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Synthesis and Testing of 5-Benzyl-2,4-diaminopyrimidines as Potential Inhibitors of Leishmanial and Trypanosomal Dihydrofolate Reductase

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Dihydrofolate reductase is a drug target that has not been thoroughly investigated in leishmania and trypanosomes. Work has previously shown that 5-benzyl-2,4-diaminopyrimidines are selective inhibitors of the leishmanial and trypanosome enzymes. Modelling predicted that alkyl/aryl substitution on the 6-position of the pyrimidine ring should increase enzyme activity of 5-benzyl-2,4-diaminopyrimidines as inhibitors of leishmanial and trypanosomal dihydrofolate reductase. Various compounds were prepared and evaluated against both the recombinant enzymes and the intact organisms. The presence of a substituent had a small or negative effect on activity against the enzyme or intact parasites compared to unsubstituted compounds.

Keywords: Dihydrofolate reductase; *Trypanosoma brucei*; *Trypanosoma cruzi*; Leishmania

INTRODUCTION

There is an urgent need for new drugs for the treatment of parasitic diseases caused by protozoa, such as leishmaniasis, African trypanosomiasis and Chagas' disease. The current drugs available to treat these diseases are increasingly subject to resistance, show poor clinical efficacy in some cases, and often show severe side effects. We are interested in exploiting dihydrofolate reductase (DHFR) as a potential drug target against the causative organisms of these diseases. Although this enzyme is a well-studied and proven drug target for a number of diseases (cancer, bacterial infections, malaria, toxoplasmosis, etc), little has been done to investigate it as a drug target for these diseases.[1] Modelling suggests that there are significant differences in the structures of the actives sites of the leishmanial and trypanosomal enzymes compared to the human enzyme, which should allow selective drug design.[2] Most common DHFR inhibitors, such as pyrimethamine and trimethoprim, are not suitable as drug candidates for these diseases as they are selective for the human enzyme over the parasite enzyme. However following some preliminary data by Sirawaraporn and co-workers[3] we prepared an extensive series of substituted 5-benzyl-2,4-diaminopyrimidines,[4] (with general structure **1**) and evaluated these against the enzymes from *Leishmania major* (a causative organism of leishmaniasis), *Trypanosoma cruzi* (the causative organism of Chagas' disease) and *Trypanosoma brucei* (the causative organism of African trypanosomiasis) and also against the human enzyme. Modelling studies suggested that there may be a hydrophobic pocket adjacent to the 6-position of the pyrimidine ring. Preliminary studies with substituents at this position (**2**, **3** and **4**), indicated that an ethyl substituent may increase the inhibitor activity and selectivity (Fig. 1, Table I).[4] This paper describes the design of a set of 6-substituted pyrimidines, and the preparation and evaluation of new compounds (**5**, **7**, **8**, **9** and **10**).

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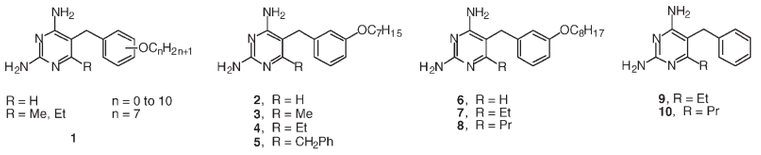


FIGURE 1

MATERIALS AND METHODS

Chemistry

Chemicals were obtained from Sigma-Aldrich, Fluka and Lancaster. Where applicable, glassware was oven dried and reactions were carried out under a nitrogen atmosphere. Dry solvents were purchased from Aldrich or Fluka. Reactions were monitored by t.l.c. using silica gel 60 F254 plates (Merck). NMR spectra were recorded on a Bruker avance 300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Infrared spectra were recorded on a Perkin–Elmer 1600 series FTIR spectrometer. Low-resolution mass spectra were recorded on a Micromass Platform 2 spectrometer. Accurate mass spectra were determined by the EPSRC Mass Spectrometry Centre, Swansea, UK. Elemental analysis was carried out by Medac Limited.

5-(1-Hydroxy-2-phenylethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11)

To a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (4.0 g, 28 mmol, 1 eq) in dry dichloromethane (200 ml), phenyl acetylchloride (4.36 ml, 33 mmol, 1.2 eq) and dry pyridine (4.5 ml, 56 mmol, 2 eq) were added and the reaction mixture was stirred at 0°C for 1 h and then at room temperature for another 2 h under an atmosphere of nitrogen. After acylation was complete, DCM was washed with dilute HCl (0.5 M) and water and the organic layer was separated, dried over $MgSO_4$ and concentrated *in vacuo* to yield the crude product. The crude product was purified by flash column chromatography on silica gel using an elution of 5% EtOAc in hexane to yield the title compound (**11**) as colourless crystals (4.5 g, 61.6%).

TLC R_f 0.38 (5% MeOH in EtOAc); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.8 (6H, s, $2\times CH_3$), 2.2 (1H, s, O*H*), 4.6 (2H, s, CH_2–Ar), 7.4 (5H, m, $5\times$ Ar*H*); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 27.2 ($2\times CH_3$), 41.2 (CH_2), 91.8 ($OC(CH_3)_2$), 105.4 (C = *C*CO), 127.7 (Ar–C), 127.9 (Ar–C), 129.0 (Ar–C), 129.7 (Ar–C), 130.0 (Ar–C), 134.5 (Ar–C1), 160.7 (CO), 170.9 (CO), 195.0 (OH*C* = C); IR (KBr) 3064 (C–H aromatic), 2870 (C–H aliphatic), 1742 (CO of ester) cm^{-1}; *m/z* (ES^+) 263.4 ($M + H^+$, 100%); HRMS (CI^+) 280.1185 ($M + NH_4^+$, $C_{14}H_{18}NO_5$ requires 280.1185); Found: C, 64.17; H, 5.27. Calc for $C_{14}H_{14}O_5$; C, 64.12; H, 5.38%.

Ethyl-3-oxo-4-phenylbutanoate (12)

A solution of compound **11** (4.5 g) in dry ethanol (500 ml) was heated to reflux for 5 h. The reaction mixture was allowed to cool and reduced *in vacuo* to yield the title compound (**12**) as colourless crystals (2.2 g, 62.1%).

TLC R_f 0.32 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.2 (3H, t, $J = 7.1$ Hz, CH_3), 3.5 (2H, s, $COCH_2CO$), 3.8 (2H, s, $ArCH_2$), 4.2 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 7.4 (5H, m, $5\times$ Ar*H*); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 14.5 (CH_3), 48.7 (CH_2), 50.4 ($COCH_2CO$), 61.8 (OCH_2), 127.7 (Ar–C), 127.7 (Ar–C), 129.2 (Ar–C), 129.8 (Ar–C), 130.0 (Ar–C), 133.8 (Ar–C1), 177.4 (CO), 201.0 (CO); IR (liquid film) 2983.4 (C–H aromatic), 1734.1 (CO of ester), 1718.6 (CO of ketone) cm^{-1}; *m/z* ($APCI^+$)

TABLE I Inhibition constant (K_i, μM) of compounds against the recombinant enzyme

Compound	6-Substitutent	3′-Substitutent	DOCK contact score	*L. major*	*T. cruzi*	*T. brucei*	Human
2	H	O-Heptyl	188	0.22 (2.8)	1.34 (0.4)	0.13 (4.7)	0.62
3	Me	O-Heptyl	N.D.	0.36 (2.7)	1.51 (0.66)	0.07 (14)	1.0
4	Et	O-Heptyl	N.D.	0.04 (8.0)	0.71 (0.56)	0.02 (21)	0.4
5	CH_2Ph	O-Heptyl	254	0.08 (54)	7.10 (0.65)	N.D.	4.58
6	H	O-Octyl	188	0.09 (25)	1.13 (2.1)	0.024 (100)	2.42
7	Et	O-Octyl	196	0.03 (27)	1.49 (0.59)	N.D.	0.88
8	Pr	O-Octyl	195	0.06 (21)	6.70 (0.21)	N.D.	1.42
9	Et	H	N.D.	8.09 (0.42)	5.59 (0.60)	N.D.	3.33
10	Pr	H	N.D.	0.49 (1.2)	0.53 (1.1)	N.D.	0.58

The selectivity of the compounds is shown in parenthesis, where selectivity is defined as: K_i (human enzyme)/K_i (parasite enzyme).

206.84 ($M + H^+$, 30%); HRMS (CI^+) 207.1021 ($M + H^+$, $C_{12}H_{15}O_3$ requires 207.1021).

3-Octyloxy-benzaldehyde (13)

To a solution of 3-hydroxy benzaldehyde (10 g, 81.9 mmol, 1 eq) in dry EtOH (500 ml) potassium hydroxide (9.19 g, 163.8 mmol, 2 eq) was added. Iodooctane (17.69 ml, 98 mmol, 1.2 eq) was added to the reaction mixture dropwise after 10 min. The mixture was heated to reflux for 5 h and was allowed to cool to room temperature. The reaction mixture was extracted with Et_2O (1000 ml), dried over $MgSO_4$ and reduced *in vacuo* to yield the crude product as a brown oil. The crude product was purified by flash column chromatography on silica gel using gradient elution of 5 to 15% EtOAc in hexane to yield the title compound as a faintly yellow oil (**13**) (11 g, 57%).

TLC R_f 0.45 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, $J = 6.3$ Hz, CH_3), 1.3–1.6 (10H, m, 5 × CH_2), 1.8 (2H, quintet, $J = 6.7$ Hz, OCH$_2$CH_2), 3.9 (2H, t, $J = 6.5$ Hz, OCH_2), 7.1–7.5 (4H, m, 4 × ArH), 9.9 (IH, s, CHO); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 16.5 (CH_3), 25.1 (CH_2), 28.5 (CH_2), 31.6 (CH_2), 31.7 (CH_2), 32.3 (CH_2), 34.3 (CH_2), 70.7 (OCH_2), 115.2 (Ar–C), 124.3 (Ar–C), 125.6 (Ar–C), 132.4 (Ar–C), 140.2 (Ar–C1), 162.1 (Ar–C3), 194.5 (CHO); IR (liquid film) 2932 (C–H aromatic), 2864 (C–H aliphatic), 1687 (CHO) cm^{-1}; m/z (APCI, + ve) 235.03 ($M + H^+$, 100%); HRMS(CI^+): m/z 252.1964 ($M + NH_4^+$, $C_{15}H_{26}NO_2$ requires 252.1963).

3-(Octyloxy)-phenylmethanol (14)

To an ethanolic solution of **13** (11 g, 47 mmol, 1 eq) sodium borohydride (0.91 g, 24 mmol, 0.5 eq) was added and the reaction was stirred at room temperature for 20 min. The reaction mixture was reduced *in vacuo* and extracted with EtOAc, dried over $MgSO_4$ and concentrated *in vacuo* to yield the title compound (**14**) as a clear oil (5.145 g, 59%).

TLC R_f 0.36 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, $J = 6.6$ Hz, CH_3), 1.3–1.6 (10H, m, 5 × CH_2), 1.8 (1H, s, CH$_2$OH), 1.9 (2H, quintet, $J = 6.5$ Hz, OCH$_2$CH_2), 3.8 (2H, t, $J = 6.5$ Hz, OCH_2), 4.8 (2H, s, CH_2Ar), 7.1–7.5 (4H, m, 4 × ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 14.5 (CH_3), 23.1 (CH_2), 23.6 (CH_2), 26.4 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 32.2 (CH_2), 65.5 (CH_2OH), 68.3 (OCH_2), 113.2 (Ar–C), 114.2 (Ar–C), 119.3 (Ar–C), 129.9 (Ar–C), 142.8 (Ar–C1), 159.8 (Ar–C3); IR (liquid film) 3348 (OH), 2990 (C–H aromatic), 2855 (C–H aliphatic) cm^{-1}; m/z (ES^+) 218.96 (M–OH, 100%); HRMS (CI^+) 237.1854 ($M + H^+$, $C_{15}H_{25}O_2$ requires 237.1854).

1-(3-(Bromomethyl)-phenoxy)-octane (15b)

Potassium tribromide (10.17 ml, 110 mmol, 5.5 eq) was added dropwise to a solution of **14** (1.9 g, 8.56 mmol, 1 eq) in dry dioxane (80 ml) under an atmosphere of nitrogen and the reaction mixture was stirred overnight at room temperature. The reaction mixture was partitioned between water and Et_2O and was washed with $NaHCO_3$. The solvent was removed *in vacuo* to yield the title compound (**15b**) as a light yellow oil (3.4 g, 56%).

TLC R_f 0.52 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) 1 (3H, t, $J = 6.6$ Hz, CH_3), 1.2–1.6 (10H, m, 5 × CH_2), 1.9 (2H, quintet, $J = 6.6$ Hz, OCH$_2$CH_2), 3.9 (2H, t, $J = 6.5$ Hz, OCH_2), 4.8 (2H, s, CH_2Ar), 6.8–7.4 (4H, m, 4 × ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) 15.5 (CH_3), 24.1 (CH_2), 27.5 (CH_2), 30.6 (CH_2), 30.8 (CH_2), 32.2 (CH_2), 33.2 (CH_2), 67.3 (CH_2Br), 68.4 (OCH_2), 116.1 (Ar–C), 116.4 (Ar–C), 122.5 (Ar–C), 131.2 (Ar–C), 140.5 (Ar–C1), 160.7 (Ar–C3); m/z ($APCI^+$) 298.91 (M + H, 100%), 300.85 (M + H + 2, 90%); HRMS (CI^+) 298.0932 $C_{15}H_{23}OBr$ (M +) requires 298.0932.

Ethyl-2-(3-heptoxy)-benzyl)-3-oxo-4-phenylbutanoate (16a)

Sodium ethoxide (0.639 g, 9.46 mmol, 1 eq) was added to a solution of (**12**) (1.95 g, 9.46 mmol, 1 eq) in dry ethanol (100 ml) and compound **15a** (2.67 g, 9.46 mmol, 1 eq) was added to the reaction mixture after 20 min and the mixture then heated to reflux for 4 h. It was then extracted with Et_2O, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography using an elution of 5% EtOAc in hexane to yield the title compound **16a** as a clear oil (2 g, 51.5%).

TLC R_f 0.35 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, $J = 6.5$ Hz, CH_3), 1.2 (3H, t, $J = 7.1$ Hz, CH_3), 1.2–1.8 (8H, m, 4 × CH_2), 1.8 (2H, quintet, $J = 6.6$ Hz, OCH$_2$CH_2), 3.1 (2H, d, $J = 7.6$ Hz, CH_2Ar), 3.7 (2H, s, COCH_2Ar), 3.8 (1H, t, $J = 7.3$ Hz, CHCH$_2$Ar), 3.9 (2H, t, $J = 6.2$ Hz, OCH_2CH$_2$), 4.1 (2H, q, $J = 7.1$ Hz, OCH_2CH$_3$), 6.7–7.2 (9H, m, 9 × ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 14.4 (CH_3), 14.5 (CH_3), 23.0 (CH_2), 26.4 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 30.1 (CH_2), 32.2 (CH_2), 34.6 (CH_2), 59.5 (COC HCO), 61.9 (OCH_2), 68.2 (OCH_2), 113.1 (Ar–C), 115.3 (Ar–C), 121.3 (Ar–C), 123.5 (Ar–C), 126.2 (Ar–C), 127.5 (Ar–C), 129.0 (Ar–C), 129.8 (Ar–C), 130.0 (Ar–C), 133.4 (Ar–C1), 139.9 (Ar–C1′), 159.6 (Ar–C3), 169.2 (CO), 202.6 (CO); IR (liquid film) 2930 (C–H aromatic), 2858 (C–H Aliphatic), 1743 (CO of ester), 1716 (CO of ketone) cm^{-1}; m/z (ES^+), 427.91 ($M + NH_4^+$, 100%), 448.76 ($M + K^+$, 25%); HRMS (CI^+) 428.2801 ($M + NH_4^+$, $C_{26}H_{38}NO_4$ requires 428.2801).

Ethyl-2-(3-(octyloxy)-benzyl)-3-oxopentonoate (16b)

Sodium ethoxide (0.27 g, 3.90 mmol, 1.5 eq) was added to a solution of ethylpropionyl acetate (0.376 ml, 2.64 mmol, 1 eq) in dry ethanol (25 ml) and compound **15b** (0.79 g, 2.64 mmol, 1 eq) was added to the reaction mixture after 20 min and the mixture then heated to reflux for 4 h. It was then extracted with Et_2O, washed with water, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography using an elution of 5% EtOAc in hexane to yield the title compound (**16b**) as a clear oil (0.65 g, 68.42%).

TLC R_f 0.38 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, $J = 6.14$ Hz, CH_3), 1 (3H, t, $J = 7.1$ Hz, CH_3), 1.2 (3H, t, $J = 7.1$ Hz, CH_3), 1.3–1.6 (10H, m, 5CH_2), 1.8 (2H, quintet, $J = 6.5$ Hz, OCH$_2$CH_2), 2.3–2.5 (2H, q, $J = 7.0$ Hz, CH_2CH$_3$), 3.1 (2H, d, $J = 7.6$ Hz, CH_2,Ar), 3.8 (1H, t, $J = 7.5$ Hz, CHCH$_2$,Ar), 3.9 (2H, t, $J = 6.5$ Hz, OCH_2CH$_2$), 4.2 (2H, q, $J = 7.1$ Hz, OCH_2CH$_3$), 6.7 (3H, m, 3 × ArH), 7.2 (1H, t, $J = 7.6$ Hz, ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 7.9 (CH_3), 14.4 (CH_3), 14.5 (CH_3), 23.0 (CH_2), 26.4 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 32.0 (CH_2), 34.6 (CH_2), 36.7 (CH_2), 60.6 (CO*C*HCO), 61.8 (O*C*H$_2$), 68.2 (O*C*H$_2$), 111.8 (Ar–C), 113.8 (Ar–C), 121.2 (Ar–C), 129.9 (Ar–C), 140.2 (Ar–C), 159.6 (Ar–C), 170.08 (CO), 205.6 (CO); IR (liquid film) 2929 (C–H aromatic), 2856 (C–H Aliphatic), 1745 (CO of ester), 1716 (CO of ketone) cm^{-1}; m/z (ES$^+$) 380.0 (M + NH$_4^+$, 100%), 385.1 (M + Na$^+$, 80%), 401.0 (M + K$^+$, 70%); HRMS (CI$^+$) 380.2801 (M + NH$_4^+$, $C_{22}H_{38}NO_4$ requires 380.2801).

Ethyl-2-(3-(octyloxy)-benzyl)-3-oxohexanoate (16c)

Sodium ethoxide (0.293 g, 4.32 mmol, 1.5 eq) was added to a solution of ethylbutyryl acetate (0.455 ml, 2.88 mmol, 1 eq) in dry ethanol (35 ml) and compound **15b** (0.86 g, 2.88 mmol, 1 eq) was added to the reaction mixture after 20 min and the mixture then heated to reflux for 3 h. It was then extracted with Et_2O, washed with water, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography using an elution of 20% EtOAc in hexane to yield the title compound (**16c**) as a clear oil (0.545 g, 51%).

TLC R_f 0.37 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (6H, m, 2 × CH_3), 1.1 (3H, t, $J = 7.1$ Hz, CH_3), 1.3–1.5 (10H, m, 5 × CH_2), 1.6 (2H, q, $J = 7.2$ Hz, CH_2CH$_3$), 1.8 (2H, quintet, $J = 6.5$ Hz, OCH$_2$CH_2), 2.3–2.5 (2H, m, CH_2CH$_2$CH$_3$), 3.1 (2H, d, $J = 7.6$ Hz, CH_2Ar), 3.8 (1H, t, $J = 7.5$ Hz, CHCH$_2$Ar), 3.9 (2H, t, $J = 6.5$ Hz, OCH_2CH$_2$), 4.2 (2H, q, $J = 7.1$ Hz, OCH_2CH$_3$), 6.7 (3H, m, 3 × ArH), 7.2 (1H, t, $J = 7.6$ Hz, ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 13.8 (CH_3), 14.4 (CH_3), 17.1 (CH_3), 23.0 (CH_2), 26.4 (CH_2), 29.6 (CH_2), 29.8 (CH_2), 31.4 (CH_2), 31.6 (CH_2), 34.5 (CH_2), 36.2 (CH_2), 36.6 (CH_2), 60.8 (CO*C*HCO), 61.7 (O*C*H$_2$), 68.2 (O*C*H$_2$), 111.8 (Ar–C), 115.4 (Ar–C), 120.8 (Ar–C), 129.8 (Ar–C), 140.2 (Ar–C1), 159.4 (Ar–C3), 169.5 (CO), 205.0 (CO); IR (liquid film) 2928 (C–H aromatic), 2861 (C–H Aliphatic), 1745 (CO of ester), 1715 (CO of ketone) cm^{-1}; m/z (ES$^+$) 394.2 (M + NH$_4^+$, 100%), 415.0 (M + K$^+$, 25%); HRMS (CI$^+$) 376.2614 (M$^+$, $C_{23}H_{36}O_4$ requires 376.2613).

Ethyl-2-benzyl-3-oxopentanoate (16d)

Sodium ethoxide (0.48 g, 6.94 mmol, 1 eq) was added to a solution of ethylpropionyl acetate (0.988 ml, 6.94 mmol, 1 eq) in dry ethanol (25 ml) and benzyl bromide (0.818 ml, 6.94 mmol, 1 eq) was added to the reaction mixture after 20 min and the mixture was then heated to reflux for 4 h. The reaction mixture was extracted with Et_2O (200 ml), washed with water, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography using an elution of 5% EtOAc in hexane to yield the title compound (**16d**) as a clear oil (1 gm, 61%).

TLC R_f 0.25 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, $J = 7.4$ Hz, CH$_2$CH_3), 1.15 (3H, t, $J = 7.1$ Hz, OCH$_2$CH_3), 2.2–2.5 (2H, m, CH_2CH$_3$), 3.2 (2H, d, $J = 7.7$ Hz, CH_2Ar), 3.8 (1H, t, $J = 7.5$ Hz, CHCH$_2$Ar), 4.1 (2H, q, $J = 7.0$ Hz, OCH_2CH$_3$), 7.1–7.3 (5H, m, 5 × ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 5.2 (CH_3), 8.9 (CH_3), 31.9 (CH_2Ar), 33.8 (*C*H$_2$CH$_3$), 58.4 (*C*HCH$_2$Ar), 59.3 (O*C*H$_2$CH$_3$), 123.8 (Ar–C), 126.2 (Ar–C), 126.6 (Ar–C), 136.7 (Ar–C1), 167.1 (CO), 203.2 (CO); m/z (ES$^+$) 235.04 (M + H$^+$, 25%), 257.06 (M + Na$^+$), 273.07 (M + K$^+$).

Ethyl-2-benzyl-3-oxohexanoate (16e)

Sodium ethoxide (0.645 gm, 9.48 mmol, 1.5 eq) was added to a solution of 3-oxo-hexanoic acid ethyl ester (0.999 ml, 6.32 mmol, 1 eq) in dry ethanol (25 ml) and benzyl bromide (0.76 ml, 6.32 mmol, 1 eq) was added to the reaction mixture. After 20 min the reaction mixture was heated to reflux for 4 h. The reaction mixture was extracted with Et_2O, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography using an elution of 5% EtOAc in hexane to yield the title compound (**16e**) as a clear oil (0.9 g, 57%).

TLC R_f 0.25 (10% EtOAc in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.8 (3H, t, $J = 7.4$ Hz, CH$_2$CH$_2$-CH_3), 1.2 (3H, t, $J = 7.1$ Hz, OCH$_2$CH_3), 1.5 (2H, q, $J = 7.2$ Hz, CH_2CH$_3$), 2.2–2.5 (2H, m, CH_2CH$_2$CH$_3$), 3.1 (2H, d, $J = 7.7$ Hz, CH_2Ar), 3.8 (1H, t, $J = 7.5$ Hz, CHCH$_2$Ar), 4.2 (2H, q, $J = 7.1$ Hz, OCH_2CH$_3$), 7.1–7.5 (5H, m, 5 × ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) 13.8 (CH_3), 14.4 (CH_3), 17.1 (CH_2), 34.4 (CH_2), 45.1 (CH_2), 60.9 (CO*C*HCO), 63.9 (O*C*H$_2$),

127.0 (Ar–C), 128.1 (Ar–C), 128.6 (Ar–C), 129.2 (Ar–C1), 169.5 (CO), 205.1 (CO); IR (liquid film) 2945 (C–H aromatic), 2879 (C–H Aliphatic), 1699 (CO of ketone) cm^{-1}; m/z (ES^+) 271.0 ($M + Na^+$); HRMS (CI^+) 249.1490 ($M + H^+$, $C_{15}H_{21}O_3$ requires 249.1490).

Compounds 17 (a–e): General Procedure

Solutions of guanidine hydrochloride (5 eq) in warm anhydrous ethanol (2 ml per mmol of guanidine hydrochloride) and sodium ethoxide (5 eq) in warm anhydrous ethanol (2 ml per mmol of sodium ethoxide) were prepared separately and allowed to cool to room temperature. The guanidine hydrochloride solution was added to the stirred solution of sodium ethoxide under an atmosphere of nitrogen. The mixture was stirred for 5 min, filtered and added to the corresponding β-ketoester **16a–e** (1 eq). The reaction mixture was heated to reflux for 12 to 20 h and allowed to cool, diluted with water (200 to 300 ml per mmol of 16). Concentrated HCl was added until the pH became 6.5. Then the solid from the reaction mixture was separated by vacuum filtration and washed with cold water, acetone and Et_2O to yield the title compounds (**17**) as white powders.

2-Amino-4-hydroxy-6-benzyl-5-(3-(heptoxy)-benzyl)-pyrimidine (**17a**): Using **16a** (2 g, 4.88 mmol, 1 eq) gave the title compound (**17a**) as a white powder (1.6 g, 86%)

TLC R_f 0.58 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6-DMSO) δ 0.7 (3H, t, $J = 6.6$ Hz, CH_3), 1.2–1.5 (8H, m, $4CH_2$), 1.6 (2H, quintet, $J = 6.5$ Hz, OCH_2CH_2), 3.6 (4H, s, $2 \times CH_2Ar$), 3.9 (2H, t, $J = 6.4$ Hz, OCH_2CH_2), 6.4 (2H, s, NH_2), 6.5–7.2 (9H, m, $9 \times ArH$), 10.9 (1H, s, OH); ^{13}C-NMR (75.5 MHz, d_6-DMSO) δ 14.7 (CH_3), 22.8 (CH_2), 26.3 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 30.6 (CH_2), 31.2 (CH_2), 32.0 (CH_2), 67.9 (OCH_2), 111.5 (Ar–C), 112.1 (Ar–C5), 115.0 (Ar–C), 121.0 (Ar–C), 126.2 (Ar–C), 126.7 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.4 (Ar–C), 129.8 (Ar–C), 130.4 (Ar–C), 139.5 (Ar–C1′), 143.8 (Ar–C1″), 159.4 (Ar–C2), 161.2 (Ar–C3′), 164.5 (Ar–C6); IR (KBr) 3337.3 (OH), 3112 (NH_2), 2934 (C–H aromatic), 2955 (C–H Aliphatic) cm^{-1}; m/z (ES^+) 405.88 ($M + H^+$, 100%); HRMS (FAB^+) 406.2479 ($M + H^+$, $C_{25}H_{32}N_3O_2$ requires 406.2494).

2-Amino-4-hydroxy-5-(3-(octyloxy)benzyl)-6-ethyl-pyrimidine (**17b**): Using **16b** (1 g, 2.76 mmol, 1 eq) gave the title compound (**17b**) as a white powder (0.679 g, 70.87%)

TLC R_f 0.53 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6-DMSO) δ 0.8 (3H, brs, CH_3), 0.9 (3H, t, $J = 6.8$ Hz, CH_3), 1.2–1.6 (12H, m, $6CH_2$), 2.3 (2H, q, $J = 6.7$ Hz, CH_2CH_3), 3.6 (2H, s, CH_2Ar), 3.9 (2H, brs, OCH_2CH_2), 6.4 (2H, s, NH_2), 6.7 (3H, m, $3 \times ArH$), 7.2 (1H, t, $J = 6.9$ Hz, ArH), 10.9 (1H, s, OH); ^{13}C-NMR (75.5 MHz, d_6-DMSO) δ 11.2 (CH_3), 12.7 (CH_3), 20.9 (CH_2), 24.3 (CH_2), 27.4 (CH_2), 27.5 (CH_2), 27.6 (CH_2), 28.2 (CH_2), 30.1 (CH_2), 31.9 (CH_2), 65.9 (OCH_2), 111.2 (Ar–C), 111.9 (Ar–C5), 115.0 (Ar–C), 120.8 (Ar–C), 125.8 (Ar–C6), 129.9 (Ar–C), 143.9 (Ar–C1′), 158.7 (Ar–C4), 159.3 (Ar–C2), 160.8 (Ar–C3′); m/z (ES^+) 358.19 ($M + H^+$, 100%), 380.16 ($M + Na^+$, 70%), 396.1 ($M + K^+$, 25%); IR (KBr) 3330.5 (OH), 3108.9 (NH_2), 2920.7 (C–H aromatic), 2857.7 (C–H aromatic) cm^{-1}; HRMS (CI^+) 357.2416 (M^+, $C_{21}H_{31}N_3O_2$ requires 357.2416).

2-Amino-5-(3-octyloxy)benzyl)-6-propyl-4-pyrimidinol (**17c**); Using **16c** (0.545 g, 1.45 mmol, 1 eq) gave the title compound (**17c**) as a white powder (0.355 g, 66%)

TLC R_f 0.57 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6-DMSO) δ 0.8 (6H, brs, $2 \times CH_3$), 1.2–1.6 (12H, m, $6CH_2$), 2.2 (2H, q, $J = 6.5$ Hz, CH_2CH_3), 2.3 (2H, t, $J = 7.1$ Hz, CH_2CH_2), 3.5 (2H, s, CH_2Ar), 3.9 (2H, brs, OCH_2CH_2), 6.4 (2H, s, NH_2), 6.7 (3H, m, ArH), 7.2 (1H, t, $J = 6.9$ Hz, ArH), 10.9 (1H, s, OH); ^{13}C-NMR (75.5 MHz, d_6-DMSO) δ 13.3 (CH_3), 13.7 (CH_3), 20.8 (CH_2), 21.9 (CH_2), 25.3 (CH_2), 28.5 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 30.6 (CH_2), 32.0 (CH_2), 40.5 (CH_2), 67.9 (OCH_2), 110.9 (Ar–C5), 114.0 (Ar–C), 114.2 (Ar–C), 119.8 (Ar–C), 120.1 (Ar–C), 128.8. (Ar–C), 129.0 (Ar–C), 143.1 (Ar–C1′), 158.3 (Ar–C3′), 161.3 (Ar–C6); IR (KBr) 3360 (OH), 3146 (NH_2), 2920 (C–H aromatic), 2858 (C–H Aliphatic) cm^{-1}; m/z (ES^+) 372.0 ($M + H^+$, 100%); HRMS (CI^+) 372.2651 ($M + H^+$, $C_{22}H_{34}N_3O_2$ requires 372.2651).

2-Amino-5-benzyl-6-ethyl-4-pyrimidinol (**17d**): Using **16d** (1.0 g, 4.27 mmol, 1 eq) gave the title compound (**17d**) as a white powder (0.515 g, 52%)

TLC R_f 0.48 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6-DMSO) δ 1.0 (3H, t, $J = 7.3$ Hz, CH_2CH_3), 2.3 (2H, q, $J = 7.5$ Hz, CH_2CH_3), 3.7 (2H, s, Ar–CH_2–Ar), 7.1–67.3 (5H, m, $5 \times ArH$); ^{13}C-NMR (75.5 MHz, d_6-DMSO) δ 13.2 (CH_3), 27.7 (CH_2), 30.2 (CH_2), 110.5 (Ar–C5), 114. (Ar–C), 118.2 (Ar–C), 126.2 (Ar–C), 128.6 (Ar–C), 128.9 (Ar–C), 142.3 (Ar–C1′), 154.5 (Ar–C2), 160.7 (Ar–C4), 164.8 (Ar–C6); IR (KBr) 3343 (OH), 3144 (NH_2), 3030 (C–H aromatic) cm^{-1}; m/z (ES^+) 230.03 ($M + H^+$, 100%); HRMS (CI^+) 229.1215 (M^+, $C_{13}H_{15}N_3O$ requires 229.1215).

2-Amino-5-benzyl-6-propyl-4-pyrimidinol (**17e**): Using **17e** (0.9 g, 3.63 mmol, 1 eq) gave the title compound (**17e**) as a white powder (0.4 g, 45%)

TLC R_f 0.5 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6 DMSO) δ 0.8 (3H, t, $J = 7.3$ Hz, CH_2CH_3), 1.3 (2H, sextuplet, $J = 7.5$ Hz, CH_2CH_3), 2.2 (2H, t, $J = 7.2$ Hz, CH_2CH_2), 3.4 (2H, s, CH_2Ar), 6.3 (2H, s, NH_2), 7.1–7.3 (5H, m, $5 \times ArH$), 10.9 (1H, s, OH); ^{13}C-NMR (75.5 MHz, d_6 DMSO) δ 13.3 (CH_3), 20.4 (CH_2), 28.9 (CH_2), 35.6 (CH_2), 109.5 (Ar–C5), 118.2 (Ar–C), 124.9 (Ar–C), 126.3 (Ar–C6), 127.3

(Ar–C), 127.5 (Ar–C), 141.2 (Ar–C1′), 154.9 (Ar–C2), 162.8 (Ar–C4), 164.2 (Ar–C6); IR (KBr) 3320.3 (OH), 3150 (NH_2), 3074.6 (C–H aromatic), 1644.2 (aromatic C = C) cm^{-1}; *m/z* (ES^+) 243.8 ($M + H^+$, 100%); HRMS (CI^+) 243.1372 (M^+, $C_{14}H_{17}N_3O$ requires 243.1372).

Compounds 18(a–e): General Procedure

A mixture of **17a–e** (1 eq), phosphorus oxychloride (5 eq) and phosphorus pentachloride (1 eq) were heated for 1 h at 105°C. The reaction mixture was allowed to cool to room temperature and slowly poured on to ice (100–150 ml per mmol of **17**) with vigorous stirring to give an oil which gradually solidified upon standing. The title compounds (**18**) were isolated by filtration as a white powdery solids usually contaminated with small amounts of impurities.

2-Amino-4-chloro-6-benzyl-5-(3-(heptoxy)benzyl)-4-pyrimidine (**18a**): Using **17a** (0.6 g, 1.48 mmol, 1 eq), the title compound (**18c**) was isolated by filtration as a white powdery solid with impurities (0.607 g, 97%)

^{1}H-NMR (300 MHz, d_4-MeOH) δ 0.8 (3H, t, J = 6.5 Hz, CH_3), 1.2–1.5 (8H, m, $4CH_2$), 1.7 (2H, quintet, J = 6.6 Hz, OCH_2CH_2), 3.8 (2H, t, J = 6.5 Hz, OCH_2CH_2), 4.1 (4H, s, $2 \times CH_2$Ar), 6.5–7.1 (9H, m, $9 \times$ ArH) (impurities at 2.8 and 4.2); IR (KBr) cm^{-1} 2924 (C–H aromatic), 2858 (C–H Aliphatic); *m/z* (ES^+) 424.24 ($M + H^+$), 426.1 ($M + 2 + H^+$); HRMS (CI^+) 424.2155 ($M + H^+$, $C_{25}H_{31}ClN_3O$ requires 424.2155).

2-Amino-4-chloro-5-(3-(octyloxy)benzyl)-6-ethylpyrimidine (**18b**): Using **17b** (0.607 g, 1.7 mmol, 1 eq), the title compound (**18b**) was isolated by filtration as a white powdery solid with impurities (0.416 g, 65.3%)

^{1}H-NMR (300 MHz, d_4-MeOH) δ 0.8 (3H, t, J = 6.5 Hz, CH_3), 1.1 (3H, t, J = 6.0 Hz, CH_3), 1.2–1.5 (12H, m, $6CH_2$), 2.6 (2H, q, J = 7.5 Hz, CH_2CH_3), 3.8 (2H, t, J = 6.3 Hz, OCH_2CH_2), 4.1 (2H, s, CH_2Ar), 6.8–7.2 (4H, m, $4 \times$ ArH) (impurities at 3.7, 4.9 and 5.1); IR (KBr) 2934 (C–H aromatic), 2854 (C–H Aliphatic) cm^{-1}; HRMS (CI^+) 375.2077 (M^+, $C_{21}H_{30}ClN_3O$ requires 375.2077).

4-Chloro-5-(3-(octyloxy)-benzyl)-6-propyl-2-pyrimidinylamine (**18c**): Using **17c** (0.355 g, 0.957 mmol, 1 eq), the title compound (**18c**) was isolated by filtration as a yellowish solid with impurities (0.22 g, 60%)

^{1}H-NMR (300 MHz, d_4-MeOH) δ 0.8 (6H, t, J = 6.5 Hz, $2 \times CH_3$), 1.2–1.6 (10H, m, $5 \times CH_2$), 1.7 (4H, m, $CH_2CH_2CH_3$ and OCH_2CH_2), 2.8 (2H, t, J = 7.5 Hz, $CH_2CH_2CH_3$), 3.9 (2H, t, J = 6.3 Hz, OCH_2CH_2), 3.8 (2H, s, CH_2Ar), 6.8–7.2 (4H, m, $4 \times$ ArH) (impurities at 3.7 and 4.2); IR (KBr) 2931.8 (C–H Aliphatic), 2863.4 (C–H aromatic) cm^{-1}; HRMS (CI^+) 390.2312 ($M + H^+$, $C_{22}H_{33}N_3OCl$ requires 390.2312).

5-Benzyl-4-chloro-6-ethyl-2-pyrimidinylamine (**18d**): Using **17d** (0.515 g, 2.25 mmol, 1 eq), the title compound (**18d**) was isolated by filtration as a white powder (0.677 g, 55%)

TLC R_f 0.6 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_4-MeOH) δ 1.0 (3H, t, J = 7.5 Hz, CH_2CH_3), 2.8 (2H, q, J = 7.4 Hz, CH_2CH_3), 4.1 (2H, s, CH_2Ar), 7–7.4 (5H, m, $5 \times$ ArH) (impurities at 3.9 and 1.2: *m/z* (ES^+) 248.1 ($M + H^+$, 100%) and 250.1 ($M + 2 + H^+$).

5-Benzyl-4-chloro-6-propyl-2-pyrimidinylamine (**18e**): Using **17e** (0.4 g, 1.65 mmol, 1 eq), the title compound (**18e**) was isolated by filtration as a white powder (0.23 g, 55%)

^{1}H-NMR (300 MHz, d_4-MeOH) 0.8 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.5 (2H, sextuplet, J = 7 Hz, CH_2CH_3), 2.5 (2H, t, J = 7.2 Hz, CH_2CH_2), 4.0 (2H, s, CH_2Ar), 6.8–7.2 (5H, m, $5 \times$ ArH) (impurities at 3.7 and 4.1); *m/z* (ES^+) 262.0 ($M + H^+$, 75%); HRMS (CI^+) 261.1033 (M^+, $C_{14}H_{16}ClN_3$ requires 261.1033).

2-4-Diamino-5-(3-(heptoxy)-benzyl)-6-benzyl-pyrimidine (5)

Ammonia (g) was bubbled for 15 min into a solution of **18a** (0.607 g, 1.43 mmol, 1 eq) in absolute EtOH (100 ml) cooled to 0°C. The reaction mixture was heated to 150°C for 16 h under an atmosphere of nitrogen in an autoclave and then allowed to cool and reduced *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an elution of 2–5% MeOH in EtOAc and yielded the title product (**5**) as a white powder (0.03 g, 5.2%), m.p. 115–117°C.

TLC R_f 0.28 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9 (3H, t, J = 6.5 Hz, CH_3), 1.2–1.3 (8H, m, $4 \times CH_2$), 1.8 (2H, quintet, J = 6.6 Hz, OCH_2CH_2), 3.7 (2H, s, CH_2Ar), 3.8 (2H, t, J = 6.5 Hz, OCH_2CH_2), 4.0 (2H, s, CH_2Ar), 4.8 (NH_2), 5 (NH_2), 6.5–7.4 (9H, m, $9 \times$ ArH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 14.5 (CH_3), 23.0 (CH_2), 26.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 32.2 (CH_2), 40.9 (CH_2), 68.2 (OCH_2), 104.7 (Ar–C), 111.8 (Ar–C), 113.0 (Ar–C5), 114.3 (Ar–C), 120.2 (Ar–C), 126.2 (Ar–C), 126.9 (Ar–C), 128.9 (Ar–C), 129.0 (Ar–C), 129.7 (Ar–C), 130.3 (Ar–C), 138.3 (Ar–C1′), 139.7 (Ar–C1″), 160.1 (Ar–C2), 160.7 (Ar–C3′), 164.2 (Ar–C6); IR (KBr) cm^{-1} 3500 and 3108 (NH_2), 2932 (C–H aromatic), 2850 (C–H Aliphatic); *m/z* (ES, +ve) 404.9 ($M + H^+$); HRMS (CI^+) 405.2653 ($M + H^+$, $C_{25}H_{33}N_4O$ requires 405.2654).

2,4-Diamino-5-(3-(octyloxy)benzyl)-6-ethylpyrimidine (7)

Ammonia (g) was bubbled into a solution of **18b** (0.4 g, 1.06 mmol, 1 eq) in absolute EtOH (100 ml) cooled to 0°C for 15 min. The reaction mixture was heated to 150°C for 24 h under an atmosphere of nitrogen in an autoclave and then allowed to cool and reduced *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an elution of 5% MeOH in EtOAc and yielded the title product (**7**) as a white powder (0.150 g, 39%), m.p. 128.4–130.2°C.

TLC R_f 0.27 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_6-DMSO) δ 0.8 (3H, t, $J = 6.5$ Hz, CH_3), 1.0 (3H, t, $J = 7.2$ Hz, CH_3), 1.3–1.5 (12H, m, $6CH_2$), 2.5 (2H, quartet, $J = 7.4$ Hz, CH_2CH_3), 3.6 (2H, s, CH_2Ar), 3.9 (2H, t, $J = 6.3$, OCH_2CH_2), 5.5 (NH_2), 6 (NH_2), 6.6 (4H, m, $3 \times$ Ar*H*), 7.1 (1H, t, $J = 7.8$ Hz, Ar*H*); ^{13}C-NMR (75.5 MHz, d_6-DMSO) δ 11.9 (CH_3), 12.8 (CH_3), 21.0 (CH_2), 24.4 (CH_2), 27.5 (CH_2), 27.6 (CH_2), 28.6 (CH_2), 29.9 (CH_2), 30.1 (CH_2), 31.1 (CH_2), 66.1 (OCH_2), 110.2 (Ar–C5), 113.1 (Ar–C), 118.8 (Ar–C), 124.4 (Ar–C), 128.2 (Ar–C), 140.8 (Ar–C1′), 157.5 (Ar–C4), 159.8 (Ar–C2), 162.2 (Ar–C3′), 166.2 (Ar–C6); IR (KBr) 3484 and 3308 (NH_2), 3120 (C–H aromatic), 2849 (C–H Aliphatic) cm^{-1}; *m/z* (ES^+) 357.14 ($M + H^+$, 100%); HRMS (CI^+) 356.2576 (M^+, $C_{21}H_{32}N_4O$ requires 356.2576).

5-(3-(Octyloxy)-benzyl)-6-propyl-2,4-pyrimidinediamine (8)

Ammonia (g) was bubbled into a solution of (**18c**) (0.22 g, 0.565 mmol, 1 eq) in absolute EtOH (50 ml) cooled to 0°C for 15 min. The reaction mixture was heated to 150°C for 24 h under an atmosphere of nitrogen in an autoclave and then allowed to cool and reduced *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an elution of 5% MeOH in EtOAc and yielded the title product (**8**) as a white powder (0.150 g, 39%), m.p. 126.5–127.4°C.

TLC R_f 0.22 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_4-MeOH) δ 0.8 (6H, t, $J = 7.2$ Hz, $2 \times CH_3$), 1.2–1.3 (10H, m, $5 \times CH_2$), 1.5 (2H, q, $J = 7.6$ Hz, CH_2CH_3) 1.6 (2H, quintet, $J = 6.5$ Hz, OCH_2CH_2), 2.4 (2H, t, $J = 7.8$ Hz, CH_2CH_2), 3.7 (2H, s, CH_2Ar), 3.8 (2H, t, $J = 6.4$ Hz, OCH_2CH_2), 6.6 (4H, m, $4 \times$ Ar*H*), 7.1 (1H, t, $J = 7.8$ Hz, Ar*H*); ^{13}C-NMR (75.5 MHz, d_4-MeOH) δ 11.0 (CH_3), 11.2 (CH_3), 14.8 (CH_2), 20.1 (CH_2), 20.5 (CH_2), 24.0 (CH_2), 27.2 (CH_2), 27.3 (CH_2), 28.0 (CH_2), 29.8 (CH_2), 33.1 (CH_2), 65.6 (OCH_2), 110.1 (Ar–C5), 111.9 (Ar–C), 113.1 (Ar–C), 114.0 (Ar–C), 117.8 (Ar–C), 127.4 (Ar–C), 138.6 (Ar–C1′), 157.0 (Ar–C2), 161.8 (Ar–C3′), 162.5 (Ar–C6); IR (KBr) 3461 and 3338 (NH_2), 3123 (C–H aromatic), 2925 (C–H Aliphatic) cm^{-1}; *m/z* (ES, +ve) 370.8 ($M + H^+$, 100%); HRMS (CI^+) 371.2811 ($M + H^+$, $C_{22}H_{35}N_4O$ requires 371.2811).

5-Benzyl-6-ethyl-2,4-pyrimidinediamine (9)

Ammonia (g) was bubbled into a solution of **18d** (0.1 g, 0.405 mmol, 1 eq) in absolute EtOH (10 ml) cooled to 0°C for 15 min. The reaction mixture was heated to 150°C for 24 h under an atmosphere of nitrogen and then allowed to cool and reduced *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an elution of 5% MeOH in EtOAc to yield the title product **9** (0.067 g, 72%).

TLC R_f 0.28 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_4-MeOH) δ 1.1 (3H, t, $J = 7.4$ Hz, CH_2CH_3), 2.6 (2H, q, $J = 7.4$ Hz, CH_2CH_3), 4.1 (2H, s, ArCH_2), 7–7.4 (5H, m, $5 \times$ Ar*H*): ^{13}C-NMR (75.5 MHz, d_4-MeOH) δ 11.2 (CH_3), 28.1 (CH_2), 33.1 (CH_2), 109.8 (Ar–C5), 118.8 (Ar–C), 121.2 (Ar–C), 126.4 (Ar–C), 127.8 (Ar–C), 128.5 (Ar–C6), 138.7 (Ar–C1′), 155.7 (Ar–C4), 161.7 (Ar–C2), 164.3 (Ar–C6); IR (KBr) 3296.3 (NH_2), 3143.6 (NH_2), 3031 (C–H aromatic) cm^{-1}; *m/z* (ES^+) 229.01 ($M + H^+$, 100%).

5-Benzyl-6-propyl-2,4-pyrimidinediamine (10)

Ammonia (g) was bubbled into a solution of **18e** (0.23 g, 0.88 mmol, 1 eq) in absolute EtOH (20 ml) cooled to 0°C for 15 min. The reaction mixture was heated to 150°C for 24 h under an atmosphere of nitrogen in an autoclave and then allowed to cool and reduced *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an elution of 5% MeOH in EtOAc to yield the title product (**10**) (0.052 g, 24%), m.p. 160–161°C.

TLC R_f 0.28 (10% MeOH in EtOAc); ^{1}H-NMR (300 MHz, d_4-MeOH) δ 0.9 (3H, t, $J = 7.4$ Hz, CH_2CH_3), 1.6 (2H, sextuplet, $J = 7.6$ Hz, CH_2CH_3), 2.5 (2H, t, $J = 7.6$ Hz, CH_2CH_2), 3.9 (2H, s, CH_2–Ar), 7–7.4 (5H, m, $5 \times$ Ar*H*); ^{13}C-NMR (75.5 MHz, d_4-MeOH) 12.7 (CH_3), 21.7 (CH_2), 29.7 (CH_2), 35.4 (CH_2), 110.3 (Ar–C5), 118.7 (Ar–C), 123.6 (Ar–C), 125.2 (Ar–C), 127.1 (Ar–C6), 127.8 (Ar–C), 139.0 (Ar–C1′), 154.3 (Ar–C), 160.2 (Ar–C4), 163.6 (Ar–C2); IR (KBr) 3495 and 3307.2 (NH_2), 2919.1 (C–H aromatic), 2834.6 (C–H Aliphatic) cm^{-1}; *m/z* (ES^+) 243.0 ($M + H^+$, 100%); HRMS (CI^+) 242.1531 (M^+, $C_{14}H_{18}N_4$ requires 242.1531).

Biological Assays

Enzyme Assays

Enzyme assays were carried out as detailed in reference[4] using recombinant enzymes.

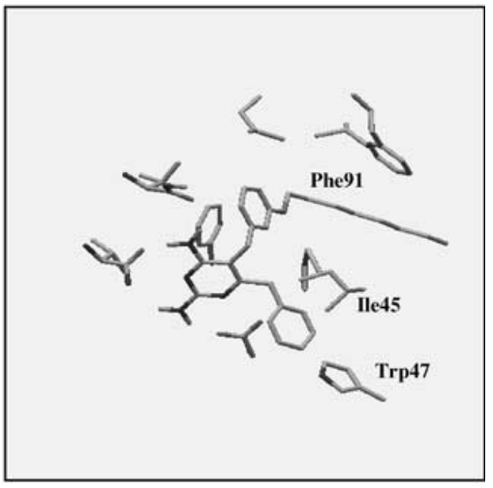


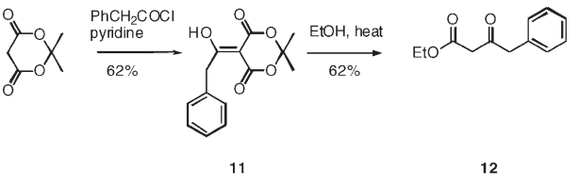
FIGURE 2 Compound 5 modelled into the active site of *L. major* DHFR.

In Vitro Assays

Compounds were assayed against the clinically relevant forms of the intact parasites: *T. brucei rhodesiense* trypomastigotes, *T. cruzi* amastigotes cultured in L6 cells and *L. donovani* amastigotes, cultured in L6 cells. The full details are given in reference.[5]

Modelling

Modelling was performed using silicon graphics O2 workstations with Macromodel 4.5 and 6.0 and DOCK 3.5. The coordinates of the *L. major* DHFR were obtained from D. A. Matthews.[6]

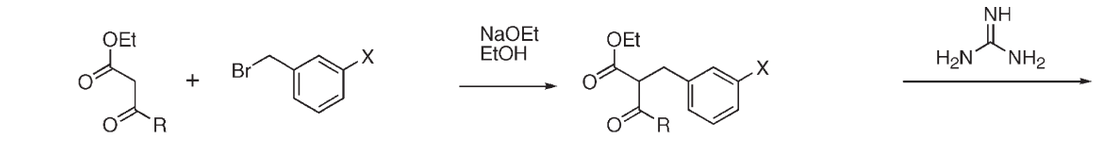


SCHEME 2

RESULTS

Modelling

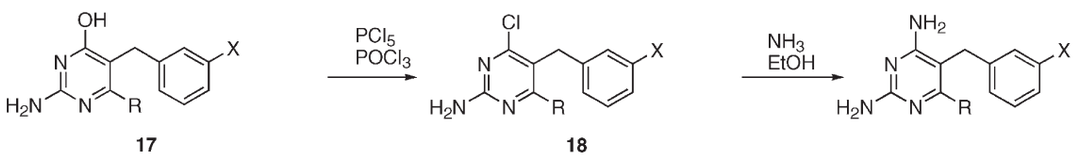
Compounds **2**, **5**, **6**, **7** and **8** were drawn into the active site of the *L. major* DHFR using the location of the pteridine ring of methotrexate (co-crystallised with the enzyme) as a template. Compounds (enzyme and ligand) were then minimised and a Monte-Carlo conformational search performed (using Macromodel 6.0[7]) in order to obtain the potential binding conformation. Modelling predicted hydrophobic interactions between the 6-benzyl substituent and the side chains of Ile45 and Trp47 present in the leishmanial DHFR (Fig. 2). In order to compare the inhibitors, the compounds in the conformations predicted by this search were then docked into the active site of the *L. major* enzyme using DOCK 3.5.[8] DOCK 3.5 was used in the "single mode" option (to find the optimum orientation of the molecule in the active site) with contact scoring which is a measure of fit in the active site (Table I). The orientations of the inhibitors in the active site predicted by the Monte-Carlo search



12 R = CH_2Ph; R = Et; R = Pr

15a, X = OC_7H_{15}; **15b**, X = OC_8H_{17}; X = H

a, R = CH_2Ph, X = OC_7H_{15}; 52%
b, R = Et, X = OC_8H_{17}; 69%
c, R = Pr, X = OC_8H_{17}; 51%
d, R = Et, X = H; 61%
e, R = Pr, X = H; 59%

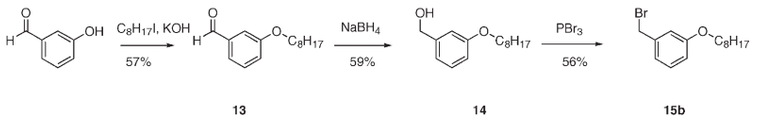


a, R = CH_2Ph, X = OC_7H_{15}; 86%
b, R = Et,X = OC_8H_{17}; 70%
c, R = Pr, X = OC_8H_{17}; 66%
d, R = Et, X = H; 52%
e, R = Pr, X = H; 45%

a, R = CH_2Ph, X = OC_7H_{15}; 97%
b, R = Et, X = OC_8H_{17}; 65%
c, R = Pr, X = OC_8H_{17}; 60%
d, R = Et, X = H; 55%
e, R = Pr, X = H; 55%

5, R = CH_2Ph, X = OC_7H_{15} 5%
7, R = Et, X = OC_8H_{17} 65%
8, R = Pr, X = OC_8H_{17} 60%
9, R = Et, X = H 73%
10, R = Pr, X = H 24%

SCHEME 1



SCHEME 3

and DOCK were very similar. The compound having the highest score (best predicted activity) was the 6-benzyl pyrimidine analogue **5**, which had a much higher score than the parent compound **2**. Similarly the ethyl and propyl derivatives **7** and **8** had a higher score than the parent octyloxy derivative **6**, although this difference was less significant.

Chemistry

The compounds were prepared as shown in Scheme 1. The preparation of compounds **2**, **3**, **4** and **6** has already been described.[4] The general method for synthesis of compounds **5**, **7**, **8**, **9**, and **10** is shown in Scheme 1. Essentially the relevant β-ketoesters (**12**) were alkylated with the corresponding substituted benzylbromides (**15**), followed by condensation with guanidine to give the pyrimidines **17**. The final products were obtained by chlorination (**18**) followed by ammonolysis. 4-Oxo-4-phenyl-butyric acid, ethyl ester (**12**) was not commercially available and was prepared as shown in Scheme 2. The preparation of 1-bromomethyl-3-heptyloxy-benzene has been described[4] and the octyloxy analogue was made using the same methodology (Scheme 3).

Biological Assays

Enzyme Assays

The compounds were assayed as described against the recombinant DHFR from *L. major*, *T. cruzi*, *T. brucei* and human.[4] The results of these assays are shown in Table I. Essentially this data shows a species dependent effect. Both the heptyloxy and octyloxy compounds showed a small increase in activity and selectivity towards *L. major* DHFR on addition of a 6-substitutent, however, the effect seemed largest with the ethyl substituent (**4** and **7**). In the case of the *T. cruzi* enzyme, there was a marginal effect on activity on addition of the ethyl group (**4** and **7**) but a large loss in activity and selectivity with larger substituents (benzyl **5** and propyl **8**). Compound **10** showed surprising activity given that it had no 3′-substitutent, but showed no selectivity.

In Vitro Assays

Compounds were also assayed against intact parasites: *L. donovani*, *T. cruzi* and *T. brucei rhodesiense*. In all cases, the life-cycle stages found in the human host were used; in the case of *L. donovani* and *T. cruzi* the amastigote stage cultured in mammalian macrophages or rat skeletal muscle cells (L-6), respectively, and in the case of *T. b. rhodesiense*, bloodstream forms cultured axenically. The results of these assays are shown in Table II. Compounds were also assayed against the L-6 cells to assess cytotoxicity. It was not possible to evaluate the compounds against *L. donovani* due to the cytotoxicity of the compounds. All of the compounds showed cytotoxic effects on the macrophages before antiparasitic activity was observed. In the case of *T. cruzi* the compounds showed moderate to good activity (**2**, **6**). Against *T. brucei* the compounds showed activity in the range 0.5–5 μM. However

TABLE II *In vitro* data against intact parasites

Compound	6-Substituent	3′-Substitutent	*L. donovani* IC_{50} (μM)	*T. cruzi* IC_{50} (μM)	*T. brucei* IC_{50} (μM)	L-6 cells IC_{50} (μM)
2	H	O-Heptyl	T	2.3 (17)	0.70 (57)	40
3	Me	O-Heptyl	T	7.3 (11)	0.73 (110)	79
4	Et	O-Heptyl	N.D.	19 (2.6)	5.0 (10)	49
5	CH_2Ph	O-Heptyl	>74	19 (7.7)	1.5 (98)	147
6	H	O-Octyl	T	1.4 (14)	0.72 (26)	19
7	Et	O-Octyl	N.D.	10	2.6	N.D.
8	Pr	O-Octyl	T	7.3 (2.2)	0.55 (29)	16
9	Et	H	N.D.	360	67 (>6)	>394
10	Pr	H	68 (3.6)	84 (2.9)	7.9 (31)	243

N.D. = not determined; T = activity less than toxicity to macrophages. Selectivity shown in parenthesis. Standards: *T. cruzi*, benznidazole, IC_{50} = 3.2 μM; *T. brucei rhodesiense*, melarsoprol IC_{50} = 4.5 nM.

the activity did not seem to be affected much by the presence of a 6-substituent.

CONCLUSION

The preliminary data suggest attachment of a hydrophobic substituent to the 6-position of the pyrimidine ring has a marginal increase on both activity and selectivity of compounds to leishmanial or trypanosomal dihydrofolate reductase. *In vitro*, the substituent does not have a significant effect on the activity against *T. brucei*. It was not possible to obtain activity data against *L. donovani* due to toxicity against the host cells, mouse macrophages.

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References

[1] Gilbert, I.H. (2002) *Biochim. Biophys. Acta* **62126**, 249–257.
[2] Zuccotto, F., Martin, A.C.R., Laskowski, R.A., Thornton, J.M. and Gilbert, I.H. (1998) *J. Comput.-Aided Mol. Des.* **12**, 241–257.
[3] Sirawaraporn, W., Sertsrivanich, R., Booth, R.G., Hansch, C., Neal, R.A. and Santi, D.V. (1988) *Mol. Biochem. Parasitol.* **31**, 79.
[4] Chowdhury, S.F., Villamor, V.B., Guerrero, R.H., Leal, I., Brun, R., Croft, S.L., Goodman, J.M., Maes, L., Ruiz-Perez, L.M., Pacanowska, D.G. and Gilbert, I.H. (1999) *J. Med. Chem.* **42**, 4300–4312.
[5] Chowdhury, S.F., Di Lucrezia, R., Guerrero, R.H., Brun, R., Goodman, J., Ruiz-Perez, L.M., Pacanowska, D.G. and Gilbert, I.H. (2001) *Bioorg. Med. Chem. Lett.* **11**, 977–980.
[6] Knighton, D.R., Kan, C.C., Howland, E., Janson, C.A., Hostomska, Z., Welsch, K.M. and Matthews, D.A. (1994) *Nat. Struct. Biol.* **1**, 186–194.
[7] Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T. and Still, W.C. (1990) *J. Comput. Chem.* **11**, 440–467.
[8] Shoichet, B.K., Stroud, R.M., Santi, D.V., Kuntz, I.D. and Perry, K.M. (1993) *Science* **259**, 1445–1450.