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Structural Studies on Bioactive Compounds. Part 36: Design, Synthesis and Biological Evaluation of Pyrimethamine-Based Antifolates Against *Pneumocystis carinii* ☆

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Abstract—As part of a research effort to improve the quality of current chemotherapy of *Pneumocystis carinii* pneumonia, we report a structure-based design project to optimise activity, species selectivity and pharmaceutical properties of the triazenyl-pyrimethamine TAB (**4**) ($IC_{50}=0.17$ μM; rat liver DHFR IC_{50}/*P. carinii* DHFR $IC_{50}=114$). This has led us to design, synthesise and evaluate four new series of pyrimethamine derivatives bearing triazole, triazolium, triazinium and amino moieties at the 3′-position of the *p*-chlorophenyl ring. Such stabilised 'triazene' derivatives address the potentially compromised pharmaceutical profile of TAB and the 3′-amine substituted agents afford conformationally flexible substitutes. The benzylamino-pyrimethamine derivative (**24a**) ($IC_{50}=0.12$ μM, rat liver DHFR IC_{50}/*P. carinii* DHFR IC_{50}: 5.26) was the most potent and the only *P. carinii*-selective antifolate of the new series.

Introduction

The fungus *Pneumocystis carinii* is one of the most prevalent opportunistic microorganisms afflicting individuals with compromised immune systems. HIV positive patients are particularly vulnerable and a substantial number will develop *P. carinii* pneumonia (PCP) which gives rise to significant morbidity and mortality. Dihydrofolate reductase (DHFR) inhibitors such as trimethoprim (**1**) and trimetrexate (**2**) (Fig. 1) have been drugs of first choice to treat PCP, but they have limitations. Maximal clinical efficacy of trimethoprim requires combination with sulphamethoxazole which leads to an increase in the frequency of adverse drug reactions. Although trimetrexate does not require the synergistic effect of sulphonamides, its lack of species selectivity renders the concomitant use of leucovorin mandatory to ameliorate severe antifolate toxicity towards the host. The goal of identifying antifolates with high species selectivity and potency remains unachieved despite the efforts of a number of research groups.[1]

Using pyrimethamine (**3**) as a starting point Stevens et al.[2] reported that the acetoxyethyl-derivative of a benzyltriazenyl-substituted pyrimethamine (**4**; TAB) has a promising species selectivity for *P. carinii* DHFR over that of the mammalian enzyme. This prompted a new search for robust, potent and species selective antifolates based on this pharmacophore.[3] The increase in species selectivity of TAB has been attributed to favourable hydrophobic contact of the benzyl group with the unique *P. carinii* DHFR Phe69 residue.[4] (Asn64 is the equivalent residue in the human enzyme.) However, 1-aryl-3,3-dialkyltriazenes (e.g., **5**) as a class are compromised as therapeutic agents, particularly in the setting where long-term administration might be required. Firstly, they are only moderately stable under physiological conditions;[5,6] moreover, they are potential substrates for metabolic dealkylation to mutagenic monoalkyltriazenes (**6**),[7–9] which, following heterolytic degradation, liberate an arylamine (**7**) and an alkylating alkanediazonium species (**8**). The biological sequelae of these events include the alkylation of nucleic acids (**9**) (Scheme 1).[9]

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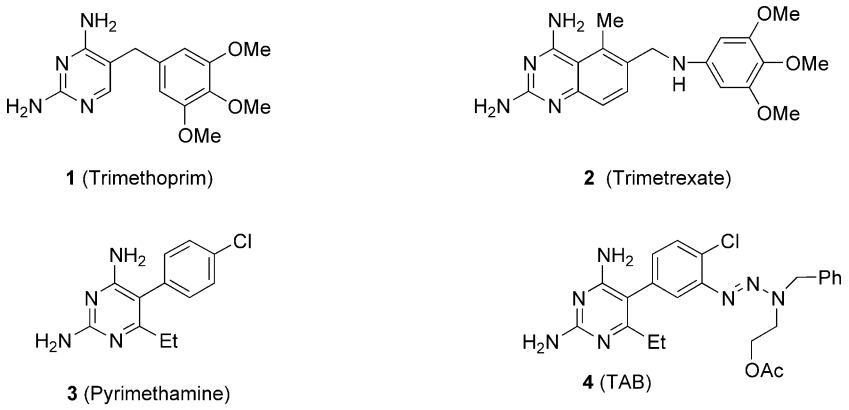
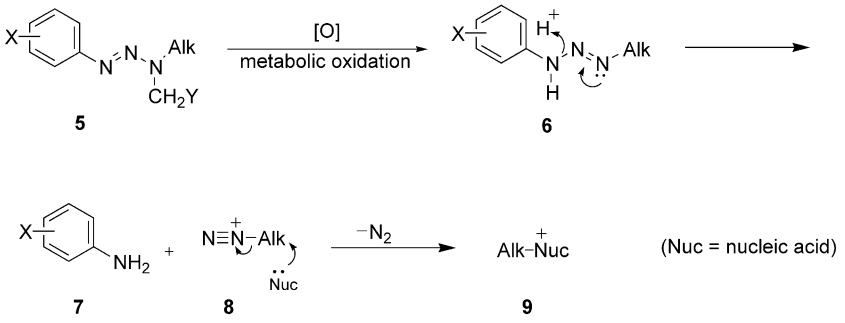


Figure 1. Structures of inhibitors of *P. carinii* DHFR.



Scheme 1. Metabolism of 3,3-dialkyl-1-aryltriazenes.

Sustaining further interest in triazene-based agents would depend on a radical redesign to remove these undesirable features whilst optimising inhibitory activity against the *P. carinii* DHFR enzyme target. To provide greater chemical stability and to remove opportunities for bio-conversion to monoalkyltriazene species for the putative second generation diaminopyrimidines, we have adopted two approaches: (i) to constrain the triazene NNN linkage within a triazole or triazine ring which removes the 'masked diazonium' character implicit in a triazene structure;[5,6] and (ii) to replace the triazene linker with a benzanilide or benzylamine moiety. In order to provide a qualitative assessment of the binding potential of these novel analogues to *P. carinii* DHFR, molecular modelling studies utilising manual docking and molecular dynamics were initiated with the prototypical benzyltriazolyl-substituted pyrimethamine (**11a;** AZO) which was chosen as a mimic of TAB (**4**) devoid of the acetoxyethyl substituent but retaining the critical benzyl function (Fig. 1).

Molecular Modelling

When this study was initiated the only structural information available was from the X-ray crystal structure of the *P. carinii* DHFR-NADPH-trimethoprim ternary complex determined by Champness et al.[10] Conformational analysis of AZO within the active site of the DHFR–NADPH complex was performed first through a manual docking study. Essentially, two orientations were predicted to be likely. In the first the torsion angle between the pyrimidine and phenyl rings led to the extended benzyl sidechain occupying the pocket above the plane of the pyrimidine ring, where it could make extensive hydrophobic contacts with residues such as Phe36, Ile65, Phe69 and Leu72. Aromatic–aromatic interactions of the π-stacking type with a centroid separation of 4 Å between the benzyl moiety of AZO and Phe69 were possible; the benzyl moiety could also establish a weaker contact of 5.7 Å with Phe36 in a face-edge manner. In the second orientation the torsion angle between the pyrimidine ring and the phenyl ring led to the benzyltriazole branch being located in the opening of the active site cleft, where there were significantly fewer possible favourable hydrophobic contacts. In this orientation the N(2) triazole nitrogen was 4.1 Å away from the centre of the nicotinamide ring of NADPH. Only one other significant hydrophobic contact appeared possible, between the phenyl ring and Leu25. On the basis of these manual docking studies, it was concluded that this second orientation for AZO within the active site should be considerably less favoured.

These predictions were then tested by application of the molecular dynamics/simulated annealing method we have previously described for performing a potentially less biased prediction of binding geometries.[4] Six MD runs were performed, each of 100 ps. The resulting final conformations of AZO are shown in Figure 2, and the total molecular mechanics energies of the *P. carinii* DHFR–AZO–NADPH complex are given in Table 1. From Figure 2 it can be seen that the MD runs led to the sampling of both classes of AZO conformations predicted from the manual docking. Runs c, d, and f led

to structures where the phenyl sidechain occupied the upper pocket (illustrated for c in Fig. 3), while a, b, and e resulted in structures where it sampled the lower pocket (illustrated for b in Fig. 4). In contrast to the expectations from the inspection of the manually docked structures, we see that the lowest energy interaction geometry actually corresponds to AZO being oriented so that the phenyl sidechain occupies the lower pocket. It is then distant from the residues, particularly Phe69, that are predicted to be important for providing species selectivity. Although these results did not augur well for the activities of these classes of compounds, the qualitative nature of the modelling study demanded its full validation through the synthesis of a range of compounds from all structural classes.

Chemistry

1,2,3-Triazole derivatives

Interaction of *m*-azidopyrimethamine (**10**), prepared by the method of Bliss et al.,[11] with an excess (5 mol equiv) of benzylacetylene in DMF at 100 °C for 48 h resulted in the isolation of two regioisomeric cycloaddition products (**11a**,**b**) in 77% total yield (Scheme 2); overall yield in the absence of solvent was 71%. The proportion of each cycloaddition product in the crude reaction mixture was estimated as 2:1 in favour of the least sterically crowded 4-benzyltriazole regioisomer (**11a**), based on integrating signals due to the α-methylene protons of the 6-ethyl group and the methylene protons of the benzyl function in the ^{1}H NMR spectra of the mixed products. Structure **11a** for the major isomer was confirmed by ^{1}H–^{1}H proximity determinations: irradiation of the triazole 5-H singlet at δ 8.27 caused NOE enhancements in two locations: at δ 4.11 (3.7%) and δ 7.20 (3.1%) which correspond to the benzyl methylene

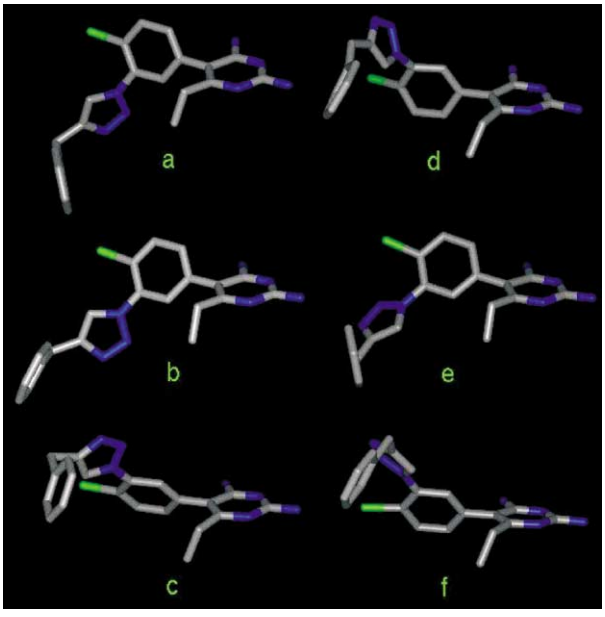


Figure 2. Binding conformations of AZO (**11a**) at *P. carinii* DHFR active site after six MD simulations. The views are relative to an identical orientation of the protein in each case, but this is not shown, for clarity.

Table 1. Summary of the total molecular mechanics energies of the *P. carinii* DHFR–AZO–NADPH complex after 100 ps simulated annealing run at *P. carinii* DHFR active site

Simulation	Energy (kcal mol^{-1})
a	−341.0
b	−366.1
c	−350.0
d	−355.4
e	−335.4
f	−311.3

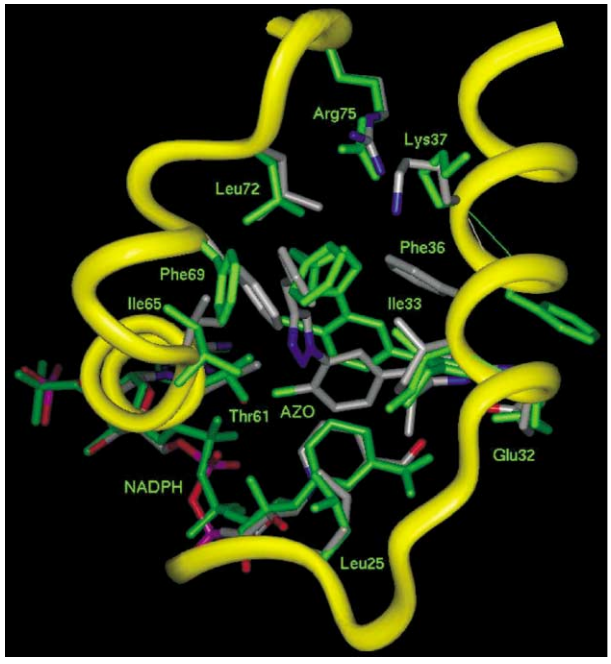


Figure 3. Orientation of AZO (**11a**) in the active site of *P. carinii* DHFR resulting from MD run c (green). The corresponding structure predicted by manual docking is also shown (grey).

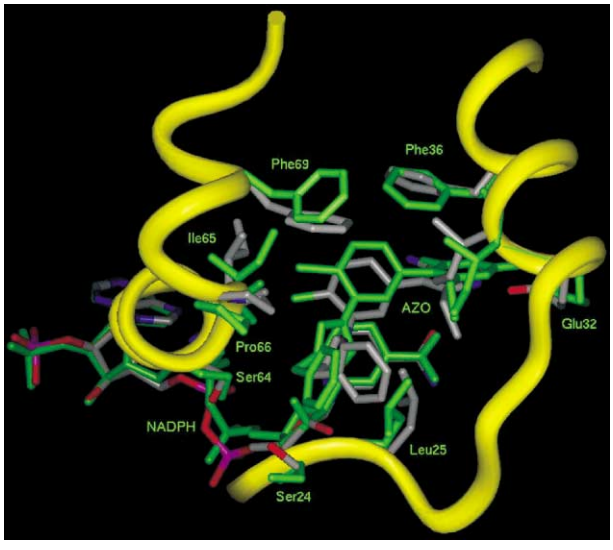
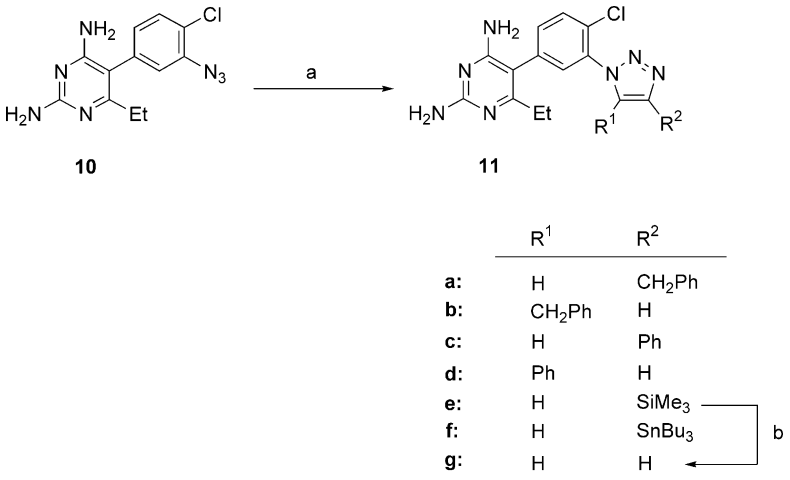


Figure 4. Orientation of AZO (**11a**) in the active site of *P. carinii* DHFR resulting from MD run b (green). The corresponding structure predicted by manual docking is also shown (grey).



Scheme 2. Reagents: (a) $R^1C{\equiv}CR^2$ in DMF, 100 °C; (b) TBAF in THF.

protons and H-2′ in the chlorophenyl ring, respectively. In the reaction of azide (**10**) with phenylacetylene the ratio of the phenyltriazoles (**11c**) and (**11d**) was 3:2 (79% overall yield) in favour of the 4-substituted isomer (**11c**). Irradiation of the 5-H singlet of **11c** at δ 9.0 gave rise to two NOE enhancements (0.5 and 2.4%) corresponding to the two 2′-aromatic protons of the chlorophenyl and phenyl rings, respectively.

The lack of efficient chromatographic methods to separate the regioisomers (**11a**,**b**) and (**11c**,**d**) thwarted the efficient isolation of pure products and led us to seek alternative routes to substituted triazoles. Reaction between azide (**10**) and the sterically-demanding (trimethylsilyl)acetylene was regiospecific, the 4-(trimethylsilyl)triazole (**11e**) being formed in 85% yield: irradiation of the triazole H-5 singlet at δ 8.52 induced the expected NOE enhancement (3.7%) at H-2′ in the chlorophenyl ring. Other cycloaddition reactions involving (trimethylsilyl)acetylene have been reported to proceed regioselectively.[12–14] Sakamoto et al. have reported an efficient two-step synthetic synthesis of 1,4-diphenyl-1,2,3-triazole[15] from the regioselective addition reaction of (trimethylstannyl)acetylene with phenyl azide to give exclusively the 4-trimethylstannyl intermediate followed by a palladium-catalysed phenylation reaction to yield the diphenyltriazole. The air-sensitive (trimethylstannyl)acetylene is not commercially available and its synthesis and storage are problematic.[16–18] The commercially available (tributylstannyl)acetylene (10 mol equiv) was chosen to replace (trimethylstannyl)acetylene and was reacted with **10** at 110 °C for 4 days in anhydrous DMF. None of the expected (tributylstannyl)-triazole (**11f**) was formed and the only product isolated was the 1-substituted triazole (**11g**) (39%). The latter triazole could be prepared more efficiently (74%) by removal of the TMS group of **11e** with a slight excess of TBAF (1.1 mol equiv) (Scheme 2).

1,2,3-Triazolium and 1,2,3-triazinium derivatives

The facile cyclisation of aryl-[3,3-bis(2-chloroethyl)]-triazenes by intramolecular displacement of a chloro group has provided access to a number of 1,2,3-triazolium salts.[19,20] In 1968, Shealy recorded this reaction following his synthesis of the experimental anti-leukemic agent 5-[3,3-bis(2-chloroethyl)triazen-1-yl]imidazole-4-carboxamide (**12**) which cyclised in the solution and solid states to the biologically inactive ionic triazolium salt (**13**) (Scheme 3).[21–23] Hansen et al. have also reported the one-pot cyclisation of hydroxyalkyl-triazenes (**14**) with mesyl chloride to the dihydrotriazolium salts (**16**): the intermediate methanesulfonyl esters (**15**) could not be isolated.[24]

In the present work, triazenes (**19a**,**b**) were formed by coupling diazonium salts derived from 2-chloroaniline (**17**; R = H) with benzylalkanolamines (**18a**,**b**) in aqueous sodium carbonate. These were then converted with thionyl chloride (as solvent and reactant) at 0 °C, via the intermediate chloroalkyltriazenes (**20a**,**b**), to the model triazolium (**21a**) and triazinium (**21b**) products, respectively (Scheme 4). The diazonium chloride (**17**; R = 2,4-diamino-6-ethylpyrimidin-5-yl)[11] was similarly coupled with substituted benzylalkanolamines (**18c**–**f**) to yield hydroxyalkyl-triazenes (**19c**–**f**) which were converted with thionyl chloride to the required diaminopyrimidinyl-substituted salts (**21c**–**f**) which were precipitated from the reaction mixtures in yields >75% by addition of excess ether. The products were freely water-soluble consistent with their ionic nature, and their ^{1}H NMR spectra did not show any sign of peak broadening of the signals of the methylene protons in the dihydrotriazolium and tetrahydrotriazinium rings. Ring-opened triazenyl-pyrimidines related in structure to intermediate chloroalkyl-triazenes (**20c**–**f**), which were not isolated, do show this characteristic feature because of restricted rotation around the N=N bond.[3]

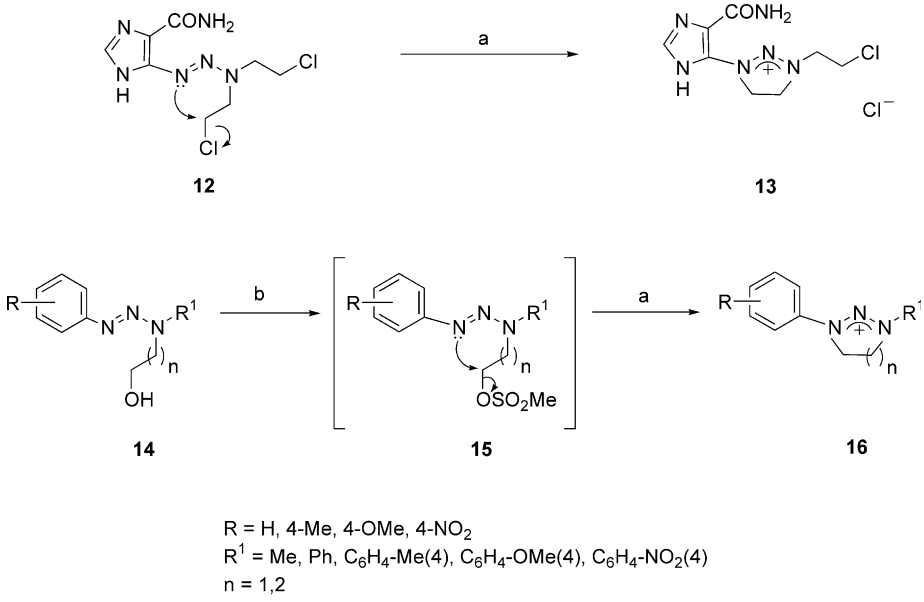
Benzanilide and benzylamine derivatives

Acylation of 5-(3-amino-4-chlorophenyl)-2,4-diamino-6-ethylpyrimidine (**22**)[11] with the appropriate acid chloride in hot pyridine furnished the anilides (**23a**,**b**). Benzylamines (**24a**,**b**) were obtained by alkylation of **22** with the requisite benzyl bromide in refluxing isopropanol (Scheme 5). Subsequently, reductive methylation reactions were carried out on the two secondary amines

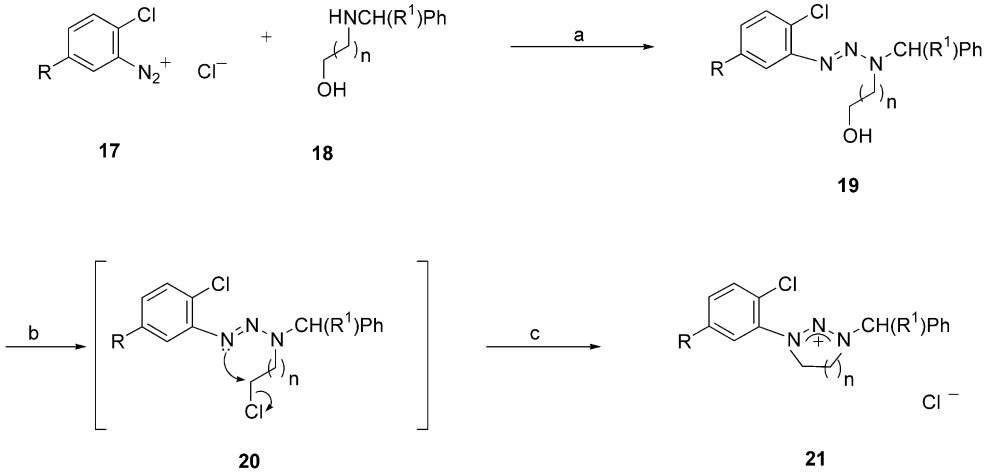
using 37% formaldehyde and sodium cyanoborohydride in an acidic medium to afford the methylated analogues (**24c**,**d**) in >90% yield.

Biological Results

The assay methods used were described in previous reports[25–27] and results are given in Table 2. The activity of four triazolyl analogues (**11a**,**c**,**e**,**g**) were evaluated as inhibitors of *P. carinii*, *T. gondii*, *M. avium* and rat liver DHFR. The two most potent compounds against the *P. carinii* enzyme were those with a benzyl (**11a**) or phenyl group (**11c**) which had IC_{50} values of 5.18 and 3.53 μM, respectively. By comparison, the TMS analogue (**11e**) and the unsubstituted triazole (**11g**) were less active (IC_{50} values 10.5 and 24.8 μM, respectively). This class of compounds successfully addressed the problematic

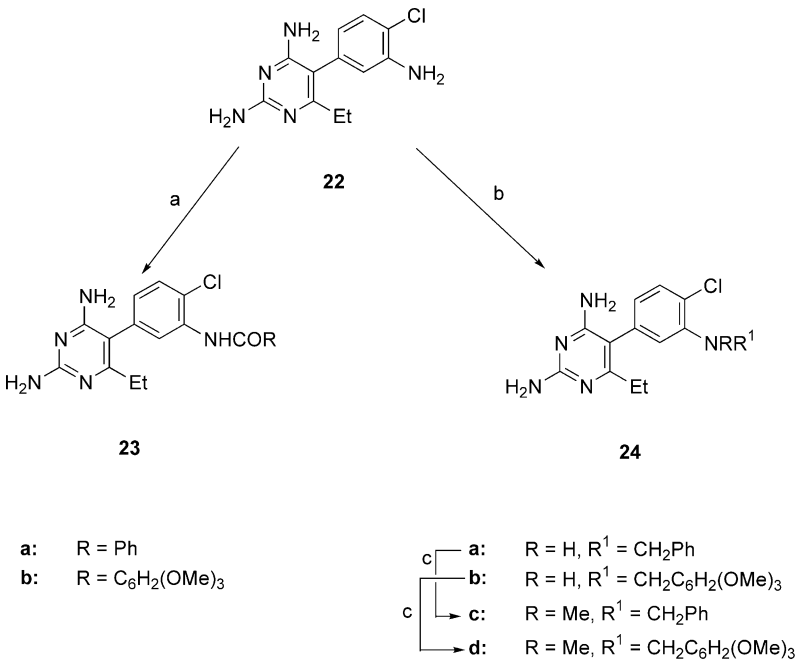


Scheme 3. (a) Spontaneous cyclisation; (b) mesyl chloride.



	n	R	R^1
a:	1	H	H
b:	2	H	H
c:	1	2,4-diamino-6-ethylpyrimidin-5-yl	H
d:	2	2,4-diamino-6-ethylpyrimidin-5-yl	H
e:	1	2,4-diamino-6-ethylpyrimidin-5-yl	Me(S)
f:	1	2,4-diamino-6-ethylpyrimidin-5-yl	Me(R)

Scheme 4. (a) Aqueous Na_2CO_3, 0 °C; (b) $SOCl_2$ in Et_2O, 0 °C; (c) spontaneous cyclisation.



Scheme 5. Reagents: (a) RCOCl, pyridine, reflux; (b) R^1CH_2Br, ^{i}PrOH, reflux; (c) HCHO/HCl/$NaBH_3CN$ in MeCN.

Table 2. Structure–activity relationship of pyrimethamine based 2,4-diaminopyrimidines towards *P. carinii* (pc), rat liver (rl) *T. gondii* (tx), and *M. avium* (ma) DHFR

Compd	IC_{50} (μM) *vs* DHFR				Selectivity ratio		
	P. carinii	rat liver	*T. gondii*	*M. avium*	rl/pc	rl/tx	rl/ma
2[a]	0.042	0.003	0.01	—	0.07	0.30	—
4[a]	0.17	19.4	0.69	—	114	28	—
11a	5.18	1.38	0.52	—	0.27	2.65	—
11c	3.53	0.15	0.37	—	0.04	0.41	—
11e	10.5	3.34	1.83	2.5	0.32	1.83	1.34
11g	24.8	0.38	0.28	3.18	0.02	1.36	0.12
21c	13.5	3.3	1.38	—	0.24	2.39	—
23a	10.8	4.1	0.51	—	0.38	8.04	—
23b	1.3	0.99	0.08	—	0.77	12.5	—
24a	0.12	0.63	0.07	—	5.26	9.0	—
24c	1.07	0.09	0.02	—	0.08	4.50	—

[a]Data reported previously, see refs 2 and 3.

stability issue associated with the triazene TAB (**4**) but did not produce superior activity nor comparable species selectivity to the lead structure. This result was predicted from modelling studies which suggested that aromatic interaction with Phe69 alone cannot dictate significant species selectivity. Nevertheless, it is apparent from the structure–activity relationships that substitution at the 4-position of the triazole ring gives rise to compounds with improved activity compared with previously reported triazenyl-pyrimethamine derivatives,[3] presumably through an increasingly optimal interaction with the enzyme active site residues. Only one analogue with a triazolium moiety (**21c**) was evaluated for activity (IC_{50} 13.5 μM against *P. carinii* DHFR; 3.3 μM against rat DHFR). Although the DHFR inhibitory activity is not substantial, our intuitive concern that positioning a positively charge species in a hydrophobic pocket would severely impede binding was not realised. Four compounds with a nitrogen bridge (benzanilides and benzylamines) were tested for activities. Compounds in the benzanilide series (**23a**,**b**) are less potent than the benzylamines (**24a**,**c**). In the former series, addition of trimethoxy groups (**23b**) resulted in a near 10-fold increase in activity over that of the unsubstituted benzanilide (**23a**). With an IC_{50} value of 0.12 μM compound (**24a**) is the most potent member of the group as an inhibitor of *P. carinii* DHFR and is also the only agent with species selectivity (5.26 vs rat liver DHFR). Interestingly, methylation of the amino bridge (**24c**) resulted a 10-fold reduction of activity towards the *P. carinii* enzyme and nearly a 7-fold gain of potency towards the rat liver DHFR.

Overall, the compounds listed in Table 2 were consistently more potent against the *T. gondii* enzyme than the *P. carinii* enzyme. The most potent compounds were the benzylamines (**24a**,**c**) but the trimethoxybenzoyl derivative (**23b**) displayed the greatest selectivity (selectivity ratio of 12.5 vs rat liver DHFR).

Conclusion

The outcome of the manual docking study of the benzyltriazolyl-substituted pyrimethamine (**11a;** AZO) correlates well with the result of the dynamics simulations. Although the ligand orientations generated from dynamics simulations are expected to be only qualitatively correct, these results have shown that automated docking using simulated annealing can produce an effective search of conformational space. Deriving useful quantitative data such as binding

constants of the designed compounds was beyond the scope of the present molecular modelling study, because high quality ternary X-ray crystal data of the *P. carinii* DHFR–TAB–NADPH ternary complex was not available at the beginning of this project. Four series of structurally diverse analogues with varying chemical and physical properties have been designed, synthesised and assayed against DHFR enzymes from different species. Although compound (**11a**) was thought to be a promising mimic of TAB by molecular modelling analysis, it did not display any substantial potency or species selectivity towards *P. carinii* DHFR. Compounds belonging to the triazole (**11**), triazolium and triazinium (**21**) series showed activities consistently weaker than the benzylamine series (**24**). Although the benzylamine (**24a**) (IC_{50} 0.12 μM; rat liver DHFR IC_{50}/*P. carinii* DHFR IC_{50} = 5.26) was the most active (and the only selective) antifolate towards *P. carinii* within the four series of compounds, it was not as potent or selective as the original lead compound, (acetoxyethyl)benzyltriazenyl-pyrimethamine (**4**; TAB).

Experimental

Molecular modelling

Comprehensive details have been given elsewhere.[4] Briefly, the docking study was performed using the DISCOVER module of the molecular modelling package Insight II[28] as an interface to the AMBER 3.5 force field while the stand alone AMBER 4.0 package[29] was used for the molecular dynamics simulations.

Synthetic chemistry

Chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and other commercial sources and were used without further purification. Melting points were determined in open capillary tubes on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured in KBr on a Mattson 2020 Galaxy Series FT-IR spectrometer. The optical measurements were made in tubes with length 20 cm on a Bellingham & Stanley ADP 220 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer in DMSO-d_6 solutions at 250.1 and 62.9 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) with tetramethysilane as an internal standard. ^{13}C assignments for secondary and quaternary carbons were based on DEPT135 experiments. MS were recorded on an ANI MS-902, a VG Micromass 7070E or a VG platform spectrometer. Elemental analyses were performed on either a Perkin-Elmer PE240B Elemental Analyser or an Exeter Analytical CE-440 Elemental Analyser by the Microanalysis service at the Department of Chemistry, University of Nottingham, UK. Samples were dried in vacuo overnight in the presence of phosphorous pentoxide at room temperature prior to the submission for elemental analysis. Precoated silica gel $60F_{254}$ plates were used as the absorbent for thin layer chromatography, with the developing solvent being either chloroform–methanol or hexane–ethylacetate mixture. TLC spots were visualised using UV light. Flash column chromatography was performed using silica gel C60H.

2,4-Diamino-5-[3-(4-benzyl-1,2,3-triazol-1-yl)-4-chlorophenyl]-6-ethylpyrimidine (11a; AZO) and regioisomer (11b). To *m*-azidopyrimethamine[11] (**10**; 1.5 g, 5.2 mmol) in anhydrous DMF (10 mL) was added benzylacetylene (3.0 g, 25.9 mmol, 5 mol equiv). The suspension was heated at 100 °C for 48 h and the brown solution was then cooled to 25 °C and was shaken with hexane (3×50 mL) to remove the excess benzylacetylene. Ice cold water was added to the mixture to precipitate a mixture of **11a** and **11b** in the ratio 2:1 (total yield 77%). A pure sample of **11a** (120 mg) could be isolated from the regioisomeric mixture (600 mg) by flash column chromatography in silica with chloroform/methanol/ammonium acetate mixture (400:10:1) as eluent. Crystallisation from ethanol gave creamy needles, mp 122–123 °C; IR (KBr) ν_{max}/cm^{-1} 3381, 3149 (NH), 2972, 1601, 1551, 1431, 1040, 721; ^{1}H NMR δ 0.97 (3H, t, J = 7.5 Hz, CH_3), 2.15 (2H, q, J = 7.5 Hz, CH_2), 4.11 (2H, s, CH_2Ph), 5.80 (2H, br s, NH_2), 5.97 (2H, br s, NH_2), 7.20–7.74 (7H, m, Ar-H), 7.76 (1H, d, J = 8.2, Ar-H), 8.27 (1H, s, CH); ^{13}C NMR δ 13.2 (CH_3), 27.7 (CH_2), 31.2 (CH_2), 104.4 (C), 124.8 (CH), 126.4 (CH), 127.0 (C), 128.7 (C), 128.8 (C), 130.3 (CH), 131.1 (CH), 133.9 (CH), 135.1 (C), 136.9 (C), 139.5 (C), 146.2 (C), 162.1 (C), 162.5 (C), 166.7 (C); FAB$^+$-HRMS calcd for $C_{21}H_{21}N_7Cl$: 406.154697, 408.151747 $[M+H]^+$; found: 406.154751, 408.153182 $[M+H]^+$. The regioisomeric mixture could be prepared by heating azidopyrimethamine (**10**) (50 mg, 0.17 mmol) in benzylacetylene (0.5 mL) at 100 °C for 18 h in a sealed tube. After heating for ca. 2 h the off-white suspension went dark and subsequently formed a deep brown solution. The reaction mixture was then cooled to room temperature and washed with several portions of hexane (20 mL) until precipitation occurred. The yellow solid was filtered and characterised by ^{1}H NMR. The spectrum corresponds to a sample of that of the regioisomeric mixture of **11a** and **11b** in the ratio 2:1 (total yield 71%).

2,4-Diamino-5-[4-chloro-3-(4-phenyl-1,2,3-triazol-1-yl)-phenyl]-6-ethylpyrimidine (11c) and regioisomer (11d). Similarly prepared, from *m*-azidopyrimethamine[11] (**10**; 2.0 g, 6.9 mmol) in anhydrous DMF (15 mL) and phenylacetylene (3.53 g, 34.5 mmol; 5 mol equiv) at 100 °C for 48 h was a mixture of **11c** and **11d** (79%) in a ratio 3:2 (by ^{1}H NMR). A pure sample (190 mg) of **11c** could be isolated from the regioisomeric mixture (1.0 g) by flash column chromatography in silica with chloroform/methanol/ammonium acetate mixture (400:10:1) as eluent. Crystallisation of **11c** from acetone gave white needles, mp 174–176 °C; IR (KBr) ν_{max}/cm^{-1} 3395, 3324, 1601, 1553, 1433, 1239, 1099, 764; ^{1}H NMR δ 1.01 (3H, t, J = 7.5 Hz, CH_3), 2.20 (2H, q, J = 7.5 Hz, CH_2), 5.85 (2H, br s, NH_2), 5.99 (2H, br s, NH_2), 7.35–7.53 (5H, m, Ar–H), 7.55 (1H, d, J = 2.0, Ar–H), 7.80 (1H, d, J = 8.3, Ar–H_5), 7.80 (1H, d, J = 7.0, Ar–H), 8.99 (1H, s, CH); ^{13}C NMR δ 13.2 (CH_3), 27.6 (CH_2), 104.4 (C), 123.6 (CH), 125.6 (CH), 127.2 (C), 128.4 (CH), 129.2

(CH), 130.3 (C), 130.4 (CH), 131.2 (CH), 134.2 (C), 135.0 (C), 136.9 (C), 146.6 (C), 162.1 (C), 162.2 (C), 166.5 (C); ES^+-MS m/z 392, 394 $[M+H]^+$. Anal. calcd for $C_{20}H_{18}N_7Cl$ $0.65H_2O$: C, 59.53; H, 4.82; N, 24.29; found: C, 59.87; H, 4.58; N, 23.89%.

2,4-Diamino-5-[4-chloro-3-(4-trimethylsilyl-1,2,3-triazol-1-yl)phenyl]-6-ethylpyrimidine (11e). Similarly prepared, from *m*-azidopyrimethamine[11] (**10**; 2 g, 6.9 mmol) in anhydrous DMF (25 mL) and (trimethylsilyl)acetylene (2.03 g, 20.7 mmol, 3 mol equiv) in an inert atmosphere, the chalky pink solid was purified using flash column chromatography on silica with chloroform/methanol mixture as eluent (9:1). Crystallisation from ethanol afforded colourless needles (85%), mp 223–225 °C; IR (KBr) ν_{max}/cm^{-1} 3397, 3327, 3167 (NH), 1601, 1561, 1431, 1250, 843; ^1H NMR δ 0.33 (9H, s, $Si(CH_3)_3$), 1.02 (3H, t, $J=7.5$ Hz, CH_3), 2.17 (2H, q, $J=7.5$ Hz, CH_2), 5.78 (2H, br s, NH_2), 5.94 (2H, br s, NH_2), 7.39–7.43 (2H, m, Ar–H), 7.78 (1H, d, $J=8.2$ Hz, Ar–H), 8.52 (1H, s, CH); ^{13}C NMR δ −0.8 (CH_3), 13.2 (CH_3), 27.7 (CH_2), 79.4 (CH), 104.4 (CH), 127.0 (C), 130.3 (CH), 131.2 (CH), 132.5 (CH), 133.8 (CH), 135.1 (C), 136.8 (C), 145.1 (C), 162.1 (C), 162.5 (C), 166.8 (C); ES^+-MS m/z 388, 390 $[M+H]^+$. Anal. calcd for $C_{17}H_{22}N_7ClSi$ $0.2H_2O$ 0.1 EtOH: C, 52.15; H, 5.85; N, 24.75; found: C, 51.84; H, 5.64; N, 24.76%.

2,4-Diamino-5-[4-chloro-3-(1,2,3-triazol-1-yl)phenyl]-6-ethylpyrimidine (11g). (i) 2,4-Diamino-5-[4-chloro-3-(4-trimethylsilyl-1,2,3-triazol-1-yl)phenyl]-6-ethylpyrimidine (**11e**; 450 mg, 1.15 mmol) was dissolved in THF (5 mL) and tetrabutylammonium fluoride (TBAF; 0.47 mL 75% w/v, 1.1 mol equiv) was added dropwise. A fine precipitate was observed after ca. 3 h of stirring and the reaction mixture was stirred at 25 °C overnight. Excess solvent was removed under reduced pressure and the off-white solid obtained was suspended in boiling water and recovered by filtration. The triazole was crystallised from ethanol to give an off-white solid (74%), mp 243–244 °C; IR (KBr) ν_{max}/cm^{-1} 3426, 3320, 3163 (NH), 1634, 1557, 1439, 1235, 1099; ^1H NMR δ 0.99 (3H, t, $J=7.5$ Hz, CH_3), 2.16 (2H, q, $J=7.5$ Hz, CH_2), 5.80 (2H, br s, NH_2), 5.96 (2H, br s, NH_2), 7.39–7.45 (2H, m, Ar–H), 7.78 (1H, d, $J=8.1$ Hz, Ar–H), 7.98 (1H, s, CH), 8.51 (1H, s, CH); ^{13}C NMR δ 13.2 (CH_3), 27.7 (CH_2), 104.3 (C), 127.2 (CH), 130.4 (CH), 131.1 (CH), 133.6 (CH), 134.0 (CH), 135.0 (C), 137.0 (C), 162.1 (C), 162.5 (C), 166.8 (C); ES^+-MS m/z 316, 318 $[M+H]^+$. Anal. calcd for $C_{14}H_{14}N_7Cl$ $0.25H_2O$: C, 52.50; H, 4.56; N, 30.61; found: C, 52.72; H, 4.44; N, 30.92%.

(ii) The same triazole (39%) was isolated when *m*-azidopyrimidine was reacted with (tributylstannyl)acetylene (10 mol equiv) in DMF under an inert atmosphere at 110 °C for 4 days.

General procedure for the synthesis of compound (21a–f). To ice cold thionyl chloride (5 mL) was added the hydroxyalkyltriazene (**19**; 2.5 g, 5.9 mmol) portionwise over a period of 5 min. An amber suspension was formed and the reaction mixture was allowed to warm to room temperature slowly whereupon a solution was formed. The reaction mixture was stirred at room temperature overnight before the addition of anhydrous diethyl ether (20 mL). An orange coloured suspension was formed. The reaction mixture was stirred for a further 12 h and the orange solid was collected and washed well with diethyl ether under a stream of nitrogen. Compounds were dissolved in isopropanol and re-precipitated by addition of anhydrous cyclohexane. The cream solid triazolium and triazinium chloride salts were collected under an inert atmosphere. The following compounds were prepared.

1-Benzyl-3-(2-chlorophenyl)-4,5-dihydro-1,2,3-triazolium chloride hydrochloride (21a). From 3-benzyl-1-(2-chlorophenyl)-3-(2-hydroxyethyl)triazene (**19a**), yield (88%); IR (NaCl) ν_{max}/cm^{-1} 3407, 1633, 1510, 1485, 1256, 860, 764, 704; ^{1} NMR δ 4.63 (2H, t, $J=12.3$ Hz, NCH_2), 4.87 (2H, t, $J=12.3$ Hz, NCH_2), 4.47 (2H, s, CH_2Ph), 7.46–7.84 (9H, m, Ar–H); ^{13}C NMR δ 54.9 (CH_2), 55.0 (CH_2), 56.7 (CH_2), 127.0 (CH), 127.6 (C), 129.0 (CH), 129.3 (CH), 129.5 (CH), 129.9 (CH), 131.4 (C), 131.5 (CH), 132.2 (CH), 134.0 (C); EI^+-HRMS m/z 272.09593 (M); calcd for $C_{15}H_{15}N_3Cl$, 272.09546.

1-Benzyl-3-(2-chlorophenyl)-3,4,5,6-tetrahydro-1,2,3-triazinium chloride hydrochloride (21b). From 3-benzyl-1-(2-chlorophenyl)-3-(3-hydroxypropyl)triazene (**19b**), yield (73%); ^1H NMR δ 2.28 (2H, quintet, $J=5.5$ Hz, $CH_2CH_2CH_2$), 3.95 (2H, t, $J=5.5$ Hz, NCH_2), 4.16 (2H, t, $J=5.5$ Hz, NCH_2), 5.32 (2H, s, CH_2Ph), 7.31–7.90 (9H, m, Ar–H); ^{13}C NMR 14.3 (CH_2), δ 46.9 (CH_2), 49.0 (CH_2), 62.2 (CH_2), 128.5 (CH), 128.8 (C), 129.1 (CH), 129.3 (CH), 129.4 (CH), 130.4 (CH), 131.0 (C), 132.1 (CH), 132.5 (CH), 139.8 (C); FAB^+-HRMS matrix NBA m/z 286.111458 (M); calcd for $C_{16}H_{17}N_3Cl$, 286.111100.

1-Benzyl-3-[2-chloro-5-(2,4-diamino-6-ethylpyrimidin-5-yl)]phenyl-4,5-dihydro-1,2,3-triazolium chloride hydrochloride (21c). From 2,4-diamino-5-{3-[3-benzyl-3-(2-hydroxyethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (**19c**),[2] yield 82%; mp 178 °C (efferv); IR (KBr) ν_{max}/cm^{-1} 3324, 3156, 1655, 1508, 1456, 1252), 1045, 704; ^1H NMR δ 1.09 (3H, t, CH_3), 2.26 (2H, q, CH_2), 4.60 (2H, t, $J=12.5$ Hz, NCH_2), 4.77 (2H, t, $J=12.5$ Hz, NCH_2) 5.43 (2H, s, CH_2Ph), 7.09 (1H, s, NH, exch D_2O), 7.46–7.88 (m, 8H, Ar–H), 8.31 (1H, br s, NH, exch D_2O), 13.22 (1H, br s, NH, exch D_2O); ^{13}C NMR δ 12.6 (CH_3), 24.0 (CH_2), 55.1 (CH_2), 55.2 (CH_2), 56.9 (CH_2), 106.1 (C), 128.2 (C), 128.8 (CH), 129.3 (CH), 129.6 (CH), 130.0 (CH), 130.6 (CH), 131.3 (C), 132.1 (C), 132.5 (CH), 134.3 (CH), 134.9 (C), 154.7 (C), 155.4 (C), 163.9 (C); ES^+-MS m/z 408, 410 $[M-H]^+$; FAB^+-HRMS calcd for $C_{21}H_{24}N_7Cl$: 408.170347, 410.167397 $[M-H]^+$; found: 408.171020, 410.169142 $[M-H]^+$.

1-Benzyl-3-[2-chloro-5-(2,4-diamino-6-ethylpyrimidine-5-yl)]phenyl-3,4,5,6-tetrahydro-1,2,3-triazinium chloride (21d). From 2,4-diamino-5-{3-[3-benzyl-3-(3-hydroxypropyl)-triazene-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (**19d**),[3] yield 77%; mp 178 °C (decomp); IR (KBr) ν_{max}/cm^{-1} 3310, 3146 (N–H), 1657, 1535, 1481, 1290, 1194,

700; ^{1}H NMR δ 1.09 (3H, t, J = 7.5 Hz, CH_3), 2.28 (4H, m, CH_2CH_3 and $CH_2CH_2CH_2$), 3.95 (2H, br s, NCH_2), 4.25 (2H, br s, NCH_2), 7.15 (1H, br s, NH_2), 7.44–7.94 (m, 8H, Ar–H), 8.27 (1H, br s, NH, exch D_2O), 13.3 (1H, br s, NH); ^{13}C NMR δ 12.3 (CH_3), 14.3 (CH_2), 24.0 (CH_2), 47.2 (CH_2), 49.2 (CH_2), 64.3 (CH_2), 106.0 (C), 128.7 (CH), 129.2 (C), 129.3 (CH), 129.4 (CH), 129.5 (CH), 130.5 (CH), 130.6 (CH), 132.0 (CH), 132.1 (C), 132.2 (C), 134.6 (CH), 140.4(C), 154.5(C), 155.4(C), 163.9 (C); FAB$^+$-HRMS calcd for $C_{22}H_{25}N_7Cl$: 422.185997, 424.183047 $[M-H]^+$; found: 422.187354, 424.184595 $[M-H]^+$.

***S*-(+)-1-(α-Methylbenzyl)-3-[2-chloro-5-(2,4-diamino-6-ethylpyrimidin-5-yl)]phenyl-3,4-dihydro-1,2,3-triazolium chloride hydrochloride (21e).** From *S*-(+)-2,4-diamino-5-{3-[3-(2-hydroxyethyl)-3-(α-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (**19e**),[3] yield 93%; mp 132 °C (efferv); $[\alpha]_D^{28.0}$ 56.0 ° (c 0.804 mg mL^{-1}, EtOH); IR (KBr)ν_{max}/cm^{-1} 3341, 3169, 1655, 1485, 1452, 1240, 702; ^{1}H NMR δ 1.11 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.83 (3H, d, J = 7.5 Hz, $CHCH_3$), 2.28 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.40–4.88 (4H, m, 2×CH_2), 5.63 (1H, q, J = 7.5 Hz, $CHCH_3$), 7.11 (1H, br s, NH, exch D_2O), 7.45–7.90 (m, 8H, Ar–H), 8.32 (1H, br s, NH, exch D_2O), 13.36 (1H, br s, NH, exch D_2O); ^{13}C NMR δ 12.5 (CH_3), 19.8 (CH_3), 24.0 (CH_2), 54.2 (CH_2), 54.9 (CH_2), 63.6 (CH), 106.0 (C), 127.8 (CH), 128.9 (C), 129.4 (2×CH), 132.0 (C), 132.5 (CH), 134.0 (CH), 134.9 (C), 136.6 (C), 154.5 (C), 155.4 (C), 163.8 (C); FAB$^+$-HRMS calcd for $C_{22}H_{25}N_7Cl$: 422.185997, 424.183047 $[M-H]^+$; Found: 422.187316, 424.184664 $[M-H]^+$.

***R*-(−)-3-(α-Methylbenzyl)-1-[2-chloro-5-(2,4-diamino-6-ethylpyrimidin-5-yl)]phenyl-3,4-dihydro-1,2,3-triazolium chloride hydrochloride (21f).** From *R*-(−)-2,4-diamino-5-{3-[3-(2-hydroxyethyl)-3-(α-methylbenzyl)triazen-1-yl]-4-chloro-phenyl}-6-ethylpyrimidine (**19f**),[3] yield 89%; mp 122 °C (efferv); $[\alpha]_D^{27.8}$ −53.4° (c 0.562 mg mL^{-1}, EtOH); IR (KBr) ν_{max}/cm^{-1} 3324, 3167, 1657, 1485, 1452, 1246, 704; ^{1}H NMR δ 1.11 (3H, t, J = 7.5 Hz CH_2CH_3), 1.83 (3H, d, J = 7.5 Hz, $CHCH_3$), 2.31 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.39–4.90 (m, 4H, 2×CH_2), 5.68 (1H, q, J = 7.5 Hz, $CHCH_3$), 7.13 (1H, br s, NH, exch D_2O), 7.44–7.92 (m, 8H, Ar–H), 8.28 (1H, br s, NH, exch D_2O), 13.36 (1H, br s, NH, exch D_2O); ^{13}C NMR δ 12.5 (CH_3), 19.8 (CH_3), 24.0 (CH_2), 54.3 (CH_2), 54.8 (CH_2), 63.7 (CH), 106.0 (C), 127.8 (CH), 128.8 (CH), 129.4 (CH), 129.5 (CH), 132.0 (C), 132.5 (CH), 134.0 (CH), 134.9 (C), 136.5 (C), 154.5 (C), 155.4 (C), 163.8 (C); FAB$^+$-HRMS calcd for $C_{22}H_{25}N_7Cl$: 422.185997, 424.183047 $[M-H]^+$; found: 422.186106, 424.184254 $[M-H]^+$.

2,4-Diamino-5-(3-benzoylamino-4-chlorophenyl)-6-ethylpyrimidine (23a). A mixture of aminopyrimethamine (**22**)[11] (2.64 g, 10 mmol), benzoyl chloride (2 mL, 17 mmol), and pyridine (15 mL) was heated at 95 °C for 3 h and then cooled to room temperature. The reaction mixture was quenched with ice-ammonia and the resultant precipitate was collected. The recovered solid was washed successively with hexane and water and crystallised from ethanol gave the benzoylamino-pyrimidine (**23a**), 3.53 g (95%); mp 164–165 °C; IR (KBr) ν_{max}/cm^{-1} 3385, 3169 (NH), 1647 (C=O), 1574, 1439, 1406, 1283, 706; ^{1}H NMR δ 0.99 (3H, t, J = 7.5 Hz, CH_3), 2.15 (2H, q, J = 7.5 Hz, CH_2), 5.64 (2H, br s, NH_2), 5.96 (2H, br s, NH_2), 7.10 (1H, dd, J = 2.5, 7.5 Hz, Ar–H), 7.47 (1H, d, J = 2.5 Hz, Ar–H), 7.51–7.68 (4H, m, Ar–H), 7.99 (2H, d, J = 7.5 Hz, Ar–H), 10.11 (1H, br s, NH); ^{13}C NMR δ 13.4 (CH_3), 27.7 (CH_2), 105.3 (CH), 127.7 (C), 128.7 (CH), 129.6 (CH), 129.8 (CH), 130.3 (CH), 132.1 (CH), 134.2 (C), 135.5 (C), 135.6 (C), 162.1 (C), 162.3 (C), 165.6 (C), 166.8 (C); ES$^+$-MS m/z 368, 370 $[M+H]^+$. Anal. calcd for $C_{19}H_{18}N_5OCl$ 0.3 EtOH 0.2H_2O: C, 61.11; H, 5.28; N, 18.18; found: C, 60.94; H, 4.99; N, 18.11%.

2,4-Diamino-5-[3-(3,4,5-trimethoxybenzoyl)amino-4-chlorophenyl]-6-ethylpyrimidine (23b). Prepared from **22** and 3,4,5-trimethoxybenzoyl chloride in pyridine (35 mL) as above, the trimethoxybenzoylamino-pyrimidine (14%) had mp 265–267 °C (decomp) (from ethanol); IR (KBr) ν_{max}/cm^{-1} 3434, 3179 (N–H), 1630, 1574, 1503, 1335, 1120, 997; ^{1}H NMR δ 0.95 (3H, t, J 7.5 Hz, CH_3), 2.18 (2H, q, J = 7.5 Hz, CH_2), 3.73 (3H, s, OCH_3), 3.86 (6H, s, 2×OCH_3), 5.60 (2H, br s, NH_2), 5.95 (2H, br s, NH_2), 7.11 (1H, dd, J = 2.5, 7.5 Hz, Ar–H), 7.33 (2H, s, Ar–H), 7.37 (1H, d, J = 2.5 Hz, Ar–H), 7.64 (1H, d, J = 7.5 Hz, Ar–H), 10.03 (1H, s, NH); ^{13}C NMR δ 13.3 (CH_3), 27.7 (CH_2), 56.2 (CH_3), 60.3 (CH_3), 105.2 (C), 105.5 (CH), 128.1 (C), 129.3 (C), 129.8 (CH), 130.3 (CH), 135.5 (C), 135.6 (C), 140.7 (C), 152.7 (C), 162.1 (C), 162.4 (C), 165.0 (C), 166.8 (C); ES$^+$-MS m/z 458, 460 $[M+H]^+$. Anal. calcd for $C_{22}H_{24}N_5O_4Cl$ 0.2H_2O 0.2 EtOH: C, 57.15; H, 5.48; N, 14.88; found: C, 57.15; H, 5.16; N, 14.85%.

2,4-Diamino-5-{4-chloro-3-[*N*-(benzyl)amino]phenyl}-6-ethylpyrimidine (24a). (i) Aminopyrimethamine (**22**) (1.32 g, 5 mmol) was suspended in isopropanol (30 mL) and benzyl bromide (1.71 g, 10 mmol) was added. The mixture was refluxed for 36 h and upon cooling, an off-white precipitate formed. The product was collected, stirred with aqueous ammonia to furnish the free base. Flash column chromatography (dichloromethane/methanol 10:1) followed by crystallisation from acetone gave the benzylamine (**24a**) (66%); R_f (chloroform/methanol 5:1) 0.71; mp 180–182 °C; IR (KBr)ν_{max}/cm^{-1} 3454, 3318, 3162 (NH), 1630, 1570, 1433, 1030, 690; ^{1}H NMR δ 0.72 (3H, t, J = 7.5 Hz, CH_3), 1.90 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.42 (2H, dd, J = 6.6 Hz, CH_2Ph), 5.61 (2H, br s, NH_2), 5.90 (2H, br s, NH_2), 6.19 (1H, t, J = 6.1 Hz, NH), 6.25 (1H, s, Ar–H), 6.34 (1H, dd, J = 1.3, 6.8 Hz, Ar–H), 7.15–7.32 (6H, m, Ar–H); ^{13}C NMR δ 13.2 (CH_2), 27.4 (CH_2), 46.2 (CH_2), 106.4 (C), 144.2 (CH), 117.0 (C), 118.7 (CH), 126.8 (2×CH), 128.5 (CH), 129.5 (CH), 135.7 (C), 39.7 (C), 144.2 (C), 162.0 (C), 166.5 (C); ES$^+$-MS m/z 354, 356 $[M+H]^+$; EI$^+$-HRMS calcd for $C_{19}H_{21}N_5Cl$: 353.14072; found: 353.14047 $[M+H]^+$.

(ii) The benzylamine (**24a**) could also be prepared (59%) by treating aminopyrimethamine (0.66 g, 2.5 mmol) with benzyl chloride (0.86 mL, 3 mol equiv) in ethanol (4 mL) for 24 h.

2,4-Diamino-5-{4-chloro-3-[*N*-(3,4,5-trimethoxybenzyl)-amino]phenyl}-6-ethylpyrim-idine (24b). Similarly prepared (above) from **22** (2.64 g, 10 mmol) and 3,4,5-trimethoxybenzyl bromide (3.2 g, 20 mmol) in refluxing isopropanol (60 mL) (5 days). Flash column chromatography (dichloromethane/methanol 10:1) followed by crystallisation from acetone gave the pyrimidine as a white solid (12%); mp 93–94 °C; R_f (chloroform/methanol 5:1) 0.49; IR (KBr) ν_{max}/cm^{-1} 3395, 3160 (NH), 2936, 1593, 1460, 1414, 1120, 810; ^1H NMR δ 0.73 (3H, t, $J=7.5$ Hz, CH_3), 1.90 (2H, q, $J=7.5$ Hz, CH_2), 3.59 (3H, s, OCH_3), 3.68 (6H, s, $2\times OCH_3$), 4.26–4.37 (2H, m, CH_2Ph), 5.49 (2H, br s, NH_2), 5.82 (2H, br s, NH_2), 6.11 (1H, t, $J=6.3$ Hz, NH), 6.32–6.38 (2H, m, Ar–H), 6.62 (2H, s, Ar–H), 7.28 (1H, d, $J=7.8$ Hz, Ar–H); ^{13}C NMR δ 13.1 (CH_3), 27.4 (CH_2), 46.4 (CH_2), 55.9 (CH_3), 60.0 (CH_3), 104.0 (CH), 106.5 (C), 114.4 (CH), 117.0 (C), 118.8 (CH), 129.5 (CH), 135.4 (C), 135.7 (C), 136.3 (C), 144.3 (C), 153.1 (C), 162.0 (C), 162.1 (C), 166.3 (C); ES^+-MS m/z 444, 446 $[M+H]^+$. Anal. calcd for $C_{22}H_{26}N_5O_3Cl{\cdot}0.1(CH_2)_3CO{\cdot}0.4H_2O$: C, 58.62; H, 6.04; N, 15.32; found: C, 58.94; H, 5.80; N, 15.40.

2,4-Diamino-5-{3-[*N*-(benzyl)-*N*-methylamino]-4-chlorophenyl}-6-ethylpyrimidine (24c). To benzylamino-pyrimethamine (**24a**) (500 mg, 1.4 mmol) suspended in acetonitrile (100 mL) was added formaldehyde (47 mg, 1.54 mmol, 1.1 mol equiv) with constant stirring. Sodium cyanoborohydride (150 mg, 2.4 mmol) was added followed by the dropwise addition of concentrated hydrochloric acid until the pH of the reaction mixture reached 2–3. After 30 min solvents were removed by vacuum evaporation. The residue was basified with aqueous ammonia solution, collected and crystallised from ethanol to yield **24c** (90%); mp 121–122 °C; IR (KBr) ν_{max}/cm^{-1} 3453, 3310, 3152 (NH), 1624, 1557, 1435, 1107, 698; ^1H NMR δ 0.90 (3H, t, $J=7.5$ Hz, CH_2CH_3), 2.02 (2H, q, $J=7.5$ Hz, CH_2CH_3), 2.63 (3H, s, NCH_3), 4.22 (2H, m, CH_2Ph), 5.73 (2H, br s, NH_2), 6.04 (2H, br s, NH_2), 6.81 (2H, m, Ar–H), 7.20–7.48 (6H, m, Ar–H); ^{13}C NMR δ 13.2 (CH_3), 27.2 (CH_2), 40.1 (CH_3), 58.8 (CH_2), 105.9 (CH), 124.5 (CH), 125.9 (CH), 126.8 (C), 127.2 (CH), 128.4 ($2\times$CH), 131.0 (CH), 135.4 (C), 138.0 (C), 149.3 (C), 161.5 (C), 162.3 (C), 165.5 (C); ES^+-MS m/z 368, 370 $[M+H]^+$. Anal. calcd for $C_{20}H_{22}N_5OCl$ 0.3 EtOH $0.2H_2O$: C, 64.06; H, 6.27; N, 18.31; found: C, 64.00; H, 6.12; N, 18.18%.

2,4-Diamino-5-{4-chloro-3-[*N*-(3,4,5-trimethoxybenzyl)-*N*-methylamino]phenyl}-6-ethylpyrimidine (24d). Similarly prepared (above) from **24b**, formaldehyde, sodium cyanoborohydride in acetonitrile. The product was crystallised from acetone to give **24d** (93%); mp 161–162 °C; IR (KBr) ν_{max}/cm^{-1} 3455, 3169 (NH), 1632, 1553, 1447, 1362, 1233, 1125; ^1H NMR δ 0.87 (3H, t, $J=7.5$ Hz, CH_2CH_3), 1.98 (2H, q, $J=7.5$ Hz, CH_2CH_3), 2.67 (3H, s, NCH_3), 3.61 (3H, s, OCH_3), 3.70 (6H, s, $2\times OCH_3$), 4.17 (2H, d, CH_2Ph), 5.54 (2H, br s, NH_2), 5.87 (2H, br s, NH_2), 6.64 (2H, s, Ar–H), 6.82 (1H, dd, J 2.0 6.1 Hz, Ar–H), 6.87 (1H, d, $J=1.9$ Hz, Ar–H), 7.46 (1H, d, $J=4.0$ Hz, Ar–H); ^{13}C NMR δ 13.1 (CH_3), 27.5 (CH_2), 30.8 (CH_3), 55.8 (CH_2), 58.7 (CH_3), 60.0 (CH_3), 105.3 (CH), 105.7 (C), 124.5 (CH), 125.9 (CH), 126.7 (C), 130.8 (CH), 133.8 (C), 135.9 (C), 136.5 (C), 149.2 (C), 152.9 (C), 162.0 (C), 162.2 (C), 166.7 (C); ES^+-MS m/z 458, 460 $[M+H]^+$. Anal. calcd for $C_{23}H_{28}N_5O_3Cl$ $(CH_3)_2CO$: C, 60.52; H, 6.64; N, 13.57; found: C, 60.11; H, 6.42; N, 13.81.

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