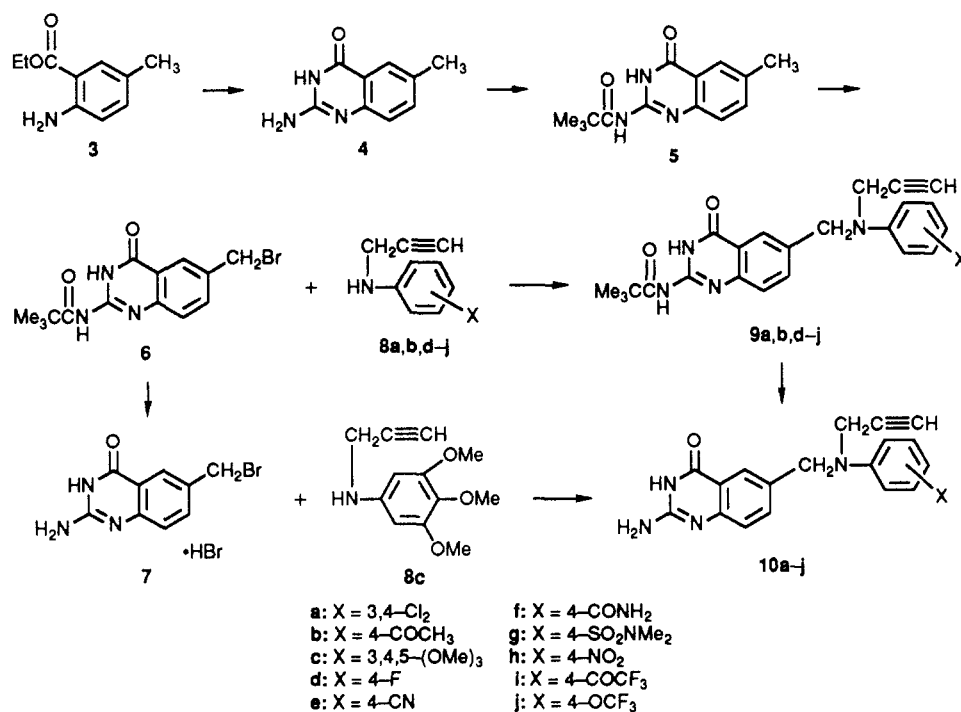


Scheme I



our laboratory to investigate further the role of the glutamate moiety in TS inhibition and cytotoxicity. Our curiosity was, in part, prompted by two observations. Firstly, the role of the glutamate in the transport of 1 is unclear. Compound 1 does not use the reduced-folate carrier system required for methotrexate transport.^{2a-c} Transport of trimetrexate is in part attributable to passive diffusion, although some energy-dependent component also has been suggested.^{2a} We extrapolated from this to investigate quinazolinone-based TS inhibitors which would not necessarily require active transport. This seemed appealing since active transport mechanisms can open the door to the development of resistance. Interestingly, this does not seem to be the case for 1. Secondly, and more importantly, it has been demonstrated that the polyglutamates of 1 are more potent inhibitors of TS than the parent drug, in particular the compound with three additional glutamates attached to 1.^{6a-c} For this reason, it has always been assumed that the glutamic acid moiety was required for potent activity, if not for transport.

With this in mind, we wondered whether potent TS inhibition could be achieved with a nonclassical quinazolinone analogue which did not bear the appended amino acid. Toward that end, we synthesized two series of nonclassical quinazolinone analogues, one (10a-j) analogous to 1 and bearing a 2-amino substituent, the other (13a-c,k) analogous to 2, lacking the 2-amino substituent. Although we will develop expanded structure-activity relationships in these two series, our early emphasis was to maintain the electron-withdrawing property of the 4-carboxamide substituent of the natural cofactor, N⁵,N¹⁰-methylene tetrahydrofolate, while increasing its lipophilicity and decreasing its steric bulk. Herein we report on the first nonclassical quinazolines to achieve potent TS inhibition.¹⁴

Chemistry

The 2-amino-6-[(phenyl-2-propynylamino)methyl]-4-(3H)-quinazolinone target compounds 10a-j were syn-

Table I. Preparation of Intermediates 9a,b,d-j and 12a-c,k

no.	% yield	mp, °C	no.	% yield	mp, °C
9a	66	a	9i	41.3	b
9b	63	190-192	9j	65	174-176
9d	80.5	187-190	12a	88	c
9e	66.6	183-186	12b	42	b
9f	44	147-153	12c	100	d
9g	58.6	217-220	12k	60	c
9h	32.6	210-215			

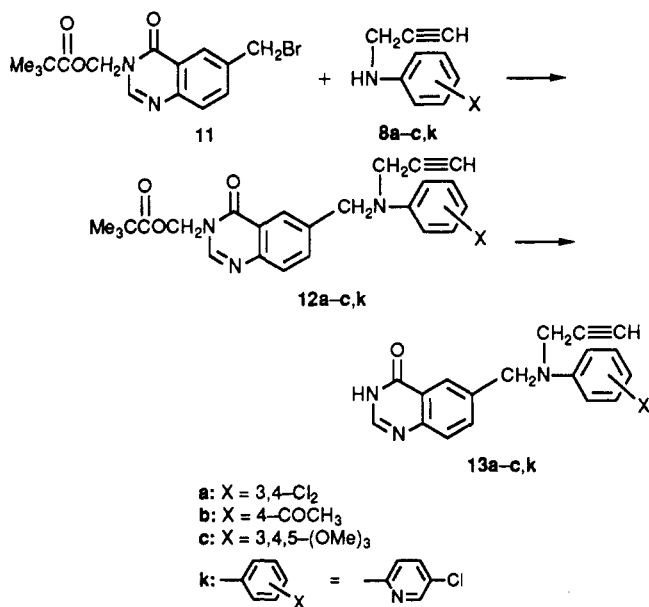
^aNot recorded. ^bOff-white foam. ^cColorless oil. ^dLight brown oil.

thesized by one of the two published variations illustrated in Scheme I.⁷ In Scheme I, the 2-amino-6-methyl-4-(3H)-quinazolinone (4) had previously been made in yields of 79-82% by treating ethyl 2-amino-5-methylbenzoate (3) with guanidine.¹⁵ By substituting chloroformamide hydrochloride¹⁶ for guanidine in this reaction we increased the yield of 4 to 99%.¹⁷ In the first variation, treatment of 3,4,5-trimethoxy-N-2-propynylbenzenamine (8c) with 2-amino-6-(bromomethyl)-4-(3H)-quinazolinone hydrobromide (7)¹⁸ gave the target compound 10c. Purification of 10c made by this route required both chromatography and recrystallization. When other N-2-propynylbenzenamines (8) were similarly treated with 7, purification of the target compounds was more difficult due to their decreased solubility. Therefore the variation in Scheme I where 8 was treated with the protected bromomethyl derivative 6¹⁵ rather than the deprotected bromomethyl derivative 7 was employed. This reaction gave the pen-

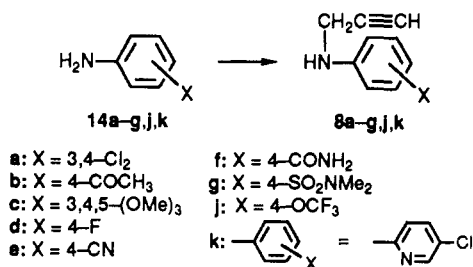
(14) Preliminary communication of this work appeared previously: Fry, D. W.; McNamara, D. J.; Werbel, L. M.; Berman, E. M. *Proc. Am. Assoc. Cancer Res.* 1989, 30, Abstract 1905, 479.

(15) Acharya, S. P.; Hynes, J. B. *J. Heterocycl. Chem.* 1975, 12, 1283.
 (16) Johnson, T. B.; Sprague, J. M. *J. Am. Chem. Soc.* 1939, 61, 176. Hantzsch, A.; Vagt, A. *Annalen* 1900, 314, 339.
 (17) While this manuscript was in preparation, essentially this same improvement was reported: Jones, T. R.; Betteridge, R. F.; Newell, D. R.; Jackman, A. L. *J. Heterocycl. Chem.* 1989, 26, 1501.
 (18) Calvert, A. H.; Jones, T. R.; Jackman, A. L.; Brown, S. J.; Harrap, K. R. In *Human Cancer, Its Characterization and Treatment*; Davis, W.; Harrap, K. R.; Stathopoulos, G., Eds.; Excerpta Medica: Amsterdam, 1980; p 272.

Scheme II



Scheme III

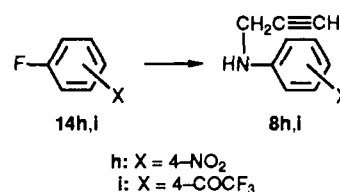


ultimate intermediates **9**, which were very soluble and easily purified by chromatography. The yields and melting points of **9a,b,d-j** are listed in Table I. These intermediates **9** were then treated with NH₃/MeOH to provide the target compounds **10**, which generally required no purification. Target compounds **10a,b,d-j** were prepared by this variation.

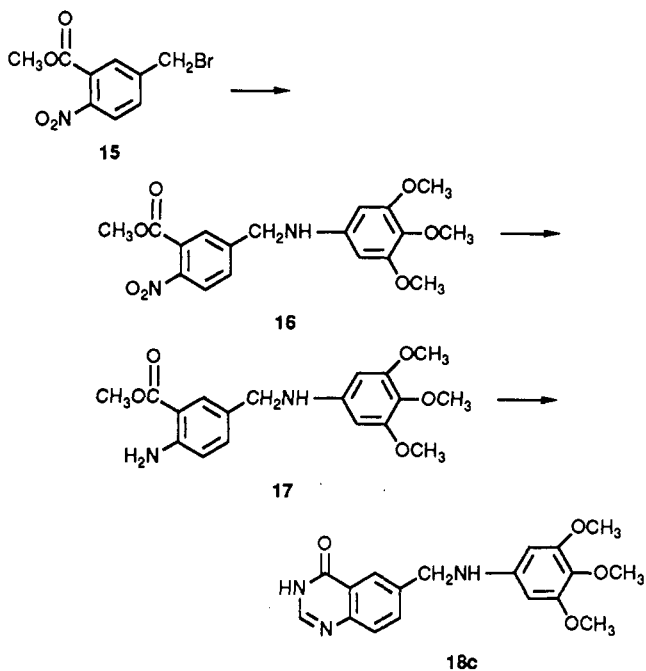
The 6-[(phenyl-2-propynylamino)methyl]-4(3*H*)-quinazolinone target compounds **13a-c,k**, lacking the 2-amino group, were synthesized by the published route illustrated in Scheme II.¹² The yields and appearances of the intermediates **12a-c,k** are listed in Table I. Our only departure from the literature procedures was the use of NH₃/MeOH rather than aqueous NaOH in the conversion of the penultimate intermediates **12** to the target compounds **13**.

The required *N*-2-propynylbenzenamines (**8**) were made by one of two different methods (Scheme III or IV). Compounds **8a-g,j,k** were synthesized as illustrated in Scheme III by alkylating the appropriately substituted benzenamine (**14a-g,j,k**) with 3-bromo-1-propyne. The desired monoalkylated products were usually contaminated by dialkylated byproducts, but these were easily removed by chromatography. Compounds **8h,i** could not be synthesized by this method due to the low nucleophilicity of the starting benzenamines. They were therefore synthesized as illustrated in Scheme IV by displacing the aromatic fluorine atom from 4-fluoronitrobenzene (**14h**)¹⁹ and 2,2,2-trifluoro-1-(4-fluorophenyl)ethanone (**14i**), re-

Scheme IV



Scheme V



spectively, with 2-propynylamine.

The despropargylated 2-desamino compound **18c** was prepared by the novel route illustrated in Scheme V. Thus the known 5-(bromomethyl)-2-nitrobenzoic acid methyl ester (**15**)²⁰ was treated with 3,4,5-trimethoxybenzenamine (**14c**) to afford 2-nitro intermediate **16**. This was catalytically reduced to 2-amino intermediate **17**, which was then cyclized with formamidine²¹ to yield the target **18c**. As discussed above, the other 2-desamino compounds **13a-c,k** were not obtained by this method but rather by that in Scheme II, which was more amenable to the synthesis of analogues.

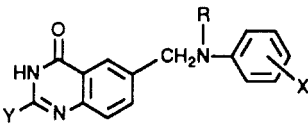
Results and Discussion

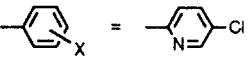
The nonclassical target compounds **10a-j**, **13a-c,k**, and **18c** synthesized here, as well as the classical reference compound **1**, were tested for inhibition of L1210 TS, inhibition of L1210 growth in culture, and inhibition of WI-L2 DHFR. These results are summarized in Table II. Also included are the literature values for the 2-desamino reference compound **2**.¹² The 2-amino target compounds **10a-j** were generally potent inhibitors of L1210 TS, with IC₅₀s within the narrow range of 0.51–11.5 μM. Compound **10i** (X = 4-COCF₃) exhibited the most potent inhibition with an IC₅₀ of 0.51 μM, which shows it to be 10-fold less potent than **1**. These compounds also inhibited the growth of L1210 cells in culture, with IC₅₀s of 2.4–>>20 μM. Compound **10h** (X = 4-NO₂) exhibited the most potent

(19) The synthesis of **8h** by this method has been reported in Garito, A. F.; Horner, C. J.; Kalyanaraman, P. S.; Desai, K. N. *Makromol. Chem.* 1980, 181, 1605.

(20) Zrihen, M.; Labia, R.; Wakselman, M. *Eur. J. Med. Chem.-Chim. Ther.* 1983, 18, 307.

(21) The use of formamidine acetate to effect the ring closure of anthranilic acid esters to 4(3*H*)-quinazolinones has been reported: Stevenson, T. M.; Kazmierczak, F.; Leonard, N. J. *J. Org. Chem.* 1986, 51, 616.

Table II. Biological Properties of 5,8-Dideazafolates


compd	Y	R	X	IC ₅₀ , μM		
				TS (L1210) inhibn	L1210 growth inhibn	DHFR (WI-L2) inhibn
10a	NH ₂	CH ₂ C≡CH	3,4-Cl ₂	insol ^a	insol	insol
10b	NH ₂	CH ₂ C≡CH	4-COCH ₃	0.77	4.3	37.3
10c	NH ₂	CH ₂ C≡CH	3,4,5-(OMe) ₃	insol	insol	insol
10d	NH ₂	CH ₂ C≡CH	4-F	11.5	26.1	insol
10e	NH ₂	CH ₂ C≡CH	4-CN	1.3	5.9	66.6
10f	NH ₂	CH ₂ C≡CH	4-CONH ₂	0.64	>>20	72.6
10g	NH ₂	CH ₂ C≡CH	4-SO ₂ NMe ₂	1.0	4.1	20.3
10h	NH ₂	CH ₂ C≡CH	4-NO ₂	0.62	2.4	22.6
10i	NH ₂	CH ₂ C≡CH	4-COCF ₃	0.51	11.8	10.2
10j	NH ₂	CH ₂ C≡CH	4-OCF ₃	11.3	25.1	ND ^c
13a	H	CH ₂ C≡CH	3,4-Cl ₂	11	4.0	ND
13b	H	CH ₂ C≡CH	4-COCH ₃	2.4	7.0	100
13c	H	CH ₂ C≡CH	3,4,5-(OMe) ₃	96	>10	66.6
13k	H	CH ₂ C≡CH		>>100	>10	ND
18c	H	H	3,4,5-(OMe) ₃	>>500	>10	63.3
1	NH ₂	CH ₂ C≡CH	4-COglu	0.05	3.0	7.2
2	H	CH ₂ C≡CH	4-COglu	0.16 ^b	0.4 ^b	ND

^a Insol = not tested due to insolubility. ^b Literature¹² value. ^c ND = not determined.

inhibition with an IC₅₀ of 2.4 μM, comparable to that of 1 with an IC₅₀ of 3.0 μM.

The less well represented 2-desamino target compounds 13a-c,k also exhibited significant inhibition of L1210 TS and L1210 growth in culture. Compound 13b (X = 4-COCH₃) exhibited the most potent TS inhibition with an IC₅₀ of 2.4 μM, which was 15-fold less potent than the literature value for 2. Within this 2-desamino series, a comparison of the modest TS inhibition of the propargylated 13c (X = 3,4,5-(OCH₃)₃) with the complete lack of inhibition of despropargylated 18c (X = 3,4,5-(OCH₃)₃) emphasizes the importance of the N-10 propargyl group. This is similar to that reported in the 2-amino- and 2-desaminoglutamate-containing series.^{7,12} Compound 13a (X = 3,4-Cl₂) exhibited the most potent L1210 growth inhibition in this series with an IC₅₀ of 4.0 μM, 10-fold less potent than the literature value for the reference compound 2.

2-Amino compounds 10a-j and 2-desamino compounds 13a-c,k did inhibit WI-L2 DHFR as shown in Table II, but with much less potency than TS. Furthermore, the cytotoxicity of both classes of these compounds against L1210 cells in culture was completely reversed by the addition of thymidine to the culture medium as illustrated in Table III. These studies indicate that 10 and 13 are cytotoxic by virtue of their inhibition of TS and not some other enzyme, for example DHFR.

These results indicate that the glutamate moieties of 1 and 2 are not absolutely necessary for potent inhibition of L1210 TS and L1210 growth in culture. We are presently expanding the scope of this finding in an effort to discover more potent nonclassical inhibitors of TS.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Mass spectra were determined on a Finnigan 4000 mass spectrometer with an INCOS 2300 data system using direct introduction and electron impact at 70 eV and 150 °C. ¹H NMR spectra were determined at 90 MHz on a Varian EM-390, at 100 MHz on an IBM WP100SY, or at 200 MHz on a Varian XL-200 spectrometer in Me₂SO-*d*₆ or CDCl₃

Table III. Effects of Thymidine on L1210 Growth Inhibition of 5,8-Dideazafolates

compd ^a	without thymidine, % of control	with thymidine, ^b % of control
10b	34	102
10d	69	101
10g	20	96
10h	7	95
13b	35	90
1	11	92

^a Drug concentration was 10 μM. ^b Thymidine concentration was 10 μM.

with tetramethylsilane as an internal standard. All compounds with reported NMR spectra also had IR spectra consistent with their structure as determined on a Nicolet 205X FT/IR. Elemental analyses were determined by the microanalytical laboratory at Parke-Davis. TLC was performed with E. Merck silica gel 60 F-254 precoated glass plates (0.25 mm). Flash column chromatography was performed with E. Merck silica gel (230-400 mesh). Concentrations were performed on a rotary evaporator at ≤45 °C (20 Torr) unless otherwise noted.

Scheme I. 2-Amino-6-methyl-4(3H)-quinazolinone (4). A suspension of ethyl 2-amino-5-methylbenzoate (17.9 g, 0.1 mol, 3), chloroformamide hydrochloride¹⁸ (17.2 g, 0.15 mol), and dimethyl sulfone (50 g) was stirred and heated at 150 °C for 1 h. Water was added to the thick mixture, followed by concentrated NH₄OH to make the suspension basic. The solid was filtered off, washed with H₂O, and dried to give 17.32 g (99%) of the product as a white solid, mp >250 °C. This material was identical with that prepared with guanidine.¹⁵

2-Amino-6-[[2-propynyl(3,4,5-trimethoxyphenyl)amino]-methyl]-4(3H)-quinazolinone 0.3-Hydrate (10c). A suspension of 2-amino-6-(bromomethyl)-4(3H)-quinazolinone hydrobromide (1.00 g, 2.99 mmol, 7),¹⁸ 3,4,5-trimethoxy-N-2-propynylbenzenamine (0.66 g, 2.99 mmol, 8c), and dry CaCO₃ (0.30 g, 2.99 mmol) in *N,N*-dimethylacetamide (25 mL, DMA) was stirred at room temperature for 18 h. The suspension was filtered and the solid was washed with DMA. The filtrate and washings were concentrated (50 °C, 1 mm) to give a brown syrup. This was dissolved in EtOH and treated with silica gel (15 g, 230-400 mesh). The EtOH was evaporated and the powder was applied to a column of silica gel (225 g, 230-400 mesh) packed in CH₂Cl₂/MeOH (10:1). Elution with the same solvent gave 0.75 g of

material. Recrystallization from EtOH gave 0.61 g (52%) of the product as a grey solid: mp 236–238 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.20 (distorted t, 1, C \equiv CH), 3.57 (s, 3, 4-OCH₃), 3.70 (s, 6, 3- and 5-(OCH₃)₂), 4.13 (distorted d, 2, CH₂C \equiv CH), 4.50 (s, 2, NCH₂), 6.17 (s, 2, H2 and -6 of Ph), 6.40 (br s, 2, NH₂, exchangeable), 7.20 (d, *J* = 9.2 Hz, 1, H8), 7.57 (dd, *J* = 9.2 Hz, 2.5 Hz, 1, H7), 7.87 (distorted d, 1, H5), 10.97 (br s, 1, NH, exchangeable); MS *m/e* = 394 (M⁺). Anal. (C₂₁H₂₂N₄O₄·0.3H₂O) C, H, N, H₂O.

N-[6-[(3,4-Dichlorophenyl)-2-propynylamino]methyl]-3,4-dihydro-4-oxo-2-quinazolinyl]-2,2-dimethylpropanamide (9a). A suspension of *N*-[6-(bromomethyl)-3,4-dihydro-4-oxo-2-quinazolinyl]-2,2-dimethylpropanamide (1.00 g, 2.96 mmol, 6),¹⁵ 3,4-dichloro-*N*-2-propynylbenzeneamine (0.65 g, 3.2 mmol, 8a), and dry CaCO₃ (0.59 g, 5.9 mmol) in DMA (20 mL) was stirred at 60 °C for 24 h. The suspension was poured into an H₂O/ice mixture and stirred. The suspension was extracted with EtOAc. The EtOAc solution was dried (MgSO₄) and concentrated to give 1.79 g of an oil. Crystallization from EtOH (50 mL) gave 0.89 g (66%) of the product as an off-white solid. This was not characterized but used directly in the next reaction.

Compounds 9b,d-j were prepared similarly to 9a. Their melting points and yields are listed in Table I.

2-Amino-6-[[3,4-dichlorophenyl]-2-propynylamino]methyl]-4(3H)-quinazolinone (10a). To MeOH (150 mL) saturated with NH₃ at 0 °C was added 9a (0.89 g, 1.9 mmol). The solution was stirred at room temperature for 18 h, then cooled, resaturated with NH₃, and stirred at room temperature another 18 h. The solution was filtered and concentrated to give a solid. This was dissolved in boiling EtOH (500 mL). The solution was concentrated to 100 mL, cooled, and filtered to give 0.61 g (84%) of the product as an off-white solid: mp 251–253 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.24 (t, *J* = 2.1 Hz, 1, C \equiv CH), 4.25 (d, *J* = 2.0 Hz, 2, CH₂C \equiv CH), 4.60 (s, 2, NCH₂), 6.40 (br s, 2, NH₂, exchangeable), 6.80 (dd, *J* = 9.1 Hz, 2.9 Hz, 1, H6 of Ph), 7.01 (d, *J* = 2.9 Hz, 1, H2 of Ph), 7.18 (d, *J* = 8.5 Hz, 1, H8), 7.38 (d, *J* = 9.0 Hz, 1, H5 of Ph), 7.51 (dd, *J* = 8.5 Hz, 2.2 Hz, 1, H7), 7.79 (d, *J* = 2.0 Hz, 1, H5), 11.07 (br s, 1, NH, exchangeable); MS *m/e* = 372 (M⁺). Anal. (C₁₈H₁₄Cl₂N₄O) C, H, N.

The following compounds (10b,d-j) were prepared similarly to 10a.

6-[[4-Acetylphenyl]-2-propynylamino]methyl]-2-amino-4(3H)-quinazolinone (10b): 84.8% yield; off-white solid; mp 255–258 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.45 (s, 3, CH₃), 3.27 (distorted t, 1, C \equiv CH), 4.37 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.70 (s, 2, NCH₂), 6.40 (br s, 2, NH₂, exchangeable), 6.87 (d, *J* = 10 Hz, 2, H2 and -6 of Ph), 7.18 (d, *J* = 9 Hz, 1, H8), 7.50 (dd, *J* = 2 Hz, 9 Hz, 1, H7), 7.67 (distorted s, 1, H5), 7.82 (d, *J* = 9 Hz, 2, H3 and -5 of Ph), 10.89 (br s, 1, NH, exchangeable); MS *m/e* = 346 (M⁺). Anal. (C₂₀H₁₈N₄O₂) C, H, N.

2-Amino-6-[[4-fluorophenyl]-2-propynylamino]methyl]-4(3H)-quinazolinone (10d): 56.8% yield; white solid; mp 251–252 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.17 (t, *J* = 2.1 Hz, 1, C \equiv CH), 4.11 (d, *J* = 2.1 Hz, 2, CH₂C \equiv CH), 4.49 (s, 2, NCH₂), 6.35 (br s, 2, NH₂, exchangeable), 6.88 (m, 2, H2 and -6 of Ph), 7.04 (m, 2, H3 and -5 of Ph), 7.18 (d, *J* = 8.5 Hz, 1, H8), 7.50 (dd, *J* = 2.1 Hz, 8.4 Hz, 1, H7), 7.81 (d, *J* = 1.6 Hz, 1, H5), 11.0 (br s, 1, NH, exchangeable); MS *m/e* = 322 (M⁺). Anal. (C₁₈H₁₅FN₄O) C, H, N.

4-[[2-Amino-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-2-propynylamino]benzotrile hemihydrate (10e): 82.2% yield; cream solid; mp 251–253 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.27 (distorted t, 1, C \equiv CH), 4.35 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.70 (s, 2, NCH₂), 6.37 (br s, 2, NH₂, exchangeable), 6.87 (d, *J* = 10 Hz, 2, H3 and -5 of Ph), 7.17 (d, *J* = 9 Hz, 1, H8), 7.48 (dd, *J* = 2 Hz, 10 Hz, 1, H7), 7.59 (d, *J* = 10 Hz, 2, H2 and -6 of Ph), 7.77 (d, *J* = 2 Hz, 1, H5), 11.03 (br s, 1, NH, exchangeable); MS *m/e* = 329 (M⁺). Anal. (C₁₈H₁₅N₅O·0.5H₂O) C, H, N (insoluble for Karl Fischer H₂O analysis).

4-[[2-Amino-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-2-propynylamino]benzamide (10f): 76% yield; off-white solid; mp >275 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.21 (distorted t, 1, C \equiv CH), 4.28 (d, *J* = 1.7 Hz, 2, CH₂C \equiv CH), 4.66 (s, 2, NCH₂), 6.34 (br s, 2, NH₂, exchangeable), 6.82 (d, *J* = 8.7 Hz, 2, H3 and -5 of Ph), 7.00 (br s, 1, CONH, exchangeable), 7.18 (d, *J* = 8.5 Hz, 1, H8), 7.51 (dd, *J* = 2.1 Hz, 8.5 Hz, 1, H7), 7.67 (br s, 1,

CONH, exchangeable), 7.73 (d, 2, *J* = 8.9 Hz, 2, H2 and -6 of Ph), 7.80 (d, *J* = 2.7 Hz, 1, H5), 11.0 (br s, 1, NH, exchangeable); MS *m/e* = 347 (M⁺). Anal. (C₁₉H₁₇N₅O₂) H, N, C: calcd, 65.69; found, 65.14.

4-[[2-Amino-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-2-propynylamino]-*N,N*-dimethylbenzenesulfonamide 1.7-hydrate (10g): 72.9% yield; white solid; mp 247–250 °C dec; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.28 (distorted t, 1, C \equiv CH), 3.36 (s, 6, N(CH₃)₂), 4.35 (distorted d, 2, CH₂C \equiv CH), 4.72 (s, 2, NCH₂), 6.36 (br s, 2, NH₂, exchangeable), 6.97 (d, *J* = 9.0 Hz, 2, H3 and -5 of Ph), 7.21 (d, *J* = 8.4 Hz, 1, H8), 7.55 (m, 3, H2 and -6 of Ph and H7), 7.81 (distorted d, 1, H5), 11.01 (br s, 1, NH, exchangeable). Anal. (C₂₀H₂₁N₅O₃S·1.7H₂O) C, H, N, S, H₂O.

2-Amino-6-[[4-nitrophenyl]-2-propynylamino]methyl]-4(3H)-quinazolinone 0.25-hydrate (10h): 47% yield; yellow solid; mp 254–259 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.30 (distorted t, 1, C \equiv CH), 4.43 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.78 (s, 2, NCH₂), 6.35 (br s, 2, NH₂, exchangeable), 6.88 (d, *J* = 9 Hz, 2, H2 and -6 of Ph), 7.17 (d, *J* = 9 Hz, 1, H8), 7.49 (dd, *J* = 9 Hz, 2 Hz, 1, H7), 7.77 (d, *J* = 2 Hz, 1, H5), 8.10 (d, *J* = 9 Hz, 2, H3 and -5 of Ph); MS *m/e* = 349 (M⁺). Anal. (C₁₈H₁₅N₅O₃·0.25H₂O) C, H, N (insoluble for Karl Fischer H₂O analysis).

2-Amino-6-[[2-propynyl[4-(trifluoroacetyl)phenyl]amino]methyl]-4(3H)-quinazolinone 0.3-hydrate (10i): 94.5% yield; off-white solid; mp 231–234 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.33 (distorted t, 1, C \equiv CH), 4.47 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.82 (s, 2, NCH₂), 6.40 (br s, 2, NH₂, exchangeable), 7.00 (d, *J* = 9 Hz, 2, H2 and -6 of Ph), 7.18 (d, *J* = 9 Hz, 1, H8), 7.50 (dd, *J* = 2 Hz, 9 Hz, 1, H7), 7.78 (d, *J* = 2 Hz, 1, H5), 7.88 (d, *J* = 9 Hz, 2, H3 and -5 of Ph), 10.89 (br s, 1, NH, exchangeable); MS *m/e* = 400 (M⁺). Anal. (C₂₀H₁₅F₃N₄O₂·0.3H₂O) C, H, N (insoluble for Karl Fischer H₂O analysis).

2-Amino-6-[[2-propynyl[4-(trifluoromethoxy)phenyl]amino]methyl]-4(3H)-quinazolinone (10j): 95% yield; white solid; mp 247–249 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.23 (distorted t, 1, C \equiv CH), 4.23 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.58 (s, 2, NCH₂), 6.68 (br s, 2, NH₂, exchangeable), 6.87 (d, *J* = 10 Hz, 2, H2 and -6 of Ph), 7.20 (d, *J* = 10 Hz, 3, H3 and -5 of Ph and H8), 7.53 (dd, *J* = 2 Hz, 8 Hz, 1, H7), 7.83 (d, *J* = 2 Hz, 1, H5), 11.12 (br s, 1, NH, exchangeable); MS *m/e* = 388 (M⁺). Anal. (C₁₉H₁₅F₃N₄O₂) C, H, N.

Scheme II. [4-Oxo-6-[[2-propynyl(3,4,5-trimethoxyphenyl)amino]methyl]-3(4H)-quinazolinyl]methyl 2,2-Dimethylpropanoate (12c). A suspension of [6-(bromomethyl)-4-oxo-3(4H)-quinazolinyl]methyl 2,2-dimethylpropanoate (1.00 g, 2.16 mmol, 11),¹² 8c (0.64 g, 2.88 mmol), and dry CaCO₃ (0.58 g, 5.8 mmol) in DMA (20 mL) was stirred at room temperature for 18 h. The suspension was poured into an H₂O/ice mixture and extracted with EtOAc. The EtOAc solution was dried (MgSO₄) and concentrated to give 1.84 g of a brown oil. This was dissolved in a minimum amount of PhCH₃ and applied to a column of silica gel (100 g, 230–400 mesh) packed in PhCH₃/EtOAc (5:1). Elution with the same solvent gave 1.08 g (100% assuming 75% pure 11) of the product as a light brown oil. This was not characterized but used directly in the next reaction.

Compounds 12a,b,k were prepared similarly to 12c. Their appearance and yields are listed in Table I.

The following compounds (13a–c,k) were prepared similarly to 10a.

6-[[3,4-Dichlorophenyl]-2-propynylamino]methyl]-4(3H)-quinazolinone (13a): 79% yield; white solid; mp 211–213 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.26 (t, *J* = 2.3 Hz, 1, C \equiv CH), 4.30 (d, *J* = 2.4 Hz, 2, CH₂C \equiv CH), 4.74 (s, 2, NCH₂), 6.78 (dd, *J* = 9.0 Hz, 3.0 Hz, 1, H6 of Ph), 7.01 (d, *J* = 3.0 Hz, 1, H2 of Ph), 7.38 (d, *J* = 9.0 Hz, 1, H5 of Ph), 7.65 (d, *J* = 8.5 Hz, 1, H8), 7.73 (dd, *J* = 8.5 Hz, 2.1 Hz, 1, H7), 8.01 (d, *J* = 1.5 Hz, 1, H5), 8.07 (d, *J* = 2.8 Hz, 1, H2, coalesces to s upon D₂O addition), 12.2 (br s, 1, NH, exchangeable); MS *m/e* = 357 (M⁺). Anal. (C₁₈H₁₃Cl₂N₃O) C, H, N.

6-[[4-Acetylphenyl]-2-propynylamino]methyl]-4(3H)-quinazolinone (13b): 73.0% yield; pink solid; mp 197–199 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 2.42 (s, 3, CH₃), 3.27 (t, *J* = 2 Hz, 1, C \equiv CH), 4.42 (d, *J* = 2 Hz, 2, CH₂C \equiv CH), 4.85 (s, 2, NCH₂), 6.85 (d, *J* = 9.3 Hz, 2, H2 and -6 of Ph), 7.69 (m, 2, H7 and -8), 7.81 (d, *J* = 9.3 Hz, 2, H3 and -5 of Ph), 8.00 (distorted s, 1, H5), 8.07 (s, 1, H2), 12.2 (br s, 1, NH, exchangeable); MS *m/e* = 331

(M⁺). Anal. (C₂₀H₁₇N₃O₂) C, H, N.

6-[[2-Propynyl(3,4,5-trimethoxyphenyl)amino]methyl]-4-(3H)-quinazolinone (13c): 73% yield; white solid; mp 167–169 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.20 (t, *J* = 2.1 Hz, 1, C≡CH), 3.56 (s, 3, 4-OCH₃), 3.69 (s, 6, 3- and 5-(OCH₃)₂), 4.19 (d, *J* = 2.1 Hz, 2, CH₂C≡CH), 4.64 (s, 2, NCH₂), 6.15 (s, 2, H₂ and -6 of Ph), 7.66 (d, *J* = 8.4 Hz, 1, H₈), 7.80 (dd, *J* = 8.4 Hz, 2.0 Hz, 1, H₇), 8.07 (s, 1, H₂), 8.10 (d, *J* = 1.9 Hz, 1, H₅), 12.2 (br s, 1, NH, exchangeable); MS *m/e* = 379 (M⁺). Anal. (C₂₁H₂₁N₃O₄) C, H, N.

6-[[5-Chloro-2-pyridinyl]-2-propynylamino]methyl]-4-(3H)-quinazolinone (13k): 64.5% yield; white solid; mp 178–181 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.15 (t, *J* = 2.3 Hz, 1, C≡CH), 4.39 (d, *J* = 2.2 Hz, 2, CH₂C≡CH), 4.91 (s, 2, NCH₂), 6.77 (d, *J* = 9.1 Hz, 1, H₃), of pyridine, 7.66 (m, 2, H₈ and H₄ of pyridine), 7.74 (dd, *J* = 8.4 Hz, 1.9 Hz, 1, H₇), 8.01 (d, *J* = 1.6 Hz, 1, H₅), 8.07 (s, 1, H₂), 8.17 (d, *J* = 2.6 Hz, 1, H₆ of pyridine); 12.24 (br s, 1, NH, exchangeable); MS *m/e* = 324 (M⁺). Anal. (C₁₇H₁₃-ClN₄O) C, H, N.

Scheme III. 4-(2-Propynylamino)benzotrile (8e). A suspension of 4-aminobenzotrile (23.63 g, 0.20 mol), 3-bromo-1-propyne (80% solution in PhCH₃, 32.72 g, 0.22 mol), and *N*-ethyl-*N*-(1-methylethyl)-2-propanamine (38.3 mL, 0.22 mol) in PhCH₃ (500 mL) was heated at 90 °C for 18 h. EtOAc was added to the cooled reaction suspension, and it was washed three times with H₂O. The organic layer was dried (MgSO₄) and concentrated to give 32.5 g of a dark brown oil which crystallized on standing. This was dissolved in a minimum amount of PhCH₃ and applied to a column of silica gel (1 kg, 230–400 mesh) packed in PhCH₃/EtOAc (20:1). Elution with the same solvent gave 24.66 g (78.9%) of the product as an off-white solid: mp 68–70 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.15 (t, *J* = 2 Hz, 1, C≡CH), 3.95 (dd, *J* = 2 Hz, 7 Hz, 2, CH₂), 6.70 (d, *J* = 9 Hz, 2, H₃ and -5), 7.03 (t, *J* = 7 Hz, 1, NH), 7.50 (d, *J* = 9 Hz, 2, H₂ and -6); MS *m/e* = 156 (M⁺). Anal. (C₁₀H₈N₂) C, H, N.

The following compounds (8a–d, f, g, j, k) were prepared similarly to 8e.

3,4-Dichloro-*N*-2-propynylbenzenamine (8a): 79% yield; yellow liquid; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.13 (t, *J* < 3 Hz, 1, C≡CH), 3.92 (d, *J* < 3 Hz, 2, CH₂), 6.48 (br s, 1, NH, exchangeable), 6.64 (dd, *J* = 8.9 Hz, 2.8 Hz, 1, H₆), 6.84 (d, *J* < 3 Hz, 1, H₂), 7.32 (d, *J* = 8.9 Hz, 1, H₅); MS *m/e* = 199 (M⁺). Anal. (C₉H₇Cl₂N) C, H, N.

1-[4-(2-Propynylamino)phenyl]ethanone (8b): 47% yield; yellow solid; mp 97–100 °C; NMR (90 MHz, CDCl₃) δ = 2.33 (t, *J* = 3 Hz, 1, C≡CH), 2.56 (s, 3, CH₃), 4.07 (d, *J* = 3 Hz, 2, CH₂), 4.25 (br s, 1, NH), 6.73 (m, 2, H₃ and -5); 7.92 (m, 2, H₂ and -6); MS *m/e* = 173 (M⁺). Anal. (C₁₁H₁₁NO) C, H, N.

3,4,5-Trimethoxy-*N*-2-propynylbenzenamine (8c): 82.5% yield; yellow solid; mp 62–63 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.07 (t, *J* = 2.5 Hz, 1, C≡CH), 3.54 (s, 3, 4-OCH₃), 3.70 (s, 6, 3- and 5-(OCH₃)₂), 3.85 (dd, *J* = 2.5 Hz, 7.0 Hz, 2, CH₂, coalesces to d, *J* = 2.5 Hz, on D₂O addition), 5.76 (t, *J* = 7.0 Hz, 1, NH, exchangeable), 5.95 (s, 2, H₂ and -6); MS *m/e* = 221 (M⁺). Anal. (C₁₂H₁₅NO₃) C, H, N.

4-Fluoro-*N*-2-propynylbenzenamine (8d): 61% yield; yellow oil; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.04 (t, *J* = 2.3 Hz, 1, C≡CH), 3.86 (dd, *J* = 2.4 Hz, 6.3 Hz, 2, CH₂, coalesces to d, *J* = 2.3 Hz, on D₂O addition), 5.90 (t, *J* = 6.2 Hz, 1, NH, exchangeable), 6.61 (m, 2, H₂ and -6), 7.0 (m, 2, H₃ and -5); MS *m/e* = 149 (M⁺). Anal. (C₉H₈FN-0.15EtOAc) C, H, N.

4-(2-Propynylamino)benzamide (8f): 38.7% yield; yellow solid; mp 139–141 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.10 (t, *J* = 2 Hz, 1, C≡CH), 3.90 (dd, *J* = 2 Hz, 7 Hz, 2, CH₂, coalesces to d, *J* = 2 Hz, upon D₂O addition), 6.50 (t, *J* = 6 Hz, 1, NH, exchangeable), 6.60 (d, *J* = 9 Hz, 2, H₃ and -5), 6.92 (br s, 1, CONH, exchangeable), 7.60 (br s, 1, CONH, exchangeable), 7.67 (d, *J* = 9 Hz, 2, H₂ and -6); MS *m/e* 174 (M⁺). Anal. (C₁₀H₁₀N₂O) C, H, N.

***N,N*-Dimethyl-4-(2-propynylamino)benzenesulfonamide (8g)**: 50% yield from *N,N*-dimethyl-4-aminobenzenesulfonamide;²² off-white solid; mp 107–111 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 2.5 (s, 6, N(CH₃)₂), 3.1 (t, *J* = 2.7 Hz, 1, C≡CH),

3.9 (dd, *J* = 2.7 Hz, 6.3 Hz, 2, CH₂, coalesces to d, *J* = 2.7 Hz, upon D₂O addition), 6.76 (m, 2, H₃ and -5), 6.98 (t, *J* = 6.3 Hz, 1, exchangeable), 7.5 (m, 2, H₂ and -6); MS *m/e* = 238 (M⁺). Anal. (C₁₁H₁₄N₂O₂S) C, H, N.

***N*-2-Propynyl-4-(trifluoromethoxy)benzenamine (8j)**: 66.2% yield; brown liquid; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.07 (t, *J* = 2.3 Hz, 1, C≡CH), 3.89 (dd, *J* = 2.4 Hz, 6.2 Hz, 2, CH₂, coalesces to d, *J* = 2.4 Hz, upon D₂O addition), 6.28 (t, *J* = 6.2 Hz, 1, NH, exchangeable), 6.70 (d, *J* = 9.0 Hz, 2, H₂ and -6), 7.11 (d, *J* = 8.2 Hz, 2, H₃ and -5); MS *m/e* = 215 (M⁺). Anal. (C₁₀H₈F₃NO) C, H, N.

5-Chloro-*N*-2-propynyl-2-pyridinamine (8k): 4% yield; white solid; mp 68–70 °C; NMR (90 MHz, CDCl₃) δ = 2.15 (t, *J* = 2 Hz, 1, C≡CH), 4.0 (dd, *J* = 2 Hz, 7 Hz, 2, CH₂), 4.6 (br s, 1, NH), 6.34 (d, *J* = 9 Hz, 1, H₃), 7.3 (dd, *J* = 3 Hz, 9 Hz, 1, H₄), 8.02 (d, *J* = 3 Hz, 1, H₆).

Scheme IV. 4-Nitro-*N*-2-propynylbenzenamine (8h). A suspension of 4-fluoronitrobenzene (26.6 g, 0.189 mol, 14h), 2-propynylamine (20.8 g, 0.377 mol), potassium fluoride (11 g, 0.189 mol), and potassium carbonate (26 g, 0.188 mol) in DMSO (600 mL) was stirred at room temperature under N₂ for 20 h. The suspension was poured into H₂O (1.5 L) and stirred for 2 h. The yellow solid was collected and washed with H₂O and then 2-propanol to give 16.27 g (49%) of the product as a yellow solid: mp 150–153 °C (lit.¹⁹ mp 149–151 °C); NMR (90 MHz, CDCl₃) δ = 2.30 (t, *J* = 2.4 Hz, 1, C≡CH), 4.04 (dd, *J* = 2.3 Hz, 5.7 Hz, 2, CH₂), 4.74 (br s, 1, NH), 6.65 (m, 2, H₂ and -6), 8.14 (m, 2, H₃ and -5). MS *m/e* = 176 (M⁺). Anal. (C₉H₈N₂O₂) C, H, N.

2,2,2-Trifluoro-1-[4-(2-propynylamino)phenyl]ethanone (8i). To a solution of 2,2,2-trifluoro-1-(4-fluorophenyl)ethanone²³ (8.64 g, 0.045 mol, 14i) and Et₃N (7.53 mL, 0.054 mol) in CH₃CN (150 mL), cooled by an ice bath, was added 2-propynylamine (31.13 mL, 0.45 mol). The ice bath was removed and the solution was heated under reflux for 18 h. The reaction solution was cooled and concentrated to give a solid, which was partitioned between EtOAc and H₂O. The EtOAc layer was dried (MgSO₄) and concentrated to give 10.23 g of a red-brown oil. This was applied to a column of silica gel (250 g, 230–400 mesh) packed in *n*-heptane/CH₂Cl₂ (2:1). Elution with the same solvent gave 4.05 g (39.6%) of the product as a fluffy white solid: mp 96–98 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.22 (t, *J* = 2.4 Hz, 1, C≡CH), 4.06 (dd, *J* = 2.5 Hz, 5.9 Hz, 2, CH₂, coalesces to s, *J* = 2.5 Hz, upon D₂O addition), 6.80 (d, *J* = 9.1, 2, H₃ and -5), 7.71 (t, *J* = 5.7 Hz, 1, NH, exchangeable), 7.86 (d, *J* = 9.0 Hz, 2, H₂ and -6); MS *m/e* = 227 (M⁺).

Scheme V. 2-Nitro-5-[[3,4,5-trimethoxyphenyl]amino]methyl]benzoic Acid Methyl Ester (16). To a solution of 5-(bromomethyl)-2-nitrobenzoic acid methyl ester²⁰ (2.74 g, 0.01 mol, 15) in MeOH (50 mL) was added Et₃N (2.79 mL, 0.02 mol) and 3,4,5-trimethoxybenzenamine (1.83 g, 0.01 mol, 14c). The resulting solution was stirred at 25 °C for 18 h and then concentrated. The residue was dissolved in EtOAc, and the solution was washed with water, dried (MgSO₄), and concentrated. The residue was dissolved in a minimum amount of PhCH₃ and applied to a column of silica gel (100 g, 230–400 mesh) packed in PhCH₃/EtOAc (5:1). Elution with the same solvent gave 1.76 g (46.9%) of the product as a red gum which solidified on standing: mp 109–113 °C; NMR (200 MHz, Me₂SO-*d*₆) δ = 3.51 (s, 3, 4-OCH₃), 3.66 (s, 6, 3- and 5-(OCH₃)₂), 3.85 (s, 3, OCH₃), 4.42 (s, 2, CH₂), 5.89 (s, 2, H₂ and -6 of Ph), 6.3 (br s, 1, NH, exchangeable), 7.76 (d, *J* = 1.3 Hz, 1, H₆), 7.82 (d, *J* = 6.9 Hz, 1, H₄), 8.07 (d, *J* = 8.2 Hz, 1, H₃); MS *m/e* = 376 (M⁺).

2-Amino-5-[[3,4,5-trimethoxyphenyl]amino]methyl]benzoic Acid Methyl Ester (17). A suspension of 16 (1.64 g, 4.36 mmol) in THF (50 mL) and MeOH (50 mL) was shaken with Raney Ni (0.5 g) in a stainless steel Parr apparatus at an initial pressure of 51 psi for 21 h. The mixture was filtered to remove the catalyst and then concentrated to a gum. This was dissolved in a minimum amount of CH₂Cl₂ and applied to a column of silica gel (125 g, 230–400 mesh) packed in CH₂Cl₂/MeOH (50:1). Elution with the same solvent gave 1.0 g (67%) of the product as a light yellow oil which solidified on standing: mp 131–134 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.53 (s, 3, 4-OCH₃), 3.68 (s,

(22) Walker, J. J. *Chem. Soc.* 1940, 686.

(23) Herkes, F. E.; Burton, D. J. *J. Org. Chem.* 1967, 32, 1311.

6, 3- and 5-(OCH₃)₂, 3.80 (s, 3, OCH₃), 4.07 (d, *J* = 6 Hz, 2, CH₂, coalesces to s on D₂O addition), 5.90 (s, 2, H₂ and -6 of Ph), 5.9 (m, 1, NH, exchangeable), 6.56 (s, 2, NH₂, exchangeable), 6.73 (d, *J* = 8 Hz, 1, H₃), 7.30 (d, *J* = 8 Hz, 1 H₄), 7.73 (s, 1, H₆); MS *m/e* = 346 (M⁺).

6-[[[(3,4,5-Trimethoxyphenyl)amino]methyl]-4(3H)-quinazolinone (18c). A suspension of 17 (0.73 g, 2.1 mmol) and formamidine acetate (2.0 g, 19.2 mmol) in EtOH (75 mL) was heated under reflux for 5 h. More formamidine acetate (2.2 g, 2.1 mmol) was added to the reaction and it was heated under reflux for another 18 h. TLC (SiO₂: CH₂Cl₂/MeOH (10:1)) indicated the presence of much starting material. More formamidine acetate (10 g, 96.1 mmol) was added to the reaction, and it was again heated under reflux for 18 h. Formamidine acetate (10 g, 96.1 mmol) was again added to the reaction, and it was heated under reflux for another 18 h. The suspension was concentrated to give a semisolid which was partitioned between H₂O and EtOAc. Some material did not dissolve and this was collected to give 0.11 g of a white solid. The resulting layers were separated, and the H₂O layer was extracted with EtOAc. The organic layers were combined, dried (MgSO₄), and concentrated to give 1.46 g of a red oil which solidified on standing. This material was combined with the 0.11 g of white solid above and recrystallized first from 95% EtOH and then from CH₃CN to give 0.27 g (38%) of the product as a white solid: mp 205–208 °C; NMR (100 MHz, Me₂SO-*d*₆) δ = 3.49 (s, 3, 4-OCH₃), 3.61 (s, 6, 3- and 5-(OCH₃)₂), 4.39 (d, *J* = 6 Hz, 2, CH₂, coalesces to s on D₂O addition), 5.88 (s, 2, H₂ and -6 of Ph), 6.24 (t, *J* = 6 Hz, 1, NHCH₂, exchangeable), 7.63 (d, *J* = 8 Hz, 1, H₈), 7.84 (dd, *J* = 8 Hz, 2 Hz, 1, H₇), 8.04 (s, 1, H₂), 8.14 (d, *J* = 2 Hz, 1, H₅), 12.20 (br s, 1, NH, exchangeable); MS *m/e* = 341 (M⁺). Anal. (C₁₈H₁₉N₃O₄) C, H, N.

Purification and Assay of Thymidylate Synthase. Thymidylate synthase was isolated from a strain of L1210 cells (L1210:C15, kindly supplied by Dr. Hilary Calvert) which overproduce the enzyme by 45-fold.²⁴ Purification was achieved by affinity chromatography by the method described by Jackman et al.²⁵ Enzyme activity was determined by a modification of

the tritium release assay as described by Roberts.²⁶ In brief, the reaction was performed at 37 °C over 30 min in 13 × 75 mm glass tubes containing reaction buffer (0.275 mL; 10 mM Na₂HPO₄, 10 mM NaF, 10 mM β-mercaptoethanol, pH 7.4), 5,10-methylenetetrahydrofolate (50 μL, final concentration 100 μmol), [³H]deoxyuridine monophosphate (50 μL, final concentration 50 μmol, specific activity 40 μCi/μmol), appropriately diluted enzyme (100 μL), and inhibitor or solvent (25 μL). The reaction was terminated by the addition of acid-washed charcoal suspended in 1 N HCl (200 μL). The tubes were thoroughly mixed and spun for 5 min at 2000g, the supernatant (0.5 mL) was removed and added to Ready-gel (Beckman, 10 mL), and the radioactivity was determined in a Beckman LS 6800 scintillation counter.

Purification and Assay of Dihydrofolate Reductase. Dihydrofolate reductase was isolated from a strain of human lymphoblastoid cells (WI-L2/MTX, kindly supplied by Dr. James Freisheim) which overproduces the enzyme by 50-fold. The enzyme was purified by affinity chromatography by the method of Whiteley et al.²⁷ Enzyme assays were performed by the following procedure: in a 3-mL quartz cuvette, 0.05 M Tris Cl buffer (2.73 mL), 5 mM NADPH (0.02 mL), inhibitor or solvent (0.1 mL), and appropriately diluted enzyme (0.1 mL) were mixed and warmed to 37 °C. The reaction was started by addition of 2 mM dihydrofolate (0.05 mL) and the decrease in absorbance was monitored at 340 nm with a Cary Model 219 spectrophotometer.

Cell Growth Inhibition and Protection Assays. All experiments employed L1210 mouse leukemia cells grown as a suspension culture in RPMI 1640 medium (Gibco Laboratories) supplemented with 10% fetal bovine serum and 50 μg/mL gentamycin. The doubling time was 12–13 h and viability was greater than 95% by trypan blue exclusion. Dilutions of test compounds in 20 μL were placed into wells of 24-well Linbro plates (1.7 × 1.6 cm, flat bottom) followed by the addition of L1210 cells (3 × 10⁴/mL) in 2 mL of media. Plates were incubated for 3 days at 37 °C in a humidified atmosphere containing 5% CO₂ in air. Cell growth was determined by cell count with a Coulter Model ZM electronic cell counter (Coulter Electronics, Inc., Hialeah, FL). Protection experiments were performed in an identical manner except protecting agents were included along with the drug.

(24) Jackman, A. L.; Alison, D. L.; Calvert, A. H.; Harrap, K. R. *Cancer Res.* 1986, 46, 2810.

(25) Jackman, A. L.; Calvert, A. H.; Hart, L. I.; Harrap, K. R. In *Advances in Experimental Medical Biology 165B*; DeBruyn, C. H. M. M., Simmonds, H. A., Muller, M. M., Eds.; Plenum: New York, 1984; pp 375–378.

(26) Roberts, D. *Biochemistry* 1966, 5, 3546.

(27) Whiteley, J. M.; Jackson, R. C.; Mell, G. P.; Drais, J. H.; Hu-
ennekens, F. M. *Arch. Biochem. Biophys.* 1972, 150, 15.