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Brief Article

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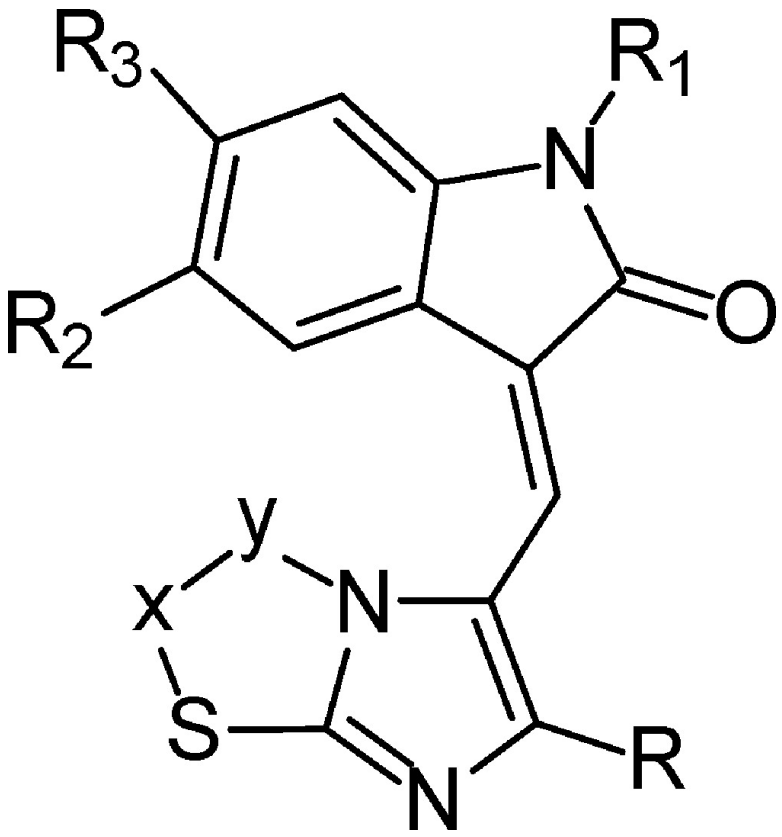


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# Antitumor Activity of New Substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones and Study of Their Effect on the Cell Cycle[1]

Aldo Andreani,*,† Massimiliano Granaiola,† Alberto Leoni,† Alessandra Locatelli,† Rita Morigi,† Mirella Rambaldi,† Vida Garaliene,‡ William Welsh,§ Sonia Arora,§ Giovanna Farruggia,|| and Lanfranco Masotti||

*Dipartimento di Scienze Farmaceutiche, Universitá di Bologna, Via Belmeloro 6, 40126 Bologna, Italy, Lithuanian Institute of Cardiology, Kaunas, Lithuania LT-3007, Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine & Dentistry of New Jersey (UMDNJ), 675 Hoes Lane, Piscataway, New Jersey 08854, and Dipartimento di Biochimica "G. Moruzzi", Universitá di Bologna, Via Irnerio 48, 40126 Bologna, Italy*

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This paper reports the synthesis of a new series of 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones which were tested as potential antitumor agents at the National Cancer Institute. Two derivatives are now under review by BEC (Biological Evaluation Committee of NCI). To investigate the mechanism of action, the effect on cell cycle progression was studied by monitoring them in colon adenocarcinoma HT-29: both were able to block HT-29 in mitosis. 3-[(2,6-Dimethylimidazo[2,1-*b*]thiazol-5-yl)methylene]-5-chloro-2-indolinone was the most active compound.

## Introduction

In 1997 we published a paper on the antitumor activity of 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones. The compounds reported in that paper[2] were mainly formed by connecting an imidazo[2,1-*b*]thiazole moiety (bearing at the 6 position Cl, $CH_3$ or $C_6H_5$) by means of a methine bridge to 2-indolinone or its 5-methoxy analogue. The promising results obtained prompted us to prepare a new series of analogues including, as starting materials, several indolinones and imidazothiazoles different at positions 2 and 3. The study[3] confirmed that the most potent compounds bear a methoxy group at the 5 position of the indole system; moreover, in the imidazothiazole portion the substitution at the 2 position is much more important than at the 6 position, since all the most interesting compounds are 2-methyl derivatives.

In this paper we describe new analogues with substitutions in the indole as well in the imidazothiazole portion. The antitumor activity of all new compounds was evaluated on three human tumor cell lines according to the protocols available at the National Cancer Institute (NCI, Bethesda, MD), and the active compounds were tested on the sixty tumor cell lines as well. Since it is well-known that several indole derivatives can induce G2/M arrest[4,5] and apoptosis in certain cell lines,[6] the effect on cell proliferation and cell cycle progression in the colon adenocarcinoma cell line HT-29 was also studied for the most potent antitumor derivatives. Finally, they were tested as positive inotropic agents in a search for potential coanthracyclinic activity.[7]

**Chemistry.** The substitutions (Scheme 1 and Table 1) have been performed according to the following rationale: (1) in order to understand the influence of the substituents at the 5 and 6 positions of the indole system, we condensed two imidazothiazole aldehydes (**5**, **6**) with four different indolinones (**10**−**13**). This reaction gave compounds **16**−**23**; (2) compound **24** was synthesized in order to study the effect of the N-substitution in the indole moiety of the most active compound of the previous series;[2] (3) new substituents have been introduced in the imidazothiazole moiety while maintaining the same indolinone: chlorine at the 2 position (**25** and **26**) and trifluoromethyl at the 6 position (**27**); (4) finally, a single compound was prepared (**28**) with the shift of the substituent from the 2 to the 3 position of the imidazothiazole.

Compounds **16**−**28** were prepared by means of the Knoevenagel reaction between the aldehydes **4**−**9** and the indolinones **10**−**15** in the presence of piperidine. The aldehyde **4** (starting compound for the synthesis of **27**) was obtained by means of the Vilsmeier reaction on the corresponding imidazo[2,1-*b*]thiazole **3**, prepared in turn from 2-amino-5-methylthiazole **1** and 1-bromo-3,3,3-trifluoroacetone. The intermediate compound **2** was isolated and used in the subsequent step without further purification. The structures of the final compounds (**16**−**28**) were confirmed by means of IR and $^1$H NMR spectra (see Supporting Information). They were obtained as pure geometrical isomers which, according to the usual NOE experiments described in the previous papers,[2,3] were assigned to the *E* configuration.

## Results and Discussion

**(a) Antitumor Activity (in vitro growth inhibition and cytotoxicity).** As a preliminary screening, compounds **16**−**28** were evaluated for their cytotoxic potency on three human cell lines: NCI-H460 lung cancer, MCF7 breast cancer, and SF-268 glioma. A

* Corresponding author: tel +39-051-2099714, fax +39-051-2099734, e-mail aldo.andreani@unibo.it.
† Universitá di Bologna.
‡ Lithuanian Institute of Cardiology.
§ University of Medicine & Dentistry of New Jersey.
|| Universitá di Bologna.

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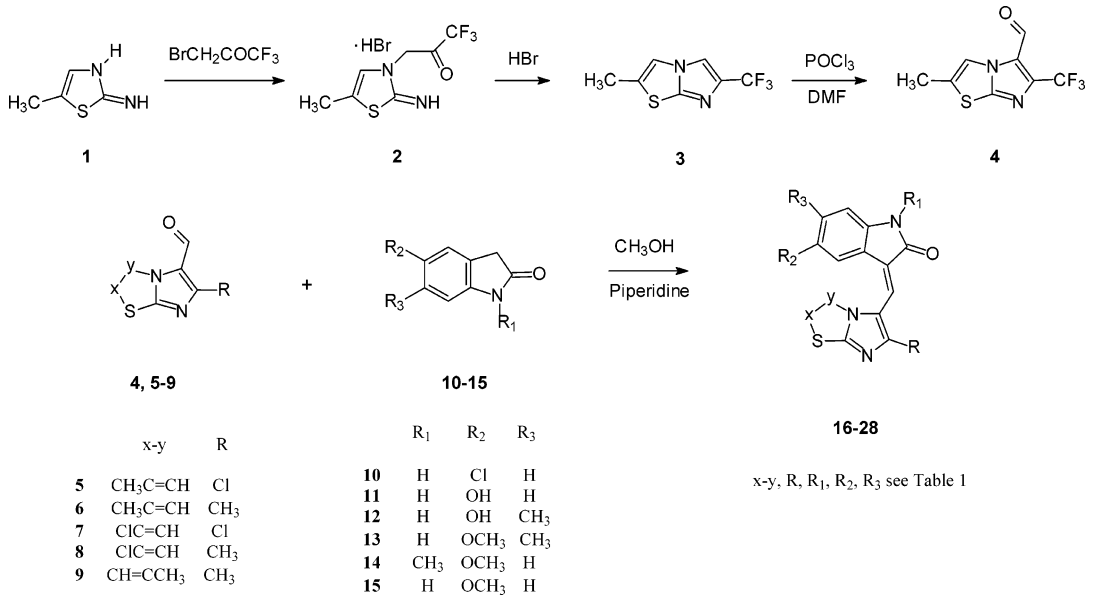
**Scheme 1**




| | x-y | R |
|---|---|---|
| **5** | $CH_3C{=}CH$ | Cl |
| **6** | $CH_3C{=}CH$ | $CH_3$ |
| **7** | $ClC{=}CH$ | Cl |
| **8** | $ClC{=}CH$ | $CH_3$ |
| **9** | $CH{=}CCH_3$ | $CH_3$ |

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **10** | H | Cl | H |
| **11** | H | OH | H |
| **12** | H | OH | $CH_3$ |
| **13** | H | $OCH_3$ | $CH_3$ |
| **14** | $CH_3$ | $OCH_3$ | H |
| **15** | H | $OCH_3$ | H |

**Table 1.** Compounds **16**−**28**

| compd | x−y | R | $R_1$ | $R_2$ | $R_3$ | formula | MW | mp, °C |
|---|---|---|---|---|---|---|---|---|
| **16** | $CH_3C{=}CH$ | Cl | H | Cl | H | $C_{15}H_9Cl_2N_3OS$ | 350.2 | 268−270 dec |
| **17** | $CH_3C{=}CH$ | Cl | H | OH | H | $C_{15}H_{10}ClN_3O_2S$ | 331.4 | 288−290 dec |
| **18** | $CH_3C{=}CH$ | Cl | H | OH | $CH_3$ | $C_{16}H_{12}ClN_3O_2S$ | 345.8 | 300−303 dec |
| **19** | $CH_3C{=}CH$ | Cl | H | $OCH_3$ | $CH_3$ | $C_{17}H_{14}ClN_3O_2S$ | 359.4 | 289−290 dec |
| **20** | $CH_3C{=}CH$ | $CH_3$ | H | Cl | H | $C_{16}H_{12}ClN_3OS$ | 329.8 | 295−298 dec |
| **21** | $CH_3C{=}CH$ | $CH_3$ | H | OH | H | $C_{16}H_{13}N_3O_2S$ | 311.3 | 300−305 dec |
| **22** | $CH_3C{=}CH$ | $CH_3$ | H | OH | $CH_3$ | $C_{17}H_{15}N_3O_2S$ | 325.4 | 310−313 dec |
| **23** | $CH_3C{=}CH$ | $CH_3$ | H | $OCH_3$ | $CH_3$ | $C_{18}H_{17}N_3O_2S$ | 339.4 | 264−266 dec |
| **24** | $CH_3C{=}CH$ | $CH_3$ | $CH_3$ | $OCH_3$ | H | $C_{18}H_{17}N_3O_2S$ | 339.4 | 163−168 |
| **25** | $ClC{=}CH$ | Cl | H | $OCH_3$ | H | $C_{15}H_9Cl_2N_3O_2S$ | 366.2 | 265−267 dec |
| **26** | $ClC{=}CH$ | $CH_3$ | H | $OCH_3$ | H | $C_{16}H_{12}ClN_3O_2S$ | 345.8 | 231−233 |
| **27** | $CH_3C{=}CH$ | $CF_3$ | H | $OCH_3$ | H | $C_{17}H_{12}F_3N_3O_2S$ | 379.4 | 260−263 |
| **28** | $HC{=}CCH_3$ | $CH_3$ | H | $OCH_3$ | H | $C_{17}H_{15}N_3O_2S$ | 325.4 | 255−257 |

**Table 2.** Sixty Cell Line Panel (growth inhibition and cytostatic and cytotoxic activity of the selected compounds)

| NSC | compd[a] | modes | leukemia | NSCLC | colon | CNS | melanoma | ovarian | renal | prostate | breast | MG-MID[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 725098 | **20** | $GI_{50}$ | −7.70 | −6.17 | −6.72 | −6.13 | −6.21 | −5.91 | −6.54 | −6.98 | −6.51 | −6.42 |
| | | TGI | − | −4.65 | −5.06 | −5.08 | −4.60 | −4.82 | −4.85 | −5.62 | −4.85 | −4.81 |
| | | $LC_{50}$ | − | −4.16 | −4.34 | −4.16 | −4.06 | −4.30 | −4.06 | −4.36 | −4.02 | −4.15 |
| 725100 | **21** | $GI_{50}$ | −6.28 | −5.40 | −5.78 | −5.41 | −5.41 | −5.16 | −5.30 | −5.52 | −5.56 | −5.48 |
| | | TGI | −5.11 | −4.36 | −4.35 | −4.50 | −4.50 | −4.09 | −4.32 | −4.42 | −4.66 | −4.44 |
| | | $LC_{50}$ | −4.47 | −4.02 | −4.03 | −4.02 | −4.08 | − | − | − | − | −4.05 |
| 725102 | **23** | $GI_{50}$ | −4.47 | −4.24 | −4.21 | −4.08 | −4.22 | −4.12 | −4.09 | −4.23 | −4.33 | −4.22 |
| 725099 | **24** | $GI_{50}$ | −5.64 | −5.11 | −5.49 | −5.46 | −5.26 | −5.16 | −5.32 | −5.06 | −5.60 | −5.35 |
| | | TGI | − | −4.40 | −4.55 | −4.59 | −4.45 | −4.42 | −4.40 | −4.10 | −4.87 | −4.46 |
| | | $LC_{50}$ | − | − | −4.09 | − | −4.13 | −4.02 | − | − | −4.03 | −4.03 |
| 67574 | vincristine sulfate[c] | $GI_{50}$ | −7.00 | −6.60 | −7.00 | −6.90 | −6.80 | −6.50 | −6.50 | −6.90 | −6.50 | −6.70 |
| | | TGI | −4.80 | −4.80 | −5.40 | −5.20 | −5.10 | −4.70 | −4.70 | −5.20 | −5.10 | −5.00 |
| | | $LC_{50}$ | −3.20 | −3.60 | −4.10 | −3.70 | −3.60 | −3.50 | −3.60 | −3.50 | −3.50 | −3.60 |

[a] All values are log. Highest concentration = $10^{-4}$ M; only modes showing values < −4.00 are reported for the new compounds. [b] Calculated mean panel. [c] Highest concentration = $10^{-3}$ M.

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 is promoted for evaluation in the full panel of 60 cell lines. Compounds **20, 21**, **23,** and **24** were 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 were dissolved in DMSO and 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.

Table 2 reports the results obtained expressed as log, taking into consideration the 50% growth inhibitory power ($GI_{50}$), the cytostatic effect (TGI = total growth inhibition), and the cytotoxic effect ($LC_{50}$). The results obtained by vincristine sulfate are reported for comparison.

The introduction of chlorine at position 2 of the imidazothiazole system (**25** and **26**) as well as the shift of the methyl group from position 2 to 3 (**28**) led to inactive compounds, thus confirming that the most active compounds are 2-methylimidazothiazole derivatives.

**Table 3.** Percentage of Cells in G2/M Phase after 24 Hours of Treatments (mean ± SD of three experiments)

| compound | control | $10^{-8}$ M | $10^{-7}$ M | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M |
|---|---|---|---|---|---|---|
| **20** | 10.0 ± 6.0 | 11.1 ± 3.5 | 9.7 ± 3.5 | 72.0 ± 3.7 | 86.2 ± 3.9 | 61.6 ± 8.0 |
| **21** | 12.8 ± 3.0 | 12.2 ± 3.5 | 13.6 ± 5.4 | 13.4 ± 3.9 | 72.7 ± 9.0 | 75.2 ± 3.5 |

As far as the position 6 of the imidazothiazole moiety is concerned, the introduction of Cl (**16**−**19**, **25**) or $CF_3$ (**27**) would seem unfavorable, but with the appropriate substitution on the indole system it can be favorable, as for example in the analogue of compound **19** lacking the methyl group at the 6 position of the indole portion: this compound[3] was active in the three cell lines and showed significant antitumor activity in the 60 cell lines.

In conclusion, the introduction of new substituents on the indolinone moiety while maintaining the same imidazothiazole substituents gave rise to active derivatives (**20**, **21**, **23**) even when the indole nitrogen was substituted (**24**). The most interesting derivatives (**20** and **21** bearing at the 5 position Cl and OH, respectively) were selected by BEC (Biological Evaluation Committee of the NCI) for possible further development. Compound **20** was the most active of the whole series with values very similar to those of vincristine and was selective toward some cell lines such as K-562 and SR (leukemias, $GI_{50} < -8$), HCC-2998 (colon cancer, $GI_{50} < -8$), and PC-3 (prostate cancer, $GI_{50} = -7.61$).

These and previous[2,3] results confirm that the most favorable substituent for positions 2 and 6 of the imidazothiazole moiety is a methyl group, and position 5 of the indole portion should be susbstituted. In the design of forthcoming derivatives, even additional N-substituted derivatives will be taken into account.

**(b) Effect on the Cell Cycle and Tubulin Polymerization.** To investigate the mechanism of action of these compounds, the effect on cell viability and cell cycle of compounds **20** and **21** was studied by monitoring them in the colon adenocarcinoma cell line HT29, chosen considering the interesting activity shown in the colon cancer cell lines.

The cytotoxic effects of compounds **20** and **21** were evaluated in the range of $10^{-8}$ to $10^{-4}$ M: they showed a limited effect on cell viability, because only the concentrations $10^{-5}$ and $10^{-4}$ M of compound **20** were able to induce a cell death respectively of 26.1 ± 7.3% and 31.1 ± 8.0% after 24 h of treatments, whereas only the concentration $10^{-4}$ M of compound **21** was effective, inducing cell death in 24.0 ± 7.0% of cell population (see Figure 1S in Supporting Information for details). Despite this low cytotoxicity in the HT29 cell line, both compounds were able to inhibit cell proliferation at concentration $10^{-7}$ M (see Figure 2S in Supporting Information for details), even if after 48 h the lower doses of compound **20** seem to lose the inhibitory effect.

Besides the antiproliferative effect, these compounds are able to induce a clear arrest in G2/M phase, as reported in Table 3. Morphological evaluation revealed that cells are blocked in M phase, as shown in Figure 1, where the microphotography of the control cells and of the cells treated for 24 h with compound **20** $10^{-6}$ M are reported: in fact, in treated cells (Figure 1b) chromatin appears condensed, with the typical feature of early mitotic cells, very similar to nuclei of cells treated with the M-blocking agent vincristine sulfate 5 × $10^{-9}$ M (Figure 1c).

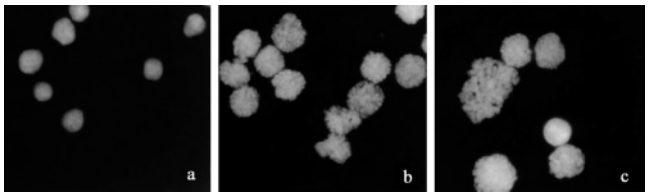



**Figure 1.** Morphological evaluation of nuclei stained with Hoechst 33258 after 24 h of treatment: (a) control cells; (b) compound **20** $10^{-6}$ M; (c) vincristine sulfate 5 × $10^{-9}$ M.

However, no significant effect on tubulin polymerization was observed (data not shown), suggesting that these compounds are able to block cells in M phase without interfering with microtubule dynamics. We can conclude that compounds **20** and **21** are new M-blocking agents, characterized by antiproliferative effects accompanied by low cytotoxicity.

**(c) Positive Inotropic Activity.** The positive inotropic activity was evaluated only for the most potent antitumor agents **20** and **21** according to the procedure described under the Experimental Section. Compound **21** was inactive whereas compound **20** at $10^{-6}$ M was as potent as amrinone ($P/P_0 = 106 \pm 5.0$) and maintained about the same activity at $10^{-7}$ M whereas amrinone was inactive.

## Experimental Section

**(A) Chemistry.** The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values. TLC was performed on Bakerflex plates (Silica gel IB2−F); the eluent was a mixture of petroleum ether/acetone in various proportions. The IR spectra were recorded in Nujol on a Nicolet Avatar 320 E.S.P.; $\nu_{max}$ is expressed in $cm^{-1}$. The $^1H$ NMR spectra were recorded on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in $\delta$(ppm) and $J$ in Hz (see Supporting Information). 2-Amino-5-methylthiazole (**1**), 1-bromo-3,3,3-trifluoroacetone, and 5-cloro-2-indolinone (**10**) are commercially available whereas the other substituted 2-indolinones (**11**−**15**)[8−12] and five of the starting aldehydes (**5**−**9**)[13−17] were prepared according to the literature.

The synthesis of 2-methyl-6-trifluoromethylimidazo[2,1-*b*]-thiazole (**3**) and its corresponding aldehyde (**4**) are reported below.

**Synthesis of 2-Methyl-6-trifluoromethylimidazo[2,1-*b*]thiazole 3.** 2-Amino-5-methylthiazole (50 mmol) was dissolved in acetone (100 mL) and treated with 1-bromo-3,3,3-trifluoroacetone (55 mmol). The reaction mixture was refluxed for 5 h, and the resulting precipitate was collected by filtration. This intermediate was treated, without further purification, with 200 mL of 2 N HBr and refluxed for 1 h. The solution thus obtained was basified with 15% $NH_4OH$, and the resulting free base was crystallized from ethanol with a yield of 40%. $C_7H_5F_3N_2S$ (206.2) mp 117−120 °C. IR: 3160, 3053, 1557, 1219, 953. $^1H$ NMR: 2.43 (3H, d, $CH_3$, $J = 1.2$), 7.74 (1H, q, im, $J = 1.2$), 8.30 (1H, q, th, $J = 1.2$).

**Synthesis of 2-Methyl-6-trifluoromethylimidazo[2,1-*b*]thiazole-5-carbaldehyde 4.** The Vilsmeier reagent was prepared at 0−5 °C by dropping $POCl_3$ (54 mmol) into a stirred solution of DMF (65 mmol) in $CHCl_3$ (5 mL). 2-Methyl-6-trifluoromethylimidazo[2,1-*b*]thiazole **3** (10 mmol) 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 25 h. Chloroform was removed under reduced pressure, and the resulting oil was poured into ice. The crude aldehyde thus obtained was collected by filtration and crystallized from ethanol with a yield of 60%. $C_8H_5F_3N_2OS$ (234.2) mp 115−122 °C. IR: 3119, 1655, 1537, 1030, 861. $^1$H NMR: 2.55 (3H, d, $CH_3$, $J = 1.2$), 8.31 (1H, q, th, $J = 1.2$), 9.93 (1H, s, CHO).

**General Procedure for the Synthesis of Compounds 16−28.** The appropriate aldehyde **4**−**9** (10 mmol) was dissolved in methanol (100 mL) and treated with the appropriate indolinone **10**−**15** (10 mmol) and piperidine (1 mL). The reaction mixture was refluxed for 3−5 h (according to a TLC test). The precipitate formed on cooling was collected by filtration and purified by crystallization from ethanol with a yield of 20−30% (**23**, **26**, **28**), 40−50% (**21**, **22**, **24**, **27**), 70−80% (**16**−**20**, **25**).

**(B) Pharmacology. (a) In vitro Growth Inhibition and Cytotoxicity.** It was determined by the NCI according to standard procedures.[18]

**(b) Cell Cycle Analysis.** The experiments were carried out on HT-29 cells according to the procedures previously described.[19] Compound **20** and **21** were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL, and a solution $10^{-4}$ M was prepared in complete RPMI 1640. Ten-fold serial dilutions were used to obtain the other concentrations, and DMSO was added to control cells. Commercial vincristine sulfate was diluted in the complete medium. Cell cycle distribution was performed on nuclei isolated and stained according to Nusse et al.[20] Flow cytometric plots were analyzed using the ModFit software (Verity). Nuclear morphology was evaluated according to Comin-Anduix et al.[4] All the data presented are expressed as the mean ± SD of three independent experiments.

**(c) Effect on Tubulin Polymerization.** The tubulin polymerization assay was performed using CytoDYNAMIX ScreenTM kit (Cytoskeleton Inc., CO) as per manufacturers' protocol. Briefly, a 96-well microplate was prewarmed at 37 °C for 1 h. HTS-Tubulin (>97% pure) was reconstituted to 3 mg/mL in G-PEM buffer (80 mM PIPES pH 6.8, 0.5 mM EGTA, 2.0 mM $MgCl_2$, 1.0 mM GTP, and 5% glycerol). Polymerization reaction was initiated by mixing 100 $\mu$L of reconstituted tubulin with vehicle or test compound (10 $\mu$M final concentration) into each well of the prewarmed plate. Polymerization kinetics were then measured at 37 °C for 1 h at 340 nm using a TECAN GENiospro microplate reader (TECAN U.S. Inc., Research Triangle Park, NC).

**(d) Positive Inotropic Activity.** The experiments were carried out on the guinea-pig papillary muscles according to a procedure previously described.[7]

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**Supporting Information Available:** Additional data on biological effects (Figure 1S: percentage of cell death. Figure 2S: time-dependent antiproliferative effect of compound **20** and **21**. Figure 3S: cytograms of BrdU incorporation versus PI fluorescence. Figure 4S**:** cell cycle phase distribution at different times). C, H, N analytical data. IR and $^1$H NMR spectroscopic data. This material is available free of charge via Internet at http://pubs.acs.org.

## References

(1) Potential Antitumor Agents 38. For part 37 see 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.

(2) Andreani, A.; Locatelli, A.; Leoni, A.; Rambaldi, M.; Morigi, R.; Bossa, R.; Chiericozzi, M.; Fraccari, A.; Galatulas, I. Synthesis and Potential *Coanthracyclinic* Activity of Substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones. *Eur. J. Med. Chem.* **1997**, *32*, 919−924.

(3) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Giorgi, G.; Salvini, L.; Garaliene, V. Synthesis and Antitumor Activity of Substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones. *Anticancer Drug Des.* **2001**, *16*, 167−174.

(4) Comin-Anduix, B.; Agell, N.; Bachs, O.; Ovadi, J.; Cascante, M. A New Bis-indole, KARs, Induces Selective M Arrest with Specific Spindle Aberration in Neuroblastoma Cell Line SH−SY5Y. *Mol. Pharmacol.* **2001**, *60*, 1235−1242.

(5) Mahboobi, S.; Pongratz, H.; Hufsky, H.; Hockmeyer, J.; Frieser, M.; Lyssenko, A.; Paper D. H.; Burgermeister, J.; Bohmer F. D.; Fiebig H. H.; Burger, A. M.; Baasner, S.; Beckers, T. Synthetic 2-Aroylindole Derivatives as a New Class of Potent Tubulin-inhibitory, Antimitotic Agents. *J. Med. Chem*. **2001**, *44*, 4535−4553.

(6) Fan, M.; Goodwin, M. E.; Birrer, M. J.; Chambers, T. C. The c-Jun NH(2)-terminal Protein Kinase/AP-1 Pathway is Required for Efficient Apoptosis Induced by Vinblastine. *Cancer Res.* **2001**, *61*, 4450−4458.

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

(8) Beer, R. J. S.; Davenport, H. F.; Robertson, A. Extensions of the Synthesis of Hydroxyindoles from p-Benzoquinones. *J. Chem. Soc.* **1953**, 1262−1264.

(9) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garaliene, V.: Synthesis and Antitumor Activity of 1,5,6-Substituted *E*-3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones. *J. Med. Chem.* **2002**, *45*, 2666−2669.

(10) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Greci, L.; Andreani, F. Potential Antitumor Agents. III. Hydrazone Derivatives of 5-substituted 2-chloro-3-formyl-6-methylindole. *Farmaco, Ed. Sci*. **1979**, *34*, 132−138.

(11) Porter, J. C.; Robinson, R.; Wyler, M. Monothiophthalimide and Some Derivatives of Oxindole. *J. Chem. Soc.* **1941**, 620−624.

(12) Koelsch C. F. A Synthesis of Ethyl Quininate from *m*-Cresol. *J. Am. Chem. Soc.* **1944**, *66*, 2019−2020.

(13) Andreani, A.; Rambaldi, M.; Carloni, P.; Greci, L.; Stipa, P. Imidazo[2,1-*b*]thiazole Carbamates and Acylureas as Potential Insect Control Agents. *J. Heterocycl. Chem.* **1989**, *26*, 525−529.

(14) Andreani, A.; Rambaldi, M.; Mascellani, G.; Rugarli, P. Synthesis and Diuretic Activity of Imidazo[2,1-*b*]thiazole Acetohydrazones. *Eur. J. Med. Chem.* **1987**, *22*, 19−22.

(15) Andreani, A.; Rambaldi, M.; Leoni, A.; Locatelli, A.; Bossa, R.; Fraccari, A.; Galatulas, I.; Salvatore, G.: Potential Antitumor Agents. 24. Synthesis and Pharmacological Behavior of Imidazo-[2,1-*b*]thiazole Guanylhydrazones Bearing at Least One Chlorine. *J. Med. Chem.* **1996**, *39*, 2852−2855.

(16) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Recanatini, M.; Lenaz, G.; Fato, R.; Bergamini, C. Effects of New Ubiquinone-imidazo[2,1-*b*]thiazoles on Mitochondrial Complex 1 (NADH-ubiquinone reductase) and on Mitochondrial Permeability Transition Pore. *Bioorg. Med. Chem.* **2004**, *12*, 5525−5532.

(17) Seki, T.; Tasaka, N.; Hoshino, R.; Kojima, J.; Fujii, K. Preparation of Imidazo[2,1-*b*]thiazoles for Improvement of Cerebral Function and as Antiulcer Agents. Jpn. Kokai Tokkyo Koho JP 02178289, 1990. *Chem. Abstr. 113*, 191361.

(18) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. 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.

(19) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garaliene, V.; Farruggia, G.; Masotti L. Substituted *E*-3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones with Antitumor Activity. *Bioorg. Med. Chem.* **2004**, *12*, 1121−1128.

(20) Nusse, M.; Beisker, W.; Hoffman, C.; Tarnok, A. Flow Cytometric Analysis of G1- and G2/M-phase Subpopulations in Mammalian Cell Nuclei using Side Scatter and DNA Content Measurements. *Cytometry* **1990**, *11*, 813−821.

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