Conformationally Restricted Analogues of Trimethoprim: 2,6-Diamino-8-substituted Purines as Potential Dihydrofolate Reductase Inhibitors from *Pneumocystis carinii* and *Toxoplasma gondii*[1]

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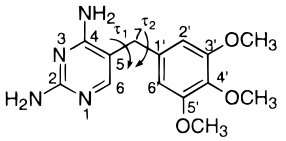
Twenty-two 2,6-diamino-8-substituted purines (**2**−**23**) were synthesized, in which rotation around the two flexible bonds of trimethoprim (TMP), linking the pyrimidine ring to the side chain phenyl ring, was restricted by incorporation into a purine ring, in an attempt to increase the potency and selectivity of TMP against dihydrofolate reductase (DHFR) from the organisms that often cause fatal opportunistic infections in patients with AIDS, *i.e.*, *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg). The syntheses of analogues **2**−**20** were achieved *via* a one-pot reaction of 2,4,5,6-tetraaminopyrimidine and the appropriately substituted benzaldehyde or phenyl acetaldehyde, in acidic methoxyethanol. Analogues **21**−**23** were synthesized *via* nucleophilic displacement of 2,6-diamino-8-(chloromethyl)purine with the appropriate anilines or 2-naphthalenethiol. The compounds were evaluated as inhibitors of pcDHFR and tgDHFR with rat liver (rl) DHFR as the mammalian reference enzyme. Compound **11**, the 3′,4′-dichlorophenyl analogue, was as potent as TMP and had a selectivity ratio of 13 for pcDHFR, which ranked it as one of the three most selective inhibitors of pcDHFR (compared to rlDHFR) known to date. It also displayed a selectivity ratio of 38 for tgDHFR. None of the other analogues showed any improvement compared to TMP in potency or selectivity. In the preclinical *in vitro* screening program of the National Cancer Institute, compound **11** showed a GI_{50} of 10^{-6} M for the inhibition of the growth of 17 tumor cell lines.

Infections with *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg) are a major cause of morbidity and mortality in patients with acquired immunodeficiency syndrome (AIDS), as well as in patients with other immunosuppresive conditions.[2,3] Regimens with documented efficacy for the treatment of these infections include trimethoprim sulfamethoxazole,[4] pentamidine,[4] trimethoprim dapsone,[5] and trimetrexate[6] for *P. carinii* infections and pyrimethamine plus a sulfonamide[7] for the treatment of *T. gondii* infections. The treatment of *P. carinii* and *T. gondii* infections with antifolates such as trimethoprim (TMP), trimetrexate (TMQ), and pyrimethamine takes advantage of the fact that these organisms are permeable to lipophilic, nonclassical antifolates and, unlike mammalian cells, lack the carrier-mediated active transport system(s) required for the uptake of classical antifolates with polar glutamate side chains.[8] Thus host tissues can be selectively protected by the coadministration of the reduced folate leucovorin, which is selectively taken up by mammalian cells and reverses toxicity associated with nonselective dihydrofolate reductase (DHFR) inhibitors such as TMQ.[9] Further, these lipophilic agents can penetrate into the central nervous system (CNS) where *T. gondii* infections are a major cause of focal encephalitis.



TMP

TMP and pyrimethamine are weak inhibitors of pcDHFR and tgDHFR and hence have to be used with sulfonamides to augment their inhibition of folate metabolism. TMQ and piritrexim (PTX), which are 100−10000 times more potent than TMP and pyrimethamine against DHFR from *P. carinii* and *T. gondii*, are also potent inhibitors of mammalian DHFR[10] and hence lack selectivity, and their use is associated with considerable toxicity. As a result, TMQ has to be coadministered with leucovorin. Unfortunately, adverse effects are often observed, and side effects associated with the use of sulfa drugs often necessitate discontinuation of therapy.[11]

As part of a program aimed at identifying potent and selective inhibitors of *P. carinii* and/or *T. gondii* DHFR as potential treatment of these infections, we have reported the design and synthesis of a variety of 6-6 ring-fused analogues,[12−16] as well as 6-5 ring-fused analogues,[17,18] with the objective of increasing the selectivity of the significantly potent, nonselective bicyclic DHFR inhibitors TMQ and PTX for pcDHFR and tgDHFR. Some of these compounds[15] were potent and selective inhibitors of pcDHFR and tgDHFR, with one analogue, 2,4-diamino-6-[*N*-(2′,5′-dimethoxybenzyl)-*N*-methylamino]pyrido[2,3-*d*]pyrimidine (**1**, X = H, Y = NCH_3, Z = CH_2, R = 2,5-OCH_3), displaying selectivity ratios of 100 and 309 for pcDHFR and tgDHFR (compared to human (h) DHFR), respectively. These compounds were designed as analogues of TMQ and PTX, with the specific objective of increasing the selectivity of these drugs for pcDHFR and/or tgDHFR. An alternate approach for the design of drugs useful for the

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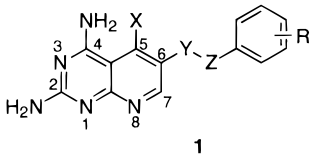

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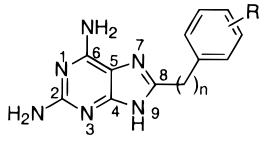
X = H, CH_3
Y = CH_2, NH, $N(CH_3)$
Z = NH, $N(CH_3)$, CH_2

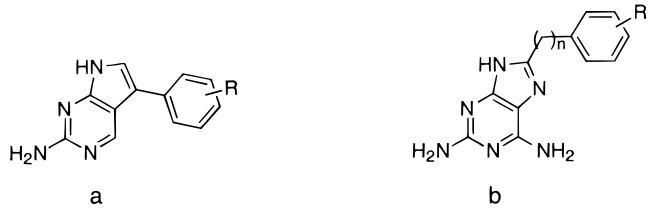
treatment of these opportunistic infections is to attempt to improve the potency of the selective, clinically useful agent TMP. As mentioned previously, TMP is a selective but weak inhibitor of pcDHFR and hence has to be used along with sulfa drugs for synergistic effects against DHFR. Since the sulfa drug is the principal culprit[11] of adverse effects associated with TMP sulfamethoxazole, a DHFR inhibitor which would combine the selectivity of TMP and the high potency of TMQ or PTX would be a viable single agent in the treatment of these opportunistic infections.

The side chain of TMP can adopt a variety of different conformations due to rotational flexibility around torsion angles τ_1 and τ_2, several of which are unfavorable for the inhibition of pcDHFR and/or tgDHFR. It is well known that the conformation of the trimethoxyphenyl side chain of TMP is responsible, in part, for its selectivity for bacterial DHFR.[19] One method of increasing the potency and/or selectivity of TMP is to introduce conformational restriction of the side chain in an appropriate orientation to optimally interact with pcDHFR and/or tgDHFR. The literature contains several examples of TMP analogues with bridging atoms other than a methylene moiety.[20,21] Chan and Roth[22] have reported elegant efforts to improve the antibacterial activity of TMP by conformational restriction of the side chain *via* incorporation into a dihydroindane ring. However, no significant improvement in *Escherichia coli* DHFR inhibition was obtained, presumably due to the steric crowding caused by the extra atoms needed to orient the side chain in the desired conformation. Recently, Kuyper *et al.*[23] reported attempts to restrict rotation around τ_1 of TMP by linking the 4-amino moiety with the methylene bridge, to afford either a pyrrolo[2,3-*d*]pyrimidine or a pyrido[2,3-*d*]pyrimidine. We elected to restrict rotation around τ_1 and τ_2 by incorporation of the C5−C7 and C7−C1′ bonds of TMP into a purine



2-23

ring, as in analogues **2**−**23** (Table 1). The choice of the ring structure (*i.e.*, purine) for conformational restriction was based on the knowledge that unsubstituted 2,6-diaminopurines have been reported[24] to be competitive inhibitors of chicken liver (cl) DHFR, with K_i values similar to that for 2,4-diamino-6-methylpteridines and more than that of 2,4-diaminopyrimidines. Nonclassical 2,4-diamino-substituted pyrido[2,3-*d*]pyrimidine antifolates are potent inhibitors of pcDHFR and tgDHFR, and since the potency against DHFR of unsubstituted purines and pyrido[2,3-*d*]pyrimidines was similar,[24] it was reasoned that conformational restriction of the side chain bridge of TMP *via* incorporation in a purine ring



a b

Figure 1. (a) Observed mode of binding of pyrrolo[2,3-*d*]pyrimidine antifolates to *E. coli* DHFR.[24] (b) Possible mode of binding of analogues **2**−**23** to DHFR.

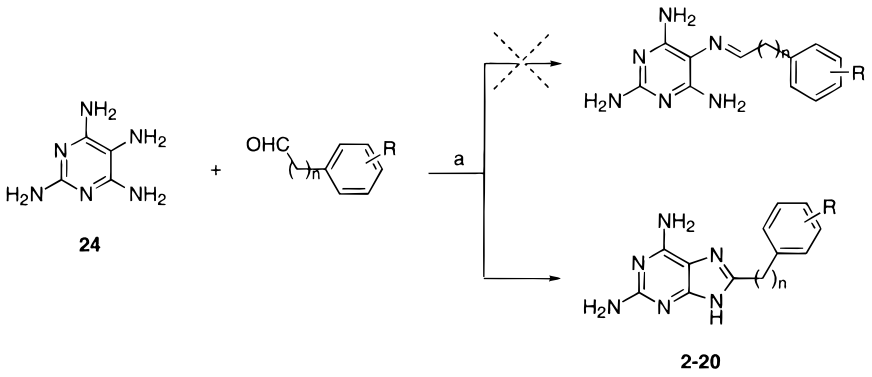
should afford analogues with defined restricted geometry. Such analogues could result in improved potency while maintaining selectivity and were worth exploring. The pK_a of 2,6-diaminopurines (5.09)[25] is similar to that of 2,4-diaminopteridines (5.32) such as methotrexate (MTX), which is a potent DHFR inhibitor, further supporting the hypothesis that appropriately substituted 2,6-diaminopurine analogues could be potent inhibitors of DHFR. Elion *et al.*[26] reported several 2,6-diamino-8-substituted purines in the 1950s and evaluated them against the growth of *L. casei* cells in culture. However, to our knowledge, no DHFR inhibition studies against *P. carinii* or *T. gondii* were reported for these analogues.

Molecular modeling using SYBYL 6.0,[27] and its SEARCH and MAXIMIN options, and superimposition of the pyrimidine ring of the energy-minimized conformations of analogues **2**−**19** onto the pyrimidine ring of the *E. coli* DHFR-bound conformation of TMP[19,28] indicated that the side chain aryl ring of these analogues occupied similar but not identical orientations as the side chain of TMP in its bound conformation. This supports the premise that though there is an extra atom between the pyrimidine ring and the side chain of analogues **2**−**19** as compared to TMP, the distance between the center of the pyrimidine ring and the phenyl ring in TMP and analogues **2**−**19** is quite similar but not identical. The crystal structure of TMP with pcDHFR[29] showed that TMP binds to pcDHFR in a conformation similar to its binding mode in *E. coli* DHFR; thus the use of *E. coli* DHFR-bound TMP as the template was a good approximation for TMP bound to pcDHFR. The coordinates of pcDHFR with TMP were unavailable at the time the target compounds of this study were designed.

Analogues **20**−**23** were synthesized with the intent of extending the distance between the side chain phenyl ring and the pyrimidine moiety, thus probing alternate binding sites in pcDHFR and tgDHFR. Molecular superimposition indicates that the 6-substituent of 6-5 ring-fused analogues lies between the 6- and 7-substituents of a 6-6 ring-fused system. Most 6-substituted 6-6 ring-fused nonclassical compounds of the pyrido[2,3-*d*]pyrimidine series synthesized in our laboratory[12−16] have been found to potently inhibit DHFR. Hence, it was reasoned that extension of the bridge length as in analogues **20**−**23** would allow for an increased distance between the heterocycle and the hydrophobic side chain compared to that in analogues **2**−**19** and would approximate the distance in the pyrido[2,3-*d*]pyrimidine analogues.

Kuyper *et al.*[24] have shown *via* X-ray crystallography that 4-desamino pyrrolo[2,3-*d*]pyrimidine analogues bind to *E. coli* DHFR in the 'folate mode' (Figure 1). Analogues **2**−**23** may also bind to DHFR in the 'folate mode' (Figure 1), with the N9-H functioning as the

Scheme 1[a]



[a] Key: (a) methoxyethanol, HCl.

4-amino moiety and interacting with Ile 10 (pcDHFR numbering). The N1-nitrogen (purine numbering) is speculated[30,31] to be the most basic nitrogen in the purines. Using semiempirical methods[32] for calculating the electron density of each atom of a 2,6-diaminopurine, the N1-nitrogen of the purine was calculated to have a Mulliken charge density greater than the N3-nitrogen. This suggested that binding of the purine analogues in the 'folate mode' would enable the N1-nitrogen of the purine, upon protonation, in conjunction with the 2-amino moiety, to participate in the salt bridge with Glu 32 and Wat 403 (pcDHFR numbering),[29] similar to that observed in the binding of TMP with *E. coli* DHFR[28,33] and pcDHFR.[29]

The side chain substituents for the target analogues include some substituents such as the methoxyphenyl rings which we have found in prior studies[12−16] to be conducive to potency and/or selectivity. Other groups including electron-withdrawing moieties and naphthyl and fluorenyl moieties were included to provide a diverse group of side chains, some of which we have also reported to possess high potency and/or selectivity for pcDHFR and/or tgDHFR in the pyrido-[15] and pyrrolo[2,3-*d*]pyrimidine[18] series. The use of different side chains was important for exploring tgDHFR inhibition in particular, since the crystal structure of tgDHFR is not yet available.

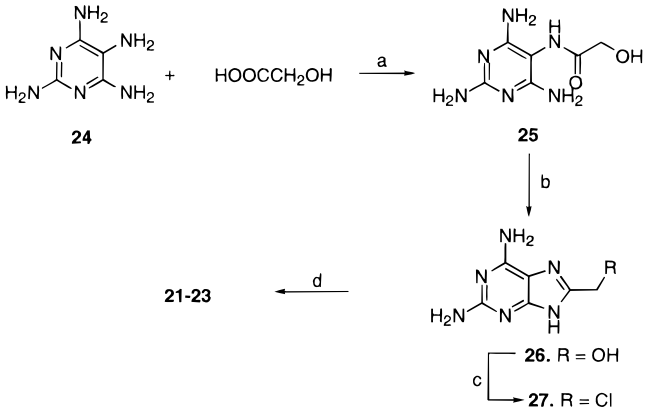
Chemistry

A search of the literature revealed that a variety of methods exist for the synthesis of 8-substituted purines.[34] Among the most widely used method is the reaction of an appropriately substituted benzoic acid and a 5,6-diaminopyrimidine followed by acid-catalyzed ring closure.[35] This methodology requires two steps for the assembly of the purine ring, involving initial formation of the 4-amino-5-(acylamino)pyrimidine, followed by ring closure. The synthetic scheme utilized in this study for the synthesis of 8-substituted purine analogues is outlined in Scheme 1 and is a modification of the Traube synthesis[36] between a 4,5-diaminopyrimidine and a substituted benzaldehyde.[37] This method has been widely used for the synthesis of benzimidazoles[38] and has been employed for purine synthesis only for xanthine derivatives.[39] This methodology theoretically requires two steps, with the initial formation of the Schiff base between the 5-amino moiety and the aldehyde followed by oxidative cyclization in the presence of ferric chloride or copper acetate.[38,39] Kuznetsov and Remizov[40] reported an attempted one-step cyclization of the intermediate Schiff base between 2,4,5,6-tetraaminopyrimidine and a benzaldehyde without isolation, in an acetate buffer at pH 4.5. However, they isolated a number of other side products and reported that this method did not have any preparative utility. We decided to investigate the *one-step* cyclization of a 4,5-diaminopyrimidine and substituted benzaldehydes in weakly acidic media with concomitant air oxidation to afford the desired purines.

2,4,5,6-Tetraaminopyrimidine (**24**) was heated in methoxyethanol, and at reflux 1 N hydrochloric acid was added dropwise to solubilize the pyrimidine. This was followed by the addition of the appropriately substituted benzaldehyde, following which the reaction was refluxed overnight. TLC analyses indicated the presence of an intense, UV-absorbing product along with some unreacted aldehyde. In all the reactions performed (except for the synthesis of analogues **12**, **16**, and **17**), at the end of the reaction period, a thick precipitate had formed, which was filtered and collected to afford the desired purines in 35−40% yield. No other products were observed on TLC, and thus, analytically pure target compounds were obtained in a single step, using comparatively mild conditions. That purine ring formation had occurred was confirmed by the ^{1}H NMR in deuterated dimethyl sulfoxide, which indicated the presence of an exchangeable proton at 11.80−13.00 ppm, corresponding to the purine N9-H. Further, the presence of only the 2- and 4-amino proton, indicated that the 5- and 6-amino moieties had participated in the reaction. Had the reaction stopped at the Schiff base, the ^{1}H NMR would have indicated the presence of three sets of amino groups along with the presence of an imine proton of the Schiff base. The absence of these features, as well as corroboration *via* mass spectrometry, confirmed purine ring formation. For analogues **12**, **16**, and **17**, at the end of the reaction, the solvent was evaporated and chromatographic purification was necessary to afford the desired analogues. In all the compounds synthesized in this study, the exhangeable proton at 11.80−13.00 ppm was assigned to the N9-H rather than the N7-H based on the comparison of the ground-state energies[41] of an N9-H purine as compared to an N7-H purine. Even though the N7-H purine has been observed predominantly in crystal structures of purines, the N9-H isomer is more stable than the N7-H isomer by 2 kcal/mol.[42]

For the synthesis of analogue **20**, a similar reaction with phenylacetaldehyde which involved refluxing the reaction mixture overnight in acidic methoxyethanol resulted in a complex mixture of products on TLC. However when the reaction was performed in acidic

Scheme 2[a]



[a] Key: (a) 110 °C, 2 h; (b) 1 N NaOH; (c) $SOCl_2$, DMF; (d) ArXH, K_2CO_3.

methoxyethanol for 6 h, followed by workup and purification, compound **20** was obtained in 12% yield.

The synthesis of analogues **21**−**23** required the versatile and important intermediate, 2,6-diamino-8-(chloromethyl)purine (**27**). Weinstock *et al.*[43] have reported an improved synthesis of the original method utilized by Baker and Santi[44] for the synthesis of this intermediate. Adoption of this method using 2,4,5,6-tetraaminopyrimidine (**24**) fused with glycolic acid for 4 h (Scheme 2) followed by workup and basification afforded 2,4,6-triamino-5-(glycoamido)pyrimidine, **25**. Base-catalyzed ring closure followed by acidification afforded 2,6-diamino-8-(hydroxymethyl)purine (**26**) in 76% yield. Chlorination of **26** with thionyl chloride in DMF afforded the desired 8-chloromethyl derivative **27**, which was sufficiently pure to be used in subsequent transformations.

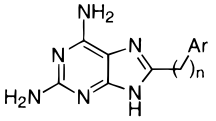
3,4,5-Trimethoxyaniline and 2,5-dimethoxyaniline were alkylated with **27** in *N*,*N*-dimethylacetamide using potassium carbonate to afford, after purification, analogues **21** and **22** in 56% and 52% yields, respectively. Similarly, 2-naphthalenethiol was alkylated with **27** to afford **23** in 65% yield.

Biological Evaluation and Discussion

Analogues **2**−**23** were evaluated against pcDHFR, tgDHFR, and rlDHFR which served as the mammalian source, and the results are reported in Table 2. These assays were performed at 37 °C, under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NADPH) saturation; tgDHFR and rlDHFR were assayed in the presence of 150 mM KCl.[45,46] Selectivity ratios, determined as the ratio of the inhibition constant (IC_{50}) of rlDHFR *vs* pcDHFR or tgDHFR, are also reported in Table 2. The corresponding values for TMP, TMQ, and PTX are included in Table 2 for comparison.

Compound **2**, which has an unsubstituted phenyl ring, displayed micromolar activity against pcDHFR. Against tgDHFR, it was 10-fold less potent than TMP and showed a selectivity ratio of 2.1. This suggested that substitution of suitable functional groups on the phenyl ring may provide analogues with improved potency and/or selectivity against pcDHFR and tgDHFR. Addition of a 3,4,5-trimethoxy substituent on the phenyl ring to afford compound **3** resulted in a 5-fold increase in potency against rlDHFR compared to **2**. Against tgDHFR, compound **3** was 12-fold more potent and twice as selective as compound **2** and was as potent as TMP against tgDHFR. These encouraging results prompted the synthesis of other trimethoxyphenyl-substituted regioisomers (**4**−**6**). Compound **5**, with a 2,4,6-trimethoxyphenyl substituent, was 33-fold more potent than **2** against tgDHFR, which could be attributed to a favorable interaction of the methoxy substituent at the ortho positions with tgDHFR or the effect of conformational restriction induced by the ortho substituents, or both. The activity of compounds **4**−**7** suggests that substitution at the ortho position with a methoxy group is beneficial for tgDHFR inhibition, since compounds **4**−**7** are more potent than **2** and **3**, with compound **5** being the most potent tgDHFR inhibitor of this series.

Table 1. Target Compounds of This Study



compd	*n*	Ar
2	0	C_6H_5
3	0	3′,4′,5′-$OCH_3C_6H_2$
4	0	2′,3′,4′-$OCH_3C_6H_2$
5	0	2′,4′,6′-$OCH_3C_6H_2$
6	0	2′,4′,5′-$OCH_3C_6H_2$
7	0	2′,5′-$OCH_3C_6H_3$
8	0	3′,5′-$OCH_3C_6H_3$
9	0	3′,4′-$OCH_3C_6H_3$
10	0	2′,4′-$OCH_3C_6H_3$
11	0	3′,4′-$Cl_2C_6H_3$
12	0	2′,6′-$Cl_2C_6H_3$
13	0	3′,5′-OCH_3-4′-OHC_6H_2
14	0	2′-NO_2-4′,5′-$OCH_3C_6H_2$
15	0	4′-$C_6H_5C_6H_4$
16	0	4-C_5H_4N
17	0	2′-naphthyl
18	0	4′-OCH_3-1′-naphthyl
19	0	9′-fluorenyl
20	1	C_6H_5
21	1	NH-3′,4′,5′-$OCH_3C_6H_2$
22	1	NH-2′,5′-$OCH_3C_6H_3$
23	1	S-2-naphthyl

Table 2. Inhibitory Concentrations (IC_{50}, μM) and Selectivity Ratios against *P. carinii* (pc), *T. gondii* (tg), and Rat Liver (rl) DHFR[45,46]

compd	pcDHFR	rlDHFR	rl/pc	tgDHFR	rl/tg
2	153	59.9	0.4	28	2.1
3	59.4 (34%)	13		2.2	5.9
4	17.0 (15%)	3.7		1.8	2.1
5	31.5 (4%)	1.88		0.84	2.2
6	22 (10%)	7		1.5	4.7
7	37.4 (9%)	3.5		1.7	2.0
8	9.0 (17%)	32.4		3.0	10.8
9	12.0 (6%)	52.3		14.2	3.7
10	14.0 (11%)	0.9		2.4	0.38
11	19.5	252	**13**	6.7	**38**
12	11.0 (11%)	11.0 (4%)		11.0 (6%)	
13	68 (6%)	15.3		1	15.3
14	25 (15%)	25 (14%)		16.8	
15	605	30.1	0.05	32.9	0.91
16	18 (5%)	18 (0%)		20 (14%)	
17	113 (10%)	280		27	10.37
18	13 (0%)	13 (15%)		13 (10%)	
19	35 (12%)	29.8		30.5	1.0
20	9.8	1.6	0.16	0.5	3.2
21	34 (6%)	34 (19%)		34 (5%)	
22	29 (7%)	29 (15%)		49	
23	105	107	1.02	45	2.38
TMP	12[a]	133[a]	11.1[a]	2.7[a]	49[a]
TMQ	0.042[a]	0.003[a]	0.07[a]	0.010[a]	0.29[a]
PTX	0.031[a]	0.0015[a]	0.048[a]	0.017[a]	0.09[a]

[a] Data from ref 47. Values in parentheses are the percent inhibition at the highest concentration tested.

Comparison of the inhibition of compounds **7**−**10** indicates the effect of dimethoxy-substituted compounds on all three DHFRs. Once again, substitution at the ortho position with a methoxy moiety, such as in compounds **7** and **10**, showed better inhibitory potency against rlDHFR and tgDHFR, compared to **8** and **9** which are unsubstituted in the ortho positions. Compound **8**, with a selectivity ratio of 10.8 against tgDHFR, was 36- and 77-fold more selective than TMQ and PTX, respectively, as an inhibitor of tgDHFR. Comparison of the activity of compounds **3** and **8** suggests that substitution at the para position with a methoxy group is somewhat detrimental to the inhibition of rlDHFR, but not for tgDHFR, resulting in a 2-fold increase in the selectivity of **8** for tgDHFR.

Compounds **11** and **12** were synthesized to evaluate the effect of electron-withdrawing chloro groups. Replacement of the methoxy groups of **9** with chloro groups to afford compound **11** resulted in a 2-fold increase in tgDHFR inhibition and a 5-fold decrease in rlDHFR inhibition. As a result, compound **11** had selectivity ratios of 13 and 38 for the inhibition of pcDHFR and tgDHFR, respectively. This analogue is almost equipotent with TMP as an inhibitor of pcDHFR. Further, it is one of the three most selective inhibitors of pcDHFR (compared to rlDHFR) reported to date (the most selective inhibitor of pcDHFR reported to date, 2,4-diamino-6-[(thiophenyl)methyl]pteridine, has a selectivity ratio of 25[47] under the conditions of the assay used in these studies). The selectivity ratio of compound **11** for tgDHFR was also impressive, and it is the most selective inhibitor of pcDHFR and tgDHFR of this entire series. The solubility of **12** precluded its evaluation beyond 11 μM. Replacement of the 4′-methoxy group of compound **3** with a hydroxy group to afford **13** results in a 2-fold increase in inhibition against tgDHFR. Further, this analogue displays a 15-fold selectivity for tgDHFR. Replacement of the *o*-methoxy group of analogue **6** with an electron-withdrawing nitro group (**14**) results in decreased inhibition against both rlDHFR and tgDHFR. Substitution of a phenyl ring at the para position of compound **2** also results in decreased inhibition of pcDHFR and tgDHFR. Further, replacement of the phenyl ring of **2** with a 2-naphthyl ring as in compound **17** results in a 5-fold increase in selectivity against tgDHFR. On the other hand, replacing the phenyl ring of **2** with a fluorenyl moiety in **19** results in increased inhibition of rlDHFR, with no change against tgDHFR.

To study the effect of increasing the distance between the purine nucleus and the hydrophobic side chain to explore accessory binding sites on DHFR, analogues **20**−**23** were synthesized. Compound **20** displayed a 16-fold increase in inhibition of pcDHFR and a 56-fold increase in inhibition of tgDHFR compared to **2**. Unfortunately, this improvement in potency did not translate into selective inhibition of pcDHFR or tgDHFR, since the inhibition of rlDHFR was also improved compared to **2**. However, since extension of the side chain afforded an increase in potency, this suggested that suitable extension of the side chain might provide for analogues with not only improved potency but also selectivity. Hence, the 2-atom-bridged analogues **21**−**23** were synthesized. However, none of these analogues were significantly potent against either pcDHFR or tgDHFR. Compound **20** could be an interesting starting point for structural variation on account of its potency against tgDHFR.

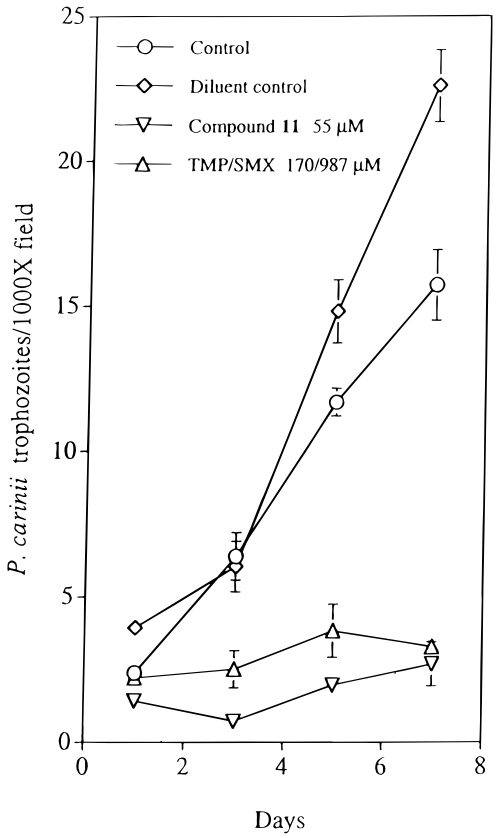


Figure 2. Inhibition of *P. carinii* growth in culture. Test compound or diluent was added to the medium at the time of inoculation of monolayers of HEL cells with *P. carinii* trophozoites, according to a standard protocol. Plates were harvested at the times shown, and numbers of *P. carinii* were determined by direct counting. Each point represents the mean of four replicate wells ± standard error of the mean.

On the basis of its impressive selectivity for pcDHFR, analogue **11** was further evaluated as an inhibitor of the growth of *P. carinii* trophozoites in culture, according to established methods.[48] Compound **11** was inhibitory to the growth of *P. carinii* at a concentration of 55 μM, and the inhibition was similar to that produced by TMP/SMX at 170/990 μM. Compound **11** was not tested in further culture studies due to its toxicity to host cells, but analogues **8**, **13**, and **17** were evaluated in culture studies, since they showed little evidence of cytotoxicity toward host cells. IC_{50} values could not be determined for **8** and **13** but were above 10 and 50 μM, respectively; the IC_{50} value for **17** was 16 μM, a value similar to its rlDHFR IC_{50}, suggesting that this compound penetrates well into infected cells.

Selected analogues were chosen to be evaluated in the preclinical *in vitro* anticancer screening program of the National Cancer Institute.[49] While most of the analogues displayed poor inhibition of the growth of tumor cells in culture ($GI_{50} > 10^{-5}$ M), reflecting their poor inhibition of rlDHFR, analogue **11** displayed a GI_{50} value of 10^{-6} M for the inhibition of the growth of 17 tumor cell lines. This was surprising, since its IC_{50} for the inhibition of rlDHFR was 252 μM, and suggests that **11** might have additional or alternate mechanism(s) of action as an inhibitor of the growth of tumor cells.

In summary, conformational restriction of the side chain of TMP analogues by incorporation of an imidazole ring did not improve the activity relative to TMP. The

poor solubility of most of these analogues precluded their evaluation as inhibitors of pcDHFR. This, in turn, could be due to the increased hydrophilicity of these analogues compared to TMP, which might, in turn, contribute to expenditure of energy (and a subsequent decrease in potency) required for desolvation of these molecules to bind to the active site of DHFR. This effect is more pronounced against pcDHFR as compared to rlDHFR and tgDHFR, suggesting significant differences in the active site of these DHFRs. Analogue **11** displayed excellent selectivity ratios for the inhibition of both pcDHFR and tgDHFR and is superior to the currently used combination of TMP/SMX in the inhibition of the growth of *P. carinii* cells in culture. Using compound **11** as a lead analogue, studies are currently underway to improve its potency and selectivity against pcDHFR and tgDHFR by conformationally restricting the side chain in 6-5 rings, other than the purine ring.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus or a Mel Temp apparatus and are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Brucker WH-300 spectrometer (300 MHz). The data were accumulated by 16K size with 0.5 s delay time with internal standard TMS; s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Low-resolution mass spectra were obtained on an LKB-9000 instrument. Thin layer chromatography was performed on silica gel plates with fluorescent indicator and were visualized with light at 254 and 366 nm, unless indicated otherwise. Column chromatography was performed with 230−400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. Elution was performed using a gradient, and 10 mL fractions were collected, unless mentioned otherwise. All anhydrous solvents were purchased from Aldrich Chemical Co. and were used without further purification. Samples for microanalysis were dried *in vacuo* over phosphorous pentoxide at 70 or 110 °C. Microanalyses were performed by Atlantic Microlabs, Norcoss, GA.

General Procedure for the Synthesis of Compounds 2−20. To a suspension of 2,4,5,6-tetraaminopyrimidine (**24**) in 25 mL of methoxyethanol at 80 °C was added concentrated HCl (∼2−3 mL) dropwise to effect solution. To this solution was added the appropriate aldehyde (substituted or unsubstituted benzaldehyde, substituted or unsubstituted naphthaldehyde, fluorene aldehyde, pyridinecarboxaldehyde or phenylacetaldehyde), and the mixture was refluxed for 6 h to 2 days. The precipitate formed was filtered and washed with hexanes and ether to afford the desired compound. In the case of compounds **12**, **16**, and **17**, at the end of the reaction, 1.0 g of silica gel was added to the reaction mixture and the solvent evaporated to afford a dry plug. This plug was applied on the surface of a silica gel column (1.05 in. × 23 in.) and eluted with $CHCl_3$:CH_3OH (5:1). Fractions corresponding to the desired product were pooled and evaporated to afford the desired compound.

2,6-Diamino-8-phenylpurine, 2. Compound **2** was obtained from **24** (0.10 g, 0.71 mmol) and benzaldehyde (0.07 g, 0.71 mmol) to afford 0.11 g (68%) as a white solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.51; lit.[40] mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 5.72 (s, 2 H, 2-NH_2), 6.67 (br s, 2 H, 4-NH_2), 7.10−7.60 (m, 5 H), 12.56 (s, 1 H, NH). Anal. ($C_{11}H_{10}N_6$·0.2H_2O) C, H, N.

2,6-Diamino-8-(3′,4′,5′-trimethoxyphenyl)purine, 3. Compound **3** was obtained from **24** (0.10 g, 0.71 mmol) and 3,4,5-trimethoxybenzaldehyde (0.14 g, 0.71 mmol) to afford 0.15 g (58%) as a light yellow solid: TLC (CH_2Cl_2:CH_3OH:NH_4OH, 5:2:0.5) R_f = 0.43; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3 H, 4′-OCH_3), 3.84 (s, 6 H, 3′,5′-OCH_3), 5.71 (s, 2 H, 2-NH_2), 6.66 (s, 2 H, 4-NH_2), 7.37 (s, 2 H, 2′,6′-H), 12.50 (s, 1 H, NH). Anal. ($C_{14}H_{16}N_6O_3$·0.2H_2O·0.2 HCl) C, H, N.

2,6-Diamino-8-(2′,3′,4′-trimethoxyphenyl)purine, 4. Compound **4** was obtained from **24** (0.31 g, 2.21 mmol) and 2,3,4-trimethoxybenzaldehyde (0.43 g, 2.21 mmol) to afford 0.43 g (62%) as a yellow solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.41; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3 H, 2′-OCH_3), 3.90 (s, 3 H, 4′-OCH_3), 4.08 (s, 3 H, 3′-OCH_3), 6.86 (d, 1 H, 5′-H), 7.41 (s, 2 H, 2-NH_2), 7.69 (d, 1 H, 6′-H), 8.31 (s, 2 H, 4-NH_2), 12.31 (s, 1 H, NH). Anal. ($C_{14}H_{16}N_6O_3$·0.5H_2O·0.7HCl) C, H, N.

2,6-Diamino-8-(2′,4′,6′-trimethoxyphenyl)purine, 5. Compound **5** was obtained from **24** (0.25 g, 1.79 mmol) and 2,4,6-trimethoxybenzaldehyde (0.35 g, 1.79 mmol) to afford 0.40 g (62%) as a cream solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 6:1:0.5) R_f = 0.38; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 6 H, 2′,6′-OCH_3), 3.90 (s, 3 H, 4′-OCH_3), 5.75 (s, 2 H, 2-NH_2), 6.05 (s, 2 H, 3′,5′-H), 6.75 (s, 2 H, 4-NH_2), 12.55 (s, 1 H, NH). Anal. ($C_{14}H_{16}N_6O_3$·0.4H_2O·1.0HCl) C, H, N.

2,6-Diamino-8-(2′,4′,5′-trimethoxyphenyl)purine, 6. Compound **6** was obtained from **24** (0.25 g, 1.79 mmol) and 2,4,5-trimethoxybenzaldehyde (0.35 g, 1.79 mmol) to afford 0.35 g (61%) as a cream solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3 H, 2′-OCH_3), 3.91 (s, 3 H, 4′-OCH_3), 4.08 (s, 3 H, 5′-OCH_3), 6.89 (s, 1 H, 3′-H), 7.34 (s, 2 H, 2-NH_2), 7.68 (s, 1 H, 6′-H), 8.29 (s, 2 H, 4-NH_2), 12.30 (s, 1 H, NH). Anal. ($C_{14}H_{16}N_6O_3$·0.3H_2O) C, H, N.

2,6-Diamino-8-(2′,5′-dimethoxyphenyl)purine, 7. Compound **7** was obtained from **24** (0.25 g, 1.79 mmol) and 2,5-dimethoxybenzaldehyde (0.29 g, 1.79 mmol) to afford 0.31 g (61%) as a white solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.58; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3 H, 2′-OCH_3), 4.02 (s, 3 H, 5′-OCH_3), 7.13 (d, 1 H, 4′-H), 7.23 (d, 1 H, 3′-H), 7.39 (s, 2 H, 2-NH_2), 7.71 (s, 1 H, 6′-H), 8.39 (s, 2 H, 4-NH_2), 12.35 (s, 1 H, NH). Anal. ($C_{13}H_{14}N_6O_2$·0.1H_2O·0.1HCl) C, H, N.

2,6-Diamino-8-(3′,5′-dimethoxyphenyl)purine, 8. Compound **8** was obtained from **24** (0.10 g, 0.71 mmol) and 3,5-dimethoxybenzaldehyde (0.12 g, 0.71 mmol) to afford 0.14 g (68%) as a white solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.53; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 6 H, 3′,5′-OCH_3), 6.65 (s, 1 H, 4′-H), 7.28 (s, 2 H, 2′,6′-H), 7.50 (s, 2 H, 2-NH_2), 8.50 (s, 2 H, 4-NH_2), 12.90 (s, 1 H, NH). Anal. ($C_{13}H_{14}N_6O_2$·0.8H_2O·0.7HCl) C, H, N.

2,6-Diamino-8-(3′,4′-dimethoxyphenyl)purine, 9. Compound **9** was obtained from **24** (0.31 g, 2.21 mmol) and 3,4-dimethoxybenzaldehyde (0.37 g, 2.21 mmol) to afford 0.38 g (60%) as a yellowish solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.51); mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3 H, 3′-OCH_3), 3.96 (s, 3 H, 4′-OCH_3), 5.72 (s, 2 H, 2-NH_2), 6.73 (s, 2 H, 4-NH_2), 6.80 (d, 1 H, 5′-H), 7.50−7.60 (m, 2 H, 2′-H, 6′-H), 12.60 (s, 1 H, NH). Anal. ($C_{13}H_{14}N_6O_2$·0.3HCl) C, H, N.

2,6-Diamino-8-(2′,4′-dimethoxyphenyl)purine, 10. Compound **10** was obtained from **24** (0.30 g, 2.14 mmol) and 2,4-dimethoxybenzaldehyde (0.36 g, 2.14 mmol) to afford 0.36 g (59%) as a cream solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.52; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 2 H, 2′-OCH_3), 4.06 (s, 3 H, 4′-OCH_3), 6.75 (s, 1 H, 3′-H), 6.82 (d, 1 H, 5′-H), 7.29 (s, 2 H, 2-NH_2), 8.13 (d, 1 H, 6′-H), 8.29 (s, 2 H, 4-NH_2), 12.00 (s, 1 H, NH). Anal. ($C_{13}H_{14}N_6O_2$·0.5HCl) C, H, N.

2,6-Diamino-8-(3′,4′-dichlorophenyl)purine, 11. Compound **11** was obtained from **24** (0.50 g, 3.57 mmol) and 3,4-dichlorobenzaldehyde (0.62 g, 3.57 mmol) to afford 0.36 g (59%) as a fluffy, white solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.65; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 7.37 (s, 2 H, 2-NH_2), 7.62 (s, 1 H, 2′-H), 7.89 (d, 1 H, 6′-H), 8.03 (d, 1 H, 5′-H), 8.72 (s, 2-NH_2), 12.96 (s, 1 H, NH). Anal. ($C_{11}H_8N_6Cl_2$·0.5H_2O) C, H, N, Cl.

2,6-Diamino-8-(2′,6′-dichlorophenyl)purine, 12. Compound **12** was obtained from **24** (0.50 g, 3.57 mmol) and 2,6-dichlorobenzaldehyde (0.62 g, 3.57 mmol) to afford 0.48 g (46%) as a light yellow solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) R_f = 0.7; TLC ($CHCl_3$:CH_3OH:NH_4OH, 4:1:0.5) R_f = 0.69; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 6.43 (br m, 4 H, 2-NH_2, 4-NH_2), 6.93 (s, 1 H, 4′-H), 7.11 (s, 2 H, 3′,5′-H), 12.61 (s, 1 H, NH). Anal. ($C_{11}H_8N_6Cl_2$·0.4H_2O) C, H, N, Cl.

2,6-Diamino-8-(3′,5′-dimethoxy-4′-hydroxyphenyl)purine, 13. Compound **13** was obtained from **24** (1.0 g, 7.14 mmol) and 3,5-dimethoxy-4-hydroxybenzaldehyde (vanillin)

(1.3 g, 7.14 mmol) to afford 0.60 g (28%) as a white solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) $R_f = 0.37$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 3.93 (s, 6 H, 3′,5′-OCH_3), 5.75 (s, 2 H, 2-NH_2), 6.35 (s, 1 H, OH), 6.75 (s, 2 H, 4-NH_2), 7.00 (s, 2 H, 2′,6′-H), 12.60 (s, 1 H, NH). Anal. ($C_{13}H_{14}N_6O_3 \cdot 1.0H_2O$) C, H, N.

2,6-Diamino-8-(2′-nitro-4′,5′-dimethoxyphenyl)purine, 14. Compound **14** was obtained from **24** (0.30 g, 2.14 mmol) and 2-nitro-4,5-dimethoxybenzaldehyde (0.45 g, 2.14 mmol) to afford 0.32 g (45%): mp > 300 °C; ^1H NMR (DMSO-d_6) δ 3.63−3.65 (overlapping s, 6 H, 4′,5′-OCH_3), 5.00 (s, 2 H, 2-NH_2), 6.10 (s, 2-H, 4-NH_2), 6.87 (s, 1 H, 6′-H), 8.04 (s, 1 H, 3′-H), 11.9 (s, 1 H, NH). Anal. ($C_{13}H_{13}N_7O_4 \cdot 0.5H_2O$) C, H, N.

2,6-Diamino-8-(4′-biphenylyl)purine, 15. Compound **15** was obtained from **24** (0.41 g, 2.92 mmol) and 4-phenylbenzaldehyde (0.53 g, 2.92 mmol) to afford 0.45 g (51%): TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) $R_f = 0.59$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 5.81 (s, 2 H, 2-NH_2), 6.70 (s, 2 H, 4-NH_2), 7.4−7.5 (m, 3 H, 3″,4″,5″-H), 7.70 (m, 2 H, 6″,2″-H), 7.78 (d, 2 H, 3′, 5′-H), 8.00 (d, 2 H, 2′, 6′-H), 12.56 (s, 1 H, NH). Anal. ($C_{17}H_{15}N_6 \cdot 1.0H_2O$) C, H, N.

2,6-Diamino-8-(4′-pyridinyl)purine, 16. Compound **16** was obtained from **24** (1.0 g, 7.14 mmol) and pyridine-4-carboxaldehyde (0.85 g, 7.14 mmol) to afford 0.38 g (22%) as a yellow solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) $R_f = 0.3$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 5.65 (s, 2 H, 2-NH_2), 6.75 (s, 2 H, 4-NH_2), 7.72 (d, 2 H, 2′,6′-H), 8.90 (d, 2 H, 3′,5′-H), 12.55 (s, 1 H, NH). Anal. ($C_{10}H_9N_7 \cdot 0.1HCl$) C, H, N.

2,6-Diamino-8-(2′-naphthyl)purine, 17. Compound **17** was obtained from **24** (0.21 g, 1.5 mmol) and 2-naphthaldehyde (0.17 g, 1.5 mmol) to afford 0.25 g (60%) as a cream solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) $R_f = 0.72$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 5.71 (s, 2 H, 2-NH_2), 6.70 (s, 2 H, 4-NH_2), 7.31−7.40 (m, 3 H), 7.66−7.74 (m, 4 H), 12.50 (s, 1 H, NH). Anal. ($C_{15}H_{12}N_6 \cdot 0.5H_2O$) C, H, N.

2,6-Diamino-8-(4′-methoxy-1′-naphthyl)purine, 18. Compound **18** was obtained from **24** (0.28 g, 2.0 mmol) and 4-methoxy-1-naphthaldehyde (0.31 g, 2.0 mmol) to afford 0.31 g (51%) as a white solid: mp > 300 °C; ^1H NMR (DMSO-d_6) δ 3.71 (s, 3 H, 4′-OCH_3), 5.91 (s, 2 H, 2-NH_2), 6.11 (d, 1 H, 3′-H), 6.21 (d, 1 H, 2′-H), 6.61 (d, 1 H, 5′-H), 6.66 (d, 1 H, 8′-H), 6.71 (m, 2 H, 6′, 7′-H), 7.14 (s, 2 H, 4-NH_2), 12.10 (s, 1 H, NH). Anal. ($C_{10}H_{14}N_6O \cdot 0.5H_2O$) C, H, N.

2,6-Diamino-8-(9′-fluorenyl)purine, 19. Compound **19** was obtained from **24** (0.48 g, 3.42 mmol) and 9-fluorenecarboxaldehyde (0.66 g, 3.42 mmol) to afford 0.52 g (48%) as a yellow solid: TLC ($CHCl_3$:CH_3OH:NH_4OH, 4:1:0.5) $R_f = 0.61$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 4.20 (s, 2 H, CH_2), 7.46 (m, 4 H, 2-NH_2, 4′,7′-H), 7.58 (d, 1 H, 8′-H), 8.01 (d, 1 H, 9′-H), 8.28 (m, 2 H, 5′,6′-H), 8.40 (s, 1 H, 2′-H), 8.60 (s, 2 H, 4-NH_2), 12.66 (s, 1 H, NH). Anal. ($C_{18}H_{13}N_6 \cdot 0.5H_2O$) C, H, N.

2,6-Diamino-8-benzylpurine, 20. Compound **20** was obtained from **24** (1.0 g, 7.14 mmol) and phenylacetaldehyde (0.86 g, 7.14 mmol) to afford 0.21 g (12%): TLC ($CHCl_3$:CH_3OH:NH_4OH, 5:1:0.5) $R_f = 0.54$; mp > 300 °C; ^1H NMR (DMSO-d_6) δ 3.69 (s, 2 H, CH_2), 5.72 (s, 2 H, 2-NH_2), 6.67 (s, 2 H, 4-NH_2), 7.10−7.50 (m, 5 H), 12.56 (s, 1 H, NH). Anal. ($C_{12}H_{12}N_6 \cdot 0.5HCl$) C, H, N, Cl.

2,6-Diamino-8-(hydroxymethyl)purine, 26. A mixture of 2,4,5,6-tetraaminopyrimidine (**24**) (4.8 g, 20 mmol) and glycolic acid (7.6 g, 100 mmol) was heated at 110 °C for 2 h. At the end of this period, the syrupy reaction mixture was washed with 2 × 50 mL portions of ethyl ether, and the residue was dissolved in 30 mL of water. The pH of the solution was adjusted to 9 with NH_4OH and the precipitated solid collected and dried. The product was recrystallized from water to afford 2,4,6-triamino-5-(glycolamido)pyrimidine, **25**.

A solution of **25** (3.5 g, 0.018 mol) in 25 mL of 2 N NaOH was refluxed for 8 h. The reaction mixture was cooled and acidified to pH 5 with acetic acid. The precipitated solid was collected to afford 2.3 g (70%) of **26** as a white solid: mp > 360 °C; ^1H NMR (DMSO-d_6) δ 3.57 (br s, 2 H, CH_2), 4.5 (s, 1 H, OH), 5.57 (s, 2 H, 2-NH_2), 6.49 (s, 2 H, 4-NH_2), 11.89 (s, 1 H, NH). Anal. ($C_6H_8N_6O \cdot 1.2H_2O$) C, H, N.

2,6-Diamino-8-(chloromethyl)purine, 27. To a suspension of **26** (3.00 g, 0.016 mol) in 20 mL of *N,N*-dimethylformamide was added 3.3 mL (5.6 g, 0.05 mol) of thionyl chloride, dropwise over a 10 min period. The resulting solution was heated at 60−70 °C for 3 h and allowed to stand at room temperature overnight. The solvents were evaporated, and 25 mL of anhydrous ether was added to the residue which precipitated a yellow solid: ^1H NMR (DMSO-d_6) δ 4.87 (s, 2 H, CH_2), 6.74 (br s, 2 H, 2-NH_2), 7.67 (br s, 2 H, 4-NH_2), 12.4 (s, 1 H, NH). Due to the hygroscopic nature of this intermediate, it was not purified further but carried directly to the next step.

2,6-Diamino-8-[(3′,4′,5′-trimethoxyanilino)methyl]purine, 21. To a solution of **27** (0.50 g, 2.71 mmol) in 20 mL of anhydrous *N,N*-dimethylacetamide was added 3,4,5-trimethoxyaniline (0.49 g, 2.71 mmol) followed by potassium carbonate (0.30 g). The reaction mixture was stirred at room temperature under nitrogen for 24 h. TLC analyses ($CHCl_3$:CH_3OH, 5:1) indicated the presence of a new product at a $R_f = 0.6$ along with trace amounts of both starting materials and a baseline spot. The reaction mixture was filtered, 1.0 g of silica gel added to the filtrate, and the solvent evaporated to afford a dry plug. This plug was eluted with $CHCl_3$:CH_3OH on a 1.05 in. × 23 in. silica gel column, using a gradient elution (95:5 to 80:20), and fractions containing the product were pooled and evaporated to afford pure **21** as a white solid (0.52 g, 56%): mp = 298 °C dec; ^1H NMR (DMSO-d_6) δ 3.50 (s, 3 H, 4′-OCH_3), 3.66 (s, 6 H, 3′,5′-OCH_3), 4.24 (d, 2 H, CH_2), 5.55 (s, 2 H, 2-NH_2), 5.82 (t, 1 H, NH), 5.96 (s, 2 H, 2′,6′-H), 6.48 (s, 2 H, 4-NH_2), 11.95 (s, 1 H, NH). Anal. ($C_{15}H_{19}N_7O_3 \cdot 1.1H_2O$) C, H, N.

2,6-Diamino-8-[(2′,5′-dimethoxyanilino)methyl]purine, 22. To a solution of **27** (0.40 g, 1.65 mmol) in 20 mL of anhydrous *N,N*-dimethylacetamide was added 2,5-dimethoxyaniline (0.25 g, 1.65 mmol) followed by potassium carbonate (0.30 g). The reaction mixture was stirred at room temperature under nitrogen for 24 h. TLC analyses ($CHCl_3$:CH_3OH, 5:1) indicated the presence of a new product at a $R_f = 0.7$ along with trace amounts of **27** and a baseline spot. The reaction mixture was filtered, 1.0 g of silica gel added to the filtrate, and the solvent evaporated to afford a dry plug. This plug was placed on a silica gel column and eluted with $CHCl_3$:CH_3OH using gradient elution (95:5 to 80:20), and fractions containing the product were pooled and evaporated to afford pure **22** (0.27 g, 52%) as a light yellow solid: mp = 290 °C dec; ^1H NMR (DMSO-d_6) δ 3.59 (s, 3 H, 2′-OCH_3), 3.74 (s, 3 H, 5′-OCH_3), 4.30 (d, 2 H, CH_2), 5.42 −5.55 (overlapping peaks, 3 H, 2-NH_2, NH), 6.10 (m, 2 H, 4′,6′-H), 6.40 (s, 2 H, 4-NH_2), 6.69 (d, 1 H, 3′-H), 11.89 (s, 1 H, NH). Anal. ($C_{14}H_{17}N_7O_2 \cdot 0.3H_2O$) C, H, N.

2,6-Diamino-8-[(2′-naphthylthio)methyl]purine, 23. To a solution of **27** (0.50 g, 2.71 mmol) in 20 mL of anhydrous *N,N*-dimethylacetamide was added 2-naphthalenethiol (0.35 g, 2.71 mmol) followed by potassium carbonate (0.30 g). The reaction mixture was stirred at room temperature under nitrogen for 12 h. TLC analysis ($CHCl_3$:CH_3OH, 5:1) indicated the presence of a new product at a $R_f = 0.8$ along with trace amounts of **27** and a baseline spot. The reaction mixture was filtered, 1.0 g of silica gel added to the filtrate, and the solvent evaporated to afford a dry plug. This plug was loaded on a silica gel column and eluted with $CHCl_3$:CH_3OH using gradient elution (95:5 to 80:20), and fractions containing the product were pooled and evaporated to afford pure **23** as a light yellow solid (0.50 g, 68%): mp = 282 °C dec; ^1H NMR (DMSO-d_6) δ 4.36 (s, 2 H, CH_2), 5.61 (s, 2 H, 2-NH_2), 6.53 (s, 2 H, 4-NH_2), 7.49 (m, 3 H), 7.89 (m, 3 H), 7.96 (s, 1 H, 1′-H), 12.12 (s, 1 H, NH). Anal. ($C_{16}H_{14}N_6S \cdot 0.3H_2O$) C, H, N, S.

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