Brief Articles

Synthesis and Antitumor Activity of Guanylhydrazones from 6-(2,4-Dichloro-5-nitrophenyl)imidazo[2,1-*b*]thiazoles and 6-Pyridylimidazo[2,1-*b*]thiazoles[1]

Aldo Andreani,*,† Silvia Burnelli,† Massimiliano Granaiola,† Alberto Leoni,† Alessandra Locatelli,† Rita Morigi,† Mirella Rambaldi,† Lucilla Varoli,† Giovanna Farruggia,‡ Claudio Stefanelli,‡ Lanfranco Masotti,‡ and Mark W. Kunkel§

Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy, Dipartimento di Biochimica "G. Moruzzi", Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy, and Developmental Therapeutics Program, Information Technology Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 6130 Executive Boulevard, Room 8008, Rockville, Maryland, 20892-7444

Received September 13, 2006

The design and synthesis of antitumor imidazothiazole guanylhydrazones are reported. The compounds were submitted to NCI for testing. All but one were more active than methyl-GAG. A few compounds were selected for further studies in search of a possible mechanism of action. The results from these studies and a final search with the NCI COMPARE algorithm suggest that the guanylhydrazones described in this paper are acting through a novel mechanism of action.

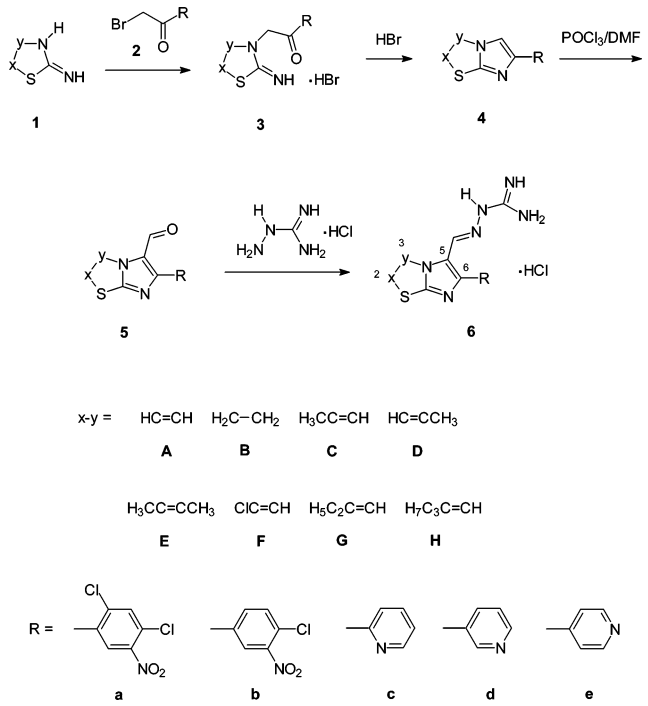
Introduction

In our third communication on the synthesis of antitumor guanylhydrazones,[2] we reported that in a series of 2,3-unsubstituted imidazo[2,1-*b*]thiazoles, 4-nitrophenyl and 4-chloro-3-nitrophenyl at the 6 position are suitable pharmacophoric groups giving rise to three potent antitumor agents. In a fourth communication,[3] we described the synthesis and antitumor activity of new imidazothiazole guanylhydrazones bearing a 3-nitrophenyl, 4-nitrophenyl, or 4-chloro-3-nitrophenyl group at the 6 position. The most active compounds were those bearing chlorine and nitro groups on the phenyl ring. Encouraged by the results with the compound unsubstituted at the 2,3-position, we prepared the 2,3-dihydro analogue to evaluate the effect of this change on the antitumor activity. We also performed the synthesis of new analogues bearing an additional chlorine atom on the phenyl ring, i.e., compounds with a 6-(2,4-dichloro-5-nitrophenyl) group. An additional series of analogues bearing a pyridyl group (i.e., a phenyl group bioisostere) at the 6 position were also prepared.

Chemistry

The guanylhydrazones **6** reported in Scheme 1 and Table S1 (see Supporting Information) were prepared by reaction of aminoguanidine with the appropriate aldehydes **5**, obtained in turn by means of the Vilsmeier reaction on the corresponding imidazo[2,1-*b*]thiazoles **4**, which were prepared from the appropriate 2-aminothiazoles **1** and bromoketones **2**. The intermediate compounds **3** were isolated and used in the subsequent step without further purification. The IR and ^{1}H NMR spectra of the new compounds (Table S2) are in agreement with the assigned structures. Analyses (C, H, N) were within ±0.4% of the theoretical values (Table S3).

Scheme 1



Biology

All the guanylhydrazones **6** were submitted to the NCI cell line screen for evaluation of their antitumor activity. For some insights into the biological effects of these derivatives, a few selected compounds were submitted to additional studies: **6Ha** (a member of the 2,4-dichloro-5-nitrophenyl derivatives and a very potent cytotoxic agent on the leukemia panel); **6Ec** and **6Fe** (members of the pyridyl derivatives).

a. Antitumor Activity. In a preliminary test at a single concentration against three human cell lines (see Supporting Information) a compound is considered active when it reduces the growth of any of the cell lines to 32% or less, and it will be

* To whom correspondence should be addressed. Phone and fax: +39-051-2099714. E-mail: aldo.andreani@unibo.it.
† Dipartimento di Scienze Farmaceutiche, Università di Bologna.
‡ Dipartimento di Biochimica "G. Moruzzi", Università di Bologna.
§ National Cancer Institute.

10.1021/jm061077m CCC: $33.50
Published on Web 12/21/2006

Table 1. Sixty-Cell Panel: Growth Inhibition and Cytostatic and Cytotoxic Activity of the Compounds That Passed the Three-Line Test

compd[a]	modes	leukemia	NSCLC	colon	CNS	melanoma	ovarian	renal	prostate	breast	MG-MID[b]
6Aa	pGI_{50}	5.84	5.75	5.99	5.81	5.80	5.73	5.70	5.68	5.55	5.76
	pTGI	5.37	5.46	5.67	5.46	5.52	5.45	5.35		5.22	5.44
	pLC_{50}	4.22	5.20	4.99		5.29	5.20	4.62			4.64
6Ba	pGI_{50}	6.47	5.94	6.35	6.52	6.12	5.88	5.93	6.09	6.21	6.19
	pTGI	5.50	5.59	5.66	5.02	5.60	5.55	5.54	5.51	4.98	5.45
	pLC_{50}						5.35	5.28			4.38
6Ca	pGI_{50}	5.79	5.60	6.18	5.67	5.64	5.68	5.68	5.63	5.38	5.67
	pTGI		4.71	4.31			4.71	4.93			4.48
	pLC_{50}							4.22			4.03
6Da	pGI_{50}	5.60	4.77	4.82	4.75	4.79	4.77	4.77	4.78	4.81	4.87
	pTGI	4.59	4.45	4.48	4.46	4.50	4.43	4.48	4.45	4.49	4.48
	pLC_{50}	4.21	4.15	4.14	4.16	4.22	4.14	4.19	4.11	4.17	4.17
6Ea	pGI_{50}	5.68	4.96	5.37	4.95	5.30	5.28	5.06	5.04	5.14	5.20
	pTGI	4.65	4.32	4.53	4.17	4.79	4.62	4.44	4.42	4.45	4.49
	pLC_{50}		4.01	4.02		4.06	4.15	4.07		4.01	4.04
6Fa	pGI_{50}	5.45	4.64	5.24	4.97	4.95	4.71	4.53	4.72	4.98	4.91
	pTGI	4.35	4.14	4.30	4.19	4.27	4.18	4.09	4.03	4.41	4.23
	pLC_{50}		4.01				4.02	4.01			
6Ga	pGI_{50}	6.03	5.77	5.86	5.84	5.82	5.80	5.82	5.80	5.78	5.83
	pTGI	5.49	5.50	5.53	5.55	5.55	5.51	5.52	5.51	5.37	5.50
	pLC_{50}		4.62	5.22	4.84	5.27	5.22	5.19	5.26	4.95	5.07
6Ha	pGI_{50}	6.14	5.76	5.83	5.78	5.78	5.79	5.78	5.76	5.76	5.82
	pTGI	5.71	5.49	5.56	5.51	5.51	5.52	5.51	5.47	5.46	5.53
	pLC_{50}	5.38		5.27	5.26	5.26	5.25	5.27	5.17	5.03	5.27
6Bb	pGI_{50}	5.51	4.98	5.18	5.16	5.08	5.01	5.00	5.18	4.94	5.10
	pTGI	4.88	4.41	4.76	4.59	4.62	4.51	4.55	4.68	4.56	4.61
	pLC_{50}	4.12	4.15	4.31	4.09	4.22	4.30	4.19	4.33	4.19	4.20
6Cc	pGI_{50}	5.44	4.99	5.09	5.09	5.01	4.76	5.10	5.10	5.05	5.05
	pTGI		4.49	4.53	4.57	4.54	4.20	4.71	4.40	4.43	4.46
	pLC_{50}		4.13	4.05	4.08	4.13	4.04	4.27			4.09
6Ec	pGI_{50}	4.28	4.74	4.74	4.68	4.77	4.73	5.18	4.65	4.64	4.74
	pTGI	4.08	4.43	4.39	4.38	4.48	4.43	4.50	4.24	4.38	4.39
	pLC_{50}		4.14	4.13	4.12	4.19	4.12	4.24	4.10	4.13	4.14
6Fc	pGI_{50}	5.36	5.06	5.15	5.25	5.11	5.15	5.10	5.03	5.05	5.13
	pTGI	4.88	4.73	4.82	4.94	4.82	4.84	4.82	4.75	4.68	4.80
	pLC_{50}	4.31	4.48	4.49	4.59	4.54	4.54	4.54	4.46	4.45	4.49
6Cd	pGI_{50}	5.32	4.86	5.25	4.92	4.99	4.80	5.02	5.16	5.15	5.02
	pTGI	4.29	4.44	4.68	4.55	4.59	4.44	4.56	4.61	4.65	4.53
	pLC_{50}		4.12	4.21	4.17	4.21	4.16	4.21	4.16	4.22	4.17
6Fd	pGI_{50}	4.86	4.70	5.07	4.82	4.74	4.81	4.74	4.82	4.78	4.80
	pTGI	4.37	4.38	4.66	4.50	4.44	4.44	4.45	4.55	4.33	4.44
	pLC_{50}	4.06	4.15	4.28	4.20	4.20	4.14	4.16	4.27	4.12	4.17
6Ce	pGI_{50}	5.01	4.78	4.80	4.79	4.71	4.78	4.63	4.76	4.46	4.75
	pTGI	4.55	4.48	4.46	4.51	4.40	4.46	4.37	4.49	4.32	4.44
	pLC_{50}	4.14	4.21	4.18	4.23	4.18	4.15	4.14	4.23	4.13	4.17
6Ee	pGI_{50}	4.52	4.41	4.45	4.41	4.42	4.27	4.40	4.26	4.36	4.40
	pTGI	4.02	4.01	4.10	4.03	4.08	4.01	4.05		4.04	4.04
6Fe	pGI_{50}	5.42	5.17	5.09	5.11	5.23	4.99	5.18	4.76	4.88	5.13
	pTGI	4.93	4.77	4.56	4.70	4.86	4.68	4.80	4.48	4.39	4.71
	pLC_{50}	4.27	4.28	4.19	4.32	4.30	4.19	4.35	4.20	4.18	4.26
methyl-GAG[c]	pGI_{50}	5.10	4.60	4.90	4.70	4.40	4.80	4.70	4.00	4.80	4.67
	pTGI	3.20	3.50	3.30	3.60	3.50	3.50	3.30	2.80	3.50	3.36
	pLC_{50}	2.60	2.80	2.90	3.00	3.00	2.80	2.70	2.60	2.80	2.80

[a] Highest concn = 10^{-4} M unless otherwise reported; only modes showing a value of >4.00 are reported. [b] Mean graph midpoint, i.e., the calculated mean panel. [c] Highest concn = $10^{-2.6}$ M.

passed on for evaluation in the full panel of 60 cell lines. Table 1 reports the results obtained with this test (methyl-GAG is reported for comparison purposes) expressed as −log of the molar concentration that inhibited cell growth by 50% (pGI_{50}), caused total cytostasis (pTGI = total growth inhibition), or killed half of the cells (pLC_{50}).

The dose response data from the NCI 60 cell line screen showed a broader response range for **6Fe** than for **6Ha** and **6Ec**; the difference between the average concentration that caused 50% growth inhibition and the concentration that killed 50% of the cells was almost 1 log (10-fold) for **6Fe** and only 0.5 log for **6Ha** and **6Ec**. Within each of the nine cell panels there was a greater difference between the least-sensitive and the most-sensitive cell lines in response to **6Fe** than to the other two compounds. For **6Fe** about half of the cell lines were more sensitive and half less sensitive than the average sensitivity of all cell lines. For **6Ha** and **6Ec** all cell lines showed almost the same sensitivity.

b. Effects on Cell Death of Leukemia HL60 Cells. The leukemic cell line HL60 was used in these experiments. First, we determined whether the guanylhydrazones induced apoptosis. Figure 1A shows that none of the aforementioned compounds (guanylhydrazones were tested at 1−100 μM) caused the activation of caspase proteases acting on the substrate sequence Asp-Glu-Val-Asp (DEVD), i.e., mainly effector caspases 3 and 7.[4] Activation of this DEVDase activity represents a marker of apoptotic cell death.[4] On the other hand, etoposide, a classical inducer of apoptosis[5] that we used as a positive control, substantially activated caspase activity. Also, cytofluorimetric analysis of hypodiploid DNA content failed to detect any form of apoptosis in guanylhydrazone-treated cells (data not shown). Nevertheless, guanylhydrazones caused cell death, even if they

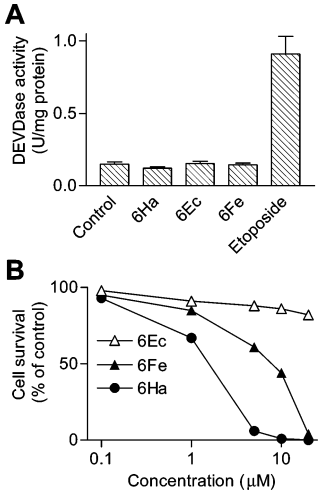


Figure 1. Induction of cell death by guanylhydrazones. (A) The activity of caspase proteases acting on the peptide sequence DEVD (DEVDase activity) was measured in HL60 cells treated for 24 h with 25 μM guanylhydrazones or 10 μM etoposide. Guanylhydrazone concentrations ranging from 1 to 100 μM were tested, with similar results. Data represent the means ± SEM of triplicate measurements. (B) HL60 cells were incubated for 24 h with 0.1−20 μM indicated guanylhydrazones. Afterward cell death was evaluated by flow cytometry. The panel reports the results obtained in one experiment repeated three times.

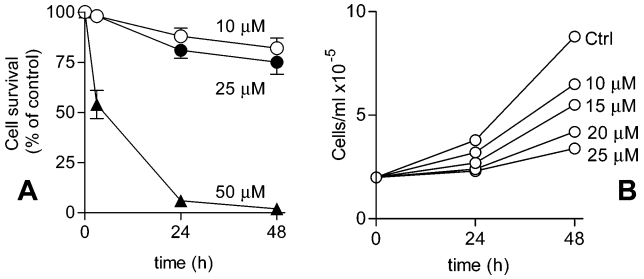


Figure 2. Effect of **6Ec** on viability and growth of HL60 cells. Cells were incubated for 24 h in the presence of the indicated concentrations of **6Ec**. Afterward the amount of cell survival (A) and the total cell number (B) were determined by flow cytometry. The panels depict the results obtained in one experiment repeated three times with comparable results.

displayed different potencies. Figure 1B shows that **6Ha** was more toxic than **6Fe**, whose cytotoxicity in turn was much higher than that of **6Ec**. Since **6Ec** causes cell growth inhibition (see the 60-cell panel), its effect must be mainly cytostatic. Actually, sublethal concentrations of **6Ec** were able to reduce cell proliferation; a 25 μM concentration elicited only a 25% of cell death after 48 h of treatment (Figure 2A), but cell proliferation was reduced more than 80% (Figure 2B). Higher doses of **6Ec** were required to cause a marked cytotoxic effect; at 50 μM, **6Ec** led to death about 45% of the cell populations within 3 h and the whole culture after 24 h of incubation. Again, no sign of apoptosis could be observed, indicating that all the tested compounds exert their cytotoxic effect by inducing mainly necrotic cell death.

c. Effects on Cell Cycle and on the Activity of *S*-Adenosylmethionine Decarboxylase. To verify whether the inhibition of cell growth by **6Ec** might be attributable to interference with the cell cycle program, HL60 cells were treated with graded concentrations of the drugs for 24 h. Then the analysis of DNA profiles was performed using propidium iodide staining and flow cytometry. Figure 3A illustrates that the majority of control cells were in the S phase or in the G0/G1 phase of the cell cycle and less than 10% were in the G2/M

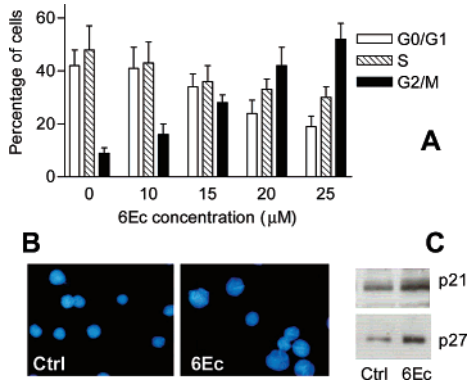


Figure 3. Effects of **6Ec** on cell cycle related events. (A) Cell cycle distribution of HL60 cells after 24 h of treatments with different amounts of **6Ec**. Results are the mean ± SEM of three determinations. (B) Morphological evaluation of nuclei stained with Hoechst 33258 obtained from untreated control cells or from cells treated for 72 h with 25 μM **6Ec**. (C) After 24 h in the absence (ctrl) or presence of **6Ec** (25 μM), HL60 cells were collected and the cell cycle inhibitors p21 and p27 were determined in cell extracts by Western blotting. All the experiments shown in the figure were repeated at least twice with similar results.

phase. Increasing the concentration of **6Ec** resulted in a dose-dependent increase in the percentage of cells in the G2/M phases, while concomitantly the G0/G1 and S populations decreased. At 25 μM **6Ec**, 52% of HL60 cells were in the G2/M phase. This block of the cell cycle in the G2/M phase was maintained even after 48 and 72 h (data not shown). The morphological evaluation of the nuclei, stained with Hoechst 33458 and examined by fluorescence microscopy, revealed that in **6Ec**-treated cells, the nuclei were bigger than in control cells, but chromosomes were not detectable and the chromatin appeared to be completely decondensed (Figure 3B). This observation suggests that the cells were blocked in the G2 rather than in the M phase.

At concentrations higher than 10 μM, **6Ec** also caused an increase in the content of the cell cycle inhibitors p21 and p27 (Figure 3C). Since these proteins are able to inhibit several cyclin-dependent kinases,[6] it appears likely that high concentrations of **6Ec** can also decrease the rate of cell cycle. The other guanylhydrazones **6Ha** and **6Fe** did not cause any of these cell cycle-related effects at sublethal concentrations (0.5 μM).

Some guanylhydrazones can block cell growth by inhibiting the enzyme *S*-adenosylmethionine decarboxylase (SAMDC), which is necessary for the synthesis of the polyamines spermidine and spermine.[7] The polyamines in turn are required for cell cycle progression and cell proliferation. However, **6Ha**, **6Ec**, and **6Fe** did not inhibit the enzymatic activity of SAMDC in extracts obtained from growing HL60 cells (data not shown), in contrast to 4-amidinoindan-1-one-2′-amidinohydrazone (CGP48664) and methylglyoxal bisguanylhydrazone (MGBG), which are established inhibitors of the enzyme.[8]

d. Effects on the Mitochondrial Membrane Potential. Previous studies have shown that the inhibition of mitochondrial functions is a feature of many guanidino-containing drugs.[9,10] Therefore, we investigated whether disruption of the mitochondrial transmembrane potential $\Delta\Psi_m$ was involved in the antiproliferative action of these novel guanylhydrazones. HL60 cells were treated for 24 h with sublethal concentrations of the drugs and then analyzed cytofluorimetrically by using the potentiometric fluorescent dye $DiOC_6$ (3,3′-dihexyloxacarbocyanine iodide) to measure mitochondrial membrane depolarization in intact viable cells. Sublethal dosages of **6Ha** and **6Fe** (0.5 μM) did not affect the mitochondrial potential, whereas **6Ec**

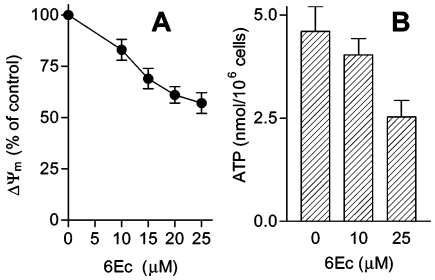


Figure 4. Effect of **6Ec** on the mitochondrial membrane potential $\Delta\Psi_m$ (A) and ATP levels (B) in HL60 cells. Cells were cultured in the presence of various concentrations of **6Ec** for 24 h. (A) At the end of the incubation the cells were stained with $DiOC_6$ and analyzed immediately by flow cytometry to measure $\Delta\Psi_m$. (B) Acid extracts were obtained, and the content of ATP was measured by HPLC. Results are the mean ± SEM of triplicate determinations.

dose-dependently decreased the value of $\Delta\Psi_m$, which was reduced by about 50% when the drug concentration was 25 μM (Figure 4A). In Figure 4B it is depicted that the collapse of $\Delta\Psi_m$ was accompanied by a decrease of cellular ATP content, indicating an interference of **6Ec** with cellular energetics. It is worth noting that mitochondria depolarization often defines an early but already irreversible stage of apoptosis,[11] but this is not the case because at toxic concentrations **6Ec** appears to trigger necrotic cell death, since no sign of apoptosis could be detected.

e. COMPARE. We have used the NCI COMPARE algorithm[12] to search the NCI databases of public 60-cell screening results and molecular target expression data to look for additional effects of the guanylhydrazones that may occur upstream of their effects on apoptosis and necrosis. COMPARE can suggest compounds that might induce cytostasis and cytotoxicity similar to our compounds and molecular targets whose levels of expression in the cell lines are correlated with the activity of our compounds. COMPARE identified compounds with correlations to the GI_{50} data for the guanylhydrazones. These results included several other compounds that we had previously submitted to the NCI 60-cell screen and a few modestly correlated compounds whose mechanisms of action are not known (see Supporting Information). There were no strongly correlated molecular targets for any of the compounds individually, but COMPARE did identify several members of different classes of molecular targets that were common to the results for nine or more of the compounds: receptor tyrosine kinases and phosphatases, components of cell structure and attachment, and components of the cell cycle machinery. COMPARE cannot identify targets or mechanisms; it can only make suggestions. The dearth of high correlations in the public data set suggests that these guanylhydrazones may be acting through a novel mechanism of action.

Conclusion

The biological activity of the guanylhydrazones **6** is not correlated to their clogP (see Table S4). When the 6 position bears a pyridyl instead of a phenyl ring, a substituent at the 2 position is also necessary because the unsubstituted (**6Ac**−**e**, **6Bc**−**e**) and the 3-substituted derivatives (**6Dc**−**e**) did not reach, in the 3 cell line test, the minimum potency necessary to enter the full 60 cell line panel. The methyl disubstitution at positions 2 and 3 leads to improvement of activity because compounds **6Ec**,**e** were active on the 3 cell line test and therefore entered the following step. The best results were obtained with chlorine at the 2 position, but compounds with a methyl group in the same position were also active. Seven of the eight derivatives tested on the 60 cell line panel, showing pGI_{50} values between 4.74 and 5.13, were more active than methyl-GAG.

The introduction of a second chlorine atom on the phenyl ring did not cause a significant change in the antitumor activity, compared with the analogues with a single chlorine atom.[3] The substitution at the 2 position is more favorable if compared with 3-substitution and 2,3-disubstitution; the substituents leading to higher growth inhibition were the ethyl (**6Ga**) and propyl group (**6Ha**), but the corresponding methyl derivative (**6Ca**) showed a more favorable ratio between growth inhibition and lethality (pGI_{50}/pLC_{50}). Generally speaking, this class of 6-(2,4-dichloro-5-nitrophenyl) derivatives is very interesting because all of them entered the 60 cell line test and growth inhibition (pGI_{50} = 4.87−6.19) was more effective than that induced by methyl-GAG.

Eliminating the double bond at the 2,3 position (**6Bb**, mean pGI_{50} = 5.10) while maintaining the same substituent at the 6 position leads to a derivative that was a little less active than the unsaturated parent compound previously published (mean pGI_{50} = 5.79 [2]), but the introduction of a second chlorine atom on the 6-phenyl ring leads to a compound (**6Ba**) that was much more active and less toxic (pGI_{50}/pLC_{50} = 1.41) than the corresponding unsaturated derivative (**6Aa**, pGI_{50}/pLC_{50} = 1.24). Compound **6Ba**, with mean pGI_{50} of 6.19, was also the most active derivative of the whole series presented in this paper.

The antiproliferative activity of these novel guanylhydrazones can be mediated through cytotoxic or cytostatic effects. Among the selected compounds that we tested against leukemic HL60 cells, **6Ha** and **6Fe** are mainly cytotoxic and trigger a nonapoptotic cell death at relatively low concentrations (in the low micromolar range). On the other hand, **6Ec** is only cytotoxic at high concentrations and its antiproliferative effect is largely due to cytostasis. We found that the antiproliferative effect of **6Ec** was associated with a block in cell cycle progression, with cell accumulation in the G2/M phase and with a marked reduction in the mitochondrial transmembrane potential $\Delta\Psi_m$ and a decrease in the intracellular ATP content. Other guanylhydrazones have been reported to disturb cell cycle progression and mitochondrial function.[2,9,10] We were not able to detect these effects in cells treated with the powerful inducers of cell death **6Ha** or **6Fe**. However, we cannot exclude that cell cycle- and mitochondria-related effects are common to many of these novel guanylhydrazones but, being exerted at concentrations of about 10 μM, could be seen only in the case of compounds with low toxicity. As regards the cytotoxic effect, the tested compounds trigger cell death that does not appear to be apoptotic.

Experimental Section

1. Chemistry. Most of the starting compounds were prepared according to the literature.[13−20]

Synthesis of the Guanylhydrazones 6. The appropriate aldehyde **5** (10 mmol) was dissolved in ethanol and treated with the equivalent of aminoguanidine hydrochloride, prepared in turn from an ethanol suspension of aminoguanidine bicarbonate and excess of 37% hydrochloric acid. The reaction mixture was refluxed for 5−10 h according to a TLC test, and the resulting precipitate was collected by filtration with a yield of 50−70% (**6Ba, 6Ea, 6G**−**Ha, 6Bb, 6Ac, 6Ec, 6C**−**Fd, 6De, 6Fe**) and 80−95% (**6Aa, 6C**−**Da, 6Fa, 6B**−**Cc, 6Ec, 6A**−**Bd, 6Dd, 6Ae, 6Ce, 6Ee**).

2. Biology. 2.a. Antitumor Activity. The antitumor tests (see Supporting Information) were performed by the National Cancer Institute (Bethesda, MD) as in our previous papers.[21]

2.b. Effects on Cell Death of Leukemia HL60 Cells. Human leukemia HL60 cells were routinely grown in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37 °C in air/5% CO_2.

Compounds were dissolved in dimethylsulfoxide and diluted to the required concentration in complete medium. Control cells received the corresponding volume of dimethylsulphoxide. Cell death was evaluated by flow cytometry with propidium iodide staining as previously described[10] and by conventional trypan blue exclusion.

2.c. Effects on Cell Cycle and on the Activity of *S*-Adenosylmethionine Decarboxylase. 2.c.1. Cell Cycle. At the indicated time points, nuclei were isolated (ref 22 in Supporting Information) and kept at 4 °C in the dark. Cells were stained with 50 μg/mL propidium iodide immediately prior to analysis. Cell cycle profiles were determined using an Epics Elite (Beckman Coulter) and analyzed by Modfit software (Verity). Nuclear morphology was evaluated according to Comin-Anduix et al. (ref 23 in Supporting Information). To determine the content of p21 and p27 proteins, at the end of the incubation the cellular proteins were extracted and assayed by Western blotting as previously described (ref 24 in Supporting Information). For immunodetection, specific antibodies from Santa Cruz were used.

2.c.2. Enzyme Activity Assays. The activity of SAMDC was assayed by measuring the release of radioactive CO_2 from [^{14}C-carboxyl]*S*-adenosylmethionine.[8] The activity of caspase enzymes hydrolyzing the peptide sequence DEVD was measured in cell extracts by a fluorimetric assay (ref 25 in Supporting Information). One unit (U) of enzyme activity catalyzes the formation of 1 nmol of product per minute.

2.d. Effects on the Mitochondrial Membrane Potential. To assess the mitochondrial transmembrane potential $\Delta\Psi_m$, 10^6 cells were collected by centrifugation (200*g* for 10 min) and resuspended in complete medium containing 4 nM $DiOC_6$ (ref 26 in Supporting Information). The cells were incubated for 40 min at 37 °C and then analyzed by flow cytometry using a log scale as described.[10] The cellular content of ATP was determined by HPLC after extraction in perchloric acid (ref 27 in Supporting Information).

Acknowledgment. This work was supported by a grant from MIUR-COFIN 2002. We are grateful to NCI for the antitumor tests and to Dr. Matteo Masetti (Dipartimento di Scienze Farmaceutiche, Università di Bologna) for the helpful discussions about clogP.

Supporting Information Available: Detailed experimental procedures with analytical and spectroscopic data for all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Potential Antitumor Agents. 40. For part 39 see: Andreani, A.; Burnelli, S.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Varoli, L.; Kunkel, M. W. Antitumor Activity of Substituted *E*-3-(3,4,5-Trimethoxybenzylidene)-1,3-dihydroindol-2-ones. *J. Med. Chem.* **2006**, *49*, 6922−6924.

(2) Andreani, A.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Recanatini, M.; Garaliene, V. Potential Antitumor Agents. Part 29: Synthesis and Potential Coanthracyclinic Activity of Imidazo[2,1-*b*]thiazole Guanylhydrazones. *Bioorg. Med. Chem.* **2000**, *8*, 2359−2366.

(3) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Giorgi, G.; Garaliene, V. Potential Antitumor Agents. 34. Synthesis and Antitumor Activity of Guanylhydrazones from Imidazo[2,1-*b*]thiazoles and from Diimidazo[1,2-*a*:1,2-*c*]pyrimidine. *Anticancer Res.* **2004**, *24*, 203−212.

(4) Lavrik, I. N.; Golks, A.; Krammer, P. H. Caspases: Pharmacological Manipulation of Cell Death. *J. Clin. Invest.* **2005**, *115*, 2665−2672.

(5) Kaufmann, S. H. Cell Death Induced by Topoisomerase-Targeted Drugs: More Questions Than Answers. *Biochim. Biophys. Acta* **1998**, *1400*, 195−211.

(6) Pei, X. H.; Xiong, Y. Biochemical and Cellular Mechanisms of Mammalian CDK Inhibitors: A Few Unresolved Issues. *Oncogene* **2005**, *24*, 2787−2795.

(7) Stanek, J.; Caravatti, G.; Capraro, H. G.; Furet, P.; Mett, H.; Schneider, P.; Regenass, U. *S*-Adenosylmethionine Decarboxylase Inhibitors: New Aryl and Heteroaryl Analogues of Methylglyoxal Bis(guanylhydrazone). *J. Med. Chem.* **1993**, *36*, 46−54.

(8) Regenass, U.; Mett, H.; Stanek, J.; Mueller, M.; Kramer, D.; Porter, C. W. CGP 48664, a New *S*-Adenosylmethionine Decarboxylase Inhibitor with Broad Spectrum Antiproliferative and Antitumor Activity. *Cancer Res.* **1994**, *54*, 3210−3217.

(9) Ekelund, S.; Nygren, P.; Larsson, R. Guanidino-Containing Drugs in Cancer Chemotherapy: Biochemical and Clinical Pharmacology. *Biochem. Pharmacol.* **2001**, *61*, 1183−1193.

(10) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Lenaz, G.; Fato, R.; Bergamini, C.; Farruggia, G. Potential Antitumor Agents. 37. Synthesis and Antitumor Activity of Guanylhydrazones from Imidazo[2,1-*b*]thiazoles and from the New Heterocyclic System Thiazolo[2′,3′:2,3]imidazo[4,5-*c*]quinoline. *J. Med. Chem.* **2005**, *48*, 3085−3089.

(11) Ly, J. D.; Grubb, D. R.; Lawen, A. The Mitochondrial Membrane Potential (deltapsi(m)) in Apoptosis: An Update. *Apoptosis* **2003**, *8*, 115−128.

(12) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and Analysis of Patterns of Differential Activity of Drugs against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.

(13) 2-Aminothiazole and 2-Amino-5-alkylthiazoles. GB 571653, 1945; *Chem. Abstr.* **1947**, *41*, 15308.

(14) Erlenmeyer, H.; Jung, J. P. Derivatives of 2-Aminothiazoles. *Helv. Chim. Acta* **1949**, *32*, 35−38.

(15) Garg, H. G. Synthesis of Nitrophenacyl Bromides. *J. Indian Chem. Soc.* **1961**, *38*, 59.

(16) Andreani, A.; Rambaldi, M.; Andreani, F.; Bossa, R.; Galatulas, I. 6-Pyridinylimidazo[2,1-*b*]thiazoles and Thiazolines as Potential Cardiotonic Agents. *Eur. J. Med. Chem.* **1985**, *20*, 93−94.

(17) Andreani, A.; Rambaldi, M.; Locatelli, A.; Andreani, F. 5-Formylimidazo[2,1-*b*]thiazoles and Derivatives with Herbicidal Activity. *Collect. Czech. Chem. Commun.* **1991**, *56*, 2436−2447.

(18) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M. Synthesis and Antitubercular Activity of Imidazo[2,1-*b*]thiazoles. *Eur. J. Med. Chem.* **2001**, *36*, 743−746.

(19) Andreani, A.; Rambaldi, M.; Andreani, F.; Hrelia, P.; Cantelli Forti, G. Synthesis and Mutagenic Activity of Imidazo[2,1-*b*]thiazoles Bearing at Least One Nitro or Nitroso Group. *Arch. Pharm. Chem.* **1987**, *15*, 41−49.

(20) Andreani, A.; Rambaldi, M.; Leoni, A.; Locatelli, A.; Andreani, F.; Gehret, J. C. Synthesis of Imidazo[2,1-*b*]thiazoles as Herbicides. *Pharm. Acta Helv.* **1996**, *71*, 247−252.

(21) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; et al. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.

JM061077M