Supporting Information

Chemistry

General: All chemicals (reagent grade) were purchased from Sigma–Aldrich. Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, Merck). The quantity of silica gel used was 50–100 times the weight charged on the column. Then, the eluates were monitored using thin-layer chromatography (TLC). Melting points (mp) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra (MS) were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer. Elemental analyses were performed on a Heraeus CHN-O-Rapid instrument and were within ± 0.4% of the theoretical values.

Methyl 3,4-dihydroxybenzoate (B)

A suspension of 3,4-dihydroxybenzoic acid (9.24 g, 60 mmol) was prepared in MeOH (100 mL). H_2SO_4 (2.5ml) was added and the mixture was heated to reflux and allowed to stir for 12 h. The solvent was removed by rotary evaporation and the residue was quenched with saturated aqueous $NaHCO_3$. Extraction was performed with ethyl acetate (3 x 100 mL) and the combined organic layers were dried with sodium sulfate and concentrated in vacuo. The crude product was purified by column chromatography on SiO_2 giving a white solid. Yield 98%. R_f= 0.6 (EtOAc/hexane, 1:3); mp: 134.5–135.1ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (s, 1H), 9.38 (s, 1H), 7.34 (t, J = 1.7 Hz, 1H), 7.32 – 7.25 (m, 1H), 6.79 (dd, J = 8.3, 1.4 Hz, 1H), 3.74 (s, 3H). ^{13} C NMR (101 MHz, DMSO-d_6) δ 166.6, 150.8, 145.5, 122.2, 120.9, 116.7, 115.7, 52.0. MS (ESI): m/z 169.2 $[M+H]^{+}$. Anal. calcd for $C_8H_8O_4$: C 57.14, H 4.80, O 38.06; found: C 57.02, H 4.87, O 38.11.

Methyl 3,4-dihydro-*2H*-benzo[*b*][1,4]dioxepine-7-carboxylate (C)

1,3-Dibromopropane (8.08g, 40mmol) was added dropwise to a mixture of ethyl methyl 3,4-dihydroxybenzoate (1.68g, 10mmol), N,N-dimethylformamide (DMF; 20ml), and potassium carbonate (4.14 g, 30mmol) at 80°C. The reaction mixture was stirred for

13h at 100°C, poured into water (100ml), and extracted with ethyl acetate (3 x 50ml). The organic layer was washed successively with water (50ml) and brine (50ml), and then dried over anhydrous sodium sulfate. After evaporating the solvent, the residue was purified by column chromatography on SiO_2 giving a colorless oil. Yield 89%. R_f = 0.7 (EtOAc/hexane, 1:4). 1H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.59 (m, 1H), 7.58 – 7.54 (m, 1H), 6.95 – 6.90 (m, 1H), 4.25 (t, J = 5.7 Hz, 2H), 4.20 (t, J = 5.8 Hz, 2H), 3.83 (d, J = 0.5 Hz, 3H), 2.21 – 2.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 155.1, 150.4, 125.0, 123.2, 121.2, 70.3, 70.2, 51.9, 31.1. MS (ESI): m/z 209.1 $[M+H]^+$. Anal. calcd for $C_{11}H_{12}O_4$: C 63.45, H 5.81, O 30.74; found: C 63.34, H 5.83, O 30.85.

3,4-dihydro-2H-benzo[b][1,4]dioxepine-7-carbohydrazide (D)

To a solution of Compound **C** (1.25 g, 6.0 mmol) in EtOH (30 mL) was added hydrazine hydrate (2.91 mL, 60 mmol), and the mixture was heated at reflux for 1 day. After cooling to room temperature, pure crystals are formed, collected by filtration, and washed several times with Et_2O giving a white solid. Yield 72%. R_f = 0.2 (EtOAc/hexane, 4:1); mp: 238.2–238.9 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 7.40 (dd, J = 8.6, 1.3 Hz, 2H), 7.00 – 6.91 (m, 1H), 4.40 (d, J = 4.0 Hz, 2H), 4.15 (dt, J = 9.0, 5.6 Hz, 4H), 2.17 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.4, 153.7, 150.8, 128.7, 122.7, 121.6, 120.9, 70.8, 70.8, 31.5. MS (ESI): m/z 209.2 $[M+H]^+$. Anal. calcd for $C_{10}H_{12}N_2O_3$: C 57.41, H 6.26, N 13.39, O 22.94; found: C 57.34, H 6.30, N 13.39, O 22.97.

Compounds F

To a stirred solution of hydrazide (1 mmol) and appropriate benzaldehyde in ethanol (25 mL), and then glacial acetic acid (0.2 mL) was dropwise added. The mixture was refluxed for 5 h, after which the solution was poured into ice water. The mixture was stirred until precipitate formed, which was collected using suction filtration and dried, followed by recrystallization in anhydrous ethanol, giving compounds **F** as a solid.

(*E*)-N'-benzylidene-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide

(F1): white solid; yield: 96%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 110.3–111.0 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.43 (s, 1H), 7.70 (d, J = 6.5 Hz, 2H), 7.53 (dd, J = 11.9, 3.6 Hz, 2H), 7.44 (q, J = 5.7 Hz, 3H), 7.06 (d, J = 8.3 Hz, 1H), 4.20 (dt, J = 8.9, 5.6 Hz, 4H), 2.24 – 2.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.3, 150.8, 147.9, 134.8, 130.4, 129.3, 128.6, 127.5, 123.6, 121.9, 121.5, 70.9, 70.8, 31.5. MS (ESI): m/z 297.1 [M+H] $^+$. Anal. calcd for $C_{17}H_{16}N_2O_3$: C68.91, H 5.44, N 9.45, O 16.20; found: C 68.94, H 5.38, N 9.47, O 16.25.

(*E*)-N'-(4-methylbenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F2): beige solid; yield: 93%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 124.9–125.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.41 (s, 1H), 7.59 (dd, J = 27.4, 10.8 Hz, 4H), 7.28 (d, J = 7.1 Hz, 2H), 7.08 (d, J = 8.2 Hz, 1H), 4.23 (s, 4H), 2.36 (s, 3H), 2.16 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.3, 150.9, 148.0, 140.3, 132.2, 129.9, 128.8, 127.5, 123.6, 121.9, 121.5, 70.9, 70.9, 31.5, 21.5. MS (ESI): m/z 311.2 [M+H] $^+$. Anal. calcd for $C_{18}H_{18}N_2O_3$: C 69.66, H 5.85, N 9.03, O 15.47; found: C 69.71, H 5.83, N 9.06, O 15.47.

(*E*)-N'-(3-methylbenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F3): white solid; yield: 91%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 135.2–135.8 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 8.41 (s, 1H), 7.64 – 7.44 (m, 4H), 7.35 (t, J = 7.5 Hz, 1H), 7.26 (d, J = 7.3 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 4.22 (dd, J = 13.5, 5.7 Hz, 4H), 2.37 (s, 3H), 2.23 – 2.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.4, 150.9, 148.0, 138.5, 134.8, 131.2, 129.2, 128.7, 127.8, 124.9, 123.7, 121.9, 121.5, 70.9, 70.9, 31.5, 21.4. MS (ESI): m/z 311.2 [M+H] $^+$. Anal. calcd for $C_{18}H_{18}N_2O_3$: C 69.66, H 5.85, N 9.03, O 15.47; found: C 69.74, H 5.85, N 8.97, O 15.41.

(*E*)-N'-(2-methylbenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F4): white solid; yield: 94%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 154.6–155.1 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.74 (s, 1H), 7.85 (d,

J = 7.4 Hz, 1H), 7.65 – 7.50 (m, 2H), 7.30 (dt, J = 15.1, 6.9 Hz, 3H), 7.09 (d, J = 8.3 Hz, 1H), 4.32 – 4.12 (m, 4H), 2.45 (s, 3H), 2.24 – 2.08 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.4, 150.9, 146.6, 137.3, 132.8, 131.3, 130.2, 128.7, 126.7, 126.2, 123.6, 121.9, 121.5, 70.9, 70.9, 31.5, 19.5. MS (ESI): m/z 311.1 $[M+H]^+$. Anal. calcd for $C_{18}H_{18}N_2O_3$: C 69.66, H 5.82, N 9.03, O 15.47; found: C 69.74, H 5.82, N 9.09, O 15.41.

(*E*)-N'-(2-hydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F5): white solid; yield: 94%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 221.5-221.7 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 11.28 (s, 1H), 8.60 (s, 1H), 7.64 – 7.44 (m, 3H), 7.28 (t, J = 7.8 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 6.90 (t, J = 7.9 Hz, 2H), 4.20 (dt, J = 9.4, 5.6 Hz, 4H), 2.23 – 2.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.1, 157.9, 154.5, 150.8, 148.5, 131.7, 130.0, 128.0, 123.6, 121.9, 121.5, 119.8, 119.1, 116.8, 70.9, 70.8, 31.4. MS (ESI): m/z 313.2 $[M+H]^+$. Anal. calcd for $C_{17}H_{16}N_2O_4$: C 65.38, H 5.16, N 8.97, O 20.49; found: C 65.43, H 5.19, N 8.97, O 20.54.

(*E*)-N'-(2-chlorobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F6): white solid; yield : 95%. R_f = 0.4 (EtOAc/ hexane, 1:1); mp: 122.6-123.4 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.86 (s, 1H), 8.02 (s, 1H), 7.57 (m, 3H), 7.51 – 7.32 (m, 2H), 7.10 (d, J = 8.3 Hz, 1H), 4.33 – 4.10 (m, 4H), 2.25 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.5, 150.9, 143.9, 133.7, 132.1, 131.8, 130.4, 128.3, 128.0, 127.3, 123.8, 121.9, 121.6, 70.9, 70.9, 31.5. MS (ESI): m/z 331.2 $[M+H]^+$. Anal. calcd for $C_{17}H_{15}ClN_2O_3$: C 61.73, H 4.57, Cl 10.72, N 8.47, O 14.51; found: C 61.68, H 4.61, Cl 10.77, N 8.49, O 14.55.

(*E*)-N'-(2-bromobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F7): beige solid; yield : 81%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 124.9–125.5 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.82 (s, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.65 – 7.53 (m, 2H), 7.48 (t, J = 7.4 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 4.32 – 4.13 (m, 4H), 2.23 – 2.10 (m,

2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.5, 150.8, 146.2, 133.6, 133.6, 132.1, 128.5, 128.3, 127.7, 124.0, 123.8, 121.9, 121.6, 70.9, 70.9, 31.5. MS (ESI): m/z 375.3 [M+H] $^+$. Anal. calcd for $C_{17}H_{15}BrN_2O_3$: C 54.42, H 4.03, Br 21.30, N 7.47, O 12.79; found: C 54.41, H 4.05, Br 21.28, N 7.49, O 12.83.

(*E*)-N'-(2-formylbenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F8): beige solid; yield: 91%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 113.2–113.8 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (s, 1H), 11.98 (s, 1H), 9.19 (s, 1H), 8.07 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.59 (m, 4H), 7.08 (d, J = 8.0 Hz, 1H), 4.41 – 4.06 (m, 4H), 2.17 (d, J = 5.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.6, 162.6, 154.4, 150.8, 146.7, 135.2, 132.4, 131.1, 130.8, 130.0, 128.5, 127.1, 123.8, 121.9, 121.6, 71.0, 70.9, 31.5. MS (ESI): m/z 325.1 [M+H] $^+$. Anal. calcd for $C_{18}H_{16}N_2O_4$: C 66.66, H 4.97, N 8.64, O 19.73; found: C 66.67, H 4.98, N 8.61, O 19.76.

(*E*)-N'-(4-(dimethylamino)benzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F9): brown solid; yield 73%. R_f = 0.3 (EtOAc/hexane, 1:1); mp: 115.8–116.2 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.30 (s, 1H), 7.54 (d, J = 9.2 Hz, 4H), 7.07 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 8.2 Hz, 2H), 4.21 (dd, J = 11.6, 5.7 Hz, 4H), 2.98 (s, 6H), 2.25 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ162.0, 154.1, 151.9, 150.8, 148.8, 129.1, 128.9, 123.5, 122.1, 121.8, 121.4, 112.3, 70.9, 70.9, 37.4, 31.5. MS (ESI): m/z 340.2 [M+H] $^+$. Anal. calcd for $C_{19}H_{21}N_3O_3$: C67.24, H 6.24, N 12.38, O 14.14; found: C 67.31, H 6.23, N 12.42, O 14.17.

(*E*)-N'-(4-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F10): pale yellow solid; yield: 95%. R_f = 0.3 (EtOAc/hexane, 1:1); mp: 189.4–190.1 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 8.54 (s, 1H), 8.32 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 7.8 Hz, 2H), 7.65 – 7.49 (m, 2H), 7.10 (d, J = 8.2 Hz, 1H), 4.33 – 4.12 (m, 4H), 2.16 (dd, J = 10.9, 5.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.7, 154.6, 150.8, 148.2, 145.4, 141.2, 128.4, 128.2, 124.5, 123.8, 121.9, 121.6,

70.9, 70.9, 31.4. MS (ESI): m/z 342.1 $[M+H]^+$. Anal. calcd for $C_{17}H_{15}N_3O_5$: C 59.82, H 4.43, N 12.31, O 23.44; found: C 59.83, H 4.38, N 12.27, O 23.51.

(*E*)-N'-(4-(trifluoromethyl)benzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F11): white solid; yield: 89%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 192.6–193.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.49 (s, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.61 – 7.47 (m, 2H), 7.07 (d, J = 8.2 Hz, 1H), 4.28 – 4.12 (m, 4H), 2.22 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.6, 154.4, 150.8, 146.1, 138.8, 130.2, 128.4, 128.0, 126.1, 126.1, 125.9, 123.7, 123.2, 121.9, 121.6, 70.9, 70.8, 31.4. MS (ESI): m/z 365.3 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}F_3N_2O_3$: C 59.34, H 4.15, F 15.64, N 7.69, O 13.17; found: C 59.38, H 4.15, F 15.71, N 7.72, O 13,24.

(*E*)-N'-(4-fluorobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F12): white solid; yield: 94%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 196.8–197.4 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 8.44 (s, 1H), 7.87 – 7.72 (m, 2H), 7.62 – 7.49 (m, 2H), 7.31 (t, J = 8.7 Hz, 2H), 7.09 (d, J = 8.3 Hz, 1H), 4.33 – 4.13 (m, 4H), 2.25 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.8, 162.5, 154.4, 150.8, 146.8, 131.5, 129.7, 128.6, 123.7, 121.9, 121.5, 116.5, 71.0, 70.9, 31.5. MS (ESI): m/z 315.4 $[M+H]^+$. Anal. calcd for $C_{17}H_{15}FN_2O_3$: C 64.96, H 4.81, F 6.04, N 8.91, O 15.27; found: C 65.02, H 4.83, F 6.01, N 8.91, O 15.33.

(*E*)-N'-(4-iodobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F13): white solid; yield: 91%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 135.4–136.1 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 8.38 (s, 1H), 7.84 (d, J = 8.2 Hz, 2H), 7.54 (m, 4H), 7.08 (d, J = 8.3 Hz, 1H), 4.22 (dt, J = 9.4, 5.6 Hz, 4H), 2.25 – 2.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.4, 150.8, 146.9, 138.1, 134.4, 129.3, 128.5, 123.7, 121.9, 121.6, 97.2, 71.0, 70.9, 31.5. MS (ESI): m/z 423.3 $[M+H]^+$. Anal. calcd for $C_{17}H_{15}IN_2O_3$: C 48.36, H 3.58, I 30.06, N 6.63, O 11.37; found: C 48.38, H 3.59, I 30.10, N 6.69, O 11.41.

(*E*)-N'-(biphenyl-4-ylmethylene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F14): white solid; yield: 86%. R_f = 0.4 (EtOAc/hexane, 1:1); mp: 128.5–129.4 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H), 8.49 (s, 1H), 7.81 (q, J = 8.1 Hz, 4H), 7.74 (d, J = 7.5 Hz, 2H), 7.63 – 7.53 (m, 2H), 7.50 (t, J = 7.5 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 4.23 (dd, J = 13.4, 5.8 Hz, 4H), 2.24 – 2.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.4, 150.9, 147.5, 142.0, 139.8, 134.0., 129.5, 128.7, 128.3, 128.1, 127.5, 127.1, 123.7, 121.9, 121.6, 71.0, 70.9, 31.5. MS (ESI): m/z 373.3 [M+H] $^+$. Anal. calcd for $C_{23}H_{20}N_2O_3$: C 74.18, H 5.41, N 7.52, O 12.89; found: C 74.08, H 5.43, N 7.54, O 12.93.

(*E*)-N'-(4-hydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F15): beige solid; yield: 95%. R_f = 0.4 (EtOAc/hexane, 4:1); mp: 245.6–246.2 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H), 9.93 (s, 1H), 8.33 (s, 1H), 7.60 – 7.46 (m, 4H), 7.07 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 4.21 (dt, J = 8.2, 5.7 Hz, 4H), 2.20 – 2.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 159.9, 154.2, 150.8, 148.4, 129.3, 128.9, 125.8, 123.6, 121.9, 121.5, 116.2, 70.9, 70.9, 31.5. MS (ESI): m/z 313.2 [M+H] $^+$. Anal. calcd for $C_{17}H_{16}N_2O_4$: C 65.38, H 5.16, N 8.97, O 20.49; found: C 65.30, H 5.17, N 9.01, O 20.53.

(*E*)-N'-(4-methoxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F16): beige solid; yield: 98%. R_f = 0.5 (EtOAc/hexane, 2:1); mp: 118.7.4–119.5 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 8.36 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.56 – 7.43 (m, 2H), 7.02 (m, 3H), 4.25 – 4.10 (m, 4H), 3.78 (d, J = 8.2 Hz, 3H), 2.20 – 2.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.2, 161.2, 154.2, 150.8, 147.8, 129.1, 128.8, 127.4, 123.5, 121.8, 121.4, 114.7, 70.9, 70.8, 55.7, 31.5. MS (ESI): m/z 327.1 [M+H] $^+$. Anal. calcd for $C_{18}H_{18}N_2O_4$: C 66.25, H 5.56, N 8.58, O 19.61; found: C 66.25, H 5.54, N 8.61, O 19.55.

(*E*)-N'-(3,4-dimethoxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-

carbohydrazide (F17): white solid; yield: 92%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 138.6–139.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.37 (s, 1H), 7.64 – 7.48 (m, 2H), 7.33 (s, 1H), 7.20 (d, J = 8.1 Hz, 1H), 7.06 (m, 2H), 4.22 (dd, J = 12.5, 6.0 Hz, 4H), 3.82 (d, J = 4.6 Hz, 6H), 2.23 – 2.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.3, 151.2, 150.9, 149.5, 148.2, 128.8, 127.6, 123.6, 122.3, 121.9, 121.5, 111.9, 108.6, 70.9, 70.9, 56.0, 55.9, 31.5. MS (ESI): m/z 356.2 [M+H] $^+$. Anal. calcd for $C_{19}H_{20}N_2O_5$: C 64.04, H 5.66, N 7.86, O 22.45; found: C 64.04, H 5.68, N 7.85, O 22.51.

(*E*)-N'-(3,4,5-trimethoxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F18): white solid; yield: 92%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp: 110.2–110.8 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 8.36 (s, 1H), 7.62 – 7.45 (m, 2H), 7.08 (d, J = 8.2 Hz, 1H), 7.01 (s, 2H), 4.21 (dd, J = 12.2, 6.1 Hz, 4H), 3.84 (s, 6H), 3.71 (s, 3H), 2.26 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.3, 153.7, 150.8, 148.0, 139.7, 130.4, 128.7, 123.6, 121.9, 121.5, 104.7, 71.0, 70.9, 60.6, 56.4, 31.5. MS (ESI): m/z 387.2 [M+H] $^+$. Anal. calcd for $C_{20}H_{20}N_2O_6$: C 62.17, H 5.74, N 7.25, O 24.84; found: C 61.92, H 5.72, N 7.29, O 24.91.

(*E*)-N'-(4-hydroxy-3,5-dimethoxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F19): white solid; yield: 87%. R_f = 0.5 (EtOAc/ hexane, 4:1); mp: 154.3–155.1 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.91 (s, 1H), 8.32 (s, 1H), 7.62 – 7.45 (m, 2H), 7.08 (d, J = 8.1 Hz, 1H), 6.97 (s, 2H), 4.22 (dd, J = 11.8, 5.9 Hz, 4H), 3.83 (s, 6H), 2.25 – 2.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.2, 150.8, 148.6, 138.4, 128.9, 125.1, 123.6, 121.9, 121.5, 105.1, 71.0, 70.9, 56.5, 31.5. MS (ESI): m/z 383.3 [M+H] $^+$. Anal. calcd for $C_{19}H_{20}N_2O_6$: C 61.28, H 5.41, N 7.52, O 25.78; found: C 61.32, H 5.40, N 7.48, O 25.79.

(*E*)-N'-(3,4-dihydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F20): beige soild; yield: 84%. R_f = 0.4 (EtOAc/ hexane, 4:1); mp:

256.1–256.7 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 9.30 (s, 2H), 8.22 (s, 1H), 7.60 – 7.44 (m, 2H), 7.21 (s, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 4.19 (dt, J = 8.4, 5.6 Hz, 4H), 2.18 – 2.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.0, 162.4, 154.6, 150.8, 144.6, 140.4, 127.8, 126.9, 124.3, 123.8, 122.0, 121.6, 120.4, 117.6, 70.9, 70.9, 31.4. MS (ESI): m/z 329.2 [M+H] $^{+}$. Anal. calcd for $C_{17}H_{16}N_2O_5$: C 62.19, H 4.91, N 8.53, O 24.37; found: C 62.21, H 4.90, N 8.56, O 24.32.

(*E*)-N'-(4-hydroxy-3-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F21): yellow solid; yield:78%. R_f = 0.4 (EtOAc/ hexane, 2:1); mp:128.7–129.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 11.48 (s, 1H), 8.40 (s, 1H), 8.19 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.67 – 7.43 (m, 2H), 7.22 (d, J = 8.7 Hz, 1H), 7.08 (d, J = 8.2 Hz, 1H), 4.22 (dt, J = 9.1, 5.6 Hz, 4H), 2.30 – 2.07 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.4, 153.7, 150.8, 145.8, 137.6, 133.4, 128.6, 126.4, 124.4, 123.7, 121.9, 121.5, 120.2, 71.0, 70.9, 31.5. MS (ESI): m/z 358.3 [M+H] $^{+}$. Anal. calcd for $C_{17}H_{15}N_3O_6$: C 57.14, H 4.23, N 11.76, O 26.87; found: C 57.12, H 4.21, N 11.83, O 26.92.

(*E*)-N'-(5-chloro-2-hydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F22): white solid; yield 89%. R_f= 0.5 (EtOAc/ hexane, 1:1); mp: 210.1-211.0 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 11.28 (s, 1H), 8.58 (s, 1H), 7.64 (s, 1H), 7.58 – 7.48 (m, 2H), 7.30 (dd, J = 8.7, 2.5 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.8 Hz, 1H), 4.28 – 4.10 (m, 4H), 2.19 – 2.06 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 156.5, 154.5, 150.8, 146.1, 131.1, 128.2, 127.9, 123.7, 123.4, 121.9, 121.6, 121.1, 118.6, 70.9, 70.8, 31.4. MS (ESI): m/z 347.3 [M+H] $^{+}$. Anal. calcd for $C_{17}H_{15}ClN_2O_4$: C 58.88, H 4.36, Cl 10.22, N 8.08, O 18.46; found: C 58.95, H 4.33, Cl 10.24, N 8.11, O 18.49.

(*E*)-N'-(4-(dimethylamino)-2-hydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F23): gray solid; yield: 90%. R_f = 0.4

(EtOAc/ hexane, 1:1); mp: 205.7-208.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 11.49 (s, 1H), 8.40 (s, 1H), 7.65 – 7.40 (m, 2H), 7.18 (d, J = 8.7 Hz, 1H), 7.08 (d, J = 8.2 Hz, 1H), 6.27 (d, J = 8.7 Hz, 1H), 6.13 (s, 1H), 4.22 (dd, J = 12.0, 5.9 Hz, 4H), 2.24 – 2.02 (m, 2H), 1.11 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.6, 160.2, 154.3, 150.9, 150.6, 150.2, 132.1, 128.4, 123.5, 121.9, 121.4, 106.9, 104.1, 98.0, 71.0, 70.9, 44.3, 31.5, 13.0. MS (ESI): m/z 356.4 $[M+H]^+$. Anal. calcd for $C_{19}H_{21}N_3O_4$: C 64.21, H 5.96, N 11.82, O 18.21; found: C 64.16, H 5.99, N 11.84, O 18.26.

(*E*)-N'-(2-hydroxy-5-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F24): yellow solid; yield: 83%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp: 214.4- 215.2 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 12.18 (s, 1H), 8.73 (s, 1H), 8.60 (s, 1H), 8.19 (d, J = 8.6 Hz, 1H), 7.70 – 7.49 (m, 2H), 7.12 (t, J = 8.0 Hz, 2H), 4.33 – 4.11 (m, 4H), 2.30 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.1, 162.4, 154.6, 150.9, 144.6, 140.4, 127.8, 126.9, 124.3, 123.8, 122.0, 121.6, 120.4, 117.6, 71.0, 70.9, 31.4. MS (ESI): m/z 358.1 $[M+H]^+$. Anal. calcd for $C_{17}H_{15}N_3O_6$: C 57.14, H 4.23, N 11.76, O 26.87; found: C 57.19, H 4.28, N 11.81, O 26.85

(*E*)-N'-(3,5-dichloro-2-hydroxybenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F25): beige solid; yield: 92%. R_f = 0.4 (EtOAc/ hexane, 1:1); mp: 180.4- 181.2 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H), 12.38 (s, 1H), 8.53 (s, 1H), 7.65 (s, 1H), 7.62 – 7.51 (m, 3H), 7.09 (d, J = 8.3 Hz, 1H), 4.21 (dt, J = 11.1, 5.5 Hz, 4H), 2.23 – 2.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.7, 152.7, 150.8, 147.1, 130.6, 128.8, 127.3, 123.8, 123.3, 122.0, 121.9, 121.6, 121.2, 70.9, 70.8, 31.3. MS (ESI): m/z 381.3 $[M+H]^+$. Anal. calcd for $C_{17}H_{14}Cl_2N_2O_4$: C 53.56, H 3.70, Cl 18.60, N 7.35, O 16.79; found: C 53.62, H 3.71, Cl 18.57, N 7.38, O 16.82.

(*E*)-N'-(2,4-dichlorobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F26): white solid; yield : 92%. R_f = 0.6 (EtOAc/ hexane, 1:1); mp: 128.1-128.5 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 8.79 (s, 1H), 8.00 (d,

J = 8.7 Hz, 1H), 7.71 (s, 1H), 7.54 (m, 3H), 7.07 (d, J = 8.3 Hz, 1H), 4.28 – 4.12 (m, 4H), 2.20 – 2.10 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.5, 150.8, 142.8, 142.7, 135.4, 134.2, 131.2, 129.8, 128.4, 128.2, 123.7, 121.9, 121.5, 70.9, 70.8, 31.4. MS (ESI): m/z 365.4 [M+H] $^+$. Anal. calcd for $C_{17}H_{14}Cl_2N_2O_3$: C 55.91, H 3.86, Cl 19.42, N 7.67, O 13.14; found: C 55.86, H 3.89, Cl 19.45, N 7.70, O 13.18.

(*E*)-N'-(2-chloro-6-fluorobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F27): white solid; yield: 96%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 200.6-201.3 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.92 (s, 1H), 8.68 (s, 1H), 7.55 (dd, J = 12.8, 5.2 Hz, 2H), 7.50 – 7.37 (m, 2H), 7.36 – 7.27 (m, 1H), 7.06 (d, J = 8.2 Hz, 1H), 4.25 – 4.14 (m, 4H), 2.18 – 2.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 161.9, 159.3, 154.5, 150.8, 141.3, 132.0, 131.9, 128.3, 126.5, 123.7, 121.9, 116.2, 115.9, 70.9, 70.8, 31.4. MS (ESI): m/z 349.2 [M+H] $^+$. Anal. calcd for $C_{17}H_{14}ClFN_2O_3$: C 58.55, H 4.05, Cl 10.17, F 5.45, N 8.03, O 13.76; found: C 58.52, H 4.07, Cl 10.20, F 5.48, N 8.01, O 13.73.

(*E*)-N'-(4-fluoro-3-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F28): beige solid; yield: 81%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp:193.1–194.0 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.56 – 8.40 (m, 2H), 8.15 (s, 1H), 7.70 (t, J = 9.8 Hz, 1H), 7.57 (d, J = 14.4 Hz, 2H), 7.10 (d, J = 8.0 Hz, 1H), 4.22 (dd, J = 9.0, 4.8 Hz, 4H), 2.23 – 2.12 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.6, 156.9, 154.5, 150.8, 144.5, 137.8, 134.7, 132.4, 128.3, 124.4, 123.8, 121.9, 121.6, 119.8, 71.0, 70.9, 31.4. MS (ESI): m/z 360.1 [M+H] $^+$. Anal. calcd for $C_{17}H_{14}FN_3O_5$: C 56.83, H 3.93, F 5.29, N 11.69, O 22.26; found: C 56.72, H 3.93, F 5.27, N 11.73, O 22.33.

(*E*)-N'-(4-chloro-3-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F29): white solid; yield: 87%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp: 198.3–199.1 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 8.49 (s, 1H), 8.38 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.68 – 7.46 (m, 2H), 7.10 (d, J = 8.3 Hz, 1H), 4.40 – 4.06 (m, 4H), 2.26 – 2.02 (m, 2H). ^{13}C NMR (101 MHz,

DMSO-d_6) δ 162.6, 154.6, 150.8, 148.4, 144.4, 135.6, 132.7, 132.0, 128.2, 126.1, 124.0, 123.8, 122.0, 121.6, 71.0, 70.9, 31.4. MS (ESI): m/z 376.4 $[M+H]^+$. Anal. calcd for $C_{17}H_{14}ClN_3O_5$: C 54.34, H 3.76, Cl 9.43, N 11.18, O 21.29; found: C 54.33, H 3.70, Cl 9.46, N 11.19, O 21.28.

(*E*)-N'-(2-chloro-5-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F30): white solid; yield: 85%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp:182.4–183.3 ºC.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 8.89 (s, 1H), 8.73 (s, 1H), 8.25 (dd, J = 8.9, 2.8 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.70 – 7.48 (m, 2H), 7.11 (d, J = 8.3 Hz, 1H), 4.24 (dt, J = 11.0, 5.6 Hz, 4H), 2.18 (dd, J = 11.1, 5.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.6, 154.7, 150.9, 147.2, 141.9, 139.5, 133.6, 132.1, 128.0, 125.6, 123.8, 122.1, 121.6, 121.6, 71.0, 70.9, 31.4. MS (ESI): m/z 376.4 $[M+H]^+$. Anal. calcd for $C_{17}H_{14}ClN_3O_5$: C 54.34, H 3.76, Cl 9.43, N 11.18, O 21.29; found: C 54.35, H 3.77, Cl 9.44, N 11.22, O 21.31.

(*E*)-N'-(2-fluoro-5-nitrobenzylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F31): white solid; yield : 88%. R_f = 0.5 (EtOAc/ hexane, 1:1); mp: 198.6- 199.1 ºC. 1H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 8.69 (s, 2H), 8.43 – 8.26 (m, 1H), 7.62 (m, 3H), 7.11 (d, J = 8.3 Hz, 1H), 4.35 – 4.13 (m, 4H), 2.25 – 2.10 (m, 2H). 13C NMR (101 MHz, DMSO-d_6) δ 165.3, 162.8, 154.7, 150.9, 144.9, 138.5, 128.0, 127.3, 124.0, 123.8, 122.1, 121.6, 118.5, 118.3, 71.0, 70.9, 31.4. MS (ESI): m/z 360.1 $[M+H]^+$. Anal. calcd for $C_{17}H_{14}FN_3O_5$: C 56.83, H 3.93, F 5.29, N 11.69, O 22.26; found: C 56.88, H 3.95, F 5.32, N 11.73, O 22.24.

(*E*)-N'-(naphthalen-2-ylmethylene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F32): white solid; yield: 91%. R_f = 0.4 (EtOAc/ hexane, 1:1); mp: 144.1- 144.8 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.60 (s, 1H), 8.15 (s, 1H), 8.06 – 7.89 (m, 4H), 7.67 – 7.48 (m, 4H), 7.11 (d, J = 8.2 Hz, 1H), 4.23 (dd, J = 12.4, 6.1 Hz, 4H), 2.29 – 2.05 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.5, 154.4, 150.9, 147.9, 134.2, 133.4, 132.6, 129.1, 129.0, 128.8, 128.7, 128.3, 127.6, 127.2,

123.7, 123.2, 121.9, 121.6, 71.0, 70.9, 31.5. MS (ESI): m/z 347.2 [M+H] $^{+}$. Anal. calcd for $C_{21}H_{18}N_2O_3$: C 72.82, H 5.24, N 8.09, O 13.86; found: C 72.84, H 5.27, N 8.13, O 13.85.

(*E*)-N'-(naphthalen-1-ylmethylene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F33): white solid; yield : 89%. R_f = 0.4 (EtOAc/ hexane, 1:1); mp: 143.5- 143.9 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 9.11 (s, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 6.7 Hz, 2H), 7.94 (d, J = 7.2 Hz, 1H), 7.79 – 7.50 (m, 5H), 7.12 (d, J = 8.2 Hz, 1H), 4.24 (dd, J = 12.0, 5.9 Hz, 4H), 2.26 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.4, 154.4, 150.9, 147.9, 134.0, 131.0, 130.7, 130.1, 129.3, 128.7, 128.2, 127.8, 126.8, 126.1, 124.7, 123.7, 122.0, 121.5, 71.0, 70.9, 31.5. MS (ESI): m/z 347.2 [M+H] $^{+}$. Anal. calcd for $C_{21}H_{18}N_2O_3$: C 72.82, H 5.24, N 8.09, O 13.86; found: C 72.80, H 5.25, N 8.11, O 13.86.

(*E*)-N'-((E)-3-phenylallylidene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F34): white solid; yield: 86%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp: 222.4- 223.2 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.23 (d, J = 5.1 Hz, 1H), 7.64 (d, J = 7.6 Hz, 2H), 7.58 – 7.50 (m, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.37 – 7.30 (m, 1H), 7.12 – 7.01 (m, 3H), 4.29 – 4.14 (m, 4H), 2.23 – 2.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.3, 150.8, 149.9, 139.3, 136.4, 129.3, 128.7, 127.6, 126.2, 123.7, 121.9, 121.5, 71.0, 70.9, 31.5. MS (ESI): m/z 323.2 [M+H] $^{+}$. Anal. calcd for $C_{19}H_{18}N_2O_3$: C 70.79, H 5.63, N 8.69, O 14.89; found: C 70.84, H 5.61, N 8.72, O 14.85.

(*E*)-N'-((5-methylfuran-2-yl)methylene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F35): white solid; yield: 81%. R_f = 0.3 (EtOAc/ hexane, 1:1); mp: 112.8- 113.5 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.17 (s, 1H), 7.47 (d, J = 9.6 Hz, 2H), 7.03 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 2.5 Hz, 1H), 6.23 (d, J = 2.2 Hz, 1H), 4.24 – 4.10 (m, 4H), 2.31 (s, 3H), 2.19 – 2.03 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.6, 155.2, 154.3, 150.8, 148.2, 137.9, 128.4, 123.5, 121.9, 121.4,

116.0, 109.1, 71.0, 70.9, 31.3, 13.8. MS (ESI): m/z 301.1 $[M+H]^+$. Anal. calcd for $C_{16}H_{16}N_2O_4$: C 63.99, H 5.37, N 9.33, O 21.31; found: C 63.92, H 5.39, N 9.37, O 21.29.

(*E*)-N'-(thiophen-2-ylmethylene)-3,4-dihydro-2H-benzo[*b*][1,4]dioxepine-7-carbohydrazide (F36): beige solid; yield: 84%. R_f = 0.4 (EtOAc/ hexane, 1:1); mp: 116.3- 117.7 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.65 (s, 1H), 7.68 (d, J = 4.9 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.46 (d, J = 3.1 Hz, 1H), 7.15 (dd, J = 4.9, 3.7 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 4.22 (dt, J = 8.8, 5.6 Hz, 4H), 2.21 – 2.10 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.3, 154.4, 150.9, 143.1, 139.7, 131.3, 129.3, 128.6, 128.3, 123.6, 121.9, 121.5, 71.0, 70.9, 31.5. MS (ESI): m/z 303.2 $[M+H]^+$. Anal. calcd for $C_{15}H_{14}N_2O_4S$: C 59.59, H 4.67, N 9.27, O 15.88, S 10.61; found: C 59.63, H 4.66, N 9.31, O 15.83, S 10.60.

Biology

Bacterial suppressive assay: The antibacterial activity of the synthesized compounds was tested against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 6538 and *B. subtilis* ATCC 530 (kindly provided by pro Chang-Hong Liu, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing) using Mueller-Hinton medium (MH medium: 17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). The minimum inhibitory concentration (MIC) values of the tested compounds were determined by a colorimetric method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). A stock solution of the test compound (100 μg/mL) in dimethyl sulfoxide (DMSO) was prepared, and graded quantities were added to a specified volume of sterilized liquid MH medium. A specified volume of the compound-containing medium was then poured into microtiter plates. A suspension of the microorganism was prepared to contain approx. 105 cfu/mL and applied to microtiter plates with serially. diluted compounds in DMSO and same amount blank DMSO to be tested and incubated at 37 ºC for 24 h. After the MIC values

were visually determined on each of the microtiter plates, phosphate buffered saline (PBS; 50 μL, 0.01M, pH 7.4; 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4, 8.0 g NaCl, 0.2 g KCl, 1000 mL distilled H_2O) containing MTT (2 mg/mL) was added to each well. Incubation was continued at RT for 4–5 h. The content of each well was removed, and 100 mL of isopropanol containing 5% HCl (final concentration 1M) was added to extract the dye. After 12 h of incubation at RT, the optical density (OD) was measured with a microplate reader at 550 nm.

E. coli FabH inhibitory assay: Native *E. coli* FabH protein was overexpressed in *E. coli* DH10B cells using the pET30 vector (pET30 vector was kindly supplied by the State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University) and purified to homogeneity in three chromatographic steps (Q-Sepharose, MonoQ, and hydroxyapatite) at 4°C. The selenomethionine-substituted protein was expressed in *E. coli* BL21(DE3) cells and purified in a similar way. Harvested cells containing FabH were lysed by sonication in 20 mM Tris (pH 7.6) containing 5 mM imidazole and 0.5M NaCl, and centrifuged (20000 rpm, 30 min, 4°C). The supernatant was applied to a Ni-NTA agarose column, washed, and eluted using a 5–500 mm imidazole gradient over 20 column volumes. Eluted protein was dialyzed against 20 mM Tris (pH 7.6) containing 1 mM dithiothreitol (DTT) and 100 mM NaCl. Purified FabHs were concentrated to 2 mg/mL and stored at -80°C in 20 mM Tris (pH 7.6) containing 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzymatic assay.

In a final 20 μL reaction, 20 mM Na_2HPO_4 (pH 7.0) containing 0.5 mM DTT, 0.25 mM $MgCl_2$, and 2.5 μM holo-ACP were mixed with 1 nM FabH, and H_2O was added to 15 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA, 0.5 mm NADH, and 0.5 mM NADPH was added for FabH reaction for 25 min. The reaction was stopped by adding 20 μL of ice-cold 50% trichloroacetic acid (TCA), incubating for 5 min on ice, and centrifuging to pellet the protein. The pellet was washed with 10% ice-cold TCA and resuspended in 0.5M NaOH (5 μL). The incorporation of the ^3H signal in the final product was read by liquid scintillation. When determining the IC_{50} values,

inhibitors were added from a concentrated DMSO stock such that the final concentration of DMSO did not exceed 2%.

Cytotoxicity test: The cytotoxic activity in vitro was measured against mouse fibroblast NIH-3T3 cells using the MTT assay. Cells were cultured in a 96-well plate at a density of 5×10^{-5} cells and different concentrations of compounds were respectively added to each well. The incubation was permitted at 37°C, 5% CO_2 atmosphere for 24 h before the cytotoxicity assessments. 20 μL MTT reagent (4 mg/mL) was added per well 4 h before the end of the incubation. Four hours later, the plate was centrifugated at 1200 rcf for 5 min and the supernatants were removed, each well was added with 200 μL DMSO. The absorbance was measured at a wavelength of 570 nm (OD570 nm) on an ELISA microplate reader. Three replicate wells were used for each concentration and each assay was measured three times, after which the average of IC_{50} was calculated. The cytotoxicity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (IC_{50}).

Docking study

Molecular docking of compound **2E** into the three-dimensional Xray structure of *E. coli* FabH (PDB: 1HNJ) [24] was carried out using Discovery Studio (v3.1) as implemented through the graphical user interface DS-CDOCKER protocol.[22]

The 3D structures of the aforementioned compounds were constructed using Chem3D ultra 12.0 (Chemical Structure Drawing Standard; Cambridge Soft corporation, USA) and energy minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. The crystal structures of *E. coli* FabH (PDB: 1HNJ) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/). All bound waters and ligands were eliminated from the protein and the polar hydrogen was added to the proteins.