2,4-Diamino-5-chloroquinazoline Analogues of Trimetrexate and Piritrexim: Synthesis and Antifolate Activity

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Ten heretofore undescribed 2,4-diamino-5-chloroquinazoline analogues of trimetrexate (TMQ) and piritrexim (PTX) were synthesized and tested as inhibitors of dihydrofolate reductase (DHFR) from rat liver, *Pneumocystis carinii*, and *Toxoplasma gondii*. The most active quinazolines against both the *P. carinii* and the *T. gondii* enzyme were those with an $ArCH_2$-NH or $ArNHCH_2$ side chain. Among $ArNH(CH_2)_n$ compounds with $n = 1-3$ and either 2′,5′-dimethoxyphenyl or 3′,4′,5′-trimethoxyphenyl as the Ar moiety, activity decreased in the order $n = 1 > n = 2 > n = 3$. The best inhibitor of *P. carinii* DHFR, 2,4-diamino-5-chloro-6-[(*N*-methyl-3′,4′,5′-trimethoxyanilino)methyl]quinazoline (**10**) had an IC_{50} of 0.012 μM and was slightly more potent than TMQ and PTX. Compound **10** was also the best inhibitor of *T. gondii* DHFR, with an IC_{50} of 0.0064 μM corresponding again to a minor increase in activity over TMQ and PTX. However, as with these standard agents, **10** showed no appreciable selectivity for either the *P. carinii* or *T. gondii* enzyme relative to the rat liver enzyme. The highest selectivity achieved in this limited series was with 2,4-diamino-5-chloro-6-[*N*-(3′,4′,5′-trimethoxybenzyl)-*N*-methylamino]quinazoline (**17**) against *T. gondii* DHFR. While **17** (IC_{50} = 0.016 μM) was somewhat less potent than **10**, its selectivity, as defined by the ratio IC_{50}(rat liver)/IC_{50}(*T. gondii*) was ca. 30-fold higher than that of TMQ or PTX. Two compounds, 2,4-diamino-5-chloro-6-[(3′,4′,5′-trimethoxyanilino)methyl]quinazoline (**9**) and 2,4-diamino-5-chloro-6-[*N*-(3′,4′,5′-trimethoxybenzyl)amino]quinazoline (**15**), were also tested against human DHFR and were found to have an IC_{50}/[E] of 0.5, indicating that their binding was near-stoichiometric.

Pneumocystis carinii and *Toxoplasma gondii* ordinarily pose little health risk in persons with a normal immune system but can become life-threatening opportunistic pathogens in individuals infected by the human immunodeficiency virus (HIV) or receiving high-dose immunosuppressant therapy for organ transplantation.[1] *Pneumocystis carinii* pneumonia (PCP) is frequently the earliest clinical sign of progression from latent HIV infection to full-blown AIDS.[2,3] This very debilitating type of pneumonia is also seen in a small percentage of cancer patients as a complication of cancer chemotherapy.[4] *T. gondii* is thought to be present in a completely benign form in as much as 40% of the general U.S. population, typically in a permanently encysted form in muscle and/or brain tissue.[5] Upon "endogenous reactivation" in the AIDS patient, however, toxoplasmosis becomes a very complex disease because the parasite can colonize virtually any tissue.

Therapeutic regimens currently used to treat PCP include systemic or aerosolized pentamidine isethionate,[6,7] antifolate combinations such as trimethoprim (**1**) and sulfamethoxazole,[8] trimethoprim and dapsone,[9] and more recently trimetrexate (TMQ, **2**) or piritrexim (PTX, **3**) with leucovorin in patients who cease responding to, or cannot tolerate, the more traditional drugs.[10–12] Standard treatment for toxoplasmosis, on the other hand, generally relies on pyrimethamine (**4**) sulfadiazine or other sulfa drugs.[13,14] TMQ and PTX, in combination with leucovorin as a host tissue-protective agent, have been used as a salvage regimen to treat refractory disease.[15] Although all these antifolate-based regimens typically show high initial success rates, their efficacy tends to diminish over time. Moreover, serious side effects occur in a number of patients, necessitating interruption or discontinuation of treatment. For this reason, vigorous efforts have been made over the past several years to discover alternative treatment approaches for both PCP and toxoplasmosis. Drugs against other parasitic disease such as malaria are often effective; for example, activity in experimental models of *P. carinii* or in patients with PCP has been reported recently with primaquine and several other 8-aminoquinolines,[16,17] and with the naphthoquinone antimalarial 566C80 (atovaquone).[18,19] Acridones,[20] as well as two extracts from Chinese herbs, bilobalide[21] and artemisinin,[22] have likewise shown promise. Activity against *T. gondii* has been reported recently with clindamycin,[23] several macrolide antibiotics,[24] doxycycline,[25] atovaquone,[26] artemisinin,[27] and synthetic 1,2,4-trioxane analogues of artemisinin.[28] A second-generation trimethoprim analogue, RO 11-8958, was recently also discovered to have remarkable selectivity for *P. carinii* DHFR and is synergistic with sulfonamides and dapsone.[29] It seems likely that, as the number of these new agents expands, strategies for the therapy and prophylaxis of *P. carinii* and *T. gondii* infections in AIDS patients will follow the lead of cancer chemotherapy in moving increasingly toward multidrug regimens.

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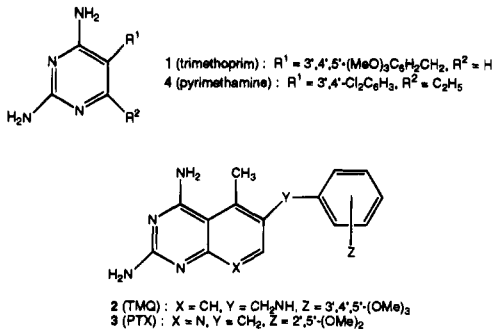


The rationale for using TMQ or PTX in combination with leucovorin[10-12,15] is that, when used alone for extended periods at the doses required for significant parasite eradication, these potent dihydrofolate reductase (DHFR) inhibitors produce unacceptable toxicity to host proliferative tissues such as the marrow and the oral and intestinal mucosa. Since *P. carinii* and *T. gondii*, unlike mammalian cells, cannot actively transport reduced folates and rely instead on *de novo* synthesis to meet their metabolic cofactor requirements,[30] leucovorin can be used to selectively rescue the host. An unfortunate property of TMQ and PTX, however, is that, in contrast to trimethoprim and pyrimethamine, neither compound binds as well to *P. carinii* and *T. gondii* DHFR as it does to the mammalian enzyme (Table 1). Thus there exists a need to develop second-generation TMQ and/or PTX analogues that bind more tightly to *P. carinii* or *T. gondii* DHFR and less tightly to mammalian DHFR. Recent evidence suggests that selective binding to the enzyme from these parasites is likely to be an achievable goal.[31-34]

In the preceding paper of this series several 2,4-diaminothieno[2,3-*d*]pyrimidines structurally related to TMQ and PTX were synthesized and tested as inhibitors of *P. carinii* and *T. gondii* DHFR versus rat DHFR.[35] Two of these compounds, 2,4-diamino-6-[2-(2',5'-dimethoxyphenyl)ethyl]-5-methylthieno[2,3-*d*]pyrimidine (**5**) and 2,4-diamino-6-(3',4',5'-trimethoxy-phenyl)-5-methylthieno-[2,3-*d*]pyrimidine (**6**), showed ca. 5-fold selectivity for the *P. carinii* and *T. gondii* enzyme, respectively. A third, 2,4-diamino-5-methyl-6-(3',4',5'-trimethoxybenzyl)thieno[2,3-*d*]pyrimidine (**7**), had ca. 80-fold selectivity for the *T. gondii* enzyme. In the present paper we report the synthesis and anti-DHFR activity of 10 previously unknown TMQ and PTX analogues (**8**–**17**) based on the 2,4-diamino-5-chloroquinazoline nucleus and describe their structure–activity and structure–selectivity features relative to TMQ and PTX.

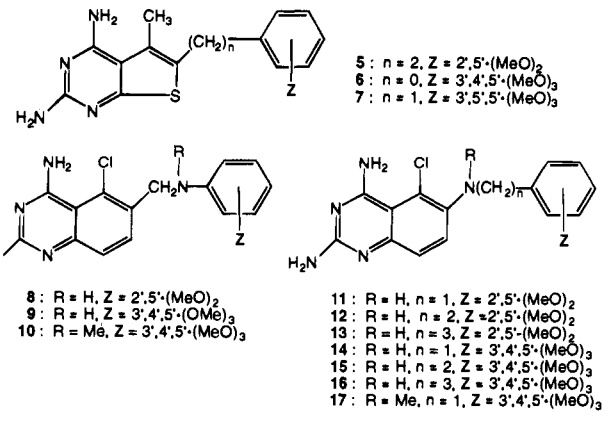


Table 1. Activity and Selectivity of Nonclassical Antifolate Drugs as Inhibitors of Dihydrofolate Reductase

drug	IC_{50} (μM)[a]		
	rat liver	*P. carinii*	*T. gondii*
trimethoprim (**1**)	130	12 (11)	2.7 (44)
pyrimethamine (**4**)	2.3	3.7 (0.62)	0.39 (5.9)
trimetrexate (TMQ, **2**)	0.003	0.042 (0.071)	0.010 (0.29)
piritrexim (PTX, **3**)	0.0015	0.031 (0.048)	0.017 (0.088)

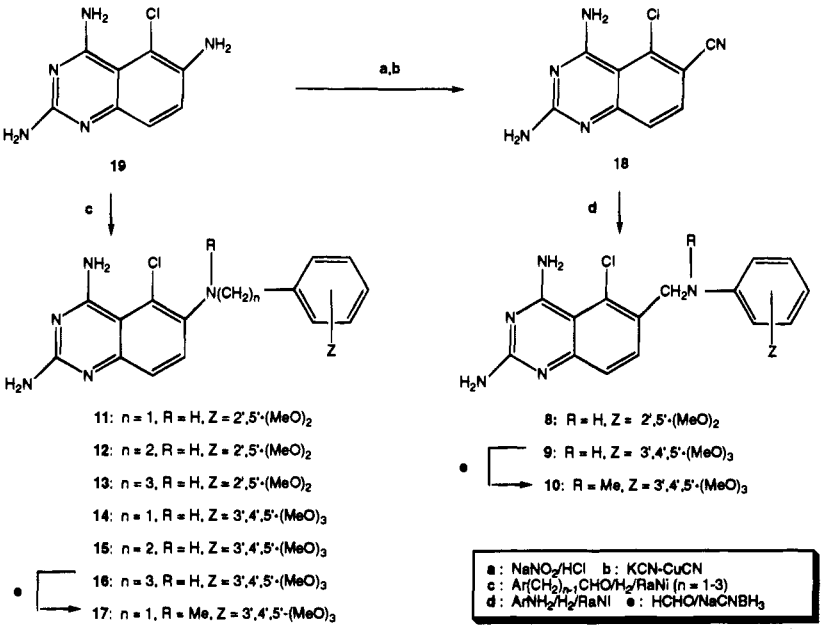
[a] Data are from ref 33. Numbers in parentheses are enzyme selectivity ratios, defined as IC_{50}(rat liver)/IC_{50}(*P. carinii*) or IC_{50}(rat liver)/IC_{50}(*T. gondii*). Values of 1.0 or less indicate absence of selectivity for the *P. carinii* or *T. gondii* enzyme versus the rat liver enzyme.

Chemical Synthesis. The synthetic route followed to obtain the title compounds is summarized in Scheme 1. 2,4-Diamino-5-chloroquinazoline-6-carbonitrile (**18**) has been used previously to prepare classical DHFR inhibitors with an *N*-(4-aminobenzoyl)-L-glutamic acid,[36-39] *N*-(4-aminobenzoyl)-L-aspartic acid,[36] or *N*-(aminobenzoyl)-L-ornithine side chain,[39,40] and nonclassical inhibitors with a substituted aralkylamino or (arylamino)alkyl side chain[41,42] at the 6-position. However, none of the reported examples among the latter group contained the 3',4',5'-trimethoxyphenyl or 2',5'-dimethoxyphenyl substitution pattern of TMQ and PTX, respectively. Use has likewise been made of 2,4,6-triamino-5-chloroquinazoline (**19**) to obtain classical and nonclassical analogues with an "inverted" carbon–nitrogen bridge.[37-42] Again, however, reported examples of this class do not, up to now, include 3',4',5'-trimethoxyphenyl or 2',5'-dimethoxyphenyl analogues. The synthesis of **18** from **19** via a Sandmeyer-type reaction using KCN and $CuSO_4$ as described in the classical paper by Davoll and Johnson[36] was found to give erratic yields. Therefore, we developed a modified process, based on recent work in a different context by Hynes and co-workers.[43] In this modification, treatment of the diazotized solution of **19** with CuCl/KCN instead of $CuSO_4$/KCN allowed **18** to be reliably obtained in >70% yield.

Reductive condensation of **18** with 2,5-dimethoxyaniline or 3,4,5-trimethoxyaniline in glacial AcOH under 1 atm of hydrogen in the presence of Raney nickel afforded **8** and **9** in 20–25% yield (nonoptimized) after silica gel chromatography and recrystallization. Substantial amounts of dark polymeric materials always formed, accounting for these modest yields. Moreover, the coupling reaction occurred in the desired manner only when performed at atmospheric pressure, using a hydrogen-filled balloon and a magnetically stirred Raney nickel suspension, rather than by shaking in a Parr apparatus.

Condensation of **19** with 2,5-dimethoxybenzaldehyde in refluxing EtOH afforded the expected imine (72%), which was reduced directly with $NaCNBH_3$ in MeOH at room temperature to obtain **11** (43%). The same two-step sequence starting from 2-(2,5-dimethoxyphenyl)-acetaldehyde and 3-(2,5-dimethoxyphenyl)propanal led to the homologous 2,5-dimethoxy derivatives **12** and **13**. Compound **11** was obtained satisfactorily as a free base after chromatography on silica gel and recrystallization, whereas **12** and **13** could only be isolated in crystalline form as HCl salts. As expected from the strong propensity of the starting aldehyde to undergo aldol-type

Scheme 1



self-condensation, the yield of **12** was also quite low (9%) in comparison with **11** (43%) and **13**·HCl (34%). Reaction of **19** proceeded better with 3,4,5-trimethoxybenzaldehyde than 2,5-dimethoxybenzaldehyde, giving a 66% yield of **14** and 29% recovery of unchanged **19**. In this case, $NaCNBH_3$ was added directly to the amine and aldehyde mixture, without isolation of the imine. Thus it is not known whether recovery of **19** was due to incomplete formation of the imine or hydrolysis of nonreduced imine during the acidification step. Use of 2-(3,4,5-trimethoxyphenyl)acetaldehyde and 3-(3,4,5-trimethoxyphenyl)propanal in place of 3,4,5-trimethoxybenzaldehyde produced **15** and **16**, respectively. The yield of **15** (20%) was lower than that of **14** but higher than that of **12**. In the case of **16**, isolation of a crystalline product was possible only after converting it to the HCl salt (31% yield).

The ^1H NMR spectrum of the anilinomethyl derivative **8** contained a singlet at δ 6.1 for the 6'-proton and a multiplet at δ 6.50–6.75 for the 3'- and 4'-protons on the phenyl ring, whereas in the corresponding aralkylamino derivative **11** these protons appeared as a broad multiplet at δ 6.8–6.9, reflecting the change from NH to CH_2 at the 10-position. Similarly the 2'- and 6'-protons in the trimethoxyanilino derivative **9** gave rise to a two-proton singlet at δ 5.95, whereas in the (trimethoxybenzyl)amino derivative **14** there was a considerable upfield shift to δ 6.6.

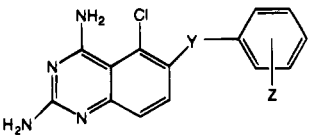
Further reaction of **9** and **14** with 37% HCHO in the presence of $NaCNBH_3$ in a MeCN–THF–AcOH mixture, followed by silica gel chromatography and recrystallization, yielded the N^{10}- and N^9-methyl derivatives **10** (39%) and **17** (63%), respectively. The ^1H NMR spectra of **10** and **17** contained the expected three-proton singlet at δ 3.05 and 2.70 for the N^{10}-methyl and N^9-methyl group, respectively, confirming that reductive alkylation had occurred on the bridge nitrogen in both **9** and **14**.

Biological Activity. The 2,4-diamino-5-chloroquinazolines **8**–**17** were tested as inhibitors of DHFR from rat liver, *P. carinii*, and *T. gondii* as described previously.[44–46] The results are shown in Table 2. In addition, the (3',4',5'-trimethoxybenzyl)amino derivative **9** and the (3',4',5'-trimethoxyanilino)methyl derivative **14** were tested as inhibitors of human DHFR and as inhibitors of the growth of tumor cells in culture.

The IC_{50} values of the quinazolines as inhibitors of DHFR from rat liver ranged from 0.0059 and 0.0060 μM for **9** and **14**, respectively, to 19 μM for the 3-(3',4',5'-trimethoxyphenyl)propyl analogue **16**. Other things being equal (e.g., in **8** versus **11** and in **10** versus **17**), activity was minimally affected by a change in the bridge from CH_2NH to $NHCH_2$. On the other hand, among the [(2',5'-dimethoxyphenyl)alkyl]amino derivatives **11**–**13** as well as among the [(3',4',5'-trimethoxyphenyl)alkyl]amino derivatives **14**–**16**, the most active were consistently those with a single CH_2 group in the bridge (**11**, **14**), whereas two or three CH_2 groups resulted in an activity decrease of 2 orders of magnitude. Methylation of **9** on N^{10} and of **14** on N^9 decreased binding in both instances, although N^9-methylation appeared to have a somewhat larger effect. The reported IC_{50} values of TMQ and PTX against rat liver DHFR under the same assay conditions are 0.003 and 0.0015 μM, respectively (Table 1).[32] Thus, **8** and **9**, the best analogues among those in this study against the mammalian enzyme, were 2- and 4-fold less active than these key reference compounds.

Against DHFR from *P. carinii*, the IC_{50} values observed with the quinazolines ranged from 0.012 μM for **10** to 95 μM for **16**. Thus the weakest inhibitor of human DHFR was also the weakest inhibitor of *P. carinii* DHFR, but the most potent inhibitor of the human enzyme was not the best inhibitor of the parasite enzyme. This would be consistent with the view that the amino acid sequence and topological feature of the active site are not the same in the two enzymes. The reported IC_{50} values of TMQ and PTX against *P. carinii* DHFR are 0.042 and 0.031 μM, respectively (Table 1).[32] Thus the best compound of those tested was more potent than TMQ or PTX, although the difference was less than 4-fold. In previous studies comparing the effect of 5-substitution on rat liver DHFR inhibition by classical quinazoline inhibitors with an *N*-(4-aminobenzoyl)-L-

Table 2. Inhibition of Dihydrofolate Reductase by 2,4-Diamino-5-chloroquinazoline Analogues of Trimetrexate (TMQ) and Piritrexim (PTX)



compd	Y	Z	IC_{50} (μM)[a] rat liver	*P. carinii*	*T. gondii*
8	CH_2NH	2,5-$(MeO)_2$	0.044	0.051 (0.86)	0.03 (1.5)
9	CH_2NH	3,4,5-$(MeO)_3$	0.0059	0.033 (0.18)	0.0052 (1.1)
10	$CH_2N(Me)$	3,4,5-$(MeO)_3$	0.012	0.012 (1.0)	0.0064(1.9)
11	$NHCH_2$	2,5-$(MeO)_2$	0.028	0.053 (0.53)	0.017 (1.6)
12	$NH(CH_2)_2$	2,5-$(MeO)_2$	2.1	12 (0.18)	0.99 (2.1)
13	$NH(CH_2)_3$	2,5-$(MeO)_2$	1.9	26 (0.073)	1.0 (1.9)
14	$NHCH_2$	3,4,5-$(MeO)_3$	0.0060	0.033 (0.18)	0.007 (0.86)
15	$NH(CH_2)_2$	3,4,5-$(MeO)_3$	5.4	43 (0.13)	2.6 (2.1)
16	$NH(CH_2)_3$	3,4,5-$(MeO)_3$	19	95 (0.20)	14 (1.4)
17	$N(Me)CH_2$	3,4,5-$(MeO)_3$	0.038	0.17 (0.22)	0.016 (2.4)

[a] See Table 1, footnote *a*.

glutamate side chain, the difference in IC_{50} values between 5-chloro and 5-methyl analogues was reported to be less than 2-fold.[47] In another more recent study, the IC_{50} values of the 6-unsubstituted model compounds 2,4-diamino-5-chloroquinazoline and 2,4-diamino-5-methylquinazoline, while substantially higher than those of **9** and TMQ, likewise differed by less than 2-fold.[48] Thus the influence of 5-chloro versus 5-methyl substitution on DHFR binding appears to be a minor one in nonclassical, as well as classical, quinazoline inhibitors.

As with the rat liver enzyme, activity against *P. carinii* DHFR diminished markedly as the bridge was lengthened by more than one CH_2 group. Moreover, there was, again, a smaller loss of activity in going from two CH_2 groups to three than in going from one to two. On the other hand, while N^9-methylation resulted in decreased binding to both the rat liver and *P. carinii* enzyme (compare **14** and **17**), N^{10}-methylation produced a small increase in binding to the *P. carinii* enzyme (compare **9** and **10**). Nonetheless, the selectivity index for the group as a whole, expressed as the ratio IC_{50}(rat liver)/IC_{50}(*P. carinii*), proved to be only in the 0.1–1.0 range. Since the selectivity index of **9** was 0.18 as compared with the reported value of 0.071 for TMQ,[33] it appears that replacement of the 5-methyl by a 5-chloro substituent does not strongly affect the selectivity of *P. carinii* DHFR binding.

Against *T. gondii* DHFR, the most potent compounds, with IC_{50} values in the 0.005–0.007 μM range, were **9**, **10**, and **14**, and the least active compound was again **16**, with an IC_{50} of 14 μM. The reported IC_{50} values for TMQ and PTX against the *T. gondii* enzyme are 0.010 and 0.017 mM, respectively (Table 1).[33] Thus the best compounds in our group were more active than TMQ and PTX, but the difference, as with the *P. carinii* enzyme, was less than 4-fold. In agreement with the trend observed with both *P. carinii* and rat liver DHFR, binding diminished considerably among the (2′,5′-dimethoxybenzyl)- as well as (3′,4′,5′-trimethoxybenzyl)-amino compounds when the length of the bridge extended beyond one CH_2 group. A small decrease in binding was also observed upon N^{10}- or N^9-methylation. For the group as a whole, selectivity for the *T. gondii* enzyme was in the 0.86–2.4 range. The reported selectivity ratios TMQ and PTX are 0.29 and 0.088, respectively (Table 1).[33] Although our ratios were slightly higher, they cannot be viewed as a significant improvement and were not as favorable as those of trimethoprim and pyrimethamine.

Compounds **9** and **14** were also found to be potent inhibitors of human DHFR, with IC_{50} values comparable to those against rat liver enzyme and an IC_{50}/[E] ratio very close to 0.5, indicative of near-stoichiometric inhibition. We therefore considered it worthwhile to test these compounds for *in vitro* antitumor activity. However, when **9** and **14** were tested as inhibitors of the growth of cultures of SCC VII murine squamous carcinoma cells (one of several mammalian tumor cell lines currently in routine use in our laboratory),[49] their IC_{50} values for a 72-h exposure were determined to be 0.187 and 1.79 μM, respectively. This was in marked contrast to TMQ, which had an IC_{50} of 0.0063 μM against these cells. It thus appears that replacement of 5-methyl by 5-chloro in TMQ leads to a 30-fold decrease in potency against intact cells and that this structural modification, while it marginally increases DHFR binding, is probably detrimental for cellular uptake. Moreover, while C^9–N^{10} interchange in the 2,4-diamino-5-chloroquinazolines reported here is also tolerated by the enzyme, this is likewise unfavorable where transport across the cell membrane is concerned.

The fact that cells were 30-fold less sensitive to **9** than to TMQ was in contrast to what has been observed with the 5-chloro and 5-methyl derivatives of classical 2,4-diaminoquinazolines, whose IC_{50} values as inhibitors of cell growth in culture are essentially the same (ca. 0.02 μM).[47] It thus appears that when a glutamate side chain is present, allowing active transport via the reduced folate carrier followed by conversion to nonefluxing polyglutamates, small differences in DHFR binding can be negated. In the case of **9**, even though DHFR binding was better than that of TMQ, replacement of the 5-methyl by a 5-chloro substituent was clearly detrimental to uptake. It appears from several studies that, while the precise mechanism of TMQ uptake is not yet fully understood, it occurs, at least in some cells, via a process other than simple diffusion. Observations supporting this view are that (a) TMQ transport in WI-L2 human leukemic lymphoblasts is

temperature dependent and completely blocked by p-(chloromercuri)benzenesulfonic acid, a known inhibitor of the active transport of methotrexate, probably via irreversible reaction with a thiol group on a membrane protein;[50] (b) WI-L2 cells with 40–60-fold TMQ resistance show only a 2-fold increase in DHFR activity while remaining fully sensitive to methotrexate;[50] (c) MOLT-3 human lymphoblastoid cells 200-fold resistant to TMQ but not cross-resistant to doxorubicin (i.e., not multidrug resistant via high P-glycoprotein expression) show a 40% decrease in TMQ influx rate and approximately 4-fold reduction in steady-state accumulation of TMQ as well as a similar reduction in steady-state accumulation of methotrexate;[51] and (d) other cell lines such as soft tissue sarcomas show low TMQ uptake which correlates with intrinsically low TMQ sensitivity.[52] Our finding that **9** is 30-fold less active than TMQ in culture even though it binds more tightly to DHFR lends further support for an uptake mechanism involving, at some stage, an interaction between the drug and a transport-associated membrane protein. This interaction appears to be influenced by the nature of the 5-substituent. In the case of the 2,4-diamino-5-chloroquinazoline analogues reported here, a reasonable possibility is that a decrease in basicity of the 2,4-diaminopyrimidine moiety due to the electron-withdrawing effect of the halogen atom could diminish binding to this protein.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; only peaks with wavenumbers greater than 1400 cm^{-1} are reported. Quantitative UV absorbance spectra were measured on a Varian Model 210 instrument. ^1H NMR spectra were recorded on a Varian EM360 or in some instances a Varian Model VXR500 instrument, using Me_4Si as the reference. TLC analyses were done on Baker Si250F silica gel plates, with spots being visualized under 254-nm illumination. Column chromatography was on Baker 7024 flash silica gel (40 μm particle size). Solvents for reactions sensitive to moisture were purchased from Aldrich (Milwaukee, WI) in Sure-Seal bottles. Melting points were determined in capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Microanalyses were by QTI Laboratories, Whitehouse, NJ. Except where indicated, elemental analyses for C, H, N, and Cl were within ±0.4% of calculated values.

2,4-Diamino-5-chloroquinazoline-6-carbonitrile (18). To a solution of **19** (600 mg, 1.66 mmol) in 2 N HCl (8 mL) cooled in an ice bath was added a solution of $NaNO_2$ (204 mg, 1.44 mmol) in H_2O (1.5 mL). The mixture was stirred at 2 °C for 20 min, then added to a stirred solution of KCN (1.48 g, 11.4 mmol) and CuCl (325 mg, 3.28 mmol) in H_2O (3 mL). After being stirred for another 5 min, the reaction mixture was diluted with ice-water (20 mL), stirred at 2 °C for an additional 30 min, warmed to 55 °C for 40 min, and finally left to stir at room temperature for 1 h. The pH was adjusted to 10 with aqueous ammonia while cooling in ice, and the red precipitate was filtered and air-dried overnight. The solid was stirred in 15% AcOH (40 mL), a small amount of insoluble material was removed by filtration, and the filtrate was diluted with $MeOCH_2CH_2OH$ (40 mL). The pH was readjusted to 10 with ammonia while cooling, and the reddish-brown precipitate was filtered and dried *in vacuo* at 60 °C to obtain a brown powder: yield 425 mg (71%); R_f 0.29 (silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH); mp 285 °C dec (lit.[36] mp 287 °C dec); IR (KBr) ν 3500, 3340, 3180, 2210, 1610, 1550, 1510, 1475, 1445 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.70 (br s, 2H, NH_2), 7.20 (d, 1H, J = 9 Hz, C_7-H), 7.70 (br s, 2H, NH_2), 7.80 (d, 1H, J = 9 Hz, C_8-H).

2,4-Diamino-5-chloro-6-[(2′,5′-dimethoxyanilino)methyl]quinazoline (8). A slurry of Raney Ni (100 mg, 50% in H_2O) was added to a solution of **18** (220 mg, 1.0 mmol) and 2,5-dimethoxyaniline (184 mg, 1.2 mmol) in glacial AcOH (10 mL), and the mixture was stirred under hydrogen at atmospheric pressure for 1.5 h. The reaction mixture was filtered through a pad of Celite, the pad washed with 15% AcOH, and the filtrate cooled in an ice bath and neutralized with concentrated NH_4OH. The precipitated solid was collected and purified by chromatography on silica gel with 10:1 $CHCl_3$/MeOH as the eluent. The desired product (R_f 0.29; silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH) was isolated as a brown powder, which was recrystallized from hot MeOH to obtain white needles (94 mg, 20%): mp 203–205 °C; IR (KBr) ν 3460, 3430, 3390, 3200, 1615, 1565, 1530 cm^{-1}; ^1H NMR ($CDCl_3$ + 10% CD_3OD) δ 3.60 (s, 3H, 5′-OMe), 3.80 (s, 3H, 2′-OMe), 4.35 (s, 2H, CH_2N), 6.1 (br s, 1H, 6′-H), 6.50–6.75 (m, 2H, 3′- and 4′-H), 7.25 (d, 1H, C_7-H), 7.60 (d, 1H, C_8-H). Anal. ($C_{17}H_{18}ClN_5O_2 \cdot 0.4H_2O$) C,H,N; Cl: calcd, 9.65; found, 10.39.

2,4-Diamino-5-chloro-6-[(3′,4′,5′-trimethoxyanilino)methyl]quinazoline (9). Reductive coupling of **18** (120 mg, 0.54 mmol) and 3,4,5-trimethoxyaniline (85 mg, 0.46 mmol) was performed as in the preceding experiment to obtain off-white needles (52 mg, 22%): mp 203–206 °C; IR (KBr) ν 3480, 3990, 2920, 1610, 1555, 1540, 1505 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.70 (s, 9H, 3′-, 4′-, and 5′-OMe), 4.25–4.50 (m, 2H, CH_2N), 5.95 (s, 2H, 2′- and 6′-H), 6.0–6.2 (br m, 2H, NH_2), 7.20–7.55 (br m, 4H, NH_2, C_7- and C_8-H). Anal. ($C_{11}H_{20}ClN_5O_3 \cdot H_2O \cdot CH_3OH$ (C, H, Cl, N).

2,4-Diamino-5-chloro-6-[(*N*-methyl-3′,4′,5′-trimethoxyanilino)methyl]quinazoline (10). A stirred solution of **9** (68 mg, 0.17 mmol) and 37% formaldehyde (0.34 mL, 0.34 mmol) in MeCN (3 mL) at room temperature was treated with 1 M $NaCNBH_3$ in THF (0.27 mL). Glacial AcOH (0.1 mL) was then added slowly over 20 min. After 1.5 h, the mixture was treated with 2 N HCl (5 mL) and H_2O (20 mL), stirred for 15 min, and poured into an equal volume of H_2O. The solution was extracted with Et_2O (2 × 20 mL), and the aqueous layer was basified (pH > 9) with solid NaOH and reextracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to dryness by rotary evaporation. Chromatography on silica gel with 200:10:1 CH_2Cl_2/MeOH/28% NH_4OH yielded the desired product (R_f 0.40; silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH) as a yellow powder, which was recrystallized in warm Et_2O: 27 mg (39%); mp 104–112 °C dec; IR (KBr) ν 3420 br, 2920, 1610, 1555, 1545, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.05 (NMe), 3.80 (s, 9H, 3′-, 4′-, and 5′-OMe), 4.55 (s, 2H, CH_2N), 4.8–5.0 (br m, 2H, NH_2), 5.95 (s, 2H, 2′- and 6′-H), 6.50–6.75 (br m, 2H, NH_2), 7.40 (br s, 2H, C_7- and C_8-H). Anal. ($C_{19}H_{12}ClN_5O_3 \cdot 0.8H_2O$) C, H, N.

2,4-Diamino-5-chloro-6-[(*N*-(2′,5′-dimethoxybenzyl)amino]quinazoline (11). A solution of 2,5-dimethoxybenzaldehyde (250 mg, 1.5 mmol) and **19** (210 mg, 1.0 mmol) in EtOH (12 mL) was refluxed for 36 h. The yellow precipitate which formed on cooling was filtered, washed with Et_2O, and dried *in vacuo* to obtain the imine as a yellow powder (245 mg, 72%). The imine was dissolved directly in MeOH (10 mL), and $NaCNBH_3$ (27 mg) was added with stirring followed by 5 N HCl in MeOH (0.1 mL). After 16 h of sirring at room temperature, the reaction mixture was diluted with aqueous 4 N HCl (12 mL) and H_2O (20 mL). After another 15 min, the acidic solution was washed with Et_2O (2 × 25 mL) and basified (pH > 10) with solid NaOH. The precipitate was collected and chromatographed on silica gel with 100:10:1 $CHCl_3$/MeOH/28% NH_4OH as the eluent. Fractions showing a single TLC spot with R_f 0.36 (silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH) were pooled and evaporated, and the residue was reycrstallized from hot i-PrOH to obtain yellow prisms (112 mg, 43%): mp 192–193 °C; IR (KBr) ν 3460, 3410, 3310, 3150, 1625, 1570, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.7 (s, 3H, 5′-OMe), 3.8 (s, 3H, 2′-OMe), 4.4 (d, 2H, CH_2), 4.5–4.8 (br m, 3H, NH, NH_2), 6.3–6.6 (br m, 2H, NH_2), 6.8–6.9 (m, 3H, 3′-, 4′-, and 6′-H), 7.3 (br s, 2H, C_7- and C_8-H). Anal. ($C_{17}H_{17}ClN_5O_2 \cdot 0.1H_2O$ (C, H, Cl, N).

2,4-Diamino-5-chloro-6-[*N*-[2-(2′,5′-dimethoxyphenyl)ethyl]amino]quinazoline hydrochloride (12·HCl). 9% (prepared analogously to **16**·HCl); R_f 0.35 (silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH); mp 105–107 °C; IR (KBr) ν 3460

br, 3340, 3200, 2915, 1635, 1590 cm^{-1}; ^1H NMR (CD_3OD) δ 2.85–3.20 (m, 2H, CH_2Ar), 3.3–3.7 (m, 2H, CH_2N), 3.80 (s, 3H, 5′-OMe), 3.90 (s, H, 2′-OMe), 6.80–6.95 (m, 3H, 3′-, 4′-, and 6′-H), 7.3–7.4 (m, 2H, C_7- and C_8-H). Anal. ($C_{18}H_{20}Cl_5O_2$·HCl·0.5H_2O) C, H, N.

2,4-Diamino-5-chloro-6-[*N*-[3-(2′,5′-dimethoxyphenyl)propyl]amino]quinazoline hydrochloride (13·HCl): 34% (prepared analogously to **16**·HCl); R_f 0.41 (silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH); mp 90–93 °C; IR (KBr) ν 3470 br, 3210, 2910, 1645, 1580 cm^{-1}; ^1H NMR ($CDCl_3$ + 10% CD_3OD) δ 1.7–2.0 (m, 2H, $CH_2CH_2CH_2$), 2.45–2.7 (m, 2H, CH_2Ar), 3.2–3.5 (m, 2H, CH_2N), 3.75 (s, 3H, 5′-OMe), 3.80 (s, 3H, 2′-OMe), 6.6–6.8 (br m, 3H, 3′-, 4′-, and 6′-H), 7.3 (br, 2H, C_7- and C_8-H). Anal. ($C_{19}H_{22}ClN_5O_2$·HCl·0.3H_2O) C, H, Cl, N.

2,4-Diamino-5-chloro-6-[*N*-(3′,4′,5′-trimethoxybenzyl)amino]quinazoline (14). A solution of 3,4,5-trimethoxybenzaldehyde (250 mg, 1.5 mmol) and **19** (210 mg, 1.0 mmol) in MeOH (12 mL) under an argon atmosphere was treated with $NaCNBH_3$ (38 mg, 0.6 mmol) followed by 5N HCl in MeOH (0.1 mL). The resultant yellow solution was stirred at room temperature for 24 h, then diluted with 4 N HCl (5 mL) and water (25 mL). After another 15 min the acidic solution was washed with Et_2O (2 × 25 mL), and the aqueous layer basified (pH > 10) with solid KOH and chilled at 2 °C for 1 h. The yellow precipitate was collected and chromatographed on silica gel with 4:1 $CHCl_3$/MeOH as the eluent. The fast-moving fraction (R_f 0.73; silica gel, 28:12:1 $CHCl_3$/MeOH/28% NH_4OH) contained the desired product (258 mg, 66%): mp 215–216 °C; IR (KBr) ν 3490, 3390, 3380, 3150, 1605, 1565, 1540 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (br, 3′-, 4′-, and 5′-OMe), 4.3 (d, 2H, CH_2), 4.40–4.75 (br m, 3H, NH, NH_2), 6.3–6.5 (br m, 2H, NH_2), 6.6 (s, 2H, 2′- and 6′-H), 7.0–7.5 (m, 2H, C_7- and C_8-H). A slower-moving fraction (R_f 0.32) was identified as unchanged **19** (61 mg, 29% recovery). Anal. ($C_{18}H_{20}ClN_5O_3$) C, H, Cl, N.

2,4-Diamino-5-chloro-6-[*N*-[2-(3′,4′,5′-trimethoxyphenyl)ethyl]amino]quinazoline (15). A solution of 3,4,5-trimethoxyphenylacetaldehyde (300 mg, 1.43 mmol) and **19** (235 mg, 1.1 mmol) in MeOH (15 mL) was stirred with 3 Å molecular sieves (400 mg) at room temperature for 3 days under an argon atmosphere. The molecular sieves were filtered off and washed with EtOAc, and the filtrate was concentrated to a viscous oil under reduced pressure. The crude oil was dissolved in MeOH (6 mL) under argon and the solution treated consecutively with 1 N $NaCNBH_3$ in THF (1.4 mL) and 3 N HCl in MeOH (0.5 mL). The reaction mixture was stirred at room temperature for 18 h and then diluted with 2 N HCl (10 mL) and H_2O (20 mL). After 15 min of stirring, the acidic solution was washed with Et_2O (3 × 20 mL), and the aqueous layer was basified to pH 8 with 28% NH_4OH while cooling in an ice bath. The aqueous layer was extracted with $CHCl_3$ (3 × 40 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated to a yellow oil by rotary evaporation. The oil was then applied onto a silica gel column which was eluted with 100:10:1 $CHCl_3$/MeOH/28% NH_4OH to obtain a fast-moving fraction, containing the desired product (R_f 0.35; silica gel, 100:10:1 $CHCl_3$/MeOH/28% NH_4OH) and a slower fraction (R_f 0.17), consisting of unchanged **19** (91 mg, 39% recovery). Recrystallization of the fast-moving material from boiling i-PrOH yielded a yellow powder (78 mg, 20%): mp 105–107 °C; IR (KBr) ν 3500, 3440 br, 3390, 2920, 1610, 1595, 1570, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (s, 9H, 3′-, 4′-, and 5′-OMe), 2.60–3.05 (m, 2H, CH_2CH_2NH), 3.60–4.05 (m, CH_2CH_2NH), 4.4–4.7 (br m, 2H, NH_2), 6.1–6.3 (br m, 2H, NH_2), 6.35 (s, 2H, 2′- and 6′-H), 7.05 (br s, 1H, C_7- or C_8-H), 7.15 (br s, 1H, C_7- or C_8-H). Anal. ($C_{19}H_{22}ClN_5O_2$·H_2O·0.5Me_2CHOH) C, H, Cl, N.

2,4-Diamino-5-chloro-6-[*N*-[3-(3′,4′,5′-trimethoxyphenyl)propyl]amino]quinazoline Hydrochloride (16·HCl). A solution of 3-(3,4,5-trimethoxyphenyl)propanal (375 mg, 1.67 mmol) and **19** (300 mg, 1.4 mmol) in MeOH (15 mL) was stirred with 3 Å molecular sieves (400 mg) at room temperature under argon for 3 days. The mixture was filtered, the sieves were washed with EtOAc, the filtrate was concentrated under reduced pressure to a semisolid, and the latter was redissolved in MeOH (15 mL) under argon. The solution was treated with 1 M $NaCNBH_3$ in THF (2 mL), stirred at room temperature for 18 h, and was then diluted with 3 N HCl (10 mL) and H_2O (20 mL) and stirred again for 15 min. The acidic solution was washed with EtOAc (2 × 20 mL) and basified to pH > 9 with 4 N NaOH. Extraction with $CHCl_3$ (3 × 30 mL), drying (Na_2SO_4), and rotary evaporation yielded a yellow oil. Chromatography on silica gel with 100:10:1 $CHCl_3$/MeOH/28% NH_4OH separated the desired product (142 mg; R_f 0.31) from unreacted **19** (101 mg; R_f 0.17). The product was dissolved in 2 N HCl (15 mL) at 45 °C, and the solution was chilled for 1 h at 5 °C. The precipitate was collected, washed with cold 1 N HCl, and dried *in vacuo* at 45 °C for 48 h to obtain a yellow powder (135 mg, 31%): mp 97–99 °C dec; IR (KBr) ν 3420 br, 3210, 2910, 1645, 1580 cm^{-1}; ^1H NMR ($CDCl_3$ + 10% CD_3OD) δ 1.85–2.20 (m, 2H, $CH_2CH_2CH_2$), 2.55–2.85 (m, 2H, CH_2Ar), 3.30–3.65 (m, 2H, $NHCH_2$), 3.75 (s, 3H, 4′-OMe), 3.85 (s, 6H, 3′- and 5′-OMe), 6.4 (s, 2H, 2′- and 6′-H), 7.5 (br s, 2H, C_7- and C_8-H). Anal. ($C_{20}H_{24}ClN_5O_3$·H_2O·HCl) C, H, Cl, N.

2,4-Diamino-5-chloro-6-[*N*-(3′,4′,5′-trimethoxybenzyl)-*N*-methylamino]quinazoline (17). A stirred solution of **14** (200 mg, 0.51 mmol) and 37% formaldehyde (0.86 mL, 1.02 mmol) in MeCN (5 mL) at room temperature was treated with 1 M $NaCNBH_3$ in THF (0.82 mL), glacial AcOH (0.5 mL) was added slowly over 30 min, and stirring was continued for another 2 h. The reaction was quenched by addition of 2 N HCl (5 mL) and H_2O (20 mL), and after being stirred for 15 min the mixture was poured into an equal volume of H_2O. The aqueous solution was washed with Et_2O (2 × 20 mL), basified (pH > 9) with solid NaOH, and reextracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to dryness by rotary evaporation. Recrystallization from warm Et_2O afforded yellow prisms (131 mg, 63%): mp 179–180 °C; IR (KBr) ν 3450, 3310, 2920, 1675, 1595, 1555, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H, NMe), 3.75 (s, 9H, 3′-, 4′-, and 5′-OMe), 4.05 (s, 2H, CH_2N), 4.65–4.80 (br m, 2H, NH_2), 6.75 (s, 2H, 2′- and 6′-H), 6.45–6.80 (br m, 2H, NH_2), 7.40–7.45 (m, 2H, C_7- and C_8-H). Anal. ($C_{19}H_{12}ClN_5O_3$·0.8H_2O) C, H, N.

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