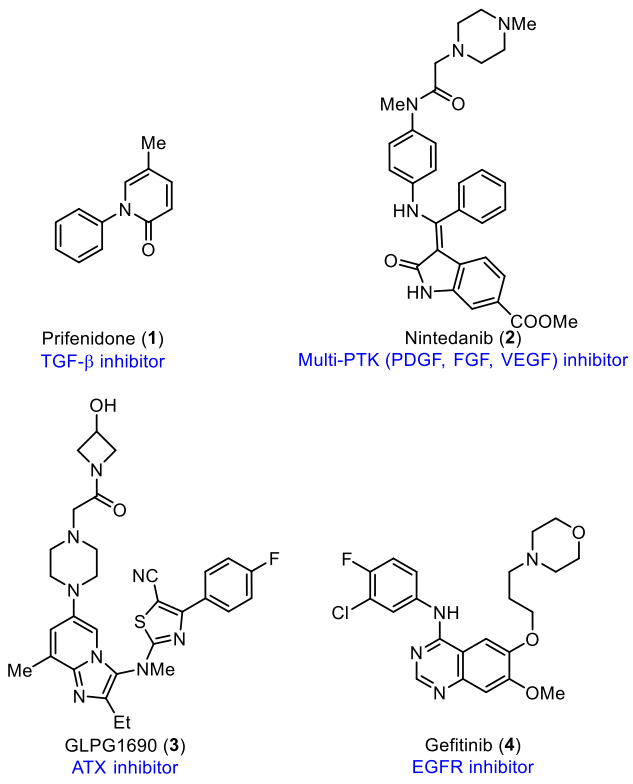


Fig. 1. Representative compounds for the treatment of pulmonary fibrosis.

lesions in mouse lung tissue models.[20–22] Selenium is an essential component of glutathione peroxidase (GSH-px), which catalyzes the reduction of hydrogen peroxide by the concomitant oxidation of glutathione (GSH) to form glutathione disulfide as a means to prevent oxidative damage. Administering an appropriate amount of selenium can objectively alleviate oxidative stress-related diseases.[23,24] Studies have shown that selenium also exhibits potent anti-inflammatory properties.[25] Considering the increases in oxidative stress and inflammation in IPF patients, selenium was incorporated into the structure of gefitinib to develop a novel EGFR inhibitor, referred to as Selenogefitinib, which was synthesized and evaluated for its activity against EGFR as a potential to treat the progression of bleomycin-induced pulmonary fibrosis (Fig. 2).

The synthesis of selenogefitinib is shown in Scheme 1. First, commercially available 3-hydroxy-4-methoxybenzaldehyde was reacted with hydroxylamine hydrochloride in an acetate buffer solution to afford **7**. After substituting the bromide in 1-bromo-3-chloropropane with **7**, the resulting intermediate **8** was nitrated to introduce an aromatic NO_2 group, which was then smoothly reduced to the corresponding aniline using Zn/aq. NH_4Cl to afford intermediate **10**. After reacting the aniline with DMF-DMA, the resulting *N,N*-dimethylformamidine-protected aniline underwent subsequent cyclization with 3-chloro-4-fluoroaniline (Dimroth rearrangement) to generate the corresponding quinazoline **12**.[26,27] Finally, the Cl atom in **12** was substituted with the secondary amine of **13** to generate the final compound **5**.

The inhibitory activity of selenogefitinib (**5**) against EGFR was evaluated using an ADP-Glo^{TM} kinase assay, with gefitinib as the positive control. The data in Table 1 showed that gefitinib and **5** both displayed strong inhibitory potencies against EGFR, with IC_{50} values of 15.5 nM and 25.3 nM, respectively. The results indicated that the presence of the selenium atom did not cause the target compound to

Table 1
In vitro inhibition of kinase and cellular toxic assay of **5** and gefitinib.

Compd.	Enzymatic activity (IC_{50}, nM)[a]	Toxicity to L-02, HBE (IC_{50}, μM)[b]	
	EGFR	L-02	HBE
5	25.3 ± 1.26	13.79 ± 0.85	21.77 ± 2.10
Gefitinib	15.5 ± 2.01	10.08 ± 1.20	18.40 ± 0.96

a) Dose-response curves were determined at three concentrations. The IC_{50} values are the concentrations in nanomolar needed to inhibit cell growth by 50% as determined from these curves. b) Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations in micromolar needed to inhibit cell growth by 50% as determined from these curves.

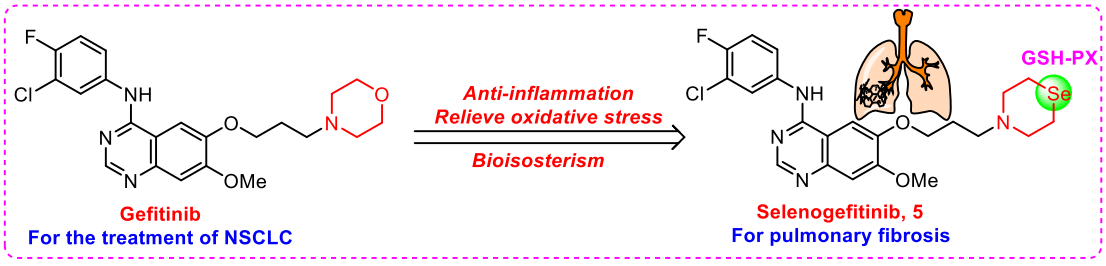
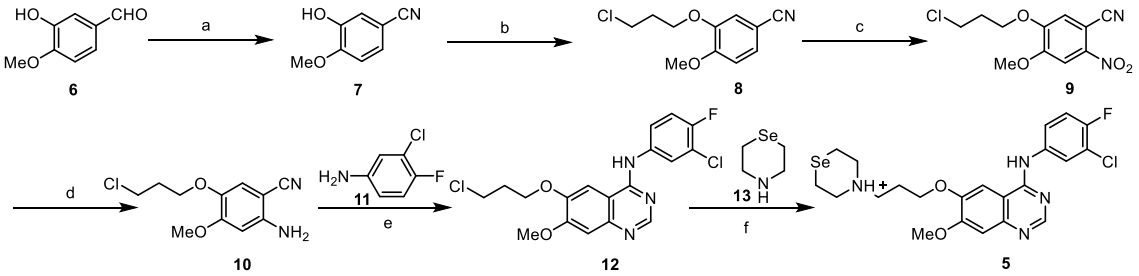
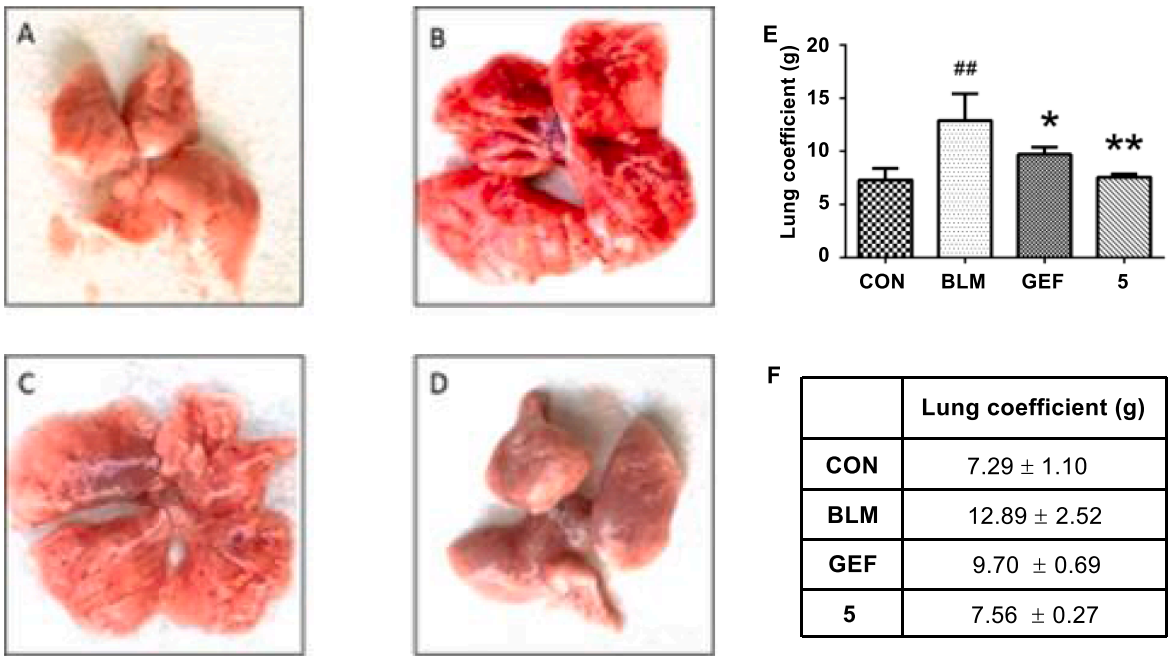


Fig. 2. Design of the title compound.



Reagents and condition: a) HCOONa, HCOOH, NH_2OH HCl, 85 °C, 2 h, 75%; b) 1-bromo-3-chloropropane, K_2CO_3, DMF, 60 °C, 5 h, 80%; c) 70% HNO_3, con. H_2SO_4, rt, 2 h, 85%; d) Zn, aq. NH_4Cl. 60 °C, 30 min, 75%; e) DMF-DMA, acetic acid (catalyst), Toluene, 4 h, 110 °C, then 3-chloro-4-fluoroaniline, acetic acid, 4 h, 130 °C, 78%; f) Selenomorpholine, K_2CO_3, KI, CH_3CN, 2 h, 70 °C, 80%.

Scheme 1. Synthesis of selenogefitinib.



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	Lung coefficient (g)
CON	7.29 ± 1.10
BLM	12.89 ± 2.52
GEF	9.70 ± 0.69
5	7.56 ± 0.27

Fig. 3. The effects of gefitinib and **5** on the lung morphology and lung coefficient in the mouse model. Groups C and D were exposed to gefitinib and **5**, respectively, once daily for 14 days after BLM exposure (n = 10). (A: control group; B: BLM-induced group; C: BLM-induced group plus gefitinib (60 mg/kg); D: BLM-induced group plus **5** (60 mg/kg); E and F: The lung coefficient of the mice) Data are reported as the mean ± SEM. $^{*}p < 0.05$ vs. BLM group; $^{**}p < 0.01$ vs. BLM group; $^{\#\#}p < 0.01$ vs. control group.

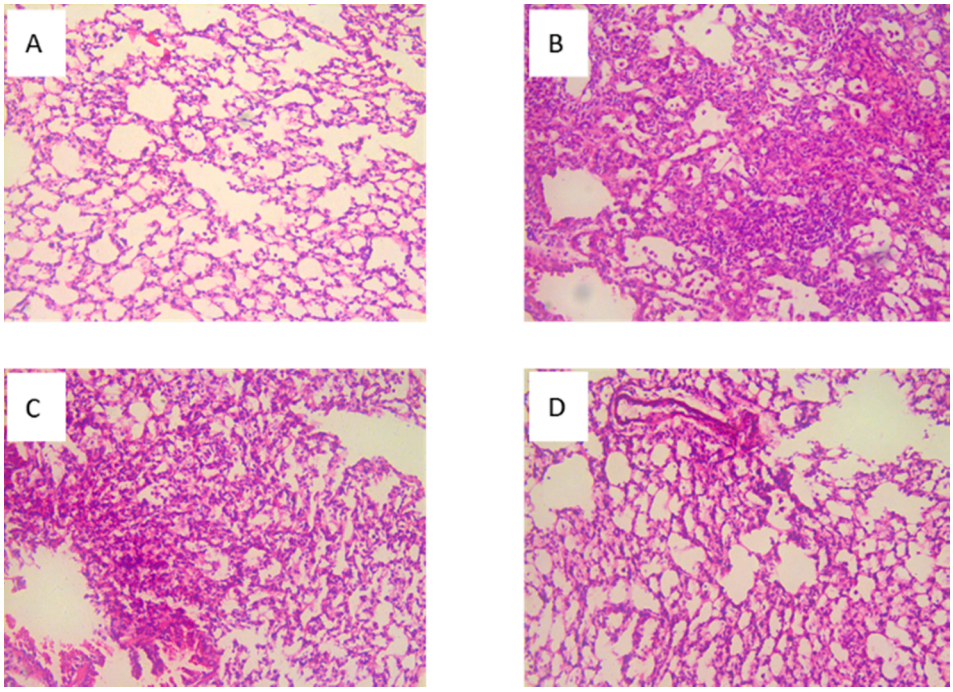

Fig. 4. Representative histological lung sections stained with H&E (200 ×). (A: control group; B: BLM group; C: BLM group plus Gefitinib (60 mg/kg); D: BLM group plus **5** (60 mg/kg)).

interfere with the binding of ATP to EGFR. The cytotoxicity of **5** in L-02 and HBE cells was evaluated using the CCK-8 cell viability assay. Compared to gefitinib, substitution of the oxygen atom with selenium led to a significantly lower cytotoxicity in L-02 and HBE cells, with IC_{50} values (corresponding to the inhibition of growth of the cells) of 13.79 μM and 21.77 μM, respectively, which indicated that **5** was less hepatotoxic and cytotoxic than gefitinib.

The therapeutic effects of **5** on the progression of BLM-induced pulmonary inflammation and pulmonary fibrosis model was investigated *in vivo* using C57BL mice and compared to gefitinib, which has been shown to be efficacious in a lung fibrosis model. In general, higher lung coefficients corresponded to less efficacy of the inhibitor on reduce lung fibrosis and inflammation. As shown in Fig. 3, the color of the lung tissue had obviously darkened in the mice after being exposed to BLM, and the lung coefficient increased significantly. H&E staining (Fig. 4) demonstrated that treatment with BLM led to the accumulation of

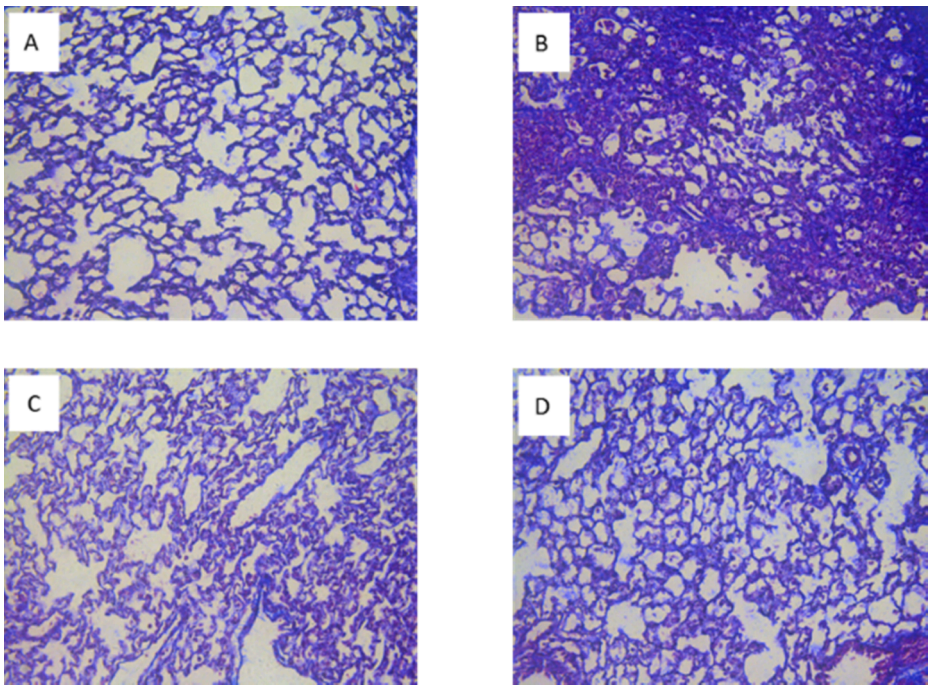

Fig. 5. The effects of gefitinib and **5** on the changes in collagen disposition in the mouse model (200 ×). (A: control group; B: BLM group; C: BLM group plus gefitinib (60 mg/kg); D: BLM group plus **5** (60 mg/kg)).

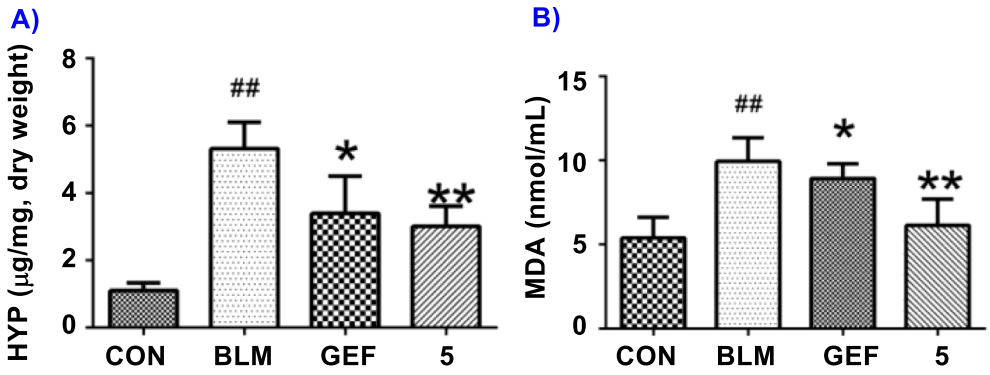

Fig. 6. A: HYP content in model mice lung tissue (n = 10); B: MDA content in model mice lung tissue (n = 10). Data are reported as the mean ± SEM. *p < 0.05 vs. BLM group; **p < 0.01 vs. BLM group; ##p < 0.01 vs. control group.

inflammatory cells in the alveolar space, enhancing the thickness of the alveolar wall. These results indicated that the lung tissue had become significantly damaged in mice after being exposed to BLM. When the mice were administered 60 mg/kg of **5**, H&E staining of the lung tissue showed that the lung coefficient decreased significantly, which suggested that **5** protected the structure of the alveolar tissue and mitigated the infiltration of inflammatory cells. Therefore, replacing the oxygen atom in gefitinib with selenium appeared to manifest an enhanced anti-IPF efficacy.

The deposition of a large amount of collagen in lung tissue is characteristic of pulmonary fibrosis, and the amount of hydroxyproline (HYP) is an well-established indicator of the formation of collagen fibers. Masson staining results (Fig. 5) showed that BLM increased the deposition of collagen in the lung tissue of the mice, while both **5** and gefitinib inhibited the deposition of collagen in the lung tissue. Accordingly, the HYP content in the lung tissue of the mice was measured using a HYP analysis kit (Fig. 6A). After administration of gefitinib or **5** to the mice at a dose of 60 mg/kg, the HYP content in the lung had significantly reduced compared to the untreated BLM group. In addition, compared to the gefitinib group, treatment with **5** more-

Table 2
The typical values of the evaluation of MDA, HYP and inflammatory factors.

	HYP (μg/mg)	MDA (nmol/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Control	1.09 ± 0.23	5.38 ± 1.22	0.46 ± 0.17	0.62 ± 0.11	0.86 ± 0.20
BLM	5.31 ± 0.79	9.93 ± 1.41	2.72 ± 0.14	2.36 ± 0.16	3.12 ± 0.11
Gefitinib	3.39 ± 1.11	8.92 ± 0.86	2.17 ± 0.04	1.77 ± 0.02	2.39 ± 0.13
5	3.01 ± 0.61	6.13 ± 1.57	1.72 ± 0.18	1.00 ± 0.01	1.56 ± 0.09

significantly reduced the deposition of collagen and the content of HYP in the lung tissue (Table 2).

Malondialdehyde (MDA) is a critical marker of oxidative stress, and the levels of MDA have been used as an indicator of the degree of oxidative damage to cells in various organisms. After the mice were treated with **5** at a dosage of 60 mg/kg for 14 days, the levels of MDA determined in the blood were lower than the gefitinib-treated mice

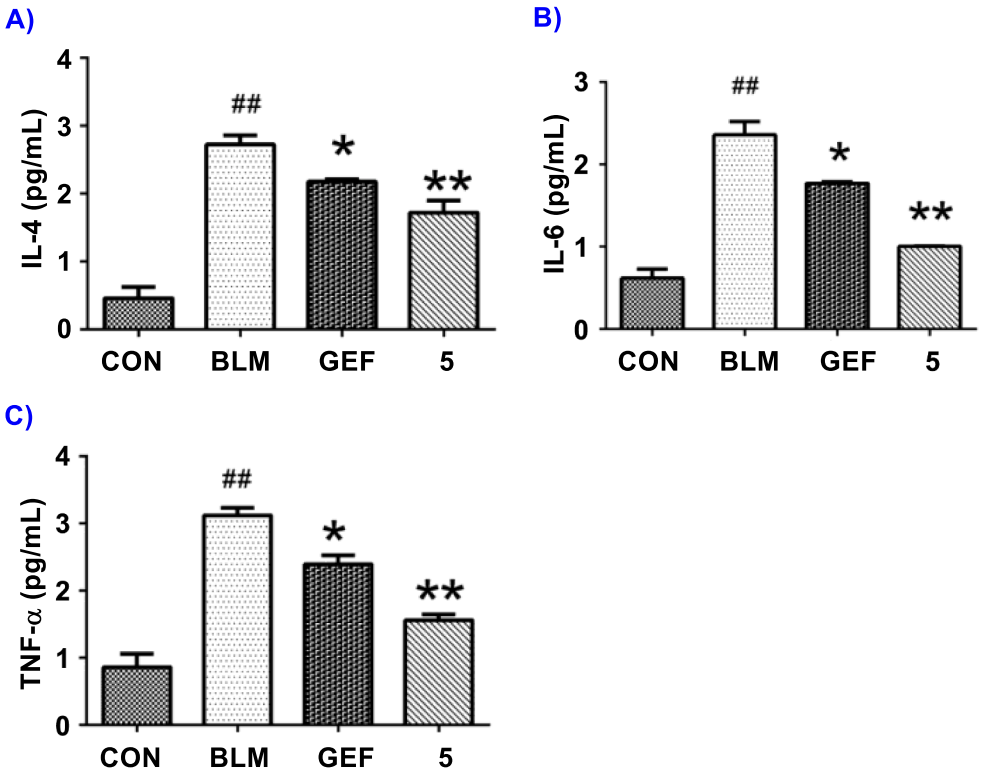

Fig. 7. The effect of gefitinib and **5** on the changes in the expression of inflammatory markers in mice blood (A: IL-4 content; B: IL-6 content; C: TNF-α content. n = 10). Data are reported as the mean ± SEM. *p < 0.05 vs. BLM group; **p < 0.01 vs. BLM group; ##p < 0.01 vs. control group.

group with the same dosage (Fig. 6**B**). IL-4, IL-6, and TNF-α are quintessential pro-inflammatory mediators in various inflammatory diseases. As such, the levels of IL-4, IL-6 and TNF-α in the blood of the BLM-induced mice were significantly upregulated based on flow cytometry analysis. As shown in Fig. 7, compared to the gefitinib-treated group, the expression of the inflammatory factors significantly decreased after administering **5** to the mice for 14 days, where the levels of IL-6 returned to baseline values (control group). The decreased expression of TNF-α in the mice treated with **5** was comparable to that of the gefitinib-treated group. These results clearly validated that the substitution of the oxygen atom in gefitinib with selenium manifested significant alleviation of oxidative stress and a reduction in inflammation, thereby slowing down the progression of IPF (Table 2).

Herein, a potential EGFR inhibitor, selenogefitinib, was synthesized based on the FDA-approved gefitinib, and the efficacy of selenogefitinib in reducing the progression of bleomycin-induced pulmonary fibrosis was evaluated *in vitro* and *in vivo*. Compared to gefitinib, selenogefitinib effectively reduced the deposition of collagen fibers in lung tissue, significantly reduced inflammation through a reduction in the expression of pro-inflammatory markers, and lowered the content of HYP and MDA in mice. These results supported the potential of Selenogefitinib to be further studied for the treatment of pulmonary fibrosis. Overall, this work provided a new insight into the treatment of IPF by mitigating the exacerbation of oxidative damage and inflammation by inhibiting EGFR and interfering in its signaling cascade.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128238.

References

1 King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet.* 2011;378:1949–1961.
2 Chapman HA. Disorders of lung matrix remodeling. *J Clin Investig.* 2004;113:148–157.
3 Costabel U. The changing treatment landscape in idiopathic pulmonary fibrosis. *Eur Respir Rev.* 2015;24:65–68.
4 Ley B, Collard HR. Epidemiology of idiopathic pulmonary fibrosis. *Clin Epidemiol.* 2013;5:483–492.
5 Fernandez IE, Eickelberg O. New cellular and molecular mechanisms of lung injury and fbrosis in idiopathic pulmonary fbrosis. *Lancet.* 2012;380:680–688.
6 Wynn TA. Integrating mechanisms of pulmonary fbrosis. *J Exp Med.* 2011;208:1339–1350.
7 Degryse AL, Lawson WE. Progress toward improving animal models for IPF. *Am J Med Sci.* 2011;341:444–449.
8 King Jr TE, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fbrosis. *N Engl J Med.* 2014;370:2083–2092.
9 Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fbrosis. *N Engl J Med.* 2014;370:2071–2082.
10 Inomata M, Kamio K, Azuma A, et al. Pirfenidone inhibits fibrocyte accumulation in the lungs in bleomycin-induced murine pulmonary fibrosis. *Respir Res.* 2014;15:16. https://doi.org/10.1186/1465-9921-15-16.
11 Ley B, Swigris J, Day B-m, et al. Pirfenidone reduces respiratory-related hospitalizations in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2017;196:756–761.

12 Ebina M, Shimizukawa M, Shibata N, et al. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2004; 169:1203–1208.
13 Gabasa M, Ikemori R, Hilberg F, Reguart N, Alcaraz J. Nintedanib selectively inhibits the activation and tumour-promoting effects of fibroblasts from lung adenocarcinoma patients. *Br J Cancer*. 2017;117:1128–1138.
14 Rangarajan S, Kurundkar A, Kurundkar D, et al. Novel mechanisms for the antifibrotic action of nintedanib. *Am J Respir Cell Mol Biol*. 2016;54:51–59.
15 Roth GJ, Binder R, Colbatzky F, et al. Nintedanib: From Discovery to the Clinic. *J Med Chem*. 2015;58:1053–1063.
16 Bando M, Yamauchi H, Ogura T, et al. Clinical experience of the long-term use of pirfenidone for idiopathic pulmonary fibrosis. *Intern Med*. 2016;55:443–448.
17 Nathan SD, Albera C, Bradford WZ, et al. Effect of continued treatment with pirfenidone following clinically meaningful declines in forced vital capacity: analysis of data from three phase 3 trials in patients with idiopathic pulmonary fbrosis. *Thorax*. 2016;71:429–435.
18 Richeldi L, Cottin V, du Bois RM, et al. Nintedanib in patients with idiopathic pulmonary fbrosis: combined evidence from the TOMORROW and INPULSIS((R)) trials. *Respir Med*. 2016;113:74–79.
19 Desroy N, Housseman C, Bock X, et al. Discovery of 2-[[2-Ethyl-6-[4-[2-(3-hydroxyazetidin-1-yl)-2-oxoethyl]piperazin-1-yl]-8-methylimidazo[1,2-a]pyridin-3-yl] methylamino]-4-(4-fluorophenyl)thiazole-5-carbonitrile (GLPG1690), a First-in-Class Autotaxin Inhibitor Undergoing Clinical Evaluation for the Treatment of Idiopathic Pulmonary Fibrosis. *J Med Chem*. 2017;60:3580–3590.
20 Suzuki H, Aoshiba K, Yokohori N, et al. Epidermal growth factor receptor tyrosine kinase inhibition augments a murine model of pulmonary fibrosis. *Cancer Res*. 2003; 63:5054–5059.
21 Ishii Y, Fujimoto S, Fukuda T. Gefitinib prevents bleomycin-induced lung fibrosis in mice. *Am J Respir Crit Care Med*. 2006;174:550–556.
22 Hardie WD, Davidson C, Ikegami M, et al. EGF receptor tyrosine kinase inhibitors diminish transforming growth factor-α-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L1217–L1225.
23 Rohr-Udilova N, Sieghart W, Eferl R, et al. Antagonistic effects of selenium and lipid peroxides on growth control in early hepatocellular carcinoma. *Hepatology*. 2012;55: 1112–1121.
24 Laird MD, Wakade C, Alleyne CH, Dhandapani KM. Hemin-induced necroptosis involves glutathione depletion in mouse astrocytes. *Free Radical Biol Med*. 2008;45: 1103–1114.
25 Khan HA. Selenium partially reverses the depletion of striatal dopamine and its metabolites in MPTP-treated C57BL mice. *Neurochem Int*. 2010;57:489–491.
26 Chandregowda V, Venkateswara Rao G, Chandrasekara Reddy G. Convergent Approach for Commercial Synthesis of Gefitinib and Erlotinib. *Org Process Res Dev*. 2007;11:813–816.
27 Wahren M. *Z Chem*. 1969;9:241–252.