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Potential Antitumor Agents. Part 29[1]: Synthesis and Potential Coanthracyclinic Activity of Imidazo[2,1-b]thiazole Guanylhydrazones

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Abstract—This paper reports the synthesis of new imidazo[2,1-b]thiazole guanylhydrazones which were tested as potential antitumor agents. Three of these derivatives (those bearing a 3- or 4-nitrophenyl group) were the most potent and one of these showed a mild effect as CDK1 inhibitor. These same three derivatives were also tested as positive inotropic agents and two of them were more potent than amrinone at 10^{-5} M. These two guanylhydrazones could be useful coanthracyclinic agents.

Introduction

In 1998,[1,2] we suggested the term 'coanthracyclinic activity' to indicate the pharmacological behavior of a molecule endowed with both antitumor activity (in order to reduce the anthracycline toxicity by reducing its dosage) and positive inotropic activity (in order to counteract the heart depression induced by anthracyclines). We believe in this 'bivalent' approach even though others have been suggested, such as the possibility to prevent anthracycline induced apoptosis of cardiac myocytes.[3]

In our first search on imidazo[2,1-b]thiazole guanylhydrazones endowed with antitumor[4] and cardiotonic[5] activity, we described the synthesis and pharmacological profile of compounds related to structure **1** (Chart 1). The results showed that the active compounds had a chlorine at the 6 position or at the phenyl ring of the 6 position. In the subsequent paper,[6] the substitution at the 2 and 3 positions was also considered and two potential coanthracyclinic agents were described.

In this paper we wish to report the synthesis and pharmacological activity of new analogues. The first series bears a nitro (**2–5**) or amino group (**6**) at the phenyl ring of the 6 position. In compounds **7–8** the guanylhydrazone chain is at the 6 position whereas a nitro group is present at position 5. A different electron attracting group was considered at position 6 of compounds **9–10** whereas the guanylhydrazone chain at position 5 bears an additional methyl group. Compounds **11–14** belong to a series where two of the previous molecules are connected by means of a couple of amide (**11–12**) or ester (**13–14**) bonds to a 2, 4 or 6 carbon spacer.

Compounds **2–14** were subjected to the screening developed by the National Cancer Institute (NCI, Bethesda, MD) and the most active compounds were also evaluated for their positive inotropic activity, in search of potential coanthracyclinic agents. These same compounds were tested as potential inhibitors of cyclin-dependent kinase 1 (CDK1), in order to verify if the antitumor activity was related to this mechanism of action.

Chemistry

The guanylhydrazones **2–8** (Scheme 1a) and **9–14** (Scheme 1b) were prepared by reaction of aminoguanidine with the appropriate aldehydes **19–24**, **27** and ketones **28**, **29**, **31–34**. Some of these compounds (**19**, **20**, **24**, **28**) are reported in the literature.[7–9] The starting aldehydes **21–22** which are necessary for the preparation of compounds **4–5** were prepared by means of the Vilsmeier

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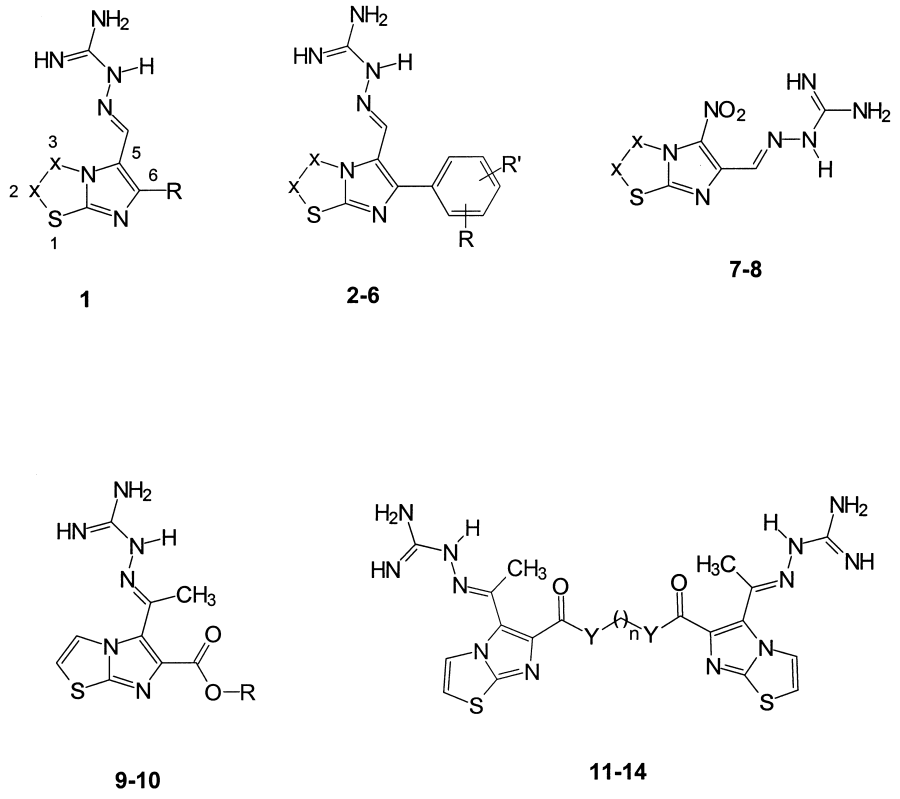


Chart 1. Design of the guanylhydrazones.

reaction from the corresponding imidazo[2,1-b]thiazoles **17–18**. The aminoaldehyde **23** (starting material for **6**) was obtained by catalytic hydrogenation of the nitroderivative **20**. The aldehyde **27** (starting material for **8**) was prepared by oxidation with osmium tetroxide of 5-nitro-6-(2-phenylethenyl)-2,3-dihydroimidazo[2,1-b]thiazole **26** obtained in turn from 6-methyl-5-nitro-2,3-dihydroimidazo[2,1-b]thiazole **25**.[7] The dimeric ketones **31–34** (for the synthesis of **11–14**) were prepared with two different approaches. The acid **29** (which is also the starting material for **10**) was obtained by hydrolysis of the ester **28**: it was activated[10] in the form of the *N*-succinimidyl ester **30** and condensed with the suitable diamine to afford the amides **31–32**.

The acid **29** was also treated with 1,1′-carbonyldiimidazole (CDI) to yield the acylimidazole, which in the presence of 1,4-butanediol or 1,6-hexanediol and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) as activator of the alcoholic function[11] gave the esters **33–34**.

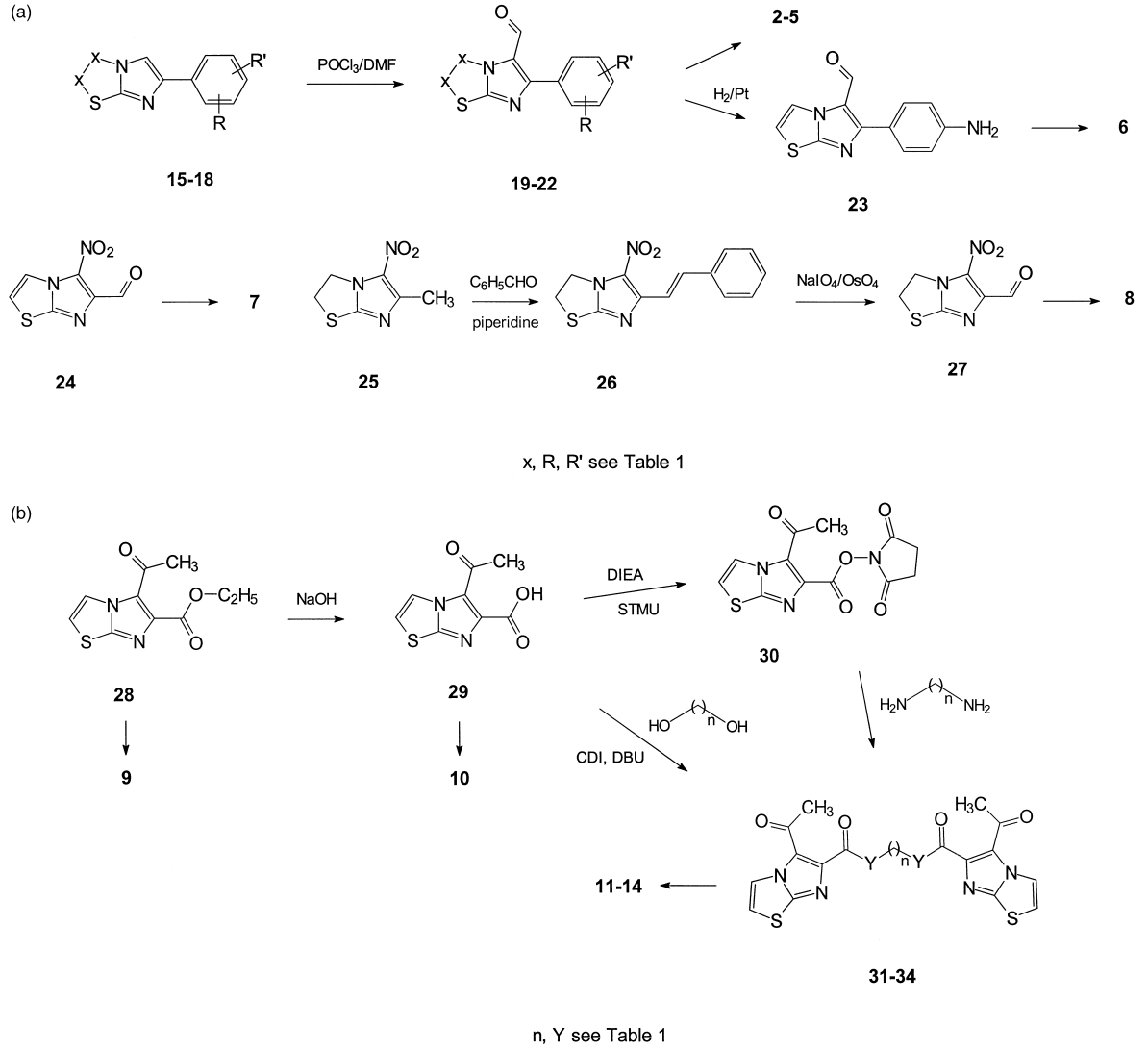
Pharmacological Results and Discussion

In vitro growth inhibition and cytotoxicity[12]

As a primary screening, compounds **2–14** were evaluated for their cytotoxic potency on three human cell lines, such as NCI-H460 lung cancer, MCF7 breast cancer and SF-268 glioma. A compound is considered active when it reduces the growth of any of the cell lines to 32% or less (negative numbers indicate cell kill) and it is passed on for evaluation in the full panel of 60 cell lines. As shown in Table 3, all the guanylhydrazones reported in Scheme 1a (**2–8**, i.e. those arising from aldehydes) were active, whereas among the guanylhydrazones arising from ketones (Scheme 1b), only compound **14** was active.

The panel of 60 human tumor cell lines is organized into subpanels representing leukemia, melanoma and cancers of lung, colon, kidney, ovary, breast, prostate and central nervous system. The test compounds (**2–8**, **14**) were evaluated using five concentrations at 10-fold dilutions, the highest being 10^{-4} M and the others 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M.

Compounds **3–5**, i.e. those bearing a nitro group at the position 3 or 4 of the phenyl ring in position 6 of the imidazothiazole, were the most active. Table 4 reports the results obtained (only for these compounds) expressed as $\log_{10}$, taking into consideration the growth inhibitory power (GI_{50}), the cytostatic effect (TGI) and the cytotoxic effect (LC_{50}). For comparison purposes, the results obtained by DTIC (4-dimethyltriazenoimidazole-5-carboxamide) and Methyl-GAG (methylglyoxal bis-guanylhydrazone dihydrochloride) are also reported. The structure of the latter compound is related to that of the guanylhydrazones described here but the highest dose tested was $10^{-2.6}$ instead of 10^{-4}. The antitumor



Scheme 1. (a) Synthesis of the guanylhydrazones **2–8**. (b) Synthesis of the guanylhydrazones **9–14**.

data from these and other reference drugs are available at the address: http://dtp.nci.nih.gov/docs/cancer/searches/standard_agent_table.html

As shown in Table 4, compounds **3–5** are about equipotent with DTIC. Compound **4** showed cytostatic effect at concentrations between 10^{-4} and 10^{-5} M. Both compounds **3** and **5** were approximately 10 times more potent, since they showed a good cytostatic activity in the majority of the cell lines at concentrations in the range of 10^{-6}–10^{-5} M. Both compounds showed a peculiar profile of cytotoxicity since in several cell lines the increase of concentration from 10^{-5} to 10^{-4} determined a decrease of activity. This paradoxical effect does not appear to be related to technical errors since it was evident in both separate experiments (graphs not shown).

Moreover we wish to point out that, in most of the cellular lines, the concentrations of compounds **3–4** endowed with cytostatic activity are very near to the cytotoxic doses. By contrast, the pharmacological profile of compound **5** was more promising since in six cellular lines (leukemia, non-small cell lung cancer, colon, melanoma, ovarian and breast cancer) it is possible to note a significant difference between cytostatic and cytotoxic doses.

CDK1 inhibitory activity[13]

As a first attempt to investigate the mechanism of the antitumor activity of compounds **3**, **4** and **5**, they were tested in a CDK1/cyclin B kinase inhibition assay. This kinase and other members of the CDK family play a central role in the control of the cellular cycle, and it has been demonstrated that the deregulation of their activity may be involved in various human tumors.[14] Compounds **3** and **4** did not result to be CDK1 inhibitors, whereas compound **5** exhibited a slight inhibitory activity ($IC_{50} = 60\,\mu M$). Even if this is too weak an evidence to assume a correlation between the antitumor activity of **5** and its intervention on the CDK-controlled mechanisms, we will take it into account in order to further explore the mechanism of action of the imidazo[2,1-b]thiazole guanylhydrazones as possible CDK1 inhibitors.

Positive inotropic activity

The positive inotropic activity of the most potent antitumor agents **3–5** is reported in Table 5 in comparison with amrinone.

These compounds, at a concentration of 10^{-6}–5×10^{-5} M increased the contraction force of the guinea-pig papillary muscle. Compound **4**, in the range of abovementioned doses, showed only tendency to increase this force. The inotropic action of amrinone accrued within 40–50 s and peak drug effect was within 4–5 min. By contrast, the inotropic action of compounds **3**, **5** accrued slowly (2–3 min) and the maximal response was within 10–12 min.

In Table 5 it is possible to notice that compound **3** showed a dose-related effect and both the guanylhydrazones (**3**, **5**) are more potent than amrinone.

Conclusions

In the present work we pointed out that a 3- or 4-nitrophenyl group is a suitable pharmacophoric group in the imidazo[2,1-b]thiazole guanylhydrazone series, giving rise to three potent antitumor agents. Even though the mechanism of action of this activity has not been determined, an interesting concomitant cardiotonic effect was found in two of these compounds (**3**, **5**). We therefore suggest that these guanylhydrazones could be endowed with the coanthracyclinic activity we were looking for. It has been recently reported[15] the antitumor effect of vesnarinone, an oral positive inotropic agent that has been used for the treatment of patients with congestive heart failure. There are no doubts that the mechanisms of action involved in the pharmacological behavior of compounds endowed with both cardiotonic and antitumor properties require accurate investigations in order to determine whether such agents can be introduced into practical therapy. It is important to note that the concentrations causing the useful cardiotonic and antitumor effects should not be considerably different, and this does not seem to be the case with vesnarinone whereas this condition was verified in the compounds we tested.

Experimental

Chemistry

The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values. Bakerflex plates (silica gel IB2-F) were used for TLC: the eluent was petroleum ether:acetone 50:50 (with 0.1% of concd NH_4OH for the analysis of the guanylhydrazones as

Table 1. Compounds **2–34**

Compound	x	R, R′ or Y, n	Formula	Mw	Mp, °C or (lit.)	ν_{max} (cm^{-1})[a] or (lit.)
2	–CH=	2-NO_2, H	$C_{13}H_{11}N_7O_2S\cdot HCl\cdot H_2O$	383.8	280–284 dec	1675, 1660, 1515, 1155
3	–CH=	4-NO_2, H	$C_{13}H_{11}N_7O_2S\cdot HCl\cdot H_2O$	383.8	334–337 dec	1680, 1635, 1500, 1250
4	–CH_2–	4-NO_2, H	$C_{13}H_{13}N_7O_2S\cdot 2HCl\cdot H_2O$	422.3	296–298 dec	1670, 1620, 1510, 855
5	–CH=	3-NO_2, 4-Cl	$C_{13}H_{10}ClN_7O_2S\cdot 2HCl$	436.7	303–305 dec	1675, 1640, 1525, 1150
6	–CH=	4-NH_2, H	$C_{13}H_{13}N_7S\cdot 2HCl\cdot H_2O$	390.3	258–263 dec	1660, 1245, 1090, 820
7	–CH=	—	$C_7H_7N_7O_2S\cdot 4HCl$	399.1	285–290 dec	1665, 1245, 1135, 1025
8	–CH_2–	—	$C_7H_9N_7O_2S\cdot 2HCl$	328.2	275–280 dec	1675, 1575, 1180, 1140
9	—	C_2H_5	$C_{11}H_{14}N_6O_2S\cdot HCl\cdot H_2O$	348.8	272–275 dec	1710, 1670, 1590, 1195
10	—	H	$C_9H_{10}N_6O_2S\cdot HCl\cdot H_2O$	320.8	275–280 dec	1720, 1675, 855, 720
11	—	NH, 2	$C_{20}H_{24}N_{14}O_2S_2\cdot 2HCl\cdot 3H_2O$	683.6	297–300 dec	1670, 1640, 1595, 715
12	—	NH, 4	$C_{22}H_{28}N_{14}O_2S_2\cdot 2HCl\cdot H_2O$	675.6	288–292 dec	1640, 1585, 1210, 860
13	—	O, 4	$C_{22}H_{26}N_{12}O_4S_2\cdot 2HCl\cdot 2H_2O$	695.6	235–238 dec	1670, 1585, 1210, 1040
14	—	O, 6	$C_{24}H_{30}N_{12}O_4S_2\cdot 2HCl\cdot H_2O$	705.6	300–305 dec	1670, 1590, 1260, 855
15	–CH=	2-NO_2, H	$C_{11}H_7N_3O_2S$	245.3		6
16	–CH=	4-NO_2, H	$C_{11}H_7N_3O_2S$	245.3		7
17	–CH_2–	4-NO_2, H	$C_{11}H_9N_3O_2S$	247.3		7
18	–CH=	3-NO_2, 4-Cl	$C_{11}H_6ClN_3O_2S$	279.7	202–205	1535, 1510, 1195, 725
19	–CH=	2-NO_2, H	$C_{12}H_7N_3O_3S$	273.3		6
20	–CH=	4-NO_2, H	$C_{12}H_7N_3O_3S$	273.3		6
21	–CH_2–	4-NO_2, H	$C_{12}H_9N_3O_3S$	275.3	192–194	1650, 1500, 1250, 860
22	–CH=	3-NO_2, 4-Cl	$C_{12}H_6ClN_3O_3S$	307.7	251–253 dec	1645, 1095, 830, 715
23	—	—	$C_{12}H_9N_3OS$	243.3	178–180 dec	3340-3060, 1610, 1250
24	—	—	$C_6H_3N_3O_3S$	197.2		6
25	—	—	$C_6H_7N_3O_2S$	185.2		7
26	—	—	$C_{13}H_{11}N_3O_2S$	273.3	145–150	1680, 1255, 1165, 710
27	—	—	$C_6H_5N_3O_3S$	199.2	128–130 dec	1680, 1150, 870, 795
28	—	—	$C_{10}H_{10}N_2O_3S$	238.3		8
29	—	—	$C_8H_6N_2O_3S$	210.2	177–178 dec	3140-2400, 1685, 1630
30	—	—	$C_{12}H_9N_3O_5S$	307.3	227–228 dec	1800, 1770, 1650, 1310
31		NH, 2	$C_{18}H_{16}N_6O_4S_2$	444.5	275–278 dec	3380, 1660, 1640, 970
32		NH, 4	$C_{20}H_{20}N_6O_4S_2$	472.5		b
33		O, 4	$C_{20}H_{18}N_4O_6S_2$	474.5		b
34		O, 6	$C_{22}H_{22}N_4O_6S_2$	502.6	135–138	1705, 1640, 1185, 1020

[a]In all the guanylhydrazones the NH groups give broad bands in the range 3600–2300 cm^{-1}.

Table 2. ^{1}H NMR of compounds **2–14**, **18**, **21–23**, **26**, **27**, **29–34**

Compound	δ, ppm
2	7.58 (1H, d, th, $J=4.5$), 7.74 (2H, m, ar), 7.80 (3H, broad, NH + NH_2), 7.83 (1H, t, ar, $J=7.7$), 8.05 (1H, d, ar, $J=7.7$), 8.29 (1H, s, -CH=), 8.74 (1H, d, th, $J=4.5$), 11.99 (1H, s, NH)
3	7,56 (1H, d, th, $J=4.5$), 7.80 (3H, broad, NH + NH_2), 7.98 (2H, d, ar, $J=7$), 8.3 (2H, d, ar, $J=7$), 8.60 (1H, s, CH=), 8.68 (1H, d, th, $J=4.5$), 12.09 (1H, s, NH)
4	4.02 (2H, t, thn, $J=7.2$), 4.66 (2H, t, thn, $J=7.2$), 5.52 (2H, broad, NH_2), 7.79 (1H, broad s, NH), 7.87 (2H, d, ar, $J=8.8$), 8.27 (2H, d, ar, $J=8.8$), 8.45 (1H, s, CH=), 12.22 (1H, s, NH)
5	7.57 (1H, d, th, $J=4.4$), 7.80 (3H, broad, NH + NH_2), 7.91 (1H, d, ar-5, $J=8.4$), 8.03 (1H, dd, ar-6, $J=2$, $J=8.4$), 8.38 (1H, d, ar-2, $J=2$), 8.54 (1H, s, -CH=), 8.70 (1H, d, th, $J=4,4$), 11.84 (1H, s, NH)
6	7.18 (2H, s, $C_6H_4NH_2$), 7.42 (2H, d, ar, $J=8.5$), 7.55 (1H, d, th, $J=4.4$), 7.77 (2H, d, ar, $J=8.5$), 7.80 (3H, broad, NH + NH_2), 8.51 (1H, s, -CH=), 8.71 (1H, d, th, $J=4.4$), 12.00 (1H, s, NH)
7	7.76 (1H, d, th, $J=4.4$), 7.95 (3H, s, NH_2 + NH), 8.41 (1H, d, th, $J=4.4$), 8.81 (1H, s, -CH =), 12.61 (1H, s, NH)
8	4.06 (2H, t, thn, $J=7.9$), 4.63 (2H, t, thn, $J=7.9$), 7.91 (3H, s, NH_2 + NH), 8.62 (1H, s, -CH=), 12.68 (1H, s, NH)
9	1.28 (3H, t, CH_2CH_3, $J=7$), 2.37 (3H, s, CH_3), 4.28 (2H, q, CH_2CH_3, $J=7$), 7.49 (1H, d, th, $J=4.5$), 7.91 (3H, s, NH_2 + NH), 7.97 (1H, d, th, $J=4.5$), 11.46 (1H, s, NH)
10	2.42 (3H, s, CH_3), 5.30 (1H, broad, OH), 7.50 (1H, d, th, $J=4.4$), 8.00 (1H, d, th, $J=4.4$), 8.04 (3H, s, NH_2 + NH), 11.72 (1H, s, NH)
11	2.42 (6H, s, CH_3), 3.47 (4H, broad, CH_2), 7.46 (2H, d, th, $J=4.5$), 7.85 (6H, s, NH_2 + NH), 8.01 (2H, d, th, $J=4.5$), 8.58 (2H, s, CONH), 11.31 (2H, s, NH)
12	1.54 (4H, broad, CH_2), 2.42 (6H, s, CH_3), 3.28 (4H, broad, $NHCH_2$), 7.45 (2H, d, th, $J=4.5$), 7.82 (6H, s, NH_2 + NH), 8.01 (2H, d, th, $J=4.5$), 8.47 (2H, t, CONH), 11.25 (2H, s, NH)
13	1.79 (4H, broad, CH_2), 2.38 (6H, s, CH_3), 4.28 (4H, broad, OCH_2), 7.49 (2H, d, th, $J=4.5$), 7.89 (6H, s, NH_2 + NH), 7.98 (2H, d, th, $J=4.5$), 11.45 (2H, s, NH)
14	1.40 (4H, broad, CH_2), 1.67 (4H, broad, CH_2), 2.38 (6H, s, CH_3), 4.25 (4H, t, OCH_2, $J=6$), 7.49 (2H, d, th, $J=4.5$), 7.89 (6H, s, NH_2 + NH), 7.98 (2H, d, th, $J=4.5$), 11.45 (2H, s, NH)
18	7.33 (1H, d, th, $J=4.5$), 7.78 (1H, d, ar-5, $J=8.5$), 8.00 (1H, d, th, $J=4.5$), 8.13 (1H, dd, ar-6, $J=2$, $J=8.5$), 8.46 (2H: 1H, s, H-5 + 1H, d, ar-2, $J=2$)
21	4.05 (2H, t, thn, $J=7.6$), 4.53 (2H, t, thn, $J=7.6$), 8.08 (2H, d, ar, $J=8.6$), 8.30 (2H, d, ar, $J=8.6$), 9.79 (1H, s, -CH=)
22	7.65 (1H, d, th, $J=4.3$), 7.92 (1H, d, ar, $J=8.5$), 8.24 (1H, d, ar, $J=8.5$), 8.44 (1H, d, th, $J=4.3$), 8.57 (1H, s, ar), 9.96 (1H, s, -CH=)
23	6.95 (2H, d, ar, $J=8.5$), 7.54 (1H, d, th, $J=4.4$), 7.73 (2H, d, ar, $J=8.5$), 8.38 (1H, d, th, $J=4.4$), 8.60 (2H, broad, $C_6H_4NH_2$), 9.87 (1H, s, CHO)
26	4.02 (2H, t, thn, $J=7.5$), 4.60 (2H, t, thn, $J=7.5$), 7.41 (3H, m: 2H, ar + 1H, CH), 7.66 (4H, m: 3H, ar + 1H, CH)
27	4.07 (2H, t, thn, $J=7.8$), 4.65 (2H, t, thn, $J=7.8$), 10.16 (1H, s, CHO)
29	2.62 (3H, s, CH_3), 3.40 (1H, broad, OH), 7.63 (1H, d, th, $J=4.4$), 8.36 (1H, d, th, $J=4.4$)
30	2.64 (3H, s, CH_3), 2.92 (4H, s, CH_2), 7.75 (1H, d, th, $J=4.5$), 8.42 (1H, d, th, $J=4.5$)
31	2.66 (6H, s, CH_3), 3.53 (4H, broad, CH_2), 7.62 (2H, d, th, $J=4.5$), 8.39 (2H, d, th, $J=4.5$), 8.81 (2H, broad, NH)
32	1.58 (4H, broad, CH_2), 2.63 (6H, s, CH_3), 3.30 (4H, broad, $NHCH_2$), 7.61 (2H, d, th, $J=4.5$), 8.38 (2H, d, th, $J=4.5$), 8.74 (2H, t, NH)
33	1.74 (4H, broad, CH_2), 2.58 (6H, s, CH_3), 4.32 (4H, t, OCH_2, $J=6.5$), 7.61 (2H, d, th, $J=4.5$), 8.32 (2H, d, th, $J=4.5$)
34	1.45 (4H, broad, CH_2), 1.74 (4H, broad, CH_2), 2.59 (6H, s, CH_3), 4.33 (4H, t, OCH_2, $J=6.4$), 7.63 (2H, d, th, $J=4.5$), 8.34 (2H, d, th, $J=4.5$)

Table 3. Growth inhibition percentages of three human tumor cell lines in the presence of compounds **2–14**

Compound (10^{-4} M)	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)
2	−4	−53	29
3	−27	3	−50
4	−51	−26	−65
5	12	17	4
6	8	6	5
7	9	1	24
8	1	8	6
9	100	78	86
10	111	88	97
11	109	102	73
12	68	82	88
13	82	44	54
14	59	−19	8

free bases). Kieselgel 60 (Merck) was used for column chromatography. The IR spectra were recorded in Nujol on a Perkin–Elmer 683; ν_{max} is expressed in cm^{-1} (Table 1). The ^{1}H NMR spectra were recorded in $(CD_3)_2SO$ on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in δ (ppm) and J in Hz, with the following abbreviations: th = thiazole, thn = thiazoline, ar = aromatic (Table 2).

General procedure for the synthesis of the guanylhydrazones 2–14

The appropriate aldehyde or ketone (10 mM) 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 30 min and the resulting precipitate was collected by filtration with a yield of 80–90%. For the synthesis of guanylhydrazones **11–14** two equivalents of aminoguanidine hydrochloride were employed and the yield was 30–40%.

6-(4-Chloro-3-nitrophenyl)imidazo[2,1-b]thiazole (18). 2-Aminothiazole (11 mM) was dissolved in acetone (50 mL) and treated with 2-bromo-1-(4-chloro-3-nitrophenyl)-ethanone[8] (11 mM). The reaction mixture was refluxed for 1 h, the resulting salt was separated by filtration and treated, without further purification, with 20 mL of

Table 4. Growth inhibition, cytostatic and cytotoxic activity of compounds **3–5** on the 60 cell panel[a]

	3[b]			**4**[b]			**5**[b]			DTIC[b]			Methyl-GAG[c]		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia	−5.9	−5.2	−4.3	−5.3	−4.7	−4.1	−5.9	−5.4	−4.0	−4.8	−4.1	−4.0	−5.1	−3.2	−2.6
NSCLC	−5.7	−5.5	−5.1	−4.8	−4.4	−4.2	−5.7	−5.5	−4.9	−4.4	−4.2	−4.0	−4.6	−3.5	−2.8
Colon	−5.9	−5.5	−5.1	−5.2	−4.8	−4.3	−5.9	−5.6	−5.0	−4.2	−4.0	−4.0	−4.9	−3.3	−2.9
CNS	−5.8	−5.5	−5.5	−5.2	−4.8	−4.4	−5.8	−5.5	−5.2	−4.2	−4.0	−4.0	−4.7	−3.6	−3.0
Melanoma	−5.7	−5.5	−5.0	−4.9	−4.5	−4.2	−5.7	−5.4	−4.6	−4.5	−4.0	−4.0	−4.4	−3.5	−3.0
Ovarian	−5.7	−5.3	−4.8	−4.9	−4.5	−4.1	−5.8	−5.5	−4.5	−4.3	−4.0	−4.0	−4.8	−3.5	−2.8
Renal	−5.7	−5.4	−4.9	−4.9	−4.6	−4.2	−5.8	−5.5	−5.2	−4.3	−4.0	−4.0	−4.7	−3.3	−2.7
Prostate	−5.8	−5.5	−5.3	−4.9	−4.6	−4.3	−5.8	−5.5	−5.2	−4.1	−4.0	−4.0	−4.0	−2.8	−2.6
Breast	−5.5	−5.3	−4.9	−4.9	−4.6	−4.1	−5.7	−5.4	−4.4	−4.1	−4.0	−4.0	−4.8	−3.5	−2.8

[a]All values are $\log_{10}$.
[b]Highest concn 10^{-4} M.
[c]Highest concn $10^{-2.6}$ M.

Table 5. Positive inotropic activity of compounds **3–5**

Compound	Concn (M)	Force of contraction (P/P_0±SEM,%)[a]	EC_{50} (M)
3	10^{-6}	120.6±8.6	$(4.0\pm0.56)\times10^{-6}$
	10^{-5}	138.3±19.0	
	5×10^{-5}	168.0±18.0	
4	10^{-6}	112.0±6.3	—
	10^{-5}	118.0±9.7	
	5×10^{-5}	111.0±5.7	
5	10^{-6}	136.5±12.0	$(1\pm0.6)\times10^{-6}$
	10^{-5}	149.5±14.4	
	5×10^{-5}	124.5±11.8	
Amrinone	10^{-6}	105.0±2.3	$(2.5\pm0.4)\times10^{4}$
	10^{-5}	114.7±5.9	
	5×10^{-5}	124.0±4.2	

[a]Mean of five experiments for **3** and amrinone, four for **4–5**. P = force of contraction at a given concentration; P_0 = control (100%).

ethanol and 400 mL of 2N HCl. After 1 h of reflux, the solution was cautiously basified by dropwise addition of 15% NH_4OH. The resulting base was collected by filtration and crystallized from ethanol with a yield of 80%.

6-(4-Nitrophenyl)-2,3-dihydroimidazo[2,1-b]thiazole-5-carbaldehyde (21) and 6-(4-chloro-3-nitrophenyl)imidazo[2,1-b]thiazole-5-carbaldehyde (22). The Vilsmeier reagent was prepared at 0–5 °C by dropping $POCl_3$ (54 mM) into a stirred solution of DMF (65 mM) in $CHCl_3$ (5 mL). 6-(4-Nitrophenyl)-2,3-dihydroimidazo[2,1-b]thiazole **17** or 6-(4-chloro-3-nitrophenyl)imidazo[2,1-b]thiazole **18** (10 mM) in $CHCl_3$ (60 mL) was added dropwise to the Vilsmeier reagent while maintaining stirring and cooling. The reaction mixture was kept for 3 h at room temperature and under reflux for 20 h. Chloroform was removed under reduced pressure and the resulting oil was poured onto ice. The crude aldehyde thus obtained was collected by filtration and crystallized from ethanol with a yield of 55% (**21**) and 90% (**22**).

6-(4-Aminophenyl)imidazo[2,1-b]thiazole-5-carbaldehyde (23). The nitroderivative **20** (1.8 mM), dissolved in 30 mL of DMF:MeOH (0.1:10 v/v), was hydrogenated in the presence of 10% Pd/C (10 mg) at atmospheric pressure and room temperature for 8 h. The mixture was filtered through Celite and methanol was evaporated under reduced pressure. The residue was purified by column chromatography with a mixture of CH_2Cl_2:toluene:MeOH (7.5:1:1.5) as the eluent. A yellow solid was obtained which was crystallized from acetone/petroleum ether with a yield of 25%.

5-Nitro-6-(2-phenylethenyl)-2,3-dihydroimidazo[2,1-b]thiazole (26). A mixture of 6-methyl-5-nitro-2,3-dihydroimidazo[2,1-b]thiazole **25** (3 g, 16 mM), benzaldehyde (10 mL) and piperidine (2.5 mL) was heated at 120–130 °C for 6 h under stirring. After cooling, the mixture was concentrated under reduced pressure and the resulting yellow solid was purified by column chromatography (eluent petroleum ether:acetone 70:30) and crystallized from ethanol with a yield of 85%.

5-Nitro-2,3-dihydroimidazo[2,1-b]thiazole-6-carbaldehyde (27). A solution of sodium periodate (18 mM) in 15 mL H_2O was added to a stirred solution of compound **26** (9 mM) and osmium tetroxide (0.2 mM) in 50 mL THF. The mixture was stirred at room temperature for 48 h. The suspension was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography with petroleum ether:acetone 70:30 as the eluent. Compound **27** was obtained as a yellow solid with a yield of 65%.

5-Acetylimidazo[2,1-b]thiazole-6-carboxylic acid (29). A solution of compound **28**[9] (4.2 mM) in 0.1N NaOH (20 mL) and MeOH (30 mL) was refluxed for 5 h. The solvent was evaporated under reduced pressure and the residue was dissolved in water (40 mL). The solution was treated with 10% HCl until pH 2 was reached. The suspension was extracted with $CHCl_3$ which was evaporated under reduced pressure. The residue, crystallized from acetone/petroleum ether, gave a white solid with a yield of 34%.

1-([(5-Acetylimidazo[2,1-b]thiazol-6-yl)carbonyl]oxy)pirrolidine-2,5-dione (30). A mixture of the carboxylic acid **29** (1.4 mM), diisopropylethylamine (DIEA, 1.4 mM) and *O*-(N-succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (STMU, 1.4 mM) in anhydrous DMF was stirred at room temperature for 10 h. The mixture was diluted with H_2O (20 mL) and extracted with

CH_2Cl_2. The organic phase was dried and evaporated under reduced pressure. The crude product was crystallized from acetone/petroleum ether to afford a white solid with a yield of 68%.

***N,N′*-1,2-Ethanediylbis(5-acetylimidazo[2,1-b]thiazole-6-carboxamide) (31) and *N,N′*-1,4-butanediylbis(5-acetylimidazo[2,1-b]thiazole-6-carboxamide) (32).** A mixture of compound **30** (1 mM) and 0.5 mM of 1,2-diaminoethane (or 1,4-diaminobutane) in 5 mL of DMF was refluxed under stirring for 2 h. MeOH (10 mL) was added to the cooled solution and the solid obtained was filtered and used as such in the subsequent step. The yield was 9% (**31**) and 37% (**32**).

5-Acetylimidazo[2,1-b]thiazole-6-carboxylic acid 1,4-butanediyl ester (33) and 5-acetylimidazo[2,1-b]thiazole-6-carboxylic acid 1,6-hexanediyl ester (34). 1,1′-Carbonyldiimidazole (CDI, 2.4 mM) was added to a suspension of compound **29** (2.4 mM) in anhydrous CH_2Cl_2 (35 mL) and refluxed until effervescence ceased. Stirring at reflux was maintained for 2 h. The cooled acylimidazole was treated with 1,4-butanediol (or 1,6-hexanediol, 1.2 mM) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2.4 mM). The reaction mixture was stirred under reflux for 4 h, cooled, washed with water, dried and concentrated under reduced pressure to afford a crude residue which was used as such in the subsequent step. The yield was 12% (**33**) and 35% (**34**).

Pharmacology

Antitumor activity

Three cell panel. Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B (SRB), a protein-binding dye. Results for each test compound are reported as the percent of growth of the treated cells when compared to the untreated control cells.

Sixty cell panel. Each cell line was inoculated onto microtiter plates, then preincubated for 24–28 h at 37 °C for stabilization. Subsequently, test agents were added in five 10-fold dilutions and the culture was incubated for an additional 48 h in 5% CO_2 atmosphere and 100% humidity. For each test agent, a dose–response profile was generated. End-point determinations of cell growth were performed by in situ fixation of cells, followed by staining with SRB, which binds to the basic amino acids of cellular macromolecules. The solubilized stain was measured spectrophotometrically in order to determine relative cell growth in treated and untreated cells.

A plate reader was used to read the optical densities, and a microcomputer processed the optical densities in the special concentration parameters: GI_{50}, TGI, LC_{50}. The optical density of the test well after 48 h period of exposure to the test drugs is T, the optical density at time zero is T_0 and the control optical density is C.

GI_{50} is the concentration of test drug where $100\times(T-T_0)/(C-T_0)=50$.

TGI is the concentration of test drug where $100\times(T-T_0)/(C-T_0)=0$.

LC_{50} is the concentration of test drug where $100\times(T-T_0)/T_0=-50$ (the control optical density is not used in the calculation of LC_{50}).

Positive inotropic activity. Guinea-pig right ventricle papillary muscle was mounted in an organ bath with a circulation of physiological saline solution of following composition (mM): NaCl 144; KCl 4; $CaCl_2$ 1.8; Tris.Cl 10; $MgCl_2$ 1; glucose 5; pH 7.3–7.4. The solution was aerated with pure oxygen and the temperature was maintained at 36–37 °C. The maximal perfusion value of the papillary muscle was about 4.5 mL/min.

Electrostimulator ESU-2 (Russia) electrically stimulated the muscle by square pulses with a voltage of 20% above threshold, 10 ms duration at a frequency of 1.0 Hz. The force of the isometric contraction was measured by the 6MX2B force displacement transducer (Russia) and recorded by the H3030-4 recorder (Russia).

In order to obtain a concentration–response curve, each papillary muscle, after 1 h equilibration period, was tested in cumulative doses of the agents under test. Each compound was dissolved in a minimum amount (0.1–0.3 mL) of DMSO and diluted with physiological solution. Quantitatively, the contraction force (P) was expressed as respective ratio to the initial value of the contraction force (P_0) under normal conditions. From concentration–response curves the negative logarithm of the concentration of the test agent required for a 50% increase in the maximal force of contraction (EC_{50}) was determined. Results are presented as mean ± SEM. Statistical analyses were performed using Student's t-test.

CDK1 inhibitory activity. CDK1-cyclin B was extracted in homogenization buffer from M-phase starfish (*Marthasterias glacialis*) oocytes and purified by affinity chromatography on p9CKShs1-Sepharose beads, from which it was eluted by free p9CKShs1 as described.[13] The kinase activity was assayed with 1 mg mL^{-1} histone H1 (Sigma type III-S), in the presence of 15 μM [γ-^{32}P]ATP (3000 Ci mM^{-1}; 1 mCi mL^{-1}) in a final volume of 30 μL. After a 10 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5×3 cm pieces of Whatman P81 phosphocellulose paper and, 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid per liter water. The wet filters were counted in the presence of 1 mL scintillation fluid (Amersham). The kinase activity was expressed as a percentage of maximal activity.

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References and Notes

1. For part 28 see Andreani, A.; Leoni, A.; Locatelli, A.; Morigi, R.; Chiericozzi, M.; Fraccari, A.; Galatulas, I. *Anticancer Res*. **1998**, *18*, 3407.
2. Andreani, A.; Locatelli, A.; Leoni, A.; Morigi, R.; Chiericozzi, M.; Fraccari, A.; Galatulas, I.; Salvatore, G. *Eur. J. Med. Chem*. **1998**, *33*, 905.
3. Andrieu Abadie, N.; Jaffrezou, J. P.; Hatem, S.; Laurent, G.; Levade, T.; Mercaider, J. J. *FASEB J*. **1999**, *13*, 1501.
4. Andreani, A.; Rambaldi, M.; Locatelli, A.; Bossa, R.; Galatulas, I.; Salvatore, G. *In Vivo* **1994**, *8*, 1031.
5. Andreani, A.; Rambaldi, M.; Locatelli, A.; Bossa, R.; Fraccari, A.; Galatulas, I. *J. Med. Chem*. **1992**, *35*, 4634.
6. Andreani, A.; Rambaldi, M.; Leoni, A.; Locatelli, A.; Bossa, R.; Fraccari, A.; Galatulas, I.; Salvatore, G. *J. Med. Chem*. **1996**, *39*, 2852.
7. Andreani, A.; Leoni, A.; Rambaldi, M.; Locatelli, A.; Bossa, R.; Galatulas, I.; Chiericozzi, M.; Cozzi, R. *Eur. J. Med. Chem*. **1997**, *32*, 151.
8. Andreani, A.; Rambaldi, M.; Andreani, F.; Hrelia, P.; Cantelli Forti, G. *Arch. Pharm. Chem., Sci. Ed*. **1987**, *15*, 41.
9. Canestrari S.; Sgarabotto P.; Andreani A.; Greci L. *J. Chem. Research (S)* **1999**, 412; *(M)* **1999**, 1750.
10. Singh, M. A.; Plouvier, B.; Hill, G. C.; Gueck, J.; Pon, R. T.; Lown, J. W. *J. Am. Chem. Soc*. **1994**, *116*, 7006.
11. Andreani, A.; Rambaldi, M.; Leoni, A.; Locatelli, A.; Andreani, F.; Bossa, R.; Chiericozzi, M.; Cozzi, R.; Galatulas, I. *Eur. J. Med. Chem*. **1996**, *31*, 741.
12. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst*. **1991**, *83*, 757.
13. Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. *Eur. J. Biochem*. **1997**, *243*, 527.
14. Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem*. **2000**, *43*, 1.
15. Yokozaki, H.; Ito, R.; Ono, S.; Hayashi, K.; Tahara, E. *Cancer Lett*. **1999**, *140*, 121.