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SYNTHESIS OF PYRROLO[2,3-*d*]PYRIMIDINE ANALOGUES OF THE POTENT ANTITUMOR AGENT *N*-{4-[3-(2,4-DIAMINO-7H-PYRROLO-[2,3-*d*]PYRIMIDIN-5-YL)PROPYL]BENZOYL}-L-GLUTAMIC ACID (TNP-351)

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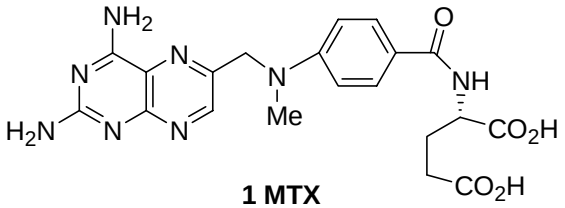
Abstract – Two three-atom-bridged analogues of TNP-351, *N*-(4-{[(2-amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}-benzoyl)-L-glutamic acid (**4**) and *N*-(4-{[(2,4-diamino-7H-pyrrolo[2,3-*d*]-pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid (**5**) were synthesized as anticancer agents, and their biological activities were evaluated.

INTRODUCTION

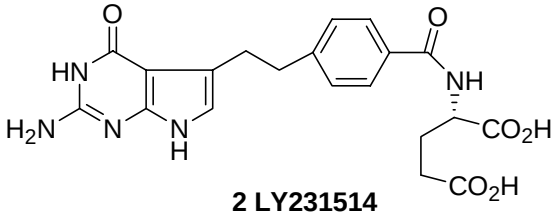
Folate metabolism is an attractive chemotherapeutic target since it plays a crucial role in the biosynthesis of nucleic acid precursors.[1] Dihydrofolate reductase (DHFR) is one of the most important enzymes involved in the biosynthesis.[2] Thymidylate synthase (TS) is a crucial enzyme that catalyzes the conversion of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) utilizing the cofactor 5,10-methylenetetrahydrofolate (5,10-CH_2FH_4) as the source of the one carbon as well as the reductant.[3] TS and DHFR have long been recognized as important targets for cancer chemotherapy.

MTX (**1**) (Figure 1) strongly inhibits DHFR and has been used as an anticancer agent since 1953. However, it has limitations in clinical use because of toxicity to patients, drug resistance from tumor cells and lack of efficacy against most human solid tumors.[4] LY231514 (**2**, Alimta, Pemetrexed) was approved as a multitargeted antitumor agent by FDA in 2004 for the treatment of malignant pleural or peritoneal mesothelioma in combination with cisplatin and as a single-agent in the treatment of locally advanced or metastatic nonsmall cell lung cancer (NSCLC).[5] TNP-351 (**3**) was very potent against DHFR, which was

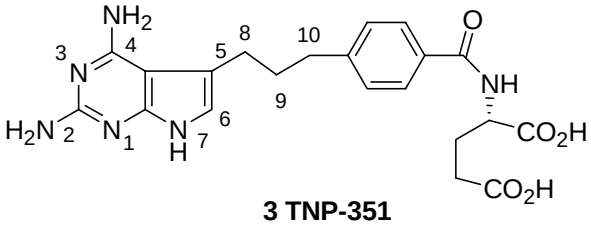
almost 8 times more active than MTX in inhibition of tumor cell growth. It indicates that introduction of the pyrrolo[2,3-*d*]pyrimidine ring together with a three-carbon bridge would greatly improve antitumor activity.[6] It was of interest to replace the C8-C9-C10 bridge of TNP-351 with a C8-N9-C10 bridge. We designed and synthesized novel 2-amino-4-oxo-5-substituted–pyrrolo[2,3-*d*]pyrimidine (**4**) and 2,4-diamino-5-substituted-pyrrolo[2,3-*d*]pyrimidines (**5**), and evaluated their biological activities.



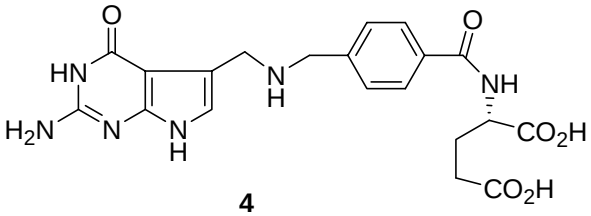
1 MTX



2 LY231514



3 TNP-351



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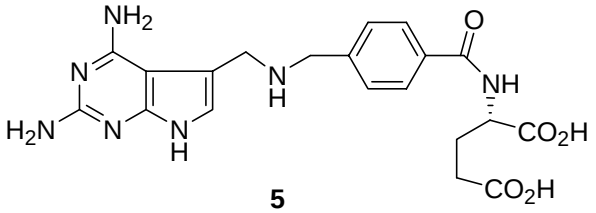
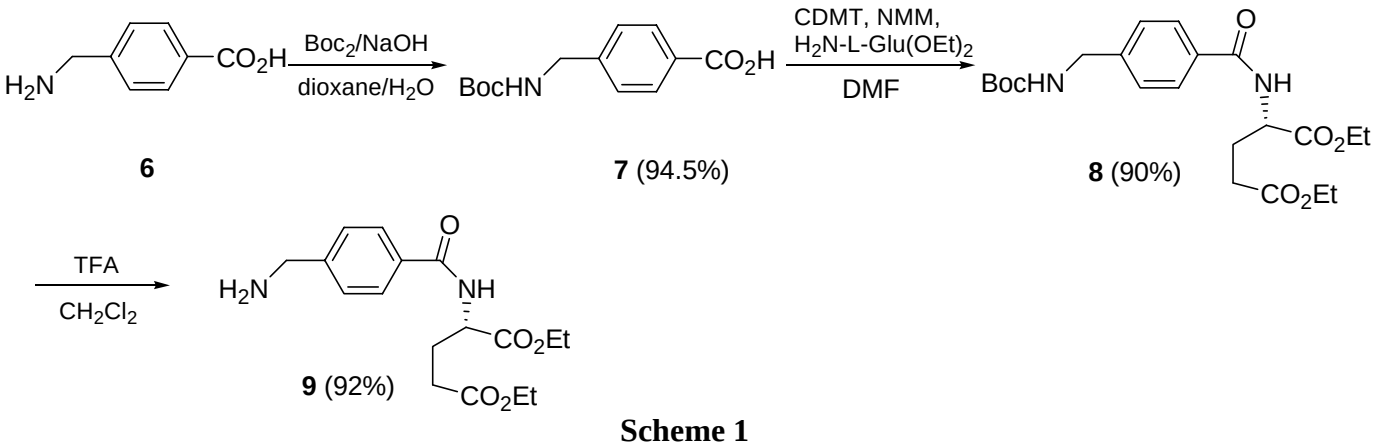


5

RESULTS AND DISCUSSION

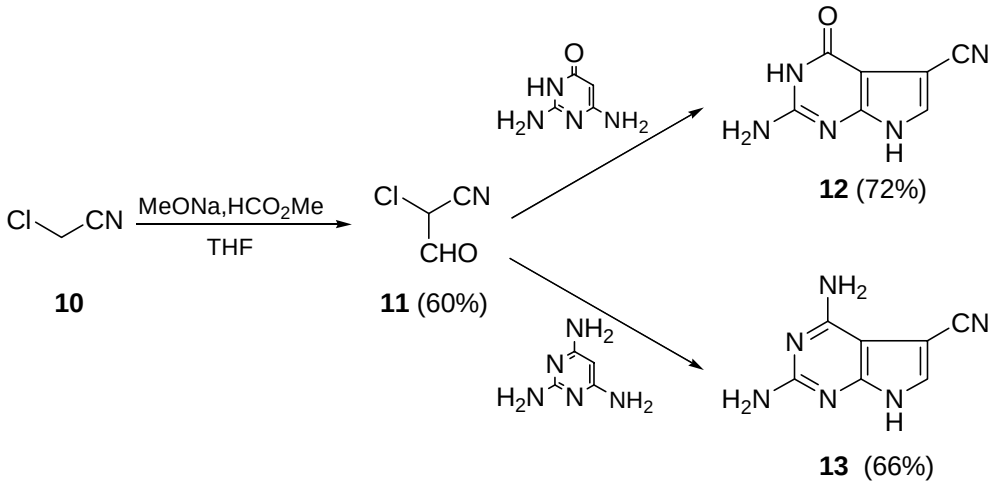
In our synthesis of analogues **4** and **5**, the key intermediate amine **9** was prepared according to Gangjee's procedure[7] (Scheme 1).



6 **7** (94.5%) **8** (90%) **9** (92%)

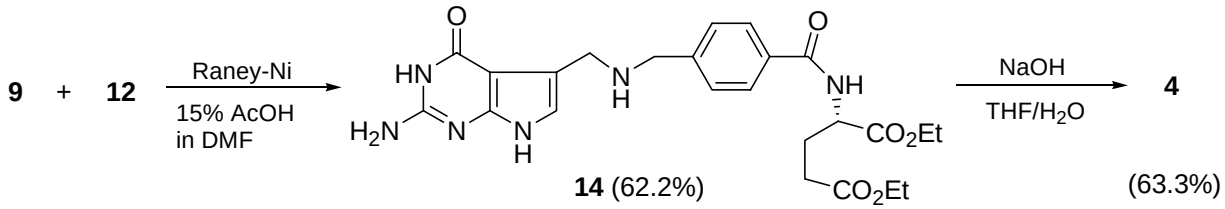
Scheme 1

Compound **12** was prepared by the method reported by Migawa;[8] condensation of 2,4-diamino-6-hydroxypyrimidine with chloroformylacetonitrile (**11**) (Scheme 2). Compound **13** was originally synthesized by Gangjee[9] by condensation of 2-amino-3,4-dicyanopyrole with chlorformamidine. We synthesized **13** by the condensation reaction of pyrimidine-2,4,6-triamine with **11**.



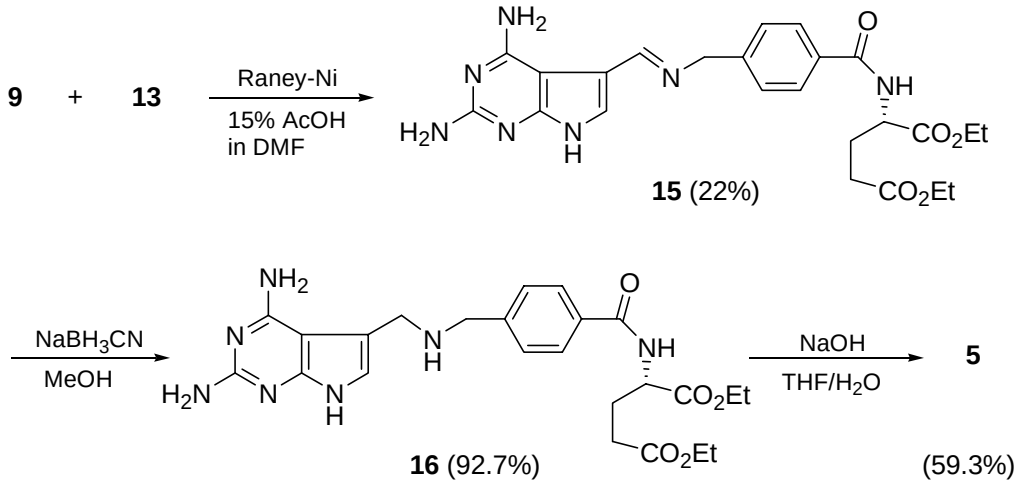
Scheme 2

Reductive condensation[10,11] of the nitrile **12** with amine **9** in 15% AcOH in DMF using Raney nickel as catalyst afforded the compound *N*-(4-{[(2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid diethyl ester (**14**) (Scheme 3), and final saponification[12,13] then gave the target **4**.



Scheme 3

However, reductive condensation[9,10] of the nitrile **13** with amine **9** in the same condition did not afford the expected compound *N*-(4-{[(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}-benzoyl)-L-glutamic acid diethyl ester (**16**) (Scheme 4), but rather the intermediate *N*-(4-{[(2,4-diamino-7H-pyrrolo[2,3-*d*]pyrimidin-5-ylmethylene)amino]methyl}benzoyl)-L-glutamic acid diethyl ester (**15**). Further reduction[9] of the Schiff base **15** with $NaBH_3CN$ proceeded smoothly within 4 h in MeOH at room temperature, amine **16** was formed. The target **5** was obtained from **16** on hydrolysis[12,13] of the diethyl ester in 59.3% yield.



Scheme 4

Biological evaluation of compounds (**4**) and (**5**) revealed that they were inactive in antiproliferative assay at 20 μg/mL.

EXPERIMENTAL

General. ^{1}H NMR and ^{13}C NMR were measured on MERCURYplus 400 and MERCURYplus 300 spectrometers with chemical shifts reports as parts per million (in DMSO-d_6/$CDCl_3$, TMS as an internal standard). Mass spectra were measured on Agilent 1100 LC-MSD VL and HP 1100 LC-MS spectrometers. Melting points were determined with a RY-2 melting point apparatus and were uncorrected.

4-(*tert*-Butoxycarbonylaminomethyl)benzoic acid (7). To a solution of 4-(aminomethyl)benzoic acid (6) (10.0 g, 66.2 mmol) in dioxane/water (1:1, 130 mL) was added 1 N NaOH (67 mL). Di-*tert*-butyl dicarbonate (17.3 g, 79.5 mmol) was added to this solution and the mixture stirred at room temperature for 12 h. The reaction mixture was evaporated to 1/3 its original volume under reduced pressure. The pH of the solution was adjusted to 3 by dropwise addition of 50% aqueous HCl while maintaining the temperature below 10 ^{o}C with an ice bath. The precipitate was collected by filtration, washed with H_2O (3×10 mL), and dried in vacuum to afford 15.72 g (94.5%) of **7** as a white solid. mp 163-165 ^{o}C. MS (ESI) *m/z* 250 (MH^-). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.38 (s, 9H, $C(CH_3)_3$), 4.16 (d, 2H, CH_2, J = 6.0 Hz), 7.32 (d, 2H, 3'-, 5'-CH, J = 8.0 Hz), 7.46 (br s, 1H, NH), 7.87 (d, 2H, 2'-, 6'-CH, J = 8.0 Hz), 12.83 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 28.89, 43.87, 78.61, 127.54, 129.86, 130.03, 146.03, 156.49, 167.88.

***N*-[4-(*tert*-Butoxycarbonylaminomethyl)benzoyl]-L-glutamic acid diethyl ester (8).** To a suspension of **7** (5.0 g, 19.9 mmol) in 100 mL of dry DMF under nitrogen were added 4-methylmorpholine (NMM) (2.42 g, 23.9 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (4.20 g, 23.9 mmol). The

resulting mixture was stirred at rt for 2 h, and 4-methylmorpholine (2.42 g, 23.9 mmol) and diethyl L-glutamate hydrochloride (5.73 g, 23.9 mmol) were added. The reaction mixture was stirred for another 4 h at rt. Solvent was evaporated under vacuum and the residue was purified by column chromatography to give 7.86 g (90%) of **8** as a white solid. mp 129-131 °C. MS (ESI) *m/z* 437 (MH^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.22 (t, 3H, CH_3, *J* = 7.2 Hz), 1.30 (t, 3H, CH_3, *J* = 7.2 Hz), 1.46 (s, 9H, $C(CH_3)_3$), 2.15, 2.30 (m, 2H, β-CH_2), 2.46 (m, 2H, γ-CH_2), 4.10 (q, 2H, COO-C$\underline{H}_2$CH$_3$, *J* = 7.2 Hz), 4.23 (q, 2H, COO-C$\underline{H}_2$CH$_3$, *J* = 7.2 Hz), 4.35 (d, 2H, CH_2, *J* = 6.2 Hz), 4.78 (m, 1H, glu-NH-C$\underline{H}$), 4.93 (br s, 1H, NH), 7.02 (d, 1H, glu-NH, *J* = 7.2 Hz), 7.35 (d, 2H, 3'-, 5'-CH, *J* = 8.2 Hz), 7.77 (d, 2H, 2'-, 6'-CH, *J* = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.36, 27.46, 28.60, 29.89, 30.73, 44.51, 52.61, 60.99, 61.91, 79.93, 127.60, 127.65, 132.91, 143.34, 156.10, 166.99, 172.19, 173.43.

***N*-(4-Aminomethyl-benzoyl)-L-glutamic acid diethyl ester (9).** Trifluoroacetic acid (6.4 mL, 85.8 mmol) was added dropwise to a stirred solution of **8** (3.74 g, 8.58 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at rt for 2 h, evaporated to dryness under reduced pressure, and coevaporated twice with absolute EtOH (100 mL). The residue was purified by column chromatography to afford 2.65 g (92%) of **9** as a white solid. mp 110-112 °C. MS (ESI) *m/z* 337 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.14 (t, 3H, CH_3, *J* = 7.2 Hz), 1.19 (t, 3H, CH_3, *J* = 7.2 Hz), 2.03 (m, 2H, β-CH_2), 2.42 (m, 2H, γ-CH_2), 4.02 (q, 2H, COO-C$\underline{H}_2$CH$_3$, *J* = 7.2 Hz), 4.08 (q, 2H, COO-C$\underline{H}_2$CH$_3$, *J* = 7.2 Hz), 4.10 (s, 2H, NH$_2$C$\underline{H}_2$), 4.44 (m, 1H, glu-NH-C$\underline{H}$), 7.54 (d, 2H, 3'-, 5'-CH, *J* = 8.2 Hz), 7.90 (d, 2H, 2'-, 6'-CH, *J* = 8.2 Hz), 8.42 (br s, 2H, NH_2), 8.81 (d, 1H, glu-NH, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.25, 25.87, 30.40, 42.08, 52.31, 60.16, 60.82, 127.91, 128.84, 133.76, 137.70, 166.45, 171.93, 172.43.

Chloro(formyl)acetonitrile (11). While the temperature was maintained between 0 and 5 °C, methyl formate (14.4 g, 0.24 mol) was added to a stirred mixture of MeONa (10.8 g, 0.2 mol) in 180 mL of THF. This was followed by dropwise addition of chloroacetonitrile (**10**) (15.0 g, 0.2 mol) over a period of 1 h. The mixture was allowed to stir for an additional 3 h, and then 15 mL of 12 N HCl was added dropwise, while maintaining the temperature below 10 °C. The mixture was filtered, and the filtrate was concentrated under reduced pressure to afford 12.4 g (60%) of **11**, which was used without further purification.

2-Amino-5-cyanopyrrolo[2,3-*d*]pyrimidin-4-one (12). NaOAc (7.9 g, 96.0 mmol) was dissolved in 180 mL of distilled water. 2,4-Diamino-6-hydroxypyrimidine (6.0 g, 48.0 mmol) was added, and the mixture was heated to 50 °C, at which time chloro(formyl)acetonitrile (**11**) (6.0 g, 57.6 mmol) was added over 1 h period. The solution was allowed to stir for 12 h, and then heated at reflux for 1 h. The mixture was cooled to rt and then filtered. The solid was washed with distilled water and acetone to yield 6.0 g (72%) of **12** as a white solid. mp > 300 °C. MS (EI) *m/z* 175 (M^+). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.70 (s, 2H, 2-NH_2), 7.54 (s, 1H, 6-CH), 10.70 (s, 1H, 7-NH), 11.80 (s, 1H, 3-NH). ^{13}C NMR (DMSO-d_6, 75

MHz) δ: 85.26, 99.04, 116.51, 128.55, 152.63, 154.74, 159.16.

2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (13).

Synthesized from **11** (6.0 g, 57.6 mmol) and pyrimidine-2,4,6-triamine (6.0 g, 48.0 mmol) by method described above for **12**, afforded **13** (5.5 g, 66%) as a brown solid. mp > 300 ^{o}C. MS (EI) *m/z* 174 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.90 (s, 2H, 2-NH_2), 6.21 (s, 2H, 4-NH_2), 7.68 (s, 1H, 6-CH), 11.82 (s, 1H, 7-NH).

***N*-(4-{[(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid diethyl ester (14).** 2-Amino-5-cyanopyrrolo[2,3-*d*]pyrimidin-4-one (**12**) (1.05 g, 6 mmol) and *N*-(4-Aminomethylbenzoyl)-L-glutamic acid diethyl ester (**9**) (2.40 g, 7 mmol) were dissolved in 140 mL of 15% AcOH in DMF with heating. To this solution was added 4.0 g of damp Raney nickel and the mixture was hydrogenated at 85 psi for a period of 6 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography to afford 1.8 g (62.2%) of **14** as an off-white solid. mp 155-157 ^{o}C. MS (ESI) *m/z* 499 (MH^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.14 (t, 3H, COO-$CH_2C\underline{H}_3$, J = 7.2 Hz), 1.17 (t, 3H, COO-$CH_2C\underline{H}_3$, J = 7.2 Hz), 2.00, 2.09 (m, 2H, β-CH_2), 2.43 (m, 2H, γ-CH_2), 4.03 (q, 2H, COO-$C\underline{H}_2CH_3$, J = 7.2 Hz), 4.10 (q, 2H, COO-$C\underline{H}_2CH_3$, J = 7.2 Hz), 4.16 (s, 2H, 8-CH_2), 4.24 (s, 2H, 10-CH_2), 4.42 (m, 1H, glu-NH-C$\underline{H}$), 6.45 (s, 2H, 2-NH_2), 6.83 (s, 1H, 6-CH), 7.56 (d, 2H, 3'-,5'-CH, J = 8.2 Hz), 7.91 (d, 2H, 2'-,6'-CH, J = 8.2 Hz), 8.79 (d, 1H, glu-NH, J = 7.2 Hz), 9.59 (s, 1H, 7-NH), 11.30 (s, 1H, 3-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.73, 21.19, 26.34, 30.83, 43.60, 49.43, 52.75, 60.61, 61.28, 98.92, 109.13, 117.98, 128.53, 130.20, 134.79, 136.36, 153.07, 153.46, 161.26, 166.85, 172.36, 172.86. Anal. Calcd for $C_{24}H_{30}N_6O_6$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.92; H, 6.17; N, 16.65.

***N*-(4-{[(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid (4).** A suspension of **14** (0.10 g, 0.2 mmol) in 2 mL of 1 N NaOH and 8 mL of THF was stirred at rt for 24 h. The THF was evaporated under reduced pressure, and the residual solution was acidified with 1 N HCl to pH 4. The precipitate was collected by filtration, washed with H_2O (3 × 5 mL), and dried in vacuum to afford 56 mg (63.3%) of **4** as a white solid. mp 238-241 ^{o}C (decomp). MS (ESI) *m/z* 443 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.93-2.06 (m, 2H, β-CH_2), 2.28 (m, 2H, γ-CH_2), 4.01 (s, 2H, 8-CH_2), 4.06 (s, 2H, 10-CH_2), 4.29 (m, 1H, glu-NH-C$\underline{H}$), 6.56 (s, 2H, 2-NH_2), 6.72 (s, 1H, 6-CH), 7.49 (d, 2H, 3'-,5'-CH, J = 8.2 Hz), 7.84 (d, 2H, 2'-,6'-CH, J = 8.2 Hz), 8.37 (d, 1H, glu-NH, J = 7.2 Hz), 11.12 (s, 1H, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 27.82, 31.97, 43.97, 50.13, 53.75, 99.01, 111.38, 117.15, 128.12, 129.74, 134.90, 138.27, 152.99, 153.60, 161.19, 166.17, 174.75, 175.27. Anal. Calcd for $C_{20}H_{22}N_6O_6$: C, 54.29; H, 5.01; N, 19.00. Found: C, 54.38; H, 5.16; N, 18.86.

***N*-(4-{[(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethylene)amino]methyl}benzoyl)-L-glutamic**

acid diethyl ester (15). Synthesized from **13** (1.05 g, 6 mmol) and **9** (2.40 g, 7 mmol) by method described above for **14**, afforded **15** (0.65 g, 22%) as a gray-white solid. mp 164-166 °C. MS (ESI) *m/z* 496 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.14 (t, 3H, COO-$CH_2C\underline{H}_3$, *J* = 7.2 Hz), 1.17 (t, 3H, COO-$CH_2C\underline{H}_3$, *J* = 7.2 Hz), 2.00, 2.09 (m, 2H, β-CH_2), 2.42 (m, 2H, γ-CH_2), 4.02 (q, 2H, COO-$C\underline{H}_2CH_3$, *J* = 7.2 Hz), 4.08 (q, 2H, COO-$C\underline{H}_2CH_3$, *J* = 7.2 Hz), 4.39 (m, 1H, glu-NH-C<u>H</u>), 4.73 (s, 2H, 10-CH_2), 5.60 (br s, 2H, 2-NH_2), 6.52, 8.91 (br s, 2H, 4-NH_2), 7.28 (s, 1H, 6-CH), 7.38 (d, 2H, 3'-,5'-CH, *J* = 8.0 Hz), 7.85 (d, 2H, 2'-,6'-CH, *J* = 8.0 Hz), 8.29 (s, 1H, 8-CH), 8.69 (d, 1H, glu-NH, *J* = 7.2 Hz), 11.25 (br s, 1H, 7-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.74, 26.45, 27.10, 30.89, 52.70, 60.59, 61.22, 63.63, 93.82, 115.73, 127.16, 128.07, 128.36, 132.93, 144.36, 155.98, 158.95, 159.40, 161.59, 167.18, 172.41, 172.86. Anal. Calcd for $C_{24}H_{29}N_7O_5$: C, 58.17; H, 5.90; N, 19.79. Found: C, 58.32; H, 5.98; N, 19.89.

***N*-(4-{[(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid diethyl ester (16).** $NaCNBH_3$ (0.13 g, 2 mmol) was added to a solution of **15** (0.7 g, 1.41 mmol) in MeOH (50 mL), the pH was adjusted to 2 with 50% MeOH/HCl, and stirring was continued at rt for 4 h. The solvent was evaporated to dryness, and the residue was purified by column chromatography to afford 0.65 g (92.7%) of **16** as a gummy solid. MS (ESI) *m/z* 498 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.14 (t, 3H, COO-$CH_2C\underline{H}_3$, *J* = 7.2 Hz), 1.16 (t, 3H, COO-$CH_2C\underline{H}_3$, *J* = 7.2 Hz), 2.00, 2.09 (m, 2H, β-CH_2), 2.42 (m, 2H, γ-CH_2), 4.02 (q, 2H, COO-$C\underline{H}_2CH_3$, *J* = 7.2 Hz), 4.08 (q, 2H, COO-$C\underline{H}_2CH_3$, *J* = 7.2 Hz), 4.26 (s, 2H, 8-CH_2), 4.39 (m, 1H, glu-NH-C<u>H</u>), 4.41 (s, 2H, 10-CH_2), 7.26 (s, 1H, 6-CH), 7.32 (br s, 2H, 2-NH_2), 7.68 (d, 2H, 3'-,5'-CH, *J* = 8.0 Hz), 7.90 (d, 2H, 2'-,6'-CH, *J* = 8.0 Hz), 8.45 (br s, 2H, 4-NH_2), 8.81 (d, 1H, glu-NH, *J* = 7.2 Hz), 9.64 (s, 1H, 7-NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.74, 14.75, 26.34, 30.91, 42.13, 49.45, 52.77, 60.58, 61.22, 63.50, 107.16, 119.58, 128.17, 130.62, 134.43, 136.37, 151.92, 158.96, 159.38, 166.86, 172.32, 172.85. Anal. Calcd for $C_{24}H_{31}N_7O_5$: C, 57.94; H, 6.28; N, 19.71. Found: C, 57.84; H, 6.38; N, 19.78.

***N*-(4-{[(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-ylmethyl)amino]methyl}benzoyl)-L-glutamic acid (5).** Synthesized from **16** (0.13 g, 0.26 mmol) by method described above for **4**, afforded **5** (68 mg, 59.3%) as a white solid. mp 188-191 °C (decomp). MS (ESI) *m/z* 440 (MH^-). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.97, 2.07 (m, 2H, β-CH_2), 2.33 (m, 2H, γ-CH_2), 3.70 (s, 2H, 8-CH_2), 3.76 (s, 2H, 10-CH_2), 4.37 (m, 1H, glu-NH-C<u>H</u>), 5.77 (br s, 2H, 2-NH_2), 6.61 (s, 1H, 6-CH), 7.41 (d, 2H, 3'-,5'-CH, *J* = 8.0 Hz), 7.50 (br s, 2H, 4-NH_2), 7.83 (d, 2H, 2'-,6'-CH, *J* = 8.0 Hz), 8.50 (d, 1H, glu-NH, *J* = 7.2 Hz), 10.69 (s, 1H, 7-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 27.02, 31.39, 45.38, 51.96, 52.96, 96.29, 113.41, 117.33, 128.06, 128.78, 133.34, 143.58, 153.77, 158.03, 158.59, 166.85, 174.58, 174.82. Anal. Calcd for $C_{20}H_{23}N_7O_5$: C, 54.42; H, 5.25; N, 22.21. Found: C, 54.22; H, 5.35; N, 22.12.

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