Supporting Information

Synthesis and evaluation of dibenzothiophene analogues as Pin1 inhibitors for cervical cancer therapy

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Experimental Section

Synthesis and characterization.

4-bromodibenzo[b,d]thiophene (Yield: 68%). This compound is synthesized according to the former reported method.\textsuperscript{1}\textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 400 MHz). δ ppm: 8.45-8.38 (m, 2 H), 8.13-8.09 (m, 1 H), 7.80-7.76 (dd, J= 7.6, 0.8 Hz, 1 H), 7.62-7.55 (m, 2 H), 7.50 (t, J= 8 Hz, 1 H). \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 101 MHz), δ ppm: 140.00, 137.84, 136.49, 135.48, 129.55, 127.73, 126.59, 125.24, 123.16, 122.86, 115.44, 39.50. GC-MS, m/z calculated. 263.9, experimental. 263.9.

1-(dibenzo[b,d]thiophen-4-yl)-1H-imidazole (Yield: 80%). A mixture of 4-bromodibenzo[b,d]thiophene (20 mmol), 1H-imidazole (24 mmol), K\textsubscript{2}CO\textsubscript{3} (40 mmol), Cul (0.5 mmol), and L-proline (4 mmol) in 50 mL of DMF was heated for 48 hours at 90 °C. The cooled mixture was partitioned between water and dichloromethane. The organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated in vacuo. The residual oil was loaded on a silica gel column and eluted with the mixture solution of dichloromethane/petroleum ether to afford purified product. \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 400 MHz). δ ppm: 8.50-8.45 (m, 2 H), 8.20 (d, J=0.8 Hz, 1 H), 8.11-8.00 (m, 1 H), 7.75 (d, J=1.2 Hz, 1 H), 7.72-7.64 (m, 2 H), 7.60-7.56 (m, 2 H), 7.24 (d, J=0.8 Hz, 1 H). \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 101 MHz), δ ppm: 138.54, 137.74, 137.45, 135.38, 133.86, 132.84, 130.19, 128.33, 126.57,
125.77, 123.62, 123.11, 123.05, 122.09, 120.02. GC-MS, m/z calculated 250.1, experimental, 250.1.

1-(dibenzo[b,d]thiophen-4-yl)-1H-benzo[d]imidazole (Yield: 78%). This compound is synthesized using the same procedure of 1-(dibenzo[b,d]thiophen-4-yl)-1H-imidazole with 1H-benzo[d]imidazole in the place of 1H-imidazole. 1H NMR (CDCl₃, 400 MHz). δ ppm: 8.35-8.20 (m, 3 H), 7.97 (d, J=8 Hz, 1 H), 7.86-7.83 (m, 3 H), 7.70-7.66 (t, J=8 Hz, 1 H), 7.60-7.53 (m, 3 H), 7.45-7.32 (m, 3 H). 13C NMR (CDCl₃, 101 MHz). δ ppm: 143.73, 142.31, 139.13, 138.18, 136.51, 135.28, 133.67, 131.08, 127.69, 125.57, 125.01, 124.13, 123.62, 123.00, 122.95, 122.06, 121.79, 120.73, 110.97.

1-(dibenzo[b,d]thiophen-2-yl)-1H-imidazole (Yield: 77%). This compound is synthesized using the same procedure of 1-(dibenzo[b,d]thiophen-4-yl)-1H-imidazole with 2-bromodibenzo[b,d]thiophene in the place of 4-bromodibenzo[b,d]thiophene. 1H NMR (DMSO-d₆, 400 MHz). δ ppm: 8.71 (d, J=2 Hz, 1 H), 8.53-8.49 (m, 1 H), 8.417 (s, 1 H), 8.18 (d, J=8.4 Hz, 1 H), 7.92 (t, J=1.2 Hz, 1 H), 7.85-7.82 (dd, J=8.4, 2.4 Hz, 1 H), 7.58 (m, 2 H), 7.18 (s, 1 H). 13C NMR (DMSO-d₆, 101 MHz), δ ppm: 140.04, 137.07, 136.67, 136.31, 135.15, 134.92, 130.36, 128.12, 125.29, 124.74, 124.66, 123.11, 120.19, 118.87, 114.16.

4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide (Yield: 93%). 15 g hydrogen peroxide (30 wt%) was slowly dropped into 30 mL glacial acetic acid solution of 1-(dibenzo[b,d]thiophen-4-yl)-1H-imidazole (14 mmol) at 0°C. After the addition, the temperature is raised to room temperature and the mixture solution are still kept stirring until the starting materials are almost consumed (TLC detection). The mixture solution was neutralized with 10wt% aq. NaHCO₃ solution. Then, the solution was extracted with dichloromethane and the organic phase are dried with anhydrous Na₂SO₄. The concentrated organic phase is purified using silica gel column chromatography with an eluent solution of dichloromethane/petroleum ether and the ideal product is received as white powder. Notice. A few amount of 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5,5-dioxide (1c) was also eluted out from the silica gel column and collected before the 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. 1H NMR (CDCl₃, 400 MHz). δ ppm: 8.12 (s, 1 H), 8.00 (d, J=7.6 Hz, 1 H), 7.91-7.86 (m, 2 H), 7.75 (t, J=8 Hz, 1 H), 7.70-7.65 (m, 2 H), 7.60 (m, 1 H), 7.43 (dd, J=8, 0.8 Hz, 1 H), 7.33 (s, 1 H). 13C NMR (CDCl₃, 101 MHz). δ ppm: 144.65, 140.12, 138.47, 138.09, 136.31, 134.38, 132.84, 130.74, 130.51, 127.53, 125.24, 122.27, 121.21, 121.14. Tof-MS, m/z calculated 267.0587, experimental, 267.0592.

4-(1H-benzo[d]imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide (Yield: 87%). This compound was synthesized using the same procedure of 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide just with 1H-benzo[d]imidazole in the place of 1H-imidazole. 1H NMR (CDCl₃, 400 MHz). δ ppm: 8.69 (s, 1 H), 8.05-7.98 (m, 3 H), 7.93 (d, J=8 Hz, 1 H), 7.85 (t, J=8 Hz, 1 H), 7.70 (t, J=7.2 Hz, 1 H), 7.61 (m, 2 H), 7.48-7.40 (m, 3 H). 13C NMR (CDCl₃, 101 MHz). δ ppm: 144.74, 140.71, 140.49, 136.06, 134.60, 133.08, 130.78, 127.74, 126.99, 125.31, 124.90, 122.48, 119.60, 111.17.

2-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide (Yield: 90%). This compound was synthesized using the same procedure of the 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide just with 1-(dibenzo[b,d]thiophen-2-yl)-1H-imidazole in the place 1-(dibenzo[b,d]thiophen-4-yl)-1H-imidazole. 1H NMR (CDCl₃, 400 MHz). δ ppm: 8.10 (t, J=8.4 Hz, 2 H), 8.04 (d, J=8 Hz, 1 H), 7.90 (d, J=7.2 Hz, 1 H), 7.85 (d, J=2 H, 1 H), 7.68 (td, J=7.6, 1.2, 1 H), 7.61 (td, J=7.6, 1.2, 1 H), 7.54 (dd, J=8.4, 2 Hz, 1 H), 7.42 (s, 1 H), 7.31 (s, 1 H). 13C NMR (CDCl₃, 101 MHz). δ ppm: 146.07, 143.78,
Compound 1a (Yield: 95%). 5 mmol (bromomethyl)benzene was added to 20 mL acetonitrile solution of compound 1c (1 mmol) and the mixture solution was stirred for 24 hours under argon atmosphere at room temperature. And then, removing CH$_3$CN from the mixture solution under vacuum and adding methanol and 5 mmol KI to the residues, which was followed by stirring for another 12 hours at room temperature. Then, the solvent was removed by rotary evaporator. The inorganic salt in the residual oil was removed by extraction and the organic phase was collected together and dried over anhydrous Na$_2$SO$_4$. After the filtration and concentrated under reduced pressure, the crude product was purified by recrystallization with n-hexane/methanol. $^1$H NMR (DMSO-d$_6$, 400 MHz). δ ppm: 9.96 (s, 1 H), 8.52 (d, J=8 Hz, 1 H), 8.27 (s, 1 H), 8.17-8.06 (m, 3 H), 8.00 (d, J=8 Hz, 1 H), 7.92 (t, J=8 Hz, 1 H), 7.75(t, J=7.6 Hz, 1 H), 7.46 (m, 5 H), 5.66 (s, 2 H). $^{13}$C NMR (DMSO-d$_6$, 101 MHz). δ ppm: 138.03, 136.52, 136.36, 135.35, 134.20, 133.19, 132.06, 131.33, 130.34,
129.43, 129.10, 129.06, 128.95, 128.32, 125.13, 125.08, 123.61, 123.22, 122.33, 52.53. Tof-MS. [M-I]⁺, m/z calculated 373.1005, experimental 373.1006.

**Compound 1b** (Yield: 99%). 5 mmol iodomethane was added into 10 mL acetonitrile solution of compound 1c (1 mmol) and the mixture solution was stirred for overnight at room temperature under argon atmosphere. Then, there is a large amount of precipitate in the solution. After filtration and the precipitate was washed with THF and n-hexane and dried under vacuum. Compound 1b was then received as white powder finally.

**1H NMR (DMSO-d$_6$, 400 MHz).** δ ppm: 9.75 (s, 1 H), 8.52 (dd, J=8, 0.8 Hz, 1 H), 8.37 (d, J=8 Hz, 1 H), 8.22 (t, J=2 Hz, 1 H), 8.15-8.06 (m, 3 H), 7.94-7.90 (m, 2 H), 7.76 (td, J=7.6, 0.8 Hz, 1 H), 4.05 (s, 3 H). **13C NMR (DMSO-d$_6$, 101 MHz).** δ ppm: 138.17, 136.45, 136.23, 135.24, 133.18, 131.95, 131.05, 130.29, 129.32, 128.84, 124.93, 124.28, 124.23, 123.48, 122.18, 36.36.

**Compound 1c** (Yield: 98%). 15 g hydrogen peroxide (30 wt%) was slowly dropped to 30 mL glacial acetic acid solution of 4-((1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide (10 mmol) at 0 °C. After the addition, the temperature is raised to room temperature and the mixture solution are still kept stirring until the starting materials
are almost consumed (TLC detection, new product was detected upon the original material in the TLC plate). The mixture solution was neutralized with 10wt% aq. NaHCO₃ solution. Then, the solution was extracted with dichloromethane and the organic phase are dried with anhydrous Na₂SO₄. The concentrated organic phase is purified using silica gel column chromatography with an eluent solution of dichloromethane/petroleum ether and the ideal product is received as white powder. ¹H NMR (CDCl₃, 400 MHz). δ ppm: 8.10 (s, 1 H), 7.90-7.85 (m, 3 H), 7.79-7.72 (m, 2 H), 7.65-7.60 (m, 2 H), 7.46 (dd, J=8, 0.8 Hz, 1 H), 7.33 (s, 1 H). ¹³C NMR (CDCl₃, 101 MHz). δ ppm: 135.09, 134.12, 131.17, 130.74, 126.59, 122.26, 121.80, 120.73. Tof-MS, m/z calculated 283.0536, experimental, 283.0532.

**Compound 1d** (Yield: 98%). This compound was synthesized by the same procedure of compound 1a just substituting the original material of (bromomethyl)benzene with bromoethane. ¹H NMR (DMSO-d₆, 400 MHz). δ ppm: 9.82 (s, 1 H), 8.52 (d, J=7.2 Hz,
1 H), 8.36 (d, J=7.6 Hz, 1 H), 8.24 (t, J=2 Hz, 1 H), 8.18 (t, J=2 Hz, 1 H), 8.13 (t, J=8 Hz, 1 H), 8.06 (d, J=7.6 Hz, 1 H), 7.97-7.89 (m, 2 H), 7.76 (t, J=7.6 Hz, 1 H), 4.41 (q, J=3.2 Hz, 2 H), 1.54 (t, J=3.2 Hz, 3 H). \(^{13}\)C NMR (DMSO-d\(_6\), 101 MHz). \(\delta\) ppm: 138.03, 137.03, 136.83, 135.84, 133.74, 132.55, 131.72, 130.94, 129.90, 129.43, 125.53, 124.99, 124.08, 123.41, 122.78, 45.57, 15.45.

**Compound 1e** (Yield: 96%). This compound was synthesized using the same procedure of compound 1b just substituting the starting material of compound 1c with 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. \(^{1}\)H NMR (DMSO-d\(_6\), 400 MHz). \(\delta\) ppm: 9.77 (s, 1 H), 8.43 (dd, J=7.6, 0.8 Hz, 1 H), 8.33 (t, J=2 Hz, 1 H), 8.28 (d, J=7.2 Hz, 1 H), 8.15 (d, J=2 Hz, 1 H), 8.08 (t, J=1.6 Hz, 1 H), 8.03 (t, J=7.6 Hz, 1 H), 7.84-7.80 (m, 2 H), 7.71-7.67 (td, J=7.6, 1.2 Hz, 1 H), 4.04 (s, 3 H). \(^{13}\)C NMR (DMSO-d\(_6\), 101 MHz). \(\delta\) ppm: 144.13, 139.46, 139.33, 138.04, 135.84, 135.15, 134.44, 133.54, 130.96, 127.78, 126.84, 124.73, 124.65, 123.63, 123.55, 36.46.
Compound 2a (Yield: 95%). This compound was synthesized using the same procedure of compound 1c just with 4-(1H-benzo[d]imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide in the place of 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. $^1$H NMR (CDCl$_3$, 400 MHz). δ ppm: 8.66 (s, 1 H), 8.00-7.90 (m, 3 H), 7.88-7.82 (m, 2 H), 7.76-7.71 (td, J=7.6, 1.2 Hz, 1 H), 7.64-7.61 (m, 2 H), 7.44-7.38 (m, 3 H). $^{13}$C NMR (CDCl$_3$, 101 MHz). δ ppm: 143.26, 137.83, 135.13, 134.56, 134.21, 133.14, 131.27, 130.20, 128.10, 124.50, 123.87, 122.33, 121.91, 121.54, 120.46, 110.66.
Compound 2b (Yield: 99%). This compound was synthesized using the same procedure of 1e just substituting the starting material of 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide with 4-(1H-benzo[d]imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. \( ^1\)H NMR (DMSO-d\(_6\), 400 MHz). \( \delta \) ppm: 10.28 (s, 1 H), 8.53 (dd, \( J=8 \) Hz, 1 H), 8.31 (dd, \( J=8 \) Hz, 1 H), 8.23 (dd, \( J=8.4 \) Hz, 1 H), 8.09 (m, 2 H), 7.92 (d, \( J=7.6 \) Hz, 2 H), 7.81 (q, \( J=7.6 \) Hz, 2 H), 7.75 (t, \( J=8 \) Hz, 1 H), 7.68 (t, \( J=7.6 \) Hz, 1 H), 4.28 (s, 3 H). \( ^{13}\)C NMR (DMSO-d\(_6\), 101 MHz). \( \delta \) ppm: 144.95, 144.27, 141.56, 140.05, 136.43, 135.74, 133.87, 132.42, 132.03, 131.29, 128.81, 128.15, 127.82, 125.85, 123.95, 114.58, 114.50, 104.99, 34.37.
Compound 2c (Yield: 98%). This compound was synthesized using the same procedure of 1b just substituting the starting material of compound 1c with compound 2a. $^1$H NMR (DMSO-d$_6$, 400 MHz). δ ppm: 10.29 (s, 1 H), 8.62 (d, J=8 Hz, 1 H), 8.39 (d, J=8 Hz, 1 H), 8.22-8.15 (m, 2 H), 8.00-7.90 (m, 3 H), 7.84 (t, J=8 Hz, 1 H), 7.76-7.69 (m, 3 H), 4.31 (s, 3 H). $^{13}$C NMR (DMSO-d$_6$, 101 MHz). δ ppm: 144.37, 137.34, 136.96, 135.82, 134.02, 132.86, 132.51, 131.70, 131.03, 130.01, 128.35, 128.24, 127.81, 126.31, 124.09, 122.72, 114.54, 114.34, 34.45.
Compound 3a (Yield: 99%). 5 mmol iodomethane was added into 10 mL acetonitrile solution of compound 3b (1 mmol) and the mixture solution was stirred for overnight at room temperature under argon atmosphere. Then, there is a large amount of precipitate in the solution. After filtration and the precipitate was washed with THF and n-hexane and dried under vacuum. Compound 3a was then received as white powder finally.

$^1$H NMR (DMSO-d$_6$, 400 MHz). $\delta$ ppm: 9.93 (s, 1 H), 8.69 (d, J=2 Hz, 1 H), 8.44 (t, J=6 Hz, 1 H), 8.36 (d, J=8 Hz, 1 H), 8.25 (d, J= 8 Hz, 1 H), 8.09-7.99 (m, 3 H), 7.94 (t, J=8 Hz, 1 H), 7.76 (t, J=8 Hz, 1 H), 4.00 (s, 3 H).

$^{13}$C NMR (DMSO-d$_6$, 101 MHz). $\delta$ ppm: 139.88, 137.88, 137.63, 137.11, 135.44, 133.65, 132.54, 129.93, 125.18, 124.65, 124.43, 123.62, 122.85, 121.51, 116.90, 36.88.
Compound 3b (Yield: 95%). This compound was synthesized using the same procedure of the compound 1c just with 2-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide in the place of 4-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. $^1$H NMR (CDCl$_3$, 400 MHz). $\delta$ ppm: 8.01 (s, 1 H), 7.96 (d, J=8 Hz, 1 H), 7.88 (t, J=7.2 Hz, 2 H), 7.80 (d, J=1.6 Hz, 1 H), 7.73 (t, J=8 Hz, 1 H), 7.64 (t, J= 8 Hz, 1 H), 7.57 (dd, J=8, 2 Hz, 1 H), 7.41 (s, 1 H), 7.32 (s, 1 H). $^{13}$C NMR (CDCl$_3$, 101 MHz). $\delta$ ppm: 141.96, 138.34, 136.24, 134.16, 131.40, 130.21, 124.17, 122.74, 122.49, 121.86, 114.15.
**Compound 3c** (Yield: 99%). This compound was synthesized using the same procedure of 3a just substituting the starting material of compound 3b with 2-(1H-imidazol-1-yl)dibenzo[b,d]thiophene 5-oxide. $^1$H NMR (DMSO-d$_6$, 400 MHz). δ ppm: 9.92 (s, 1 H), 8.61 (d, J=2 Hz, 1 H), 8.42 (m, 2 H), 8.18 (dd, J=8, 7.2 Hz, 2 H), 8.02 (t, J=2 Hz, 1 H), 7.98 (dd, J=8.4, 2.4 Hz, 1 H), 7.84 (td, J=8, 1 Hz, 1 H), 7.69 (td, J=7.6, 1 Hz, 1 H), 4.00 (s, 3 H). $^{13}$C NMR (DMSO-d$_6$, 101 MHz). δ ppm: 145.94, 139.22, 138.71, 136.97, 135.73, 133.65, 131.33, 129.92, 128.36, 125.14, 123.55, 123.08, 121.50, 116.59, 36.83.
Cellular thermal shift assay

Cellular thermal shift assay was performed to monitor the target engagement of compounds in HeLa cells. Briefly, cell lysates from $2 \times 10^6$ HeLa cells were collected, diluted in PBS and separated in same aliquots. Each aliquot was treated with $1a$ (10 µM) or DMSO. 30 min after incubation at room temperature, the complex-treated lysates were divided into 50 µl in each of tubes and heated individually at different temperatures (Veriti thermal cycler, Applied Biosystems/Life Technologies). The heated lysates were centrifuged and the supernatants were analyzed by SDS-PAGE followed by immunoblotting analysis by probing with Pin1 antibody and GAPDH antibody (Cell Signaling Technology).

Co-immunoprecipitation

The inhibition of Pin1-p65 interactions was investigated using a co-immunoprecipitation assay following the manufacturer’s instructions. Briefly, HeLa
cells (1x10^6 cells/well) were treated with indicated concentrations of 1a (1 and 10 µM) and DMSO for 12 h. After cell lysis and protein lysate separation, 100 µg of total protein was incubated with p65 antibody at 4 °C overnight. The proteins were immunoprecipitated using agarose beads. The levels of co-precipitated Pin1 were analyzed using Western blotting.

**Transfection with plasmids.**

Transfection with plasmids was performed as previously. Briefly, 293T cells were seed at a density of 6 × 10^5 cell in a culture dish overnight. The cells were co-transfected with recombinant plasmids pCMV6-Pin1-wild-type for 36 h in serum-free DMEM medium using TurboFect Transfection Reagent. Then, the transfected cells were treated with an indicated concentration of 1a in 1% FBS medium for 72h.

**Western blotting**

HeLa cells were seeded at a density of 6 × 10^5 cell in a 6-well plate overnight. Cells were treated with 1a (in 0.1% DMSO), PiB, or vehicle control in 1% FBS medium for an additional 12h. Cells were lysed, and protein samples were collected. Protein concentration was determined by BCA protein assay kit (Thermo Scientific). 30 µg of proteins samples were resolved on a 10% SDS/PAGE gel and transferred to a polvinylidene fluoride (PVDF) membrane. Blots were blocked in 5% none-fat dry milk with TBS containing 0.1% Tween-20 for 1 h, the membranes were subsequently treated with primary antibodies to p65, phospho-p65(Thr254), Erk1/2, c-Jun, PKM2, Histone H3 and GAPDH with gentle agitation overnight at 4 °C. Then, membranes were washed five time with TBST. After incubation with secondary antibody for 1 h. Proteins bands were detected using enhanced chemiluminescent Plus reagents (GE Healthcare) and analyzed by Image Lab.

**Knockdown assay**

HeLa cells were seeded in 6-well plates at 80% confluence in DMEM medium for 24 h. Lipo2000 reagent and Pin1 siRNA was gently mixed and incubated for 20 min at room temperature. Then, 500 µL of the Lipo2000/siRNA mixture were added to each well. Cells were incubated at 37 °C in a CO_2 incubator for 48 h post-transfection before further research.

**Half-life assay**

After treated with 1a for 12h, HeLa cells were treated with 50 µg/ml cycloheximide (CHX, Sigma-Aldrich) for the indicated time periods. p65 levels were determined by Western blot analysis and quantified by densitometry analysis.

**Cytotoxicity assay**

HeLa, 293T and LO2 cells were seeded at 5,000 cells per well in 96-well plates and incubated overnight at 37 °C. The cells were treated with 1a at final concentration from 0.1 to 100 µM for 72 h. Then MTT reagent was added to each well at a final concentration of 0.5 mg/mL for a further 4 h. After then, the medium was replaced with 100 µL DMSO. The viability of the cells was measured by recording the absorbance of each well at 490 nm using a SpectraMax M5 microplate reader after shaking the plate for 10 min at room temperature in the dark.
Cell imaging

HeLa cells were seeded into a glass-bottomed dish (35 mm dish with 20 mm wells) for 24 h, then the cells were incubated with doxorubicin in the presence or absence of 1a for the indicated time periods or concentrations and then washed with phosphate-buffered saline three times. The luminescence imaging of complexes in cells was carried out by a Leica TCS SP8 confocal laser scanning microscope system. The excitation wavelength was 488 nm.

Flow cytometry analysis

HeLa cells were seeded at density of 1 × 10⁶ in a 6-well plate, and were treated with 1a (50 μM) for 6 h and then in the presence or absence of doxorubicin (5 μM) for 6 h at 37 °C. Cells were harvested and washed twice with ice-cold PBS, and then resuspended in 1× binding buffer. The samples were immediately analyzed by flow cytometry using a C6 Flow Cytometer™ system (BD Biosciences). At least 2 × 10⁵ cells were analyzed for each sample. Doxorubicin was determined by fluorescence (488 nm excitation and 585 nm emission). Data were analyzed using FlowJo7.6 software.

Data analysis

All data were reported as the means of at least three separate experiments. Group comparisons between the control group and various drug treatment groups were done by a one-way ANOVA using GraphPad Prism software (Prism).

References

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2. C. Yang, W. Wang, L. Chen, J. Liang, S. Lin, M.-Y. Lee, D.-L. Ma and C.-H. Leung, Chemical Communications, 2016, 52, 12837-12840.
Supplementary Figures

Figure S1. $^1$H NMR spectra of compound 1a in DMSO-d$_6$ recorded at 0, 3 and 7 days.

Figure S2. Pin1 activity was determined by a PPlase activity assay. Inhibition of Pin1 activity by compounds 1a and PiB was dose-dependent. Error bars represent standard deviation of the means of the results from three independent experiments.
Figure S3. Compound 1a stabilizes Pin1 in Hela cells. (a) Stabilization of Pin1 by 1a at 0-100 μM at 61°C as revealed by Western blotting. (b) The band intensity of Pin1 in the soluble fraction at different concentration of 1a. The data were normalized to the Pin1 level of the control group (1a at 100 μM) and are expressed as the means ± SD of three individual experiments. The data were analyzed using Image Lab.

Figure S4. The cytotoxicity effect of 1a on HeLa and 293T cells as determined by the MTT assay. (a) HeLa cells and Pin1 knockdown HeLa cells were exposed to the indicated concentrations of 1a for 72 h. (b) Pin1 overexpressing 293T cells and 293T cells were exposed to the indicated concentrations of 1a for 72 h. Error bars represent standard deviation mean of the results from three independent experiments.
Figure S5. The cytotoxicity effect of 1a on LO2 cells as determined by the MTT assay. LO2 cells were exposed to the indicated concentrations of 1a for 72 h. Error bars represent standard deviation of the means of the results from three independent experiments.

Figure S6. HeLa cells were treated with 1a (10 μM) for 12 h, and then the cells were treated with cycloheximide (CHX) for 0, 30, 90, 180 min. (a) Equal amounts of whole cell lysates were analyzed by Western blot with a p65 antibody. GAPDH was used as an internal control. (b) The band intensity was analysed using Image Lab. Error bars represent standard deviation of the means of the results from three independent experiments.
Figure S7. 1a regulated multiple oncogenic pathways associated with Pin1 inhibition in HeLa cells. (a) Western blot analysis of Erk1/2, PKM2, c-Jun and Pin1 levels in the equal amounts of whole cell lysates of HeLa cells treated with PiB or 1a for 12 h. (b) Western blot analysis of Erk1/2, PKM2, c-Jun and Pin1 levels in the equal amounts of whole cell lysates of Pin1 knockdown HeLa cells treated with PiB or 1a for 12 h.

Figure S8. 1a can’t enhance cisplatin efficacy. HeLa cells were treated with cisplatin in the presence or absence of 1a for 72 h, and cell viability was measured using the MTT. Combination index (CI) values are calculated. D1 and D2 are the concentrations of 1a and cisplatin used in the combination, and DL_{X,1} and DL_{X,2} are the concentrations of a single drug to produce the same effect.
Figure S9. 1a decreased the intranuclear accumulation of p65 in HeLa cells. Hela cells incubated with compound 1a (10μM) for 6h and then in the presence or absence of DOX (5 μM) for 6 h at 37°C. Western blot analysis of p65 levels in intranuclear and cytoplasm.

Figure S10. Luminescence and bright-field images of HeLa cells stained with 1μM of complexes 1a for 12 h. Scale bar = 25 μm.
Figure S11. 1a increased the intracellular accumulation of DOX in HeLa cells. Hela cells incubated with compound 1a (50μM) for 6h and then in the presence or absence of DOX (5 μM) for 6 h at 37°C. Flow cytometric histograms of HeLa cells incubated with DOX or 1a with DOX.