Supplementary Information for

N-Phenyl-2-Pyridone-Derived Endoperoxide Exhibiting Dual Activity by Suppressing both Lung Cancer and Idiopathic Pulmonary Fibrosis

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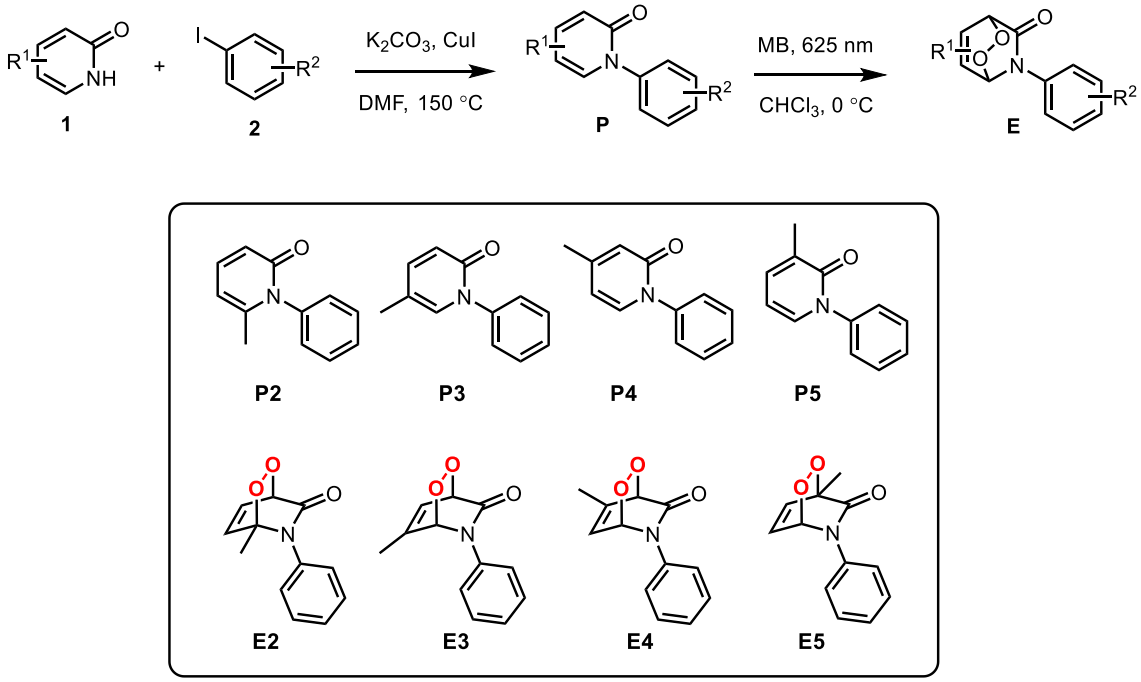
1. Syntheses of N-phenyl-2-pyridone and endoperoxides



Supplementary Fig. 1 Synthetic route of endoperoxides and chemical structure of precursor **P2-P5** and endoperoxide **E2-E5**.

To a solution of 2-pyridone **1** (5 mmol) and iodobenzene **2** (7.5 mmol) in DMF, anhydrous K_2CO_3 (10 mmol) and CuI (0.5 mmol) were added. The reaction mixture was stirred at 150 °C. After the reaction was completed, the reaction mixture was cooled to room temperature, quenched with H_2O, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude residue was purified by column chromatography (Hex/EtOAc, 1/1) to afford the desired products. To a solution of *N*-phenyl-2-pyridone (**P2-P5**) in $CHCl_3$, catalytic amount of methylene blue was added. The solution was irradiated with red light (625 nm) at 0 °C until starting material was consumed completely. The solution was

subjected to a short silica gel to remove methylene blue and the eluent was concentrated under vacuo to afford the product.

P2 (Yield: 12.8%) : ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (t, J = 7.4 Hz, 2H), 7.44 (t, J = 7.4 Hz, 1H), 7.32 – 7.28 (m, 1H), 7.21 – 7.19 (m, 2H), 6.54 (d, J = 9.2 Hz, 1H), 6.10 (d, J = 6.6 Hz, 1H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 146.5, 139.7, 138.9, 129.8, 128.8, 127.9, 118.5, 106.1, 21.6.

P3 (Yield: 72.4%) : ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, J = 7.3 Hz, 2H), 7.42 – 7.36 (m, 3H), 7.27 – 7.24 (m, 1H), 7.11 (s, 1H), 6.60 (d, J = 9.3 Hz, 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 142.6, 141.1, 135.3, 129.2, 128.3, 126.5, 121.3, 114.8, 17.0.

P4 (Yield: 71.2%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, J = 7.3 Hz, 2H), 7.41 – 7.35 (m, 3H), 7.22 (d, J = 7.0 Hz, 1H), 6.45 (s, 1H), 6.08 (d, J = 7.2 Hz, 1H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 151.6, 140.8, 136.8, 129.2, 128.3, 126.5, 119.9, 108.6, 21.3.

P5 (Yield: 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, J = 7.4 Hz, 2H), 7.42 – 7.36 (m, 3H), 7.27 (d, J = 5.3 Hz, 1H), 7.22 (d, J = 6.8 Hz, 1H), 6.16 (t, J = 6.8 Hz, 1H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8, 141.3, 137.0, 135.4, 130.8, 129.2, 128.2, 126.6, 105.6, 17.4.

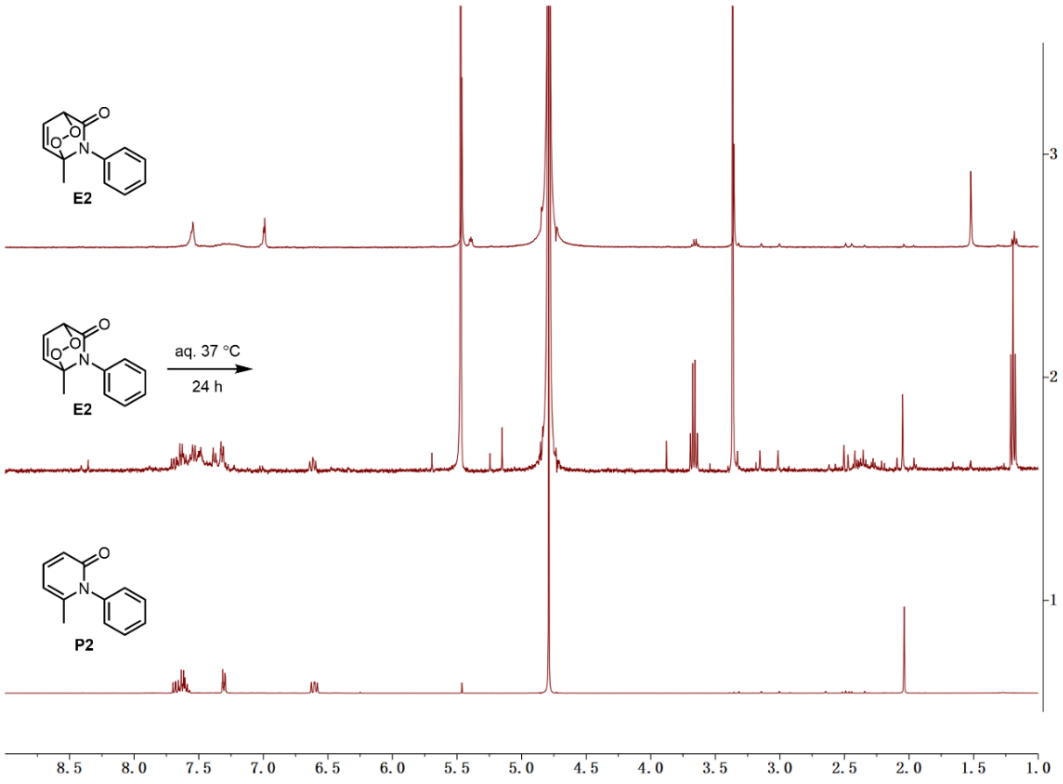
E2 (Yield: 94.2%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.39 (m, 3H), 7.17 (s, 2H), 6.88 – 6.84 (m, 1H), 6.76 – 6.73 (m, 1H), 5.13 – 5.11 (m, 1H), 1.45 (s, 3H).

E3 (Yield: 95.5%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.40 (m, 2H), 7.32 – 7.28 (m, 3H), 6.46 – 6.43 (m, 1H), 5.85 (d, J = 2.3 Hz, 1H), 5.05 (d, J = 6.2 Hz, 1H), 2.12 (s, 3H).

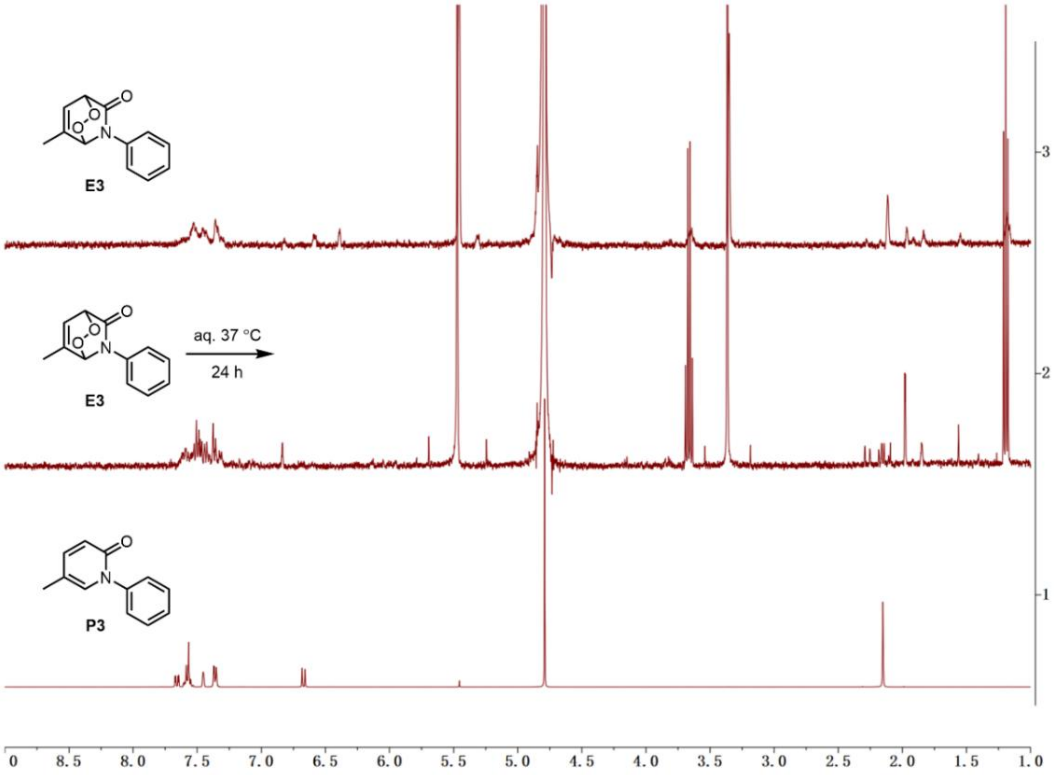
E4 (Yield: 94.6%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, J = 7.7 Hz, 2H), 7.32 – 7.27 (m, 3H), 6.59 – 6.56 (m, 1H), 6.04 (d, J = 5.6 Hz,1H), 4.94 (s, 1H), 2.12 (s, 3H).

E5 (Yield: 96.9%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, J = 7.8 Hz, 2H), 7.32 – 7.27 (m, 3H), 6.96 – 6.93 (m, 1H), 6.59 – 6.57 (m, 1H), 6.08 – 6.06 (m, 1H), 1.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 138.2, 134.0, 129.4, 126.9, 123.7, 86.1, 82.1, 14.7.

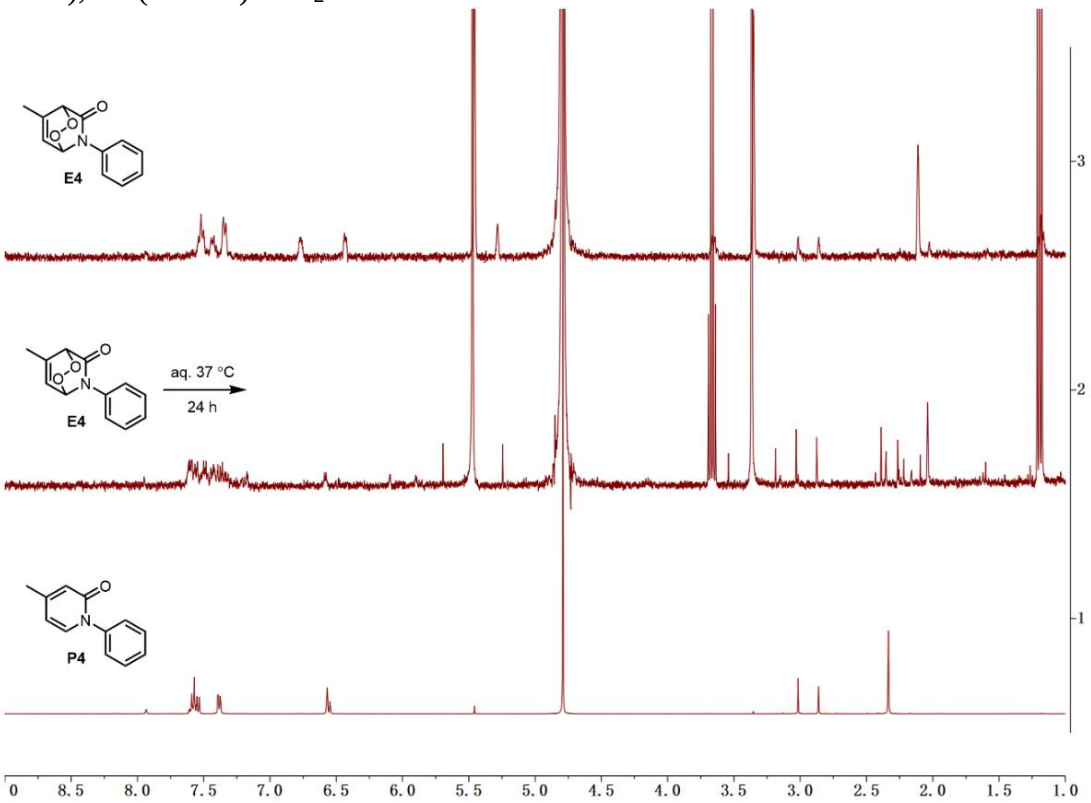
2. Cycloreversion of endoperoxides measured by ^{1}H NMR



Supplementary Fig. 2 ^{1}H NMR spectra of **E2** (top), **E2** incubated at 37 °C for 24 h (middle), **P2** (bottom) in D_2O.



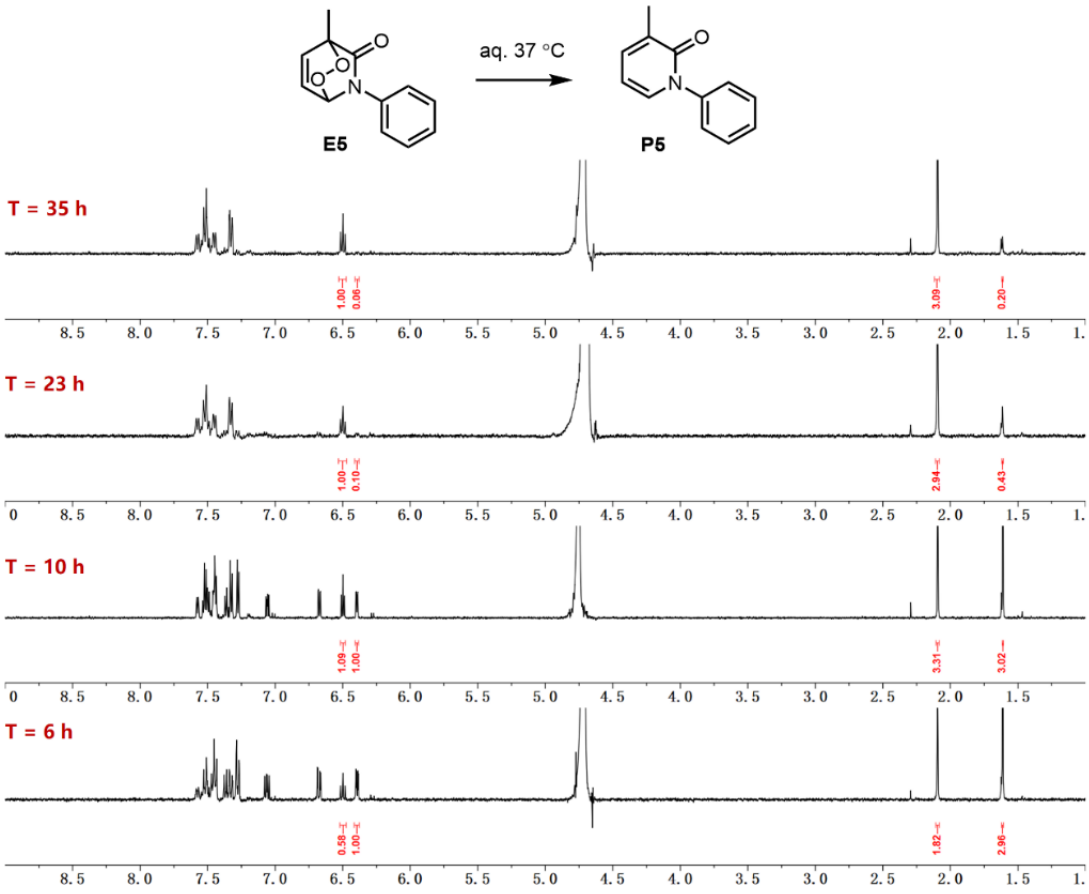
Supplementary Fig. 3 ^{1}H NMR spectra of **E3** (top), **E3** incubated at 37 °C for 24 h (middle), **P3** (bottom) in D_2O.



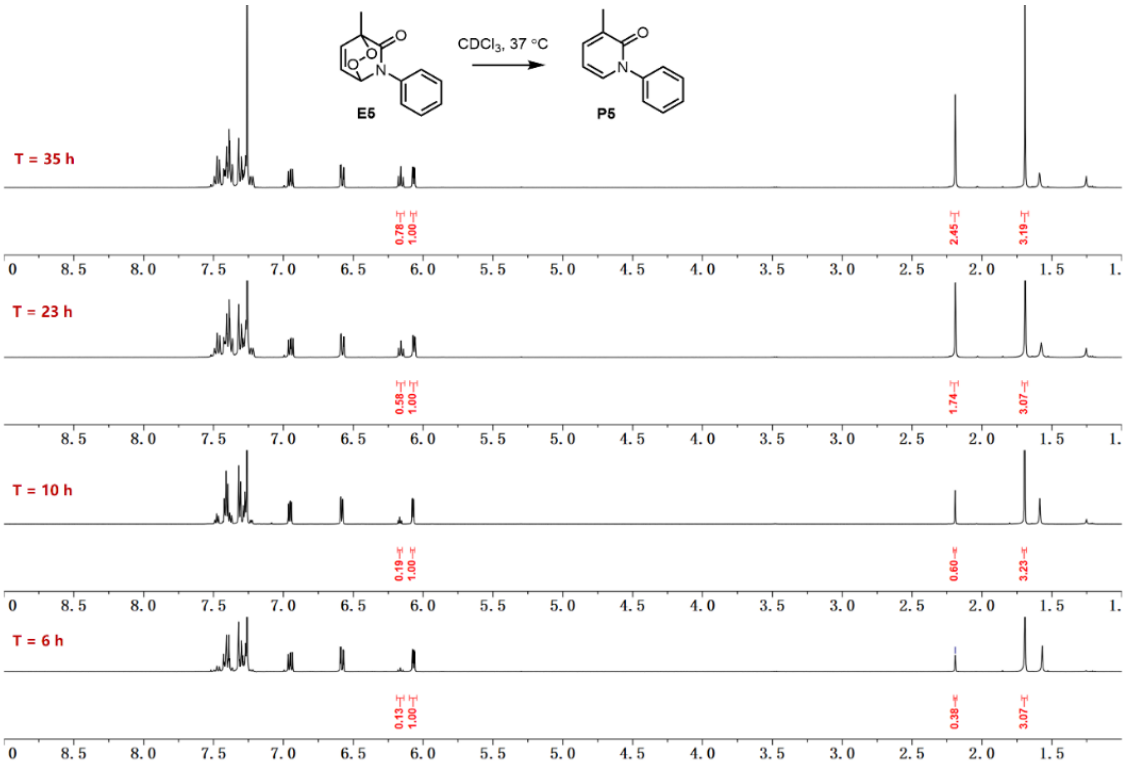
Supplementary Fig. 4 ^{1}H NMR spectra of **E4** (top), **E4** incubated at 37 °C for 24 h (middle), **P4** (bottom) in D_2O.

The cycloreversion rate and half-life time calculation were done by ^{1}H NMR integration of specific signals in accordance to the first-order reaction rate equations[1]. The equation is given below:

$\ln[A] = -kt + \ln[A]_0$, $t_{1/2} = 0.693/k$



Supplementary Fig. 5 ^{1}H NMR spectra of **E5** in D_2O at 37 °C for different times and its half life time was calculated based on the NMR integration. Peaks at 6.39 and 1.61 belong to **E5**, while peaks at 6.50 and 2.10 belong to **P5**. $t_{1/2}$ = 8.3 hours (37 °C in D_2O).



Supplementary Fig. 6 ^{1}H NMR spectra of **E5** in $CDCl_3$ at 37 °C for different times and its half life time was calculated based on the NMR integration. Peaks at 6.06 and 1.69 belong to **E5**, while peaks at 6.16 and 2.19 belong to **P5**. $t_{1/2}$ = 42.3 hours (37 °C in $CDCl_3$).

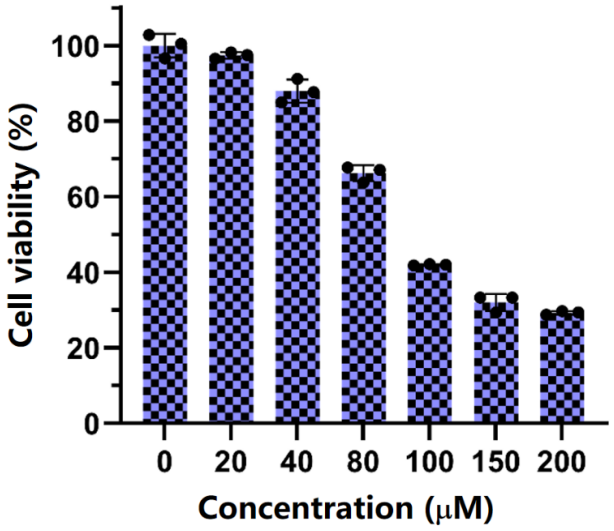
3. Extracellular release of singlet oxygen and measurement of dissolved oxygen.

To calculate the ratio of singlet oxygen released from **E5**, tetramethylethylene was used as a probe which was incubated with **E5** in a 1-1 molar ratio. After **E5** was consumed, the generated **P5** and trapping product **A** were analyzed by ^{1}H NMR. Peak at 6.17 belong to **P5**, while peaks at 4.99 and 4.94 belong to **A**. ^{1}H NMR integrations suggest that 1 mol of **E5** could release 0.5 mol of singlet oxygen in $CDCl_3$.

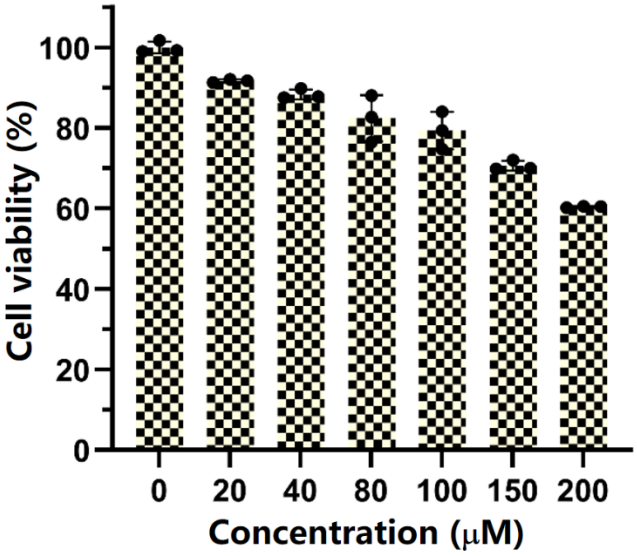
Oxygen release was study using a pocket size portable (FireString GO_2) which is based on the changes of luminescence induced by interference with oxygen[2]. Briefly, a

solution of **E5** in 0.5 mL DMSO was added to PBS buffer (4.5 mL) until the baseline achieve stability, and the dissolved oxygen was directly measured. The final concentration of **E5** is 20 mM and the measurement was done at 37 ℃.

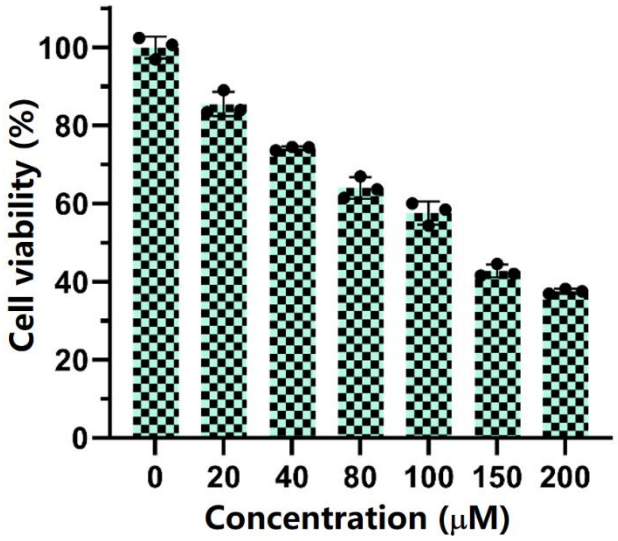
4. MTT assays



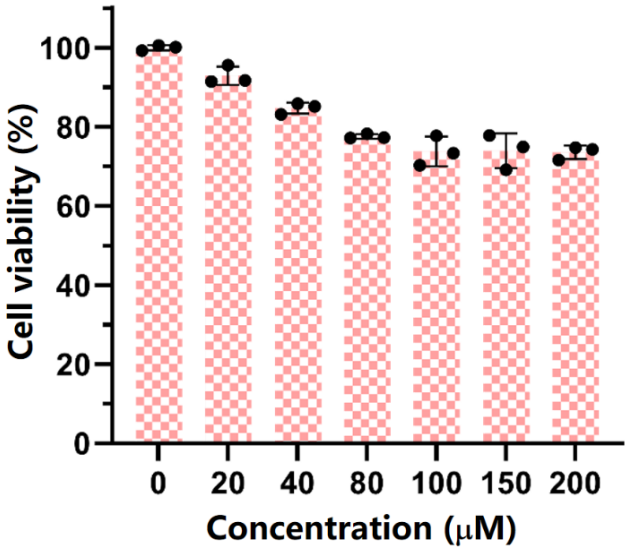
Supplementary Fig. 7 Cell viability of Hela cell treated with **E5** at various concentrations (0, 20, 40, 80, 100, 150, 200 μM).



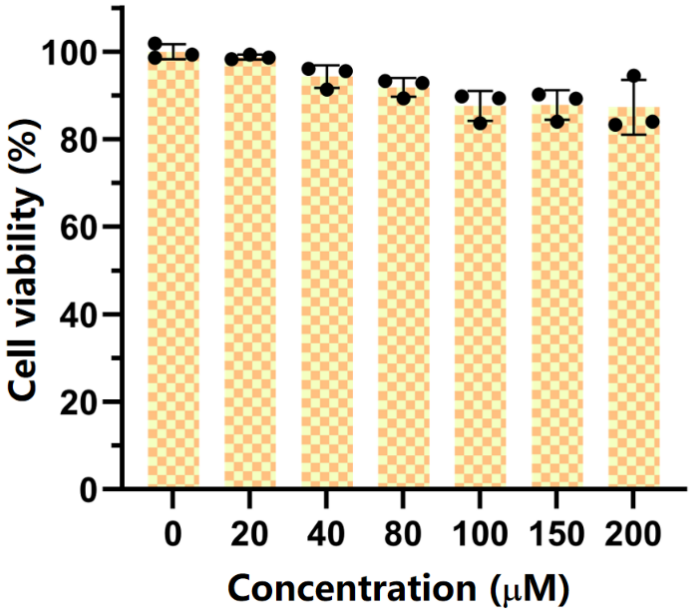
Supplementary Fig. 8 Cell viability of HepG2 cell treated with **E5** at various concentrations (0, 20, 40, 80, 100, 150, 200 μM).



Supplementary Fig. 9 Cell viability of SK-OV-3 cell treated with **E5** at various concentrations (0, 20, 40, 80, 100, 150, 200 μM).



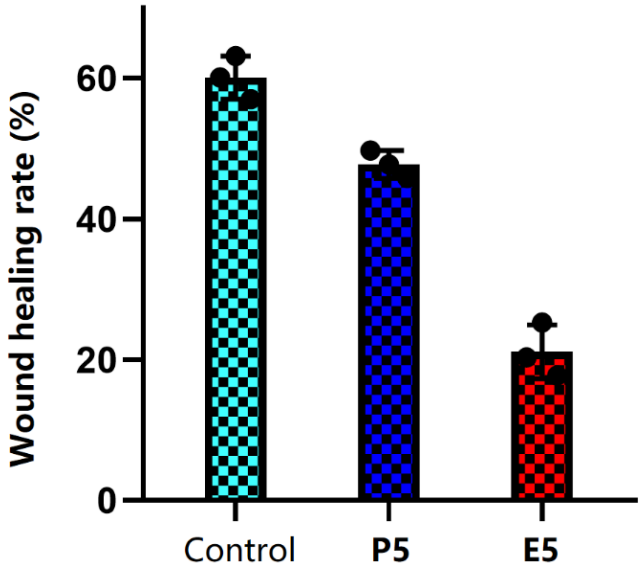
Supplementary Fig. 10 Cell viability of HUVEC cell line treated with **E5** at various concentrations (0, 20, 40, 80, 100, 150, 200 μM).



Supplementary Fig. 11 Cell viability of A549 cell line treated with **P5** at various concentrations (0, 20, 40, 80, 100, 150, 200 μM).

5. Wound healing and transwell invasion assay

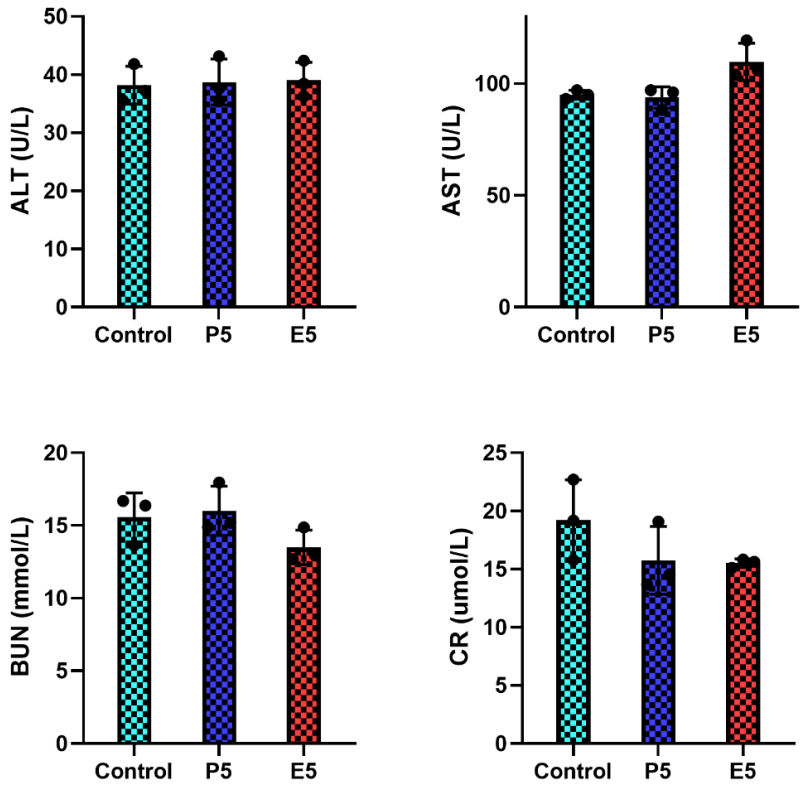
A549 cells (1.5×10^5 cells per dish) were cultured in full growth media at 37 °C with 5% CO_2 in order to achieve proper adhesion of the cells to the glass bottomed dishes. Subsequently, the medium in the well was removed, and a 20 μL of pipette was used to scratch cell monolayers for creating a wound artificially. Then the cells were washed with PBS for 3 times and replaced with fresh medium. Next, 100 μM of **P5** or **E5** were added respectively and further incubated for 48 h, and the photograph at 0 h and 48 h were observed using fluorescence microscope (Nikon Eclipse Ts2-FL).



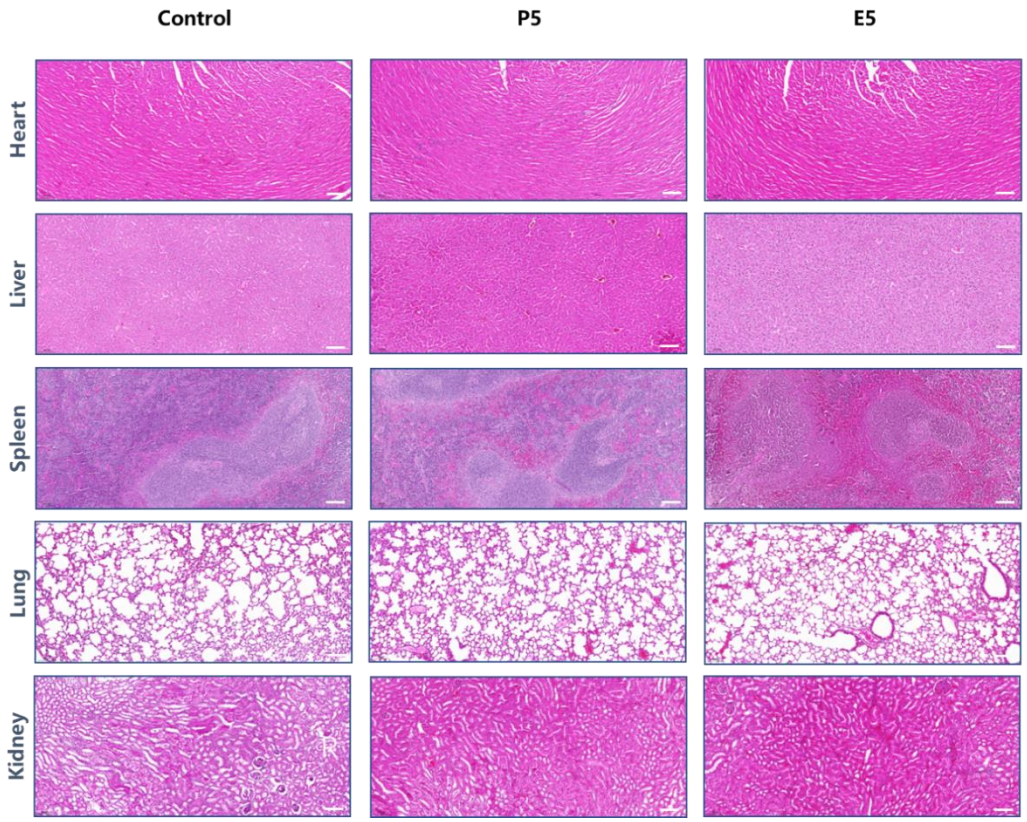
Supplementary Fig. 12 Wound healing rate of A549 cells under different treatments (**E5** or **P5**, 100 μM).

Transwell invasion assay chamber was prepared following the manufacturer's protocol. A549 cells (5×10^5 cells/mL) were seeded at a density of into the upper chambers and incubated at 37 °C for 24 hours. After removal of the medium, **P5** or **E5** (100 μM) were added respectively and further incubated for 24 hours. The bottom cells were washed with PBS for three times, fixed with 4% paraformaldehyde and air dry the chamber properly. The cells were stained with 0.1% crystal violet for 20 minutes and washed with PBS for 3 times, the upper layer of the cells were wiped off with cotton swab and the invasion was analyzed using fluorescence microscope (Nikon Eclipse Ts2-FL).

6. Biosafety assay in anticancer study

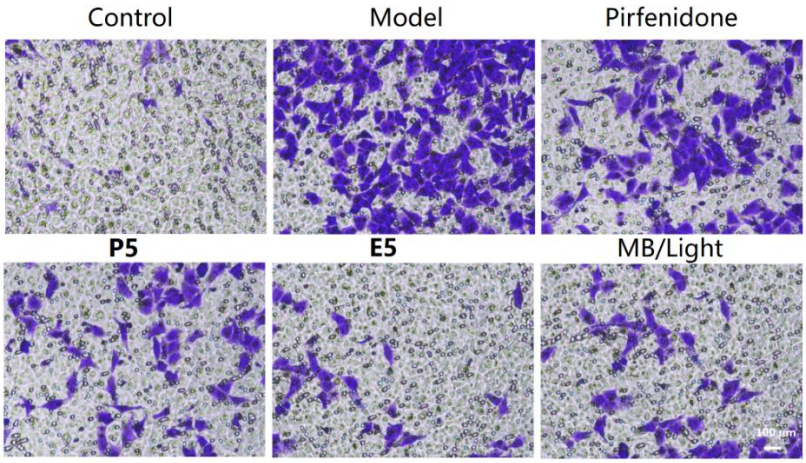


Supplementary Fig. 13 Blood biochemistry analysis of the mice after the treatment of **P5** and **E5** including alanine transferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine (CR) .

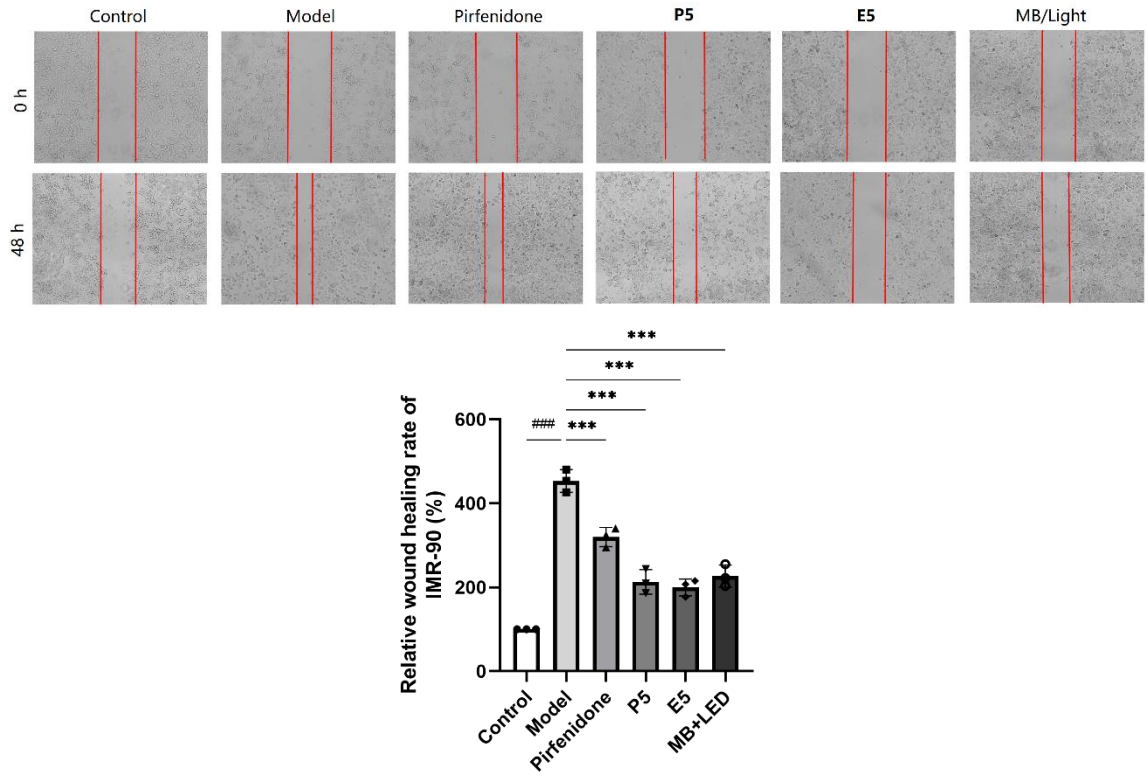


Supplementary Fig. 14 H&E staining of different organs in various groups of mice. Scale bar: 100 μm.

7. *In vitro* Anti-IPF study

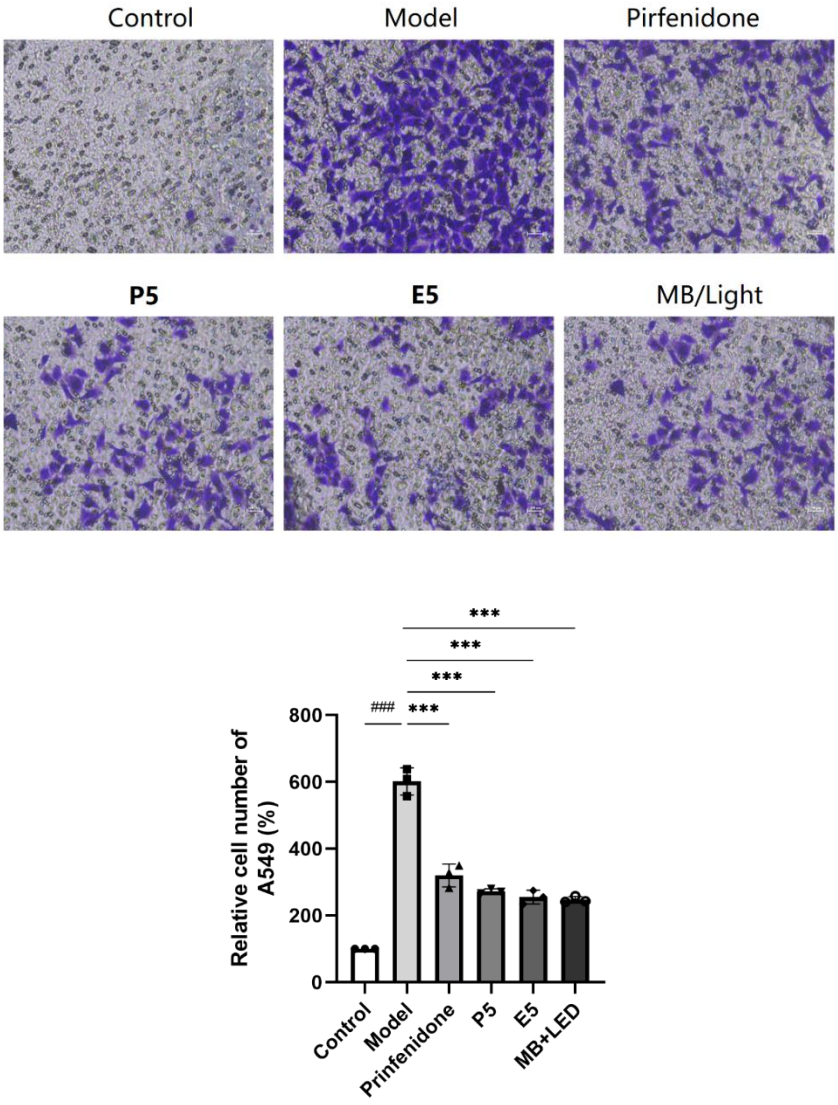


Supplementary Fig. 15 Transwell migration assay of TGF-β1 induced IMR-90 cell line under different treatments (pirfenidone, **E5** or **P5**: 80 μM, MB/light: 17.5 uM, 625 nm LED, 20 min). Control: IMR-90 cell without any treatment. Scale bar: 100 μm.



Supplementary Fig. 16 Wound healing study of TGF-β1 induced IMR-90 cells under different treatments (pirfenidone, **E5** or **P5**: 80 μM, MB/light: 17.5 uM, 625 nm LED, 20 min). Control: IMR-90 cell without any treatment.

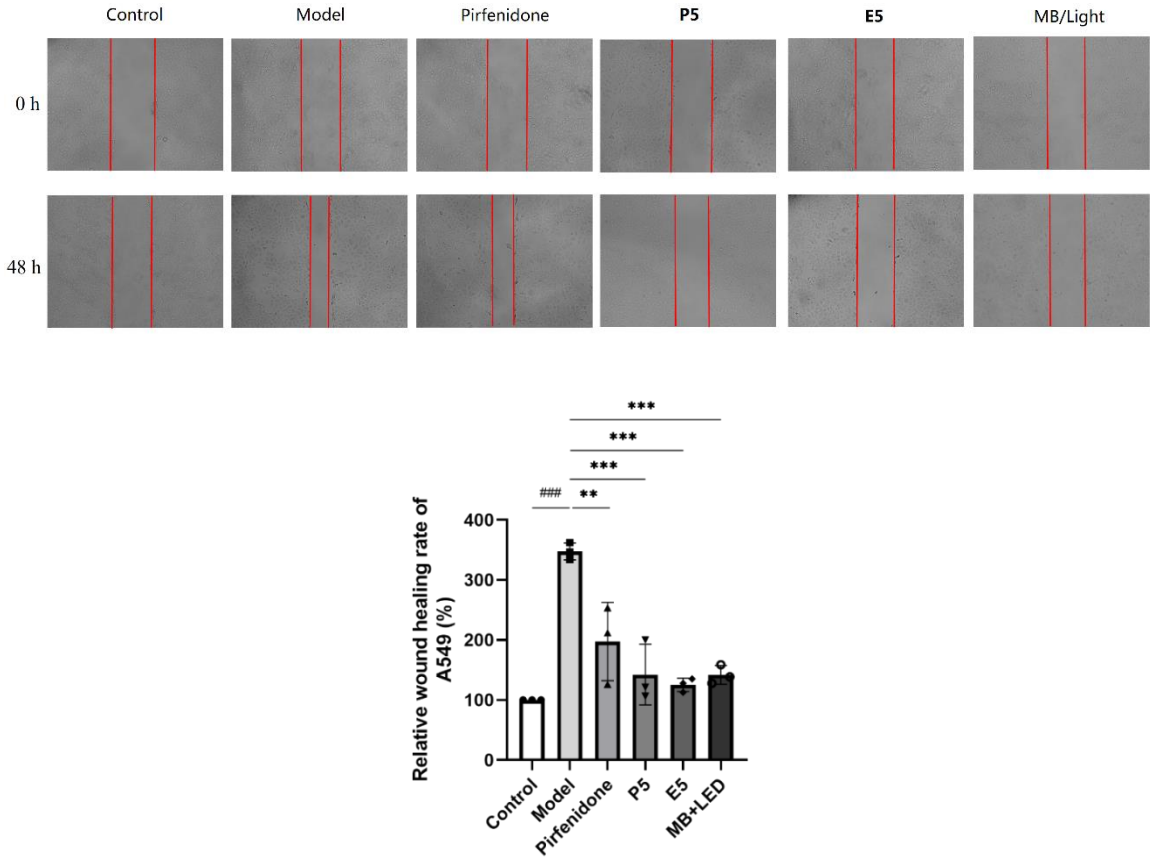
Transwell migration assay[3]: A549 cells seeded into the upper chamber were induced by TGF-β1 under different treatments (pirfenidone, **P5**, **E5** at 80 uM concentration or a MB-mediated photoreaction: MB/light: 17.5 uM, 625 nm LED, 20 min) in DMEM medium containing 2% FBS. After 48 h incubation, the chambers were washed with PBS, fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet dye solution at room temperature for 30 min. After rinsed by PBS, the cells were scraped with a cotton swab and each chamber was air-dried and imaged using Fluorescence microscope (Nikon Eclipse Ts2-FL).



Supplementary Fig. 17 Transwell migration assay of TGF-β1 induced A549 cell line under different treatments (pirfenidone, **E5** or **P5**, 80 μM, MB/light: 17.5 uM, 625 nm LED, 20 min). Control: A549 cell without any treatment. Scale bar: 100 μm.

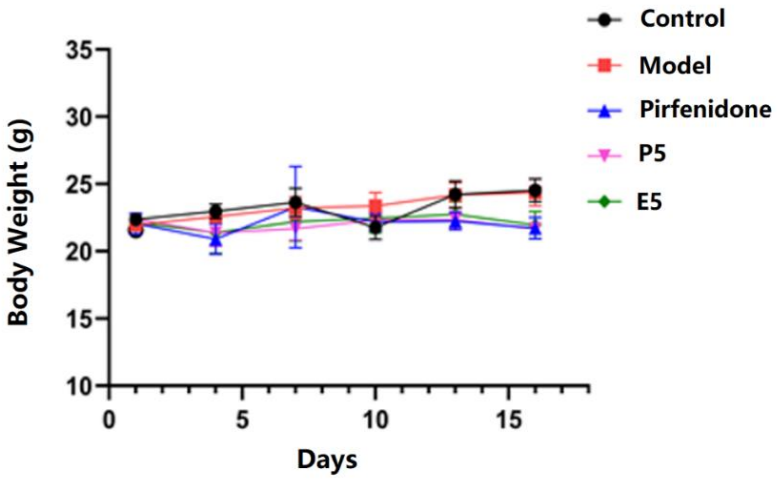
Wound healing assay: A549 cells were cultured in 6-well plates at 37 °C for 24 h. Then, 200 μL of pipette was used to scratch cell monolayers for creating a wound artificially. After washed with PBS and replaced with fresh medium, TGF-β1 (5 ng/ml) was added and the cells was further incubated for 6 hours. After the treatment by pirfenidone, **P5**, **E5** (80 μM) or MB-mediated photoreaction, the cells was further

incubated for 48 h. Cells migrating from the edge of the wound were photographed at 0 and 48 h time points by (Olympus IX71).

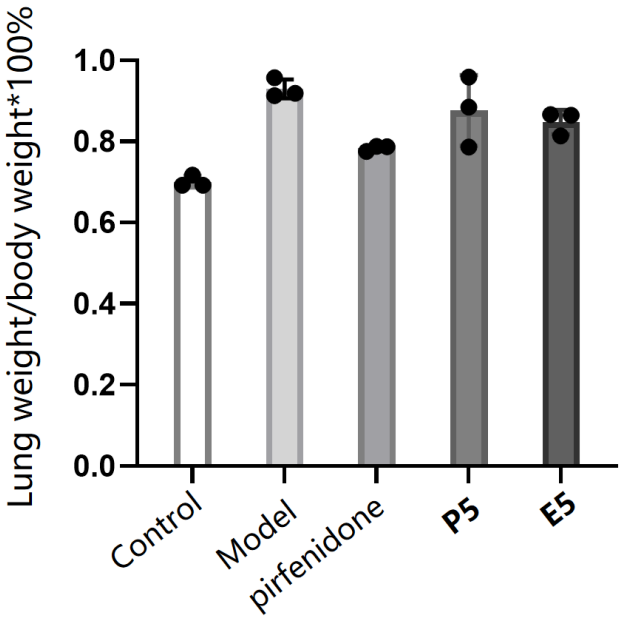


Supplementary Fig. 18 Wound healing assay of TGF-β1 induced A549 cell line under different treatments (pirfenidone, **E5** or **P5**, 80 μM, MB/light: 17.5 uM, 625 nm LED, 20 min). Control: A549 cell without any treatment.

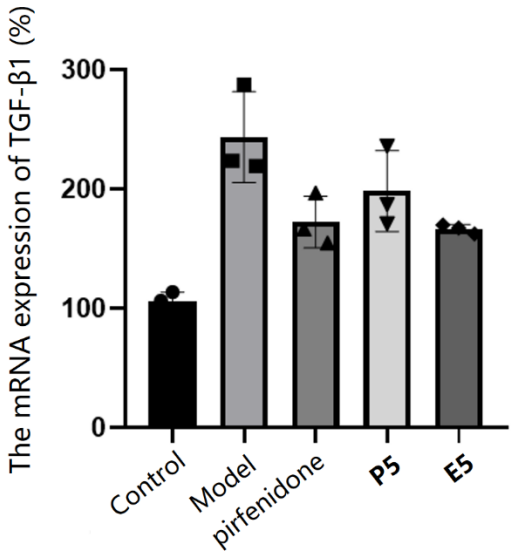
8. *In vivo* Anti-IPF study



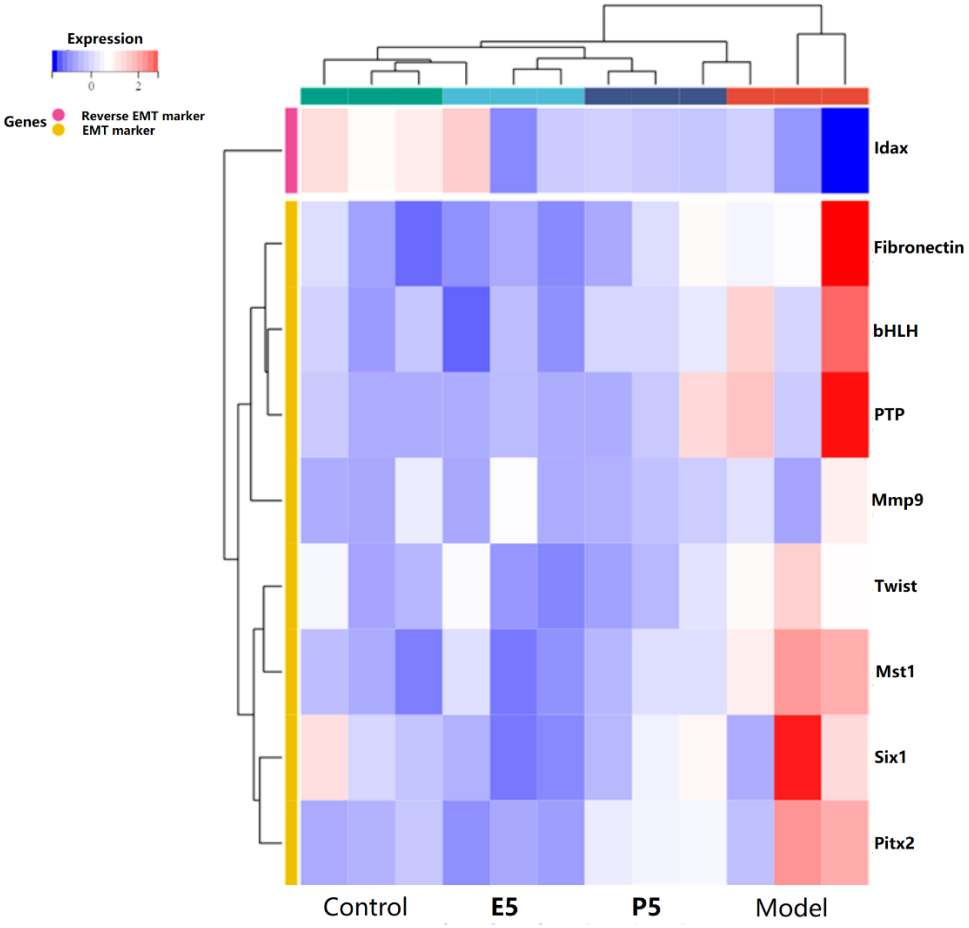
Supplementary Fig. 19 Body weight of mice under different treatment.



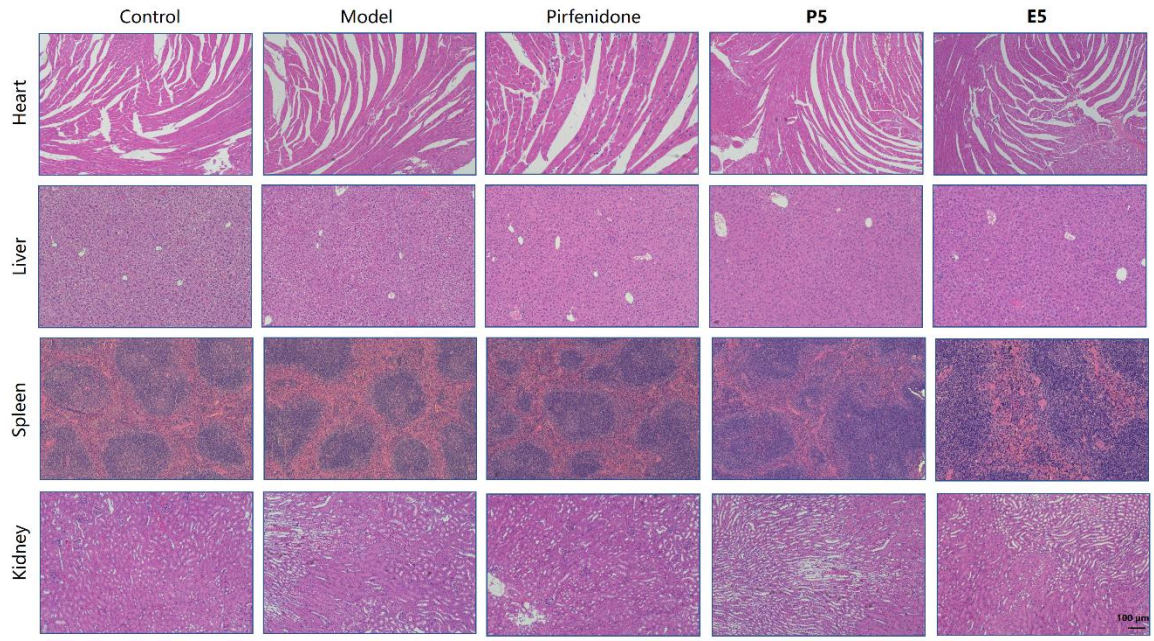
Supplementary Fig. 20 Lung index of mice (lung weight/body weight*100%) in different groups.



Supplementary Fig. 21 The mRNA expression of TGF-β1 expression of mice in different groups.



Supplementary Fig. 22 Heat map of the EMT related markers in different groups.

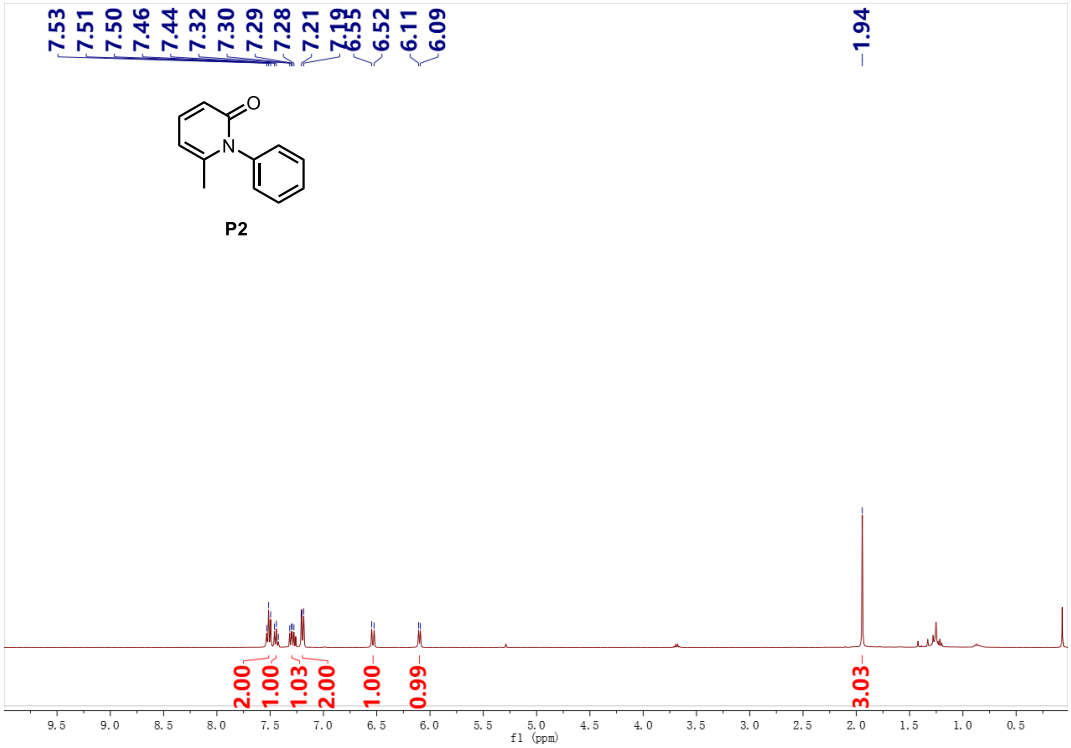


Supplementary Fig. 23 H&E staining of different organs in various groups of mice. Scale bar: 100 μm.

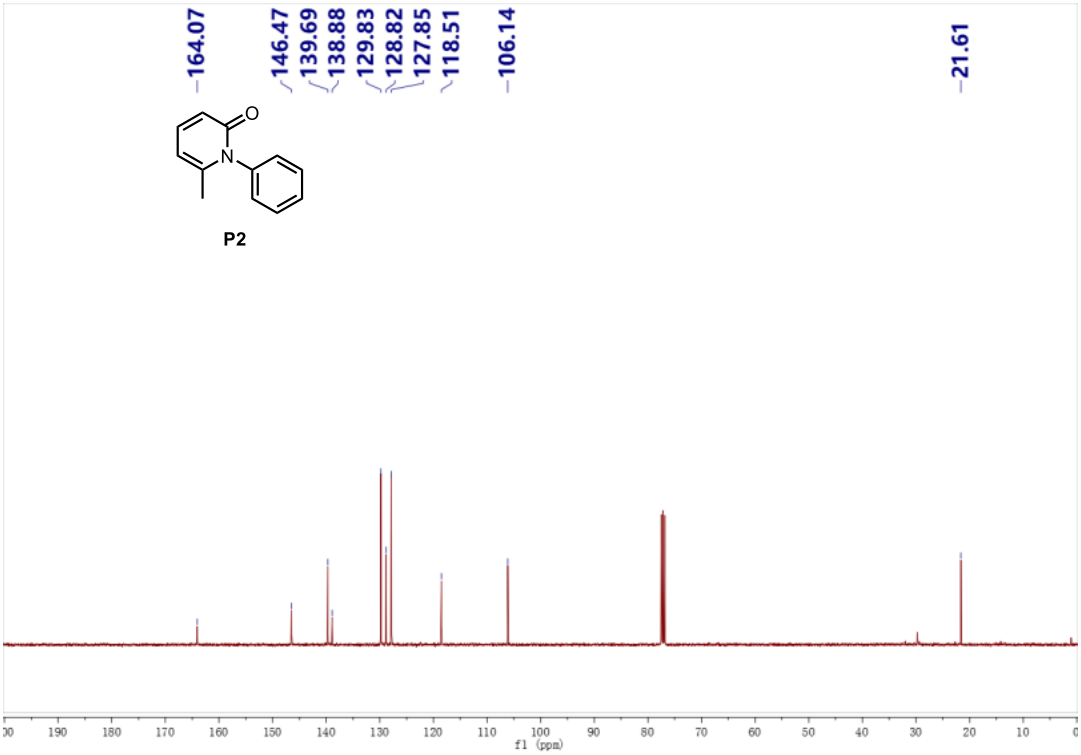
9. References

1. Ucar, E. et al. “Off–on” switching of intracellular singlet oxygen release under biocompatible conditions. *Chem. Commun*. **55**, 13808–13811 (2019).
2. Li, J. et al. Taming of Singlet Oxygen: Towards Artificial Oxygen Carriers Based on 1,4-Dialkylnaphthalenes. *Chemistry-A European Journal* **28**, e202200506 (2022).
3. Fujiwara, A. et al. Pirfenidone plays a biphasic role in inhibition ofepithelial-mesenchymal transition in non-small cell lung cancer. *Lung Cancer* **106**, 8–16 (2017).

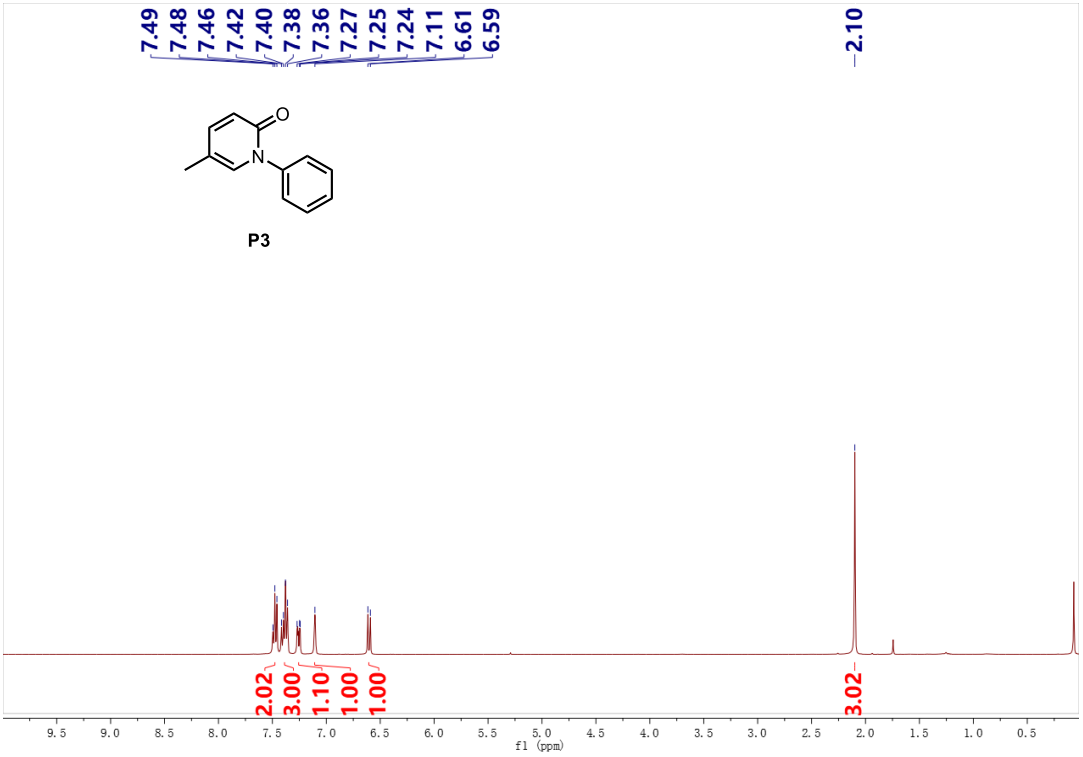
10. NMR spectra



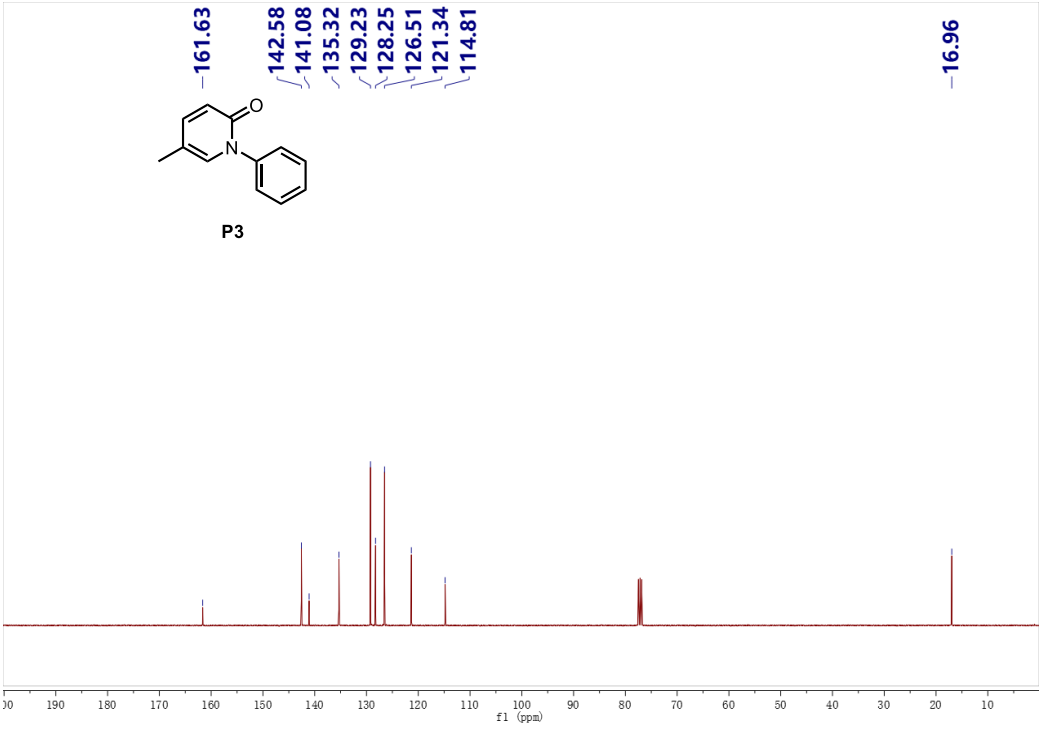
Supplementary Fig. 24 ^{1}H NMR spectrum of compound **P2**



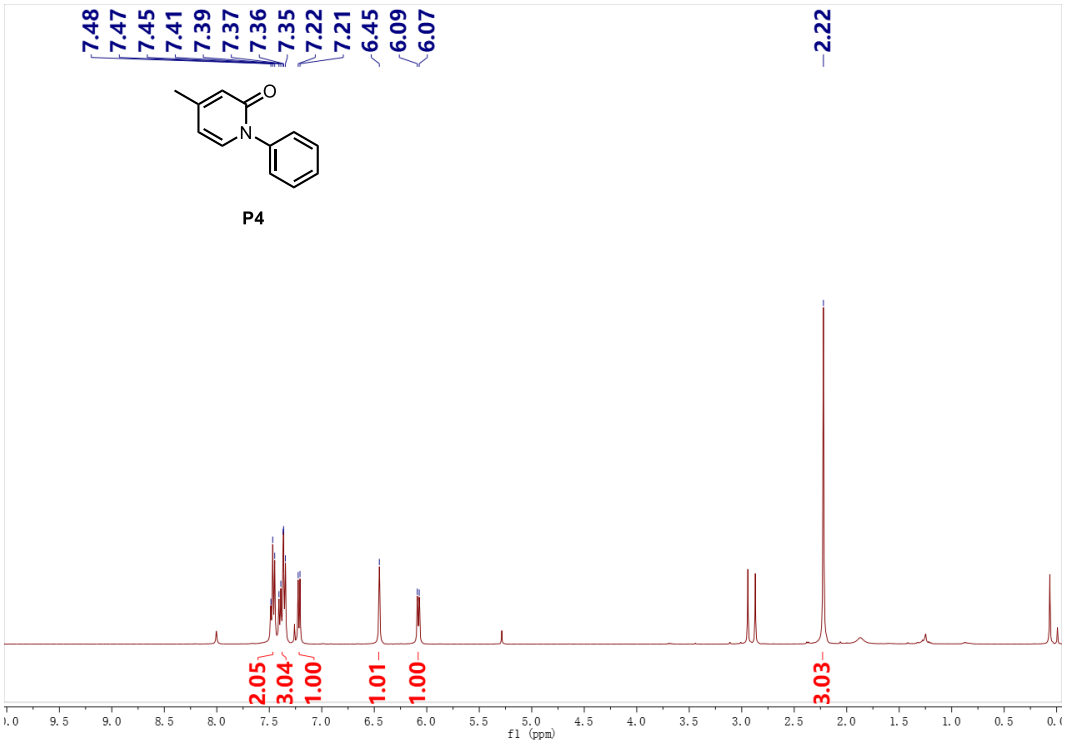
Supplementary Fig. 25 ^{13}C NMR spectrum of compound **P2**



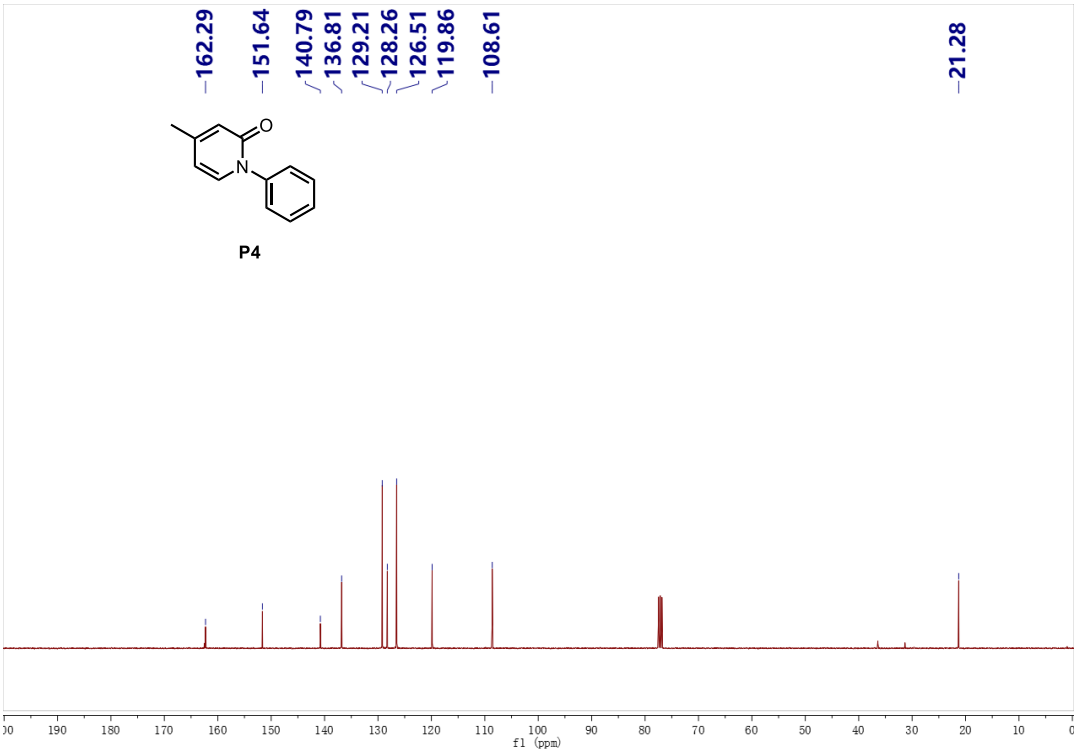
Supplementary Fig. 26 ^{1}H NMR spectrum of compound **P3**



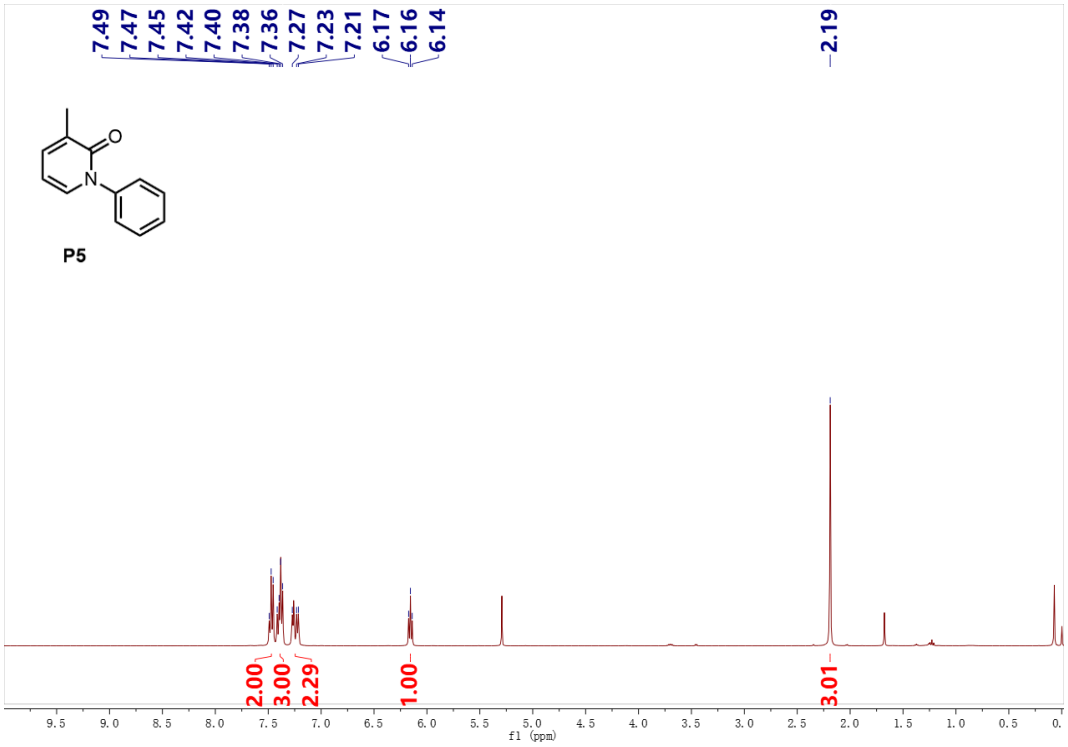
Supplementary Fig. 27 ^{13}C NMR spectrum of compound **P3**



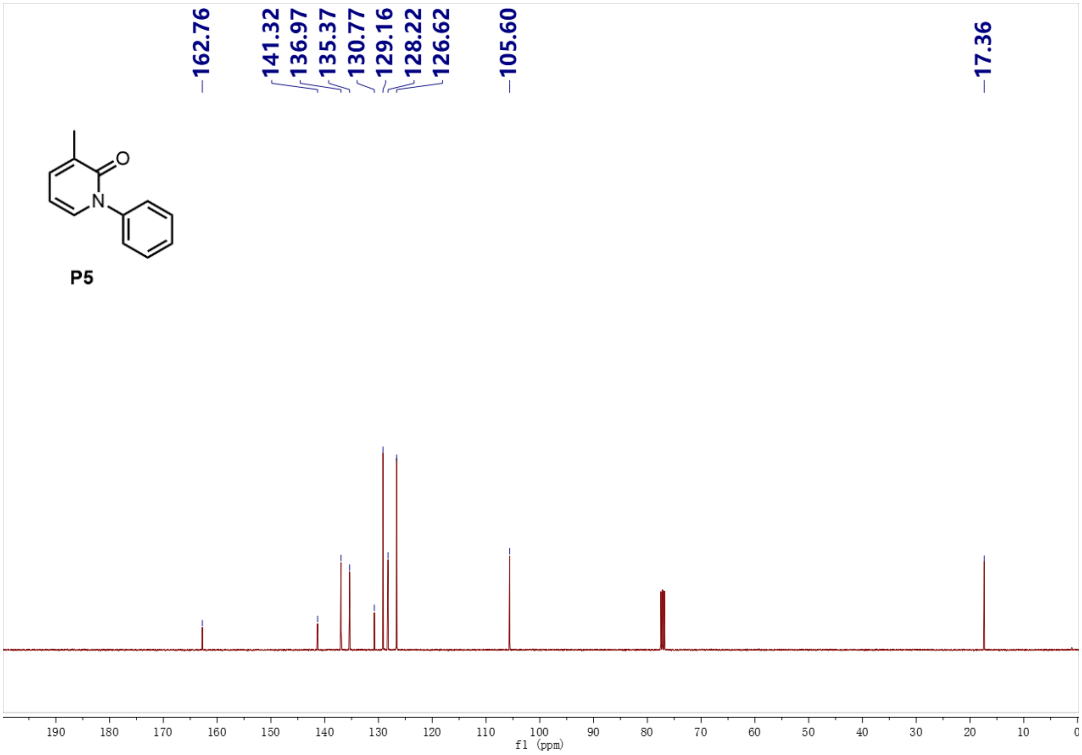
Supplementary Fig. 28 ^{1}H NMR spectrum of compound **P4**



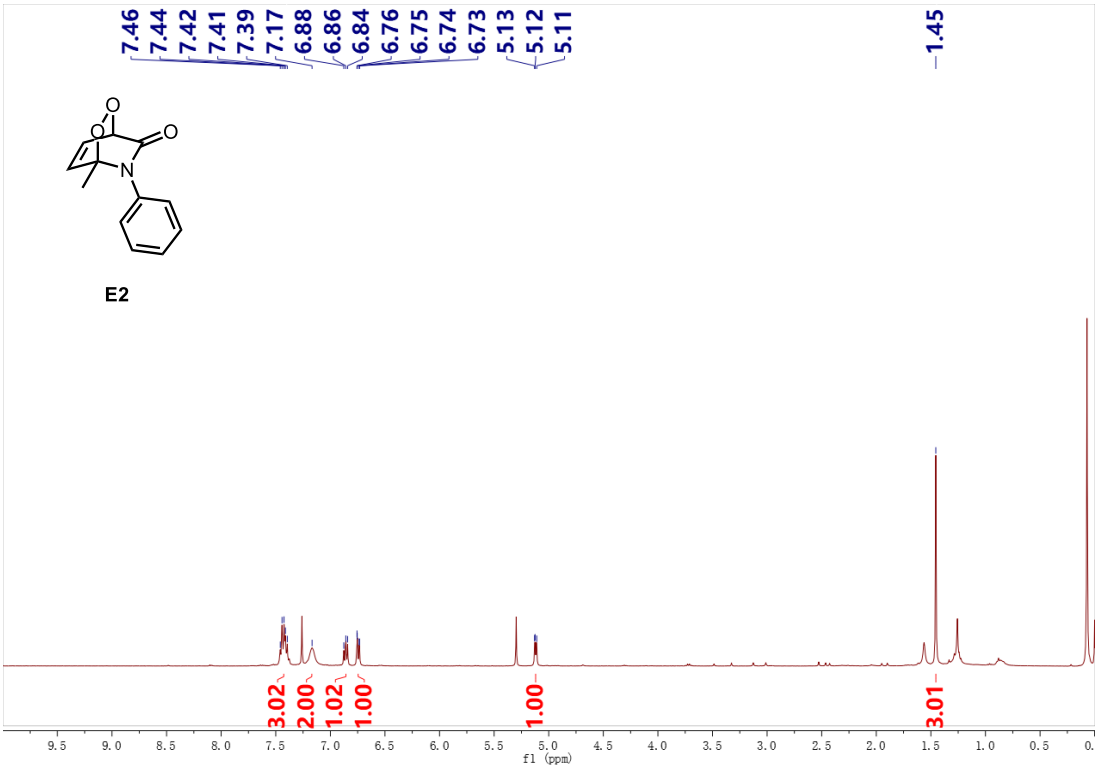
Supplementary Fig. 29 ^{13}C NMR spectrum of compound **P4**



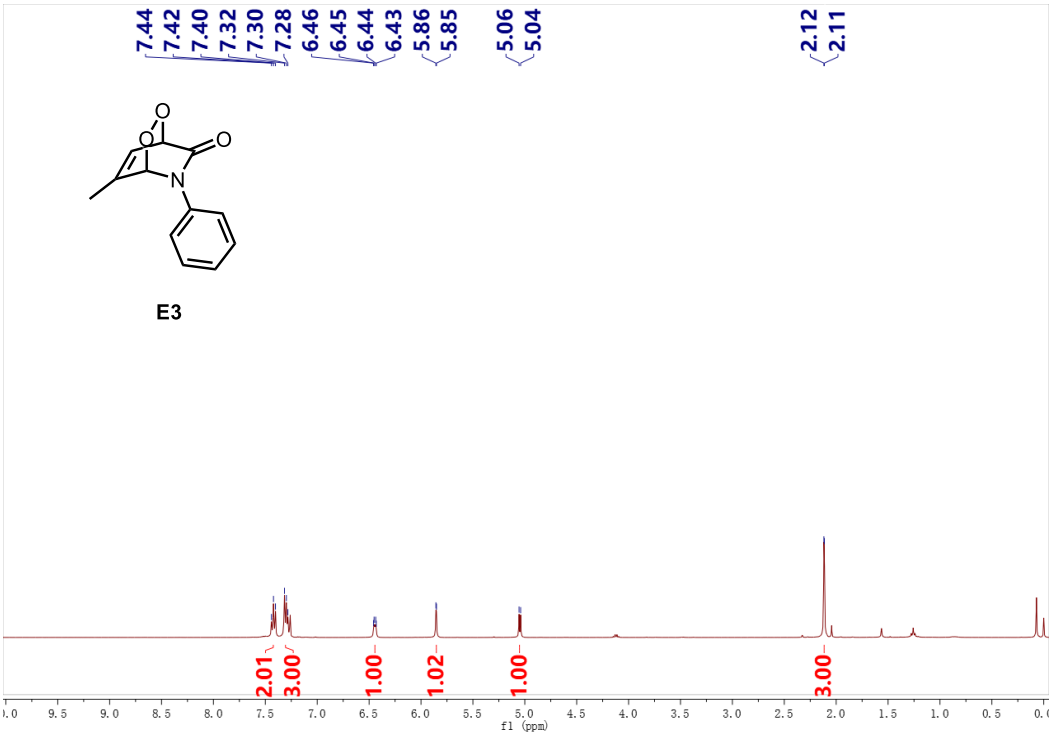
Supplementary Fig. 30 ^{1}H NMR spectrum of compound **P5**



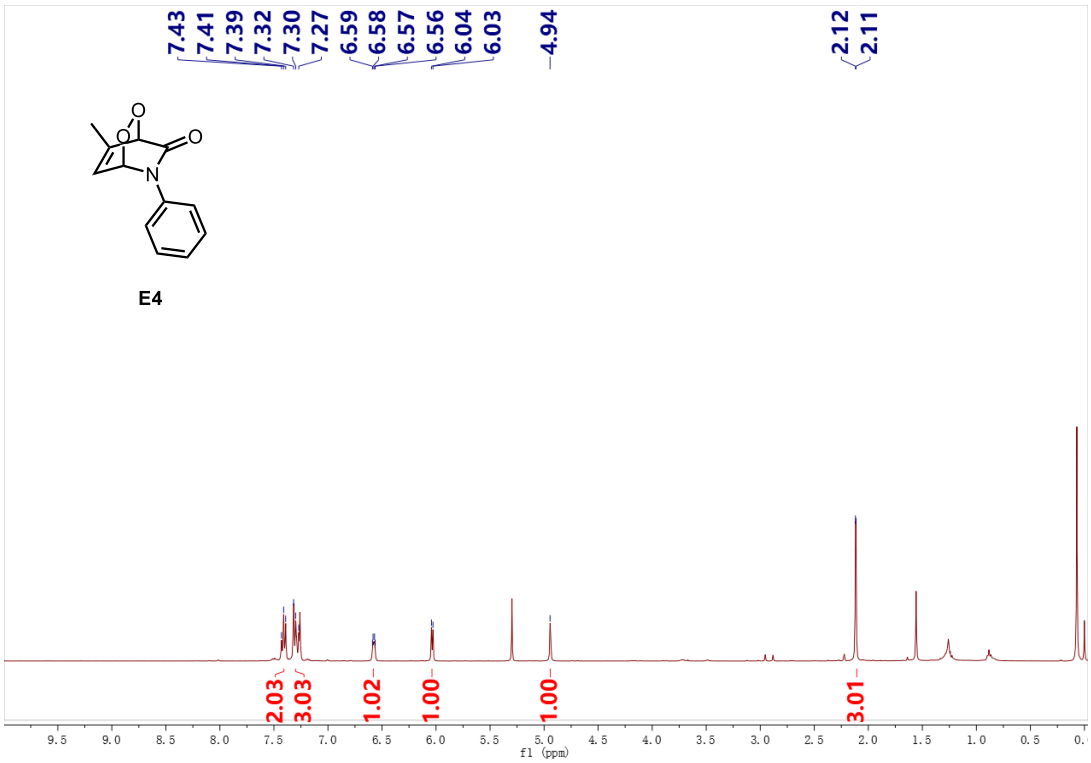
Supplementary Fig. 31 ^{13}C NMR spectrum of compound **P5**



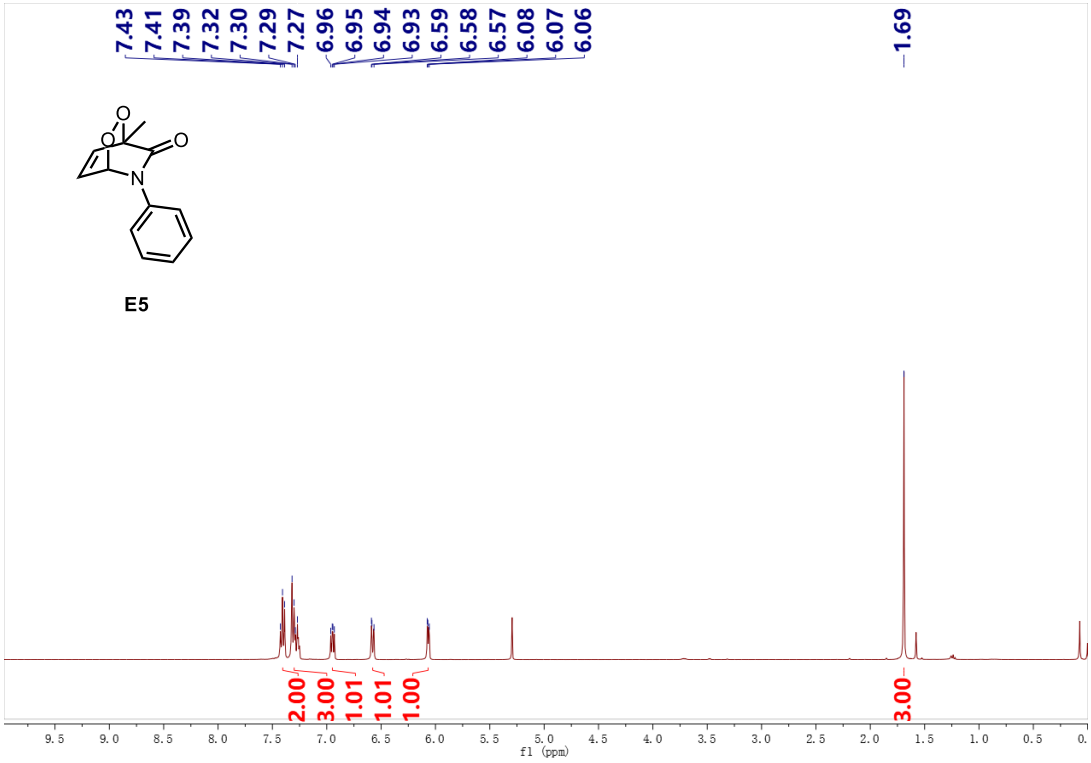
Supplementary Fig. 32 ^{1}H NMR spectrum of compound **E2**



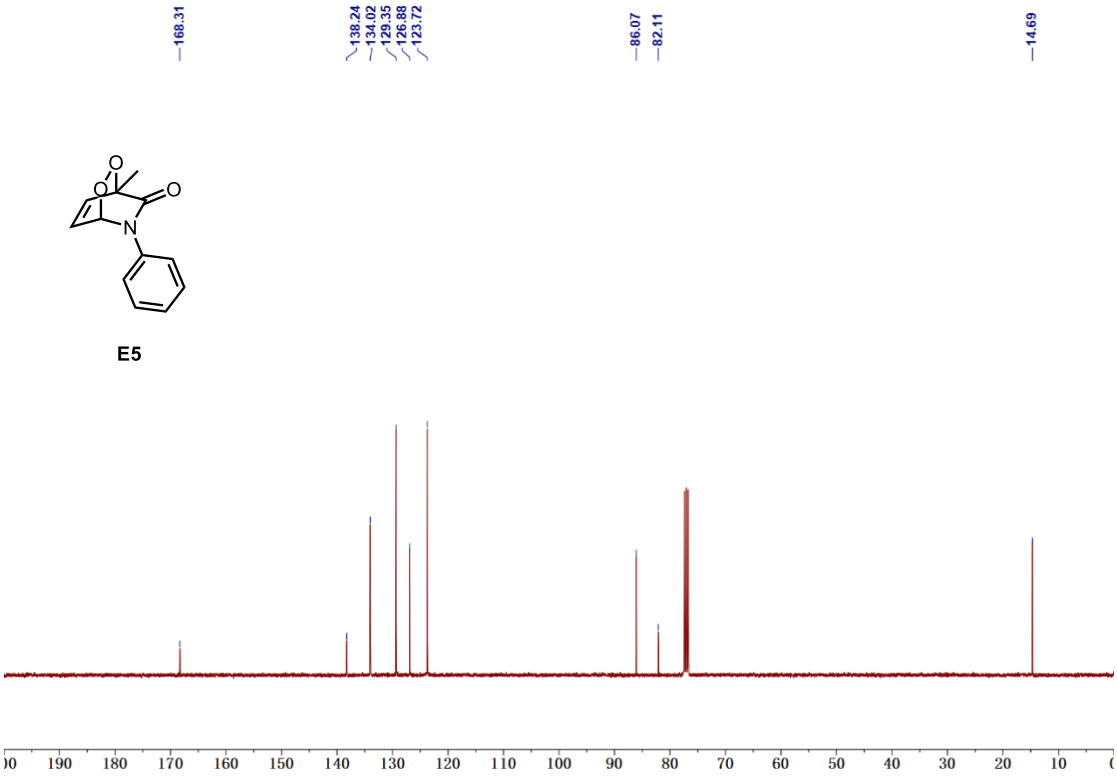
Supplementary Fig. 33 ^{1}H NMR spectrum of compound **E3**



Supplementary Fig. 34 ^{1}H NMR spectrum of compound **E4**



Supplementary Fig. 35 ^{1}H NMR spectrum of compound **E5**



Supplementary Fig. 36 ^{13}C NMR spectrum of compound **E5**