# Inhibition of Human Dihydrofolate Reductase by 2,4-Diaminoquinazolines Bearing Simple Substituents on the Aromatic Ring

John B. Hynes*, Alenka Tomažič, Arvind Kumar and Veena Kumar

Department of Pharmaceutical Sciences,
Medical University of South Carolina,
Charleston, SC 29425 USA

James H. Freisheim

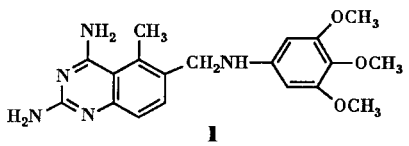
Department of Biochemistry and Molecular Biology,
Medical College of Ohio,
Toledo, OH 43699 USA

Received September 23, 1991

A series of thirty eight 2,4-diaminoquinazolines having diverse substitution patterns on the aromatic ring was evaluated for inhibitory activity against dihydrofolate reductase (DHFR) obtained from a human lymphoblast cell line. Many of these compounds were also evaluated as inhibitors of rat liver DHFR under the same experimental conditions. In most instances the results obtained with each enzyme were comparable indicating that the rodent enzyme is a suitable model for the human DHFR as far as the determination of $I_{50}$ values is concerned. The results demonstrate that relatively simple 5-substituted- or 5,6-disubstituted-2,4-diaminoquinazolines can be potent DHFR inhibitors. The presence of a nonpolar substituent at position 7 or 8 was highly detrimental to inhibitory potency.

*J. Heterocyclic Chem.*, **28**, 1981 (1991).

Nonclassical 2,4-diaminoquinazoline analogues of folic acid have been of interest as potential chemotherapeutic agents for over two decades. For example, it was shown that 2,4-diaminoquinazolines bearing simple substituents on the aromatic possessed interesting levels of antibacterial activity [1,2]. It was suggested that for a given substituent, the antibacterial potency followed the order of 5 >6 >7 [1,2]. A plethora of 2,4-diaminoquinazolines having large hydrophobic substituents attached through a suitable spacer at position six have been synthesized. Many of these possessed remarkable antimalarial effects [3,4]. In some instances the presence of a small nonpolar substituent such as chlorine or methyl at position five caused a substantial enhancement of antimalarial activity [3-7]. The drug trimetrexate, **1**, which arose from these synthetic efforts, was found to display potent antimalarial, antibacterial and antitumor activities and was subsequently introduced into clinical trials as an anticancer agent [7-9]. Compound **1** was reported to have a similar level of inhibitory potency toward L1210 dihydrofolate reductase (DHFR) as methotrexate, the standard of comparison for potent inhibitors of this enzyme [8]. More recently, compound **1** has also been undergoing clinical trials for the treatment of




**1**

*Pneumocystis carinii* infections in AIDS patients, being employed in combination with the rescue agent calcium leucovorin [10-12]. The latter drug is required in the therapeutic regimen because **1** is actually a better inhibitor of mammalian DHFR than the DHFR from *Pneumocystis carinii* [11].

Interest in 2,4-diaminoquinazolines as potential antimycobacterial agents was initiated by the work of DeGraw and coworkers who synthesized a series of 6-alkyl-2,4-diamino-5-methylquinazolines [13]. These compounds were found to be moderate to potent inhibitors of the growth of *Mycobacterium sp.* 607 with 2,4-diamino-6-(*n*-propyl)-5-methylquinazoline, **31**, being the most effective of the analogues studied [13]. When **31** was used in combination with 4,4'-diaminodiphenylsulfone, dapsone, marked drug synergy was observed against *M. sp.* 607 indicating that DHFR was its enzymatic target. Later, compounds of this type as well as structurally related 5-deazapteridines were shown to be potent inhibitors of the DHFR isolated from *M. sp.* 607 [14]. Gelber and Levy tested a series of these 2,4-diaminoquinazolines against *M. leprae* growing in the mouse foot pad [15]. Several analogues produced bacteriostatic effects, but growth of *M. leprae* resumed upon removal of drug from the diet. Compound **31** and the 5,6-dimethyl derivative, **30**, were also evaluated in combination with dapsone. In each case the antibacterial activity was enhanced as compared to that produced by dapsone alone. In spite of the interesting activity shown by quinazolines of this type in this cumbersome and protracted evaluation system, none of these compounds was introduced into clinical trials for the treatment of leprosy. It should be noted that other promising inhibitors of DHFR have not shown impressive activity in the mouse foot pad test system [16].

In this laboratory, we have been involved in the search

for new DHFR inhibitors as potential antileprotic agents and more recently for the treatment of *M. avium intracellulare* infections in AIDS patients. During the course of these efforts a wide variety of 2,4-diaminoquinazolines have been evaluated as inhibitors of rat liver DHFR and more recently the human enzyme obtained from WIL2 fibroblasts. This paper summarizes our findings using the DHFRs from both sources. It is believed that this data will be of value in predicting the potential host toxicity of newly synthesized compounds of this type. In addition, the results may be of value in the design of DHFR inhibitors having enhanced selectivity for the DHFRs of opportunistic microorganisms.

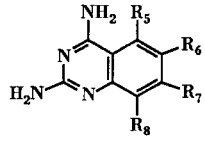
A total of thirty-eight 2,4-diaminoquinazolines were employed in this study. Of these, eleven compounds were prepared for the first time and their physical properties are summarized in Table 1. The key intermediates, 5-methyl-2,4,6-triaminoquinazoline, **33**, and 5-methyl-2,4,8-triaminoquinazoline, **35**, were prepared by the literature methods [17]. The 6-chloro, 6-bromo, 8-chloro and 8-bromo analogues, **2**, **3**, **5** and **6**, were obtained from the requisite amines under Sandmeyer conditions. The diazotization of **33** or **35** followed by treatment with potassium iodide afforded 2,4-diamino-6-iodo-5-methylquinazoline, **4**, and 2,4-diamino-8-iodo-5-methylquinazoline, **7**, respectively. The 2,4-diamino-6-iodoquinazoline, **8**, and 5-chloro-2,4-diamino-6-iodoquinazoline, **9**, were obtained in an analogous fashion from 2,4,6-triaminoquinazoline [17] and 5-chloro-2,4,6-triaminoquinazoline [17]. The 2,4-diaminoquinazolines bearing a trifluoromethyl group at position six (**10**), seven (**11**), or eight (**12**) were obtained using the guanidine carbonate cyclization of the requisite 2-fluorobenzonitrile [18].

The inhibition results obtained against human and rat liver DHFRs are presented in Table 2. All thirty-eight compounds were evaluated against the human tumor enzyme, while many were also studied using the rat liver enzyme using the same enzyme concentration as determined by methotrexate titration. The results obtained with the antiprotozoan agent, pyrimethamine, are also included for reference purposes. The values in parentheses are $I_{50}$ values from an earlier study conducted in this laboratory which also employed rat liver DHFR [19].

It will be noted that in nearly every instance there is good correlation between the $I_{50}$ values for the two enzymes. This confirms the earlier assumption that the rodent enzyme was an acceptable model for the human enzyme as far as drug screening is concerned. With the ex-

Table 1

Physical and Analytical Data for 2,4-Diaminoquinazolines Synthesized

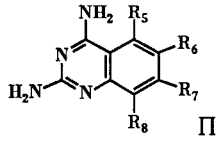



| Compound No. | $R_5$ | $R_6$ | $R_7$ | $R_8$ | Method | Yield % | MP °C | MS m/e [a] | Empirical Formula | Analyses %, Calcd./Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2** | $CH_3$ | Cl | | | A | 78 | 281-284 | 208 | $C_9H_9ClN_4$ | 51.81 | 4.35 | 26.85 |
| | | | | | | | | | | 51.78 | 4.39 | 26.73 |
| **3** | $CH_3$ | Br | | | B | 75 | 303-305 dec | 253 | $C_9H_9BrN_4$ | 42.70 | 3.58 | 22.13 |
| | | | | | | | | | | 42.90 | 3.66 | 22.03 |
| **4** | $CH_3$ | I | | | C | 70 | 291-293 dec | 300 | $C_9H_9IN_4$ | 36.01 | 3.02 | 18.67 |
| | | | | | | | | | | 36.22 | 3.22 | 18.47 |
| **5** | $CH_3$ | | | Cl | A | 45 | 260-262 | 208 | $C_9H_9ClN_4$ | 51.81 | 4.35 | 26.85 |
| | | | | | | | | | | 51.93 | 4.37 | 26.72 |
| **6** | $CH_3$ | | | Br | B | 35 | 275-276 | 253 | $C_9H_9BrN_4$ | 42.70 | 3.58 | 22.13 |
| | | | | | | | | | | 42.75 | 3.69 | 21.93 |
| **7** | $CH_3$ | | | I | C | 80 | 226-230 dec | 300 | $C_9H_9IN_4$ | 36.01 | 3.02 | 18.67 |
| | | | | | | | | | | 36.40 | 3.16 | 18.70 |
| **8** | | I | | | C | 38 | 250-255 | 286 | $C_8H_7IN_4$ | 33.59 | 2.47 | 19.57 |
| | | | | | | | | | | 33.86 | 2.67 | 19.32 |
| **9** | Cl | I | | | C | 43 | 226-228 | 320 | $C_8H_6ClIN_4$ | 29.97 | 1.88 | 17.48 |
| | | | | | | | | | | 29.81 | 2.00 | 17.22 |
| **10** | | $CF_3$ | | | D | 81 | 234-236 | 228 | $C_9H_7F_3N_4$ | 47.37 | 3.09 | 24.55 |
| | | | | | | | | | | 47.29 | 3.07 | 24.49 |
| **11** | | | $CF_3$ | | D | 74 | 244-246 | 228 | $C_9H_7F_3N_4$ | 47.37 | 3.09 | 24.55 |
| | | | | | | | | | | 47.46 | 3.11 | 24.46 |
| **12** | | | | $CF_3$ | D | 73 | 195-197 | 228 | $C_9H_7F_3N_4$ | 47.37 | 3.09 | 24.55 |
| | | | | | | | | | | 47.31 | 3.10 | 24.47 |

[a] Electron impact, off probe.

Table 2

Inhibition of Human and Rat Liver Dihydrofolate Reductases by 2,4-Diaminoquinazolines Bearing Simple Aromatic Substitutents



II

| Compound No. | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $I_{50}$, μM | |
|---|---|---|---|---|---|---|
| | | | | | Rat Liver [a] | WIL2 cells |
| **13** | $CH_3$ | | | | (0.82) | 0.63 |
| **14** | F | | | | 0.44 | 0.35 |
| **15** | Cl | | | | 0.65 (0.56) | 0.57 |
| **16** | Br | | | | | 1.5 |
| **17** | I | | | | | 1.1 |
| **18** | $CF_3$ | | | | | 2.1 |
| **19** | $OCH_3$ | | | | 0.33 | 0.31 |
| **20** | $OCH_2CH_3$ | | | | | 0.069 |
| **21** | $OCH_2CF_3$ | | | | | 0.027 |
| **22** | $SCH_3$ | | | | | 1.2 |
| **23** | $SCH_2CH_3$ | | | | | 0.38 |
| **24** | $N(CH_3)_2$ | | | | | 11.0 |
| **25** | | F | | | | 96.0 |
| **26** | | Cl | | | (4.0) | 1.5 |
| **27** | | Br | | | (2.5) | 3.2 |
| **8** | | I | | | 0.84 | 2.0 |
| **10** | | $CF_3$ | | | | 3.2 |
| **28** | Cl | Cl | | | 0.33 (0.20) | 0.093 |
| **29** | Cl | Br | | | 0.16 (0.10) | 0.14 |
| **9** | Cl | I | | | 0.082 | 0.048 |
| **2** | $CH_3$ | Cl | | | 0.21 | 0.15 |
| **3** | $CH_3$ | Br | | | 0.11 | 0.13 |
| **4** | $CH_3$ | I | | | 0.037 | 0.043 |
| **30** | $CH_3$ | $CH_3$ | | | 0.54 | 0.36 |
| **31** | $CH_3$ | $n$-$C_3H_7$ | | | 0.0079 | 0.0067 |
| **32** | $CH_3$ | $NO_2$ | | | 0.56 | 1.08 |
| **33** | $CH_3$ | $NH_2$ | | | 2.9 | 1.4 |
| **34** | F | $NH_2$ | | | | 9.4 |
| **5** | $CH_3$ | | | Cl | 22.0 | 46.0 |
| **6** | $CH_3$ | | | Br | 17.0 | 30.0 |
| **7** | $CH_3$ | | | I | 19.0 | 23.0 |
| **35** | $CH_3$ | | | $NH_2$ | 0.74 | 1.8 |
| **36** | F | | | $NH_2$ | | 17.0 |
| **37** | | | F | | | 81.0 |
| **11** | | | $CF_3$ | | | >100.0 [b] |
| **38** | | | | F | | 146.0 |
| **12** | | | | $CF_3$ | | >100.0 [b] |
| **39** | F | F | F | F | | 112.0 |
| Pyrimethamine | | | | | 0.083 (0.07) | 0.18 |

[a] Values in parentheses were reported earlier in Ref [19]. [b] Compound absorbs at 340 mμ preventing the determination of an $I_{50}$ value.

ception of the 5-dimethylamino analogue, **24**, each of the 5-substituted compounds is a moderate to good inhibitor of the human enzyme with the 2,2,2-trifluoroethoxy compound, **21**, being the most effective inhibitor of this set. In general, these results are consistent with the earlier suggestion that there is a hydrophobic binding region adjacent to position five of 2,4-diaminoquinazolines [19]. 2,4-Diaminoquinazolines having small nonpolar substituents located at position six are less potent or have similar levels of activity as their 5-position isomers. However, the 6-fluoro derivative, **25**, is 274-fold less inhibitory than 2,4-diamino-5-fluoroquinazoline, **14**.

The addition of a small hydrophobic group at position 6 of 2,4-diamino-5-chloroquinazoline, **15**, or 2,4-diamino-5-methylquinazoline, **13**, enhances binding to the human enzyme with iodo showing the greatest effect of the halogens. However, the 5-methyl-6-*n*-propyl derivative, **31**, is the most potent of the compounds studied being only about 2-fold less potent than methotrexate. The presence of nitro, **32**, or amino, **33** and **34**, at position six decreases inhibitory potency modestly in the case of 5-methyl derivatives but more dramatically in the case of the 5-fluoro derivative, **34**.

For the three 5-methylquinazolines bearing a halogen at position eight (**5–7**), the inhibitory potencies are decreased markedly in comparison to the parent compound **13**. However, 2,4,8-triamino-5-methylquinazoline, **35**, is only 3-fold less potent than 2,4-diamino-5-methylquinazoline, **13**, suggesting that the region adjacent to position eight is polar in nature. However, 5-fluoro-2,4,8-triaminoquinazoline, **36**, is approximately 50-fold less effective than 2,4-diamino-5-fluoroquinazoline, **14**, implying that electronic factors may also be involved. Finally, monosubstituted 2,4-diaminoquinazolines containing fluorine or trifluoromethyl at positions seven or eight, **37**, **38**, **11** and **12**, are all extremely weak inhibitors of human DHFR.

Based upon the results presented in Table 2, three of the best inhibitors of human DHFR were evaluated for potential antitumor activity against the growth of L1210 leukemia cells *in vitro*. The $IC_{50}$ ($\mu M$) values obtained were as follows: **4** (0.10), **9** (0.30), **21** (0.011). The $IC_{50}$ value for compound **1** under the same conditions was 0.0044 $\mu M$ and that for methotrexate was 0.0047 $\mu M$. Thus, the cytotoxicity of 2,4-diamino-5-(2,2,2-trifluoroethoxy)quinazoline, **21**, approaches that of trimetrexate as well as methotrexate suggesting that it should be evaluated in animal models of cancer.

## EXPERIMENTAL

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. or Atlantic Microlab, Inc., Norcross, GA. Analytical samples were dried under vacuum at 100°. The $^1H$ and $^{19}F$ nmr spectra were determined by using a Varian EM 390 spectrometer. The $^1H$ chemical shifts are presented in ppm downfield from tetramethylsilane as the internal standard. The $^{19}F$ chemical shifts are presented in ppm upfield from fluorotrichloromethane as the internal standard. Relative peak areas are given to the nearest whole number. The electron impact mass spectra were obtained off probe using a Finnigan 4521 mass spectrometer at the Department of Chemistry, University of South Carolina, Columbia, SC.

DHFR activity was assayed spectrophotometrically at 340 nm using homogeneous enzyme obtained from human WIL2 lymphoblasts as described earlier [20]. The assay was performed at 22° after a preincubation time of 2 minutes. The assay mixture contained DHFR (0.0076 $\mu M$), dihydrofolate (9 $\mu M$), NADPH (30 $\mu M$), and potassium chloride (0.15 $M$) in 0.05 $M$ Tris buffer (*p*H 7.4) in a final volume of 1 ml. The final concentration of DHFR was determined by methotrexate titration. The methotrexate was a gift from Dr. Suresh Kerwar, Lederle Laboratories, Pearl River, NY. Partially purified DHFR from rat liver was obtained as described previously [21]. It was assayed in an identical manner as described for the human enzyme with the enzyme activity being adjusted by dilution with buffer as determined by titration with methotrexate. An *in vitro* colorimetric assay was used to measure the cytoxicity of selected compouds toward L1210 cells [22].

Compounds **14**, **15**, **16**, **17**, **19**, **20**, **21**, **22**, **24**, **25**, **37**, **38**, and **39** were available for this study by virtue of recent synthetic efforts emanating from this laboratory [18]. The syntheses of **26**, **27**, **28**, and **29**, were described earlier [19]. Compound **23** was prepared in essentially the same manner as recently reported [23]. Compounds **30** and **31** were obtained as generous gifts from Dr. William T. Colwell, Stanford Research Institute, Palo Alto, CA. The preparation of **32**, **33**, and **35** have been described earlier [17]. The chemistry of **10** [24] and **34** [25] have recently been reported from this laboratory. The synthesis of compound **36** will be presented in a forthcoming communication [26].

2,4-Diamino-5-methylquinazoline (**13**).

An improved method for preparing **13** was developed. A mixture of 6-amino-*o*-toluonitrile [27], (12.0 g, 0.075 mole) and chloroformamidine hydrochloride (9.2 g, 0.080 mole) in 35 ml of diethyleneglycol dimethyl ether was heated with stirring at *ca.* 130° for 15 minutes, 140° for 20 minutes and then 150° for 1 hour. The reaction mixture was diluted with 100 ml of diethyl ether and the resulting solid was separated by filtration. The crude product was dissolved in boiling water (charcoal) and filtered through a Celite bed. After cooling the filtrate was basified with 6*N* ammonium hydroxide to *p*H 10. The solid was separated by filtration, washed with water and then dried under vacuum at 100° for 6 hours to yield 9.5 g (73%) of yellow crystals, mp 211-212° (lit [1] mp 210-211°); $^1H$ nmr (deuteriotrifluoroacetic acid): $\delta$ 7.96 (dd, 1, $H_7$, J = 8 Hz), 7.53 (d, 1, $H_6$ or $H_8$, J = 8 Hz), 7.43 (d, 1, $H_6$ or $H_8$, J = 8 Hz), 3.0 (s, 3, $CH_3$).

Method A for Preparing Compounds **2** and **5**.

6-Chloro-2,4-diamino-5-methylquinazoline (**2**).

To a stirred solution of 5-methyl-2,4,6-triaminoquinazoline, **33**, [17] (0.29 g, 1.5 mmoles) in 4 ml of 2*N* hydrochloric acid maintained in an ice bath was added a solution of sodium nitrite (0.11 g, 1.6 mmoles) in 0.5 ml of water. After 0.5 hour this mixture was added to a cold (ice bath) solution of cuprous chloride (0.16 g, 1.6 mmoles) in 2 ml of 37% hydrochloric acid. The resulting mixture

was allowed to warm to room temperature and stirred for 6 hours. The mixture was basified with 6*N* ammonium hydroxide and the resulting solid was separated by filtration, washed with water and then ether. The solid was recrystallized from dimethylformamide-water to give 0.24 g (78%) of yellow solid; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.05 (d, 1, $H_7$, J = 9 Hz), 7.43 (d, 1, $H_8$, J = 9 Hz), 3.03 (s, 3, $CH_3$).

8-Chloro-2,4-diamino-5-methylquinazoline (**5**).

This compound was obtained from **35** [17] as a brown colored solid by recrystallization from ethanol: $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 7.93 (d, 1, $H_7$, J = 8 Hz), 7.46 (d, 1, $H_6$, J = 8 Hz), 2.96 (s, 3, $CH_3$).

Method B for Preparing Compounds **3** and **6**.

6-Bromo-2,4-diamino-5-methylquinazoline (**3**).

To a stirred solution of 5-methyl-2,4,6-triaminoquinazoline, **33**, [17] (0.29 g, 1.5 mmoles) in 4 ml of 2*N* methanesulfonic acid maintained in an ice bath was added a cold solution of sodium nitrite (0.11 g, 1.6 mmoles) in 0.5 ml of water. After stirring for 0.5 hour this mixture was added to a cold (ice bath) solution of cuprous bromide (0.23 g, 1.6 mmoles) in 2 ml of 48% hydrobromic acid. This mixture was allowed to warm to ambient temperature over 3 hours and then heated to 70° for 0.5 hour. After cooling the reaction mixture was basified with 6*N* ammonium hydroxide and the resulting solid separated by filtration and washed with water and ether. The crude solid was recrystallized from dimethylformamide-water and then dried under vacuum at 100° to give 0.28 g (75%) of yellow crystals; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.20 (d, 1, $H_7$, J = 9 Hz), 7.35 (d, 1, $H_8$, J = 9 Hz), 3.06 (s, 3, $CH_3$).

8-Bromo-2,4-diamino-5-methylquinazoline (**6**).

This compound was obtained from **35** [17] as a tan solid by recrystallization from ethanol; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.1 (d, 1, $H_7$, J = 8 Hz), 7.36 (d, 1, $H_6$, J = 8 Hz), 2.96 (s, 3, $CH_3$).

Method C for Preparing Compounds **4**, **7**, **8** and **9**.

2,4-Diamino-6-iodo-5-methylquinazoline (**4**).

To a stirred solution of 5-methyl-2,4,6-triaminoquinazoline, **33**, [17] (0.29 g, 1.5 mmoles) in 4 ml of 2*N* hydrochloric acid maintained in an ice bath was added sodium nitrite (0.11 g, 1.6 mmoles) in 0.5 ml of water. After stirring for 0.5 hour a solution of potassium iodide (0.26 g, 1.6 mmoles) in 2 ml was added and the resulting mixture was allowed to warm to ambient temperature over 2 hours and then heated briefly to 70°. Next, the mixture was basified with 6*N* ammonium hydroxide and the crude product was separated by filtration and washed with water. After drying the product was recrystallized from dimethylformamide-water to yield 0.31 g (70%) of yellow-green solid; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.46 (d, 1, $H_7$, J = 9 Hz), 7.10 (d, 1, $H_8$, J = 9 Hz), 3.0 (s, 3, $CH_3$).

2,4-Diamino-8-iodo-5-methylquinazoline (**7**).

This compound was obtained from **35** [17] as a light brown solid by recrystallization from dimethylformamide; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.33 (d, 1, $H_7$, J = 7.5 Hz), 7.26 (d, 1, $H_6$, J = 7.5 Hz), 2.93 (s, 3, $CH_3$).

2,4-Diamino-6-iodoquinazoline (**8**).

This compound was obtained from 2,4,6-triaminoquinazoline [17] as a tan solid after recrystallization from ethanol; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.66 (d, 1, $H_5$, J = 2.5 Hz), 8.30 (dd, 1, $H_7$, J = 8 Hz, J = 2.5 Hz), 7.30 (d, 1, $H_8$, J = 8 Hz).

5-Chloro-2,4-diamino-6-iodoquinazoline (**9**).

This compound was obtained from 5-chloro-2,4,6-triaminoquinazoline [17] as a brown solid after recrystallization from dimethylformamide-water; $^1$H nmr (deuteriotrifluoroacetic acid): $\delta$ 8.48 (d, 1, $H_7$, J = 8.5 Hz), 7.30 (d, 1, $H_8$, J = 8.5 Hz).

Method D for Preparing Compounds **10**, **11** and **12**.

2,4-Diamino-6-(trifluoromethyl)quinazoline (**10**).

This compound was prepared from 2-fluoro-5-(trifluoromethyl)-benzonitrile by reaction with guanidine carbonate in dimethylacetamide according to the procedure recently described from this laboratory [18]. It was obtained as a white crystalline solid by recrystallization from ethanol-water; $^1$H nmr (DMSO-$d_6$): $\delta$ 8.22 (br s, 1, $H_5$), 7.52 (dd, 1, $H_7$, J = 9 Hz, J = 2.5 Hz), 7.36 (br s, 2, 4-$NH_2$), 7.12 (d, 1, $H_8$, J = 9 Hz), 6.08 (br s, 2, 2-$NH_2$); $^{19}$F nmr (DMSO): 59.5 ($CF_3$).

2,4-Diamino-7-(trifluoromethyl)quinazoline (**11**).

This compound was obtained as a white crystalline solid from ethanol-water; $^1$H nmr (DMSO-$d_6$): $\delta$ 8.14 (d, 1, $H_5$, J = 9 Hz), 7.52 (br s, 2, 4-$NH_2$), 7.49 (br s, 1, $H_8$), 7.26 (dd, 1, $H_6$, J = 9 Hz, J = 3 Hz), 6.18 (br s, 2, 2-$NH_2$); $^{19}$F nmr (DMSO): $\delta$ 61.7 ($CF_3$).

2,4-Diamino-8-(trifluoromethyl)quinazoline (**12**).

This compound was obtained as a white crystalline solid from ethanol-water; $^1$H nmr (DMSO-$d_6$): $\delta$ 8.25 (d, 1, $H_5$, J = 7.5 Hz), 7.90 (d, 1, $H_7$, J = 7.5 Hz), 7.48 (br s, 2, 4-$NH_2$), 7.13 (dd, 1, $H_6$, J = 7.5 Hz), 6.18 (br s, 2, 2-$NH_2$); $^{19}$F nmr (DMSO): $\delta$ 59.5 ($CF_3$).

Acknowledgement.

This investigation received financial support from the UNDP/-World Bank/WHO Special Programme for Research and Training in Tropical Diseases. It was also supported (in part) by PHS Grants CA 25014 (J.B.H.) and 41461 (J.H.F.) awarded by the National Cancer Institute, DHHS. We thank Alpana Pathak for expert technical assistance.

## REFERENCES AND NOTES

[1] A. Rosowsky, J. L. Marini, M. E. Nadel and E. J. Modest, *J. Med. Chem.*, **13**, 882 (1970).

[2] A. Rosowsky and N. Papathanasopoulos, *J. Heterocyclic Chem.*, **9**, 1235 (1972).

[3] E. F. Elslager and J. Davoll in Lectures in Heterocyclic Chemistry, Vol **2**, R. N. Castle and L. B. Townsend, eds, HeteroCorporation, Orem, Utah, 1974, pp S-97-S-133.

[4] J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke and E. F. Elslager, *J. Med. Chem.*, **15**, 812 (1972).

[5] E. F. Elslager, M. P. Hutt, P. Jacob, J. Johnson, B. Temporelli, L. M. Werbel and D. F. Worth, *J. Med. Chem.*, **22**, 1247 (1979).

[6] J. B. Hynes and W. T. Ashton, *J. Med. Chem.*, **18**, 263 (1975).

[7] E. F. Elslager, J. L. Johnson and L. M. Werbel, *J. Med. Chem.*, **26**, 1753 (1983).

[8] J. R. Bertino, W. L. Sawicki, B. A. Moroson, A. R. Cashmore and E. F. Elslager, *Biochem. Pharmacol.*, **28**, 1983 (1979).

[9] P. J. O'Dwyer, D. D. Shoemaker, J. Plowman, J. Cradock, A. Grillo-Lopez and B. Leyland-Jones, *Invest. New Drugs*, **3**, 71 (1985).

[10] C. J. Allegra, J. A. Kovacs, J. C. Drake, J. C. Swan, B. A. Chabner and H. Masur, *J. Exptl. Med.*, **165**, 926 (1987).

[11] S. F. Queener, M. S. Bartlett, M. A. Jay, M. M. Durkin and J. W. Smith, *Antimicrob. Agents Chemother.*, **31**, 1323 (1987).

[12] C. J. Allegra, B. A. Chabner, C. U. Tuazon, D. Ogata-Arakaki, B. Baird, J. C. Drake, J. T. Simmons, E. E. Lack, J. H. Shelhamer, F. Balis, R. Walker, J. A. Kovacs, H. C. Lane and H. Masur, *N. Engl. J. Med.*, **317**, 978 (1987).

[13] J. I. DeGraw, V. H. Brown, W. T. Colwell and N. E. Morrison, *J. Med. Chem.*, **17**, 762 (1974).

[14] W. T. Colwell, V. H. Brown, J. I. DeGraw and N. E. Morrison in Chemistry and Biology of Pteridines, R. L. Kisliuk and G. M. Brown, eds, Elsevier/North Holland, Inc., Amsterdam, 1979, p 215.

[15] R. H. Gelber and L. Levy, *Internat. J. Leprosy*, **44**, 124 (1976).

[16] R. H. Gelber, *Prog. Drug Res.*, **34**, 421 (1990).

[17] J. Davoll and A. M. Johnson, *J. Chem. Soc.* C, 997 (1970).

[18] J. B. Hynes, A. Pathak, C. H. Panos and C. C. Okeke, *J. Heterocyclic Chem.*, **25**, 1173 (1988).

[19] W. T. Ashton, F. C. Walker, III and J. B. Hynes, *J. Med. Chem.*, **16**, 694 (1973).

[20] T. J. Delcamp, S. S. Susten, D. T. Blankenship and J. H. Freisheim, *Biochemistry*, **22**, 633 (1983).

[21] B. R. Baker and C. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967).

[22] T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).

[23] N. V. Harris, C. Smith and K. Bowden, *J. Med. Chem.*, **33**, 434 (1990).

[24] S. K. Singh, M. Govindan and J. B. Hynes, *J. Heterocyclic Chem.*, **27**, 2101 (1990).

[25] A. Tomažič, J. B. Hynes, G. R. Gale and J. H. Freisheim, *J. Heterocyclic Chem.*, **27**, 2081 (1990).

[26] A. Tomažič and J. B. Hynes, unpublished results.

[27] S. Gabriel and A. Thieme, *Chem. Ber.*, **52**, 1079 (1919).