Synthesis and Biological Evaluation of 2-Styrylquinazolin-4(3*H*)-ones, a New Class of Antimitotic Anticancer Agents Which Inhibit Tubulin Polymerization

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A novel series of 2-styrylquinazolin-4(3*H*)-ones which inhibited tubulin polymerization and the growth of L1210 murine leukemia cells was discovered. Extensive structure–activity relationship studies suggest that the entire quinazolinone structure was required, but activity was further enhanced by halide or small hydrophobic substituents at position 6. These analogues did not substantially interfere with the binding of radiolabeled colchicine, vinblastine, or GTP to tubulin and weakly stimulated GTP hydrolysis uncoupled from polymerization. Several analogues have shown in vivo tumor growth inhibitory activity in the L1210 leukemia model, with the lead compound **5o** exhibiting good antitumor activity against murine solid tumors as well as human tumor xenografts.

Evaluation of synthetic compounds and natural products for potential antineoplastic activity continues to be an important method for the initial identification of new drugs with possible clinical value. Such studies in our laboratories demonstrated that 2-styrylquinazolin-4(3*H*)-one (**5j**) had significant growth inhibitory activity against L1210 murine leukemia cells. We decided to prepare a series of analogues of this compound to see if agents with still greater potency could be obtained. In this report we describe the synthesis and initial evaluation of this group of compounds as antitumor agents.

Studies to determine the mechanism of action of this new class of cytotoxic compounds were also conducted. We found that the most active members of the group caused cells to accumulate in metaphase arrest. Like virtually all drugs with this property, the 2-styrylquinazolin-4(3*H*)-ones interact with the major component of the mitotic spindle, the protein tubulin, and inhibit its polymerization into microtubules.[1] Clinically, the most important inhibitors of tubulin polymerization remain the vinca alkaloids vincristine and vinblastine. An additional mitotic inhibitor with clinical promise is taxol, which does not inhibit microtubule assembly. Instead, taxol, through an interaction with tubulin, causes formation of highly stable microtubules which cannot depolymerize.[2] A new class of mitotic inhibitors thus is of interest both for its potential as a source of chemotherapeutic agents and for insights it may provide into the structure and function of microtubules and the key role they play in cell division.

Synthesis

The construction of the quinazolinone ring has been extensively described in the literature.[3] In this work, three different general approaches to the 2-styrylquinazolin-4-(3*H*)-one system have been employed, depending on starting material availability (Scheme I). Tables I–V summarize the synthetic routes, yields, and biological properties of the compounds whose synthesis is described below.

Amidation of anthranilonitrile (**1**) or anthranilic acid (**2**) gave rise to the respective cinnamide intermediates **3** and **4**. Oxidative ring closure of **3** under acidic conditions afforded quinazolinone **5** in various yields (method A).[4] The same product can be obtained by condensation reaction of **4** with formamide at 160 °C (method C).[5] When substituted aniline **6** was used, the corresponding cinnamide intermediate **7** was treated with urethane **8** in the presence of P_2O_5 at elevated temperatures to give, via acylation followed by dehydration, quinazolinone **5** (method B).[6]

Water-soluble analogues were sought because of the highly insoluble nature of these quinazolinones. An attempt to convert chloro analogue **5a** directly into amino analogues resulted in Michael adduct **9** (method D, Scheme II). This process was reversed, however, upon treatment with acid. Thus a number of water-soluble hydrochloride salts of analogues bearing a cyclic amino group were synthesized in two steps (method E, Scheme II).

Reduction of the 6-nitro analogue gave rise to the anilino quinazolinone **5i** whose HCl salt failed to show appreciable water solubility (method F, Scheme III).

Since the 6-MeO analogue **5o** appeared to be the most active compound in vivo, efforts were made to synthesize its prodrugs in the hope of improving the solubility and bioavailability of **5o**. Thus **5o** was hydrolyzed in refluxing aqueous HBr to give hydroxy analogue **5p**, which upon treatment with acetic anhydride or isotoic anhydride furnished the respective acetate **5u** and anthranate **5v** (method G, Scheme IV).

The synthesis of 2-alkylquinazolin-4(3*H*)-ones is exemplified by the synthesis of analogue **15a** (method H, Scheme V) via benzoxazinone **14**, which gave us an entry to the heteroaromatic analogues[7] of the 2-styryl compounds as shown (method I, Scheme V). Benzoxazinone **16a** was converted to the 3-*N*-amino analogue **16b** upon treatment with hydrazine hydrate at 110 °C. Heating the benzoxazinone at 110 °C before the addition of hydrazine appeared to be crucial, as otherwise Michael adduct **16c** dominated as shown in Scheme V.

Modification at the carbonyl position resulted in several structurally interesting analogues. 4-Thione analogue **17** was obtained from a reaction of **1a** with P_2S_5 in refluxing pyridine (method J, Scheme VI). Other examples include the formation of imidoyl chloride (**18**) followed by ami-

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‡ Du Pont.

§ NIH.

(1) Hamel, E. In *Microtubule Proteins*, Avila, J., Ed.; CRC Press: Boca Raton, FL, 1990; p 89.
(2) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
(3) For example: Armarego, W. L. F.; Adv. Heterocyc. Chem. **1979**, *24*, 16.
(4) Brown, D. J. Fused Pyrimidines. In *The Chemistry of Heterocyclic Compounds*; Vol. 23, Page 80, Interscience: New York, 1967; Vol 23, p 80, references cited therein.
(5) Patel, V. S.; Patel, S. R. *J. Indian Chem. Soc.* **1965**, *42*, 531.
(6) Chakraborty, D. P.; Mandal, A. K.; Roy, S. K. *Synthesis* **1981**, 977–979.
(7) Misra, R. S.; Dwivedi, C.; Parmar, S. S. *J. Heterocycl. Chem.* **1980**, *17*, 1337–1338; Varma, R. S.; Bahadur, S.; Agnihotri, A. K. *J. Chem. Eng. Data* **1981**, *26*, 103–104.

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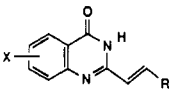
Table I. 2-Styrylquinazolin-4(3*H*)-ones

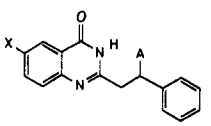


5a-w

entry	X	R	synthetic method	% yield[a]	mp, °C	formula[b]	inhibn tubulin polym IC_{50} ± SD, μM	L1210 in vitro IC_{50}, μg/mL	L1210 in vivo % T/C (mg/kg)
5a	6-Cl	Ph	A	61	315–320	$C_{16}H_{11}ClN_2O$	2.5 ± 0.2	0.001	146 (50)
5b	5-Cl	Ph	A	6.5	288–293	$C_{16}H_{11}ClN_2O$	>40	>1	100 (100)
5c	7-Cl	Ph	C	8.3	257–262	$C_{16}H_{11}ClN_2O$	17 ± 6	>1	NT[d]
5d	8-Cl	Ph	C	17.7	296–301	$C_{16}H_{11}ClN_2O$	>40	>1	NT
5e	6-Br	Ph	C	3.8	334–338	$C_{16}H_{11}BrN_2O$	2.0 ± 0.1	0.01	133 (25)
5f	6-I	Ph	C	12	336–342	$C_{16}H_{11}IN_2O$	1.4 ± 0.05	0.3	117 (200)
5g	6-F	Ph	C	4.7	274–275	$C_{16}H_{11}FN_2O$	5.4 ± 0.9	0.05	129 (100)
5h	6-Cl	Me	A	2.3	216–222	$C_{11}H_9ClN_2O$	>40	>1	95 (200)
5i	6-NH_2	Ph	F	5.2	257–260	$C_{16}H_{13}N_3O$	>40	0.6	NT
5j	H	Ph	A	28.8	220–228	$C_{16}H_{12}N_2O$	11 ± 0.8	0.02	133 (240)
5k	6-Me	Ph	C	3.5	274–276	$C_{17}H_{14}N_2O$	2.0 ± 0.1	0.02	107 (25)
5l	6-F,7-Cl	Ph	C	15.0	296–301	$C_{16}H_{10}ClFN_2O$	>40	0.007	111 (100)
5m	6,7-$(MeO)_2$	Ph	C	13.0	296–301	$C_{18}H_{16}N_2O_3$	3.3 ± 0.6	0.03	96 (200)
5n	5-Me	Ph	C	4.5	275–279	$C_{17}H_{14}N_2O$	>40	0.03	100 (200)
5o	6-MeO	Ph	B	8.9	287–291	$C_{17}H_{14}N_2O_2$[c]	2.0 ± 0.4	0.001	173 (50)
5p	6-HO	Ph	G	6.7	323–327	$C_{16}H_{12}N_2O_2$	>40	0.006	99 (100)
5q	6-F,7-piperidinyl	Ph	E	2.6	237–239	$C_{21}H_{20}FN_3O$	>40	0.2	85 (200)
5r	6-EtO	Ph	B	4.2	307–311	$C_{18}H_{16}N_2O_2$	>40	0.4	NT
5s	6-F,7-*N*-Me-piperazinyl	Ph	E	8.0	288–295	$C_{21}H_{21}FN_4O$	>40	0.2	100 (50)
5t	7-MeO	Ph	B	6.7	275–278	$C_{17}H_{14}N_2O_2$	5.7 ± 0.7	0.001	100 (200)
5u	6-AcO	Ph	G	4.6	293–298	$C_{18}H_{14}N_2O_3$	4.0 ± 1.0	0.3	78 (200)
5v	6-anthra	Ph	G	5.3	304–306	$C_{23}H_{17}N_3O_3$	>40	>1	99 (150)
5w	H	Me	A	13.4	195–198	$C_{11}H_{10}N_2O$	>40	>1	NT

[a] Overall yields from commerical starting materials; products are spectroscopically and chromatographically homogeneous (0.95%). [b] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds, unless otherwise indicated. [c] C: calcd, 73.36; found, 72.90. [d] NT = not tested.

Table II. Michael Adducts of 2-Styrylquinazolinones

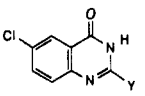


9a-c

entry	X	A	synthetic method	% yield[a]	mp, °C	formula[b]	inhibn tubulin polym IC_{50} ± SD μM	L1210 in vitro IC_{50}, μg/mL	L1210 in vivo % T/C (mg/kg)
9a	6-Cl	*N*-Me-piperazinyl	D	100	158–160	$C_{21}H_{23}ClN_4O$	5.3 ± 0.5	NT[c]	100 (100)
9b	6-Cl	*N*-morpholino	D	60	177–179	$C_{20}H_{20}ClN_3O_2$	>40	NT	125 (200)
9c	6-MeO	*N*-piperidyl	D	100	284–287	$C_{22}H_{25}N_3O_2$	14 ± 0.8	0.001	107 (100)

[a] One-step yields from styrylquinazolinones; products are spectroscopically and chromatographically homogeneous (0.95%). [b] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds, unless otherwise indicated. [c] NT = not tested.

Table III. 2-Substituted Quinazolin-4(3*H*)-ones

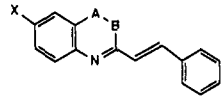


15a-e

entry	Y	synthetic method	% yield[a]	mp, °C	formula[b]	inhibn tubulin polym IC_{50} ± SD μM	L1210 in vitro IC_{50}, μg/mL	L1210 in vivo % T/C (mg/kg)
15a	Me	H	41.3	>325	$C_9H_7ClN_2O$	>40	>1	NT[d]
15b	phenylethynyl	A	18.8	296–298	$C_{16}H_9ClN_2O$	>40	0.002	125 (200)
15c	$Ph(CH_2)_2$	A	76.0	245–250	$C_{16}H_{13}ClN_2O$	7.3 ± 0.1	0.01	113 (200)
15d	$PhCH_2$	A	9.3	256–260	$C_{15}H_{11}ClN_2O$	>40	>1	NT
15e		I	28.1	334–337	$C_{15}H_{10}ClN_3O$[c]	>40	0.61	91 (200)

[a] Overall yields from commerical starting materials; products are spectroscopically and chromatographically homogeneous (0.95%). [b] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds, unless otherwise indicated. [c] C: calcd, 63.49; found, 62.98. [d] NT = not tested.

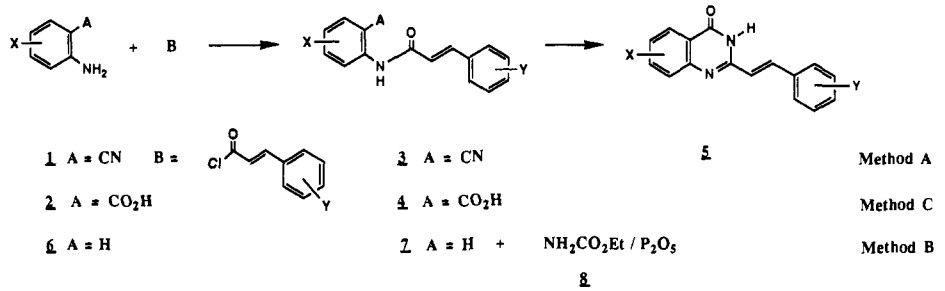
Table IV. Modification at the 3,4-Positions



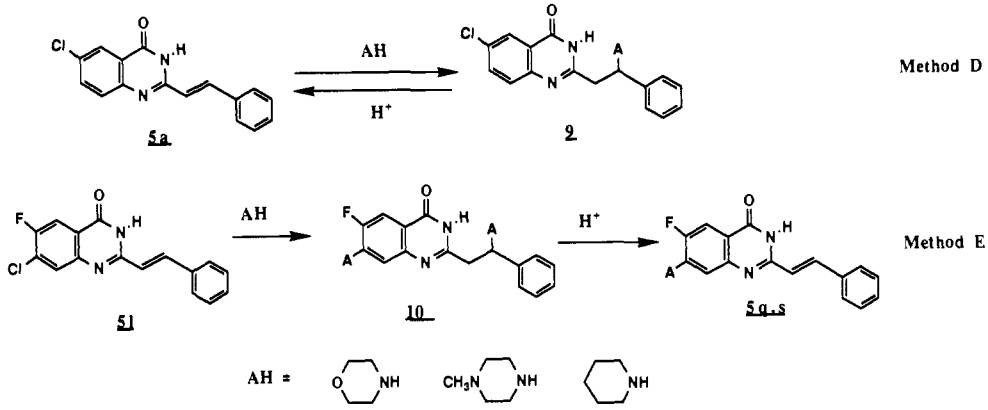
entry	X	A–B	synthetic method	% yield[a]	mp, °C	formula[c]	inhibn tubulin polym IC_{50}, μM	L1210 in vitro IC_{50}, μg/mL	L1210 in vivo % T/C (mg/kg)
16b	Cl	CONNH	H	30.7	171–174	$C_{16}H_{12}ClN_3O$	>40	>1	90 (50)
17	Cl	CSNH	J	25.4	284–289	$C_{16}H_{11}ClN_2S$	>40	>1	94 (50)
19	Cl	RNC=N[b]	K	24.0	275–280	$C_{25}H_{31}ClN_4$	>40	NT[d]	105 (12)
22	H	SO_2NH	L	35.2	344–350	$C_{15}H_{12}N_2O_2S$	>40	>1	91 (200)

[a] Overall yields from commerical starting materials; products are spectroscopically and chromatographyically homogeneous (0.95%). [b] R: $CH(CH_3)(CH_2)_3NEt_2$ HCl. [c] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds, unless otherwise indicated. [d] NT = not tested.

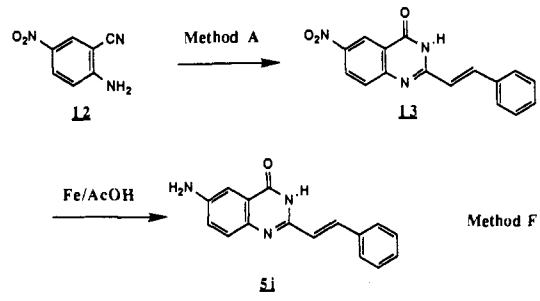
Scheme I



Scheme II



Scheme III



nation, which gave rise to 4-amino analogue **19** (method K, Scheme VI). Benzothiadiazine **22**, an analogue with the carbonyl replaced by a sulfoxide group, was synthesized according to the reaction shown in Scheme VII (method L). When cinnamide intermediate **3a** was N-methylated, the corresponding *N*-methyl 2-styrylquinazolin-4(3*H*)-one (**24**) was obtained after ring closure under the conditions described in method A (Scheme VII).

To study the debenzo system, the pyrimidine analogue was synthesized by reacting amidine **32**[8] with keto ester **33** to give dimethylpyrimidine **29** (Scheme VIII).

Biological Studies

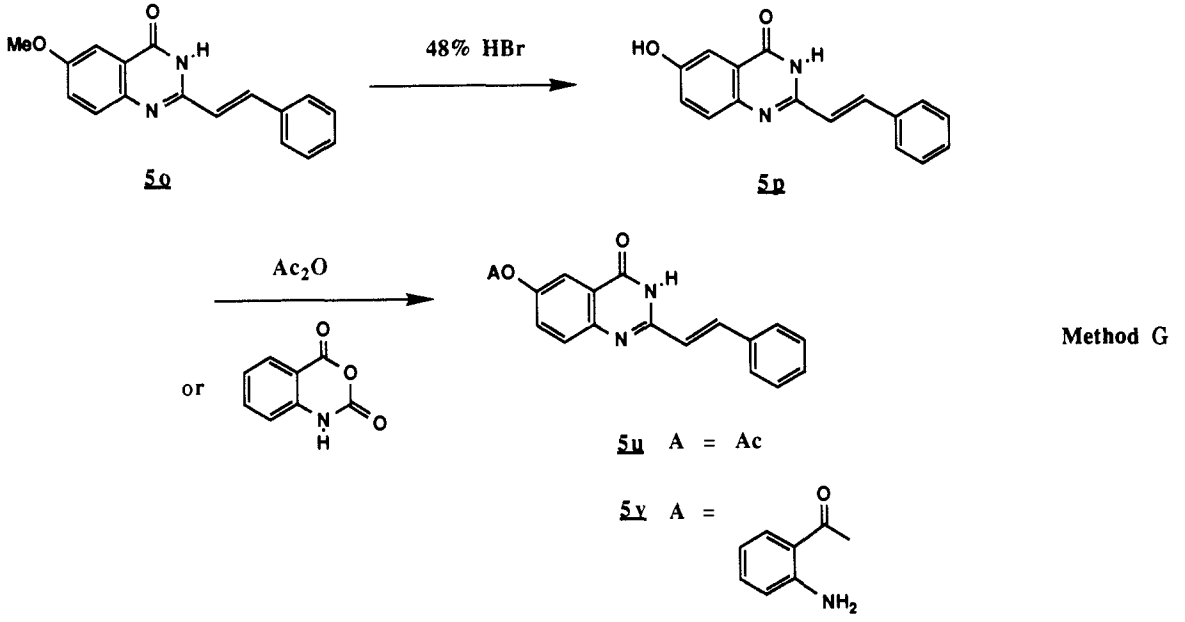
Estimation of the cytotoxicity of the 2-styrylquinazolin-4(3*H*)-ones and related compounds for L1210 murine leukemia cells in culture (the MTT assay[9]) provided the initial evaluation of these new agents. Our data are summarized in Tables I–V. Of the compounds examined, with only three exceptions (**9c**, **15c**, and **24**), all agents with significant tumor cell inhibitory activity (IC_{50} value less than 0.1 μg/mL), contained an intact 2-styrylquinazolin-4(3*H*)-one structure.

These studies were followed by an examination of the therapeutic efficacy of the 2-styrylquinazolin-4(3*H*)-ones for L1210 murine leukemia in vivo, with the drug injected into the peritoneum one day after the ip innoculation of tumor cells. These data, too, are summarized in Table I–V. Only five compounds (**5a**, **5e**, **5g**, **5j**, and **5o**) showed significant prolongation of life-span in the treated as compared to the control animals, with compound **5o** yielding the best results. This compound was therefore examined

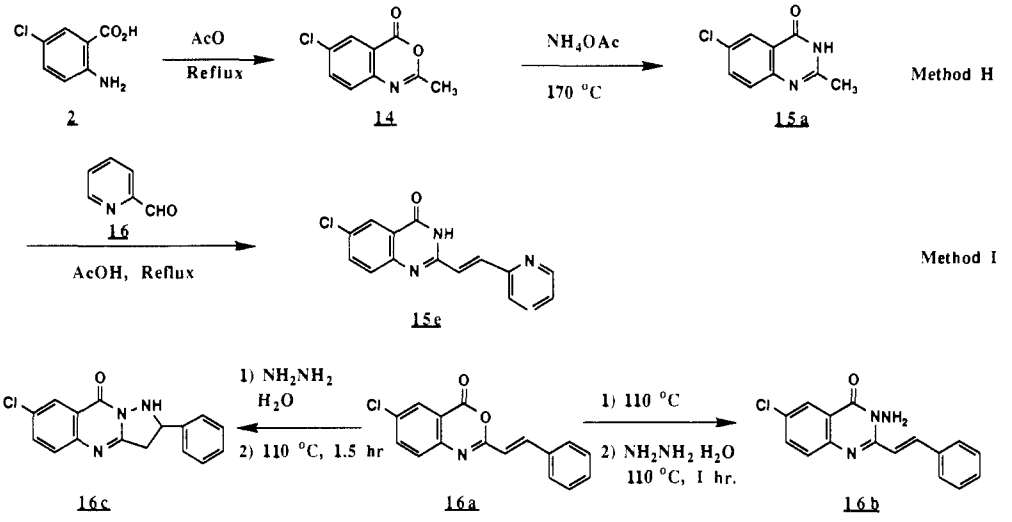
(8) Schaefer, F. C.; and Peters, G. A. *J. Org. Chem.* **1961**, *26*, 412.

(9) Ruben, R. L. Cell culture for testing anticancer compounds. In *Advances in Cell Culture*; Maramorosch, K., Ed.; Academic Press: New York, 1988; Vol. 6.

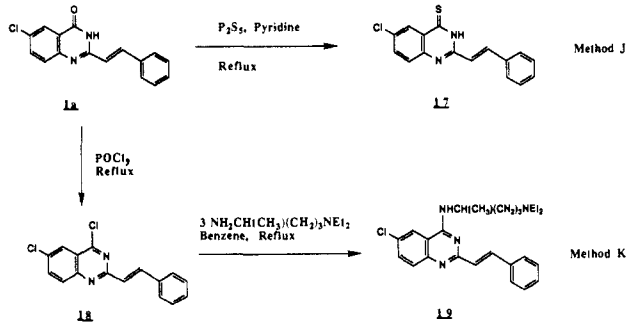
Scheme IV



Scheme V



Scheme VI



in a number of additional murine tumor systems, including human tumor xenografts in nude mice, and significant activity was observed in five additional tumor models (Table VI)–P388 leukemia, B16 melanoma, M531 sarcoma, the MX-1 mammary xenograft, and the LOX melanoma xenograft.

When studies to determine the mechanism of action of the 2-styrylquinazolin-4(3*H*)-ones demonstrated potent inhibition of both microtubule assembly dependent on microtubule-associated proteins and the polymerization of purified tubulin (see below), several of the most inhibitory compounds were reexamined in L1210 murine leukemia cells to determine whether they were mitotic inhibitors. At cytotoxic concentrations compounds **5a**, **5e**, **5k**, **5m**, and **5o** all caused the accumulation of large numbers of cells arrested in metaphase. Mitotic cells ranged from 24 to 42%, compared to the control value of 4%, confirming that these compounds represent a new class of mitotic inhibitors which act at the level of tubulin polymerization.

Initial studies on the effects of the 2-styrylquinazolin-4(3*H*)-ones on tubulin polymerization were performed with compound **5o**. We found that low concentrations of the drug inhibited microtubule assembly dependent on microtubule-associated proteins (Figure 1A). In reaction mixtures containing 15 μM tubulin, microtubule assembly was progressively inhibited as the concentration of compound **5o** was increased from 2 to 10 μM. No further decrease in turbidity development occurred at higher compound concentrations, but the residual reaction probably represents nonspecific tubulin aggregation since it was not cold-reversible (Figure 1A, curves 4 and 5).

The glutamate-dependent polymerization of purified tubulin (Figure 1B) was even more sensitive to inhibition by compound **5o**, confirming that tubulin is the specific drug target.[10] The extent of the polymerization reaction

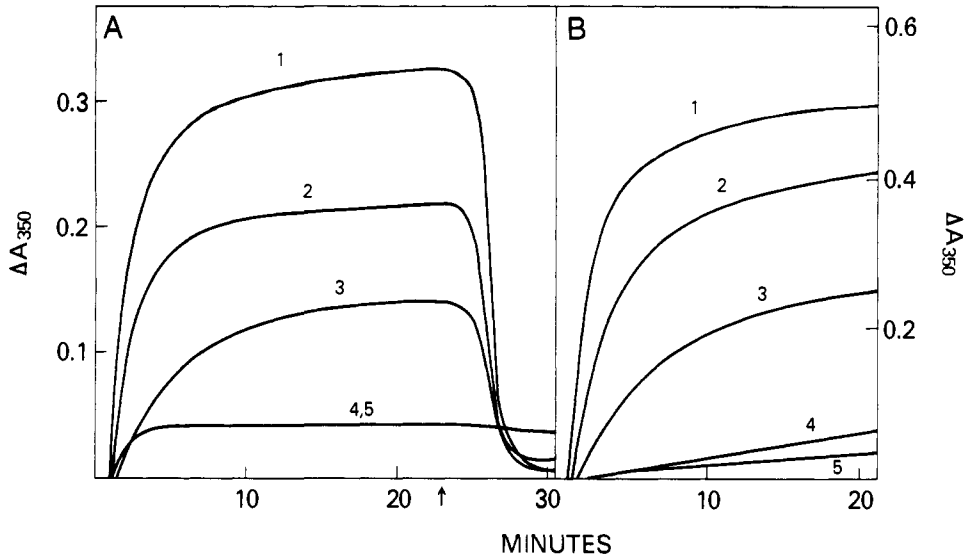
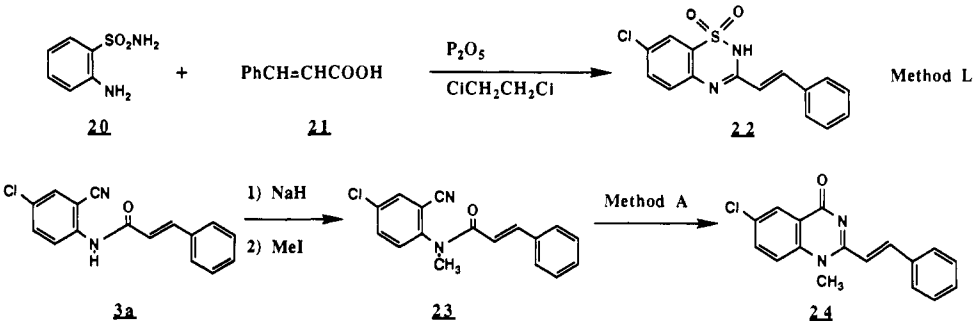
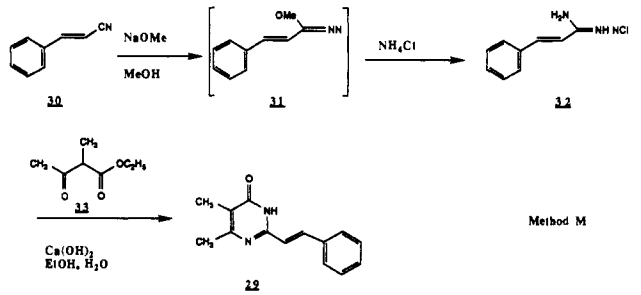


Figure 1. Inhibition of tubulin polymerization by compound **5o**. (A) Polymerization dependent on microtubule-associated proteins is shown. Each 0.25 mL of reaction mixture contained 1.5 mg/mL (15 μM) purified tubulin and 0.5 mg/mL heat-treated microtubule-associated proteins (both protein preparations were purified as described in ref 4), 0.1 M 4-morpholineethane sulfonate adjusted to pH 7.0 with NaOH, 0.5 mM $MgCl_2$, 0.5 mM GTP, 4% (v/v) dimethyl sulfoxide, and the following concentrations of compound **5o**: curve 1, none; curve 2, 2 μM; curve 3, 6 μM; curve 4, 10 μM; curve 5, 20 μM. Samples were placed in cuvettes maintained at 0 °C by an electronic temperature controller. At zero time the controller was set at 37 °C (temperature equilibration complete in about 70–75 s). At the time indicated by the arrow on the abcissa, the controller was set again at 0 °C (temperature equilibration complete in about 6 min). (B) Polymerization dependent on 1.0 M glutamate is shown. Final reaction volume was 0.25 mL, and all concentrations are expressed in terms of this final volume. Each reaction mixture contained 1.0 mg/mL (10 μM) purified tubulin, 1.0 M monosodium glutamate adjusted to pH 6.6 with HCl, 1.0 mM $MgCl_2$, 4% (v/v) dimethyl sulfoxide, and the following concentrations of compound **5o**: curve 1, none; curve 2, 1 μM; curve 3, 2 μM; curve 4, 3 μM; curve 5, 4 μM. The 0.24-mL reaction mixtures were preincubated for 15 min at 37 °C in a water bath and chilled on ice, and 10 μL of 10 mM GTP was added to each reaction. They were transferred to cuvettes held at 0 °C by the temperature controller, which was set at 37 °C at zero time.

Scheme VII



Scheme VIII



with 10 μM tubulin was inhibited by about 50% by 2μM compound, and total inhibition (as judged by cold-reversibility of low-grade-turbidity readings) occurred with 4 μM compound **5o**.

The glutamate system was chosen for detailed quantitative comparison of the series of 2-styrylquinazolin-4-(3*H*)-ones and related compounds. The data are summarized in Tables I–V and are presented in terms of an IC_{50} value representing the drug concentration required to inhibit the extent of polymerization by 50%. Methodology has been described previously,[11] except that in the studies presented here IC_{50} values were obtained graphically in triplicate experiments.

In most cases there was good correlation between cytotoxicity and inhibition of tubulin polymerization. Nine compounds inhibited both tubulin polymerization and the growth of L1210 cells, and 21 analogues were not inhibitory in either system. A few analogues either were good tumor cell growth inhibitors with little activity as inhibitors of polymerization (compounds **5l**, **5n**, **5p**, and **24**) or effective inhibitors of polymerization with little growth inhibitory effect against tumor cells (compounds **5c**, **5f**, and **5u**). These discrepancies are not understood at present, but may result from differences in drug transport or intracellular metabolism.

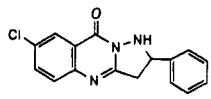
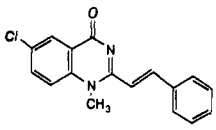
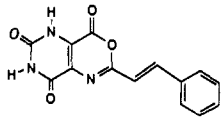
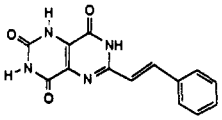
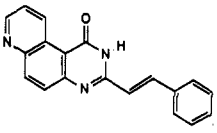
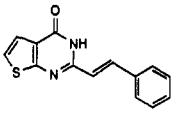
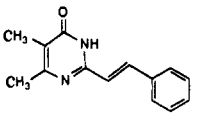
Discussion—Structure-Activity Correlates

The quantitative comparisons of the 2-styrylquinazolin-4(3*H*)-ones and related compounds using the

(10) Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173.

(11) Batra, J. K.; Kang, G. J.; Jurd, L., Hamel, E. *Biochem. Pharmacol.* **1988**, *37*, 2595.

Table V. Other Ring Systems

entry	synthetic method	% yield[a]	mp, °C	formula[b]	inhibn tubulin polym IC_{50}, μM	L1210 in vitro IC_{50}, μg/mL	L1210 in vivo % T/C (mg/kg)
 1.6c	H	11.3	241–246	$C_{16}H_{11}ClN_2S$	>40	>1	NT[c]
 2.4	A	64.9	241–246	$C_{17}H_{13}ClN_2O$	>40	0.04	96 (50)
 2.5	H	70.6	311–315	$C_{14}H_9N_3O_4$	>40	>1	94 (50)
 2.6	H	22.0	>360	$C_{14}H_{10}N_4O_3$	>40	>1	93 (50)
 2.7	A	4.5	350–355	$C_{19}H_{13}N_3O$	>40	0.4	95 (50)
 2.8	A	2.0	252–256	$C_{14}H_{10}N_2OS$	>40	>1	92 (200)
 2.9	M	22.1	237–240	$C_{14}H_{14}N_2O$	>40	>1	89 (50)

[a] Overall yields from commerical starting materials; products are spectroscopically and chromatographyically homogeneous (0.95%). [b] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained for all new compounds, unless otherwise indicated. [c] NT = not tested.

Table VI. Antitumor Activities of 6-Methoxy-2-styrylquinazolin-4(3*H*)-one (**5o**)

in vivo tumor[a]	schedule (ip)	% T/C (dose, mg/kg)
P388 (ip)[1]	Q1D × 5	174 (120), 135 (60)
L1210 (ip)[1]	Q1D × 5	225 (120), 158 (60)
B16 (ip)[2]	Q1D × 9	197 (200), 145 (100), 129 (50)
	Q4D × 3	190 (400), 158 (300), 141 (200)
M531(ip)[3]	Q4D × 4	154 (240), 136 (120)
MX-1 (SRC)[4]	Q4D × 3	-81 (240), 16 (120), 21 (60)
LOX (SC)[5]	Q4D × 3	153 (240), 142 (120), 142 (60)

[a] 1: Murine leukemia, active T/C > 125%; 2: murine melanoma, active T/C > 125%; 3: murine sarcoma, active T/C > 125%; 4: human mammary tumor xenograft, active T/C < 20%; 5: human melanoma xenograft, active T/C > 125%.

tubulin polymerization assay, as summarized in Tables I–V, permit a structure–activity analysis of the interactions of this class of compounds with their probable intracellular target. The reaction examined is extremely simple, for in glutamate only GTP is required for polymerization to occur. Aside from compounds **9a**, **9c**, and **15c** (discussed below), inhibition of polymerization only occurred with agents that contained the unmodified 2-styrylquinazolin-4(3*H*)-one structure. Even apparently minor changes in the basic structure of the parent molecule (**5j**), such as replacing the ketone oxygen with a sulfur (**17**) or the phenyl ring with a pyridine ring (**15e**), yielded inactive analogues. Further, within the limits of available analogues, substituents at position 3 (**16b**), 5 (**5b** and **5n**), or 8 (**5d**) resulted in complete loss of inhibitory effects on the polymerization reaction.

Two analogues with substituents at position 7 were prepared, and they had opposite effects relative to the activity of the parent compound **5j** (IC_{50}, 11 μM). A methoxy group at position 7 yielded compound **5t** (IC_{50}, 5.7 μM) that was twice as active as **5j**, but a chlorine atom at position 7 resulted in about a 50% loss of activity (**5c**; IC_{50}, 17 μM).

Many analogues with substituents at position 6 had inhibitory activity significantly greater than that of the unsubstituted compound **5j**. All halide substituents had enhanced activity, and inhibition increased as the halide atom increased in size: from an IC_{50} value of 5.4 μM with fluorine (**5g**) to 2.5 μM with chlorine (**5a**) to 2.0 μM with bromine (**5e**) to 1.4 μM with iodine (**5f**). In fact, the iodinated compound was the most inhibitory agent in the whole group in the tubulin polymerization assay, although it appeared to have only limited growth inhibition toward L1210 cells. A second group of substituents at position

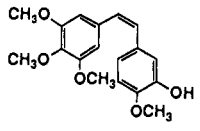
6 that enhanced activity of the 2-styrylquinazolin-4-(3*H*)-ones, relative to the parent compound **5j**, consisted of small hydrophobic groups—methyl, methoxy, and acetyl (**5k**, **5o**, and **5u**), with IC_{50} values of 2.0, 2.0, and 4.0 μM, respectively. Bulkier groups (including ethoxy, **5r**) or small hydrophilic substituents (**5p**) yielded inert compounds.

Two compounds with substituents at both positions 6 and 7 should be noted. Compound **5m** with methoxy groups at both positions was a potent inhibitor of polymerization, with an IC_{50} value of 3.3 μM, intermediate between the 6-substituted (**5o**) and the 7-substituted (**5t**) compounds. Compound **5l** has a fluorine at position 6 and a chlorine at position 7, and it was noninhibitory. Apparently the undesirable effect of the chlorine substituent at position 7 (see above) is dominant in compound **5l**.

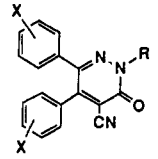
The sole exception for the requirement of an intact 2-styrylquinazolin-4(3*H*)-one structure for inhibition of tubulin polymerization occurred in compounds with additions across the 2-styryl double bond bridge. Simply reducing the double bond of compound **5a** (IC_{50}, 2.5 μM) resulted in about a 3-fold drop in activity (**15c**; IC_{50}, 7.3 μM). Much more damaging was either introduction of a triple bond in the bridge (**15b**) or elimination of one carbon atom from the bridge (**15d**). Interestingly, the Michael adduct of compound **5a** with an *N*-methylpiperazinyl group (**9a**) was about $^1/_2$ as active as **5a** itself, while introduction of a morpholino group (**9b**) yielded an inactive analogue. A Michael adduct derivative (**9c**) of compound **5o** had only 15% the activity of **5o**.

In summary, optimum activity in the inhibition of tubulin polymerization was observed in compounds which contained the 2-styrylquinazolin-4(3*H*)-one core structure intact, with either a halide (iodine optimal) or small hydrophobic (methyl or methoxy optimal) substituent at position 6.

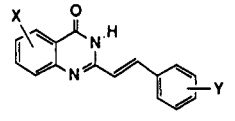
It is worth comparing the structure–activity relationships observed in the 2-styrylquinazolin-4(3*H*)-one class of antimitotic compounds with those observed in two superficially similar series of agents: natural products derived from the South African tree *Combretum caffrum*[12] and synthetic derivative of 5,6-diphenylpyridazin-3(2*H*)-one.[13] The most active agents in these two series and the most active 2-styrylquinazolin-4(3*H*)-ones have quantitatively similar inhibitory effects on tubulin polymerization.



Combretastatin A-4



5,6-Diphenyl-pyridazin-3-ones



2-Styryl-3H-quinazolin-4-ones

Like the 2-styrylquinazolin-4(3*H*)-one series, the *C. caffrum* agents contain a two-carbon bridge, separating two aromatic rings. The two-carbon separation of the aromatic rings is probably optimal. Maximally active *C. caffrum* compounds, however, are *cis*-stilbenes, with the trans and saturated analogues significantly less potent. Furthermore, the *trans*-stilbenes were less active than the saturated derivatives. A major difference between the *C. caffrum* agents and the 2-styrylquinazolin-4(3*H*)-ones is that the former are potent competitive inhibitors of the binding of colchicine to tubulin, while the latter have only weak effects on colchicine binding (data not presented).

The active derivatives of 5,6-diphenylpyridazin-3-(2*H*)-one contain a six-membered heterocyclic ring with two nitrogen atoms and a ketone function, although it is not identical with the comparable ring of the 2-styrylquinazolin-4(3*H*)-ones. Both series of compounds do not interfere greatly with the binding of other radiolabeled ligands (colchicine, vinblastine, GTP) to tubulin and perhaps share a specific, and different, drug binding site on the tubulin molecule. In addition, halide and methyl substituents on the phenyl rings enhance inhibitory effects of the 5,6-diphenylpyridazin-3(2*H*)-one derivatives, as occurs with the 2-styrylquinazolin-4(3*H*)-ones. The substituent effects, however, are not entirely identical: with the pyridazinone compounds chlorine is more effective than fluorine, which is in turn more effective than bromine; a second halide substituent further enhances inhibitory effects; and methoxy, in contrast to methyl, substituents result in inactive compounds.

Thus, we have discovered a novel series of antitumor agents with good solid-tumor activities in mice. Inhibition of microtubule formation appears to be the mechanism of action. Detailed characterization of the interactions of this class of agents with tubulin is in progress.

Experimental Section

General Procedures. Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover melting point apparatus. IR spectra were determined with either a Perkin-Elmer 1600 Series FTIR spectrophotometer or a Perkin-Elmer 1710 FTIR spectrophotometer. NMR spectra were determined with an IBM/Bruker 200SY (200 mHz) spectrometer containing tetrmethylsilane as internal standard. Microanalyses were performed by Micro-analysis, Inc., Wilmington, DE, and were within 0.4% of the calculated values, unless stated otherwise. Mass spectra were obtained on a Finnigan MAT 8230 double-focusing mass spectrometer. Chromatography was done using the flash column technique on silica gel 60 supplied by EM Science.

General Procedures for Preparing Cinnamide Intermediates 3, 4, and 7.[7] A solution of cinnamoyl chloride (25 mmol) in benzene (75 mL) was gradually added at 5 °C into a solution of the substituted aniline **1**, **2**, or **6** (25 mmol) and DMAP (2.5 mmol) in pyridine (200 mL) with stirring under nitrogen. The resulting yellowish slurry was stirred at 25 °C for 20 h and filtered, and the white solid was washed with benzene to give the corresponding cinnamide intermediate in good yields. The purity and structure of the products were determined by thin-layer chromatography, NMR, IR, MS, and melting point measurement.

Synthesis and Structural Modification of Quinazolinone. Data for all the analogues synthesized according to each method are summarized in Tables I–V.

Method A. 6-Chloro-2-styrylquinazolin-4(3*H*)-one (5a). To a mixture of amide intermediate **7a** (151.5 g, 54 mmol), 6 N NaOH (100 mL), and 95% EtOH (1200 mL) at 20–25 °C was added 30% H_2O_2 (182.2 mL, 1.6 M). The resultant mixture was stirred at reflux for 1 h, cooled, and acidified to pH 1.5 with concentrated HCl. The solid was filtered, washed with MeOH, and air-dried. Recrystallization from DMF gave the final product **5a** (115 g, 75%): NMR (DMSO-d_6) δ 7.90 (1 H, d, J = 16 Hz), 7.50–7.75 (8 H, m), 7.0 (1 H, d, J = 16 Hz); MS m/e 282. Anal. ($C_{16}H_{11}ClN_2O$) C, H, N.

Method B. 6-Methoxy-2-styrylquinazolin-4(3*H*)-one (5o). A mixture of amide intermediate **7o** (14 g, 50 mmol), ethyl carbamate (4.9 g, 50 mmol), and P_2O_5 (7.8 g, 50 mmol) in xylene (250 mL) was stirred at reflux for 6 h. The clear xylene supernatant was decanted while still hot, and the gummy residue was triturated

(12) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* 1988, *34*, 200.
(13) Batra, J. K.; Powers, L. J.; Hess, F. D.; Hamel, E. *Cancer Res.* 1986, *46*, 1889.

with hot methanol. After filtration, a yellowish solid was obtained, which was suspended in warm DMSO with stirring. The warm mixture was filtered, and the filtrate was treated with methanol, stirred at 25 °C for 30 min, and filtered to give the final product **5o** (1.9 g, 14%): mp 287–291 °C; TLC (5% methanol in methylene chloride on silica gel) showed one spot; NMR (DMSO-d_6) δ 7.89 (1 H, d, J = 16 Hz), 7.40–7.66 (8 H, m), 6.98 (1 H, d, J = 16 Hz), 3.88 (3 H, s); IR (KBr) 3062 (M), 1677 (S), 1646 (S) cm^{-1}; MS m/e 278. Anal. ($C_{17}H_{14}N_2O_2$) H, N; C: calcd, 73.36; found, 72.90.

Method C. 6-Iodo-2-styrylquinazolin-4(3*H*)-one (5f). A mixture of amide intermediate **7f** (40 g, 102 mmol) and formamide (300 mL) was stirred at 160 °C for 6 h. The white solid was filtered and recrystallized from DMF to afford product **5f** (7.9 g, 20.7%): NMR (DMSO-d_6) δ 7.95 (1 H, d, J = 15.5 Hz), 7.48–7.80 (8 H, m), 7.08 (1 H, d, J = 15.5 Hz); MS m/e 374. Anal. ($C_{16}H_{11}IN_2O$) C, H, N.

Method D. 6-Chloro-2-[2-phenyl-2-(*N*-methylpiperazin-1-yl)ethyl]quinazolin-4(3*H*)-one (9a). A mixture of quinazolinone **5a** (2.8 g, 10 mmol) and *N*-methylpiperazine (30 mL, 270 mmol) was stirred at reflux under nitrogen for 12 h, cooled to 25 °C, evaporated in vacuo, treated with ether, and filtered. Recrystallization from ether gave the product **9a** (2.0 g): NMR ($CDCl_3$) δ 14.0 (1 H, s), 7.95 (1 H, d, J = 12 Hz), 7.40–7.00 (7 H, m), 4.10 (1 H, d, J = 2 Hz), 3.45 (1 H, m), 3.15–2.10 (12 H, m); MS m/e 382. Anal. ($C_{21}H_{23}ClN_4O$) C, H, N.

Method E. 6-Fluoro-7-piperidinyl-2-styrylquinazolin-4-(3*H*)-one (5q). A mixture of quinazolinone **5l** (5 g, 16.7 mmol) and piperidine (50 mL) was stirred at reflux under nitrogen for 48 h and evaporated to yield a yellow semisolid, which was treated with acetone. The yellow, insoluble solid was filtered off and the filtrate was purified by chromatography (silica gel/EtOAc) to give intermediate **10**: NMR ($CDCl_3$) δ 13.25 (1 H, s), 7.80 (1 H, d, J = 13 Hz), 7.38–7.01 (6 H, m), 4.00 (1 H, d, J = 2 Hz), 3.5 (1 H, m), 3.20 (4 H, m), 2.84–1.59 (17 H, m).

Crude **10** was dissolved in methanol and treated with 1 N HCl to pH 1. After 30 min at 25 °C, the reaction mixture was evaporated in vacuo and the residue was chromatographed (silica gel/ethyl acetate) to afford product **5q** (0.9 g, 6.4%): NMR (DMSO-d_6) δ 8.10 (1 H, d, J = 16 Hz), 7.72–7.22 (8 H, m), 7.01 (1 H, d, J = 16 Hz), 3.18 (4 H, s), 1.66 (6 H, s); MS m/e 349. Anal. ($C_{21}H_{20}FN_3O$) C, H, N.

Method F. 6-Amino-2-styrylquinazolin-4(3*H*)-one (5i). Iron powder (1.5 g, 27 mmol) was added in portions to a mixture of 6-nitro-2-styrylquinazolin-4(3*H*)-one (2 g, 6.8 mmol) and acetic acid (35 mL) at 50 °C under nitrogen. An exothermic reaction was observed. The yellow suspension was then stirred at 110 °C for 3 h, and the dark brown mixture was diluted after cooling with methanol (50 mmol). The solid thus obtained was filtered, rinsed with methanol, and air-dried. The crude product was chromatographed (silica gel/5% methanol in methylene chloride) to give a solid which upon recrystallization from methylene chloride and ether afforded product **5i** (180 mg, 12%): NMR (DMSO-d_6) δ 7.88 (1 H, d, J = 16 Hz), 7.38–7.70 (8 H, m), 6.95 (1 H, d, J = 16 Hz); MS m/e 263. Anal. ($C_{16}H_{13}N_3O$) C, H, N.

Method G. 6-Hydroxy-2-styrylquinazolin-4(3*H*)-one (5p). A suspension of **5o** (5.0 g, 18 mmol) in 48% aqueous HBr (100 mL) and acetic acid (100 mL) was stirred at reflux for 24 h. The solution was cooled to 25 °C and the yellow solid was collected by filtration and rinsed with water (2 × 50 mL) followed by methanol (2 × 25 mL) to give product **5p** (3.0 g, 63.3%): NMR (DMSO-d_6) δ 7.8–7.4 (9 H, m), 6.90 (1 H, d, J = 16 Hz); MS m/e 264. Anal. ($C_{16}H_{12}N_2O_2$) C, H, N.

6-(Acetyloxy)-2-styrylquinazolin-4(3*H*)-one (5u). A mixture of **5p** (1.3 g, 5 mmol) and AcONa (100 mg) in acetic anhydride (30 mL) was stirred at reflux for 8 h, cooled, and filtered, and the resultant solid was rinsed with methanol and dried to give product **5u** (1.02 g, 66.6%): NMR (DMSO-d_6) δ 7.85 (1 H, d, J = 16 Hz), 7.45–7.80 (8 H, m), 6.90 (1 H, d, J = 16 Hz), 2.05 (3 H, s); MS m/e 306. Anal. ($C_{18}H_{14}N_2O_3$) C, H, N.

Method H. 6-Chloro-2-methylquinazolin-4(3*H*)-one (15a). A mixture of 5-chloroanthranilic acid (**2**, 50.0 g, 0.29 mol) and acetic anhydride (170 mL) was heated at reflux under nitrogen for 2 h, cooled to 25 °C, and filtered. The solid was then rinsed with ether (2 × 100 mL) and dried to afford benzoxazinone **14** (43 g, 75.8%): mp 123–126 °C; NMR (DMSO-d_6) 7.97 (2 H, m), 7.58 (1 H, d, J = 4 Hz), 2.40 (3 H, s). This product was then stirred with ammonium acetate (18.5 g, 0.24 mol) at 150 °C for 30 min under nitrogen, cooled to 50 °C, and diluted with hot methanol (50 mL). The resultant solution was stirred at reflux for 1 h, cooled to 25 °C, and filtered. The solid was rinsed with methanol and dried to yield a white solid **15a** (23.3 g, 54.4%): NMR (DMSO-d_6) δ 12.26 (1 H, s), 8.0 (1 H, d, J = 2 Hz), 7.8 (1 H, dd, J_1 = 9 Hz, J_2 = 2 Hz), 7.59 (1 H, d, J = 9 Hz), 2.35 (3 H, s); MS m/e 194. Anal. ($C_9H_7ClN_2O$) C, H, N.

Method I. 6-Chloro-2-styrylquinazoline-4(3*H*)-thione (15e). A mixture of **15a** (2 g, 10 mM) and **16** (1.1 g, 10 mmol) in glacial acetic acid (10 mL) was stirred at reflux for 12 h, cooled at 25 °C, and filtered, and the solid was rinsed with warm methanol and dried to give product **15e** (1.93 g, 68.2%): NMR (DMSO-d_6) δ 8.68 (1 H, d, J = 1 Hz), 7.37–8.04 (8 H, m); MS m/e 283. Anal. ($C_{15}H_{10}ClN_3O$) H, N; C: calcd, 63.49; found, 62.98.

Method J. 6-Chloro-2-styrylquinazoline-4(3*H*)-thione (17). A mixture of **5a** (8.5 g, 30 mM) and P_2S_5 (5.3 g, 12 mmol) in pyridine (90 mL) was stirred at reflux for 1.5 h. The hot reaction mixture was poured into hot water (40 mL). After cooling, the yellowish suspension was filtered, and the solid was rinsed with water (100 mL), methanol (100 mL), and acetone (100 mL) to yield the product **17** (4.3 g, 48.1%): NMR (DMSO-d_6) δ 8.46 (1 H, d, J = 2 Hz), 7.97–7.85 (2 H, m), 7.75–7.43 (6 H, m), 7.22 (1 H, d, J = 16 Hz); MS m/e 298. Anal. ($C_{16}H_{11}ClN_2S$) C, H, N.

Method K. 4-[[5-(Diethylamino)pentan-2-yl]amino]-6-chloro-2-styrylquinazoline Hydrochloride (19). A mixture of **5a** (5.0 g, 17.7 mmol) and $POCl_3$ (500 mL) was heated at reflux for 7 h. The excess $POCl_3$ was distilled in vacuo and the residual oil was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed several times with cold saturated aqueous Na_2CO_3 and dried over $MgSO_4$, filtered, and evaporated in vacuo. The oily imidoyl chloride **18** was heated in refluxing benzene (50 mL) for 8 h and cooled to 25 °C to give a gummy solid, which was resolidified from methanol/acetone to give the crude product. The solid was dissolved in hot water and then basified with saturated $NaHCO_3$ and extracted with CH_2Cl_2, and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give the free base, which upon acidification with concentrated HCl in acetone gave desired product **19** (3.3 g, 45.4%): NMR (DMSO-d_6) δ 8.02 (1 H, d, J = 16 Hz), 7.60–7.80 (8 H, m), 7.20 (1 H, d, J = 16 Hz), 3.50–4.00 (8 H, m), 1.50–1.76 (12 H, m); MS m/e 422. Anal. ($C_{25}H_{31}ClN_4 \cdot HCl$) C, H, N.

Method L. 3-(2-Phenylethenyl)-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22). To a mixture of P_2O_5 (28.4 g, 163 mmol), 1,2-ethylene dichloride (50 mL), and hexamethyldisiloxane (42.6 mL) was added cinnamic acid (3.7 g, 20 mM) followed by 2-aminobenzenesulfonamide (4.2 g, 20 mmol). The resultant mixture was stirred at reflux for 5 h, cooled, filtered, and rinsed with acetone to give the white, solid final product **22** (2.0 g): NMR (DMSO-d_6) δ 7.68–7.92 (6 H, m), 7.50–7.39 (5 H, m), 6.89 (1 H, d, J = 16 Hz); MS m/e 284. Anal. ($C_{15}H_{12}N_2O_2S$) C, H, N.

Method M. 5,6-Dimethyl-2-styrylpyrimidin-4(3*H*)-one (29). A mixture of amidine **32** (1.4 g, 10 mmol), prepared according to a literature procedure,[9] $Ca(OH)_2$ (0.8 g, 11 mmol), and ethyl 2-methylacetate (1.4 g, 10 mmol) in EtOH (25 mL) and H_2O (25 mL) was stirred at 25 °C for 72 h and filtered. The solid was rinsed with EtOH and air-dried to give product **29** (0.5 g, 22%): NMR (DMSO-d_6) δ 7.80 (1 H, d, J = 16.1 Hz), 7.63–7.42 (5 H, m), 6.87 (1 H, d, J = 16.1 Hz), 2.25 (3 H, s), 1.94 (3 H, s); MS m/e 226. Anal. ($C_{14}H_{14}N_2O$) C, H, N.

Acknowledgment. We thank A. E. Knight for preparing compound **15c** and Drs. K. Paull and J. Plowman at the National Cancer Institute for evaluating some of the analogues in the P388 model as well as selected tumor panel models.