# Design, Synthesis, and Biological Evaluation of Hybrid Molecules Containing α-Methylene-γ-butyrolactones and Polypyrrole Minor Groove Binders

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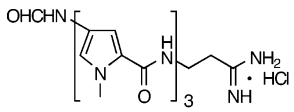
We have synthesized and evaluated a series of hybrids of polypyrrole minor groove binders structurally related to the natural antitumor agent distamycin A, and α-methylene-γ-butyrolactones with methyl, phenyl, and 4-substituted phenyl groups at the lactone C(γ) position, denoted **5**−**17**, for in vitro cytotoxic activity against a variety of cancer cell lines. The apoptotic and cytotoxic activities against several tumor cell lines are reported and discussed in terms of their structural differences in relation to both the number of *N*-methylpyrrole rings and the type of the alkylating unit tethered to the oligopeptidic frame. It may be noted that in general, and especially for **11**, **12**, and **17**, the cytotoxicity of the hybrids was much greater than that of the α-methylene-γ-butyrolactone units **24a**−**g** alone. Using the human leukemia cell line HL-60, we have tested the effects of a selected series of compounds on programmed cell death (apoptosis). The results clearly indicate that **11**, **12**, and **17**, but not **9**, are able to induce apoptosis as demonstrated from (i) identification of nuclear changes associated with apoptosis using fluorescence microscopy and (ii) by DNA laddering on agarose gel electrophoresis. Compound **12** was the most potent, especially after a short incubation period. It induced extensive hydrolysis of poly ADP-ribose polymerase (PARP), considered to be a hallmark of apoptosis, which plays a critical role in chromatin architecture and DNA metabolism.

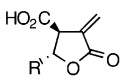
## Introduction

Many natural and synthetic anticancer agents with the ability to interact with DNA have been discovered, but most have little sequence specificity and often exhibit severe toxicity to normal tissues.[1] For these reasons, DNA minor groove binders have been an attractive source of novel antitumor agents.[2] The increasing interest in this group of compounds stems from their ability to interact in a sequence-selective fashion at quite long DNA binding sites, suggesting the possibility of targeting specific DNA sequences within the genome. Distamycin A **1** is a naturally occurring antibiotic characterized by the presence of an oligopeptidic pyrrolecarbamoyl frame ending with an amidino moiety,[3,4] which binds to the DNA minor groove, preferentially to the AT-rich sequence, and in a reversible manner.[5−7] Distamycin A **1** can be used as a DNA sequence selective vector for both alkylating and nonalkylating agents leading to a substantial increase in cytotoxicity against cancer cell lines in comparison to that of distamycin alone.[8−10]

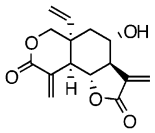
α-Methylene-γ-butyrolactone derivatives have attracted much attention over the years because the α-methylene-γ-butyrolactone ring is an important functional structure in a wide range of natural products,[11,12] particularly cytotoxic sesquiterpene lactones such as methylenolactocin **2**,[13] protolichesterinic acid **3**,[14] and vernolepin **4**.[15] 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 in a Michael-type reaction with biological cellular nucleophiles or sulfydryl-containing enzymes.[16] Because of the interesting biological activities and their unique structural features, α-methylene-γ-butyrolactones present an important scientific theme.[17]




Distamycin A, **1**




R=$C_5H_{11}$, Methylenolactocin, **2**
R=$C_{13}H_{27}$, Protolichesterinic acid, **3**




Vernolepin, **4**

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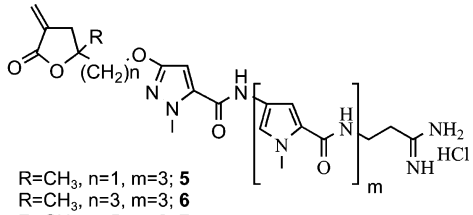
Here, we report on the preparation and biological evaluation of a novel series of conjugates, **5**−**17**, that have two moieties in their structure. One is a distamycin-like moiety, to acquire DNA minor groove binding activity, which is joined by a pyrazole, which acts as a rigid linker, to the α-methylidene-γ-butyrolactone residues **24a**−**g** to acquire alkylation with biological nucleophiles. In fact, via the hydroxy and carbonyl functions present in the 3- and 5-position, respectively, on the pyrazolic moiety, it was possible to join the α-methylidene-γ-butyrolactone residues **24a**−**g** and the aminopolypyrrolic amides **25**−**28**.

We have synthesized three different series of compounds. In the first series of hybrids **5**, **9**, and **10**, an α-methylidene-γ-methyl-γ-butyrolactone residue joined by a methylene residue to a pyrazole moiety (derivative **24a)** was linked to an oligopyrrole consisting of a varying number of pyrrole amide units (from one to three). For the derivative characterized by the presence of deformyl distamycin A as the oligopyrrole carrier (compound **5**), the effect of the replacement of the C-terminal amidino moiety by a more basic and ionizable guanidino group (derivative **16**) was also evaluated. In the second series of derivatives **6**−**8**, we have evaluated the effects in terms of cytotoxic activity by varying the length of the methylene chain, from three to seven methylene units, between the α-methylidene-γ-methyl-γ-butyrolactone residue and the pyrazole moiety (compounds **24b**−**d**, respectively).

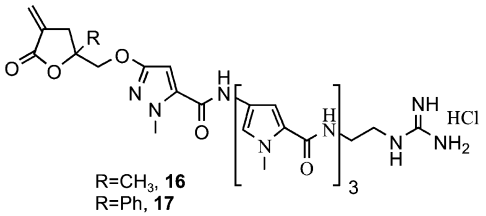
In an attempt to better understand the effect of structural modifications on optimal cytotoxic activity, we have finally synthesized analogues of **24a**, in which the C(γ)-methyl of the lactone ring was replaced by a more lipophilic moiety, such as a phenyl (compound **24e**) or a para-substituted phenyl (compounds **24f** and **24g**). For the latter derivatives, the effects of the presence of both electron-withdrawing (Cl, compound **24f**) and electron-donating (Ph, compound **24g**) substituents on the γ-phenyl moiety of the lactone skeleton were determined. The derivative **24e** has been used for the synthesis of the third novel series of hybrids **11**, **12**, **13** containing, respectively, three, two, and one pyrrole amide units, while compounds **24f** and **24g** were linked to the N-terminal pyrrole residue of distamycin A to afford distamycin conjugates **14** and **15**, respectively. By the synthesis of **17**, we have evaluated the biological effects due to the substitution in compound **11** of the C-terminal amidine moiety with a guanidine function.

## Chemistry

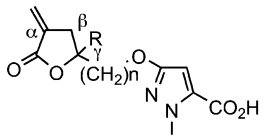
The synthetic route followed for the synthesis of hybrid compounds **5**−**17** is outlined in Scheme 1. The key step was the assembly between pyrazole carboxylic acids **24a**−**g** bearing the α-methylidene-γ-methyl/aryl-γ-butyrolactone as the alkylating moiety and the aminopolypyrroleamide moieties **25**−**28**.[18] The condensation of the acylating agents **24a**−**g** with amines **25**−**28** was performed using an excess (2 equiv) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCl) as the coupling agent, in dry DMF as solvent, in the presence of Hunig's base at room temperature and with identical reaction times (18 h). Compounds **5**−**17** were prepared in acceptable yields, after purification by silica gel flash-chromatography.



R=$CH_3$, n=1, m=3; **5**
R=$CH_3$, n=3, m=3; **6**
R=$CH_3$, n=5, m=3; **7**
R=$CH_3$, n=7, m=3; **8**
R=$CH_3$, n=1, m=2; **9**
R=$CH_3$, n=1, m=1; **10**
R=Ph, n=1, m=3; **11**
R=Ph, n=1, m=2; **12**
R=Ph, n=1, m=1; **13**
R=p-Cl-$C_6H_4$, n=1, m=3; **14**
R=p-$C_6H_5$-$C_6H_4$, n=1, m=3; **15**



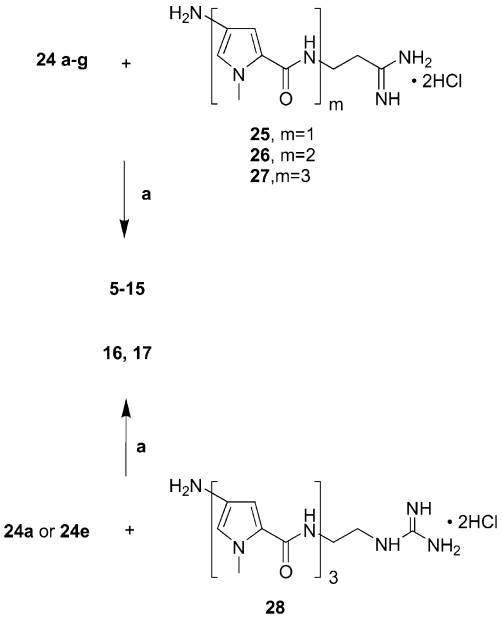
R=$CH_3$, **16**
R=Ph, **17**



R=$CH_3$, n=1; **24a**
R=$CH_3$, n=3; **24b**
R=$CH_3$, n=5; **24c**
R=$CH_3$, n=7; **24d**
R=Ph, n=1; **24e**
R=p-Cl-$C_6H_4$, n=1; **24f**
R=p-$C_6H_5$-$C_6H_4$, n=1; **24g**

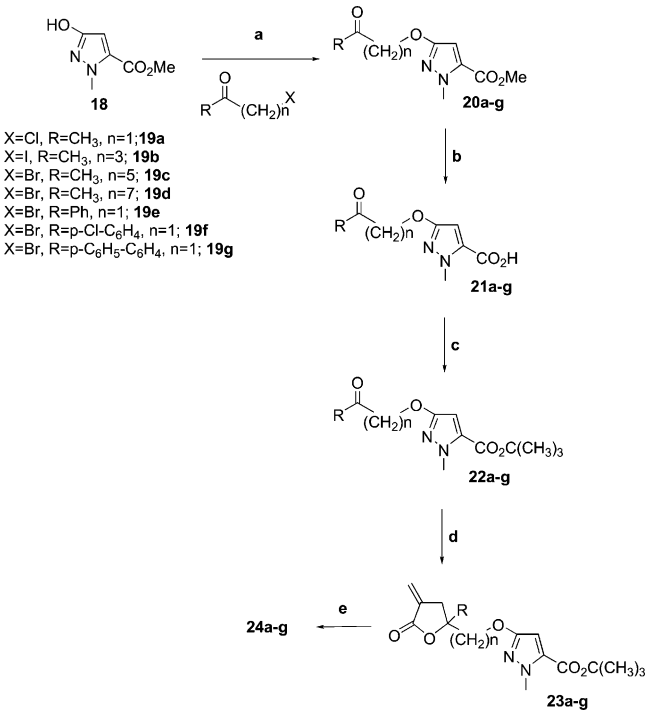
The synthesis of these hybrid compounds **5**−**17** required the preparation of pyrazole α-methylidene-γ-butyrolactone bearing a carboxylic acid moiety to pro-

**Scheme 1**[a]



[a] Reagents: (a) EDCl, Hunig's base, DMF.

**Scheme 2**[a]




[a] Reagents: (a) **19a**−**g**, NaH, DMF; (b) LiOH THF/MeOH, $H_2O$; (c) $(CH_3)_3CBr$, $K_2CO_3$, BTEAC, DMF; (d) $CH_2{=}C(CH_2Br)CO_2Et$, Zn, THF; (e) TFA, $CH_2Cl_2$.

vide a point of attachment for the appropriate aminopolypyrroleamide moieties. O-alkylation of the methyl ester of 5-hydroxy-2-methyl-2*H*-pyrazole-3-carboxylic acid **18**[19] with the appropriate ketone **19a**−**g**[20] using sodium hydride (NaH, 50% in mineral oil) in dry DMF afforded the corresponding derivatives **20a**−**g**, which were then transformed into the corresponding *tert*-butyl esters **22a**−**g** after mild alkaline hydrolysis of the methyl ester derivatives **20a**−**g** with LiOH, to give the corresponding carboxylic acids **21a**−**g**, and subsequent reaction with *tert*-butyl bromide in $K_2CO_3$.[21] The intermediates **22a**−**g** were then cyclized by the reaction with ethyl 2-(bromomethyl)acrylate[22] and zinc powder in dry THF (Reformatsky-type condensation) to form the γ-lactones **23a**−**g**. Subsequent treatment with trifluoroacetic acid (TFA) in dichloromethane, at room temperature, afforded the carboxylic acids **24a**−**g** (Scheme 2).

## Results and Discussion

**Antitumor Activity.** In Table 1 we have reported the in vitro cytostatic activity of hybrid compounds **5**−**17** and α-methylene γ-substituted γ-butyrolactone pyrazole derivatives **24a** and **24e**−**g** against a panel of tumor cell lines, using distamycin A as reference compound. Several compounds demonstrated profound growth-inhibitory effects on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), and human T-lymphoblastoid (Molt/4 and CEM) cells.

In the series of lactones **24a** and **24e**−**g**, compound **24e**, possessing a phenyl substituent at the γ-position of the lactone, was more active against all cell lines than its methyl counterpart **24a**. However, a significant increase in cytostatic activity was observed when the γ-phenyl group of **24e** was replaced by a 4′-chloro or a 4′-phenyl-substituted phenyl group (compounds **24f** and **24g**, respectively), where compound **24f** bearing the 4′-Cl-substituted phenyl moiety exhibited in most cases a lower potency than its 4′-Ph counterpart **24g**. The good antiproliferative activity of the latter compound implies that a lipophilic and bulky substituent at the benzene increases the cytostatic potency. Otherwise, tethering **24f** and **24g** to deformyl distamycin A **27** to give the corresponding hydrids **14** and **15**, respectively, proved to be less cytotoxic than the alkylating unit alone with respect to all cell lines, with the exception of the murine mammary carcinoma (FM3A) cells.

**Table 1.** In Vitro Activity of Hybrids **5**−**17** and Alkylating Units **24a**,**e**−**g** against the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), and Human T-Lymphoblast (Molt/4 and CEM) Cells[a]

| | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| compd | L1210/0 | FM3A/0 | Molt4/C8 | CEM/0 |
| **5** | 14.7 ± 4.9 | 49.0 ± 3.7 | 14.7 ± 2.5 | 15.9 ± 1.2 |
| **6** | 195 ± 12 | 243 ± 21 | 254 ± 11 | 255 ± 22 |
| **7** | 109 ± 10 | 92 ± 16 | 106 ± 13 | 74.3 ± 3.8 |
| **8** | 26.7 ± 1.1 | 63.3 ± 6.7 | 71.1 ± 2.2 | 35.6 ± 5.6 |
| **9** | 8.02 ± 2.9 | 27.6 ± 11 | 6.8 ± 0.3 | 9.6 ± 4.7 |
| **10** | 24.3 ± 4.0 | 32.4 ± 6.1 | 22.3 ± 10.1 | 26.3 ± 2.0 |
| **11** | 4.2 ± 0.5 | 17.5 ± 1.2 | 5.2 ± 0.1 | 9.7 ± 4.2 |
| **12** | 4.1 ± 0.1 | 18.5 ± 1.3 | 4.6 ± 0.4 | 5.7 ± 1.1 |
| **13** | 12.5 ± 5.8 | 36.3 ± 20 | 15.8 ± 13 | 18.9 ± 3.2 |
| **14** | 25.2 ± 2.4 | 64.7 ± 29 | 79.1 ± 31 | 86.3 ± 3.6 |
| **15** | 33.2 ± 1.1 | 21.8 ± 9.2 | 65.3 ± 8.0 | 95.0 ± 0 |
| **16** | 9.6 ± 4.5 | 42.5 ± 23 | 12.3 ± 7.7 | 15.9 ± 2.7 |
| **17** | 5.1 ± 1.4 | 20.5 ± 4.8 | 10.1 ± 7.1 | 10.0 ± 1.1 |
| **24a** | 522 ± 26 | 755 ± 12 | 746 ± 21 | 402 ± 143 |
| **24e** | 107 ± 24 | 189 ± 46 | 189 ± 58 | 174 ± 36 |
| **24f** | 13.0 ± 0 | 99.2 ± 44.1 | 46.9 ± 5.5 | 57.9 ± 8.3 |
| **24g** | 14.8 ± 0.5 | 42.0 ± 2.5 | 9.4 ± 1.5 | 18.5 ± 4.7 |
| distamycin A (**1**) | 133 ± 18 | 150 ± 29 | 64.6 ± 15 | 113 ± 26 |

[a] $IC_{50}$ is the 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.

It can be seen in Table 1 that tethering the lactones **24a**−**e** to the DNA binders **25**−**28** (to afford the hybrids **5**−**13**, **16**, and **17**) resulted, with only a few exceptions, in improved cytotoxic potency against most of the tumor cell lines compared to the alkylating units alone. Compounds **11**, **12**, and **17**, which possess the phenyl at the γ-position of the α-methylene-γ-butyrolactone moiety (**24e**) as the alkylating moiety, were found to have both the best and broadest antiproliferative activity. As previously reported, the lower inhibitory potency of **14** and **15** with respect to **11** implies that both an electron-donating substituent (Ph, compound **15**) and an electron-withdrawing substituent (Cl, compound **14**) at the benzene moiety of the lactone reduced their antiproliferative activity.

In compounds **5**−**8**, we set out to investigate the influence of the length (*n*) of the polymethylene chain between the lactone ring and the pyrazole moiety on the cytotostatic activity. With an increase in the number of methylene units from one to three (compounds **5** and **6**, respectively), the cytostatic activity dramatically decreased. When the methylene linker length was increased from three (compound **6**) to five (compound **7**) methylene units, the in vitro antiproliferative activity increased at least 2-fold but proved to be lower than that with $n = 1$ (derivative **5**). Compound **8** with the longest linker length in the series ($n = 7$) was more active with respect to the derivatives with a propyl and pentyl chain

(compounds **6** and **7**, respectively) and comparable to the compound (**5**) with the shortest spacer ($n = 1$).

A correlation between cytostatic activity and the length of the polypyrrolic frame was observed for the compounds bearing **24a** and **24e** as alkylating moieties. In the series of derivatives **5**, **9**, and **10**, which possess an α-methylene-γ-methyl-γ-butyrolactone moiety, the derivative **9** with two *N*-methylpyrrolic units was almost 2-fold more cytostatic than the corresponding pyrrole homologue **5**. The derivative **5** showed an activity comparable to that of compound **10** characterized by the presence of a single pyrrole ring in its structure. The same behavior was not observed for the hybrids **11**−**13**, characterized by the presence of an α-methylene-γ-phenyl-γ-butyrolactone moiety as the alkylating unit. Compounds **11** and **12**, which possess three and two pyrrolic rings, respectively, showed a similarly pronounced antiproliferative activity and proved to be 3-fold higher than that observed for the monopyrrolic compound **13**. Of compounds **11**−**13**, the hybrids **11** and **12** exhibited the highest potency across the panel of tumor cell lines, especially against L1210, Molt/4, and CEM cells, with $IC_{50}$ values ranging between 4 and 10 μM.

Taken together, for all tumor cell lines, compounds possessing the same number of pyrrole rings and the alkylating unit **24e** appeared to be more cytostatic than those containing **24a** as the alkylating agent.

The nature of the substituent at the C(γ)-position of the lactone moiety had a great effect on the antiproliferative activity of compounds having the same oligopeptide frame. From a comparison of the cytostatic activity of the compounds with a different alkylating unit (**24a** and **24e**) and with the same number of pyrrolic rings, the derivatives **5**, **9**, and **10** characterized by the presence of three, two, and one pyrrole units, respectively, and with the aliphatic methyl substituent at the γ-position of the lactone were less active than their γ-phenyl counterparts **11**, **12**, **13**, respectively. Otherwise, the data show that the two series of derivatives **5**, **9**, **10** and **11**−**13** retain a higher antiproliferative activity when compared to the lactones **24a** and **24e** alone. Modification of the C-terminal amidine group in compounds **5** and **11**, with a guanidino moiety to furnish the corresponding derivatives **16** and **17**, substantially maintained the cytostatic activity.

**Induction of Apoptosis by Compounds 9, 11, 12, and 17.** To determine whether a selected series of the most active compounds **9**, **11**, **12**, and **17** induced apoptosis, we incubated HL-60 cells with 10 or 30 μM of these agents for 24 h. Compounds **11**, **12**, and **17** were able to induce apoptosis, judging by the appearance of typical morphological changes that included chromatin condensation, its compaction along the periphery of the nucleus, and nuclear segmentation into three or more chromatin fragments visualized by fluorescence microscopy. As shown in Figure 1A, the percentage of apoptotic cells increased in a dose-dependent manner, with the exception of derivative **9**. During this period, **11** and **12** caused an increase in the percentage of apoptotic cells to the same extent, approximately 16% with 10 μM and about 26% with 30 μM. These values are significantly higher than those obtained with control cells (7.4 ± 0.1% of apoptotic cells). Compound **17** also induced apoptosis at both concentrations tested, although it was slightly less effective. Figure 1B shows the morphological changes associated with the cells in response to 10 μM of the compounds and their comparison with those from cells incubated with the vehicle alone.

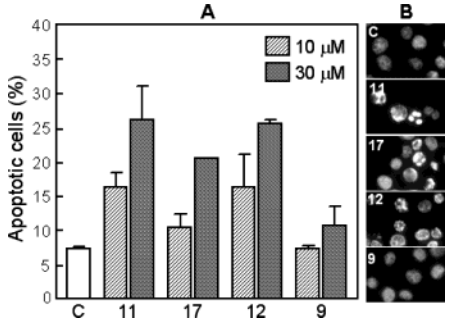



**Figure 1.** Effects of compounds **9**, **11**, **12**, and **17** on induction of apoptosis in HL-60 cells. Cells were incubated with no drug (C, control) or with 10−30 μM of the indicated compounds for 24 h. After treatment they were stained with bisbenzimide trihydrochloride, and apoptotic cells were quantitated by fluorescence microscopy (A). The results of a representative experiment are shown, and each point represents the average ± SE of triplicate determinations. Also shown are photomicrographs of representative fields of cells treated with 10 μM of the indicated products and stained as above (B).

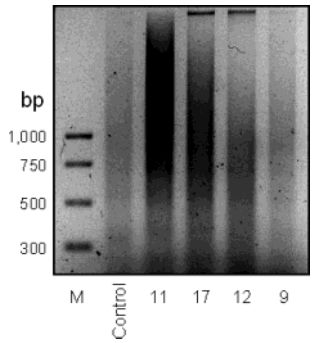



**Figure 2.** Analysis of DNA fragmentation induced by compounds **9**, **11**, **12**, and **17** in HL-60 cells. Cells were incubated in the absence (control) or presence of 30 μM of the indicated compounds for 8 h. Low molecular weight DNA was isolated, electrophoresed on 2% agarose gel containing ethidium bromide, and visualized under UV light: M, size marker; bp, base pairs.

We also examined whether fragmentation of DNA, which is considered as the end point of the apoptotic pathway, is activated in response to compounds **9**, **11**, **12**, and **17**. As demonstrated in Figure 2, DNA fragments formed by intranucleosomal hydrolysis of chromatin was observed after 8 h of treatment with 30 μM of each compound. In agreement with the results obtained in Figure 1, only **9** did not induce DNA laddering, as revealed by agarose gel electrophoresis.

We further performed an immunoblot assay to examine whether the compounds induced poly ADP-ribose polymerase (PARP) cleavage. This protein plays an important role in chromatin architecture and DNA metabolism.[23] It is also considered to be a hallmark of apoptosis and a recognized caspase substrate whose degradation from 116 kDa to an 85 kDa fragment is indicative of activation of the apoptotic process. In dose−response experiments, PARP cleavage was detected in **12**-treated cells after 4 h of exposure at a concentration as low as 10 μM and increased in a dose-dependent manner (Figure 3). On the other hand, PARP cleavage

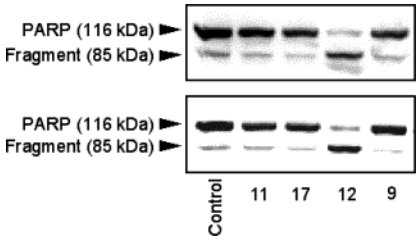



**Figure 3.** Western blot analysis of poly ADP-ribose polymerase (PARP) cleavage. Cells were incubated in the presence of 10 $\mu$M (top) or 30 $\mu$M (bottom) of the indicated compounds **9**, **11**, **12**, and **17** for 4 h, and cell lysates were subjected to SDS−PAGE. The PARP level was determined by immunoblotting with a monoclonal antibody that also recognizes the 85 kDa fragment.

was minimal in cells exposed to **11** and **17**, although the percentage of apoptotic cells was comparable (see Figure 1A). These results indicate that PARP cleavage was involved in the apoptosic process induced by **12** but was minimally, if at all, involved in apoptosis induced by **11** and **17** within the time frame of the study.

## Conclusions

The present work demonstrated not only the molecular design and the chemical synthesis of novel α-methylene-$\gamma$-butyrolactones−lexitropsin hybrids but also their apoptotic profiles and cytostatic activities. Many of the compounds prepared in this study were shown to demonstrate a significant antiproliferative activity toward four different tumor cell lines. We found that compounds **11**, **12**, and **17** activate apoptosis on HL-60 cells. Treatment of the tumor cells with these compounds induced morphological changes and DNA fragmentation characteristic of apoptotic cell death. We have also demonstrated that only **12** induces PARP hydrolysis, which is considered a marker of apoptosis. PARP is an important nuclear chromatin-associated enzyme that plays an important role in mediating the normal cellular response to DNA damage, and its cleavage implicates activation of caspase(s).

## Experimental Section

**Chemistry. Materials and Methods.** $^1$H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts ($\delta$) are given in ppm upfield from tetramethylsilane as the internal standard, and the spectra were recorded in appropriate deuterated solvents indicated in the procedure. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed $^1$H NMR spectra in agreement with the assigned structures. Elemental analyses were conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated $F_{254}$ Merck plates) and visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230−400 mesh silica gel and the solvent system indicated in the procedure. All commercially available compounds were used without further purification. Organic solutions were dried over anhydrous $MgSO_4$. Dioxane was distilled from calcium hydride, and dry DMF was distilled from calcium chloride and stored over molecular sieves (3 Å).

**General Procedure A for the Synthesis of 20a−g.** Compound **18** (156 mg, 1 mmol), NaH (50% in mineral oil, 1 mmol, 48 mg), and dry DMF (5 mL) were stirred at 0 °C for 30 min and 1 h at room temperature. To this solution was added the appropriate $\omega$-halo ketone (1−4 mmol) dissolved in dry DMF (2 mL), and the resulting mixture continued to stir at room temperature for 18 h and then concentrated in vacuo. The resulting residue was dissolved in EtOAc (10 mL) and washed with water (2 × 5 mL). The recombined organic phases were dried on $Na_2SO_4$ and concentrated under vacuum, and the crude product was purified by flash chromatography (EtOAc/petroleum ether).

**Methyl Ester of 2-Methyl-5-(2-oxopropoxy)-2*H*-pyrazole-3-carboxylic Acid (20a).** Following the general procedure A, starting from **18** (5.8 g, 37 mmol) and 1-chloropropan-2-one **19a** (13.6 g, 11.7 mL, 147 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20a** was obtained as a white solid (4.1 g, 52% yield), mp 56−57 °C. $^1$H NMR ($CDCl_3$) $\delta$: 2.22 (s, 3H), 3.85 (s, 3H), 3.99 (s, 3H), 4.70 (s, 2H), 6.23 (s, 1H).

**Methyl Ester of 2-Methyl-5-(4-oxopentyloxy)-2*H*-pyrazole-3-carboxylic Acid (20b).** Following the general procedure A, starting from **18** (781 mg, 5 mmol) and 5-iodopentan-2-one **19b** (4.24 g, 20 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 1:9), compound **20b** was obtained as a white solid (650 mg, 54% yield), mp 49−51 °C. $^1$H NMR ($CDCl_3$) $\delta$: 2.06 (m, 2H), 2.17 (s, 3H), 2.64 (t, $J$ = 7.2 Hz, 2H), 3.66 (s, 3H), 4.03 (s, 3H), 4.11 (t, $J$ = 6.1 Hz, 2H), 6.17 (s, 1H).

**Methyl Ester of 2-Methyl-5-(6-oxoheptyloxy)-2*H*-pyrazole-3-carboxylic Acid (20c).** Following the general procedure A, starting from **18** (520 mg, 3.3 mmol) and 7-bromoheptan-2-one **19c** (1.95 g, 10 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20c** was obtained as a white solid (894 mg, 95% yield), mp 54−56 °C. $^1$H NMR ($CDCl_3$) $\delta$: 1.65 (m, 6H), 2.14 (s, 3H), 2.45 (t, $J$ = 7.4 Hz, 2H), 3.86 (s, 3H), 4.03 (s, 3H), 4.08 (t, $J$ = 6.5 Hz, 2H), 6.16 (s, 1H).

**Methyl Ester of 2-Methyl-5-(8-oxononyloxy)-2*H*-pyrazole-3-carboxylic Acid (20d).** Following the general procedure A, starting from **18** (99 mg, 0.63 mmol) and 9-bromononan-2-one **19d** (420 mg, 1.9 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20d** was obtained as a white solid (187 mg, 95% yield), mp 55−57 °C. $^1$H NMR ($CDCl_3$) $\delta$: 1.34 (m, 10H), 2.13 (s, 3H), 2.42 (t, $J$ = 7.2 Hz, 2H), 3.66 (s, 3H), 4.03 (s, 3H), 4.08 (t, $J$ = 6.7 Hz, 2H), 6.17 (s, 1H).

**Methyl Ester of 2-Methyl-5-(2-oxo-2-phenylethoxy)-2*H*-pyrazole-3-carboxylic Acid (20e).** Following the general procedure A, starting from **18** (4 g, 25 mmol) and 2-bromo-1-phenylethanone **19e** (5.47 g, 27.5 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20e** was obtained as a white solid (6.85 g, 70% yield), mp 102−105 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.85 (s, 3H), 3.98 (s, 3H), 5.54 (s, 2H), 6.37 (s, 1H), 7.60 (m, 2H), 7.68 (t, $J$ = 7.6 Hz, 1H), 7.98 (d, $J$ = 7.6 Hz, 2H).

**Methyl Ester of 5-[2-(4-Chlorophenyl)-2-oxoethoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (20f).** Following the general procedure A, starting from **18** (1 g, 6.4 mmol) and 2-bromo-1-(4-chlorophenyl)ethanone **19f** (2.68 g, 19 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20f** was obtained as a yellow solid (1.97 g, 95% yield), mp 118−120 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.86 (s, 3H), 3.98 (s, 3H), 5.41 (s, 2H), 6.28 (s, 1H), 7.45 (d, $J$ = 7.6 Hz, 2H), 7.95 (d, $J$ = 7.6 Hz, 2H).

**Methyl Ester of 5-(2-Biphenyl-4-yl-2-oxoethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (20g).** Following the general procedure A, starting from **18** (510 mg, 3.26 mmol) and 1-biphenyl-4-yl-2-bromoethanone **19g** (1.23 g, 4.5 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **20f** was obtained as a yellow solid (750 mg, 71% yield), mp 168−170 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.66 (s, 3H), 3.99 (s, 3H), 5.49 (s, 2H), 6.31 (s, 1H), 7.45 (m, 3H), 7.62 (m, 2H), 7.69 (d, $J$ = 7.8 Hz, 2H), 8.04 (d, $J$ = 7.8 Hz, 2H).

**General Procedure B for the Synthesis of 21a−g.** Lithium hydroxide monohydrate (46 mg, 1.1 mmol, 1.1 equiv)

was added to a solution of **20a**−**g** (1 mmol) in 6 mL of THF/MeOH/$H_2O$ (4:1:1) at room temperature. The reaction mixture was stirred for 1 h and then concentrated in vacuo. The crude residue was dissolved in water (5 mL), and the solution was acidified with 10% aqueous HCl and extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (5 mL), dried over $Na_2SO_4$, and concentrated in vacuo. The products obtained were used without any purification for the next reaction.

**2-Methyl-5-(2-oxopropoxy)-2*H*-pyrazole-3-carboxylic Acid (21a).** Following the general procedure B, starting from **20a** (4.03 g, 19 mmol), after workup compound **21a** was obtained as a yellow oil (3.7 g, 97% yield). $^1$H NMR ($CDCl_3$) $\delta$: 2.25 (s, 3H), 4.02 (s, 3H), 4.74 (s, 2H), 6.36 (s, 1H), 7.7 (bs, 1H).

**2-Methyl-5-(4-oxopentyloxy)-2*H*-pyrazole-3-carboxylic Acid (21b).** Following the general procedure B, starting from **20b** (240 mg, 1 mmol), after workup compound **21b** was obtained as a colorless oil (220 mg, 97% yield). $^1$H NMR ($CDCl_3$) $\delta$: 2.05 (m, 2H), 2.18 (s, 3H), 2.65 (t, $J$ = 7.2 Hz, 2H), 4.04 (s, 3H), 4.12 (t, $J$ = 6.2 Hz, 2H), 6.30 (s, 1H), 10.3 (bs, 1H).

**2-Methyl-5-(6-oxoheptyloxy)-2*H*-pyrazole-3-carboxylic Acid (21c).** Following the general procedure B, starting from **20c** (894 mg, 1 mmol), after workup compound **21c** was obtained as an oil (620 mg, 74% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.65 (m, 6H), 2.14 (s, 3H), 2.45 (t, $J$ = 7.4 Hz, 2H), 4.03 (s, 3H), 4.11 (t, $J$ = 6.5 Hz, 2H), 6.16 (s, 1H), 8.5 (bs, 1H).

**2-Methyl-5-(8-oxononyloxy)-2*H*-pyrazole-3-carboxylic Acid (21d).** Following the general procedure B, starting from **20d** (990 mg, 3.3 mmol), after workup compound **21d** was obtained as a colorless oil (860 mg, 92% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.32 (m, 10H), 2.14 (s, 3H), 2.43 (t, $J$ = 7.6 Hz, 2H), 4.05 (s, 3H), 4.12 (t, $J$ = 6.6 Hz, 2H), 6.29 (s, 1H), 8.3 (bs, 1H).

**2-Methyl-5-(2-oxo-2-phenylethoxy)-2*H*-pyrazole-3-carboxylic Acid (21e).** Following the general procedure B, starting from **20e** (4.79 g, 17.5 mmol), after workup compound **21e** was obtained as a white solid (4.33 g, 96% yield), mp 136−138 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.96 (s, 3H), 5.51 (s, 2H), 6.25 (s, 1H), 7.53 (m, 2H), 7.65 (m, 2H), 7.99 (m, 1H), 10.8 (bs, 1H).

**5-[2-(4-Chlorophenyl)-2-oxoethoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (21f).** Following the general procedure B, starting from **20f** (1.80 g, 5.80 mmol), after workup compound **21f** was obtained as a white solid (1.58 g, 92% yield), mp 147−150 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.87 (s, 3H), 5.55 (s, 2H), 6.27 (s, 1H), 7.65 (d, $J$ = 7.4 Hz, 2H), 8.01 (d, $J$ = 7.4 Hz, 2H), 10.8 (bs, 1H).

**5-(2-Biphenyl-4-yl-2-oxoethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (21g).** Following the general procedure B, starting from **20g** (702 mg, 2 mmol), after workup compound **21g** was obtained as a white solid (504 mg, 75% yield), mp 208−210 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.88 (s, 3H), 5.60 (s, 2H), 6.30 (s, 1H), 7.52 (m, 3H), 7.78 (d, $J$ = 7.4 Hz, 2H), 7.88 (d, $J$ = 7.4 Hz, 2H), 8.09 (d, $J$ = 8.2 Hz, 2H), 10.7 (bs, 1H).

**General Procedure C for the Synthesis of 22a−g.** Compounds **21a**−**g** (1 mmol) dissolved in a solution of BTEAC (228 mg, 1 mmol) in dry DMF (10 mL) was treated with $K_2CO_3$ (3.59 g, 26 mmol) and *tert*-butyl bromide (6.57 g, 5.4 mL, 48 mmol). After 24 h at 55 °C, the mixture was poured into cold water (10 mL) and extracted with EtOAc (3 × 15 mL). The recombined organic phases were dried ($Na_2SO_4$), and after concentration in vacuo, the crude product (**22a**−**g**) was used without purification for the next step.

***tert*-Butyl Ester of 2-Methyl-5-(2-oxopropoxy)-2*H*-pyrazole-3-carboxylic Acid (22a).** Following the general procedure C, starting from **21a** (1.23 g, 6 mmol), after workup **22a** was obtained as a brown oil (400 mg, 27% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.56 (s, 9H), 2.23 (s, 3H), 3.97 (s, 3H), 4.70 (s, 2H), 6.16 (s, 1H).

***tert*-Butyl Ester of 2-Methyl-5-(4-oxopentyloxy)-2*H*-pyrazole-3-carboxylic Acid (22b).** Following the general procedure C, starting from **21b** (724 mg, 3.2 mmol), after workup **22b** was obtained as an orange oil (850 mg, 94% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.56 (s, 9H), 2.05 (m, 2H), 2.16 (s, 3H), 2.63 (t, $J$ = 7.2 Hz, 2H), 4.02 (s, 3H), 4.09 (t, $J$ = 6.2 Hz, 2H), 6.09 (s, 1H).

***tert*-Butyl Ester of 2-Methyl-5-(6-oxoheptyloxy)-2*H*-pyrazole-3-carboxylic Acid (22c).** Following the general procedure C, starting from **21c** (620 mg, 2.4 mmol), after workup **22c** was obtained as a brown oil (500 mg, 67% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.63 (m, 6H), 1.56 (s, 9H), 2.14 (s, 3H), 2.45 (t, $J$ = 7.2 Hz, 2H), 4.03 (s, 3H), 4.07 (t, $J$ = 6.4 Hz, 2H), 6.10 (s, 1H).

***tert*-Butyl Ester of 2-Methyl-5-(8-oxononyloxy)-2*H*-pyrazole-3-carboxylic Acid (22d).** Following the general procedure C, starting from **21d** (840 mg, 2.97 mmol), after workup **22d** was obtained as a brown oil (940 mg, 94% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.35 (m, 10H), 1.55 (s, 9H), 2.13 (s, 3H), 2.42 (t, $J$ = 7.2 Hz, 2H), 3.99 (s, 3H), 4.06 (t, $J$ = 6.4 Hz, 2H), 6.09 (s, 1H).

***tert*-Butyl Ester of 2-Methyl-5-(2-oxo-2-phenylethoxy)-2*H*-pyrazole-3-carboxylic Acid (22e).** Following the general procedure C, starting from **21e** (1 g, 3.8 mmol), after workup **22e** was obtained as a brown oil (1.13 g, 94% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.54 (s, 9H), 3.94 (s, 3H), 5.44 (s, 2H), 6.33 (s, 1H), 7.59 (m, 2H), 7.68 (t, $J$ = 7.2 Hz, 1H), 8.00 (d, $J$ = 7.2 Hz, 2H).

***tert*-Butyl Ester of 5-[2-(4-Chlorophenyl)-2-oxoethoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (22f).** Following the general procedure C, starting from **21f** (1.53 g, 5.2 mmol), after workup compound **22f** was obtained as a brown oil (1.60 g, 89% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.56 (s, 9H), 3.95 (s, 3H), 5.40 (s, 2H), 6.22 (s, 1H), 7.49 (d, $J$ = 8.0 Hz, 2H), 7.95 (d, $J$ = 8.00 Hz, 2H).

***tert*-Butyl Ester of 5-(2-Biphenyl-4-yl-2-oxoethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (22g).** Following the general procedure C, starting from **21g** (504 mg, 1.5 mmol), after workup **22g** was obtained as a yellow oil (410 mg, 78% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.56 (s, 9H), 3.97 (s, 3H), 5.49 (s, 2H), 6.25 (s, 1H), 7.45 (m, 3H), 7.62 (m, 2H), 7.74 (d, $J$ = 8.2 Hz, 2H), 8.07 (d, $J$ = 8.4 Hz, 2H).

**General Procedure D for the Synthesis of 23a−g.** To a solution of **22a**−**g** (1 mmol) in dry THF (7 mL), activated Zn powder (261 mg, 4 mmol), hydroquinone (2 mg), and ethyl 2-(bromomethyl)acrylate (503 mg, 0.26 mL, 2.6 mmol) were added. The mixture was refluxed under nitrogen for 24 h. After cooling, the reaction mixture was filtered on Celite and the filtrate was diluted with $CH_2Cl_2$ (15 mL). The organic solution washed with 1 N HCl solution (5 mL) and water (5 mL), dried ($Na_2SO_4$), and then evaporated gave a residual solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether).

***tert*-Butyl Ester of 2-Methyl-5-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-2*H*-pyrazole-3-carboxylic Acid (23a).** Following the general procedure D, starting from **22a** (400 mg, 1.6 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **23a** was obtained as a colorless oil (240 mg, 50% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.51 (s, 3H), 1.55 (s, 9H), 2.74 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 3.11 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 3.99 (s, 3H), 4.13 (s, 2H), 5.63 (t, $J$ = 2.4 Hz, 1H), 6.10 (s, 1H), 6.25 (t, $J$ = 2.8 Hz, 1H).

***tert*-Butyl Ester of 2-Methyl-5-[3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)propoxy]-2*H*-pyrazole-3-carboxylic Acid (23b).** Following the general procedure D, starting from **22b** (800 mg, 3 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 3:7), compound **23b** was obtained as a colorless oil (852 mg, 81% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.43 (s, 3H), 1.56 (s, 9H), 1.84 (m, 4H), 2.60 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.12 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.99 (s, 3H), 4.14 (t, $J$ = 7.4 Hz, 2H), 5.63 (t, $J$ = 2.0 Hz, 1H), 6.09 (s, 1H), 6.24 (t, $J$ = 2.8 Hz, 1H).

***tert*-Butyl Ester of 2-Methyl-5-[5-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)pentyloxy]-2*H*-pyrazole-3-carboxylic Acid (23c).** Following the general procedure D, starting from **22c** (500 mg, 1.6 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether

3/7), compound **23c** was obtained as a colorless oil (460 mg, 76% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.33 (t, $J$ = 2.8 Hz, 2H), 1.40 (s, 3H), 1.56 (s, 9H), 1.76 (m, 6H), 2.72 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 3.23 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 4.04 (s, 3H), 4.10 (t, $J$ = 7.4 Hz, 2H), 5.61 (t, $J$ = 2.2 Hz, 1H), 6.10 (s, 1H), 6.23 (t, $J$ = 2.6 Hz, 1H).

***tert*-Butyl Ester of 2-Methyl-5-[7-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)heptyloxy]-2*H*-pyrazole-3-carboxylic Acid (23d).** Following the general procedure D, starting from **22d** (950 mg, 2.8 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **23d** was obtained as a colorless oil (790 mg, 70% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.01 (t, $J$ = 4.6 Hz, 2H), 1.39 (s, 3H), 1.56 (s, 9H), 1.66 (m, 10H), 2.71 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.21 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.99 (s, 3H), 4.10 (t, $J$ = 6.5 Hz, 2H), 5.61 (t, $J$ = 2.4 Hz, 1H), 6.10 (s, 1H), 6.22 (t, $J$ = 2.6 Hz, 1H).

***tert*-Butyl Ester of 2-Methyl-5-(4-methylene-5-oxo-2-phenyltetrahydrofuran-2-ylmethoxy)-2*H*-pyrazole-3-carboxylic Acid (23e).** Following the general procedure D, starting from **22e** (2.26 g, 7.0 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 3/7), compound **23e** was obtained as a colorless oil (2.69 g, 80% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.54 (s, 9H), 3.20 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 3.66 (dt, $J$ = 17.2 and 2.6 Hz, 1H), 3.97 (s, 3H), 4.33 (s, 3H), 5.64 (t, $J$ = 2.4 Hz, 1H), 6.08 (s, 1H), 6.26 (t, $J$ = 2.8 Hz, 1H), 7.40 (m, 4H).

***tert*-Butyl Ester of 5-[2-(4-Chlorophenyl)-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (23f).** Following the general procedure D, starting from **22f** (410 mg, 1.2 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 2:8), compound **23f** was obtained as a colorless oil (280 mg, 60% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.54 (s, 9H), 3.12 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.56 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.97 (s, 3H), 4.30 (s, 2H), 5.66 (t, $J$ = 2.40 Hz, 1H), 6.07 (s, 1H), 6.28 (t, $J$ = 2.78 Hz, 1H), 7.40 (m, 4H).

***tert*-Butyl Ester of 5-(2-Biphenyl-4-yl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (23g).** Following the general procedure D, starting from **22g** (410 mg, 1 mmol), after workup and purification by flash chromatography (EtOAc/petroleum ether 3:7), compound **23g** was obtained as a colorless oil (400 mg, 71% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.55 (s, 9H), 3.21 (dt, $J$ = 16.8 and 2.6 Hz, 1H), 3.62 (dt, $J$ = 16.8 and 2.6 Hz, 1H), 3.98 (s, 3H), 4.37 (s, 2H), 5.66 (t, $J$ = 2.4 Hz, 1H), 6.10 (s, 1H), 6.28 (t, $J$ = 2.8 Hz, 1H), 7.51 (m, 9H).

**General Procedure E for the Synthesis of 24a−g.** To a solution of the *tert*-butyl esters **23a−g** (1 mmol) in $CH_2Cl_2$ (5 mL) was added $CF_3COOH$ (1 mL), and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and triturated with petroleum ether (5 mL) to furnish **23a−g**, which were collected by filtration.

**2-Methyl-5-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-2*H*-pyrazole-3-carboxylic Acid (24a).** Following the general procedure E, starting from compound **23a** (240 mg, 0.7 mmol), after workup, compound **24a** was obtained as a light-yellow powder (210 mg, 95% yield), mp 131−134 °C. $^1$H NMR (DMSO) $\delta$: 1.42 (s, 3H), 2.81 (dt, $J$ = 17 and 2.4 Hz, 1H), 3.08 (dt, $J$ = 17 and 2.4 Hz, 1H), 3.91 (s, 3H), 4.14 (s, 2H), 5.73 (t, $J$ = 2.0 Hz, 1H), 6.04 (t, $J$ = 2.8 Hz, 1H), 6.22 (s, 1H), 13.2 (bs, 1H).

**2-Methyl-5-[3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)propoxy]-2*H*-pyrazole-3-carboxylic Acid (24b).** Following the general procedure E, starting from compound **23b** (858 mg, 2.5 mmol), after workup, compound **24b** was obtained as a colorless oil (720 mg, 95% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.43 (s, 3H), 1.86 (m, 4H), 2.76 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 3.00 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 4.04 (s, 3H), 4.14 (t, $J$ = 7.21 Hz, 2H), 5.64 (t, $J$ = 2.04 Hz, 1H), 6.24 (t, $J$ = 2.85 Hz, 1H), 6.29 (s, 1H), 8.7 (bs, 1H).

**2-Methyl-5-[5-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)pentyloxy]-2*H*-pyrazole-3-carboxylic Acid (24c).** Following the general procedure E, starting from compound **23c** (460 mg, 1.2 mmol), after workup, compound **24c** was obtained as a colorless oil (228 mg, 59% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.26 (t, $J$ = 7.01 Hz, 2H), 1.40 (s, 3H), 1.74 (m, 6H), 2.72 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.02 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 4.05 (s, 3H), 4.13 (t, $J$ = 7.43 Hz, 2H), 5.62 (t, $J$ = 2.33 Hz, 1H), 6.24 (t, $J$ = 2.63 Hz, 1H), 6.29 (s, 1H), 8.4 (bs, 1H).

**2-Methyl-5-[7-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)heptyloxy]-2*H*-pyrazole-3-carboxylic Acid (24d).** Following the general procedure E, starting from compound **23d** (406 mg, 1 mmol), after workup, compound **24d** was obtained as a colorless oil (350 mg, 77% yield). $^1$H NMR ($CDCl_3$) $\delta$: 1.17 (t, $J$ = 4.6 Hz, 2H), 1.39 (s, 3H), 1.71 (m, 10H), 2.72 (dt, $J$ = 17.0 and 2.6 Hz, 1H), 3.12 (dt, $J$ = 17.0 and 2.6 Hz, 1H), 4.05 (s, 3H), 4.12 (t, $J$ = 2.4 Hz, 2H), 5.62 (t, $J$ = 2.4 Hz, 1H), 6.25 (t, $J$ = 2.6 Hz, 1H), 6.30 (s, 1H), 8.5 (bs, 1H).

**2-Methyl-5-(4-methylene-5-oxo-2-phenyltetrahydrofuran-2-ylmethoxy)-2*H*-pyrazole-3-carboxylic Acid (24e).** Following the general procedure E, starting from compound **23e** (2.17 g, 5.6 mmol), after workup, compound **24e** was obtained as a colorless oil (1.71 g, 95% yield). $^1$H NMR ($CDCl_3$) $\delta$: 3.19 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.63 (dt, $J$ = 17.2 and 2.4 Hz, 1H), 3.86 (s, 3H), 4.38 (s, 3H), 5.66 (t, $J$ = 2.4 Hz, 1H), 6.11 (s, 1H), 6.24 (t, $J$ = 2.8 Hz, 1H), 7.42 (m, 4H), 8.4 (bs, 1H).

**5-[2-(4-Chlorophenyl)-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (24f).** Following the general procedure E, starting from compound **23f** (280 mg, 0.67 mmol), after workup, compound **24f** was obtained as a colorless oil (242 mg, 95% yield). $^1$H NMR ($CDCl_3$) $\delta$: 3.10 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 3.61 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 3.95 (s, 3H), 4.28 (s, 2H), 5.69 (t, $J$ = 2.4 Hz, 1H), 6.10 (s, 1H), 6.31 (t, $J$ = 2.8 Hz, 1H), 7.38 (m, 4H), 8.7 (bs, 1H).

**5-(2-Biphenyl-4-yl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (24g).** Following the general procedure E, starting from compound **23g** (400 mg, 0.87 mmol), after workup, compound **24g** was obtained as a white solid (351 mg, 95% yield), mp 101−103 °C. $^1$H NMR ($CDCl_3$) $\delta$: 3.25 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 3.61 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 4.01 (s, 3H), 4.40 (s, 2H), 5.67 (t, $J$ = 2.4 Hz 1H), 6.10 (s, 1H), 6.28 (t, $J$ = 2.8 Hz, 1H), 7.51 (m, 9H), 8.6 (bs, 1H).

**General Procedure F for the Coupling of Pyrazole Carboxylic Acid (24a−g) with Pyrrole Oligomers (25−28).** To a stirred 0.4 M solution of the pyrrole oligomer **25−28** in anhydrous DMF under argon atmosphere Hünig's base (1 equiv) was added at 0 °C. After 5 min, the acids **24a−g** (1.1 equiv) followed by EDCl (2 equiv) were added. The resulting mixture was stirred for 18 h as it warmed to room temperature, acidified with 10% HCl to pH 3, and then evaporated to dryness in a vacuum. The resulting residue was purified by column chromatography ($CH_2Cl_2$/$CH_3OH$ 8:2 v/v) and recrystallized ($CH_3OH$/diethyl ether) to give **5−17**.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (5).** Yield, 33%; white solid; mp 220−221 °C. $^1$H NMR (DMSO) $\delta$: 1.45 (s, 3H), 2.65 (t, $J$ = 6.3 Hz, 2H), 2.94 (dt, $J$ = 17.6 and 2.4 Hz, 1H), 3.22 (dt, $J$ = 17.6 and 2.4 Hz, 1H), 3.43 (m, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 3.95 (s, 3H), 4.17 (s, 2H), 5.74 (t, $J$ = 2.32 Hz, 1H), 6.05 (t, $J$ = 2.77 Hz, 1H), 6.42 (s, 1H), 6.97 (s, 1H), 7.07 (s, 1H), 7.08 (s, 1H), 7.19 (s, 1H), 7.25 (s, 1H), 7.30 (s, 1H), 8.25 (t, $J$ = 7.2 Hz, 1H), 8.62 (bs, 2H), 8.99 (bs, 2H), 9.95 (s, 1H), 10.02 (s, 1H), 10.32 (s, 1H). Anal. ($C_{33}H_{40}ClN_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)-propoxy]-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (6).** Yield, 36%; yellow solid; mp 146−149 °C. $^1$H NMR (DMSO) $\delta$: 1.36 (s, 3H), 1.74

(m, 4H), 2.51 (t, $J$ = 6.3 Hz, 2H), 2.80 (dt, $J$ = 17.0 and 2.6 Hz, 1H), 2.92 (dt, $J$ = 17.0 and 2.6 Hz, 1H), 3.49 (m, 2H), 3.61 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 3.94 (s, 3H), 4.07 (s, 2H), 5.73 (t, $J$ = 2.4 Hz, 1H), 6.05 (t, $J$ = 2.8, 1H), 6.37 (s, 1H), 6.96 (s, 1H), 7.06 (s, 1H), 7.08 (s, 1H), 7.18 (s, 1H), 7.24 (s, 1H), 7.28 (s, 1H), 8.22 (t, $J$ = 7.2 Hz, 1H), 8.58 (bs, 2H), 8.92 (bs, 2H), 9.94 (s, 1H), 10.00 (s, 1H), 10.27 (s, 1H). Anal. ($C_{35}H_{44}ClN_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[5-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)-pentyloxy]-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (7).** Yield, 29%; yellow solid; mp 112−114 °C. $^1$H NMR (DMSO) $\delta$: 1.25 (t, $J$ = 2.89 Hz, 2H), 1.33 (s, 3H), 1.73 (m, 6H), 2.49 (t, $J$ = 6.3 Hz, 2H), 2.77 (m, 2H), 3.46 (m, 2H), 3.81 (s, 3H), 3.87 (s, 3H), 3.94 (s, 3H), 4.02 (s, 3H), 4.08 (s, 2H), 5.71 (t, $J$ = 2.3 Hz, 1H), 6.03 (t, $J$ = 2.8, 1H), 6.45 (s, 1H), 6.95 (s, 1H), 7.07 (s, 1H), 7.11 (s, 1H), 7.21 (s, 1H), 7.25 (s, 1H), 7.30 (s, 1H), 8.27 (t, $J$ = 7.2 Hz, 1H), 8.74 (bs, 2H), 9.05 (bs, 2H), 9.96 (s, 1H), 10.03 (s, 1H), 10.37 (s, 1H). Anal. ($C_{37}H_{48}ClN_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[7-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-yl)-heptyloxy]-2*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (8).** Yield, 40%; yellow solid; mp 132−136 °C. $^1$H NMR (DMSO) $\delta$: 1.20 (t, $J$ = 4.61 Hz, 2H), 1.32 (s, 3H), 1.68 (m, 10H), 2.63 (t, $J$ = 6.1 Hz, 2H), 2.71 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 2.82 (dt, $J$ = 16.8 and 2.4 Hz, 1H), 3.49 (m, 2H), 3.72 (s, 3H), 3.75 (s, 3H), 3.81 (s, 3H), 3.94 (s, 3H), 4.17 (s, 2H), 5.70 (t, $J$ = 2.4 Hz, 1H), 6.03 (t, $J$ = 2.4 Hz, 1H), 6.37 (s, 1H), 6.96 (s, 1H), 7.07 (s, 1H), 7.09 (s, 1H), 7.19 (s, 1H), 7.25 (s, 1H), 7.29 (s, 1H), 8.23 (t, $J$ = 7.2 Hz, 1H), 8.61 (bs, 2H), 8.92 (bs, 2H), 9.94 (s, 1H), 10.00 (s, 1H), 10.27 (s, 1H). Anal. ($C_{39}H_{52}ClN_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]propionamidine Hydrochloride (9).** Yield, 43%; white solid; mp 154−156 °C. $^1$H NMR (DMSO) $\delta$: 1.44 (s, 3H), 2.64 (t, $J$ = 6.1 Hz, 2H), 2.82 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.05 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.48 (m, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 3.94 (s, 3H), 4.16 (s, 2H), 5.73 (t, $J$ = 2.2 Hz, 1H), 6.05 (t, $J$ = 2.6 Hz, 1H), 6.43 (s, 1H), 6.94 (s, 1H), 7.06 (s, 1H), 7.19 (s, 1H), 7.28 (s, 1H), 8.25 (t, $J$ = 7.2 Hz, 1H), 8.66 (bs, 2H), 9.00 (bs, 2H), 9.99 (s, 1H), 10.32 (s, 1H). Anal. ($C_{27}H_{34}ClN_9O_6$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (10).** Yield, 48%; white solid; mp 112−114 °C. $^1$H NMR (DMSO) $\delta$: 1.44 (s, 3H), 2.62 (t, $J$ = 2.6 Hz, 2H), 3.17 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.48 (m, 2H), 3.62 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.81 (s, 3H), 3.93 (s, 3H), 4.16 (s, 2H), 5.73 (t, $J$ = 2.2 Hz, 1H), 6.05 (t, $J$ = 2.7 Hz, 1H), 6.43 (s, 1H), 6.95 (s, 1H), 7.24 (s, 1H), 8.33 (t, $J$ = 7.2 Hz, 1H), 8.70 (bs, 2H), 9.04 (bs, 2H), 10.33 (s, 1H). Anal. ($C_{21}H_{28}ClN_7O_5$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-(2-phenyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (11).** Yield, 33%; white solid; mp 225−228 °C. $^1$H NMR (DMSO) $\delta$: 2.61 (t, $J$ = 6.1 Hz, 2H), 2.93 (m, 2H), 3.49 (m, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 3.93 (s, 3H), 4.36 (d, $J$ = 11 Hz, 1H), 4.48 (d, $J$ = 11 Hz, 1H), 5.76 (t, $J$ = 2.2 Hz, 1H), 6.10 (t, $J$ = 2.7 Hz 1H), 6.40 (s, 1H), 6.96 (s, 1H), 7.06 (s, 1H), 7.19 (s, 1H), 7.25 (s, 1H), 7.28 (s, 1H), 7.38 (s, 1H), 7.49 (m, 5H), 8.24 (t, $J$ = 7.2, 1H), 8.58 (bs, 2H), 8.97 (bs, 2H), 9.94 (s, 1H), 10.02 (s, 1H), 10.27 (s, 1H). Anal. ($C_{38}H_{42}ClN_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-3-(2-phenyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]propionamidine Hydrochloride (12).** Yield, 49%; white solid; mp 132−134 °C. $^1$H NMR (DMSO) $\delta$: 2.62 (t, $J$ = 2.6 Hz, 2H), 3.11 (dt, $J$ = 17.4 and 2.5 Hz, 1H), 3.49 (m, 2H), 3.52 (dt, $J$ = 17.4 and 2.5 Hz, 1H), 3.81 (s, 3H), 3.85 (s, 3H), 3.93 (s, 3H), 4.36 (d, $J$ = 11 Hz, 1H), 4.48 (d, $J$ = 11 Hz, 1H), 5.79 (t, $J$ = 2.2 Hz, 1H), 6.10 (t, $J$ = 2.7 Hz, 1H), 6.37 (s, 1H), 6.94 (s, 1H), 7.03 (s, 1H), 7.18 (s, 1H), 7.26 (s, 1H), 7.49 (m, 5H), 8.22 (t, $J$ = 7.2 Hz, 1H), 8.54 (bs, 2H), 8.91 (bs, 2H), 9.97 (s, 1H), 10.23 (s, 1H). Anal. ($C_{32}H_{36}ClN_9O_6$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-3-(2-phenyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (13).** Yield, 45%; white solid; mp 114−117 °C. $^1$H NMR (DMSO) $\delta$: 2.58 (t, $J$ = 2.6 Hz, 2H), 3.11 (dt, $J$ = 17.4 and 2.5 Hz, 1H), 3.49 (m, 2H), 3.64 (dt, $J$ = 17.4 and 2.5 Hz, 1H), 3.81 (s, 3H), 3.91 (s, 3H), 4.35 (d, $J$ = 11 Hz, 1H), 4.47 (d, $J$ = 11 Hz, 1H), 5.78 (t, $J$ = 2.2 Hz, 1H), 6.10 (t, $J$ = 2.7 Hz, 1H), 6.37 (s, 1H), 6.92 (s, 1H), 7.20 (s,1H), 7.48 (m, 5H), 8.27 (t, $J$ = 7.2, 1H), 8.56 (bs, 2H), 8.91 (bs, 2H), 10.20 (s, 1H). Anal. ($C_{26}H_{30}ClN_7O_5$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[2-(4-chlorophenyl)-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy]-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (14).** Yield, 32%; white solid; mp 215−218 °C. $^1$H NMR (DMSO) $\delta$: 2.61 (t, $J$ = 6.1 Hz, 2H), 2.71 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 2.93 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 3.43 (m, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 3. 86 (s, 3H), 3.93 (s, 3H), 4.37 (d, $J$ = 11 Hz, 1H), 4.42 (d, $J$ = 11 Hz, 1H), 5.80 (t, $J$ = 2.2 Hz, 1H), 6.11 (t, $J$ = 2.7 Hz, 1H), 6.40 (s, 1H), 6.96 (s, 1H), 7.06 (s, 1H), 7.19 (s, 1H), 7.25 (s, 1H), 7.28 (s, 1H), 7.50 (s, 1H), 7.52 (m, 4H), 8.24 (t, $J$ = 7.2 Hz, 1H), 8.62 (bs, 2H), 8.99 (bs, 2H), 9.94 (s, 1H), 10.01 (s, 1H), 10.29 (s, 1H). Anal. ($C_{38}H_{41}Cl_2N_{11}O_7$) C, H, Cl, N.

**3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[2-biphenyl-4-yl-4-methylene-5-oxotetrahydrofuran-2-yl methoxy]-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (15).** Yield, 38%; white solid; mp 227−230 °C. $^1$H NMR (DMSO) $\delta$: 2.89 (t, $J$ = 6.1 Hz, 2H), 3.04 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.48 (m, 2H), 3.64 (dt, $J$ = 17.4 and 2.4 Hz, 1H), 3.81 (s, 3H), 3.84 (s, 3H), 3. 86 (s, 3H), 3.93 (s, 3H), 4.14 (d, $J$ = 11 Hz, 1H), 4.47 (d, $J$ = 11 Hz, 1H), 5.80 (t, $J$ = 2.24 Hz, 1H), 6.20 (t, $J$ = 2.7 Hz, 1H), 6.43 (s, 1H), 6.96 (s, 1H), 7.06 (s, 1H), 7.24 (s, 1H), 7.27 (s, 1H), 7.44 (s, 1H), 7.48 (s, 1H), 7.56 (m, 9H), 8.26 (t, $J$ = 7.2 Hz, 1H), 8.60 (bs, 2H), 8.98 (bs, 2H), 9.94 (s, 1H), 10.01 (s, 1H), 10.29 (s, 1H). Anal. ($C_{44}H_{46}ClN_{11}O_7$) C, H, Cl, N.

**2-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-(2-methyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethyl Guanidine Hydrochloride (16).** Yield, 47%; yellow solid; mp 128−132 °C. $^1$H NMR (DMSO) $\delta$: 1.45 (s, 3H), 2.82 (dt, $J$ = 17.6 and 2.5 Hz, 1H), 3.02 (dt, $J$ = 17.6 and 2.5 Hz, 1H), 3.15 (m, 2H), 3.43 (m, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 3.95 (s, 3H), 4.17 (s, 2H), 5.76 (t, $J$ = 2.3 Hz, 1H), 6.12 (t, $J$ = 2.8 Hz, 1H), 6.42 (s, 1H), 6.96 (s, 1H), 7.07 (s, 1H), 7.12 (s, 1H), 7.15 (bs, 2H), 7.20 (s, 1H), 7.25 (s, 1H), 7.29 (s, 1H), 7.38 (bs, 2H), 7.54 (bs, 1H), 8.25 (t, $J$ = 7.2 Hz, 1H), 9.96 (s, 1H), 10.02 (s, 1H), 10.32 (s, 1H). Anal. ($C_{33}H_{41}ClN_{12}O_7$) C, H, Cl, N.

**2-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-(2-phenyl-4-methylene-5-oxotetrahydrofuran-2-ylmethoxy)-1*H*-pyrazole-5-carboxamido]pyrrole-2-carboxamido]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethyl-guanidine Hydrochloride (17).** Yield, 51%; white solid; mp 161−162 °C. $^1$H NMR (DMSO) $\delta$: 3.09 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 3.15 (m, 2H), 3.55 (m, 2H), 3.64 (dt, $J$ = 17.0 and 2.4 Hz, 1H), 3.82 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 3.93 (s, 3H), 4.36 (d, $J$ = 11 Hz, 1H), 4.48 (d, $J$ = 11 Hz, 1H), 5.79 (t, $J$ = 2.2 Hz, 1H), 6.10 (t, $J$ = 2.7 Hz 1H), 6.41 (s, 1H), 6.96 (s, 1H), 7.07 (s, 1H), 7.15 (bs, 2H), 7.20 (s, 1H), 7.25 (s, 1H), 7.28 (s, 1H), 7.38 (s, 1H), 7.41 (bs, 2H), 7.45 (m, 5H), 7.52 (bs, 1H),

8.15 (t, $J = 7.2$, 1H), 9.96 (s, 1H), 10.02 (s, 1H), 10.28 (s, 1H). Anal. ($C_{38}H_{43}ClN_{12}O_7$) C, H, Cl, N.

**Cytostatic Assays**. Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte Molt 4/ C8, and CEM cells were suspended at 300000−500000 cells/mL of culture medium, and an amount of 100 $\mu$L of these cell suspensions was added to 200 $\mu$L microtiter plate wells containing 100 $\mu$L of an appropriate dilution of the test compounds. After 2 days (L1210 and FM3A) or 3 days (Molt 4/ C8 and CEM) of incubation at 37 °C, the cell number was determined using a Coulter counter. The 50% cytostatic concentration ($IC_{50}$) was defined as the compound concentration required to inhibit cell proliferation by 50%.

**Biological Assays for the Apoptotic Studies. Cell Culture.** The human promyelocytic leukemia HL-60 cell line established by Gallagher et al.[24] was used in this study. Cells were cultured in a suspension in RPMI-1640 medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (100 units $mL^{-1}$), and streptomycin (100 $\mu g\ mL^{-1}$) in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C. Cells were maintained at a density of $<1 \times 10^6$ cells/mL. Compounds were dissolved in dimethyl sulfoxide (DMSO) and stored as stock solutions at −20 °C. Further dilutions were made in culture medium immediately prior to use. In all experiments, the final concentration of DMSO did not exceed 1% (v/v), a concentration that was nontoxic to the host cells. Cell viability was determined using the trypan blue exclusion test. To ensure an exponential growth, cells were resuspended in fresh medium 24 h before each treatment.

**Quantitative Fluorescence Microscopy.** Cells ($\sim 1 \times 10^6$) were fixed in 3% paraformaldehyde for 10 min at room temperature and then stained with 10 $\mu$g/mL bisbenzimide trihydrochloride (Hoechst 33258) for 30 min at 37 °C before fluorescence microscopy analysis. Apoptotic cells were identified by their fragmented chromatin.[25] Stained nuclei with condensed chromatin (supercondensed chromatin at the nuclear periphery) or nuclei that were fragmented into multiple smaller dense bodies were considered as apoptotic. Nuclei with uncondensed and dispersed chromatin were considered as nonapoptotic. A minimum of 500 cells were counted for each sample, and each experiment was done in triplicate.

**Determination of DNA Fragmentation.** Low molecular weight DNA was extracted following the method described by Colotta et al.[26] Briefly, the cells ($\sim 2 \times 10^6$) were washed with phosphate-buffered saline and incubated in 100 $\mu$L of hypotonic detergent buffer (10 mM Tris-HCl, 1 mM EDTA, 0.2% Triton X-100, pH 7.5) for 30 min at 4 °C. The supernatants obtained after centrifugation were sequentially incubated with 40 $\mu$g of DNase-free RNase and 40 $\mu$g of proteinase K at 37 °C for 30 min. The samples were mixed with 100 $\mu$L of phenol, and the aqueous phase containing the fragmented DNA was incubated with 250 $\mu$L of ethanol in the presence of 5 $\mu$g of t-RNA for 24 h at −20 °C. After centrifugation, the DNA precipitate was resuspended in 20 $\mu$L of 10 mM Tris-HCl (pH 7.5) and aliquots (10 $\mu$L) were electrophoresed at 40 V for 4 h through a 2% agarose gel in TAE buffer (40 mM Tris-acetate and 1.0 mM EDTA, pH 8.3). DNA bands were visualized under UV light after staining with ethidium bromide (0.5 $\mu$g/mL), and the images were captured by a digital camera (Kodak).

**Western Blot Analysis of PARP Hydrolysis.** Exponentially growing HL-60 cells ($\sim 7 \times 10^5$) were treated at the indicated concentrations of the compounds at 37 °C. Cells lysates were prepared in a buffer containing 20 mM Hepes (pH 7.4), 250 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 $\mu$g/mL leupeptin, aprotinin, and pepstatin A. Insoluble materials were removed by centrifugation at 10000*g* for 10 min. Extracted proteins (50 $\mu$g/well) were separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) on 7.5% polyacrylamide gels and were electrophoretically transferred onto an Immobilon-P membrane. The loading and transfer of equal amounts of protein were confirmed by staining the membrane with Ponceau S. Membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h, followed by overnight incubation with anti-PARP monoclonal antibody (BD PharMingen; 1:1000 dilution in TBST supplemented with 3% nonfat milk). After being washed and incubated with anti-mouse antibody conjugated to horseradish peroxidase (HRP) (Amersham Pharmacia Biotech), the antigen−antibody complexes were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) using the manufacturer's protocol.

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