of part of the $POCl_3$, mixing with ice, and neutralizing with NH_4OH. The product was extracted into CH_2Cl_2 and purified on a silica gel column, eluted with hexane/EtOAc, 5:1, giving 2.76 g (93%) of **45**, mp 140–142 °C. Anal. ($C_{11}H_9BrClNO$) C, H, N.

6-Bromo-4,8-dimethoxy-2-methylquinoline (46). Compound **45** (0.85 g, 3.0 mmol) was dissolved in 40 mL of MeOH plus 0.8 g (15 mmol) of NaOMe and heated in a steel bomb at 120 °C for 5 h. The solvent was removed, water added, and the product extracted into CH_2Cl_2 and purified on a silica gel column, which was eluted with hexane/EtOAc, 1:3, giving 0.72 g (86%) of **46**, mp 167–168 °C. Anal. ($C_{12}H_{12}BrNO_2$) C, H, N.

6-Formyl-4,8-dimethoxy-2-methylquinoline (48). Compound **46** (1.88 g, 6.66 mmol) was dissolved in 60 mL of freshly distilled dry THF and chilled to −70 °C under N_2. Then 5.0 mL (1.2 equiv) of 1.6 M *n*-BuLi in hexane was added dropwise with a syringe, followed by stirring 2 min, and then addition of 0.78 mL (1.5 equiv) of dry DMF. The reaction was allowed to warm to −40 °C and then quenched with 8 mL of 1 N hydrochloric acid. The solution was extracted with Et_2O and the aqueous fraction rendered basic with 1 N NaOH to pH 12, followed by extraction with CH_2Cl_2 and evaporation of the solvent. The residue was purified on a silica gel column, eluted with 2% MeOH in CH_2Cl_2, which produced 0.74 g (48%) of **48**: NMR ($CDCl_3$) δ 2.73 (s, 3, Me), 4.01 (s, 3, OMe), 4.06 (s, 3, OMe), 6.70 (s, 1, Q-3-H), 7.40 (d, 1, Ar), 8.14 (d, 1, Ar), 9.98 (s, 1, CHO). A second fraction which separated from the column, **49** (0.28 g), was considered to be 6-formyl-4,8-dimethoxy-2-(*n*-pentyl)quinoline on the basis of an analogous experiment with the 4-(dimethylamino) analogue. This structure was not proven, but it was successfully converted to **54** (Table I).

Acknowledgment. We thank Dr. Lee Kuyper for valuable suggestions concerning synthetic methods and Dr. David Henry for his support of this investigation. The elementary analyses were carried out by Atlantic Microlab, Atlanta, GA. NMR spectroscopy was performed under the direction of Dr. Stuart Hurlbert of these laboratories. Enzyme assays were supervised by Robert Ferone and the in vitro antibacterial screens by Dr. Lynn Elwell. We thank Pauline Baker for expert technical assistance.

Registry No. **3**, 106-40-1; **4**, 591-19-5; **5**, 583-75-5; **6**, 674-82-8; **7**, 38418-24-5; **8**, 61579-06-4; **9**, 23939-48-2; **10**, 89446-19-5; **11**, 89446-51-5; **12**, 89446-45-7; **13**, 3913-19-7; **14**, 89446-52-6; **15**, 89446-46-8; **16**, 89446-53-7; **17**, 99471-78-0; **18**, 89446-20-8; **19**, 121269-15-6; **20**, 121269-16-7; **21**, 89446-47-9; **22**, 89446-54-8; **23**, 121269-17-8; **24**, 121269-18-9; **25**, 89446-21-9; **26**, 121269-19-0; **27**, 89446-48-0; **28**, 1075-76-9; **29**, 89446-55-9; **30**, 121269-20-3; **31**, 121269-21-4; **32**, 89446-22-0; **33**, 121269-22-5; **34**, 89446-49-1; **35**, 89446-56-0; **36**, 121269-23-6; **37**, 121269-24-7; **38**, 89446-23-1; **39**, 121269-25-8; **40**, 89446-50-4; **41**, 59557-91-4; **42**, 141-97-9; **43**, 89446-10-6; **44**, 121269-26-9; **45**, 89446-12-8; **46**, 89446-13-9; **47**, 89446-16-2; **48**, 89446-14-0; **49**, 121269-27-0; **50**, 89446-17-3; **51**, 121269-28-1; **52**, 89446-15-1; **53**, 89446-18-4; **54**, 121269-29-2; $HN(CH_2CH_2)_2O$, 110-91-8; guanidine hydrochloride, 50-01-1; *o*-anisidine, 90-04-0.

2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 12. 1,2-Dihydroquinolylmethyl Analogues with High Activity and Specificity for Bacterial Dihydrofolate Reductase

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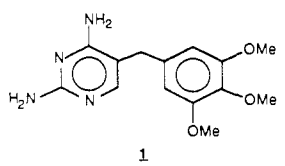
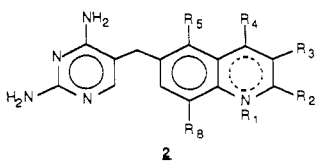
Received October 24, 1988

Twelve 2,4-diamino-5-[(1,2-dihydro-6-quinolyl)methyl]pyrimidines containing *gem*-dimethyl or fluoromethyl substituents at the 2-position of the dihydroquinoline ring were prepared by condensations of dihydroquinolines with 2,4-diamino-5-(hydroxymethyl)pyrimidine. The dihydroquinolines were produced from the reaction of anilines with mesityl oxide or fluoroacetone. In some cases, 1-aryl-2,4-dimethylpyrroles were obtained as byproducts. Most of these pyrimidines were highly inhibitory to *Escherichia coli* dihydrofolate reductase (DHFR) and also had high specificity for the bacterial enzyme. 2,4-Diamino-5-[[1,2-dihydro-2,4-dimethyl-3-fluoro-2-(fluoromethyl)-8-methoxy-6(1*H*)quinolyl]methyl]pyrimidine (**13**) had an apparent K_i value for *E. coli* DHFR 13 times lower than that of the control, trimethoprim (1), and was 1 order of magnitude more selective for the bacterial enzyme. It had outstanding activity against Gram-positive organisms in vitro, as well as broad-spectrum antibacterial activity equivalent to that of 1. The results of in vivo testing will be reported elsewhere. The *gem*-dimethyl substituents of the dihydroquinoline derivatives are considered to be responsible for the high selectivity, as well as contributing to potent bacterial DHFR inhibition. Molecular models are presented which suggest the probable interactions with the bacterial enzyme.

The previous two papers of this series described analogues of trimethoprim[1] (TMP, 1) where the aromatic



1



2

moiety was replaced by a quinoline or 1,2,3,4-tetrahydroquinoline, which was usually bridged to the pyrimidylmethyl moiety at the 6-position of the quinoline ring system, as in **2**.[2,3] In the aromatic series, the presence of 4-methyl-8-methoxy substituents conveyed greater inhibitory activity against bacterial dihydrofolate reductase (DHFR, EC 1.5.1.3) than was the case with 1, but high selectivity was only achieved when basic substituents were present in the 2-position.[2,3] The 1,2,3,4-tetrahydro ana-

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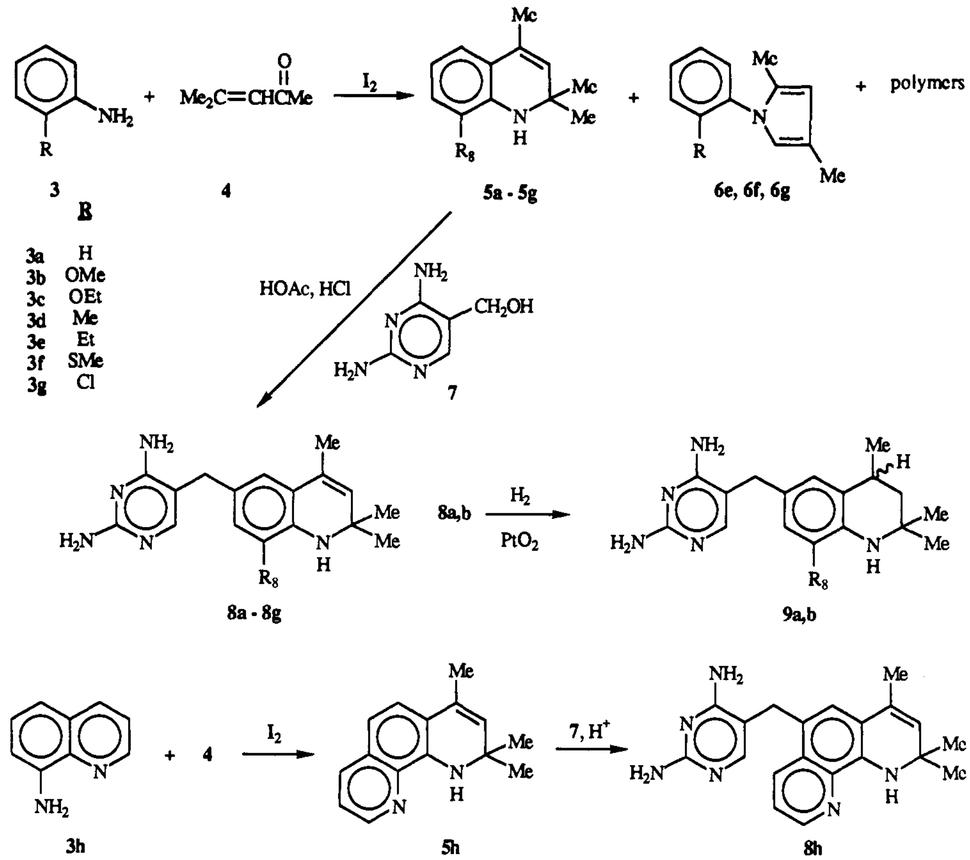
(1) Roth, B.; Falco, E. A.; Hitchings, G. H.; Bushby, S. R. M. *J. Med. Pharm. Chem.* **1962**, *5*, 1103.
(2) Rauckman, B. S.; Tidwell, M. Y.; Johnson, J. V.; Roth B. *J. Med. Chem.* first of four papers in this issue.
(3) Davis, S. E.; Rauckman, B. S.; Chan, J. H.; Roth, B. second of four papers in this issue.

0022-2623/89/1832-1942$01.50/0

Scheme I



logues were slightly less inhibitory and less selective for *Escherichia coli* DHFR than was the standard (1) unless the quinoline nitrogen was alkylated; such derivatives then matched these properties of 1.

Our purpose was to broaden the scope of the antibacterial activity of 1, to increase the activity, particularly against Gram-positive organisms, and also to maintain the selectivity for bacterial, as opposed to vertebrate, DHFR, in order to prevent toxicity for the host. To accomplish these things we needed a better understanding of the factors that created selectivity for the bacterial enzyme, the three-dimensional structure of which is now known for *E. coli* and *Lactobacillus casei* DHFRs, as well as L1210 mouse lymphoma and human DHFR.[4-7] In this paper, we describe 1,2-dihydroquinoline analogues of **2** which are stabilized by *gem*-dimethyl substitution at the 2-position or by analogous fluorinated derivatives. The DHFR inhibitory activities and selectivities are compared with the previous series and analyzed in an effort to provide further clues as to specificity requirements. In vitro antibacterial activities are also presented.

Chemistry

Since 1,2,3,4-tetrahydroquinolines had been found to condense readily with 2,4-diamino-5-(hydroxymethyl)pyrimidine (**7**) to produce adducts of type **2**,[2] we decided to try this reaction with the commercially available 1,2-dihydro-2,2,4-trimethylquinoline (**5a**) (Scheme I). The reaction was very successful in producing the desired pyrimidylmethyl adduct, **8a**. However, we needed substituents at the 8-position of the dihydroquinoline if we were to obtain optimal binding to bacterial DHFR.[2] This then required synthesis of the desired dihydroquinoline intermediates.

The preparation of dihydroquinoline **5a** is described in *Organic Syntheses*,[8] in a very unappealing reaction which involves heating an aniline–iodine mixture to 170–175 °C and slowly adding several moles of acetone under the surface at a rate such that two drops of liquid distills per second. A black, thick mass is formed, from which the products are distilled and redistilled several times in vacuo. The yield, based on aniline recovery, is stated to be high. Various modifications have been described, which include the use of polymerization inhibitors[9] and various activators, as well as a continuous-flow apparatus.[10] One review[11] suggests that mesityl oxide may be formed as an intermediate in this reaction, but we found no report indicating that this acetone derivative had actually been used. Since this high-boiling dimer would be expected to aid in the mechanics of the reaction, we investigated it with several ortho-substituted anilines, and found that the reaction was much easier to manage, although much dimerization or polymerization of the product still occurred. Yields were

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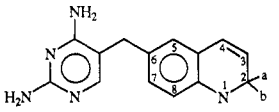
(11) Elderfield, R. C. *Heterocyclic Compounds*; John Wiley & Sons: New York, 1952; Vol. 4, p 271.

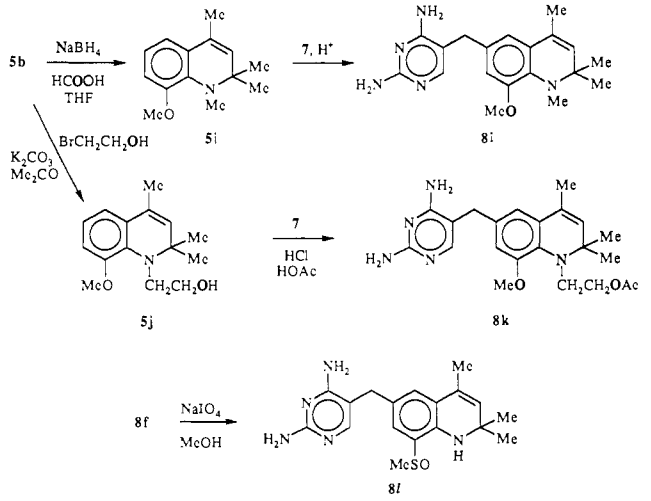
Table I. 2,4-Diamino-5-[(1,2-dihydro-6-quinolyl)methyl]pyrimidines Prepared by Condensation of 1,2-Dihydroquinolines with 2,4-Diamino-5-(hydroxymethyl)pyrimidine



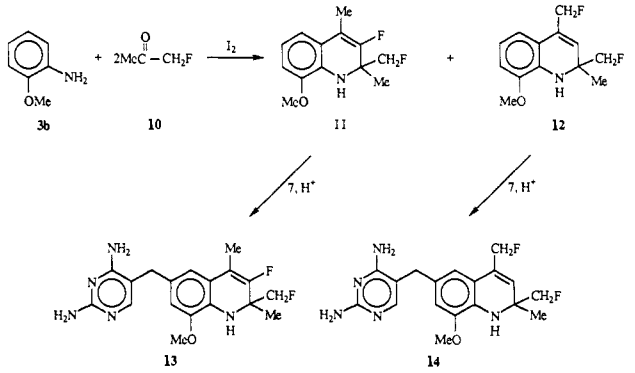
	dihydroquinoline substituents								recryst			
no.	1	2a	2b	3	4	7	8	MP, °C	solv[a]	% yield	empirical formula	analysis
8a[b]		Me	Me		Me			260–264	A		$C_{17}H_{21}N_5 \cdot 2HCl \cdot 0.5H_2O$	C, H, N
8b[c]		Me	Me		Me		OMe	186–188		79	$C_{18}H_{23}N_5O$	C, H, N
8c		Me	Me		Me		OEt	211–216	A	27	$C_{19}H_{25}N_5O$	C, H, N
8d		Me	Me		Me		Me	193–195	A	9	$C_{18}H_{23}N_5$	C, H, N
8e		Me	Me		Me		Et	217–220	B		$C_{19}H_{25}N_5 \cdot 2HCl \cdot H_2O$	C, H, N
8f		Me	Me		Me		SMe	182.5–185	A	66	$C_{18}H_{23}N_5S$	C, H, N
8g[d]		Me	Me		Me		Cl	211–214	C	4	$C_{17}H_{20}ClN_5$	C, H, N
8h		Me	Me		Me	—HC=CHCH=N—		226–230	E	73	$C_{20}H_{22}N_6 \cdot 0.5H_2O$	C, H, N
8i	Me	Me	Me		Me		OMe	245–248	D	21	$C_{19}H_{25}N_5O \cdot 0.75H_2O$	C, H, N
8k	$(CH_2)_2OAc$	Me	Me		Me		OMe	180–184	B	11	$C_{22}H_{29}N_5O_3 \cdot 2HCl$	C, H, N
13		Me	CH_2F	F	Me		OMe	258–261	F	66	$C_{18}H_{21}F_2N_5O \cdot HCl$	C, H, N, Cl
14		Me	CH_2F		CH_2F		OMe	215–216	G	34	$C_{18}H_{21}F_2N_5O \cdot HCl \cdot 0.5H_2O$	C, H, N

[a] Recrystallization solvent: A, EtOH; B, EtOH–HCl; C, MeOH–Et_2O (1:1); D, absolute EtOH; E, EtOAc; F, EtOH–EtOAc–HCl; G, EtOH–Et_2O–HCl. [b] From 1,2-dihydro-2,2,4-trimethylquinoline, purchased from Aldrich Chemical Co., ref 8. [c] From 1,2-dihydro-2,2,4-trimethyl-8-methoxyquinoline, ref 22. [d] The intermediate dihydroquinoline was isolated as an intractable 1:2 mixture with 1-(2-chlorophenyl)-2,4-dimethylpyrrole (**6g**), which was separated only after the final condensation with **6**.

Scheme II



Scheme III



usually low, but were not based on recovered anilines, as is the case with most of the literature reports.

1-Aryl-2,4-dimethylpyrroles were identified as byproducts or even as major products, in a few instances. The parent 1-phenyl-2,4-dimethylpyrrole has been reported, from aniline plus various six-carbon agents, but not including mesityl oxide.[12,13] With *o*-ethylaniline we obtained an 18% yield of the pyrrole **6e** and only 2% of the dihydroquinoline **5e**. The *o*-(methylthio)aniline produced approximately equivalent amounts of the two products, but *o*-anisidine produced 25% of the dihydroquinoline, with no pyrrole being detected. The best results were obtained in the condensation of 8-aminoquinoline (**3h**) with 4, which gave 53% **5h**.

Table I describes the pyrimidylmethyl adducts obtained by condensations of the dihydroquinolines with **7**. Illustrative reaction conditions are provided in the Experimental Section. The pyrimidine condensation products **8a** and **8b** were readily reduced catalytically to the 1,2,3,4-tetrahydroquinolines **9a** and **9b**.

Scheme II shows various dihydroquinoline conversions to give N-1 substitution products, and the oxidation of the 8-methylthio derivative **8f** to the sulfoxide.

Scheme III illustrates the reaction of fluoroacetone with *o*-anisidine, which produced the two isomeric dihydroquinolines **11** and **12**, with the former in considerable preponderance. Since fluoroacetone is a very expensive reagent, very mild reaction conditions were developed for optimal recovery of this ketone, and dihydroquinoline yields were in the 25% range, based on starting anisidine.

Condensations to the pyrimidine derivatives **13** and **14** proceeded smoothly. It should perhaps be mentioned that the aromatization of 1,2-dihydro-2,2-dimethylquinolines by heating with sodamide, dry HCl, or other reagents has been described, which caused methane to be lost.[14] In this case it could be methyl fluoride. We did not investigate this reaction, but mention it as a possible source for 3-fluoroquinolines.

Biological Results and Discussion

Table II lists the inhibitory activities of the 2,4-diamino-5-[(dihydro- or -tetrahydro-6-quinolyl)methyl]pyrimidines against three DHFR enzymes, compared to **1**. As expected, 8-substitution was required for potent in-

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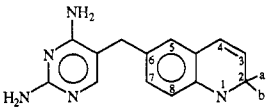
Table II. Inhibitory Activities of 2,4-Diamino-5-[(1,2-dihydro-6-quinolyl)methyl]pyrimidines and Tetrahydro Analogues against Dihydrofolate Reductase Enzymes



no.	reduced-quinoline substituents							inhibition vs DHFR, $I_{50} \times 10^8$ M			app K_i, nM,
	1	2a	2b	3	4	7	8	*E. coli*	rat liver	chicken	*E. coli*
8a		Me	Me		Me			17	24000		
8b		Me	Me		Me		OMe	<0.2	30000 (I_{32})	130000	0.30
8c		Me	Me		Me		OEt	<0.2	25000		
8d		Me	Me		Me		Me	1.54	50000		
8e		Me	Me		Me		Et	0.26	17400		
8f		Me	Me		Me		SMe	<0.2	11800		0.43
8g		Me	Me		Me		Cl	0.34	7600		
8h		Me	Me		Me	—CH=CHCH=N—		<0.2	3500		0.30
8i	Me	Me	Me		Me		OMe	0.31	34000		
8k	$(CH_2)_2OAc$	Me	Me		Me		OMe	5.4	16400		
8l		Me	Me		Me		SOMe	<0.2	5600		
13		Me	CH_2F	F	Me		OMe	<0.2	23300		0.10
14		Me	CH_2F		CH_2F		OMe	<0.2	23000		
9a		Me	Me		Me, H			15	30000 (I_{41})		
9b		Me	Me		Me, H		OMe	0.7	43000		
15[a]		H			Me		OMe	<0.2	2000		
16[b]		H	H		Me, H		OMe	0.81	3300		
17	Me	H	H		Me, H		OMe	0.68	25000		
1 (standard)								0.5–0.7	26000–34000	76000	1.3

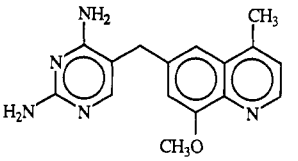
[a] Aromatic quinoline, ref 2. [b] Tetrahydro analogue, ref 2.

hibition of *E. coli* DHFR.[2] This position mimics one of the meta positions of **1**, as does the 4-quinoline locus, which is always substituted here by methyl or fluoromethyl. All three of the methoxy groups of **1** are in excellent van der Waals contact with *E. coli* DHFR, as demonstrated by the three dimensional structure of its complex with **1** and NADPH.[4b,15] Compound **1** is approximately 10-fold more inhibitory to *E. coli* DHFR than its 3,4-disubstituted counterpart.[16] In the quinoline case, a 4-methyl substituent would mimic a *m*-methoxy group of **1**, as can be seen by superposition of the two rings.[2]

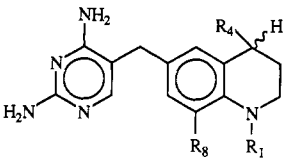
One could predict here that an 8-substituent would be optimal as methoxy or a similar unsymmetrical-top shape which would preferably lie in plane with the benzene ring.[2,15] This is clearly the case here when the 1-NH function is present. An 8-methyl substituent (**8d**) has insufficient bulk, although the 8-chloro derivative (**8g**) is more active than expected. The two compounds may be situated slightly differently in the enzyme, due to the electronic effect of the 8-chloro atom.

Quantitation of affinity differences for some of the more active *E. coli* DHFR inhibitors ($I_{50} < 0.2 \times 10^{-8}$ M) was made on the basis of apparent K_i determination. For example, **8b**, **8f**, and **8h** bind approximately 4 times more tightly to *E. coli* DHFR than does the control, **1**, and **13** has a 13-fold greater affinity than **1**. The effect of the *gem*-dimethyl groups on enzyme binding must be considered in the light of specificity for bacterial DHFR, as well as for inhibitory activity against *E. coli* DHFR per se. For example, **13** and **1** have similar rat liver DHFR I_{50} values. Therefore, their differences in *E. coli* K_i values translates into 1 order of magnitude increase in selectivity for **13**. Several of the other compounds in Table II also have a higher degree of *E. coli* DHFR specificity than **1**.

These results are quite different from those we observed with quinolines and tetrahydroquinolines in a previous paper.[2] The most direct comparisons are with the 4-methyl-8-methoxyquinoline derivative (numbered **15** here)



15



	R_1	R_4	R_8
16	H	CH_3	OCH_3
17	CH_3	CH_3	OCH_3

and its 1,2,3,4-tetrahydro analogue (**16**) (Table II). Neither were highly selective. Addition of a 1-methyl substituent to that tetrahydro derivative (**17**) increased the selectivity to give IC_{50} values comparable to those of **1**. However, adding a 1-methyl substituent to **8b** to produce **8i** had little effect on the binding to either enzyme. Reducing **8b** to the tetrahydro derivative (**9b**) reduced the *E. coli* DHFR binding severalfold.

Compound **8b** was also compared to **1** for its binding to chicken liver DHFR, and it was found that **1** was bound about twice as tightly as was **8b**. On this basis, the selectivity of **8b** for bacterial DHFR was 8-fold greater than was the case for **1**.

Another point concerning **8b** which should be mentioned is the comparison of its *E. coli* DHFR inhibition to that of its 8-unsubstituted counterpart, **8a**. Note that it becomes fully 100 times more active just by adding a methoxy group, a 10-fold greater effect than is the case with the benzylpyrimidines.[16]

(15) Roth, B.; Rauckman, B. S.; Ferone, R.; Baccanari, D. P.; Champness, J. N.; Hyde, R. M. *J. Med. Chem.* **1987**, *30*, 348.
(16) Baccanari, D. P.; Daluge, S.; King, R. W. *Biochemistry* **1982**, *21*, 5068.

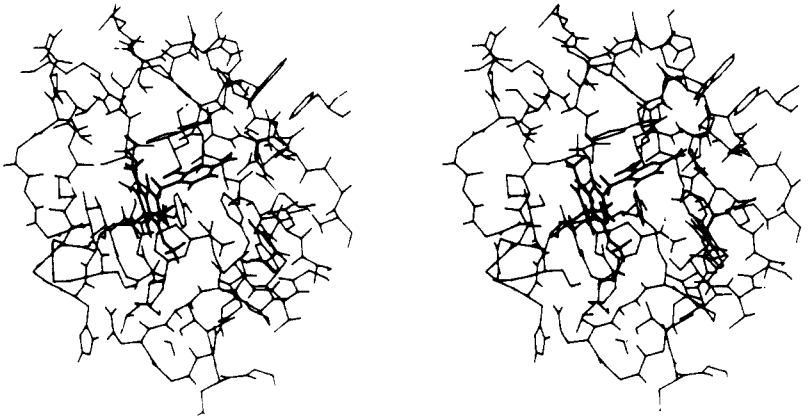


Figure 1. Stereo view of the active site region of *E. coli* DHFR–NADPH modeled with compound **8b** after deletion of 1. Enzyme–NADPH–pyrimidine ring coordinates are from ref 4b. The inhibitor is shown near the center in heavier lines, with the pyrimidine ring buried to the upper right, and the quinoline ring is shown toward the front, with the *gem*-dimethyl groups nearest to the viewer. The nicotinamide, ribose, and first phosphate of the coenzyme are shown buried near the bottom center.

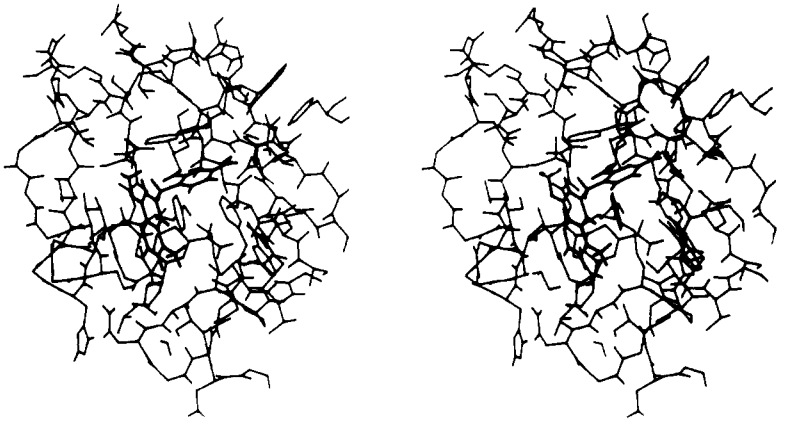


Figure 2. Stereo view showing compound **15** modeled as for the *E. coli* DHFR complex described for Figure 1.

In the absence of 1,2-dihydroquinolines which do not contain *gem*-dimethyl substitution, one cannot make a direct statement about their effect. It seemed more useful to model a dihydro and aromatic quinoline in *E. coli* DHFR to see how they might best bind. Compound **8b** and the aromatic derivative **15** were chosen for this purpose. They were both fitted into the ternary complex of *E. coli* DHFR–NADPH–TMP, with coordinates of Champness et al.[4b], by removal of the TMP and placement of the new inhibitors in the same pyrimidine locus. AMBER molecular mechanics[17] was then employed for estimating optimal fitting into the active site. The calculations employed about 40% of the enzyme in the active-site region, as shown in Figures 1 and 2.

Figure 1 shows the calculated lowest energy conformation taken by **8b**, in stereo view, and Figure 2 shows the result for **15**, using the above methodology. What will be noted immediately in these models is that the outer ring of **8b** is in an "up" position in the enzyme, whereas it is "down" in the case of **15**—nearly a 180° flip. Attempts to substantiate these calculations by X-ray crystallography were unsuccessful, because the complexes of these compounds with *E. coli* DHFR and NADPH did not crystallize satisfactorily.[18]

An inspection of the interactions of the substituents of the two compounds with the protein, as suggested by these models, provides useful guidance. The positions of the *gem*-dimethyl groups in Figure 1 are of particular interest. They are sandwiched between Leu-28 in the upper right and Ile-50 on the left, appearing to be in good van der Waals contact with each side chain. The 4-methyl group lies with Ile-50 on the left, Leu-54 above it, Phe-31 somewhat behind, and Leu-28 on the right. The 8-methoxy group lies snugly in a hydrophobic pocket with the methyl group interacting with the nicotinamide ring of the cofactor and lying between the Met-20 side chain on the right and Ser-49 on the left. The absence of the 8-methoxy group would create an aqueous void. These contacts may be compared with corresponding trimethoprim interactions by reference to Figure 1 in Champness et al.[4b] and Figure 2 in Matthews et al.[4a] The presence of the *gem*-dimethyl groups at the entrance to the cleft may create a more tightly closed hydrophobic pocket than is the case with **1**, with additional van der Waals interactions, thus producing the tighter binding.

In Figure 2, the 8-methoxy group of **15** lies in the hydrophobic pocket described for the 4-methyl of **8b**. The methyl moieties of these functions appear to be situated very similarly. The 4-methyl group faces the nicotinamide, and the Met-20 side chain offers a protective arm. The aromatic ring, as modeled, is situated optimally between the two sides of the cleft, with the ring nitrogen facing outward.

One would wish for similar models with vertebrate DHFR, but at the time the work was done we did not have appropriate coordinates. It is known that the out-of-plane 4-methoxy group of TMP contributes to poor binding to rat and chicken DHFR.[4a,19,15] A 4-isopropenyl analogue,

(17) Weiner, P. K.; Kollman, P. A. *J. Comput. Chem.* **1981**, *2*, 287.
(18) Champness, J. N. Unpublished data.
(19) Matthews, D. A.; Bolin, J. T.; Burridge, J. M.; Filman, D. J.; Volz, K. W.; Kraut, J. *J. Biol. Chem.* **1985**, *260*, 392.

Table III. pK_a Values of Dihydro- and Tetrahydroquinolines[a]

no.	substituents 1	2	4	8	pK_a(20°C)
5a		$(CH_3)_2$	CH_3		3.69
5b		$(CH_3)_2$	CH_3	OCH_3	3.36
5i	CH_3	$(CH_3)_2$	CH_3	OCH_3	6.82
17[b]	CH_3	H_2	CH_3, H	OCH_3	6.51
18[b]	C_2H_5	H_2	CH_3, H		4.18
19[b]	CH_3	H_2	H_2		4.10

[a] Roth, B.; Strelitz, J. Z. *J. Org. Chem.* **1969**, *34*, 821. [b] Reference 2.

with two out-of-plane carbon atoms, binds even more weakly.[20] The out-of-plane *gem*-dimethyl substituents may then contribute to the poor vertebrate-DHFR inhibition and resultant very high selectivity.

In the case of the tetrahydroquinolines of a previous paper,[2] the addition of a methyl group at N-1 increased the selectivity significantly.[2] In that case, we considered the possibility that increased basicity, with resultant protonation, might have caused this effect. We measured the pK_a values of compounds **5a**, **5b**, and **5i**, as shown in Table III, and compared them with three previously prepared tetrahydro compounds (**17**–**19**).

1-Methylation of **5b** increased the pK_a by 3.5 units. That this increase is not due merely to 1-methylation is indicated by comparison with the tetrahydro derivatives **17**–**19**. These show that an 8-methoxy substituent, as well as a 1-methyl group, is required to increase the basicity significantly. Hydrogen bonding to the 8-methoxy group, as well as steric crowding at N-1, probably holds the proton firmly in position.

It is clear from these pK_a data that protonation is not involved in decreasing binding to vertebrate DHFR in the case of the *gem*-dimethyl derivatives. Whether protonation can be invoked for the compounds of the previous two papers remains an open question, which cannot be ruled out at this point.

Table IV depicts relative in vitro antibacterial activities of representative [(dihydro- and tetrahydroquinolyl)-methyl]pyrimidines of this paper. Although many of the compounds were less active than the standard TMP for the Gram-negative organisms, some were considerably more active for the Gram-positive *Staphylococci* and *Streptococci*. The fluorodihydroquinoline **13** was outstanding in this regard. This compound maintains an across-the-board highly active antibacterial profile, which is comparable to that of **1** for the Gram-negative organisms such as *E. coli*, although it might have been expected to be superior, on the basis of its very high *E. coli* DHFR inhibition. We have observed with many compounds that superior *E. coli* DHFR activity is not matched with the whole-cell data, although it usually is with *Staphylococcus aureus* DHFR. Curiously, the close analogue **14** is quite a bit less active than **13**.

Compound **8b** was highly active in vitro, although slightly inferior to the standard against the Gram-negative organisms. Its 1-methyl derivative **8i** was less active (data not shown). The remaining compounds were less active on the Gram-negative organisms, due possibly to increased lipophilicity or bulk, but also in some cases to poor DHFR inhibitory activity. Note that lipophilicity per se cannot be cited as the cause for low activity; **8b**, with a log *P* value of 3, is highly active.

Since compounds **13** and **8b** show considerable advantages over trimethoprim for the Gram-positive organisms, as well as high broad-spectrum activity, these two derivatives are being evaluated in depth for their in vivo antibacterial activity, toxicity, and pharmacokinetic parameters. The results will be reported elsewhere.

(20) Kompis, I.; Then, R.; Boehni, E.; Rey-Bellet, G.; Zanetti, G.; Montavon, M. *Eur. J. Med. Chem.* **1980**, *15*, 7.

Experimental Section

A. Chemistry. Melting points were determined with a Hoover apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, these results were within ±0.04% of the theoretical values. Nuclear magnetic resonance (^1H NMR) spectra were recorded on Varian XL-100 and FT-80 spectrometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane.

Preparation of 1,2-Dihydro-2,2-dimethylquinolines. These were prepared by a much simplified modification of the method described in *Organic Syntheses*,[8] in which an aniline and mesityl oxide were heated together at 100 °C while adding iodine slowly to the mixture over approximately a 2-h period. After cooling, the dark product was purified on a silica gel column, with solvents such as hexane, hexane–0.5% EtOAc, or CH_2Cl_2–3% MeOH for elution. In most cases, the yields (based on starting anilines) were very low (less than 10%), and part of the product was dimeric or polymeric. There were exceptions, however, which are described below. Most of the products were oils, which were identified by NMR spectroscopy and in some cases by analysis. 1-Aryl-2,4-dimethylpyrroles were also isolated as byproducts in some instances. In some cases partially purified products were used in the next reaction; no separation difficulties were encountered.

1,2-Dihydro-2,2,4-trimethyl-1,10-phenanthroline (5h). 8-Aminoquinoline (**3h**) (7.21 g, 0.05 mol) and mesityl oxide (23 mL, 0.2 mol) were heated together at 100 °C, while iodine was added gradually over 2 h. The reaction was cooled, and it was then purified on a silica gel column, eluting with hexane–0.5% EtOAc, to give 5.91 g (52.7%) of **5h** as a red oil. NMR ($CDCl_3$): δ 1.34 (s, 6, Me_2), 2.04 (s, 3, Me), 5.29 (s, 1, CH, dihydropyridine-β-H), 5.96 (br, 1, NH), 6.86 (d, 1, Ar), 7.2 (dd, 1, pyridine-β-H), 7.27 (d, 1, Ar), 7.89 (dd, 1, pyridine-γ-H), 8.60 (dd, 1, pyridine-α-H). Anal. ($C_{15}H_{16}N_2$) C, H, N.

1,2-Dihydro-2,2,4-trimethyl-8-ethoxyquinoline (5c). *o*-Phenetidine (20 g, 0.15 mol) was reacted with mesityl oxide (4 equiv) and iodine (0.2 equiv) in the manner described above for **5h**. The reaction was purified on a silica gel column that was eluted with hexane–0.3% Et_2O, giving 2.81 g (12%) of **5c** as yellow crystals. NMR (Me_2SO-d_6): δ 1.23 (s, 6, Me_2), 1.34 (tr, 3, Me), 1.90 (d, 3, Me), 4.01 (q, 2, CH_2), 4.65 (br s, 1, NH), 5.29 (s, 1, CH), 6.43–6.70 (m, 3, Ar). Anal. ($C_{14}H_{19}NO$) C, H, N.

1,2-Dihydro-2,4-dimethyl-3-fluoro-2-(fluoromethyl)-8-methoxyquinoline (11) and 1,2-Dihydro-2,4-(difluoromethyl)-8-methoxy-2-methylquinoline (12). To 9.6 g (0.078 mol) of *o*-anisidine (**3b**) contained in a round-bottomed flask equipped with condenser, stirrer, and nitrogen inlet tube was added 30 g (0.39 mol) of fluoroacetone (**10**). The mixture was slowly brought to reflux temperature, and a total of 3.3 g (0.013 mol) of iodine was then added slowly through the top of the condenser to avoid loss of fluoroacetone. The mixture was allowed to heat for 3.5 h under reflux, after which the excess fluoroacetone was removed, and the residue was purified by chromatography on silica gel, using a gradient elution with hexane–(1 to 5% EtOAc). This resulted in the separation of 3.4 g (18%) of **11** and 1.1 g (6%) of **12** as oils, in addition to 1.86 g of a mixture of **11** and **12**. In a second, similar reaction, there was obtained 4.56 g (24%) of **11** and 0.60 g of **12**. NMR of **11** in Me_2SO-d_6: δ 1.33 (dd, 3, 2-Me), 1.89 (d, 3, 4-Me, J = 3.1 Hz), 3.78 (s, 3, OMe), 4.35 (d, 2, 2-CH_2F, J = 47.6 Hz), 5.52 (br d, 1, NH, J = 7.3 Hz), 6.4–6.8 (m, 3, ArH_3). NMR of **12** in Me_2SO-d_6: δ 1.24 (d, 3, 2-Me, J = 2.3 Hz), 3.78 (s, 3, OMe), 4.27 (dd, 2, CH_2F, J = 1 and 48 Hz), 5.16 (br, 1, NH), 5.20 (dd, 2, 4-CH_2F, J = 0.7 and 47 Hz), 5.61 (d, 4.2, pyridine-3-H), 6.37–6.83 (m, 3, ArH_3). Anal. **11** ($C_{13}H_{15}F_2NO$) C, H, N. Anal. **12** ($C_{13}H_{15}F_2NO$) C, H, N.

1,2-Dihydro-8-methoxy-1,2,2,4-tetramethylquinoline (5i). The method used was a modification of that of Gribble and Heald.[21] Compound **5b** (1.02 g, 5 mmol) was dissolved in dry

(21) Gribble, G. W.; Heald, P. W. *Synthesis* **1975**, 650.

Table IV. Relative in Vitro Antibacterial Activities of Representative 2,4-Diamino-5-[(1,2-dihydro-6-quinolyl)methyl]pyrimidines and Tetrahydro Analogues Compared to Trimethoprim (MIC Compound/MIC Trimethoprim)[a-d]

organism	8b	8f	8g	8h	13[e]	14	9b
Streptococcus pyogenes CN10	0.1	0.1	10	0.03	0.03 0.0001	0.1	1
Streptococcus faecalis CN478	0.1	≤0.03	300	0.3	0.0001	0.3	1
S. agalactiae CN1143	0.3	0.3	100	0.3	0.1 0.03	0.3	1
Staphylococcus aureus CN491	0.3	0.3	30	0.1	0.001	0.3	3
Vibrio cholerae ATCC14035	3	1	100	1	3	3	3
Salmonella typhimurium S8587	10	30	30	100	3 3	3	30
Salmonella typhosa CN512	10	30	30	30	1 30	10	100
Shigella flexneri CN6007	3	30	100	100	3 0.3	10	30
E. coli CN314	3	10	100	30	1 10	10	30
Klebsiella pneumoniae CN3632	10	100	30	>300	10 1	10	
Enterobacter aerogenes 2200/86	3	10	300	10	1 10	30	10
Proteus vulgaris CN329	3	>100	30	>100	3 3	10	>100

[a] Numbers less than 1 indicate activity greater than TMP. Differences of one dilution (1:3) are not considered significant. MIC = minimum inhibitory concentration. [b] Data for all compounds against 17 organisms are available in the supplementary material. [c] MIC values against *P. aeruginosa* were >100 μg/mL for all compounds, including 1. [d] Representative MIC values for 1 are as follows, respectively: 0.3, 0.1, 0.3, 0.1, 0.3, 0.1, 0.1, 0.1, 0.1, 0.3, 0.1, and 1.0 μg/mL for the organisms as listed. [e] The second set of numbers represents repeat assays on a different day.

THF under N_2 and chilled to 0 °C, followed by the addition of 2.0 g (10 equiv) of $NaBH_4$. Then 20 mL of freshly distilled formic acid was added slowly over 30 min, keeping the temperature at 0 °C. The reaction was allowed to warm to 25 °C and then stirred overnight, and then it was refluxed at 60 °C for 1.5 h. The solvent was removed and the residue was neutralized with cold NH_4OH to pH 9 and extracted into CH_2Cl_2. The product was purified on a silica gel column which was eluted with hexane–EtOAc/(50:1 and then 19:1), giving 0.76 g (70%) of **5i**. NMR ($CDCl_3$): δ 1.20 (s, 6, Me_2), 2.02 (s, 3, 4-Me), 2.49 (s, 3, NMe), 3.81 (s, 3, OMe), 5.34 (s, 1, 3-H), 6.80 (m, 3, Ar). Anal. ($C_{14}H_{19}NO$) C, H, N.

2-(1,2-Dihydro-8-methoxy-2,2,4-trimethyl-1-quinolyl)-ethanol (5j). Compound **5b** (2.36 g, 12 mmol) was dissolved in acetone with 2-bromoethanol (25 equiv) and anhydrous K_2CO_3 (3 g). The mixture was stirred and refluxed until no more starting material could be seen by TLC. The reaction mixture was then filtered through silica gel and the product was purified on a silica gel column, eluting with 2–50% EtOAc–hexane, to give 0.80 g (28%) of a light tan solid which was recrystallized from Et_2O; mp 94.5–96 °C. NMR (Me_2SO-d_6): δ 1.11 (s, 6, Me_2), 1.97 (d, 3, 4-Me, J = 1.4 Hz), 3.13 and 3.16 (2s, 4, $(CH_2)_2$), 3.80 (s, 3, OMe), 4.19 (br s, 1, OH), 5.52 (d, 1, 3-H, J = 1.4 Hz), 6.86–6.93 (m, 3, 5–7-H). Anal. ($C_{15}H_{21}NO_2$) C, H, N.

General Procedure for Condensation of Dihydroquinolines with 2,4-Diamino-5-(hydroxymethyl)pyrimidine (7). The procedures used were practically identical with those described previously for the tetrahydroquinolines.[2] Compounds prepared and physical data are listed in Table I. Several examples follow.

2,4-Diamino-5-[(1,2-dihydro-2,2,4-trimethyl-8-methoxy-6-quinolyl)methyl]pyrimidine (8b). A mixture of 4.0 g (0.02 mol) of 1,2-dihydro-2,2,4-trimethyl-8-methoxyquinoline (**3b**),[22] 2.76 g (0.02 mol) of **7**, 50 mL of glacial HOAc, and 1.64 mL (0.02 mol) of concentrated hydrochloric acid was heated under reflux for 2 h. The solvent was removed, and the residue was dissolved in water and made basic with NH_4OH. The product was extracted into CH_2Cl_2–MeOH (3:1) and purified on a silica gel column which was eluted with CH_2Cl_2–MeOH (19:1). There was recovered 5.06 g (79%) of **8b**, mp 186–188 °C. NMR ($CDCl_3$): δ 1.25 (s, 6, Me_2), 1.94 (s, 3, Me), 3.57 (s, 2, CH_2), 3.73 (s, 3, OMe), 4.17 (br s, 1, NH), 4.63 (br s, 2, NH_2), 4.80 (br s, 2, NH_2), 5.28 (s, 1, CH), 6.40 (d, 1, Ar), 6.53 (d, 1, Ar), 7.72 (s, 1, pyrimidine-6-H). log P (octanol–0.01 N NaOH) = 3.03. Anal. ($C_{18}H_{23}N_5O$) C, H, N.

(22) Elliott, I. W., Jr.; Dunathan, H. C. *Tetrahedron* **1963**, *19*, 833.

2,4-Diamino-5-[(1,2-dihydro-2,2,4-trimethyl-1,10-phenanthrolin-5-yl)methyl]pyrimidine (8h). Compound **5h** (2.33 g, 10.4 mmol), compound **7** (1.46 g, 10.4 mmol), and 1 equiv of concentrated hydrochloric acid in 25 mL of glacial HOAc were refluxed for 4 h, and treated as for **8b**. After purification on a silica gel column which was eluted with CH_2Cl_2–3% MeOH, 2.62 g (73%) of **8h** was obtained, mp 226–230 °C (EtOAc). NMR (Me_2SO-d_6): δ 1.32 (s, 6, Me_2), 1.96 (d, 3, Me), 3.85 (s, 2, CH_2), 5.35 (br s, 1, NH), 5.62 (br s, 2, NH_2), 6.18 (br s, 2, NH_2), 6.34 (q, 1, CH), 7.11 (s, 1, Ar), 7.14 (s, 1, pyrimidine-6-H), 7.41 (dd, 1, pyridine-β-H), 8.12 (dd, 1, pyridine-γ-H), 8.69 (dd, 1, pyridine-α-H). Anal. ($C_{20}H_{22}N_6 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-[(1,2-dihydro-2,4-dimethyl-3-fluoro-2-(fluoromethyl)-8-methoxy-6-quinolyl)methyl]pyrimidine Hydrochloride (13). A mixture of 3.41 g (0.014 mol) of **11**, 1.96 g (0.014 mol) of **7**, 1.25 mL of concentrated HCl, and 50 mL of glacial HOAc was heated under reflux for 2 h, followed by removal of solvent, neutralization, and extraction into 3:1 CH_2Cl_2–MeOH. After drying and removal of the solvent, there was recovered 5.0 g of crude **13**, which was purified by chromatography as for **8h**, followed by recrystallization from EtOAc plus 1 equiv of HCl in EtOH, to yield 3.66 g (65%) of **13** hydrochloride, mp 258–261 °C. NMR (Me_2SO-d_6): δ 1.30 (d, 3, 2-Me, J = 1.6 Hz), 1.86 (d, 3, 4-Me, J = 3 Hz), 3.51 (s, 2, CH_2), 3.75 (s, 3, OMe), 4.33 (d, 2, 2-CH_2F, J = 48 Hz), 5.50 (d, 1, NH, J = 6 Hz), 6.62 (d, 1, Ar, J = 1 Hz), 6.68 (d, 1, Ar, J = 1 Hz), 7.40 (s, 1, pyrimidine-6-H), 7.57 (s, 2, NH_2), 7.75 and 8.25 (2 br, 2, NH_2), 12.12 (br 2, 1, NH^+). Anal. ($C_{18}H_{21}F_2N_5O \cdot HCl$) C, H, N, Cl.

2,4-Diamino-5-[(1,2-dihydro-2,4-bis(difluoromethyl)-2-methyl-8-methoxy-6-quinolyl)methyl]pyrimidine Hydrochloride (14). This compound was prepared in the manner of **13**; the product, obtained as the hydrochloride hemihydrate, melted at 215–216 °C. NMR (Me_2SO-d_6): δ 1.22 (d, 3, 2-Me), 3.50 (s, 2, CH_2), 3.76 (s, 3, OMe), 4.25 (d, 2, 2-CH_2F, J = 47 Hz), 5.13 (br s, 1, NH), 5.15 (d, 2, 4-CH_2F, J = 47 Hz), 5.60 (d, 1, 3-H, J = 4 Hz), 6.63 (d, 1, Ar), 6.72 (d, 1, Ar), 7.38 (s, 1, pyrimidine-6-H), 7.58 (br s, 2, NH_2), 7.75 and 8.30 (2 br s, 4-NH_2), 11.9 (br s, 1, NH^+). Anal. ($C_{18}H_{21}F_2N_5O \cdot HCl \cdot 0.5H_2O$) C, H, N.

2-[6-[(2,4-Diamino-5-pyrimidyl)methyl]-1,2-dihydro-8-methoxy-2,2,4-trimethyl-1-quinolyl]ethyl Acetate Dihydrochloride (8k). Compound **5j** (0.63 g, 3 mmol) was treated with **7** as in the previous examples except that it was heated for 27 h. Purification as above followed by crystallization from absolute EtOH–Et_2O–HCl gave 0.13 g (11%) of **8k**, mp 180–184 °C. NMR (Me_2SO-d_6): δ 1.24 (br s, 6, Me_2), 1.77 (s, 3, COMe), 1.98 (s, 3, 4-Me), 3.68 (s, 2, CH_2 bridge), 3.83 (br s, 7, OMe and $(CH_2)_2$), 5.63

(s, 1, 3-H), 6.96 and 7.00 (2s, 2, 5-H and 7-H), 7.56 and 7.65 (2s, 3, NH_2 and pyrimidine 6-H), 7.90 and 8.31 (2s, 2, NH and NH). Anal. ($C_{22}H_{31}Cl_2N_5O_3$) C, H, N.

2,4-Diamino-5-[(1,2-dihydro-2,2,4-trimethyl-8-(methylsulfinyl)-6-quinolyl)methyl]pyrimidine (8l). Compound **8f** (1.0 g, 2.9 mmol) was dissolved in MeOH and added to a solution of $NaIO_4$ (0.66 g, 3.07 mmol) in water at 0 °C. The reaction was then allowed to stir at room temperature overnight. The inorganic precipitate was removed and the product was extracted into CH_2Cl_2 and dried. It was then purified by column chromatography on silica gel, eluting with CH_2Cl_2–MeOH (19:1) to yield 0.47 g (45%) (EtOH) of **8l**, mp 246–248 °C. NMR (Me_2SO-d_6): 1.24 (d, 6, Me_2), 1.90 (d, 3, Me, J = 1.2 Hz), 2.73 (s, 3, SOMe), 3.48 (s, 2, CH_2), 5.42 (br s, 1, quinoline-3-H), 5.65 (br s, 2, NH_2), 6.04 (br s, 2, NH_2), 6.11 (m, 1, NH), 7.02 (s, 2, Ar), 7.50 (s, 1, pyrimidine-6-H). Anal. ($C_{18}H_{23}N_5OS \cdot 0.3H_2O$) C, H, N, S.

2,4-Diamino-5-[(1,2,3,4-tetrahydro-8-methoxy-2,2,4-trimethyl-6-quinolyl)methyl]pyrimidine (9b). Compound **8b** (0.65 g, 2 mmol) was dissolved in 50 mL of EtOH with 2 equiv of hydrochloric acid and reduced on a Parr hydrogenator with 0.2 g of PtO_2. After removal of the catalyst and solvent, a 0.5 M $NaHCO_3$ solution was added, and the product was extracted into CH_2Cl_2, followed by column chromatography as above described; yield 0.41 g (63%), mp 214–216 °C (absolute EtOH). NMR ($CDCl_3$): δ 1.14–1.30 [m, 9, Me, Me_2 (2 different isomers)], 1.41–1.80 (m, 2, CH_2), 2.88 (m, 1, C*H*Me), 3.60 (d, 2, CH_2), 3.75 (s, 3, OMe), 4.01 (br, 1, NH), 4.58 (br, 2, NH_2), 4.67 (br, 2, NH_2), 6.38 (d, 1, Ar), 6.65 (d, 1, Ar), 7.77 (s, 1, pyrimidine-6-H). Anal. ($C_{18}H_{25}N_5O$) C, H, N.

2,4-Diamino-5-[(1,2,3,4-tetrahydro-2,2,4-trimethyl-6-quinolyl)methyl]pyrimidine (9a). This compound was prepared from **8a** as described for **9b** and isolated as the hydrochloride; mp 245–250 °C dec (absolute EtOH–Et_2O). Anal. ($C_{17}H_{23}N_5 \cdot 2HCl \cdot 0.33H_2O$) C, H, N.

B. Enzyme Assays. Assay conditions for *E. coli* and rat liver DHFR were as previously described.[15,23] Homogeneous chicken liver DHFR was obtained from J. Freisheim (Medical College of Ohio) and assayed like the rat liver enzyme. I_{50} is the concentration of inhibitor that decreases the velocity of the standard assay by 50%. The enzyme, NADPH (65 μM), and varying concentrations of inhibitor were preincubated for 2 min at 30 °C, and the reaction was initiated by the addition of dihydrofolate (45 μM final concentration). Steady-state velocities were measured, and plots of the percentage inhibition vs the logarithm of inhibitor concentration were used to estimate I_{50} values. In two cases the inhibition measured at the solubility limit of the compound is given as I_{32} or I_{41}. Since the *E. coli* DHFR assay contained 1 nM enzyme, a 2 nM lower limit was arbitrarily set for I_{50} values. With this cutoff, error due to depletion of free inhibitor by enzyme binding[24] was limited to 25%. Apparent K_i values for some of the more potent *E. coli* DHFR inhibitors were determined by the method of Henderson[25] as described by Baccanari and Joyner.[26]

Acknowledgment. We wish to thank Dr. Lee Kuyper and Dr. Joseph Chan for the molecular mechanics calculations and for providing Figures 1 and 2. We thank Pauline Baker for expert technical assistance. The determination of I_{50} values for *E. coli* and rat liver DHFR inhibition were carried out under the direction of Robert Ferone, and Dr. Lynn Elwell's staff conducted the antibacterial assays. The NMR spectral determinations were carried out by Dr. Stuart Hurlbert and his staff. The log *P* measurements were carried out under the aegis of Dr. David Brent. Microanalyses were carried out by Atlantic Microlabs, Inc., Norcross, GA.

Supplementary Material Available: A table listing NMR data for certain 1,2-dihydroquinolines and 1-aryl-2,4-dimethylpyrroles isolated as oils are available, as well as a table of MIC data for all compounds of this paper against 17 organisms (2 pages). Ordering information is given on any current masthead page.

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2,4-Diamino-5-benzylpyrimidines as Antibacterial Agents. 13. Some Alkenyl Derivatives with High in Vitro Activity against Anaerobic Organisms

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A series of 2,4-diamino-5-(3,5-dialkenyl-4-methoxy- or -4-hydroxybenzyl)pyrimidines was prepared from [(allyloxy)benzyl]pyrimidines by Claisen rearrangements, and the resulting allyl phenols were further modified by methylation and rearrangement to 1-propenyl analogues. Analogous 3,4-dimethoxy-5-alkenyl derivatives were prepared by similar techniques. High in vitro antibacterial activity was obtained against certain anaerobic organisms, such as *Bacteroides* species and *Fusobacterium*, which was equal to or better than the control, metronidazole, in several cases. The profile was similar against *Neisseria gonorrhoeae* and *Staphylococcus aureus*. The 3,5-bis(1-propenyl)-4-methoxy derivative **8** was 1 order of magnitude more active against *Escherichia coli* dihydrofolate reductase than its saturated counterpart, and it was also more active than trimethoprim, **1**. However, it was considerably less active in vitro against the Gram-negative organisms. The 3,4-dimethoxy-5-alkenyl, -5-alkyl, and -5-alkoxy analogues had very high broad-spectrum antibacterial activity. However, pharmacokinetic studies of four of the compounds in dogs and rats and in vivo studies with an abdominal sepsis model in rats showed no advantages over trimethoprim.

Although trimethoprim (TMP, **1**)[1-4] has excellent broad-spectrum antibacterial activity, it is not useful against all types of organisms. The search has continued for other inhibitors of dihydrofolate reductase (DHFR, E.C. 1.5.1.3) which will show greater activity against

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0022-2623/89/1832-1949$01.50/0