Supplementary Information

Structure based design and synthesis of antiparasitic pyrrolopyrimidines targeting pteridine reductase 1

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Synthetic Chemistry

General

^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX- 400 MHz spectrometer with chemical shifts given in ppm (δ values), relative to proton and carbon traces in solvent. Coupling constants are reported in Hz. IR spectra were recorded on Shimadzu, IRAffinity-1 spectrometer. Elemental analysis was carried out on a Perkin Elmer 2400, analyser series 2. Accurate mass was measured using Thermo Exactive MS and a Thermo U3000 HPLC system. Ionisation is carried out by an ESI source (not heated). Anhydrous solvents were obtained from a Puresolv purification system, from Innovative Technologies, or purchased as such from Aldrich. Melting points were recorded on a Reichert hot-stage microscope, and are uncorrected. Chromatography was carried out using 200–400 mesh silica gels, or using reverse-phase HPLC on a Waters system using a C18 Luna column.

Declaration of Purity: All final compounds were equal or more than 95% pure by HPLC

Contents:

Synthesis of:

***N*-[3-(2-Amino-5-cyano-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)phenyl]methanesulfonamide 3a**

2-Amino-6-bromo-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (0.108 g, 0.43 mmol), 3-(methylsulfonylaminobenzene)boronic acid (0.140 g, 0.65 mmol), $Pd(PPh_3)_4$ (0.049 g, 0.042 mmol) in isopropanol:H_2O (3:1.5 mL) was degassed under nitrogen. *t*-Butylamine (0.18 mL, 1.71 mmol) was added and the reaction mixture was irradiated with microwaves at 160°C for 40 minutes. The resulting solution was washed with ethyl acetate, water and brine. Organics were filtered through a pad of Celite and concentrated under reduced

pressure. The resulting solid was triturated with hot methanol, acetone and ether to afford the title compound as a brown solid (15 mg, 10%). ^{1}H NMR (DMSO-d_6): 3.10 (3H, s), 6.49 (2H, br, s), 7.23 (1H, m), 7.48 (2H, m), 7.63 (1H, s), 10.05 (1H, br, s), 10.84 (1H, br, s), 12.57 (1H, br, s). IR: 1316, 1422, 1563, 1581, 1624, 1756, 2220, 3175, 3320, 3337 cm^{-1} HRESIMS: Found: 343.0621 Calculated for $C_{14}H_{11}O_3N_6S$ 343.0619.

2-Amino-6-[3-(methylsulfonyl)phenyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3b

2-Amino-6-bromo-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (0.105 g, 0.42 mmol), 3-(methylsulfonylbenzene)boronic acid (0.129 g, 0.64 mmol), $Pd(PPh_3)_4$ (0.041 g, 0.035 mmol) in isopropanol:H_2O (3:1.5 mL) was degassed under nitrogen. *t*-Butylamine (0.18 mL, 1.71 mmol) was added and the reaction mixture was irradiated with microwaves at 160 °C for 40 minutes. The resulting solution was washed with ethyl acetate, water and brine. Organics were filtered through a pad of Celite and concentrated under reduced pressure. The resulting solid was triturated with hot methanol, acetone and diethyl ether to afford the title compound as an off-white solid (74 mg, 55%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 3.27 (3H, s), 6.76 (2H, br, s), 7.78 (1H, t, J = 7.7 Hz), 7.91 (1H, d, J = 7.5 Hz), 8.17 (1H, d, J = 7.5 Hz), 8.37 (1H, s), 10.87 (2H, br, s). IR: 1315, 1421, 1566, 1580, 1627, 1760, 2220, 3177, 3322, 3338 cm^{-1} HRESIMS: Found: 328.0513 Calculated for $C_{14}H_{10}O_3N_5S$ 328.0509.

2-Amino-4-oxo-6-[(*E*)-2-phenylethenyl]-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3c

(*E*)-2-Phenylethenylboronic acid (93 mg, 0.630 mmol), 2-amino-6-bromo-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (80 mg, 0.315 mmol) and cesium carbonate (513 mg, 1.575 mmol) were suspended in isopropanol:water (2:1) (6 mL) to which (1,1'-bis(diphenylphosphino)ferrocene)-dichloropalladium(II) [Pd(dppf)Cl_2] (29 mg, 0.0395 mmol) was added. The reaction mixture was purged with nitrogen for 20 min then heated overnight at 100 ^{o}C [oil bath temperature] in a sealed tube. Silica gel was added to the reaction mixture and the solvents were removed under reduced pressure. The residue was partitioned between ethyl acetate and water and then the organic layer was collected and the solvent removed. The crude material was purified by HPLC. Fractions containing the required material were collected and freeze-dried to give the desired product as a yellow solid (20 mg, 23%), mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6): 12.28 (1H, s), 10.73 (1H, s), 7.55 (2H, d, J = 7.4 Hz), 7.42-7.30 (4H, m), 6.99 (1H, d, J = 16.4 Hz), 6.50 (2H, br). IR (KBr): 2209, 1701, 1609, 1580, 1488, 1419, 1273, 1037, 954, 791, 753, 695 cm.$^{-1}$ HRESIMS: Found: 278.1039 calculated for $C_{15}H_{12}N_5O$ 278.1036.

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

(a) 2-Chloro-3-oxopropanenitrile[1]

While the temperature was maintained between 0 and 5 ^{o}C, methyl formate (4.45 mL, 4.317 g; 71.9 mmol) was added to a stirred mixture of sodium methoxide (3.600 g, 66.6 mmol) in toluene (100 mL, dry). This was followed by dropwise addition of chloroacetonitrile (4.2 mL, 5.011 g; 66.6 mmol) over a period of 1 h. The mixture was left stirring for an additional 3 h. Water (100 mL) was added, and the mixture was extracted with ethyl acetate (100 mL). The aqueous layer was cooled to 0 ^{o}C and acidified to pH 4 using concentrated hydrochloric acid. This was extracted with ethyl acetate (3 x 100 mL), and the combined organic layers was dried (Na_2SO_4)

and the solvent was removed under reduced pressure to give the crude product (2.620 g, 38%) as brown oil, which was used in the next step without further purification.

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

Sodium acetate (3.10 g, 37.8 mmol, 3.3 eq.) was dissolved in water (40 mL) to which pyrimidine-2,4,6-triamine (1.427 g, 11.40 mmol) was added. The reaction mixture was heated to 100 ^{o}C for a few minutes. The solution became homogeneous. 2-Chloro-3-oxopropanenitrile (1.18 g, 14.2 mmol, 1.2 eq.) was dissolved in water (20 mL) then the aqueous solution was added to the reaction mixture. The reaction mixture was heated to 100 ^{o}C with stirring for 17 h. The reaction mixture was cooled to 0 ^{o}C and the brown precipitate was filtered, washed with water and acetone and dried to give (0.640 g, 32%) as dark brown solid. When the filtrate was left at room temperature overnight an additional amount (77 mg) was obtained as light brown solid (0.717 g, 36%) mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.79 (1H, br, NH, exchangeable), 7.69 (1H, s), 6.14 (2H, br, NH_2, exchangeable), 5.86 (2H, NH_2, exchangeable). IR (KBr): 2217, 1629, 1582, 1529, 1480, 1410, 1187, 1100, 831, 807, 790 cm.$^{-1}$ HRESIMS: Found: 175.0727 calculated for $C_7H_7N_6$ 175.0727

2,4-Diamino-6-(3-formylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 6a

3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (172 mg, 0.74 mmol), 2,4-diamino-6-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile and cesium carbonate (619 mg, 1.9 mmol) were suspended in isopropanol:water (2:1) (6 mL) to which (1,1'-bis(diphenylphosphino)ferrocene)-dichloropalladium(II) [Pd(dppf)Cl_2] (27 mg, 0.037 mmol) was added. The reaction mixture was purged with nitrogen for 20 min then heated overnight at 100 ^{o}C [oil bath temperature] in a sealed tube. Solvents were removed under reduced pressure and the residue was applied to a silica gel column chromatography and eluted with [1] *n*-hexane [2] ethyl acetate [3] methanol:ethyl acetate [10%]. The required product was obtained as a yellow solid (41 mg, 20%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 12.45 (1H, br), 10.09 (1H, s), 8.39 (1H, t, J = 1.6 Hz), 8.18 (1H, d, J = 7.7 Hz), 7.99 (1H, d, J = 7.7 Hz), 7.81 (1H, t, J = 7.7 Hz), 6.30 (2H, br), 6.07 (2H, br). IR (KBr): 3435, 2212, 1691, 1622, 1579, 1488, 1416, 1200, 792, 741 cm^{-1}.

2,4-Diamino-6-(phenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 6c

4,4,5,5-Tetramethyl-2-(phenylethynyl)-1,3,2-dioxaborolane [alternative name: 2-phenyl-1-ethynylboronic acid pinacol ester] (180 mg, 0.790 mmol, 2 mol eq), 2,4-diamino-6-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (100 mg, 0.395 mmol) and *t*-butylamine [145 mg, 1.975 mmol, 5 mol eq] Pd(dppf)Cl_2 (29 mg, 0.0395 mmol, 10% mol eq) in isopropanol:water (6 mL, 4:2). The reaction mixture was purged with N_2 for 20 min then heated overnight at 100 ^{o}C [oil bath temperature] in a sealed tube. The reaction mixture was filtered over cotton and the solvents were removed under reduced pressure. This material was purified by HPLC. Fractions containing the desired material were collected and freeze-dried to give required product as a pale yellow solid with no distinct melting point (20 mg, 22%). ^{1}H NMR (DMSO-d_6): 12.89 (1H, br), 7.60-6.89 (9H, m). IR: 2210, 1630, 1602, 1541, 1487, 1378, 1178, 1112, 1013, 786, 754 cm.$^{-1}$ HRESIMS: Found: 275.1044 Calculated for: $C_{15}H_{11}N_6$ 275.1040.

2,4-Diamino-6-[(*E*)-2-(4-methylphenyl)ethenyl]-7*H*-pyrrolo[2,3-*d*] pyrimidine-5-carbonitrile 6d

(*E*)-2-(4-Methylphenyl)ethenylboronic acid (130 mg, 0.790 mmol, 2 mol eq), 2,4-diamino-6-bromo-7*H*-pyrrolo[2,3-d]pyrimidine-5-carbonitrile (100 mg, 0.395 mmol) and *t*-butylamine [208

mg, 2.00 mmol, 5 mol eq,] Pd(dppf)Cl_2 (29 mg, 0.0395 mmol, 10% mol eq) in isopropanol:water (6 mL, 4:2). The reaction mixture was purged with N_2 for 20 min then heated in a microwave oven at 140 ^oC for 40 min. The reaction mixture was filtered over cotton and the solvents were removed under reduced pressure. The crude product was applied to a silica gel column chromatography using methanol:ethyl acetate 1:10 [R_F = 0.5]. The required product was obtained as light brown solid (17 mg, 15%) with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 12.08 (1H, s), 7.46 (2H, d, *J* = 8.1 Hz), 7.37 (1H, d, *J* = 16.4 Hz), 7.23 (2H, d, *J* = 8.1 Hz), 6.99 (1H, d, *J* = 16.4 Hz), 6.19 (2H, s, br), 5.99 (2H, s, br), 2.32 (3H, s). IR (KBr): 2209, 1705, 1610, 1582, 1490, 1416, 1273, 1040, 954, 793, 755, 693 cm.$^{-1}$ HRESIMS: Found: 291.1349 Calculated for $C_{16}H_{15}N_6$ 291.1353.

2,4-Diamino-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 6e

2,4-Diamino-6-[(*E*)-2-phenylethenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (24 mg, 86.9 mmol) was dissolved in methanol (25 mL), cooled to 0 ^oC. Pd:C-10% (20 mg) was added under N_2 with stirring. The reaction mixture was hydrogenated for 3 h at room temperature and atmospheric pressure. The catalyst was removed over Kieselguhr and the solvent was removed under reduced pressure. The crude material obtained was purified by HPLC. Fractions containing the required product were collected and freeze-dried to give the pure product as a white solid (9 mg, 26%) with no distinct melting point. ^{1} ^{1}H-NMR (DMSO-d_6): 12.45 (1H, br, s), 7.43-7.29 (2H, br), 7.28-7.18 (5H, m), 7.17-6.98 (2H, br), 3.02-2.98 (4H, m). IR (KBr): 2209, 1700, 1604, 1581, 1489, 1416, 1270, 1040, 955, 793, 754, 696 cm. HRESIMS: Found: 279.1349 Calculated for $C_{15}H_{15}N_6$ 279.1353.

4-(4-Thiomorpholinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine 8b

A mixture of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (100 mg, 0.593 mmol), thiomorpholine (109 mg, 1.06 mmol, 100 µL, d1.088) and triethylamine (63 mg, d0.726, 87 µL, 0.720 mmol) were dissolved in 1,4-dioxane (2 mL, dry). The reaction mixture was heated in a microwave reactor for 1 h at 200 ^oC. The reaction mixture was diluted with methanol then silica gel was added. Solvents and excess reagents were removed under reduced pressure. The residue was applied to a silica gel column and eluted with methanol:ethyl acetate (1:9), R_F = 0.1. The required product was obtained as light brown solid (60 mg, 43%), mp > 230 ^oC. ^{1}H NMR (DMSO-d_6): 10.84 (1H, s), 6.75 (1H, q, *J* = 2.3 Hz), 6.28 (1H, q, *J* = 1.8 Hz), 5.51 (2H, s), 4.07 (4H, qt, *J* = 2.8 Hz), 2.64 (4H, qt, *J* = 2.8 Hz). IR (KBr): 1598, 1562, 1493, 1475, 1436, 1357, 1311, 1071, 952, 814, 787 cm.$^{-1}$ HRESIMS: Found: 236.0964 Calculated for $C_{10}H_{14}N_5S$ 236.0964.

***N*-(4-Chloro-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide 9**

Dimethylaniline (3.3 ml, 26.03 mmol), TEBACl (0.731 g, 3.2 mmol), and $POCl_3$ (5.5 ml, 60 mmol) were added to a stirred suspension of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (1.570 g, 6.09 mmol) at room temperature under nitrogen. The resultant suspension was heated to 90 ^oC for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to give brown oil. Ice was added to the resultant oil and the pH adjusted to 4 using 7N ammonia in methanol resulting in the precipitation of a brown solid. The resultant solid was filtered and washed with water and ether to give the required product as a brown solid (0.700 g, 41%), mp > 300 ^oC. ^{1}H NMR (DMSO-d_6): 1.24 (9H, s), 7.93 (1H, d, *J* = 2.5 Hz), 10.97 (1H, s), 12.57 (1H, s). IR: 1280, 1560, 1657,

1703, 2229, 2835, 2975, 3301 cm^{-1}. LRESIMS: Found: 276.00 calculated for $C_{12}H_{12}ClN_5O$ 277.07.

Leading to 12b

6-Isopropoxy-2,4-pyrimidinediamine[3]

6-Chloro-2,4-pyrimidinediamine (1.037 g, 7.17 mmol) was dissolved in isopropanol (25 mL) to which sodium hydride (1.062 g, 60% in oil) was added. The reaction mixture was heated overnight under nitrogen. The reaction mixture was cooled to 0 ^oC then diluted with brine and extracted with ethyl acetate. The organic layer was collected, dried (Na_2SO_4), filtered and the solvents removed under reduced pressure. The crude material obtained was applied to a silica gel column chromatography and the product was eluted with ethyl acetate, R_F = 0.1. The required product was obtained as white crystalline material (0.760 g, 63%), mp 106-108 ^oC; [Lit. mp[3] 105-107 ^oC.] ^{1}H-NMR (DMSO-d_6): 5.92 (2H, s), 5.77 (2H, s), 5.15 (1H, septet, J = 6.2Hz), 4.98 (1H, s), 1.20 (6H, d, J = 6.2Hz). IR (KBr):1574, 1555, 1532, 1495, 1427, 1408, 1366, 1346, 1238, 1097, 961, 889, 793 cm^{-1} HRESIMS: Found: 169.1088 Calculated for $C_7H_{13}N_4O$ 169.1084.

***N*-(5-Cyano-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide 12a**

(a) Sodium methoxide (95 mg, 3.6 mmol) was added to a stirred suspension of *N*-(4-chloro-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide (0.100 g, 0.361 mmol) in methanol (10 mL, dry). The resultant solution was stirred at 80 ^oC for 48 h after which time the resultant off white solid was triturated with dilute HCl and filtered under reduced pressure and dried to give the required product as an off white solid (17 mg, 25%), mp > 300 ^oC. ^{1}H NMR (DMSO-d_6): 3.96 (3H, s), 6.43 (2H, s), 7.80 (1H, s), 12.06 (1H, s). ^{13}C NMR (DMSO-d_6): 53.71, 82.84, 95.88, 116.59, 130.77, 155.36, 161.17, 163.17. IR: 1394, 1483, 1489, 1591, 2227, 2846, 3364, 3467 cm^{-1}. HRESIMS: Found: 190.0723 Calculated for $C_8H_7N_5O$ 189.0710.

(b) Potassium hydroxide (0.295 g, 5.25 mmol) was added to a stirred suspension of *N*-(4-chloro-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide (0.100 g, 0.36 mmol) in methanol (5 ml, dry). The suspension was heated at 80 ^oC for 96 h after which time the resultant solid was triturated with HCl, filtered under reduced pressure and dried to give the required product as an off white solid (30 mg, 44%).

2-Amino-4-isopropoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 12b

Sodium (83 mg, 3.6 mmol) was added to a solution of isopropanol (7 mL, anhydrous) and stirred at room temperature under nitrogen for 2 h. The solution was heated until the solid and Na metal dissolved and *N*-(4-chloro-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide (0.100 g, 0.36 mmol) was added. The reaction mixture was heated to 90 ^oC for 24 h and concentrated under reduced pressure to give a brown solid. The resultant solid was dry loaded onto silica and purified by flash column chromatography using 100% ethyl acetate to give the required product as a white solid (70 mg, 90%), mp > 300 ^oC. ^{1}H NMR (DMSO-d_6): 1.34 (6H, d, J = 6.2 Hz), 5.39-5.45 (1H, m), 6.36 (2H, s), 7.78 (1H, s), 12.01 (1H, s). ^{13}C NMR (DMSO-d_6): 22.3, 68.64, 83.05, 96.11, 116.40, 130.49, 155.36, 161.23, 162.55 IR: 1314, 1420, 1562, 1580, 1625, 1759, 2220, 3176, 3320, 3336 cm^{-1}. HRESIMS: Found: 218.1035 Calculated for $C_{10}H_{11}N_5O$ 217.1010.

2-Amino-4-(cyclopentyloxy)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 12c

To a solution of cyclopentanol (7 mL, anhydrous), was added sodium metal (83 mg, 0.36 mmol). The solution was heated to 30 ^{o}C under nitrogen until the solid had dissolved, *N*-(4-chloro-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pivalamide (0.100 g, 0.36 mmol) was then added. The solution heated to 150 ^{o}C for 24 h and the solvent evaporated under reduced pressure to give an off white solid. The solid was dry loaded onto silica and purified by column chromatography using 0-10% methanol-ethyl acetate to give the required product as a white solid (53 mg, 59 %), mp > 300 ^{o}C. ^{1}H NMR (DMSO-d_6): 1.61-1.98 (9H, m), 5.55 (1H, m), 6.35 (2H, s), 7.75 (1H, s), 11.9 (1H, s). ^{13}C NMR (DMSO-d_6): 23.15, 32.36, 71.58, 77.54, 95.94, 115.81, 129.83, 154.78, 160.74, 162.10. IR: 1146, 1302, 1398, 1575, 1603, 2220, 3398, 3517 cm^{-1} HRESIMS: Found 244.1101 Calculated for $C_{12}H_{13}N_6O$ 244.1100.

N^4,N^4-Dimethyl-2,4,6-pyrimidinetriamine 25

6-Chloro-2,4-pyrimidinediamine (2.000 g, 13.83 mmol) was dissolved in DMF (6 mL, dry). The reaction mixture was heated at 185 ^{o}C for 18 h. The excess of DMF was removed *in vacuo* and the residue was dissolved in hydrochloric acid (2N, 10 mL) then made alkaline with 5% aqueous ammonia. The solution was extracted with chloroform. The organic layer was collected, dried (Na_2SO_4) and the solvent removed under reduced pressure. Trituration with ethyl acetate gave the required product (1.020 g, 48%) as pale yellow solid, mp 190-194 ^{o}C. ^{1}H NMR (DMSO-d_6): 5.67 (2H, s), 5.49 (2H, s), 4.93 (1H, s), 2.88 (6H, s). ^{13}C NMR (DMSO-d_6): 164.60, 163.86, 162.37, 73.22, 36.58. IR: 741, 760, 789, 883, 968, 1007, 1059, 1142, 1184, 1246, 1300, 1404, 1445, 1508, 1557, 1645 cm^{-1}. HRESIMS: Found: 154.1087 Calculated for $C_6H_{12}N_5$ 154.1087.

2,6-Diamino-5-[1-(4-methylphenyl)-2-nitroethyl]-4(3*H*)-pyrimidinone 26a

2,6-Diamino-3*H*-pyrimidin-4-one (1.16 g, 9.20 mmol) and 1-methyl-4-[(*E*)-2-nitroethenyl]benzene (1.71 g, 10.5 mmol) were suspended in a mixture of (20 mL) of water and (20 mL) of ethyl acetate at room temperature. The resulting mixture was heated for 18 h at 50 ^{o}C. The reaction mixture was left to cool to room temperature and solid material was formed; this was filtered, washed with small amount of ethyl acetate and dried to give the required product as light pink microcrystalline solid (2.50 g, 96%), mp 188-190 ^{o}C. ^{1}H NMR (DMSO-d_6): 9.72 (1H, s), 7.38 (2H, d, J = 8.1 Hz), 7.06 (2H, d, J = 8.1 Hz), 5.97 (2H, s), 5.85 (2H, s), 5.42 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 5.27 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 4.54 (1H, t, J = 7.6 Hz), 2.25 (3H, s). IR (KBr): 1676, 1631, 1589, 1483, 1553, 1437, 1372, 840, 790, 695 cm.$^{-1}$ HRESIMS: Found: 290.1243 calculated for $C_{13}H_{16}O_3N_5$ 290.1248.

2,6-Diamino-5-[1-(4-fluorophenyl)-2-nitroethyl]-4(3*H*)-pyrimidinone 26b

2,6-Diamino-3*H*-pyrimidin-4-one (1.16g, 9.20 mmol) and 1-fluoro-4-[(*E*)-2-nitroethenyl]benzene (1.75 g, 10.5 mmol) were suspended in a mixture of water (20 mL) and ethyl acetate (20 mL) at room temperature. The resulting mixture was heated for 18 h at 5 ^{o}C. The reaction mixture was left to cool to room temperature but no solid material was formed; the mixture was extracted with ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give a crude product (3.0 g, 100%) of product as light pink solid. Purification by column chromatography [1:9 methanol:ethyl acetate] afforded the pure material as white microcrystalline solid (2.22g, 82%), mp 110-115 ^{o}C. ^{1}H NMR (DMSO-d_6): 9.82 (1H, s), 7.57-7.54 (2H, m), 7.09 (2H, dd, J = 6.7 Hz & J = 9.0 Hz), 6.14 (2H, s), 6.07 (2H, s), 5.45 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 5.27 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 4.54 (1H, t, J = 7.6 Hz). IR

(KBr): 1622, 1547, 1506, 1436, 1375, 1342, 1226, 1161, 832, 787 cm.$^{-1}$ HRESIMS: Found: 294.0992 calculated for $C_{12}H_{13}O_3N_5F$ 294.0997.

2,6-Diamino-5-(2-nitro-1-phenylethyl)-4(3*H*)-pyrimidinone 26c

2,6-Diamino-3*H*-pyrimidin-4-one (1.16g, 9.20 mmol) and [(*E*)-2-nitroethenyl]benzene (1.56g, 10.5 mmol) were suspended in a mixture of water (20 mL) and ethyl acetate (20 mL) at room temperature. The resulting mixture was heated for 18 h at 50 ^{o}C. The reaction mixture was left to cool to room temperature solid material was formed, which was filtered, washed with small amount of ethyl acetate, and dried to give the required product as light pink microcrystalline solid (1.98 g, 78%), mp 140-142 ^{o}C ^{1}H NMR (DMSO-d_6): 9.81 (1H, s), 7.51 (2H, d, J = 7.2 Hz), 7.26-7.15 (3H, m), 6.08 (2H, s), 6.06 (2H, s), 5.48 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 5.32 (1H, dd, J = 8.0 Hz & J = 12.7 Hz), 4.57 (1H, t, J = 7.6 Hz). IR (KBr): 1675, 1629, 1589, 1549, 1484, 1437, 1373, 833, 770, 747, 703 cm.$^{-1}$ HRESIMS: Found: 276.1086 calculated for $C_{12}H_{14}O_3N_5$ 276.1091.

[(3*E*)-4-Nitro-3-butenyl]benzene – intermediate for (26d) and 28d[4]

To a solution of 1-nitro-4-phenyl-2-butanol (1.462 g, 7.50 mmol) in dichloromethane (20 mL, dry) at 0^{o}C was added methanesulfonyl chloride (1.719 g, 15.0 mmol) followed by addition of triethylamine (3.036 g, 30.0 mmol, dry). The mixture was warmed up to room temperature, stirred for 20 min, then poured into water (20 mL) and extracted with dichloromethane (25 mL). The organic layer was separated, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product as a yellow oil (1.250 g, 94%). TLC: R_F = 0.5 (9:1 ethyl acetate:*n*-hexane). ^{1}H NMR ($CDCl_3$): 7.34-7.23 (m, 5H), 7.18 (1H, d, J = 7.7 Hz), 6.96 (1H, d, J = 13.3 Hz), 2.84 (2H, t, J = 7.4 Hz), 2.60 (2H, dd, J = 7.5 & 14.7 Hz). IR: 1648, 1555, 1524, 1497, 1454, 1351, 1176, 953, 931, 750, 700 cm^{-1} HRESIMS: Found: 178.0865 Calculated for $C_{10}H_{12}NO_2$ 178.0868.

2,6-Diamino-5-[1-(nitromethyl)-3-phenylpropyl]-4(3*H*)-pyrimidinone 26d

[(3*E*)-4-Nitro-3-butenyl]benzene (0.620 g, 3.50 mmol) and 2,4,6-pyrimidinetriamine (0.441 g, 3.50 mmol) were dissolved in ethyl acetate (20 mL) and water (20 mL). The reaction mixture was heated in a sealed tube at 50 ^{o}C overnight. The organic layer was collected, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude material was purified by silica gel column chromatography using methanol:dichloromethane (1:9), R_F = 0.4. The required product was obtained as a pale yellow solid (0.640 g, 60%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 1.27 (1H, br), 7.29-7.17 (5H, m), 5.07-5.03 (1H, m), 4.88 (2H, s), 4.62-4.57 (3H, m), 3.15-3.11 (1H, m), 2.73-2.68 (1H, m), 2.61-2.55 (1H, m), 2.46-2.42 (1H, m), 1.94-1.89 (1H, m). IR (KBr): 1720, 1685, 1660, 1210, 1181, 1146, 763, 723, 703 cm.$^{-1}$ HRESIMS: Found: 304.1401 Calculated for $C_{14}H_{18}N_5O_3$ 304.1404.

2-Amino-5-(4-methylphenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 27a

To a solution of NaOH (0.500 g, 12.5 mmol) in water (5mL) was added (0.500 g, 1.73 mmol) of 2,6-diamino-5-[1-(4-methylphenyl)-2-nitroethyl]-4(3*H*)-pyrimidinone at room temperature. The mixture was stirred for 2 h, and then was slowly added to an aqueous solution of 1.37 g (14 mmol) of sulfuric acid (98%) in 5 mL of water at 0°C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried. The crude material was purified by HPLC,

fractions containing the required product were collected and freeze dried to give lilac colored solid (60 mg, 16%), mp > 230 °C. ^{1}H NMR (DMSO-d_6): 11.16 (1H, s), 10.31 (1H, s), 7.84 (2H, d, *J* = 8.0 Hz), 7.10 (2H, d, *J* = 8.0 Hz), 6.96 (1H, d, *J* = 2.4 Hz), 6.13 (2H, br), 2.29 (3H, s). IR (KBr): 1718, 1684, 1658, 1213, 1177, 1141, 833, 723, 707, 686 cm.$^{-1}$ HRESIMS: Found: 241.1083 calculated for $C_{13}H_{13}ON_4$ 241.1084.

2-Amino-5-(4-fluorophenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 27b

To a solution of NaOH (0.330 g, 8.25 mmol) in water (5mL) was added (0.450 g, 1.53 mmol) of 2,6-diamino-5-[1-(4-methylphenyl)-2-nitroethyl]-4(3*H*)-pyrimidinone at room temperature. The mixture was stirred for 2 h, and then was slowly added to an aqueous solution of 1.37 g (14 mmol) of sulfuric acid (98%) in 5 mL of water at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried. The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give lilac colored solid (70 mg, 19%), mp > 230°C. ^{1}H NMR (DMSO-d_6): 11.25 (1H, s), 10.42 (1H, s), 8.01 (2H, dd, *J* = 5.6 Hz & *J* = 8.9 Hz), 7.13 (2H, t, *J* = 9.0 Hz), 7.03 (1H, d, *J* = 2.4 Hz), 6.21 (2H, br). IR (KBr): 1721, 1682, 1655, 1505, 1209, 1186, 1137, 845, 724 cm.$^{-1}$ HRESIMS: Found: 245.0830 Calculated for $C_{12}H_{10}ON_4F$ 245.0833.

2-Amino-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 27c[5]

To a solution of NaOH (0.330 g, 8.25 mmol) in of water (5mL) was added (0.398 g, 1.53 mmol) of 2,6-diamino-5-(2-nitro-1-phenylethyl)-4(3*H*)-pyrimidinone at room temperature. The mixture was stirred for 2 h, and then was slowly added to an aqueous solution of 1.37 g (14 mmol) of sulfuric acid (98%) in 5 mL of water at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried. The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give lilac colored solid (50 mg, 15%), mp > 230 °C. ^{1}H NMR (DMSO-d_6): 11.26 (1H, s), 10.43 (1H, s), 7.95 (2H, dd, *J* = 1.4 Hz & *J* = 8.4 Hz), 7.31 (2H, t, *J* = 7.5 Hz), 7.16 (1H, t, 7.3 Hz), 7.03 (1H, d, *J* = 2.4 Hz), 6.25 (2H, br). IR (KBr): 1719, 1684, 1657, 1210, 1181, 1146, 763, 723, 705 cm.$^{-1}$ HRESIMS: Found: 227.0925 Calculated for $C_{12}H_{11}ON_4$.227.0927.

2-Amino-5-(2-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 27d

To a solution of NaOH (0.500 g, 12.5 mmol) in water (5mL) was added (0.444 g, 1.469 mmol) of 2,6-diamino-5-[1-(nitromethyl)-3-phenylpropyl]-4(3*H*)-pyrimidinone at room temperature. The mixture was stirred for 2 h, and then was slowly added to an aqueous solution of 1.37 g (14 mmol) of sulfuric acid (98%) in 5 mL of water at 0°C[the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried. The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give lilac colored solid (72 mg, 20%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 10.78 (1H, s), 10.47 (1H, s), 7.28-7.09 (6H, m), 6.38 (2H, d, *J* = 2.0 Hz), 2.92-2.81 (4H, m). IR (KBr): 1720, 1684, 1656, 1504, 1210, 1188, 1135, 844, 725 cm.$^{-1}$ HRESIMS: Found: 255.1244 Calculated for $C_{14}H_{15}N_4O$ 255.1240.

2-Amino-4-oxo-5-methylpyrrolo[2,3-*d*]pyrimidine 27e[6]

To a solution of 2,4-diamino-6-oxopyrimidine (5 g, 39 mmol) in DMSO (30 mL) was added 2-bromopropanal (5 mL, 48 mmol) and 10 mL of triethylamine (10 mL). The reaction mixture was stirred vigorously at room temperature for 1 h and then loaded on to a dry silica gel column. The column was eluted with chloroform (1.5 L) and then with chloroform:methanol (1.5L, 10:1). The fractions containing the product (TLC) were pooled and the solvent was evaporated to afford the required product (4.60 g, 71%), mp > 230 ^{o}C. TLC: R_f = 0.45 ($CHCl_3$:CH_3OH 5:1). ^{1}H NMR (DMSO-d_6): 10.59 (1H, s), 10.13 (1H, s), 6.33 (1H, q, J = 1.2 Hz), 5.99 (2H, br), 2.17 (3H, d, J = 1.1 Hz). IR (KBr): 1720, 1649, 1604, 1436, 1370, 1212, 1177, 1138, 757, 728, 699 cm.$^{-1}$ HRESIMS: Found: 165.0770 calculated for $C_7H_9ON_4$ 165.0771.

5-[1-(4-Methylphenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine 28a

2,4,6-Pyrimidinetriamine (0.575 g, 4.60 mmol) and 1-methyl-4-[(*E*)-2-nitroethenyl]benzene (0.856 g, 5.25 mmol) were suspended in a mixture of water (20 mL) and ethyl acetate (20 mL) at room temperature. The resulting mixture was left stirring at room temperature for 18 h. The reaction mixture was extracted with ethyl acetate, and dried (Na_2SO_4). The crude material obtained was purified by column chromatography using silica gel, methanol:ethyl acetate [1:9, R_F = 0.5] to give the required product as yellow solid (1.25 g, 94%), mp 100-103 ^{o}C. ^{1}H NMR (DMSO-d_6): 7.14 (4H, s), 5.50-5.3 (7H, m, containing 3 x NH_2, exchangeable), 5.14-4.99 (2H, m), 2.27 (3H, s). IR (KBr): 1616, 1568, 1439, 1375, 1250, 1042, 802 cm.$^{-1}$ HRESIMS: Found: 289.1405 Calculated for $C_{13}H_{17}N_6O_2$ 289.1408.

5-[1-(4-Fluorophenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine 28b

2,4,6-Pyrimidinetriamine (0.575 g, 4.60 mmol) and 1-fluoro-4-[(*E*)-2-nitroethenyl]benzene (0.877 g, 5.25 mmol) were suspended in a mixture of water (20 mL) and ethyl acetate (20 mL) at room temperature. The resulting mixture was left stirring at room temperature for 18 h. The reaction mixture was extracted with ethyl acetate, and dried (Na_2SO_4). The crude material obtained was purified by column chromatography using silica gel, methanol:ethyl acetate [1:9, R_F = 0.5] to give the required product as yellow solid (1.130 g, 84%), mp 104-107 ^{o}C. ^{1}H NMR (DMSO-d_6): 7.30 (1H, d, J = 8.5 Hz), 7.29 (1H, d, J = 8.5 Hz), 7.18 (2H, t, J = 8.9 Hz), 5.48-5.38 (7H, m, containing 3 x NH_2, exchangeable), 5.18-5.00 (2H, m). IR (KBr): 1618, 1567, 1508, 1440, 1376, 1228, 1162, 1042, 834, 798 cm.$^{-1}$ HRESIMS: Found: 293.1160 Calculated for $C_{12}H_{14}FN_6O_2$ 293.1157.

5-(2-Nitro-1-phenylethyl)-2,4,6-pyrimidinetriamine 28c

2,4,6-Pyrimidinetriamine (0.575 g, 4.60 mmol) and [(*E*)-2-nitroethenyl]benzene (0.783 g, 5.25 mmol) were suspended in a mixture of water (20 mL) and ethyl acetate (20 mL) at room temperature. The resulting mixture was left stirring at room temperature for 18 h. The reaction mixture was extracted with ethyl acetate, and dried (Na_2SO_4). The crude material obtained was purified by column chromatography using silica gel, methanol:ethyl acetate [1:9, R_F = 0.5] to give the required product as yellow solid (0.500 g, 40%), mp 110-113 ^{o}C (transparent). ^{1}H NMR (DMSO-d_6): 7.36-7.22 (5H, m), 5.48-5.41 (7H, m, containing 3 x NH_2, exchangeable), 5.18-5.04 (2H, m). IR (KBr): 1619, 1567, 1442, 1377, 1254, 1032, 802, 743, 701 cm.$^{-1}$ HRESIMS: Found: 275.1244 Calculated for $C_{12}H_{15}O_2N_6$ 275.1251.

5-[1-(Nitromethyl)-3-phenylpropyl]-2,4,6-pyrimidinetriamine 28d

[(3*E*)-4-Nitro-3-butenyl]benzene (0.627 g, 3.538 mmol) and 2,4,6-pyrimidinetriamine (0.443 g, 3.538 mmol) were dissolved in ethyl acetate (20 mL) and water (20 mL). The reaction mixture was heated in a sealed tube at 50 ^{o}C overnight. The organic layer was collected, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude material was purified by silica gel column chromatography using methanol:dichloromethane (1:9), R_F = 0.4. The required product was obtained as a pale yellow solid (0.510 g, 48%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 7.32 (2H, t, *J* = 7.3 Hz), 7.24 (1H, t, *J* = 7.3 Hz), 7.16 (2H, d, *J* = 7.3 Hz), 4.67-4.57 (2H, m), 4.51 (3H, br), 4.43 (3H, br), 3.48-3.42 (1H, m), 2.75-2.57 (2H, m), 2.09-2.08 (2H, m). IR: 1620, 1566, 1442, 1377, 1254, 1032, 802, 743, 703 cm.$^{-1}$ HRESIMS: Found: 303.1566 Calculated for $C_{14}H_{19}N_6O_2$303.1564.

5-(4-Methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 29a

To a solution of NaOH (0.330 g, 8.25 mmol) in water (5mL) was added (0.441 g, 1.53 mmol) of 5-[1-(4-methylphenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine at room temperature. The mixture was stirred for 2 h, and then was slowly added to a solution of sulfuric acid (98%, 1.37 g, 14 mmol) in water (5 mL) at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried to give the crude product as brown solid (400 mg). The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give lilac colored solid (30 mg, 13%), mp 210-213 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.90 (1H, s, NH, exchangeable), 7.34 (2H, d, *J* = 8.1 Hz), 7.29 (2H, d, *J* = 8.1 Hz), 7.20 (4H, br, 2 x NH_2 exchangeable), 7.03 (1H, d, *J* = 1.9 Hz), 2.36 (3H, s). IR (KBr): 1647, 1458, 1206, 1132, 831, 798, 771, 724 cm.$^{-1}$ HRESIMS: Found: 240.1239 Calculated for $C_{13}H_{14}N_5$ 240.1244.

5-(4-Fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 29b

To a solution of NaOH (0.500 g, 12.5 mmol) in water (5mL) was added (0.400 g, 1.39 mmol) of 5-[1-(4-fluorophenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine at room temperature. The mixture was stirred for 2 h, and then was slowly added to a solution of sulfuric acid (98%, 1.37 g, 14 mmol) in water (5 mL) at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried to give the crude product as brown solid (285 mg). The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give pale yellow solid (40 mg, 35%), mp 130-134 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.90 (1H, s, NH, exchangeable), 7.48-7.44 (2H, dd, *J* = 3.2 Hz & *J* = 8.7 Hz), 7.31 (2H, t, *J* = 9.0 Hz), 7.17 (4H, br, 2 x NH_2, exchangeable), 7.07 (1H, d, *J* = 1.9 Hz). IR (KBr): 1661, 1547, 1503, 1459, 1190, 1124, 844, 799, 725 cm.$^{-1}$ HRESIMS: Found: 244.0989 Calculated for $C_{12}H_{11}N_5F$ 244.0993.

5-Phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 29c[5]

To an aqueous solution of NaOH (0.330 g, 8.25 mmol) in 5 mL of water was added (0.441 g, 1.53 mmol) of 5-(2-nitro-1-phenylethyl)-2,4,6-pyrimidinetriamine at room temperature. The mixture was stirred for 2 h, and then was slowly added to a solution of sulfuric acid (98%, 1.37 g, 14 mmol) in water (5 mL) at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried to give the crude product as brown solid (285 mg). The crude material was purified by HPLC, fractions containing the required product were collected

and freeze dried to give pale yellow solid (50 mg, 29%), mp 200-203 ^{o}C ^{1}H NMR (DMSO-d_6): 11.91 (1H, s), 7.48-7.34 (5H, m), 7.20 (4H, br), 7.08 (1H, d, *J* = 1.8 Hz.) IR (KBr): 1694, 1651, 1543, 1453, 1390, 1207, 1131, 826, 800, 759, 725 cm.$^{-1}$ HRESIMS: Found: 226.1082 Calculated for $C_{12}H_{12}N_5$ 226.1087.

5-(2-Phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 29d

To a solution of NaOH (0.500 g, 12.5 mmol) in water (5 mL) was added (0.444 g, 1.469 mmol) of 5-[1-(nitromethyl)-3-phenylpropyl]-2,4,6-pyrimidinetriamine at room temperature. The mixture was stirred for 2 h and then was slowly added to a solution of sulfuric acid (98%, 1.37 g, 14 mmol) in water (5 mL) at 0 °C [the sequence of the addition is important]. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solid material was filtered, washed with water and dried. The crude material was purified by HPLC, fractions containing the required product were collected and freeze dried to give lilac colored solid (72 mg, 20%) with no distinct melting point. ^{1}H NMR (DMSO-d_6): 11.45 (1H, s), 7.82 (2H, br), 7.29-7.12 (7H, m), 6.69 (1H, s), 3.02 (2H, t, *J* = 8.3 Hz), 2.90 (2H, t, *J* = 8.3 Hz). IR: 1650, 1455, 1209, 1133, 833, 800, 777, 725 cm.$^{-1}$ HRMS: Found: 254.1396 calculated for $C_{14}H_{16}N_5$ 254.1400.

5-[1-(4-Fluorophenyl)-2-nitroethyl]-6-isobutoxy-2,4-pyrimidinediamine 30a

6-Isobutoxy-2,4-pyrimidinediamine (195 mg, 1.070 mmol) and 1-fluoro-4-[(*E*)-2-nitroethenyl]benzene (179 mg, 1.070 mmol) were dissolved in ethyl acetate (20 mL). The reaction mixture was placed in a sealed tube and heated at 50 ^{o}C for 2 d. Solvent was removed under reduced pressure and the crude material was applied to a silica gel column chromatography. Elution with ethyl acetate:*n*-hexane (1:1, R_F = 0.1) gave the required product (240 mg, 64%) as pale yellow solid, mp 145-147 ^{o}C. ^{1}H-NMR (DMSO-d_6):7.35 (2H, dd, *J* = 8.8 Hz & *J* = 5.5 Hz), 7.13 (2H, t, *J* = 8.8 Hz), 6.14 (2H, s), 5.84 (2H, s), 5.32-5.17 (2H, m), 4.89 (1H, t, *J* = 8.0 Hz), 3.93 (2H, 2xd, *J* = 6.7 Hz & *J* = 6.7 Hz), 1.97 (1H, septet, *J* = 6.7 Hz), 0.89 (6H, 2xd, J_1 = 6.7 Hz & J_2 = 6.7 Hz). IR (KBr): 1619, 1566, 1510, 1441, 1376, 1228, 1015, 800 cm^{-1} HRESIMS: Found: 350.1621 Calculated for $C_{16}H_{21}O_3N_5F$ 350.1623.

***N*^{4}-Cyclohexyl-5-[1-(4-fluorophenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine 30b**

N^4-Cyclohexyl-2,4,6-pyrimidinetriamine (200 mg, 0.965 mmol) and 1-fluoro-4-[(*E*)-2-nitroethenyl]benzene (161 mg, 0.965 mmol) were dissolved in ethyl acetate (20 mL). The reaction mixture was placed in a sealed tube and heated at 50 ^{o}C for 2 d. Solvent was removed under reduced pressure and the crude material was applied to a silica gel column chromatography. Elution with ethyl acetate (R_F = 0.1) gave the required product (210 mg, 58%) as light brown solid, mp 77-77 ^{o}C. Some of this material was further purified by HPLC to give the required material as white solid. ^{1}H-NMR (DMSO-d_6): 11.10(1H, br), 7.37-7.19 (6H, m), 6.81 (2H, s), 6.25 (2H, br), 5.63-5.55 (1H, m), 5.24-5.16 (2H, m), 3.89 (1H, br), 1.52-1.17 (10H, m). IR (KBr): 1618, 1567, 1508, 1440, 1376, 1228, 1015, 797 cm^{-1} HRESIMS: Found: 375.1936 Calculated for $C_{18}H_{24}O_2N_6F$ 375.1939.

5-(4-Fluorophenyl)-4-isobutoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine 31a

5-[1-(4-Fluorophenyl)-2-nitroethyl]-6-isobutoxy-2,4-pyrimidinediamine (105 mg, 0.301 mmol) was dissolved in a solution of NaOH (215 mg, 5.375 mmol) in water (25 mL) at room temperature with stirring. The stirring was continued for 2 h at room temperature. Sulfuric acid

(98%, 0.700 g, 7.00 mmol) was diluted with water (5 mL) and cooled to 0 ^{o}C. The sodium hydroxide solution was added to the acid dropwise with stirring at 0 ^{o}C, after which time it was left stirring at room temperature overnight. The white solid material formed was collected by filtration. This material was purified by HPLC. The starting material was recovered as well as the required product (5 mg, 4%) as white solid after freeze drying with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.41 (1H, s), 7.66 (2H, dd, *J* = 8.7 Hz, *J* = 5.7 Hz), 7.16 (2H, t, *J* = 8.9 Hz), 7.07 (1H, d, *J* = 2.15 Hz), 4.14 (2H, d, *J* = 6.4 Hz), 1.98 (1H, septet, *J* = 6.7 Hz), 0.91 (6H, d, *J* = 6.7 Hz). IR (KBr): 1720, 1686, 1658, 1213, 1179, 1140, 833, 727, 705, 688 cm.$^{-1}$ HRESIMS: Found: 301.1454 calculated for $C_{16}H_{18}ON_4F$ 301.1459.

***N*^{4}-Cyclohexyl-5-(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 31b**

N^4-Cyclohexyl-5-[1-(4-fluorophenyl)-2-nitroethyl]-2,4,6-pyrimidinetriamine (150 mg, 0.400 mmol) was dissolved in a solution of NaOH (215 mg, 5.375 mmol) in water (25 mL) at room temperature with stirring. The stirring was continued for 2 h at room temperature. Sulfuric acid (98%, 0.700 g, 7.00 mmol) was diluted with water (5 mL) and cooled to 0 ^{o}C. The sodium hydroxide solution was added to the acid dropwise with stirring at 0 ^{o}C, after which time it was left stirring at room temperature overnight. The white solid material formed was collected by filtration. This material was purified by HPLC. The starting material was recovered as well as the required product (34 mg, 19%) as white solid after freeze drying with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.93 (1H, s), 7.52-7.28 (6H, m), 7.04 (1H, s), 5.97 (1H, br, s), 4.41 (1H, m), 1.87-1.21 (10H, m). IR (KBr): 1718, 1684, 1658, 1213, 1177, 1141, 833, 723, 707, 686 cm.$^{-1}$ HRESIMS: Found: 326.1779 Calculated for $C_{18}H_{21}FN_5$ 326.1776.

Leading to 34d, 35c, 36a

1,2-Bis(4-fluorophenyl)ethanone[7]

Aluminium chloride (4.635 g, 34.76 mmol, 1.2 mol eq) was added to fluorobenzene (13.92g, 144.85 mmol, 5.0 mol eq) with stirring and cooling with ice-water, under nitrogen. 2-chloro-1-(4-fluorophenyl)ethanone (5.00 g, 28.97 mmol, 1.05 mol eq) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was heated at 50 ^{o}C for 5 h, after which time the reaction mixture was left stirring at room temperature for 9 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture onto crushed ice (50 g) and extracting the resulting suspension with 2N aqueous HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give solid material which was washed with *n*-hexane. The desired material (6.260 g, 93%) was obtained as a white crystalline solid, mp 100-102 ^{o}C [Lit. mp 94-96 ^{o}C][7], R_F = 0.4 (1:9 ethyl acetate:*n*-hexane). ^{1}H NMR (DMSO-d_6): 8.16 (2H, dd, *J* = 8.9 Hz & *J* = 5.6 Hz), 7.40 (2H, t, *J* = 8.9 Hz), 7.32 (2H, dd, *J* = 8.7 Hz & *J* = 5.7 Hz), 7.17(2H, t, *J* = 8.9 Hz), 4.42 (2H, s). IR: 709, 721, 744, 792, 829, 860, 989, 1093, 1147, 1190, 1234, 1332, 1411, 1500, 1593, 1678 cm.$^{-1}$ HRESIMS: Found: 233.0775 Calculated for $C_{14}H_{11}F_2O$ 233.0772.

2-Bromo-1,2-bis(4-fluorophenyl)ethanone[8] 33d

1,2-Bis(4-fluorophenyl)ethanone (5.46 g, 23.51 mmol) was dissolved in chloroform (58 mL) to which hydrobromic acid solution 30% in acetic acid (0.140 mL, 1 mol eq) was added at room temperature with stirring. Bromine (1.32 mL) dissolved in chloroform (5 mL) and added to the

reaction mixture dropwise with stirring. At the end of the reaction, a slight bromine coloration should remain. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (6.460 g, 88%) as a colorless oil. R_F = 0.4 [1:9 ethyl acetate:*n*-hexane]. ^{1}H NMR (DMSO-d_6): 8.19 (2H, dd, *J* = 5.5 Hz & 8.7 Hz), 7.62 (2H, dd, *J* = 5.5 Hz & 8.7 Hz), 7.40 (2H, t, *J* = 8.7 Hz), 7.25 (2H, t, 8.7 Hz), 7.19 (1H, s). IR: 683, 717, 735, 752, 791, 822, 833, 852, 993, 1097, 1157, 1192, 1213, 1288, 1300, 1408, 1504, 1593, 1688 cm.$^{-1}$ HRESIMS: Found: 310.9878 Calculated for $C_{14}H_{10}{}^{79}BrF_2O$ 310.9878

Leading to 34e, 35d, 36b

1,2-Bis(4-chlorophenyl)ethanone[9]

Aluminium chloride (16.00 g, 0.119 mol) was added to chlorobenzene (50 mL) with stirring and cooling with ice-water, under nitrogen. 2-chloro-1-(4-chorophenyl)ethanone (14 mL) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was heated at 50 ^{o}C for 5 h, after which time the reaction mixture was left stirring at room temperature for 9h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture onto crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give solid material which was washed with *n*-hexane. The desired material (24.79 g, 98%) was obtained as a white crystalline solid, mp 113-115 ^{o}C [Lit. mp 114-115 ^{o}C][9], R_F = 0.4 (1:9 ethyl acetate:*n*-hexane). ^{1}H NMR (DMSO-d_6): 8.05 (2H, d, *J* = 8.5 Hz), 7.62 (2H, d, *J* = 8.5 Hz), 7.38 (2H, d, *J* = 8.5 Hz), 7.28 (2H, d, *J* = 8.5 Hz), 4.42 (2H, s). IR: 709, 721, 744, 792, 829, 860, 989, 1093, 1147, 1190, 1234, 1332, 1411, 1500, 1593, 1678 cm.$^{-1}$ HRESIMS: Found: 265.0180 Calculated for $C_{14}H_{11}{}^{35}Cl_2O$ 265.0181.

2-Bromo-1,2-bis(4-chlorophenyl)ethanone[10] 33e

1,2-Bis(4-chlorophenyl)ethanone (5.50 g, 20.70 mmol) was dissolved in chloroform (60 mL) to which hydrobromic acid solution 30% in acetic acid (0.140 mL, 1 mol eq) was added at room temperature with stirring. Bromine (1.32 mL) dissolved in chloroform (5 mL) and added to the reaction mixture dropwise with stirring. At the end of the reaction, a slight bromine coloration should remain. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (7.05 g, 99%) as a pale yellow oil. R_F = 0.4 [1:9 ethyl acetate:*n*-hexane]. ^{1}H NMR ($CDCl_3$): 7.96 (2H, d, *J* = 8.7 Hz), 7.50 (2H, d, *J* = 8.2 Hz), 7.48 (2H, d, *J* = 8.4 Hz), 7.39 (2H, d, *J* = 8.7 Hz), 6.26(1H, s). ^{13}C NMR ($CDCl_3$): 189.12, 140.01, 134.88, 133.48, 132.33, 131.77, 130.07, 130.01, 128.76, 128.73, 127.67, 48.41. IR: 660, 733, 743, 797, 829, 845, 990, 1013, 1090, 1179, 1213, 1398, 1489, 1568, 1587, 1688 cm^{-1}

Leading to 34f, 34e, 36e

(4-Methoxyphenyl)acetyl chloride[11]

(4-Methoxyphenyl)acetic acid (3.018 g, 18.161 mmol) was dissolved in dichloromethane (25 mL, dry) to which thionyl chloride (10 mL) was added dropwise at 0 ^{o}C with stirring. The reaction mixture was left stirring at room temperature overnight. Solvent and excess of thionyl chloride were removed under reduced pressure and the crude product was used in the next experiment without further purification. The required product was obtained as orange oil in quantitative yield.

1-(4-Fluorophenyl)-2-(4-methoxyphenyl)ethanone[11]

Aluminium chloride (5.416 g, 40.625 mmol, 1.2 mol eq) was added to fluorobenzene (10.411 g, 108.331 mmol; 4.0 mol eq) with stirring and cooling with ice-water, under nitrogen. (4-Methoxyphenyl)acetyl chloride (5.00 g, 27.083 mmol) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was heated at 50 ^{o}C for 5 h, after which time the reaction mixture was left stirring at room temperature for 9 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture onto crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give white solid material which was washed with *n*-hexane (4.800 g, 73%), mp 97-100 ^{o}C, [Lit. mp 112-113 ^{o}C].[11] ^{1}H-NMR (DMSO-d_6): 8.12 (2H, dd, J = 5.6 Hz & J = 8.9 Hz), 7.36 (2H, t, J = 8.9 Hz), 7.37 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.29 (2H, s), 3.71 (3H, s). ^{13}C-NMR (DMSO-d_6): 197.0, 166.5, 164.5, 158.5, 133.5, 131.9, 131.8, 131.1, 127.2, 116.3, 116.1, 114.3, 55.5, 44.3 IR: 704, 741, 783, 812, 829, 1032, 1229, 1300, 1333, 1406, 1499, 1589, 1686 cm^{-1} HRESIMS: Found: 245.0973 Calculated for $C_{15}H_{14}O_2F$ 245.0972

2-Bromo-1-(4-fluorophenyl)-2-(4-methoxyphenyl)ethanone[12] 33f

1-(4-Fluorophenyl)-2-(4-methoxyphenyl)ethanone (2.500 g, 10.233 mmol) was dissolved in chloroform (50 mL) to which HBr in acetic acid 30% (1 mL) was added at room temperature. Bromine (0.7 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (3.190 g, 96%) as pale yellow oil. ^{1}H-NMR ($CDCl_3$): 8.04 (2H, dd, J = 5.2 Hz & J = 8.9 Hz), 7.47 (2H, d, 8.9 Hz), 7.14 (2H, t, J = 8.9 Hz), 6.91 (2H, d, J = 8.9 Hz), 6.34 (1H, s), 3.81 (3H, s). IR: 721, 831, 997, 1032, 1119, 1179, 1225, 1506, 1597, 1684 cm^{-1} HRESIMS: Found: 323.0085 calculated for $C_{15}H_{13}{}^{79}BrFO_2$ 323.0083.

Leading to 34g, 35f

(4-Methylphenyl)acetyl chloride

(4-Methylphenyl)acetic acid (10.196 g, 0.068 mol) was dissolved in dichloromethane (20 mL, dry) to which thionyl chloride (15 mL) was added dropwise at 0 ^{o}C with stirring. The reaction mixture was left stirring at room temperature overnight. Solvent and excess of thionyl chloride were removed under reduced pressure and the crude product was used in the next experiment without further purification. The required product was obtained as orange oil (11.44 g, 100%).

1,2-Bis(4-methylphenyl)ethanone[13]

Aluminium chloride (4.635 g, 34.76 mmol, 1.2 mol eq) was added to toluene (13.345 g, 144.83 mmol; 5.0 mol eq) with stirring and cooling with ice-water, under nitrogen. (4-Methylphenyl)acetyl chloride (5.435 g, 28.97 mmol) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was heated at 50 ^{o}C for 5 h, after which time the reaction mixture was left stirring at room temperature for 9 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give white solid material which was washed with *n*-hexane (6.02 g, 93%), mp 95-98 ^{o}C, [Lit. mp 102.5-103.5 ^{o}C].[13] ^{1}H-NMR (DMSO-d_6): 7.93 (2H, d, J = 8.2 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.14 (2H, d, J = 8.0 Hz), 7.10 (2H, d, J = 8.0 Hz), 4.26 (2H, s), 2.36 (3H, s), 2.25 (3H, s). IR: 736, 771, 806, 987, 1001, 1039, 1083, 1182, 1209, 1327, 1402, 1487, 1597, 1681cm^{-1} HRESIMS: Found: 225.1271 calculated for $C_{16}H_{17}O$ 225.1274.

2-Bromo-1,2-bis(4-methylphenyl)ethanone 33g

1,2-Bis(4-methylphenyl)ethanone (2.500 g, 11.146 mmol) was dissolved in chloroform (50 mL) to which HBr in acetic acid 30% (1 mL) was added at room temperature. Bromine (0.7 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (3.090 g, 91%) as a yellow solid after trituration with *n*-hexane. ^{1}H-NMR ($CDCl_3$): 7.90 (2H, d, J = 8.2 Hz), 7.43 (2H, d, J = 8.1 Hz), 7.25 (2H, d, J = 8.1 Hz), 7.18 (2H, d, J = 8.1 Hz), 6.37 (1H, s), 2.40 (3H, s), 2.34 (3H, s).^{13}C-NMR ($CDCl_3$): 190.7, 144.7, 139.2, 133.2, 131.7, 129.8, 129.5, 129.3, 128.9, 51.5, 21.7, 21.2. IR: 783, 851, 986, 1063, 1163, 1267, 1371, 1454, 1557, 1674 cm^{-1}. HRESIMS: Found: 303.0383 calculated for $C_{16}H_{16}{}^{79}BrO$ 303.0379.

Leading to 34h, 35g

1-(4-Bromophenyl)-2-phenylethanone[14]

Aluminium chloride (4.635 g, 34.76 mmol) was added to bromobenzene (5.374 g, 34.23 mmol) in dichloromethane (20 mL, dry) with stirring and cooling with ice-water, under nitrogen. Phenylacetyl chloride (5.374 g, 34.76 mmol) in dichloromethane (10 mL, dry) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was heated at 50 ^{o}C for 5 h, after which time the reaction mixture was left stirring at room temperature for 9 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give yellow solid material which was washed with n-hexane (8.86 g, 94%), mp 104-106 ^{o}C, [Lit mp106-108 ^{o}C].[14] ^{1}H-NMR ($CDCl_3$): 7.88 (2H, d, J = 8.5 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.34-7.25 (5H, m), 4.26 (2H,

s). IR: 740, 770, 808, 986, 1003, 1040, 1085, 1183, 1210, 1327, 1402, 1487, 1599cm^{-1} HRESIMS: Found: 275.0061 calculated for $C_{14}H_{12}{}^{79}BrO$ 275.0066.

2-Bromo-1-(4-bromophenyl)-2-phenylethanone 33h

1-(4-Bromophenyl)-2-phenylethanone (2.500 g, 9.088 mmol) was dissolved in chloroform (50 mL) to which HBr (1 mL) was added at room temperature. Bromine (0.7 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (3.300 g, 97%) as light brown oil. Trituration with *n*-hexane gave pale yellow solid, mp 90-93 ^{o}C. ^{1}H-NMR ($CDCl_3$): 7.86 (2H, d, J = 8.6 Hz), 7.61 (2H, d, J = 8.7 Hz), 7.52-7.35 (5H, m), 6.30 (1H, s). IR: 693, 734, 752, 842, 989, 1070, 1178, 1213, 1269, 1394, 1448, 1485, 1566, 1581, 1598, 1689cm^{-1} HRESIMS: Found: 352.9176 calculated for $C_{14}H_{11}{}^{79}Br_2O$ 352.9171.

Leading to 34i

2-Bromo-1,4-diphenyl-1-butanone 33a

1,4-Diphenyl-1-butanone (3.015 g, 13.44 mmol) was dissolved in chloroform (25 mL) to which HBr (in acetic acid 30%) 1 mL was added at room temperature. Bromine (0.7 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (4.00 g, 98%) as light brown oil. ^{1}H NMR ($CDCl_6$): 7.95 (2H, dd, J = 8.1 Hz & J = 1.0 Hz), 7.51-7.46 (2H, m), 7.33-7.21 (6H, m), 5.09-5.06 (1H, m), 2.89-2.79 (2H, m), 2.55-2.43 (2H, m). IR: 802, 854, 960, 979, 1001, 1157, 1176, 1232, 1259, 1446, 1494, 1579, 1595, 1681 cm^{-1}. HRESIMS: Found: 303.0380 Calculated for $C_{16}H_{16}{}^{79}BrO$ 303.0379.

Leading to 34j and 35h

1-(4-Isobutylphenyl)-2-phenylethanone

Aluminium chloride (4.635 g, 34.76 mmol) was added to isobutylbenzene (4.665 g, 34.76 mmol) with stirring and cooling with ice-water, under nitrogen. Phenylacetyl chloride (5.374 g, 34.76 mmol) was added dropwise while keeping the temperature below 20 ^{o}C. The reaction mixture was stirred for a further 15 min and then it was it was stirred at room temperature for 2.5 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product as yellow solid after trituration with n-hexane (2.500 g, 29%), mp 48-50 ^{o}C, R_T = 0.5 [1:10 ethyl acetate:*n*-hexane]. ^{1}H-NMR (DMSO-d_6): 7.97 (2H, d, J = 7.2 Hz), 7.31-7.21 (7H, m), 4.34 (2H, s), 2.52 (2H, d, J = 7.1 Hz), 1.91-1.82 (1H, m), 0.86 (6H, d, J = 6.5 Hz). ^{13}C-NMR (DMSO-d_6): 197.7, 147.6, 135.7, 134.7, 130.2, 129.8, 128.9, 128.7, 126.9, 45.0, 44.9, 29.9, 22.6. IR: 694, 713, 731, 796, 837, 985, 1076, 1118, 1176, 1217, 1315,

1336, 1409, 1456, 1494, 1566, 1600, 1678 cm^{-1}. HRESIMS: Found: 253.1588 calculated for $C_{18}H_{21}O$ 253.1587.

2-Bromo-1-(4-isobutylphenyl)-2-phenylethanone

1-(4-Isobutylphenyl)-2-phenylethanone (2.025 g, 8.025 mmol) was dissolved in chloroform (25 mL) to which HBr (in acetic acid 33%, 1 mL) was added at room temperature. Bromine (0.7 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (2.600 g, 98%) as pale yellow oil. ^{1}H-NMR ($CDCl_3$): 7.92 (2H, d, J = 8.2 Hz), 7.56 (2H, d, J = 8.2 Hz), 7.39-7.37 (5H, m), 7.23 (2H, d, J = 8.2 Hz), 6.38 (1H, s), 2.53 (2H, d, J = 7.2 Hz), 1.91 (1H, m), 0.91 (6H, d, J = 6.7 Hz). IR: 694, 763, 808, 858, 993, 1078, 1120, 1180, 1219, 1411, 1454, 1566, 1600, 1687, 2922, 2954 cm^{-1}. HRESIMS: Found: 331.0690 calculated for $C_{18}H_{20}BrO$ 331.0692.

Leading to 34k and 35i

1-[4-(Methylsulfanyl)phenyl]-2-phenylethanone[15]

Aluminium chloride (5.20 g, 38.25 mmol) was added to (methylsulfanyl)benzene (3.65 g, 29.40 mmol) in dichloromethane (20 mL, dry) with stirring and cooling with ice-water, under nitrogen. Phenylacetyl chloride (5.00 g, 32.35 mmol) in dichloromethane (10 mL, dry) was added dropwise while keeping the temperature below 20 ^oC. The reaction mixture was stirred for a further 15 min and then it was it was stirred at room temperature for 2.5 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture onto 50 g of crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give pale yellow solid material which was washed with *n*-hexane to give the required product as pale yellow solid (6.900 g, 97%), mp 95-97 ^oC, [Lit. mp 96-97 ^oC][15] ^{1}H-NMR (DMSO-d_6): 7.97 (2H, d, J = 8.5 Hz), 7.36 (2H, d, J = 8.5 Hz), 7.31-7.20 (5H, m), 4.32 (2H, s), 2.52 (3H, s). ^{13}C-NMR (DMSO-d_6): 197.1, 146.1, 135.7, 132.9, 130.1, 129.4, 128.8, 126.9, 125.4, 44.9, 14.4. IR: 704, 725, 746, 796, 812, 960, 987, 1028, 1087, 1182, 1200, 1212, 1332, 1398, 1431, 1452, 1494, 1552, 1583, 1678 cm^{-1}. HRESIMS: Found: 243.0840 Calculated for $C_{15}H_{15}OS$ 243.0838.

1-[4-(Methylsulfonyl)phenyl]-2-phenylethanone[15]

1-[4-(Methylsulfanyl)phenyl]-2-phenylethanone (2.290 g, 9.45 mmol) was dissolved in THF (50 mL) to which Oxone^R [Sigma-Aldrich] [$2KHSO_5.KHSO_4.K_2SO_4$] (13.74 g) in water (50 mL) was added at room temperature with stirring. The stirring was continued overnight. THF was removed under reduced pressure and the resulting mixture was diluted with dichloromethane and extracted. The organic layer was collected, dried (Na_2SO_4), filtered and the solvent removed to give the required product as pale yellow solid (2.290 g, 88%), mp 168-170 ^oC [Lit. mp 169-170 ^oC].[15] ^{1}H-NMR (DMSO-d_6): 8.27 (2H, d, J = 8.4 Hz), 8.08 (2H, d, J = 8.4 Hz), 7.33-7.22 (5H, m), 4.47 (2H, s), 3.27 (3H, s). ^{13}C-NMR (DMSO-d_6): 197.7, 144.8, 140.6, 135.0, 130.3, 129.7, 128.8, 127.9, 127.1, 45.6, 43.6. IR: 692, 705, 738, 771, 825, 962, 989, 1087, 1149, 1203, 1292,

1303, 1325, 1344, 1396, 1456, 1498, 1693 cm^{-1}. HRESIMS: Found: 275.0740 Calculated for $C_{15}H_{15}O_3S$ 275.0736.

2-Bromo-1-[4-(methylsulfonyl)phenyl]-2-phenylethanone

1-[4-(Methylsulfonyl)phenyl]-2-phenylethanone (1.002 g, 3.645 mmol) was dissolved in chloroform (25 mL) to which HBr (in acetic acid 33%, 0.5 mL) was added at room temperature. Bromine (0.5 mL) in chloroform (5 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product as white solid (1.250 g, 97%), mp 117-12 ^oC. ^1H-NMR ($CDCl_3$): 8.17 (2H, d, J = 8.5 Hz), 8.05 (2H, J = 8.5 Hz), 7.54-7.52 (2H, m), 7.43-7.39 (3H, m), 6.32 (1H, s), 3.08(3H, s). IR: 690, 860, 1122, 1220, 1455, 1604, 1677, 1683 cm^{-1}. HRESIMS: Found: 352.9841:366.9998 calculated for $C_{15}H_{14}O_3BrS$ 352.9842:366.9998.

Leading to 34l and 35j

2-(3-Chlorophenyl)-1-(4-fluorophenyl)ethanone

Aluminium chloride (5.20 g, 38.25 mmol) was added to fluorobenzene (5.00 g, 52.03 mmol) in dichloromethane (20 mL, dry) with stirring and cooling with ice-water, under nitrogen. (3-Chlorophenyl)acetyl chloride (5.53 g, 29.31 mmol) in dichloromethane (10 mL, dry) was added dropwise while keeping the temperature below 20 ^oC. The reaction mixture was stirred for a further 15 min and then it was it was stirred at room temperature for 2.5 h. Hydrolysis was carried out by diluting with dichloromethane and pouring the reaction mixture onto crushed ice (50 g) and extracting the resulting suspension with 2N HCl (30 mL). The organic phase was then cautiously washed with a saturated solution of sodium hydrogen carbonate and brine. The organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product as colorless oil (3.930 g, 54%). ^1H-NMR ($CDCl_3$): 8.05-8.02 (2H, m), 7.17-6.86 (6H, m), 4.24 (2H, s). IR: 682, 756, 788, 833, 991, 1078, 1095, 1155, 1201, 1224, 1328, 1409, 1446, 1477, 1487, 1506, 1595, 1681 cm^{-1}. HRESIMS: Found: 249.0480 Calculated for $C_{14}H_{11}{}^{35}ClFO$ 249.0477.

2-Bromo-2-(3-chlorophenyl)-1-(4-fluorophenyl)ethanone

2-(3-Chlorophenyl)-1-(4-fluorophenyl)ethanone (1.081 g, 4.347 mmol) was dissolved in chloroform (25 mL). Hydrobromic acid in acetic acid (33%) (1 mL) was added at room temperature with stirring. Bromine (0.5 mL) in chloroform (10 mL) was added to the reaction mixture at room temperature with stirring. The dropwise addition continued until slight bromine coloration remained. The stirring was continued for further 30 minutes. Sodium sulfite (10% aqueous) solution was added and the reaction mixture was then extracted. The organic layer was collected, washed with a saturated solution of sodium hydrogen carbonate followed by brine then the organic layer was dried (Na_2SO_4), filtered and the solvent removed under reduced pressure to give the required product (1.200 g, 84%) as pale yellow oil. ^1H NMR ($CDCl_3$): 8.05-8.02 (2H, m), 7.55 (1H, s), 7.43-7.40 (1H, m), 7.34-7.33 (1H, m), 7.18 (2H, t, J = 8.6 Hz), 6.24 (1H, s). IR: 680, 1506, 1487, 1409, 1213, 1157, 1099, 1080, 991, 850, 837, 779, 752, 711, 1591, 1689 cm^{-1}. HRESIMS: Found: 326.9580 Calculated for $C_{14}H_{10}{}^{79}Br^{35}ClFO$ 326.9582.

2-Amino-5-methyl-6-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-(34b) and 2,6-Diamino-5-(1-methyl-2-oxo-2-phenylethyl)-4(3*H*)-pyrimidinone

2-Bromo-1-phenyl-1-propanone (0.500 g, 2.35 mmol) and 2,6-diamino-4(3*H*)-pyrimidinone (0.290 g, 2.00 mmol) were dissolved in DMF (6 mL, dry). The reaction mixture was heated at 80^{o}C for 4 d with stirring. The solvent was reduced to around 2 mL *in vacuo* and the residue was purified by HPLC. Two products were isolated: fraction one: (175 mg, 34%): off-white solid, no distinct mp; uncyclised material and fraction two (the required product) (5 mg, 1%) as off-white solid, no distinct melting point.

Product 34b ^{1}H NMR (DMSO-d_6): 11.12 (1H, s), 10.23 (1H, s), 7.55-7.40 (5H, m), 6.68 (2H, br), 2.29 (3H, s). IR: 700, 724, 758, 783, 810, 835, 879, 1157, 1225, 1377, 1441, 1506, 1516, 1544, 1600, 1633 cm^{-1}. HRESIMS: Found: 241.1081 calculated for $C_{13}H_{13}N_4O$ 241.1084

By-product: 2,6-diamino-5-(1-methyl-2-oxo-2-phenylethyl)-4(3*H*)-pyrimidinone

IR: 692, 795, 966, 1036, 1084, 1192, 1229, 1379, 1422, 1451, 1562, 1587, 1630, 1659, 1680 cm^{-1}. HRESIMS: Found: 259.1195 calculated for $C_{13}H_{15}N_4O_2$ 259.1190

2-Amino-5,6-bis(4-fluorophenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34d

2-Bromo-1,2-bis(4-fluorophenyl)ethanone (1.00 g, 3.214 mmol) and 2,6-diamino-4(3*H*)-pyrimidinone (0.405 g, 3.214 mmol) were dissolved in DMF (4 mL, dry). The reaction mixture was heated at 60 ^{o}C for 4 d with stirring under nitrogen. DMF was removed *in vacuo* and the residue was applied to a silica gel column chromatography and eluted with methanol:ethyl acetate (1:9; R_F = 0.5). The product was obtained as yellow solid (0.540 g, 50%), mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6) 400 MHz: 11.50 (1H, s), 10.32 (1H, s), 7.32-7.22 (4H, m), 7.14 (2H, t, J = 9.0 Hz), 7.10 (2H, t, J = 9.0 Hz), 6.16 (2H, s). IR: 698, 723, 758, 783, 810, 835, 879, 1157, 1225, 1377, 1441, 1506, 1516, 1543, 1599, 1634 cm^{-1}. HRESIMS: Found: 339.1053 Calculated for $C_{18}H_{13}ON_4F_2$ 339.1052.

2-Amino-5,6-bis(4-chlorophenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34e

2-Bromo-1,2-bis(4-chlorophenyl)ethanone (1.00 g, 2.907 mmol) and 2,6-diamino-4(3*H*)-pyrimidinone (0.367 g, 126.12 mmol) were dissolved in DMF (4 mL, dry). The reaction mixture was heated at 60^{o}C for 4 d with stirring under nitrogen. DMF was removed *in vacuo* and the residue was applied to a silica gel column chromatography and eluted with methanol:ethyl acetate (1:9; R_F = 0.2). The product was obtained as yellow solid (0.350 g, 32%), mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.56(1H, s), 10.34(1H, s), 7.34-7.21 (8H, m), 6.18 (2H, s). IR: 656, 681, 735, 787, 826, 878, 1092, 1350, 1385, 1433, 1493, 1533, 1593, 1653 cm^{-1}. HRESIMS: Found: 371.0463 Calculated for $C_{18}H_{13}ON_4Cl_2$ 371.0461.

2-Amino-6-(4-fluorophenyl)-5-(4-methoxyphenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34f

2,6-Diamino-4(3*H*)-pyrimidinone (0.367 g, 2.909 mmol) and 2-Bromo-1-(4-fluorophenyl)-2-(4-methoxyphenyl)ethanone (0.939 g, 3.965 mmol) were dissolved in DMF (4 mL, dry) with stirring to which KI (0.541 g, 3.26 mmol) and cesium carbonate (1.062 g, 3.26 mmol) were added. The reaction mixture was heated at 60 ^{o}C overnight. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate and extracted with water. The organic layer was collected, dried (Na_2SO_4) and solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give impure material (0.400 g, 39%). This material was further purified by HPLC to give the required

product as a pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.40 (1H, s), 10.27 (1H, s), 7.26 (2H, dd, *J* = 5.6 Hz & *J* = 8.7 Hz), 7.19 (2H, d, *J* = 8.7 Hz), 7.10 (2H, t, *J* = 8.7 Hz), 6.82 (2H, d, *J* = 8.7 Hz), 6.14 (2H, br), 3.74 (3H, s). IR: 702, 733, 769, 803, 850, 875, 1072, 1184, 1386, 1442, 1514, 1537, 1564, 1600, 1658, 1672 cm^{-1}. HRESIMS: Found: 351.1256 calculated for $C_{19}H_{16}FN_4O_2$ 351.1252.

2-Amino-5,6-bis(4-methylphenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34g

2,6-Diamino-4(3*H*)-pyrimidinone (0.166 g, 1.319 mmol) and 2-bromo-1,2-bis(4-methylphenyl)ethanone (0.400 g, 1.319 mmol) were dissolved in DMF (4 mL, dry) with stirring to which KI (0.218 g, 1.319 mmol) and cesium carbonate (0.430 g, 1.319 mmol) were added. The reaction mixture was heated at 60 ^{o}C for 48 h. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.200 g, 46%). This material was further purified by HPLC to give the required product as light green solid. ^{1}H-NMR (DMSO-d_6):11.35 (1H, s), 10.25 (1H, s), 7.17-7.02 (8H, m), 6.12 (2H, br), 2.28 (3H, s), 2.24 (3H, s). IR: 736, 771, 806, 987, 1001, 1039, 1083, 1182, 1209, 1327, 1402, 1487, 1597, 1681 cm^{-1}. HRESIMS: Found: 331.1557 calculated for $C_{20}H_{19}N_4O$ 331.1553.

2-Amino-6-(4-bromophenyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34h

2,6-Diamino-4(3*H*)-pyrimidinone (0.712 g, 5.65 mmol) and 2-bromo-1-(4-bromophenyl)-2-phenylethanone (1.00 g, 2.82 mmol) were dissolved in DMF (10 mL, dry) with stirring. The reaction mixture was heated at 60 ^{o}C for 4 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.200 g, 19%) as yellow solid. This material was further purified by HPLC to give the required product as light green solid with no distinct melting point. ^{1}H NMR (DMSO-d_6): 11.52 (1H, s), 10.31 (1H, s), 7.42 (2H, d, *J* = 8.6 Hz), 7.29-7.20 (5H, m), 7.15 (2H, d, *J* = 8.6 Hz), 6.15 (2H, s). IR: 698, 717, 767, 825, 873, 1008, 1072, 1388, 1427, 1438, 1500, 1537, 1562, 1585, 1627, 1664 cm^{-1}. HRESIMS: Found: 381.0348 Calculated for $C_{18}H_{14}{}^{79}BrN_4O$ 381.0346.

2-Amino-6-phenyl-5-(2-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34i

2,6-Diamino-4(3*H*)-pyrimidinone (1.664 g, 13.193 mmol) and 2-bromo-1,4-diphenyl-1-butanone (2.00 g, 6.596 mmol) were dissolved in DMF (6 mL, dry) with stirring. The reaction mixture was heated at 25 ^{o}C for 4 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.390 g, 18%) as yellow solid. This material was further purified by HPLC to give the required product as pale yellow solid, mp > 230^{o}C. ^{1}H NMR (DMSO-d_6): 11.10 (1H, s), 10.25 (1H, s), 7.39-7.35 (4H, m), 7.27-7.14 (6H, m), 6.16 (2H, br), 2.95 (4H, s). IR: 694, 721, 771, 798, 844, 1066, 1139, 1195, 1386, 1444, 1494, 1589, 1664, 1697 cm^{-1}. HRESIMS: Found: 331.1550 Calculated for $C_{20}H_{19}N_4O$ 331.1553.

2-Amino-6-(4-isobutylphenyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34j

2,6-Diamino-4(3*H*)-pyrimidinone (0.523 g, 4.152 mmol) and 2-bromo-1-(4-isobutylphenyl)-2-phenylethanone (1.375 g, 4.152 mmol) were dissolved in DMF (6 mL, dry) with stirring. Cesium carbonate (1.062 g, 3.26 mmol) was added to the reaction mixture. The reaction mixture was heated at 60 ^{o}C for 2 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.800 g, 54%) as yellow solid. This material was further purified by HPLC to give the required product as pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.35 (1H, s), 10.23 (1H, s), 7.28-7.17 (5H, m), 7.13 (2H, d, J = 8.2 Hz), 7.01 (2H, d, J = 8.2 Hz), 2.39 (2H, d, J = 7.2 Hz), 1.82 (1H, m), 0.84 (6H, d, J = 6.7 Hz). IR: 700, 731, 767, 800, 848, 875, 1072, 1184, 1386, 1442, 1514, 1537, 1564, 1600, 1658, 1672 cm^{-1}. HRESIMS: Found: 359.1870 calculated for $C_{22}H_{23}N_4O$ 359.1866.

2-Amino-6-[4-(methylsulfonyl)phenyl]-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34k

2,6-Diamino-4(3*H*)-pyrimidinone (0.223 g, 1.769 mmol) and 2-bromo-1-[4-(methylsulfonyl)phenyl]-2-phenylethanone (0.625 g, 1.769 mmol) were dissolved in DMF (5 mL, dry) with stirring. Cesium carbonate (0.156 g, 0.479 mmol) was added to the reaction mixture. The reaction mixture was heated at 60 ^{o}C for 2 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:8 methanol:ethyl acetate) to give an impure material (0.170 g, 25%) as yellow solid. This material was further purified by HPLC to give the required product as pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.68 (1H, s), 10.36 (1H, s), 7.73 (2H, d, J = 8.5 Hz), 7.42 (2H, d, J = 8.5 Hz), 7.30-7.26 (5H, m), 6.26 (2H, br), 3.18 (3H, s). IR: 688, 729, 769, 783, 846, 875, 979, 1087, 1143, 1274, 1319, 1357, 1386, 1535, 1583, 1631, 1660 cm^{-1}. HRESIMS: Found: 381.1019 calculated for $C_{19}H_{17}N_4O_3S$ 381.1016.

2-Amino-5-(3-chlorophenyl)-6-(4-fluorophenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 34l

2,6-Diamino-4(3*H*)-pyrimidinone (0.231 g, 1.832 mmol) and 2-bromo-2-(3-chlorophenyl)-1-(4-fluorophenyl)ethanone (0.600 g, 1.832 mmol) were dissolved in DMF (5 mL, dry) with stirring. The reaction mixture was heated at 60 ^{o}C for 24 h. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate to which water was added. The reaction mixture was extracted and the organic layer was collected, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate; R_F = 0.3) to give an impure material as yellow solid. This material was further purified by HPLC to give the required product as pale yellow solid (0.288 g, 34%), with no distinct melting point. ^{1}H NMR (DMSO-d_6): 11.57 (1H, s), 10.33 (1H, s), 7.36 (1H, s), 7.26-7.23 (4H, m), 7.17-7.11 (3H, m), 6.20 (2H, br). IR: 686, 725, 765, 790, 810, 837, 1099, 1139, 1161, 1195, 1433, 1512, 1583, 1662, 1693 cm^{-1}. HRESIMS: Found: 355.0755 Calculated for $C_{18}H_{13}{}^{35}ClFN_4O$ 355.0756.

5,6-Bis(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35c

2-Bromo-1,2-bis(4-fluorophenyl)ethanone (1.00 g, 3.214 mmol) and 2,4,6-pyrimidinetriamine (0.402 g, 3.214 mmol) were dissolved in DMF (4 mL, dry). The reaction mixture was heated at

60 ^{o}C for 4 d with stirring under nitrogen. DMF was removed *in vacuo* and the residue was applied to a silica gel column chromatography and eluted with methanol:ethyl acetate (1:9; R_F = 0.4). The product was obtained as yellow solid (0.110 g, 10%), mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6: 400MHz): 11.33 (1H, s), 7.37-7.23 (6H, m), 7.12 (2H, t, *J* = 8.9 Hz), 5.65 (2H, s), 5.31 (2H, br). IR: 689, 721, 773, 791, 837, 883, 1074, 1096, 1155, 1215, 1317, 1393, 1412, 1439, 1475, 1514, 1553, 1609 cm^{-1}. HRESIMS: Found: 338.1212 Calculated for $C_{18}H_{14}N_5F_2$ 338.1212.

5,6-Bis(4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35d

2-Bromo-1,2-bis(4-chlorophenyl)ethanone (1.00 g, 2.907 mmol) and 2,4,6-pyrimidinetriamine (0.364 g, 2.907 mmol) were dissolved in DMF (4 mL, dry). The reaction mixture was heated at 60^{o}C for 4 d with stirring under nitrogen. DMF was removed *in vacuo* and the residue was applied to a silica gel column chromatography and eluted with methanol:ethyl acetate (1:9; R_F = 0.2). The product was obtained as yellow solid (0.120 g, 11%), mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.38 (1H, s), 7.49 (2H, d, *J* = 8.4 Hz), 7.33 (2H, d, *J* = 8.4 Hz), 7.32 (2H, d, *J* = 8.4 Hz), 7.22 (2H, d, *J* = 8.4 Hz), 5.66 (2H, s), 5.34 (2H, br). ^{13}C NMR (DMSO-d_6): 158.62, 152.73, 152.02, 133.26, 132.28, 131.96, 131.12, 130.83, 130.74, 129.34, 128.36, 127.59, 125.13, 115.25, 99.25. IR: 720, 737, 795, 829, 1013, 1092, 1395, 1416, 1447, 1568, 1614, 1543, 1478 cm^{-1}. HRESIMS: Found: 370.0625 Calculated for $C_{18}H_{14}N_5Cl_2$ 370.0621.

6-(4-Fluorophenyl)-5-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35e

2,4,6-Pyrimidinetriamine (0.364 g, 2.907 mmol) and 2-bromo-1-(4-fluorophenyl)-2-(4-methoxyphenyl)ethanone (0.939 g, 3.965 mmol) were dissolved in DMF (4 mL, dry) with stirring to which KI (0.541 g, 3.26 mmol) and cesium carbonate (1.062 g, 3.26 mmol) were added. The reaction mixture was heated at 60 ^{o}C overnight. Solvent was removed *in vacuo* and the crude dried Na_2SO_4 and solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give impure material (0.1760 g, 17%). This material was further purified by HPLC to give the required product as a pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 12.21 (1H, s), 11.65 (1H, br), 7.29-7.23 (6H, m), 7.17 (2H, t, *J* = 8.7 Hz), 7.03 (2H, d, *J* = 8.7 Hz), 6.80 (2H, br), 3.80 (3H, s). IR: 701, 732, 766, 805, 846, 876, 1072, 1184, 1386, 1442, 1514, 1537, 1564, 1600, 1658, 1672 cm^{-1}. HRESIMS: Found: 350.1415 calculated for $C_{19}H_{17}FN_5O$ 350.1412.

5,6-Bis(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35f

2,4,6-Pyrimidinetriamine (0.165 g, 1.319 mmol) and 2-bromo-1,2-bis(4-methylphenyl)ethanone (0.400 g, 1.319 mmol) were dissolved in DMF (4 mL, dry) with stirring to which KI (0.218 g, 1.319 mmol) and cesium carbonate (0.430 g, 1.319 mmol) were added. The reaction mixture was heated at 60 ^{o}C for 48 h. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.120 g, 27%). This material was further purified by HPLC to give the required product as pale yellow solid. ^{1}H-NMR (DMSO-d_6): 12.18 (1H, br), 11.63 (1H, br), 7.27-7.08 (10H, m), 6.80 (2H, br), 2.36 (3H, s), 2.25 (3H, s). IR: 700, 733, 766, 805, 846, 877, 1074, 1184, 1386, 1442, 1515, 1540, 1564, 1600, 1660, 1673 cm^{-1}. HRESIMS: Found: 330.1711 calculated for $C_{20}H_{20}N_5$ 330.1713.

6-(4-Bromophenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35g

2,4,6-Pyrimidinetriamine (0.706 g, 5.64 mmol) and 2-bromo-1-(4-bromophenyl)-2-phenylethanone (1.00 g, 2.82 mmol) were dissolved in DMF (10 mL, dry) with stirring. The reaction mixture was heated at 60 ^{o}C for 7 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.165 g, 15%). This material was further purified by HPLC to give the required product as pale yellow solid with no distinct melting point. ^{1}H NMR (DMSO-d_6): 11.33 (1H, s), 7.45 (2H, d, J = 8.6 Hz), 7.41-7.32 (5H, m), 7.17 (2H. d, J = 8.6 Hz), 5.65 (2H, s), 5.25 (2H, br). IR: 702, 765, 796, 831, 1006, 1085, 1323, 1388, 1411, 1440, 1477, 1543, 1560, 1610 cm^{-1}. HRESIMS: Found: 380.0508 Calculated for $C_{18}H_{15}{}^{79}BrN_5$ 380.0505.

6-(4-Isobutylphenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35h

2,4,6-Pyrimidinetriamine (0.519 g, 4.152 mmol) and 2-Bromo-1-(4-isobutylphenyl)-2-phenylethanone (1.375 g, 4.152 mmol) were dissolved in DMF (10 mL, dry) with stirring. Cesium carbonate (0.256 g, 0.786 mmol) was added to the reaction mixture. The reaction mixture was heated at 60 ^{o}C for 2 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give an impure material (0.310 g, 21%). Trituration with ethyl acetate gave the pure material as pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 11.20 (1H, s), 7.44-7.31 (5H, m), 7.17 (2H, d, J = 8.2 Hz), 6.99 (2H, d, J = 8.2 Hz), 2.37 (2H, d, J = 7.2 Hz), 1.80 (1H, m), 0.83 (6H, d, J = 6.6 Hz). IR: 700, 732, 767, 792, 846, 877, 1020, 1070, 1400, 1435, 1481, 1546, 1564, 1597, 1616 cm^{-1}. HRESIMS: Found: 358.2029 calculated for $C_{22}H_{24}N_5$ 358.2026.

6-[4-(Methylsulfonyl)phenyl]-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35i

2,4,6-Pyrimidinetriamine (0.2219 g, 1.769 mmol) and 2-bromo-1-[4-(methylsulfonyl)phenyl]-2-phenylethanone (0.625 g, 1.769 mmol) were dissolved in DMF (5 mL, dry) with stirring. Cesium carbonate (0.225 g, 0.691 mmol) was added to the reaction mixture. The reaction mixture was heated at 60 ^{o}C for 2 d. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:8 methanol:ethyl acetate) to give an impure material as light brown solid (0.170 g, 25%). HPLC purification gave the pure material as pale yellow solid with no distinct melting point. ^{1}H-NMR (DMSO-d_6): 12.49 (1H, s), 7.81 (2H, d, J = 8.5Hz), 7.51-7.36 (9H, m), 7.24 (2H, br), 3.19 (3H, s). ^{13}C-NMR (DMSO-d_6): 139.6, 136.1, 133.2, 130.3, 130.1, 128.9, 128.2, 127.5, 116.8. IR: 707, 723, 777, 798, 839, 956, 1147, 1199, 1305, 1390, 1436, 1595, 1647, 1656 cm^{-1}. HRESIMS: Found: 380.1177 calculated for $C_{19}H_{18}N_5O_2S$ 380.1176.

5-(3-Chlorophenyl)-6-(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 35j

2,4,6-Pyrimidinetriamine (0.229 g, 1.832 mmol) and 2-bromo-2-(3-chlorophenyl)-1-(4-fluorophenyl)ethanone (0.600 g, 1.832 mmol) were dissolved in DMF (5 mL, dry) with stirring. The reaction mixture was heated at 60 ^{o}C for 24 h. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate to which water was added. The reaction mixture was

extracted and the organic layer was collected, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate; R_F = 0.3) to give an impure material as yellow solid. This material was further purified by HPLC to give the required product as pale yellow solid (0.096 g, 11%), with no distinct melting point. ^{1}H NMR (DMSO-d_6): 12.34 (1H, br), 11.71 (1H, br), 7.47-7.46 (2H, m), 7.34-7.16 (10H, m). IR: 688, 723, 765, 796, 837, 1138. 1161, 1199, 1473, 1512, 1550, 1599, 1643, 1658 cm^{-1}. HRESIMS: Found: 354.0919 Calculated for $C_{18}H_{14}{}^{35}ClFN_5$ 354.0916.

5,6-Bis(4-fluorophenyl)-*N*^{4},*N*^{4}-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 36a

N^4,*N*^{4}-Dimethyl-2,4,6-pyrimidinetriamine (0.200 g, 1.306 mmol) and 2-bromo-1,2-bis(4-fluorophenyl)ethanone (0.406 g, 1.306 mmol) were dissolved in DMF (4 mL, dry) to which Hunig's base (1 mL, dry) was added. The reaction mixture was heated at 60 ^{o}C for 24 h. DMF and Hunig's base were removed *in vacuo* and the residue was dissolved in ethyl acetate and methanol to which silica gel was added and the solvents were removed under reduced pressure. The residue was applied to a silica gel column chromatography and eluted with 1:9 methanol:ethyl acetate (R_F = 0.5). Fractions containing the required product were combined and the solvents removed under reduced pressure gave the crude material which was triturated with a small amount of methanol and filtered to give the desired product (60 mg, 13%) as a pale yellow solid, mp > 230^{o}C. ^{1}H NMR (DMSO-d_6): 11.37 (1H, s), 7.23-7.08 (8H, m), 5.74 (2H, s), 2.51 (6H, s). IR: 731, 793, 810, 835, 882, 1030, 1067, 1159, 1225, 1398, 1487, 1508, 1547, 1587, 1609 cm^{-1}. HRESIMS: Found: 366.1526 Calculated for $C_{20}H_{18}N_5F_2$ 366.1525.

5,6-Bis(4-chlorophenyl)-*N*^{4},*N*^{4}-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 36b

N^4,*N*^{4}-Dimethyl-2,4,6-pyrimidinetriamine (0.200 g, 1.306 mmol) and 2-bromo-1,2-bis(4-chlorophenyl)ethanone (0.449 g, 1.306 mmol) were dissolved in DMF (4 mL, dry) to which Hunig's base (1 mL, dry) was added. The reaction mixture was heated at 60 ^{o}C for 24 h. DMF and Hunig's base were removed *in vacuo* and the residue was dissolved in ethyl acetate and methanol to which silica gel was added and the solvents were removed under reduced pressure. The residue was applied to a silica gel column chromatography and eluted with 1:9 methanol:ethyl acetate (R_F = 0.5). Fractions containing the required product were combined and the solvents removed under reduced pressure gave the crude material which was triturated with a small amount of methanol and filtered to give the desired product (60 mg, 12%) as a pale yellow solid, mp > 230 ^{o}C. ^{1}H NMR (DMSO-d_6): 11.48 (1H, s), 7.42 (2H, d, *J* = 8.4 Hz), 7.35 (2H, d, *J* = 8.6 Hz), 7.23 (2H, d, *J* = 8.4 Hz), 7.19 (2H, d, *J* = 8.6 Hz), 5.83 (2H, s), 2.52 (6H, s). ^{13}C NMR (DMSO-d_6): 132.54, 131.80, 131.69, 131.61, 130.27, 128.90, 128.73, 40.92. IR: 733, 789, 828, 880, 1011, 1057, 1057, 1092, 1398, 1449, 1472, 1545, 1593 cm^{-1}. HRESIMS: Found: 398.0938 Calculated for $C_{20}H_{18}N_5Cl_2$ 398.0934.

6-(4-Fluorophenyl)-*N*^{4},*N*^{4}-dimethyl-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 36c

N^4,*N*^{4}-Dimethyl-2,4,6-pyrimidinetriamine (0.200 g, 1.306 mmol) and 2-bromo-1-(4-fluorophenyl)-2-phenylethanone (0.383 g, 1.306 mmol) were dissolved in DMF (4 mL, dry) to which Hunig's base (1 mL, dry) was added. The reaction mixture was heated at 60 ^{o}C for 24 h. DMF and Hunig's base were removed *in vacuo* and the residue was dissolved in ethyl acetate and methanol to which silica gel was added and the solvents were removed under reduced

pressure. The residue was applied to a silica gel column chromatography and eluted with 1:9 methanol:ethyl acetate (R_F = 0.2). Fractions containing the required product were combined and the solvents removed under reduced pressure gave the crude material which was triturated with a small amount of methanol and filtered to give the desired product (60 mg, 13%) as a pale yellow solid, mp > 230 ^oC. ^1H NMR (DMSO-d_6): 11.34 (1H, s), 7.35-7.27 (3H, m), 7.21-7.17 (4H, m), 7.07 (2H, t, *J* = 8.9 Hz), 2.49 (6H, s). IR: 700, 774, 837, 1069, 1153, 1227, 1400, 1439, 1481, 1506, 1547, 1591 cm^{-1}. HRESIMS: Found: 348.1621 Calculated for $C_{20}H_{19}N_5F$ 348.1619.

N^4,N^4-Dimethyl-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 36d

N^4,N^4-Dimethyl-2,4,6-pyrimidinetriamine (0.500 g, 3.26 mmol) and 2-bromo-1,2-diphenylethanone [desyl bromide] (0.898 g, 3.26 mmol) were dissolved in DMF (4 mL, dry) to which potassium iodide (0.541 g, 3.26 mmol) and cesium carbonate (1.062 g, 3.26 mmol) were added. The reaction mixture was heated at 60 ^oC for 24 h. DMF was removed *in vacuo* and the residue was dissolved in ethyl acetate and methanol to which silica gel was added and the solvents were removed under reduced pressure. The residue was applied to a silica gel column chromatography and eluted with ethyl acetate (R_F = 0.3). The required product was obtained as a yellow solid (0.310 g, 29%), mp > 230 ^oC. ^1H NMR (DMSO-d_6): 11.35 (1H, s), 7.36-7.17 (10H, m), 5.74 (2H, s), 2.50 (6H, s). IR: 1591, 1541, 1479, 1433, 1394, 1323, 1276, 1058, 1028, 869, 767, 690 cm^{-1}. HRESIMS: Found: 330.1710 calculated for $C_{20}H_{20}N_5$ 330.1713.

6-(4-Fluorophenyl)-5-(4-methoxyphenyl)-N^4,N^4-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 36e

N^4,N^4-Dimethyl-2,4,6-pyrimidinetriamine (0.445 g, 2.907 mmol) and 2-Bromo-1-(4-fluorophenyl)-2-(4-methoxyphenyl)ethanone (0.939 g, 3.965 mmol) were dissolved in DMF (4 mL, dry) with stirring to which KI (0.541 g, 3.26 mmol) and cesium carbonate (1.062 g, 3.26 mmol) were added. The reaction mixture was heated at 60 ^oC overnight. Solvent was removed *in vacuo* and the crude material was dissolved in ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. This material was applied to a silica gel column chromatography and eluted with (1:9 methanol:ethyl acetate) to give impure material (0.390 g, 35%). This material was further purified by HPLC to give the required product as a pale yellow solid with no distinct melting point. ^1H-NMR (DMSO-d_6): 12.41 (1H, br), 7.23-7.12 (8H, m), 6.95 (2H, d, *J* = 8.7 Hz), 3.77 (3H, s), 2.73 (6H, s). IR: 705, 730, 767, 802, 844, 876, 1072, 1185, 1386, 1442, 1514, 1535, 1563, 1602, 1658 cm^{-1}. HRESIMS: Found: 378.1723 calculated for $C_{21}H_{21}FN_5O$ 378.1725

N^4-Cyclohexyl-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine 37

N^4-Cyclohexyl-2,4,6-pyrimidinetriamine (0.150 g, 0.724 mmol) and desyl bromide (0.199 g, 0.724 mmol) were dissolved in DMF (2 mL, dry). The reaction mixture was heated at 60 ^oC for 4 d. The solvent was removed in vacuo and the residue was applied to a silica gel column chromatography and eluted with 1:10 methanol:ethyl acetate, R_F = 0.5. The product was obtained as yellow solid (30 mg, 11%) after recrystallization from ethyl acetate:*n*-hexane. ^1H-NMR (DMSO-d_6): 11.25 (1H, s), 7.49-7.09 (10H, m), 5.66 (2H, s), 4.39 (1H, d, *J* = 8.1 Hz), 3.96-3.92 (1H, m), 1.68-0.94 (10H, m). IR: 725, 760, 784, 811, 835, 879, 1155, 1225, 1441, 1508, 1516, 1543, 1600, 1633 cm^{-1}. HRESIMS: Found: 384.2180 Calculated for $C_{24}H_{26}N_5$ 384.2183

HPLC methods used to assess the purity of compounds:

First method:

*All the compounds showed purity of > 95%.

The retention time of these compounds was between (15 and 25 minutes).

Reverse phase HPLC on a water system using a C18 Luna column with the gradient given in the table.

Waters 717plus Autosampler, Waters 1525 Binary HPLC Pump, Waters 2487 Dual lambda Absorbance Detector

Programme for HPLC purification:

Time (min)	A%	B%	Flow rate (mL/min)
0	90	10	6
28	30	70	6
33	10	90	6
38	90	10	6
40	90	10	0

Second method:

*All the compounds showed purity of equal or more than 95%.

The retention time of these compounds was between (8 and 20 minutes).

Instrument used:

Agilent: 1200LC coupled to 6130 Dual source MS, single wavelength detector @ 254nm ChemStation software,

Method: The material was dissolved in 0.2 mL methanol then 4 drops were taken up into 0.4 mL mobile phase (1:1 acetonitrile/water containing 0.1% formic acid). Mass Range 100-1000 Da, UV@254. Time of run was 30 min.

Zorbax Eclipse column C_{18} 150mm x 4.6mm, 5um

Retention time and purity of selected compounds as measured by HPLC and HPLC/MS

Compound	Method One		Method Two	
	Retention Time (minutes): HPLC	% Purity	Retention Time (minutes) HPLC/MS	% Purity
6c	18.7	95	10.3	98.4
6d	18.6	99	10.8	96.0
6e	18.7	99	9.4	100.0
20	31.5	98	---	---
27d	12.9	100	10.3	95.4
28d	14.4	98	8.4	95.0
29b	14.6	96	---	---

29d	16.4	99	Formed suspension	---
30a	16.6	98	11.2	100.0
30c	19.6	95	10.6	95.6
31c	19.9	96	11.9	99.2
34a	16.6	99	11.4	100.0
34b	16.8	99	10.1	100.0
34c	22.6	98	---	---
34i	20.4	97	---	---
34l	23.3	96	---	---
35a	18.2	95	10.5	100.0
35b	16.6	100	10.7	100.0
35c	16.8	99	10.8	97.6
35d	17.3	98	Formed suspension	---
36b	18.2	95	12.8	99.4
36c	18.0	97	11.6	95.7

Protein Purification and Crystallography

Reagents

PTR1 cofactors $NADP^+$ and NADPH were purchased from Melford while all pterin substrates were obtained from Schircks Laboratories. Ligands were prepared in 100 % (v:v) dimethyl sulfoxide (DMSO) to 100 mM based on dry weight. The insolubility of any compound at this concentration was established following the application of sonication and heat (42°C). Solubilized compounds were stored in glass vials at -20°C and 10-fold serial dilutions (in DMSO) made as working stocks.

Recombinant protein purification

Expression and purification of *Tb*PTR1 by a single affinity chromatography step, was carried out following a published protocol.[16] The protein was buffer exchanged using a PD-10 desalting column (GE Healthcare) to 20 mM Tris-HCl pH 7.5 for crystallization or 50 mM Tris-HCl pH 7.5, 250 mM NaCl, 20 % (v:v) glycerol for assay. Following concentration to 15-20 mg mL^{-1} in a centrifugal concentrator with 100 kDa molecular mass limit, aliquots of *Tb*PTR1 were flash cooled in liquid nitrogen and stored at -80 °C until required.

Spectrophotometric assay

Inhibition of *Tb*PTR1 activity was carried out according to an established spectrophotometric assay.[16] In brief, 1 mL samples containing 30 μg *Tb*PTR1 (0.96 μM), 20 μM dihydrobiopterin and 0-1 mM compound of interest in 20 mM sodium citrate pH 3.7 were warmed to 30 °C in acrylic cuvettes (Sarstedt). Each reaction was started by the addition of 100 μM NADPH. The decrease in absorbance at 340 nm was monitored for 120 s using a Shimadzu UV-2450 spectrophotometer coupled to *UVProbe* (Shimadzu). Dihydrobiopterin stocks were prepared in 0.1 M NaOH to 10 mM and stored at -20 °C. Fresh NADPH was dissolved in water to 10 mM immediately prior to each set of approximately 50 measurements. DMSO to a final concentration of 1 % (v:v) was present throughout. All potential inhibitors were initially assessed in duplicate at 10 μM and 50 μM. Compounds showing inhibition of greater than 60-70 % at 50 μM were assayed in triplicate at a range of concentration points (9-12) to produce a full dose-response curve. Mean PTR1 inhibition (%) was plotted against the log compound concentration and a sigmoidal curve fit using four-parameter non-linear regression in *SigmaPlot*

(Systat Software). IC_{50} values were extracted and K_i values calculated using the Cheng-Prusoff equation for competitive inhibition where $K_i = IC_{50} : (1 + [S]:K_m)$.[17] Note that this assumes stoichiometry of 1:1 and that all inhibitor-binding reactions are reversible. Substrate K_m was used as published (10.9 μM)[16]

***Tb*PTR1-ligand co-crystallization and structure solution**

*Tb*PTR1 was crystallized in the presence of cofactor and ligand of interest following published methods.[18] A mixture of 4-6 mg mL^{-1} *Tb*PTR1, 1 mM $NADP^+$, 20 mM DTT and 1 mM compound was incubated on ice for 1 h prior to crystallization. The solution was centrifuged to remove any insoluble material. 1-2 μL protein-ligand solution was then mixed with 1 μL crystallization solution containing 1.7-2.7 M NaOAc and 20-50 μM sodium citrate pH 4.5-5.0. Drops were suspended on siliconized glass coverslips above reservoirs containing the latter solution at 18°C.

Crystals grown in an excess of 2.6 M NaOAc were placed in a nylon loop and flash-cooled directly in liquid nitrogen. Otherwise, a solution of 3 M NaOAc was used to cryoprotect the *Tb*PTR1-ligand co-crystal prior to cooling to -173°C. Diffraction data were collected in-house or at Diamond light source (Supplementary **Table 1**). Data were collected to 1.7-2.4 Å resolution and typically covered approximately 180 degrees of rotation. X-ray images were integrated using *XDS*[19] or *MOSFLM* [20] and scaled in *SCALA*[21] or *AIMLESS*.[22] Molecular replacement was performed by *MOLREP*[23] or data were refined directly against the *Tb*PTR1 tetrameric starting model using *REFMAC5* (PDB 2c7v).[16,24] Electron and difference density map inspection and model manipulation with *Coot* [25] was combined with multiple further refinement cycles. Non-crystallographic symmetry restraints were not applied and geometry restraint weightings were manually adjusted in later refinement calculations. The subset of data used to calculate R_{free} was maintained in all structure analyses. Cofactor and solvent molecules were added to the model in *Coot*[25] from the associated monomer library.[26] Novel ligands were drawn using *JME Molecule Editor*[27] and coordinates generated using *PRODRG*.[28] Geometry restraints were obtained from *PRODRG*[28] or *eLBOW*[29] and each ligand placed according to difference density Fourier maps. For compounds **8b** and **29a**, two conformations were modelled and refined, each with occupancy set to 0.5. All coordinates and structure factors have been deposited in the Protein Data Bank.

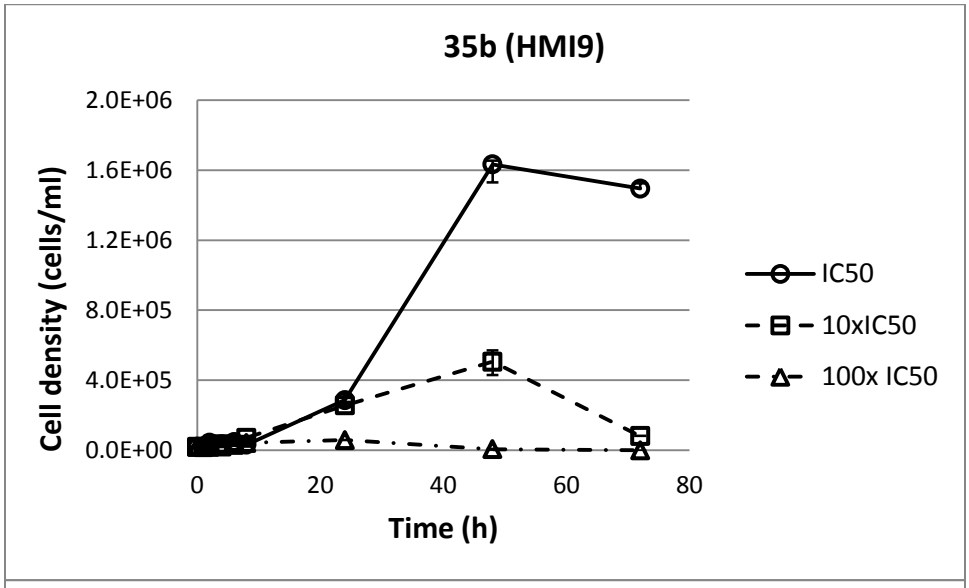
Representative kill curves for active compounds against *T. brucei* in culture

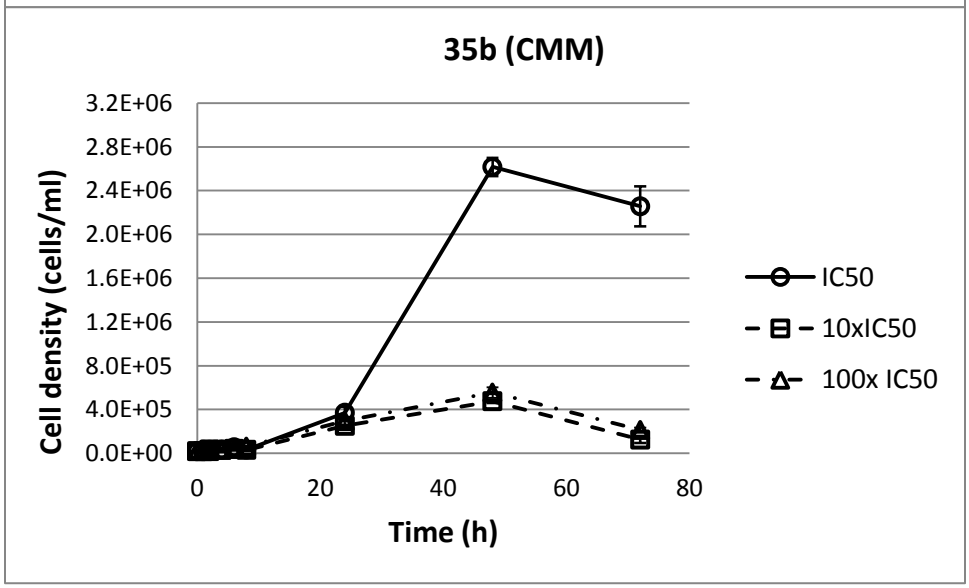
Time to kill *T. brucei* protocol:

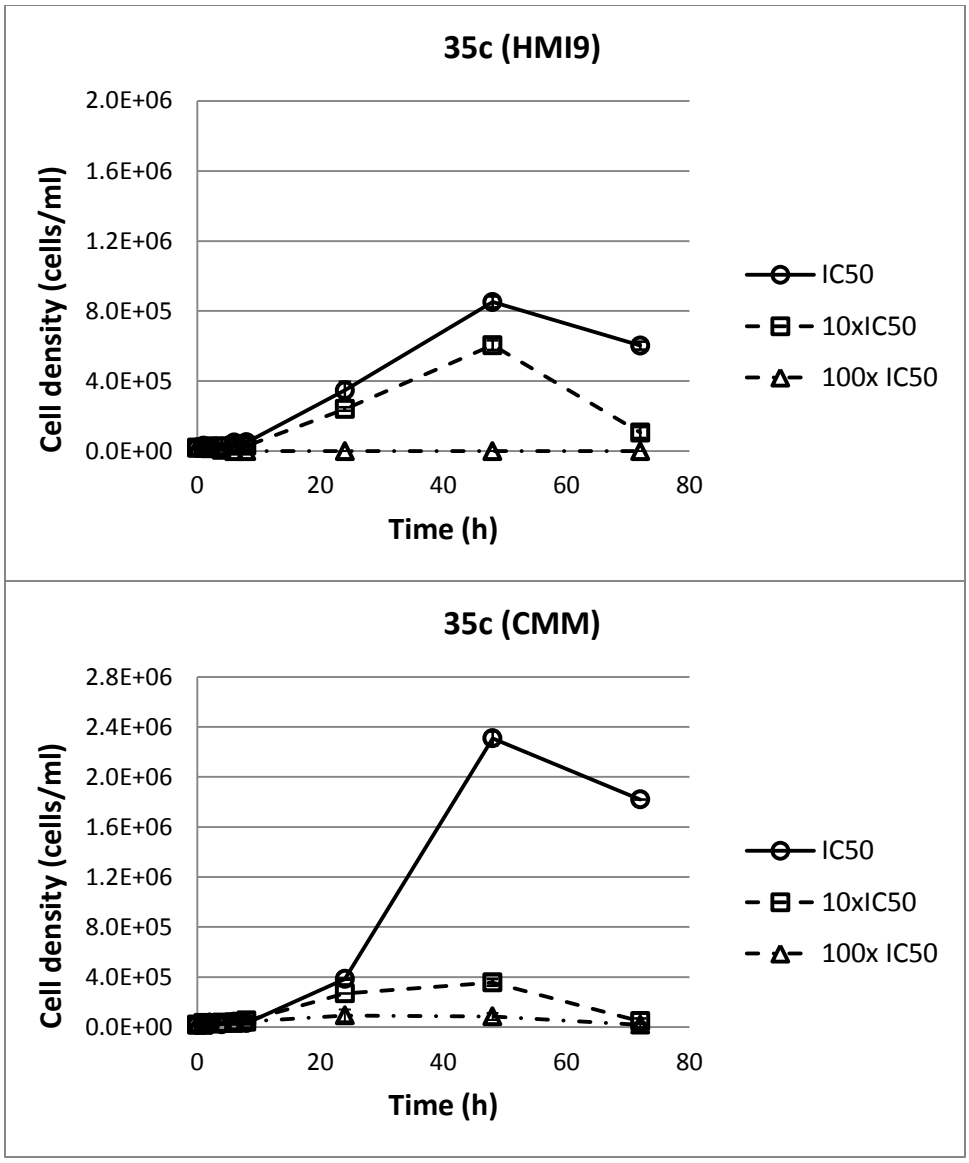
To determine the *in vitro* compounds' exposure required to kill trypanosomes, parasites were seeded at 2×10^4 cells/ml in either HMI-9 or CMM and cell concentration measured at regular intervals for 48-72 hours after exposure to a range of concentrations (IC_{50}, $10 \times IC_{50}$ and $100 \times IC_{50}$) of selected compounds. Untreated trypanosomes were monitored in parallel as negative control.

Growth curves of bloodstream *T. b. brucei* strain 427 treated with selected compounds at different doses in either HMI-9 or CMM. Data represent the average of at least two independent experiments and are given ± SEM.

Pyrrolopyrimidines






35c (HMI9)
Cell density (cells/ml)
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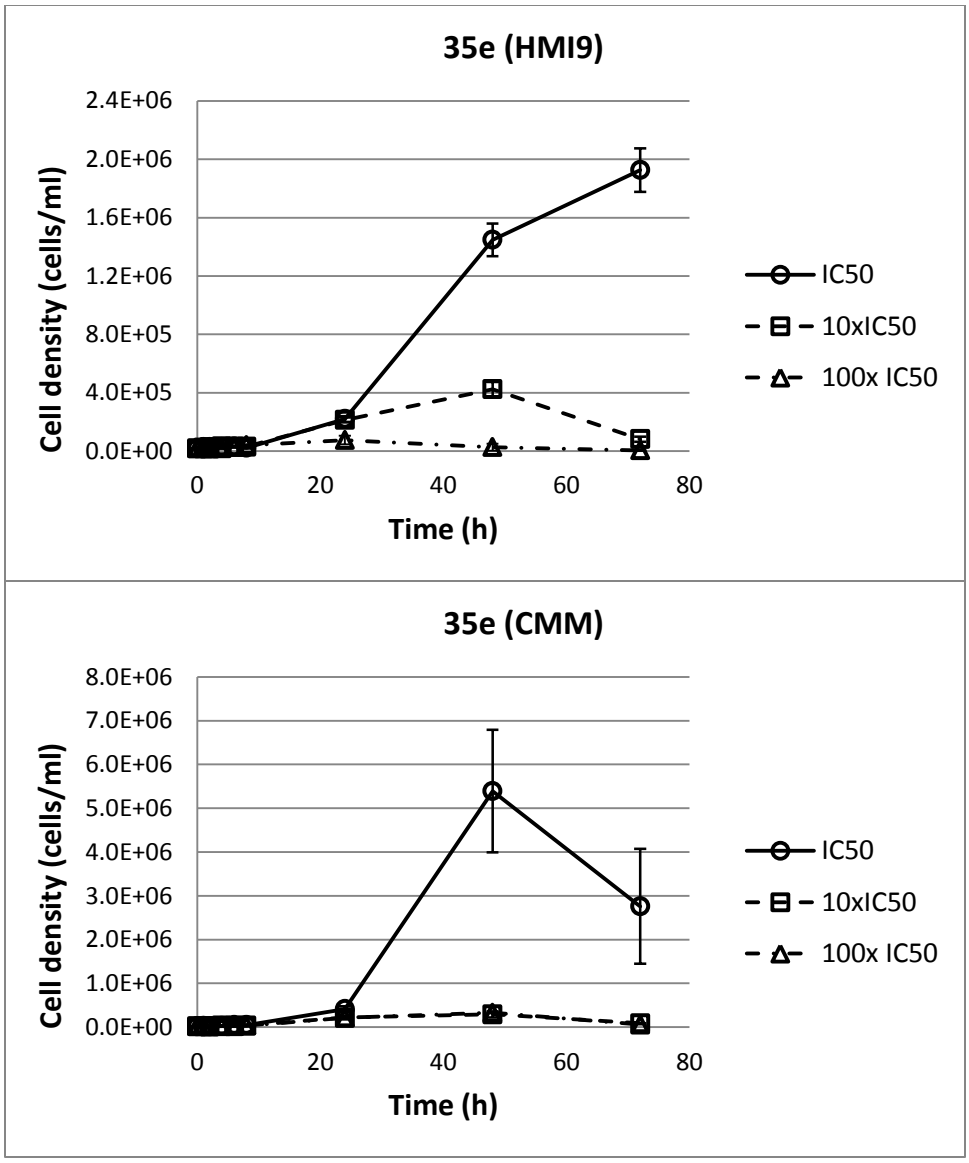
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
0
20
40
60
80
Time (h)
IC50
10xIC50
100x IC50
35c (CMM)
Cell density (cells/ml)
2.8E+06
2.4E+06
2.0E+06
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
0
20
40
60
80
Time (h)
IC50
10xIC50
100x IC50


35e (HMI9)
Cell density (cells/ml)
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1.2E+06
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4.0E+05
0.0E+00
0
20
40
60
80
Time (h)
IC50
10xIC50
100x IC50
35e (CMM)
Cell density (cells/ml)
8.0E+06
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
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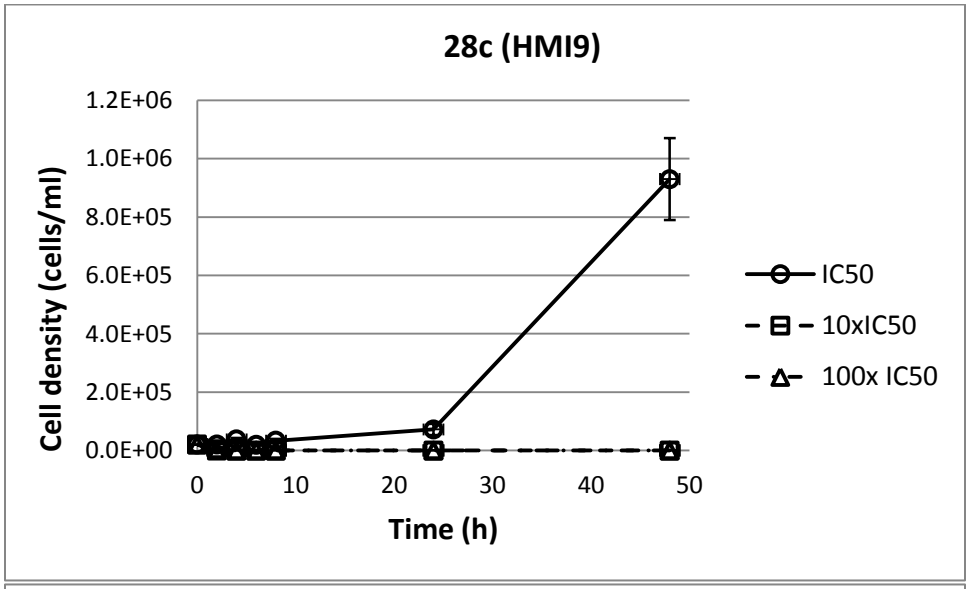
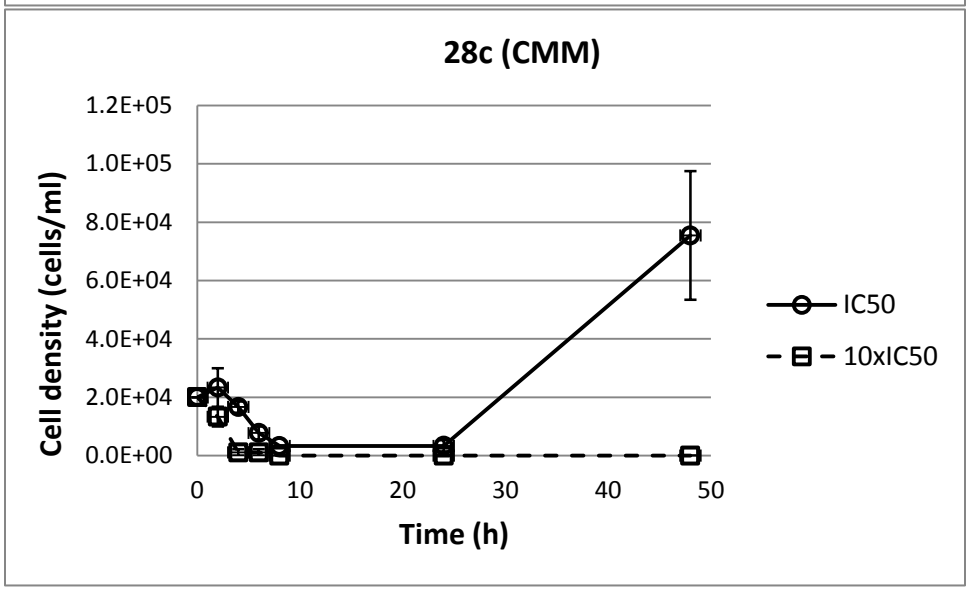
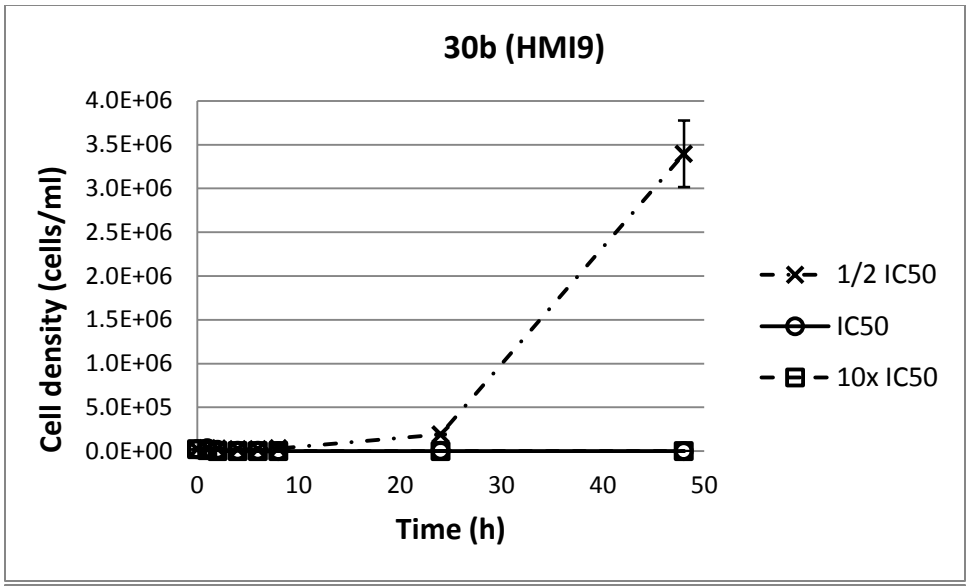
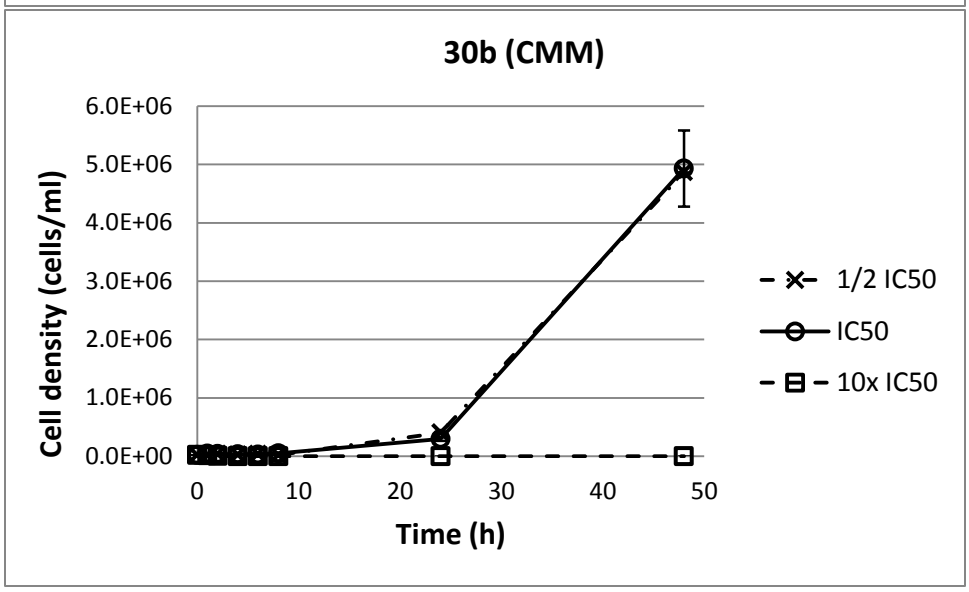
0
20
40
60
80
Time (h)
IC50
10xIC50
100x IC50

Nitro intermediates









Estimation of physicochemical parameters

Pipeline Pilot ® (Accelrys Inc.) uses an atom-based method to calculate logP.[30] Estimation of pK_a is carried out using the path fingerprints for the identified ionization sites and comparing them with comparable sites of known pK_a.[31]

References for supplementary information

1. Gangjee, A.; Vidwans, A.; Elzein, E.; McGuire, J.J.; Queener,S.F.; Kisliuk. R.L. Synthesis, antifolate, and antitumor activities of classical and nonclassical 2-amino-4-oxo-5-substituted-pyrrolo[2,3-d]pyrimidines, *J. Med. Chem*., **2001**, *44*, 1993-2003.

2. Gaojun, Z.; Liu, Z.; Xu, Y.; Mao, Z.. 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), *Heterocycles* **2008**, *75*, 1631-1638.

3. Pfleiderer,.W.; Lohrmann, Rolf.; Pteridines. XII. Synthesis of 2-amino-4-alkoxypteridines *Chemische Berichte* **1961**, *94*, 12-18.

4. Nakano, Y.; Kato, Y.; Imai, K.; Ochiai, E.; Namekawa, J.I.; Ishizuka, S.; Takenouchi, K.; Tanatani,A.; Hashimoto,Y.; Kazuo Nagasawa, K. Practical synthesis and evaluation of the biological activities of 1α,25-dihydroxyvitamin D3 Antagonists, 1α,25-dihydroxyvitamin D3-26,23-lactams designed on the basis of the helix 12-folding inhibition hypothesis, *J. Med. Chem.*, **2006**, *49*, 2398–2406.

5. Taylor, E.C.; Liu, B., A New and efficient synthesis of pyrrolo[2,3-d]pyrimidine anticancer agents: Alimta (LY231514, MTA), Homo-Alimta, TNP-351, and some aryl 5-substituted pyrrolo[2,3-d]pyrimidines, *J. Org. Chem.* **2003**, *68*, 9938-9947.

6. Gangjee, A.; Lin, X.; Kisliuk, R.L.; McGuire, J.J. Synthesis of N-{4-[(2,4-Diamino-5-methyl-4,7-dihydro-3H- pyrrolo[2,3-d]pyrimidin-6-yl)thio]benzoyl}-L-glutamic Acid and N-{4-[(2-Amino-4-oxo-5-methyl-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin- 6-yl)thio]benzoyl}-L-glutamic acid as dual Inhibitors of dihydrofolate reductase and thymidylate synthase and as potential antitumor agents, *J. Med. Chem*. **2005**, *48*, 7215-7222.

7. Cutulic, S.P.Y.; Findlay, N.J.; Zhou, S-Z; Chrystal, E.J.T.; Murphy, J.A., Metal-free reductive cleavage of C-O σ-bonds in acyloin derivatives by an organic neutral super-electron-donor, *J. Org. Chem*. **2009**, *74*, 8713-8718. Chatterjee, A.K.; Nagle, A.; Wu, T.; Tully, D.; Kuhen, K.L. Compounds and compositions for the treatment of parasitic diseases, WO **201**1:006143.

8. Baader, E; Jendralla, H; Kerekjarto, B; Beck, G Preparation and testing of carboxydihydroxyhexenylpyridazines and lactone analogs as cholesterol lowering substances. EP0324347 **1989**.

9. Ramajayam, R.; Giridhar, R.; Yadav, M. R.; Balaraman, R.; Djaballah, H; Shum, D.; Radu, C., Synthesis, antileukemic and antiplatelet activities of 2,3-diaryl-6,7-dihydro-5H-1,4-diazepines, Eur. *J. Med. Chem*. **2008**, *43*, P2004-2010.

10. Nyitrai, J., Markovits-Kornis, R., Lempert, K.,Hydantoins, thiohydantoins, and glycocyamidines. XXVII. Reductive rearrangements of the retrobenzilic acid type induced by Lewis acids. 4. Scope of the aluminum chloride induced reactions of dithiohydantoins with arenes, *Acta Chimica Academiae Scientiarum Hungaricae* **1969**, *60*, 141-149.

11. Wataru, F.; Teiichi, A.; Kazumi,K.; Saneshisa, K. Organic fluorine compounds. II. Some triarylethylenes containing fluorine, *Yuki Gosei Kagaku Kyokaishi*, **1959**, *17*, 717-721.

12. Gadad, Andanappa K.; Palkar, Mahesh B.; Anand, K.; Noolvi, Malleshappa N.; Boreddy, Thippeswamy S.; Wagwade, J., Synthesis and biological evaluation of 2-trifluoromethyl/sulfonamido-5,6-diaryl substituted imidazo[2,1-b]-1,3,4-thiadiazoles: A novel class of cyclooxygenase-2 inhibitor, *Bioorg. Med. Chem*., **2008**, *16*, 276-283.

13. Newman, Melvin S.; George, M. V., Synthesis of 3',6-dimethyl-1,2-benzanthracene, *J. Org. Chem*., **1961**, *26*, 4306-4307.

14. Prakash, O. Kumar, R., Sharma, D., Pannu, K., Kamal, R., The chemistry of α,β-ditosyloxyketones: novel routes for the synthesis of desoxybenzoins and α-aryl-β-ketoaldehyde dimethylacetals from α,β-chalcone ditosylates, *Synlett*, **2007**, 2189-2192.

15. Zarghi, A.; Argaei, S.; Ghodsi, R. Design and synthesis of new 2,4,5-triarylimidazole derivatives as selective cyclooxygenase (COX-2) inhibitors, *Med. Chem. Res*. **2012**, *21*, 1803-1810.

16. Dawson, A., Gibellini, F., Sienkiewicz, N., Tulloch, L.B., Fyfe, P.K., McLuskey, K., Fairlamb, A.H., Hunter, W.N., Structure and reactivity of *Trypanosoma brucei* pteridine reductase: inhibition by the archetypal antifolate methotrexate, *Mol. Microbiol.* **2006**, *61*, 1457–1468.

17. Cheng, Y., Prusoff, W.H., Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction, *Biochem. Pharmacol*. **1973**, *22*, 3099–3108.

18. Tulloch, L.B., Martini, V.P., Iulek, J., Huggan, J.K., Lee, J.H., Gibson, C.L., Smith, T.K., Suckling, C.J., Hunter, W.N., Structure-based design of pteridine reductase inhibitors targeting African sleeping sickness and the leishmaniases, *J. Med. Chem.* **2010**, *53*, 221–229.

19 Kabsch, W., Software XDS for image rotation, recognition and crystal symmetry assignment, *Acta Crystallogr. D, Biol. Crystallogr.,* **2010**, *66*, 125-132.

20. Battye, T.G.G., Kontogiannis, L., Johnson, O., Powell, H.R., Leslie, A.G.W., iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr. D Biol. Crystallogr*. **2011**, *67*, 271–281.

21. Evans, P., Scaling and assessment of data quality. *Acta Crystallogr. D Biol. Crystallogr*. **2006**, *62*, 72–82.

22. Evans, P.R., Murshudov, G.N., How good are my data and what is the resolution? *Acta Crystallogr. D Biol. Crystallogr.* **2013**, *69*, 1204–1214.

23. Vagin, A., Teplyakov, A. MOLREP: an automated program for molecular replacement. *J. Appl. Cryst.* **1997**, *30*, 1022-1025

24. Murshudov, G.N., Skubák, P., Lebedev, A.A., Pannu, N.S., Steiner, R.A., Nicholls, R.A., Winn, M.D., Long, F., Vagin, A.A. REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 355–367.

25. Emsley, P., Lohkamp, B., Scott, W.G., Cowtan, K. Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 486–501.

26. Vagin, A.A., Steiner, R.A., Lebedev, A.A., Potterton, L., McNicholas, S., Long, F., Murshudov, G.N. REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2184–2195.

27. Ertl, P. Molecular structure input on the web. *J. Cheminform.* **2010**, *2*, 1.

28. Schüttelkopf, A.W., van Aalten, D.M.F. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 1355–1363.

29. Moriarty, N.W., Grosse-Kunstleve, R.W., Adams, P.D.,Electronic ligand Builder and optimization workbench (eLBOW): a tool for ligand coordinate and restraint generation. *Acta Crystallogr. D Biol. Crystallogr.* **2009**, *65*, 1074–1080.

30. Ghose, A.K., Viswanadhan, V.N., Wendoloski, J.J. Prediction of hydrophobic (lipophilic) properties of small organic molecules using fragment methods: an analysis of AlogP and ClogP methods. *J. Phys. Chem. A* **1998**, *102*, 3762-3772.

31. www.biobyte.com. Lange's Handbook of Chemistry, 16th edition, McGraw-Hill Education, New York, **2005**.