Supporting Information

Development of tetrahydropyrido[4,3-d]pyrimidine-2,4-diones as novel anti-cancer agents

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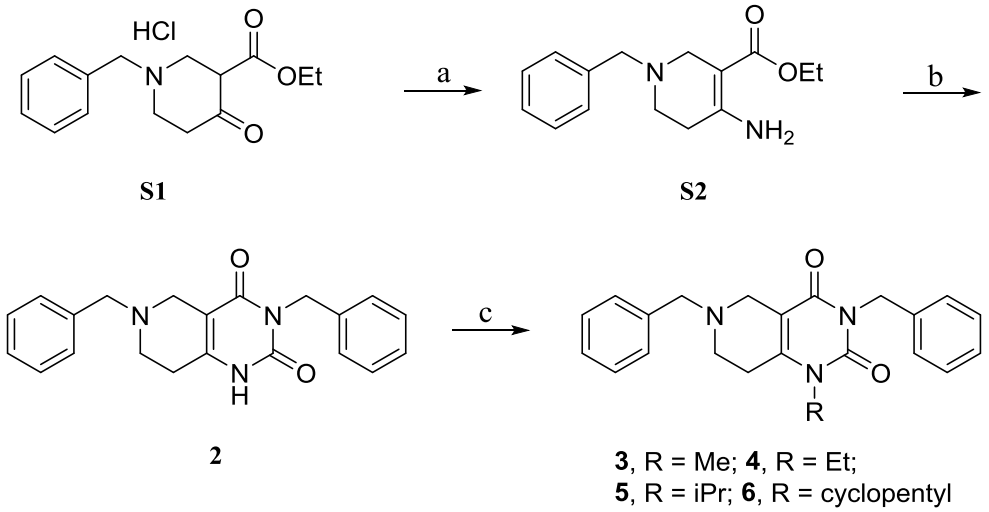
The synthesis of compound **2-6** is shown in **Scheme S1**. Briefly, condensation of commercially available compound **S1** with ammonium acetate in ethanol gave intermediate **S2**.[1] Addition of the amino group in **S2** with benzyl isocyanate followed by cyclization of the resulted urea with the ester group provided compound **2**.[1,2] Introduction of various alkyl group to N_1 in **2** by substitution with corresponding halide furnished the designed compounds **3-6**, respectively.

Scheme S1. Synthesis of compounds **2-6**.

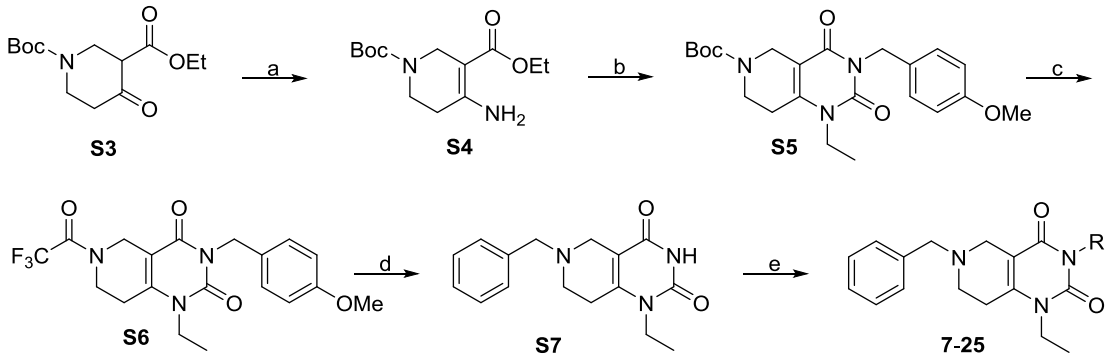


Reagents and conditions: (a) NH_4OAc, EtOH, room temperature, 1 h, 99%; (b) (i) $PhCH_2NCO$, DIPEA, toluene, 60 ℃, 20 h; (ii) NaOMe, MeOH, room temperature, 1 h 90% over two steps; (c) For **3**: DMF, NaH, CH_3I, 60%; For **4-6**: halides, K_2CO_3, MeCN, reflux, 6-12 h, 40%-50%.

The synthesis of compounds **7-25** is shown in **Scheme S2**. Condensation of commercially available compound **S3** with ammonium acetate in ethanol gave intermediate **S4**. Addition of the amino group in **S4** with 4-methyoxybenzyl isocyanate followed by cyclization of the resulted urea with the ester group provided a pyrimidine-2,4-dione. Introduction of an ethyl group to N_1 in this pyrimidine-2,4-dione furnished

intermediate **S5**. Removal of the Boc protecting group in **S5** followed by the protection of the resulted amine with trifluoroacetyl group yielded compound **S6**. After removal of the 4-methoxybenzyl group in **S6** by treatment with $AlCl_3$ and the subsequent cleavage of the trifluoroacetyl group with K_2CO_3 in methanol, a benzyl group was selectively introduced to the resulted amino group to give key intermediate **S7**. Various substituted groups were introduced to N_3 in **S7** by substitution with corresponding halide furnished the designed compounds **7**-**25**, respectively.

Scheme S2. Synthesis of compounds **7**-**25**.

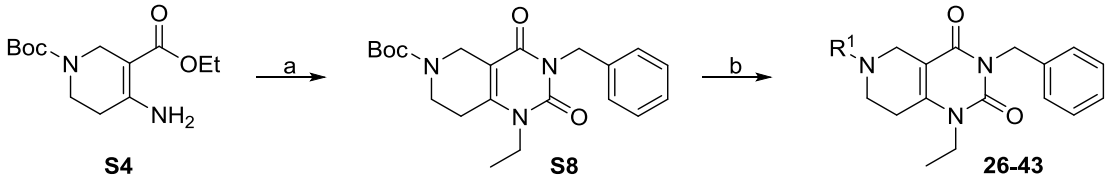


Reagents and conditions: (a) NH_4OAc, EtOH, room temperature, 1 h, 98%; (b) i. PMBNCO, toluene, 80 ℃, 24 h; ii. NaOMe, MeOH, room temperature, 1 h; iii. bromoethane, K_2CO_3, MeCN, reflux, 4 h, 25% over three steps; (c) i. TFA, CH_2Cl_2, room temperature, 2 h; ii. TFAA, DIPEA, CH_2Cl_2, 24 h, 68%, two steps; (d) i. $AlCl_3$, CH_2Cl_2, room temperature, 1 h; ii. K_2CO_3, MeOH, room temperature, 3 h; iii. benzyl bromide, DIPEA, DMF, room temperature, 1 h, 31% over three steps; (e) halides, K_2CO_3, DMF, room temperature, 12 h, 40-50%.

The synthesis of compounds **26**-**43** is outlined in **Scheme S3**. Addition of the amino group in **S4** with benzyl isocyanate followed by cyclization of the resulted urea with the ester group yielded a pyrimidine-2,4-dione. Introduction of an ethyl group to N_1 in

this pyrimidine-2,4-dione furnished intermediate **S8**. After removal of the Boc protecting group in **S8**, various substituted groups were introduced to the resulted amino group by substitution with different halide to provide the designed compounds **26-43**.

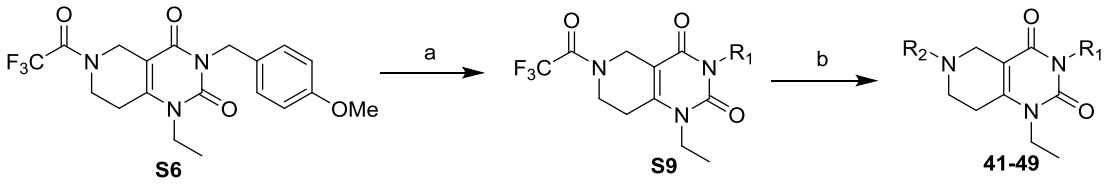
Scheme S3. Synthesis of compounds **26-43**.



S4 S8 26-43

Reagents and conditions: (a) i. $PhCH_2NCO$, toluene, 80 ℃, 24 h; ii. NaOMe, MeOH, room temperature, 1 h, 80% over two steps; iii. bromoethane, K_2CO_3, MeCN, reflux, 4 h, 60%. (b) i. TFA, CH_2Cl_2, room temperature, 2 h, 75%; ii. halides, DIPEA, DMF, room temperature, 12-24 h, 40-50%.

The synthesis of compounds **41-49** is described in **Scheme S4**. Removal of the 4-methoxybenzyl group in **S6** by treatment with $AlCl_3$ followed by introduction of different substituted benzyl groups to the resulted imide group provided intermediates **S9**. Cleavage of the trifluoroacetyl group in **S9** by treatment with K_2CO_3 in methanol and the following substitution of the resulted amines with various *meta* substituted benzyl bromide gave the target compounds **41-49**.



S6 S9 41-49

Reagents and conditions: (a) i. $AlCl_3$, CH_2Cl_2, room temperature, 1 h, 85%; ii. K_2CO_3, substituted benzyl bromide, DMF, room temperature, 2 h, 60-70%; (b) i. K_2CO_3, MeOH, room temperature, 3 h, 90%; ii. Substituted benzyl bromide, DIPEA, DMF, room

temperature, 1 h, 40-60%.

Experimental Section

Chemistry.

General Methods. ^{1}H NMR spectra were measured at 300 MHz using tetramethylsilane as the internal standard. The purity of the final products was checked by analytical HPLC using the C_{18} reverse phase column.

3,6-dibenzyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (2). 25.9 g (336 mmol) of ammonium acetate was added to a solution of ethyl 1-benzyl-4-oxo-piperidine-3-carboxylate hydrochloride **S1** (10 g, 33.6 mmol) in ethanol (100 mL). The mixture was stirred at room temperature until complete consumption of the starting material. After removal of the solvent in vacuo, the residue was neutralized with 1 *N* NaOH and extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated to give ethyl-4-amino-1-benzyl-1,2,5,6-tetrahydropyridine-3-carboxylate **S2** as a beige oil (8.6 g, 99%) which was used in the following reaction without purification. A solution of **S2** (5 g, 19.2 mmol), benzyl isocyanate (2.9 mL, 21.1 mmol), and diisopropylethylamine (0.7 mL, 3.84 mmol) in toluene (50 mL) was warmed to 60℃ and stirred at this temperature for 18 h. The solvent was evaporated and the residue was dissolved in 50 mL of methanol. After carefully adding 3.1 g (57.6 mmol) of sodium methoxide, the solution was stirred at room temperature for 1 h and then concentrated. The residue was dissolved in 150 mL of water and the solution was acidified to pH value is 4 with 1 N aqueous HCl. A pale yellow solid was precipitated during

neutralization. The solid was collected by filtration and then purified by crystallization in ethyl acetate to give pure product (6.0 g, 90% from **S2**). The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.15 (s, 1H), 7.48–7.09 (m, 10H), 5.06 (s, 2H), 3.68 (s, 2H), 3.32 (s, 2H), 2.65 (t, J = 5.3 Hz, 2H), 2.42 (t, J = 5.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.15, 150.39, 145.78, 137.00, 131.31, 129.50, 128.82, 128.34, 127.67, 127.26, 98.98, 57.98, 46.85, 45.29, 42.74, 22.92. ESI-MS *m/z* calc'd for $C_{21}H_{22}N_3O_2$ $[M+H]^+$ 348.17, found 348.42.

3,6-dibenzyl-1-methyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H) -dione (3). 35 mg of sodium hydride (60%, 0.86 mmol) was added to a solution of **2** (200 mg, 0.58 mmol) in dimethylformamide (2.0 mL) at 0 ℃. After stirring for 5 min, 54 μL of methyl iodide (0.86 mmol) was added and the resulting solution was stirred at room temperature for 0.5 h. The solution was poured into ice-water and extracted with dichloromethane for three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography to yield compound **3** as a yellow solid (125 mg, 60%). The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.21 (m, 10H), 5.14 (s, 2H), 3.70 (s, 2H), 3.40 (s, 2H), 3.35 (s, 3H), 2.72 (t, J = 5.5 Hz, 2H), 2.62 (t, J = 5.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.26, 151.70, 146.99, 137.48, 136.97, 129.03, 128.86, 128.39, 128.28, 127.44, 127.36, 107.36, 62.09, 49.53, 48.65, 44.42, 30.59, 27.35. ESI-MS *m/z* calc'd for $C_{22}H_{24}N_3O_2$ $[M+H]^+$ 362.18, found 362.45.

General procedure for preparation of compounds (4-6).

To a suspension of **2** (200 mg, 0.58 mmol) and anhydrous potassium carbonate (120 mg, 0.86 mmol) in 5.0 mL of acetonitrile, was added different halides (1.16 mmol) at room temperature. After addition, the mixture was refluxed for 6-12 h until the reaction was completed. The reaction mixture was cooled to room temperature, filtered through celite, and then concentrated. The residue was purified by column chromatography to give **4-6** respectively.

3,6-dibenzyl-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (4). Yellow solid, yield 51%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.19 (m, 10H), 5.14 (s, 2H), 3.87 (q, *J* = 7.0 Hz, 2H), 3.69 (s, 2H), 3.39 (s, 2H), 2.68 (m, 4H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.24, 151.09, 146.30, 137.35, 136.92, 128.91, 128.72, 128.24, 128.14, 127.26, 127.21, 107.20, 61.99, 49.50, 48.57, 44.14, 39.08, 26.47, 13.95. ESI-MS *m/z* calc'd for $C_{23}H_{26}N_3O_2$ $[M+H]^+$ 376.19, found 376.47.

3,6-dibenzyl-1-isopropyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (5). Yellow solid, yield 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.17 (m, 10H), 5.31 (m, 1H), 5.15 (s, 2H), 3.73 (s, 2H), 3.47 (s, 2H), 2.71 (t, *J* = 5.5 Hz, 2H), 2.63 (t, *J* = 5.5 Hz, 2H), 1.31 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.81, 157.32, 153.55, 138.07, 136.81, 129.17, 128.59, 128.35, 128.32, 127.52, 127.20, 112.33, 72.09, 62.61, 50.18, 49.41, 44.15, 31.96, 21.66. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.21, found 390.50.

3,6-dibenzyl-1-cyclopentyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-

2,4(1H,3H)-dione (6). Yellow solid, yield 41%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.08 (m, 10H), 5.42 (m, 1H), 5.10 (s, 2H), 3.68 (s, 2H), 3.40 (s, 2H), 2.75–2.48 (m, 4H), 1.71 (m, 4H), 1.59 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.92, 157.45, 153.84, 138.11, 136.82, 129.29, 128.46, 128.45, 128.30, 127.59, 127.30, 112.33, 81.55, 62.73, 50.30, 49.50, 44.34, 32.67, 32.09, 23.65. ESI-MS *m/z* calc'd for $C_{26}H_{30}N_3O_2$ $[M+H]^+$ 416.23, found 416.54.

1-(tert-butyl) 3-ethyl 4-amino-5,6-dihydropyridine-1,3(2H)-dicarboxylate (S4). To a solution of **S3** (17.5 g, 64.5 mmol) in 200 mL of ethanol was added ammonium acetate (49.7 g, 645 mmol). The mixture was stirred at room temperature until completive consumption of starting material. After removal of the solvent, the residue was neutralized with 1 *N* NaOH and extracted with dichloromethane for three times. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The solvent was evaporated to give **S4** as a beige oil (17.1 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (q, *J* = 7.1 Hz, 2H), 4.07 (s, 2H), 3.51 (t, *J* = 5.9 Hz, 2H), 2.29 (t, *J* = 5.9 Hz, 2H), 1.47 (s, 9H), 1.27 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z* calc'd for $C_{13}H_{23}N_2O_4$ $[M+H]^+$ 271.16, found 271.33.

tert-butyl 1-ethyl-3-(4-methoxybenzyl)-2,4-dioxo-1,3,4,5,7,8-hexahydropyrido[4,3-d]pyrimidine-6(2H)-carboxylate (S5). To a solution of **S4** (10.0 g, 37.0 mmol) in 100 mL of dry toluene was added 4-methoxybenzyl isocyanate (18.1 g, 111 mmol). The resulting mixture was stirred at 80 ℃ for 24 h and then concentrated. The residue was dissolved in 100 mL of methanol. After careful addition

of sodium methoxide (6.0 g, 111 mmol), the solution was stirred at room temperature for 1 h and then concentrated. The residue was partitioned between dichloromethane (200 mL) and 0.5 M citric acid (200 mL). The organic phase was separated and the water phase was extracted by dichloromethane for two times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography to provide a light yellow solid. To a solution of this solid in 100 mL of acetonitrile was added anhydrous potassium carbonate (5.1 g, 37.2 mmol) and bromoethane (4.2 mL, 55.8 mmol). The resulted mixture was refluxed for 4 h, filtered through celite and then concentrated. The residue was purified by column chromatography on silica gel to give **S5** as yellow oil (3.9 g, 25% over three steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.07 (s, 2H), 4.24 (s, 2H), 3.88 (q, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 3.65 (t, *J* = 5.8 Hz, 2H), 2.59 (t, *J* = 5.8 Hz, 2H), 1.47 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z* calc'd for $C_{22}H_{30}N_3O_5$ $[M+H]^+$ 416.21, found 416.49.

1-ethyl-3-(4-methoxybenzyl)-6-(2,2,2-trifluoroacetyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (S6).

10 mL of trifluoroacetic acid was added to a solution of **S5** (3.9 g, 9.4 mmol) in 10 mL of dichloromethane. The solution was stirred at room temperature for 2 hours and then concentrated. After the residue was partitioned between 50 mL of dichloromethane and 50 mL of 1 M NaOH, the organic layer was separated and the water layer was extracted with dichloromethane for two times. The combined organic layers were washed with

brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give an amine as a yellow oil. This amine was dissolved in 20 mL of dichloromethane. After adding 1.4 mL of diisopropylethylamine (8.4 mmol) to this solution, 1.5 mL of trifluoroacetic anhydride (10.5 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 24 h and concentrated. The residue was purified by column chromatography to yield **S6** as a yellow solid (2.6 g, 68% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (dd, *J* = 9.0, 3.0 Hz, 2H), 6.83 (dd, *J* = 9.0, 3.0 Hz, 2H), 5.07 (s, 2H), 4.47 (m, 2H), 3.95 – 3.80 (m, 4H), 3.78 (s, 3H), 2.77 – 2.65 (m, 2H), 1.26 (t, J = 6.0 Hz, 3H). ESI-MS *m/z* calc'd for $C_{19}H_{21}F_3N_3O_4$ $[M+H]^+$ 412.14, found 412.38.

6-benzyl-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione(S7). To a solution of **S6** (2.6 g, 6.3 mmol) in 30 mL of dichloromethane was added anhydrous aluminum chloride (2.5 g, 19 mmol). The mixture was stirred at room temperature for 1 h and then partitioned between 50 mL of dichloromethane and 50 mL of 1 N hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane for two times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to provide a white solid (1.6 g, 85%). To a solution of this solid 20 mL of methanol was added 2.3 g of K_2CO_3 (16.5 mmol). The resulted mixture was stirred at room temperature for 3 h and then filtered to remove the solid. The solvent was evaporated and the residue was purified by column chromatography to yield a yellow solid (0.96 g, 90%). To a solution of this solid in 10 mL of dimethylformamide was added 1.2 mL of diisopropylethylamine (7.4 mmol) and 0.7

mL of benzyl bromide (0.7 mL, 5.9 mmol). The resulted solution was stirred at room temperature for 1 h and then partitioned between 20 mL of water and 20 mL of dichloromethane. The organic layer was separated and the aqueous layer was extracted by dichloromethane for two times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to afforded **S7** (0.6 g, 31% over three steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.40 – 7.24 (m, 5H), 3.86 (q, *J* = 7.1 Hz, 2H), 3.70 (s, 2H), 3.39 (s, 2H), 2.78 – 2.68 (m, 2H), 2.72 – 2.60 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z* calc'd for $C_{16}H_{20}N_3O_2$ $[M+H]^+$ 286.15, found 286.35.

General procedure for the synthesis of compounds (7-25). To a solution of 100 mg of **S7** (0.35 mmol) in 2 mL of dimethylformamide was added 70 mg of K_2CO_3 (0.53 mmol) and corresponding halide (0.4 mmol). The mixture was stirred at room temperature for 12 h and then partitioned between 10 mL of water and 10 mL of dichloromethane. The organic layer was separated and the aqueous layer was extracted with dichloromethane for two times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and then concentrated. The residue was purified by column chromatography to afford the desired compound.

6-benzyl-1-ethyl-3-methyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (7). Light yellow solid, yield 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H), 3.88 (q, *J* = 7.0 Hz, 2H), 3.69 (s, 2H), 3.38 (s, 2H), 3.34 (s, 3H) 2.75 – 2.56 (m, 4H), 1.26 (t, *J* = 7.0 Hz, 3H). ESI-MS *m/z* calc'd for $C_{17}H_{22}N_3O_2$ $[M+H]^+$ 300.16,

found 300.21. HRMS (ESI-TOF) *m/z* calc'd for $C_{17}H_{22}N_3O_2$ $[M+H]^+$ 300.1712, found 300.1733.

6-benzyl-1,3-diethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (8). Light yellow solid, yield 47%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.87 (q, *J* = 7.1 Hz, 2H), 3.68 (s, 2H), 3.37 (s, 2H), 2.74 – 2.57 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.84, 150.58, 145.49, 137.08, 128.58, 127.93, 126.88, 107.02, 61.70, 49.27, 48.23, 38.61, 35.81, 26.22, 13.67, 12.35. ESI-MS *m/z* calc'd for $C_{18}H_{24}N_3O_2$ $[M+H]^+$ 314.19, found 314.30. HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{24}N_3O_2$ $[M+H]^+$ 314.1869, found 314.1881.

6-benzyl-3-(cyclopropylmethyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (9). Light yellow solid, yield 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 5H), 3.92 – 3.79 (m, 4H), 3.69 (s, 2H), 3.38 (s, 2H), 2.75 – 2.58 (m, 4H), 1.26 (t, *J* = 7.0 Hz, 4H), 0.48 – 0.35 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.21, 151.02, 145.54, 137.08, 128.60, 127.93, 126.89, 107.05, 61.73, 49.34, 48.24, 45.05, 38.67, 26.27, 13.66, 9.30, 3.28. ESI-MS *m/z* calc'd for $C_{20}H_{26}N_3O_2$ $[M+H]^+$ 340.20, found 340.41. HRMS (ESI-TOF) *m/z* calc'd for $C_{20}H_{26}N_3O_2$ $[M+H]^+$ 340.2025, found 340.2047.

6-benzyl-3-(cyclobutylmethyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (10). Light yellow solid, yield 44%. The purity of the

compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.24 (m, 5H), 4.01 (d, *J* = 7.2 Hz, 2H), 3.85 (q, *J* = 6.9 Hz, 2H), 3.68 (s, 2H), 3.37 (s, 2H), 2.69 (m, 2H), 2.68 – 2.58 (m, 2H), 1.96 (m, 2H), 1.87 – 1.72 (m, 5H), 1.24 (t, *J* = 6.9 Hz, 3H). ESI-MS *m/z* calc'd for $C_{21}H_{28}N_3O_2$ $[M+H]^+$ 354.21, found 354.36. HRMS (ESI-TOF) *m/z* calc'd for $C_{21}H_{28}N_3O_2$ $[M+H]^+$ 354.2182, found 354.2201.

6-benzyl-3-(cyclohexylmethyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (11). Light yellow solid, yield: 43%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 5H), 3.90 – 3.83 (m, 2H), 3.83 – 3.75 (m, 2H), 3.68 (s, 2H), 3.37 (s, 2H), 2.74 – 2.57 (m, 4H), 1.85 – 1.66 (m, 3H), 1.64-1.60 (m, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.18-1.14 (m, 3H), 1.09 – 0.95 (m, 2H). ESI-MS *m/z* calc'd for $C_{23}H_{32}N_3O_2$ $[M+H]^+$ 382.25, found 382.39. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{32}N_3O_2$ $[M+H]^+$ 382.2495, found 382.2507.

6-benzyl-1-ethyl-3-(2-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (12). Light yellow solid, yield 46%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.35 (m, 2H), 7.37 – 7.33 (m, 3H), 7.28 – 7.17 (m, 2H), 7.12 – 6.98 (m, 2H), 5.24 (m, 2H), 3.91 (q, *J* = 7.6 Hz, 2H), 3.71 (s, 2H), 3.41 (s, 2H), 2.76 – 2.64 (m, 4H), 1.29 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.41, 160.74, 151.22, 146.52, 137.45, 129.30, 129.14, 129.98, 128.52, 128.46, 127.44, 124.00, 115.40, 107.53, 62.26, 49.74, 48.74, 39.89, 39.33, 26.82, 14.15. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found

394.33. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found 394.1940.

6-benzyl-1-ethyl-3-(3-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (13). Light yellow solid, yield 47%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.44 (m, 1H), 7.37 – 7.35 (m, 2H), 7.34 – 7.31 (m, 3H), 7.24 (m, 1H), 6.97 – 6.88 (m, 2H), 5.15-5.09 (m, 2H), 3.94 – 3.83 (m, 2H), 3.68 (s, 2H), 3.37 (m, 2H), 2.74 – 2.60 (m, 4H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.44, 161.37, 151.29, 146.52, 139.41, 137.48, 129.86, 129.11, 128.46, 127.43, 124.56, 115.76, 114.42, 107.61, 62.22, 49.71, 48.71, 44.08, 39.36, 26.81, 14.15. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found 394.31. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found 394.1949.

6-benzyl-1-ethyl-3-(4-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (14). Light yellow solid, yield: 42%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.42 (m, 3H), 7.36 (m, 1H), 7.33 – 7.30 (m, 3H), 6.99 – 6.91 (m, 2H), 5.08 (m, 2H), 3.86 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 2H), 3.37 (m, 2H), 2.69 (m, 2H), 2.64 (m, 2H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.24, 161.42, 151.31, 146.40, 137.48, 131.23, 131.06, 129.10, 128.46, 127.43, 115.12, 107.62, 62.23, 49.71, 48.73, 43.65, 39.32, 26.79, 14.15. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found 394.33. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found 394.1953.

6-benzyl-3-(2-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (15). Light yellow solid, yield: 43%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.32 (m, 5H), 7.30 – 7.27 (m, 1H), 7.16 – 7.13 (m, 2H), 7.01 – 6.94 (m, 1H), 5.26 (s, 2H), 3.89 (q, *J* = 7.1 Hz, 2H), 3.69 (s, 2H), 3.40 (s, 2H), 2.75 – 2.66 (m, 4H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.41, 151.25, 146.68, 137.42, 134.16, 133.07, 129.54, 129.17, 128.48, 128.17, 127.47, 127.09, 126.78, 107.54, 62.29, 49.77, 48.74, 42.13, 39.37, 26.87, 14.18. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.37. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1653.

6-benzyl-3-(3-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (16). Light yellow solid, yield: 41%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 – 7.41 (m, 1H), 7.40 – 7.37 (m, 1H), 7.36 – 7.30 (m, 5H), 7.23 – 7.17 (m, 2H), 5.07 (s, 2H), 3.86 (q, *J* = 7.1 Hz, 2H), 3.68 (s, 2H), 3.37 (s, 2H), 2.79 – 2.55 (m, 4H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.40, 151.27, 146.60, 137.45, 134.70, 133.20, 130.24, 129.15, 128.48 128.28, 128.26, 128.20, 126.90, 107.60, 62.26, 49.70, 48.74, 42.60, 39.36, 26.85, 14.16. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.29. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1641.

6-benzyl-3-(4-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (17). Light yellow solid, yield: 45%. The purity of the compound

was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.40 (m, 1H), 7.40 – 7.38 (m, 1H), 7.37 – 7.35 (m, 1H), 7.32 (m, 3H), 7.28 (m, 1H), 7.26 – 7.22 (m, 2H), 5.07 (s, 2H), 3.86 (q, *J* = 8.0 Hz, 2H), 3.67 (s, 2H), 3.36 (s, 2H), 2.69 (m, 2H), 2.67 – 2.59 (m, 2H), 1.25 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.39, 151.28, 146.47, 137.47, 135.54, 133.38, 130.61, 129.10, 128.48, 128.46, 127.44, 107.62, 62.23, 49.69, 48.73, 43.73, 39.34, 26.80, 14.14. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.30. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1651.

6-benzyl-3-(2-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (18). Light yellow solid, yield: 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (m, 1H), 7.38 – 7.27 (m, 5H), 7.19 (m, 1H), 7.06 (m, 1H), 6.92 (m, 1H), 5.23 (s, 2H), 3.89 (q, *J* = 7.1 Hz, 2H), 3.69 (s, 2H), 3.40 (s, 2H), 2.77 – 2.65 (m, 4H), 1.27 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.38, 151.24, 146.71, 137.41, 135.67, 132.83, 129.17, 128.48, 128.42, 127.47, 127.43, 126.81, 122.93, 107.55, 62.29, 49.77, 48.74, 44.69, 39.38, 26.88, 14.19. ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.23. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1147.

6-benzyl-3-(3-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (19). Light yellow solid, yield: 50%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (m, 1H), 7.40 – 7.35 (m, 2H), 7.34 – 7.31 (m, 4H), 7.28 (m, 1H), 7.15 (m, 1H), 5.07 (s,

2H), 3.87 (q, *J* = 7.1 Hz, 2H), 3.68 (s, 2H), 3.38 (s, 2H), 2.74 – 2.66 (m, 2H), 2.68 – 2.60 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.34, 151.27, 146.54, 139.25, 137.49, 131.84, 130.67, 129.88, 129.11, 128.46, 127.74, 127.44, 122.39, 107.61, 62.22, 49.72, 48.70, 43.83, 39.37, 26.81, 14.15. ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.30. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1151.

6-benzyl-3-(4-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (20). Light yellow solid, yield: 40%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.38 (m, 2H), 7.36 – 7.31 (m, 6H), 7.29 (m, 1H), 5.06 (s, 2H), 3.86 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 2H), 3.36 (s, 2H), 2.69 (m, 2H), 2.66 – 2.60 (m, 2H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.38, 151.27, 146.48, 137.47, 136.05, 131.45, 130.95, 129.10, 128.46, 127.44, 121.56, 107.62, 62.22, 49.69, 48.73, 43.78, 39.34, 26.80, 14.14. ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.29. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1144.

6-benzyl-1-ethyl-3-(3-methoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (21). Light yellow solid, yield: 43%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 7.19 (m, 1H), 7.06 – 6.96 (m, 2H), 6.78 (m, 1H), 5.10 (s, 2H), 3.86 (q, *J* = 6.9 Hz, 2H), 3.78 (s, 3H), 3.67 (s, 2H), 3.37 (s, 2H), 2.74 – 2.52 (m, 4H), 1.24 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.44, 159.57, 151.36, 146.29, 138.58, 137.54, 129.32, 129.10, 128.44, 127.41, 121.24, 114.31, 113.14, 107.61,

62.21, 55.21, 49.77, 48.72, 44.34, 39.30, 26.77, 14.17. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.21, found 406.39. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.2131, found 406.2145.

6-benzyl-1-ethyl-3-(4-methoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (22). Light yellow solid, yield: 46%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (m, 2H), 7.39 – 7.23 (m, 5H), 6.81 (m, 2H), 5.05 (s, 2H), 3.85 (q, *J* = 6.8 Hz, 2H), 3.76 (s, 3H), 3.67 (s, 2H), 3.36 (s, 2H), 2.70 – 2.56 (m, 4H), 1.24 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.49, 159.00, 151.36, 146.18, 137.55, 130.70, 129.40, 129.10, 128.44, 127.40, 113.67, 107.63, 62.23, 55.23, 49.75, 48.76, 43.80, 39.26, 26.76, 14.16. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.21, found 406.37. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.2121, found 406.2129.

6-benzyl-1-ethyl-3-(2-methylbenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (23). Light yellow solid, yield: 44%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H), 7.15 – 7.06 (m, 3H), 7.01 (m, 1H), 5.13 (s, 2H), 3.87 (q, *J* = 6.8 Hz, 2H), 3.68 (s, 2H), 3.39 (s, 2H), 2.75 – 2.58 (m, 4H), 2.44 (s, 3H), 1.25 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.58, 151.44, 146.40, 137.48, 135.84, 134.81, 130.24, 129.15, 128.46, 127.44, 126.97, 126.12, 125.96, 107.57, 62.27, 49.82, 48.73, 41.80, 39.32, 26.83, 19.35, 14.19. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.21, found 390.33. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.2182, found 390.2203.

6-benzyl-1-ethyl-3-(4-methylbenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (24). Light yellow solid, yield: 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.22 (m, 7H), 7.13 – 7.04 (m, 2H), 5.08 (s, 2H), 3.85 (q, *J* = 6.9 Hz, 2H), 3.67 (s, 2H), 3.36 (s, 2H), 2.71 – 2.51 (m, 4H), 2.30 (s, 3H), 1.23 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.48, 151.36, 146.18, 137.54, 137.14, 134.14, 129.11, 129.00, 128.44, 127.40, 107.62, 62.23, 49.76, 48.76, 44.11, 39.26, 26.76, 21.14, 14.16. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.21, found 390.35. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.2182, found 390.2199.

6-benzyl-1-ethyl-3-(4-(trifluoromethyl)benzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (25). Light yellow solid, yield: 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.49 (m, 5H), 7.37 – 7.28 (m, 4H), 5.16 (s, 2H), 3.86 (q, *J* = 6.9 Hz, 2H), 3.68 (s, 2H), 3.37 (s, 2H), 2.74 – 2.58 (m, 4H), 1.25 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.37, 151.28, 146.64, 137.44, 132.46, 130.96, 129.24, 129.17, 129.06, 128.47, 127.45, 125.32, 107.63, 62.22, 49.68, 48.72, 43.96, 39.39, 26.83, 14.13. ESI-MS *m/z* calc'd for $C_{24}H_{25}F_3N_3O_2$ $[M+H]^+$ 444.19, found 444.35. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{25}F_3N_3O_2$ $[M+H]^+$ 444.1899, found 444.1907.

tert-butyl 3-benzyl-1-ethyl-2,4-dioxo-1,3,4,5,7,8-hexahydropyrido[4,3-d]pyrimidine-6(2H)-carboxylate (S8). To 1-(tert-butyl) 3-ethyl 4-amino-5,6-dihydropyridine-1,3(2H)-dicarboxylate **S4** (6.0 g, 22.2 mmol) in dry toluene (60 mL) was added benzyl isocyanate (9.1 mL, 66.6 mmol). The resulting mixture was stirred

at 80 ℃ for 24 h and then concentrated in vacuo. The residue was dissolved in 60 mL of methanol. After carefully adding 3.6 g of sodium methoxide (66.6 mmol), the solution was stirred at room temperature for 1 h and then concentrated. The residue was partitioned between dichloromethane (100 mL) and 0.5 M citric acid (100 mL). The organic phase was separated and the aqueous phase was extracted with dichloromethane for two times. The combined dichloromethane extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography to provide a light yellow solid. To a suspension of this solid in 65 mL of acetonitrile was added bromoethane (4.0 mL, 52.9 mmol) and anhydrous potassium carbonate (4.9 g, 35.3 mmol). The resulted mixture was refluxed for 4 h and then filtered through celite. The filtrate was concentrated and the residue was purified by column chromatography to give **S8** as yellow oil (4.1 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.45 (m, 2H), 7.34 – 7.23 (m, 3H), 5.13 (s, 2H), 4.24 (s, 2H), 3.89 (q, *J* = 7.1 Hz, 2H), 3.66 (t, *J* = 5.8 Hz, 2H), 2.61 (t, *J* = 5.8 Hz, 2H), 1.47 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z* calc'd for $C_{21}H_{28}N_3O_4$ $[M+H]^+$ 386.20, found 386.46.

General procedure for preparation of compounds (26-40). To tert-butyl 3-benzyl-1-ethyl-2,4-dioxo-1,3,4,5,7,8-hexahydropyrido[4,3-d]pyrimidine-6(2H)-carboxylate (4.1 g, 10.6 mmol) in dichloromethane (40 mL) was added trifluoroacetic acid (7.9 mL, 106.4 mmol). The resulting mixture was stirred for 2 hours at room temperature and then concentrated in vacuo. The residue was partitioned between dichloromethane (50 mL) and 1 M NaOH (50 mL), after which the organic layer was separated and the water layer was extracted by dichloromethane for two times. The combined organic layers

were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the free amine 3-benzyl-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione as a yellow oil (2.3 g, 75%). It was used without further purification in the next step.

To 3-benzyl-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (0.20 g, 0.70 mmol) in dimethylformamide (2.0 mL) was added diisopropylethylamine (127 μL, 0.77 mmol), followed by different benzyl halides (0.84 mmol). The resulting solution was stirred at room temperature for 12 h. Upon completion, water was added and the mixture was extracted by dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography to afford the title compound as a light yellow solid in 40-50% yield.

3-benzyl-1-ethyl-6-methyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (26). Light yellow solid, yield: 43%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.40 (m, 2H), 7.34 – 7.19 (m, 3H), 5.14 (s, 2H), 3.87 (q, *J* = 7.0 Hz, 2H), 3.29 (s, 2H), 2.67 (s, 4H), 2.45 (s, 3H), 1.24 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.43, 151.33, 145.89, 137.07, 129.01, 128.35, 127.47, 107.49, 51.38, 51.35, 45.41, 44.37, 39.27, 26.68, 14.14. ESI-MS *m/z* calc'd for $C_{17}H_{22}N_3O_2$ $[M+H]^+$ 300.17, found 300.32. HRMS (ESI-TOF) *m/z* calc'd for $C_{17}H_{22}N_3O_2$ $[M+H]^+$ 300.1712, found 300.1729.

3-benzyl-1,6-diethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (27). Light yellow solid, yield: 45%. The purity of the compound was detected by

analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.43 (m, 2H), 7.34 – 7.24 (m, 3H), 5.15 (s, 2H), 3.89 (q, *J* = 7.1 Hz, 2H), 3.35 (m, 2H), 2.76 – 2.66 (m, 4H), 2.60 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.18 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z* calc'd for $C_{18}H_{24}N_3O_2$ $[M+H]^+$ 314.19, found 314.30. HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{24}N_3O_2$ $[M+H]^+$ 314.1869, found 314.1877.

3-benzyl-6-(cyclopropylmethyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (28). Light yellow solid, yield: 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.50 – 7.39 (m, 2H), 7.35 – 7.15 (m, 3H), 5.14 (s, 2H), 3.87 (q, *J* = 6.0 Hz, 2H), 3.42 (s, 2H), 2.78 (t, *J* = 5.6 Hz, 2H), 2.70 (t, *J* = 5.6 Hz, 2H), 2.42 (m, 2H), 1.24 (t, *J* = 6.0 Hz, 3H), 0.91 (m, 1H), 0.58 (m, 2H), 0.16 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.51, 151.34, 146.17, 137.06, 128.95, 128.35, 127.47, 107.36, 62.90, 49.41, 49.34, 44.41, 39.29, 26.56, 14.14, 8.47, 3.99. ESI-MS *m/z* calc'd for $C_{20}H_{26}N_3O_2$ $[M+H]^+$ 340.20, found 340.39. HRMS (ESI-TOF) *m/z* calc'd for $C_{20}H_{26}N_3O_2$ $[M+H]^+$ 340.2025, found 340.2041.

3-benzyl-1-ethyl-6-phenethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (29). Light yellow solid, yield: 50%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 – 7.46 (m, 2H), 7.38 – 7.29 (m, 5H), 7.31 – 7.20 (m, 3H), 5.18 (s, 2H), 3.91 (q, *J* = 6.0 Hz, 2H), 3.48 (s, 2H), 2.91 (m, 2H), 2.86 – 2.75 (m, 4H), 2.71 (t, *J* = 5.5 Hz, 2H), 1.29 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.56, 151.33, 146.20, 139.87, 137.07, 129.04, 128.69, 128.48, 128.36, 127.50, 126.21, 107.42, 59.75, 49.73, 49.33,

44.41, 39.30, 33.90, 26.72, 14.16. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.22, found 390.37. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$ 390.2182, found 390.2197.

3-benzyl-1-ethyl-6-(2-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (30). Light yellow solid, yield: 42%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (m, 2H), 7.37 (m, 1H), 7.28 – 7.17 (m, 4H), 7.16 – 6.97 (m, 2H), 5.10 (s, 2H), 3.83 (q, *J* = 6.9 Hz, 2H), 3.73 (s, 2H), 3.38 (s, 2H), 2.73 (t, *J* = 5.6 Hz, 2H), 2.64 (t, *J* = 5.6 Hz, 2H), 1.22 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.46, 161.43, 151.35, 146.20, 137.06, 131.51, 129.19, 129.01, 128.35, 127.48, 125.79, 124.09, 115.46, 107.50, 54.61, 49.37, 48.80, 44.40, 39.30, 26.72, 14.15. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found 394.34. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found 394.1953.

3-benzyl-1-ethyl-6-(3-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (31). Light yellow solid, yield: 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 2H), 7.30 (m, 1H), 7.27 – 7.20 (m, 3H), 7.08 (m, 2H), 7.02 – 6.89 (m, 1H), 5.11 (s, 2H), 3.85 (q, *J* = 7.0 Hz, 2H), 3.65 (s, 2H), 3.34 (s, 2H), 2.77 – 2.56 (m, 4H), 1.24 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.03, 161.46, 151.34, 146.24, 140.39, 137.05, 129.89, 129.07, 128.35, 127.50, 124.48, 115.67, 114.33, 107.45, 61.62, 49.69, 48.87, 44.41, 39.33, 26.76, 14.17. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found 394.35. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found

394.1949.

3-benzyl-1-ethyl-6-(4-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (32). Light yellow solid, yield: 47%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 2H), 7.35 – 7.16 (m, 5H), 7.00 (m, 2H), 5.11 (s, 2H), 3.85 (q, *J* = 6.7 Hz, 2H), 3.63 (s, 2H), 3.33 (s, 2H), 2.73 – 2.53 (m, 4H), 1.24 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.20, 161.46, 151.34, 146.24, 137.05, 133.29, 130.58, 129.08, 128.34, 127.50, 115.27, 107.49, 61.43, 49.62, 48.77, 44.40, 39.31, 26.76, 14.16. ESI-MS *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.19, found 394.46. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}FN_3O_2$ $[M+H]^+$ 394.1931, found 394.1942.

3-benzyl-6-(2-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (33). Light yellow solid, yield: 41%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 3H), 7.36 (m, 1H), 7.27 (m, 2H), 7.25 – 7.16 (m, 3H), 5.11 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 2H), 3.42 (s, 2H), 2.76 (t, *J* = 5.6 Hz, 2H), 2.65 (t, *J* = 5.6 Hz, 2H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.47, 151.36, 146.24, 137.07, 135.31, 134.47, 130.81, 129.65, 129.08, 128.56, 128.35, 127.49, 126.80, 107.54, 58.64, 49.64, 49.05, 44.40, 39.32, 26.76, 14.18. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.32. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1647.

3-benzyl-6-(3-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (34). Light yellow solid, yield: 43%. The purity of the compound

was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (m, 3H), 7.38 (m, 1H), 7.34 – 7.29 (m, 3H), 7.26 – 7.23 (m, 2H), 5.15 (s, 2H), 3.89 (q, *J* = 7.0 Hz, 2H), 3.67 (s, 2H), 3.38 (s, 2H), 2.77 – 2.60 (m, 4H), 1.28 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.45, 151.33, 146.22, 139.82, 137.04, 134.40, 129.70, 129.07, 128.61, 128.35, 127.62, 127.50, 127.09, 107.42, 61.61, 49.69, 48.87, 44.41, 39.33, 26.75, 14.17. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.34. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1655.

3-benzyl-6-(4-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (35). Light yellow solid, yield: 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (m, 2H), 7.29 – 7.25 (m, 4H), 7.23 – 7.16 (m, 3H), 5.09 (s, 2H), 3.84 (q, *J* = 7.0 Hz, 2H), 3.61 (s, 2H), 3.32 (s, 2H), 2.73 – 2.46 (m, 4H), 1.23 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.46, 151.33, 146.24, 139.26, 137.03, 133.17, 130.34, 129.07, 128.35, 127.45, 127.27, 107.43, 61.44, 49.65, 48.79, 44.40, 39.33, 26.74, 14.16. ESI-MS *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.16, found 410.29. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}ClN_3O_2$ $[M+H]^+$ 410.1635, found 410.1639.

3-benzyl-6-(2-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (36). Light yellow solid, yield: 47%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.55 (m, 1H), 7.45 (m, 3H), 7.34 – 7.19 (m, 4H), 7.13 (m, 1H), 5.12 (s, 2H), 3.92 – 3.80 (m, 2H), 3.77 (s, 2H), 3.43 (s, 2H), 2.78 (t, *J* = 5.6 Hz, 2H), 2.70 – 2.59 (m, 2H), 1.25 (t, *J*

= 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.47, 151.37, 146.23, 137.08, 137.01, 132.95, 130.83, 129.11, 128.82, 128.34, 127.49, 127.42, 124.81, 107.55, 61.17, 49.60, 49.04, 44.40, 39.32, 26.77, 14.18. ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.37. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1152.

3-benzyl-6-(3-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (37). Light yellow solid, yield: 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 1H), 7.45 (m, 2H), 7.40 (m, 1H), 7.32 – 7.15 (m, 5H), 5.11 (s, 2H), 3.86 (q, *J* = 6.9 Hz, 2H), 3.63 (s, 2H), 3.34 (s, 2H), 2.73 – 2.48 (m, 4H), 1.25 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.46, 151.33, 146.25, 140.11, 139.22, 137.02, 130.56, 130.01, 129.05, 128.62, 128.36, 127.46, 127.29, 107.41, 61.55, 49.69, 48.84, 44.41, 39.34, 26.74, 14.17. ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.31. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1147.

3-benzyl-6-(4-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (38). Light yellow solid, yield: 48%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.50 – 7.38 (m, 4H), 7.32 – 7.26 (m, 2H), 7.23 – 7.16 (m, 3H), 5.11 (s, 2H), 3.84 (q, *J* = 7.1 Hz, 2H), 3.61 (s, 2H), 3.33 (s, 2H), 2.71 – 2.54 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.44, 151.33, 146.21, 137.04, 136.66, 131.57, 130.69, 129.09, 128.65, 128.35, 127.51, 107.43, 61.49, 49.67, 48.80, 44.40, 39.32, 26.74, 14.16.

ESI-MS *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.11, found 454.29. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{25}BrN_3O_2$ $[M+H]^+$ 454.1130, found 454.1152.

3-benzyl-1-ethyl-6-(3-methoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (39). Light yellow solid, yield: 46%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.41 (m, 2H), 7.30 (m, 1H), 7.25 – 7.18 (m, 3H), 6.91 (m, 2H), 6.80 (m, 1H), 5.11 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.80 (d, *J* = 5.7 Hz, 3H), 3.64 (s, 2H), 3.37 (s, 2H), 2.73 – 2.58 (m, 4H), 1.24 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.49, 159.79, 151.36, 146.29, 139.24, 137.09, 129.40, 129.07, 128.34, 127.48, 121.39, 114.54, 112.87, 107.62, 62.14, 55.24, 49.82, 48.70, 44.39, 39.30, 26.77, 14.16. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.21, found 406.45. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.2131, found 406.2149.

3-benzyl-1-ethyl-6-(4-methoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (40). Light yellow solid, yield: 42%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.40 (m, 2H), 7.32 – 7.17 (m, 5H), 6.85 (m, 2H), 5.11 2(s, 2H), 3.84 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 3.60 (s, 2H), 3.33 (s, 2H), 2.71 – 2.56 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.49, 158.99, 151.36, 146.30, 137.09, 130.33, 129.50, 129.05, 128.34, 127.47, 113.84, 107.64, 61.61, 55.29, 49.61, 48.64, 44.39, 39.29, 26.78, 14.15. ESI-MS *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.21, found 406.44. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_3O_3$ $[M+H]^+$ 406.2131, found 406.2152.

General procedure for the preparation of intermediates (S9).

To a solution of **S6** (6.0 g, 14.6 mmol) in 60 mL of dichloromethane was added anhydrous aluminum chloride (5.8 g, 43.8 mmol) at 0 ℃. The mixture was stirred at temperature for 1 h and then partitioned between 100 mL of dichloromethane and 100 mL of 1 N hydrochloric acid. The organic layer was separated and the aqueous layer was extracted with dichloromethane (50 mL×2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to provide a white solid (3.6 g, 85%). To a solution of this solid (3.4 mmol) in 10 mL of dimethylformamide was added 0.71 g of K_2CO_3 (5.2 mmol) and corresponding halide (4.1 mmol). The mixture was stirred at room temperature for 2 h and then partitioned between 50 mL of water and 50 mL of dichloromethane. The organic layer was separated and the aqueous layer was extracted with dichloromethane (30 mL×2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and then concentrated. The residue was purified by column chromatography to afford **S9** (60-70%).

General procedure for the synthesis of compounds (41-49).

To a solution of **S9** (3.0 mmol) in 20 mL of methanol was added 6.0 mmol of K_2CO_3. The resulted mixture was stirred at room temperature for 3 h and then filtered to remove the solid. The solvent was evaporated and the residue was purified by column chromatography to yield a yellow solid. To a solution of this solid (1.0 mmol) in 3 mL of dimethylformamide was added 2 mmol of diisopropylethylamine and 1.2 mmol of substituted benzyl bromide. The resulted solution was stirred at room temperature for

1 h and then partitioned between 20 mL of water and 20 mL of dichloromethane. The organic layer was separated and the aqueous layer was extracted by dichloromethane (20 mL × 2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to afford the desired compounds **41-49** (40-60%).

1-ethyl-6-(3-fluorobenzyl)-3-(4-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (41). Light yellow solid, yield 45%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51-7.42 (m, 2H), 7.28 (m, 1H), 7.13-7.03 (m, 2H), 7.01-6.90 (m, 3H), 5.07 (s, 2H), 3.87 (q, *J* = 7.0 Hz, 2H), 3.66 (s, 2H), 3.35 (s, 2H), 2.74-2.60 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.34, 161.01, 162.22,151.25, 146.29, 140.35, 132.87, 131.04, 129.84, 124.41, 115.60, 115.07, 114.28, 107.44, 61.58, 49.61, 48.83, 43.63, 39.29, 26.73, 14.10. ESI-MS *m/z* calc'd for $C_{23}H_{24}F_2N_3O_2$ $[M+H]^+$ 412.18, found 412.23. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}F_2N_3O_2$ $[M+H]^+$ 412.1837, found 412.1844.

6-(3-chlorobenzyl)-1-ethyl-3-(4-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (42). Light yellow solid, yield 50%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52-7.41 (m, 2H), 7.34 (m, 1H), 7.29-7.16 (m, 3H), 7.01-6.90 (m, 2H), 5.07 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.64 (s, 2H), 3.34 (s, 2H), 2.69-2.59 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.59, 160.59, 151.25, 146.28, 139.81, 134.38, 132.85, 131.04, 129.66, 128.91, 127.58, 127.03, 115.07, 107.40, 61.55, 49.61,

48.83, 43.63, 39.29, 26.72, 14.11. ESI-MS *m/z* calc'd for $C_{23}H_{24}ClFN_3O_2$ $[M+H]^+$ 428.15, found 428.18. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}ClFN_3O_2$ $[M+H]^+$ 428.1541, found 428.1553.

6-(3-bromobenzyl)-1-ethyl-3-(4-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (43). Light yellow solid, yield 56%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53-7.36 (m, 4H), 7.28-7.14 (m, 2H), 7.01-6.90 (m, 2H), 5.07 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.63 (s, 2H), 3.34 (s, 2H), 2.72 – 2.60 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.58, 160.59, 151.24, 146.27, 140.10, 132.85, 131.82, 131.03, 130.52, 129.96, 127.50, 122.63, 115.07, 107.39, 61.50, 49.61, 48.82, 43.63, 39.29, 26.72, 14.11. ESI-MS *m/z* calc'd for $C_{23}H_{24}BrFN_3O_2$ $[M+H]^+$ 472.10, found 472.13. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}BrFN_3O_2$ $[M+H]^+$ 472.1036, found 472.1045.

3-(4-chlorobenzyl)-1-ethyl-6-(3-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (44). Light yellow solid, yield 54%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.36 (m, 2H), 7.32-7.21 (m, 3H), 7.14-7.03 (m, 2H), 6.95 (m, 1H), 5.07 (s, 2H), 3.86 (q, *J* = 6.9 Hz, 2H), 3.66 (s, 2H), 3.35 (s, 2H), 2.76 – 2.59 (m, 4H), 1.25 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.51, 161.31, 151.23, 146.35, 140.34, 135.52, 133.36, 130.59, 129.85, 128.44, 124.42, 115.61, 114.29, 107.44, 61.58, 49.60, 48.82, 43.70, 39.31, 26.74, 14.10. ESI-MS *m/z* calc'd for $C_{23}H_{24}ClFN_3O_2$ $[M+H]^+$ 428.15, found 428.14. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}ClFN_3O_2$

$[M+H]^+$ 428.1541, found 428.1549.

6-(3-chlorobenzyl)-3-(4-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (45). Light yellow solid, yield 46%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.37 (m, 2H), 7.34 (m, 1H), 7.29-7.15 (m, 5H), 5.07 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.64 (s, 2H), 3.34 (s, 2H), 2.73-2.60 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.82, 150.74, 145.87, 139.32, 135.04, 133.90, 132.87, 130.11, 129.18, 128.42, 127.96, 127.11, 126.54, 106.92, 61.07, 49.12, 48.34, 43.22, 38.83, 26.25, 13.63. ESI-MS *m/z* calc'd for $C_{23}H_{24}Cl_2N_3O_2$ $[M+H]^+$ 444.12, found 444.19. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}Cl_2N_3O_2$ $[M+H]^+$ 444.1246, found 444.1252.

6-(3-bromobenzyl)-3-(4-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (46). Light yellow solid, yield 52%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 1H), 7.45-7.36 (m, 3H), 7.28-7.14 (m, 4H), 5.07 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.63 (s, 2H), 3.34 (s, 2H), 2.74-2.60 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.82, 150.74, 145.87, 139.61, 135.03, 132.87, 131.34, 130.10, 130.05, 129.48, 127.96, 127.02, 122.15, 106.91, 61.02, 49.12, 48.33, 43.22, 38.83, 26.25, 13.63. ESI-MS *m/z* calc'd for $C_{23}H_{24}BrClN_3O_2$ $[M+H]^+$ 488.07, found 488.10. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}BrClN_3O_2$ $[M+H]^+$ 488.0740, found 488.0746.

3-(4-bromobenzyl)-1-ethyl-6-(3-fluorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-

d]pyrimidine-2,4(1H,3H)-dione (47). Light yellow solid, yield 47%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.31 (m, 4H), 7.25 (m, 1H), 7.15-7.02 (m, 2H), 6.95 (m, 1H), 5.05 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.66 (s, 2H), 3.35 (s, 2H), 2.77-2.59 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.53, 160.82, 150.73, 145.89, 139.86, 135.55, 130.93, 130.45, 129.37, 123.94, 121.04, 115.13, 113.81, 106.95, 61.09, 49.11, 48.34, 43.27, 38.83, 26.26, 13.62. ESI-MS *m/z* calc'd for $C_{23}H_{24}BrFN_3O_2$ $[M+H]^+$ 472.10, found 472.15. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}BrFN_3O_2$ $[M+H]^+$ 472.1036, found 472.1043.

3-(4-bromobenzyl)-6-(3-chlorobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (48). Light yellow solid, yield 56%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.32 (m, 5H), 7.28-7.17 (m, 3H), 5.05 (s, 2H), 3.86 (q, *J* = 7.0 Hz, 2H), 3.64 (s, 2H), 3.34 (s, 2H), 2.70 – 2.60 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.82, 150.73, 145.89, 139.31, 135.54, 133.90, 130.93, 130.44, 129.19, 128.43, 127.11, 126.55, 121.05, 106.91, 61.06, 49.11, 48.34, 43.28, 38.84, 26.25, 13.63. ESI-MS *m/z* calc'd for $C_{23}H_{24}BrClN_3O_2$ $[M+H]^+$ 488.07, found 488.09. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}BrClN_3O_2$ $[M+H]^+$ 488.0740, found 488.0743.

6-(3-bromobenzyl)-3-(4-bromobenzyl)-1-ethyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,4(1H,3H)-dione (49). Light yellow solid, yield 43%. The purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 1H), 7.43-7.31 (m, 5H), 7.28-7.14 (m, 2H), 5.05 (s, 2H), 3.86 (q, *J*

= 7.0 Hz, 2H), 3.63 (s, 2H), 3.34 (s, 2H), 2.73-2.59 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.81, 150.73, 145.88, 139.61, 135.54, 131.34, 130.93, 130.44, 130.05, 129.49, 127.02, 122.15, 121.05, 106.91, 61.02, 49.12, 48.33, 43.28, 38.84, 26.25, 13.63. ESI-MS *m/z* calc'd for $C_{23}H_{24}Br_2N_3O_2$ $[M+H]^+$ 532.02, found 532.05. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{24}Br_2N_3O_2$ $[M+H]^+$ 532.0235, found 532.0240.

Biological Characterization

Cell growth inhibition determined by the WST method

The tumor cell lines were seeded in 96-well plates at a density of 2,000-3,000 cells per well. After adding different concentrations of the compound, the cells were incubated for 3 days in a 5% CO_2 incubator at 37 ℃. After incubation, (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazoliummonosodium salt (WST-8; Dojindo Molecular Technologies Inc., Gaithersburg, MD) was added to each well to a final concentration of 10%, and then the plates were incubated at 37 ℃ for 2-3 h. The absorbance of the samples was measured at 450 nm using a TECAN ULTRA reader. Concentration of the compounds that inhibited cell growth by 50% (IC_{50}) was calculated by comparing absorbance in the untreated cells and the cells treated with the compounds.

Western blot detection of biomarkers

The tumor cell lines were seeded in 96-well plates at a density of 500,000-700,000 cells per well. After adding different concentrations of the compound, the cells were incubated for 24 hours in a 5% CO_2 incubator at 37 ℃. The cells were harvested and

then lysed using radio immune-precipitation assay (RIPA) lysis buffer and lysates were then cleared by centrifugation prior to protein concentration determination using the Bio-Rad protein assay kit according to the manufacturer's instructions. Proteins were electrophoresed onto 18% SDS-PAGE gels and transferred to PVDF membranes. Following blocking in 5% milk, membranes were incubated with a specific primary antibody, washed, and incubated with horseradish peroxidase linked secondary antibody. The signals were visualized with the chemiluminescent HRP antibody detection reagent. When indicated, the blots were stripped and reprobed with a different antibody.

The change of TRAIL concentration

Tumor cell lines were seeded in 6-well plates at a density of 200,000 to 300,000 cells per well. After adding different concentrations of the compound, the cells were incubated for 3 days in a 5% CO_2 incubator at 37 ℃. The TRAIL concentration in the cell culture medium was measured by ELISA kit (Human TRAIL ELISA Kit).

The detection of SubG1

Tumor cell lines were seeded in 6-well plates at a density of 200,000 to 300,000 cells per well. After adding different concentrations of the compound, the cells were incubated for 3 days in a 5% CO_2 incubator at 37 ℃. By coloring with propidium iodide, the concentration of SubG1 was measured by flow cytometry.

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