2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 11. Quinolylmethyl Analogues with Basic Substituents Conveying Specificity[1]

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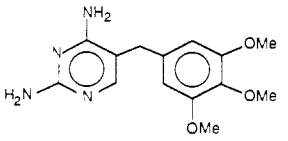
A series of nine 2,4-diamino-5-[6-(or 7-)quinolylmethyl]pyrimidines has been prepared by condensations of quinolinecarboxaldehydes with β-anilinopropionitriles, followed by treatment with guanidine. All compounds had basic or methoxy substituents at the 2- or 4-positions of the quinoline ring. All of the 6-quinolylmethyl derivatives were highly inhibitory against *Escherichia coli* dihydrofolate reductase (DHFR), provided that an 8-substituent was present in the quinoline ring. Those compounds that had basic substituents in the 2-position of the quinoline ring were also highly specific for bacterial dihydrofolate DHFR, relative to a vertebrate counterpart. Protonation on the quinoline ring nitrogen is a possible cause of specificity.

The previous paper in this series described analogues of trimethoprim (TMP, **1**) in which the benzyl function was replaced by the bicyclic 6-quinolylmethyl ring system or its 1,2,3,4-tetrahydroquinolyl derivatives (**2**).[2] Some of these compounds were highly inhibitory to *Escherichia coli* dihydrofolate reductase (DHFR, EC 1.5.1.3), in particular the aromatic quinoline **2**, where R_4 = methyl and R_8 = methoxy. However, such quinolines lacked the high specificity of **1** for bacterial DHFR. The tetrahydro derivatives, while not quite as potent in their inhibition of the *E. coli* enzyme, were highly selective in certain cases, particularly with 1,4-dimethyl-8-methoxy substitution. The fact that this combination of functions increased the quinoline pK_a value by about 2 units, resulting in partial protonation at physiological pH, suggested that this might play a role in the selectivity pattern. Since selectivity is an essential element in the design of useful antibacterial agents, we deemed it important to pursue this clue.



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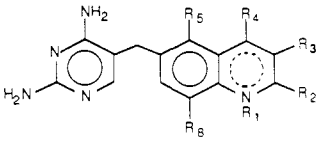

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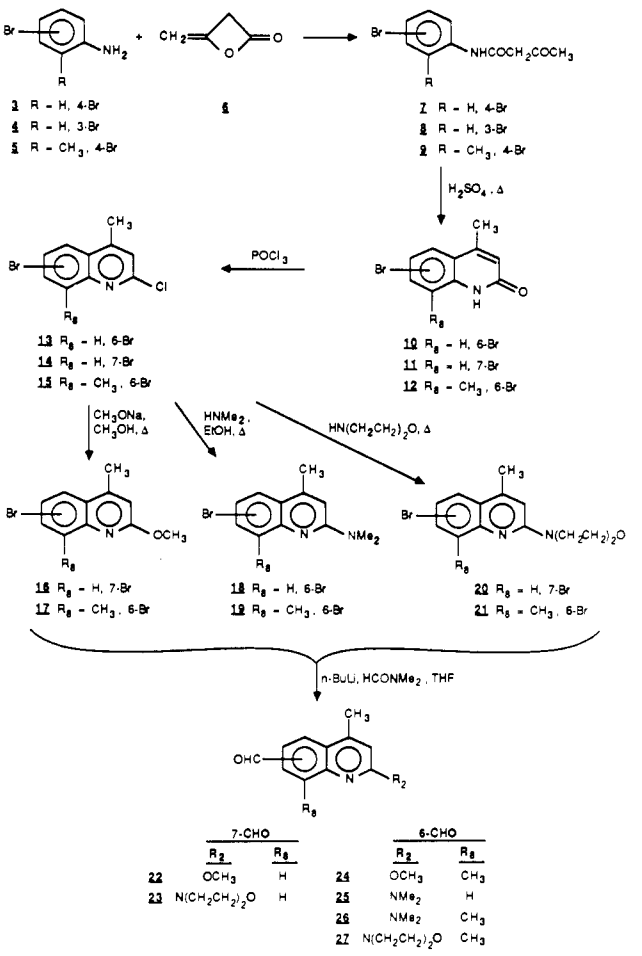
In this paper we describe quinoline analogues with basic or polar substituents at the 2- or 4-position of the quinoline ring. In the majority of cases, the pyrimidylmethyl moiety was attached at C-6 of the quinoline, but we also explored C-7 substitution.

Chemistry

The chief synthetic route to the compounds of general type **2**, which involved condensation of 2,4-diamino-5-(hydroxymethyl)pyrimidine with 1,2,3,4-tetrahydroquinolines in acidic medium, followed by oxidation in some instances, did not permit polar substitution (such as amino or methoxy) in the 2- or 4-positions of the tetrahydroquinoline ring.[2] In fact, all compounds prepared had either hydrogen or methyl at these positions. We then required new methodology.

Scheme I illustrates the routes to several quinolinecarboxaldehyde intermediates which contain 2-methoxy or dialkylamino substitution. The strategy involved condensation of appropriately substituted bromoanilines with diketene to produce 6- or 7-bromo-2-quinolones. Conversion of the quinolones to their chloro analogues resulted in ready transformation to their desired 2-methoxy or substituted amino counterparts. The bromo-substituted

Scheme I



quinolines were treated with butyllithium, followed by dimethylformamide, to create the corresponding aldehydes.

Scheme II illustrates the procedure used for condensation of the aldehydes to produce pyrimidylmethyl derivatives in two steps. This is a standard route used for the synthesis of trimethoprim and many other benzylpyrimidines.[3]

Scheme III shows the routes to 4-methoxy- or 4-(dialkylamino)quinoline derivatives. The methodology was similar to that of Scheme I. Bromoanilines were condensed

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(1) Roth, B.; Rauckman, B. S. U.S. Patent 4,587,341, May 6, 1986.
(2) Rauckman, B. S.; Tidwell, M. Y.; Johnson, J. V.; Roth, B. *J. Med. Chem.* preceding paper in this issue.
(3) Cresswell, R. M.; Mentha, J. W.; Seaman, R. L. Ger. Offen. 2,010,166, 1970; *Chem. Abstr.* **1971**, *74*, 128562.

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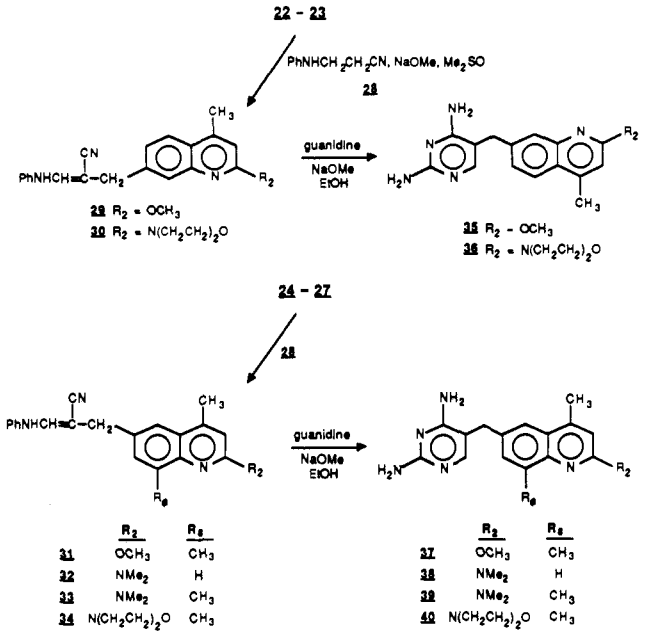
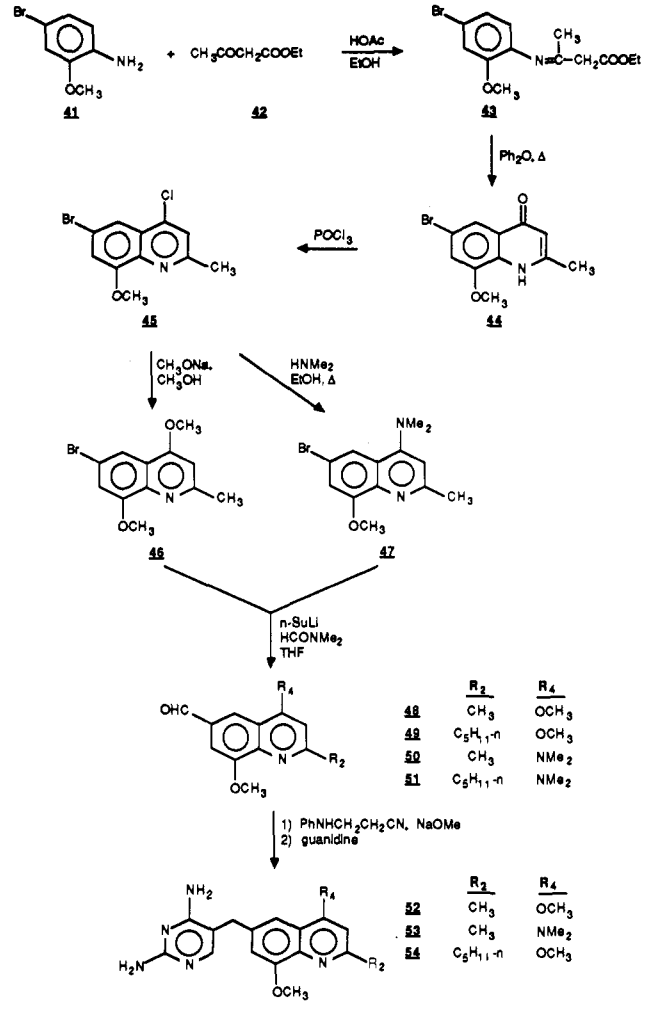
Scheme II



Scheme III



with acetoacetic ester in two steps to produce 4-quinolones, which were then treated in the manner of Scheme I to produce the desired aldehydes. In a few cases the butyllithium reaction did not proceed completely as desired; attack on the 2-methyl function as well as the bromo substituent occurred, to produce 2-*n*-amylquinoline-carboxaldehydes. The 2-methyl group was apparently converted to its anion in part, which attacked butyl bromide produced from the butyllithium and bromoquinoline. Decreasing the amount of butyllithium obviated the problem for the most part.

The compounds prepared, yields, and physical constants are listed in Table I, with notes on methodology in certain cases. Examples of each procedure are described in detail under Experimental Section.

Biological Data and Discussion

Table II presents the inhibitory activities of the (quinolylmethyl)pyrimidines of Table I against DHFR from bacterial and vertebrate sources. The most interesting compounds of this table are **39** and **40**, both of which have basic substituents at the 2-position of the quinoline ring. Both of these compounds show high inhibitory activity and selectivity for bacterial DHFR from *E. coli*, which are of a similar order of magnitude to that of the prototype standard **1**. The corresponding 2-methoxyquinolyl derivative, **37**, while a potent inhibitor of *E. coli* DHFR, was also several times more active against the rat liver enzyme. This suggests that the basic 2-substituents may be responsible for conveying the high specificity of **39** and **40**.

4- and 8-substituents in the quinoline ring may be equated to the 3- and 5-substituents of benzylpyrimidines, which probably occupy very similar positions in space when bound to DHFR.[2] Appropriate substituents at both of these positions are important to tight binding to the bacterial enzyme.[4] By comparison of compounds **38** and **39**, it will be noted that an 8-methyl substituent added appreciably to the inhibitory activity. Although we considered a methoxy substituent to be optimal at this position, earlier studies with (3,5-dialkylbenzyl)pyrimidines demonstrated that a 3-methyl-5-ethyl derivative was nearly as active as its 3,5-diethyl or 3,5-dimethoxy analogue; however 3,5-dimethyl substitution was quite inferior.[5]

Moving the dimethylamino or methoxy substituent to the 4-quinolyl position did not achieve the desired result. A 4-methoxy derivative (**52**) was an excellent inhibitor of *E. coli* DHFR but showed poor selectivity. The dimethylamino analogue was neither very active nor very selective. Very likely the decreased activity against bacterial DHFR was due to the bulk of the dimethylamino function. In a series of (3,5-dialkylbenzyl)pyrimidines, 3,5-diisopropyl derivatives were found to suffer spatial crowding in contact with the bacterial enzyme.[5]

The 2-*n*-amylquinolyl derivative **54** was a powerful *E. coli* DHFR inhibitor, probably as a result of additional van der Waals contacts with the enzyme. Insolubility precluded obtaining useful data with the rat liver DHFR, but normally lipophilic compounds of this type bind more tightly to vertebrate DHFR, so high selectivity is not to be expected.[5]

The two (7-quinolylmethyl)pyrimidines **35** and **36** showed quite the reverse of the desired trend in bacterial, compared to mammalian, DHFR activity; this series has not been pursued further. Possibly, appropriate substitution would provide improved results.

A few compounds were tested against *Neisseria gonorrhoeae* DHFR. Compound **53** was 3 times as active as **1**, but not nearly as active as certain (alkylbenzyl)pyrimidines.[5]

The literature pK_a values for several quinolines related to our investigation are given in Table IV,[6-9] and pK_a

(4) Roth, B.; Cheng, C. C. *Prog. Med. Chem.* **1982**, *19*, 270–331.
(5) Roth, B.; Rauckman, B. S.; Ferone, R.; Baccanari, D. P.; Champness, J. N.; Hyde, R. M. *J. Med. Chem.* **1987**, *30*, 348.

Table I. Quinolines and Their Intermediates

no.	type of compd	substitution (Bz or Q) 2	3	4	6	7	8	yield, %	mp, °C	recrystn solvent[a]	empirical formula	anal.
7	*N*-aryl-3-oxobutyramide			Br				43	135.5–137	A	$C_{10}H_{10}BrNO_2$	C, H, N, Br
8			Br					48	94–95	B	$C_{10}H_{10}BrNO_2$	C, H, N, Br
9		CH_3		Br				52.6	93.8–94.9	C	$C_{11}H_{12}BrNO_2$	C, H, N, Br
43	(arylimino)butyrate	OCH_3		Br				79	oil	D	$C_{13}H_{16}BrNO_3$	C, H, N
10	2-quinolone	=O		CH_3	Br			25.6	292–299	C	$C_{10}H_8BrNO$	C, H, N, Br
11		=O		CH_3		Br		84	275–276	E	$C_{10}H_8BrNO$	C, H, N, Br
12		=O		CH_3	Br		CH_3	60.8	246–250	F	$C_{11}H_{10}BrNO$	C, H, N, Br
44	4-quinolone	CH_3		=O	Br		OCH_3	71[b]	293–296	C	$C_{11}H_{10}BrNO_2$	C, H, N
13	chloroquinoline	Cl		CH_3	Br			97	139–139.8	C	$C_{10}H_7BrClN$	C, H, N, Br, Cl
14		Cl		CH_3		Br		71	73–74	E	$C_{10}H_7BrClN$	C, H, N
15		Cl			Br		CH_3	100	168.5–170.5		$C_{11}H_9BrClN$	C, H, N, Br, Cl
45		CH_3		Cl	Br		OCH_3	93	140–142	D	$C_{11}H_9BrClNO$	C, H, N
16	2-methoxy- or 2-(subst amino)bromoquinoline	OCH_3		CH_3		Br		87	58–60	B	$C_{11}H_{10}BrNO$	C, H, N, Br
17		OCH_3		CH_3	Br		CH_3	94.9	104–106.5		$C_{12}H_{12}BrNO$	C, H, N, Br
18		NMe_2		CH_3	Br			74	81.9–84.1	E	$C_{12}H_{13}BrN_2$	C, H, N, Br
19		NMe_2		CH_3	Br		CH_3	39	95–98	C	$C_{13}H_{15}BrN_2$	C, H, N, Br
20		Morph		CH_3		Br		83	115–116	C	$C_{14}H_{15}BrN_2O$	C, H, N, Br
21		Morph		CH_3	Br		CH_3	98	155–158		$C_{15}H_{17}BrN_2O$	C, H, N, Br
46	4-methoxy- or 2-(subst amino)bromoquinoline	CH_3		OCH_3	Br		OCH_3	86	167–168	D	$C_{12}H_{12}BrNO_2$	C, H, N
47		CH_3		NMe_2	Br		OCH_3	89	115–117	D	$C_{13}H_{15}BrN_2O$	C, H, N
22	quinolinecarboxaldehyde	OCH_3		CH_3		CHO		76	111–112	B	$C_{12}H_{11}NO_2$	C, H, N
23		Morph		CH_3		CHO						
24		OCH_3		CH_3	CHO		CH_3	73.3	148.5–150.2	C	$C_{13}H_{13}NO_2$	C, H, N
25		NMe_2		CH_3	CHO			17	116–117.8	D, G	$C_{13}H_{14}N_2O$	C, H, N
26		NMe_2		CH_3	CHO		CH_3	79	148–150	C	$C_{14}H_{16}N_2O$	C, H, N
27		Morph		CH_3	CHO		CH_3	59	161.5–162.5	D, C	$C_{16}H_{18}N_2O_2$	C, H, N
48		CH_3		OCH_3	CHO		OCH_3	48[c] 58[e]		D		*d*
49		C_5H_{11}-*n*		OCH_3	CHO		OCH_3	14.6[c]		D		*f*
50		CH_3		NMe_2	CHO		OCH_3	39[g] 58[h]	127–129	D	$C_{14}H_{16}N_2O_2 \cdot 0.3H_2O$	C, H, N[i]
51		C_5H_{11}-*n*		NMe_2	CHO		OCH_3	30[g] 19[h]		D	$C_{18}H_{24}N_2O_2$	C, H, N[j]
29	adduct with anilinopropionitrile	OCH_3		CH_3		$CH_2C(=NHPh)CN$		46	170–172	C	$C_{21}H_{19}BrN_3O$	C, H, N
35	(pyrimidylmethyl)quinolines	OCH_3		CH_3		CH_2P[k]		14	205–206	D	$C_{16}H_{17}N_5O \cdot 0.6H_2O$	C, H, N
36		Morph		CH_3		CH_2P		30	204–205	D	$C_{19}H_{22}N_6O \cdot 0.5H_2O$	C, H, N
37		OCH_3		CH_3	CH_2P		CH_3	28.4	246.5–250	D	$C_{17}H_{19}N_5O$	C, H, N
38		NMe_2		CH_3	CH_2P			31	218.1–219	D, E	$C_{17}H_{20}N_6$	C, H, N
39		NMe_2		CH_3	CH_2P		CH_3	46	251–253	C	$C_{18}H_{22}N_6 \cdot 0.9EtOH$	C, H, N
40		Morph		CH_3	CH_2P		CH_3	42	248–251	D, C	$C_{20}H_{24}N_6O$	C, H, N
52		CH_3		OCH_3	CH_2P		OCH_3		288–293	D, C	$C_{17}H_{19}N_5O_2 \cdot 0.75H_2O$	C, H, N[l]
53		CH_3		NMe_2	CH_2P		OCH_3	33	169–172	D, C	$C_{18}H_{22}N_6O$	C, H, N
54		C_5H_{11}-*n*		OCH_3	CH_2P		OCH_3	19	220–222	D, C	$C_{21}H_{27}N_5O_2$	C, H, N

[a] A, absolute EtOH + toluene; B, toluene; C, absolute EtOH; D, column chromatography; E, 95% EtOH; F, DMF; G, dilute EtOH. [b] Crude yield; TLC, pure product, EtOAc/MeOH, 9:1, R_f = 0.1. [c] 1.1 equiv of *n*-BuLi; no pentylaldehyde found on column chromatography. [d] Product was not analyzed. See Experimental Section for NMR spectrum. [e] 1.2 equiv of *n*-BuLi. Both **48** and **49** were separated by chromatography. [f] Structure not proved; but see comments in text. [g] 2 equiv of *n*-BuLi. Both **50** and **51** were separated on chromatography. [h] 1.5 equiv of *n*-BuLi. More of **50** than **51** was separated, compared with the previous experiment. [i] NMR. ($CDCl_3$) δ 2.75 (s, 3, Me), 3.10 (s, 6, NMe_2), 4.11 (s, 3, OMe), 6.73 (s, 1, Q-3-H), 7.38 (d, 1, Ar), 8.05 (d, 1, Ar), 10.00 (s, 1, CHO). [j] NMR ($CDCl_3$) δ 0.93 (t, 3, CH_3), 1.43 [m, 4, $(CH_2)_2$], 1.83 (m, 2, CH_2), 3.01 (t, 2, CH_2Bu, J = approximately 7 Hz), 3.06 (s, 6, NMe_2), 4.09 (s, 3, OMe), 6.79 (s, 1, Q-3H), 7.42 (d, 1, Ar), 8.09 (d, 1, Ar), 9.98 (s, 1, CHO); TLC, hexane/EtOAc, 1:2, R_f = 0.3. [k] P = 2,4-diamino-5-pyrimidyl. [l] TLC, CH_2Cl_2/MeOH, 6:1, R_f = 0.2.

Table II. Inhibition of Dihydrofolate Reductase Enzymes by 2,4-Diamino-5-[6-(or 7)quinolylmethyl]pyrimidines of Table I

	quinoline substituents					I_{50}, M × 10^8, vs DHFR		
no.	2	4	6	7	8	*E. coli*	rat liver	*N. gonorrhoeae*
35	OMe	Me		CH_2P[a]		11	450	30
36	$N(CH_2CH_2)_2O$	Me		CH_2P		8.8	1600	63
37	OMe	Me	CH_2P[a]		Me	0.52	6200	180
38	NMe_2	Me	CH_2P			3.3		
39	NMe_2	Me	CH_2P		Me	0.77	28000	
40	$N(CH_2CH_2)_2O$	Me	CH_2P		Me	0.75	41000	
52	Me	OMe	CH_2P		OMe	0.31	4900	54
53	Me	NMe_2	CH_2P		OMe	3.5, 1.0	7000	14
54	C_5H_{11}-*n*	OMe	CH_2P		OMe	<0.2	*b*	
1[c]						0.5	34000	45

[a] P = 2,4-diamino-5-pyrimidyl. [b] Insoluble. [c] Trimethoprim standard.

Table III. Comparative in Vitro Antibacterial Activity (MIC Compound/MIC 1) of 2,4-Diamino-5-[6-(or 7)quinolylmethyl]pyrimidines[a]

	compound								
organism	**35**	**36**	**37**	**38**	**39**	**40**	**52**	**53**	**54**
Streptococcus pyogenes CN10	3	10	3	10	3	1	0.3	10	3
Streptococcus faecalis CN478	3	3	1	3	1	0.3	1	100	1
Streptococcus agalactiae CN1143	3	30	1	10	1	0.3	1	30	0.3
Staphylococcus aureus CN491	10	3	1	3	1	0.3	0.3	30	0.3
Bordetella bronchiseptica CN385	>100	>100	100	10	30	3	10	30	10
Vibrio cholerae ATCC 14035	3	10	3	1	1	3	1	10	3
Pasteurella multocida ATCC 6587	10	300	10	1	3	10	3	100	10
Mycobacterium smegmatis S 3254	3	3	3	1	3	3	3	100	10
Salmonella typhimurium S 8587	100	1000	30	10	10	30	3	100	30
Salmonella typhosa CN512	1000	10000	10	10	3	30	10	100	30
Shigella flexneri CN6007	300	1000	30	10	10	30	3	30	30
Escherichia coli CN314	300	300	10	3	10	30	3	>10	10
Serratia marcescens CN2398	>100[b]	>10	10	3	10	100	3	>300	30
Klebsiella pneumoniae CN3632	100	300	10	10	3	10	10	>300	30
Enterobacter aerogenes 2200/86	300[c]	300	30	3	10	30	10	300	10
Citrobacter freundii 2200/77	100[d]	100	30	10	10	10	3	>300	10
Proteus vulgaris CN329	30	>100	30	3	10	100	3	>100	100
Proteus mirabilis S2409	30	>30	10	3	10	30	10	>30	30

[a] Numbers greater than 1 signify lower activity than trimethoprim (1). Differences of ± one dilution (1:3) are not considered significant. [b] Strain CN8712. [c] Strain S3770. [d] Strain P1436.

Table IV. Literature pK_a Values for Quinolines

quinoline substituent	pK_a (20 °C)	ref
unsubstituted	4.90	6
2-NH_2	7.30	6
4-NH_2	9.13	6
2-OCH_3	3.16	7
8-OCH_3	4.99	8
6-Br	3.91	9

values for five of our intermediate 6-bromoquinolines are shown in Table V. From this information we inferred that our 6-(pyrimidylmethyl)-2-aminoquinoline analogues would be more than half protonated at physiological pH, and about 99% protonated with 4-amino substitution. Heterocyclic bases, such as 2- or 4-aminopyridines, -quinolines, and -pyrimidines, are protonated on the N-1 ring nitrogen, as has been well demonstrated from their UV spectra, and also from NMR studies.[10] The amino substituents are very weak bases. 2,4-Diaminopyrimidine has a pK_a value of approximately 7.4.[11] Although the two pK_a values of the pyrimidine and quinoline moieties are expected to influence each other slightly, no major changes are to be expected in individual pK_a values, since the two functions are insulated from each other. Comparative UV spectra are found in Table VI.

Table V. Some pK_a Values for Intermediate Bromoquinolines

	quinoline substituents				
no.	2	4	6	8	pK_a
17	OCH_3	CH_3	Br	CH_3	2.96 ± 0.03 (25 °C)[a]
18	$N(CH_3)_2$	CH_3	Br		7.34 ± 0.03 (25 °C)[a]
21	$N(CH_3)_2$	CH_3	Br	CH_3	6.37 ± 0.04 (25 °C)[a]
46	CH_3	OCH_3	Br	OCH_3	6.39 ± 0.01 (20 °C)[b]
47	CH_3	$N(CH_3)_2$	Br	OCH_3	8.18 ± 0.02 (20 °C)[b]

[a] Minick, D. J.; Sabatka, J. J.; Hurlbert, B. S.; Brent, D. A. In *Automation of Pharmaceutical Operations*; Frank, D. J., Ed.; Aster Publ.: Springfield, Oregon, 1985; Supplement, Chapter 3. These pK_a values were determined by Mr. Minick. [b] Method of ref 11.

This evidence, coupled with that gleaned from paper 10, would suggest that protonation at the quinoline N-1 of compounds **39** and **40** may be responsible for the specificity of inhibition of these compounds for bacterial DHFR.[2] This charged center is expected to be surrounded by a hydration sheath. Furthermore, this center is expected to lie in approximately the same locus as the oxygen of the 4-methoxy group of 1 in interaction with *E. coli* DHFR.[2] This oxygen atom is positioned right at the edge of the enzyme cleft, where it is exposed to solvent. This may be

(6) Albert, A.; Goldacre, R.; Phillips, J. *J. Chem. Soc.* **1948**, 2240.
(7) Albert, A.; Phillips, J. N. *J. Chem. Soc.* **1956**, 1294.
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(10) (a) Albert, A. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; Vol. I. (b) Mason, S. F. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: 1963; New York, Vol. II. (c) Batterham, T. J. In *The Pyrimidines*; Brown, D. J., Wiley-Interscience: New York, 1970; Suppl. I, pp 386–398. (d) Threadgill, M. D.; Griffin, R. J.; Stevens, M. F. G.; Wong, S. K. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2229.
(11) Roth, B.; Strelitz, J. Z. *J. Org. Chem.* **1969**, *34*, 821.

Table VI. Comparison of UV Spectra for Two Quinolines (**46** and **47**) and Their Pyrimidylmethyl Adducts (**52** and **53**): λ_{max} (nm) or Shoulders (sh) and Extinction Coefficients (ϵ)

46		52		47		53	
λ_{max}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ
			Cationic Species (pH 2, 0.01 N HCl)				
				sh 354	13 400	sh 355	14 000
				347	14 000	347.5	14 400
326.5	4 500	324.5	4 900	sh 321	6 850	sh 315	7 000
		sh 270	8 300			sh 274	10 000
248	51 400	247.5	56 100	247.5	37 000	246	46 400
sh 217	20 300	sh 214	43 000	sh 226	15 700	sh 215	32 400
210	22 000	207	46 100				
			Free Base (pH 12, 0.01 N NaOH)				
sh 321	2 200	sh 316	4 450	320	8 700	sh 316	9 100
296.5	6 800	289	15 000			292	13 510
236.5	49 400	239	53 000	242	47 000	242	49 120
sh 221	28 500	217	42 500				

seen by reference to stereo drawings in paper 7.[5] However, in chicken liver DHFR, this oxygen is buried in a hydrophobic environment.[5]

Although the structure of rat liver DHFR is not known, it is expected to be very similar to that of the avian enzyme, judged by the closely similar X-ray data on the mouse lymphoma DHFR,[12] and from the fact that all known vertebrate DHFRs have high sequence homology.[13] Furthermore, a vast array of inhibitory data from our laboratory has shown that vertebrate DHFR inhibitory data follow the same pattern, if not the same absolute numbers. We would then expect energy to be expended for a protonated quinoline either to enter the hydrophobic environment of the vertebrate enzyme or, alternatively, to assume a different conformation relative to the enzyme.

Despite these arguments, we cannot conclude from the available evidence that protonation is required at this site for high specificity, since our basic 2-substituents are more bulky than the methoxy function.

Although the [[4-(dimethylamino)quinolyl]methyl]pyrimidine **53** is a strong base, it shows surprisingly high activity against the vertebrate DHFR enzyme, which would seem contrary to our previous argument for the 2-(dialkylamino) derivatives. However, its conformation may be sufficiently different from that of **39** in rat liver DHFR to permit the basic center at N-1 to lie closer to the surface. Trimethoprim (1), when bound to chicken DHFR, has one *m*-methoxy group completely buried in a tight pocket which would require some conformational adjustment of the protein or ligand to accommodate a larger group. Assuming that **53** has the same torsional angles as 1 about the methylene group in vertebrate DHFR, the 4-(dimethylamino) function of **53**, rather than the 8-methoxy group, would probably prefer the inner hydrophobic pocket, on the basis of two considerations. First, it is somewhat more lipophilic than a methoxy group (π = 0.18, compared to −0.02 for methoxy in octanol/water).[14] In the second place, the proton at N-1 probably also interacts with the lone pair on the 8-methoxy oxygen, so that the methoxy group is included in the solvation sheath, which would prefer an aqueous environment. (Note that the 2-substituted amino derivatives did not have a methoxy group in the 8-position.) However, it is possible that the bulk of the 4-(dimethylamino) function causes a repositioning of the inhibitor to assume torsional angles about the methylene group similar to those observed for 1 in *E. coli* DHFR (177°, 76°), as opposed to −85° and 102° for 1 in chicken DHFR.[15,16] This would cause the quinoline moiety to reside in a lower hydrophobic cleft, as described by Matthews for chicken DHFR.[15,16] We postulated that this might occur with bulky (3,5-dialkylbenzyl)pyrimidines in vertebrate DHFR in order to explain relative inhibitory activities.[5] The number of factors involved, including the effect of water molecules and ionization, makes it difficult to arrive at the truth without actual experimental data.

In retrospect, a less bulky 4-(monomethylamino) function may have provided a better test of whether a basic 4-substituent could assist in creating selectivity. However, it would not answer the question of whether protonation at N-1 was responsible for this result, or whether the much more hydrophilic 4-(methylamino) substituent (π = −0.47)[14] provided the repulsive force in its occupation of a hydrophobic site on the enzyme. The problem is complex, and our paucity of information on this type of compound does not permit further speculation at this point.

Compounds **39** and **40** were somewhat disappointing in their antibacterial activity, compared with their enzyme binding. None of the compounds of Table III quite matched the control in overall activity, although **52** came very close to doing so. To try to relate enzyme activity with antibacterial potency with this type of compound continues to be somewhat of a black box, particularly with the Gram-negative bacteria. Recent NMR experiments with liposomes interacting with DHFR inhibitors suggest that these types of compounds do not enter bacteria by passive diffusion, but require a carrier.[17] Much previous information suggests that simplicity of ring substitution or lack of any substitution aids the process.[2]

The following paper in this series builds on the theme of this paper in trying to relate specificity and activity to shape and charge.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Where analyses are indi-

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cated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Nuclear magnetic resonance (NMR) spectra were recorded on Varian XL-100 and T60 spectrophotometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Ultraviolet spectra were recorded on a Cary 118 spectrophotometer. Thin-layer chromatography was carried out on silica gel with CH_2Cl_2/MeOH, $CHCl_3$/MeOH, $CHCl_3$/EtOH–NH_3, hexane/EtOAc, or EtOAc/MeOH as solvent mixtures in varying proportions, depending on the polarity of the compound. Column chromatographic separations were carried out on silica gel, normally with CH_2Cl_2/MeOH mixtures, or as otherwise stated. Yields quoted refer to products that were chromatographically homogeneous except as described. The biological assays were carried out according to methods previously detailed.[18]

General Methods. Several types of compounds were synthesized in this paper, which are categorized in Table I. In all cases an aniline was converted by one or another means to a quinoline, which could eventually be converted to a quinoline-6- or -7-carboxaldehyde. This in turn could be converted in two steps to a (quinolylmethyl)pyrimidine. The synthetic sequences are shown in Schemes I–III and are exemplified below for the conversion of aniline **4** to the 7-quinolinecarboxaldehyde **22**, followed by its condensation to the pyrimidine derivative **35**. The conversion of aniline **41** to the 6-quinolinecarboxaldehyde **48** as well as byproduct **49** is also described. The conversion of bromoquinolines to the aldehydes necessitated varying amounts of *n*-BuLi, depending on the substituents, to optimize the yields of the desired products. Footnotes to Table I illustrate such variations. In the penultimate step (**29–34**) the anilinoacrylonitriles were normally not purified, except for **29**. They usually existed as a mixture of isomers, in which the double bond could be shifted to give a quinolinoacrylonitrile. It was often necessary to purify the final products by chromatography to remove intermediates that did not condense properly.

***N*-(3-Bromophenyl)-3-oxobutyramide (8).** To a warm solution of 20.0 g (0.12 mol) of 3-bromoaniline (**4**) in 200 mL of dry toluene in an oil bath at 80 °C was added dropwise 12 g (0.14 mol) of diketene in 100 mL of dry toluene over 0.5 h. When the addition was completed, the reaction mixture was heated under reflux for 5 h. The toluene was then removed in vacuo, which left a yellow residue: mp 94–95 °C (toluene); yield, 48%; NMR (Me_2SO-d_6) δ 2.20 (s, 3, Me), 3.55 (s, 2, CH_2), 7.15–7.65 (m, 3, Ar), 7.95 (dd, 1, 2-H, *J* = 2 Hz), 11.10 (br s, 1, NH). Anal. ($C_{10}H_{10}BrNO_2$) C, H, N, Br.

7-Bromo-4-methyl-2-quinolone (11). A mixture of 6.65 g (26 mmol) of **8** and 30 mL of concentrated H_2SO_4 was heated to 120 °C for 1.5 h. The solution was then poured on ice, yielding a light precipitate which was filtered and washed well with water. After recrystallization from 95% EtOH, 5.21 g (84%) of **11** was obtained as a white solid: mp 275–276 °C; NMR (Me_2SO-d_6) δ 2.35 (d, 3, 4-Me, *J* = 1 Hz), 6.70 (d, 1, 3-H, *J* = 1 Hz), 7.30 (dd, 1, Ar-6H, *J* = 9, 2 Hz), 7.45 (d, 1, Ar-8H, *J* = 2 Hz), 7.60 (d, 1, Ar-5H, *J* = 9 Hz), 11.65 (br s, 1, 1-NH). Anal. ($C_{10}H_8BrNO$) C, H, N, Br.

7-Bromo-2-chloro-4-methylquinoline (14). Five grams of **11** was refluxed in 50 mL of $POCl_3$ for 2 h, after which it was cooled and poured on ice and neutralized with NH_4OH. The resultant precipitate was isolated and washed well with water, followed by recrystallization from 95% EtOH, to give 3.82 g (71%) of **14**: mp 73–74 °C; NMR (Me_2SO-d_6) δ 2.70 (d, 3, 4-Me, *J* = 1 Hz); 7.55 (d, 1, 3-H, *J* = 1 Hz), 7.75 (dd, 1, 6-H, *J* = 9, 2 Hz), 8.10 (d, 1, 5-H, *J* = 9 Hz), 8.17 (d, 1, 8-H, *J* = 2 Hz). Anal. ($C_{10}H_7BrClN$) C, H, N.

7-Bromo-2-methoxy-4-methylquinoline (16). A mixture of 3.0 g (12 mmol) of **14** and 0.63 g (12 mmol) of NaOMe in 30 mL of dry MeOH was refluxed for 48 h. The solvent was then removed and the residue taken up in CH_2Cl_2. The solution was washed repeatedly with saturated NaCl, dried over $MgSO_4$, taken to dryness, and recrystallized from toluene; 2.63 g (87%) of **16** was obtained as white crystals: mp 58–60 °C; NMR (Me_2SO-d_6) δ 2.55 (d, 3, 4-Me, *J* = 1 Hz), 3.80 (s, 3, OMe), 6.80 (d, 1, 3-H, *J* = 1 Hz), 7.55 (dd, 1, 6-H, *J* = 9, 2 Hz), 7.80 (d, 1, 5-H, *J* = 9 Hz), 7.85 (d, 1, 8-H, *J* = 2 Hz). Anal. ($C_{11}H_{10}BrNO$) C, H, N, Br.

7-Formyl-2-methoxy-4-methylquinoline (22). A 1.50 g (5.90 mmol) portion of **16** was dissolved in 20 mL of freshly distilled dry THF in a 50-mL flame-dried three-necked flask under N_2. The mixture was cooled in a dry ice/acetone bath to −78 °C, followed by the dropwise addition (via a syringe) of 7.65 mL of *n*-BuLi in hexane (1.56 M, 2 equiv). After stirring for 5 min, 1.26 mL (16 mmol) of dry DMF was added via a syringe. The reaction mixture was then brought to −20 °C. Water was slowly added, followed by 1 N HCl. Following Et_2O extraction and recrystallization from toluene, 0.90 g (76%) of **22** was obtained as a white solid: mp 111–112 °C; NMR (Me_2SO-d_6) δ 2.65 (d, 3, 4-Me, *J* = 1 Hz), 4.00 (s, 3, OMe), 7.08 (br s, 1, 3-H), 7.85 (dd, 1, 6-H, *J* = 8, 2 Hz), 8.15 (d, 1, 5-H, *J* = 8 Hz), 8.37 (d, 1, 8-H, *J* = 2 Hz), 10.24 (s, 1, CHO). Anal. ($C_{12}H_{11}NO_2$) C, H, N.

2-[(2-Methoxy-4-methyl-7-quinolyl)methyl]-3-anilinoacrylonitrile (29). To a stirred solution of 0.65 g (3.20 mmol) of **22** and 0.52 g (3.50 mmol) of anilinopropionitrile (**28**) in 10 mL of dry Me_2SO was added 0.19 g (3.50 mmol) of NaOMe. The mixture was then heated to 90–95 °C for 2 h, followed by removal of the solvent in vacuo. Water was added to the residue, which yielded a brown precipitate; this was isolated and washed well with water, followed by recrystallization from absolute EtOH, to give 0.49 g (46%) of **29**, mp 170–172 °C. Anal. ($C_{21}H_{19}N_3O$) C, H, N.

2,4-Diamino-5-[(2-methoxy-4-methyl-7-quinolyl)methyl]-pyrimidine (35). A mixture of 0.17 g (1.82 mmol) of guanidine hydrochloride, 10 mL of absolute EtOH, and 0.13 g (2.44 mmol) of NaOMe was stirred for 5 min under N_2, filtered from salt, and added to a round-bottomed flask containing 0.20 g (0.61 mmol) of **29**. The mixture was heated to refluxing under N_2 overnight, followed by the addition of an equivalent amount of guanidine, prepared as above. The mixture was heated for an additional 24 h and the solvent then removed in vacuo. The residue was then taken up in 90% MeOH and the precipitate isolated. This was then purified by flash chromatography using 20–26 g of silica gel, 230–400 mesh, and eluting with CH_2Cl_2/MeOH, 9:1. This was repeated a second time. A 14% yield (0.025 g) of **35** was obtained as an off-white solid: mp 205–206 °C; NMR (Me_2SO-d_6) δ 2.57 (d, 3, Q-4-Me, *J* = 1 Hz), 3.79 (s, 2, CH_2), 3.92 (s, 3, OMe), 5.76 (br s, 2, NH_2), 6.16 (br s, 2, NH_2), 6.80 (d, 1, Q-3-H, *J* = 1 Hz), 7.35 (dd, 1, Q-6-H, *J* = 8.50, 2 Hz), 7.60 (d, 1, Q-8-H, *J* = 2 Hz), 7.65 (s, 1, pyr-6-H), 7.90 (d, 1, Q-5-H, *J* = 8.50 Hz). Anal. ($C_{16}H_{17}N_5O{\cdot}0.6H_2O$) C, H, N.

4-Bromo-2-methoxyaniline (41).[19] *o*-Anisidine (15 g, 0.122 mol) was brominated with 2,4,4,6-tetrabromo-2,5-cyclohexadienone[19] (50 g, 0.122 mol) by dissolving the aniline in 250 mL of CH_2Cl_2, chilling the solution to −10 °C, and slowly adding the brominating agent, while keeping the temperature below −5 °C. The reaction was allowed to warm to room temperature, then washed with 2 N NaOH, followed by water, and then dried over $MgSO_4$. The solvent was removed and the residue purified on a silica gel column, which was eluted with CH_2Cl_2, giving 23.7 g (96%) of **41**, mp 56.5–58 °C (petroleum ether). Anal. (C_7H_8BrNO) C, H, Br, N.

Ethyl 3-[(4-Bromo-2-methoxyphenyl)imino]butyrate (43). A mixture of 5.25 g (26 mmol) of **41**, 3.39 g (26 mmol) of ethyl acetoacetate (**42**), 20 mL of absolute EtOH, 0.06 mL of glacial AcOH, and 7 g of Drierite were heated under reflux for 4 h. The Drierite was filtered off and the solvent removed, followed by column purification of the product. Elution with hexane/EtOAc, 19:1, separated 6.47 g (79%) of **43** as a colorless oil: NMR ($CDCl_3$) δ 1.28 (tr, 3, CH_2*Me*), 1.97 (s, 3, =CH*Me*), 3.85 (s, 3, OMe), 4.17 (q, 2, C*H*$_2$Me), 4.75 (s, 1, =CH), 7.00 (s, 3, Ar). Anal. ($C_{13}H_{16}BrNO_3$) C, H, N.

6-Bromo-8-methoxy-2-methyl-4-quinolone (44). The above product (**43**) (6.32 g, 20.1 mmol) was cyclized in diphenyl ether (30 mL) by heating at 255 °C for 25 min. A precipitate separated (3.80 g, 70.5% crude), which was washed well with Et_2O, mp 293–296 °C (absolute EtOH). Anal. ($C_{11}H_{10}BrNO_2$) C, H, N.

6-Bromo-4-chloro-8-methoxy-2-methylquinoline (45). Chlorination of **44** was accomplished by mixing 2.79 g (10.4 mmol) with 13 mL of $POCl_3$ and refluxing for 2 h, followed by removal

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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).

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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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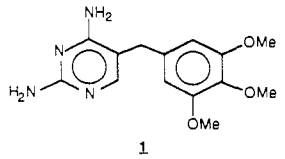
Twelve 2,4-diamino-5-[(1,2-dihydro-6-quinolyl)methyl]pyrimidines containing *gem*-dimethyl or fluoromethyl substituents at the 2-position of the dihydroquinoline ring were prepared by condensations of dihydroquinolines with 2,4-diamino-5-(hydroxymethyl)pyrimidine. The dihydroquinolines were produced 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



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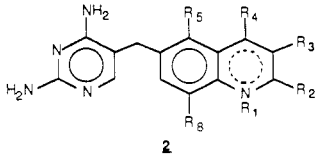

2

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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