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ANTIRETROVIRAL ACTIVITY OF SYNTHETIC HYPERICIN AND RELATED ANALOGS

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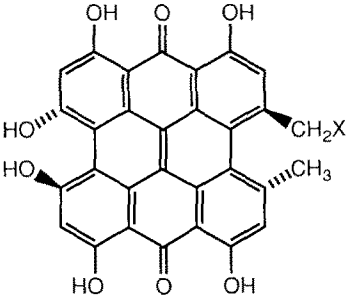
Hypericin and pseudohypericin are naturally occurring polycyclic quinones which have recently been shown to inhibit the infectivity of several retroviruses, including human immunodeficiency virus. To better understand the antiviral mechanisms of these compounds, hypericin and a series of analogous quinones were synthesized and tested for anti-retroviral activity against equine infectious anemia virus (EIAV). Treatment of EIAV-infected cells with hypericin reduced the production of infectious virus by 99.99%. None of the analogs were found to inhibit virus replication. These results suggest that the complete ring structure of hypericin is required, but not sufficient, for antiviral activity.

Recently, Meruelo and coworkers demonstrated that hypericin (**1**), a natural product found in plants (1), inhibits the spread of Friend leukemia virus and radiation leukemia virus both in vitro and in vivo. Meruelo has also reported that **1** can directly inactivate human immunodeficiency virus (HIV) as measured by reverse transcriptase (RT) activity (2). Interestingly, hypericin does not directly inhibit purified RT (2, 3).

Despite the promising biological profile of hypericin, only one analog of hypericin, the natural product pseudohypericin (**2**), has been tested.



1: X=H **2**: X=OH

0006-291X/90 $1.50

Although pseudohypericin differs from hypericin only by virtue of a benzylic alcohol group, pseudohypericin is somewhat less active than **1** in vitro. As part of our program for evaluating animal retroviruses as models for HIV, we report herein both the synthesis of **1** and selected analogs and the retroviral inhibitory activity of these compounds against equine infectious anemia virus (EIAV), a lentivirus structurally, genetically and antigenically related to HIV (4, 5, 6).

MATERIALS AND METHODS

Materials: Emodin (**3**), emodin anthrone **4** and anthraquinone **5** were prepared by standard procedures (7). Hypericin was generated from **4** by a two step route (8). Quinone **6** was prepared from perylene by nitration and hydrolysis with concentrated sulfuric acid (9) Quinone **7** was made from anthrone (10).

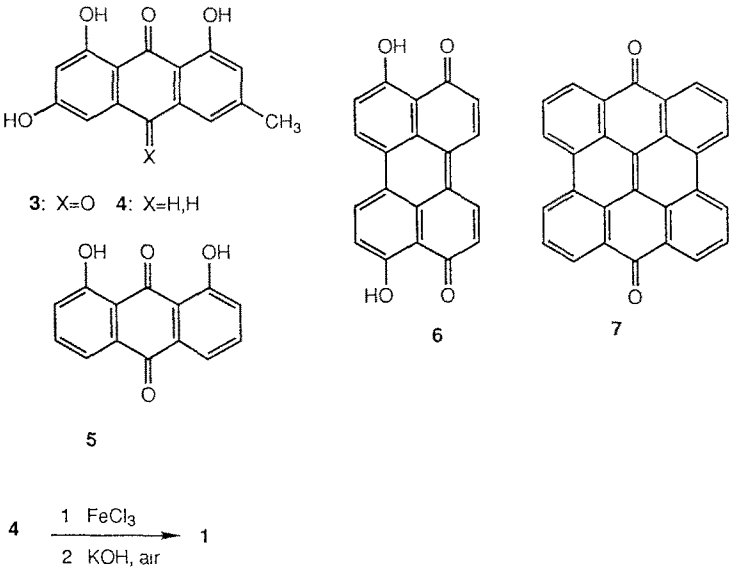
Cell culture and virus: Equine dermal (ED) cells (ATCC CCL57) chronically infected with the MA-1 strain of EIAV (11, 12) were grown in Dulbecco minimum essential medium supplemented with 20% fetal bovine serum (FBS), penicillin (200 units/ml) and streptomycin (100 μg/ml).

Treatment of cells with quinones: Cells were seeded in 24 well tissue culture plates at a concentration of 5 X 10^4 cells per well. Twenty-four hours post seeding, culture media was aspirated and replaced with media containing 10 μg/ml of compounds **1**, **3**, **5**, **6** or **7** in 1% DMSO. Media containing 1% DMSO was used as a control. Cells were incubated 30 min at 37°C, washed three times in phosphate-buffered balanced salt solution containing 2% FBS and 1.0 ml culture media was added. Following treatment, culture fluid was removed from triplicate wells at 24 hour intervals and assayed for both RT activity and infectious virus.

Assays for virus: RT assays were performed as described previously (12, 13, 14). Briefly, 10 μl of clarified culture supernatant was incubated in 30 μl of RT reaction buffer (12) containing 20 μCi/ml [a-^{32}P]dTTP and enzyme activity was detected by autoradiography. Titers of infectious virus were determined using a focal immunofluorescence assay as previously described (11, 12, 15). Titers of virus are expressed as fluorescent-focus units (FFU) per ml of inoculum.

RESULTS AND DISCUSSION

In order to gain some insight into the structural requirements for antiretroviral activity in the hypericin series, simple quinones which mimicked various parts of the hypericin ring system were tested. Quinone **3** represents the top half of hypericin, while quinone **7** contains the entire hypericin ring system but lacks the hydroxyl substitution. Quinone **6** has the aromatic ring system of calphostin C, a natural product which, like hypericin, is a specific inhibitor of protein kinase C (16).



We examined the effect of quinones **1**, **3**, **5**, **6** and **7** on the production of infectious virus in cells chronically infected with EIAV. Treatment of cells with concentrations of quinone at 10 μg/ml did not affect cell viability and morphology. At the same concentration, hypericin displayed a dramatic antiviral effect: production of infectious EIAV was reduced by 99.99% in cells treated with hypericin (Figure 1). Moreover, the antiviral effect of hypericin was prolonged, as evidenced by continued viral inhibition at 96 hours post treatment. In contrast to the striking antiviral activity of hypericin, none of the synthetic analogs were able to reduce viral infectivity.

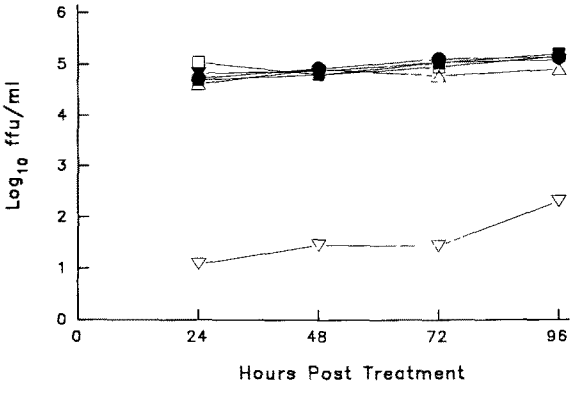


Figure 1. Titers of infectious EIAV produced from chronically infected cells following treatment with quinones. Each point represents mean titers of triplicate wells sampled at 24 h intervals. Quinone 1 (▽); 3 (▼); 5 (□); 6 (■); 7 (△); 1% DMSO (●).

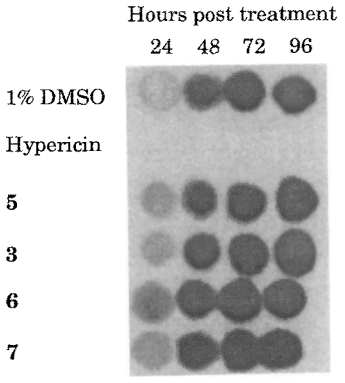


Figure 2. Reverse transcriptase activity in supernatant collected from EIAV-infected cells treated with 10 µg/ml of 1, 3, 5 6 or 7 in 1% DMSO.

Supernatants from quinone-treated cultures were also tested for RT activity. No RT activity was detected in supernatants from hypericin-treated cells, while supernatants from cells treated with various analogs contained RT levels comparable to controls (Figure 2). Thus, inhibition of viral infectivity correlated with inhibition of RT activity. However, the infectivity assay is more sensitive than the RT assay and it is not clear if the antiviral activity of hypericin is wholly dependent on inactivation of RT.

The antiviral effect of hypericin in cells chronically infected with retrovirus indicates that hypericin acts on a post-integration step in the virus life cycle. Seminal studies by Meruelo and coworkers suggest that hypericin may act at steps involving virus assembly (3). In the present study, electron microscopic examination of both hypericin-treated and non-treated controls indicated that hypericin did not prevent the production of EIAV virions (data not shown). In contrast to previous studies (3), however, we could not readily distinguish EIAV virions produced by hypericin-treated cells from those produced by non-treated control cells. However, lentiviruses are considerably more pleiomorphic than are type-C murine retroviruses and differences in core morphology among lentiviruses may not be as readily apparent as with murine retroviruses.

Hypericin is reported to not directly inactivate the purified enzyme reverse transcriptase (2), yet the quinone is able to inhibit virion-associated enzyme activity. Presumably, hypericin is able to interact with cellular and viral membranes and it has been suggested that hypericin interferes with activation of the reverse transcriptase enzyme following viral uncoating (3). Thus the antiviral effect may be dependent on the hydrophobic nature imparted by the aromatic ring system of hypericin. The present results suggest that the ring structure by itself is not sufficient to inhibit retroviral replication. It is more likely that the reduction potential of the quinone, which is markedly affected by hydroxyl

substitution, plays the dominant role. Hydroxyl substitution is not sufficient for activity, as evidenced by the fact that quinone **3** did not inhibit EIAV. Both the complete ring system and the substituents are required for antiviral activity.

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