evaporated. The residue was purified by column chromatography on silica gel. Elution with ethyl acetate–light petroleum (40–60 °C) (1:1) gave first a colorless oil. Attempted crystallization of this compound from ethanol was unsuccessful and evaporation of the solvent afforded the α-anomer **15** (0.143 g, 87%) as a white foam: UV (EtOH) λ_{max} 224.4 nm (ε 45 300), 230 (sh 36 000), 244.8 (sh 14 900), 282.4 (4400), 309.6 (6900); IR ($CHBr_3$) 3525, 3425, 1730, 1265 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.71 (2 H, d, 2H-5′), 4.93 (1 H, q, H-4′), 5.08 (2 H, bs, NH_2), 5.77 (1 H, dd, J_{HF} = 17 Hz, H-3′), 6.10 (1 H, d, J_{HF} = 48 Hz, H-2′), 6.41 (1 H, d, J_{HF} = 14 Hz, H-1′), 7.3–7.7 (6 H, m, 6 phenyl protons), 7.72 (2 H, d, 2 ortho phenyl protons), 8.02 (1 H, s, H-8), 8.12 (2 H, d, 2 ortho phenyl protons). Anal. ($C_{24}H_{19}ClFN_5O_5$·0.25H_2O·EtOH) C, H, N; Cl: calcd, 6.31; found, 6.91.

This was followed by the β-anomer **16**, which was crystallized from dichloromethane–petroleum (40–60 °C) and dried in vacuo over P_2O_5 at room temperature for 48 h to give white fluffy hygroscopic crystals (543 mg, 35%): mp 84–87 °C; UV (EtOH) λ_{max} 222.4 nm (ε 43 700), 228 (sh 33 900), 244 (sh 11 100), 277.8 (3200), 309 (7900); IR ($CHBr_3$) ν_{max} 3520, 3415, 1720, 1286 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.58 (1 H, q, H-4′), 4.7–4.9 (2 H, m, 2H-5′), 5.13 (2 H, bs, NH_2), 5.34 (1 H, dd, J_{HF} = 50 Hz, H-2′), 5.77 (1 H, dd, J_{HF} = 17 Hz, H-3′), 6.46 (1 H, dd, J_{HF} = 22 Hz, H-1′), 7.40–7.80 (6 H, m, 6 phenyl protons), 8.04 (1 H, d, J_{HF} = 4 Hz, H-8), 8.09 (4 H, d, 4 ortho phenyl protons). Anal. ($C_{24}H_{19}ClFN_3O_5$·0.25CH_2Cl_2) C, H, N.

2-Amino-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one (17). To a solution of **16** (0.458 g, 0.89 mmol) in dioxane (8 mL) was added 0.5 N sodium hydroxide solution (13.5 mL, 6.75 mmol) and the mixture heated at 100 °C for 1 h. The mixture was cooled to room temperature and 1 N hydrochloric acid added to adjust the solution to pH 7. The mixture was evaporated and the residue purified by preparative HPLC (elution 4:1 MeCN–H_2O, 10 mL/min) to give **17** (51 mg, 20%), which was crystallized from water–ethanol (9:1): mp 245–247 °C; $[\alpha]^{22}_D$ = +41.6° (*c* 0.3, MeOH); UV (MeOH) λ_{max} 253 nm (ε 14 300), 262.4 (sh, 11 400); IR (Nujol) ν_{max} 3510–3000, 1720, 1675, 1633, 1602 cm^{-1}. Anal. ($C_{10}H_{12}FN_5O_4$·0.2EtOH·H_2O) C, H, N. This data and the NMR parameters (Table I) are consistent with those previously reported for **17**.[21,22]

Antiviral Activity. (A) Anti-Herpes Activity. Anti-herpes activity was measured in a plaque-reduction assay.[25] Confluent monolayers of Vero cells in 24-well plates (NUNC) were infected with 30–40 plaque forming units of either HSV-1 (strain KOS) or HSV-2 (strain 186). The infected monolayers were incubated at 37 °C for 1 h and then overlaid with maintainence medium containing 0.75% (carboxymethyl)cellulose and various concentrations of test compound. The monolayers were incubated for a further 2 days at 37 °C, after which the cells were fixed and stained, the plaques were counted, and the concentration of compound causing 50% inhibition of plaque formation was calculated.

(B) Cytotoxicity. The cytotoxic effects of the test compounds on Vero cells were determined by examination of mock infected cell monolayers incubated with the compounds. Gross changes in cell staining, number, or morphology were noted and scored. Cytotoxic doses quoted were those that caused 50% of the cell monolayer to be affected.

(C) Animal Studies. Efficacy Tests. Female albino mice weighing 15–18 g (Charles River UK Ltd.) were inoculated intraperitoneally with 0.2 mL of virus suspension equivalent to (5–15) × LD_{50} dose. One hour after the challenge the mice were treated (0.2 mL sc) with solutions of test compounds in saline. Mice were treated 2× daily for 4 days, in groups of 5–10 animals, for a range of doses. The experiment was terminated on day 21 and the number of mice survivors in the various treatment groups was used to calculate the ED_{50} dose. The median effective dose (ED_{50}, mg/kg per dose) was calculated by logit transformation from the numbers of animals surviving at each dose level on day 21 as described by Litchfield and Wilcoxon.[31]

Acknowledgment. We thank Dr. V. E. Wilson for assistance in interpreting the spectroscopic data and Janet Horn for technical assistance with the antiviral evaluations.

Registry No. **6a**·HCl, 110312-76-0; **6b**·HCl, 111001-52-6; **6c**·HCl, 131043-35-1; **6d**·HCl, 131101-17-2; **7a**, 110289-10-6; **7b**, 131101-18-3; **7c**, 131043-36-2; **7d**, 131101-19-4; **8a**, 110289-09-3; **8b**, 131101-20-7; **8c**, 131043-37-3; **8d**, 131101-21-8; **9a**, 110289-11-7; **9b**, 131101-22-9; **9c**, 131043-38-4; **9d**, 131101-23-0; **10a**, 110289-21-9; **10c**, 131043-39-5; **10d**, 131101-24-1; **11a**, 110289-24-2; **11b**, 131101-25-2; **11c**, 131043-40-8; **11d**, 131101-26-3; **12c**, 123238-61-9; **12d**, 110567-26-5; **13**, 97614-44-3; **15**, 118373-60-7; **16**, 118373-61-8; **17**, 103884-98-6; 2-amino-6-chloropurine, 10310-21-1; 2-amino-4,6-dichloropyrimidine, 56-05-3; 4-chlorobenzenediazonium chloride, 2028-74-2.

(30) This experiment was carried out by Dr. D. E. Lake of our Chemical Development Department.
(31) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

N-(5-Fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide. A Novel, Systemically Active Antitumor Agent Effective against 3LL Lewis Lung Carcinoma

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N-(5-Fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide (1) is a member of a series of amides found to substantially increase lifespan in mice bearing established micrometastatic 3LL Lewis lung carcinoma. Amide 1 is effective after either oral or intraperitoneal dosing in acute, subacute, or chronic regimens. 1 is well tolerated in this model with an excellent therapeutic index relative to the cytotoxic anticancer drug adriamycin.

The control of disseminated tumor growth by systemically active chemotherapeutants remains a major challenge for cancer chemotherapy despite decades of focused effort. Although there are some notable successes with certain forms of cancer, drug therapy has had only limited impact against the three major killers: carcinoma of the lung, breast, and colorectal system.

Considerable data support the usefulness of transplantable tumor systems for discovery of clinically active cancer therapeutants.[1] Among these certain tumor models have been shown to have higher predictivity for clinical success.[2] In our analysis of this literature we concluded that it was important to employ a model system for discovering anticancer drugs that embraced systemic drug treatment, i.e., administration of drug at a site distal to

(1) (a) Overjera, A. A.; Johnson, R. K.; Goldin, A. *Cancer Chemother. Rep.* **1975**, *5*, 111. (b) Carter, S. K.; Goldin, A. *Natl. Cancer Inst. Monogr.* **1977**, *45*, 63. Goldin, A.; Kline, I.; Sofina, Z. P. (Eds) *Natl. Cancer Inst. Monogr.* **1980**, *55*. (c) Goldin, A.; Venditti, J. M.; MacDonald, J. S.; Muggia, F. M.; Henry, J. E.; DeVita, V. T., Jr. *Eur. J. Cancer* **1975**, *2*, 1.

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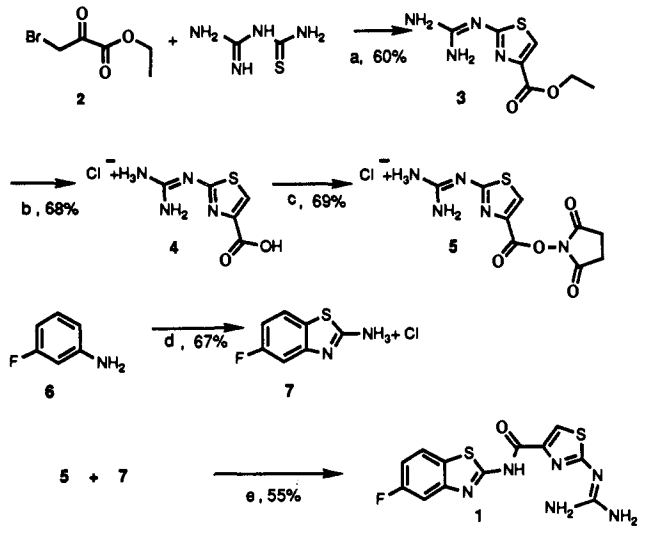
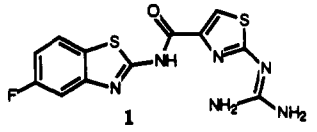
Scheme I. Synthesis of Guanidinothiazolecarboxamide (GTC) 1[a]



[a] (a) 3 h reflux in ethanol. (b) 0.5 h reflux in 3 N KOH then acidification with 6 N HCl. (c) 1 equiv of DCC and 1 equiv of *N*-hydroxysuccinimide in DMF. (d) 1.1 equiv of NH_4NCS and 1.1 equiv of benzoyl chloride in acetone, reflux 1.5 h, then 1 equiv of Br_2 in dichloroethane <32 °C for 18 h. (e) Stoichiometric at 120 °C with 0.3% hydroquinone in NMP, then $NaHCO_3$.

tumor implantation, in contrast to "local" therapy, e.g., intraperitoneal (ip) drug treatment after ip tumor implantation. In addition our goals embraced identifying compounds that were effective against solid tumors without prescreening for efficacy versus hemopoetic tumors, since activity against P388 or L1210 was not a particularly good predictor for effect on carcinomas in either animal models or human therapy.[2] Thus, we initially chose to evaluate the anticancer potential of new synthetics arising from in vitro assays in the in vivo model of intravenous (iv) induced experimental pulmonary metastasis of Lewis lung carcinoma (3LL).[1a]

Among compounds found efficacious in this system was the guanidinothiazolecarboxamide (GTC), *N*-(5-fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide (1).[3]



1 is structurally related to a serine protease inhibitor tested and found active in the 3LL model.[4,5] Our efforts focused on optimization of in vivo efficacy in the structural class. We observed that effects against proteases were *not* concordant with efficacy in the 3LL model and thus concluded that the antitumor effects exhibited by 1 were not likely to arise from protease inhibition.

We report here that compound 1 possesses therapeutic activity in the iv 3LL model using survival as the endpoint. GTC 1 substantially increases lifespan of mice bearing nascent and established micrometastatic disease of 3LL Lewis lung carcinoma in a dose-dependent manner by

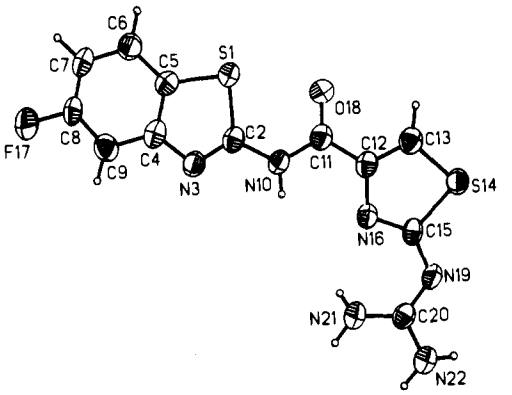


Figure 1. X-ray structure of compound 1.

intraperitoneal (ip) and oral (po) routes of administration. Amide 1 was well tolerated with an excellent therapeutic index in this animal model. Its systemic activity in a predictive carcinoma model and its structural novelty, unrelated to known antitumor agents, suggest that 1 may be an important discovery among anticancer agents.

Chemistry

Compound 1 was prepared by the general method shown on Scheme I. Ethyl ester 3 was prepared from 2 according to the method of LaMattina.[6] Saponification of 3 afforded 4. It was critical to isolate the acid hydrochloride form of 4 in order to ensure high yields during the subsequent coupling reaction. Workup of the saponification reaction was complicated by precipitation of zwitterionic 4 during the acidification of the basic reaction mixture. Further acidification afforded dissolution of the zwitterion with formation of the hydrochloride salt of 4, suitable for conversion to the hydroxysuccinimide active ester hydrochloride, 5, using DCC. Compound 5 is sensitive to moisture and should be stored under anhydrous conditions in the cold and dark.

2-Amino-5-fluorobenzothiazole (7) prepared from *m*-fluoroaniline (6) by using standard procedures, was coupled as its HCl salt with 1 equiv of the active ester hydrochloride 5 in NMP (*N*-methylpyrrolidinone) at 120 °C in the dark. Trace hydroquinone was used to inhibit radical fragmentations of the ester at the higher temperature. GTC 1, isolated initially as neutral material by quenching the reaction with 5% $NaHCO_3$, was purified by recrystallization from acetic acid and then pyridine followed by trituration with methanol.

GTC 1 also was formed directly by condensation of neutral ester 3 with a metal salt of the benzothiazole 7. In a typical reaction, the anion of 7 generated with NaH was condensed with 3 in DMF at room temperature during 16 h. Potassium t-butoxide in THF also has been shown to effect the reaction. This method was general for substituted benzothiazoles that are unaffected by strong base.

Amide 1 is amphoteric. The acidity of the protonated guanidine group is pKa 5.3 while that of the amide N–H is pKa 10.3, as measured in 3:1 $DMSO/H_2O$ at 22 °C.

(2) Staquet, M. J.; Byar, D. P.; Green, S. B.; Rosencweig, M. *Cancer Treat. Rep.* **1983**, *67*, 753. Martin, D. S.; Balis, M. E.; Fisher, B.; Freireich, E. J.; Heppner, G. H.; Holland, J. F.; Houghton, P. J.; Johnson, R. K.; Mittelman, A.; Rustum, Y.; Sawyer, R. C.; Schnid, F. A.; Stolfi, R. L.; Young, C. W. *Cancer Res.* **1986**, *46*, 2189.

(3) Schnur, R. C.; Fliri, A. F. J. EP-343,893, 1990.

(4) For a review of this subject, see: Goldfarb, R. J. Plasminogen Activators. In *Annual Reports in Medicinal Chemistry*; Hess, H.-J., Ed.; Academic Press: New York, 1983; Vol. 18, pp 257–264 and references therein.

(5) In vivo activity has been reported for the guanidinobenzoate ester FOY-305: Ohkashi, M. *Gann* **1981**, *72*, 959. Ohkashi, M.; Fujii, S. *Gann* **1982**, *73*, 108. Ohkashi, M.; Fujii, S. *JNCI* **1983**, *71*, 1053.

(6) LaMittina, J. L.; Lipinski, C. A. US Pat., 4,374,843, Feb 22, 1983.

Thus, 1 is conveniently converted to a variety of salt forms including hydrochloride, mesylate, and sodium salts, all of which express similar activity in in vivo studies. Compound 1 appears to be relatively stable in acidic and neutral media; however, saponification occurs under vigorously basic conditions. Thus, attempts to recrystallize the sodium salt from water afforded pure material in much reduced yield with concomitant hydrolysis.

X-ray crystallographic analysis of 1 (Figure 1) using crystals obtained from DMSO, reveals a largely planar conformation with the tautomeric form of the guanidine as drawn. One molecule of solvent DMSO and one and one-half molecules of water of hydration per molecule of 1 are incorporated into the unit cell.

Biology

Experimental pulmonary metastases of 3LL Lewis lung carcinoma were induced by an intravenous injection of 3LL cells according to published procedures as modified by Pollack to afford greater reproducibility and suitability for routine screening.[1a,7] Intravenous implantation was performed in the lateral tail vein of 18–20-g female (C57BL/6 X DBA/2)F_1 hybrid mice (BDF_1 mice) with 6 × 10^5 log phase 3LL cells suspended in 0.20 mL RPMI 1640 medium. Mice were randomized upon receipt of shipment and immediately after tumor implantation. Groups of seven mice each were used for each treatment. For ip or po dosing, compound 1 was freshly formulated by dissolution in a small amount of dimethyl sulfoxide (DMSO, 20% of final volume) and dilution with sufficient sterile saline (0.15 N sodium chloride) to produce a fine suspension. Test animals, 7 animals per treatment group, were treated with 200 μL of drug solution from days 2–9 after implantation (8 treatments) and were monitored daily thereafter for survivors. The increased lifespan was measured as a ratio of treated versus control groups (T/C) calculated from the median survival times (MST), where T/C (%) = ($MST_{treated}/MST_{controls}$ × 100%). The MST for the control groups averaged 19 days with a range of medians in individual experiments of 16–23 days. The results of testing under this regimen are shown in Table I. Reference drugs adriamycin and cyclophosphamide were used in this model as positive controls during each experiment. Adriamycin (23 mg/kg iv) and cyclophosphamide (300 mg/kg ip) were given as a single injection 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, T/C ≥ 150%.

For monolayer assay determinations, exponentially growing 3LL cells, cultured in RPMI 1640 supplemented with 10% fetal calf serum with 2 mM glutamine, 25 μg/mL gentamycin, and 500 units pen/strep, were harvested by trypsinization then seeded into 6-well plates at a cell density of 2.5–5.0 × 10^4 cells/mL in 2 mL of growth media. Cells were incubated at 37 °C overnight. Then the media was exchanged for 3 mL of fresh media containing 1. Cells were collected by centrifugation at 800 rpm for 5 min after trypsin treatment, exposed to 0.4% trypan blue in 50% growth media and counted on a hemacytometer. The number of viable cells was determined in control and drug treated wells. The IC_{50} was determined as the drug concentration required to reduce by 50% the number of viable cells collected at 48 h.

(7) Pollack, V. A.; Fidler, I. J. *JNCI* 1982, *69*, 137.

Table I. In Vivo Activity of Compound 1 against 3LL Metastasis in BDF_1 Mice

dose, mg/kg	route	% T/C[a] (s.e.)	% lethality[b] (s.e.)	n[c]
6.25	ip	112.7 (7)	0.00	6
12.5		149.3 (20)	0.00	8
25.0		153.8 (16)	0.00	9
50.0		153.0 (15)	0.43 (2.4)	33
100		160.7 (16)	0.00	7
200		179.7 (18)	14.3 (12)	3
400		-	42.9	1
12.5	po	119.0 (14)	0.00	5
25.0		127.0 (10)	1.78 (4.7)	8
50.0		140.4 (12)	2.00 (7.4)	14
100		157.7 (20)	5.70 (11)	20
200		150.3 (11)	22.7 (29)	24
400		-	45.7 (46)	5

[a] % T/C = [$MST_{treated}/MST_{control}$] × 100%, where MST is the median survival time, dosing on days 2–8 after tumor challenge. [b] Drug related lethality is defined as % animals in treated groups dying prior to first tumor related death in untreated groups. [c] n = number of separate experimental determinations of T/C and lethality.

For the colony-formation inhibition assay in soft agar, 3LL cells, 2 × 10^4 cells/mL, growing in 3 mL of 0.5% agar, were incubated for 10 days at 37 °C after being layered with drug 1 dissolved in 3 mL of 0.35% agar. Colonies were counted with an Optimax densitometer.

Results and Discussion

The in vivo model of metastasis and tumor outgrowth was an iv implanted 3LL Lewis lung carcinoma with therapy on and after day 2 following tumor challenge. The level of tumor challenge was established such that drug treatment would not result in long-term survivors with positive controls adriamycin and cyclophosphamide. When the 3LL model was established by our laboratories in the above manner a graded quantitative relationship was obtainable for all controls and compounds emanating from discovery efforts.[8] A graded quantitation of data for test compounds was clearly useful when selecting those compounds that warranted fuller characterization from among closely related analogues.

Compound 1 produced significant ($P < 0.05$) and substantial (T/C > 150%) enhancement of survival in mice bearing 3LL lung metastases after either systemic ip or po dosing (Table I). The activity of 1 against Lewis lung carcinoma was remarkable for both its degree and its range of effective doses against established tumor metastases (Table I). The T/C numbers reported in Table I at each dose level are mean values ± standard error, resulting from multiple experiments (n = 3–33, depending on dose). Significantly, 1 was approximately equally effective after oral or ip dosing although it appears to be less potent orally.

Intraperitoneal administration of 1 afforded a wide margin of safety (Figure 2) with a therapeutic index (TI) of approximately 40 (TI = $[1]_{LD50\%}/[1]_{T/C150\%}$ = ~500/12.5). While the TI observed after oral dosing under this regimen was diminished, TI ~8–10, significant margin of safety exists to enable administering the drug at substantially efficacious doses. In addition, intraperitoneal administration of lower doses for longer time periods resulted in significant efficacy, e.g. at 6.25 mg/kg on days 2–17 the T/C = 143% (n = 2).

(8) Using a similar model of 3LL with slightly modified conditions, Robins observed long term survivors for tiazafurin but also, significantly, for cyclophosphamide treatment: Robins, R. K.; Srivastava, P. C.; Narayanan, V. L.; Plowman, J.; Paull, K. D. *J. Med. Chem.* 1982, *25*, 107.

Table II. Survival in the Iv 3LL Model with Anticancer Agents

drug	dose, mg/kg	route	regimen[a]	% T/C	lethality	n
adriamycin	2.5	iv	d3	120	0	3
	5.0			115	0	3
	10			137	0	3
	15			215	21	3
	18			191	0	61
	20			189	15	3
	25			197	72	3
	30			–	100	3
cisplatin	5.0	ip	d3	112	0	2
	10			116	0	2
5-fluorouracil	25	ip	d2,4	114	0	1
	50			118	0	1
	100			127	0	1
	200			141	28	1
	50	ip	d3–6	109	0	1
	100			125	14	1
etoposide	8.0	ip	d3,7,11	118	0	2
	16			128	0	2
	32			130	7	2
cyclophosphamide	300	ip	d3	192	0	61
	400			172	14	1

[a] Days after tumor challenge on which drug was dosed; see Table I for other definitions.

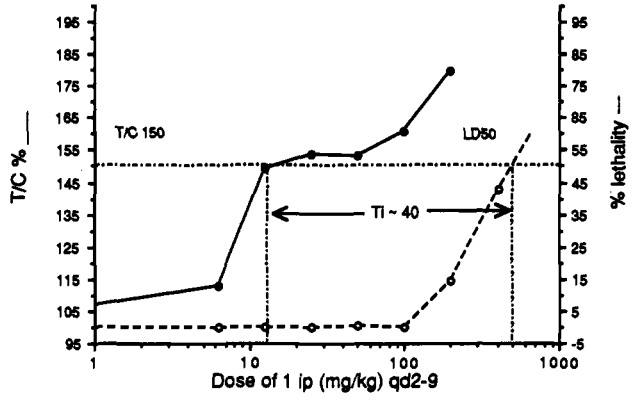


Figure 2. Efficacy of GTC 1 in 3LL experimental metastasis survival.

Substantial enhancements in survival were obtained also under different regimens of drug administration in this same 3LL model, for example, 1 was active after acute oral dosing, 200 mg/kg po on day 2 and 5 (T/C = 157%) with no observed drug toxicity.

We tested several clinically active anticancer drugs in our model in order to assess the relative promise of this new class of anticancer agent (Table II). While the values we obtained were comparable to those reported in the literature, results in our model tended to be quantitatively slightly lower with none of the standard drugs affording long-term survivors. For example, we found that cyclophosphamide dosed ip at 300 mg/kg on day 3 gave an average T/C = 192%. The NCI has reported 222%.[9] Drug dosing levels for these standards were chosen upon the basis of literature precedent for efficacy and toleration in the 3LL/BDF_1 models on either experimental or spontaneous metastasis. In our model the maximal antitumor efficacy obtained with 1 was comparable though slightly below that of the positive controls adriamycin and cyclophosphamide and superior to etoposide, 5-fluorouracil and cisplatin, where only modest effects were observed.

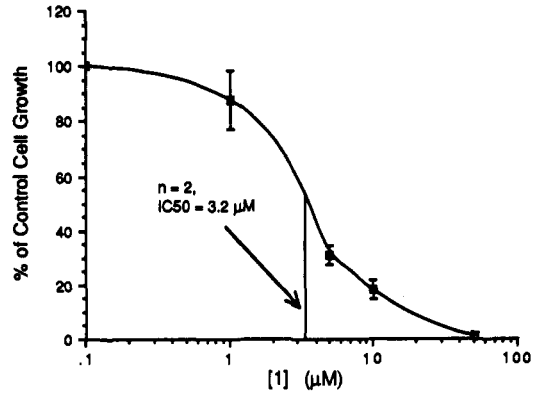


Figure 3. 3LL cell growth inhibition by compound 1.

Many of the clinically used anticancer agents including those in Table II are administered to patients at their maximally tolerated doses. In animal models also the maximum effects are seen often at the highest tolerated doses. Our experiences with adriamycin and cyclophosphamide in the 3LL model indicate that the greatest enhancements in survival are observed at these maximum doses. However, in contrast to adriamycin, compound 1 when dosed intraperitoneally appears to possess a wider margin of safety ($LD_{50}/ED_{150\%}$, ~40 vs ~2).

An important feature that distinguishes efficacious anticancer agents is their ability to inhibit tumor cell proliferation. The cessation of cell growth, usually ascribed to the perturbation of a wide variety of cellular functions, often results in cell death and is commonly referred to as "drug-related cytotoxicity". In some cases tumor growth arrest may be the consequence of altered host response or induction of terminal differentiation. We sought to evaluate whether GTC 1 was a direct acting agent with cytotoxic or cytostatic effects on tumor cells. Figure 3 depicts the antiproliferative effects of 1 in a 48-h 3LL cell monolayer growth assay. GTC 1 inhibited 3LL cell growth in a dose-dependent manner with an IC_{50} of 4 μM, with use of trypan blue exclusion as the indicator of cell viability. Compound 1 also inhibited 3LL colony formation in soft agar. The response of 3LL cells to 1 in the anchorage independent growth assay was also dose dependent with an IC_{50} of ~12 μM. Our preliminary data (not shown here) in mice suggested that these drug levels are achievable in plasma at doses where substantial efficacy is observed. While a molecular mechanism of action of compound 1 is not yet defined, these early results imply that 1 may have direct effects on tumor cells without involvement of host responses. Further studies are required to characterize more completely the mechanism of action of 1.

Conclusions

GTC 1, a member of a structurally novel class of antitumor agents, was discovered in an in vivo optimization program using survival as an endpoint in the iv 3LL metastasis model. An advantage of such a direct approach lies in obtaining at the outset sufficient animal data on each compound to assess whether it could meet one of the basic criteria of the NCI for further study, i.e. DN2 criteria of T/C ≥ 150% the 3LL model.[2]

Compound 1 is systemically active and possesses a broad range of doses where substantial efficacy is observed. In this model under certain regimens 1 shows a large thera-

(9) T/C values reported by the NCI for cyclophosphamide, adriamycin, and 5-fluorouracil are 222%, 252%, and 150%, respectively (ref 1c). In a 3LL survival assay employing intramuscular injection of 3LL cell, etoposide dosed iv at 13 mg/kg on days 8, 11, and 14 afforded a T/C of 139%. A single 40 mg/kg iv injection yielded an LD_{50}; Colombo, T.; Brogguino, M.; Vaghi, M.; Amato, G.; Erba, E.; D'Incaici, M. *Eur. J. Cancer Clin. Oncol.* **1986**, *22*, 173.

peutic index in comparison to adriamycin. In vitro studies with 1 indicate that it has direct dose dependent effects on 3LL tumor cells both in inhibition of monolayer cell growth and in reduction of colony formation in soft agar. Initial studies with 1 indicate that its potency in the antiproliferative assays is sufficient to account for the in vivo effects seen in the 3LL metastasis model. At present there has been no molecular mechanism of action ascribed for GTC 1. Effects of 1 against serine proteases appear to be insufficient to explain its antitumor action.

The combined results from acute, subacute, and chronic dosing with 1 suggest that a variety of dosing regimens may be employed with retention of substantial activity in the 3LL model. Thus it is possible that longer enhancements of lifespan might be achieved by treatment for extended periods at the lower doses than those shown to be maximally effective in subacute regimens or by repeated high-dose acute treatments. Because of the versatility of dosing regimens available GTC 1 appears attractive for studying in protocols employing combination therapy with other cytotoxic agents. Currently the most often used approach to cancer treatment in humans involves parallel or sequential therapy with combinations of two or more cytotoxic drugs. Further data on the efficacy of 1 and its analogues in this and other carcinoma models is the subject of manuscripts in preparation.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Pfizer Central Research Microanalysis Laboratory, and results for specified elements are within the ±0.4% of theoretical value unless otherwise denoted. IR spectra were obtained on a Perkin-Elmer Model 21 spectrophotometer with use of the stipulated solvent and are reported in reciprocal centimeters. ^{1}H NMR spectra in stipulated solvents were recorded on a Bruker 250-MHz or 300-MHz (where indicated) instrument. Low-resolution mass spectra were recorded on an Hitachi RMU6-E spectrometer.

2-Guanidino-*N*-(5-fluorobenzothiazol-2-yl)thiazole-4-carboxamide (1). A suspension of 7 hydrochloride (42.9 g, 21.0 mmol), 5 hydrochloride (67.0 g, 21.0 mmol), and hydroquinone (100 mg) in 300 mL of *N*-methylpyrrolidinone (NMP) was heated in the dark at 125 °C for 6 h. The reaction mixture was cooled to room temperature and diluted with 500 mL of 5% $NaHCO_3$. The resulting precipitate was filtered, washed with water (3 × 500 mL), dried, and recrystallized twice from pyridine to afford pure 1 (22.5 g, 31.4%): mp 293.7 °C (by DTC); ^{1}H NMR (DMSO-d_6) δ 6.60–7.10 (br, 2 × NH2), 7.24 (m, H-6'), 7.60 (dd, J = 2.3, 10 Hz, H-4'), 7.80 (s, H-5), 8.07 (dd, J = 5.4, 8.8 Hz, H-7'); ^{13}C NMR ppm 106.562 (d, J = 24 Hz), 111.714 (d, J = 24 Hz), 117.998, 123.052 (d, J = 10 Hz), 127.591, 143.163, 149.838, 156.651, 160.761, 161.439 (d, J = 232 Hz), 175.167; MS m/e 336 (M^+). Anal. ($C_{12}H_9FN_6OS_2 \cdot 0.5H_2O$) C, H, N; N: calcd, 2.91; found, 2.46.

2-Amino-5-fluorobenzothiazole Hydrochloride (7). The method of Gourley[10] was adapted. Ammonium thiocyanate (56.4 g, 0.742 mmol) was dissolved in 100 mL of acetone and treated with benzoyl chloride (86.0 mL, 0.742 mmol). This suspension, containing benzoyl isothiocyanate, was warmed to reflux and treated with 3-fluoroaniline (Fairfield, 65.0 mL, 0.675 mmol). The reaction mixture began to reflux more vigorously, became thicker and was diluted with 80 mL of acetone. After 1 h a solution of NaOH (84.6 g, 2.16 mmol) in 500 mL of water was added and the yellow homogeneous solution refluxed for 1.5 h. The cooled reaction mixture was concentrated in vacuo to remove the acetone, adjusted to pH 5.0 with concentrated HCl, and then to pH 11.0 with concentrated NH_4OH, and filtered. The solid 3-fluorophenylthiourea thus obtained was washed with water (2 × 100 mL) and dried in vacuo at 80 Torr and 55 °C. The thiourea [98.3 g, 86%, mp 114–115 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.90 (t, J = 6 Hz, Ar-H), 7.18 (d, J = 9 Hz, Ar-H), 7.33 (q, J = 6 Hz, Ar-H), 7.54–7.80 (br, Ar-H, NH and NH_2)] was used directly in the cyclization step without further purification.

The thiourea (98.3 g, 0.577 mmol) dissolved in 1.75 L of dichloromethane was treated dropwise with a dichloromethane solution (250 mL) of bromine (29.8 mL, 0.577 mmol), maintaining the temperature below 30 °C. The reaction was then heated at reflux for 3 h. Crude product isolated by filtration was suspended in 3 L of water made basic to pH 11.0 with NH_4OH and extracted into 4 × 500 mL of ethyl acetate. The pooled organic layers were washed with 500 mL of water and 500 mL of brine, dried ($MgSO_4$), filtered, and evaporated in vacuo to a straw colored solid (76 g) which was recrystallized from 1 L of benzene (removes trace 7-isomer contaminant) to yield white plates of 7 (64.5 g, 66%): mp 181 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.84 (dd, J = 3, 9 Hz, H-6), 7.12 (dd, J = 3, 12 Hz, H-4), 7.66 (d, J = 3, 9 Hz, H-7), 7.65 (s, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 104.301 (d, J = 25 Hz), 107.957 (d, J = 25 Hz), 121.650 (d, J = 10 Hz), 126.405 (d, J = 4 Hz), 154.085 (d, J = 13 Hz), 161.265 (d, J = 237 Hz), 168.773 (d, J = 4 Hz); MS m/e 168 (M^+). Anal. ($C_7H_5FN_2S$) C, H, N.

The hydrochloride of 7 was formed by dissolving 2-amino-5-fluorobenzothiazole in cold methanol and perfusing with excess hydrogen chloride. The residual solid obtained after evaporation in vacuo was triturated with ether and dried to afford the HCl salt of 7: mp 266–268 °C (lit.[11] mp 199–200 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.06 (m, H-6), 7.32 (dd, J = 2, 9 Hz, H-4), 7.85 (dd, J = 3, 6 Hz, H-7), 9.44 (br, NH_2).

2-Guanidinothiazole-4-carboxylic Acid Hydrochloride (4). Ester 3[6] (661 g, 3.08 mol) was added to 6 L of 3 N KOH and refluxed for 30 min. The cooled reaction mixture was acidified with 500 mL of concentrated HCl, with external cooling. Acidification was continued with 3 L of 4 N HCl during which a precipitate formed and redissolved. The mixture was filtered through supercel and concentrated in vacuo to 1 L. The precipitate that formed was collected by filtration and dried in vacuo at 70 °C to afford 464 g (68%) of 4: mp 287–289 °C; ^{1}H NMR (DMSO-d_6) δ 7.94 (s, H-5), 7.97 (br s, NH_2). Anal. ($C_5H_6N_4O_2S \cdot HCl \cdot H_2O$) C, H, N.

2-Guanidinothiazole-4-carboxylic Acid Succinimido Ester Hydrochloride (5). Acid 4 (182 g, 0.817 mol) and *N*-hydroxysuccinimide (98.7 g, 0.858 mol) in 2 L of DMF were treated with a solution of dicyclohexylcarbodiimide (180 g, 0.872 mol) in 0.5 L of DMF maintaining the temperature below 15 °C with an external ice bath. After addition was complete the reaction mixture was stirred overnight without further cooling then filtered to remove dicyclohexylurea. The filtrate was poured into 10.5 L of ether and 5 was isolated by filtration. This solid was washed with 2 × 500 mL of 10% methanol/ether and dried in vacuo 172 g (66%): mp 262 °C; ^{1}H NMR (DMSO-d_6) δ 2.86 (br s, 2[CH_2]), 8.23 (br s, 2[NH_2]), 8.57 (s, H-5). Anal. ($C_9H_9N_5O_4S \cdot HCl$) C, H, N: calc, 2.82, 21.90; found 3.40, 21.20.

X-ray Crystallographic Analysis of 1. Crystals were obtained from a concentrated solution of wet DMSO/$C_{12}H_9N_6OS_2 \cdot C_2H_6OS \cdot 1.5H_2O$; crystal size, 0.18 × 0.20 × 0.24 mm; space group $C2/c$; cell dimensions, a = 23.211 (6) Å, b = 7.576 (2) Å, c = 21.805 (5) Å, α = 90.0°, β = 96.86 (2)°, γ = 90.0°, V = 3807 (8) Å^3; 8 molecules/unit cell. A total of 1958 unique reflections were observed. Lattice constants and intensity data were measured by using graphite monochromatic Cu Kα on a Nicolet R3m/u diffractometer. The structure was solved by the SHEXTL system and refined to a final R value of 0.066.

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Supplementary Material Available: X-ray coordinate data including experimental information and tables of atomic coordinates isotropic thermal parameters, bond lengths and angles, and anisotropic thermal parameters (8 pages). Ordering information is given on any current masthead page.

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