HETEROCYCLES, Vol. 78, No. 9, 2009, pp. 2353 - 2360. © The Japan Institute of Heterocyclic Chemistry
Received, 3rd April, 2009, Accepted, 14th May, 2009, Published online, 18th May, 2009.
DOI: 10.3978/COM-09-11725

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

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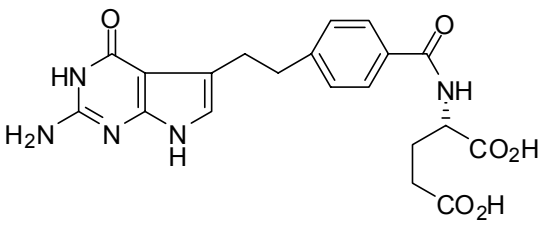
Abstract – Two analogues of pemetrexed with its phenyl ring replaced with pyridine ring as novel anticancer agents were synthesized. Preliminary *in vitro* evaluation indicated that replacement of the phenyl moiety of pemetrexed by the pyridine ring with the 6-5 bicyclic ring system showed low cytotoxicity,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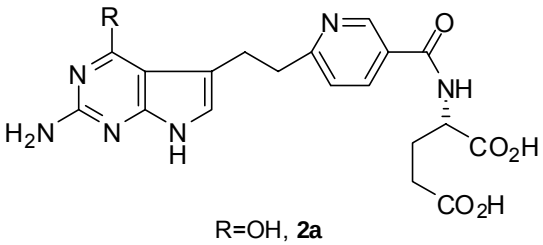
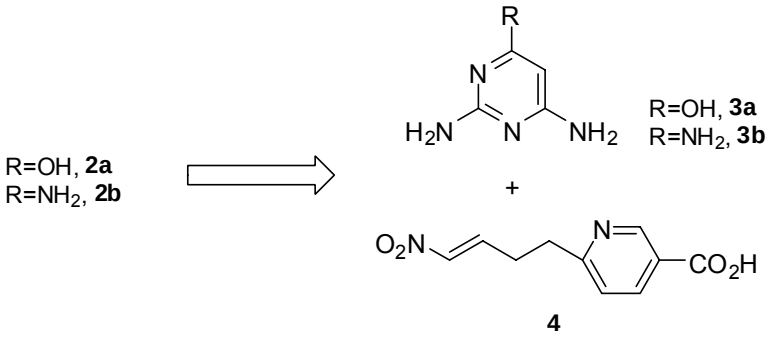


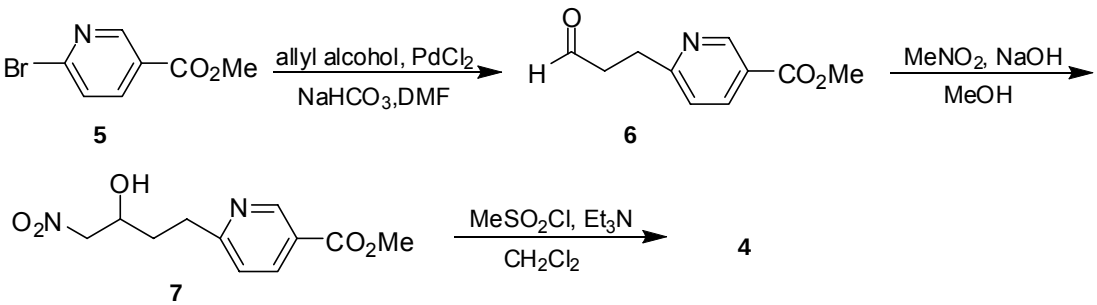
R=OH, **2a**
R=NH_2, **2b**

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]



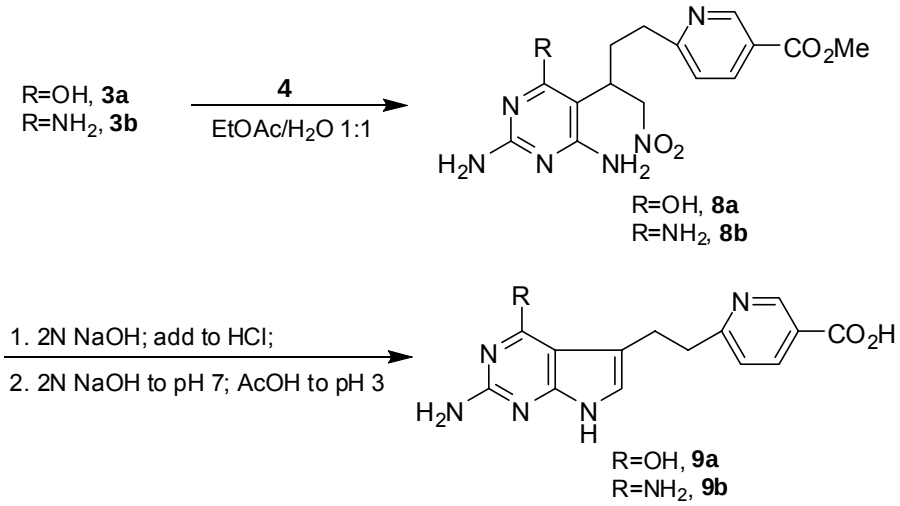
Scheme 1

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.



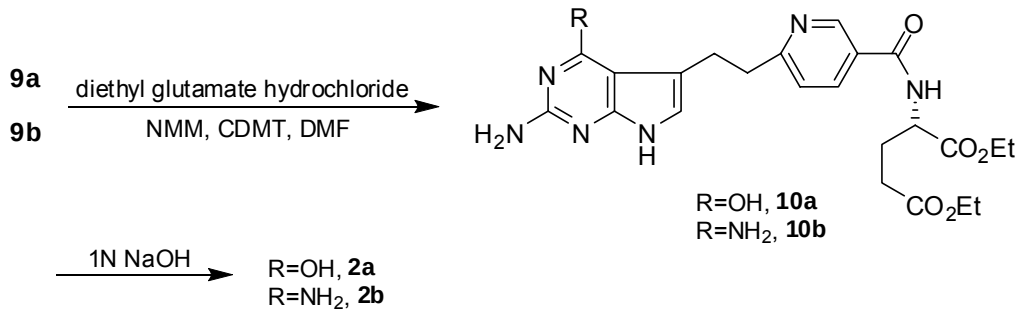
Scheme 2

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-CH_2CH_2), 2.62 (q, 2H, *J*=7.5Hz, 6-CH_2CH_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-$CHCH_2CH_2$), 2.13, 1.70 (2m, 2H, 5-$CHCH_2CH_2$). ^{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_2CH_2), 2.83 (t, 2H, *J*=6.9Hz, 5-CH_2CH_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.

ACKNOWLEDGEMENTS

We would thank to the funds of Shanghai Science and Technology Committee for the financial support (042319231). We are grateful to School of Chemistry and Chemical Engineering, Shanghai Jiaotong University, Shanghai Institute of Planned Parenthood Research and the Instrumental Analysis Center of East China University of Science and Technology for providing all the spectral data.

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