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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To a solution of 2-pyridone 1 (5 mmol) and iodobenzene 2 (7.5 mmol) in DMF, anhydrous K$_2$CO$_3$ (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$_2$O, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na$_2$SO$_4$, 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_2$O.
Supplementary Fig. 3 $^1$H NMR spectra of \textit{E}3 (top), \textit{E}3 incubated at 37 °C for 24 h (middle), \textit{P}3 (bottom) in D$_2$O.

Supplementary Fig. 4 $^1$H NMR spectra of \textit{E}4 (top), \textit{E}4 incubated at 37 °C for 24 h (middle), \textit{P}4 (bottom) in D$_2$O.
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\textsuperscript{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$_2$O 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$_2$O).](image)
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 °C.

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% CO₂ 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 × 10⁵ 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 μM, 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 assay³: 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

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