Nonclassical 2,4-Diamino-5-aryl-6-ethylpyrimidine Antifolates: Activity as Inhibitors of Dihydrofolate Reductase from *Pneumocystis carinii* and *Toxoplasma gondii* and as Antitumor Agents

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Twelve novel 2,4-diamino-5-(4′-benzylamino)- and 2,4-diamino-5-[4′-(*N*-methylbenzylamino)-3′-nitrophenyl]-6-ethylpyrimidines bearing 4-substituents on the benzylamino or *N*-methylbenzylamino aryl ring were synthesized and evaluated as nonclassical inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase (DHFR). Compounds were prepared by reaction of 2,4-diamino-5-(4′-chloro-3′-nitrophenyl)- (**8**) or 2,4-diamino-5-(4′-fluoro-3′-nitrophenyl)-6-ethylpyrimidine (**15**) with the appropriate 4-substituted (CO_2H, CO_2Me, SO_2NH_2, dioxolan-2-yl, CHO, dimethyloxazolin-2-yl) benzylamine or *N*-methylbenzylamine derivative. Compounds **25**−**29** were synthesized from 2,4-diamino-5-{4′-[*N*-(4″-carboxybenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (**10**) and the corresponding amine (NH_3, $MeNH_2$, Me_2NH, piperidine, diethyl L-glutamate) *via* isobutyl mixed anhydride coupling; hydrolysis of the diethyl L-glutamate **29** afforded the L-glutamate analogue **30**. The compounds exhibited potent inhibitory activity against *T. gondii* (IC_{50} values 0.0018−0.14 μM) and rat liver (IC_{50} values 0.0029−0.27 μM) DHFR, with a 4-substituent invariably enhancing binding to both enzymes relative to the unsubstituted benzoprim (**5**) or methylbenzoprim (**6**). Modest selectivity for *T. gondii* enzyme was observed with several analogues, whereas all of the compounds were relatively weak inhibitors of *P. carinii* DHFR and exhibited no selectivity. Selected analogues were evaluated for *in vivo* antitumor activity against the methotrexate-resistant M5076 murine reticulosarcoma, with 2,4-diamino-5-{4′-[*N*-[4″-(*N*′-methylcarbamoyl)benzyl]-*N*-methylamino]-3′-nitrophenyl}-6-ethylpyrimidine (**14**) (K_i for rat liver DHFR = 0.000 35 ± 0.000 29 nM) combining significant antitumor activity with minimal toxicity.

Introduction

The antimetabolite drug methotrexate (MTX), which acts principally *via* inhibition of folate-dependent enzymes, is the prototype of the antifolate drugs used in cancer chemotherapy.[1−3] Problems associated with the antitumor activity of MTX, a potent nonselective inhibitor of dihydrofolate reductase (DHFR), include a limited spectrum of activity and the development of resistance.[4,5] Lipophilic DHFR inhibitors, exemplified by the first-generation antifolates metoprine (DDMP) (**1**) and etoprine (**2**) lack a polar glutamate side chain and differ from MTX in not requiring a carrier-mediated active transport mechanism to gain ingress to cells, entering by passive or facilitated diffusion.[6,7] As a consequence, these agents exhibit activity against MTX-resistant tumors and also central nervous system (CNS) malignancies inaccessible to the more hydrophilic MTX.[8] The lipophilic antifolates piritrexim (PTX) (**3**)[9,10] and trimetrexate (TMQ) (**4**)[11,12] were developed to overcome toxicity problems encountered with DDMP, attributed to the prolonged biological half-life[7] and inhibition of histamine metabolism observed for this highly lipid soluble compound.[13] Interestingly, PTX and TMQ, but not DDMP or the antimalarial antifolate pyrimethamine (PYM), are substrates for the membrane-bound P-glycoprotein (GP-170)[14,15] which effluxes a diverse range of unrelated antitumor agents from cells and is responsible for the multidrug-resistance (MDR) phenotype.[16] Baccanari *et al*. have demonstrated that TMQ and PTX do not attain appreciable CSF/plasma ratios, and the endothelial cells of the blood−brain barrier are known to overexpress GP-170.[17] By denying access of drug to CNS tumors, MDR represents a potential resistance mechanism for this class of antitumor agent, and a number of novel lipophilic antifolates which are nonsubstrates for GP-170 have reportedly been identified.[2]

Lipophilic DHFR inhibitors, including PTX and TMQ, have also enjoyed a more recent resurgence in interest as agents for the treatment of infection by opportunistic pathogens, including *Candida albicans*,[18] *Toxoplasma gondii*,[19] and *Pneumocystis carinii*,[20] in immunocompromised patients. Indeed, TMQ has recently gained clinical approval for the treatment of *P. carinii* infections in patients with acquired immune deficiency syndrome (AIDS).[21] Unfortunately, unlike PYM or the antibacterial DHFR inhibitor trimethoprim, these antifolates exhibit no selectivity for pathogen DHFR and are more potent inhibitors of the mammalian enzyme. As a consequence, the concomitant administration of leucovorin is necessary in order to ameliorate host antifolate toxicity.[22] Clearly, there is a requirement for novel and potent lipophilic antifolates with improved

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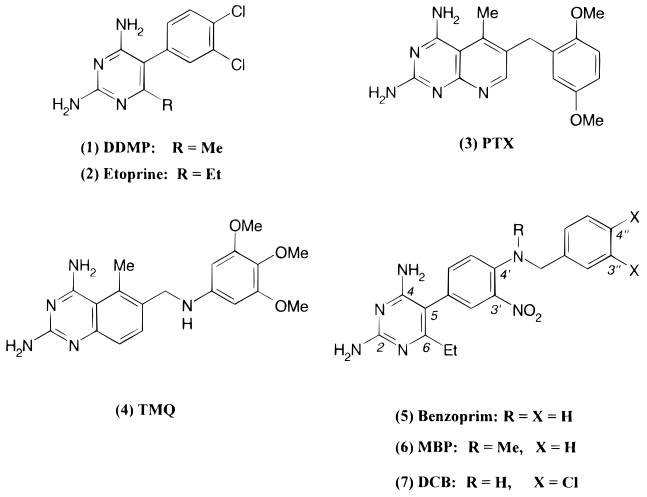
Chart 1



selectivity for pathogen DHFR, and a large number of compounds have been prepared and evaluated to this end.[23]

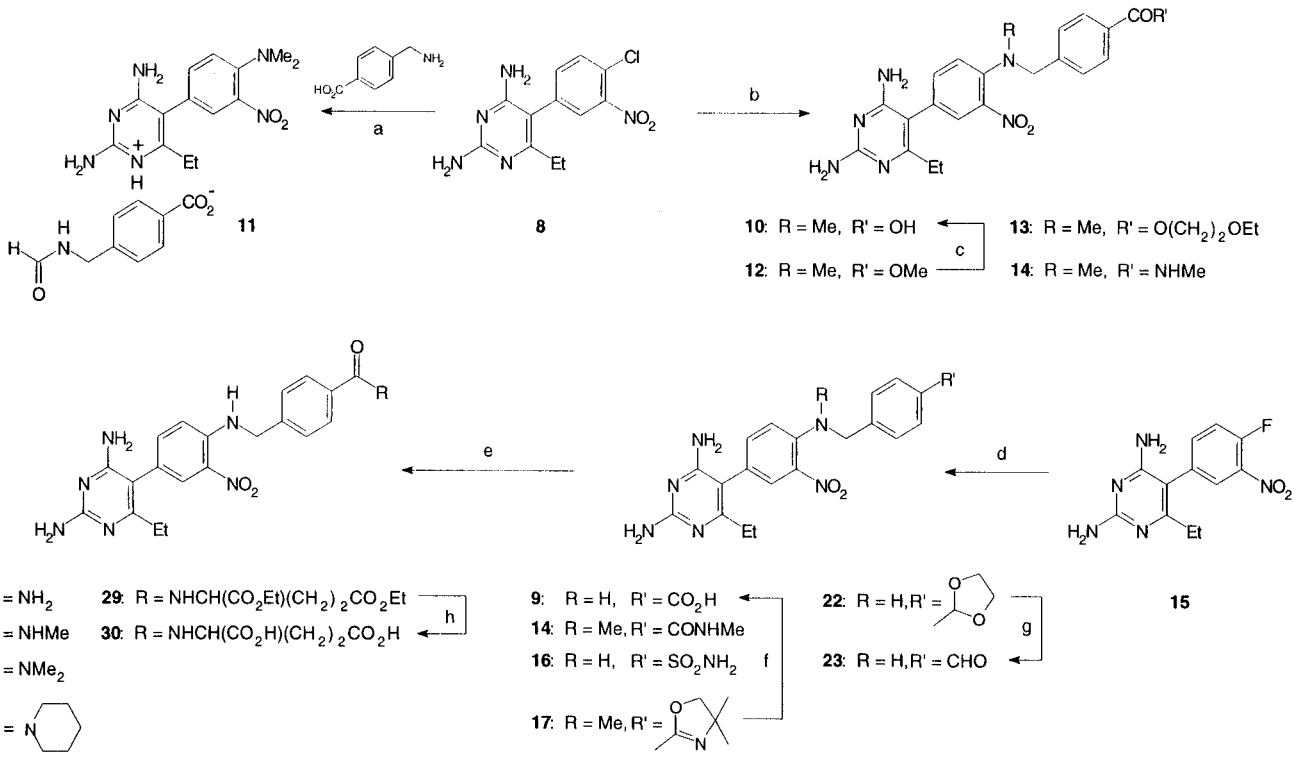
In an earlier paper we reported on the synthesis and biological properties of a series of 2,4-diamino-5-aryl-6-ethylpyrimidines encompassing secondary and tertiary amine groups on the 5-aryl ring.[24] Of particular interest were analogues bearing a benzylamino (**5**) or *N*-alkylbenzylamino substituent (*e.g.*, **6**), as these proved to be potent inhibitors of mammalian DHFR and exhibited *in vivo* antitumor activity superior to that of DDMP against the MTX-resistant M5076 murine reticulosarcoma. Methylbenzoprim (MBP, **6**) and the closely related dichlorobenzoprim (DCB, **7**) were selected for more extensive evaluation on the basis of promising biological activity combined with relatively low toxicity, and activity has been observed *in vitro* and *in vivo* for both diaminopyrimidines against a panel of MTX-sensitive and -resistant human tumor xenografts.[25]

The potent DHFR-inhibitory activity of MBP and related compounds was originally ascribed to a resemblance of the *N*-methylbenzylamino substituent to the 4-(*N*-methylamino)benzoyl moiety of MTX. This suggested that a carboxylate or carboxamide function in the *para* position of the benzyl ring should enhance binding to the enzyme. In this paper we describe the results of these studies and also the synthesis of an L-glutamate derivative, **30**, in the expectation that this "classical" analogue might also exhibit some interesting biological properties. The comparative DHFR-inhibitory activity of these diaminopyrimidines was determined against enzyme from *P. carinii*, *T. gondii*, and rat liver. Selected compounds were also evaluated for *in vivo* antitumor activity against the M5076 reticulosarcoma in mice.

Chemistry

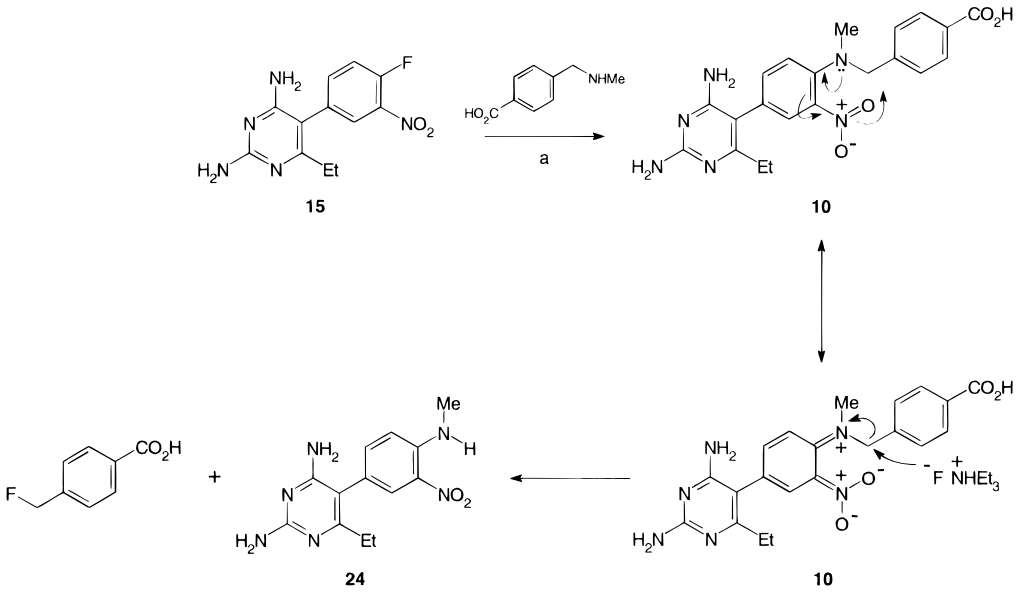
The synthetic procedures employed for the preparation of the target compounds are outlined in Scheme 1. We have previously described the use of *m*-nitropyrimethamine (**8**) as a key starting material for the synthesis of diaminopyrimidines bearing amine substituents in the 5-aryl ring, where simply heating a solution of **8** in the appropriate amine generally afforded the target antifolates in excellent yields.[24,26] Unfortunately, analogous reactions of **8** with 4-(aminomethyl)- or 4-[(*N*-methylamino)methyl]benzoic acid, which would furnish carboxylic acids **9** and **10** and enable subsequent elaboration to other derivatives, were precluded by the lack of reactivity and very poor solubility of these amines. Copper(II) chloride catalysis of the amination

Scheme 1[a]



[a] Reagents and conditions: (a) $CuCl_2 \cdot 2H_2O$, NaOAc, DMF, reflux; (b) amine, $EtO(CH_2)_2OH$, reflux; (c) NaOH, MeOH, reflux; (d) amine, NEt_3, NMP, 100 °C; (e) 1. *i*-BuOCOCl, NEt_3, DMF, 0 °C, 2. amine, 25 °C; (f) 1. NBu_4HSO_4, NaOCl, EtOAc, 25 °C, 2. NaOH, MeOH, 25 °C; (g) AcOH (aq), 25 °C; (h) 1. $Ba(OH)_2 \cdot 8H_2O$, EtOH (aq), 25 °C, 2. Na_2SO_4, H_2O.

Scheme 2[a]



[a] Reagents and conditions: (a) NEt_3, NMP, 100 °C.

of chloronitroarenes has been reported previously,[27,28] and this approach was investigated for the reaction of **8** with 4-(aminomethyl)benzoic acid in DMF. Interestingly, while none of the required product **9** was obtained, the (dimethylamino)pyrimidine **11** was isolated in excellent yield, as an *N*-formyl-4-(aminomethyl)benzoate salt. We have previously utilized DMF−ethylenediamine, as reported by Yamamoto *et al.*,[29] for the dimethylamination of nitropyrimethamine (**8**), and this reaction is clearly catalyzed by copper(II) salts, as no reaction was observed when a mixture of **8** and 4-(aminomethyl)-benzoic acid was heated in DMF, in the absence of a copper salt.

Reaction of **8** with the more soluble methyl 4-[(*N*-methylamino)methyl]benzoate, prepared from 4-(chloromethyl)benzoic acid by standard methodology, afforded the corresponding diaminopyrimidine methyl ester **12** in low yield, together with the 2-ethoxyethyl ester **13** arising from transesterification by the 2-ethoxyethanol employed as solvent. Conversion of the mixture of esters into the required **12** was achieved with methanol−sulfuric acid, and subsequent base-catalyzed hydrolysis of **12** with methanolic sodium hydroxide gave the target carboxylic acid derivative **10**, which was isolated as an ethanesulfonic acid salt. An authentic sample of **13** was also prepared from **8** and 2-ethoxyethyl 4-[(*N*-methylamino)methyl]benzoate under identical reaction conditions. The analogous reaction of **8** with *N*-methyl-4-[(*N*-methylamino)methyl]benzamide furnished the required *N*-methylcarboxamide **14**, albeit in moderate yield.

The poor product yields arising from the relatively vigorous conditions necessary to effect the reaction of **8** with amines led us to consider an alternative, more reactive pyrimidine starting material. The increased reactivity, to nucleophilic substitution, of fluoro- over chloronitroarenes is well established, and we have previously reported the preparation of 2,4-diamino-5-(4′-fluoro-3′-nitrophenyl)-6-ethylpyrimidine (**15**), the fluoro analogue of **8**.[30] Reaction of **15** with *N*-methyl-4-[(*N*-methylamino)methyl]benzamide in 2-ethoxyethanol at 80 °C afforded the required *N*-methylcarboxamide **14** in excellent yield, and similar reactions with 4-(aminomethyl)benzoic acid and 4-(aminomethyl)benzenesulfonamide gave good yields of the benzoic acid **9** and sulfonamide **16**, respectively. 2,4-Diamino-5-{4′-[*N*-[4″-(4,4-dimethyloxazolin-2-yl)benzyl]amino]-3′-nitrophenyl}-6-ethylpyrimidine (**17**), prepared analogously from **15** and 2-[4-[(*N*-methylamino)methyl]phenyl]-4,4-dimethyloxazoline (**19**), was a potential precursor to the acid **10**.[31] Unfortunately, conversion of **17** to the required acid **10** proved problematical owing to degradation of the diaminopyrimidine, although treatment with sodium hypochlorite−sodium hydroxide under phase-transfer conditions did give **10** in poor yield.[32] The aldehyde **23** was obtained by acid hydrolysis of acetal **22**, which was synthesized by the reaction of **15** with 2-[4-(aminomethyl)phenyl]dioxolane (**21**). This amine was prepared from 4-cyanobenzaldehyde,[33] with lithium triethylborohydride proving to be the most satisfactory reagent for reduction of the nitrile group without concomitant cleavage of the acetal function.

Surprisingly, the reaction of **15** with 4-[(*N*-methylamino)methyl]benzoic acid did not give **10** as expected but rather the (*N*-methylamino)pyrimidine **24** arising, presumably, from debenzylation of the initially formed **10**. In an earlier paper we have described the acid-catalyzed debenzylation of methylbenzoprim (**6**) to give **24**.[34] Although the exact mechanism of this reaction remains to be elucidated, debenzylation of **10** by nucleophilic attack of unsolvated fluoride ion ($NEt_3{}^+F^-$) in the polar aprotic solvent (NMP) employed would afford **24** and 4-(fluoromethyl)benzoic acid (Scheme 2).

Amides **25**−**28** were prepared from the diaminopyrimidinecarboxylic acid **9** in excellent yields *via* the mixed anhydride, and the analogous coupling reaction with diethyl L-glutamate gave the diester **29** in good yield.[35] The use of sodium hydroxide to effect ester hydrolysis resulted in extensive decomposition, and conversion into the target folate analogue **30** was eventually achieved using barium hydroxide,[36] the dibarium salt precipitating from solution as the reaction proceeded. Dissolution in water and addition of sodium sulfate afforded the

Table 1. Inhibition Concentration (IC_{50}, in μM) of Dihydrofolate Reductase from *P. carinii*, *T. gondii*, and Rat Liver and Selectivity Ratios[a]

no.	rlDHFR	pcDHFR[b]	tgDHFR	rl/pc	rl/tg
5	0.025	1.03[c]	0.019	0.024	1.3
6	0.0032	1.6[c]	0.091	0.002	0.04
8	0.015	0.85[c]	0.013	0.02	1.1
9	0.0029	0.27	0.0018	0.01	1.61
10	0.0038	0.27	0.0031	0.01	1.23
14	0.0068	0.46	0.0074	0.01	0.92
16	0.0031	0.46	0.0058	0.01	0.53
17	0.015	0.95	0.014	0.02	1.07
22	0.016	0.93	0.015	0.02	1.07
25	0.0075	0.53	0.0037	0.01	2.03
26	0.006	0.58	0.0043	0.01	1.40
27	0.0089	1.3	0.0079	0.01	1.13
28	0.27	2.2	0.14	0.12	1.93
29	0.0067	0.45	0.0071	0.01	0.94
30	0.013	0.1	0.0062	0.13	2.10
PTX (**3**)	0.0015	0.031	0.017	0.048	0.088
TMQ (**4**)	0.003	0.042	0.010	0.071	0.29
TMP	133.0	12.0	2.7	11.1	49.0
epiroprim	33.2	2.6	0.47	12.8	70.6
MTX	0.0025	0.0013	0.014	1.9	0.18

[a] Assays were conducted at 37 °C under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NADPH) in the presence of 150 mM KCl.[19,20] [b] Recombinant *P. carinii* DHFR employed unless indicated otherwise. [c] DHFR isolated from *P. carinii*.

disodium salt, although only a poor yield of the free acid was isolated on acidification with aqueous hydrochloric acid.

Biology

The DHFR-inhibitory activity of the benzoprim analogues was determined against enzyme from *P. carinii* (pc), *T. gondii* (tg), and rat liver (rl) as described previously,[20,37] and the results are summarized in Table 1. Published data[20,37] for benzoprim (**5**), MBP (**6**), nitropyrimethamine (**8**), and the reference compounds TMP, epiroprim, PTX (**3**), and TMQ (**4**) are included for comparison purposes. Selectivity ratios (IC_{50}(rlDHFR)/IC_{50}(pcDHFR) and IC_{50}(rlDHFR)/IC_{50}(tgDHFR)) are also presented in Table 1. Potency against pcDHFR was generally weak, although all of the novel compounds were more potent than TMP and at least equipotent with epiroprim, with IC_{50} values varying from 0.1 μM (**30**) to 2.2 μM (**28**). The differential inhibition of the various DHFRs by compounds **6**, **27**, and **30** may reflect subtle differences in the ability of the active sites of the respective DHFRs to accommodate larger substituents such as *p*-aminobenzoyl L-glutamate versus smaller substituents (*e.g.*, *N*-methylbenzylamino) as suggested previously.[38]

Where a comparison was possible, replacement of a benzylamino by an *N*-methylbenzylamino substituent had no significant effect on binding to *P. carinii* DHFR (compare **5** with **6**, **9** with **10**, and **26** with **14**). The introduction of a *para*-substituent (CO_2H, $CONH_2$, CONHMe, SO_2NH_2) on the benzylamino or *N*-methylbenzylamino ring enhanced binding 2−5-fold compared to the unsubstituted inhibitors **5** and **6**. By contrast, a dimethyloxazoline (**17**) or dioxolane (**22**) ring or a disubstituted carboxamide (**27**, **28**) at this position reduced potency, and these compounds were approximately equipotent with **5** and **6** against pcDHFR. Unfortunately, from the rlDHFR/pcDHFR selectivity indices, it is evident that all of the new compounds evaluated exhibit a 50−100-fold higher affinity for mammalian enzyme over that from *P. carinii*, and this, combined with the poor inhibitory activity observed against pathogen DHFR, mitigates against this class of inhibitor offering any advantages over existing agents.

The diaminopyrimidine derivatives exhibited potent inhibitory activity against *T. gondii* DHFR with IC_{50} values ranging from 0.0018 μM (**9**) to 0.14 μM (**28**). In contrast to pcDHFR, *N*-methylation of the benzylamino side chain marginally reduced binding to the *T. gondii* enzyme; for example, **9** and **26** are approximately 2-fold more potent than their *N*-methyl counterparts **10** and **14**, and this is consistent with the relative activities of benzoprim (**5**) and methylbenzoprim (**6**) against tgDHFR. Again, the introduction of a *para*-substituent on the aryl ring (**9**, **10**, **14**, **16**, **27**, **29**, **30**) enhanced binding compared to **5** and **6**. *Para*-substitution with a dimethyloxazoline (**17**) or dioxolane (**22**) ring had little effect on activity against tgDHFR, but a piperidino substituent (**28**) reduced activity, and this trend was also observed against mammalian DHFR. Indeed, with the exception of **6**, which was some 28 times more active against mammalian enzyme (IC_{50} = 0.0032 μM) than that from *T. gondii* (IC_{50} = 0.091 μM), inhibitory activity against rlDHFR closely paralleled that against tgDHFR, as is evident from the rlDHFR/tgDHFR selectivity indices. Consequently, the compounds exhibited little selectivity for the *T. gondii* over mammalian enzyme, although **25**, **28**, and the classical analogue **30** were approximately 2-fold more active against the tgDHFR.

The potent inhibitory activity observed against mammalian DHFR was studied in more detail by conducting inhibition constant (K_i) determinations on three selected compounds (**10**, **12**, and **14**). Inhibitory activity was determined spectroscopically at 340 nm against rat liver DHFR as described previously, employing the zone B analysis method appropriate for tight-binding inhibitors (Table 2).[39] K_i values for DDMP (**1**) and MBP (**6**) are included for comparative purposes. All three compounds were more potent than **1**, and in comparison with MBP (**6**), inhibitory activity was of the order **10** ≡ **14** > **6** > **12**, with the carboxylic acid **10** (K_i = 0.0004 ± 0.0003 nM) and *N*-methylcarboxamide **14** (K_i = 0.000 35 ± 0.000 29 nM) proving to be among the most potent lipophilic inhibitors of mammalian DHFR reported to date. The *in vivo* antitumor activity of **10** and **14** was determined against the M5076 reticulosarcoma, a murine solid tumor intrinsically resistant to MTX as a result of impaired transport,[40] in order to evaluate the potential of these novel lipophilic antifolates against MTX-resistant malignancies. The results are summarized in Table 2, and antitumor activity is expressed as the inhibition of tumor volume (T/C) of test (T) versus control (C) animals, where a T/C value of 8% represents maximal activity in this system. DDMP (**1**) exhibited activity in this tumor model but was highly toxic at the lowest dose level utilized, whereas the *N*-methylcarboxamide **14** combined good antitumor activity with no host toxicity at 12.5 mg·kg^{-1}. Some toxicity was observed with both **10** and **14** at the higher dose of 25 mg·kg^{-1}. The promising activity of **14** as an agent for the treatment of MTX-resistant malignancies is currently being investigated against a broader spectrum of tumors, including those expressing the MDR phenotype, and the results of these studies will be published subsequently.

Table 2. Dihydrofolate Reductase-Inhibitory Activity (K_i)[a] and *in Vivo* Antitumor Activity against the M5076 Reticulum Cell Sarcoma in Mice[b] for Selected 2,4-Diamino-5-aryl-6-ethylpyrimidines

			M5076 reticulum cell sarcoma		
no.	solvent	K_i (nM)	optimum treatment, mg/kg/day[c]	T/C, %[d]	survivors (test/control)[e]
MTX			3.125	92	5/5
DDMP (**1**)	A	0.12 ± 0.04	3.125	<8[f]	1/5
6	A	0.009 ± 0.002	25	37	5/5
10	B	0.0004 ± 0.0003	12.5	100	5/5
12	C	0.014 ± 0.011	ND[g]	ND	ND
14	A	0.00035 ± 0.00029	12.5	15	5/5

[a] Partially purified rat liver DHFR (EC 1.5.1.3) was prepared by the method of Bertino and Fischer.[45] Inhibitory activity was determined spectrophotometrically in duplicate at a minimum of five inhibitor concentrations, K_i values being obtained by the zone B analysis method,[39] assuming a K_m value of 0.2 μM for dihydrofolate.[46] Solvents: (A) 0.1 M hydrochloric acid; (B) 95% ethanol; (C) DMSO. [b] 10^6 cells implanted im into the left leg of BDF_1 female mice (groups of 5) on day 0. Drug as a solution in 10−20% DMSO in arachis oil was administered ip daily on days 1−17, and tumor volumes were measured on days 12, 16, 20, and 24 after tumor implantation. Control animals received vehicle only. [c] Drug administered at dose levels of 12.5, 6.25, 3.125, 1.5, and 0.75 mg·kg^{-1}, the optimum treatment dose being that nearest in value to the LD_{10}. [d] Antitumor activity expressed as a ratio of tumor volumes of test (T) animals compared to control (C) animals × 100. [e] On day 24 when all animals were sacrificed. [f] Maximal activity for this system. [g] Not determined.

The introduction of carboxylate or carboxamide substituents onto the benzylamino ring of **5** and **6** was initially predicted to enhance inhibitory activity by facilitating binding to the putative PABA binding site of DHFR. However, subsequent X-ray analysis[41] and molecular modeling studies[42] conducted with **6** have indicated that the antifolate exhibits very different binding characteristics compared with other lipophilic antifolates and MTX. Interestingly, the preferred binding orientation positions the central nitrophenyl ring of **6** into the NAD^+ binding domain, such that competition with the cofactor occurs. In addition, the pendant benzylamino substituent occupies a large hydrophobic pocket which will clearly accommodate much bulkier substituents. In light of these observations, more detailed kinetic studies are in progress with **14** and related compounds, with a view to establishing the interaction of these compounds with DHFR at the molecular level.

Experimental Section

Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Unicam SP200 infrared spectrometer or a Nicolet 205X spectrometer. Mass spectra were determined on a AE1 MS9 or Kratos MS80 spectrometer in electron impact (EI) mode or on a Kratos instrument using a *m*-nitrobenzyl alcohol matrix in fast atom bombardment (FAB) mode. UV spectra were recorded in EtOH on a Pye Unicam SP8000 or a UVIKON 810 recording spectrophotometer. ^{1}H-NMR spectra were obtained on either a Bruker Spectrospin AC 200E (200 MHz) or a Bruker WH 400 (400 MHz) spectrometer, employing TMS as internal standard. Unless indicated otherwise, spectra were recorded in [$^2H_6$]DMSO as solvent. NH signals appeared as broad singlets (br s) exchangeable with D_2O. Key: t = triplet, s = singlet, q = quartet, d = doublet, dd = double doublet, m = multiplet. The TLC systems employed aluminum sheets precoated with Kieselgel $60F_{254}$ (0.2 mm) as the adsorbent and were visualized with light at 254 and 366 nm. Column chromatography was conducted on silica gel (Kieselgel 60, 240−400 mesh). Elemental analyses were performed by Butterworth Laboratories, Middlesex, U.K., and are within ±0.4% of theory unless otherwise specified. Reagents were purchased from Aldrich Chemical Co., Gillingham, U.K., and used as received unless otherwise stated. Ethanol and methanol were dried using Mg/I_2 and stored over 3 Å molecular sieves. Diethyl ether and tetrahydrofuran were predried over $CaCl_2$ and distilled from sodium/benzophenone. Benzene was static dried over alumina. Petroleum ether refers to that fraction in the boiling range 60−80 °C.

2-[(4-Chloromethyl)phenyl]-4,4-dimethyloxazoline (18). To a stirred solution of 4-(chloromethyl)benzoic acid (5 g, 29 mmol) in dry THF (40 mL) was added thionyl chloride (2.3 mL, 32 mmol) and DMF (0.1 mL), and the reaction mixture was stirred at ambient temperature for 48 h. Evaporation of solvents *in vacuo* gave a green solid which was redissolved in dichloromethane (10 mL), and added dropwise to a stirred solution of 2-amino-2-methylpropan-1-ol (7.63 g, 57 mmol) in dichloromethane (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for a further 12 h, and the white solid which remained on removal of the solvent was triturated with H_2O and collected. Thionyl chloride (5 mL) was added dropwise to the solid with stirring, and when the vigorous reaction had subsided, the mixture was triturated with cold ether (100 mL). The white crystals which deposited were collected and dissolved in H_2O, and the solution was neutralized with 5 M NaOH solution. The solution was extracted with ether (3 × 20 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated to furnish a white solid. Recrystallization from EtOAc gave **18** (3.62 g, 55%): mp 73−75 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.39 (6H, s, CH_3 × 2), 4.22 (2H, s, OCH_2), 4.93 (2H, s, CH_2Cl), 7.61 (2H, d, J = 8.2 Hz, 2,6-ArH), 7.98 (2H, d, J = 8.2 Hz, 3,5-ArH); MS (EI) m/z 223 $[M]^+$. Anal. ($C_{12}H_{14}NOCl$) C, H, N.

2-[4-[(*N*-Methylamino)methyl]phenyl]-4,4-dimethyloxazoline (19). A suspension of the oxazoline **18** (2 g, 8.95 mmol) in 33% methylamine in EtOH (30 mL) was stirred at room temperature overnight, the solvent was removed under reduced pressure, and H_2O (15 mL) was added. The mixture was extracted with EtOAc (3 × 25 mL), and the combined organic layers were dried ($MgSO_4$). The crude product was purified by chromatography using 3:2 (v/v) CH_2Cl_2/MeOH as eluent, to give a colorless oil (1.46 g, 75%): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.37 (6H, s, CH_3 × 2), 2.34 (3H, s, NHCH_3), 3.77 (2H, s, OCH_2), 4.19 (2H, s, CH_2NHCH$_3$), 5.30 (1H, br s, NH), 7.51 (2H, d, J = 8.1 Hz, 2,6-ArH), 7.86 (2H, d, J = 8.2 Hz, 3,5-ArH); MS (EI) m/z 218 $[M]^+$. Anal. ($C_{13}H_{18}N_2O\cdot0.3H_2O$) C, H, N.

2-(4-Cyanophenyl)dioxolane (20).[43] A mixture of 4-cyanobenzaldehyde (1 g, 7.63 mmol), ethane-1,2-diol (2 g, 30.53 mmol), and *p*-toluenesulfonic acid (0.2 g) in benzene (10 mL) was stirred under reflux for 21 h, with the continuous removal of H_2O (Dean−Stark). The solvent was removed by distillation, and the residue was redissolved in EtOAc (15 mL) and washed successively with saturated sodium bicarbonate solution (10 mL) and water (10 mL), and the organic layer was dried ($MgSO_4$). Removal of the solvent gave a yellow solid, and pure **20** was obtained following recrystallization from petroleum ether (0.98 g, 73%): mp 59−61 °C; IR (KBr) 2959, 2229, 1284 cm^{-1}; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 4.18 (4H, m, OCH_2CH_2O), 5.95 (1H, s, OCHO), 7.77 (2H, d, J = 8.1 Hz, 2,6-ArH), 8.09 (2H, d, J = 8.0 Hz, 3,5-ArH); MS (EI) m/z 175 $[M]^+$. Anal. ($C_{10}H_9NO_2$) C, H, N.

2-[4-(Aminomethyl)phenyl]dioxolane (21).[44] To a solution of the acetal **20** (0.25 g, 1.45 mmol) in THF (10 mL) was added lithium triethylborohydride (1 M solution in THF, 7.15 mL, 7.15 mmol), and the mixture was stirred under a nitrogen atmosphere at ambient temperature for 12 h. H_2O (5 mL) was added cautiously, and after extraction with ether (3 × 10 mL), the organic layers were combined and dried ($MgSO_4$), and the solvent was removed to give a clear oil (0.24 g, 92%): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.80 (2H, s, CH_2NH$_2$), 4.15 (4H, m, OCH_2CH_2O), 5.20 (2H, br s, NH_2), 5.81 (1H, s, OC*H*O), 7.49 (4H, s, Ar*H*); MS (EI) *m*/*z* 179 $[M]^+$.

2,4-Diamino-5-[4′-(*N*,*N*-dimethylamino)-3′-nitrophenyl]-6-ethylpyrimidine (11). A mixture of **8** (5.0 g, 17 mmol), sodium acetate (9 g, 80 mmol), copper(II) chloride dihydrate (0.05 g, 0.03 mmol), and 4-(aminomethyl)benzoic acid (6.8 g, 80 mmol) in DMF (20 mL) was heated under reflux for 6 h. The DMF was removed *in vacuo*, and the orange residue was triturated with H_2O and collected. Recrystallization from EtOH afforded **11** as the *N*-formyl-4-(aminomethyl)benzoate salt (5.41 g, 66%): mp 237−239 °C; IR (mineral oil) 3385, 3050, 2830, 1680, 1640, 1541 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.04 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 2.20 (2H, q, J = 7.2 Hz, CH_2CH$_3$), 2.97 (6H, s, N(CH_3)$_2$), 4.48 (2H, d, 4-CHOHNC$_6$H$_4$CH_2-CO$_2^-$), 6.12 (2H, br s, NH_2), 6.54 (2H, br s, NH_2), 7.28 (1H, d, J = 8.7 Hz, 5-Ar*H*), 7.35 (1H, dd, J = 8.7 Hz, J' = 2.1 Hz, 6-Ar*H*), 7.38 (2H, d, J = 7.5 Hz, 3,5-Ar*H* of 4-CHOHNC$_6$H$_4$-CH$_2$CO$_2^-$), 7.67 (1H, d, J = 2.1 Hz, 2-Ar*H*), 8.01 (2H, d, J = 7.7 Hz, 2,6-Ar*H* of 4-CHOHNC$_6$H$_4$CH$_2$CO$_2^-$), 8.21 (1H, s, ArNHC*H*O), 8.66 (1H, t, ArN*H*CHO); MS (EI) *m*/*z* 302 $[M]^+$.

Dissolution of salt **11** in H_2O and addition of 1 M NaOH furnished orange crystals of the pyrimidine free base, which were identical (^{1}H-NMR, MS, mp) to an authentic sample of 2,4-diamino-5-[4-(*N*,*N*-dimethylamino)-3-nitrophenyl]-6-ethylpyrimidine.[24]

2,4-Diamino-5-[4′-[*N*-[4″-(methoxycarbonyl)benzyl]-*N*-methylamino]-3′-nitrophenyl]-6-ethylpyrimidine (12). A solution of methyl 4-[(methylamino)methyl]benzoate (1.06 g, 6 mmol), **8** (0.86 g, 3 mmol), and NEt$_3$ (0.8 mL, 9 mmol) in 2-ethoxyethanol (10 mL) was heated under reflux for 72 h. Evaporation of the solvent gave a red syrup, and chromatography on silica gel with 95:5 (v/v) $CHCl_3$/MeOH as eluent afforded a yellow oil characterized (^{1}H-NMR, MS) as comprising a mixture of the methyl (**12**) and 2-ethoxyethyl (**13**) esters. The mixture was redissolved in MeOH (30 mL) containing sulfuric acid (0.5 mL) and boiled for 6 h, and the solvent was evaporated under reduced pressure. The remaining orange solid was dissolved in EtOAc (50 mL), washed with 10% aqueous sodium carbonate solution, and filtered, and the solvent was removed to furnish the methyl ester **12** (0.16 g, 6.0%): mp 198−200 °C; IR (mineral oil) 3490, 3320, 3150, 1720 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.5 Hz, CH$_2$CH_3), 2.11 (2H, q, J = 7.5 Hz, CH_2CH$_3$), 2.74 (3H, s, NCH_3), 3.84 (3H, s, CO$_2$*Me*), 4.50 (2H, br s, ArCH_2N), 5.75 (2H, br s, NH_2), 5.92 (2H, br s, NH_2), 7.28 (2H, s, 5,6-Ar*H*), 7.45 (2H, d, J = 8.2 Hz, 2′,6′-Ar*H*), 7.56 (1H, s, 2-Ar*H*), 7.95 (2H, d, J = 8.2 Hz, 3′,5′-Ar*H*); MS (EI) *m*/*z* 436 $[M]^+$. Anal. ($C_{22}H_{24}N_6O_4$) C, H, N.

2,4-Diamino-5-{4′-[*N*-[4″-[(2-ethoxyethoxy)carbonyl]-benzyl]-*N*-methylamino]-3′-nitrophenyl}-6-ethylpyrimidine (13). Thionyl chloride (6 mL, 83 mmol) was added dropwise cautiously to a solution of 4-[(methylamino)methyl]-benzoic acid in 2-ethoxyethanol (100 mL). After the mixture stirred for 16 h at 50 °C, the solvent was removed under reduced pressure, the remaining cream solid was dissolved in $CHCl_3$ (50 mL) and washed with 10% aqueous potassium carbonate (2 × 50 mL), and the organic layer was dried (Na_2SO_4) and filtered. Evaporation of the solvent gave 2-ethoxyethyl 4-[(*N*-methylamino)methyl]benzoate as a colorless oil (3.5 g, 54%). Without further purification the ester (3.5 g, 15 mmol) was added to a solution of **8** (3.5 g, 12 mmol) in 2-ethoxyethanol (10 mL), and the mixture was stirred under reflux for 20 h. After evaporation of the solvent, the residual red oil was purified by chromatography on silica gel, employing 9:1 (v/v) $CHCl_3$/MeOH as eluent, to furnish the 2-ethoxyethyl ester **13** as a yellow powder (0.18 g, 3%): mp 114−116 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.97 (3H, t, J = 7.5 Hz, CH$_2$CH_3), 1.11 (3H, t, J = 7.0 Hz, OCH$_2$CH_3), 2.11 (2H, q, J = 7.5 Hz, CH_2-CH$_3$), 2.75 (3H, s, NCH_3), 3.50 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.69 (2H, m, CO$_2$CH$_2$CH_2OEt), 4.38 (2H, m, CO$_2$CH_2CH$_2$OEt), 4.51 (2H, br s, ArCH_2N), 5.69 (2H, br s, NH_2), 5.88 (2H, br s, NH_2), 7.28 (2H, m, 5,6-Ar*H*), 7.46 (2H, d, J = 8.4 Hz, 2′,6′-Ar*H*), 7.56 (1H, m, 2-Ar*H*), 7.95 (2H, d, J = 8.4 Hz, 3′,5′-Ar*H*); HRMS (EI) *m*/*z* 494.2273 $[M]^+$ ($C_{25}H_{30}N_6O_5$ requires 495.2278). Anal. ($C_{25}H_{30}N_6O_5$) C, H, N.

2,4-Diamino-5-{4′-[*N*-[4″-(*N*′-methylcarbamoyl)benzyl]-*N*-methylamino]-3′-nitrophenyl}-6-ethylpyrimidine (14).

a. From Nitropyrimethamine (8). A mixture of *N*-methyl-4-[(methylamino)methyl]benzamide (2.56 g, 14.4 mmol) and nitropyrimethamine (**8**) (2.1 g, 7.2 mmol) in 2-ethoxyethanol (25 mL) was heated under reflux for 6 h, cooled, and poured into ice−H_2O (100 mL). The solid which precipitated was collected and washed thoroughly with H_2O. Recrystallization from EtOAc−petroleum ether (bp 60−80 °C) furnished **14** as an amorphous orange powder (0.8 g, 26%): mp 235−237 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 0.97 (3H, t, J = 7.5 Hz, CH$_2$CH_3), 2.12 (2H, q, J = 7.5 Hz, CH_2CH$_3$), 2.73 (3H, s, NCH_3), 2.78 (3H, d, CONHCH_3), 4.47 (2H, br s, ArCH_2N), 5.70 (2H, br s, NH_2), 5.89 (2H, br s, NH_2), 7.28 (2H, m, 5,6-Ar*H*), 7.38 (2H, d, J = 8.2 Hz, 2′,6′-Ar*H*), 7.56 (1H, m, 2-Ar*H*), 7.81 (2H, d, J = 8.2 Hz, 3′,5′-Ar*H*); HRMS (EI) *m*/*z* 435.2104 $[M]^+$ ($C_{22}H_{25}N_7O_3$ requires 435.2019). Anal. ($C_{22}H_{25}N_7O_3$) C, H, N.

b. From Fluoronitropyrimethamine (15). A solution of **15** (0.23 g, 0.84 mmol), *N*-methyl-4-[(methylamino)methyl]-benzamide (0.36 g, 1.68 mmol), and NEt$_3$ (0.36 g, 3.6 mmol) in 2-ethoxyethanol (15 mL) was stirred at 80 °C for 16 h. The solvent was removed *in vacuo*, the residue was suspended in H_2O (30 mL) and extracted with EtOAc (3 × 15 mL), and the combined organic layers were dried ($MgSO_4$). Evaporation of the solvent and recrystallization from MeOH afforded **14** (0.28 g, 76%), which was identical (TLC, MS, NMR) to that produced by method a above.

2,4-Diamino-5-{4′-[*N*-(4″-carboxybenzyl)-*N*-methylamino]-3′-nitrophenyl}-6-ethylpyrimidine (10). A mixture of methyl ester **12** (0.92 g, 2.11 mmol) and NaOH (0.5 g, 12.5 mmol) in MeOH (25 mL) was refluxed for 16 h. After cooling, the solution was acidified to pH 6.0 with concentrated HCl, and the solid which deposited was collected and washed with cold MeOH. The orange powder (0.72 g, 80%) was dissolved in hot H_2O containing ethanesulfonic acid (0.21g 1.88 mmol), and the precipitate which formed on cooling was collected and recrystallized from H_2O to afford the benzoic acid monohydrate (0.41 g, 44%): mp 244−246 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.5 Hz, CH$_2$CH_3), 2.12 (2H, q, J = 7.5 Hz, CH_2CH$_3$), 2.74 (3H, s, NCH_3), 4.49 (2H, s, ArCH_2N), 5.86 (2H, br s, NH_2), 6.10 (2H, br s, NH_2), 7.27 (2H, m, 5,6-Ar*H*), 7.40 (2H, d, J = 8.1 Hz, 2′,6′-Ar*H*), 7.56 (1H, s, 2-Ar*H*), 7.91 (2H, d, J = 8.1 Hz, 3′,5′-Ar*H*); MS (FAB) *m*/*z* 423 $[M + 1]^+$. Anal. ($C_{21}H_{22}N_6O_4 \cdot 1H_2O$) C, H, N.

General Procedure for the Synthesis of Compounds 9, 16, 17, and 22. To a solution of fluoronitropyrimethamine (**15**) in *N*-methyl-2-pyrrolidone (15 mL) was added NEt$_3$. The solution was purged with N_2, and after addition of the appropriate benzylamine derivative, the reaction mixture was stirred under N_2 at 100 °C until TLC analysis indicated the absence of **15** (8−10 h). The solvent was evaporated *in vacuo*, and the remaining solid was triturated with H_2O, collected by filtration, and purified by recrystallization.

2,4-Diamino-5-{4′-[*N*-(4″-carboxybenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (9). Compound **9** was synthesized from **15** (0.1 g, 0.36 mmol), 4-(aminomethyl)benzoic acid (0.11 g, 0.72 mmol), and NEt$_3$ (0.36 g, 3.6 mmol) and purified by recrystallization from EtOH (0.10 g, 69%): mp 292−294 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.09 (3H, t, J = 7.4 Hz, CH$_2$CH_3), 2.23 (2H, q, J = 7.3 Hz, CH_2CH$_3$), 4.85 (2H, d, NHCH_2), 6.19 (2H, br s, NH_2), 6.49 (2H, br s, NH_2), 7.08 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.41 (1H, dd, J_{ortho} = 8.8 Hz, J_{meta} = 1.9 Hz, 6-Ar*H*), 7.63 (2H, d, J_{ortho} = 8.1 Hz, 2′,6′-Ar*H*), 7.96 (1H, d, J_{meta} = 1.9 Hz, 2-Ar*H*), 8.01 (2H, d, J_{ortho} = 8.1 Hz, 3′,5′-Ar*H*), 8.94 (1H, t, NH); high-resolution EIMS 408.1545 (calcd 408.1546). Anal. ($C_{20}H_{20}N_6O_4 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-{4′-[*N*-(4″-sulfonamidobenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (16). Compound **16** was

synthesized from **15** (1.0 g, 3.61 mmol), 4-(aminomethyl)-benzenesulfonamide (0.84 g, 3.76 mmol), and NEt_3 (1.02 g, 10.2 mmol) and purified by recrystallization from MeOH (1.12 g, 69.9%): mp 266−268 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.09 (3H, t, J = 7.5 Hz, CH_2CH_3), 2.25 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.86 (2H, d, $NHCH_2$), 5.90 (2H, br s, NH_2), 6.10 (2H, br s, NH_2), 7.03 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.41 (1H, dd, J_{ortho} = 8.8 Hz, J_{meta} = 1.9 Hz, 6-Ar*H*), 7.63 (2H, d, J_{ortho} = 8.1 Hz, 2′,6′-Ar*H*), 7.96 (1H, d, J_{meta} = 1.9 Hz, 2-Ar*H*), 8.01 (2H, d, J_{ortho} = 8.1 Hz, 3′,5′-Ar*H*), 8.94 (1H, t, NH); MS (FAB) *m*/*z* 408 $[M + 1]^+$. Anal. ($C_{19}H_{21}N_7O_4S$) C, H, N.

2,4-Diamino-5-{4′-[*N*-[4″-(4,4-dimethyloxazolin-2-yl)-benzyl]amino]-3′-nitrophenyl}-6-ethylpyrimidine (17). Compound **17** was synthesized from **15** (0.1 g, 0.361 mmol), **19** (0.16 g, 0.72 mmol), and NEt_3 (0.36 g, 3.6 mmol) and purified by recrystallization from EtOAc−petroleum ether (0.14 g, 83%): mp 173−175 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.08 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.39 (6H, s, $C(CH_3)_2$), 2.21 (2H, q, J = 7.6 Hz, CH_2CH_3), 2.85 (3H, s, NCH_3), 4.21 (2H, s, OCH_2), 5.85 (2H, d, $NMeCH_2$), 5.85 (2H, br s, NH_2), 6.04 (2H, br s, NH_2), 7.39 (2H, m, 5,6-Ar*H*), 7.48 (2H, d, J = 8.0 Hz, 2′,6′-Ar*H*), 7.68 (1H, d, J_{meta} = 1.8 Hz, 2-Ar*H*), 7.92 (2H, d, J = 8.1 Hz, 3′,5′-Ar*H*); MS (FAB) *m*/*z* 476 $[M + 1]^+$. Anal. ($C_{25}H_{29}N_7O_3 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-{4′-[*N*-(4″-dioxolan-2-ylbenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (22). Compound **22** was synthesized from **15** (0.2 g, 0.72 mmol), **21** (0.26 g, 1.44 mmol), and NEt_3 (0.72 g, 7.2 mmol) and purified by recrystallization from EtOAc−petroleum ether (0.26 g, 82%): mp 283−285 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.07 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.20 (2H, q, J = 7.4 Hz, CH_2CH_3), 4.16 (4H, m, OCH_2CH_2O), 4.80 (2H, d, J = 5.8 Hz, $NHCH_2$), 5.85 (1H, s, OC*H*O), 5.93 (2H, br s, NH_2), 6.02 (2H, br s, NH_2), 7.06 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.39 (1H, dd, J_{ortho} = 8.8 Hz, J_{meta} = 2.0 Hz, 6-Ar*H*), 7.55 (4H, s, 2′,3′,5′,6′-Ar*H*), 7.94 (1H, d, J_{meta} = 2.0 Hz, 2-Ar*H*), 8.88 (1H, t, J = 6.0 Hz, $NHCH_2$); high-resolution EIMS 436.1867 (calcd 436.1859). Anal. ($C_{22}H_{24}N_6O_4 \cdot 0.4H_2O$) C, H, N.

2,4-Diamino-5-{4′-[*N*-(4″-formylbenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (23). The acetal derivative **22** (80 mg, 0.183 mmol) was suspended in aqueous acetic acid (1.5 M, 0.8 mL), and glacial acetic acid was added dropwise until a clear solution was obtained. The reaction mixture was stirred at room temperature for 12 h, and evaporation of the solvents under reduced pressure gave the title compound as a red solid (69.5 mg, 97%): mp 271−273 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.10 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.22 (2H, q, J = 7.4 Hz, CH_2CH_3), 4.89 (2H, m, CH_2NH), 5.91 (2H, br s, NH_2), 6.23 (2H, br s, NH_2), 7.08 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.43 (1H, dd, J_{ortho} = 8.9 Hz, J_{meta} = 1.8 Hz, 6-Ar*H*), 7.60 (2H, d, J_{ortho} = 8.1 Hz, 2′,6′-Ar*H*), 7.76 (2H, d, J_{ortho} = 8.0 Hz, 3′,5′-Ar*H*), 7.99 (1H, d, J_{meta} = 2.3 Hz, 2-Ar*H*), 8.97 (1H, t, $NHCH_2$), 10.012 (1H, s, CHO); MS (FAB) *m*/*z* 393 $[M + 1]^+$. Anal. Calcd ($C_{20}H_{20}N_6O_2 \cdot 0.4H_2O$): C, 59.56; H, 5.42; N, 18.95. Found: C, 60.07; H, 5.71; N, 18.29.

2,4-Diamino-5-{4′-[*N*-(4″-carboxybenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (9) from 17. To a mixture of sodium hypochlorite solution (0.2 M, 25 mL) and EtOAc (5 mL) were added **17** (0.2 g, 0.42 mmol) and tetrabutylammonium hydrogen sulfate (14.3 mg, 0.042 mmol), and the mixture was stirred at 25 °C for 48 h. The EtOAc layer was separated, and the solvent was removed *in vacuo* to give a yellow oil, which was redissolved in MeOH. NaOH solution (1.25 M, 6 mL) was added, and the reaction mixture was stirred at room temperature for 48 h. After extraction with EtOAc (3 × 15 mL), the combined organic layers were dried ($MgSO_4$), and the solvent was removed to give an orange solid (9 mg, 5%) identical (TLC, MS) to an authentic sample of **9**.

2,4-Diamino-5-[4′-(*N*-methylamino)-3′-nitrophenyl]-6-ethylpyrimidine (24). To a suspension of **15** (0.2 g, 0.72 mmol) in *N*-methyl-2-pyrrolidone (15 mL) were added [(*N*-methylamino)methyl]benzoic acid (0.24 g, 1.44 mmol) and NEt_3 (0.72 g, 7.2 mmol). The reaction mixture was stirred at 100 °C for 2 h, and the solvent was removed under reduced pressure. Trituration of the residue with H_2O and recrystallization from EtOH gave 0.18 g (87%) of **24** as orange microcrystals: mp 263−264 °C (lit.[24] mp 262−265 °C); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.01 (3H, t, J = 7.5 Hz, CH_2CH_3), 2.22 (2H, q, J = 7.5 Hz, CH_2CH_3), 3.09 (3H, d, J = 4.8 Hz, $NHCH_3$), 5.71 (2H, br s, NH_2), 5.86 (2H, br s, NH_2), 7.23 (1H, d, J_{ortho} = 9.0 Hz, 5-Ar*H*), 7.53 (1H, dd, J_{ortho} = 8.9 Hz, J_{meta} = 2.0 Hz, 6-Ar*H*), 8.02 (1H, d, J_{meta} = 1.9 Hz, 2-Ar*H*), 8.43 (1H, q, J = 4.9 Hz, $NHCH_3$); MS (EI) *m*/*z* 288 $[M]^+$.

General Procedure for the Synthesis of Compounds 25−28. A stirred solution of the carboxylic acid **9** in anhydrous DMF (15 mL) was cooled to 0 °C, and NEt_3 was added. After the addition of isobutyl chloroformate, the solution was stirred under N_2 for 1 h at 0 °C, the appropriate amine was added, and stirring was continued at 0 °C for a further 1 h and then for 12 h at ambient temperature. The solvent was removed *in vacuo*, and the product was purified by chromatography on silica gel, using 5:1 (v/v) CH_2Cl_2/MeOH as eluent.

2,4-Diamino-5-{4′-[*N*-(4″-carbamoylbenzyl)amino]-3′-nitrophenyl}-6-ethylpyrimidine (25). Compound **25** was synthesized from **9** (0.2 g, 0.49 mmol), isobutyl chloroformate (0.08 mmol), ammonium hydroxide (30% (w/v) in H_2O, 0.5 mL), and NEt_3 (0.12 g, 1.1 mmol) and recrystallized from MeOH (0.16 g, 79%): mp 201−203 °C (0.58 mmol); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.11 (3H, t, J = 7.6 Hz, CH_2CH_3), 2.25 (2H, q, J =7.5 Hz, CH_2CH_3), 2.80 (3H, d, J = 4.4 Hz, $NHCH_3$), 4.81 (2H, d, J = 5.8 Hz, $NHCH_2$), 6.65 (4H, br s, 2 × NH_2), 7.07 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.39 (1H, dd, J_{ortho} = 8.7 Hz, J_{meta} = 2.0 Hz, 6-Ar*H*), 7.42 (1H, br s, N*H*), 7.56 (2H, d, J = 8.1 Hz, 2′/6′-Ar*H*), 7.95 (2H, d, J = 8.2 Hz, 3′/5′-Ar*H*), 8.01 (1H, d, J_{meta} = 2.0 Hz, 2-Ar*H*), 8.10 (1H, br s, NH), 8.54 (1H, m, $NHCH_3$), 8.99 (1H, m, J = 5.8 Hz, $NHCH_2$); high-resolution EIMS 407.1715 (calcd 407.1706). Anal. Calcd ($C_{20}H_{21}N_7O_3 \cdot 0.85$DMF): C, 51.16; H, 4.48; N, 20.89. Found: C, 51.88; H, 4.95; N, 20.16.

2,4-Diamino-5-{4′-[*N*-[4″-(*N*′-methylcarbamoyl)benzyl]-amino]-3′-nitrophenyl}-6-ethylpyrimidine (26). Compound **26** was synthesized from **9** (0.2 g, 0.49 mmol), isobutyl chloroformate (0.08 g, 0.58 mmol), methylamine (33% (w/v) solution in EtOH; 1 mL, 10.6 mmol), and NEt_3 (0.12 g, 1.1 mmol) and recrystallized from MeOH (0.16 g, 76%): mp 209−211 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.07 (3H, t, J = 7.5 Hz, CH_2CH_3), 2.23 (2H, q, J = 7.3 Hz, CH_2CH_3), 2.80 (3H, d, J = 4.4 Hz, $NHCH_3$), 4.84 (2H, d, $NHCH_2$), 6.65 (2H, br s, NH_2), 6.84 (2H, br s, NH_2), 7.06 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.42 (1H, dd, J_{ortho} = 8.8 Hz, J_{meta} = 1.9 Hz, 6-Ar*H*), 7.62 (2H, d, J = 7.3 Hz, 2′/6′-Ar*H*), 7.87 (2H, d, J = 7.3 Hz, 3′/5′-Ar*H*), 8.01 (1H, d, J_{meta} = 1.9 Hz, 2-Ar*H*), 8.54 (1H, m, $NHCH_3$), 8.95 (1H, m, $NHCH_2$); high-resolution EIMS 421.1860 (calcd 421.1862). Anal. Calcd ($C_{21}H_{23}N_7O_3 \cdot 2H_2O$): C, 55.13; H, 5.95; N, 21.43. Found: C, 54.67; H, 5.30; N, 20.71.

2,4-Diamino-5-{4′-[*N*-[4″-(*N*′,*N*′-dimethylcarbamoyl)-benzyl]amino]-3′-nitrophenyl}-6-ethylpyrimidine (27). Compound **27** was synthesized from **9** (0.1 g, 0.25 mmol), isobutyl chloroformate (0.10 g, 0.70 mmol), dimethylamine (33% (w/v) solution in EtOH; 2 mL, 15 mmol), and NEt_3 (0.06 g, 0.55 mmol) and recrystallized from MeOH (75 mg, 70%): mp 166−168 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.07 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.23 (2H, q, J = 7.5 Hz, CH_2CH_3), 3.017 (6H, s, $N(CH_3)_2$), 4.83 (2H, d, $NHCH_2$), 5.90 (4H, br s, 2 × NH_2), 7.19 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.41 (1H, dd, J_{ortho} = 8.8 Hz, J_{meta} = 2.0 Hz, 6-Ar*H*), 7.52 (2H, d, J = 8.3 Hz, 2′/6′-Ar*H*), 7.61 (2H, d, J = 8.3 Hz, 3′/5′-Ar*H*), 7.95 (1H, d, J_{meta} = 2.0 Hz, 2-Ar*H*), 8.951 (1H, m, $NHCH_2$); high-resolution EIMS 435.2012 (calcd 435.2019). Anal. Calcd ($C_{22}H_{25}N_7O_3 \cdot 1H_2O$): C, 58.25; H, 6.00; N, 21.63. Found: C, 58.74; H, 5.75; N, 21.03.

2,4-Diamino-5-{4′-[*N*-[4″-(1-piperidinocarbonyl)benzyl]-amino]-3′-nitrophenyl}-6-ethylpyrimidine (28). Compound **28** was synthesized from **9** (0.2 g, 0.49 mmol), isobutyl chloroformate (0.14 g, 1.0 mmol), piperidine (50 mg, 0.59 mmol), and NEt_3 (0.12 g, 1.1 mmol) and recrystallized from MeOH (0.18 g, 77%): mp 192−195 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.15 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.74 (6H, m, C_3H_6), 2.31 (2H, q, J = 7.5 Hz, CH_2CH_3), 3.20 (4H, m, CH_2NCH_2), 4.88 (2H, d, J = 5.3 Hz, $NHCH_2$), 6.45 (4H, br s, 2 × NH_2), 7.20 (1H, d, J_{ortho} = 8.9 Hz, 5-Ar*H*), 7.54 (5H, m, 6-Ar*H*, 2′/3′/5′/6′-Ar*H*), 8.03 (1H, d, J_{meta} = 1.9 Hz, 2-Ar*H*), 8.99 (1H,

t, $J = 5.3$ Hz, N*H*CH_2); high-resolution EIMS 475.2325 (calcd 475.2332). Anal. ($C_{25}H_{29}N_7O_3 \cdot 0.6H_2O$) C, H, N.

Diethyl *N*-{4-[[*N*′-[2-Nitro-4-(2,4-diamino-6-ethylpyrimidin-5-yl)anilino]]methyl]benzoyl}-L-glutamate (29). To a stirred solution of **9** (90 mg, 0.22 mmol) in anhydrous DMF (10 mL) at 0 °C was added NEt_3 (70 mg, 0.70 mmol) followed by isobutyl chloroformate (36 mg, 0.26 mmol). After the solution stirred for 1 h, L-glutamic acid diethyl ester hydrochloride (63.5 mg, 0.26 mmol) was added. Stirring was continued at 0 °C for 4 h and then overnight at ambient temperature. Evaporation of the solvent under reduced pressure furnished an orange solid which was purified by chromatography on silica gel, employing 4:1 (v/v) CH_2Cl_2/MeOH as the eluting solvent. Recrystallization from EtOH gave the title compound (101 mg, 79%): mp 119−121 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.08 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 1.37 (6H, m, 2 × $CO_2CH_2CH_3$), 2.20 (2H, q, $J = 7.5$ Hz, CH_2CH_3), 2.30 (4H, m, $CH_2CH_2CO_2Et$), 4.23 (4H, m, 2 × $CO_2CH_2CH_3$), 4.54 (1H, m, NHC*H*CO_2Et), 4.86 (2H, d, $J = 5.5$ Hz, NHCH_2), 6.68 (2H, br s, NH_2), 6.87 (2H, br s, NH_2), 7.09 (1H, d, $J_{ortho} = 9.0$ Hz, 5-Ar*H*), 7.42 (1H, dd, $J_{ortho} = 8.9$ Hz, 6-Ar*H*), 7.61 (2H, d, $J = 8.1$ Hz, 2′/6′-Ar*H*), 7.96 (2H, d, $J = 8.3$ Hz, 3′/5′Ar*H*), 8.02 (1H, d, $J_{meta} = 2.1$ Hz, 2-Ar*H*), 8.85 (1H, d, $J = 7.4$ Hz, CON*H*), 9.00 (1H, t, $J = 5.4$ Hz, N*H*CH_2); MS (FAB) *m*/*z* 594 $[M + 1]^+$. Anal. ($C_{29}H_{35}N_7O_7 \cdot 0.5H_2O$) C, H, N.

***N*-{4-[[*N*′-[2-Nitro-4-(2,4-diamino-6-ethylpyrimidin-5-yl)anilino]]methyl]benzoyl}-L-glutamatic Acid (30).** The diethyl ester **29** (30 mg, 0.05 mmol) was dissolved in EtOH (3 mL), and a solution of barium hydroxide octahydrate (31.9 mg, 0.101 mmol) in H_2O (2.5 mL) was added. The reaction mixture was stirred for 12 h at room temperature, and the solid which precipitated was collected and redissolved in H_2O (20 mL). Sodium sulfate (14 mg, 0.101 mmol) in H_2O (1 mL) was added, and the precipitate which formed after 15 min was removed by filtration through Celite. The filtrate was evaporated to dryness under reduced pressure, the remaining solid was redissolved in a minimum of H_2O, and the pH was adjusted to 2.0 with 1 M HCl. The resulting yellow precipitate was collected, washed with water, and recrystallized from propan-2-ol (8.5 mg, 32%): mp 136−138 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.10 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 2.20 (2H, q, $J = 7.5$ Hz, CH_2CH_3), 2.29 (4H, m, $CH_2CH_2CO_2Et$), 4.48 (1H, m, NHC*H*CO_2H), 4.84 (2H, d, $J = 5.5$ Hz, NHCH_2), 6.88 (4H, br s, 2 × NH_2), 7.10 (1H, d, $J_{ortho} = 8.9$ Hz, 5-Ar*H*), 7.38 (1H, dd, $J_{ortho} = 9.0$ Hz, 6-Ar*H*), 7.63 (2H, d, $J = 8.1$ Hz, 2′/6′-Ar*H*), 7.95 (2H, d, $J = 8.1$ Hz, 3′/5′-Ar*H*), 8.04 (1H, d, $J_{meta} = 2.0$ Hz, 2-Ar*H*), 8.67 (1H, d, $J = 7.5$ Hz, CON*H*), 8.90 (1H, t, $J = 5.5$ Hz, N*H*CH_2); MS (FAB) *m*/*z* 520 $[M + 1 - H_2O]^+$, 406 $[M - CH(CO_2H)CH_2CH_2CO_2H]^+$, 391 $[M - NHCH(CO_2H)CH_2CH_2CO_2H]^+$.

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