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Brief Article

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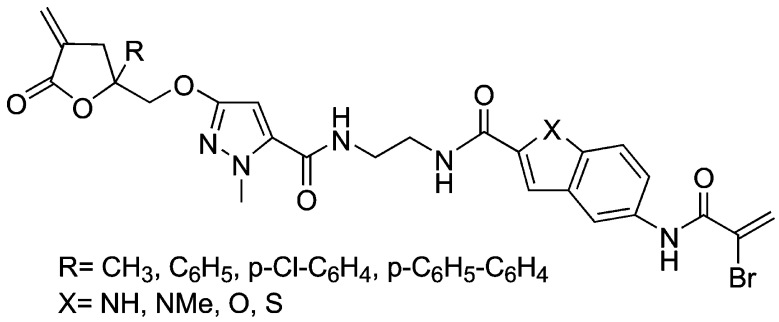

R= CH_3, C_6H_5, p-Cl-C_6H_4, p-C_6H_5-C_6H_4
X= NH, NMe, O, S

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Brief Articles

Design, Synthesis, and Biological Evaluation of Hybrid Molecules Containing α-Methylene-γ-Butyrolactones and α-Bromoacryloyl Moieties

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The synthesis and biological activity of hybrids **8**−**18** prepared combining α-methylene-γ-butyrolactones and α-bromoacryloylamides have been described and their structure−activity relationships discussed. All these heterobifunctional compounds demonstrate good antileukemic activity, significantly superior to that of both alkylating units alone. Using the human leukemia HL-60 cell line, selected compounds **10**, **11**, **13**, and **17** were found to induce morphological changes and internucleosomal DNA fragmentation characteristic of apoptotic cell death.

Introduction

Many natural and synthetic anticancer agents with the ability to interact with DNA have been discovered, but most of them display low sequence specificity and often exhibit severe toxicity to normal tissues.[1] However, cytotoxic agents will continue to represent an essential part of the therapy against tumor cells. This consideration implies a need for novel cytotoxics, exhibiting greater or broader activity and lower toxicity.

α-Methylene-γ-butyrolactone derivatives have attracted much attention over the years, since the α-methylene-γ-butyrolactone ring is an important functional structure in a wide range of natural products,[2,3] particularly cytotoxic sesquiterpene lactones such as 1-*O*-acetylbritannilactone **1**,[4] methylenolactocin **2**,[5] protolichesterinic acid **3**,[6] and helenalin **4**.[7] It was soon determined that the structural requirement for the biological activities is mainly associated with the exocyclic, conjugated double bond (the O=C=C=CH_2 moiety), which acts as an alkylating agent via Michael-type reaction with biological cellular nucleophiles or cysteine residues of functional proteins to form covalent bonds.[8] Because of the interesting biological activities and their unique structural features, α-methylene-γ-butyrolactones present an important scientific theme.[9]

The pyrroloiminoquinone cytotoxic alkaloids discorhabdin A (**5**)[10] and discorhabdin G (**6**)[11] are characterized by the presence of an α-bromoacryloyl alkylating moiety of low chemical reactivity and unusual for cytotoxic compounds; in fact, α-bromoacrylic acid is not per se cytotoxic (IC_{50} on L1210 cells being superior to120 μM). The reactivity of the α-bromoacryoyl moiety has been hypothesized to be based on a first-step Michael-type nucleophilic attack followed by a further reaction of the no longer vinylic bromo substituent α to the carbonyl, leading successively to a second nucleophilic substitution or to β elimination.[12] The same moiety is present in some potent anticancer distamycin-like minor groove binders, for example, PNU-166196 (brostallicin, **7**), an α-bromoacrylamido derivative of a four-pyrrole distamycin homologue ending with a guanidino moiety, which is presently undergoing phase II clinical trials.[13]

On the basis of these considerations, we report here the preparation and biological evaluation of novel conjugates **8**−**18** that have two moieties in their structures acting as Michael acceptors. One is an α-bromoacrylic derivative of benzoheterocyclic rings (**19**−**22**), such as indole, *N*-methylindole, benzofuran, and benzothiophene, tethered via a flexible ethylenediamino chain to a pyrazole moiety linked by a methylene unit to an α-methylidene-γ-methyl/aryl-γ-butyrolactone residue (derivatives **23**−**26**).

To better understand the relationship between the antiproliferative effects and the structural modifications of the γ position in the α-methylidene-γ-butyrolactone residue, we have synthesized analogues of **8**, in which the C(γ)-methyl of the lactone ring was replaced by a more lipophilic moiety, such as a phenyl (**9**−**12**) or a para-substituted phenyl (**13**−**18**). For the latter derivatives, the effects of the presence of electron-donating (Ph, **13**−**16**) and electron-withdrawing (Cl, **17** and **18**) substituents on the γ-phenyl moiety of the lactone skeleton were determined.

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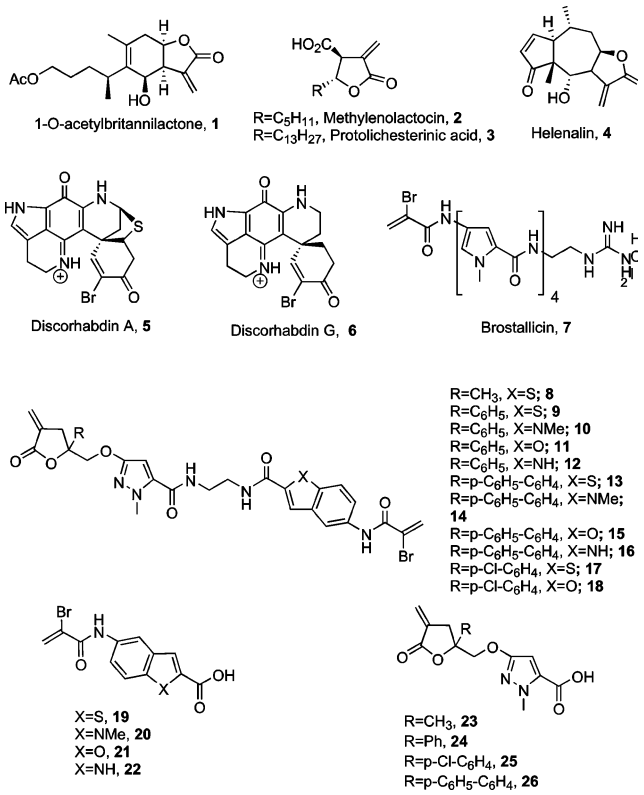


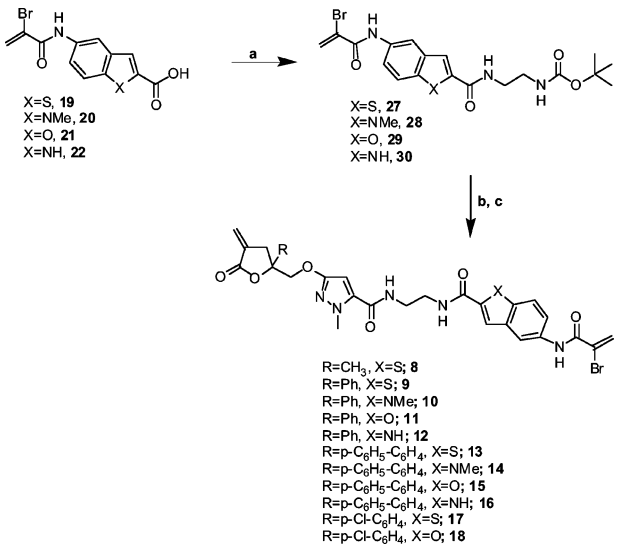
Chemistry

The synthetic strategy for the synthesis of hybrid compounds **8**−**18** is outlined in Scheme 1, in which the two parts of each conjugate, **19**−**22**[14] and **23**−**26**,[15] were prepared separately before coupling, according to previously described methods. The α-bromoacryloyl benzoheterocyclic carboxylic acid derivatives **19**−**22**, bearing the α-bromoacryloyl moiety as alkylating moiety, were transformed into the corresponding acyl chloride by treatment with refluxing thionyl chloride and then condensed with *N*-*tert*-butoxycarbonyl-1,2-ethanediamine[16] in the presence of TEA as base, giving rise to products **27**−**30** in good yields. The subsequent acid hydrolysis with dry HCl in ethyl acetate at room temperature allowed removal of the *N*-Boc protecting group and furnished the unstable chlorhydrate salts of **27**−**30**. The hybrid compounds **8**−**18** were achieved by condensation with the pyrazole 5-carboxylic acids **23**−**26** bearing the α-methylidene-γ-methyl/aryl-γ-butyrolactone. This condensation was performed using an excess (2 equiv) of 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (EDCl) as coupling agent in dry DMF as solvent, in the presence of Hünig's base, at room temperature and with identical reaction times (18 h).

Results and Discussion

In Table 1 we have reported the in vitro antiproliferative activity of hybrid compounds **8**−**18**, α-bromoacrylamido benzoheterocycles **19**−**22**, and α-methylene-γ-substituted-γ-butyrolactone pyrazole derivatives **23**−**26** against mouse L1210[17] and human K562[18] leukaemia cells. For these latter compounds, the nature of the substituent at the C(γ) position of the lactone moiety had no effect on the antiproliferative activity, being active at a concentrations superior to 10 μM on both cell lines.

Scheme 1[a]



[a] Reagents: (a) $SOCl_2$, 1 h, reflux, then $NH_2CH_2CH_2NHBoc$, TEA, DCM, room temp, 24 h; (b) HCl/EtOAc, room temp, 2 h; (c) **23**−**26**, EDCl, Hünig's base, DMF, room temp, 24 h.

Table 1. In Vitro Activity of Hybrids **8**−**18** and Alkylating Units **19**−**22** and **23**−**26** against the Proliferation of Murine L1210 and Human K562 Leukemia Cell Lines

	IC_{50} (μM)[a]			IC_{50} (μM)[a]	
compd	L1210	K562	compd	L1210	K562
8	0.84 ± 0.14	1.02 ± 0.09	**18**	0.55 ± 0.03	0.71 ± 0.09
9	0.29 ± 0.06	0.27 ± 0.04	**19**	4.50 ± 0.70	5.00 ± 0.14
10	0.05 ± 0.01	0.08 ± 0.00	**20**	2.07 ± 0.24	3.37 ± 0.17
11	0.31 ± 0.08	0.42 ± 0.17	**21**	2.95 ± 1.34	7.25 ± 0.35
12	0.85 ± 0.03	0.63 ± 0.08	**22**	4.30 ± 0.50	5.60 ± 1.32
13	0.29 ± 0.01	0.38 ± 0.01	**23**	>10	>10
14	0.28 ± 0.01	0.36 ± 0.06	**24**	>10	>10
15	0.27 ± 0.02	0.41 ± 0.01	**25**	>10	>10
16	0.36 ± 0.05	0.48 ± 0.03	**26**	>10	>10
17	0.26 ± 0.01	0.41 ± 0.01			

[a] IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose response curves of at least three independent experiments.

As evident from Table 1, the growth inhibitory activity of each hybrid compound **8**−**18**, obtained by tethering α-bromoacryloyl derivatives **19**−**22** and α-methylene-γ-butyrolactones **23**−**26**, proved to be much greater than that of the alkylating units tested alone. Derivatives **8**−**18** showed activity between 50 and 800 nM against murine L1210 leukaemia cell line, while for the human K562 leukaemia cell line the IC_{50} ranged between 80 and 1000 nM. These results indicated that all these conjugates were consistently more active against murine with respect to human leukemic cell line.

The greatest potency was shown by **10**, consisting of α-methylene-γ-phenyl-γ-butyrolactone **24** and the α-bromoacrylamido-*N*-methylindole derivative **20**, with IC_{50} of 55 and 82 nM against L1210 and K-562 cell lines, respectively. The least potent compound of this series was the conjugate **8**, which comprises the α-methylene-γ-methyl-γ-butyrolactone **23** joined to derivative **19**. This hybrid molecule was found to be more than 10-fold less active with respect to **10**.

A significant increase in antiproliferative activity was observed when the γ-methyl group of **8** was replaced with a phenyl group to give **9**, which exhibited the same activity against both cell lines but were 3- and 4-fold

more active than **8** against L1210 and K 562 cells, respectively. The good antiproliferative activity of the latter compound implies that a lipophilic and bulky substituent at the γ position of the lactone increases the cytostatic potency.

The cytostatic activity against L-1210 cells was unmodified when the γ-phenyl group of **9** was substituted at the para position by an electron-withdrawing group (chloro, Cl, **17**) or electron-donating substituent (phenyl, Ph, **13**), while it was slightly decreased against K-562 cells, in which **17** bearing the 4′-Cl-phenyl moiety exhibited a lower potency than its 4′-Ph counterpart **13**.

Replacement of benzothiophene with a benzofuran furnished derivative **11**, which exhibited cytostatic activity comparable with that of **9** against L1210 cells but was 1.5-fold lower against K-562 cells. The antiproliferative activity decreased when benzothiophene was replaced with an indole, furnishing **12**, which was found to be 3- and 2.5-fold less active than **11** against L1210 and K-562 cells, respectively. Replacement of benzothiophene with an *N*-methylindole gave **10**, which resulted in a hybrid derivative more active than the whole series. A 2-fold decrease in cytostatic activity was observed when the γ-phenyl group of **11** was replaced by a 4′-chlorophenyl group (**18**), while the potency increased when the 4′-chlorophenyl group was replaced with a 4′-biphenyl moiety (**15**).

When **13**−**16**, which possess the 4′-biphenyl at the γ position of the butyrolactone moiety, were compared with their phenyl counterparts **9**−**12**, the antiproliferative activity for the benzothiophene **9** and **13** and benzofuran **11** and **15** derivatives was unmodified when replacing the γ-phenyl with a γ-4′-biphenyl. For the *N*-methylindole derivative **10**, its 4′-biphenyl counterpart **14** was 5-fold less active with respect to both cell lines. Finally, the 4′-biphenylindole derivative **16** was 2- and 1.5-fold more active than the counterpart **12** against L-1210 and K-562 cells, respectively.

These data do not allow the identification of the molecular target(s) of these novel antiproliferative compounds. Preliminary experiments sustain the concept that these molecules retain low ability to alkylate DNA (data not shown; methodology and conditions used are reported in the Supporting Information). This is expected, since brostallicin (**7**) exhibits low DNA alkylating activity.[13] On the other hand, α-methylene-γ-butyrolactones are known to exert their main action through chemical reactions with cysteine residues of proteins.[8]

To better characterize the biological activity of these compounds, we have tested the effects of a selected series of derivatives on programmed cell death (apoptosis), using the human leukemia cell line HL-60 as the experimental system.[19] As shown in Figure 1A, **10**, **11**, **13** and **17** (1 μM, 12 h) induced morphological changes typical of apoptotic cells. Cells exposed to 1 μM compounds for 12 h displayed condensation of chromatin[20] and the appearance of apoptotic bodies by fluorescence microscopy after DNA staining with Hoechst 33258. From quantitative fluorescence microscopy, the less potent compound was **13**. Meanwhile, **10**, **11**, and **17** display similar values in the percentage of apoptotic cells (Figure 1B). We also examined whether these compounds induced DNA fragmentation, which is considered the end point of the apoptotic pathway.[21] DNA fragments formed by intranucleosomal hydrolysis of chromatin were evident after 12 h of treatment with 1 μM **10**, **11**, **13**, and **17** (Figure 1C).

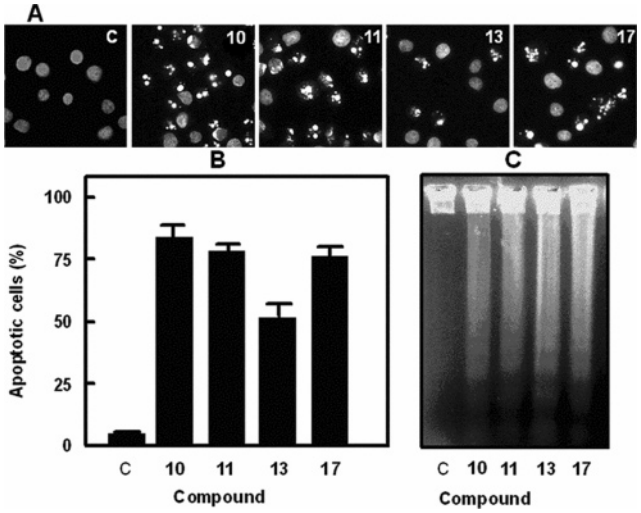


Figure 1. Apoptosis induction by **10**, **11**, **13**, and **17** on HL-60 cells. (A) Shown are photomicrographs of representative fields of HL-60 cells cultured in the absence (C, control) or presence of 1 μM of the indicated compounds for 12 h, which were then stained with Hoechst 33258. (B) Cells were treated as above, and apoptotic nuclei were quantified by fluorescence microscopy. Values represent the mean ± standard error of an experiment run in triplicate; similar results were obtained in a separate experiment. (C) Cells were treated with 1 μM of compounds for 12 h. Total cellular DNA was isolated and stained with ethidium bromide after electrophoresis on a 2% agarose gel. Internucleosomal DNA fragmentation was visualized under UV light.

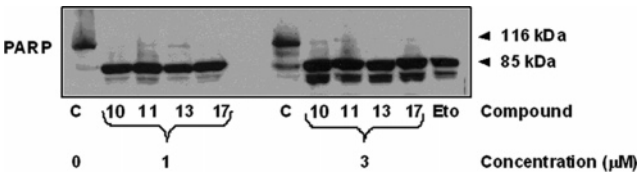


Figure 2. Cleavage of PARP. The cells were treated, and equal amounts of proteins from cell lysates were loaded in each lane and subjected to SDS−PAGE followed by blotting with an anti-PARP monoclonal antibody. Etoposide (Eto) was used as a positive control.

Caspase-3 is one of the key executioners of apoptosis, being responsible partially or totally for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly(ADP-ribose)polymerase (PARP). This protein plays an important role in chromatin architecture and DNA metabolism.[22] PARP, which is a 116 kDa DNA repair enzyme, is cleaved to produce an 85 kDa fragment during apoptosis. By an immunoblot assay, we have examined whether these compounds also induced PARP cleavage, indicating activation of caspase. As expected, the typical apoptotic 85 kDa fragment was visualized (Figure 2). Etoposide, a cancer chemotherapeutic drug that induces apoptosis in most cell lines, was used as positive control (Figure 2, last lane).

We also examined the effect of these compounds on cytochrome *c* translocation from the mitochondria into the cytosol. The representative Western blot analysis showed that the monoclonal antibody for cytochrome *c* produced a single band at the expected size of 15 kDa

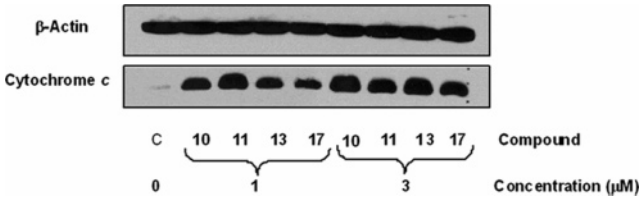


Figure 3. Western blot analysis of cytochrome *c* release. HL-60 cells were treated with 1 or 3 μM compounds and harvested at 12 h. Cytosolic lysates were analyzed by immunoblotting with an anti-cytochrome *c* antibody. β-Actin was used as the loading control.

Table 2. In Vitro Activity of Hybrids **10**, **11**, **13**, and **17** against the Proliferation of Human HL-60 Myeloid Leukaemia and PBM Cell Lines

	IC_{50} (μM)[a]	
compd	HL-60	PBMC
10	0.11 ± 0.02	2.52 ± 0.18
11	0.27 ± 0.03	5.44 ± 0.63
13	0.36 ± 0.03	8.61 ± 0.43
17	0.29 ± 0.02	6.82 ± 0.52

[a] IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose response curves of at least two independent experiments with three determinations in each.

(Figure 3). Cytochrome *c* release was observed in the presence of doses as low as 1 μM **10**, **11**, **13**, and **17**.

When considered together, Figures 1−3 consistently indicate that **10**, **11**, **13**, and **17** are able to induce apoptosis as demonstrated by fulfilling two criteria: (i) identification of nuclear changes associated with apoptosis using fluorescence microscopy; (ii) DNA laddering on agarose gel electrophoresis. All these derivatives induced extensive PARP hydrolysis, considered to be one of the major hallmarks of apoptosis.

Selectivity toward cancer cells is an important criterion, as it is for all the agents used or developed for cancer treatment. We therefore compared the effects of selected compounds for inducing antiproliferative activity on cancer cells (HL-60) with those on human peripheral blood mononuclear cells (PBMCs).

Compounds **10**, **11**, **13**, and **17** were found to inhibit the growth of human HL-60 myeloid leukemia cells in a dose-dependent manner as determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) dye-reduction assay. The inhibitory concentrations (IC_{50}) of these compounds were 0.11, 0.27, 0.36, and 0.29 μM, respectively.

To investigate the antiproliferative activities of **10**, **11**, **13**, and **17** on human PBMCs, the cells were isolated from anticoagulant-treated blood of healthy human donors. Growth of cells cultured in the presence or absence of these compounds for 24 h was measured by MTT assay. When tested in normal human lymphocytes, the IC_{50} values of these compounds were 20 times higher (Table 2).

Conclusions

The present work demonstrates not only the molecular design and the chemical synthesis of novel hybrid compounds but also their ability to induce cytostatic activities and apoptosis. By comparison of the inhibitory effects on in vitro cell growth of **9**−**12** and **13**−**16**, possessing the phenyl and the 4′-diphenyl substituents, respectively, at the γ position of the lactone, the heteroatom present in the benzoheterocyclic ring has a superior effect on derivatives **9**−**12** with respect to **13**−**16**. We have observed that **10**, **11**, **13**, and **17** suppress proliferation of HL-60 cells by triggering morphological changes and internucleosomal DNA fragmentation, which are well-known features of apoptotic cell death. Finally, our results indicate that the compounds investigated induce apoptosis via activation of caspase. The selectivity to inhibit the growth of HL-60 cells is of interest, compared with normal lymphocytes. Human HL-60 cells were more sensitive to these compounds than PBMCs.

Although further investigations are necessary to determine the detailed pathway of programmed cell death induced by these derivatives, these derivatives should be considered as interesting lead compounds for potential application to human health.

Experimental Section

General Procedure A for the Synthesis of 27−30. A suspension of the appropriate α-bromoacryloylamido benzoheterocycle carboxylic acids **19**−**22** (2 mmol) in thionyl chloride (5 mL) and DMF (2 drops) was boiled under reflux for 2 h. The remaining thionyl chloride was removed under reduced pressure, and the residue was dissolved in dry CH_2Cl_2 (5 mL) and added dropwise to an ice-cooled solution of *N-tert*-butoxycarbonyl-1,2-ethanediamine (2 equiv) and TEA (2 equiv) in dry CH_2Cl_2 (10 mL). The resulting mixture was stirred at ambient temperature for 24 h, then washed with water (5 mL) and brine (5 mL), dried with Na_2SO_4, and evaporated at reduced pressure. The residue purified by flash chromatography on silica gel (EtOAc/CH_2Cl_2 4:6 v/v as eluent) gave derivatives **27**−**30**.

General Procedure B for the Synthesis of 8−18. Compounds **27**−**30** (1 mmol) were dissolved in 3 N HCl in EtOAc, and the solution was stirred at room temperature for 2 h. The solvent was removed. The residue was dissolved in anhydrous DMF under argon atmosphere and then ice-cooled. Hünig's base (diisopropylethylamine, 1 equiv), the acids **23**−**26** (1.1 equiv), and EDCl (2 equiv) were added sequentially. The mixture was stirred overnight, was warmed to ambient temperature, and then evaporated to dryness in a vacuum. The resulting residue was purified by flash chromatography and recrystallized (CH_3OH/diethyl ether), affording **8**−**18**.

Growth Inhibitory Activity on Murine L1210 and Human K562 Cells. The murine lymphocytic L1210 leukemia and the human chronic myelogenous K562 cell lines were obtained from the American Type Culture Collection (ATCC). All tested compounds were dissolved in DMSO at 1 mg/mL immediately before the use and diluted in medium before addition to the cells. Both cell lines were cultured in RPMI 1640 medium (GIBCO) supplemented with 10% FCS (Flow, Irvine, U.K.), 2 mM L-glutamine (GIBCO), 10 mM β-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin. To determine the effects of the studied compounds on in vitro cell growth, exponentially growing L1210 and K562 cells were exposed to increasing concentrations of drugs and the cell number per milliliter was determined after 48 h using a model ZBI Coulter counter (Coulter Electronics, Hialeah, FL). Results were expressed as IC_{50} (dose causing 50% inhibition of cell growth in treated cultures relative to untreated controls). All experiments were repeated at least three times. For each drug concentration, duplicate cultures were used.

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Supporting Information Available: Experimental Section containing characterization of **8**−**18** and **27**−**30** and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hartley, J. A.; Lown, J. W.; Mattes, W. B.; Kohn, K. W. DNA sequence specificity of antitumor agents. Oncogenes as possible targets for cancer therapy. *Acta Oncol.* **1998**, *27*, 503−510.

(2) Lee, K. H.; Hall, I. H.; Mar, E. C.; Starnes, C. O.; El Gebaly, S. A.; Waddell, T. G.; Hadgraft, R. I.; Ruffner, C. G.; Weidner, I. Sesquiterpene antitumor agents: inhibitors of cellular metabolism. *Science* **1977**, *196*, 533−536.

(3) Spring, O.; Albert, K.; Gradmann, W. Annuithrin, a new biologically active germacranolide from Helianthus annus. *Phytochemistry* **1981**, *20*, 1883−1885.

(4) Liu, S.; Liu, H.; Yan, W.; Zhang, L.; Bai, N.; Ho, C.-T. Studies on 1-acetylbritannilactone and its derivative, (2-*O*-butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1101−1104.

(5) Park, B. K.; Nakagawa, M.; Hirota, A.; Nakayama, M. Methylenolactocin, a novel antitumor antibiotic from *Penicillium* sp. *J. Antibiot.* **1988**, *41*, 751−758.

(6) Cavallito, C. J.; Fruehauf, D. M.; Bailey, J. H. Lactone aliphatic acids as antibacterial agents. *J. Am. Chem.* Soc. **1948**, *70*, 3724−3726.

(7) Willuhn, G.; Merfort, I.; Pabreiter, C. M.; Schimdt, T. J. In *Advances in Compositae Systematics*; Hind, D. J. N., Jeffrey, C., Pope, G. V., Eds.; Royal Botanic Gardens: Kew, U.K., 1995; pp 167−195.

(8) Kupchan, S. M.; Fessler, C. D.; Eakin, M.; Giacobbe, T. J. Reactions of alpha methylene lactone tumor inhibitor with model biological nucleophiles. *Science* **1970**, *168*, 376−377.

(9) Kupchan, S. M. *Recent Advances in Phytochemistry*; Plenum Press: New York, 1975; pp 167−176.

(10) Perry, N. B.; Blunt, J. W.; Munro, H. M. G. Cytotoxic pigments from New Zealand sponges of the genus *Lantraculia*: Dischorhabdins A, B and C. *Tetrahedron* **1988**, *44*, 1727−1734.

(11) Antunes, E. M.; Beukes, D. R.; Kelly, M.; Samaai, T.; Barrows, L. R.; Marshall, K. M.; Sincich, C.; Davies-Coleman, M. T. Cytotoxic pyrroloimiquinones from four new species of South African Latrunculid sponges. *J. Nat. Prod.* **2004**, *67*, 1268−1276.

(12) Cozzi, P.; Beria, I.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Monelli, N. Cytotoxic halogenoacrylic derivatives of distamycin A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1269−1272.

(13) Geroni, C.; Marchini, S.; Cozzi, P.; Galliera, E.; Ragg, E.; Colombo, T.; Battaglia, R.; Howard, M.; D'Imcalci, M.; Broggini, M. Brostallicin, a novel anticancer agent whose activity is enhanced upon binding to glutathione. *Cancer Res.* **2002**, *62*, 2332−2336.

(14) Compound **19** was prepared by the procedure reported in the following: Baraldi, P. G.; Beria, I.; Cozzi, P.; Bianchi, N.; Gambari, R.; Romagnoli, R. Synthesis and growth inhibition activity of α-bromoacrylic heterocyclic and benzoheterocyclic derivatives of distamycin A modified on the amidino moiety. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 965−975. Compounds **20**−**22** were synthesized using the procedure reported in the following: Baraldi, P. G.; Romagnoli, R.; Beria, I.; Cozzi, P.; Geroni, G.; Mongelli, N.; Bianchi, N.; Mischiati, C.; Gambari, R. Synthesis and antitumor activity of new benzoheterocyclic derivatives of distamycin A. *J. Med. Chem.* **2000**, *43*, 2675−2684.

(15) Compound **23**−**26** were synthesized using the procedure reported in the following: Baraldi, P. G.; Nunez, M. C.; Tabrizi, M. A.; De Clercq, E.; Balzarini, J.; Bermejo, J.; Estévez, F.; Romagnoli, R. Design, synthesis and biological evaluation of hybrid molecules containing α-methylene-γ-butyrolactones and polypyrrole minor groove binders. *J. Med. Chem.* **2004**, *47*, 2877−2886.

(16) Krapcho, A. P.; Kuell, C. S. Mono-protected diammines, *N-tert*-butoxycarbonyl-α,ω-alkanediammines from α,ω-alkanediammines. *Synth. Commun.* **1990**, *20*, 2559−2264.

(17) Gambari, R.; Feriotto, G.; Rutigliano, C.; Bianchi, N.; Mischiati, C. Biospecific interaction analysis (BIA) of low-molecular weight DNA-binding drugs. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 370−377.

(18) Bianchi, N.; Ongaro, F.; Chiarabelli, C.; Gualandi, L.; Mischiati, C.; Bergamini, P.; Gambari, R. Induction of erythroid differentiation of human K562 cells by cisplatin analogs. *Biochem. Pharmacol.* **2000**, *60*, 31−40.

(19) Gallagher, R.; Collins, S.; Trujillo, J.; McCredie, K.; Ahearn, M.; Tsai, S.; Metzgar, R.; Aulakh, G.; Ting, R.; Ruscetti, F.; Gallo, R. Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia. *Blood* **1979**, *54*, 713−733.

(20) Schwartz, G. K.; Farsi, K.; Maslak, P.; Kelsen, D. P.; Spriggs, D. Potentiation of apoptosis by flavopiridol in mitomycin-C-treated gastric and breast cancer cells. *Clin. Cancer Res.* **1997**, *3*, 1467−1472.

(21) Benyahia, S.; Benayache, S.; Benayache, F.; Quintana, J.; López, M.; León, F.; Hernández, J. C.; Estévez, F.; Bermejo, J. Isolation from *Eucalyptus occidentalis* and identification of a new kaempferol derivative that induces apoptosis in human myeloid leukemia cells. *J. Nat. Prod.* **2004**, *67*, 527−531.

(22) Tewari, M.; Quan, L. T.; O'Rourke, K.; Desnoyers, S.; Zeng, Z.; Beidler, D. R.; Poirier, G. G.; Salvesen, G. S.; Dixit, V.M. YAMA/CPP32-beta, a mammalian homolog of CED-3, is a CRMA-inhibitable protease that cleaves the death substrate poly (ADP-ribose) polymerase. *Cell* **1995**, *81*, 801−809.

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