Supplementary Materials

ALTERNATE SYNTHESIS OF HSP90 INHIBITOR AT13387

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Materials and General Methods

¹H and ¹³C NMR spectra were measured on a Bruker 500 MHz NMR spectrometer using TMS as the internal standard. High resolution mass spectrometry (HRMS) was performed on an Agilent 6230 Series using ESI techniques. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F₂₅₄ plates. Flash column chromatography was performed with silica gel (300-400 mesh). All yields were given refer to as isolated yields. All chemical reagents were purchased from commercial sources and used as received. All moisture-sensitive reactions were carried out using dry solvents and under a slight pressure of ultra-pure quality argon.

Glassware was dried in an oven at 120°C for at least 12h prior to use, and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Anhydrous tetrahydrofuran (THF) was refluxed and distilled from sodium metal, and all the other chemical reagents were purchased from commercial sources and used as received. The melting points are uncorrected.

N-Boc-propargylamine (2)

To a stirred solution of propargyl amine (2.0 g, 36.3 mmol) in THF (30 mL) was added di-*tert*-butyl dicarbonate (8.78 g, 40.2 mmol, 1.1 equiv) at rt. The solution was stirred at the same temperature for 4h, and then concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (100 mL), washed with water (3×20 mL) and brine (20 mL) and then dried over anhydrous Na₂SO₄. After removal of the solvent *in vacuo*, N-Boc-propargylamine (5.11 g, 32.9 mmol, 90%) was obtained as a yellow solid, which was pure enough and used in the subsequent reaction without further purification. mp 38-39 °C (Lit.¹³ 41-42 °C). ¹H NMR (500 MHz, CDCl₃) δ 4.81 (brs, 1H), 3.89 (s, 2H), 2.19 (t, *J* = 2.5 Hz, 1H), 1.42 (s, 9H); ¹³C NMR (125 MHz, CDCl₃, ppm): δ 155.92, 80.76, 71.86, 30.99, 28.97, 28.04.

N-Boc-dipropargylamine (3)

To a stirred solution of N-Boc-propargylamine (**2**) (3.99 g, 25.7 mmol) in anhydrous THF (70 mL) was added 60% NaH (1.43 g, 35.7 mmol, 1.4 equiv). The

suspension was stirred for 0.5h and then propargyl bromide (4.87 g, 40.9 mmol, 1.6 equiv) was added dropwise. The mixture was stirred at rt for 8h, and then quenched with saturated NH₄Cl solution. The resulting mixture was extracted with EtOAc (3 × 100 mL). The combined organic phases were then washed with water (3 × 30 mL) and brine (30 mL) and dried (Na₂SO₄). After the solvent was removed *in vacuo*, the crude product was subjected to flash column chromatography (eluting with petroleum ether/EtOAc=120:1) to give **3** as pale yellow oil (3.78 g, 19.6 mmol, 76%). ¹H NMR (500 MHz, CDCl₃) δ 4.17 (brs, 4H), 2.23 (s, 2H), 1.48 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 154.85, 81.77, 79.43, 72.56, 35.81, 28.92.

***tert*-Butyl-5-hydroxymethyl-1,3-dihydro-2H-isoindole-2-carboxylate **4**:**

General Procedure

To a stirred solution of **3** (1.0 equiv) and propargyl alcohol (3.0 equiv) in a solvent (Table 1) was added a catalyst (0.03 equiv) at rt. After the mixture was stirred at the given temperature for certain period of time, the solvent was concentrated to dryness *in vacuo*, and the crude product was purified by flash column chromatography to give **4** as white solid. When the [2+2+2] cycloaddition of **3** with propargyl alcohol was performed in THF at ambient temperature and in the presence of (PPh₃)₃RhCl, **4** was obtained in 70% yield. Mp 105-106 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.15-7.24 (m, 3H), 4.66 (s, 2H), 4.55 (br, 4H), 2.75 (brs, 1H), 1.50 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 155.28, 141.29 (+141.26, rotamer) 138.16 (+137.83), 137.05 (+136.79), 126.94 (+126.86), 123.29 (+123.11), 121.96 (+121.83), 80.52

(+80.49), 65.59 (+65.54), 52.79 (+52.70), 52.47 (+52.34), 29.17; HRMS/ESI: m/z [M+Na]⁺ calcd. for C₁₄H₁₉NNaO₃: 272.1257; found: 272.1254.

***tert*-Butyl-5-formyl-1,3-dihydro-2*H*-isoindole-2-carboxylate (5)**

To a solution of **4** (500 mg, 2.0 mmol) in DCM (15 mL) was added MnO₂ (2.0 g), and the mixture was stirred at rt 4h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated *in vacuo*, and the residue was purified by flash column chromatography on silica gel (petroleum ether/EtOAc = 5:1) to give **5** as colorless solid (415 mg, 1.7 mmol, 84%). mp 116-117 °C; ¹H NMR (500 MHz, CDCl₃) δ 10.00 (s, 1H), 7.79-7.81 (m, 2H), 7.42 (m, 1H), 4.75 (s, 2H), 4.72 (s, 2H), 1.53 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 192.28, 154.97 (+154.93, rotamer), 144.98 (+144.66), 139.23 (+138.82), 136.78, 130.53 (+130.09), 124.67, 124.08, (+123.87), 80.75 (+80.73), 52.93 (+52.66), 52.48 (+52.22), 29.12; HRMS/ESI: m/z [M+Na]⁺ calcd. for C₁₄H₁₇NNaO₃: 270.1101; found: 270.1105.

***tert*-Butyl 5-[(4-methylpiperazin-1-yl)methyl]-isoindoline-2-carboxylate (6)**

To a mixture of **5** (1.83 g, 7.40 mmol) and 1-methylpiperazine (1.0 g, 10.0 mmol, 1.3 equiv) in DCM (45 mL) was added acetic acid (0.85 mL, 14.8 mmol, 2.0 equiv). The mixture was stirred at rt for 10 min, and then was added sodium triacetoxyborohydride (2.41 g, 11.4 mmol, 1.5 equiv). The mixture was stirred at rt under N₂ atmosphere for 3h. The reaction mixture was quenched by adding saturated NaHCO₃ solution, and the resulting mixture was extracted with DCM (3×100 mL).

The combined organic phases were then washed with water (20 mL) and brine (2×30 mL) and dried (Na₂SO₄). After the solvent was removed *in vacuo*, the crude product was purified by flash column chromatography on silica gel (DCM/MeOH = 20:1) to give **6** (2.18 g, 6.6 mmol, 90%). mp 45-47 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.14-7.24 (m, 3H), 4.67 (s, 2H), 4.62 (s, 2H), 3.50 (s, 2H), 2.47 (brs, 8H), 2.29 (s, 3H), 1.51 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 155.19, 138.21 (+138.16, rotamer), 138.09, 137.74, 136.80 (+136.45), 129.03, 124.10 (+123.85), 123.10 (+122.86), 80.26, 63.46, 55.65, 53.63, 52.83 (+52.73), 52.53 (+52.42), 46.57, 29.17; HRMS/ESI : *m/z* [M+Na]⁺ calcd. for C₁₉H₂₉N₃NaO₂: 354.2152; found: 354.2159.

5-[(4-Methylpiperazin-1-yl)methyl]isoindoline trihydrochloride (7)

To a solution of **6** (710 mg, 2.1 mmol) in DCM (20 mL) was bubbled hydrogen chloride gas at rt for about 10 min, and the mixture was stirred for 4 h. The solvent was evaporated to give a gray solid (almost quantitatively), which was pure enough and used in the subsequent reaction without further purification, mp >300 °C (Lit.¹³ >300 °C); ¹H NMR (500 MHz, D₂O) δ 7.57 (s, 1H), 7.57 (s, 2H), 4.72 (s, 4H), 4.54(s, 2H), 3.71 (br, 8H), 3.03 (s, 3H); ¹³C NMR (125 MHz, D₂O) δ 136.72, 135.53, 131.80, 128.20, 125.79, 123.97, 59.97, 50.06, 50.59, 50.19, 48.18, 42.83; HRMS/ESI: *m/z* [M+H]⁺ calcd. for C₁₄H₂₂N₃: 232.1808; found: 232.1814.

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(4-methyl-piperazin-1-ylmethyl)-1,3-dihydro-isoindol-2-yl]-methanone (10)

From 9: To a mixture of **9** (786 mg, 2.1 mmol), HOBt (312 mg, 2.3 mmol, 1.1 equiv), EDC·HCl (442 mg, 2.3 mmol, 1.1 equiv) in anhydrous DMF (30 mL) was added **7** (715 mg, 2.1 mmol, 1.0 equiv), and the mixture was stirred at rt for 0.5h, then DIEA (1.4 mL, 8.4 mmol, 4.0 equiv) was added at 0°C and the mixture was stirred at the same temperature for 0.5h. The mixture was allowed to warm to ambient temperature, and the stirring was continued for additional 10h. Water (30 mL) was added, and the resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with water and brine and dried (Na₂SO₄). The crude product was purified by flash column chromatography on silica gel (DCM/MeOH = 20:1) to afford **10** as pale-yellow solid (1.08 g, 1.8 mmol, 87%). mp 43-44 °C; ¹H NMR (500 MHz, CD₃OD) δ 7.08-7.42 (m, 14H), 6.83 (d, *J* = 3.35 Hz, 1H), 5.13 (s, 4H), 5.07(d, *J* = 11.65 Hz, 2H), 4.83 (s, 3H), 4.58 (s, 2H), 3.5 (d, *J* = 20.25 Hz, 2H), 2.47 (s, 8H), 2.25 (d, *J* = 10.05 Hz, 3H), 2.07 (s, 3H); ¹³C NMR (125 MHz, CD₃OD) δ 170.74, 159.28 (+159.27, rotamer), 156.16 (+156.15), 144.38, 138.41 (+138.30), 138.24, 138.09 (+138.06), 137.98, 137.39, 136.93, 136.38, 130.29 (+130.14), 129.62, 129.59, 129.53, 129.39, 129.05, 129.00 (+128.97), 128.56, 128.39 (+128.30), 127.56, 125.06 (+124.83), 123.79 (+123.57), 119.91, 115.81, 100.48, 71.84 (+71.78), 71.64, 63.52, 55.67 (+55.65), 54.61 (+54.53), 53.47, 53.02 (+52.93), 45.95, 23.63; HRMS/ESI: *m/z* [M+Na]⁺ calcd. for C₃₈H₄₁N₃NaO₃: 610.3040; found: 610.3047.

From 14: To a mixture of 1-methylpiperazine (192 mg, 1.9 mmol, 1.2 equiv) and K₂CO₃ (267 mg, 1.9 mmol, 1.2 equiv) in anhydrous DMF (20 mL) was added

14 (950 mg, 1.6 mmol), and the mixture was stirred at rt for 12h. Then the mixture was diluted with water (30 mL) and extracted with DCM (3×100 mL). The combined organic layers were washed with water and brine, and dried (Na_2SO_4). After the solvent was removed *in vacuo*, the resulting material was subjected to flash column chromatography (DCM/MeOH = 15:1) to give **10** as pale-yellow solid (850 mg, 1.4 mmol, 90%). The NMR spectra of the product were identical to those obtained from **9**.

2,4-Bis-benzyloxy-5-isopropenyl-N-propargyl benzamide (11)

To a mixture of **9** (3.0 g, 8.0 mmol), HOBt (1.20 g, 8.8 mmol, 1.1 equiv), EDC·HCl (1.71 g, 8.8 mmol, 1.1 equiv) in anhydrous DMF (25 mL) was added dropwise 2-propynylamine (0.55 g, 10.0 mmol, 1.2 equiv), and the mixture was stirred at rt for 0.5h, then DIEA (4.0 mL, 24.2 mmol, 3.0 equiv) was added at 0°C, and the mixture was stirred at the same temperature for 0.5h. The mixture was allowed to warm to ambient temperature, and the stirring was continued for additional 13h. Water (60 mL) was added, and the resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with water and brine and dried (Na_2SO_4). The crude product was purified by flash column chromatography on silica gel (petroleum ether/EtOAc=10:1) to afford **11** as pale-yellow solid (3.27 g, 7.9 mmol, 99%). mp 101-102 °C; ^1H NMR (500 MHz, CDCl_3) δ 8.12 (s, 1H), 7.99(s, 1H), 7.44-7.33 (m, 10H), 6.56 (s, 1H), 5.16 (s, 2H), 5.12 (s, 4H), 4.15 (d, $J = 2.2$ Hz, 2H), 2.12 (s, 4H); ^{13}C NMR (125 MHz, CDCl_3) δ 164.71, 159.28, 157.12, 142.18, 136.34,

135.36, 133.25, 128.99, 128.69, 128.11, 127.79, 127.12, 126.34, 115.79, 113.51, 98.14, 79.86, 71.68, 71.15, 70.59, 29.39, 23.33. HRMS/ESI: m/z $[M+H]^+$ calcd. for $C_{27}H_{26}NO_3$: 412.1907; found: 412.1906.

2,4-Bis(benzyloxy)-5-isopropenyl-N,N-dipropargylbenzamide (12)

To a stirred solution of **11** (3.27 g, 8.0 mmol) in anhydrous DMF (20 mL) was added 60% NaH (0.41 g, 9.6 mmol, 1.2 equiv). The suspension was stirred for 0.5h and then propargyl bromide (1.31 g, 11.0 mmol, 1.4 equiv) was added dropwise. The mixture was stirred at rt for 16h, and then quenched with saturated NH_4Cl solution. The solvent was removed *in vacuo* and the resulting residue was triturated with DCM. The organic phase was washed with water and brine, and dried (Na_2SO_4). After the solvent was removed *in vacuo*, the resulting material was subjected to flash column chromatography (DCM/petroleum ether = 3:1) to give **12** as yellow oil (3.5 g, 7.8 mmol, 98%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.40-7.28 (m, 10H), 7.22 (s, 1H), 6.51 (s, 1H), 5.12 (d, $J = 3.2$ Hz, 2H), 5.05 (s, 4H), 4.70 (s, 1H), 4.36 (br, 1H), 4.12 (br, 2H), 2.21 (s, 1H), 2.18 (s, 1H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.36, 157.80, 154.79, 142.57, 136.51, 129.29, 128.63, 128.52, 128.02, 127.88, 127.17, 127.05, 126.38, 117.10, 115.53, 98.98, 78.23, 78.07, 73.01, 72.07, 70.77, 70.59, 37.65, 37.94, 23.25; HRMS/ESI: m/z $[M+Na]^+$ calcd. for $C_{30}H_{27}NNaO_3$: 472.1883; found: 472.1887.

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(hydroxymethyl)-1,3-dihydro-

isoindol-2-yl]-methanone (13)

To a solution of **12** (500 mg, 1.1 mmol) in THF (15 mL) was added dropwise propargyl alcohol (250 mg, 4.4 mmol, 4.0 equiv), followed by the addition of tris(triphenylphosphine) rhodium (I) chloride (30 mg, 0.032 mmol, 0.03 equiv). The reaction was stirred at rt for 4h, and then the solvent was removed *in vacuo* to give a red oil, which was subjected to flash column chromatography (petroleum ether/EtOAc = 1:3) to give **13** as solid (405 mg, 0.8 mmol, 72%). mp 162-164 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.37-7.29 (m, 5H), 7.24-7.03 (m, 9H), 6.56 (s, 1H), 5.10 (s, 2H), 5.04 (s, 4H), 4.87 (s, 2H), 4.61-4.65 (m, 4H), 2.92 (br, 1H), 2.10 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 168.38, 157.50, 154.73 (+154.70, rotamer), 142.65, 141.09 (+140.88), 136.92, 136.65, 136.58 (+136.57), 136.53, 135.69 (+135.52), 128.68 (+128.66), 128.61 (+128.57), 127.98 (+127.94), 127.16, 126.96 (+126.94), 126.45 (+126.23), 122.87 (+122.39), 121.45 (+121.02), 119.56 (+119.51), 115.49, 99.55, 77.07 (+77.06), 64.71 (+64.68), 53.41 (+53.32), 51.97 (+51.86), 23.29; HRMS/ESI: *m/z* [M+Na]⁺ calcd. for C₃₃H₃₁NNaO₄: 528.2145; found: 528.2152.

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(methanesulfonyloxy-methyl)-1,3-dihydro-isoindol-2-yl]-methanone (14)

To a solution of **13** (650 mg, 1.3 mmol) in anhydrous DCM (25 mL) was added Et₃N (273 mg, 2.6 mmol, 2.0 equiv), followed by the addition of MsCl (186 mg, 1.6 mmol, 1.2 equiv) at 0°C. The mixture was stirred at 0-5°C for 0.5h followed by 12h at rt. The reaction mixture was diluted with DCM (100 mL), and then washed with water

(30 mL) and brine (30 mL), and dried (Na₂SO₄). After the solvent was removed *in vacuo*, the resulting material was subjected to flash column chromatography (petroleum ether/EtOAc = 3:1) to give **14** as yellow oil (474 mg, 0.81 mmol, 63%). ¹H NMR (500 MHz, CDCl₃) δ 7.38-7.28 (m, 8H), 7.25-7.22 (m, 5H), 7.13-7.08 (m, 1H), 6.56 (d, *J* = 1.5 Hz, 1H), 5.11 (s, 2H), 5.06 (dd, *J*₁ = 5.95 Hz, *J*₂ = 1.1 Hz, 4H), 4.95 (s, 2H), 4.64 (s, 2H), 4.58 (s, 1H), 4.54 (s, 1H), 2.10 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 168.24, 157.50, 154.73, 142.66, 137.55, 137.27, 137.22 (+137.17, rotamer), 136.99 (+136.94), 136.60 (+136.56), 137.54, 128.73, 128.62 (+128.58), 128.13, 127.98 (+127.96), 127.16 (+126.97), 126.52, 123.33(+123.30), 122.83 (+122.78), 119.63 (+119.61), 115.49, 99.60, 71.11 (+70.59), 53.27, 51.87 (+51.85), 46.03, 29.68, 23.30.

(2,4-Dihydroxy-5-isopropylphenyl)-(5-((4-methylpiperazin-1-yl)methyl)isoin-dolin-2-yl)methanone (AT13387)

10 (3.2 g, 5.5 mmol) was dissolved in MeOH (30 mL), and 10% Pd/C (0.35g) was added. The mixture was hydrogenated under an atmosphere of H₂ at rt for 12h. After the mixture was filtered through a Celite pad, rinsing the filter cake with MeOH (30 mL), the combined filtrates were concentrated *in vacuo*, and the crude product was purified by flash column chromatography on silica gel (DCM/MeOH = 25:1) to give **AT13387** as a white solid (1.78 g, 4.3 mmol, 80%). mp 105-106 °C (Lit.¹⁴ 93-94 °C); ¹H NMR (500 MHz, CD₃OD) δ 7.24 (brs, 3H), 7.16 (s, 1H), 6.36 (s, 1H), 4.91 (s, 4H), 3.51 (s, 2H), 3.16-3.22 (m, 1H), 2.49 (brs, 8H), 2.28 (s, 3H), 1.20 (d, *J* = 6.95 Hz,

6H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.32, 159.07, 155.87, 138.15, 130.16, 127.95, 126.93, 124.83, 123.57, 114.34, 103.67, 63.47, 55.59, 53.33, 45.84, 27.71, 23.13;
HRMS/ESI: m/z $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{32}\text{N}_3\text{O}_3$: 410.2438; found: 410.2447.

Copies of ^1H and ^{13}C NMR spectra of compounds are on the following pages

N-Boc-propargylamine (2)

^1H NMR

^{13}C NMR

N-Boc-dipropargylamine (3)

^1H NMR

^{13}C NMR

***tert*-Butyl-5-hydroxymethyl-1,3-dihydro-2H-isindole-2-carboxylate (4)**

¹H NMR

¹³C NMR

^{13}C NMR (DMSO- d_6 , 293K)

^{13}C NMR (DMSO- d_6 , 333K)

***tert*-Butyl -5-formyl-1,3-dihydro-2*H*-isoindole-2-carboxylate (5)**

¹H NMR

¹³C NMR

***tert*-Butyl 5-[(4-methylpiperazin-1-yl)methyl]-isoindoline-2-carboxylate (6)**

¹H NMR

¹³C NMR

5-[(4-Methylpiperazin-1-yl)methyl]isoindoline trihydrochloride (7)

^1H NMR

^{13}C NMR

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(4-methyl-piperazin-1-ylmethyl)-1,3-dihydro-isoindol-2-yl]-methanone (10)

¹H NMR

¹³C NMR

2,4-Bis-benzyloxy-5-isopropenyl-N-propargyl benzamide (11)

^1H NMR

^{13}C NMR

2,4-Bis(benzyloxy)-5-isopropenyl-N,N-dipropargylbenzamide (12)

¹H NMR

¹³C NMR

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(hydroxymethyl)-1,3-dihydro-isoindol-2-yl]-methanone (13)

^1H NMR

^{13}C NMR

[2,4-Bis(benzyloxy)-5-isopropenylphenyl]-[5-(methanesulfonyloxy-methyl)-1,3-dihydro-isoindol-2-yl]-methanone (14)

^1H NMR

^{13}C NMR

**(2,4-Dihydroxy-5-isopropylphenyl)-(5-((4-methylpiperazin-1-yl)methyl)isoin-
dolin-2-yl)methanone (AT13387)**

¹H NMR

¹³C NMR