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SYNTHESIS OF PYRROLO[2,3-*d*]PYRIMIDINE ANALOGUES: "PYRIDINE RING" ANALOGUES OF PEMETREXED

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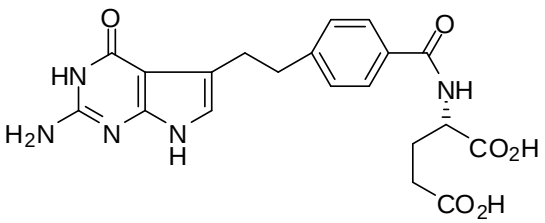
Abstract – Two analogues of pemetrexed with its phenyl ring replaced with pyridine ring as novel anticancer agents were synthesized. Preliminary *in vitro* evaluation indicated that replacement of the phenyl moiety of pemetrexed by the pyridine ring with the 6-5 bicyclic ring system showed low cytotoxicity, that departs from the findings with antifolates bearing 6-6 bicyclic ring system.

Pemetrexed (LY231514, **1**) is a novel 6-5 bicyclic pyrrolo[2,3-*d*]pyrimidine antifolate that shows remarkable activity against a broad spectrum of solid tumors. Pemetrexed was approved as a multitargeted antifolate (MTA) by FDA in 2004 for the treatment of malignant pleural mesothelioma in combination with cisplatin and as a single-agent in the treatment of non-small-cell lung cancer.[1] The clinical success of pemetrexed has generated renewed interest in the design and synthesis of antifolates that function as multi-inhibitors for folate-dependent enzymes.[2, 3]

Antifolates bearing classical 6-6 bicyclic ring system, such as pteridine, deazapteridine and quinazoline, could tolerate changes in phenyl group in some extent without the loss of cytotoxic potency.[4] It is of interests to further explore the effect of change of phenyl ring with other ring system, such as pyridine in pemetrexed, while retain its 6-5 bicyclic pyrrolo[2,3-*d*]pyrimidine moiety. We describe herein the synthesis and biological activity of two close structural analogues of pemetrexed with the substitution of phenyl ring by pyridine, 2-amino-4-oxo-5-substituted-pyrrolo[2,3-*d*]pyrimidine (**2a**) and 2,4-diamino-5-substituted-pyrrolo[2,3-*d*]pyrimidine (**2b**).



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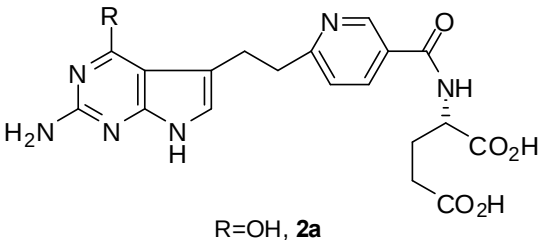
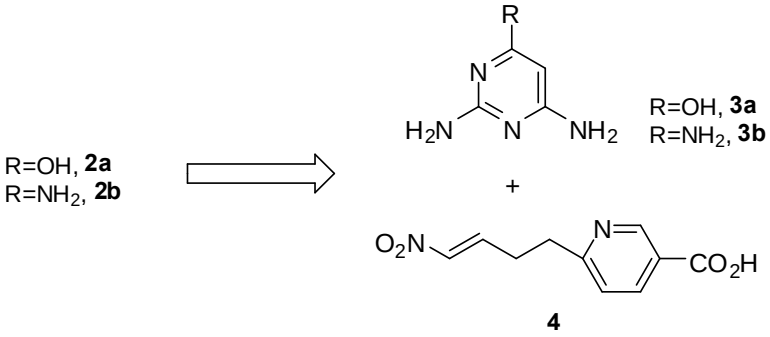


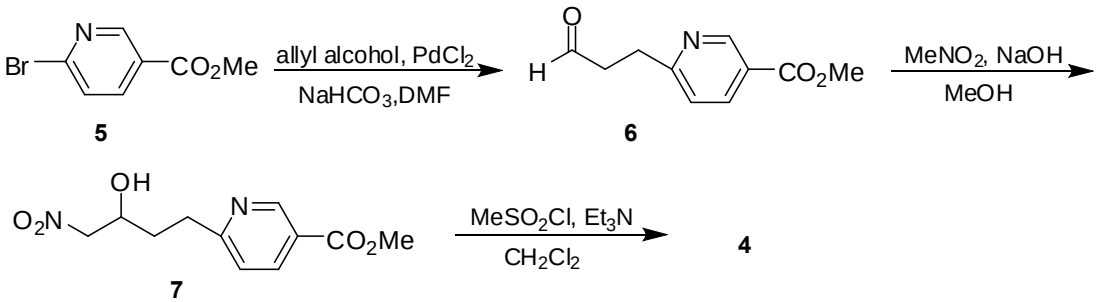
R=OH, **2a**
R=NH_2, **2b**

Retrosynthetic analysis suggested that structures **3a**, **3b** and **4** were key precursors to the synthesis of target compounds **2a** and **2b** (Scheme 1). Taylor and Liu[5] have demonstrated a synthetic approach for construction of the bicyclic pyrrolo[2,3-*d*]pyrimidine ring system through the coupling of aminopyrimidine and nitroolefin structure in the presence of strong base. The synthesis employs a Michael addition and a following Nef reaction. The Michael addition happens between the unsubstituted C-5 positions of 2,6-diaminopyrimidin-4(3*H*)-one (**3a**) and 2,4,6-triaminopyrimidine (**3b**) to nitroolefin group of **4**. The adducts then are undergone Nef reaction by converting the nitro group into a aldehyde which cyclizes with the C-6 amino substituent to form the 6-5 bicyclic ring system.[6]



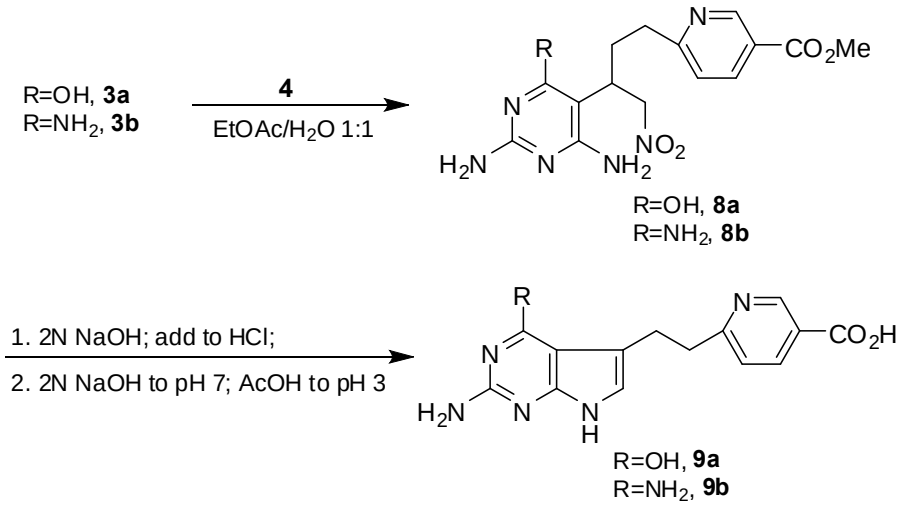
Scheme 1

The synthesis of the requisite precursor 6-(4-nitrobut-3-enyl)nicotinic acid methyl ester (**4**) was started from a palladium-catalyzed coupling of methyl 6-bromonicotinate (**5**) with allyl alcohol to form 6-(3-oxopropyl)nicotinic acid methyl ester (**6**) in 91% yield,[7] which underwent aldol condensation with nitromethane to give the nitro alcohol **7** in 50% yield, followed by dehydration[8] through conversion of hydroxyl to methanesulfonyl ester with MsCl and subsequent elimination with triethylamine to afford the key nitroolefin intermediate **4** in 96% yield.



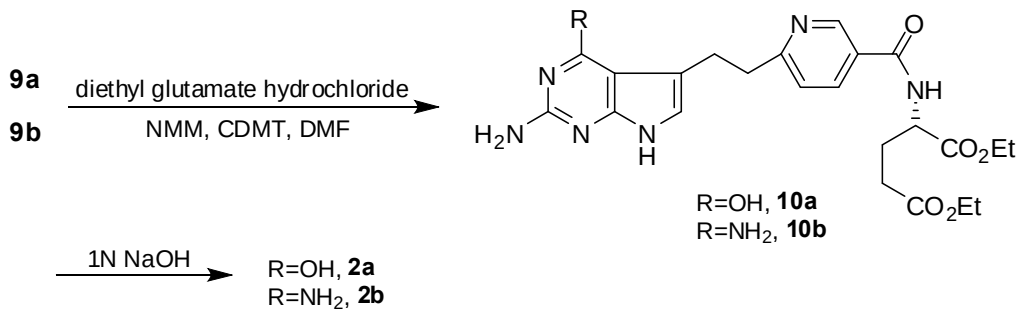
Scheme 2

Michael addition of 2,6-diaminopyrimidin-4(3*H*)-one (**3a**) to the nitroolefin group of **4** was proceeded at 50 °C to yield the adduct **8a**. The crucial intramolecular cyclization of **8a** to the pyrrolo[2,3-*d*]pyrimidine **9a** was achieved by stirring with NaOH at 25 °C for 2 h followed by addition into HCl at -5 °C for 3 h, the mixture was neutralized by addition of NaOH and then acidified with AcOH in excellent overall yield. This procedure includes a three-step conversion: a Nef reaction which transform the nitro group to aldehyde with treatment of NaOH and subsequent HCl, followed by a intramolecular condensation between the aldehyde and the 6-amino group in acidic condition and a final aromatization.[5] This one-pot operation efficiently resulted in not only the formation of the desired 6-5 bicyclic ring system, but also a necessary saponification of the carbonyl eater to carboxylic acid for the later coupling with glutamate in this case.



Scheme 3

The synthesis of penultimate **10a** was accomplished by coupling of the carboxylic acid **9a** to glutamate with the activation of the carboxylic acid by 2,4-dimethoxy-6-chloro-1,3,5-triazine and *N*-methylmorpholine.[9] Final hydrolysis of **10a** with 1N NaOH followed by acidification with AcOH yielded the target compound **2a**. The 4-amino substituted target compound **2b** was synthesized from 2,4,6-triaminopyrimidine (**3b**) by procedures similar to those of **2a**.



Scheme 4

In vitro cell culture screening of target compounds **2a** and **2b,** however, shown that both target compounds were not active against many cancer cells with low cytotoxicity (>20 μg/mL). This result might indicate that the pyridine substitution of phenyl ring in antifolates bearing the 6-5 bicyclic ring system is not well tolerated, that departs from the findings with antifolates bearing 6-6 bicyclic ring system.

EXPERIMENTAL

General. ^{1}H NMR and ^{13}C NMR were recorded on a Varian-300 instrument. The chemical shift values were expressed in ppm (parts per million) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on Agilent 1100 series LC-MSD and HP 1100 LC-MS spectrometers. Melting points were determined on a RY-2 microscopic melting point apparatus and were uncorrected. Column chromatography was carried out with silica gel (200-300 mesh).

6-(3-Oxopropyl)nicotinic acid methyl ester (6). A mixture of methyl 6-bromonicotinate **5** (1.1 g, 5.0 mmol), allyl alcohol (0.5 mL, 7.5 mmol), $NaHCO_3$ (1.1 g, 10.0 mmol), $Pd(OAc)_2$ (56.1 mg, 0.25 mmol), (n-Bu)$_4$NBr (1.9 g , 6.0 mmol) in DMF (10 mL) was stirred for 72 h at 25 °C under nitrogen. The reaction mixture was filtered, and the filtrate was poured into H_2O and extracted with hexane (5×20 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel/hexanes: EtOAc = 10: 1) to give 0.88 g (91%) of **6** as a clear oil. MS (ESI): *m/z* 194 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.82 (s, 1H, CHO), 8.81 (d, 1H, J=2.4Hz, 2-H), 7.97 (dd, 1H, J=2.4, 8.4Hz, 4-H), 7.26 (d, 1H, J=8.4Hz, 5-H), 3.90 (s, 3H, CH_3), 3.01 (t, 2H, J=7.5Hz, 6-CH_2CH$_2$CHO), 2.81 (t, 2H, J=7.5Hz, 6-CH$_2$CH_2CHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.9, 167.1, 161.0, 146.0, 130.1, 128.6, 128.5, 52.2, 45.0, 28.3. HRMS: Calcd for $C_{10}H_{11}NO_3$: 193.0739; found: 193.0737.

6-(3-Hydroxy-4-nitrobutyl)nicotinic acid methyl ester (7). To a solution of **6** (0.55 g, 2.85 mmol) in MeOH (5 mL) at 25 °C was added nitromethane (0.21 g, 3.44 mmol) followed by addition of sodium hydroxide (6 mg, 0.15 mmol) in MeOH (1 mL) at 0 °C. The mixture was stirred at 35 °C for 48 h. Then the mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel/hexanes: EtOAc = 5: 1) to give 0.36 g (50%) of **7** as a light yellow solid, mp 65~67 °C. MS (ESI): *m/z* 255 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.82 (d, 1H, J=2.4Hz, 2-H), 7.99 (dd, 1H, J=2.4, 7.8Hz, 4-H), 7.29 (d, 1H, J=7.8Hz, 5-H), 4.36 (d, 2H, J=6.6Hz, CH_2NO_2), 4.25 (m, 1H, CHOH), 3.92 (s, 3H, CH_3), 2.88 (m, 2H, 6-CH_2CH$_2$), 2.50~3.00 (br s, 1H, OH), 1.86 (m, 2H, 6-CH$_2$CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.3, 161.9, 146.4, 130.2, 128.7, 128.6, 80.7, 67.8, 52.3, 34.9, 31.6. HRMS: Calcd for $C_{11}H_{14}N_2O_5$: 254.0903; found: 254.0910.

6-(4-Nitrobut-3-enyl)nicotinic acid methyl ester (4). To a solution of **7** (0.36 g, 1.42 mmol) in dry

CH_2Cl_2 (5 mL) at 0 °C was added methanesulfonyl chloride (0.15 mL, 1.92 mmol) followed by addition triethylamine (0.4 mL, 2.84 mmol). After 3 h, the mixture was warmed to 25 °C, and poured into H_2O (10 mL) and extracted with CH_2Cl_2 (2×10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel/hexanes: EtOAc = 10: 1) to give 0.32 g (96%) of **4** as a white solid, mp 69~71 °C. MS (ESI): *m/z* 237 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.82 (d, 1H, *J*=2.4Hz, 2-H), 7.99 (dd, 1H, *J*=2.4, 8.4Hz, 4-H), 7.26 (m, 2H, 5-H, CHC*H*NO_2), 6.96 (d, 1H, *J*=13.5Hz, C*H*$CHNO_2$), 3.91 (s, 3H, CH_3), 2.90 (t, 2H, *J*=7.5Hz, 6-C*H*$_2$$CH_2$), 2.62 (q, 2H, *J*=7.5Hz, 6-CH_2C*H*$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.1, 160.2, 145.1, 141.0, 140.4, 130.3, 128.9, 128.6, 52.4, 34.1, 29.9. HRMS: Calcd for $C_{11}H_{12}N_2O_4$: 236.0797; found: 236.0798.

6-[3-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-4-nitrobutyl]nicotinic acid methyl ester (8a). A mixture of **4** (0.30g, 1.27 mmol) and 2,6-diaminopyrimidin-4(3*H*)-one **3a** (0.20 g, 1.59 mmol) in H_2O (7.5 mL) and EtOAc (7.5 mL) was stirred at 50 °C for 24 h. The reaction mixture was poured into EtOAc (50 mL) and washed with H_2O (2×10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel/CH_2Cl_2: MeOH = 20: 1) to give 0.30 g (65%) of **8a** as a yellow solid, mp 216~218 °C. MS (ESI): *m/z* 363 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.76 (s, 1H, 3-H), 8.66 (d, 1H, *J*=2.4Hz, pyridine 2-H), 7.83 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.25 (d, 1H, *J*=8.4Hz, pyridine 5-H), 6.03 (s, 2H, 6-NH_2), 5.93 (s, 2H, 2-NH_2), 5.00, 4.75 (2m, 2H, CH_2NO_2), 3.81 (s, 3H, CH_3), 3.40 (m, 1H, 5-C*H*CH_2CH_2), 2.63, 2.50 (2m, 2H, 5-$CHCH_2$C*H*$_2$), 2.13, 1.70 (2m, 2H, 5-CHC*H*$_2$$CH_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 166.9, 163.6, 162.6, 162.5, 154.2, 148.9, 129.9, 129.1, 127.8, 112.6, 78.3, 52.6, 35.7, 33.7, 31.9. HRMS: Calcd for $C_{15}H_{18}N_6O_5$: 362.1339; found: 362.1342.

6-[2-(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinic acid (9a). A mixture of **8a** (0.10 g, 0.28 mmol) in 2 N NaOH (2.5 mL) was stirred at 25 °C for 2 h and then was slowly added into 2.5 N HCl (3 mL) at -5 °C. After 3 h, the pH of the reaction mixture was adjusted to 7 with 2 N NaOH. The mixture was warmed to 25 °C, and stirred for another 1 h and added AcOH to adjust the pH to 3. The solid was filtered, washed with H_2O, EtOAc and dried under vacuum to give **9a** (0.80 g, 95%) as a green solid without further purification for the next reaction, mp >300 °C. MS (ESI): *m/z* 300 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.40~13.20 (br s, 1H, COOH), 10.60 (s, 1H, 7-H), 10.13 (s, 1H, 3-H), 8.65 (d, 1H, *J*=2.4Hz, pyridine 2-H), 7.82 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.30 (d, 1H, *J*=8.4Hz, pyridine 5-H), 6.30 (s, 1H, 6-H), 5.98 (s, 2H, 2-NH_2), 2.97 (t, 2H, *J*=6.9Hz, 5-CH_2C*H*$_2$), 2.83 (t, 2H, *J*=6.9Hz, 5-C*H*$_2$$CH_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.0, 159.9, 152.9, 152.0, 149.8, 148.5, 129.9, 129.2, 128.9, 118.3, 114.1, 99.4, 37.0, 28.6. HRMS: Calcd for $C_{14}H_{13}N_5O_3$: 299.1018; found: 299.1012.

***N*-{6-[2-(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinoyl}-L-glutamic**

acid diethyl ester (10a). To a suspension of acid **9a** (50 mg, 0.17 mmol) in DMF (5 mL) was added *N*-methylmorpholine (20 μL, 0.18 mmol) followed by 2-chloro-4,6-dimethoxy-1,3,5–triazine (32 mg, 0.18 mmol), and the solution was stirred at 25 °C for 2 h. Another portion of *N*-methylmorpholine (20 μL, 0.18 mmol) was added to the solution followed by diethyl L-glutamate hydrochloride (44 mg, 0.18 mmol), and the mixture was stirred at 25 °C for 4 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel/CH_2Cl_2: MeOH = 20: 1) to give **10a** (73 mg , 89%) as a white solid, mp 137~139 °C. MS (ESI): *m/z* 485 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.59 (s, 1H, 7-H), 10.13 (s, 1H, 3-H), 8.61 (d, 2H, *J*=6.9Hz, CONH, pyridine 2-H), 7.76 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.27 (d, 1H, *J*=8.4Hz, pyridine 5-H), 6.29 (d, 1H, 6-H), 5.99 (s, 2H, 2-NH_2), 4.40 (m, 1H, glutamate α-CH), 4.08, 4.03 (2q, 4H, *J*=6.9Hz, 2C$H_2$$CH_3$), 2.96 (m, 2H, 5-$CH_2CH_2$), 2.84 (m, 2H, 5-C$H_2$$CH_2$), 2.42 (t, 2H, *J*=7.5Hz, glutamate γ-CH_2), 2.04 (m, 2H, glutamate β-CH_2), 1.16 (m, 6H, 2CH_2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.3, 172.9, 167.7, 156.1, 155.2, 151.9, 151.1, 146.5, 132.3, 129.5, 128.4, 117.7, 116.2, 99.4, 61.6, 61.0, 53.1, 36.4, 31.3, 28.1, 26.8, 15.1. HRMS: Calcd for $C_{23}H_{28}N_6O_6$: 484.2070; found: 484.2069.

***N*-{6-[2-(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinoyl}-L-glutamic acid (2a).** To a solution of **10a** (50 mg, 0.10 mmol) in THF (5 mL) was added 1 N NaOH (2 mL). The mixture was stirred at 25 °C for 3 h. The solvent was evaporated under reduced pressure, and the residual solution was acidified with AcOH. The precipitate was collected by filtration, washed with H_2O, EtOAc, and Et_2O, and dried under reduced pressure to give **2a** (30 mg, 70%) as a green solid, mp 190~192 °C. MS (ESI): *m/z* 429 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.58 (s, 1H, 7-H), 10.13 (s, 1H, 3-H), 8.58 (d, 1H, *J*=2.4Hz, pyridine 2-H), 8.41 (d, 1H, *J*=8.1Hz, CONH), 7.75 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.26 (d, 1H, *J*=8.4Hz, pyridine 5-H), 6.28 (d, 1H, 6-H), 5.98(s, 2H, 2-NH_2), 4.35 (m, 1H, glutamate α-CH), 2.94 (m, 2H, 5-CH_2CH_2), 2.83 (m, 2H, 5-C$H_2$$CH_2$), 2.32 (t, 2H, *J*=7.8Hz, glutamate γ-CH_2), 1.96 (m, 2H, glutamate β-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 174.8, 174.2, 166.8, 160.0, 152.9, 146.7, 132.2, 128.9, 127.9, 118.3, 114.1, 99.4, 52.8, 36.8, 31.7, 28.7, 27.2. HRMS: Calcd for $C_{19}H_{20}N_6O_6$: 428.1444; found: 428.1450.

6-[4-Nitro-3-(2,4,6-triaminopyrimidin-5-yl)butyl]nicotinic acid methyl ester (8b). Prepared from **4** (0.56 g, 2.36 mmol) and pyrimidine-2,4,6-triamine **3b** (0.36 g, 2.88 mmol) as described for the preparation of **8a**, obtained **8b** (0.56 g, 66%) as a yellow solid, mp 89~91 °C. MS (ESI): *m/z* 362 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.86 (d, 1H, *J*=2.4Hz, pyridine 2-H), 7.83 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.27 (d, 1H, *J*=8.4Hz, pyridine 5-H), 5.64 (br s, 4H, 4-NH_2, 6-NH_2), 5.36 (s, 2H, 2-NH_2), 4.84 (d, 2H, *J*=7.5Hz, CH_2NO_2), 3.79 (s, 3H, CH_3), 3.61 (m, 1H, 5-C*H*$CH_2$$CH_2$), 2.62, 2.48 (2m, 2H, 5-CHCH_2CH_2), 2.00, 1.84 (2m, 2H, 5-CHC$H_2$$CH_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 166.9, 164.2, 161.6, 148.7, 129.9, 129.2, 127.9, 83.3, 77.6, 52.6, 34.7, 33.7, 31.6. HRMS: Calcd for $C_{15}H_{19}N_7O_4$:

361.1499; found: 361.1506.

6-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinic acid (9b). Prepared from **8b** (0.25 g, 0.69 mmol) as described for the preparation of **9a,** yielded **9b** (0.20 g, 97%) as a gray solid, mp > 300 °C. MS (ESI): *m/z* 299 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.41 (s, 1H, 7-H), 8.64 (d, 1H, *J*=2.4Hz, pyridine 2-H), 7.80 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.73 (br s, 2H, 4-NH_2), 7.32 (d, 1H, *J*=8.4Hz, pyridine 5-H), 7.06 (s, 2H, 2-NH_2), 6.59 (s, 1H, 6-H), 2.98 (t, 2H, *J*=7.2Hz, 5-CH_2CH_2), 2.90 (t, 2H, *J*=7.2Hz, 5-CH_2CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.0, 154.4, 152.6, 150.1, 147.4, 129.9, 129.4, 129.1, 118.1, 116.5, 94.5, 35.9, 27.3. HRMS: Calcd for $C_{14}H_{14}N_6O_2$: 298.1178; found: 298.1175.

***N*-{6-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinoyl}-L-glutamic acid diethyl ester (10b).** The acid **9b** (0.22 g, 0.74 mmol) was condensed with diethyl L-glutamate hydrochloride as described for the preparation of **10a** to give **10b** (0.33 g, 93%) as a light yellow solid, mp 102~104 °C. MS (ESI): *m/z* 484 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.10 (s, 1H, 7-H), 8.69(m, 2H, CONH, pyridine 2-H), 7.86 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.34 (d, 1H, *J*=8.4Hz, pyridine 5-H), 7.22 (br s, 2H, 4-NH_2), 6.54 (br s, 3H, 6-H, 2-NH_2), 4.42 (m, 1H, glutamate α-CH), 4.10, 4.04 (2q, 4H, *J*=6.9Hz, $2CH_2CH_3$), 3.03 (m, 2H, 5-CH_2CH_2), 2.92 (m, 2H, 5-CH_2CH_2), 2.43 (t, 2H, *J*=7.5Hz, glutamate γ-CH_2), 2.05 (m, 2H, glutamate β-CH_2), 1.17 (m, 6H, $2CH_2CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.9, 172.5, 167.3, 155.7, 154.8, 151.5, 150.7, 146.1, 131.9, 129.1, 128.0, 117.3, 115.8, 95.0, 61.2, 60.6, 52.7, 36.0, 30.9, 27.7, 26.4, 14.8. HRMS: Calcd for $C_{23}H_{29}N_7O_5$: 483.2230; found: 483.2234.

***N*-{6-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]nicotinoyl}-L-glutamic acid (2b).** Hydrolysis of **10b** (0.10 g, 0.2 mmol) as described above for the preparation of **2a**, gave **2b** (58 mg, 68%) as a light yellow solid, mp 253~255 °C. MS (ESI): *m/z* 428 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.46 (s, 1H, 7-H), 8.60 (d, 1H, *J*=2.4Hz, pyridine 2-H), 8.36 (d, 2H, *J*=7.5Hz, CONH), 7.77 (dd, 1H, *J*=2.4, 8.4Hz, pyridine 4-H), 7.32 (d, 1H, *J*=8.4Hz, pyridine 5-H), 6.38 (s, 1H, 6-H), 6.20 (s, 2H, 4-NH_2), 5.54(br s, 2H, 2- NH_2), 4.35 (m, 1H, glutamate α-CH), 3.30~4.90 (br s, 2H, 2COOH), 2.95 (m, 4H, 5-CH_2CH_2), 2.32 (t, 2H, *J*=6.9Hz, glutamate γ-CH_2), 2.00 (m, 2H, glutamate β-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 175.0, 174.5, 166.7, 159.5, 158.3, 154.4, 146.1, 132.3, 129.0, 127.9, 115.5, 114.5, 96.0, 53.0, 36.5, 31.8, 28.3, 27.3. HRMS: Calcd for $C_{19}H_{21}N_7O_5$: 427.1604; found: 427.1612.

Measurement of cytotoxicity (*in vitro* cell culture screening).

Cell culture: The target compounds **2a** and **2b** were evaluated for their cytotoxic activity against L1210 and A549 cells respectively.[4]

Methods[10]**:** The cell viability was estimated by the MTT method. Cells were plated into 96-well tissue culture plates at a density of 4~5×10^3 cells/well and incubated for 24 h under hydroxic conditions. They were treated with the target compounds and incubated for another 48 h. Twenty microliters MTT (5 μg/mL in PBS) was added to the culture medium. After 4 h, the blue reaction product was yielded. The

medium was removed and the residual product was dissolved with DMSO (100 μL). The absorbance was measured with Thermo Multiskan MK3 at 570nm.

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