Quantitative Structure–Activity Relationships of Antitumor Guanidinothiazolecarboxamides with Survival Enhancement for Therapy in the 3LL Lewis Lung Carcinoma Model

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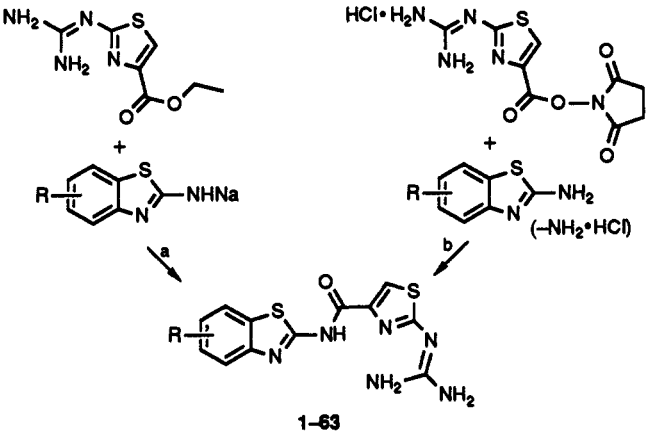
Guanidinothiazolecarboxamides (GTCs) are a novel class of antitumor agents found to be systemically active against experimental pulmonary metastases of 3LL Lewis lung carcinoma. A series of substituted benzothiazole GTCs were found to produce enhancement of survival in this model by using 8 days of intraperitoneal dosing initiated 2 days after intravenous tumor challenge. Quantitative structure–activity relationships have been discovered in the GTC series with survival enhancement correlated to substituent parameters. Optimal correlations were found between the probit transform of the drug-induced increased lifespan (ILS) and field and π parameters. Among the most effective analogues in this series was *N*-(5-fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide (**19**).

While structure–activity relationship (SAR) studies involving in vivo evaluation of antitumor agents against adenocarcinomas have been reported in the literature,[1] in vivo quantitative structure–activity relationship (QSAR) reports on anticancer drugs are rare and focus almost exclusively on leukemia models.[2] Unfortunately drug activity in many of the commonly used murine models of leukemia has been an inefficient predictor of human clinical efficacy against solid tumors. However, there are considerable data supporting the usefulness of transplantable tumor systems, particularly certain carcinomas, for the discovery of clinically active cancer therapeutants.[3] Of particular interest are studies involving 3LL Lewis lung carcinoma, where clinical predictivity for human activity was reported to be 80%.[3b] Recently, Baguley and coworkers reported in vivo QSAR studies of amsacrine analogues used in systemic treatment in the metastatic Lewis lung model, relating in vivo efficacy to in vitro cytotoxicity rather than to physicochemical parameters.[4] This approach limits the predictivity promised by QSAR to compounds already synthesized and tested in vitro. Nevertheless, Baguley's correlation stands as a pioneer study for in vivo QSAR with anticancer drug effects on carcinomas.

Scheme I. Synthesis of Guanidinothiazolecarboxamides (GTCs)[a]



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[a] (a) Sodium hydride generated anion in THF, GTC isolated as the HCl salt (method A1) or the neutral (method A2); (b) (method B1) neutral benzothiazole heated at 120 °C in NMP with hydroquinone in the dark, GTC isolated as the HCl salt; (method B2) benzothiazole hydrochloride reacted as in B1, GTC isolated as the neutral.

We report here the synthesis and QSAR analyses of a series of guanidinothiazolecarboxamides (GTCs) found to be systemically and significantly active for enhancement of survival of mice bearing nascent and established micrometastatic disease of 3LL. One example of this series, **19**, already has been reported[5] to enhance lifespan in the 3LL model after either oral or intraperitoneal dosing and to be well tolerated. The GTCs of this study provide the first example of antitumor QSAR where survival efficacy against carcinomas have been related to analogue substituent parameters.

Chemistry. Guanidinothiazolecarboxamides (GTCs), Table I, were prepared by two general methods as depicted in Scheme I, either by condensation of the anion of a 2-aminobenzothiazole with ethyl 2-guanidinothiazole-4-carboxylate (methods A1 and A2) in tetrahydrofuran or by condensation of the 2-aminobenzothiazole or its hydrochloride salt (methods B1 and B2, respectively) with the 2-guanidinothiazole-4-carboxylate *N*-hydroxysuccinimide ester in *N*-methyl-2-pyrrolidinone at 110–160 °C.

N-Hydroxysuccinimide esters employed in methods B1 and B2 can undergo homolytic degradation at elevated

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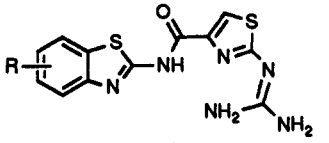
Table I. Physical and Survival (ILS) Data of Guanidinothiazolecarboxamides (GTCs) Evaluated in 3LL Lewis Lung Adenocarcinoma



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compd	R	method[a]	mp, °C	recrys[b]	HPLC[c]	formula	% yield	mean ILS	benzthiaz mp, °C[d]
1	H	A1	285	M	5.4/98.5	$C_{12}H_{10}N_6OS_2 \cdot HCl \cdot 3H_2O$[f]	7.7	15.0	
2	6-NO_2	A1	295	DE	6.5/94.3	$C_{12}H_9N_7O_3S_2 \cdot HCl \cdot H_2O$	52.7	51.7	
3	6-Cl	A1	295	M	12.5/98.1	$C_{12}H_9ClN_6OS_2 \cdot HCl \cdot H_2O$	19.5	58.0	
4	6-F	B1/H	285	M	6.6/97.2	$C_{12}H_8FN_6OS_2 \cdot Na$	27.5	38.8	
5	6-OCH_3	A2	253–254	C	5.2/99.7	$C_{13}H_{12}N_6O_2S_2 \cdot H_2O$	8.2	7.5	
6	6-$CONH_2$	B1/H	265	M	2.0/90.7	$C_{13}H_{10}N_7O_2S_2 \cdot Na \cdot 8H_2O$[g]	41.9	1.0	226
7	6-OCH_2CH_3	A2/D	259–260	DE	9.2/99.2	$C_{14}H_{13}N_6O_2S_2 \cdot Na \cdot 0.75H_2O$	17.1	6.0	
8	6-CH_3	A2/D	260	M	9.8/99.1	$C_{13}H_{11}N_6OS_2 \cdot Na \cdot 1.5H_2O$[h]	91.0	37.3	
9	6-CN	B1	309–310	DE	4.0/97.6	$C_{13}H_9N_7OS_2 \cdot HCl \cdot 1.5H_2O$	50.5	53.0	
10	6-$CH(CH_3)_2$	A2/D	240–242	MT	24.0/96.2	$C_{15}H_{16}N_6OS_2 \cdot Na \cdot 4.5H_2O$[i]	46.9	38.0	
11	6-SO_2NH_2	B1	240–242	M	1.7/94.7	$C_{12}H_{11}N_7O_3S_3 \cdot HCl \cdot 2.25H_2O$	50.5	1.0	
12	6-SCH_3	B1/D	238–240	DE	10.6/98.6	$C_{13}H_{11}N_6OS_3 \cdot Na \cdot 2H_2O$[j]	16.0	25.5	
13	6-CH_2CH_3	B1	325–326	M	16.0/94.7	$C_{14}H_{14}N_6OS_2 \cdot HCl \cdot H_2O$[k]	62.1	33.0	
14	6-Ph	B2	288–290	P	33.5/96.0	$C_{18}H_{14}N_6OS_2 \cdot 0.25H_2O$	74.2	67.0	
15	6-$O(CH_2)_3CH_3$	B1/H	308–310	M	4.2/96.7*	$C_{16}H_{18}N_6O_2S_2 \cdot HCl \cdot 2H_2O$[m]	36.3	59.0	
16	6-$C(CH_3)_3$	B1	320–322	M	4.5/95.4*	$C_{16}H_{18}N_6OS_2 \cdot HCl \cdot H_2O$[n]	73.7	61.0	142–143
17	6-$(CH_2)_2CH_3$	B1	307	M	22.3/94.9	$C_{15}H_{16}N_6OS_2 \cdot HCl \cdot 0.5H_2O$	73.2	21.5	
18	6-$SO_2(CH_2)_4CH_3$	B1/H	265–267	A	2.2/95.7*	$C_{17}H_{20}N_6O_3S_3 \cdot Na \cdot 2.5H_2O$[o]	29.6	30.0	98–100
19	5-F	B2	294	P	6.0/96.5	$C_{12}H_9FN_6OS_2 \cdot 0.5H_2O$[p]	41.0	67.6	287–290
20	5-SCH_3	B1/H	214–216	M	10.6/96.4	$C_{13}H_{12}N_6OS_3 \cdot HCl \cdot 0.5H_2O$	41.7	1.0	149–151
21	5-SO_2Ch_3	B1/H	258–260	A	2.3/96.2	$C_{13}H_{11}N_6O_3S_3 \cdot Na \cdot 1.25H_2O$	62.1	1.0	240
22	5-NO_2	B1	>350	M	6.6/95.5	$C_{12}H_9N_7O_3S_2 \cdot HCl \cdot 0.5H_2O$	81.6	57.0	
23	5-OCH_2CH_3	B1	185–188	M	8.6/97.7	$C_{14}H_{14}N_6O_2S_2 \cdot HCl \cdot 2H_2O$[q]	42.3	44.0	
24	5-Ph	B1	264–265	M	31.2/96.6	$C_{18}H_{14}N_6OS_2 \cdot HCl \cdot 1.5H_2O$	62.4	18.0	225
25	5-$O(CH_2)_3CH_3$	B1	236–239	M	32.4/97.7	$C_{16}H_{18}N_6O_2S_2 \cdot HCl \cdot H_2O$[r]	67.4	34.5	87–89
26	5-$OCH(CH_3)_2$	B1	235	M	11.4/96.8	$C_{15}H_{16}N_6O_2S_2 \cdot HCl \cdot H_2O$	29.5	23.0	113
27	5-OH	B2	299–301	M	3.0/96.8	$C_{12}H_{10}N_6O_2S_2 \cdot HCl \cdot 1.5H_2O$	31.8	10.0	
28	4-OCH_3	A1	298	M	5.7/96.2	$C_{13}H_{12}N_6O_2S_2 \cdot HCl \cdot 3H_2O$[s]	34.8	45.0	
29	4-Cl	A2/H	285	A	10.6/97.6	$C_{12}H_8ClN_6OS_2 \cdot Na \cdot 4H_2O$[t]	63.0	18.5	
30	4-CH_3	B1	254	M	10.6/96.5	$C_{13}H_{12}N_6OS_2 \cdot HCl \cdot 1.5H_2O$[u]	34.6	44.0	
31	4-NO_2	A2/H	234–236	C	6.3/97.5	$C_{12}H_8N_7O_3S_2 \cdot Na \cdot 5H_2O$[v]	63.0	30.0	
32	4-F	B1/H	298–300	H	5.8/99.0	$C_{12}H_8FN_6OS_2 \cdot Na \cdot 2.5H_2O$	34.5	38.0	
33	4-$CH(CH_3)_2$	B1	205–206	M	23.7/96.9	$C_{15}H_{16}N_6OS_2 \cdot HCl \cdot H_2O$[w]	68.2	24.0	122–124
34	4-SCH_3	B1	184–185	M	9.5/96.3	$C_{13}H_{12}N_6OS_3 \cdot HCl \cdot 0.5H_2O$	57.5	9.0	164–167
35	4-OH	B2	311–313	P	2.1/99.9*	$C_{12}H_{10}N_6O_2S_2 \cdot HCl \cdot 0.75H_2O$[x]	25.1	14.0	
36	7-CF_3	B1/H	255–258	A	15.0/96.6	$C_{13}H_9F_3N_6OS_2 \cdot Na$[y]	14.5	35.0	
37	7-Ph	B1	333–335	M	3.7/98.8*	$C_{18}H_{14}N_6OS_2 \cdot HCl$[z]	72.2	49.0	175–177
38	5,6-di-OCH_3	A2	186–189	M	3.9/93.8	$C_{14}H_{14}N_6O_3S_2 \cdot CH_3OH$[aa]	8.2	5.0	
39	5,6-di-CH_3	B1	289–290	M	13.9/95.3	$C_{14}H_{14}N_6OS_2 \cdot HCl \cdot 2H_2O$	75.2	18.0	
40	5,6-di-F	B2	294	P/M	3.8/99.2*	$C_{12}H_9F_2N_6OS_2 \cdot CH_3OH$	26.5	62.0	170–173
41	5,6-di-Cl	B2	305–308	P/AC	5.0/98.5*	$C_{12}H_8Cl_2N_6OS_2$[ab]	38.7	71.0	
42	5-F,6-OH	B2	>350	M	2.5/93.8	$C_{12}H_9FN_6O_2S_2 \cdot 7H_2O$[ac]	31.6	23.0	287–290
43	5-OCH_3,6-Cl	B2	264–265	PA	3.1/98.3*	$C_{13}H_{11}ClN_6O_2S_2 \cdot H_2O$	32.3	21.0	239–241
44	5-F,6-Cl	B2	299–302	AC	4.0/99.4*	$C_{12}H_8ClFN_6OS_2$[ad]	11.6	75.0	282–284[am]
45	5-Cl,6-$CONH_2$	B2	319–322	M	1.9/97.0*	$C_{13}H_{10}ClN_7O_2S_2 \cdot 5H_2O$[ae]	43.9	42.0	228–229
46	5-Cl,6-F	B2	290–292	PA	3.5/96.9*	$C_{12}H_8ClFN_6OS_2 \cdot H_2O$[af]	26.0	41.0	
47	5,7-di-F	B1	297–299	M	12.6/95.0	$C_{12}H_8F_2N_6OS_2 \cdot HCl \cdot 1.5H_2O$[ag]	43.8	22.0	184–186
48	6-Cl,7-CF_3	B2[e]	339–340	M	4.5/94.2*	$C_{13}H_8ClF_3N_6OS_2 \cdot HCl$	35.9	69.0	
49	4-CH_3,5-OCH_3	B2	270	P	12.6/96.0	$C_{13}H_{11}FN_6OS_2 \cdot 1.5H_2O$	32.9	57.0	211–213
50	4-CH_3,5-F	B1	284–285	M	10.0/96.5	$C_{14}H_{14}N_6O_2S_2 \cdot HCl \cdot 2H_2O$[ah]	76.9	62.0	206–208
51	4-CH_3,5-Cl	B2	264	P	20.9/96.4	$C_{13}H_{11}ClN_6OS_2 \cdot HCl$	39.0	73.0	
52	4-OMe,6-NO_2	B1	289–291	M	7.9/99.0	$C_{13}H_{11}N_7O_4S_2 \cdot 0.5H_2O$	30.1	20.0	
53	4,6-di-CH_3	B2	276–277	P	3.8/94.9*	$C_{14}H_{14}N_6OS_2 \cdot 1.25H_2O$	30.0	64.0	
54	4-CH_3,6-F	B1	271–273	M	12.0/99.1	$C_{13}H_{11}FN_6OS_2 \cdot HCl \cdot 2H_2O$[ai]	65.6	29.0	160–162
55	4-CH_2CH_3,6-CN	B1	294–297	M	9.2/99.8	$C_{15}H_{13}N_7OS_2 \cdot HCl \cdot 2H_2O$[aj]	65.0	38.0	199–201
56	4-CH_3,6-CF_3	B2	294	P	5.2/98.8*	$C_{14}H_{11}F_3N_6OS_2 \cdot 5H_2O$[ah]	26.7	19.0	169–171
57	4-CH_3,6-Cl	B2	262–263	PA	4.6/98.6*	$C_{13}H_{11}ClN_6OS_2 \cdot H_2O$	19.0	15.0	
58	4-OCH_3,7-Cl	A1/H	228–230	H	13.1/99.6	$C_{13}H_{10}N_6O_2S_2 \cdot Na \cdot H_2O$	64.0	69.0	
59	4-CH_3,7-F	B1	312–315	M	15.5/97.5	$C_{13}H_{11}FN_6OS_2 \cdot HCl \cdot 1.5H_2O$	76.6	53.0	195–197
60	4,7-di-F	B1/H	340	A	10.0/98.8	$C_{12}H_7F_2N_6OS_2 \cdot Na \cdot H_2O$	48.2	26.0	190–191
61	4-OCH_2CH_3,7-F	B2/D	179–182	H	3.7/98.1*	$C_{14}H_{12}FN_6O_2S_2 \cdot Na \cdot 0.5H_2O$	18.9	53.0	209–211
62	4-OCH_2CH_3,7-Cl	B2/D	273–275	P	3.8/95.8*	$C_{14}H_{12}ClN_6O_2S_2 \cdot Na$	59.6	75.0	209–211
63	4-OCH_3,7-CF_3	B2	265–266	P	4.0/98.7*	$C_{14}H_{11}F_3N_6O_2S_2 \cdot 2H_2O$[al]	36.3	17.0	168–169

[a] Method of synthesis/salt preparation. [b] Recrystallization solvent: M = CH_3OH trituration, DE = DMSO solution was precipitated with Et_2O, P = pyridine A = aqueous NaOH, C = CH_3CN trituration, H = H_2O, AC = HOAc, PA = pyridine/acetone, MT = CH_3OH solution was precipitated with Et_2O. [c] HPLC retention time/percent purity at 60% CH_3OH or * = 80% CH_3OH. [d] Melting points of novel 2-aminobenzothiazole intermediates. [e] See Experimental Section for HCl salt formation. [f] Calcd: H, 4.19. Found: H, 3.209. [g] Calcd: H, 4.96; N, 18.59. Found: H, 2.30; N, 17.83. [h] Calcd: N, 22.03. Found: N, 21.29. [i] Calcd: H, 5.41; N, 18.09. Found: H, 3.70; N, 18.63. [j] Calcd: N,

Table I (Continued)

19.89. Found: 18.83. [k] Calcd: C, 41.94; H, 4.24; N, 20.96. Found: C, 41.52; H, 3.55; N, 21.49. [l] Deleted. [m] Calcd: H, 5.00. Found: H, 4.47. [n] Calcd: H, 4.93; N, 19.59. Found: H, 4.27; N, 20.06. [o] Calcd: H, 4.65. Found: H, 3.93. [p] Calcd: H, 2.91. Found: H, 2.46. [q] Calcd: C, 38.66; H, 4.40. Found: C, 37.93; H, 3.92. [r] Calcd: H, 4.76; N, 18.89. Found: H, 4.23; N, 18.19. [s] Calcd: H, 4.36. Found: H, 3.21. [t] Calcd: C, 32.25; H, 3.61. Found: C, 32.96; H, 2.75. [u] Calcd: C, 39.44. Found: C, 38.98. [v] Calcd: N, 20.62. Found: N, 17.18. [w] Calcd: N, 20.25. Found: N, 21.06. [x] Calcd: C, 37.25. Found: C, 37.77. [y] Calcd: H, 1.97. Found: H, 2.56. [z] Calcd: C, 50.17. Found: C, 48.80. [aa] Calcd: N, 20.47. Found: N, 19.79. [ab] Calcd: C, 37.22. Found: C, 37.68. [ac] Calcd: H, 4.84; N, 17.56. Found: H, 2.35; N, 16.69. [ad] Calcd: N, 2.97; N, 20.66. Found: H, 2.11; N, 19.90. [ae] Calcd: H, 4.15. Found: H, 2.22. [af] Calcd: H, 2.59; N, 21.61. Found: H, 1.99: N, 21.01. [ag] Calcd: N, 20.10. Found: N, 19.35. [ah] Calcd: H, 4.40. Found: H, 3.97. [ai] Calcd: H, 3.48. Found: H, 2.89. [aj] Calcd: C, 40.56. Found: C, 39.83. [ak] Calcd: H, 4.32; N, 17.13. Found: H, 2.57; N, 16.46. [al] Calcd: H, 3.35. Found: H, 2.67. [am] Hydrobromide salt used in method B2.

temperatures. Thus, it was found advantageous to include an inhibitor of radical reactions, hydroquinone, and to conduct the experiments shielded from light. For some examples conducted early in our investigations, the neutral benzothiazole was used (method B1); however, it was found during the course of this research that employing the neutral amine led to the generation of contaminating 2:1 adducts. This was avoided by using the hydrohalide salt of the amine (B2). The 2:1 adducts arose formally from the attack of the guanidine group in the GTC product on another molecule of the active ester. The observations are not surprising in view of the similarity in acidity between the protonated 2-aminobenzothiazoles (2-amino-6-methylbenzothiazole has $pK_a = 4.6$[6]) and that of the guanidinothiazoles ($pK_a \approx 5.3$[5]). While in certain cases, e.g., 6, 11, 18, the substituents on the benzothiazole starting material prohibited employing method A1 or A2, the active ester method was general and successful in all cases where it was attempted.

No attempt was made to optimize synthetic yields for the individual analogues. Yields recorded reflect idiosyncracies of isolation and purification of this relatively insoluble class of compounds rather than the efficiency of coupling. Purity was assessed by reversed-phase HPLC (Table I) and NMR for all analogues. The GTCs tenaciously retained molecules of solvation, particularly protic solvents, even after prolonged heating above 110 °C at <1 Torr as evidenced by microanalysis and NMR data.

GTC analogues could be isolated as either neutral compounds, anionic salts of metal ions, or acid addition salts, as anticipated by the observation of two pK_a values for this class, as typified by compound 19; $pK_a = 5.3$ for the protonated guanidinium group and $pK_a = 10.3$ for the amide NH.[5] Methods for generating a particular salt form are detailed in Table I and the Experimental Section. Biological test data was accumulated on analogues without concern for the salt form employed since all drugs were formulated in a dimethyl sulfoxide (DMSO)/saline suspension buffered to pH 7.4.

Many of the substituted 2-aminobenzothiazole intermediates were previously unknown. These novel compounds were prepared as neutral or HCl salts from the appropriate anilines by adaptation of the Gourley[7] procedure, involving cyclization of intermediate *N*-arylthioureas with bromine. Table I records the melting points of these new intermediate benzothiazoles.

Biology. Experimental pulmonary metastases of 3LL Lewis lung carcinoma were obtained by an intravenous injection of 3LL cells according to published procedures as modified by Pollack to afford greater reproducibility and suitability for routine screening.[3a,8] Intravenous implantation was performed in the lateral tail vein of 18–20-g female BDF1 mice with 6×10^5 log phase 3LL cells suspended in 0.2 mL of RPMI 1640 medium. Mice were randomized upon receipt of shipment and again immediately after tumor implantation. Seven mice were used for each treatment group. For intraperitoneal (ip) testing, compounds were freshly formulated each day by dissolution in a small amount of DMSO (20% of final volume) and dilution with sufficient sterile saline (0.15 N sodium chloride) to afford a fine suspension. Groups of test animals were treated once a day from days 2 to 9 after implantation (8 treatments) and were monitored daily thereafter for survivors. The increased lifespan (ILS) was calculated from the median survival times (MST) of the treated versus vehicle control groups, where ILS (%) = $[(MST_{treated}/MST_{controls} \times 100) - 100]$. The MST for the control groups averaged 19 days with a range of medians in individual experiments of 16–23 days. Adriamycin and cyclophosphamide have been used in this model as positive controls, resulting in mean ILS values of 91% (SD = 24, CV = 13%, $n = 61$) and 92% (SD = 24, CV = 13%, $n = 61$), respectively. Adriamycin was given as a single iv injection of 18 mg/kg on day 3 after tumor implantation. Cyclophosphamide was dosed as a single ip treatment of 300 mg/kg on day 3 after tumor implantation. Under these conditions long-term survivors in any of the control, treated, or positive control groups were very exceptional. The results of survival studies were analyzed for statistical significance by using the Armitage test for analysis of trends in proportions. For significant therapy, $P \leq 0.05$. For substantial therapeutic activity, ILS ≥ 50%.

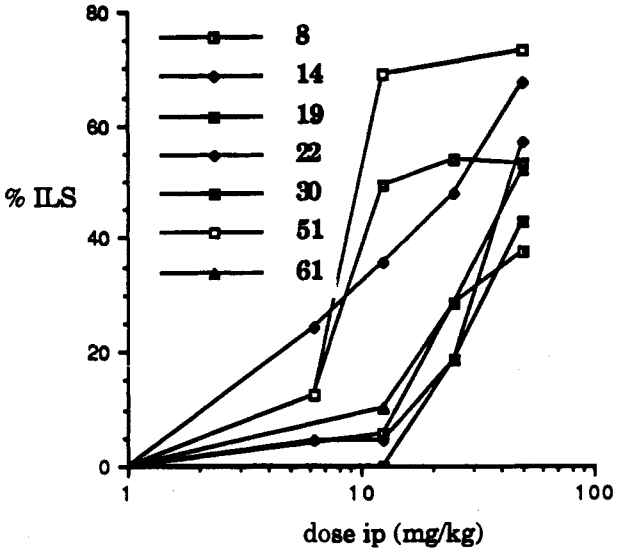

Figure 1. Survival dose–response curves for GTC analogues dosed ip qd 2–9 after iv 3LL tumor challenge.

Lethality from drug treatment with individual analogues was ascribed to those deaths that were observed in treated groups before any deaths occurred in the vehicle-treated tumor bearing control groups. A typical experiment comprised three control groups, two positive control groups, and 12 drug treated groups (total animals = 119).

Structure–Activity Correlations. Usually QSAR analyses are performed on databases where the dependent variable is expressed as the logarithm of the dose necessary to produce a standard response, e.g., log ED_{50}. Because of the impracticality of obtaining ED_{50} values from dose–

(6) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965; pp 268–9.
(7) Gourley, R. N. U.S. Patent 4,052,379 (Oct. 4, 1977).
(8) Pollack, V. A.; Fidler, I. J. *JNCI* 1982, *69*, 137.

response testing in survival studies with a large number of moderately effective antitumor analogues, analyses in this study were carried out on the measurement of biological response (ILS) at a standard dose. A probit transformation [probit = log (100/100 - ILS)] was taken as the index of efficacy.[9] The probit function provided better discrimination among the more active compounds and obviated biasing the regression equations with data from a few weakly active analogues. The general character of the QSAR using the log ILS as the dependent variable afforded qualitatively similar regression equations of lower correlation. QSAR analyses were performed by considering a variety of substituent parameters representing properties of size, steric constraints, lipophilicity, electronic character, dipole moments, and polarizability. Values for π, F, R, and MR were taken from Norrington.[10] Dipole moment substituent constants were taken from Lein.[11] E_s values were taken from the Hansch compilation.[12] Calculated log P (Clog$P$) was obtained by using the computer program of MedChem Software Release.[13] Regression analyses were carried out by using the RS3 computational program.[14]

Results

The physical properties, methods of preparation, and biological results of testing at 50 mg/kg (ip) for 63 GTC analogues are recorded in Table I. Compounds have been grouped according to their substitution patterns. Dose–response titrations with substantially active analogues prepared at the beginning of this study indicated that compounds could be efficiently screened for activity by testing at this single intraperitoneal dose level. Those compounds that elicited an ILS ≥ 50% at the 50 mg/kg dose level were investigated more carefully for effects of dosage regimen, lethality, and potency characteristics. In Figure 1, dose–response curves for several analogues depict the qualitative similarity of survival responses among congeners of differing potency. In general those compounds that were more effective at 50 mg/kg also demonstrated survival enhancement at 25 mg/kg and often 12.5 mg/kg. Parallelism in the dose–response curves is suggestive though not proof of a homogeneous mechanism of action within a narrow series. Significant deviations in maximal effects achieved or slope of the dose–response curves could indicate differences in bioavailability or additional mechanisms of action for an individual analogue or subset within the series. When the biological response measured lies on parallel portions of the dose–response curves, then variances in degree of response should be directly proportional to potency.

In considering the inclusion of weakly active compounds into our QSAR analyses, we recognize that the traditional definition of "activity" in the 3LL assay has been a T/C = 125% or more. But this definition was chosen by Geran (*Cancer Chemother. Rep.* **1972**, *3*, 13) to solve a different problem. A cut-off like that was useful when screening compounds since it tends to produce a minimal number of false positives. However, when exploring the structure–activity relationships, it is useful to have structures associated with minimal or no activity, since the regression analysis is an attempt to identify important characteristics of the molecule, and it is important to have examples where those characteristics are reduced and where activity is also minimal. Statistical theory indicates that, when in the estimation mode as we are here in QSAR, it is best to use the point estimates from all the data rather than throw out those data that are not sufficiently strong enough to stand by themselves. The failure of a compound to show statistically significant "activity" in a single trial is due to the number of animals used, the degree of intrinsic activity that may be there, and the random noise that surrounds any such study. Nevertheless, these authors recognize that data determined to be minimally active are less precisely graded than those found in the center of the response curves and thus a transformation was sought that would include but not over emphasize these influence points. The probit transformation described above performed such a

Table II. Substituent Parameters and Efficacy for 18 6-Substituted GTC analogues[a]

compd	R	ILS (%)	F	π	log [100/100 - ILS] obsd	log [100/100 - ILS] calcd
1	H	15.0	0.00	0.00	0.0706	0.0705
2	NO_2	51.7	1.09	0.11	0.316	0.295
3	Cl	58.0	0.68	0.77	0.377	0.301
4	F	38.8	0.68	0.22	0.213	0.230
5	OCH_3	7.5	0.41	0.12	0.0339	0.165
6	$CONH_2$	1.0	0.40	-1.51	0.00437	-0.0498
7	OCH_2CH_3	6.0	0.36	0.62	0.0269	0.219
8	CH_3	37.3	-0.05	0.52	0.203	0.127
9	CN	53.0	0.83	-0.31	0.328	0.191
10	$CH(CH_3)_2$	38.0	-0.07	1.33	0.208	0.227
11	SO_2NH_2	1.0	0.67	-1.86	0.00437	-0.0379
12	SCH_3	25.5	0.33	0.64	0.128	0.216
13	CH_2CH_3	33.0	-0.06	0.99	0.174	0.186
14	phenyl	67.0	0.14	1.92	0.481	0.343
15	$O(CH_2)_3CH_3$	59.0	0.40	1.62	0.387	0.355
16	$C(CH_3)_3$	61.0	-0.10	1.70	0.409	0.269
17	$(CH_2)_2CH_3$	21.5	-0.03	1.45	0.105	0.250
18	$SO_2(CH_2)_4CH_3$	30.0	0.88	0.15	0.155	0.260

[a] 6 = meta.

(9) A complete description of the activity of a series of compounds is a curved surface in three dimensions in its simplest form, dose, response (a dependent variable), and an independent variable which may be, for example, a field effect. The QSAR often reported in the literature can be depicted as a plane passing through this three-dimensional surface at a constant response level and relating a dose to the value of the independent variable. In the analysis herein the plane chosen was orthogonally related to those literature examples such that its intersection occurred at a fixed dose and the connection of the response to the independent variable was assessed. Among the compounds present in any series are those with low levels of activity at a given dose. In fact, for some, the activity may be so low that they form extremes of the data, called "influential points" because they greatly influence the nature of straightforward regression analyses. However, differing degrees of low or randomly spurious activity may mean very little in terms of structure. One way of addressing this problem is to transform the activity measures. The often-used log transformation adjusts for influential points where activity is higher than the bulk of the data. Because the influential points considered here were among the compounds with low activity we used the transformation log [100/(100 - ILS)]. This transformation has been termed a "probit". Cramer, R. D. In *Annual Reviews in Medicinal Chemistrry*, Vol. 11; Clarke, F. H., Ed.; Academic Press: New York, 1976; p 301. Thomas, J.; Berkoff, C. E.; Flagga, W. B.; Gallo, J. J.; Haff, R. F.; Pinto, C. A. *J. Med. Chem.* **1975**, *18*, 245. Tukey, J. W. "on the Comparative Anatomy of Transformations", *Ann. Math. Stat.* **1957**, *28*, 602.

(10) Norrington, F. E.; Hyde, R. M.; Williams, S. G.; Wooton, R. *J. Med. Chem.* **1975**, *18*, 604.

(11) Lein, E. J.; Guo, Z.-R.; Li, R.-L.; Su, C.-T. *J. Pharmaceut. Sci.* **1982**, *71*, 641.

(12) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

(13) MedChem Software Release 3.54; Claremont Information Systems, Inc.: Claremont, CA, January, 1989, p 14.1. This program is an adaptation of the work found in ref 4 and that of Jurs; Chou, J.; Jurs, P. J. *J. Chem. Inf. Comput. Sci.* **1979**, *19*, 172.

(14) BNN Software Products Corporation, 10 Fawcett St., Cambridge, MA 02238.

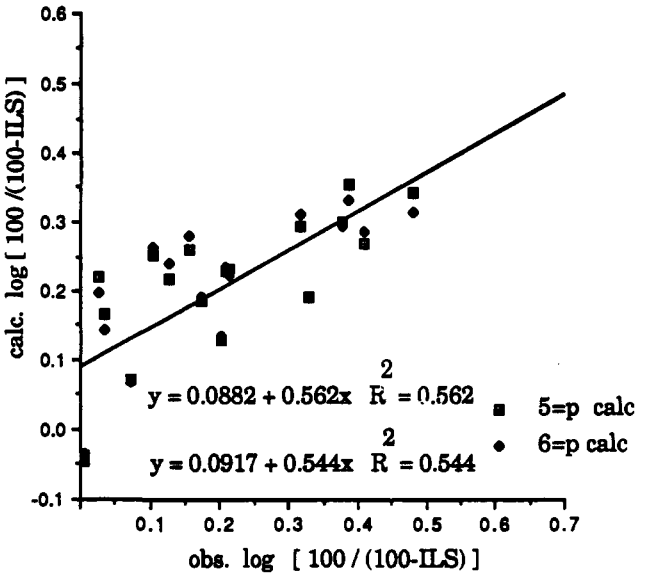

Figure 2. Comparison of correlations using para- and meta-substituent parameters for 18 6-substituted GTC analogues.

function admirably. It thus permits the reporting of novel relationships such as this one and fulfills the purpose of bringing this relationship to the attention of scientists so that it might be independently tested and added to the armamentarium of weapons at their disposal if appropriate.[9]

Thus, within limits, this single-dose measurement of lifespan enhancement in the 3LL model was practical for both identifying the best analogues of this series and using the QSAR approach to predict more refined targets for synthesis.

For QSAR analysis, the values of substituent parameters, especially electronic factors, are often positionally dependent.[15] We sought to determine the importance of correctly assigning the positions on the bicyclic benzothiazole ring, e.g., should para or meta parameters be used for 6-substituents. Initially, numerous sets of regression analyses were performed on singly substituted GTC analogues using substituent parameters where either the 6-position substituents were considered as meta while the 5-position were para or vice versa. For example, the analysis using meta parameters for 18 6-substituted GTC analogues, see Table II, resulted in eq 1 where 56% of the

$$\log [100/(100 - \text{ILS})] = 0.193F + 0.128\pi + 0.0705 \quad (1)$$

$$n = 18; \quad r^2 = 0.56; \quad p = 0.0021$$

variance ($r^2 = 0.56$) in the survival data was accounted for by an equation incorporating an electronic parameter (F) and a lipophilicity factor (π). Figure 2 shows the correlation graphically of the observed versus calculated log (100/100 - ILS) values, see curve for 5 = p. A similar analysis for F and π using para parameters gave $r^2 = 0.54$ (curve 6 = p of Figure 2). The results of this analysis and other similar ones indicated that very marginally better mathematical fits were obtained from assigning the 5-position of the benzothiazole ring as the para position. Thus the results of QSAR reported here arbitrarily employ para parameters for the 5-position and meta for the 6-position, since the precision in the efficacy measurement does not support such a subtle distinction. Table III records the statistical significance for inclusion of each variable in the respective equations 1–4.

(15) See refs 10 and 12 and Hansch, C.; Silipo, C. *J. Med. Chem.* 1974, *17*, 661.

Table III. Statistical Significance for Inclusion of Parameters in Equations 1–4

eq	parameter	significance level
1	F	0.027 6
1	π	0.000 544
2	F	0.006 54
2	π	0.004 33
2	MR_5	0.005 49
3	F_6	0.088 3
3	π_6	0.000 100
3	F_5	0.000 219
3	π_5	0.001 89
3	MR_5	0.001 98
4	F_6	0.019 1
4	R_6	0.001 52
4	π_6	0.000 100
4	F_5	0.000 100
4	π_5	0.000 187
4	MR_5	0.000 114

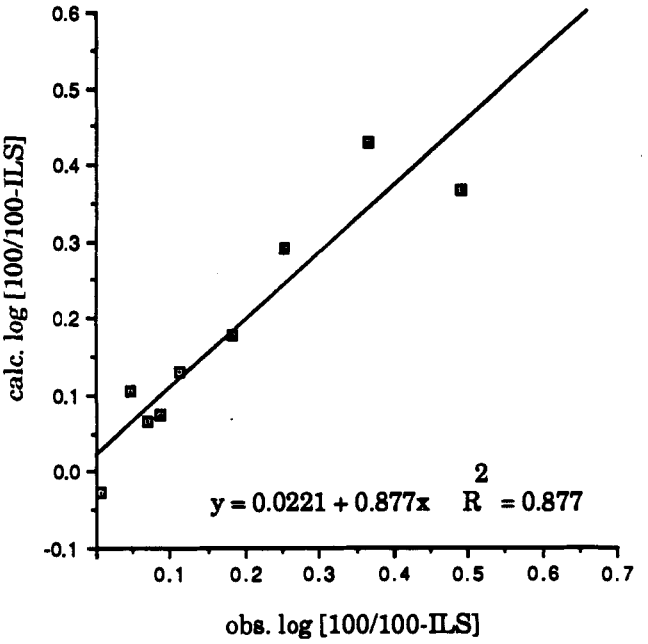

Figure 3. QSAR correlation using eq 2 with nine 5-substituted GTC analogues.

Regression analyses on the Table II set of 6-substituted GTC analogues incorporating log ILS, π^2, dipole moment (μ), μ^2, σ, Clog$P$, MR, and/or E_s led to equations that accounted for less of the variance (i.e., $r^2 < 0.56$) in the observed efficacy data. Use of the logit transform,[9] log [ILS/(100 - ILS)], led to inferior correlation equations than the probit transform.

Nine GTC analogues singly substituted at the 5-position were analyzed in a similar manner. The parameters that best accounted ($r^2 = 0.88$) for the observed variance in the in vivo efficacy were again F and π with the addition of MR_5 (eq 2). Figure 3 depicts the correlation of observed

$$\log [100/(100 - \text{ILS})] = 0.401F + 0.183\pi - 0.0159MR_5 + 0.0447 \quad (2)$$

$$n = 9; \; r^2 = 0.88; \; p = 0.01$$

with calculated values for the 5-substituted GTC analogues using eq 2 and para substituent parameters. As in the case for 6-substituted analogues the QSAR analysis of the 5-substituted benzothiazoles showed no significant difference in assigning the 5-position as para compared to assigning it as meta ($r^2 = 0.88$ versus 0.86, respectively).

The combined QSAR of the 35-member set of 5-substituted and 6-substituted analogues along with 5,6-di-

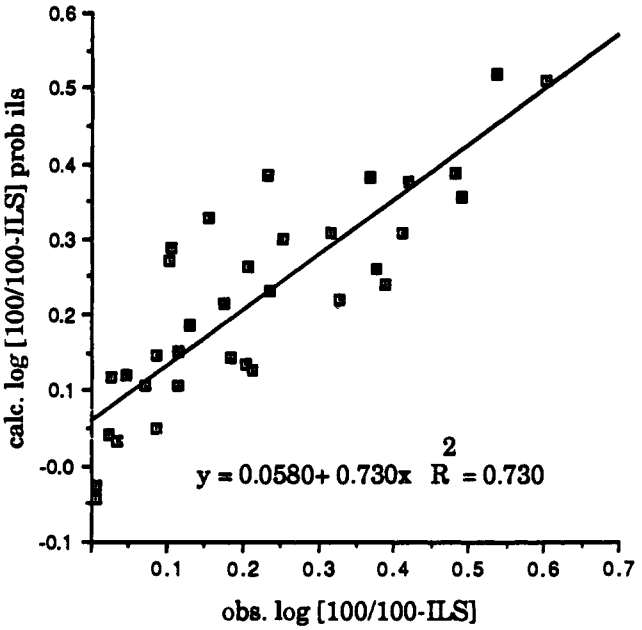

Figure 4. QSAR correlation using eq 4 with 35 5- and 6-substituted GTC analogues.

substituted GTCs demonstrated the additivity of the individual QSARs (eq 3). Further, it was found in analyzing

$$\log [100/(100 - \mathrm{ILS})] = 0.109F_6 + 0.115\pi_6 + 0.261F_5 + 0.166\pi_5 - 0.0149\mathrm{MR}_5 + 0.0942 \quad (3)$$

$$n = 35; \quad r^2 = 0.61; \quad p = 0.000\,027$$

this larger data set that inclusion of the resonance term R_6 into the regression analysis accounted for a larger percentage of the variance ($r^2 = 0.73$, $p = 0.000\,000\,7$, eq 4). Figure 4 depicts the correlation of observed efficacy with that calculated by using substituent parameters (eq 4). Substitution of ClogP for π_5 and π_6 in eq 4 resulted in a slightly poorer fit, $r^2 = 0.67$, $p = 0.000003$.

$$\log [100/(100 - \mathrm{ILS})] = 0.131F_6 + 0.861R_6 + 0.151\pi_6 + 0.303F_5 + 0.177\pi_5 - 0.0168\mathrm{MR}_5 + 0.107 \quad (4)$$

$$N = 35; \quad r^2 = 0.73; \quad p = 0.000\,000\,7$$

In completing the study of positional effects of substituents on bioactivity, GTC analogues monosubstituted at the 4-position were prepared and evaluated in the manner above. No correlations were observed between substituent parameters and efficacy. As Table I shows, numerous examples of significantly active (ILS ≥ 25%) 4-substituted GTCs were found. QSAR analyses of larger data sets including 4,5-disubstituted, 4,6-disubstituted, and/or 4,7-disubstituted analogues along with the above groups also resulted in poor correlations of questionable significance and predictive utility. An insufficient number of analogues monosubstituted at the 7-position were prepared to afford a separate QSAR analysis for this set.

Discussion

The observation of QSAR in an in vivo model of micrometastatic carcinoma is remarkable in the experience of antitumor drug discovery. While Denny and Baguley have reported QSAR for in vivo activity using 3LL with a series of amsacrine analogues[4] they found that enhancement of lifespan was related to potency in vitro. In the current work, the enhancement of survival was related to electronic, lipophilic, and steric substituent parameters for certain subsets in the series of GTC analogues rather than translation of a measured in vitro activity to in vivo activity.

In the course of this work, two factors limited scope of the QSAR analyses: (a) insignificant lifespan enhancement and (b) drug-induced lethality. ILS values for inactive compounds lie off the parallel portion of the dose–response curve and thus were not considered in the analyses. Other analogues were not included in these analyses because of significant lethality arising from drug treatment. Where substantial survival enhancement coincided with significant drug lethality, repetitive testing of these toxic analogues revealed wide variations in the observed ILS values.

It is clear that ILS values in the lower range of the data, 0–15%, are less accurately determined and may not be significant. Nevertheless, weakly active compounds can be important components of QSAR analyses where it is desirable to obtain maximum variance in the data set. Thus some weakly active analogues were included in this QSAR study when the values had been multiply determined or where survival enhancement had been observed at a higher dose, i.e., 100 mg/kg, suggesting that the response at 50 mg/kg was at the threshold of significantly measurable efficacy. Effort was made to include as many marginally active compounds as possible in order to maximize the range of biological activity and physical chemical properties embraced in the analysis.

Enhanced survival was favored (Table I) by electron-withdrawing substituents like halogens, CF_3 groups, nitro, cyano, and π-deficient aromatics substituted at the 5-, 6-, and 7-positions of the benzothiazole ring. Electron-releasing substituents like alkyl and alkoxy groups were acceptable at the 4-position. Steric constraints appeared to be most stringent at the 5-position with greater latitude observed at the 4-, 6-, and 7-positions. In general, activity increased with increasing lipophilicity; however, active compounds were observed over a broad range of lipophilicity as measured by reversed-phase HPLC retention times (Table I). For example, the 5-F analogues **19** had $t_R = 6.0$ min while the 6-phenyl analogue **14** had $t_R = 33.5$ min; both were substantially active at 50 mg/kg.

For both 5- and 6-substituted GTCs, the general observations of SAR could be more precisely quantitated, affording QSAR. The QSAR showed that a strong electron-withdrawing effect of a substituent was a somewhat more important determinant of biological activity than lipophilic factors as measured by the magnitude of the parameter coefficient. There appears to be a more than additive impact of the electronic effect as evidenced by the fact that the coefficients for F_6 and F_5 of eq 3 are decreased relative to those corresponding coefficients in eqs 1 and 2. In contrast, increased lipophilicity at both 5- and 6-positions affords enhanced survival in an additive manner as shown by the similarity of the coefficients found in eqs 1–4. Similarly, the significances to include all respective parameters are similar in the series of equations presented, see Table III. The QSAR indicates that steric bulk at the 5-position is disfavored from the negative coefficient for MR_5. This trend was further exemplified by other compounds bearing bulky substituents (data not shown). This is in contrast to the lack of correlation of MR_6 in 6-substituted GTCs.

In spite of occult structure–activity relationships embraced by 4-substituted GTCs, our empirical search among these analogues revealed several with significant activity, e.g., **28** and **30**. Substituent parameters of the 4-substituted analogues could not be found to correlate with survival, either using the parameters already shown to correlate or others also considered (vide supra). One possibility might be that these analogues undergo a different metabolic fate in comparison to the 5- and 6-sub-

stituted derivatives. Nevertheless, since disubstituted analogues with a substituent at the 4-position were readily synthesized, several were prepared by using the more active 4-substituted GTCs in combination with electron-withdrawing groups, especially at the 5- and 7-positions. A number of these disubstituted GTCs were substantially active, e.g., **49**, **50**, **51**, **53**, **58**, **59**, **61**, and **62**. As anticipated, these analogues also did not yield a QSAR equation with significant variance accounted for by physical chemical parameters.

A number of highly active analogues described in Table I were significantly active when dosed orally, e.g., **2**, **9**, **14**, **17**, **19**, **30**, **40**, **49**, **51**, and **59**. However, other very active congeners were markedly less effective with the once a day oral dosing regimen, e.g., **15**, **16**, **41**, **48** or **61**. In general, these latter were not characterized further. There were no obvious consistent structural features or substituent patterns that favored oral activity in this series.

Other analogues that were synthetic targets, for example, the 5-chloro derivative, were prepared but were not considered in this analysis because they caused substantial lethality when dosed at 50 mg/kg. Efficacy without lethality was observed at lower doses in the 5-chloro compound (ILS = 56% at 25 mg/kg) as well as in a number of others.

Conclusions

These results exemplify some of the advantages and limitations of the analytical approach to drug design afforded by the application of QSAR techniques. Significant among these limitations is the time and expense required to determine adequately the in vivo biological data on all compounds with equal quality regardless of their level of activity. Antitumor testing in vivo at its most rapid takes 10–20 days, with substantially effective compounds requiring longer periods for quantitation. In addition, cures, the most desirable result of anticancer research, are particularly unsuited for quantitative evaluation since a discontinuity arises between weakly active and strongly active compounds. Weakly active compounds are often as important as potent ones for understanding the factors that contribute to activity. Obtaining QSAR in vivo for a series of analyses requires not only significant antitumor effects but also relative homogeneity with respect to other pharmacologic and metabolic properties.

Nevertheless, limited in vivo data sets as described here can be important for establishing quantitative relationships. The systemic model for screening, with survival as an endpoint, was particularly attractive because of its greater potential clinical relevance. The consideration, individually, of subsets of a larger database permitted the discovery of important components of QSAR when the larger set was unresponsive to regression analysis techniques. The emerging QSAR identified in the GTC series led to a systematic and more efficient synthetic strategy for discovering the optimal analogues in a series, as well as identifying situations where empiricism was the most appropriate approach. Using these principles we have discovered a number of promising, systemically active, relatively potent, well-tolerated guanidinothiazolecarboxamides, exemplified particularly by compound **19**, with substantial and significant efficacy in the clinically predictive model of 3LL Lewis lung carcinoma.

Experimental Section

All melting points less than 250 °C were determined on a Thomas-Hoover apparatus and are uncorrected. All melting points greater than 250 °C were determined on a Mel-Temp hot stage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on either Varian XL-300 or Brucker AM-300 MHz spectrometers, using DMSO-d_6 as solvent, unless otherwise noted. Coupling constants were obtained by measuring spectral spacings judged to be first order. Infrared (IR) spectra were taken on a Perkin-Elmer Model 283 spectrometer in KBr pellets. Thin layer chromatography (TLC) was used to monitor all reactions and was performed on EM Science silica gel-60 F254 precoated glass plates. For flash column chromatography, Universal Scientific Corporation Silica Woelm (32–63 μm) was used. High performance liquid chromatography (HPLC) was performed on a Waters Nova-Pak C18 15-cm column, using either 60% or 80% CH_3OH/buffer (0.012 M TEA/0.013 M TFA adjusted to pH 3.5 with 1 N NaOH) as eluant at a flow of 1 mL/min. Detection was obtained on a Gilson Holochrome UV detector at 254 nm. Mass spectra were recorded on a Hitachi RMU6-E spectrometer. Microanalyses were performed by the Pfizer Central Research microanalysis laboratory, and results obtained for specified elements are within ±0.4% of the theoretical values, unless otherwise noted.

2-Guanidino-*N*-(6′-chlorobenzothiazol-2′-yl)thiazole-4-carboxamide Hydrochloride (3) [Method A1]. Sodium hydride (0.450 g, 18.7 mmol, 60% oil dispersion) was washed free of its oil with 3 × 25 mL of hexanes and then suspended in 30 mL of anhydrous tetrahydrofuran. 2-Amino-6-chlorobenzothiazole (3.45 g, 18.7 mmol) was added in one portion (*CAUTION*: vigorous H_2 evolution) followed by addition of 20 mL of tetrahydrofuran. After 1 h, 2-guanidinothiazole-4-carboxylic acid, ethyl ester (2.00 g, 9.34 mmol) was added as a solid followed by 20 mL of tetrahydrofuran. The mixture was refluxed for 6 h and quenched onto 400 mL of 5% $NaHCO_3$. The basic phase was extracted with 3 × 200 mL of ethyl acetate. The pooled organic phases were shaken with 500 mL of 1 N HCl and filtered. The solid thus obtained was triturated with methanol and then dried at 110 °C and 0.2 Torr, affording **3**, 0.708 g (19.5%): mp 295 °C; ^{1}H NMR δ 7.51 (dd, J = 3 Hz, 10 Hz, H-5′), 7.80 (d, J = 10 Hz, H-4′), 8.35 (s, H-5), 8.45 (br, H-7′ and 2 × NH_2); ms, m/e 352 (M^+). Anal. ($C_{12}H_9ClN_6OS_2 \cdot HCl \cdot H_2O$) C, H, N.

2-Guanidino-*N*-(6′-methoxybenzothiazol-2′-yl)thiazole-4-carboxamide (5) [Method A2]. Sodium hydride (0.450 g, 18.7 mmol, 60% oil dispersion) was washed free of its oil with 3 × 25 mL of hexanes and then suspended in 30 mL of anhydrous tetrahydrofuran. 2-Amino-6-methoxybenzothiazole (3.63 g, 18.7 mmol) was added in one portion (*CAUTION*: vigorous H_2 evolution) followed by addition of 20 mL of tetrahydrofuran. After 1 h, 2-guanidinothiazole-4-carboxylic acid, ethyl ester (2.00 g, 9.34 mmol) was added as a solid followed by 20 mL of tetrahydrofuran. The mixture was refluxed for 6 h, quenched onto 10 mL of water, diluted with 500 mL of ether, and extracted with 4 × 100 mL of 1 N NaOH. The combined base layers were adjusted to pH 7.2 with 6 N HCl and extracted with 5 × 150 mL of ethyl acetate. The pooled ethyl acetate layers were washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo to afford a light tan solid. Trituration with 100 mL of acetonitrile afforded **5**, 1.12 g (34.3%): mp 253–254 °C; ^{1}H NMR δ 3.82 (s, 3 H, CH_3), 6.7–7.0 (br, 4 H, 2 × NH_2), 7.05 (dd, J = 3, 10 Hz, 1 H, H-5′), 7.60 (d, J = 3 Hz, 1 H, H-7′), 7.65 (d, J = 10 Hz, 1 H, H-4′), 7.71 (s, 1 H, H-5), 12.49 (br s, 1 H, NH); ms, m/e 348 (M^+). Anal. ($C_{13}H_{12}N_6O_2S_2 \cdot H_2O$) C, H, N.

2-Guanidino-*N*-(6′-cyano-4′-ethylbenzothiazol-2′-yl)thiazole-4-carboxamide Hydrochloride (55) [Method B1]. A suspension of 2-amino-6-cyano-4-ethylbenzothiazole (2.03 g, 10.0 mmol), 2-guanidinothiazole-4-carboxylic acid, *N*-hydroxysuccinimide ester hydrochloride (3.06 g, 10.0 mmol), and a trace of hydroquinone (approximately 10 mg) in 25 mL of *N*-methyl-2-pyrrolidone (NMP) was heated in the dark under N_2 at 125 °C for 6 h. The tan precipitate, formed upon the addition of the cooled reaction mixture to 1.5 L of ether, was filtered, washed with 2 × 50 mL of methanol, and dried in vacuo at 2 Torr and 65 °C, affording **55**, 2.62 g (64%): mp 294–297 °C; ^{1}H NMR δ 1.29 (t, J = 6 Hz, 3 H, CH_3), 3.06 (q, J = 6 Hz, 2 H, CH_2), 7.69 (s, 1 H, H-5′), 8.34 (s, 1 H, H-7′), 8.42 (s, 1 H, H-5), 8.48 (br, 4 H, 2 × NH_2); ms, m/e 372 (M^+). Anal. ($C_{15}H_{13}N_7OS_2 \cdot HCl \cdot 2H_2O$) C, H, N.

***N*-(6′-Phenylbenzothiazol-2′-yl)-2-guanidinothiazole-4-carboxamide (14) [Method B2].** A suspension of 2-amino-6-phenylbenzothiazole hydrochloride (29.3 g, 0.100 mol), 2-guanidinothiazole-4-carboxylic acid, *N*-hydroxysuccinimide ester

hydrochloride (32.0 g, 0.100 mol), and hydroquinone (100 mg, 0.91 mmol) in 300 mL of NMP was heated in the dark under N_2 at 125 °C for 6 h. The tan precipitate, formed upon the addition of 1 L of 5% $NaHCO_3$ to the cooled reaction mixture, was filtered, washed with 2 × 100 mL of water, and dried in vacuo at 80 Torr and 55 °C. Recrystallization from 175 mL of pyridine afforded **14**, 14.6 g (74.2%): mp 288–290 °C; ^{1}H NMR δ 6.86 (br, 4 H, 2 × NH_2), 7.34 (t, J = 9 Hz, 1 H, H-4″), 7.46 (t, J = 9 Hz, 2 H, 2 × H-3″), 7.74 (m, 4 H, H-5, 2 × H-2″, H-5′), 7.82 (d, J = 9 Hz, 1 H, H-4′), 8.30 (s, 1 H, H-7′); ms, m/e 395 (M^+). Anal. ($C_{18}H_{14}N_6OS_2$·0.25H_2O) C, H, N.

***N*-(6′-Methylbenzothiazol-2′-yl)-2-guanidinothiazole-4-carboxamide, Sodium Salt (8) [Method D].** Compound **8** (0.539 g, 1.62 mmol), prepared by method A2, was dissolved in 20 mL of dimethyl sulfoxide. A solution of $NaOCH_3$, prepared from sodium (37.3 mg, 1.62 mmol) in methanol (2 mL, 49.4 mmol), was added under N_2. After 20 min, the red solution was added to 300 mL of ether and then filtered. The residual solid was redissolved in 2 × 10 mL of dimethyl sulfoxide and refiltered. The filtrate was poured onto 300 mL of ether, affording a precipitate. The filtered solid was washed with 1 × 40 mL of methanol and dried in vacuo at 2 Torr and 111 °C, affording **8**, 0.525 g (91%): mp 260 °C; ^{1}H NMR δ 2.33 (s, 3 H, CH_3), 7.00 (dd, J = 2 Hz, 10 Hz, 1 H, H-5′), 7.26 (s, 1 H, H-5), 7.30 (d, J = 10 Hz, 1 H, H-4′), 7.42 (d, J = 2 Hz, 1 H, H-7′); ms, m/e 332 (M^+). Anal. ($C_{13}H_{11}H_6OS_2$·Na·1.5H_2O) C, H, N.

2-Guanidino-*N*-(7′-(trifluoromethyl)benzothiazol-2′-yl)-thiazole-4-carboxamide, Sodium Salt (36) [Method H]. The hydrochloride salt of **36**, prepared by method B1 (0.960 g, 2.36 mmol), was suspended in 60 °C water (75 mL) and adjusted to pH 12 by addition of 5 N NaOH. From the cooled aqueous, a precipitate was filtered, washed with 1 × 15 mL of water, and dried in vacuo (2 Torr) at 111 °C, yielding **37** (24%): mp 255–258 °C; ^{1}H NMR δ 7.76 (m, 2 H, H-6′, H-7′), 7.84 (s, 1 H, H-5), 7.96 (br, 4 H, 2 × NH_2), 8.12 (d, 1 H, J = 3 Hz, H-5′); ms, m/e 387 (M^+). Anal. ($C_{13}H_9N_6OS_2F_3N$·H_2O) C, H, N.

2-Guanidino-*N*-(6′-chloro-7′-(trifluoromethyl)benzothiazol-2′-yl)thiazole-4-carboxamide Hydrochloride (48). Neutral **48** (1.28 g, 3.27 mmol) was dissolved in 50 mL of methanol and treated with 3.4 mL of anhydrous 1 N HCl/methanol. The solid resulting from addition of 300 mL of ether was filtered and dried in vacuo at 110 °C overnight, affording pure **48**, 0.580 g (39%): mp 339–340 °C; ^{1}H NMR δ 7.72 (d, J = 9 Hz, 1 H, H-5′), 8.00 (d, J = 9 Hz, 1 H, H-4′), 8.07 (s, 1 H, H-5), 8.40 (br s, 4 H, 2 × NH_2); ms, m/e 422 (M^+). Anal. ($C_{13}H_8ClF_3N_6OS_2$·HCl) C, H, N.

2-Amino-5-fluoro-6-hydroxybenzothiazole Hydrochloride. 2-Amino-5-fluoro-6-methoxybenzothiazole (16.1 g, 58.0 mmol) was refluxed in 150 mL of 48% HBr for 16 h. The solid, obtained after filtration of the cooled reaction mixture, was neutralized with $NaHCO_3$ in 300 mL of water and extracted into 3 × 300 mL of ethyl acetate. The pooled organic extracts were washed with 50 mL each of water and brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to a residue. Conversion to the hydrochloride salt was accomplished in anhydrous HCl/methanol followed by precipitation with ether, 6.20 g (49%): mp 287–290 °C; ^{1}H NMR δ 7.33 (d, J = 12 Hz, 1 H, H-4), 7.49 (d, J = 6 Hz, 1 H, H-7), 9.9 (br, 3 H, NH_2 and OH): ms, m/e 184 (M^+). Anal. ($C_7H_5FN_2OS$·HCl) C, H, N.

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Registry No. **1**, 134056-79-4; **1**·HCl, 126611-48-1; **2**, 134056-80-7; **2**·HCl, 126611-45-8; **3**, 134056-81-8; **3**·HCl, 126611-47-0; **4**, 126612-40-6; **4**·Na, 134057-22-0; **5**, 126612-41-7; **6**, 126612-43-9; **6**·Na, 134057-23-1; **7**, 126612-44-0; **7**·Na, 134057-24-2; **8**, 126612-45-1; **8**·Na, 134057-25-3; **9**, 134056-82-9; **9**·HCl, 126611-51-6; **10**, 126612-46-2; **10**·Na, 134057-26-4; **11**, 134056-83-0; **11**·HCl, 134057-27-5; **12**, 134056-84-1; **12**·Na, 134057-28-6; **13**, 134056-85-2; **13**·HCl, 126611-54-9; **14**, 134056-86-3; **15**, 134056-87-4; **15**·HCl, 126611-59-4; **16**, 134056-88-5; **16**·HCl, 126611-79-8; **17**, 134056-89-6; **17**·HCl, 126611-81-2; **18**, 126612-57-5; **18**·Na, 134057-29-7; **19**, 126612-52-0; **20**, 134056-90-9; **20**·HCl, 126611-55-0; **21**, 126612-55-3; **21**·Na, 134057-30-0; **22**, 134056-91-0; **22**·HCl, 126611-62-9; **23**, 134056-92-1; **23**·HCl, 126611-77-6; **24**, 134056-93-2; **24**·HCl, 126611-65-2; **25**, 134056-94-3; **25**·HCl, 126611-66-3; **26**, 134056-95-4; **26**·HCl, 126611-67-4; **27**, 134056-96-5; **27**·HCl, 134057-31-1; **28**, 134056-97-6; **28**·HCl, 126611-46-9; **29**, 126612-42-8; **29**·Na, 134057-32-2; **30**, 134056-98-7; **30**·HCl, 134057-33-3; **31**, 126612-48-4; **31**·Na, 134057-34-4; **32**, 126612-56-4; **32**·Na, 134057-35-5; **33**, 134056-99-8; **33**·HCl, 126611-63-0; **34**, 126612-49-5; **34**·HCl, 126611-64-1; **35**, 134057-00-4; **35**·HCl, 134057-36-6; **36**, 126612-51-9; **36**·Na, 134057-37-7; **37**, 134057-01-5; **37**·HCl, 126611-68-5; **38**, 126612-47-3; **38**·CH_3OH, 134057-38-8; **39**, 134057-02-6; **39**·HCl, 126611-61-8; **40**, 134057-03-7; **40**·CH_3OH, 134057-39-9; **41**, 134057-04-8; **42**, 134057-05-9; **43**, 134057-06-0; **44**, 134057-07-1; **45**, 134057-08-2; **46**, 134057-09-3; **47**, 134057-10-6; **47**·HCl, 134057-40-2; **48**, 134057-11-7; **48**·HCl, 126637-54-5; **49**, 134057-12-8; **50**, 134057-13-9; **50**·HCl, 126611-78-7; **51**, 134057-14-0; **51**·HCl, 126611-72-1; **52**, 134057-15-1; **53**, 134057-16-2; **54**, 126612-61-1; **54**·HCl, 134057-41-3; **55**, 134057-17-3; **55**·HCl, 126611-00-5; **56**, 126612-64-4; **57**, 134057-18-4; **58**, 126612-54-2; **58**·Na, 134057-42-4; **59**, 134057-19-5; **59**·HCl, 126611-75-4; **60**, 126612-62-2; **60**·Na, 134057-43-5; **61**, 134057-20-8; **61**·Na, 134057-44-6; **62**, 134057-21-9; **62**·Na, 134057-45-7; **63**, 126612-65-5; 2-aminobenzothiazole, 136-95-8; 2-amino-6-chlorobenzothiazole, 95-24-9; 2-amino-6-methoxybenzothiazole, 1747-60-0; 2-amino-6-cyano-4-ethylbenzothiazole, 134057-46-8; 2-amino-6-phenylbenzothiazole hydrochloride, 134057-47-9; 2-amino-5-fluoro-6-methoxybenzothiazole, 134057-48-0; ethyl 2-guanidinothiazole-4-carboxylate, 82982-26-1; 2-guanidinothiazole-4-carboxylate *N*-hydroxysuccinamide ester, 134057-49-1.