Structural Studies on Bioactive Compounds. 34.[1] Design, Synthesis, and Biological Evaluation of Triazenyl-Substituted Pyrimethamine Inhibitors of *Pneumocystis carinii* Dihydrofolate Reductase

David C. M. Chan,[†] Charles A. Laughton,[†] Sherry F. Queener,[‡] and Malcolm F. G. Stevens*[,†]

Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, U.K., and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202

Received March 7, 2001

The triazenyl-pyrimethamine derivative **3a** (TAB), a potent and selective inhibitor of *Pneumocystis carinii* DHFR, was selected as the starting point for a lead optimization study. Molecular modeling studies, corroborated by a recent crystal structure determination of the ternary complex of *P. carinii* DHFR−NADPH bound to TAB, predicted that modifications to the acetoxy residue of the lead inhibitor could exploit binding opportunities in the vicinity of an active site pocket bounded by residues Ile33, Lys37, and Leu72. Substitutions in the benzyl moiety with electron-donating and electron-withdrawing groups were predicted to probe face−edge interactions with amino acid Phe69 unique to the *P. carinii* enzyme. New triazenes **10a**−**v** and **12a**−**f** were prepared by coupling the diazonium tetrafluoroborate salt **6b** of aminopyrimethamine with substituted benzylamines or phenethylamines. The most potent of the new inhibitors against *P. carinii* DHFR was the naphthylmethyl-substituted triazene **10t** (IC_{50}: 0.053 μM), but a more substantial increase in potency against the rat liver DHFR led to a reduction in selectivity (ratio rat liver DHFR IC_{50}/*P. carinii* DHFR IC_{50}: 5.36) compared to the original lead structure **3a** (ratio rat liver DHFR IC_{50}/*P. carinii* DHFR IC_{50}: 114).

Introduction

Pneumocystis carinii (PC) is an ubiquitous microorganism that causes life-threatening pneumonia (PCP) in individuals with severe immunodeficiency. In the early stages of the AIDS epidemic, up to 75% of individuals infected with HIV developed PCP. At the end of 1998, the number of individuals carrying HIV had soared to 33.4 million with 6 million new infections reported every year.[2]

The dihydrofolate reductase (DHFR) inhibitor trimethoprim **1** (Figure 1) is a first-line therapy for PCP but its moderate potency and poor species selectivity toward *P. carinii* DHFR render it effective only in combination with the sulfa drug sulfamethoxazole. Many patients are unable to tolerate the combined therapy due to life-threatening adverse drug reactions.[3] Recently, trimetrexate **2**, a potent lipophilic quinazoline inhibitor of both human and *P. carinii* DHFR, has been shown to be an effective but toxic alternative treatment, requiring expensive concomitant leucovorin rescue therapy to counter the antifolate effects on the host.[4] Despite enormous efforts to improve existing compounds from various research groups, the goal of identifying an agent possessing high potency and species selectivity for the clinical management of PCP remains unfulfilled.

In addition to *P. carinii*, a coccidian protozoan *Toxoplasma gondii* causes an opportunistic infection associated with AIDS[5] which can lead to encephalitis,[6] and treatment regimes for *T. gondii* infections are similar to those for PCP.

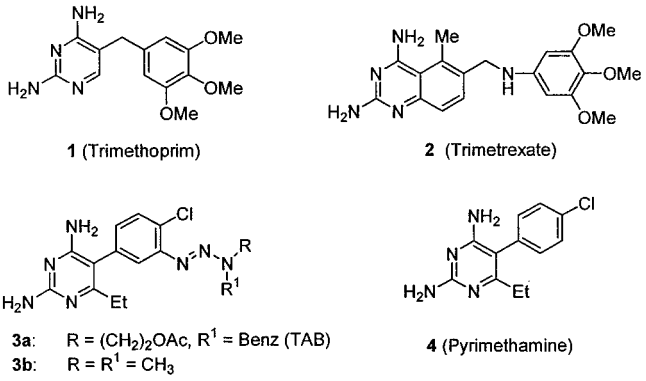


Figure 1. Structures of inhibitors of *Pneumocystis carinii* dihydrofolate reductase.

In 1997 we reported the exquisite species selectivity (ratio rat liver DHFR IC_{50}/*P. carinii* DHFR IC_{50}: 114) displayed by a triazenyl-pyrimethamine derivative (TAB; **3a**); this compound was also selectively toxic to the *T. gondii* DHFR enzyme (ratio rat liver IC_{50}/*T. gondii* IC_{50}: 28).[7] This agent emerged from an analogue development program based on the dimethyltriazene **3b** with modest species selectivity (ratio rat liver IC_{50}/*P. carinii* IC_{50}: 6.75). Although the IC_{50} value for **3a** of 0.17 μM toward the *P. carinii* enzyme is an order of magnitude less than that of the currently used agent trimetrexate **2** (IC_{50} 0.042 μM), there are no more potent PC DHFR-inhibitory chemical entities (i.e., IC_{50} value < 1 μM) reported in the literature possessing a better species selectivity profile than that exhibited by TAB.[7] In a search for more potent and selective *P. carinii* DHFR inhibitors than the existing compounds, we have exploited new structural insights developed from molecular modeling and crystallographic studies to guide

* Tel: (115) 951 3414. Fax: (115) 951 3412. E-mail: malcolm.stevens@nottingham.ac.uk.
[†] University of Nottingham, Nottingham.
[‡] Indiana University School of Medicine.

10.1021/jm0108698 CCC: $20.00
Published on Web 06/30/2001

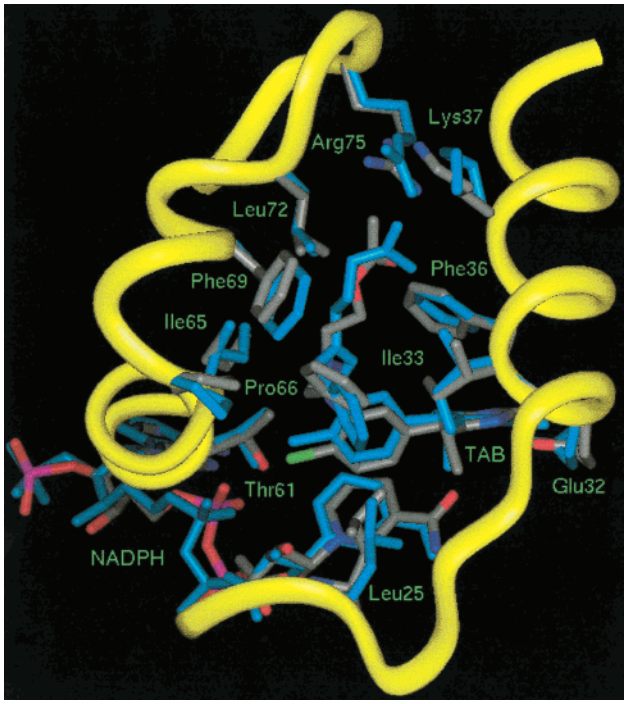


Figure 2. Comparison of the modeled (colored gray) and X-ray derived structures[10] (colored blue) of the *P. carinii* DHFR-TAB-NADPH ternary complex. Backbone C_α atoms are shown as the yellow ribbon. Side chains from the modeled structure are depicted colored by atom type.

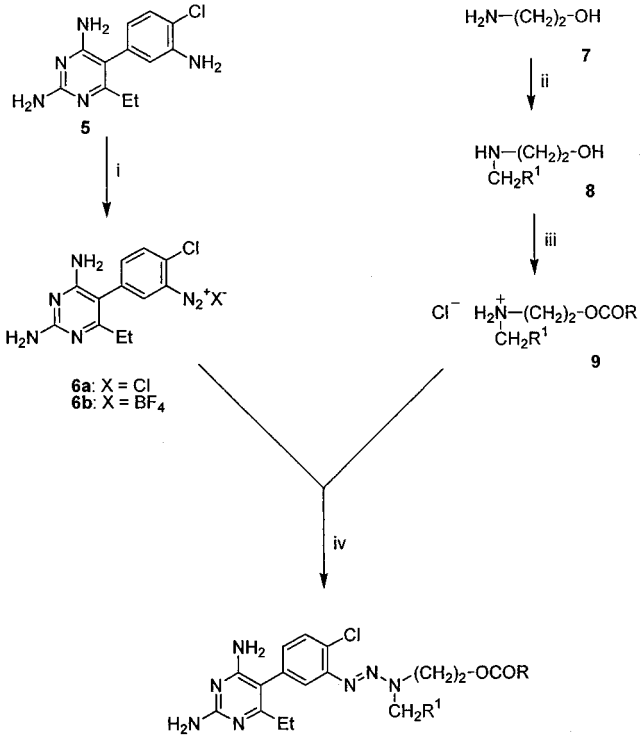
a program of further lead optimization based on the structure of TAB **3a**.

Molecular Modeling

The design process commenced with a manual docking study of TAB bound to the *P. carinii* DHFR utilizing the only X-ray determined coordinates of *P. carinii* DHFR available at the time this program was initiated.[8] The benzyl function of TAB was predicted to form a face−edge aromatic−aromatic interaction with the active site residue Phe69. Such an interaction, which is a common phenomenon in proteins and protein−ligand complexes, requires in this case the rotation of the N(2)−N(3) triazenyl bond by 47° from the planar conformation. This simultaneously steers the acetoxy branch of TAB toward the side chain of Lys37 which is located on the surface of the active site cleft. Phe69 and Lys37 are two of the most significant points of difference between the human and *P. carinii* DHFR active sites. In a hydrophobic reversal, *P. carinii* Phe69 is replaced by Asn64 in the human variant; also Lys37 (*P. carinii*) is substituted with Gln35 (human). Favorable interactions with Phe69 have been implicated as the basis of species selectivity of other *P. carinii* DHFR inhibitors of the 2,4-diamino-5-substituted furo[2,3-*d*]pyrimidine class.[9]

The validity of the manual docking model was confirmed unequivocally by our subsequent crystal structure determination of the *P. carinii* DHFR-TAB-NADPH ternary complex.[10] Least-squares superimposition of the two sets of Cα atoms gave an RMS deviation of 0.94 Å (Figure 2). The modeled structure (colored gray) predicted accurately the orientations of several important active site residues (Glu32, Phe36, Phe69, and Leu72) and particularly the crucial dispositions of the benzyl and acetoxyethyl substituents of the flexible TAB ligand.

Scheme 1[a]



	R	R^1		R	R^1
a:	Et	Ph	**l:**	Me	C_6H_4Cl-3
b:	^{i}Pr	Ph	**m:**	Me	C_6H_4Cl-4
c:	Ph	Ph	**n:**	Me	C_6H_4Me-2
d:	Me	C_6H_4OMe-4	**o:**	Me	C_6H_4Me-3
e:	Me	$C_6H_3(OMe)_2$-3,4	**p:**	Me	C_6H_4Me-4
f:	Me	$C_6H_3(OMe)_2$-3,5	**q:**	Me	$C_6H_4CF_3$-4
g:	Me	$C_6H_2(OMe)_3$-3,4,5	**r:**	Me	$C_6H_4NO_2$-3
h:	Me	C_6H_4F-2	**s:**	Me	$C_6H_4NO_2$-4
i:	Me	C_6H_4F-3	**t:**	Me	naphth-1-yl
j:	Me	C_6H_4F-4	**u:**	Me	naphth-2-yl
k:	Me	C_6H_4Cl-2	**v:**	Me	pyridin-4-yl

[a] Reagents: (i) 3 M HCl/$NaNO_2$ (**6a**) or HBF_4/$NaNO_2$ (**6b**); (ii) R^1CHO/MeOH/$NaBH_4$; (iii) RCO_2H/HCl or RCOCl/HCl; (iv) aq Na_2CO_3/0 °C.

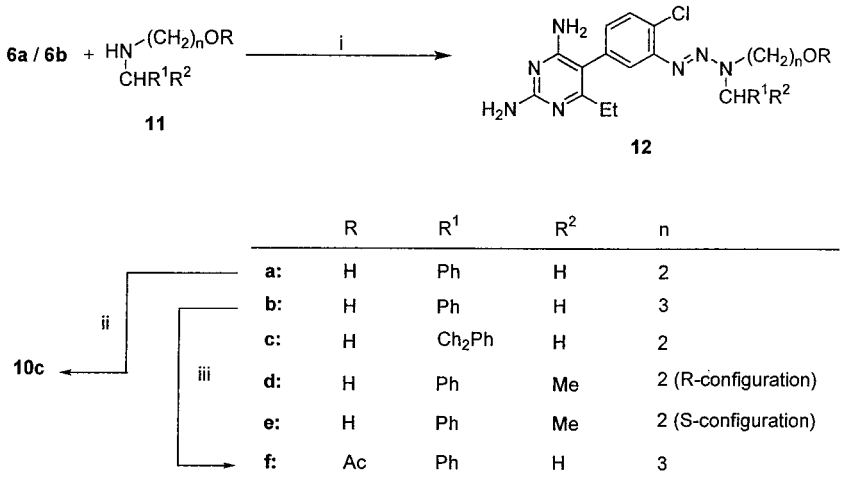
We anticipated that, by modifying the alkyl branches attached to the terminal triazenyl N(3) atom in a new cycle of synthesis/evaluation, a structure with enhanced potency and selectivity toward the *P. carinii* DHFR enzyme might be discovered.

In this work we have concentrated on two strategies: (i) exploring binding opportunities in the vicinity of a potential binding pocket delineated by Ile33, Lys37, and Leu72 through new variants at the acetoxy group of TAB and (ii) probing the proposed face−edge interaction of the benzyl group of TAB with Phe69 by incorporating electron-donating and electron-withdrawing substitutents in the benzyl moiety.

Chemistry

Aminopyrimethamine **5**, available by reduction of the precursor nitro compound,[11] was the starting material for the synthesis of new triazenyl-pyrimethamine derivatives **10** (Scheme 1). Diazotization of **5** in aqueous HCl gave solutions of the diazonium chloride **6a** suitable for coupling reactions, but in practice it proved to be more efficient to prepare (and store at 4 °C) the diazonium tetrafluoroborate **6b** as a stable hydrotet-

Scheme 2[a]



[a] Reagents: (i) aq Na_2CO_3/0 °C; (ii) BzCl/Pyridine/DMAP; (iii) AcOH/HCl.

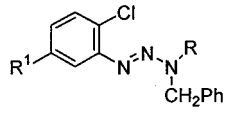
rafluoroborate salt.[12] N-Substituted ethanolamines **8** were usually synthesized from ethanolamine **7** by initial formation of a Schiff base with appropriate benzaldehydes followed by sodium borohydride reduction.[13] Alternatively, the nitrobenzyl-ethanolamines **8r**,**s** were prepared by direct alkylation of ethanolamine with the appropriate nitrobenzyl chlorides, compound **11c** from ethanolamine and 2-phenethyl chloride, and the chiral ethanolamines **11d**,**e** from the alkylation of *R*-(+)-α-methylbenzylamine and *S*-(−)-α-methylbenzylamine, respectively, with 2-chloroethanol.

Esterification of secondary amines **8** was achieved either by treatment with a carboxylic acid-HCl gas, or by interaction with an acid chloride. The water-soluble hydrochloride salts **9** were coupled with diazonium salts **6** in an aqueous sodium carbonate medium at 0 °C to afford the required triazenes **10** in high yields.

Further variations in substituents at the triazene N(3) position **12** were obtained by coupling diazonium salts **6** with the free bases of *N*-benzylethanolamine **11a**, *N*-benzylpropanolamine **11b**, *N*-phenethylethanolamine **11c**, and the *N*-hydroxyethyl derivatives of (*R*)- and (*S*)-α-methylbenzylamines **11d**,**e** (Scheme 2). Esterification of **12a** with benzoyl chloride-pyridine-DMAP gave the benzoate **10c**. Because of difficulties experienced in synthesizing the benzoate salt **9c** required for the direct coupling method (Scheme 1), this was the preferred route to **10c**. Esterification of the triazenyl-substituted *N*-benzylpropanolamine **12b** with acetic acid-HCl gas afforded the acetate **12f**.

The triazenes **10a**−**v** and **12b**−**f** were purified either by flash chromatography ($CHCl_3$−MeOH, 10:1) or by crystallization from acetone or ethanol, and they were isolated as stable solids which effervesced vigorously (−N_2) at their melting points. In common with many other diamino-pyrimidines,[11,12] several compounds gave unsatisfactory elemental analyses because of tenacious entrapment of solvent. However, an analytically pure sample of the monoethanesulfonic acid salt of **3a** was prepared (98%) from the free base and ethanesulfonic acid (1 mol equiv) in ice-cold propanol-2-ol. This salt was surprisingly stable, being undegraded in boiling ethanol after 2 weeks.

In all cases, triazenes were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy and MS. The ^{1}H chemical shifts can be compared directly with those of related first generation compounds,[7] simple 3-alkyl-3-benzyltriazenes of the triazenyl-pyrimethamine series **13a**,**b,** and model reference triazenes **13c**−**e** prepared from diazotized 2-chloroaniline. Although the terminal nitrogen atom in 1-aryl-3,3-dialkyltriazenes is (formally) tetrahedral, X-ray crystal studies have shown it has sp^2 character and the terminal N−N linkage is intermediate in length between a single and a double bond.[14] This leads to restricted rotation about the N(2)−N(3) triazenyl bond and significant temperature-dependent line broadening of the alkyl protons in 3,3-dialkyltriazenes is often observed.[15,16] Thus the ^{1}H NMR spectrum of **13a** in DMSO-d_6 at 289 K shows two broadened singlets for the methyl protons at δ 3.13 and 3.52 whereas the methylene absorption of the benzyl group is a slightly broadened singlet at δ 5.01. However, in the ^{1}H NMR spectrum of the simple model triazene **13c**, only one sharp methyl resonance is observed, indicating that N−N rotation is too fast to be observed by this technique at ambient temperature. The methylene signals in the ^{1}H NMR spectrum of TAB free base (**3a**; Figure 3) compare closely with those of the model compound **13e**; the methylene absorptions of the acetoxyethyl group of **3a** appear as three multiplets between δ 3.91 and 4.28 (δ 3.91−4.30 for **13e**).



	R	R^1
a:	Me	2,4-diamino-6-ethylpyrimidin-5-yl
b:	Et	2,4-diamino-6-ethylpyrimidin-5-yl
c:	Me	H
d:	Et	H
e:	$(CH_2)_2OAc$	H

Biological Results

The assay methods used in this study were as previously described.[17−19] Although a full sequence of

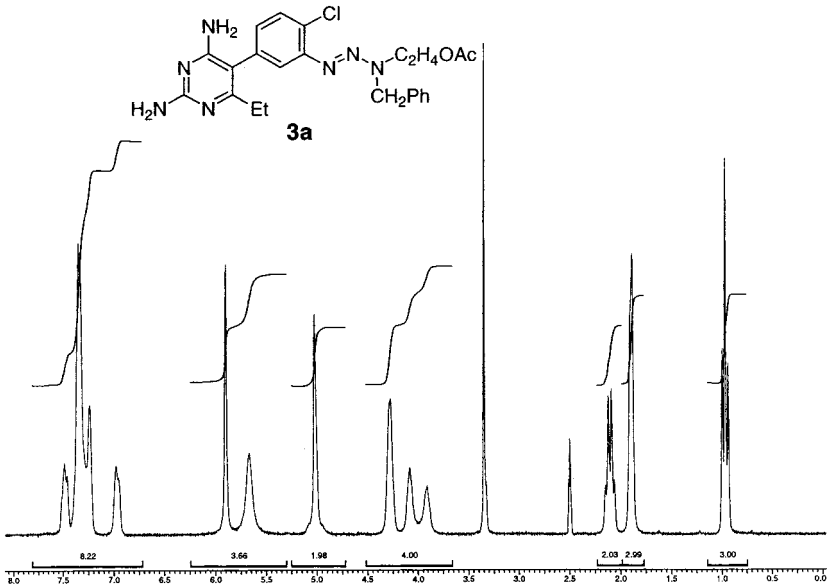


Figure 3. ^{1}H NMR spectrum of compound **13a** in DMSO-d_6 at 289 K. The methylene protons of the benzyl group are at δ 5.01, and the methylene protons of the acetoxyethyl group are between δ 3.91 and 4.28. Numbers above the chemical shift (δ) scale refer to integral ratios.

the rat liver DHFR has never been published, it has been accepted as a reasonable in vitro model for the human DHFR.[20] The ability of the triazenyl-substituted pyrimethamine derivatives of general structure **10** and **12** to inhibit *P. carinii* and rat liver DHFR was compared with that of the lead triazene TAB **3a** and other reference compounds. These results are presented as inhibitory concentrations (IC_{50}) in Table 1.

The three compounds **10a**−**c** were designed to explore the bulk tolerance of the pocket created by the *P. carinii* enzyme residues Lys37, Leu72, and Arg75. Leaving the benzyl substituent constant, propionyl, isobutyryl, and benzoyl moieties replace the acetyl function of **3a**. Compared to TAB these modifications resulted in a 5- to 8-fold decrease in activity toward *P. carinii* DHFR. Similarly, the hydroxyalkyltriazenes **12a**−**e** were less potent as inhibitors of the *P. carinii* enzyme; increasing the length of the hydroxyalkyl chain from methylene **12a** to propylene **12b** also led to a reduction in potency. As all the aforementioned compounds were marginally more potent against the mammalian enzyme than TAB, this conjunction of activities reduced their selectivity (rat liver/*P. carinii* (rl/pc)) ratio in comparison to the starting structure **3a**.

Methoxy groups were introduced into the phenyl substituent of **3a** in the expectation that these bulky functions would better occupy the hydrophobic pocket bounded by *P. carinii* enzyme residues Phe36, Pro66, and Phe69, thus enhancing potency. However, none of these compounds **10d**−**g** displayed any increased potency over the lead compound **3a** or increase in selectivity. From manual docking studies of TAB, face−edge aromatic interactions with *P. carinii* DHFR Phe69 have been identified as one of the factors contributing to its species selectivity. We hypothesized that, as this type of interaction is partially electrostatic, an electron-withdrawing group (EWG) on the phenyl ring of TAB would give rise to an optimal interaction with the π electrons of Phe69. Therefore EWGs and electron-donating groups were introduced onto the phenyl ring of the benzyl group **10h**−**s** to evaluate both electronic and steric effects on potency and selectivity. However, confounding the original hypothesis, all three fluoroben-

Table 1. Structure−Activity Relationship of Triazenyl-Substituted 2,4-Diaminopyrimidines toward *P. carinii* (pc), *T. gondii* (tg), and *M. avium* (ma) DHFR

	IC_{50} (μM) vs DHFR[a]				selectivity ratio[b]		
cpd	*P. carinii*	rat liver	*T. gondii*	*M. avium*	rl/pc	rl/tg	rl/ma
2[c]	0.042	0.003	0.01		0.071	0.003	
3a[d]	0.17	19.4	0.69		114	28	
3b[d]	2.8	18.9	0.31		6.8	61	
4[d]	3.65	2.3	0.39		0.63	5.9	
10a	1.3	3.3	0.7		2.54	4.71	
10b	1.2	3.7	0.66		3.08	5.61	
10c	0.85	0.69	0.22		0.81	3.14	
10d	2.1	2.1	0.44		1.00	4.77	
10e	1.4	0.39	0.08		0.28	4.88	
10f	0.86	0.845	0.378	4.36	0.98	2.24	0.19
10g	1.5	0.45	0.07		0.30	6.43	
10h	92	14.9	7.8		0.16	1.91	
10i	2.22	3.87	1.25	4.07	1.74	3.10	0.95
10j	1.91	3.83	4.4	7.46	2.01	0.87	0.51
10k	4.7	8.4	6.1		1.79	1.38	
10l	0.58	1.29	1.16		2.23	1.11	
10m	0.94	1.8	0.8		1.91	2.25	
10n	11.08	11.23	0.83	24.54	1.24	1.93	
10o	5.29	5.8	5.39	25.2	1.10	1.08	0.23
10p	1.7	3.85	0.63	4.43	2.26	6.14	0.87
10q	1.35	6.62	2.54		4.90	2.61	
10r	1.3	1.61	0.83	3.69	1.24	1.93	0.44
10s	1.21	3.33	0.85	5.34	2.75	3.94	0.85
10t	0.053	0.28	0.20		5.36	1.44	
10u	0.59	0.62	1.05		1.04	0.58	
10v	3.46	6.19	0.64	2.03	1.79	9.72	3.05
12a[d]	0.26	7			27		
12b	0.68	7.35	2.01	3.9	10.87	3.66	2.01
12c	0.48	4.67	1.79	5.42	9.71	2.61	0.86
12d	1.09	6.44	1.47		5.91	4.38	
12e	1.26	6.51	2.7		5.17	2.41	

[a] In vitro enzymatic assays were performed according to previously described methods.[17−19] [b] Selectivity is defined by ratio of IC_{50} (rat liver DHFR)/IC_{50} (*P. carinii*, *T. gondii*, or *M. avium* DHFR); values of >1 indicate a preferential binding to the corresponding enzyme in the denominator. [c] Data from Chio et al.[18] [d] Data from Stevens et al.[7]

zyltriazenes **10h**−**j** were less potent against the *P. carinii* enzyme than their unfluorinated precursor **3a**. The general trend showing that potency in the isomeric fluoro series is in the order 2-F < 3-F or 4-F was repeated in the chlorobenzyltriazenes **10k**−**m** and the methyl-substituted benzyltriazenes **10n**−**p**. We conclude that the steric effect imposed by the 2′-substitution impedes an optimal aromatic interaction between Phe69 and the substituted benzyl moiety. Substitution of the benzyl moiety by trifluoromethyl **10q** or nitro substituents **10r**,**s** gave compounds which were 10-fold less active than **3a**.

Overall, all new compounds were less potent inhibitors of *P. carinii* DHFR than TAB **3a** with the exception of **10t** where the benzyl substituent has been replaced by a naphth-1-ylmethyl moiety (IC_{50} 0.053 μM). The potency of this analogue is comparable to that of the quinazoline trimetrexate **2** but because **10t** is less selective for the mammalian inhibitor, it shows a superior rl/pc selectivity ratio (5.36) to **2** (0.071). The isomeric naphth-2-ylmethyl triazene **10u** was 10-fold less active (IC_{50} 0.59 μM) than the isomer **10t**.

The triazenyl-pyrimethamines were also tested for activity against *T. gondii* DHFR (Table 1). Overall they were more active toward the DHFR enzyme from this protozoan than *P. carinii*, exhibiting a potency predominantly in the submicromolar range. Those compounds with a 2′-substituted phenyl ring were again generally less active than their 3′- and 4′-substituted isomers. Where supplies of compound allowed, new triazenes were evaluated against the DHFR from *Mycobacterium avium* (Table 1). IC_{50} values observed for these compounds spanned only a 10-fold inhibitory range (2.03 to 25.2 μM).

Conclusion

A structure-based design process to optimize the selectivity and potency of a promising lead compound TAB **3a**[7] against DHFR from *P. carinii* has been initiated. The approach encompasses molecular modeling studies, organic syntheses, and biological evaluation. Manual docking studies of TAB **3a** utilizing the published X-ray crystallographically determined coordinates of the *P. carinii* enzyme[8] have enabled us to investigate the structural basis for the species selectivity observed. It was predicted that the benzyl side chain of TAB might interact favorably with the hydrophobic region occupied by Phe69 which is unique to *P. carinii* DHFR. The veracity of the model was subsequently confirmed by the X-ray determination of the *P. carinii* DHFR-TAB-NADPH ternary complex.[10]

Both benzyl and acetoxyethyl appendages of the triazenyl moiety were predicted to be important for determining affinity and species selectivity and were therefore investigated through rational structural modification. Disappointingly, all adjustments to the acetoxyethyl branch, including increasing its length and steric bulk, or increasing its polarity by deacetylation had a dyschemotherapeutic effect, despite predictions from modeling and crystal structure analysis which suggested that there were opportunities for enhancing enzyme interactions made by the acyl moiety. Modifications to the benzyl group were also generally detrimental, but this was not so unexpected as the structural data indicated little room to further optimize interaction with Phe69. However, replacement of the benzyl substituent of **3a** with a naphth-1-ylmethyl group **10t** resulted in a 3-fold improvement in potency toward the *P. carinii* DHFR, placing this analogue in an activity range similar to that of trimetrexate **2**.

All the triazenyl analogues were tested for activity against *T. gondii* DHFR. Generally, the new triazenyl-pyrimethamines were more potent toward the DHFR enzyme from this protozoa than *P. carinii*, exhibiting IC_{50} values predominantly in the submicromolar range; the most potent compound with the bulky trimethoxybenzyl substituent **10g** gave an IC_{50} value of 0.053 μM. Selected triazenyl analogues of TAB were less effective against the DHFR from *M. avium* than *P. carinii* and *T. gondii*, suggesting that the size of the active site pocket in the mycobacterium DHFR is more restricted.

Experimental Section

Molecular Modeling. The docking study was performed using the DISCOVER module within the molecular graphics program INSIGHT II[21] utilizing the AMBER 3.5 force field[22] for energy minimization. Full details have been given elsewhere.[10]

Synthetic Chemistry. Melting points were determined in open capillaries using a Gallenkamp melting point apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra of synthetic intermediates and final products were recorded in DMSO-d_6 solutions on a Bruker spectrometer observing ^{1}H at 250.13 MHz and ^{13} C at 62.9 MHz. Chemical shifts (δ) are reported in parts per million (ppm) with tetramethysilane as an internal standard; s = singlet, d = doublet, dd = doublet of doublets, t = triplet, brs = broad singlet, m = multiplet. Mass spectra (MS) in the atmospheric pressure chemical ionization (APCI), electrospray ionization (ES), or fast atom bombardment (FAB) mode were recorded using an ANI MS-902, a VG Micromass 7070E, or a VG platform spectrometer. Optical rotations were recorded on a Bellingham Stanley ADP220 polarimeter. Elemental analyses (C, H, N) were performed using either a Perkin-Elmer PE240B elemental analyzer or an Exeter Analytical CE-440 elemental analyzer by the Microanalysis service at the School of Chemistry, University of Nottingham, and element compositions are within ±0.4% of the calculated values unless otherwise stated. To monitor reaction mixtures by TLC, precoated silica gel $60F_{254}$ plates were used with the developing solvent being either chloroform−methanol or hexanes−ethyl acetate mixtures; TLC spots were visualized with UV irradiation. All new triazenyl-pyrimethamines and model compounds were purified initially by flash column chromatography using silica gel C60H from Merck, and crystallized unchanged from acetone, methanol, or ethanol. Samples were dried in vacuo overnight over P_2O_5 at room temperature prior to submission for elemental analysis. No other special procedures were undertaken to remove residues of solvents, and fractional moles of water and/or organic solvents were found in some analytical samples. The presence of these contaminations was detected in the ^{1}H NMR spectra but are not recorded in the listed spectral data.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-benzyltriazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine ethanesulfonic acid salt (3a): This salt (98%), mp 170−171 °C (efferv.), was formed from **3a** and ethanesulfonic acid (1.1 mol equiv) in 2-propanol at 0 °C; IR (KBr) 3401, 3144, 1738 (C=O), 1657, 1451, 1186, 1038, 740 cm^{-1}; ^{1}H NMR 1.02 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.09 (t, J = 7.5 Hz, 3H, SCH_2CH_3), 1.90 (s, 3H, C=OCH_3), 2.23 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.46 (q, J = 7.5 Hz, 2H, SCH_2CH_3), 3.93−4.32 (m, 4H, CH_2CH_2), 5.04 (brs, 2H, CH_2Ph), 7.00−7.61 (m, 11H), 8.20 (s, 1H), 12.21 (brs, NH and NH^+ absorptions); ^{13}C NMR δ 9.9 (CH_3), 20.7 (CH_3), 23.8 (CH_2), 45.4 (CH_2), 46.3 (CH_2), 50.6 (CH_2), 53.2 (CH_2), 59.3 (CH_2), 61.9 (CH_2), 107.5 (C), 120.6 (CH), 127.3 (CH), 128.0 (CH), 128.4

(CH), 128.5 (CH), 128.7 (CH), 128.9 (CH), 130.9 (C), 131.2 (CH), 136.2 (C), 136.9 (C), 146.9 (C), 154.6 (C), 154.9 (C), 164.2 (C), 170.3 (C); MS (CI) *m/z* 468, 470 (M + H, $-EtSO_3$). Anal. ($C_{25}H_{32}ClN_7O_5S$) C, H, N.

General Method for the Synthesis of 2-[*N*-(Substituted-benzyl)amino]-ethanol Derivatives 8a−q, 8t−v, 11a,b. The substituted benzaldehyde was dissolved in a solution of ethanolamine (1.2 mol equiv) in methanol. Sodium hydrogen carbonate (approximately 2 mol equiv) was added to the solution which was then heated at reflux for 4 h. The reaction mixture was cooled to 5 °C, and sodium borohydride (1.2 mol equiv) was added portionwise during a period of 2 h while keeping the temperature at 10 °C during which hydrogen gas was evolved, and an off-white precipitate was formed. The reaction mixture was maintained at room temperature overnight. Any insoluble material was filtered off, and the filtrate was collected and evaporated to dryness. The colored oily residue was dissolved in chloroform and washed successively with water and brine. The organic layer was collected and concentrated in vacuo. The crude 2-[*N*-(substituted-benzyl)-amino]ethanol derivatives (**8**) were purified by distillation under reduced pressure, or by crystallization from benzene if solid, and used directly for the next step.

The ethanolamines **8r**,**s** were prepared from ethanolamine and 3-nitro- and 4-nitrobenzyl chloride, respectively; [23] ethanolamine **11c** from ethanolamine and 2-phenethyl chloride;[24] and ethanolamine **11d**,**e** from 2-chloroethanol and *R*-(+)-α-methylbenzylamine and *S*-(−)-α-methyl-benzylamine, respectively.[25]

General Method for the Synthesis of Hydrochloride Salts of Esters of 2-[*N*-(Substituted-benzyl)amino]ethanols 9a−v. Esterification of 2-[*N*-(substituted-benzyl)amino]-ethanols **8a−v** have been achieved by two methods. The ethanol **8** was dissolved in a minimum of the appropriate carboxylic acid, and hydrogen chloride gas was bubbled through the solution for 1 h. The reaction mixture was stirred at room temperature for a further 24 h to give a pale colored solution. The off-white solid isolated when the solvent was removed (vacuum distillation) was washed well with diethyl ether and collected. Crystallization from either acetonitrile or ethanol gave pure hydrochloride salts of the required esters **9**.

Alternatively the 2-[*N*-(substituted-benzyl)amino]ethanol **8** was dissolved in 1,2-dichloro-ethane, and anhydrous hydrochloride gas was passed through the solution with stirring for 10 min. The acyl or benzoyl chloride (1 mol equiv) was added, and the reaction mixture was heated at 60 °C for 4 h. The precipitated hydrochloride salts (**9**) were collected by filtration.

General Procedure of the Synthesis of Triazenyl-Substituted 2,4-Diamino-5-(4-chlorophenyl)-6-ethylpyrimidines 10a−v, 12a−e, and 13a,b. (i) A stirred mixture of aminopyrimethamine **5**[11] (1.32 g, 5 mmol) in 3 M HCl (17.5 mL) was diazotized with a solution of sodium nitrite (375 mg) in water (2.5 mL) at 0 °C for 1 h. A pale green solution of the diazonium chloride **6a** was obtained. An aqueous solution (5 mL) of the appropriate amine salt **9** (1 mol equiv) was added dropwise to the above mixture. Solid sodium carbonate was added to achieve a pH between 9 and 10. The reaction mixture was stirred for a further 1 h between 0 and 5 °C, and the creamy precipitate was collected and washed well with copious amounts of cold water. Analytically pure samples of triazenes could be obtained either from flash column chromatography (chloroform−methanol 10:1) and/or crystallization from acetone, methanol, or ethanol.

(ii) The diazonium tetrafluoroborate hydrotetrafluoroborate **6b** (0.5 g, 1.11 mmol) was dissolved in water (30 mL) at 0 °C and coupled with an aqueous solution of the appropriate amine (or amine salt) (1 mol equiv) which was added dropwise. Sodium carbonate solid was added (to pH 9−10), and the reaction mixture was then stirred for a further hour between 0 and 5 °C. The creamy precipitate was collected and purified as above.

The following triazenyl-substituted 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidines were prepared by this general method.

2,4-Diamino-5-{3-[3-benzyl-3-[2-(propionyloxy)ethyl]-triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10a): From **6b** and **9a**; 87% (from acetone), mp 127−128 °C (decomp.); IR (KBr) 3451, 3322, 3169 (N−H), 1720 (C═O), 1638, 1557, 1449, 1154 cm^{-1}; ^1H NMR δ 0.88 (t, J = 7.4 Hz, 3H, $CH_2CH_2CH_3$), 0.96 (t, J = 7.4 Hz, 3H, CH_2CH_3), 2.06−2.21 (m, 4H), 3.92−4.30 (m, 4H, CH_2CH_2), 5.03 (brs, 2H, CH_2Ph), 5.71 (brs, 2H, NH_2), 5.92 (brs, 2H, NH_2), 6.95−7.49 (m, 8H); ^{13}C NMR δ 8.9 (CH_3), 13.3 (CH_3), 26.8 (CH_2), 27.7 (CH_2), 46.2 (CH_2), 50.4 (CH_2), 53.1 (CH_2), 58.8 (CH_2), 59.4 (CH_2), 61.7 (CH_2), 105.7 (C), 120.7 (CH), 127.2 (CH), 128.3 (CH), 128.5 (CH), 128.8 (CH), 129.1 (CH), 130.5 (CH), 136.3 (C), 136.9 (C), 146.6 (C), 162.2, (C, C), 166.6 (C), 173.5 (C); MS (ES) *m/z* 482, 484 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-benzyl-3-[2-(isobutyryloxy)ethyl]-triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10b): From **6b** and **9b**; 78% (from acetone), mp 134−136 °C (decomp.); IR (KBr) 3451, 3322, 3169 (N−H), 1720 (C═O), 1630, 1557, 1449, 1154 cm^{-1}; ^1H NMR δ 0.99 (m, 9H), 2.08 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.33 (m, 1H, CH), 3.94−4.30 (m, 4H, CH_2CH_2), 5.04 (brs, 2H, CH_2Ph), 5.68 (brs, 2H, NH_2), 5.92 (brs, 2H, NH_2), 6.94−7.48 (m, 8H); ^{13}C NMR δ 13.3 (CH_3), 18.8 (CH_3), 27.7 (CH_2), 33.3 (CH), 46.1 (CH_2), 50.2 (CH_2), 53.2 (CH_2), 58.7 (CH_2), 59.6 (CH_2), 61.6 (CH_2), 105.7 (C), 120.7 (CH), 127.4 (CH), 128.3 (CH), 128.5 (CH), 129.1 (CH), 130.5 (CH), 135.8 (C), 136.3 (C), 136.9 (C), 146.6 (C), 162.1 (C), 162.3 (C), 166.6 (C), 176.0 (C); MS (ES) *m/z* 496, 498 (M + 1). Anal. ($C_{25}H_{30}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(benzoyloxy)ethyl]-3-benzyltriazen-1-yl]-4-chloro-phenyl}-6-ethylpyrimidine (10c): From **6b** and **9c**; 89% (from acetone), mp 98 °C (decomp.); IR (KBr) 3451, 3322, 3179 (N−H), 1709 (C═O), 1620, 1553, 1449, 1285 cm^{-1}; ^1H NMR δ 0.98 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.06 (q, J = 7.5 Hz, 2H, CH_2CH_3), 4.10−4.57 (m, 4H, CH_2CH_2), 5.57 (brs, 2H, CH_2Ph), 5.76 (brs, 2H, NH_2), 5.90 (brs, 2H, NH_2), 7.08−7.84 (m, 13H); ^{13}C NMR δ 13.3 (CH_3), 27.3 (CH_2), 46.3 (CH_2), 50.3 (CH_2), 53.2 (CH_2), 58.7 (CH_2), 60.5 (CH_2), 62.5 (CH_2), 105.9 (CH), 120.8 (CH), 127.3 (CH), 128.5 (CH), 128.7 (CH), 129.0 (CH), 129.2 (CH), 129.5 (C), 130.6 (CH), 133.5 (CH); MS (ES) *m/z* 496, 498 (M + 1). Anal. ($C_{28}H_{28}ClN_7O_2$) C, H, N.

This compound was also prepared from 2,4-diamino-5-{4-chloro-3-[3-benzyl-3-(3-hydroxyethyl)triazen-1-yl]phenyl}-6-ethylpyrimidine **12a** and benzoyl chloride (1.5 mol equiv) in pyridine containing DMAP (0.2 mol equiv) at 50 °C for 2 h.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-methoxybenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10d): From **6b** and **9d**; 90% (from acetone), mp 146−148 °C (efferv.); IR (KBr) 3432, 3325, 3187 (N−H), 1730 (C═O), 1618, 1559, 1441, 1235 (C−O) cm^{-1}; ^1H NMR δ 0.97 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.91 (d, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.72 (s, 3H, OCH_3), 3.88−4.25 (m, 4H, CH_2CH_2), 4.94 (brs, 2H, CH_2Ph), 5.62 (brs, 2H, NH_2), 5.89 (brs, 2H, NH_2), 6.90−6.98 (m, 3H), 7.24−7.37 (m, 3H), 7.49 (d, J = 8.3 Hz, 1H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 45.9 (CH_2), 49.7 (CH_2), 52.7 (CH_2), 55.2 (CH_3), 58.3 (CH_2), 59.4 (CH_2), 61.9 (CH_2), 105.7 (C), 113.9 (CH), 114.3 (CH), 120.7 (CH), 127.4 (C), 128.2 (C), 128.6 (C), 129.1 (CH), 129.8 (CH), 130.1 (CH), 130.6 (CH), 135.8 (C), 146.6 (C), 158.7 (C), 159.1 (C), 162.2 (C), 162.3 (C), 166.7 (C), 170.3 (C); MS (CI) *m/z* 498, 500 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_3$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3,4-dimethoxybenzyl)-triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10e): From **6b** and **9e**; 73% (from acetone), mp 123−124 °C (efferv.); IR (KBr) 3445, 3167 (N−H), 1736 (C═O), 1630, 1572, 1443, 1230 (C−O), 1138 cm^{-1}; ^1H NMR δ 0.97 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.91 (d, 3H, C═OCH_3), 2.10 (q, J = 7.6 Hz, 2H, CH_2CH_3), 3.72 (s, 6H, 2 × OCH_3), 3.89−4.26 (m, 4H, CH_2CH_2), 4.93 (brs, 2H, CH_2Ph), 5.70 (brs, 2H, NH_2), 5.94 (brs, 2H, NH_2), 6.75−7.34 (m, 5H), 7.50 (d, J = 8.0 Hz 1H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_2), 27.7 (CH_3), 46.0 (CH_2), 49.9 (CH_2), 52.5 (CH_2), 55.6 (CH_3), 55.7 (CH_3), 58.7 (CH_2), 59.4 (CH_2), 61.9

(CH_2), 105.7 (C), 111.9 (CH), 112.8 (CH), 120.7 (CH), 121.1 (CH), 127.3 (C), 128.6 (C), 129.0 (CH), 130.6 (CH), 135.8 (C), 146.6 (C), 148.2 (C), 148.8 (C), 162.1 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 529, 531 (M + 1). Anal. ($C_{25}H_{30}ClN_7O_4$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3,5-dimethoxybenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10f): From **6b** and **9f**; 46% (from acetone), mp 153−154 °C (efferv.); IR (KBr) 3408, 3152 (N−H), 1742 (C═O), 1601 (C−O), 1441, 1353, 1231 (C−O), 1055 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.97 (d, 3H, C═OCH_3), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.70 (s, 6H, 2 × OCH_3), 3.92−4.28 (m, 4H, CH_2CH_2), 4.94 (brs, 2H, CH_2Ph), 5.64 (brs, 2H, NH_2), 5.90 (brs, 2H, NH_2), 6.40−6.55 (m, 3H), 6.97 (d, J = 7.8 Hz 1H), 7.22 (brs, 1H), 7.48 (brs, 1H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 46.3 (CH_2), 50.3 (CH_2), 52.9 (CH_2), 55.3 (CH_3), 58.7 (CH_2), 59.4 (CH_2), 99.1 (CH), 99.5 (CH), 105.7 (C), 106.2 (CH), 106.5 (CH), 120.8 (CH), 127.4 (C), 129.2 (CH), 130.6 (CH), 135.8 (C), 138.5 (C), 139.4 (C), 146.6 (C), 160.7 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 529, 531 (M + 1). Anal. ($C_{25}H_{30}ClN_7O_4$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3,4,5-trimethoxybenzyl)-triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10g): From **6b** and **9g**; 73% (from acetone), mp 151−152 °C (efferv.); IR (KBr) 3453, 3167 (N−H), 2938, 1740 (C═O), 1555, 1443, 1238 (C−O), 1120 cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.91 (d, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.62 (s, 3H, OCH_3), 3.74 (brs, 6H, 2 × OCH_3), 3.94−4.23 (m, 4H, CH_2CH_2), 4.93 (s, 2H, CH_2Ph), 5.63 (brs, 2H, NH_2), 5.88 (brs, 2H, NH_2), 6.64−7.22 (m, 4H), 7.49 (d, J = 8.0 Hz, 1H); ^{13}C NMR δ 13.3 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 46.3 (CH_2), 50.5 (CH_2), 52.8 (CH_2), 56.0 (CH_3), 59.0 (CH_2), 59.4 (CH_2), 60.1 (CH_3), 61.9 (CH_2), 105.6 (CH), 106.1 (CH), 120.8 (CH), 127.3 (C), 129.1 (CH), 130.6 (CH), 131.9 (C), 132.5 (C), 135.9 (C), 136.9 (C), 146.6 (C), 153.0 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 559, 561 (M + 1). Anal. ($C_{26}H_{32}ClN_7O_5$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(2-fluorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10h): From **6b** and **9h**; 68% (from acetone), mp 183−184 °C (efferv.); IR (KBr) 3459, 3185 (N−H), 1724 (C═O), 1628, 1555, 1447, 1236 (C−O), 1047 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.0 (s, 3H, C═OCH_3), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.95−4.31 (m, 4H, CH_2CH_2), 5.04 (d, 2H, CH_2Ph), 5.64 (brs, 2H, NH_2), 5.91 (brs, 2H, NH_2), 6.96 (d, J = 7.8 Hz), 7.19−7.47 (m, 6H); ^{13}C NMR δ 13.4 (CH_3), 20.6 (CH_3), 27.7 (CH_2), 45.2 (CH_2), 46.4 (CH_2), 52.9 (CH_2), 53.4 (CH_2), 59.4 (CH_2), 62.0 (CH_2), 105.6 (C), 115.2 (CH), 115.5 (CH), 120.7 (CH), 123.0 (C), 124.3 (C), 127.5 (CH), 129.3 (CH), 130.6 (CH), 130.8 (CH), 135.8 (C), 146.4 (C), 158.7 (C), 162.2 (C), 162.3 (C), 162.5 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 486, 488 (M + 1). Anal. ($C_{23}H_{25}ClFN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3-fluorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10i): From **6b** and **9i**; 74% (from acetone), mp 146−147 °C (efferv.); IR (KBr) 3464, 3187 (N−H), 1720 (C═O), 1624, 1555, 1443, 1235 (C−O), 1045 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.89 (brs, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.95−4.3 (m, 4H, CH_2CH_2), 5.03 (brs, 2H, CH_2Ph), 5.66 (brs, 2H, NH_2), 5.91 (brs, 2H, NH_2), 6.96−7.49 (m, 7H); ^{13}C NMR δ 13.4 (CH_3), 20.6 (CH_3), 27.7 (CH_2), 46.5 (CH_2), 50.3 (CH_2), 53.4 (CH_2), 58.1 (CH_2), 59.4 (CH_2), 61.9 (CH_2), 105.6 (C), 113.9 (CH), 114.2 (CH),114.6 (CH), 114.9 (CH), 115.3 (CH), 120.8 (CH), 124.3 (CH), 124.3 (CH), 127.5 (C), 129.3 (CH), 130.6 (CH), 135.9 (C), 139.3 (C), 140.0 (C), 146.4 (C), 160.4 (C), 162.2 (C), 162.3 (C), 164.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 486, 488 (M + 1). Anal. ($C_{23}H_{25}ClFN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-fluorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10j): From **6b** and **9j**; 84% (from acetone), mp 161−162 °C (efferv.); IR (KBr) 3476, 3173 (N−H), 1730 (C═O), 1605, 1562, 1437, 1225 (C−O), 1059 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.89 (brs, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.91−3.44 (brs, 4H, CH_2CH_2), 5.00 (brs, 2H, CH_2Ph), 5.69 (brs, 2H, NH_2), 5.91 (brs, 2H, NH_2), 6.97 (d, J = 7.5 Hz, 1H), 7.16−7.50 (m, 6H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 39.9 (CH_2), 46.2 (CH_2), 49.8 (CH_2), 53.1 (CH_2), 57.9 (CH_2), 59.3 (CH_2), 61.9 (CH_2), 105.6 (C), 115.1 (CH), 115.4 (CH), 115.8 (CH), 120.7 (CH), 127.4 (C), 129.2 (CH), 130.4 (CH), 130.5 (CH), 132.5 (C), 133.2 (C), 135.8 (C), 146.5 (C), 162.1 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 486, 488 (M + 1). Anal. ($C_{23}H_{25}ClFN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(2-chlorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10k): From **6b** and **9k**; 76% (from acetone), mp 181−182 °C (efferv.); IR (KBr) 3464, 3171 (N−H), 1724 (C═O), 1630, 1553, 1441, 1231 (C−O), 1049 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.89 (s, 3H, C═OCH_3), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.67−4.31 (m, 4H, CH_2CH_2), 5.09 (d, J = 16.8 Hz, 2H, CH_2Ph,), 5.64 (brs, 2H, NH_2), 5.90 (brs, 2H, NH_2), 6.97 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.37−7.47 (m, 5H); ^{13}C NMR δ 13.3 (CH_3), 20.6 (CH_3), 27.6 (CH_2), 46.5 (CH_2), 49.2 (CH_2), 53.4 (CH_2), 56.4 (CH_2), 59.1 (CH_2), 59.4 (CH_2), 62.1 (CH_2), 105.6 (C), 120.6 (CH), 127.4 (CH), 128.9 (CH), 129.3 (CH), 130.5 (CH), 132.3 (C), 133.2 (C), 134.2 (C), 135.8 (C), 146.4 (C), 162.1 (C), 162.2 (C), 166.6 (C), 170.2 (C); MS (CI) *m*/*z* 502, 504 (M + 1). Anal. ($C_{23}H_{25}Cl_2N_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3-chlorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10l): From **6b** and **9l**; 75% (from acetone), mp 153−154 °C (efferv.); IR (KBr) 3464, 3162 (N−H), 1730 (C═O), 1647, 1574, 1444, 1375, 1230 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.89 (s, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 3.95−4.30 (m, 4H, CH_2CH_2), 4.99 (brs, 2H, CH_2Ph), 5.65 (brs, 2H, NH_2), 5.90 (brs, 2H, NH_2), 6.97 (d, J = 8.3 Hz, 1H), 7.21−7.48 (m, 6H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_3), 46.6 (CH_2), 50.4 (CH_2), 53.5 (CH_2), 58.0 (CH_2), 59.4 (CH_2), 61.9 (CH_2); 105.6 (C), 120.8 (CH), 126.9 (CH), 127.2 (CH), 127.5 (C), 128.4 (CH), 129.3 (CH), 130.3 (CH), 130.6 (CH), 133.1 (C), 135.9 (C), 138.9 (C), 146.4 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 502, 504 (M + 1). Anal. ($C_{23}H_{25}Cl_2N_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-chlorobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10m): From **6b** and **9m**; 82% (from acetone), mp 154−156 °C (efferv.); IR (KBr) 2917, 2769, 1730 (C═O), 1562, 1441, 1246 (C−O), 1065 (C−O), 770 cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.90 (brs, 3H, C═OCH_3), 2.10 (q, J = 7.4 Hz, 2H, CH_2CH_3), 3.92−4.29 (m, 4H, CH_2CH_2), 5.00 (brs, 2H, CH_2-Ph), 5.70 (brs, 2H, NH_2), 5.92 (brs, 2H, NH_2), 6.97 (d, J = 7.3 Hz, 1H), 7.22 (s, 1H), 7.38−7.49 (m, 5H); ^{13}C NMR δ 13.4 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 46.8 (CH_2), 50.9 (CH_2), 53.8 (CH_2), 57.9 (CH_2), 59.5 (CH_2), 62.0 (CH_2), 105.6 (C), 120.8 (CH), 123.6 (CH), 127.6 (C), 129.0 (CH), 129.5 (CH), 130.6 (CH), 135.8 (C), 144.6 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 502, 504 (M + 1). Anal. ($C_{23}H_{25}Cl_2N_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(2-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10n): From **6b** and **9n**; 61% (from acetone), mp 175−176 °C (efferv.); IR (KBr) 3459, 3177 (N−H), 1723 (C═O), 1626, 1553, 1441, 1233 (C−O), 1045 (C−O) cm^{-1}; ^1H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.90 (brs, 3H, C═OCH_3), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.28 (s, 3H, CH_3), 3.90−4.00 (m, 2H, NCH_2), 4.26 (t, J = 4.9 Hz, 2H, CH_2O), 5.09 (brs, 2H, CH_2Ph), 5.64 (brs, 2H, NH_2), 5.89 (brs, 2H, NH_2), 6.96 (d, J = 7.5 Hz, 1H), 7.14−7.47 (m, 6H); ^{13}C NMR δ 13.4 (CH_3), 19.0 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 45.9 (CH_2), 48.5 (CH_2), 52.5 (CH_2), 57.1 (CH_2), 59.3 (CH_2), 61.9 (CH_2), 105.6 (C), 120.9 (CH), 126.2 (CH), 127.3 (CH), 127.9 (CH), 128.1(CH), 129.1 (CH), 130.6 (CH), 135.8 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 482, 484 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10o): From **6b** and **9o**; 76% (from acetone), mp 155−156 °C (efferv.); IR (KBr) 3451, 3181 (N−H), 1724 (C═O), 1620, 1572, 1443, 1235 (C−O), 1045 (C−O) cm^{-1}; ^1H NMR δ 0.97 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.90 (d, 3H, C═OCH_3), 2.10 (q, J =

7.5 Hz, 2H, CH_2CH$_3$), 2.27 (s, 3H, CH$_3$), 3.90−4.27 (m, 4H, CH$_2$CH$_2$), 4.98 (brs, 2H, CH_2Ph), 5.65 (brs, 2H, NH$_2$), 5.90 (brs, 2H, NH$_2$), 6.98−7.49 (m, 7H); ^{13}C NMR δ 13.4 (CH$_3$), 20.7 (CH$_3$), 21.2 (CH$_3$), 27.7 (CH$_2$), 46.2 (CH$_2$), 50.4 (CH$_2$), 53.0 (CH$_2$), 58.8 (CH$_2$), 59.4 (CH$_2$), 61.9 (CH$_2$); 105.7 (C), 120.8 (CH), 125.4 (CH), 127.4 (C), 128.0 (CH), 128.0 (CH), 128.6 (CH), 129.1 (CH), 130.6 (CH), 135.8 (C), 136.2 (C), 136.8 (C), 137.5 (C), 138.0 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 482, 484 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10p): From **6b** and **9p**; 72% (from acetone), mp 167−169 °C (efferv.); IR (KBr) 3437, 3410, 3154 (N−H), 1728 (C=O), 1579, 1433, 1229 (C−O), 1063 (C−O) cm^{-1}; ^{1}H NMR δ 0.97 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.86 (d, 3H, C=OCH$_3$), 2.08 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 2.27 (s, 3H, CH$_3$), 3.88−4.26 (m, 4H, CH$_2$CH$_2$), 4.97 (brs, 2H, CH_2Ph), 5.67 (brs, 2H, NH$_2$), 5.92 (brs, 2H, NH$_2$), 6.97−7.50 (m, 7H); ^{13}C NMR δ 13.4 (CH$_3$), 20.7 (CH$_3$), 20.9 (CH$_3$), 27.7 (CH$_2$), 46.0 (CH$_2$), 50.0 (CH$_2$), 52.8 (CH$_2$), 58.6 (CH$_2$), 59.4 (CH$_2$), 61.9 (CH$_2$), 105.7 (C), 127.4 (C), 127.7 (CH), 128.3 (CH), 128.5 (CH), 129.1 (CH), 129.4 (CH), 130.6 (CH), 133.2 (C), 133.8 (C), 135.8 (C), 136.5 (C), 137.3 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 482, 484 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[2-(acetyloxy)ethyl]-3-(4-trifluoromethylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10q): From **6b** and **9q**; 75% (from ethanol), mp 142−143 °C (efferv.); IR (KBr) 3455, 3183 (N−H), 1726 (C=O), 1628, 1555, 1443, 1325, 1067 (C−O) cm^{-1}, ^{1}H NMR δ 0.95 (t, J = 7.5 Hz, 2H, CH$_2$CH_3), 1.87 (s, 3H, C=OCH$_3$), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.96−4.32 (m, 4H, CH$_2$CH$_2$), 5.11 (m, 2H, CH_2Ph), 5.64 (brs, 2H, NH$_2$), 5.90 (brs, 2H, NH$_2$), 6.95−7.76 (m, 7H); ^{13}C NMR δ 13.8 (CH$_3$), 21.1 (CH$_3$), 28.5 (CH$_2$), 46.7 (CH$_2$), 51.3 (CH$_2$), 53.5 (CH$_2$), 59.4 (CH$_2$), 60.6 (CH$_2$), 62.8 (CH$_2$), 76.5 (CF), 77.0 (CF), 77.5 (CF), 107.2 (C), 120.6 (CH), 125.4 (CH), 126.1 (CH), 128.3 (CH), 128.8 (CH), 129.3 (C), 130.9 (CH), 134.4 (C), 139.9 (C), 140.5 (C), 146.8 (C), 161.8 (C), 162.0 (C), 168.3 (C), 170.6 (C); MS (CI) *m*/*z* 536, 538 (M + 1). Anal. ($C_{24}H_{28}F_3ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(3-nitrobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10r): From **6b** and **9r**; 79% (from ethanol), mp 167−168 °C (efferv.); IR (KBr) 3484, 3150 (N−H), 1736 (C=O), 1601, 1549 (NO$_2$), 1437, 1349 (NO$_2$), 1240 cm^{-1}; ^{1}H NMR δ 0.95 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.89 (s, 3H, C=OCH$_3$), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.99−4.33 (m, 4H, CH$_2$CH$_2$), 5.08 and 5.17 (2 brs, 2H, CH_2Ph), 5.63 (brs, 2H, NH$_2$), 5.89 (brs, 2H, NH$_2$), 6.97 (d, J = 7.5 Hz, 1H), 7.20 (brs, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.24 (s, 1H); ^{13}C NMR δ 13.4 (CH$_3$), 20.7 (CH$_3$), 27.7 (CH$_2$), 46.8 (CH$_2$), 50.7 (CH$_2$), 53.8 (CH$_2$), 57.7 (CH$_2$), 59.4 (CH$_2$), 62.0 (CH$_2$), 105.6 (C), 120.8 (CH), 122.2 (CH), 123.1 (CH), 127.5 (C), 129.5 (CH), 129.9 (CH), 130.6 (CH), 135.0 (CH), 138.9 (C), 146.3 (C), 147.9 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 513, 515 (M + 1). Anal. ($C_{23}H_{25}ClN_8O_4$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-nitrobenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10s): From **6b** and **9s**; 72% (from acetone), mp 117−118 °C (efferv.); IR (KBr) 3455, 3165 (N−H), 1720 (C=O), 1628, 1555, 1522 (NO$_2$), 1441, 1344 (NO$_2$), 1240 (C−O) cm^{-1}; ^{1}H NMR δ 0.95 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.90 (s, 3H, C=OCH$_3$), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.98−4.38 (m, 4H, CH$_2$CH$_2$), 5.10−5.18 (m, 2H, CH_2Ph), 5.63 (brs, 2H, NH$_2$), 5.90 (brs, 2H, NH$_2$), 6.95−7.60 (m, 5H), 8.18−8.28 (m, 2H); ^{13}C NMR δ 13.3 (CH$_3$), 20.6 (CH$_3$), 27.6 (CH$_2$), 20.9 (CH$_2$), 53.7 (CH$_2$), 62.0 (CH$_2$), 105.6 (C), 120.7 (CH), 123.6 (CH), 127.5 (CH), 129.0 (CH), 129.4 (CH), 130.6 (CH), 135.8 (C), 144.6 (C), 146.3(C), 146.6 (C), 162.1 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 513, 515 (M + 1). Anal. ($C_{23}H_{25}ClN_8O_4$) C, H, N.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(naphth-1-ylmethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10t): From **6b** and **9t**; 67% (from acetone), mp 149−151 °C (efferv.); IR (KBr) 3445, 3162 (N−H), 1738 (C=O), 1624, 1553, 1445, 1341, 1233 cm^{-1}; ^{1}H NMR δ 0.98 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.79 (brs, 3H, C=OCH$_3$) 2.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.94−4.23 (m, 4H, CH$_2$CH$_2$), 5.50 (brs, 2H, CH_2-naphthyl), 5.67 (brs, 2H, NH$_2$), 5.91 (brs, 2H, NH$_2$), 6.99 (dd, J = 2.0, 8.3 Hz, 1H), 7.26−8.16 (m, 9H); ^{13}C NMR δ 13.4 (CH$_3$), 20.5 (CH$_3$), 27.7 (CH$_2$), 45.8 (CH$_2$), 47.9 (CH$_2$), 51.9 (CH$_2$), 57.0 (CH$_2$), 59.2 (CH$_2$), 61.8 (CH$_2$), 105.6 (C), 120.7 (CH), 121.0 (C), 123.7 (CH), 123.8 (CH), 125.7 (CH), 126.2 (CH), 126.6 (CH), 127.3 (C), 127.4 (C), 127.9 (CH), 128.2 (CH), 128.8 (CH), 129.2 (CH), 130.6 (CH), 131.1 (C), 131.5 (C), 132.1 (C), 133.6 (C), 133.7 (C), 135.9 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.2 (C); MS (CI) *m*/*z* 518, 520 (M + 1); HR-MS (FAB) calcd for $C_{27}H_{29}ClN_7O_2$ (M + H) *m*/*z* 518.207126, found 518.207048.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(naphth-2-ylmethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10u): From **6b** and **9u**; 84% (from acetone), mp 77−78 °C (efferv.); IR (KBr) 3449, 3163 (N−H), 1736 (C=O), 1632, 1555, 1445, 1339, 1233 (C−O) cm^{-1}; ^{1}H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.86 (brs, 3H, CH$_3$), 2.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.96−4.32 (m, 4H, CH$_2$CH$_2$), 5.19 (brs, 2H, CH_2-naphthyl), 5.64 (brs, 2H, NH$_2$), 5.90 (brs, 2H, NH$_2$), 6.95 (t, 1H), 7.27 (d, 1H), 7.50 (brs, 4H), 7.89 (brs, 4H); ^{13}C NMR δ 13.4 (CH$_3$), 20.6 (CH$_3$), 27.7 (CH$_2$), 46.3 (CH$_2$), 50.7 (CH$_2$), 53.2 (CH$_2$), 59.0 (CH$_2$), 62.0 (CH$_2$), 105.7 (C), 120.8 (CH), 126.1 (CH), 126.2 (CH), 126.4 (CH), 127.1 (CH), 127.5 (C), 127.8 (CH), 128.1 (CH), 128.6 (CH), 129.2 (CH), 130.6 (CH), 132.4 (C), 132.6 (C), 133.1 (C), 133.9 (C), 134.4 (C), 135.9 (C), 146.6 (C), 162.2 (C), 162.3 (C), 166.6 (C), 170.3 (C); MS (CI) *m*/*z* 518, 520 (M + 1); HR-MS (FAB) calcd for $C_{27}H_{29}ClN_7O_2$ (M + H) *m*/*z* 518.207126, found 518.207393.

2,4-Diamino-5-{3-[3-[2-(acetyloxy)ethyl]-3-(4-pyridin-4-ylmethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (10v): From **6b** and **9v**; 74% (from acetone), mp 168−170 °C (efferv.); IR (KBr) 3462, 3322, 3163 (N−H), 1740 (C=O), 1632, 1555, 1443, 1235 (C−O) cm^{-1}; ^{1}H NMR δ 0.96 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.89 (s, 3H, C=OCH$_3$), 2.10 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 4.01−4.32 (m, 4H, CH$_2$CH$_2$), 5.02 (brs, 2H CH_2-pyridyl), 5.70 (brs, 2H, NH$_2$), 5.91 (brs, 2H, NH$_2$), 6.96−7.47 (m, 5H), 8.51 (brs, 2H); ^{13}C NMR δ 13.4 (CH$_3$), 20.6 (CH$_3$), 27.6 (CH$_2$), 47.0 (CH$_2$), 50.4 (CH$_2$), 53.8 (CH$_2$), 57.5 (CH$_2$), 59.4 (CH$_2$), 62.0 (CH$_2$), 105.6 (C), 120.8 (CH), 122.8 (CH), 127.5 (C), 129.4 (CH), 130.6 (CH), 135.8 (C), 145.4 (C), 146.3 (C), 146.3 (C), 149.6 (CH), 162.1 (C), 162.2 (C), 166.5 (C), 170.3 (C); MS (CI) *m*/*z* 469, 471 (M + 1); HR-MS (FAB) calcd for $C_{22}H_{26}ClN_8O_2$ (M + H) *m*/*z* 469.186725, found 469.187214.

2,4-Diamino-5-{3-[3-benzyl-3-(2-hydroxyethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (12a): Prepared (68%) from **6b** and ethanolamine (**11a**), this triazene had identical physical characteristics to an authentic sample.[7]

2,4-Diamino-5-{3-[3-benzyl-3-(3-hydroxypropyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (12b): From **6b** and **11b**; 53% (from ethanol), mp 147−148 °C (efferv.); IR (KBr) 3329, 3181 (N−H), 1620, 1555, 1443, 1260, 1053, 698 cm^{-1}; ^{1}H NMR δ 0.98 (t, J = 7.5 Hz, 3H, CH$_2$CH_3), 1.81 (brs, 2H, NCH$_2$CH_2), 2.11 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.39−3.47 (m, 2H, NCH$_2$), 3.69−3.88 (m, 2H, CH_2OH), 4.53 (t, J = 5.1 Hz, 1H, OH,), 4.99 (brs, 2H, CH_2Ph), 5.62 (brs, 2H, NH$_2$), 5.89 (brs, 2H, NH$_2$), 6.94 (d, J = 8.3 Hz, 1H), 7.25−7.49 (m, 7H); ^{13}C NMR δ 13.5 (CH$_3$), 27.7 (CH$_2$), 28.4 (CH$_2$), 32.0 (CH$_2$), 44.7 (CH$_2$), 50.3 (CH$_2$), 51.7 (CH$_2$), 58.2 (CH$_2$), 58.8 (CH$_2$), 105.8 (C), 120.6 (CH), 127.3 (CH), 128.0 (CH), 128.2 (CH), 128.5 (CH), 128.7 (CH), 130.5 (CH), 135.7 (C), 146.9 (C), 137.2 (C), 136.4 (C), 162.2 (C), 162.3 (C), 166.6 (C); MS (CI) *m*/*z* 440, 442 (M + 1). Anal. ($C_{22}H_{26}ClN_7O$) C, H, N.

The acetoxy-derivative **12f** was prepared from **12b** in acetic acid/anhydrous hydrochloric acid according to the general method described earlier. Flash column chromatography (dichloromethane/methanol 10:1), followed by crystallization from ethanol, gave **12f** as an off-white solid (39%), mp 202−204 °C; IR (KBr) 3447, 3322, 3189 (N−H), 1721 (C=O), 1624, 1553, 1441, 1262 (C−O) cm^{-1}; ^{1}H NMR δ 0.97 (t, J = 7.5 Hz 3H, CH$_2$CH_3), 1.95 (d, 5H, C=OCH$_3$ and CH$_2$CH_2CH$_2$), 2.10 (q, J = 7.4 Hz, 2H, CH_2CH$_3$), 3.72−3.90 (m, 2H, NCH$_2$), 3.99 (t, J

= 5.6 Hz, 2H, CH_2O), 4.98 (brs, 2H, CH_2Ph), 5.63 (brs, 2H, NH_2), 5.90 (brs, 2H, NH_2), 6.95 (d, 2H), 7.21−7.56 (m, 7H); ^{13}C NMR δ 13.4 (CH_3), 20.8 (CH_3), 24.6 (CH_2), 27.5 (CH_2), 27.7 (CH_2), 44.3 (CH_2), 50.1 (CH_2), 51.3 (CH_2), 58.2 (CH_2), 61.5 (CH_2), 62.1 (CH_2), 105.7 (C), 120.8 (CH), 127.3 (CH), 128.3 (CH), 128.5 (CH), 128.9 (CH), 130.5 (CH), 135.8 (C), 136.3 (C), 146.7 (C), 162.1 (C), 162.3 (C), 166.6 (C), 170.4 (C); MS (CI) *m*/*z* 482, 484 (M + 1). Anal. ($C_{24}H_{28}ClN_7O_2$) C, H, N.

2,4-Diamino-5-{3-[3-phenethyl-3-(2-hydroxyethyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (12c): From **6b** and **11c**; 69% (from ethanol), mp 87−89 °C (efferv.); IR (KBr) 3341, 3156 (N−H), 1655, 1610, 1555, 1452, 1053, 700 cm^{-1}; ^{1}H NMR δ 0.98 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.11 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.00−4.08 (m, 8H, 4 × CH_2), 4.85 (brs, 1H, OH), 5.69 (brs, 2H, NH_2), 5.95 (brs, 2H, NH_2), 6.79−7.53 (m, 8H); ^{13}C NMR δ 13.4 (CH_3), 27.6 (CH_2), 30.5 (CH_2), 34.9 (CH_2), 50.2 (CH_2), 50.5 (CH_2), 56.6 (CH_2), 56.9 (CH_2), 57.4 (CH_2), 59.8 (CH_2), 105.9 (C), 120.4 (CH), 120.8 (CH), 126.3 (CH), 126.4 (CH), 127.0 (C), 127.3 (C), 128.4 (CH), 128.5 (CH), 128.6 (CH), 128.9 (CH), 129.1 (CH), 130.3 (CH), 130.5 (CH), 135.6 (C), 138.9 (C), 139.4 (C), 147.1 (C), 162.1 (C), 162.2 (C), 166.2 (C); MS (ES) *m*/*z* 440, 442 (M + 1). Anal. ($C_{22}H_{26}ClN_7O$) C, H, N.

***R*-(−)-2,4-Diamino-5-{3-[3-(2-hydroxyethyl)-3-(α-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (12d):** From **6a** and **11d**; 78% (from acetone), mp 143−144 °C (efferv.); $[\alpha]_D$ −146.7° (*c* 0.375 mg mL^{-1}, EtOH, 17.6 °C); IR (KBr) 3322, 3169 (N−H), 1632, 1553, 1439, 1381, 1059, 700 cm^{-1}; ^{1}H NMR 0.99 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.7 (d, J = 6.8 Hz, 3H, CHCH_3), 2.12 (q, J = 7.4 Hz, 2H, CH_2CH$_3$), 3.52−3.89 (m, 4H, CH_2CH_2), 4.81 (t, J = 5.2 Hz, 1H, OH), 5.2 (q, J = 6.8 Hz, 1H, CHCH$_3$), 5.67 (brs, 2H, NH_2), 5.92 (brs, 2H, NH_2), 6.93−6.97 (m, 1H), 7.22−7.39 (m, 6H), 7.48−7.51 (m, 1H); ^{13}C NMR δ 13.5 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 50.4 (CH_2), 56.7 (CH_2), 63.9 (CH), 105.9 (C), 120.7 (CH), 126.9 (CH), 127.2 (C), 127.8 (CH), 128.6 (C), 128.8 (CH), 130.5 (CH), 135.7 (C), 141.9 (C), 147.3 (C), 162.2 (C), 166.6 (C); MS (CI) *m*/*z* 440, 442 (M + 1). Anal. ($C_{22}H_{26}ClN_7O$) C, H, N.

***S*-(+)-2,4-Diamino-5-{3-[3-(2-hydroxyethyl)-3-(α-methylbenzyl)triazen-1-yl]-4-chlorophenyl}-6-ethylpyrimidine (12e):** From **6a** and **11e**; 78% (from acetone), mp 157−158 °C (efferv.); $[\alpha]_D$ +182.1° (*c* 0.302 mg mL^{-1}, EtOH, 20.3 °C); IR (KBr) 3322, 3169 (N−H), 1632, 1553, 1439, 1381, 1059, 700 cm^{-1}; ^{1}H NMR 0.99 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.7 (d, J = 6.8 Hz, 3H, CHCH_3), 2.12 (q, J = 7.4 Hz, 2H, CH_2CH$_3$), 3.52−3.89 (m, 4H, CH_2CH_2), 4.81 (t, J = 5.2 Hz, 1H, OH), 5.2 (q, J = 6.8 Hz, 1H, CHCH$_3$), 5.67 (brs, 2H, NH_2), 5.92 (brs, 2H, NH_2), 6.93−6.97 (m, 1H), 7.22−7.39 (m, 6H), 7.48−7.51 (m, 1H); ^{13}C NMR δ 13.5 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 50.4 (CH_2), 56.7 (CH_2), 63.9 (CH), 105.9 (C), 120.7 (CH), 126.9 (CH), 127.2 (C), 127.8 (CH), 128.6 (C), 128.8 (CH), 130.5 (CH), 135.7 (C), 141.9 (C), 147.3 (C), 162.2 (C), 166.6 (C); MS (ES) *m*/*z* 440, 442 (M + 1). Anal. ($C_{22}H_{26}ClN_7O$) C, H, N.

2,4-Diamino-5-[3-(3-benzyl-3-methyltriazen-1-yl)-4-chlorophenyl]-6-ethylpyrimidine (13a): From **6b** and *N*-methylbenzylamine, 69% (from ethanol), mp 190−191 °C (lit.[7] mp 196−197 °C); ^{1}H NMR δ 0.98 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.12 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.13 and 3.52 (2 × brs, 3H, NCH_3), 5.01 (brs, 2H, CH_2Ph), 5.62 (brs, 2H, NH_2), 5.88 (brs, 2H, NH_2), 6.95 (dd, J = 1.6,8.3 Hz, 1H), 7.27−7.37 (m, 6H), 7.48 (d, J = 8.5 Hz, 1H).

2,4-Diamino-5-[3-(3-benzyl-3-ethyltriazen-1-yl)-4-chlorophenyl]-6-ethylpyrimidine (13b): From **6b** and *N*-ethylbenzylamine, 75% (from ethanol), mp 153−154 °C (efferv.); IR (KBr) 3451, 3314, 3165 (NH), 1630, 1561, 1439, 1346, 1173 cm^{-1}; ^{1}H NMR δ 0.97 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.09−1.26 (m, 3H, NCH_2CH_3), 2.11 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.71−3.85 (m, 2H, NCH_2CH$_3$), 4.98 (brs, 2H, CH_2Ph), 5.62 (brs, 2H, NH_2), 5.88 (brs, 2H, NH_2), 6.94 (d, J = 8.3 Hz, 1H), 7.23−7.49 (m, 7H); ^{13}C NMR δ 10.3 (CH_3), 13.4 (CH_3), 14.3 (CH_3), 27.7 (CH_2), 42.0 (CH_2), 49.3 (CH_2), 49.8 (CH_2), 57.6 (CH_2), 105.7 (C), 120.6 (CH), 127.1 (C), 128.5 (CH), 128.7 (CH), 130.5 (CH), 135.7 (C), 137.1 (C), 146.9 (C), 162.1 (C), 162.3 (C), 166.5 (C); MS (CI) *m*/*z* 410, 412 (M + 1). Anal. ($C_{21}H_{24}ClN_7$) C, H, N.

General Procedure of the Synthesis of Triazenyl-Substituted 2-Chlorobenzenes 13c−e. 2-Chloroaniline (1.53 g) in 1 M HCl (30 mL) was diazotized at 0 °C with a solution of sodium nitrite (1.1 mol equiv). The mixture was maintained at 0 °C (1 h), and the appropriate secondary amine or amine salt (1 mol equiv) was added to the vigorously stirred mixture followed by sodium carbonate to adjust the pH to 9−10. The triazenes separated as light brown oils which were extracted into diethyl ether (2 × 25 mL). The organic layer was separated, washed successively with brine and water (50 mL), dried ($MgSO_4$), and evaporated to yield a residue which was purified by flash column chromatography employing hexanes−ethyl acetate (10:1) as eluent. The viscous triazenes decomposed when subjected to vacuum distillation.

The following triazenyl-substituted 2-chlorobenzenes were prepared by this general method.

2-(3-Benzyl-3-methyltriazen-1-yl)chlorobenzene (13c): From *N*-methylbenzyl-amine, 77%; IR (NaCl) 3965, 1464, 1348, 1179, 1053, 750, 698, 588 cm^{-1}; ^{1}H NMR δ 3.12 (s, 3H, CH_3), 5.02 (s, 2H CH_2Ph), 7.13−7.49 (m, 9H); ^{13}C NMR δ 34.7 (CH_3), 59.1 (CH_2), 118.9 (CH), 126.4 (CH), 127.7 (CH), 128.0 (CH), 128.3 (C), 128.9 (CH), 130.1 (CH), 136.8 (C), 146.9 (C); HR-MS (FAB) calcd for $C_{14}H_{14}ClN_3$ (M) *m*/*z* 259.08762, found 259.08704.

2-(3-Benzyl-3-ethyltriazen-1-yl)chlorobenzene (13d): From *N*-ethylbenzyl-amine, 79%; IR (NaCl) 2970, 1468, 1048, 1327, 1173, 1053, 754, 698 cm^{-1}; ^{1}H NMR δ 1.10−1.25 (m, 3H, NCH_2CH_3), 3.70−3.85 (m, 2H, NCH_2CH$_3$), 4.98 (s, 2H, CH_2-Ph), 7.02−7.46 (m, 9H); ^{13}C NMR δ 10.3 (CH_3), 14.3 (CH_3), 41.9 (CH_2), 49.1 (CH_2), 49.7 (CH_2), 57.6 (CH_2), 118.7 (CH), 126.3 (CH), 127.2 (CH), 128.1 (CH), 128.4 (CH), 128.7 (CH), 130.0 (CH), 136.4 (C), 137.1 (C), 146.9 (C); HR-MS (EI) calcd for $C_{15}H_{16}ClN_3$ (M) *m*/*z* 273.10327, found 273.10295.

2-[3-(2-Acetyloxyethyl)-3-benzyltriazen-1-yl]chlorobenzene (13e): From 2-(benzylamino)ethyl acetate hydrochloride, 92%; IR (NaCl) 1742 (C═O), 1463, 1375, 1348, 1236, 1163, 1053, 750 cm^{-1}; ^{1}H NMR δ 1.92 (s, 3H, CH_3), 3.91−4.30 (m, 4H, CH_2CH_2), 5.02 (s, 2H, CH_2Ph), 7.16−7.43 (m, 9H); HR-MS (EI) calcd for $C_{17}H_{18}ClN_3O_2$ (M) *m*/*z* 331.10876, found 331.10889.

Enzymatic Inhibition Assays. Spectrophotometric assays used to determined the DHFR potency have been described in detail previously.[17−19] Briefly, activity of the target compounds (series **3**, **10**, and **12**) was reported in terms of drug concentration required to reduce by 50% the rate of enzymatic reduction of dihydrofolate to tetrahydrofolate in the presence of cofactor NADPH compared to control (IC_{50}).

Each standard assay mixture was made up of phosphate buffer (40.7 mM, pH 7.4) to a total volume of 1 mL which contained the following materials: NADPH (0.117 mM), dihydrofolate (0.092 mM), 2-mercaptoethanol (8.9 mM), and DHFR enzyme (0.018 units of activity; under standard conditions each unit of DHFR reduces 1 μM dihydrofolate/min).

References

(1) Palát, K.; Stevens, M. F. G. Structural Studies on Bioactive Compounds. Part 33. Synthesis of 9-Arylacridines by Palladium-mediated Couplings. *J. Chem. Res. (S)*, **2000**, 136−137.

(2) AIDS Epidemic Update; December 1998. http://www.who.int/emchiv/december1998/wadr98e.pdf

(3) Kovacs, J. A.; Hiemenz, J. W.; Macher, A. M.; Stover, D.; Murray, H. W.; Shelhamer, J.; Lane, H. C.; Urmacher, C.; Honig, C.; Longo, D. L.; Parker, M. W.; Natanson, C.; Parrillo, J. E.; Fauci, A. S.; Pizzo, P. A.; Masur, H. *Pneumocystis carinii* Pneumonia: A Comparison Between Patients with the Acquired Immunodeficiency Syndrome and Patients with Other Immunodeficiencies. *Ann. Intern. Med.* **1984**, *100*, 663−671.

(4) Queener, S. F.; Bartlett, M. S.; Jay, M. A.; Durkin, M. M.; Smith, J. W. Activity of Lipid-soluble Inhibitors of Dihydrofolate Reductase Against *Pneumocyctis carinii* in Culture and in a Rat Model of Infection. *Antimicrob. Agents Chemother.* **1987**, *31*, 1323−1327.

(5) Klepser, M.; Klepser, T. B. Drug Treatment of HIV-related Opportunistic Infections. *Drugs* **1997**, *53*, 40−73. Brun-Pascaud, M.; Chau, F.; Garry, L.; Jacobus, D.; Derouin, F.; Girard, P. Combination of PS-15, Epiroprim, or Pyrimethamine with Dapsone in Prophylaxis of *Toxoplasma gondii* and *Pneumocystis carinii* Dual Infection in a Rat Model. *Antimicrob. Agents*

Chemother. **1996**, *40*, 2067−2070. Torre, D.; Casari, S.; Speranza, F.; Donisi, A.; Gregis, G.; Poggio, A.; Ranieri, S.; Orani, A.; Angarano, G.; Chiodo, F.; Fiori, G.; Carosi, G.; Group, T. I. C. S. Randomised Trial of Trimethoprim-Sulphamethoxazole versus Pyrimethamine-Sulfadiazine for Therapy of Toxoplasmic Encephalitis in Patients with AIDS. *Antimicrob. Agents Chemother.* **1998**, *42*, 1346−1349.

(6) Brun-Pascaud, M.; Rajagopalan-Levasseur, P.; Chau, F.; Bertrand, G.; Garry, L.; Derouin, F.; Girard, P. M. Drug Evaluation of Concurrent *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium* Complex Infections in a Rat Model. *Antimicrob. Agents Chemother.* **1998**, *42*, 1068−1072.

(7) Stevens, M. F. G.; Phillip, K. S.; Rathbone, D. L.; O'Shea, D. M.; Queener, S. F.; Schwalbe, C. H.; Lambert, P. A. Structural Studies on Bioactive Compounds. 28. Selective Activity of Triazenyl-Substituted Pyrimethamine Derivatives against *Pneumocystis carinii* Dihydrofolate Reductase. *J. Med. Chem.* **1997**, *40*, 1886−1893.

(8) Champness, J. N.; Achari, A.; Ballantine, S. P.; Bryant, P. K.; Delves, C. J.; Stammers, D. K. The Structure of *Pneumocystis carinii* Dihydrofolate Reductase to 1.9 Å Resolution. *Structure* **1994**, *2*, 915−924.

(9) Gangjee, A.; Guo, X.; Queener, S. F.; Cody, V.; Galitsky, N.; Luft, J.; Pangborn, W. Selective *Pneumocystis carinii* Dihydrofolate Reductase Inhibitors: Design, Synthesis, and Biological Evaluation of New 2,4-Diamino-5-substituted-furo[2,3-*d*]pyrimidines. *J. Med. Chem.* **1998**, *41*, 1263−1271.

(10) Cody, V.; Chan, D.; Galitsky, N.; Rak, D.; Luft, J. R.; Pangborn, W.; Queener, S. F.; Laughton, C. A.; Stevens, M. F. G. Structural Studies on Bio-active Compounds. 30. Crystal Structure and Molecular Modeling Studies on the*Pneumocystis carinii* Dihydrofolate Reductase Cofactor Complex with TAB, a Highly Selective Antifolate. *Biochemistry* **2000**, *39*, 3556−3564.

(11) Bliss, E. A.; Griffin, R. J.; Stevens, M. F. G. Structural Studies on Bio-active Compounds. Part 5. Synthesis and Properties of 2,4-Diaminopyrimidine Dihydrofolate Reductase Inhibitors bearing Lipophilic Azido Groups. *J. Chem. Soc., Perkin Trans. I* **1987**, 2217−2228.

(12) Griffin, R. J.; Schwalbe, C. H.; Stevens, M. F. G.; Wong, K. P. Structural Studies on Bio-active Compounds. Part 3. Reexamination of the Hydrolysis of the Antimalarial Drug Pyrimethamine and Related Derivatives and Crystal Structure of a Hydrolysis Product. *J. Chem. Soc., Perkin Trans. I* **1985**, 2267−2276.

(13) Morie, T.; Kato, S.; Harada, H.; Yoshida, N.; Matsumoto, J. I. Synthesis and Biological Activities of the Optical Isomers of (±)-4-Amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]-methyl]benzamide (Mosapride). *Chem. Pharm. Bull.* **1994**, *42*, 877−882.

(14) Edwards, S. L.; Sherfinski, J. S.; Marsh, R. E. The Crystal Structure of the Antitumor Agent 5-(3,3-Dimethyl-1-triazeno)-imidazole-4-carboxamide Monohydrate Hydrochloride (NSC-45388). *J. Am. Chem. Soc.* **1974**, *96*, 2593−2597.

(15) Hooper, D. L.; Pottie, I. R.; Vacheresse, M.; Vaughan, K. 1,2-Bis(1-aryl-3-alkyltriazen-3-yl)ethanes and Related Compounds. *Can. J. Chem.* **1998**, *76*, 125−135.

(16) Akhtar, M. H.; McDaniel, R. S.; Feser, M.; Oehlschlager, A. C. NMR Study of Hindered Rotation in 1-Aryl-3,3-dimethyltriazenes. *Tetrahedron* **1968**, *24*, 3899−3906.

(17) Broughton, M. C.; Queener, S. F. *Pneumocystis carinii* Dihydrofolate Reductase Used to Screen Potential Antipneumocystis Drugs. *Antimicrob. Agents Chemother.* **1991**, *35*, 1348−1355.

(18) Chio, L.-C.; Queener, S. F. Identification of Highly Potent and Selective Inhibitors of *Toxoplasma gondii* Dihydrofolate Reductase. *Antimicrob. Agents Chemother.* **1993**, *37*, 1914−1923.

(19) Chio, L.-C.; Boylard, L. A.; Nasr, M.; Queener, S. F. Identification of a Class of Sulfonamides Highly Active Against Dihydropteroate Synthase from *Toxoplasma gondii*, *Pneumosystis carinii*, and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1996**, *40*, 727−733.

(20) Rosowsky, A.; Cody, V.; Galitsky, N.; Fu, H.; Papoulis, A. T.; Queener, S. Structure-Based Design of Selective Inhibitors of Dihydrofolate Reductase: Synthesis and Antiparasitic Activity of 2,4-Diaminopteridine Analogues with a Bridged Diarylamine Side Chain. *J. Med. Chem.* **1999**, *42*, 4853−4860.

(21) Biosym, *Insight II 95.0.* **1995**. 9685 Scranton Road, San Diego, CA 9221, USA.

(22) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An All Atom Force Field for Simulations of Proteins and Nucleic Acid. *J. Comput. Chem.* **1986**, *7*, 230−252.

(23) Bárbière, J. Estérification Nitrique et Nitration d'Amino-alcools. *Bull. Soc. Chim. Fr.* **944**, *5*, 470−480.

(24) Bárbière, J. Nitration d'Aralcoylamino-alcools. *Bull. Soc. Chim. Fr.* **1940**, *7*, 621−626.

(25) Potapov, V. M.; Dem'yanovich, V. M.; Maleev, V. I. Derivatives of Optically Active (1-Phenylethyl)amine in Complexes with Lithium Aluminium Hydride for the Asymmetric Reduction of a Carbonyl Group. *J. Org. Chem. USSR Eng. Trans.* **1985**, *21*, 1606−1609.

JM0108698