# Synthesis and Biological Activity of Isoxazolidinyl Polycyclic Aromatic Hydrocarbons: Potential DNA Intercalators

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Isoxazolidinyl polycyclic aromatic hydrocarbons (isoxazolidinyl-PAHs) have been synthesized in good yields by 1,3-dipolar cycloaddition methodology promoted by microwave irradiation. The structures of the obtained cycloadducts have been determined by NOE experiments and supported by computational studies at the AM1 level. The cytotoxicity and antiviral activity of the synthesized compounds have been investigated. In particular, compounds **7c** and **7e** show high levels of cytotoxicity on MOLT-3 leukemia cells; **7e** exerts a remarkable enhancing activity on apoptosis caused by anti-fas antibody addition. Furthermore, compounds **7b** and **8b** exhibit specific antiviral effects against the Punta Toro virus.

## Introduction

Research on the development of nonpeptide-based DNA interactive drugs has been intense, since such agents offer the potential to interact with their intended target without falling hostage to cellular peptidases.[1] Of the established drug−DNA associative methods, intercalation is one of the most predictable, and a variety of natural and synthetic agents that function using this strategy show excellent antitumor activity. Pertinent examples include the anthracyclines such as daunomycin **1** and adriamycin **2**,[2] the acridines such as amsacrine **3**,[3] the ellipticines such as elliptinium acetate **4**,[4] and camptothecin **5**[5] (Figure 1).

The driving force for intercalation relies on the binding energy derived from removal of the agent from aqueous medium, together with van der Waals forces between the agent and DNA.

The primary mode of action of these intercalators is believed to be their reversible binding to nuclear DNA, which causes inhibition of the replication process[6] and hence cell death. Numerous biochemical studies, including evidence from NMR spectroscopy and X-ray crystallography, have shown that daunomycin and adriamycin intercalate into the B-form of the DNA double-stranded helix with guanine−cytosine d(CpG) site-specific interactions.[7] The base pairs above and below the drug "buckle" the conformation so as to afford a distorted DNA helix, thereby preventing association with the DNA helicase, DNA topoisomerase,[8] and DNA and RNA polymerases, i.e., enzymes required to initiate DNA replication, RNA synthesis, protein formation, and cell division.

Several factors play a role in the stabilization of the drug−DNA complex. Anthracyclines are stabilized by electrostatic hydrogen bond and stacking $\pi$-bond interactions between the electron-deficient quinone-based chromophore and the electron-rich purine−pyrimidine bases. Hydrogen bonds play an important part in the stabilization of the complex: an anthracycline

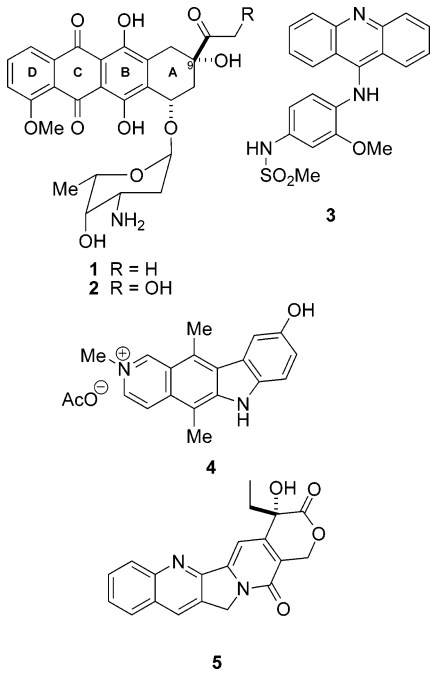


**Figure 1.** Selected DNA intercalating agents.

lacking the hydroxyl group at $C_9$ on the right side of the ring-A (Figure 1) is devoid of anticancer activity.

A wide variety of planar ring systems can intercalate with DNA, giving rise to many drugs that possess chemotherapeutic activity. In this context, new types of antitumor DNA intercalators have been designed; in particular, a series of polycyclic aromatic derivatives containing polar side chains has been systematically investigated.[9] Antitumor activity was discovered in a class of (1-pyrenylmethyl)amino alcohol derivatives, and binding studies have shown that all of these derivatives bind to some extent to DNA by intercalation.[10]

To the same context, the preparation and the intercalative ability of a series of phenanthrene derivatives, in which the side chain structure has been elaborated into a functionalized pyrrolo-[9,10-*b*] nucleus, have been also described.[11]

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**Scheme 1**

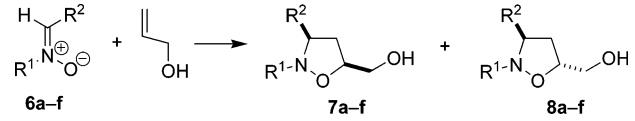



**Table 1.** Reaction of Nitrones **6** with Allyl Alcohol

| entry | nitrone | $R^1$ | $R^2$ | microwave conditions: time (min)[a] | microwave conditions: isolated yield[b] (%) | classical heating[c] (% yield) | **7**/**8** ratio |
|---|---|---|---|---|---|---|---|
| 1 | **6a** | Me | 9-anthryl | 80 | 53 | 1 | 1.1:1.0 |
| 2 | **6b** | Me | 9-phenanthryl | 20 | 83 | 30 | 1.3:1.0 |
| 3 | **6c** | Me | 1-pyrenyl | 20 | 75 | 20 | 1.5:1.0 |
| 4 | **6d** | Bn | 1-pyrenyl | 20 | 85 | 30 | 1.6:1.0 |
| 5 | **6e** | Bn | 9-phenanthryl | 20 | 39 | NR[d] | 1.5:1.0 |
| 6 | **6f** | Bn | 9-anthryl | 80 | NR[e] | NR | |

[a] Irradiation at 90 W; the temperatures reached by the reaction mixture are in the range from 150 to 160 °C. [b] Reaction performed in a pressure tube equipped with a stir bar, in the absence of solvent, using a 1:200 relative ratio of dipole/dipolarophile. [c] Reaction performed in sealed tube in absence of solvent, using a 1:200 relative ratio of dipole/dipolarophile at 130 °C for 48 h. [d] No reaction. [e] When irradiated at 120 W (200−210 °C), only decomposition products are observed.

In this paper, we report the synthesis of isoxazolidinyl-PAHs **7** and **8**, the first members of a new series of potential, nonionic, DNA intercalators featuring the presence of an isoxazolidine ring on the aromatic planar system of an anthracene, phenanthrene, and pyrene nucleus. Our design is supported by the consideration of two principal requirements: (1) to achieve effective intercalation, fused polyaromatic systems of 3−5 rings are desirable and (2) in order to perform useful SAR correlations within a family of intercalative vehicles, regioselective functionalizations of the polycyclic core would be advantageous. The presence of the isoxazolidine ring could ensure the second condition by the possibility of interesting synthetic manipulations, such as the easy cleavage of the N,O-bond leading to the release of a 1,3-amino alcoholic functionality.[12] At the same time, the presence of the hydroxyl group could play an important role in the DNA-recognizing region, because of its ability to form favorable intermolecular hydrogen bonds[13] with the DNA backbone, which could contribute to the formation of stable complexes and then to a better interaction with DNA sites.

Taking into account the pharmacological potential of this class of compounds, the cytotoxicity against a panel of tumor or normal cell lines, the ability to induce and potentiate apoptosis, and the antiviral activity of the synthesized compounds were evaluated.

## Results and Discussion

The cycloaddition of nitrones **6**, prepared by standard procedures[14] with allyl alcohol in a sealed tube at 130 °C for 48 h using a 1:200 relative ratio of dipole to dipolarophile, afforded a mixture of two isoxazolidines **7** and **8** (Scheme 1) in ratio from ca. 1.1:1 to 1.6:1 (Table 1, entries 1−4), with a yield between 1 and 30%; no reaction was observed for entries 5 and 6 (Table 1).

A marked improvement of the cycloaddition process was obtained under microwave irradiation. Thus, the reaction of nitrones **6a**−**e** with allyl alcohol, under microwave irradiation for 20−80 min, afforded the 1,3-dipolar cycloadducts **7a**−**e** and **8a**−**e** in the above-reported relative ratio, but with a total yield in the range 39−85%. No reaction was observed for nitrone **6f** (Table 1, entry 6).

As reported in Table 1, the investigated reaction was found to be regiospecific, affording a mixture of cis and trans 5-substituted isoxazolidines **7** and **8** as exclusive adducts.

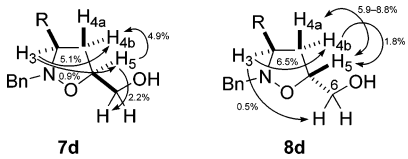



**Figure 2.** Selected observed NOEs in compounds **7d** and **8d**.

**Table 2.** AM1 Calculations Results on Transition State Structures for Isoxazolidines **7** and **8**

| compd | $\Delta H_f$[a] *Z-exo*-TS | $\Delta H_f$[a] *Z-endo*-TS | calcd ratio 7:**8** | $\Delta H^{\ddagger}$[a,b] |
|---|---|---|---|---|
| **a** | 78.78 | 78.95 | 1.3:1 | 25.95 |
| **b** | 65.27 | 65.54 | 1.6:1 | 22.06 |
| **c** | 74.93 | 75.20 | 1.6:1 | 18.96 |
| **d** | 101.93 | 102.32 | 1.9:1 | 22.35 |
| **e** | 92.28 | 92.68 | 1.9:1 | 22.34 |
| **f** | 105.79 | 106.07 | 1.6:1 | 26.30 |

[a] All values are in kcal/mol. [b] Referred to nitrone **6** + allyl alcohol with respect to *Z-exo*-TS.

All products were purified by preparative centrifuge-accelerated radial thin-layer chromatography (PCAR-TLC) and obtained as pure compounds (see Experimental Section). The molecular structure of the reaction products was assigned on the basis of analytical and spectroscopic data. The regiochemistry of the cycloaddition process was readily deduced from the $^1$H NMR measurements. In each case, there was one proton signal at $\delta$ 4.42−4.79, which corresponded to the $H_5$ proton; the alternative regioisomers are not reported to show a resonance at this chemical value.[15]

The pericyclic reaction showed a low level of cis/trans stereoselectivity with the cis isomers **7** as the major products. The relative stereochemical assignments have been performed by NOE measurements. In particular, in compound **7d** irradiation of the $H_3$ resonance induced a positive NOE effect on the downfield resonance of methylene protons at $C_4$ ($H_{4b}$) (5.1%) and on $H_5$ (0.9%), while the irradiation of $H_5$ induced a positive NOE on $H_{4b}$ (4.9%) and the methylene protons at $C_6$ (2.2%). These results indicate that $H_3$, $H_{4b}$, and $H_5$ are in a cis relationship, as reported in Figure 2.

Conversely, for trans adduct **8d**, a diagnostic NOE effect was observed for $H_6$ (0.5%) when irradiating the $H_3$ proton.

The stereochemical outcome of the cycloaddition process can be explained by considering that nitrones **6a**,**b**,**e**,**f** exist exclusively as *Z* isomers, whereas nitrones **6c**,**d** exist as a mixture of *Z*/*E* isomers: NOE data indicated that the *Z* form is predominant (*Z*/*E* ratio = 15:1 for **6c** and 10:1 for **6d**). Thus, the major product **7a**,**b**,**e** could be formed by the *Z*-nitrone reacting in an exo mode, whereas the major products **7c**,**d** could be formed by the *Z*-nitrone reacting in an exo mode or by the *E*-nitrone reacting in an endo mode. AM1 calculations give theoretical support to experimental data; in all the cases the activation enthalpy ($\Delta H^{\ddagger}$) of the cycloaddition reaction starting from *Z*-nitrones is lower than that of *E*-nitrones; therefore, we report only the results derived from the reaction of *Z*-nitrones in an exo and endo mode (Table 2, Figure 3).

The data reported in Table 2 are in satisfactory agreement with the experimental results; moreover, the activation enthalpy for the reaction that involves nitrone **6f** is the highest of the series, thus lending a possible explanation for the failure of this cycloaddition. Probably, the activation enthalpy value of about 26 kcal/mol is borderline to the energetic requirement for the occurrence of the reactions at hand and increasing the temperature causes a decomposition of the reagents and/or products.

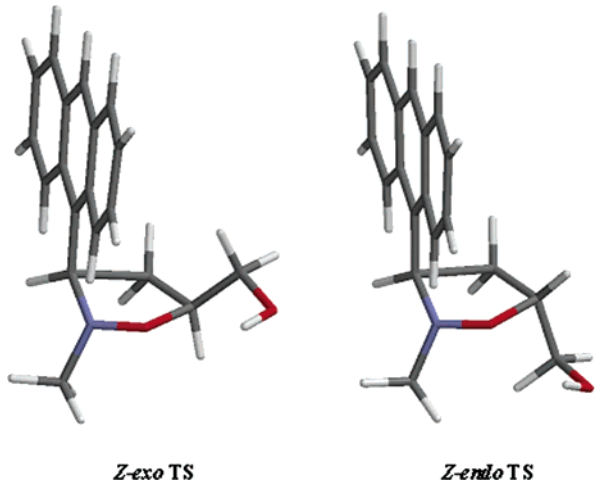
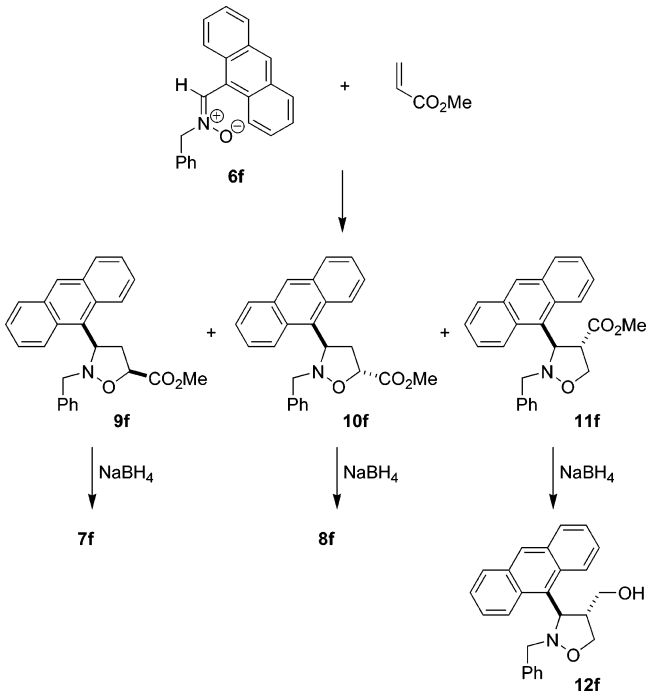



**Figure 3.** Transition states for compounds **7a** and **7b**.

**Scheme 2**




Thus, isoxazolidines **7f** and **8f**, useful for our biological screening, have been prepared by an alternative procedure. Nitrone **6f** reacted with methyl acrylate, followed by reduction with $NaBH_4$ of the methoxycarbonyl group at $C_3$, to afford the target compounds in good yields (Scheme 2).

The cycloadducts **9**−**11f** were obtained as pure compounds after PCAR-TLC in a 2.41:1.00:4.18 ratio, in accord with similar reactions,[16] with a 4/5 regioselectivity of 1.22:1; the trans/cis ratio is 1.15:1. The cis/trans stereochemistry for isoxazolidines **9**−**11f** was assigned on the basis of their methoxycarbonyl peaks in $^1$H NMR spectra[17] and subsequently confirmed by NOE experiments.

## Biological Evaluation

Since the primary hypothesized function of isoxazolidinyl-PAHs was to serve as a intercalative system, their interactions with DNA were investigated to determine their effectiveness in this role. In the UV spectral analysis of a mixture of **7c** and calf thymus DNA, characteristic red shift of 13 nm for DNA were observed on incubation, consistent with intercalation.[18] Preliminary results indicate that the examined compounds show a low binding constant for the intercalation with calf thymus DNA ($\approx 6 \times 10^3$ $M^{-1}$).[19]

Furthermore, incubation followed by electrophoretic examination of a mixture of **7c** with ΦX174 RF I DNA showed characteristic streaking of the supercoiled species, indicative of intercalation.

Additionally, preliminary docking studies of compound **7c** to the B-DNA fragment $d(CGCAATTGCG)_2$ show that it preferentially intercalates in the DNA AT region, and the resulting complex is further stabilized by a hydrogen bond between the hydroxyl group of the methanol moiety and an oxygen of a phosphate group (Figure 4).[19]

**Cytotoxicity Assays.** The cytotoxicity of all the compounds obtained was assessed in vitro against a panel of leukemia (MOLT-3, THP-1) or lymphoma (U-937) as well as against a cell line from normal African green monkey kidney (Vero). As a screening assay, the cytotoxicity was tested using a MTS tetrazolium reduction assay and expressed as $IC_{50}$ values. $IC_{50}$ is the concentration ($\mu$M) required to reduce the absorbance values, i.e., the capability of the cells to reduce MTS, by 50% after 20 h of treatment. Results shown in Table 3 indicate that Vero cells were more resistant than leukemia/lymphoma cells to the toxicity induced by the new synthesized compounds and that, among the compounds tested, particularly **7c** and **7e** showed a high level of cytotoxicity. Moreover, the cis/trans configuration is practically irrelevant for the cytotoxicity, e.g. **7a** vs **8a** and **7b** vs **8b**.

Subsequently, to investigate in deeper detail the capability of the compounds to induce cell death in leukemia cells, we tested the cytotoxicity of the two more active compounds, **7c** and **7e**, in the MTS assay, as well as that of **7d**, by means of a conventional viability assay, such as the trypan blue exclusion test, and by evaluating the percent of apoptosis in MOLT-3 cells. Results, expressed as cytotoxic concentration 50 ($CC_{50}$, the concentration that causes 50% toxicity by trypan blue) and apoptotic concentration 50 ($AC_{50}$, the concentration required to cause apoptosis in 50% of treated cells), respectively, are reported in Table 4. **7e** was confirmed as the more toxic compound, causing a high level of cell death in MOLT-3 leukemia cells at 24 h. However, at longer times, also compounds **7c** and **7d** caused high levels of cell death at concentrations comparable with those of **7e**. Moreover, results suggest that apoptosis was the main cause of cell death caused by the compounds. In any case, the actinomycin D, selected as reference compound, showed an extremely higher toxicity with respect to all the new tested compounds.

We assayed the capability of **7e** to potentiate fas-induced apoptosis. As shown in Figure 5, **7e** had a remarkable enhancing activity on apoptosis caused by anti-fas antibody addition.

**Antiviral Activity.** Compound **7b** and **8b**, chosen as model compounds, were evaluated against a wide range of DNA and RNA viruses, including herpes simplex virus 1 (HSV-1; KOS), herpes simplex virus 2 (HSV-2; G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus 1 ($TK^-$ KOS $ACV^r$), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, and respiratory syncytial virus (RSV).

The antiviral activity of the above-reported compounds was tested in vitro in HEL, HeLa, and Vero cells, where appropriate, along with the reference antiviral compounds such as brivudin, ribavirin, acyclovir, ganciclovir, and (*S*)-DHPA. Results are shown in Table 5.

Compounds **7b** and **8b** showed no specific antiviral effects (i.e. minimal antiviral effective concentration ≥5-fold lower than

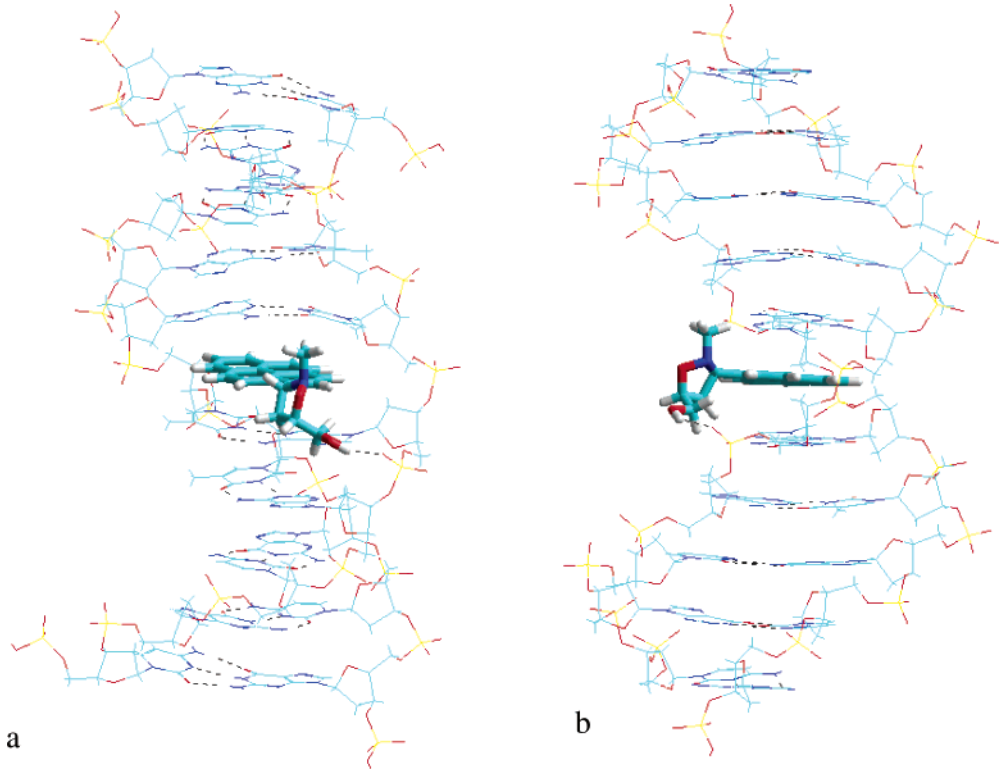



**Figure 4.** Plot representing the intercalated compound **7c** as a tube model and the DNA backbone as wireframe: (a) front view, (b) side view.

**Table 3.** Evaluation of Cytotoxicity of the Compounds **7a**−**f** and **8a**,**b** by MTS Assay[a]

| | IC$_{50}$ ($\mu$M)[b] | | | | | | |
|---|---|---|---|---|---|---|---|
| cell line | **7b** | **7c** | **7d** | **7e** | **7a, 7f, 8a, 8f** | **8b** | AcD[c] |
| Molt-3 | 125 (72−178)[d] | 112 (87−146) | 127 (107−147) | 78 (43−112) | >500 | 128 (101−155) | <1 |
| THP-1 | 169 (152−186) | 107 (65−177) | 192 (156−228) | 50 (23−110) | ND[e] | 148 (114−182) | ND |
| U937 | 153 (134−172) | 111 (88−140) | 193 (160−226) | 54 (39−74) | ND | 154 (123−185) | <1 |
| Vero | 246 (224−268) | 159 (147−172) | 301 (275−327) | 63 (49−80) | ND | 231 (198−264) | 11 (8−14) |

[a] Cells were exposed under optimal culture conditions in 96-well plates to four concentrations of the compounds (10, 100, 200, 500 $\mu$M for the new compounds and 1, 10, 100, 200 for actinomycin D, respectively) or control medium for 20 h before determining cellular metabolic activity by a MTS tetrazolium compound bioreduction assay. [b] IC$_{50}$ is the concentration of the drug required to cause 50% reduction in absorbance value in comparison with cells exposed to control medium. Each value is determined from triplicate samples using a linear (**7d**, **7d**, **8b**, and **AcD**) or a nonlinear (**7c** and **7e**) regression. [c] Actinomycin D. [d] Numbers in parentheses indicate the lower and the upper 95% confidence limits. [e] Not done.

**Table 4.** Evaluation of Cell Death Caused by the Compounds in MOLT-3 Leukemia Cells[a]

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| compd | CC$_{50}$ ($\mu$M)[b] | AC$_{50}$ ($\mu$M)[b] | CC$_{50}$ ($\mu$M) | AC$_{50}$ ($\mu$M) | CC$_{50}$ ($\mu$M) | AC$_{50}$ ($\mu$M) |
| **7c** | 278 | 118 | 168 | 71 | 133 | 67 |
| **7d** | >500 | >500 | 97 | 97 | 58 | 67 |
| **7e** | 73 | 69 | NC[c] | NC | NC | NC |
| actinomycin D | NC | NC | NC | NC | NC | NC |

[a] Cells were exposed under optimal culture conditions to concentrations ranging from 10 to 500 $\mu$M for the new compounds and from 1 to 200 $\mu$M for actinomycin D or control medium for the times indicated. [b] CC$_{50}$ and AC$_{50}$ are the concentrations of the drugs required to cause 50% toxicity, detected by trypan blue exclusion test, and 50% apoptosis, detected by microscopy analysis, respectively. [c] Not calculable, due to massive cell death at all concentrations tested.

the minimal cytotoxic concentration) except against the bunyavirus Punta Toro for which the cytotoxic/antiviral ratio was virtually 10-fold.

## Conclusions

A new class of potential DNA intercalation agents, the isoxazolidinyl-PAHs, has been synthesized according to the 1,3-dipolar cycloaddition methodology, promoted by microwave irradiation. The utility of this template in the synthesis of structures designed to capitalize on its intercalative properties has been examined.

As well-known, cytotoxicity is not only dependent on the ability to interact with DNA; the drug must be capable of interacting with DNA to form a stable ternary DNA−intercalator−enzyme complex with a relatively long half-life in such a way that the enzymatic process cannot continue forward and backward. The low level of cytotoxicity observed for the obtained compounds could be ascribed to a low binding association to DNA.

Further application of this template in antitumor agents design would seem warranted, particularly where systematic structural variations of the isoxazolidine nucleus are performed.

## Experimental Section

**General.** Melting points were determined with a Kofler apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer elemental analyzer. NMR spectra were recorded on a Varian instrument at 500 MHz ($^1$H) and at 125 MHz ($^{13}$C) using

**Table 5.** Antiviral Activity of **7b** and **8b** against Reference Antiviral Compounds

| | **7b** | **8b** | brivudin | ribavirin | acyclovir | ganciclovir | (*S*)-DHPA |
|---|---|---|---|---|---|---|---|
| min cytotoxic concn ($\mu$g/mL)[a] | 400 | 400 | >400 | >400 | >400 | >100 | ND[b] |
| min cytotoxic concn ($\mu$g/mL)[c] | 80 | 80 | >400 | >400 | ND | ND | >400 |
| min cytotoxic concn ($\mu$g/mL)[d] | 80 | 80 | >400 | >400 | ND | ND | >400 |
| herpes simplex virus 1 (HSV-1; KOS)[e] | >80 | >80 | 0.128 | 240 | 0.384 | 0.096 | ND |
| herpes simplex virus 2 (HSV-2; G)[e] | >80 | >80 | 240 | >400 | 0.384 | 0.032 | ND |
| vaccinia virus[e] | >80 | >80 | 48 | 80 | >400 | >100 | ND |
| vesicular stomatitis virus[e] | >80 | >80 | >400 | 240 | >400 | >100 | ND |
| herpes simplex virus 1 ($TK^-$ KOS $ACV^r$)[e] | 48 | >80 | >400 | >400 | 48 | 0.48 | ND |
| parainfluenza-3 virus[f] | >16 | >16 | >400 | 48 | ND | ND | 400 |
| reovirus-1[f] | >16 | >16 | >400 | 16 | ND | ND | 240 |
| Sindbis virus[f] | >16 | >16 | >400 | >400 | ND | ND | >400 |
| Coxsackie virus B4[f] | >16 | >16 | >400 | >400 | ND | ND | >400 |
| Punta Toro virus[f] | 9.6 | 9.6 | >400 | 80 | ND | ND | >400 |
| respiratory syncytial virus[g] | >16 | >16 | >400 | 9.6 | ND | ND | >400 |
| Coxsackie virus B4[g] | >16 | >16 | >400 | 9.6 | ND | ND | >400 |
| vesicular stomatitis virus[g] | >16 | >16 | >400 | 16 | ND | ND | >400 |

[a] Required to cause a microscopically detectable alteration of normal cell morphology in HEL cells. [b] Not done. [c] Required to cause a microscopically detectable alteration of normal cell morphology in Vero cells. [d] Required to cause a microscopically detectable alteration of normal cell morphology in HeLa cells. [e] Minimum inhibitory concentration ($\mu$g/mL) required to reduce virus-induced cytopathogenicity by 50% in HEL cells. [f] Minimum inhibitory concentration ($\mu$g/mL) required to reduce virus-induced cytopathogenicity by 50% in Vero cells. [g] Minimum inhibitory concentration ($\mu$g/mL) required to reduce virus-induced cytopathogenicity by 50% in HeLa cells.

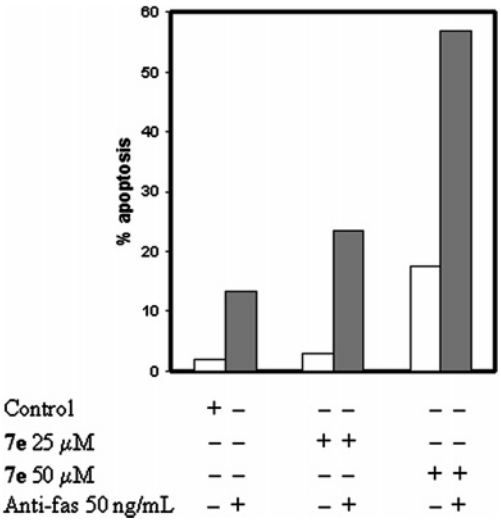



**Figure 5.** Potentiation of fas-mediated apoptosis by **7e**; Molt-3 cells were incubated with anti-fas at 25 and 50 or 100 ng/mL (data shown are for 50 ng/mL), either alone or immediately after addition of **7e** at concentrations of 25 or 50 $\mu$M. Apoptosis was detected by fluorescence microscopy analysis, following staining with acridine orange, after 20 h of culture.

deuteriochloroform or deuteriobenzene as solvents; chemical shifts are given in ppm from TMS as internal standard. Thin-layer chromatographic separations were performed on Merck silica gel 60-$F_{254}$ precoated aluminum plates. Preparative separations were carried out by flash chromatography using Merck silica gel 0.035−0.070 mm. PCAR-TLC was performed with a Chromatotron Model 7924 T (Harrison Research, Palo Alto, CA); the rotors (1 or 2 mm layer thickness) were coated with silica gel Merck grade type 7749, TLC grade, with binder and fluorescence indicator (Aldrich 34,-644-6), and the eluting solvents were delivered by the pump at a flow rate of 0.5−1.5 mL/min.

The reactions under microwave irradiations were carried out using a CEM Corp. Focused Microwave Sytem, Model Discover.

The identification of samples from different experiments was secured by mixed melting points and superimposable NMR spectra.

**Starting Materials.** PAH-aldehydes were purchased from Aldrich Co. All solvents were dried according to literature methods.

**Synthesis of Nitrones 6. General Procedure.** To a solution of sodium acetate (7.5 mmol) in dichloromethane (30 mL), cooled at 0 °C, were added the *N*-methyl- or *N*-benzylhydroxylamine hydrochloride (7.5 mmol) and successively the corresponding PAH-aldehyde (5 mmol) dropwise. The reaction mixture was then stirred for 1 h at 0 °C and then at room temperature overnight. After this time, the allyl alcohol was removed under reduced pressure and the obtained solid was purified by silica gel flash chromatography ($MeOH/CHCl_3$ 5:95) to give the pure nitrone **6**.

**(*Z*)-*C*-(9-Anthryl)-*N*-methyl nitrone (6a):** yield 90%; yellow solid, mp 92−94 °C. Anal. ($C_{16}H_{13}NO$) C, H, N.

**(*Z*)-*C*-(9-Phenanthryl)-*N*-methyl nitrone (6b):** yield 94%; white solid, mp 200−202 °C. Anal. ($C_{16}H_{13}NO$) C, H, N.

**(*Z*)-*C*-(1-Pyrenyl)-*N*-methyl nitrone (6c):** *Z*/*E* ratio = 15:1; yield 89%; yellow solid, mp 177−181 °C. Anal. ($C_{18}H_{13}NO$) C, H, N.

**(*Z*)-*C*-(1-Pyrenyl)-*N*-benzyl nitrone (6d):** *Z*/*E* ratio = 10:1; yield 96%; yellow solid, mp 128−130 °C. Anal. ($C_{24}H_{17}NO$) C, H, N.

**(*Z*)-*C*-(9-Phenanthryl)-*N*-benzyl nitrone (6e):** yield 90%; white solid, mp 164−166 °C. Anal. ($C_{22}H_{17}NO$) C, H, N.

**(*Z*)-*C*-(9-Anthryl)-*N*-benzyl nitrone (6f):** yield 90%; light yellow solid, mp 229−230 °C. Anal. ($C_{22}H_{17}NO$) C, H, N.

**Synthesis of Isoxazolidines 7 and 8. General Procedure.** A solution of nitrone **6** (0.37 mmol) in allyl alcohol (5 mL, 73.5 mmol) in a pressure tube equipped with a stir bar was inserted into the cavity of a Discover Microwave System apparatus and heated at 90 W for 20−80 min (internal temperature 150−160 °C). The mixture was evaporated and the resulting solid was purified by flash chromatography on a silica gel with cyclohexane/ethyl acetate (80:20) as eluent.

**[(3*RS*,5*SR*)-3-(9-Anthryl)-2-methylisoxazolidin-5-yl]methanol (7a):** reaction time 80 min; yield 28%; yellow solid, mp 94−98 °C. Anal. ($C_{19}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*RS*)-3-(9-Anthryl)-2-methylisoxazolidin-5-yl]methanol (8a):** yield 25%; yellow solid, mp 86−90 °C. Anal. ($C_{19}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*SR*)-2-Methyl-3-(9-phenanthryl)isoxazolidin-5-yl]methanol (7b):** reaction time 20 min; yield 47%; yellow solid, mp 43−48 °C. Anal. ($C_{19}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*SR*)-2-Methyl-3-(9-phenanthryl)isoxazolidin-5-yl]methanol (8b):** reaction time 20 min; yield 36%; brown solid, mp 35−40 °C. Anal. ($C_{19}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*SR*)-2-Methyl-3-pyren-1-ylisoxazolidin-5-yl]methanol (7c):** reaction time 20 min; yield 45%; brown solid, mp 63−67 °C. Anal. ($C_{21}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*RS*)-2-Methyl-3-pyren-1-ylisoxazolidin-5-yl]methanol (8c):** reaction time 20 min; yield 30%; brown solid, mp 58−61 °C. Anal. ($C_{21}H_{19}NO_2$) C, H, N.

**[(3*RS*,5*SR*)-2-Benzyl-3-pyren-1-ylisoxazolidin-5-yl]methanol (7d):** reaction time 20 min; yield 52.3%; yellow solid, mp 45−47 °C. Anal. ($C_{27}H_{23}NO_2$) C, H, N.

**[(3*RS*,5*RS*)-2-Benzyl-3-pyren-1-ylisoxazolidin-5-yl]methanol (8d):** reaction time 20 min; yield 32.7%; yellow solid, mp 63−65 °C. Anal. ($C_{27}H_{23}NO_2$) C, H, N.

**[(3*RS*,5*SR*)-2-Benzyl-3-(9-phenanthryl)isoxazolidin-5-yl]methanol (7e):** reaction time 20 min; yield 23.3%; yellow sticky foam. Anal. ($C_{25}H_{23}NO_2$) C, H, N.

**[(3*RS*,5*RS*)-2-Benzyl-3-(9-phenanthryl)isoxazolidin-5-yl]methanol (8e):** reaction time 20 min; yield 15.7%; yellow solid, mp 41−43 °C. Anal. ($C_{25}H_{23}NO_2$) C, H, N.

**Synthesis of Isoxazolidines 9−11f. General Procedure.** A solution of nitrone **6** (0.28 mmol) in methyl acrilate (5 mL, 55.5 mmol) in a pressure tube equipped with a stir bar was inserted into the cavity of a Discover Microwave System apparatus and heated at 90 W for 60 min (internal temperature 150−160 °C). The mixture was evaporated and the resulting solid was purified by flash chromatography on a silica gel with cyclohexane/ethyl acetate (95:5) as eluent.

**Methyl (3*RS*,5*SR*)-3-(9-anthryl)-2-benzylisoxazolidine-5-carboxylate (9f):** yield 24.2%; dark yellow solid, mp 90−95 °C. Anal. ($C_{26}H_{23}NO_3$) C, H, N.

**Methyl (3*RS*,5*RS*)-3-(9-anthryl)-2-benzylisoxazolidine-5-carboxylate (10f):** yield 10.0%; dark yellow solid, mp 105−108 °C. Anal. ($C_{26}H_{23}NO_3$) C, H, N.

**Methyl (3*RS*,4*SR*)-3-(9-anthryl)-2-benzylisoxazolidine-4-carboxylate (11f):** yield 41.8%; deep yellow solid, mp 130−134 °C. Anal. ($C_{26}H_{23}NO_3$) C, H, N.

**Synthesis of Isoxazolidines 7f, 8f, and 12f. General Procedure.** To a solution of isoxazolidine **9−11f** (20 mmol) in a 1:1 dioxane/water mixture (30 mL) was added sodium borohydride (200 mmol), at room temperature, and then the reaction was magnetically stirred for 20 h. Successively, the reaction mixture was extracted with dichloromethane (3 × 20 mL) and the reunited organic phases were dried on anhydrous sodium sulfate and evaporated at reduced pressure to give a solid that was purified by silica gel flash chromatography with cyclohexane/ethyl acetate (80:20) as eluent.

**[(3*RS*,5*SR*)-3-(9-Anthryl)-2-benzylisoxazolidin-5-yl]methanol (7f):** yield 91.48%; brown solid, mp 91−95 °C. Anal. ($C_{25}H_{23}NO_2$) C, H, N.

**[(3*RS*,5*RS*)-3-(9-Anthryl)-2-benzylisoxazolidin-5-yl]methanol (8f):** yield 93.5%; brown solid, mp 85−88 °C. Anal. ($C_{25}H_{23}NO_2$) C, H, N.

**[(3*RS*,4*RS*)-3-(9-Anthryl)-2-benzylisoxazolidin-4-yl]methanol (12f):** yield 68.5%; dark yellow solid, mp 129−134. Anal. ($C_{25}H_{23}NO_2$) C, H, N.

**Evaluation of Cytotoxicity.** The cytotoxicity of all compounds was evaluated by a commercial viability assay (CellTiter 96 Aqueous One Solution Assay, Promega Co., Madison WI), according to the manufacturer's instructions. This assay is based on the principle that cells, at death, rapidly lose the ability to reduce MTS tetrazolium. Briefly, MOLT-3, U-937, THP-1, and Vero cells were cultured under optimal culture conditions for 20 h in 96-well plates, in the absence of the compounds or in their presence, at concentrations ranging from 10 to 500 $\mu$M. At the end of the incubation time, the MTS-tetrazolium-based reagent was added to each well. After a further incubation of 1 h at 37 °C in a humidified, 5% $CO_2$ atmosphere, the absorbance of the samples was recorded at 490 nm using a 96-well spectrophotometer. The assays were performed in triplicate. The $IC_{50}$ were calculated as the concentrations of the compounds required to cause 50% reduction of absorbance values. For compounds **7c** and **7e**, which showed low $IC_{50}$ values with reference to the range of concentrations utilized, a nonlinear regression was the most adequate for their calculation, while for the other compounds a linear regression was used. The 95% confidence interval for $IC_{50}$ was also calculated.

The capability of some compounds to induce cell death was also evaluated using the trypan blue exclusion test and by detection of apoptosis using morphological analysis following staining with acridine orange, as previously described.[20] Evaluation of fas-induced apoptosis was performed by adding anti-fas antibody (50 ng/mL; CH11, Upstate Biotechnology Inc., Lake Placid, NY) to the untreated or treated cells in 96-well plates.

**Evaluation of Antiviral Activity.** Antiviral activity was determined by previously established procedures.[21]

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**Supporting Information Available:** Elemental analyses data and $^1H$ and $^{13}C$ NMR spectroscopic data of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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