The Synthesis of Novel Nonclassical Reversed Bridge Quinazoline Antifolates as Inhibitors of Thymidylate Synthase [1a,b]

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Received October 10, 1999

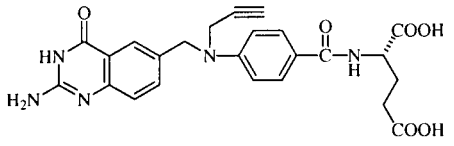
2-Amino-6-methyl-5-(pyridin-4-ylsulfanyl)-3*H*-quinazolin-4-one (**3**, AG337) a lipophilic thymidylate synthase inhibitor, is currently in clinical trials as an antitumor agent. On the basis of the crystal structure of **3** and the classical inhibitor 10-propargyl-5,8-dideazafolic acid (**1**, PDDF) with thymidylate synthase, we designed and synthesized a series of nonclassical 2-amino-6-substituted-3*H*-quinazolin-4-ones **4-13**, with a variety of electron withdrawing groups in the side chain (with the exception of compound **4**). Molecular modeling indicates that these reversed bridge (N9-C10) 6-substituted analogues orient their side chain C10-substituent such that it lies between that of **1** and **3**. These compounds were obtained by reductive amination of 6-aminoquinazoline **16** and the appropriate aryl aldehyde **17** or aryl ketone **18**. For analogues **11-13**, the yield depended on the substitutents on the aryl ketone **18** (comparison of **11** and **13**). With the exception of analogue **13**, all the compounds in the series were poor inhibitors of thymidylate synthase from *Lactobacillus casei, Pneumocystis carinii* and human sources.

J. Heterocyclic Chem., **37**, 1097 (2000).

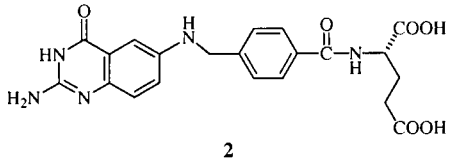
The enzyme thymidylate synthase catalyzes the conversion of deoxyuridine monophosphate to thymidylate, *via* a reductive methylation utilizing 5,10-methylenetetrahydrofolate as a cofactor [2]. This rate-limiting step is the exclusive "*denovo* source of thymidylate" for DNA synthesis. Inhibition of thymidylate synthase leads to "thymineless death." Thus, the inhibition of thymidylate synthase is an important target in cancer chemotherapy.

Efforts to discover a folate analogue active against mammalian thymidylate synthase led to the development of 10-propargyl-5,8-dideazafolic acid (**1**, PDDF) (Figure 1). Compound **1** was effective against L1210 thymidylate synthase (IC_{50} = 0.014 μ*M*) and was introduced into clinical trials [3]. Due to renal toxicity and hepatotoxicity, exhibited by **1**, resulting from its poor aqueous solubility, it was withdrawn from the clinic.

Figure 1



1, PDDF



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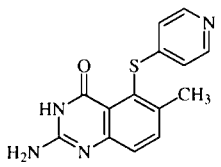
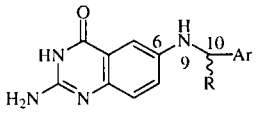


3, AG337



4-13

Concurrent to the development of **1**, the reversed bridge classical analogue **2**, where the normal C9-N10 bridge is reversed to a N9-C10, had demonstrated activity against a variety of established human colon cell lines in culture [4,5]. It was found to be highly active against methotrexate and 5-fluorodeoxyuridine resistant tumor cells *in vitro* [6]. However, large doses of **2** were required to achieve therapeutic effectiveness. This was attributed to its slow rate of influx into target cells as was demonstrated using tritiated **2** and HCT 8-cells in culture [6]. Although the affinity of **2** against thymidylate synthase was approximately 100-fold less than **1**, both compounds were equally cytotoxic against the growth of HCT-8 cells in culture [7]. Further, the cytotoxicity of **2** was reversed by thymidine, indicating its primary target, like **1**, was thymidylate synthase.

The glutamic acid side chain present in the classical antifolates such as **1** and **2** requires active transport of these compounds across cell membranes and diminished active transport is a cause of drug resistance [8]. Classical antifolates, particularly thymidylate synthase inhibitors, are often substrates for folylpolyglutamate synthetase [9,10]. Once polyglutamylated the compounds become extremely tight

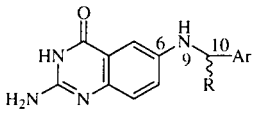
binding inhibitors of thymidylate synthase and are not effluxed from the cells [9,10]. Though polyglutamylation is often necessary for the cytotoxic effects, it has also been implicated in toxicity to normal cells, due to retention of the poly-γ-glutamate metabolites [11]. In addition, resistance also develops to these classical agents with impairment in folylpolyglutamate synthetase activity in the cell.

Using the X-ray crystal structure of *Escherichia coli* thymidylate synthase, Webber *et al.* [12] designed and synthesized a series of 2-amino-6-methyl-5-(thioaryl)-3*H*-quinazolin-4-ones as exemplified by compound **3**, (AG337). This analogue, was a potent inhibitor of human thymidylate synthase (K_i = 0.015 μ*M*). *Escherichia coli* thymidylate synthase was chosen for the crystal structure, because of the high homology of its folate binding site with that of human thymidylate synthase.

In an attempt to circumvent the disadvantages of classical thymidylate synthase inhibitors, we designed the reversed bridge nonclassical lipophilic antifolates **4-13** (Table I), as potential inhibitors of thymidylate synthase. These analogues, unlike **1** and **2**, were expected to diffuse passively into the cells, thus avoiding drug resistance due to active transport and lacking the glutamate moiety were not expected to be substrates for folylpolyglutamate synthetase and thus would also overcome resistance to tumor cells due to inefficient folyl-γ-glutamate synthetase activity.

Using molecular modeling with SYBYL6.03 [13] and superimposition of a low energy conformation of the reversed bridge analogue **9** with the thymidylate synthase bound conformation of **1** [14] and **3** [12], indicated that the 6-substituted aryl side chain of **9** oriented between that of **1** and **3**. In addition the C10-methyl substituent in the *R* -enantiomer of analogue **11**, for example, was oriented to interact with tryptophan80 of thymidylate synthase, much like that proposed for the 6-methyl moiety of **3** [12].

Table I
Designed Nonclassical Analogues **4-13**



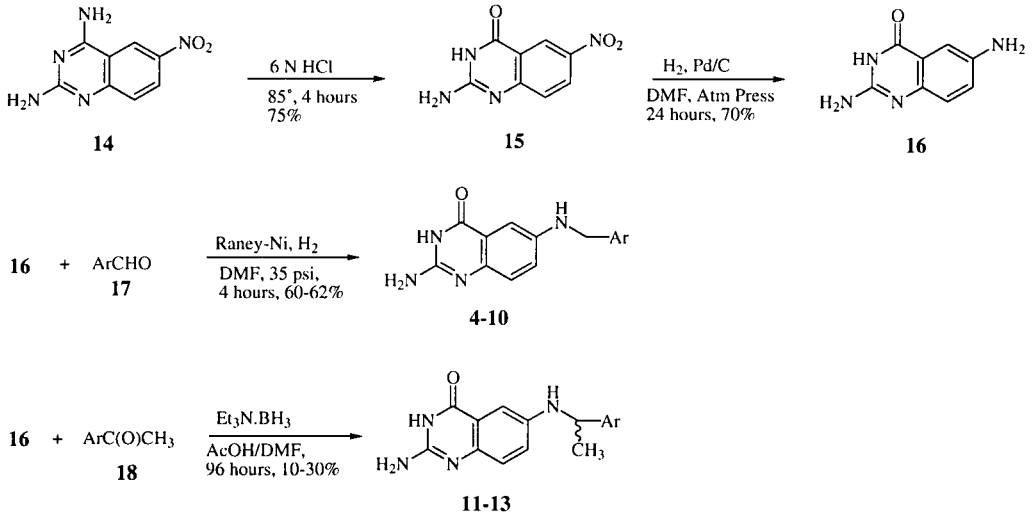
4-13

Compound	R	Ar
4	H	3,4,5-triOCH_3 Ph
5	H	3-Cl Ph
6	H	4-Cl Ph
7	H	3-F Ph
8	H	3, 4-diCl Ph
9	H	3-Pyridyl
10	H	4-NO_2 Ph
11	CH_3	Ph
12	CH_3	3-Cl Ph
13	CH_3	4-NO_2 Ph

Side chains containing electron withdrawing groups were chosen in analogues **5-13** in an attempt to mimic the side chain of the natural cofactor 5,10-methylenetetrahydrofolate [15] as well as compounds **3** [12] and pyrrolo[2,3-*d*]pyrimidines reported by Gangjee *et al.* [16,17]. In addition, the C10-methyl moiety in analogues **11-13** was expected to decrease the conformational mobility of the N9-C10 bridge and could provide for appropriate orientations of the side chains for thymidylate synthase binding. Analogue **4** with electron donating methoxy groups in the side chain was synthesized to compare its thymidylate synthase inhibitory potency to that of analogues **5-13** which had electron withdrawing groups.

The synthesis of the compounds are shown in Scheme I. Commercially available 2,4-diamino-6-nitroquinazoline **14** was hydrolysed with 6 *N* hydrochloric acid for 4 hours

Scheme I



at 85-90° to afford the 2-amino-6-nitro-3*H*-quinazolin-4-one **15** in a 75% yield [18]. The ^{1}H nmr of this compound indicated a broad singlet at δ 11.42, which was exchangeable with deuterium oxide and corresponded to the 3-NH moiety of intermediate **15**. The absence of the 4-amino protons confirmed that hydrolysis of the 4-amino group had indeed occurred. Catalytic reduction of **15** with 5% palladium over carbon and hydrogen at atmospheric pressure and room temperature for 24 hours afforded 2,6-diamino-3*H*-quinazolin-4-one **16** in a 70% yield. The ^{1}H nmr of **16** indicated a broad singlet at δ 5.00, which was exchangeable upon deuterium oxide addition, corresponding to the 6-amino group indicating the reduction had afforded compound **16**. The structures **15** and **16** were further characterized by elemental analysis.

Having obtained compound **16**, the desired reversed bridge analogues **4-10** and **11-13** could be obtained *via* the reductive amination of the appropriate benzaldehydes **17** or acetophenones **18**, with **16**. Thus, reductive amination of the appropriate aldehyde with **16** using Raney nickel as the catalyst in a 3:1 mixture of *N,N*-dimethylformamide and glacial acetic acid, afforded analogues **4-10** in 60-62% yield, following chromatographic purification. The structures of analogues **4-10** were confirmed by their ^{1}H nmr which indicated a singlet corresponding to the C10-methylene group between δ 4.19–4.35 and a broad singlet at δ 6.18–6.93 (exchangeable upon deuterium oxide addition) corresponding to the 6-amino group of analogues **4-10** respectively.

Condensation of the appropriate acetophenone with **16**, using triethylamine-borane complex as the reductant afforded analogues **11-13** in 10-30% yields. This borane complex, has been used by Gangjee *et al.* to condense hindered ketones with amines [19]. Thus, one equivalent each of **16**, the appropriate acetophenone and triethylamine-borane complex were stirred in a 3:1 mixture of *N,N*-dimethylformamide and glacial acetic acid under nitrogen for 96 hours at room temperature to afford, after column chromatography, 10-30% of the product. The substitution on the aromatic ring of the aryl ketone affected the yield of the condensed product. Thus, 4-nitroacetophenone afforded the best yield (30%) probably due to the increased electrophilic character of the ketone carbonyl as a result of the electron withdrawing ability of the 4-nitro moiety.

The designed analogues **4-13**, were evaluated as inhibitors of thymidylate synthase from *Lactobacillus casei (L. casei), Pneumocystis carinii (P. carinii)* and human sources and are reported in Table II. The compounds were poor inhibitors of thymidylate synthase as compared to **1** and **3**. Compound **13**, the most potent inhibitor of the series, was approximately 640-fold and 333-fold less active than **1** as an inhibitor of *Lactobacillus casei* and human thymidylate synthase respectively.

Table II

Inhibitory Concentrations (IC50, in μM) of Analogues **4-13** as Inhibitors of Thymidylate Synthase from *Lactobacillus casei, Pneumocystis carinii* and Human sources [21,22]

Compound	*L. casei*	*P. carinii*	human
4	>210	>210	>210
5	>25	ND	ND
6	78	ND	ND
7	>290	ND	ND
8	120	ND	ND
9	280	42	84
10	120	120	24
11	>270	ND	ND
12	>250	ND	ND
13	23	46	12
PDDF	0.095	0.09	0.18

3, Ki = 0.015 μ*M* against Human Thymidylate Synthase [12]. ND = Not Determined. *P. Carinii* and human enzyme were kindly supplied by Professor D. V. Santi University of California San Francisco.

Comparison of analogues **10** and **13**, indicated that although **13** is racemic, the C10-methyl group had a beneficial effect on thymidylate synthase inhibition. Molecular modeling predicted that, for the C10-methyl analogues, only the *R*-enantiomer would interact appropriately with the tryptophan80 present in *Escherischia coli* thymidylate synthase, as was the case with **3** [12]. As observed in previous studies from our laboratory [16,17,20] and by Webber *et al.* [12], electron withdrawing groups present on the aryl side chains were conducive to thymidylate synthase inhibition.

In summary, on the basis of molecular modeling and the inhibitory potency of the reversed bridge analogue **2**, we synthesized analogues **4-13** as nonclassical inhibitors of thymidylate synthase. These compounds were obtained by reductive amination of 6-amino quinazoline **16** and the appropriate aryl aldehyde **17** or aryl ketone **18**. For analogues **11-13**, the yield depended on the substitution of the aryl ketone **18** (as seen by comparison of analogue **11**

Table III

CHN APPENDIX

	Found				Calculated			
	% C	% H	% N	% Cl	% C	% H	% N	% Cl
4	58.49	5.72	14.58		58.77	5.75	14.28	
5	53.75	4.59	16.90	14.73	54.05	4.66	16.81	14.89
6	55.68	4.49	17.23	14.41	55.87	4.53	17.37	14.29
8	48.41	3.96	15.32	24.44	48.37	4.11	15.04	24.75
9	62.80	4.65	26.11		62.49	4.94	26.03	
10	57.45	4.18	22.06		57.21	4.29	22.24	
11	63.78	5.82	17.85		63.70	6.10	18.23	
12	56.49	5.03	16.43	13.98	56.80	4.98	16.56	13.62
13	54.80	4.93	19.94		55.13	5.08	19.84	

Analogue **7**, HRMS (EI): *m/z* Calcd. for $C_{15}H_{13}N_4OF$: 284.1073 (M^+). Found: 284.1075 (M^+).

and **13**). With the exception of analogue **13**, all the analogues in the series were poor inhibitors of thymidylate synthase from *Lactobacillus casei, Pneumocystis carinii* and human sources.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Nuclear Magnetic Resonance Spectra (^{1}H nmr) were recorded on a Brucker WH-300 (300 MHz). The data was accumulated by 16 K size with 0.5 second delay time and 70° tip angle with internal standard trimethysilane (TMS); s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution spectra (hrms) were obtained on a VG7070E-HF instrument. Thin layer chromatography (tlc) was performed on Aldrich silica gel plates with a fluorescent indicator and were visualized with uv-lamp at 254 nm and 366 nm. Column chromatography was performed with (60 Å, 230-400 mesh) silica gel from Aldrich Chemical Company, WI, employing gravity or flash columns (2.4 × 15 cm) unless otherwise stated. Elutions were performed using a gradient (specifically stated in the experimentals) and 10 ml fractions were collected. Solvents for column chromatography were purchased from Fisher Scientific, PA. Samples for microanalysis were dried under vacuum over phosphorous pentoxide at 75° for 24-48 hours utilizing the Chem-Dry apparatus. Analysis were performed by Atlantic Microlabs, Georgia and are within ± 0.4% of the calculated value. Fractional amounts of solvents could not be removed from some of the compounds despite drying under vacuum and were confirmed where possible by their presence in the ^{1}H nmr.

2-Amino-6-nitro-3*H*-quinazolin-4-one (**15**).

2,4-Diaminoquinazoline **14** (0.50 g, 1.75 mmol) and 6 *N* hydrochloric acid (50 ml) were heated at 85° for 4 hours. The mixture was concentrated under reduced pressure and neutralized with 3 *N* sodium hydroxide to afford 0.40 g (75%) of **15** (4) as a yellow solid. The residue was washed with water (5 × 25 ml) followed by diethylether (2 × 25 ml). The residue was dried over phosphorous pentoxide and carried to the next step; tlc: silica gel, chloroform:methanol (2:1), R_f 0.35, mp >300°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 7.02 (br s, 2 H, 2-NH_2), 7.27 (d, 1 H, Ar-H), 8.28 (d, 1 H, Ar-H), 8.58 (d, 1 H, Ar-H), 11.42 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_8H_6N_4O_3$•0.9H_2O: C, 43.21; H, 3.54; N, 25.19. Found: C, 43.46; H, 3.41; N, 24.88.

2,6-Diamino-3*H*-quinazolin-4-one (**16**).

Compound **15** (0.40 g, 1.20 mmol) was placed in a flask containing 10 ml of glacial acetic acid and *N,N*-dimethylformamide (50 ml). To this was added 0.04 g of (5%) palladium on activated carbon and the mixture hydrogenated for 24 hours at room temperature and pressure. The reaction was filtered through Celite and the filtrate concentrated to afford 0.35 g (70%) of **16** as a greenish-brown solid, tlc: silica gel, chloroform:methanol (2:1), R_f 0.20, mp >300° (4); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 5.00 (br s, 2 H, 6-NH_2), 5.89 (br s, 2 H, 2-NH_2), 6.92 (m, 3 H, Ar-H), 10.74 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_8H_8N_4O$•0.1CH_3COOH•0.1H_2O: C, 53.53; H, 4.71; N, 30.45. Found: C, 53.64; H, 4.60; N, 30.27.

General Procedure for the Synthesis of Analogues **4-10**.

To a solution of **16** (0.35 g, 2.00 mmol) in *N,N*-dimethylformamide (45 ml) and glacial acetic acid (15 ml) was added Raney nickel (2.50 g) followed by the addition of the appropriate aldehyde **17** (2.00 mmol). The mixture was hydrogenated in a Paar shaker at 35 psi for 4 hours. Analysis (tlc, chloroform:methanol 5:1), indicated a new product (R_f = 0.33-0.36). At the end of 4 hours, the mixture was filtered through Celite and the filtrate concentrated to afford a residue. The residue was dissolved in methanol (20 ml) followed by the addition of silica gel (0.50 g) and the methanol was then evaporated to afford a silica gel plug which was dried over phosphorous pentoxide and loaded on a silica gel column (2.4 × 20 cm). The column was eluted with 5% methanol in chloroform. Fractions containing the product were pooled to afford the final compounds **4-10** in 60-62% yield.

2-Amino-6-(3,4,5-trimethoxylbenzyl)amino-3*H*-quinazolin-4-one (**4**).

This compound was synthesized using the general procedure from **16** and 3,4,5-trimethoxybenzaldehyde to afford 0.24 g (60%) of **4** as a green solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 140°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 3.62 (s, 3 H, 4'-OCH_3), 3.74 (s, 6 H, 3',5'-OCH_3), 4.19 (s, 2 H, CH_2), 6.02 (br s, 2 H, 2-NH_2), 6.18 (br s, 1 H, 6-NH), 6.71 (s, 2 H, 2',6'-Ar-H), 6.95 (s, 1 H, Ar-H), 7.03 (s, 2 H, Ar-H).

Anal. Calcd. for $C_{18}H_{20}N_4O_4$•0.6CH_3COOH: C, 58.77; H, 5.75; N, 14.28. Found: C, 58.49; H, 5.72; N, 14.58.

2-Amino-6-(3-chlorobenzyl)amino-3*H*-quinazolin-4-one (**5**).

This compound was synthesized using the general procedure from **16** and 3-chlorobenzaldehyde to afford 0.28 g (62%) of **5** as a tan solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.35, mp 186°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.35 (s, 2 H, CH_2), 6.58 (br s, 1 H, 6-NH), 7.11 (br s, 3 H, 2-NH_2 and Ar-H), 7.14 (m, 2 H, Ar-H) 7.28 (m, 4 H, Ar-H), 11.81 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_{15}H_{13}N_4OCl$•1.0H_2O•0.4HCl: C, 54.05; H, 4.66; N, 16.81; Cl, 14.89. Found: C, 53.75; H, 4.59; N, 16.90; Cl, 14.73.

2-Amino-6-(4-chlorobenzyl)amino-3*H*-quinazolin-4-one (**6**).

This compound was synthesized using the general procedure from **16** and 4-chlorobenzaldehyde to afford 0.24 g (60%) of **6** as a green solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 192°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.28 (s, 2 H, CH_2), 6.64 (br s, 1 H, 6-NH), 6.89 (br s, 2 H, 2-NH_2), 6.90 (m, 1 H, Ar-H), 7.06 (m, 2 H, Ar-H), 7.10 (s, 4 H, Ar-H), 11.56 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_{15}H_{13}N_4OCl$•0.6H_2O•0.3HCl: C, 55.87; H, 4.53; N, 17.37; Cl, 14.29. Found: C, 55.68; H, 4.49; N, 17.23; Cl, 14.41.

2-Amino-6-(3-fluorobenzyl)amino-3*H*-quinazolin-4-one (**7**).

This compound was synthesized using the general procedure from **16** and 3-fluorobenzaldehyde to afford 0.26 g (61%) of **7** as a brown solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 238°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.33 (s, 2 H, CH_2), 6.93 (br s, 1 H, 6-NH), 7.06 (m, 2 H, Ar-H), 7.08-7.18 (m, 6 H, 2-NH_2 and Ar-H), 7.21-7.37 (m, 1 H, Ar-H); hrms (EI): m/z Calcd. for $C_{15}H_{13}N_4OF$: 284.1073 (M^+). Found 284.1075 (M^+).

2-Amino-6-(3,4-chlorobenzyl)amino-3*H*-quinazolin-4-one (**8**).

This compound was synthesized using the general procedure from **16** and 3,4-dichlorobenzaldehyde to afford 0.22 g (60%) of **8** as a green solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.36, mp 202°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.33 (s, 2 H, CH_2), 6.65 (br s, 1 H, 6-NH), 6.92 (d, 1 H, Ar-H), 7.00-7.16 (m, 4 H, 2-NH_2 and Ar-H), 7.34 (m, 1 H, Ar-H), 7.58 (t, 2 H, Ar-H), 11.88 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_{15}H_{12}N_4OCl_2 \cdot 0.8H_2O \cdot 0.6HCl$: C, 48.37; H, 4.11; N, 15.04; Cl, 24.75. Found: C, 48.41; H, 3.96; N, 15.32; Cl, 24.44.

2-Amino-6-(3-pyridylmethylene)amino-3*H*-quinazolin-4-one (**9**).

This compound was synthesized using the general procedure from **16** and 3-pyridinecarboxzaldehyde to afford 0.24 g (60%) of **9** as a brown solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 202°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.33 (s, 2 H, CH_2), 6.38 (br s, 3 H, 2-NH_2 and 6-NH), 6.94 (s, 1 H, Ar-H), 7.06 (d, 2 H, Ar-H), 7.33-7.37 (m, 1 H, pyridyl-H), 7.75 (d, 1 H, pyridyl-H), 8.44 (d, 1 H, pyridyl-H), 8.58 (s, 1 H, pyridyl-H), 11.35 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_{14}H_{13}N_5O \cdot 0.1H_2O$: C, 62.49; H, 4.94; N, 26.03. Found: C, 62.80; H, 4.65; N, 26.11.

2-Amino-6-(4-nitrobenzyl)amino-3*H*-quinazolin-4-one (**10**).

This compound was synthesized using the general procedure from **16** and 4-nitrobenzaldehyde to afford 0.29 g (62%) of **10** as a tan solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.34, mp 285° dec; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 4.28 (s, 2 H, CH_2), 6.18 (br s, 2 H, 2-NH_2), 6.53 (br s, 1 H, 6-NH), 6.85 (s, 1 H, Ar-H), 7.04 (s, 2 H, Ar-H), 7.62 (AA'BB'quartet, 2 H, Ar-H), 8.20 (AA'BB'quartet, 2 H, Ar-H).

Anal. Calcd. for $C_{15}H_{13}N_5O_3 \cdot 0.2H_2O$: C, 57.21; H, 4.29; N, 22.24. Found: C, 57.45; H, 4.18; N; 22.06.

General Procedure for the Synthesis of Analogues **11-13**.

To a solution of **16** (0.4 g, 2.27 mmol) in *N,N*-dimethylformamide (45 ml) and glacial acetic acid (15 ml) was added triethylamine-borane complex (0.50 g, 6.82 mmol) followed by the addition of the appropriate acetophenone **18** (2.27 mmol). The mixture was stirred under nitrogen for 96 hours. Analysis (tlc, chloroform:methanol 5:1), indicated a new product (R_f = 0.32-0.33). At the end of 96 hours, the reaction was concentrated under reduced pressure to afford a residue. The residue was dissolved in methanol (20 ml) followed by the addition of silica gel (0.50 g) and the mixture was concentrated to afford a silica gel plug which was dried over phosphorous pentoxide and then loaded on a silica gel column (2.4 × 20 cm). The column was eluted with 5% methanol in chloroform. Fractions containing the product were pooled to afford the final compounds **11-13** in 10-30% yield.

(*R,S*)-2-Amino-6-(1-phenylethylamino)-3*H*-quinazolin-4-one (**11**).

This compound was synthesized using the general procedure from **16** and acetophenone to afford 0.03 g (10%) of **11** as a shiny brown solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.32, mp 172°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 1.41 (d, 3 H, CH_3), 4.44 (q, 1 H, CH), 6.04 (br s, 2 H, 2-NH_2), 6.20 (br s, 1 H, 6-NH), 6.82 (m, 1 H, Ar-H), 7.01 (m, 2 H, Ar-H), 7.16 (t, 1 H, Ar-H), 7.28 (t, 2 H, Ar-H), 7.38 (d, 2 H, Ar-H), 10.94 (br s, 1 H, 3-NH).

Anal. Calcd. for $C_{16}H_{16}N_4O \cdot 1.0H_2O \cdot 0.15CH_3COOH$: C, 63.70; H, 6.10; N, 18.23. Found: C, 63.78; H, 5.82; N, 17.85.

(*R,S*)-2-Amino-6-[1-(3-chlorophenyl)ethylamino]-3*H*-quinazolin-4-one (**12**).

This compound was synthesized using the general procedure from **16** and 3-chloroacetophenone to afford 0.02 g (10%) of **12** as a brown solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 185°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 1.40 (d, 3 H, CH_3), 4.53 (q, 1 H, CH), 6.46 (br s, 1 H, 6-NH), 6.83 (br s, 3 H, 2-NH_2 and Ar-H), 7.10 (m, 2 H, Ar-H), 7.14 (m, 1 H, Ar-H), 7.24 (m, 2 H, Ar-H),), 7.42 (m, 1 H, Ar-H).

Anal. Calcd. for $C_{16}H_{15}N_4OCl \cdot 0.7H_2O \cdot 0.3HCl$: C, 56.80; H, 4.98; N, 16.56; Cl, 13.62. Found: C, 56.49; H, 5.03; N, 16.43; Cl, 13.98.

(*R,S*)-2-Amino-6-[1-(4-nitrophenyl)ethylamino]-3*H*-quinazolin-4-one (**13**).

This compound was synthesized using the general procedure from **16** and 4-nitroacetophenone to afford 0.11 g (30%) of **13** as a tan solid, tlc: silica gel, chloroform:methanol (5:1), R_f 0.33, mp 267°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 1.14 (d, 3 H, CH_3), 4.65 (q, 1 H, CH), 6.17 (br s, 1 H, 2-NH_2), 6.42 (br s, 1 H, 6-NH), 6.76 (s, 1 H, Ar-H), 7.00 (d, 2 H, Ar-H), 7.66 (AA'BB'quartet, 2 H, Ar-H), 8.18 (AA'BB'quartet, 2 H, Ar-H).

Anal. Calcd. for $C_{16}H_{15}N_5O_3 \cdot 1.2H_2O \cdot 0.1CH_3COOH$: C, 55.13; H, 5.08; N, 19.84. Found: C, 54.80; H, 4.93; N, 19.94.

Acknowledgments.

This work was supported in part by a grant from the National Institute of General Medical Sciences, GM 40998 (AG) and the National Cancer Institute, CA 10914 (RLK).

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[1a] Taken in part from the thesis submitted by M.Kotharé. to the Graduate School of Pharmaceutical Sciences, Duquesne University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, May, 1998; [1b] Presented in part at the 214th, ACS, Las Vegas, Sep 7-13, 1997 (MEDI 167).

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