Synthesis and Antiparasitic and Antitumor Activity of 2,4-Diamino-6-(arylmethyl)-5,6,7,8-tetrahydroquinazoline Analogues of Piritrexim[1]

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Nineteen previously undescribed 2,4-diamino-6-(arylmethyl)-5,6,7,8-tetrahydroquinazolines (**5a**−**m**, **10**−**12**) were synthesized as part of a larger effort to assess the therapeutic potential of lipophilic dihydrofolate reductase (DHFR) inhibitors against opportunistic infections of AIDS. Condensation of appropriately substituted (arylmethyl)triphenylphosphoranes with 4,4-ethylenedioxycyclohexanone, followed by hydrogenation (H_2/Pd−C) and acidolysis, yielded the corresponding 4-(arylmethyl)cyclohexanones, which were then condensed with cyanoguanidine to form the tetrahydroquinazolines. Three simple 2,4-diamino-6-alkyl-5,6,7,8-tetrahydroquinazoline model compounds (**9a**−**c**) were also prepared in one step from commercially available 4-alkylcyclohexanones by this method. Enzyme inhibition assays against rat liver DHFR, *Pneumocystis carinii* DHFR, and the bifunctional DHFR-TS enzyme from *Toxoplasma gondii* were carried out, and the selectivity ratios IC_{50}(rat)/IC_{50}(*P. carinii*) and IC_{50}(rat)/IC_{50}(*T. gondii*) were compared. The three most potent inhibitors of *P. carinii* DHFR were the 2,5-dimethoxybenzyl (**5j**), 3,4-dimethoxybenzyl (**5k**), and 3,4,5-trimethoxybenzyl (**5l**) analogues, with IC_{50} values of 0.057, 0.10, and 0.091 μM, respectively. The remaining compounds generally had IC_{50} values in the 0.1−1.0 μM range. However all the compounds were more potent against the rat liver enzyme than the *P. carinii* enzyme and thus were nonselective. The *T. gondii* enzyme was always more sensitive than the *P. carinii* enzyme, with most of the analogues giving IC_{50} values of 0.01−0.1 μM. Moderate 5−10-fold selectivity for *T. gondii* versus rat liver DHFR was observed with five compounds, the best combination of potency and selectivity being achieved with the 2-methoxybenzyl analogue **5d**, which had an IC_{50} of 0.014 μM and a selectivity ratio of 8.6. One compound (**5l**) was tested for antiproliferative activity against *P. carinii* trophozoites in culture at a concentration of 10 μg/mL and was found to completely suppress growth over 7 days. The suppressive effect of **5l** was the same as that of trimethoprim (10 μg/mL) + sulfamethoxazole (250 μg/mL), a standard clinical combination for the treatment of *P. carinii* pneumonia in AIDS patients. Four compounds (**5a**,**h**,**k**,**l**) were tested against *T. gondii* tachyzoites in culture and were found to have a potency (IC_{50} = 0.1−0.5 μM) similar to that of pyrimethamine (IC_{50} = 0.69 μM), a standard clinical agent for the treatment of cerebral toxoplasmosis in AIDS patients. Compound **5h** was also active against *T. gondii* infection in mice when given qdx8 by peritoneal injection at doses ranging from 62.5 (initial dose) to 25 mg/kg. Survival was prolonged to the same degree as with 25 mg/kg clindamycin, another widely used drug against toxoplasmosis. Three compounds (**5j**−**l**) were tested for antiproliferative activity against human tumor cells in culture. Among the 25 cell lines in the National Cancer Institute panel for which data were confirmed in two independent experiments, the IC_{50} for at least two of these compounds was <10 μM against 17 cell lines (68%) and in the 0.1−1 μM range against 13 cell lines (52%). One compound (**5j**) had an IC_{50} of <0.01 μM against four of the cell lines. The activity profiles of **5k**,**l** were generally similar to that of **5j** except that there were no cells against which the IC_{50} was <0.01 μM.

Introduction

As part of a larger program of evaluation of new small-molecule inhibitors of dihydrofolate reductase (DHFR) as potential drugs against *Pneumocystis carinii* and *Toxoplasma gondii*,[2,3] two major opportunistic pathogens often found in patients with AIDS and other immunodeficiency disorders, we recently synthesized the 2,4-diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidines **1**−**3**.[2h] The 3,4,5-trimethoxyphenyl group in these 6/5-fused-ring structures is also present in trimethoprim (**4**), which has been used in combination with sulfamethoxazole against *P. carinii* pneumonia (PCP) and cerebral toxoplasmosis.[4] Notable structural features of compounds **1**−**3** were the five-membered carboxyclic B-ring and the all-carbon bridge between the phenyl ring and heterocyclic moiety.

In the present paper we report a group of analogues of general structure **5**, in which the B-ring is likewise reduced but is six-membered. The one-carbon bridge of

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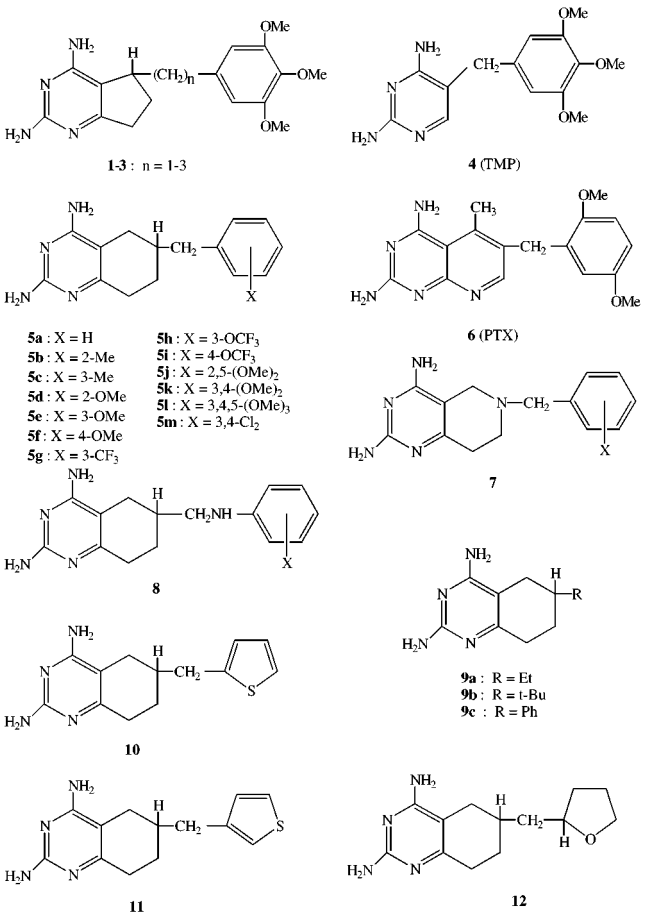
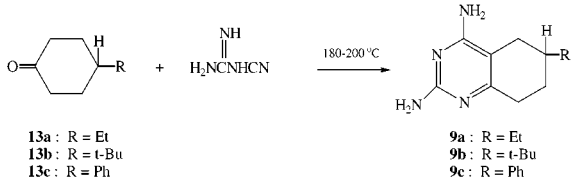
these tetrahydroquinazolines is present in both TMP and the anticancer drug piritrexim (**6**, PTX),[5] which has been reported to have potent antipneumocystis and antitoxoplasma activity in the laboratory[6a] and has been used to treat PCP in AIDS patients, with coadministration of leucovorin to prevent dose-limiting hematologic toxicity.[6b] Thus the purpose of this study was to assess the potency and selectivity of this series of CH_2-bridged analogues against *P. carinii* and *T. gondii* DHFR and to test selected examples against intact *P. carinii* and *T. gondii* organisms in culture. Because PTX has been used experimentally to treat cancer,[7a,b] it was also of interest to test selected examples of **5** as inhibitors of the growth of tumor cells in culture. It was also of interest to compare the DHFR binding properties of these compounds with those of 2,4-diamino-6-aralkyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines **7**[2c] and 2,4-diamino-6-(arylaminomethyl)-5,6,7,8-tetrahydroquinazolines **8**,[8] two other recently studied series of dicyclic small-molecule DHFR inhibitors with a saturated six-membered B-ring. Also synthesized during the present work were the tetrahydroquinazolines **9**−**12**, which contain side chains other than benzyl.



Chemistry

The synthesis of 6-substituted 2,4-diamino-5,6,7,8-tetrahydroquinazolines was accomplished by condensing cyanoguanidine (dicyandiamide) with an appropriately substituted cyclohexanone as previously described.[9] The 6-ethyl (**9a**), 6-*tert*-butyl (**9b**), and 6-phenyl (**9c**) analogues were obtained directly from the commercially available ketones **13a**−**c** (Scheme 1).

Scheme 1

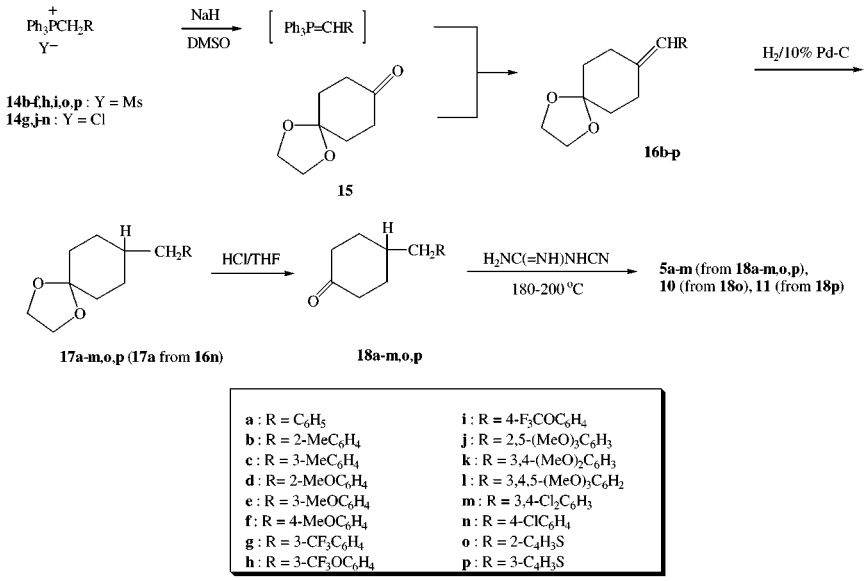


In the case of the other 6-substituted tetrahydroquinazolines, it was necessary to first develop a practical route to 4-(arylmethyl)cyclohexanones. As shown in Scheme 2, variously substituted benzyl chlorides and mesylates were heated with triphenylphosphine in xylene solution to obtain the corresponding crystalline phosphonium salts **14b**−**n.** Similarly, 2-(chloromethyl)-thiophene and 3-(chloromethyl)thiophene were converted to **14o**,**p** (Scheme 2), and 2-(chloromethyl)furan was converted to **14q** (Scheme 3).

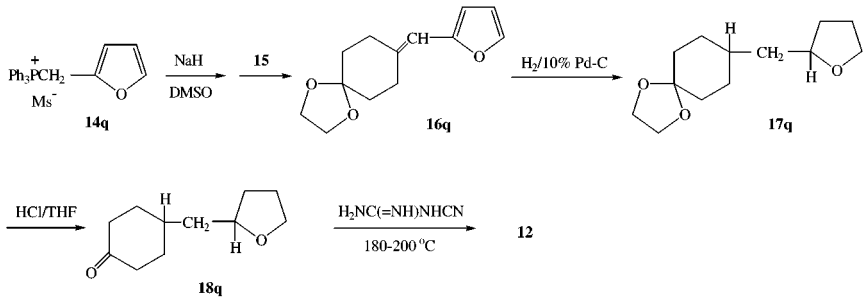
When the required substituted benzyl chlorides were not commercially available, they were prepared from the corresponding alcohols and $SOCl_2$ in toluene at room temperature, and when the alcohols were not commercially available, they were prepared from the aldehydes with $NaBH_4$. When mesylates were used instead of chlorides, they were obtained from the alcohols and $MsCl/Et_3N$. Wittig reagents were generated in situ from the phosphonium salts with dimsyl sodium (NaH/DMSO) and were condensed with 4,4-(ethylenedioxy)-cyclohexanone (**15**) to form the arylmethylene derivatives **16b**−**p** (Scheme 2) and the 2-furanylidene derivatives **16q** (Scheme 3). Catalytic hydrogenation of **16b**−**m**,**o**,**p** in the presence of 10% Pd−C and a trace of glacial AcOH afforded the arylmethyl derivatives **17b**−**m**,**o**,**p** with retention of the ketal group. Although catalytic hydrogenation of **16m** occurred without loss of the chlorine atoms, giving **17m**, reduction of the double bond in **16n** with $LiAlH_4-CoCl_2$[10] was found to cause dehalogenation, giving **17a** rather than **17n**. Thus 4-(chlorobenzyl)cyclohexanone and its cyanoguanidine fusion product were not made (catalytic hydrogenation of **16n** would presumably have worked but was not tried). Reduction of the double bond in **16q** was accompanied by reduction of the furan ring, yielding **17q** (Scheme 3); accordingly, the rest of the sequence was carried out with the furan ring in the tetrahydro form. Removal of the ketal blocking group from **17a**−**m**,**o**−**q** with HCl proceeded uneventfully, yielding the cyclohexanones **18a**−**m**,**o**−**q**. Fusion of the ketones with cyanoguanidine at 180 °C completed the synthesis. Yields, melting points, and other physical constants of the final products are summarized in Table 1. All compounds are presumably mixtures of 6*R* and 6*S* enantiomers except the tetrahydrofuranyl analogue **12**, which is assumed to be a mixture of four diastereomers.

Interestingly, when the ketal group was removed from **16f** without first reducing the double bond and the resulting ketone (**19**) was condensed directly with cyanoguanidine (Scheme 4) a product was obtained whose ^1H NMR spectrum was consistent with a 3:2 mixture of the *E* and *Z* isomers of 2,4-diamino-6-(4-methoxybenzylidene)-5,6,7,8-tetrahydroquinazoline (**20**). That the double bond was still in the exocyclic position was suggested by the location of closely spaced vinyl signals in the ^1H NMR spectrum at δ 6.47, nearly the

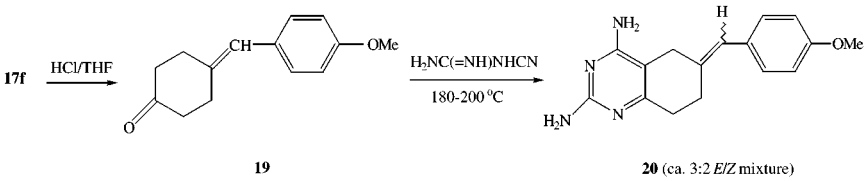
Scheme 2



Scheme 3



Scheme 4



same position as that of the vinyl proton in **19**. If the exocyclic double bond had suffered thermal rearrangement to form 2,4-diamino-6-(4-methoxybenzyl)-7,8-dihydroquinazoline, we would have expected an upfield shift due to diamagnetic shielding by the 4-amino group.

Dihydrofolate Reductase Inhibition

The ability of the tetrahydroquinazolines **5a**−**m**, **9a**−**c**, and **10**−**12** to inhibit rat liver, *P. carinii*, and *T. gondii* DHFR was determined by the standard spectrophotometric assay as described previously.[11,12] The results are shown in Table 2, along with previously reported data for trimethoprim (TMP, **4**) and piritrexim (PTX, **6**) for comparison (see, for example, ref 2e). The most potent tetrahydroquinazolines against the *P. carinii* enzyme were the 2,5-dimethoxy analogue **5j** and the 3,4,5-trimethoxy analogue **5l**. However these compounds were also the best inhibitors of the mammalian enzyme, and thus were nonselective. Interestingly, **5j** had roughly the same potency as PTX, suggesting that reduction of the B-ring is not detrimental to binding. It may be noted that since the presumed mixture of 6*R* and 6*S* enantiomers was not resolved, their individual IC_{50} values could not be determined. Nonetheless, the fact that the IC_{50} of **5j** against each enzyme was consistently higher than that of PTX suggests that one of the enantiomers probably binds more tightly than the other.

Compounds **5j**,**l** were likewise among the best inhibitors of the *T. gondii* enzyme, with IC_{50} values of 0.021 and 0.024 μM, respectively. However, even though they were more potent against this enzyme than against the *P. carinii* enzyme, the difference was not great enough to confer selectivity. Several other compounds in the series had about the same potency against *T. gondii* DHFR as **5j**,**l**, and two compounds, the 2-CH_3O and 3-CF_3O analogues **5d**,**h**, were actually a little more potent. In general, all the compounds were better inhibitors of the *T. gondii* enzyme than of the *P. carinii* enzyme, presumably reflecting more favorable contacts between the substituted benzyl moiety and hydrophobic residues in the active site.

The effect of different substituents on the phenyl ring on the binding of the tetrahydroquinazolines to DHFR

Table 1. Physical Data for 2,4-Diamino-6-substituted-5,6,7,8-tetrahydroquinazolines

compd	yield, %	mp, °C	IR (KBr) ν (cm^{-1})	^1H NMR ($CDCl_3$)[b] δ	anal.
5a	41	213−218[a]	3360, 3290, 3160, 2940, 2910, 1620, 1570, 1470, 1430, 1400	1.2−2.8 (m, 9H, CH_2, CH), 4.6 (br s, 4H, NH_2), 7.3 (m, 5H, phenyl)	C,H,N
5b	59	200−201.5	3510, 3440, 3320, 3150, 2920, 2860, 1625, 1585, 1570, 1550, 1480, 1390, 1250	1.23−2.80 (m, 9H, CH_2, CH), 2.28 (s, 3H, Me), 4.67 (br s, 4H, NH_2), 7.10 (m, 4H, phenyl)	C,H,N
5c	76	206−207.5	3460, 3440, 3300, 3060, 2920, 2820, 1645, 1620, 1575, 1560, 1420, 1240	1.10−2.70 (m, 9H, CH_2, CH), 2.20 (s, 3H, Me), 4.60 (br s, 4H, NH_2), 6.98 (m, 4H, phenyl)	C,H,N
5d	70	225−226.5	3460, 3440, 3300, 3060, 2920, 2820, 1645, 1620,1575, 1560, 1480, 1430, 1410, 1270, 1240	1.20−3.00 (m, 9H, CH_2, CH), 3.90 (s, 3.90 (s, 3H, OMe), 5.55 (s, 2H, NH_2), 6.00 (s, 2H, NH_2), 7.10 (m, 4H, phenyl)	C,H,N
5e	13	210−212	3460, 3440, 3280, 3120, 2900, 2840, 2820, 1650, 1620, 1580, 1570, 1475, 1450, 1440, 1405, 1245	1.2−2.8 (m, 9H, CH_3, CH), 3.8 (s, 3H, OMe), 4.7 (br s, 4H, NH_2), 6.8 (m, 3H, phenyl), 7.3 (m, 1H, phenyl)	C,H,N
5f	41	231	3460, 3420, 3320, 3140, 2910, 2820, 1610, 1560, 1500, 1440, 1400, 1280, 1235	0.90−2.85 (m, 9H, CH_2, CH), 3.8 (s, 3H, OMe), 4.6 (s, 4H, broad m, NH_2), 6.9 (d, J = 8 Hz, 2H, phenyl), (7.2, d, J = 8 Hz, 2H, phenyl)	C,H,N
5g	30	163−175[a]	3560, 3470, 3430, 3300, 3130, 2900, 2870, 2830, 1630, 1610, 1570, 1430, 1400, 1315	0.9−2.8 (m, 9H, CH_2, CH), 4.8 (br s, 4H, NH_2), 7.2 (m, 4H, phenyl)	C,H,N
5h	66	174−179	3480, 3330, 3280, 3105, 3060, 2910, 2870, 1630, 1610, 1570, 1430, 1250, 1210, 1200	1.00−2.85 (m, 9H, CH_2, CH), 4.65 (br s, 4H, NH_2), 6.90−7.50 (m, 4H, phenyl)	C,H,N
5i	53	180−208[a]	3460, 3330, 3180, 2940, 2840, 1630, 1560, 1540, 1510, 1440, 1430, 1275, 1200	0.95−3.50 (m, 9H, CH_2, CH), 4.40 (br s, 4H, NH_2), 7.15 (s, 4H, phenyl)	C,H,N
5j	42	172−182[a]	3440, 3210, 3120, 2910, 2810, 1645, 1620, 1550, 1490, 1425, 1340, 1210	1.2−2.8 (m, 9H, CH_2, CH), 3.8 (s, 6H, OMe), 5.0 (br s, 4H, NH_2), 6.7 (m, 3H, phenyl)	C,H,N
5k	44	224−226	3420, 3340, 3160, 2920, 2890, 2720, 1630, 1610, 1570, 1500, 1450, 1420, 1325, 1225	1.25−2.70 (m, 9H, CH_2, CH), 3.76 (s, 6H, OMe), 4.56 (br s, 4H, NH_2), 6.71−6.80 (m, 3H, phenyl)	C,H,N
5l	53	191−195[a]	3440, 3350, 3180, 2940, 2910, 2840, 1625, 1580, 1430, 1385, 1240	1.2−2.8 (m, 9H, CH_2, CH), 3.8 (s, 3H, OMe), 4.7 (broad, s, 4H, NH_2), 6.4 (s, 2H, phenyl)	C,H,N
5m	39	218−221	3460, 3300, 3120, 2910, 2890, 2910, 1640, 1605, 1520, 1510, 1425, 1250	1.10−2.80 (m, 9H, CH_2, CH), 4.60 (br s, 4H, NH_2), 6.90−7.50 (m, 3H, phenyl)	C,H,N
9a	29	209−213 dec	3360, 3280, 3140, 2980, 2900, 2890, 2730, 1640, 1565, 1470, 1425, 1400, 1260	0.95−2.95 (m, 12H, CH_3, CH_2, CH), 4.75 (s, 4H, NH_2)	C,H,N
9b	49	253−259 dec	3350, 3280, 3160, 2930, 2840, 1620, 1560, 1470, 1430, 1400, 1350, 1270, 1220	0.85−2.90 (m, 9H, CH_2, CH), 0.95 (s, 9H, t-Bu), 4.6 (s, 4H, NH_2)	C,H,N
9c	41	249−252 dec	3360, 3290, 3160, 3000, 2910, 1620, 1515, 1470, 1440, 1400, 1270, 1240	0.8−3.0 (m, 9H, CH_2, CH), 4.6 (m, 4H, NH_2), 7.4 (m, 5H, phenyl)	C,H,N
10	37	271−274	3310, 3120, 2880, 2860, 2810, 2760, 1660, 1645, 1520, 1515, 1490, 1440, 1380	1.15−2.65 (m, 9H, CH_2, CH), 3.14−4.50 (br s, 4H, NH_2), 7.0−8.0 (m, 3H, thienyl)	C,H,N
11	45	212−213	3470, 3325, 3125, 2930, 2840, 1650, 1620, 1570, 1435, 1235	0.9−3.0 (m 9H, CH_2, CH), 4.8 (br s, NH_2), 7.15 (m, 3H, thienyl)	C,H[c]
12	39	218−238 dec[d]	3420, 3290, 3130, 2940, 2900, 2810, 1645, 1615, 1570, 1560, 1430, 1400, 1310, 1255	0.8−2.4 (m, 12H, CH_2, CH), 3.5−4.0 (br s, 4H, NH_2), 4.4−4.8 (br s, 3H, CH_2O, CHO)	C,H,N
20	25	196−200 dec[e]	3440, 3330, 3170, 2900, 2830, 1620, 1525, 1510, 1440, 1295, 1245	1.80−3.49 (m, 6H, CH_2), 4.63 (br s, 4H, NH_2), 6.47 (two closely spaced singlets, 3:2 ratio, 1H, CH=), 6.88−7.18 (m, 4H, phenyl)	C,H,N

[a] Double melting point was observed within the specified temperature range. [b] The spectrum of **11** was recorded in DMSO-d_6 solution. [c] N: calcd, 20.26; found, 19.83. [d] Expectedly broad melting point of the mixed diastereomers. [e] Darkening at 180 °C.

was examined with reference to the unsubstituted benzyl derivative **5a**, along with the effect of a particular substituent at different positions. Comparing the effect of different *meta* substituents on binding to the *T. gondii* enzyme, for example, the following order of potency was noted: CF_3O (**5h**) > CH_3O (**5e**) > CH_3 (**5c**) ≈ H (**5a**) > CF_3 (**5g**), suggesting that the CF_3O group was favorable for binding whereas the CF_3 group was unfavorable. The difference in IC_{50} between the most potent congener (**5h**, 0.014 μM) and the least potent (**5g**, 0.14 μM) was 10-fold. Comparing the effect of a particular substituent (CH_3O) at different positions on the phenyl ring, the following order of potency against the *T. gondii* enzyme was observed: **5d** > **5e** > **5f**. The difference in potency between **5d** and **5f** was 3.6-fold, suggesting that *ortho* substitution was most favorable for binding. However **5d**−**f** were equipotent against the mammalian enzyme, suggesting that the effect of *ortho* substitution might be species-related. When the effect of replacing the phenyl ring with a bioisosteric thiophene ring was compared, compounds **10** and **11** proved to be slightly less potent than **5a** against all three enzymes. When the effect of deleting the CH_2 bridge while retaining the phenyl ring was examined (**9c** versus **5a**), a dramatic decrease in binding was observed against all three enzymes, suggesting that a bridge of at least one atom in length must be present for correct positioning of the phenyl ring within the active site. An even larger decrease in binding was observed when the benzyl group was replaced by a less space-filling ethyl group (**9a** versus **5a**). However when the benzyl group was replaced by a *tert*-butyl group (**9b** versus **5a**), there was

Table 2. Inhibition of *P. carinii* (Pc), *T. gondii* (Tg), and Rat Liver (RL) DHFR by 2,4-Diamino-6-substituted-5,6,7,8-tetrahydroquinazolines

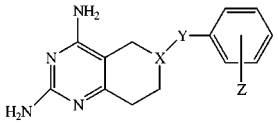
	IC_{50} (μM)			selectivity index[a]	
compd	Pc	Tg	RL	Pc	Tg
TMP (**4**)[b]	12	2.7	130	11	48
PTX (**6**)[b]	0.031	0.017	0.015	0.048	0.088
5a	0.29	0.032	0.18	0.62	5.6
5b	0.25	0.023	0.11	0.44	4.7
5c	0.34	0.036	0.11	0.32	3.1
5d	0.45	0.014	0.12	0.27	8.6
5e	0.27	0.021	0.11	0.41	5.2
5f	0.44	0.050	0.077	0.18	1.5
5g	0.40	0.14	0.19	0.48	1.4
5h	0.10	0.014	0.079	0.79	5.6
5i	0.58	0.073	0.17	0.29	2.3
5j	0.057	0.021	0.034	0.60	1.6
5k	0.10	0.023	0.063	0.63	2.7
5l	0.091	0.024	0.038	0.42	1.6
5m	15	2.6	7.3	0.49	2.8
9a	6.9	1.1	3.0	0.43	2.7
9b	0.18	0.018	0.065	0.36	3.6
9c	2.2	1.3	1.9	0.86	1.2
10	0.21	0.062	0.080	0.38	1.3
11	1.7	0.049	0.13	0.076	2.7
12	0.88	0.040	0.27	0.31	6.8

[a] Defined as the ratio IC_{50}(RL)/IC_{50}(Pc) or IC_{50}(RL)/IC_{50}(Tg). Compounds with a ratio of ≤1 are nonselective. [b] Data taken from ref 2h.

only a small difference in IC_{50}, suggesting that an aromatic ring in the side chain is not essential as long as the hydrophobic 6-substituent is of sufficient bulk.

Although the tetrahydroquinazolines **5a**−**m** were totally nonselective for *P. carinii* versus mammalian DHFR, several of them did show moderate selectivity for the *T. gondii* enzyme, the best among them being **5a**,**d**,**e**,**h**, and **12**, with a selectivity index in the 5−10-fold range. Compound **12** was notable in that its heterocyclic side chain was fully saturated. The compound with the best combination of potency (0.014 μM) and selectivity (8.6-fold) for this enzyme was the 2-CH_3O analogue **5d**, which was almost as potent with PTX and whose selectivity index was 100-fold higher. The presence of a 2-CH_3O substituent in both **5d** and PTX is worth noting. While the potency of **5d** against *T. gondii* DHFR was nearly 200-fold greater than that of TMP, its selectivity was unfortunately 5.6-fold lower. Similarly the potency of **5d** against the *P. carinii* enzyme was 27-fold greater than that of TMP, but its selectivity was 41-fold lower. Thus our hope of matching the potency of PTX with the selectivity of TMP against *T. gondii* and *P. carinii* DHFR was not fulfilled in this series.

It was of interest to compare the binding of selected 2,4-diamino-6-benzyl-5,6,7,8-tetrahydroquinazolines with that of the 2,4-diamino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines **21** and **22**[2c] and the 2,4-diamino-6-(anilinomethyl)-5,6,7,8-tetrahydroquinazolines **23** and **24**,[8] since all three ring systems contain a reduced B-ring. As can be seen in Table 3, substitution of a CH_2 group for the CH_2NH in **23** resulted in a 30-fold increase in potency against *P. carinii* DHFR and smaller increases in potency against *T. gondii* and rat liver DHFR. A similar effect was observed on substitution of a CH_2 group by a CH_2NH bridge in **24**, in this case with an increase in binding to *P. carinii* DHFR of 50-fold. However, because binding to rat liver DHFR was also increased, species selectivity was not achieved for the *P. carinii* enzyme and in fact was decreased for the *T. gondii* enzyme. When the inhibition of *P. carinii* DHFR by **5j** was compared with that of the corresponding pyrido[4,3-*d*]pyrimidine **21,** there was a 58-fold difference in potency in favor of the tetrahydroquinazoline, and when **5l** and **22** were compared this difference was 76-fold. The potency of **5j**,**l** was likewise increased against the *T. gondii* and rat liver enzymes relative to the corresponding pyrido[4,3-*d*]pyrimidines, but not to the same degree. However, there was no improvement in selectivity for the *P. carinii* enzyme and an actual decrease in selectivity for the *T. gondii* enzyme. These results reinforced our impression of a frequent inverse relationship between potency and species selectivity where inhibition of these enzymes by lipophilic antifolates is concerned.[13]



21 : X = N, Y = CH_2, Z = 2,5-$(OMe)_2$
22: X = N, Y = CH_2, Z = 3,4,5-$(OMe)_3$
23 : X = CH, Y = CH_2NH, Z = 2,5-$(OMe)_2$
24 : X = CH, Y = CH_2NH, Z = 3,4,5-$(OMe)_3$

Activity against *P. carinii* and *T. gondii*

Even though selective binding had not been achieved in the binding assays using cell-free *P. carinii* or *T. gondii* DHFR, it was of interest to test selected examples of the 2,4-diamino-6-(arylmethyl)-5,6,7,8-tetrahydroquinazolines against the intact organisms in culture. It is well-known that *P. carinii* and *T. gondii* cells lack an active transport system for exogenous folates.[14] Thus the underlying assumption in the use of cytotoxic lipophilic antifolates such as piritrexim or trimetrexate to treat *P. carinii and T. gondii* infections in AIDS patients who do not respond to, or cannot tolerate, first-line antiparasitic drugs is that hematologic toxicity can be prevented by coadministration of a folate, which would only be taken up by cells of the host. In the clinic this is done with leucovorin (5-formyl-5,6,7,8-tetrahydrofolate), whereas in tissue culture, the lung fibroblast feeder cells that are required for cultivation of these parasites are protected with folic acid, which is generally present at micromolar concentrations in standard growth media. The ability of several compounds to inhibit proliferation of *P. carinii* trophozoites in culture was assayed as previously described.[15] In the case of *T. gondii* a biochemical assay based on [^{3}H]uracil incorporation by the tachyzoite form of the organism was used.[11]

The results of an experiment comparing the effects of **5l** (10 μg/mL) and a standard combination of trimethoprim (TMP, 50 μg/mL) and sulfamethoxazole (SMX, 250 μg/mL) on the growth of *P. carinii* trophozoites in rat embryonic lung fibroblast short-term cultures are shown in Figure 1. The standard growth medium in these assays contained folic acid (10 μM) and was not supplemented with leucovorin. The average number of trophozoites in the untreated cultures increased from 1−2 to 12 per microscopic field in 7 days, whereas treatment with either **5l** or TMP+SMX at the indicated

Table 3. Comparative Activity and Selectivity of 2,4-Diamino-6-benzyl-5,6,7,8-tetrahydroquinazolines, 2,4-Diamino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines, and 2,4-Diamino-6-(arylaminomethyl)-5,6,7,8-tetrahydroquinazolines with 2,5-Dimethoxy- and 3,4,5-Trimethoxyphenyl Substitution as Inhibitors of *P. carinii*, *T. gondii*, and Rat Liver DHFR

		IC_{50} (μM)[a]					
		2,5-$(OMe)_2$			3,4,5-$(OMe)_2$		
bridge	heterocycle	Pc	Tg	RL	Pc	Tg	RL
CH_2	H_4-quinazoline[b]	0.057 (0.60)	0.021 (1.6)	0.17	0.091 (0.42)	0.024 (1.6)	0.038
CH_2	H_4-pyrido[4,3-*d*]pyrimidine[c]	3.3 (0.42)	0.3 (4.7)	1.4	6.9 (0.32)	0.20 (11)	2.2
CH_2NH	H_4-quinazoline[d]	1.7 (0.33)	0.18 (3.1)	0.56	4.6 (0.063)	0.054 (5.4)	0.29

[a] Numbers in parentheses are the selectivity index as defined in Table 2. [b] Structures **5j,l**; data from Table 2. [c] Structures **21** and **22**; data from ref 2c. [d] Structures **23** and **24**; data from ref 8.

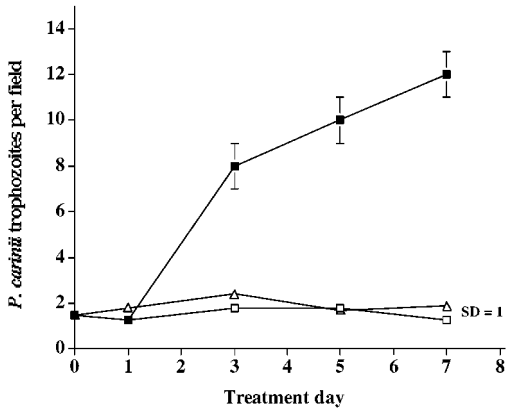


Figure 1. Suppression of the growth of *P. carinii* trophozoites in culture by **5l** (Δ, 10 μg/mL) versus untreated controls (■) or a combination of TMP+SMX (□, 50 + 250 μg/mL). The hollow symbols on days 0 and 1 are overlapped by the controls.

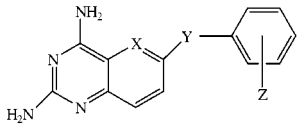
concentrations resulted in complete suppression of trophozoite growth for up to 7 days with no sign of toxicity to the mammalian host cells. Control experiments showed that neither TMP nor SMX alone retarded growth. Some suppression of growth was noted even when the concentration of **5l** was decreased to 1 μg/mL (data not shown). The fact that **5l** as a single agent was as effective as the TMP+SMX combination, and was effective at a dose that was only one-fifth the dose of TMP, suggested that this agent is taken up by *P. carinii* and may be a useful alternative to the standard TMP+SMX regimen[4] in patients who are allergic to sulfa drugs.

Four compounds (**5a**,**h**,**k**,**l**) were tested for their ability to inhibit [^{3}H]uracil incorporation by *T. gondii* in cultures of human embryonic lung (HEL) cells. The assay is based on the fact that incorporation of uracil into the nuclear DNA of dividing mammalian cells is very inefficient in comparison with that of *T. gondii*.[11] The cultures were treated with different concentrations of drug for 24 h, after which [^{3}H]uracil was added and incubation was continued for another 24 h. As shown in Table 4, the IC_{50} values for the four compounds tested were in the 0.1−0.5 μM range. The best compound of this group (**5k**, IC_{50} = 0.081 μM) was 8.5-fold more potent than pyrimethamine (IC_{50} = 0.69 μM). However it is of interest to note that the ratio [^{3}H]-uracil IC_{50}/DHFR IC_{50}, which has previously been used as an indirect measure of relative uptake,[11] was 3.5 as compared with 1.7 for pyrimethamine, suggesting that uptake of **5h** may be slightly less efficient. By way of comparison, two other compounds previously synthesized by us, 2,4-diamino-6-[(*N*-methyl-3,4,5-trimethoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (**25**)[2e] and 2,4-diamino-6-[(2-bromo-3,4,5-trimethoxyanilino)methyl]quinazoline (**26**),[2f] gave IC_{50} values in the same range as the tetrahydroquinazolines and thus are assumed to also not be transported quite as well as pyrimethamine. As noted elsewhere,[11] increased efficacy against the intact organism would presumably be achieved with all these compounds by improving uptake as well as DHFR binding.

Table 4. Inhibition of [^{3}H]Uracil Incorporation into Nuclear DNA of *T. gondii* Tachyzoites in Cultures of Human Embryonic Lung Cells

	IC_{50} (μM)		
compd	[^{3}H]uracil incorp[a]	DHFR inhib[b]	[^{3}H]uracil IC_{50}/DHFR IC_{50}
5a	0.25 (0.12−0.52)	0.032	7.8
5h	0.13 (0.073−0.19)	0.014	9.3
5k	0.081 (0.077−0.088)	0.023	3.5
5l	0.21 (0.16−0.30)	0.024	8.8
pyrimethamine[c]	0.67 (0.55−0.81)	0.39	1.7

[a] Assay performed according to ref 11. Numbers in parentheses are 95% confidence intervals. [b] Data from Table 2. [c] See ref 11.



25 : X = N, Y = CH_2NMe, Z = 3,4,5-$(OMe)_3$
26 : X = CH, Y = CH_2NH, z = 2-Br-3,4,5-$(OMe)_3$

Compound **5h** was also tested against *T. gondii* in mice in a pilot dose-finding survival experiment. The animals received a fairly high inoculum of 3×10^5 tachyzoites intraperitoneally on day 0, and intraperitoneal drug treatment was begun on day 1 and continued to day 8. To protect the mice from antifolate toxicity, 20−25 mg/kg leucovorin was also administered daily by subcutaneous injection. The initially chosen dose of **5h**, 62.5 mg/kg, appeared somewhat toxic and was therefore decreased by 50% to 31 mg/kg on day 2, raised back to 50 mg/kg on day 3, and finally stabilized at 25 mg/kg. As shown in Figure 2, 9 of the 11 mice treated with **5h** survived to day 8, the last day of drug treatment, whereas all the untreated controls died by day 6. Approximately one-half the mice treated with **5h** were still alive on day 14, but all the mice were dead by day 20. In a positive control experiment using clindamycin at 31 mg/kg for the first 2 days, followed by a 20% decrease to 25 mg/kg, the results were similar except that two of the 10 mice in this group survived to day 20. Thus, at least at the same dose, **5h** and the clinically used drug clindamycin[16a] prolonged the life of these heavily infected animals to a comparable degree. Al-

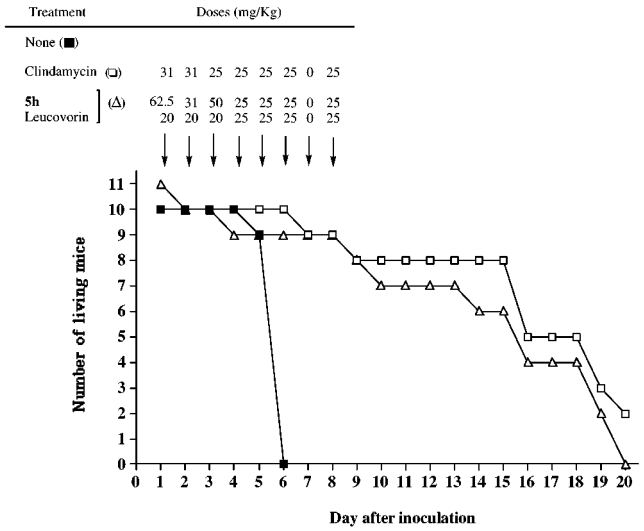


Figure 2. Suppression of the growth of *T. gondii* in mice by **5h** + leucovorin (Δ) versus controls (■) or clindamycin (□). Some of the hollow symbols on days 1−5 are overlapped by the controls.

though **5h** and clindamycin were not tested as a combination, it is noteworthy that clindamycin has been successfully combined with pyrimethamine as an antitoxoplasma regimen.[16b]

Antitumor Activity

The potent activity of compounds **5j**−**l** against rat liver DHFR suggested that these compounds would be worthwhile to evaluate as inhibitors of the growth of human cancer cells. To this end, **5j**−**l** were submitted to the National Cancer Institute for antitumor assay in culture against a standard panel of cell lines.[17] The results of growth inhibition assays using a representative number of lines of different tissue origin are given in Table 5. Data are given only for those cell lines whose average IC_{50} from two reported experiments on different days varied by no more than 0.5-fold of the range, or were <0.01 μM in both experiments. Thus, some cell lines for which there were results for only a single experiment are omitted. Since the compounds were tested as unresolved 6*R*,6*S* mixtures, each IC_{50} represents the composite effect of two individual enantiomers. Historical averages of many experiments performed at the NCI with the classical antifolate methotrexate (MTX) and the nonclassical (lipophilic) antifolate trimetrexate (TMQ) are also included in Table 5 for comparison. Among the 25 cell lines for which data satisfying the criteria defined above were obtained, the IC_{50} of at least two of the three compounds **5j**−**l** was found to be <10 μM against 17 cell lines (68%) and in the 0.1−1 μM range against 13 cell lines (52%). One compound (**5j**) was confirmed to have an IC_{50} of <0.01 μM against four of the cells (NCI-460 and A549 lung, HCT-116 colon, MDA-N breast carcinoma). The activity profiles of **5k**,**l** were similar to that of **5j** except that there were no cell lines against which the IC_{50} of these compounds was <0.01 μM.

The potency of **5j**−**l** against a number of the cell lines was similar to that reported in the NCI historical database for TMQ and MTX. Three cells lines (NCI-H226, COLO205, T-47D) that were relatively insensitive (IC_{50} > 1 μM) to TMQ and MTX were also insensitive to **5j**−**l**. Four cell lines (KM12, UACC-257, OVCAR-8, SN12C) appeared to be relatively resistant to **5j**−**l** even though they were sensitive (IC_{50} < 0.0.05 μM) to TMQ and MTX. Interestingly, the BT-549 breast carcinoma cell line, which was sensitive to TMQ and resistant to MTX, retained good sensitivity to **5j**,**k** but was less sensitive to **5l**. Against this cell line, therefore, **5j**,**k** resembled TMQ whereas **5l** was more like MTX. However the opposite was found with the EKVX cell line, which was more sensitive to TMQ than to MTX and retained sensitivity to **5l** but not to **5j**,**k**. Since lipophilic antifolates do not depend on active transport via the reduced folate carrier for their uptake into cells, differences in cytotoxicity between TMQ and the individual tetrahydoquinazolines **5j**−**l** must be due to other factors.

Summary

A series of 25 previously unknown 2,4-diamino-5,6,7,8-tetrahydroquinazolines with hydrophobic aliphatic or aromatic substituents at the 6-position was synthesized by reaction of cyanoguanidine with appropriately 4-substituted cyclohexanones. Most of the compounds were potent inhibitors of DHFR from *P. carinii*, *T. gondii*, and rat liver, but none was selective for the *P. carinii* enzyme and only a few were marginally (<10-fold) selective for the *T. gondii* enzyme relative to the mammalian enzyme. Despite this lack of enzyme selectivity, one member of the series (**5l**) was tested against *P. carinii* in culture and was found to have an antiproliferative effect comparable to the combination of trimethoprim and sulfamethoxazole (TMP+SMX), a standard clinical treatment for *P. carinii* pneumonia in AIDS patients. Four representative compounds (**5a**,**h**,**k**,**l**) were tested in culture against *T. gondii* and were found to have an effect similar to that of pyrimethamine; one of them (**5h**) was also tested for activity against *T. gondii* in mice and was found to be as potent and effective as clindamycin. Several compounds (**5j**−**l**) were tested for antitumor activity in culture and were found to be active at low micromolar or submicromolar concentrations, although they were generally less potent than TMQ or MTX.

Experimental Section

IR spectra were obtained on a Perkin-Elmer model 781 double-beam recording spectrophotometer. ^{1}H NMR spectra were recorded at 60 MHz on a Varian model EM360 spectrometer using Me_4Si as the reference or at 500 MHz on a Varian VX500 instrument. TLC analyses were done on Whatman MK6F silica gel plates, using 254-nm illumination to visualize the spots. Column chromatography was on Baker 7024 flash silica gel (40-μm particle size). Chemicals and solvents for moisture-sensitive reactions were purchased from Aldrich. Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Elemental analyses were performed by QTI Laboratories, Whitehouse, NJ, or Robertson Laboratories, Madison, NJ, and were within ±0.4% of theoretical values unless otherwise indicated.

(2,5-Dimethoxybenzyl)triphenylphosphonium Chloride (14j). A solution of 2,5-dimethoxybenzaldehyde (4.98 g, 0.03 mol) in MeOH (50 mL) was treated with $NaBH_4$ (1.14 g, 0.03 mol) and stirred at room temperature overnight. The solution was concentrated and partitioned between EtOAc and brine, and the organic layer was evaporated to an oil (5.35 g). The crude product was taken up directly in toluene (30 mL), and $SOCl_2$ (4 g, 2.64 mL, 0.0336 mol) was added dropwise over

Table 5. In Vitro Activity of **5j**−**k** against Solid Tumor Cell Lines (NCI panel)

	IC_{50} (μM)[a]				
cell line	**5j**	**5k**	**5l**	TMQ[b]	MTX[b]
			Lung		
NCI-H460	<0.01[c]	0.04 ± 0.03	0.06 ± 0.004	<0.025[c]	0.028
A549	<0.01[c]	0.06 ± 0.03	0.78 ± 0.20	0.041	0.033
EKVX	16 ± 0.80	5.7 ± 0.83	0.71 ± 0.13	0.58	8.7
NCI-H226	31 ± 23	12 ± 1.1	*d*	10	23
			Colon		
HCT-116	<0.01[c]	0.04 ± 0.009	0.05 ± 0.01	0.035	0.030
SW-620	0.32 ± 0.15	0.99 ± 0.38	*d*	<0.025[c]	0.033
KM12	2.7 ± 2.1	1.3 ± 0.30	1.3 ± 0.41	0.048	0.033
COLO205	10 ± 0.10	3.8 ± 2.4	1.6 ± 0.23	1.1	0.87
			CNS		
SF-268	*d*	0.30 ± 0.25	0.19 ± 0.08	0.026	0.052
SF-295	*d*	0.25 ± 0.19	0.25 ± 0.13	<0.025[c]	0.036
SF-539	0.43 ± 0.25	*d*	1.0 ± 0.39	0.027	0.10
			Melanoma		
LOX IMVI	0.06 ± 0.03	0.07 ± 0.02	0.08 ± 0.02	<0.025[c]	0.026
SK-MEL-5	*d*	*d*	0.08 ± 0.02	0.029	0.087
UACC-257	8.4 ± 6.0	15 ± 3.0	27 ± 10	0.14	0.79
			Ovarian		
IGROV1	*d*	0.13 ± 0.07	0.19 ± 0.13	0.032	0.069
OVCAR-3	15 ± 2.1	*d*	9.8 ± 4.0	0.29	0.40
OVCAR-8	21 ± 6.9	21 ± 15	*d*	0.026	0.031
			Renal		
786-0	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	<0.025[c]	0.033
ACHN	*d*	0.24 ± 0.17	0.27 ± 0.21	<0.025[c]	0.040
A498	10 ± 1.5	24 ± 3.2	21 ± 0.7	2.4	1.9
SN12C	15 ± 3.2	23 ± 2.6	36 ± 19	0.032	0.031
			Prostate		
PC3	0.035 ± 0.01	0.072 ± 0.005	0.19 ± 0.14	<0.025[c]	0.027
			Breast		
MDA-N	<0.01[c]	0.051 ± 0.006	0.061 ± 0.004	<0.025[c]	0.030
BT-549	0.33 ± 0.30	0.27 ± 0.12	11 ± 6.1	0.69	66
T-47D	20 ± 1.6	19 ± 1.2	22 ± 0.55	2.5	22

[a] Cells in RPMI 1640 medium containing 2 mM L-glutamine and 5% fetal bovine serum (heat-inactivated) were exposed to drug for the last 48 h of a 72-h incubation at 37 °C in a 5% CO_2 humidified atmosphere and then stained for total protein with sulforhodamine B as described.[17] Results are shown only for those cell lines whose average IC_{50} from two experiments on different days varied by no more than 0.5-fold of the range, or were <0.01 μM in both experiments. Experiment 1, #9705SR39−67 (27 May 97); experiment 2, #9707RC54−15 (22 July 97). Data kindly provided by the Developmental Therapeutics Program, NCI. [b] Averaged historical NCI data from >50 experiments with MTX and >10 experiments with TMQ. [c] Lowest concentration tested. [d] Not determined.

1 min with stirring. After being stirred for 5 min at room temperature, the solution was evaporated to obtain 2,5-dimethoxybenzyl chloride as a white solid (5.69 g): mp 64−66 °C; ^{1}H NMR δ 3.77 (s, 3H, MeO), 3.85 (s, 3H, MeO), 4.63 (s, 2H, CH_2), 6.88 (m, 2H, 3- and 4-H), 7.00 (s, 1H, 6-H). A solution of the chloride (5.68 g, 0.03 mol) and triphenylphosphine (7.86 g, 0.03 mol) in xylene (100 mL) was heated under reflux for 22 h and then cooled in an ice bath. The precipitate was collected, washed with Et_2O, and dried in vacuo at 60 °C to obtain **14j** as a white solid (11.0 g). Further refluxing of the mother liquor for 3 days afforded an additional 0.7 g; total 11.7 g (87% yield): mp 221−222 °C; IR (KBr) ν 3450, 3380, 3050, 3000, 2990, 2950, 2930, 2910, 2850, 2830, 2780, 1615, 1585, 1505, 1485, 1465, 1435, 1400 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.17 (s, 3H, MeO), 3.47 (s, 3H, MeO), 4.92 (d, J = 13 Hz, 2H, CH_2), 6.65 (d, J = 3 Hz, 1H, 6-H), 6.83 (s, 2H, 3- and 4-H), 7.38−8.15 (m, 15H, phenyl protons). Anal. C, H, Cl.

The same general method was used to prepare the following triphenylphosphonium chloride salts: 3-(trifluoromethyl)benzyl (**14g**), mp 285−287 °C (lit.[20] mp 286−288 °C); 3,4-dimethoxybenzyl (**14k**), 82%, mp 224−225 °C (Anal. C, H, Cl); 3,4,5-trimethoxybenzyl (**14l**), 77%, mp 214−215 °C (Anal. C, H, Cl); 3,4-dichlorobenzyl (**14m**), mp 300−302 °C (lit.[21] mp 326−328 °C); and 4-chlorobenzyl (**14n**), mp 278−280 °C (lit.[22] mp 289 °C).

(4-Methoxybenzyl)triphenylphosphonium Methanesulfonate (14f). A stirred solution of 4-methoxybenzyl alcohol (10 g, 0.0727 mol) and Et_3N (14.6 g, 0.145 mol) in CH_2Cl_2 (200 mL) was cooled in an ice bath and treated dropwise over 15 min with methanesulfonyl chloride (11.1 g, 0.0969 mol). When addition was complete, the bath was removed and the reaction was left to stir for 5 h. The reaction mixture was washed with H_2O (100 mL), and the organic layer was dried (Na_2SO_4) and evaporated to obtain 4-methoxybenzyl methanesulfonate. The crude product (9.50 g) was taken up directly in xylene (200 mL), and triphenylphosphine (12.7 g, 0.0484 mol) was added. The reaction mixture was stirred under reflux for 2 days, then cooled to room temperature, and filtered. The filter cake was rinsed with xylene, and the combined organic layers were evaporated to a solid, which was recrystallized from 1:1 $CHCl_3$ and EtOAc to obtain **14f** as a white powder (8.0 g, 25%): mp 233.5−234.5 °C; IR (KBr) ν 3300, 3200, 3040, 2990, 2860, 2780, 1630, 1600, 1580, 1500, 1435 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H, MsO), 3.20 (s, 3H, MeO), 5.30 (d, J = 14 Hz, 2H, CH_2), 6.5 (d, J = 8.4 Hz, 2H, 3- and 5-H), 7.05−7.95 (2-, 6-, and other phenyl protons).

The following other (arylmethyl)triphenylphosphonium mesylate salts were prepared similarly and were confirmed to be pure by TLC and by IR and ^{1}H NMR spectroscopy: 2-methylbenzyl (**14b**), 72%, mp 257−258 °C; 3-methylbenzyl (**14c**), 63%, mp 282−284 °C; 2-methoxybenzyl (**14d**), 48%, mp 243−244 °C; 3-methoxybenzyl (**14e**), 48%, 293 °C (softening above 280 °C); 3-(trifluoromethoxy)benzyl (**14h**), 75%, mp 273−275 °C; 4-(trifluoromethoxy)benzyl (**14i**), 49%, mp 251−253 °C; (2-thienyl)methyl (**14o**), 91%, mp 292−294 °C; (3-thienyl)-methyl (**14p**), 59%, mp 255−256 °C (softening above 190 °C); (2-furyl)methyl (**14q**), 18%, mp 265−268 °C.

8-(2,5-Dimethoxybenzylidene)-1,4-dioxaspiro[4.5]-decane (16j). A solution of dimsyl sodium was prepared by

stirring 0.72 g of NaH (1.2 g of 60% oil dispersion, 0.03 mol) in DMSO (75 mL) at 60 °C under N_2 for 1 h (until gas evolution ceased). The solution was cooled to room temperature, and phosphonium salt **14j** (11.6 g, 0.026 mol) was added with stirring. Formation of the ylide was indicated by the development of a red color. After 10 min, **15** (4.06 g, 0.026 mol) was added, and the reaction mixture was left to stir for 23 h and then poured over ice. The mixture was thoroughly stirred with petroleum ether (bp 30−60 °C), and the insoluble triphenylphosphine oxide was removed by filtration. The filter cake was washed with petroleum ether and the wash solution added to the original filtrate. The aqueous layer was separated and extracted with petroleum ether, and all the organic layers were combined and evaporated to an oil (7.22 g): TLC R_f 0.4 (silica gel, 4:1 hexanes−EtOAc). Flash chromatography on silica gel (50 g, 4 × 9 cm) with 8:1 followed by 4:1 hexanes−EtOAc as the eluents afforded **16j** as an oil (3.06 g, 41%): IR (NaCl) ν 2940, 2880, 2830, 2690w, 2070w, 1800w, 1735w, 1655, 1605, 1585, 1495, 1465, 1425 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.77 (m, 4H, CH_2 next to ketal group), 2.47 (m, 4H, CH_2 next to double bond), 3.72 (s, 6H, 2- and 5-MeO), 3.95 (s, 4H, OCH_2CH_2O), 6.27 (d, J = 2 Hz, 1H, =CH), 6.73 (m, 3H, aromatic). Anal. C, H.

The following benzylidene derivatives were prepared similarly from the (arylmethyl)triphenylphosphonium chlorides **14g**,**k**−**n**, and were purified by column chromatography on silica gel as described above: 3-trifluoromethyl (**16g**), 34%, oil; 3,4-dimethoxy (**16k**), 12%, mp 103−105 °C (hexane); 3,4,5-trimethoxy (**16l**), 61%, oil; 3,4-dichloro (**16m**), 68%, mp 60−61 °C (MeOH) (46−49 °C before recrystallization); 4-chloro (**16n**), 61%, oil. In addition the following benzylidene derivatives were prepared via the mesylate salts **14b**−**f**,**h**,**i**: 2-methyl (**16b**), 100%, oil; 3-methyl (**16c**), 90%, oil; 2-methoxy (**16d**), 46%, oil; 3-methoxy (**16e**), 74%, oil; 4-methoxy (**16f**), 45%, oil; 3-trifluoromethoxy (**16h**), 78%, oil; 4-trifluoromethyl (**16i**), 26%, oil. The following heteroarylidene derivatives were also prepared from **14o**−**q**: 2-thienyl (**16o**), 17%, oil; 3-thienyl (**16p**), 22%, oil; 2-furyl (**16q**), 28%, oil (unstable and therefore reduced directly to a tetrahydrofuran as shown in Scheme 3). All the ylidene derivatives were TLC homogeneous, showed the expected IR and ^1H NMR features, and were used directly for the next reaction. When the yield of product was low in some cases, as in the synthesis **16o**, a significant amount of the starting phosphonium salt could be recovered from the silica gel column as a slow-moving band.

4-(2,5-Dimethoxybenzyl)cyclohexanone (18j). A solution of **16j** (2.90 g, 0.01 mol) in MeOH (75 mL) was stirred with a small amount of sponge Ni to remove traces of sulfur impurities (remaining from the $SOCl_2$ used for the synthesis of 2,5-dimethoxybenzyl chloride). The Ni was removed by filtration, and the filtrate was treated with 2 drops of glacial AcOH and shaken with 5% Pd/C (200 mg) under 3 atm of H_2 for 20 h. The catalyst was removed, and the solvent was evaporated. The crude product (**17j**) was stirred directly in a mixture of THF (50 mL) and 2 M HCl (25 mL) at room temperature for 20 h, the organic solvent was evaporated under reduced pressure, and the product was partitioned between EtOAc and H_2O. Evaporation of the organic layer and vacuum distillation gave **18j** as a colorless liquid: 2.03 g (82%); bp 159−161 °C/0.2 Torr; IR (NaCl) ν 2990, 2950, 2850, 2830, 1715, 1605, 1585, 1495, 1465, 1445, 1425, 1330, 1295, 1285, 1260, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10−2.75 (m, 11H, cyclohexyl and benzylic CH_2), 3.73 (s, 6H, two MeO), 6.72 (m, 3H, aromatic protons). Anal. ($C_{15}H_{20}O_3$) C, H.

The same general method was also used to prepare the following 4-substituted cyclohexanones via ketals **17b**−**m**,**o**,**p**: 4-(2-methylbenzyl) (**18b**), 89%, oil; 4-(3-methylbenzyl) (**18c**), 85%; 4-(3-methylbenzyl) (**18c**), 85%, oil; 4-(2-methoxybenzyl) (**18d**), 75%, mp 72−73 °C; 4-(3-methoxybenzyl) (**18e**), 75%, oil; 4-(4-methoxybenzyl) (**18f**), 28%, oil; 4-[3-(trifluoromethyl)benzyl] (**18g**), 75%, bp 125−127 °C/0.3 Torr (Anal. C, H, F); 4-[3-(trifluoromethoxy)benzyl] (**18h**), 86%, oil; 4-[4-(trifluoromethyl)benzyl] (**18i**), 91%, oil; 4-(3,4-dimethoxybenzyl) (**18k**), 78%, bp 167−170 °C/0.2 Torr (Anal. C, H); 4-(3,4,5-trimethoxybenzyl) (**18l**), 67%, mp 97−98 °C (hexanes−EtOAc) (Anal. C, H); 4-(3,4-dichlorobenzyl) (**18m**), 87%, mp 75−76.5 °C; 4-(2-thienylmethyl) (**18o**), 68%, oil; 4-(3-thienylmethyl) (**18p**), 67%, oil. Except for the compounds that were distilled (**16g**,**k**) or crystallized (**16l**) for microchemical analysis, the ketones were purified to homogeneity by chromatography on silica gel with 3:1 or 4:1 EtOAc−heptane as the eluent and were used directly for the fusion reaction with cyanoguanidine.

4-Benzylcyclohexanone (18a). A suspension of $LiAlH_4$ (0.76 g, 20 mmol) in dry THF (40 mL) under N_2 was cooled in a dry ice−acetone bath and treated successively with **16n** (2.43 g, 9.19 mmol) and anhydrous $CoCl_2$ (2.60 g, 20 mmol) as described.[10] The mixture, which turned black immediately, was stirred and allowed to come to room temperature over 20 h. The reaction was quenched with saturated aqueous Na_2SO_4 and diluted with Et_2O. The inorganic salts were removed by filtration, the filter cake was washed thoroughly with Et_2O, and the combined Et_2O filtrates were washed with H_2O, dried ($MgSO_4$), and evaporated to an oil whose ^1H NMR spectrum no longer showed a vinyl signal. The crude product (**17a**) was taken up in a mixture of THF (20 mL) and 2 N HCl (10 mL) and stirred at room temperature for 20 h. The THF was evaporated, and the remainder was partitioned between petroleum ether (bp 30−60 °C) and H_2O. The organic layer was evaporated and the residue distilled to obtain **18a** as a colorless liquid: 1.16 g (86%); bp 107−109 °C/0.07 Torr; IR (NaCl) ν 3400, 3080, 3050, 3020, 2990, 2920, 2850, 1710, 1600, 1580, 1495, 1450, 1420, 1370, 1335, 1315, 1295, 1270, 1245, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00−2.75 (m, 11H, cyclohexyl CH_2 and CH, benzylic CH_2), 7.27 (m, 5H, aromatic protons). Anal. C, H. Essentially complete dechlorination was confirmed by combustion analysis, which showed only 0.22% chlorine.

4-(Tetrahydrofuran-2-yl)cyclohexanone (18q). A mixture of **16q** (0.560 g, 2.54 mmol) and 10% Pd−C (2 g) in EtOAc (150 mL) containing glacial AcOH (2 mL) was shaken under H_2 (50 psi) in a Parr apparatus for 1 day. The catalyst was removed by filtration through Celite, and the filtrate was washed with H_2O (2 × 30 mL) followed by 5% $NaHCO_3$ (50 mL). Drying (Na_2SO_4) and evaporation under reduced pressure yielded **17q** as a yellow oil (0.63 g). A 0.58-g portion of this oil was dissolved directly in a mixture of THF (50 mL) and 2 N HCl (14 mL), and after 1 day the solution was poured into 5% $NaHCO_3$ (200 mL). The product was extracted with EtOAc (200 mL), and the solution was dried (Na_2SO_4) and evaporated. Chromatography of the residue on silica gel with 3:1 heptane−EtOAc as the eluent gave **18q** as an oil pure enough for direct use in the next reaction: 0.304 g (71%); IR (NaCl) ν 2900, 2830, 1700, 1445, 1400, 1315, 1265, 1150, 1110, 1080, 1040, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15−2.50 (m, 15H, CH_2, CH), 3.80 (m, 3H, CH_2O, CHO).

(6*R*,6*S*)-2,4-Diamino-6-benzyl-5,6,7,8-tetrahydroquinazoline (5a). A mixture of **18a** (0.50 g, 2.66 mmol) and cyanoguanidine (0.216 g, 2.57 mmol) was stirred and heated at 180−190 °C in a round-bottom flask under a flow of N_2 for 90 min. The reaction mixture was left to cool to room temperature and taken up in a mixture of 85:15 $CHCl_3$−MeOH (200 mL). The solution was washed with H_2O (100 mL), dried over Na_2SO_4, and concentrated to dryness on a rotary evaporator. Chromatography of the residue on silica gel with 85:15 $CHCl_3$−MeOH as the eluent afforded **5a** as a yellow solid (0.331 g).

Compounds **5a**−**m**, **9a**−**c**, and **10**−**12** were prepared very similarly. The amount of ketone varied from 60 mg to 2 g, the reaction temperature was kept at 180−200 °C, and the reaction time was 80−90 min. Silica gel chromatography was performed with 85:15 or 9:1 $CHCl_3$−MeOH as the eluent. Fractions were monitored by TLC on silica gel plates, using the same solvent mixture, and those giving a single spot, typically with R_f 0.2−0.3, were pooled and evaporated. Yields, melting point values, and IR and ^1H NMR spectral data for the products are given in Table 1. Samples submitted for elemental analysis were dried in vacuo overnight at room temperature, but no other special effort was made to remove

final traces of chromatography solvents. As a result, most of the compounds appeared to retain small fractional amounts of organic solvent(s), a property of lipophilic antifolates we have noted previously (reviewed in ref 2c). Empirical C/N ratios calculated from the microanalytical data were almost always a little high but could be satisfactorily explained by assuming the presence of trace amounts, typically <0.1 mol, of $CHCl_3$ and/or MeOH (i.e., the chromatography solvents). In one instance (**5l**) the small number and upfield chemical shift of the aryl protons actually made it possible to detect a singlet at δ 7.3 whose peak height was consistent with a fractional molar amount of $CHCl_3$ in the sample.

2,4-Diamino-6-(4-methoxybenzylidine)-5,6,7,8-tetrahydroquinazoline, *E* and *Z* Isomers (60:40 ratio) (20). A mixture of **19** (400 mg, 1.87 mmol) and cyanoguanidine (156 mg, 1.87 mmol) was heated at 190−200 °C with stirring in a round-bottomed flask under a flow of N_2 for 90 m. A workup procedure similar to that of the other cyanoguanidine reactions gave a white solid: yield 130 mg (25%); mp 200 °C dec; IR (KBr) ν 3440, 3330, 3170, 2900, 2830, 1620, 1525, 1510, 1440, 1295, 1245 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80−3.49 (m, 6H, CH_2), 3.81 (s, 3H, OMe), 4.63 (m, 4H, NH_2), 6.47 (two closely spaced singlets, 60:40 ratio, 1H, vinyl CH), 6.88−7.18 (m, 4H, phenyl). Anal. C, H, N.

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