Novel Terphenyls and 3,5-Diaryl Isoxazole Derivatives Endowed with Growth Supporting and Antiapoptotic Properties

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A new study on terphenyl and diaryl-isoxazole and -isoxazoline derivatives, maintaining a common 3-adamantyl-4-hydroxyphenyl moiety, has been conducted to find compounds with growth supporting and antiapoptotic properties. Unexpectedly, diphenyisoxazole derivatives bearing a nitro group replacing the carboxylic function have been found with the highest cell protective activity within the series, in complete and in serum-free conditions. Inhibition of apoptosis induced by daunorubicin has also been observed for the most active compound.

Introduction

Apoptotic processes are involved in cell proliferation/homeostasis regulation and removal of defective and therefore harmful cells.[1] Dysfunction or deregulation of apoptosis have been identified as crucial factors that intervene in many of the most widespread serious pathologies causing death or disability in modern times. More specifically, whereas deficiency of apoptosis might result in cancer,[2,3] in autoimmune diseases or viral infections, excessive accumulation of apoptotic cells has been observed as a major cause of damages during neurodegenerative disorders such as Alzheimer, Huntington's, and Parkinson diseases, AIDS, and ischemic diseases.[4–6] Considerable evidence is available supporting a role of apoptosis in cerebral ischemia. While damaged neurons often die from necrosis, apoptosis contributes significantly to cell death subsequent to cerebral ischemia, with apoptosis being predominant when the excitotoxic insult is relatively mild.[7] Apoptosis is also implicated in heart attacks. In fact, during a heart attack, cardiomyocytes become ischemic and will die through necrotic processes if the blockage is not promptly removed. Once they are reperfused, though, many of the formerly ischemic cells will go on to die through apoptosis, causing irreversible damage to the heart muscle.[8]

Inhibition of apoptosis has been therefore envisaged as a promising target for the discovery of novel drugs for these severe pathologies in order to slow down disease progression and improve patient's prospects.[4]

During our studies on derivatives of simple natural stilbenes, known for their multiple biological activities resulting into an overall antiproliferative effect on cancer cell lines, we unexpectedly discovered that some terphenyl derivatives (**2**−**4**, Chart 1) led to a growth rate enhancement in the tested cell population with respect to control. Confirmation of such protective behavior was found by testing these compounds on HL60 cells cultured with serum-free medium and, moreover, on neuronal cells exposed to proapoptotic agents.[9]

Growth stimulating activity was known to be induced by the action of physiological metabolites of retinol, as the 14-hydroxy-retro-retinol (14-HRR,[a] **1**, Chart 1)[10,11] and 13,14-dihydroxy retinol (DHR).[12] They are present into serum and are responsible for its growth enhancing and antiapoptotic activity.

To continue the search for structural features needed for new apoptosis inhibitors, in order to obtain novel drugs active against degenerative diseases, we therefore planned further modifications on the terphenyl backbone, present in the most active derivatives found in previous studies. Terphenyl structure is reported as part also of other interesting analogues of natural compounds active on cell cycle, such as resveratrol.[13]

For the new series, we chose to replace the 3,4,5-trimethoxyphenyl with the 3-adamantyl-4-hydroxyphenyl group, a substitution set recurring in known compound CD437[14] and in more recent derivatives[15,16] that regulate the mechanisms promoting differentiation and/or apoptosis in several cancer cell lines. Moreover, we previously found that derivatives bearing such substitution are endowed with either agonistic or antagonistic behavior toward apoptotic induction.[17]

From this starting point, we explored different steps of structure modifications, maintaining at first the terphenyl backbone as in compounds **13**−**15**. The second investigation involved the change of the central phenyl into an heterocyclic moiety, as we conducted with good results in a previous work.[18] We therefore obtained 3,5-disubstituted isoxazolinyl (**24**, **25**) or isoxazolyl (**28**, **29**, **32**, **33**) derivatives. Finally, we evaluated the importance of carboxylic moiety by changing this function-

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[a] Abbreviations: 14-HRR, 14-hydroxy-retro-retinol; DHR, 13,14-dihydroxy retinol; PBS, phosphate buffered salt solution; FCS, fetal calf serum.

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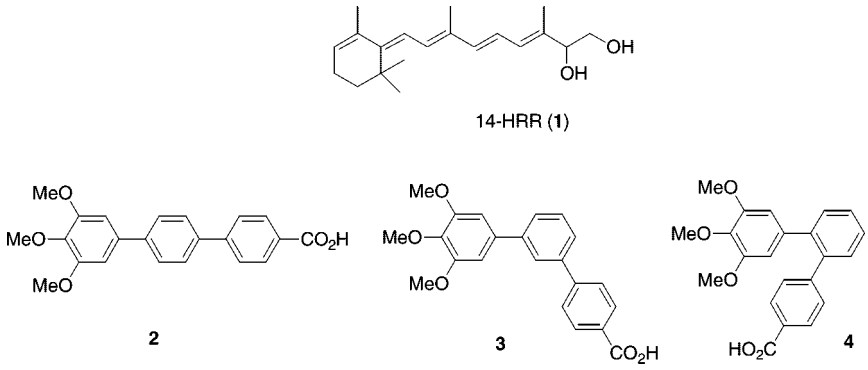
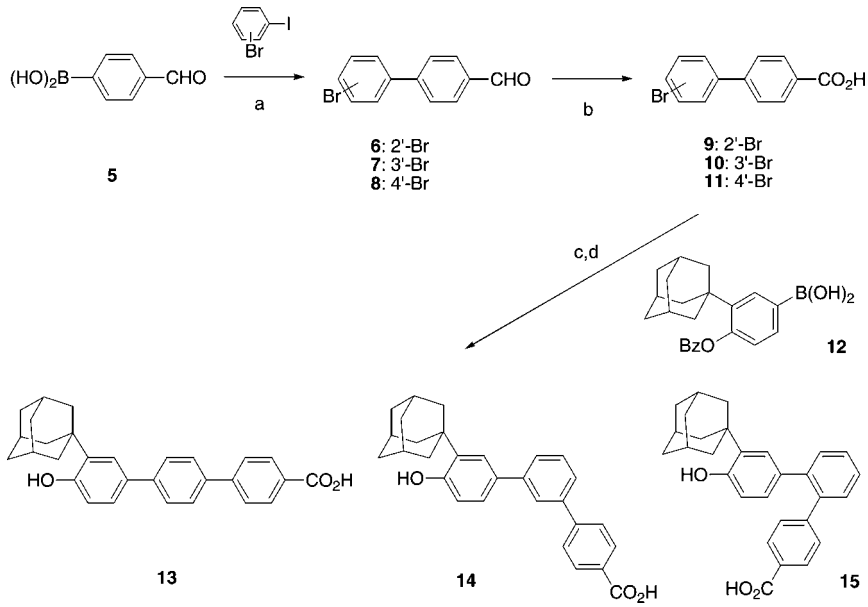
Chart 1. Natural 14-Hydroxy-retroretinol (**1**) and Known Terphenyl Derivatives (**2**−**4**) Endowed with Cell Growth-Supporting Activity



Scheme 1. Synthesis of Terphenyl Derivatives **13**−**15**[a]



[a] Reagents and conditions: (a) $Pd(Ph_3P)_4$, aq Na_2CO_3, toluene/EtOH; (b) aq Km_nO_4; (c) $Pd(Ph_3P)_4$, aq Na_2CO_3, CH_3CN; (d) H_2, 5% Pd/C,EtOH.

ality with an amino group (**31**, **35**), using as well the nitro precursors (**30**, **34**).

Chemistry

Terphenyl compounds (**13**−**15**) were synthesized through double Suzuki coupling in a similar way as previously described (Scheme 1).[9,19]

The desired 2′-, 3′-, or 4′-bromobiphenyl carboxylic acids (**9**−**11**) underwent Suzuki cross-coupling with the boronic acid **12**[16] in acetonitrile to obtain the terphenyl compounds, then deprotected at phenol moiety to give **13**−**15**.

Isoxazolines **24**,**25** and isoxazoles **28**−**31**, **32**−**35** were prepared from aldehyde **16** in good yields as described in Schemes 2 and 3.

Phenolic function of **16** was acetylated by heating in acetic anhydride at reflux temperature or methylated with iodomethane to give intermediates **17a**,**b**.

Oximes **18a**,**b** were obtained from aldehydes **17a**,**b** by reaction with hydroxylamine in high yields, while Wittig reaction's of **17a**,**b** with methyltriphenylphosphonium bromide yielded olefins **19a**,**b**.

The nitrile oxides generated from oximes **18a**,**b** following Torssell's procedure[20] underwent a [3 + 2] regioselective cycloaddition with alkenes **20a**,**b** to produce isoxazolines **21a**−**c** in good yields. In the same manner, isoxazolines **23a**−**c** were obtained from nitrile oxides generated from oximes **22a**,**b**, in turn obtained from commercial aldehydes and hydroxylamine, and alkenes **19a**,**b**. Oxidation of the isoxazoline rings of **21a**−**c** and **23a**−**c** was easily accomplished by reaction with *N*-bromosuccinimide-triethylamine in methylene chloride to the desired isoxazoles **26a**−**c** and **27a**−**c** in good yield (Scheme 3).

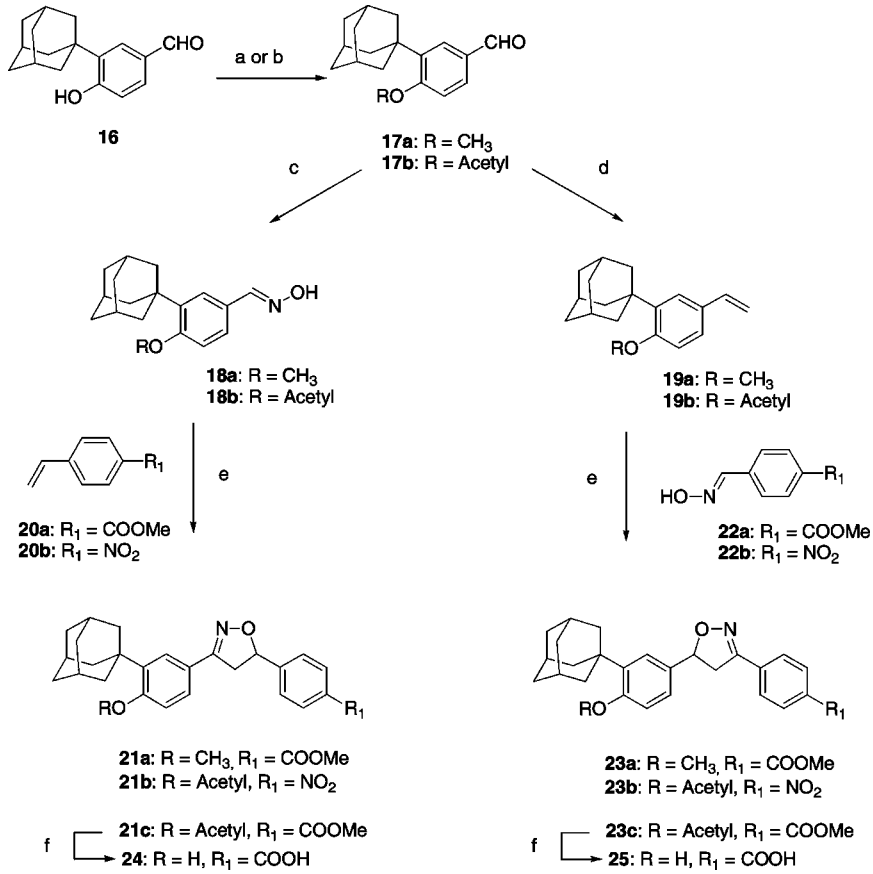
Alkaline hydrolysis of isoxazolines **21c** and **23c** yielded compounds **24** and **25**. In the same manner, isoxazoles **28**−**30** and **32**−**34** were obtained from esters **26a**−**c** and **27a**−**c**, respectively. Finally, reduction of nitro function in compounds **30** and **34** by zinc in acetic acid led to amino derivatives **31** and **35**.

Results and Discussion

Figure 1 shows the effects of novel terphenyl, diarylisoxazole, and diarylisoxazoline derivatives at different concentrations on HL60 cell growth. All compounds caused a cell growth inhibition when used at concentrations higher than 10 μM. Compounds **15** and **33** were the most active antiproliferative agents, showing an IC_{50} of 8 and 3 μM, respectively.

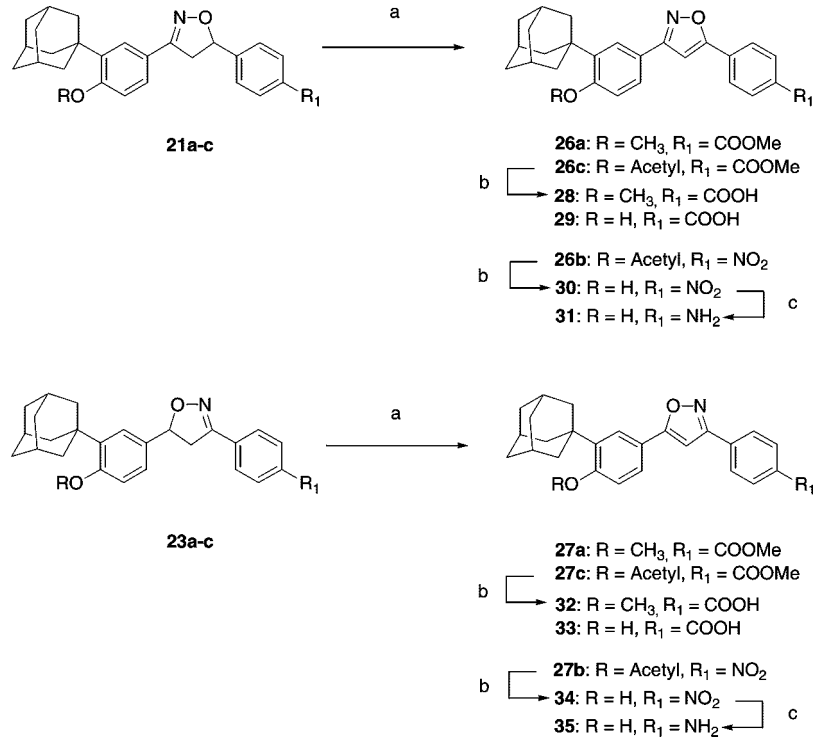
In contrast, compounds **30**, **34**, and **25** caused an increase of cell growth when used at low (<10 μM) concentration.

Scheme 2. Synthesis of 3,5-Diaryl Isoxazolines **24** and **25** and of Intermediates for 3,5-Diarylisoxazoles[a]



[a] Reagents and conditions: (a) MeI, KOH, DMSO; (b) Ac_2O reflux; (c) $NH_2OH \times HCl$, Na HCO_3, MeOH; (d) $MeP(Ph)_3Br$, NaH, THF; (e) NCS, chloroform, then triethylamine; (f) LiOH, water/MeOH.

Scheme 3. Synthesis of 3,5-Diarylisoxazoles **28**−**35**[a]



[a] Reagents and conditions: (a) NBS, CCl_4, reflux; (b) LiOH, water/MeOH; (c) Zn, AcOH.

Compound **30** used at a concentrations of 2.5 μM was the most active in improving cell growth; it was able to increase the percentage of cells in S and G_2−M phases of the cell cycle with a decrease in the percentage of cells in G_0−G_1 phase

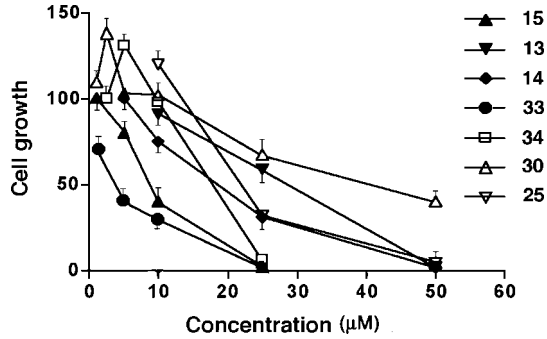


Figure 1. Effects of novel terphenyl, diarylisoxazole, and diarylisoxazoline derivatives on HL60 cell growth. Cells were growth in complete medium in presence of different concentrations (ranging from 1 to 50 μM) of each compound. The number of living cells was determined after 48 h of culture and expressed as percentage of the nontreated control. Bars represent ± SE of three independent experiments.

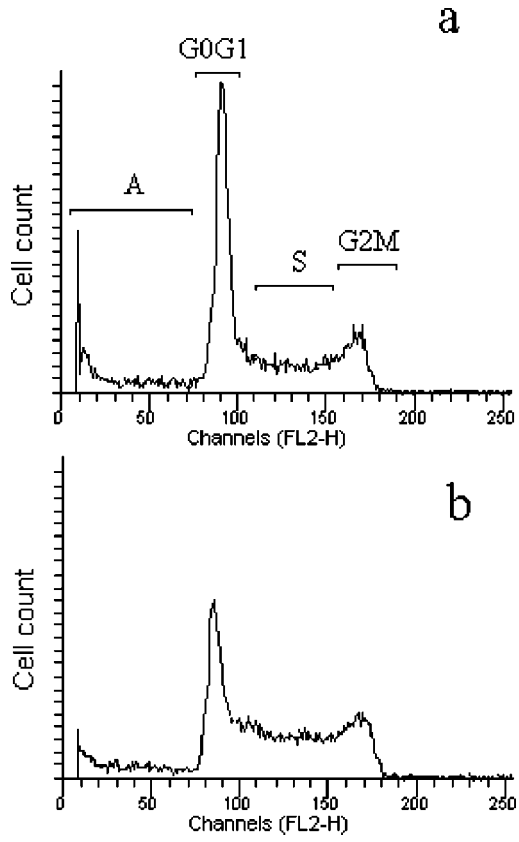


Figure 2. Cell cycle distribution of HL60 cells (a) and HL60 cells exposed 24 h to 2.5 μM compound **30** (b). Cell cycle was evaluated by flow cytometric analysis after staining cells with propidium iodide as reported in experimental section. A = apoptosis.

(Figure 2). This observation suggests that compound **30** might stimulate cell growth by promoting the G_1−S transition.

In a second phase of our study, we evaluated whether compounds **30**, **34**, and **25** were able to support growth and to avoid death of cells cultured in a serum-free medium. As is well-known, serum is an essential constituent of a cell culture medium to support the in vitro growth of continuous cell lines.

Accordingly, HL60 cells were washed twice with PBS and suspended in a serum-free medium constituted by only RPMI 1640. After 72 h, cells were harvested and the number of living cells was determined. The percentage of apoptotic cells was evaluated as reported in the Experimental Section. As reported in Figure 3a, cells cultured 72 h in serum-free medium showed

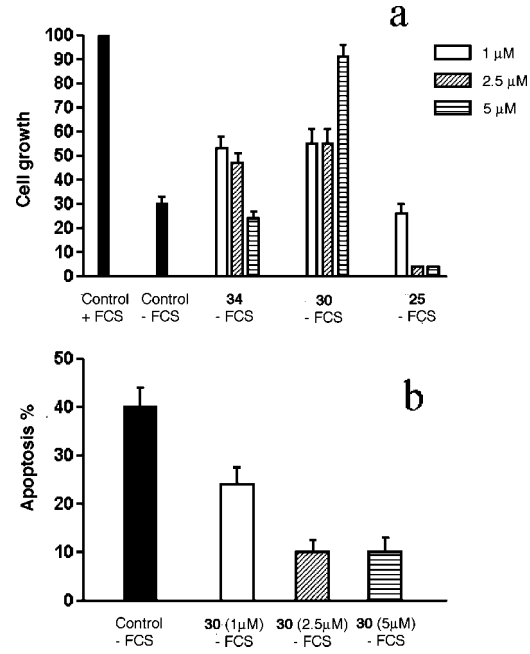


Figure 3. (a) Effects of compounds **34**, **30**, and **25** on cell growth of HL60 cells growing in serum-free medium (-FCS). (b) Effects of compound **30** on apoptosis induced by serum deprivation. Bars represent ± SE of three independent experiments.

a cell growth inhibition of 70% compared to cells growing in a complete medium.

Compound **30** was able to support growth of cells cultured in serum-free medium especially when used at the concentration of 5 μM (Figure 3a). Compound **34** was less effective than **30**, although it was able to support in part cell growth when used at 1 and 2.5 μM concentrations. In contrast, compound **25** (1 μM) did not show any cell growth stimulating activity in serum-free medium and, at 2.5 and 5 μM concentrations, it caused instead a potent cell growth inhibition.

Compound **30** was also able to inhibit apoptosis of cells cultured in serum-free medium. As shown in Figure 3b, serum deprivation induced apoptosis in about 40% of HL60 control cells (evaluation after 72 h of culture), whereas addition with 2.5 or 5 μM of **30**, reduced the percentage of apoptosis to only 10%. Compounds **2** and **3** were able to decrease the percentage of apoptosis of HL60 from about 40% to 18% and 17%,[9] resulting in being less active than **30** as antiapoptotic agent.

These data indicate that **30** is endowed with both proliferative stimulating and antiapoptotic properties. To better verify this latter property, we tested the ability of **30** to inhibit the apoptotic activity of daunorubicin, a chemotherapic agent that potently induces apoptosis in HL60 cells. After 48 h of exposure to increasing concentrations of daunorubicin alone or in combination with 2.5 μM of **30**, HL60 cells were harvested and the percentage of apoptosis was determined. As shown in Figure 4, **30** was able to halve the percentage of apoptosis induced by daunorubicin.

The anthracyclines, e.g., daunorubicin, idarubicin, epirubicin, and doxorubicin, are widely used cytotoxic drugs in the treatment of hematological malignancies and solid tumors. These agents are especially prone to causing severe tissue damage on extravasation by inducing apoptosis in skin cells. Accidental extravasation has been estimated to occur in 0.5−6% of all patients receiving chemotherapy.[21–23] The local toxicity is characterized by immediate pain, erythema, and swelling at the extravasation site. The ulceration may not appear for several

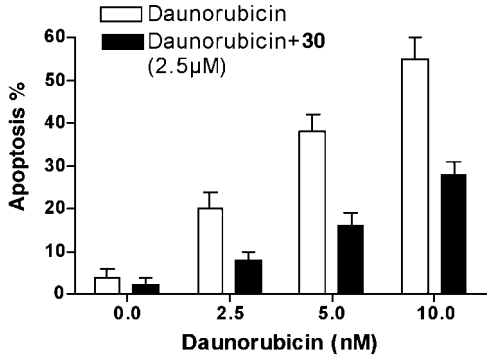


Figure 4. Percentage of apoptosis of HL60 cells after 48 h exposure to different concentrations of daunorubicin in combination or not with compound **30**. Bars represent ± SE of three independent experiments.

days or even weeks and may continue to worsen for months, probably because of drug diffusion into adjacent tissue. Indeed, it has been demonstrated that doxorubicin can persist in the tissue for at least a month. Whereas small ulcerations may heal, large ulcerations require surgical excision. The ability of **30** to inhibit apoptosis induced by daunorubicin suggests that a topical use of this compound could be useful for the treatment of tissue damage caused by extravasation of anthracyclines.

Conclusions

Recently, it has been recognized that apoptosis plays significant roles in various diseases and many trials have been conducted to induce or regulate cell apoptosis with the aim to prevent and treat these diseases. While defective apoptosis predisposes to neoplasia, inappropriate apoptosis in the brain leads to permanent neurological deficits. Disregulated apoptosis has been implicated in several neurodegenerative disorders.

Here, we observed that the modifications of a series of terphenyl compounds displays that the 3,4,5-trimethoxy and 3-adamantyl-4-hydroxy- ring A substitutions lead to derivatives with opposite activity toward cell growth.

Among compounds with carboxylic acid function at ring C, only isoxazolinic **25** has shown a small growth enhancing property, but not in serum-free conditions.

None of the amino compounds were found of interest but, unexpectedly, their nitro precursors **30** and **34** showed interesting cell growth promoting and antiapoptotic activities. In particular, compound **30** was able to support effectively the cell growth of HL60 cultured in serum-free medium, preventing apoptosis induced by serum deprivation. The apoptosis inhibitory effect of **30** was still observed when cells were exposed to the potent apoptotic inducing factor daunorubicin.

In conclusion, our data indicate that compound **30** is a potent cell growth agent endowed with antiapoptotic activity and, therefore, potentially useful in the treatment of diseases in which apoptosis plays a key pathogenetic role, including skin damage by anthracycline extravasation.

Experimental Section

Chemistry. Melting points were obtained with a Kofler apparatus and are uncorrected. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were determined in $CDCl_3$ solution, unless otherwise indicated, with a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. All drying operations were performed over anhydrous sodium sulfate. Column chromatography (medium pressure) was carried out with 60−200 mesh silica gel, using the flash technique. Microanalysis of all new final synthesized compounds agreed with calculated values within ± 0.4% of the theoretical.

4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-benzaldehyde (17a). To a suspension of KOH pulverized (450 mg, 7.9 mmol) in DMSO (10 mL) that was stirred for 15 min was added **12** (500 mg, 1.96 mmol) and then iodomethane (0.25 mL, 3.9 mmol). After stirring for 2 h at room temperature, dichloromethane (30 mL) was added and the mixture was poured into ice−water and extracted three times with dichloromethane. The combined organic extracts were washed with water (15 mL × 3), dried, and evaporated, and the residue was chromatographed on silica gel (light petroleum−ethyl acetate 9/1) to give compound **17a** (oil, 380 mg, yield 73%). ^{1}H NMR δ 1.79 (s, 6H); 2.11 (s, 9H); 6.97 (d, J = 8.4, 1H), 7.70−7.79 (m, 2H,); 9.89 (s, 1H).

Acetic Acid 4-Formyl-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (17b). A solution of **12** (4.3 g, 17 mmol) in acetic anhydride (20 mL) was refluxed for 1 h. After evaporation, diethyl ether (30 mL) was added to the residue and washed with 5% $NaHCO_3$ (15 mL), water, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography to give compound **17b** (oil, 4.28 g, yield 85%). ^{1}H NMR δ 1.77 (s, 6H), 2.12 (s, 9H), 2.40 (s, 3H), 7.17 (d, J = 8.1, 1H) 7.75 (dd, J = 8.1, J = 1, 1H), 7.90 (d, J = 1, 1H), 9.97 (s, 1H).

General Procedure for Oximes. To a solution of hydroxylamine hydrochloride (250 mg, 3.7 mmol) dissolved in water (7 mL), $NaHCO_3$ (470 mg, 5.6 mmol) was added portionwise at 0 °C, and the mixture stirred for 30 min at room temperature. The appropriate aldehyde (3.1 mmol), dissolved in methanol (5 mL), was then added to the solution and stirring was continued for additional 6 h. Methanol was evaporated in vacuo and the residue extracted with diethyl ether. The organic extracts were washed with brine, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel (diethyl ether-light petroleum).

4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-benzaldehyde Oxime (18a). Yield 75%; mp 158−160 °C. ^{1}H NMR δ 1.77 (s, 6H), 2.09 (s, 9H), 3.86 (s, 3H), 6.87 (d, J = 8.3, 1H), 7.33−7.38 (m, 2H), 7.48 (s, 1H), 8.09 (s, 1H).

Acetic Acid 4-(Hydroxyimino-methyl)-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (18b). Yield 98%, oil. ^{1}H NMR δ 1.77 (s, 6H), 2.02 (s, 9H), 2.37 (s, 3H), 7.02 (d, J = 8.1, 1H), 7.42 (dd, J = 8.1, 2, 1H), 7.58 (d, J = 2, 1H), 8.12 (s, 2H).

4-(Hydroxyiminomethyl)benzoic Acid Methyl Ester (22a). Yield 80%; mp 119−122 °C. IR (KBr) 1726, 1609, 1438, 1284, 1110, 966, 763. ^{1}H NMR δ 3.9 (s, 3H); 7.27 (s, 1H); 7.69 (d, J = 8.4, 2H); 8.06 (d, J = 8.3, 2H); 8.17 (s, 1H).

General Procedure for Olefins. NaH (1.2 eq), was added to a stirred suspension of methyltriphenylphosphonium bromide (1.07 g, 3 mmol) in dry THF (15 mL) containing the appropriate aldehyde (3 mmol). After stirring for 5 h at room temperature, diethyl ether (30 mL) was added and the mixture was poured into ice−water and extracted with Et_2O. The combined organic extracts were dried and evaporated, and the residue was chromatographed on silica gel (diethyl ether-light petroleum).

1-(2-Methoxy-5-vinyl-phenyl)-tricyclo[3.3.1.1^{0,0}]decane (19a). Yield 65%; oil. ^{1}H NMR δ 1.78 (s, 6H), 2.11 (s, 9H), 3.83 (s, 3H), 5.12 (d, J = 11.1, 1H), 5.61 (d, J = 17.6, 1H), 6.68 (dd, J = 17.6, J = 11.1, 1H), 6.84 (d, J = 8.4, 1H), 7.22−7.30 (m, 2H).

Acetic Acid 2-Tricyclo[3.3.1.1^{0,0}]dec-1-yl-4-vinyl-phenyl Ester (19b). Yield 79%; oil. ^{1}H NMR δ 1.79 (s, 6H), 2.03 (s, 9H), 2.36 (s, 3H), 5.23 (dd, J = 11, J = 0.6, 1H), 5.70 (dd, J = 17, J = 0.6, 1H), 6.71 (dd, J = 17, J = 11, 1H), 6.96 (d, J = 8.2, 1H), 7.29 (dd, J = 8.2, J = 2.1, 1H), 7.31 (d, J = 2.1, 1H).

4-Vinylbenzoic Acid Methyl Ester (20a). Yield 60%; oil. IR (neat) 1724, 1608, 1436, 1278, 1107, 782. ^{1}H NMR δ 3.91 (s, 3H), 5.38 (d, J = 10, 1H), 5.96 (d, J = 17.6, 1H), 6.75 (dd, J = 17.6, J = 10, 1H), 7.46 (d, J = 8, 2H), 7.99 (d, J = 8, 2H).

General Procedure for Terphenyl Synthesis. To a solution of bromo biphenyl acid (**9**−**11**) (100 mg, 1 eq) and boronic acid **12** (270 mg, 2 eq) in 0.4 M aqueous sodium carbonate (5 mL) and acetonitrile (5 mL) was added a catalytic amount (5% mole) of

tetrakis triphenylphosphine palladium, and the mixture was heated to reflux under argon atmosphere for 3 h. The suspension was cooled, filtered, and washed with dichloromethane (40 mL). Water phase was then acidified and extracted with diethyl ether. Purification of the residue of ether extracts gave the O-protected terphenyl products.

General Procedure for Removal of the Benzyl-Protection Group. To a solution of the appropriate benzyl protected derivative (0.11 mmol) in abs EtOH (15 mL) was added Pd/C 5% (catalytic amount). The reaction was stirred in hydrogen atmosphere for 15 h. The mixture was filtered over celite and the solution evaporated. The residue was chromatographed on silica gel (light petroleum-ethyl acetate).

4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-[1,1′;4′,1″]terphenyl-4″-carboxylic Acid (13). Yield 40%; mp 300 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 1.71 (s, 6H), 2.01 (s, 9H), 5.76 (s, 1H), 7.46−7.52 (m, 4H), 7.72−7.82 (m, 5H), 8.01 (d, J = 8.2, 2H), 12.96 (br, 1H). Anal. ($C_{29}H_{28}O_3$) C, H.

4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-[1,1′;3′,1″]terphenyl-4″-carboxylic acid (14). Yield 35%; mp 263−265 °C. ^{1}H NMR δ 1.80 (s, 6H), 2.11 (s, 3H), 2.19 (s, 6H), 5.12 (s, 1H), 6.76 (d, J = 8.0, 1H), 7.34 (dd, J = 8.0, J = 1.8, 1H), 7.48−7.60 (m, 4H), 7.73−7.77 (m, 3H), 8.20 (d, J = 8.0, 2H), 12.47 (br, 1H). Anal. ($C_{29}H_{28}O_3$) C, H.

4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-[1,1′;2′,1″]terphenyl-4″-carboxylic Acid (15). Yield 47%; mp 179−181 °C. ^{1}H NMR (CD_3OD) δ 1.86 (s, 6H), 1.92 (s, 9H), 5.49 (s, 1H), 6.60 (d, J = 8.1, 1H), 6.70 (d, J = 2.1, 1H), 6.86 (dd, J = 8.1, J = 2.1, 1H), 7.09 (d, J = 8.1, 2H), 7.35−7.40 (m, 4H), 7.86 (d, J = 8.1, 2H), 12.3 (br, 1H). Anal. ($C_{29}H_{28}O_3$) C, H.

General Procedure for Isoxazolines. A mixture of *N*-chlorosuccinimide (174 mg, 1.3 mmol), pyridine (2 drops) and oxime (1.3 mmol) in anhydrous $CHCl_3$ (15 mL) was stirred for 1 h at 50−60 °C. Olefin (1.4 mmol) was then added followed by triethylamine (0.27 mL, 1.95 mmol) in $CHCl_3$ (5 mL). After stirring at 25 °C for 20 min, water was added and the organic phase was washed with 2.5% HCl and water, then dried and evaporated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate-light petroleum).

4-[3-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-5-yl]-benzoic Acid Methyl Ester (21a). Yield 80%. ^{1}H NMR δ 1.76 (s, 6H), 2.09 (s, 9H), 3.30 (dd, J = 16.1, J = 7.9, 1H), 3.76−3.93 (m, 7H), 5.75 (dd, J = 11.2, J = 7.9, 1H), 6.87 (d, J = 8.4, 1H), 7.42−7.49 (m, 3H), 7.62 (s, 1H), 7.65 (d, J = 6.6, 2H), 8.04 (dd, J = 8.2, J = 1.8, 2H).

Acetic Acid 4-[5-(4-Nitro-phenyl)-4,5-dihydro-isoxazol-3-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (21b). Yield 42%. ^{1}H NMR δ 1.78 (s, 6H), 2.01 (s, 6H), 2.09 (s, 3H), 2.37 (s, 3H), 3.29 (dd, J = 16.4, J = 7.2, 1H), 3.88 (dd, J = 16.8, J = 11.2, 1H), 5.83 (dd, J = 10.8, J = 7.2, 1H), 7.04 (d, J = 8.4, 1H), 7.46 (dd, J = 8.4, J = 2.0, 1H), 7.55−7.58 (m, 2H), 7.74 (d, J = 2.0, 2H), 8.22−8.25 (m, 2H).

4-[3-(4-Acetoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-5-yl]-benzoic Acid Methyl Ester (21c). Yield 82%. ^{1}H NMR δ 1.77 (s, 6H), 2.02 (s, 9H), 2.37 (s, 3H), 3.30 (dd, J = 16.3, 7.7, 1H), 3.83 (dd, J = 16.5, J = 11, 1H), 3.92 (s, 3H), 5.79 (dd, J = 11, J = 7.6, 1H), 7.04 (d, J = 8.3, 1H), 7.43−7.50 (m, 3H), 7.55 (d, J = 2, 1H), 8.05 (d, J = 8.2, 2H).

4-[5-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-3-yl]-benzoic Acid Methyl Ester (23a). Yield 53%. ^{1}H NMR δ 1.76 (s, 6H), 2.07 (s, 9H), 3.36 (dd, J = 16.4, J = 8.6, 1H), 3.65−3.83 (m, 4 H), 3.93 (s, 3H), 5.72 (dd, J = 11,2, J = 8.6, 1H), 6.86 (d, J = 8.8, 1H), 7.19−7.24 (m, 2H), 7.77 (d, J = 8.1, 2H), 8.07 (d, J = 8.2, 2H).

Acetic Acid 4-[3-(4-Nitro-phenyl)-4,5-dihydro-isoxazol-5-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (23b). Yield 45%. ^{1}H NMR δ 1.76 (s, 6H), 2.0 (s, 6H), 2.08 (s, 3H), 2.35 (s, 3H), 3.44 (dd, J = 17.0, J = 8.0, 1H), 3.78 (dd, J = 17.0, J = 11.0, 1H), 5.80 (dd, J = 11.0, J = 8.0, 1H), 7.0 (d, J = 8.4, 1H), 7.22 (dd, J = 8.2, J = 1.1, 1H), 7.85 (d, J = 9.0, 2H), 8.27 (d, J = 9.0, 2H).

4-[5-(4-Acetoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-3-yl]-benzoic Acid Methyl Ester (23c). Yield 44%. ^{1}H NMR δ 1.76 (s, 6H), 2.01 (s, 9H), 2.35 (s, 3H), 3.37 (dd, J = 16.7, J = 8.6, 1H), 3.76 (dd, J = 16.7, J = 11, 1H), 3.93 (s, 3H), 5.76 (dd, J = 11, J = 8.6, 1H), 7.01 (d, J = 8.2, 1H), 7.24 (dd, J = 8.2, J = 2, 1H), 7.36 (d, J = 2, 1H), 7.65 (d, J = 6.6, 2H), 8.07 (d, J = 6.7, 2H).

General Procedure for Isoxazoles. To a solution of the isoxazoline (0.25 mmol) in carbon tetrachloride (10 mL), *N*-bromosuccinimide (66 mg, 0.37 mmol) was added and the mixture was gently refluxed for 3 h. Hydrogen bromide was slowly liberated. The cooled solution was filtered from the precipitated succinimide, washed with 5% aqueous sodium hydroxide and then with water until the organic phase became clear. The organic layer was dried and the solvent removed in vacuo. The residue was chromatographed on silica gel (ethyl acetate−light petroleum).

4-[3-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-5-yl]-benzoic Acid Methyl Ester (26a). Yield 81%. ^{1}H NMR δ 1.80 (s, 6H), 2.15 (s, 9H), 3.90 (s, 3H), 3.96 (s, 3H), 6.90 (s, 1H), 6.97 (d, J = 8.2, 1H), 7.66−7.75 (m, 2H), 7.92 (d, J = 8.4, 2H) 8.16 (d, J = 8.4, 2H).

Acetic Acid 4-[5-(4-Nitro-phenyl)-isoxazol-3-yl]-2-tricyclo-[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (26b). Yield 54%. ^{1}H NMR δ 1.72 (s, 6H), 2.02 (s, 9H), 2.33 (s, 3H), 6.92 (s, 1H), 7.07 (d, J = 8.4, 1H), 7.62 (dd, J = 8.4, J = 2.2, 1H), 7.82 (d, J = 2.2, 1H), 7.93−7.97 (m, 2H), 8.28−8.33 (m, 2H).

4-[3-(4-Acetoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-5-yl]-benzoic Acid Methyl Ester (26c). Yield 81%. ^{1}H NMR δ 1.80 (s, 6H), 2.15 (s, 9H), 3.90 (s, 3H), 3.96 (s, 3H), 6.90 (s, 1H), 6.97 (d, J = 8.2, 1H), 7.66−7.75 (m, 2H), 7.92 (d, J = 8.4, 2H) 8.16 (d, J = 8.4, 2H).

4-[5-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-3-yl]-benzoic Acid Methyl Ester (27a). Yield 62%. ^{1}H NMR δ 1.80 (s, 6H), 2.14 (s, 9H), 3.89 (s, 3H), 3.95 (s, 3H), 6.75 (s, 1H), 6.96 (d, J = 8.4, 1H), 7.65−7.70 (m, 2H), 7.95 (d, J = 8.4 Hz, 2H), 8.15 (d, J = 8.4, 2H).

Acetic Acid 4-[3-(4-Nitro-phenyl)-isoxazol-5-yl]-2-tricyclo-[3.3.1.1^{0,0}]dec-1-yl-phenyl Ester (27b). Yield 81%. ^{1}H NMR δ 1.81 (s, 6H), 2.07 (s, 6H), 2.14 (s, 3H), 2.40 (s, 3H), 6.86 (s, 1H), 7.14 (dd, J = 8.0, J = 2.0, 1H), 7.84 (d, J = 2.0, 1H), 8.05−8.07 (m, 2H), 8.34−8.37 (m, 2H).

4-[5-(4-Acetoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-3-yl]-benzoic Acid Methyl Ester (27c). Yield 65%. ^{1}H NMR δ 1.61 (s, 6H), 2.08 (s, 9H), 2.40 (s, 3H), 3.96 (s, 3H), 6.84 (s, 1H), 7.13 (d, J = 8.4, 1H), 7.69 (dd, J = 8.4, J = 2.1, 1H), 7.84 (d, J = 2.1, 1H), 7.95 (d, J = 8.3, 2H), 8.15 (d, J = 8.3, 2H).

General Procedure for Ester Hydrolysis. A mixture of ester (1 mmol), methanol (10 mL), water (6−7 mL), and lithium hydroxide (40 mg, 1.5 mmol or double amount for acetyl derivatives) was allowed to stand at 50−60 °C for 24 h. The solution was concentrated in vacuo to remove methanol, and the remaining aqueous solution was extracted with diethyl ether to separate trace amounts of unreacted ester. The aqueous solution was acidified with 1 M hydrochloric acid and extracted with three portions of ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium chloride and dried. Removal of the solvent under reduced pressure afforded a residue, which was chromatographed on silica gel (eluent: ethyl acetate−light petroleum).

4-[3-(4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-5-yl]-benzoic Acid (24). Yield 90%; mp 251−253 °C (dec). ^{1}H NMR (DMSO-d_6) δ 1.72 (s, 6H), 2.06 (s, 9H), 3.27−3.39 (m, 1H), 3.86 (dd, J = 17.1, J = 11, 1H), 5.75 (dd, J = 10.7, J = 7.4, 1H), 6.82 (d, J = 8.4, 1H), 7.34 (d, J = 8.4, 1H), 7.44−7.51 (m, 3H), 7.95 (d, J = 8, 2H), 9.86 (s, 1H), 12.98 (br, 1H). Anal. ($C_{26}H_{27}NO_4$), C, H, N.

4-[5-(4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-4,5-dihydro-isoxazol-3-yl]-benzoic Acid (25). Yield 94%; mp 240−242 °C (dec). ^{1}H NMR (DMSO-d_6) δ 1.71 (s, 6H), 2.05 (s, 9H), 3.38−3.46 (m, 1H), 3.79 (dd, J = 16.4, J = 11.2, 1H), 5.63 (dd, J = 11.2, J = 8.3, 1H), 6.77 (d, J = 8.1, 1H), 7.03−7.10 (m, 2H), 7.82 (d, J = 8.3, 2H), 8.0 (d, J = 8.3, 2H), 9.43 (s, 1H), 12.90 (br, 1H). Anal. ($C_{26}H_{27}NO_4$), C, H, N.

4-[3-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-5-yl]-benzoic Acid (28). Yield 98%; mp 230 °C (dec). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 6H), 2.09 (s, 9H), 3.87 (s, 3H), 7.14 (d, J = 8.4, 1H), 7.70−7.77 (m, 3H), 8.02−8.09 (m, 4H), 13.15 (br, 1H). Anal. ($C_{27}H_{27}NO_4$), C, H, N.

4-[5-(4-Methoxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-3-yl]-benzoic Acid (32). Yield 95%; mp 230 °C (dec). ^{1}H NMR (CD_3OD) δ 1.76 (s, 6H), 2.09 (s, 9H), 3.88 (s, 3H), 7.16 (d, J = 8.8, 1H), 7.62 (s, 1H), 7.68 (d, J = 1.8, 1H), 7.76 (d, J = 8.8, J = 1.8, 1H), 8.03−8.10 (m, 4H), 13.15 (br, 1H). Anal. ($C_{27}H_{27}NO_4$), C, H, N.

4-[3-(4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-5-yl]-benzoic Acid (29). Yield 95%; mp 251−253 °C (dec). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 6H), 2.10 (s, 9H), 6.91 (d, J = 8.1, 1H), 7.57−7.70 (m, 3H), 8.01−8.12 (m, 4H), 9.88 (s, 1H), 13.25 (br, 1H). Anal. ($C_{26}H_{25}NO_4$), C, H, N.

4-[5-(4-Hydroxy-3-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenyl)-isoxazol-3-yl]-benzoic Acid (33). Yield 95%; mp 241 °C (dec). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 6H), 2.06 (s, 9H), 6.93 (d, J = 8.1, 1H), 7.51 (s, 1H), 7.59 (d, J = 8.4, 2H), 8.04−8.09 (m, 4H), 10.06 (s, 1H), 13.20 (br, 1H). Anal. ($C_{26}H_{25}NO_4$), C, H, N.

4-[5-(4-Nitro-phenyl)-isoxazol-3-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenol (30). Yield 85%; mp 246−248 °C. ^{1}H NMR δ 1.80 (s, 6H), 2.16 (s, 9H), 6.82 (d, J = 8.0, 1H), 6.95 (s, 1H), 7.56 (dd, J = 8.0, J = 2.0, 1H), 7.72 (d, J = 2.0, 1H), 8.01 (d, J = 9.2, 2H), 8.34 (d, J = 9.2, 2H). ^{13}C NMR δ 20.7, 29.0, 29.8, 37.1, 38.0, 40.2, 40.5, 46.9, 100.1, 110.3, 117.4, 120.8, 124.5, 125.6, 126.1, 126.6, 133.1, 156.7, 167.5. Anal. ($C_{25}H_{24}N_2O_4$) C, H, N.

4-[3-(4-Nitro-phenyl)-isoxazol-5-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenol (34). Yield 84%; mp 250−253 °C. ^{1}H NMR δ 1.81 (s, 6H), 2.13−2.16 (m, 9H), 6.76 (s, 1H), 6.77 (d, J = 8.4, 1H), 7.57 (dd, J = 8.4, J = 2.0, 1H), 7.70 (d, J = 2.0, 1H), 8.03−8.07 (m, 2H), 8.32−8.37 (m, 2H). Anal. ($C_{25}H_{24}N_2O_4$) C, H, N.

General Procedure for Reduction of Nitro Compounds. To a solution of nitro compound **30** or **34** (1 mmol, 415 mg) in acetic acid (15 mL) is added Zn powder (100 mmol, 6.5 g). The suspension is stirred for 2 h at room temperature. The reaction mixture is filtered over Celite and concentrated. The crude material is dissolved in ethyl acetate (15 mL) and washed with sodium bicarbonate 5% (5 mL), brine (5 mL), dried (Na_2SO_4), and concentrated to afford the crude amino compound which is chromatographed on silica gel.

4-[5-(4-Amino-phenyl)-isoxazol-3-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenol, Hydrochloride (31). Yield 72%; mp 189−191 °C. ^{1}H NMR (CD_3OD) δ 1.83 (s, 6H), 2.07 (s, 3H), 2.20 (s, 6H), 6.83 (d, J = 8.2, 1H), 7.25 (s, 1H), 7.50−7.56 (m, 3H), 7.68 (d, J = 2.2, 2H), 8.04 (d. J = 8.6, 2H). ^{13}C NMR (CD_3OD) δ 30.6, 38.0, 38.2, 41.4, 53.6, 99.3, 117.7, 120.7, 123.7, 126.4, 126.6, 128.0, 138.2, 159.9, 165.2, 170.0. Anal. ($C_{25}H_{27}ClN_2O_2$) C, H, N.

4-[3-(4-Amino-phenyl)-isoxazol-5-yl]-2-tricyclo[3.3.1.1^{0,0}]dec-1-yl-phenol, Hydrochloride (35). Yield 80%; mp 149−152 °C. ^{1}H NMR (CD_3OD) δ 1.83 (s, 6H), 2.08 (s, 3H), 2.20 (s, 6H), 6.84 (d, J = 8.4, 1H), 7.01 (s, 1H), 7.48−7.66 (m, 4H), 8.05 (d, J = 8.6, 2H). ^{13}C NMR (CD_3OD) δ 29.4, 36.9, 37.0, 40.1, 95.3, 116.6, 118.5, 123.3, 124.5, 124.6, 128.4, 128.8, 128.9, 130.0, 131.8, 132.0, 132.6, 133.1, 137.2, 159.0, 172.3. Anal. ($C_{25}H_{27}ClN_2O_2$) C, H, N.

Materials and Methods for Biological Assays. Cell Culture and Culture Conditions. HL60 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 (Gibco Grand Island, NY) containing 10% FCS (Gibco), 100 U/mL penicillin (Gibco), 100 mg/mL streptomycin (Gibco), and 2 mM L-glutamine (Sigma Chemical Co., St. Louis, MO) in a 5% CO_2 atmosphere at 37 °C. The employed serum-free medium is constituted by RPMI 1640 and ITS+1 liquid media supplement (Sigma). ITS+1 liquid media supplement contains 0.5% delipidated bovine serum albumin, 10 g/mL insulin, 5.5 g/mL transferrin, 4.7 g/mL linoleic acid, and 2 mM L-glutamine (Sigma).

Cytotoxicity Assays. To evaluate the number of live and dead cells, cells were stained with trypan blue and counted on a hemocytometer. Cells that showed trypan blue uptake were interpreted as nonviable.

Morphological Evaluation of Apoptosis and Necrosis. Drugs effects on apoptosis and necrosis were determined morphologically by fluorescence microscopy after labeling with acridine orange and ethidium bromide. Cells (2×10^5) were centrifuged (300g), and the pellet was resuspended in 25 L of the dye mixture. An amount of 10 μL of the mixture was placed on a microscope slide, covered with a 22 mm^2 coverslip, and examined in oil immersion with a 100× objective using a fluorescence microscope. Live cells were determined by the uptake of acridine orange (green fluorescence) and exclusion of ethidium bromide (red fluorescence) stain. Live and dead apoptotic cells were identified by perinuclear condensation of chromatin stained by acridine orange or ethidium bromide, respectively, and by the formation of apoptotic bodies. Necrotic cells were identified by uniform labeling with ethidium bromide.

Determination of Apoptosis by Annexine V. Cells (1×10^6) were washed with PBS and centrifugated at 200g for 5 min. The cell pellet was suspended in 100 μL of staining solution containing annexine-V-fluorescein labeling reagent (Annexine-V-Fluos Staining Kit, Roche Molecular Biochemicals, Mannheim, Germany) and incubated for 15 min at 20 °C. Annexine V positive cells were evaluated by fluorescence microscopy and flow cytometry.

Flow Cytometric Analysis of Cell Cycle Distribution. The effects of the most active compounds of the series on cell cycle distribution were studied on K562 cells (myeloblastic leukemia) by flow cytometric analysis after staining with propidium iodide. Cells were exposed 24 h to each compound. After treatment cells were washed once in ice-cold phosphate buffered saline medium (PBS, Sigma) and resuspended at 1×10^6 mL in a hypotonic fluorochrome solution containing propidium iodide (Sigma, St Louis, MO) 50 μg/mL in 0.1% sodium citrate plus 0.03% (v/v) nonidet P-40 (Sigma). After 30 min of incubation, the fluorescence of each sample was analyzed as single-parameter frequency histograms by using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). The distribution of cells in the cell cycle was analyzed with the ModFit LT3 program (Verity Software House, Inc.).

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Supporting Information Available: Elemental analysis of target derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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