Notes

Synthesis and Biological Activities of Conformationally Restricted, Tricyclic Nonclassical Antifolates as Inhibitors of Dihydrofolate Reductases[1]

Aleem Gangjee,*,† Jufang Shi,† and Sherry F. Queener‡

Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282, and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202

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Seven novel tricyclic pyrimido[4,5-*c*][2,7]naphthyridones **5**−**8** and the corresponding naphthyridines **9**−**11** were synthesized as conformationally restricted inhibitors of dihydrofolate reductase (DHFR) and as antitumor and/or antiinfectious agents. The analogues were designed to orient the side chain trimethoxyphenyl group in different conformationally defined positions in order to explore the effect of the side chain orientation on binding affinity and selectivity for DHFR from various species. The semirigid orientations were achieved by bridging the C5 and N10 of compound **12** with a *N*-ethyl bridge and by variation of the position of double bonds in rings B and C as well as substitution at the 2′,6′- positions of the phenyl ring. The synthesis of compounds **5**−**11** were accomplished by cyclocondensation of the appropriate keto ester (as the biselectrophile) with 2,4,6-triaminopyrimidine to afford the lactam **5**. The dehydrolactams **6** and **7** were prepared by air oxidation and PtO_2-catalyzed dehydrogenation of **7**, respectively. The dichloro dehydro lactam **8** was obtained by refluxing lactam **5** and/or **6** in $POCl_3$ or a mixture of $POCl_3/PCl_5$. Compounds **9**−**1**1 were obtained by two methods, direct borane reduction of lactam **5** or **6** or thiation of the dipivoylated lactam **15** followed by reductive dethiation. Compounds **9**−**11** were interconverted by air oxidation or PtO_2-catalyzed reduction/oxidation, respectively. The compounds were evaluated as inhibitors of DHFR from *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg) with rat liver (rl) serving as the reference mammalian enzyme. In the lactam series **5**−**8**, the most unsaturated analogue **7** showed an IC_{50} of 86 nM against rlDHFR, almost 100-fold more active than **5** and 3-fold more active than **6**. The 2′,6′-dichloro dehydro lactam **8** was less active than the corresponding dehydro lactam **6** against rlDHFR. In the naphthyridine series **9**−**11**, the dehydro analogue **10** was more active than **9** against rlDHFR. The fully reduced analogue **11** (as a mixture of cis and trans isomers) was the most active in the naphthyridine series. The analogues were, in general, more inhibitory against rlDHFR than against pcDHFR, or tgDHFR, and thus lacked selectivity. In addition, they were less potent than the bicyclic compounds trimetrexate **3** (TMQ) and piritrixim **4** (PTX).

Dihydrofolate reductase (DHFR)[3] catalyzes the reduction of folate or 7,8-dihydrofolate (FH_2) to tetrahydrofolate (FH_4) and intimately couples with thymidylate synthase (TS). TS is a crucial enzyme that catalyzes the reductive methylation of 2′-deoxyuridine 5′-monophosphate (dUMP) to 2′-deoxythymidine 5′-monophosphate (dTMP) utilizing 5,10-methylenetetrahydrofolate as a cofactor which functions as the source of the methyl group as well as the reductant. This is the exclusive *de novo* source of dTMP; hence inhibition of DHFR or TS activity, in the absence of salvage, leads to "thymineless death".[4,5] Thus DHFR inhibition has long been an attractive goal for the development of chemotherapeutic agents.[6]

Opportunistic infections with *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg) remain a principal cause of death in patients with Acquired Immunodeficiency Syndrome (AIDS) in the United States. The current therapeutic regimens involve combinations of DHFR inhibitors, such as trimethoprim (**1**, TMP) or pyrimethamine (**2**), with dihydropteroate synthase inhibitors such as sulfonamides. An alternative agent for *P. carinii* infection is pentamidine.[7] These standard therapies have significant frequency and severity of toxicity. Some of the adverse reactions associated with combined therapy may be antibody-mediated because the addition of leucovorin does not prevent side effects such as neutropenia.[8] Pentamidine has systemic adverse effects, which in some cases may lead to permanent diabetes.[6,7] Trimetrexate **3** (TMQ),[9] an antineoplastic agent, is a second line drug for *P. carinii* pneumonia (PCP) and could be a more effective option for initial as well as salvage therapy. It is a potent DHFR inhibitor with an $IC_{50} = 42$ nM against pcDHFR and is 1000−1500 times more potent than TMP. Because of its potency and lack of selectivity, TMQ needs to be administered with leucovorin, which is selectively taken up by host cells and circumvents DHFR blockade, leading to selective rescue of host cells. Piritrexim **4** (PTX), which is under phase I clinical trials as an antineoplastic drug, also inhibits pcDHFR and tgDHFR

† Duquesne University.
‡ Indiana University School of Medicine.
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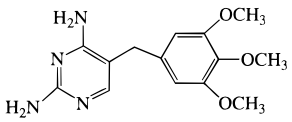
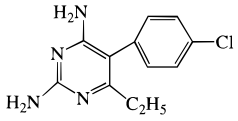
at IC_{50} = 19.3 and 17.0 nM, respectively, similar to that observed for TMQ. *P. carinii* and *T. gondii* cell culture inhibition in vitro by PTX and TMQ occurs at concentration of 0.1 −1.0 and 0.1 mg/ml, respectively. In animal models, PTX was also effective and prophylactic against PCP in rats.[10]

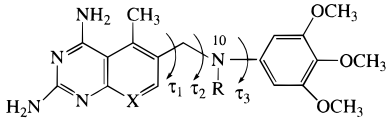
Despite their impressive potencies against pcDHFR and tgDHFR, both TMQ and PTX are nonspecific DHFR inhibitors and significantly inhibit all other sources of DHFR including human. Therefore TMQ and PTX, when used alone to treat opportunistic infections, have the potential of severe toxicity.



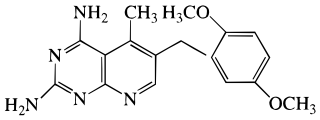
1, Trimethoprim (TMP)



2, Pyrimethamine



3, TMQ X = CH, R = H
12, X = N, R = CH_3



4, PTX

TMP is a bacterial DHFR selective inhibitor, and its binding to various DHFR has been characterized in considerable detail both biochemically and crystallographically.[11] The trimethoxyphenyl group of TMP is bound to bacterial and vertebrate DHFR in two very different conformations, which result from the different geometry of DHFR active sites and accounts, in part, for the preferential binding and hence the selectivity of TMP for *E. coli* (ec) DHFR compared to chicken liver (cl) DHFR.

Since conformations of the side chain of TMP plays an important role in the selectivity of TMP for bacterial DHFR, it was of interest to determine if restricting the flexible side chain of TMQ in different semirigid orientations would provide for selectivity against pcDHFR and/or tgDHFR. Tricyclic analogs **5, 6, 7, 9,** and **11** were designed as conformationally restricted analogues of TMQ in which τ_1 and τ_2 were partially restricted by incorporation in a six-membered ring. In addition, different orientations of the side chain were achieved by variation of the extent of saturation of the B and C rings of the resulting tricyclic ring system in **5, 6, 7, 9,** and **11**. Molecular modeling using SYBYL[12] and its MAXIMIN and SEARCH options indicated that each of the tricyclic analogues **5, 6, 7, 9,** and **11** provides a different orientation of the trimethoxyphenyl side chain, each of which is different from that of the DHFR bound conformation of TMQ.[13] We[14] have reported 2,4-diamino-5-methyl-6-[(3′,4′,5′-trimethoxy-*N*-methylanilino)-methyl]pyrido[2,3-*d*]pyrimidine (**12**) as a 8-aza-N10-methyl analogue of TMQ and found it to be more potent and significantly more selective than TMQ against tgDHFR. Thus restricting the torsion angles τ_1 and τ_2 of **12** by bridging the 5-CH_3 and 10-CH_3 of **12** to afford tricyclic analogues **5, 6, 7, 9,** and **11** was a logical and worthwhile goal.

Chemistry

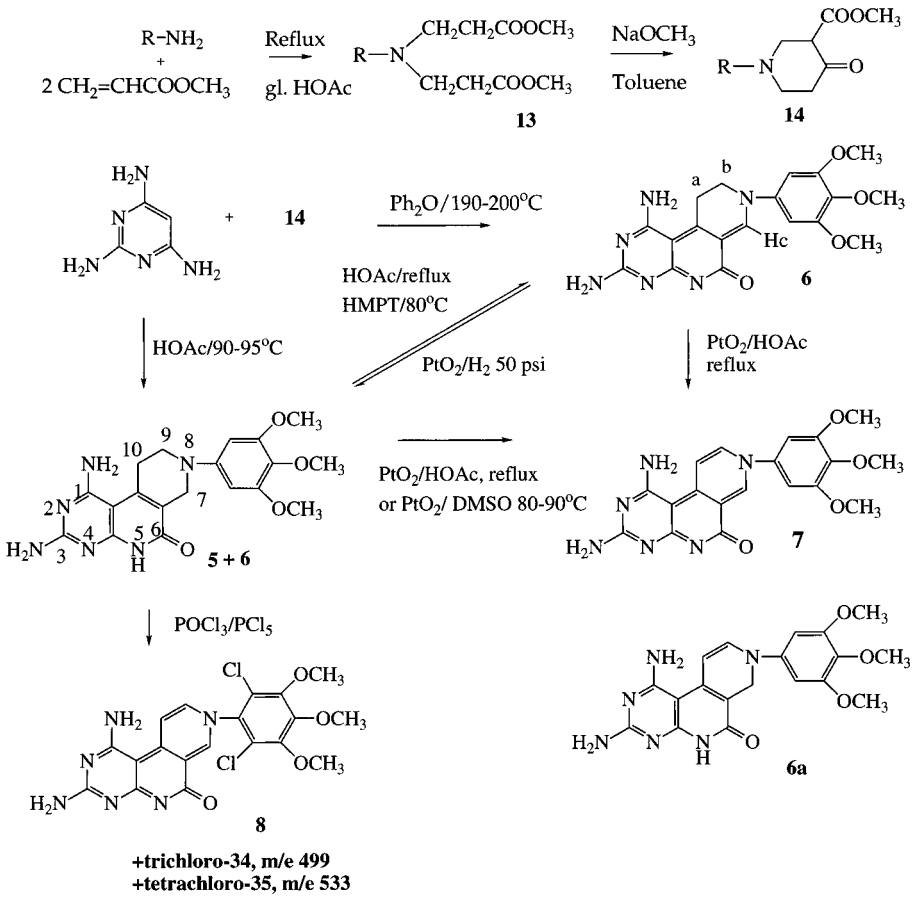
The synthesis of angular 5,6-disubstituted pyrido[2,3-*d*]pyrimidones has been reported by Grivsky *et al.*[15] and Hurlbert *et al.*,[16] and later by Gangjee *et al.*[17] and DeGraw *et al.*[18] via the cyclocondensation of substituted 6-aminopyrimidines with appropriate *β*-keto esters, followed by the conversion of the resulting pyrido[2,3-*d*]pyrimidones to the corresponding pyrido[2,3-*d*]-pyrimidines. Adoption of this procedure involved the synthesis of the keto ester **14**, which was accomplished (Scheme 1) by the Dieckmann cyclization of **13**, which in turn was obtained from the Michael addition[19] of 3,4,5-trimethoxyaniline with methyl acrylate. The cyclocondensation of **14** with 2,4,6-triaminopyrimidine in diphenyl ether at reflux (190−200 °C) afforded **6.** The FAB MS spectrum of the product **6** showed that the molecular weight was two units less than that of the lactam **5**, which suggested that the product could be the dehydrogenated lactam **6** or its isomer **6a**. Assignment of the structure of the product as the dehydro lactam **6** was based on its ^{1}H NMR spectrum, which showed a downfield nonexchangeable signal at 9.09 ppm (s, 1H), which was assigned to the olefinic proton Hc. The AA′BB′ portion of the spectrum at 4.03 (t, 2 H) and 3.27 (t, 2 H) was assigned to the vicinal methylene protons Ha and Hb.

The cyclocondensation of keto ester **14** and triaminopyrimidine in glacial HOAc at 90−95 °C afforded the lactam **5** as the major product. The reaction temperature (90−95 °C) was critical since temperatures over 95 °C afforded lower yields of **5** and temperatures lower than 90 °C afforded incomplete reaction.

Assignment of **5** as the angular structure rather than the linear isomer was achieved by comparison of similar structures in the literature.[20,21] The ^{13}C NMR of **5** showed the carbonyl carbon signal at 165.59 ppm, which was in good agreement with the chemical shifts observed for α-pyridones and similar lactams.[19,20] Further confirmation of the angular nature of **5** was provided from NOE studies. In difference NOE studies a positive NOE was observed between the 10-CH_2 (t, 2H, 3.15 ppm) and the 1-NH_2 (5.92 ppm) protons.

Attempts were made at this stage to convert the lactam **5** to the corresponding pyrido[2,3-*d*]pyrimidine **9** *via* one of the two-step literature reactions *i.e.* either chlorination−dechlorination or thiation−dethiation procedures, reported by Hurlbert *et al.*[29] and Grivsky *et al.*[28] A modified procedure of Grivsky *et al.* for chlorination of the lactam **5** using thionyl chloride in DMF afforded only the dehydrolactam **6**, which was confirmed by FAB MS. Several procedures involving time and temperature variations and different chlorinating reagents were attempted for the chlorination of the lactam **5,** including $POCl_3$ and $POCl_3$:PCl_5 (1:1).[22] The latter reaction afforded a mixture. This mixture showed three fluorescent spots very close to each other on TLC. The FAB MS of the mixture showed three clusters ([M + 1] ions) at *m/z* 463, 499, 533; each cluster had its own isotope peaks (M + 2, M + 4, M + 6), indicating that multiple chlorination had occurred. This mixture, upon being stirred in 1 N NaOH−MeOH (1:1) solution, afforded **8**

Scheme 1



as a major component, which gave an $[M + H]^+$ ion of m/z 463, 465 in its FAB mass spectrum. Its ^1H NMR showed the absence of the aliphatic methylene protons as well as the phenyl aromatic protons; however three new aromatic protons at 9.35, 8.05, and 7.06 ppm were observed, suggesting skeletal aromatization and 2,6-dichlorination on the phenyl ring.

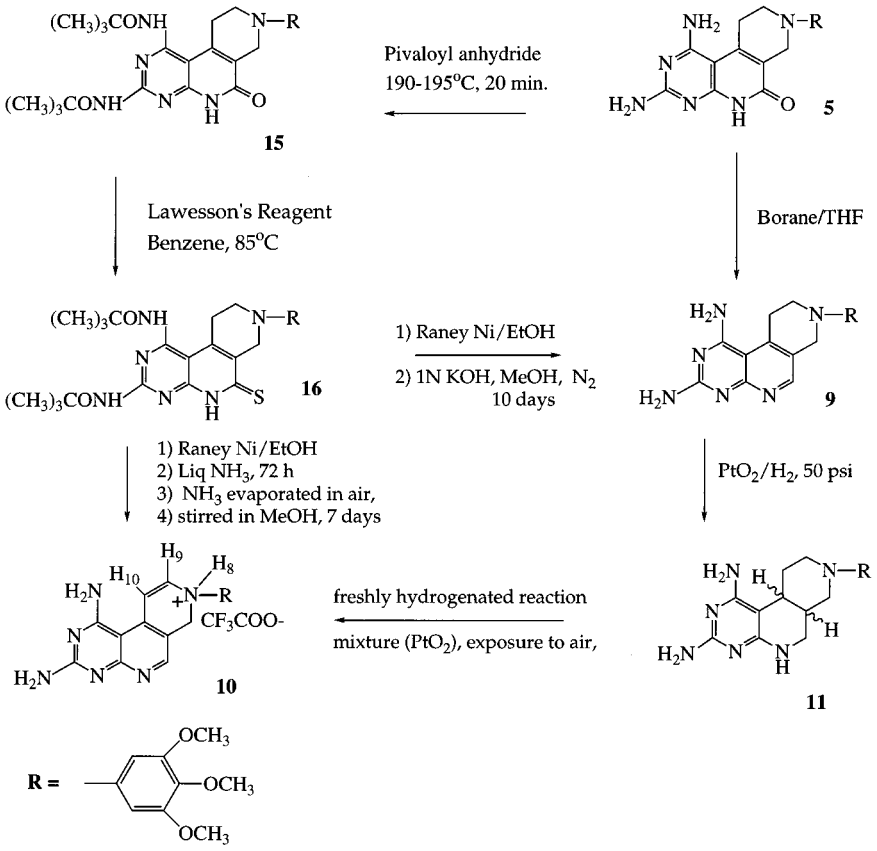
The three lactams **5**, **6**, and **7** (Scheme 1) were interconvertible. The dehydro lactam **6**, upon hydrogenation in glacial HOAc and 50 psi of H_2 with PtO_2, was reduced back to the lactam **5**. The dehydro lactam **6**, unlike the lactam **5**, was quite stable under harsh conditions such as refluxing glacial HOAc or 75% HOAc and in the presence of 5% Pd on charcoal. However, the dehydro lactam **6** and/or lactam **5** in 75% HOAc or DMSO in the presence of PtO_2 at 80−90 °C afforded compound **7**. Its ^1H NMR which showed three new aromatic signals at 9.32 (s, 1 H), 7.72 (d, 1 H, $J = 6.7$ Hz), and 6.52 ppm (d, 1 H, $J = 6.7$ Hz). This coupling pattern, especially the two sets of doublets, along with elemental analysis and FAB MS confirmed structure **7**.

The failure of the chlorination and thiation of lactam **5** was attributed to the poor solubility of **5**. Solubility of some insoluble folic acid analogues were reported[23] to be greatly improved by pivaloylation of the amino groups. The pivaloylated derivative **15** was obtained (Scheme 2) by refluxing **5** in pivalic anhydride. Thiation of the pivaloylated lactam **15** by Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide] in benzene afforded the desired thiolactam **16** in 30% yield. The structure of **16** was confirmed by FAB MS, ^1H NMR, and elemental analysis.

Desulfuration of **16** to **9** was accomplished by refluxing it in freshly prepared Raney Ni suspended in anhydrous ethanol. Depivaloylation was carried out in MeOH−1 N KOH or acetone−1 N KOH under nitrogen which was necessary to prevent air oxidation of the product. The ^1H NMR spectrum in DMSO-d_6 of compound **9** showed the desired signals, and the spectrum in TFA-*d* showed an aromatic proton at 8.83 ppm. The FAB MS has the required molecular weight (MH^+ m/z 383) for **9**. The dehydro compound **10** was obtained *via* desulfuration and depivaloylation of **16** (liquid NH_3) followed by air oxidation during workup. The structure of **10** was confirmed by ^1H NMR, elemental analysis, and FAB MS. The mass spectrum showed an $[M + H]$ ion of m/z 381, 2 units less than **9** (m/z 383, $[M + H]$), indicating that a double bond had been introduced. The ^1H NMR revealed three aromatic peaks at 9.30 (s, 1H), 7.90 (m, 1H, overlapped with NH_2 protons), and 6.94 ppm (d, 1H), corresponding to H-6, H-9, and H-10, respectively. A methylene singlet at 4.05 ppm was assigned to the CH_2 at C-7, and the peak at 3.25 ppm was assigned to MeOH, which was confirmed on elemental analysis. The coupling pattern and decoupling experiments confirmed the structure of compound **10**. The doublet of the H-10 was attributed to adjacent coupling with the H-9, which in turn occurred as a multiplet due to adjacent coupling with H-10 as well as with protonated N-8. Irradiation at the H-10 resulted in the simplification of the signal of H-9 (doublet like), further supporting the protonated form of N-8.

The hexahydro compound **11** was obtained by catalytic reduction (PtO_2/H_2) of compound **9**. On the basis of literature reports[24] and results from Gangjee *et al.*,[19]

Scheme 2



bicyclic pyrido[2,3-*d*]pyrimidine analogues were known to undergo benzylic type cleavage during hydrogenation catalyzed by PtO_2; however the tricyclic system **9** was not subject to benzylic cleavage in the presence of PtO_2. The TLC of the reaction mixture immediately after hydrogenation showed a single spot. The FAB MS of the reaction sample immediately after hydrogenation gave the desired [M + H] ion of *m*/*z* 387. However, compound **11** was unstable and air-oxidized in the presence of a trace amount of PtO_2 to afford compound **10**. After the exposure of the reaction mixture to air for less than 10 min in the presence of the catalyst, the FAB MS of the mixture showed a predominant [M + H] peak at *m*/*z* 381 (**10**) as well as at 397 (lactam **6**), indicating that three double bonds had been introduced into **11.** Thus the workup following hydrogenation of **9** to **11** needed extreme care. While this work was in progress, DeGraw *et al.*[17] reported a direct method of lactam reduction with borane. Using a modification of DeGraw's method, the mixture of the lactam **5** and the dehydrolactam **6** (obtained in Scheme 1) were subjected to borane reduction. Both lactam and dehydrolactam disappeared from the TLC, and three new components were formed (TLC). The EI MS of this mixture, after workup, showed two clusters of peaks with two molecular ions at *m*/*z* 382 (**9**) and 386 (**11**). No molecular ion for **10** at *m*/*z* 380 was observed. A single product was obtained by stirring the mixture in HOAc−MeOH. Under these condition, the tetrahydro compound **11** was aromatized by the loss of four protons of the B ring and converted to **9**. The product obtained was identical in all respects to **9** described above.

Table 1. Inhibitory Concentrations (IC_{50}, nM) against DHFRs and Selectivity Ratios (IC_{50} rlDHFR/IC_{50} pc- or tgDHFR)[a]

compd	P. carinii (pc)	T. gondii (tg)	rat liver (rl)	rl/pc	rl/tg
5	>5000		>5000		
6	4280	1120	240	0.06	0.21
7	2400	520	86	0.04	0.17
8	>1000	2300	500	<0.5	0.2
9	>2270		>2700		
10	4600	760	470	0.1	0.62
11	2200	290	160	0.07	0.55
12	13.2	0.85	7.6	0.58	8.94
TMQ	42	10	3	0.07	0.3
PTX	31	17	1.5	0.05	0.09

[a] These assay were carried out at 37 °C under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NADPH) in the presence of 150 mM KCl.[24,25]

Biological Evaluation and Discussion

Compounds **5**−**11** were evaluated as inhibitors of DHFR from *P. carinii* and *T. gondii.* Rat liver (rl) DHFR served as the mammalian reference. The IC_{50} values along with the selectivity ratios are listed in Table 1.[25,26] In the lactam series **5**−**8**, the most active analogue was the naphthyridone **7**, with the greatest extent of unsaturation and consequently planarity. Compounds **5** and **6** in which the C ring is not planar and more flexible than that of **7** results in a decrease in inhibitory potency against all three enzymes. Substitution of electron-withdrawing chlorine groups in the phenyl ring as in **8** also leads to a decrease in potency compared to **7**.

For the naphthyridines **9**−**11**, the most potent analogue against all three DHFRs was **11**, the most saturated, least planar, and consequently the most flexible analogue. This result is in contrast to the naphthyridones where planarity in the C ring resulted in the greatest potency.

None of the analogues **5**−**11** were selective for pcDHFR or tgDHFR, and the potency of the analogues was severalfold less than TMQ. Thus restriction of τ_1 and τ_2 of **12** by incorporation into a six-membered ring is detrimental to potency and does not afford selectivity for pcDHFR or tgDHFR. The effect of τ_1 and τ_2 restriction by incorporation within a six-membered ring also severely restricts the side chain trimethoxyphenyl moiety; thus the orientation of the side chain is almost entirely predicted on the conformation of the rings and the orientation of the nitrogen of the C ring. This is reflected in the observed variation in potency with the extent of unsaturation in each of the naphthyridones and naphthyridines **5**−**11**. Obviously the orientations of the trimethoxyphenyl ring in the analogues **5**−**11** are not conducive to potency or selectivity.

We have reported the 8-aza-N10-methyl analogue **12**[19] of TMQ. It was more potent than TMQ and significantly more selective than TMQ against tgDHFR (IC_{50} 0.8 nM; rl/tg = 8.14). The activity and selectivity of **12** suggests that the additional substituents on the 5- and 10-positions of **12** to afford the tricyclic analogues **5**−**11** are probably not responsible for the decrease in potency and lack of selectivity and lends further credence to the idea that the inappropriate orientation of the side chain trimethoxyphenyl ring in **5**−**11** is responsible for the lack of potency and selectivity of these analogues against pcDHFR and tgDHFR.

Experimental Section

Starting materials used in synthetic procedures were obtained from Aldrich Chemical Co., Milwaukee, WI. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Catalytic hydrogenation reactions were performed in a Parr pressure reaction apparatus. Infrared (IR) spectra were determined neat or as Nujol mulls on a Perkin-Elmer 1430 ratio recording infrared spectrophotometer and are reported in reciprocal centimeters. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-360 (60 MHz) or a Bruker WH-300 (300 MHz) spectrometer. Only 60 MHz spectra are indicated in the experimental text, and all others unspecified (^{1}H NMR) are 300 MHz. The ^{13}C NMR spectra were obtained on a Bruker WH-300 instrument at 75.46 MHz; chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard; s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra (MS) were recorded on a Varian MATCH-311 mass spectrometer in a fast-atom-bombardment (FAB) mode or as indicated. Thin layer chromatography (TLC) was performed on Eastman Kodak chromatogram sheets (silica gel) with florescent indicator. Proportions of solvents stated and used for TLC are by volume. Spots on TLC were detected by UV light at either 254 or 350 nm. Elemental analyses were carried out by Atlantic Microlab, Inc., Necross, GA, and/or Galbraith Laboratories, Knoxville, TN. Element compositions are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be removed in spite of drying in vacuo and were confirmed by their presence in the ^{1}H NMR spectrum.

Dimethyl 3′,4′,5′-Trimethoxyaniline-*β*,*β*′-dipropionate (13). A mixture of 3,4,5-trimethoxylaniline (10.90 g, 59.60 mmol), methyl acrylate (25.80 g, 300 mmol), and glacial HOAc (15 mL) was heated under reflux with stirring for 24 h. Excess methyl acrylate and HOAc were removed under reduced pressure (H_2O aspirator, bath temperature 70 °C). To the residue was added CH_2Cl_2:hexane (1:1) (500 mL). The solution was filtered through a funnel column (3.5 in. in diameter) containing 80 g of silica gel. The filtrate was concentrated to a viscous oil, diluted with ethyl ether (200 mL), and kept at room temperature for 2 h and then in a freezer overnight. Compound **13** was collected as white crystals (10.00 g, 47%) which separated (mp 77−78 °C). The crystals were washed thoroughly with ethyl ether: TLC R_f 0.57 ($CHCl_3$:Et_2O 9:2); ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.55 (t, 4 H, 2 CH_2COO), 3.47−3.80 (m, 19 H, 3 CH_3O, 2 $COOCH_3$ and 2 CH_2N), 5.95 (s, 2 H, Ar-H); IR (Nujol) 1725, 1250 (COO); MS(CI) *m*/*z* 356 (MH^+). Anal. ($C_{17}H_{25}NO_7$) C, H, N.

Methyl 4-Oxo-1-(3′,4′,5′-trimethoxyphenyl)piperidine-3-carboxylate (14). A solution of **13** (10.00 g, 28 mmol) in dry toluene (30 mL) was added to a suspension of $NaOCH_3$ (3.00 g, 56 mmol) in toluene (70 mL) under gentle reflux which was continued for 1 h. The reaction mixture was cooled in an ice bath, and the solid was collected by filtration, washed thoroughly with ethyl ether, and air-dried for 30 min. The solid was suspended in ice−H_2O (100 mL) and neutralized with 6 N HCl. Compound **14** was obtained on filtration as a white precipitate (8.20 g, 90% yield): TLC R_f 0.5 ($CHCl_3$:Et_2O, 9:2); mp 105−106 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.65 (m, 2 H, CH_2CO), 3.48 (t, 2 H, NCH_2), 3.78−3.92 (m, 15 H, 3 CH_3O and $COOCH_3$ and NCH_2CH-), 4.28 (t, 1 H, CHCOO), 6.32 (s, 2 H, Ar-H); IR (Nujol) 1670, 1630 cm^{-1}(*β*-keto ester); CI MS *m*/*z* 324, MH^+. Anal. ($C_{16}H_{21}NO_6$) C, H, N.

1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,7]naphthyridin-6-(5*H*)-one (5). A mixture of 2,4,6-triaminopyrimidine (7.50 g, 60.0 mmol) and keto ester **14** (20.00 g, 61.70 mmol) in glacial HOAc (400 mL) was stirred under gentle reflux at 90−95 °C for 36 h. After the mixture was cooled to room temperature, the precipitated acetate salt (7.30 g containing unreacted triaminopyrimidine) was collected, dissolved in hot H_2O, and left at room temperature for 12 h. The solid which separated was collected by filtration and recrystallized from MeOH. Compound **5** was obtained as pale yellow crystals (3.00 g, 12.5% yield), which were homogeneous on TLC [R_f 0.34 (EtOAc:MeOH, 4:1)]: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.05 (s, 2 H), 3.15 (t, 2 H), 3.62 and 3.73 (2 s, 9 H, 3CH_3O), 3.90 (t, 2 H), 5.51 (br s, 2 H, NH_2), 5.92 (br s, 2 H, NH_2), 6.58 (s, 2 H, ArH), 8.68 (br s, 1 H, NH); ^{13}C NMR (decoupled) showed 17 singlets (2 pairs of symmetric carbons of phenyl 2′ and 6′, and 3′ and 5′ were overlapped), 165.59 ppm (C═O); CI MS *m*/*z* 399 (MH^+). Anal. ($C_{19}H_{22}N_6O_4$·0.8H_2O) C, H, N.

1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)-9,10-dihydropyrimido[4,5-*c*][2,7]naphthyridin-6(5*H*,8*H*)-one (6). Method A. In a flask equipped with a Dean−Stark trap, a mixture of the keto ester **14** (4.85 g, 15 mmol), 2,4,6-triaminopyrimidine (1.88 g, 15 mmol), and diphenyl ether (50 mL) was heated rapidly with vigorous stirring to 195 °C and maintained at 195−200 °C until no additional water−ethanol mixture distilled (1 h). The reaction mixture was cooled to room temperature, methanol (40 mL) was added, and the solid that precipitated (2.10 g) was collected by filtration. The crude product was suspended in boiling H_2O (100 mL) and filtered, and the residue was washed with hot water, followed by methanol (40 mL) and ether (50 mL), to afford a brown solid. Recrystallization from methanol and concentrated HCl afforded **6** as its hydrochloride salt (0.74 g, 12% yield): mp >300 °C (295 °C dec); TLC R_f 0.17 (EtOAc:MeOH, 4:1); ^{1}H NMR (DMSO-d_6) δ 3.27 (t, 2 H, C$H_2$$CH_2$N), 3.67 (s, 3 H, 4′-$OCH_3$), 3.76 (s, 6 H, 3′,5′-$OCH_3$), 4.03 (t, 2 H, CH_2), 6.75 (s, 2 H, Ar-H), 7.49−7.65 (br peak, 2 H, NH_2), 8.63 (br s, 2H, NH_2), 9.09 (s, 1 H, C_7H); MS *m*/*z* 397 (MH^+). Anal. ($C_{19}H_{20}N_6O_4$·1.0HCl·1.0H_2O) C, H, N.

Method B. This dehydro lactam **6** was also obtained by gently heating **5** at 80 °C in HMPT (also in solvents or reagents such as DMSO, $POCl_3$, or glacial HOAc) for 12 h. Workup as described above afforded a pure product in 50% yield without additional purification, which was identical in all respects to **6** obtained by method A.

1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)pyrimido[4,5-*c*][2,7]naphthyridin-6-one (7). To a solution of the dehydro lactam **6** (0.10 g, 0.25 mmol) in 75% HOAc (50 mL) was added PtO_2 (0.016 g). The reaction mixture was stirred and refluxed for 7 h. After the mixture was cooled to room temperature, the catalyst was filtered and the filtrate evaporated to dryness. The residue was dissolved in MeOH (50 mL) and clarified by filtration through Celite. The filtrate was concentrated to 5

mL and left in the freezer overnight. The dark brown solid which formed was collected. The solid was then dissolved in TFA and carefully filtered through Celite and the filtrate evaporated. To the residue was added MeOH and the solution kept in a freezer overnight. The dark-red solid which separated was collected by filtration to afford 0.07 g (66% yield) of **7**: mp >300 °C; ^{1}H NMR (DMSO-d_6-D_2O) δ 3.71 (s, 3 H, 4′-OCH_3), 3.77 (s, 6 H, 3′,5′-OCH_3), 6.83 (s, 2 H, Ar-2′,6′), 6.53 (d, 1H, H9, J = 6.7 Hz), 7.72 (d, 1H, H10, J = 6.7 Hz), 9.32 (s, 1H, H7); MS m/z 395 (MH^+). Anal. ($C_{19}H_{18}O_4N_6 \cdot 0.8CF_3COOH \cdot 1.6H_2O$) C, H, N.

1,3-Diamino-8-(3′,4′,5′-trimethoxy-2′,6′-dichlorophenyl)-pyrimido[4,5-*c*][2,7]naphthyridin-6-one (8). To a solution of PCl_5 (4.00 g) and $POCl_3$ (40 mL) was added the lactam **5** (1.00 g, 2.51 mmol), and the reaction mixture was stirred and heated at 115−120 °C for 3.5 h. Excess $POCl_3$ was removed under reduced pressure (H_2O aspirator, bath temperature 65 °C). The residue was added in portions to crushed ice and neutralized carefully with MeOH−1 N NaOH (1:1). After the mixture was stirred at room temperature for 24 h, a yellow solid was obtained. The solid was collected by filtration and suspended in H_2O, stirred for an additional 24 h, and adjusted to pH 3−4 with 6 N HCl. The solid obtained was collected by filtration and dissolved in a minimum amount of AcOH, concentrated to dryness, and triturated with MeOH:EtOAc (1:5) . The solution was kept in a freezer overnight. Compound **8** separated as a brown solid (0.45 g, 36% yield) and was collected as the HCl salt: mp > 300 °C; TLC R_f 0.5 (MeOH:EtOAc, 1:9, with a few drops of NH_4OH); ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3 H, 4′-OCH_3), 3.80 (s, 6 H, 3′,5′-OCH_3), 7.06 (s, 1 H, 9- or 10-H), 8.05 (s, 1 H, 9- or 10-H), 9.35 (s, 1 H, 7H); MS m/z 463 (MH^+). Anal. ($C_{19}H_{16}N_6O_4Cl_2 \cdot 1.7HCl \cdot 0.5H_2O \cdot 0.3CH_3COOH$) C, H, N.

1,3-Bis(pivaloylamino)-8-(3′,4′,5-trimethoxyphenyl)-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,7]naphthyridin-6-(5H)-one (**15**). A mixture of **5** (1.00 g, 2.51 mmol) and pivaloyl anhydride (9.18 g, 52.7 mmole) was refluxed with stirring under nitrogen for 2 h (bath temperature 195−210 °C). The reaction mixture was then cooled to 100 °C and rapidly filtered through Celite. To the filtrate was added ethyl ether (100 mL), and the mixture was placed in a freezer overnight. Compound **15** was obtained as a white solid (1.20 g, 84%), which was collected by filtration. An analytical sample was crystallized from a mixture of methanol and ether: mp 188−190 °C; TLC R_f 0.3 (EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.18 (2 s, 18 H, 2 $(CH_3)_3C$), 2.59 (t, 2 H, CH_2CH_2N), 3.27 (s, 2 H, CH_2), 3.66 (s, 3 H, 4′-OCH_3), 3.73 (s, 6 H, 3′,5′-OCH_3), 3.75 (t, 2 H, CH_2CH_2N, overlapped with peak at 3.73 ppm), 6.56 (s, 2 H, Ar-H), 9.68 (s, 1 H, NH), 9.79 (s, 1 H, NH), 9.89 (s, 1 H, NH); MS m/z 567 (MH^+). Anal. ($C_{29}H_{38}N_6O_6 \cdot 0.5H_2O$) C, H, N.

1,3-Bis(pivaloylamino)-8-(3′,4′,5′-trimethoxyphenyl)-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,7]naphthyridine-6-(5*H*)-thione (16). A mixture of the pivaloylated lactam **15** (0.50 g, 0.88 mmol), Lawesson's reagent (0.40 g, 0.1 mmol) and 50 mL benzene in a two-necked flask equipped with a Dean−Stark trap and a nitrogen inlet was heated slowly with stirring to 85 °C and maintained at 85−90 °C for 1.5 h. During this period, about 40 mL of benzene was distilled. The reaction mixture was diluted with ethyl acetate, transferred to a separatory funnel, and washed with H_2O (50 mL × 4). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The solid residue obtained was loaded onto a column (50 g silica gel) and eluted with ethyl acetate. The fractions with a R_f 0.61 (silica gel, EtOAc) was a side product (dithiated compound). The desired fractions with TLC R_f 0.48 (EtOAc) were combined and concentrated to dryness. The yellow solid (0.28 g) obtained was washed with ethyl ether. Further purification was carried out by first dissolving it in MeOH, followed by evaporation of MeOH. To the residue was slowly added 20 mL of ethyl ether to afford **16** (0.15 g 29% yield) as a crystalline solid: TLC R_f 0.48 (EtOAc); mp 249−251 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (2s, 18 H, 2 $C(CH_3)_3$), 2.70 (t, 2 H, CH_2CH_2, J = 7.0 Hz), 3.57 (s, 2 H, CH_2), 3.65 (s, 3 H, OCH_3) 3.72 (s, 6 H, 3′,5′-OCH_3), 3.78 (t, 2 H, CH_2CH_2, J = 7.0 Hz), 6.59 (s, 2 H, Ar-H), 9.72 (s, 1 H, NH), 9.82 (s, 1 H, NH), 10.12 (s, 1 H, NH); MS m/z 583 (MH^+). Anal. ($C_{29}H_{38}N_6O_5S \cdot 0.5H_2O$) C, H, N, S.

1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)-7,8,9,10-tetrahydro-2*H*-pyrimido[4,5-*c*][2,7]naphthyridine (9). Method A. A mixture of compound **16** (0.50 g, 0.86 mmol) and anhydrous Raney Ni (3 g) were suspended in anhydrous EtOH (20 mL) and slowly heated to reflux for 1 h. The reaction mixture was cooled, the insoluble material filtered, and the filtrate evaporated to dryness. The TLC ($CHCl_3$:CH_3OH, 9:2, with 3 drops of NH_4OH) of the residue showed three products: a mixture of **9** and monopivoylated and dipivoylated derivatives which precipitated on addition of ether. The crude material (0.45 g) was collected by filtration and used for the next step without further purification.

To a solution of the above mixture (0.125 g) in acetone (15 mL) was added 1 N KOH (10 mL), and the reaction mixture was bubbled with nitrogen for 5 min, stoppered tightly, and stirred at room temperature for 10 days. The reaction mixture was then concentrated to half its volume and cooled in a freezer for about 8−10 min, and the resulting solid (0.025 g) was collected. The solid was dissolved in a minimum amount of glacial HOAc and diluted with ethanol to give **9** (0.020 g, 20% yield) as an acetate salt: TLC R_f 0.31 ($CHCl_3$:CH_3OH 9:2, with 3 drops of NH_4OH); mp >300 °C (275 °C dec); ^{1}H NMR(DMSO-d_6) δ 1.89 (s, 6 H, $2CH_3COOH$), 3.01 (t, 2 H, CH_2CH_2N), 3.57 (t, 2 H, CH_2CH_2N, overlapped with singlet at 3.56 ppm), 3.56 (s, 3 H, 4′-OCH_3), 3.77 (s, 6 H, 2-OCH_3), 4.37(s, 2 H, CH_2), 6.29 (s, 2 H, Ar-H), 6.49 (br s, 2 H, NH_2), 7.62 (br s, 2 H, NH_2, exchangeable), 8.25 (s, 1 H, C_6H); ^{1}H NMR (TFA-*d*) δ 3.84 (t, 2 H, CH_2CH_2N), 4.02 and 4.04 (2 s, 9 H, 3′-4′-5′-OCH_3), 4.40 (t, 2 H, CH_2CH_2N), 5.15 (s, 2 H, CH_2), 7.02 (s, 2 H, Ar-H) 8.83 (s, 1 H, C_6H); MS m/z 383 (MH^+). Anal. ($C_{19}H_{22}N_6O_3 \cdot 2CH_3COOH$) C, H, N.

Method B. Borane in THF (1 M, 25 mL) was added to a suspension of the lactam **5** (2.0 g, 5.01 mmol) in anhydrous THF (25 mL) under nitrogen. The reaction mixture was stirred at room temperature for 18 h, cooled in an ice bath, and adjusted to pH 2 by adding 6 N HCl dropwise. The mixture was evaporated, water (25 mL) was added, and the pH was adjusted to 7−8 with 1 N NaOH. The precipitate which formed (0.2 g) was filtered and the filtrate extracted with $CHCl_3$ (50 mL × 3). The organic extracts were combined, dried ($MgSO_4$), and concentrated to 3−5 mL. The resulting solution was kept in a freezer overnight to afford a solid (0.45 g, 23%), which separated and was collected by filtration. The TLC of this solid showed two spots, R_f 0.31 and 0.40 ($CHCl_3$:MeOH, 9:2), with 3 drops of NH_4OH). The solid was suspended in MeOH (10 mL), and glacial HOAc was added dropwise to the suspension until the solid just dissolved. The solution was stirred at room temperature (overnight) until the TLC showed a single spot. Compound **9** (0.35 g, 18% yield) was then collected by filtration and was identical in all respect to that obtained from method A.

1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)-7,8-dihydropyrimido[4,5-*c*][2,7]naphthyridine (10). Compound **16** (0.50 g, 0.86 mmol) and anhydrous Raney Ni (3.0 g) were suspended in absolute ethanol (20 mL) and gently heated to reflux for 1 h. The reaction mixture was cooled to room temperature, the Raney Ni was filtered, and the filtrate was evaporated to dryness. The TLC ($CHCl_3$:MeOH, 9:2) of this residue showed three components. The mixture was directly used for depivoylation without further purification. A solution of the mixture (0.45 g) in CH_2Cl_2 was placed in a steel vessel and NH_3 gas allowed to bubbled through at −78 °C for 10 min. The reaction mixture was sealed and stirred for 72 h at room temperature, after which the solution was allowed to evaporate at room temperature overnight in a hood. MeOH was added to the solid residue, and the solution formed was stirred for 7 days at room temperature. The resulting solid (0.20 g, 61% yield) was collected: MS m/z 381 (contaminated with about 20% m/z 383 according to the height of the peak in FAB MS), MH^+ for $C_{19}H_{20}O_3N_6$. An analytical sample was prepared by dissolving the solid in TFA followed by filtration through Celite and evaporation to dryness. To the residue was added MeOH, and the insoluble material was removed by filtration. The

filtrate was kept in a freezer overnight to afford 0.075 g, 22% yield, of **10** after filtration: TLC of this product showed a baseline-tailing spot due to its poor solubility in EtOAc:MeOH (4:1) or $CHCl_3$:MeOH (9:2) with 3 drops of NH_4OH: ^1H NMR (DMSO-d_6) δ 3.70 (s, 3 H, 4′-OCH_3), 3.77 (s, 6 H, 3′,5′-OCH_3), 4.05 (s, 2 H, CH_2), 6.70 (br s, 2 H NH_2), 6.75 (s, 2 H, ArH, overlapped with 6.70), 6.94 (d, 1 H, H-10), 7.70 (very broad peak, 2 H, NH_2), 7.90 (m, 1 H, H-9), 9.30 (s, 1 H, C7-H). Anal. ($C_{19}H_{20}N_6O_3 \cdot CF_3COOH \cdot 1.5CH_3OH$). C, H, N.

***cis,trans*-1,3-Diamino-8-(3′,4′,5′-trimethoxyphenyl)-5,6,-6a,7,8,9,10,10a-octahydropyrimido[4,5-*c*][2,7]-naphthyridine (11)**. To a solution of **9** (0.10 g, 0.26 mmole) in CF_3COOH/MeOH (1:1, 40 mL) was added platinum oxide (0.05 g). The suspension was hydrogenated in a Parr apparatus (50 psi) for 8 h at room temperature. To avoid air oxidation back to the starting material **9**, activated charcoal was added following hydrogenation and the workup performed under an atmosphere of nitrogen. The reaction mixture was rapidly filtered through Celite and the filtrate evaporated under reduced pressure (bath temperature 30 °C). The residue obtained was suspended in MeOH:acetone (1:1, 10 mL) and then kept in a freezer overnight. The solid (0.02 g) obtained was filtered (TLC of which indicated it to be the starting material **9**, and the filtrate was evaporated to dryness. To this was added acetone (4 mL), and the solution was kept in a freezer for 4 h. Compound **11** separated as a solid which was collected (0.035 g, 35% yield): mp 250 °C dec; TLC R_f 0.40 ($CHCl_3$:MeOH, 9:2, with 3 drops of NH_4OH); ^1H NMR (DMSO-d_6) δ 1.91 (m, 1H), 2.25 (br s, 1 H), 2.40 (m, 1 H), 2.62 (m, 1 H), 2.96 (s, overlapped with a multiplet, 2 H), 3.35 (br s, MeOH as solvent overlapped with a multiplet, 1 H), 3.45 (s, 1 H), 3.55 (s, 3 H, OCH_3), 3.80 (s, overlapped with multiplet (m, 8 H); MS *m*/*z* 387 (MH^+). Anal. ($C_{19}H_{26}N_6O_3 \cdot 1.2CF_3COOH \cdot 2.85CH_3OH$) C, H, N.

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