Supplementary Data

Thiosemicarbazone-based selective proliferation inactivators inhibit gastric cancer cell growth, invasion, and migration

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Experimental section

1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer respectively. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

2. General procedure for the synthesis of compounds 4a-c

A mixture of the appropriate amine derivatives **1a-c** (1mmol), CS_2 (1mmol), and triethylamine (1mmol) was stirred in methanol at room temperature for 1h. Upon completion, as judged by TLC, hydrazine hydrate 80% (4mmol) was added dropwise with stirring while maintaining the temperature of the reaction mixture. The resulting mixture was refluxed for additional 3h. The cooled reaction mixture was poured on crushed ice and the separated solid was filtered off, washed with water, dried and crystallized from aqueous ethanol to yield the pure product.

2.1. morpholine-4-carbothiohydrazide（4a）

Yield 69.5%. Yellow solid. Mp:170 –171°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.12 (s, 1H, NH, D_2O exchangeable), 4.75 (s, 2H, -NH_2), 3.70 – 3.66 (m, 4H), 3.59 – 3.54 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 182.92, 65.66, 47.72.HR-MS (ESI), calcd $C_5H_{11}N_3OS$, $[M + H]^+$ m/z, 162.0701; found, 162.0695.

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2.2. tert-butyl 4-(hydrazinecarbonothioyl)piperazine-1-carboxylate（4b）

Yield 72.4%. Yellow solid. Mp:164 –165°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.11 (s, 1H, NH, D_2O exchangeable), 4.77 (s, 2H, -NH_2), 3.74 – 3.69 (m, 4H), 3.35 – 3.31 (m, 4H), 1.41 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 182.59, 153.80, 79.12, 46.88, 28.01.HR-MS (ESI), calcd $C_{10}H_{20}N_4O_2S$, $[M + H]^+$ m/z, 261.1385; found, 261.1389.

2.3. N-phenylhydrazinecarbothioamide（4c）

Yield 60.8%. Yellow solid. Mp:144 –145°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.69 (s, 1H, NH, D_2O exchangeable), 9.15 (s, 1H, NH, D_2O exchangeable), 7.65 (d, *J* = 5.8 Hz, 2H, Ar-H), 7.29 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.09 (t, *J* = 7.2 Hz, 1H, Ar-H), 4.79 (s, 2H, -NH_2).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.29, 139.24, 128.00, 124.03, 123.47.HR-MS (ESI), calcd $C_7H_9N_3S$, $[M + H]^+$ m/z, 168.0595; found, 168.0597.

3. General procedure for the synthesis of compounds 5a-q

A mixture of **4a-c** (1mmol) and appropriate aldehyde (1mmol) was refluxed in methanol for 4h. Upon completion, the precipitated product was filtered off, washed with methanol to afford the crude product. The crude product was recrystallized from ethanol to yield the pure product.

3.1. (E)-N'-(2-hydroxybenzylidene)morpholine-4-carbothiohydrazide（5a）

Yield 73.2%. Yellow solid. Mp: 183–184°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.54 (s, 1H, OH, D_2O exchangeable), 11.50 (s, 1H, NH, D_2O exchangeable), 8.47 (s, 1H, -CH=), 7.42 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-H), 7.32 – 7.24 (m, 1H, Ar-H), 6.95 – 6.87 (m, 2H, Ar-H), 3.96 – 3.88 (m, 4H), 3.72 – 3.62 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.53, 157.06, 146.37, 130.88, 129.93, 119.07, 118.45, 116.50, 65.72, 48.91. HR-MS (ESI): Calcd. $C_{12}H_{15}N_3O_2S$, $[M+Na]^+$m/z: 288.0783, found: 288.0805.

3.2. (E)-N'-(5-chloro-2-hydroxybenzylidene)morpholine-4-carbothiohydrazide（5b）

Yield 77.3%. Yellow solid. Mp: 186–187°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.63 (s, 2H, OH, NH, D_2O exchangeable), 8.42 (s, 1H, -CH=), 7.56 (d, J =

2.6 Hz, 1H, Ar-H), 7.29 (dd, J = 8.8, 2.6 Hz, 1H, Ar-H), 6.92 (d, J = 8.8 Hz, 1H, Ar-H), 3.99 – 3.83 (m, 4H), 3.75 – 3.54 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.64, 155.73, 144.38, 130.28, 128.51, 122.59, 120.15, 118.34, 65.71, 49.04. HR-MS (ESI): Calcd. $C_{12}H_{14}ClN_3O_2S$, $[M-H]^-$m/z: 298.0417, found: 298.0416.

3.3.(E)-N'-(3,5-dibromo-2-hydroxybenzylidene)morpholine-4-carbothiohydrazide（5c）

Yield 67.5%. Yellow solid. Mp: 167–168°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 12.81 (s, 1H, OH, D_2O exchangeable), 11.86 (s, 1H, NH, D_2O exchangeable), 8.41 (s, 1H, -CH=), 7.78 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.74 (d, *J* = 2.3 Hz, 1H, Ar-H), 3.96 – 3.91 (m, 4H), 3.70 – 3.64 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.35, 152.95, 144.50, 135.03, 131.96, 120.95, 111.13, 110.01, 65.64, 48.84. HR-MS (ESI): Calcd. $C_{12}H_{13}Br_2N_3O_2S$, $[M+H]^+$m/z: 421.9173, found: 421.9170.

3.4.(E)-N'-(2-hydroxy-4-methoxybenzylidene)morpholine-4-carbothiohydrazide（5d）

Yield 75.8%. Yellow solid. Mp: 172–173°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.79 (s, 1H, OH, D_2O exchangeable), 11.37 (s, 1H, NH, D_2O exchangeable), 8.40 (s, 1H, -CH=), 7.32 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.50 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 6.46 (d, *J* = 2.4 Hz, 1H, Ar-H), 3.92 – 3.86 (m, 4H), 3.77 (s, 3H, -OCH_3), 3.68 – 3.64 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.31, 161.68, 158.97, 146.73, 131.27, 111.75, 106.11, 101.27, 65.71, 55.25, 48.76. HR-MS (ESI), calcd , HR-MS (ESI): Calcd. $C_{13}H_{17}N_3O_3S$, $[M+H]^+$m/z: 296.1069, found: 296.1070.

3.5. (E)-N'-(3,4,5-trimethoxybenzylidene)morpholine-4-carbothiohydrazide（5e）

Yield 66.4%. Yellow solid. Mp: 163–164°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.29 (s, 1H, NH, D_2O exchangeable), 8.01 (s, 1H, -CH=), 6.92 (s, 2H, Ar-H), 3.93 – 3.89 (m, 4H), 3.81 (s, 6H, -OCH_3), 3.72 – 3.69 (m, 4H), 3.69 (s, 3H, -OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 180.84, 153.14, 143.09, 138.90, 129.75, 103.81, 66.03, 60.07, 55.81, 50.92. HR-MS (ESI): Calcd. $C_{15}H_{21}N_3O_4S$, $[M+Na]^+$m/z: 362.1150, found: 362.1154.

3.6. (E)-N'-(naphthalen-2-ylmethylene)morpholine-4-carbothiohydrazide（5f）

Yield 75.6%. Yellow solid. Mp: 168–169°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.33 (s, 1H, NH, D_2O exchangeable), 8.32 (s, 1H, -CH=), 8.05 (s, 1H, Ar-H), 8.01 – 7.85 (m, 4H, Ar-H), 7.59 – 7.52 (m, 2H, Ar-H), 3.99 – 3.94 (m, 4H), 3.73 – 3.69 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 180.74, 143.89, 133.54, 132.87, 132.01, 128.53, 128.51, 128.23, 127.74, 126.99, 126.73, 122.28, 66.02, 50.60. HR-MS (ESI): Calcd. $C_{16}H_{17}N_3OS$, $[M+H]^+$m/z: 300.1171, found: 300.1173.

3.7.(E)-N'-((2-hydroxynaphthalen-1-yl)methylene)morpholine-4-carbothiohydrazide（5g）

Yield 71.8%. Yellow solid. Mp: 215–216°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 12.83 (s, 1H, OH, D_2O exchangeable), 11.55 (s, 1H, NH, D_2O exchangeable), 9.40 (s, 1H, -CH=), 8.10 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.96 – 7.86 (m, 2H, Ar-H), 7.63 – 7.57 (m, 1H, Ar-H), 7.40 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.21 (d, *J* = 9.0 Hz, 1H, Ar-H), 4.02 – 3.94 (m, 4H), 3.75 – 3.67 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 178.98, 157.12, 144.33, 132.11, 131.51, 128.94, 127.68, 127.55, 123.37, 120.20, 119.14, 108.52, 65.69, 48.63. HR-MS (ESI): Calcd. $C_{16}H_{17}N_3O_2S$, $[M+H]^+$m/z: 338.0939, found: 338.0936.

3.8.tert-butyl(E)-4-(2-(2-hydroxybenzylidene)hydrazine-1-carbonothioyl)piperazine-1-carboxylate（5h）

Yield 80.3%. Yellow solid. Mp: 197–198°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.52 (s, 2H, OH, NH, D_2O exchangeable), 8.47 (s, 1H, -CH=), 7.43 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.28 (t, *J* = 7.7 Hz, 1H, Ar-H), 6.93 – 6.87 (m, 2H, Ar-H), 3.95 – 3.90 (m, 4H), 3.47 – 3.41 (m, 4H), 1.43 (s, 9H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 179.44, 157.05, 153.79, 146.26, 130.89, 129.85, 119.08, 118.48, 116.49, 79.23, 48.05, 28.02. HR-MS (ESI): Calcd. $C_{17}H_{24}N_4O_3S$,$[M+Na]^+$m/z: 387.1467, found: 387.1469.

3.9. (E)-2-(2-hydroxybenzylidene)-N-phenylhydrazine-1-carbothioamide（5i）

Yield 73.9%. Yellow solid. Mp: 181–182°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.79 (s, 1H, OH, D_2O exchangeable), 10.07 (s, 1H, NH, D_2O exchangeable), 9.99 (s, 1H, NH, D_2O exchangeable), 8.50 (s, 1H, -CH=), 8.10 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.57 (s, 1H, Ar-H), 7.37 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.23 (dt,

J = 15.6, 4.4 Hz, 2H, Ar-H), 6.89 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.85 (t, *J* = 7.5 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 175.67, 156.56, 139.95, 139.12, 131.30, 127.99, 127.02, 125.69, 125.15, 120.22, 119.19, 115.99. HR-MS (ESI): Calcd. $C_{14}H_{13}N_3OS$, $[M+H]^+$m/z: 270.0701, found: 270.0695.

3.10. (E)-N'-(1-phenylethylidene)morpholine-4-carbothiohydrazide（5j）

Yield 67.4%. Yellow solid. Mp: 200–201°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.06 (s, 1H, NH, D_2O exchangeable), 7.95 (d, *J* = 3.0 Hz, 2H, Ar-H), 7.42 (d, *J* = 2.8 Hz, 3H, Ar-H), 3.88 – 3.83 (m, 4H), 3.66 – 3.61 (m, 4H), 2.35 (s, 3H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 178.32, 148.66, 137.49, 129.41, 128.31, 126.57, 65.73, 48.48, 14.07. HR-MS (ESI): Calcd. $C_{13}H_{17}N_3OS$, $[M+H]^+$m/z: 264.1171, found: 264.1175.

3.11. (E)-N'-(1-(2-hydroxyphenyl)ethylidene)morpholine-4-carbothiohydrazide（5k）

Yield 72.5%. Yellow solid. Mp:195–196°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 12.91 (s, 1H, NH, D_2O exchangeable), 10.38 (s, 1H, OH, D_2O exchangeable), 7.61 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.29 (t, *J* = 7.5 Hz, 1H, Ar-H), 6.89 (d, *J* = 7.2 Hz, 2H, Ar-H), 3.96 – 3.88 (m, 4H), 3.69 – 3.64 (m, 4H), 2.42 (s, 3H, -CH_3).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 188.17, 186.72, 164.80, 162.94, 136.25, 133.64, 123.74, 122.37, 71.03, 54.01, 52.98.HR-MS (ESI), calcd $C_{13}H_{17}N_3O_2S$, $[M + H]^+$ m/z, 280.1120; found, 280.1123.

3.12. (E)-N'-(1-(3-hydroxyphenyl)ethylidene)morpholine-4-carbothiohydrazide（5l）

Yield 78.3%. Yellow solid. Mp: 212 –213°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.16 (s, 1H, OH, D2O exchangeable), 9.52 (s, 1H, NH, D2O exchangeable), 7.35 (d, J = 7.9 Hz, 1H, Ar-H), 7.30 – 7.25 (m, 1H, Ar-H), 7.20 (t, J = 7.9 Hz, 1H, Ar-H), 6.82 (dd, J = 8.0, 1.8 Hz, 1H, Ar-H), 3.88 – 3.81 (m, 4H), 3.66 – 3.61 (m, 4H), 2.30 (s, 3H, -CH_3).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 181.97, 178.34, 157.28, 138.89, 129.26, 117.50, 116.49, 113.32, 65.71, 48.46, 14.23. HR-MS (ESI): Calcd. $C_{13}H_{17}N_3O_2S$, $[M - H]^-$m/z: 278.0963, found: 278.0960.

3.13. (E)-N'-(1-(4-hydroxyphenyl)ethylidene)morpholine-4-carbothiohydrazide（5m）

Yield 68.6%. Yellow solid. Mp: 181–182°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.02 (s, 1H, OH, D_2O exchangeable), 9.80 (s, 1H, NH, D_2O exchangeable), 7.78 (m, 2H, Ar-H), 6.84 – 6.68 (m, 2H, Ar-H), 3.89 – 3.81 (m, 4H), 3.69 – 3.56 (m, 4H), 2.28 (s, 3H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 181.97, 178.02, 158.88, 149.05, 128.21, 115.05, 65.72, 48.47, 13.88. HR-MS (ESI): Calcd. $C_{13}H_{17}N_3O_2S$, $[M+H]^+$m/z: 278.0963, found: 278.0958.

3.14. (E)-2-(1-(5-chloro-2-hydroxyphenyl)ethylidene)-N-phenylhydrazine-1-carbothio-amide（5n）

Yield 72.4%. Yellow solid. Mp: 177–178°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.90 (s, 1H, OH, D_2O exchangeable), 10.11 (s, 1H, NH, D_2O exchangeable), 7.65 (s, 1H, Ar-H), 7.62 (s, 2H, Ar-H), 7.37 (t, *J* = 7.7 Hz,2H, Ar-H), 7.30 (dd, *J* = 8.7, 2.1 Hz, 1H, Ar-H), 7.18 (t, *J* = 7.3 Hz, 1H, Ar-H), 6.92 (d, *J* = 8.7 Hz, 1H, Ar-H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 177.77, 157.96, 155.95, 152.28, 151.42, 138.99, 130.10, 128.39, 127.94, 124.87, 123.74, 122.26, 118.63, 15.35. HR-MS (ESI): Calcd. $C_{15}H_{14}ClN_3OS$, $[M+H]^+$m/z: 320.0624, found: 320.0618.

3.15. (E)-N'-(1-(2-aminophenyl)ethylidene)morpholine-4-carbothiohydrazide（5o）

Yield 69.5%. Yellow solid. Mp: 210–211°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 11.23 (s, 1H, NH, D_2O exchangeable), 7.35 (dd, *J* = 8.0, 1.2 Hz, 1H, Ar-H), 7.07 – 6.99 (m, 1H, Ar-H), 6.71 (dd, *J* = 8.1, 0.8 Hz, 1H, Ar-H), 6.55 (dd, *J* = 11.0, 3.9 Hz, 3H), 3.75 – 3.64 (m, 4H), 3.30 – 3.20 (m, 4H), 2.33 (s, 3H, -CH_3).^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 164.19, 146.96, 128.93, 128.79, 118.49, 115.89, 115.10, 65.27, 48.84, 15.75. HR-MS (ESI): Calcd. $C_{13}H_{18}N_4OS$, $[M+H]^+$m/z: 279.1280, found: 279.1282.

3.16.(E)-N'-(1-(5-chloro-2-hydroxyphenyl)ethylidene)morpholine-4-carbothiohydrazide（5p）

Yield 68.8%. Yellow solid. Mp: 224–225°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 11.81 (s, 1H, OH, D_2O exchangeable), 8.20 (s, 1H, NH, D_2O exchangeable), 7.34 (s,

1H, Ar-H), 7.16 (s, 1H, Ar-H), 6.87 (d, *J* = 8.6 Hz, 1H, Ar-H), 3.86 (s, 4H), 3.74 (s, 4H), 2.26 (s, 3H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 156.03, 151.02, 130.35, 126.22, 123.97, 122.80, 122.43, 118.41, 65.20, 49.81, 11.87. HR-MS (ESI): Calcd. $C_{13}H_{16}ClN_3O_2S$, $[M+H]^+$m/z: , found: 314.0732.

3.17. tert-butyl(E)-4-(2-(1-(5-chloro-2-hydroxyphenyl)ethylidene)hydrazine-1-carbonothioyl)piperazine-1-carboxylate（5q）

Yield 70.4%. Yellow solid. Mp: 212–213°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 12.99 (s, 1H, OH, D_2O exchangeable), 10.48 (s, 1H, NH, D_2O exchangeable), 7.62 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.32 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.7 Hz, 1H, Ar-H), 3.98 – 3.90 (m, 4H), 3.47 – 3.41 (s, 4H), 2.42 (s, 3H, -CH_3), 1.43 (s, 9H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 181.15, 156.62, 153.79, 135.34, 130.41, 127.55, 122.02, 119.59, 118.97, 79.20, 48.11, 28.01, 14.42. HR-MS (ESI): Calcd. $C_{18}H_{25}ClN_4O_3S$, $[M+Na]^+$m/z: 435.1234, found: 435.1239.

4. Effect of compounds on cell viability

About 3×10^3 exponentially growing cells per well were seeded into 96-well plate. After 24h incubation, the medium was removed and replaced with fresh medium containing different concentrations of candidate compound for another 72h. Then, 20μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5mg/mL) was added to each well and the cells continued incubation for more 4h. Remove the medium containing MTT, add 150mL DMSO (dimethyl sulfoxide) to each well, and agitate the plate until the dark blue crystal dissolved completely. The absorbance was measured using an ELx 800 Universal Microplate Reader (Bio-Tek, Inc.) at a wavelength of 570nm. The date of IC_{50} was analyzed by SPSS 20.

5. Colony formation assay

Exponentially growing MGC-803 cells were seeded into 6-well plate at about 1×10^3 cells per well. After 24h incubation, the medium was removed and replaced with fresh medium containing different concentrations of candidate compound for another 14days. Then, wash the cells with PBS three times, fix them with 4% paraformaldehyde for 30min, and stain the cells with 1% crystal violet staining solution for 30min at 37°C. At last, wash the cells until the colonies were clear.

6. Flow cytometric analysis of cell cycle distribution

About 2×10^5 exponentially growing MGC-803 cells were seeded into 6-well plate. After 24h incubation, the cultured medium was replaced with medium containing different concentrations of candidate compound for another 48h. Then, harvest the cells and fix the cells with cold 70% ethanol at 4°C overnight. After centrifuged, the cells were stained with 1% Triton X-100, RNase and PI cell cycle kit according to the protocol and analyzed by Accuri C6 flow cytometer.

7 Wound healing assay

Cells were seeded into 6-well plate and grown to confluence. The confluences were scratched with a sterile 200μL pipette tip and washed with PBS for three times. Then the cells were cultured with fresh medium containing only 1% FBS and different concentrations of candidate compound for another 48h, and photographed on an inverted microscope at the times of 0h and 48h.

8 Migration and Invasion Assay

Migration and invasion activity of cells were performed by transwell and matrigel-coated transwell assay, and these experiments were carried out as previously published. Briefly, MGC-803 cells were suspended with medium containing 1% FBS and different concentrations of candidate compound and seeded into the upper chamber of 24-well plate, while the medium containing 20% FBS into the bottom chamber. After 48h, wash the chamber three times with PBS and the migrating cells were fixed with methanol and stained with 20μg/mL Hoechst-33258 for 30min in the dark. Each chamber was photographed using Thermo Fisher Cellomics High Content System. As the matrigel-coated transwell assay, the upper chamber was needed paved with mateigel (BD Biosciences) previously, and following procedures were the same as transwell assay.

9 Hoechst 33258 staining

About 2×10^5 exponentially growing MGC-803 cells were seeded into 6-well plate. When the cells were adhered completely, the medium was replaced with fresh medium containing the increasing concentrations of candidate compound for another 48h. Then, the cells were fixed and stained with 20μg/mL Hoechst-33258 for 30min

in the dark. After that, the cells were washed with PBS for three times and photographed by fluorescence microscopy under UV excitation. The condensation and fragmentation of nuclei defined the apoptosis cells.

10 Flow cytometric analysis of Apoptosis

Before harvest the cells, the pretreatment was the same as flow cytometric analysis of cell cycle. Then, harvested and centrifuged, the cells were stained with FITC-conjugated Annexin V and PI apoptosis kit according to the protocol and analyzed by Accuri C6 flow cytometer.

11 Western blotting analysis

The MGC-803 cells pretreated with different concentrations of candidate compound were harvested and lysed with cell lysis buffer [1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 150 mM NaCl, 25 mM Tris-HCl, 1% deoxycholic acid sodium salt, 1% PMSF] for 30min on ice. Then, the protein lysate were denatured, and the equal amount of protein lysate were separated by SDS-PAGE and transferred onto 0.22μm nitrocellulose membrane. The membrane was blocked with PBS with 5% nonfat milk for 2h and incubated with primary antibody at 4°C overnight, followed by incubated with second antibody for 2h. The immunoblots were visualized by enhanced chemiluminescence kit from Thermo Fisher.