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Phenanthrenequinone Antiretroviral Agents

George A. Kraus,[a,*] Alex Melekhov,[a] Susan Carpenter,[b] Yvonne Wannemuhler[b] and Jacob Petrich[c]

[a]*Department of Chemistry, Iowa State University, Ames, IA 50011, USA*
[b]*Department of Microbiology, Immunology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA*
[c]*Department of Chemistry, Iowa State University, Ames, IA 50011, USA*

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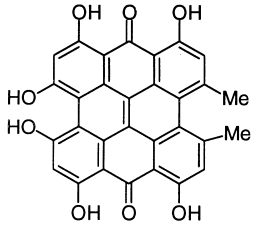
Abstract—Compounds **3** and **5** are the first phenanthrenequinones to exhibit significant virucidal activity against the retrovirus equine infectious anemia virus. They differ from hypericin in that their virucidal activity is not light dependent.

The search for new antiviral agents often involves an interplay between structure elucidation and synthetic chemistry. Natural products frequently serve as leads for the development of new drugs. Compounds such as AZT and DDI that inhibit the human immunodeficiency virus type 1 (HIV-1), the virus that leads to the onset of AIDS, have attracted intense interest in recent years. Recently, hypericin, (**1**), a naturally occurring polycyclic quinone, has been shown to exhibit antiretroviral activity against HIV-1 and a number of other enveloped viruses.[1,2] Its activity is light dependent and its mechanism of action is markedly different from that of AZT. A number of researchers have also shown that both substituted naphthoquinones and substituted anthraquinones show good activity as antiviral agents.[3,4] In particular, Cohen, Hudson and Toweres have demonstrated that anthraquinone **2** exhibits strong inhibitory activity against herpes simplex virus type 1 (HSV-1).[5,6] To date, no 1,4-phenanthrenequinone has been reported to demonstrate antiviral activity. As part of a program designed to develop new quinone-based antiretroviral and anticancer agents, we now communicate that certain synthetic 1,4-phenanthrenequinones exhibit significant inhibitory effects against the equine infectious anemia virus (EIAV), a retrovirus which has been used as a model for HIV.



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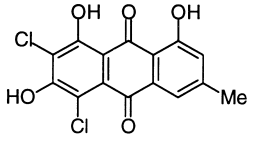

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We recently reported an efficient synthesis of 1,4-phenanthrenequinones **3**–**8** by way of stannic chloride-promoted intramolecular cyclizations of hydroxy benzoquinones.[7] In view of the structural similarity of these compounds to the active anthraquinones, their virucidal activity was assessed using the equine infectious anemia virus (EIAV), a lentivirus genetically and antigenically related to HIV-1.[8]

Compounds were solubilized in DMSO at 1 μg/mL and antiviral assays were performed as previously described.[9] Briefly, 10 μL of each compound was added to approximately 10^4 infectious units of EIAV in 1 mL Hank's buffered saline solution (HBSS) supplemented with 2% fetal calf sera. Samples were incubated in either fluorescent light or in the dark at room temperature for 30 min and 10-fold serial dilutions were inoculated onto equine dermal cells in the presence of polybrene (8 μg/mL). Hypericin and DMSO were included as positive and negative controls, respectively, in all experiments. At 5 day post-inoculation, cells were fixed in ice-cold

*Corresponding author.

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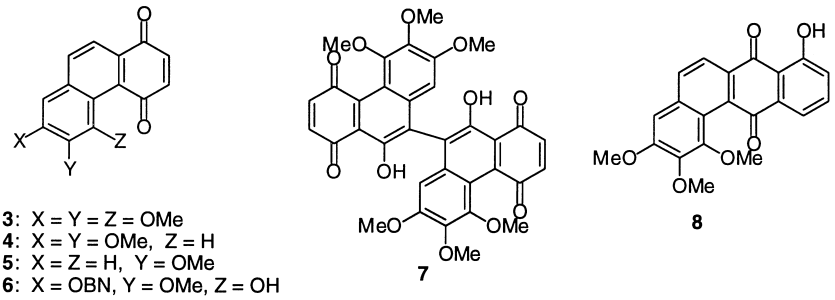

methanol and EIAV-infected cells were detected by immunocytochemistry.[10] Foci of EIAV-infected cells were enumerated and virucidal activity was calculated as a reduction in infectious virus as compared to samples treated with 1% DMSO.

Results

The results of the virucidal assays are shown in Figure 1. Similar to our previous studies, hypericin exhibited potent virucidal activity in the light, but had little activity in the dark. None of the synthetic compounds tested were as effective in virucidal activity as hypericin. Nevertheless, compounds **3** and **5** eliminated more than 90% of infectious virus. Surprisingly, both compounds **3** and **5** generated a similar level of antiretroviral activity in the absence of light.

The absence of light dependence for antiviral activity of **3** and **5** suggests that the virucidal activity of phenanthrenequinones differs from that of hypericin. A major effort in our previous studies has been directed towards the elucidation of the photophysics of hypericin and the role of light in its antiviral activity.[11–17] Therefore, it was of interest to compare the photophysics of the phenanthrenequinone **5** with that of hypericin. Hypericin absorbs strongly everywhere from the ultraviolet to the visible, having a strong maximum about 590 nm. In contrast, **5** absorbs most strongly in the blue and ultraviolet region of the spectrum (from 300 to 400 nm). The experimental conditions used to photoactivate 5 were fluorescent room light. This may have been insufficient for induction of light-activated virus killing; however, it does not account for the virucidal activity of **5** in the absence of light.

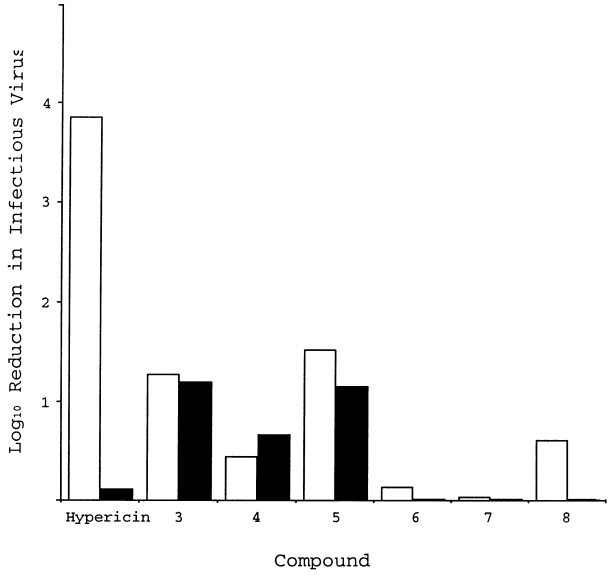

Figure 1. Virucidal activity of compounds. Compounds were solubilized in DMSO and incubated with approximately 10^4 infectious units of EIAV in 1 mL in either fluorescent light (open bars) or in the dark (solid bars). Results are expressed as the $\log_{10}$ reduction in infectious virus as compared to DMSO-treated control samples which contained 5×10^4 infectious units of EIAV. The results represent the mean of 2–3 independent experiments and standard deviation was less than 6% of mean.

In addition to the relatively weak absorbance of **5** under the conditions used for photoactivation, the excited state of **5** is decayed more rapidly than that of hypericin. Hypericin has a fluorescence lifetime of approximately **5** ns in all neat organic solvents in which it has been studied.[8–11] In comparison, **5** has an average lifetime of 0.3 ns in DMSO and 0.9 ns in 1,4-dioxane (not shown). This would suggest that the nonradiative processes deactivating the fluorescent state of **5** are more important than those processes in hypericin; however, the light-induced virucidal activity of hypericin remained much higher. While this result is consistent with the suggestion that proximate hydroxyl groups are important for the antiviral activity of hypericin, it cannot explain the virucidal activity of **5**, since **5** has no hydroxyl groups. Together, these results suggest that the virucidal activity of phenanthrenequinones is not due to photoactivation.

In summary, 1,4-phenanthrenequinones **3** and **5** exhibit antiretroviral activity independent of photoactivation, indicating that their antiretroviral activity occurs through a mechanism different from that of hypericin. Further studies to optimize virucidal activity and to elucidate the mechanism of virus killing are needed to evaluate the therapeutic potential of this promising class of compounds.

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