Supporting Information

Identification of HSP90 as a direct target of artemisinin for its anti-inflammatory activity via quantitative chemical proteomics

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1. Synthesis of the ART probe (2)

1.1 General Information. NMR spectra were taken with an Agilent-400 spectrometer (400 MHz for $^1$H NMR, 100 MHz for $^{13}$C NMR) in CDCl$_3$. All $^1$H NMR experiments were measured with tetramethylsilane (δ 0 ppm) in CDCl$_3$ as the internal reference and coupling constants were reported in Hz; $^{13}$C NMR experiments were measured in relative to the signal of CDCl$_3$ (δ 77.0 ppm). Column chromatography was performed on silicagel H (300-400 mesh). Petroleum ether (b.p. 60-90 °C), ethyl acetate and dichloromethane were purchased from Shanghai Titan Scientific Co., Ltd.; Artemisinin and 2-propynamine (98%) were purchased from Shanghai D&B Biological Science and Technology Co., Ltd.; NaBH$_4$, BzCl, pyridine, and DMAP were purchased from Sinopharm Chemical Reagent Co., Ltd.; Allyltrimethylsilane (97%) was purchased from Shanghai Shaoyuan Co., Ltd.; Oxone (95%) and EDCI (97%) were purchased from Shanghai Bide Pharmatech Co., Ltd.; Other reagents were purchased from commercial sources and used without further purification.

1.2 Synthesis of 2

![Synthesis Diagram]

Compound 2 was prepared according to the literature method with slight
1.3 Experimental details and analytical data

1.3.1 Synthesis of \( 3 \) (dihydroartemisinin, wgl-1023)

The dihydroartemisinin \( 2 \) was synthesized according to the reported procedures with slight modifications.\(^1\) To a flask was added artemisinin (2.2587 g, 98%, 8.0 mmol) and MeOH (20 mL) under argon atmosphere. Then a solution of NaBH\(_4\) (907.4 mg, 98%, 24.0 mmol) in MeOH (10 mL) was added dropwise over 10 min at 0 °C via ice-water bath. After stirred at this temperature for 3 h, the solution was slowly poured into a mixture of AcOH/H\(_2\)O (5 mL/100 mL). The resulting mixture was stirred under the ice/water bath for 0.5 h and then left standing for another 0.5 h. A white solid was isolated by filtration, washed with water and dried in vacuum to give \( 3 \) (1.9073 g, 86%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta 5.62 \) (s, 1 H), 5.35-5.25 (m, 1 H), 3.17 (s, 1 H), 2.60 (s, 1 H), 2.38 (td, \( J_1 = 14.0, J_2 = 3.7 \) Hz, 1 H), 2.11–1.97 (m, 1 H), 1.96–1.74 (m, 3 H), 1.71–1.60 (m, 1 H), 1.59–1.18 (m, 7 H), 1.05–0.82 (m, 7 H). The characterization data matches the previous reported in literature.\(^2\)

1.3.2 Synthesis of \( 4 \) (dihydroartemisinin 10\( \alpha \)-benzoate, wgl-1024)

The dihydroartemisinin 10\( \alpha \)-benzoate \( 4 \) was synthesized according to the reported procedures with slight modifications.\(^2\) To a flask was added dihydroartemisinin \( 3 \)
(1.7656 g, 6.2 mmol) and CH₂Cl₂ (25 mL) under argon atmosphere. After cooling to 0 °C via ice-water bath, pyridine (2.5 mL, d = 0.983 g.cm⁻³, 2.4575 g, 31.0 mmol) and benzoyl chloride (1.1365 g, 98%, 8.1 mmol) in CH₂Cl₂ (5 mL) were added to the stirred solution over 5 min. The resulting mixture was naturally warmed up to room temperature, stirred for 11.5 h, and quenched with a saturated aqueous ammonium chloride solution (10 mL). The organic layer was separated, and washed with H₂O (10 mL), brine (10 mL). The organic layer was then dried over anhydrous Na₂SO₄ and concentrated, and the residue was purified by column chromatography [eluent: petroleum ether/ethyl acetate = 10:1 (550 mL) to 5:1 (180 mL)] to yield 4 as a white solid (2.1826 g, 91%).¹ H NMR (400 MHz, CDCl₃) δ 8.17–8.07 (m, 2 H), 7.57 (t, J = 7.6 Hz, 1 H), 7.44 (t, J = 7.6 Hz, 2 H), 6.01 (d, J = 10.0 Hz, 1 H), 5.53 (s, 1 H), 2.83–2.68 (m, 1 H), 2.39 (td, J₁ = 13.9 Hz, J₂ = 3.9 Hz, 1 H), 2.11–1.98 (m, 1 H), 1.97–1.87 (m, 1 H), 1.86–1.64 (m, 3 H), 1.58–1.21 (m, 7 H), 1.12–0.86 (m, 7 H). The characterization data matches the previous reported in literature.²

1.3.3 Synthesis of 5 (10β-Allyldeoxoartemisinin, wgl-1-029)

The 10β-Allyldeoxoartemisinin 5 was synthesized according to the reported procedures with slight modifications.³ To an oven-dried flask was added ZnCl₂ (852.3 mg, 6.2 mmol), 4 Å molecular sieves (200 mg) in the glovebox. After cooling to 0 °C via ice-water bath, 1,2-dichloroethane (15 mL), allyltrimethylsilane (4.1 mL, 97%, d = 0.719 g.cm⁻³, 2.9479 g, 25.8 mmol) and 4 (2.0013 g, 5.2 mmol, in 15 mL 1,2-dichloroethane) were added to the mixture under argon atmosphere. The mixture was naturally warmed up to room temperature and stirred for 5 h. The mixture was quenched with a saturated aqueous NaHCO₃ solution (2 mL), and diluted with ethyl
acetate (80 mL). Then the mixture was washed with H₂O (20 mL x 2), followed by brine (20 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography [eluent: petroleum ether/ethyl acetate = 30:1 (310 mL) to 20:1 (630 mL)] to give 5 as a white solid (1.3504 g, 85%). 

\[ ^1H \text{NMR (400 MHz, CDCl}_3 \delta 6.00–5.83 \text{ (m, 1 H), 5.33 (s, 1 H), 5.18–4.99 (m, 2 H), 4.37–4.24 (m, 1 H), 2.68 (sextet, } J = 7.0 \text{ Hz, 1 H), 2.48–2.24 (m, 2 H), 2.24–2.14 (m, 1 H), 2.11–1.97 (m, 1 H), 1.96–1.87 (m, 1 H), 1.86–1.75 (m, 1 H), 1.74–1.57 (m, 2 H), 1.51–1.17 (m, 7 H), 1.03–0.92 (m, 4 H), 0.88 (d, } J = 8.0 \text{ Hz, 3 H).} \]

The characterization data matches the previous reported in literature.³

### 1.3.4 Synthesis of 10-(2-Oxoethyl)deoxoartemisinin (6, wgl-1-033)

The 10-(2-Oxoethyl)deoxoartemisinin 6 was synthesized according to the reported procedures with slight modifications.⁴ To a flask was added 5 (616.5 mg, 2.0 mmol) and acetonitrile (30 mL). Then NaIO₄ (856.2 mg, 4.0 mmol, dissolved in 5 mL of water) and RuCl₃ (14.7 mg, 97%, 0.07 mmol, dissolved in 100 μL of H₂O) was added to the mixture at room temperature. After the disappearance of 5 in 5.5 h as indicated by TLC, ethyl acetate (40 mL) was added to the reaction mixture. The organic layer was washed with water (20 mL x 2) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [eluent: petroleum ether/ethyl acetate = 4:1 (100 mL) to 2:1 (150 mL)] to give 6 (413.3 mg, 67% yield) as a colorless oil. 

\[ ^1H \text{NMR (400 MHz, CDCl}_3 \delta 9.80 \text{ (t, } J = 1.6 \text{ Hz, 1 H, CHO), 5.32 (s, 1 H), 5.01–4.89 (m, 1 H), 2.81–2.63 (m, 2 H), 2.50–2.40 (m, 1 H), 2.39–2.27 (m, 1 H), 2.09–1.99 (m,} \]
1.98–1.87 (m, 1 H), 1.84–1.75 (m, 1 H), 1.74–1.62 (m, 2 H), 1.50–1.36 (m, 4 H), 1.35–1.20 (m, 3 H), 1.04–0.93 (m, 4 H), 0.87 (d, J = 7.6 Hz, 3 H).

The characterization data matches the previous reported in literature.\(^5\)

1.3.5 Synthesis of 7 (wgl-1-040)

The intermediate 7 was synthesized according to the reported procedures with slight modifications.\(^4\) To a flask was added 6 (310.5 mg, 1.0 mmol) and acetonitrile (30 mL). Then NaHCO\(_3\) (421.7 mg, 5.0 mmol, dissolved in 5 mL of H\(_2\)O) and oxone (1.2302 g, 2.0 mmol, 95%, dissolved in 2 mL of H\(_2\)O) were added dropwise over 20 min at room temperature. After stirring for an additional 2 h, the reaction mixture was quenched with hydrochloride acid (5 mL, 2 M) and diluted with ethyl acetate (40 mL). The mixture was washed with water (20 mL x 2) and brine (10 mL), dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The residue 7 (360.3 mg) as a white solid was pure enough (which contains some EtOAc residue) for the following steps.\(^1\) H NMR (400 MHz, CDCl\(_3\)) δ 9.68 (brs, 1 H, COOH), 5.29 (s, 1 H), 4.80–4.67 (m, 1 H), 2.74–2.52 (m, 2 H), 2.49–2.36 (m, 1 H), 2.33–2.16 (m, 1 H), 1.97–1.90 (m, 1 H), 1.89–1.79 (m, 1 H), 1.78–1.67 (m, 1 H), 1.66–1.51 (m, 2 H), 1.44–1.11 (m, 7 H), 0.99–0.71 (m, 7 H). The characterization data matches the previous reported in literature.\(^4\)

1.3.6 Synthesis of 10β-(Acetamidomethylalkyne)deoxoartemisinin (2, wgl-1-043)
The compound 2 was synthesized according to the reported procedures with slight modifications. To a flask was added 7 (360.3 mg, 1.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 480.1 mg, 97%, 2.5 mmol), 4-dimethylaminopyridine (DMAP, 244.1 mg, 2.0 mmol), and CH₂Cl₂ (20 mL) at room temperature. Then 2-propynamine (0.3 mL, 98%, d = 0.86 g.cm⁻³, 252.8 mg, 4.6 mmol) was added to the stirred mixture. The resulting mixture was stirred for 18 h at room temperature. Then the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (20 mL). The resulting mixture was washed with saturated NaHCO₃ solution (5 mL), water (10 mL x 2), and brine (10 mL), then dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [eluent: petroleum ether/ethyl acetate/ethyl ether = 10:5:1 (160 mL)] and then recrystallized from CH₂Cl₂/petroleum ether to afford 2 (385.5 mg, 96%) as a white solid.¹H NMR (400 MHz, CDCl₃) δ 5.40 (s, 1 H), 4.82 (s, 1 H), 4.05 (s, 2 H), 2.85–0.45 (m, 26 H).¹³C NMR (100 MHz, CDCl₃) δ 171.3, 102.8, 90.2, 80.7, 79.7, 71.2, 69.1, 51.6, 43.2, 37.5, 37.3, 36.4, 34.1, 30.3, 28.9, 25.8, 24.73, 24.69, 19.9, 11.8. The purity of this compound was proven by ¹H and ¹³C NMR spectra.
2 Supplementary Figures

Fig S1. GO analysis of biological pathways that are potentially associated with ART target proteins.

Fig S2. Target profiles of ART are shown by a scattered plot. The dots represent proteins with competitive ratios and $p$-values above 1.3 and 0.05 (\(-\log_{10} \text{ corresponds to } 1.3\)) respectively. The proteins colored in yellow are the proteins which have been identified in malaria parasites and cancer cells.

Fig S3. Lipopolysaccharide (LPS) time-dependently induced the expression of iNOS. A representative blot of three experiments is shown.
3 Supporting Table: full list of protein targets of ART

(Please see the attached EXCEL file)

4 References


5. $^1$H NMR spectra for 2-7 and $^{13}$C NMR spectrum for 2