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ANTI-HIV ACTIVITY OF QUASSINOIDS[1]

Masayoshi Okano,[a] Narihiko Fukamiya,[a] Kiyoshi Tagahara,[b] Mark Cosentino,[c] Thomas Tung-Ying Lee,[d] Susan Morris-Natschke,[e] and Kuo-Hsiung Lee [e*]

[a]*Department of Interdisciplinary Studies of Natural Environment, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashihiroshima 739, Japan*

[b]*Faculty of Pharmaceutical Sciences, Kobe Pharmaceutical University, Kobe 658, Japan*

[c]*Biotech Research Laboratories, 3 Taft Court, Rockville, Maryland 20850*

[d]*School of Medicine, University of North Carolina, Chapel Hill, NC 27599*

[e]*Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599*

Abstract: Eighteen quassinoid glycosides and nine known quassinoids were tested for inhibitory activity against HIV replication in H9 lymphocytic cells. Of the compounds tested, shinjulactone C (**20**) demonstrated the highest anti-HIV activity (EC_{50} = 10.6 μM) in the absence of cytotoxicity with a therapeutic index of >25.

A number of terpenoids and their derivatives, such as the triterpene glycyrrhizin (glycyrrhizic acid),[2-4] salaspermic acid,[5] suberosol,[6] betulinic acid,[7] platanic acid,[7] and 15-oxocucurbitacin-F,[8] as well as the diterpene tripterifordin,[9] DASM (dehydroandrographolide succinic acid monoesters),[10] and prostratin,[11] have been reported to be anti-HIV agents.[12] These anti-HIV terpenoids, in general, possess polar functional groups. Since quassinoids are regarded as degraded triterpenes biosynthetically, an evaluation of quassinoids and glycosidic quassinoids as potential anti-HIV agents appears to be worthwhile and of current interest.

The quassinoid glycosides evaluated are those isolated previously from the authors' laboratory.[13] These include yadanzioside-F (**1**), bruceoside-A (**2**),[14] yadanzioside-A (**3**),[14] yadanzioside-G (**5**),[14] yadanzioside-C (**6**),[14] bruceoside-D (**8**),[15] bruceoside-E (**9**),[15] bruceoside-F (**10**), [15] bruceoside-B (**11**),[14] yadanzioside-B (**12**),[14] yadanzioside-L (**14**),[14] bruceoside-C (**15**),[14] and yadanzioside-E (**18**)[16-17] from *Brucea javanica*, as well as bruceantinoside-A (**4**),[18] yadanzioside-M (**7**),[19] yadanzioside-P (**13**),[19] yadanzioside-N (**16**),[20] and bruceantinoside-C(**17**),[20] from *Brucea antidysenterica* (Figure 1). In addition, nine known quassinoids were also evaluated including $\Delta^{13(18)}$-dehydroglaucarubinone (**19**), shinjulactone C (**20**), shinjulactone B (**21**), shinjulactone A (**22**), ailantinol A (**23**), ailanthone (**24**), amarolide (**25**), amarolide 11-acetate (**26**), and shinjudilactone (**27**), which were isolated from *Allanthus altissima* and whose structures were elucidated from spectral data.

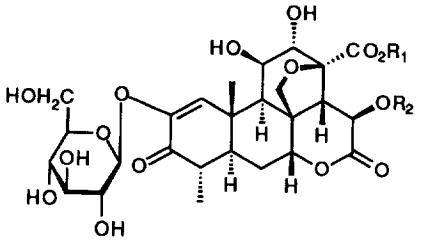
Table 1. Cytotoxicity and Anti-HIV Activity of Compounds **1**-**27**.

Compounds	IC_{50} (μM)	EC_{50} (μM)	Therapeutic Index
1	156	109	1.4
2	29	29	1
3	146	110	1.3
4	4	a	a
5	37	23	1.6
6	55	a	a
7	7	a	a
8	52	a	a
9	59	74	0.8
10	>132	112	>1.2
11	3	3	1
12	4	5	0.8
13	18	18	1
14	10	5	2
15	44	a	a
16	49	a	a
17	26	46	0.6
18	29	a	a
19	toxic at all	concentrations	tested
20	>264	10.6	>25
21	>287	28	>10
22	5	5	1
23	>246	30	>8.2
24	toxic at all	concentrations	tested
25	>275	179	>1.5
26	>246	246	>1
27	>266	43	>6.2
ddC[b]	500	0.03	16,667

[a]no suppression.

[b]ddC is the control drug

As seen in Table 1, none of the eighteen quassinoid glycosides tested showed anti-HIV activity[21] without significant toxicity. Bruceoside-B (**11**), as well as yadanzioside-B (**12**) and -L (**14**), showed good EC_{50} values of 3, 5, and 5 μM, respectively. However, their therapeutic indexes (TI) of 1, 0.8, and 2, respectively, indicate that they are too toxic as anti-HIV agents.



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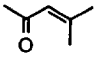
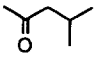
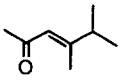
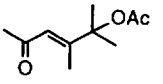
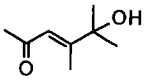
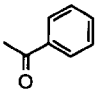
2 R_1 = Me, R_2 = 

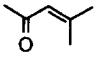
3 R_1 = Me, R_2 = 

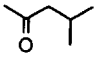
4 R_1 = Me, R_2 = 

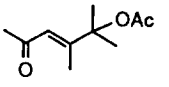
5 R_1 = Me, R_2 = 

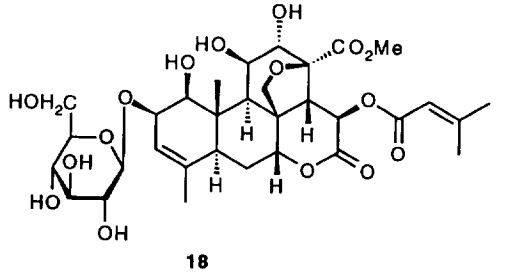
6 R_1 = Me, R_2 = 

7 R_1 = Me, R_2 = 

8 R_1 = H, R_2 = 

9 R_1 = H, R_2 = 

10 R_1 = H, R_2 = 



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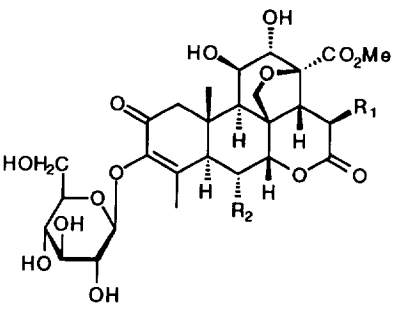
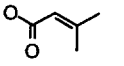
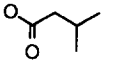
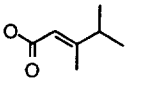
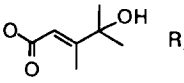
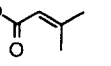


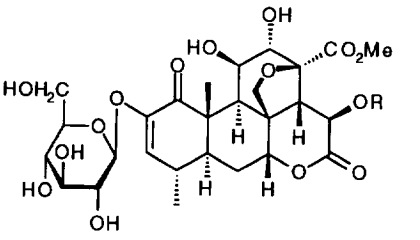
11 R_1 =  R_2 = H

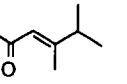
12 R_1 =  R_2 = H

13 R_1 =  R_2 = H

14 R_1 =  R_2 = H

15 R_1 = H, R_2 = 



16 R = 

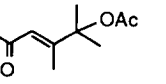
17 R = 

Figure 1

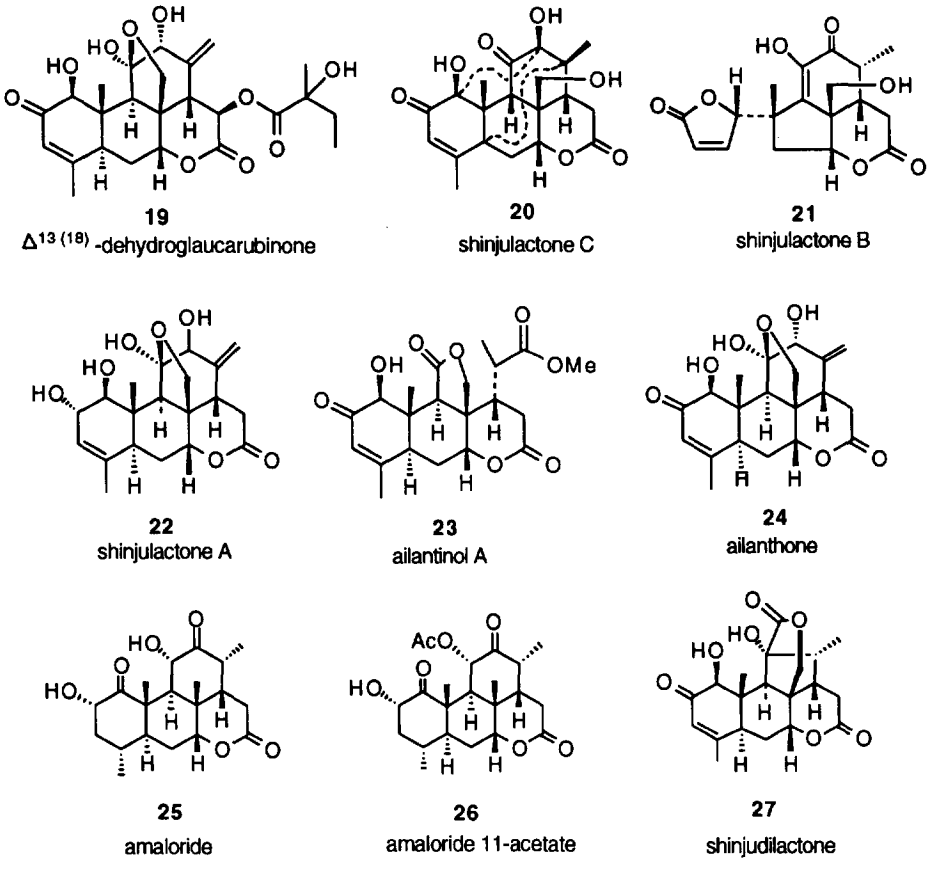


Figure 1 - Continued

Likewise, the quassinoid shinjulactone A (**22**) had a good EC_{50} value of ca. 5 μM but was also cytotoxic at this concentration. Shinjulactone B (**21**) and ailantinol A (**23**) showed no cytotoxicity and were marginally active with EC_{50} values of 28 and 30 μM, respectively. The most promising compound was shinjulactone C (**20**); this compound had a TI of >25 and showed significant anti-HIV activity with an EC_{50} value of 10.6 μM. Further studies on analogs and related compounds to increase the pharmacological profiles of **20** are in progress.

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16. Yadanzioside-E (**18**) was isolated from an ethanolic extract (45.5 kg) of *Brucea javanica* by a chromatographic method (Si gel column chromatography, repeated preparative TLC and HPLC) analogous to that described in reference 15, and identified by comparison of mp, $[\alpha]_D$, UV, and nmr spectra data with those reported in reference 17.

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21. HIV Growth Inhibition Assay: Compounds were dissolved in DMSO for a final working concentration of 100, 20, 4, and 0.8 μg/mL. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate, $TCOD_{50} = 10^{-4}$ IU/ml), while another remained uninfected (to be used for toxicity determinations). H9 cells were continuously maintained (mycoplasma-free) in RPMI-1640 with 10% FCS supplemented with L-glutamine. After a 1 hour virus adsorption at 37° C and 5% CO_2, both H9 cell populations were washed 3 times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or medium alone (positive infected control/negative drug control). In addition, ddC was also assayed during the experiment as a positive drug control. The plates were incubated at 37° C and 5% CO_2 for 4 days. Cell-free supernatants were collected on Day 4 for use in the p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was also determined by performing cell counts on the uninfected H9 cell which had either received medium alone (no toxicity), test drug or ddC. The control drug ddC showed IC_{50}, EC_{50}, and Therapeutic Index values of 500 μM, 0.03 μM, and 16,667, respectively.

Non-toxic suppressive agents are presented in the following terms: IC_{50}, the concentration of test sample toxic to 50% of the uninfected H9 cells; EC_{50}, the concentration of test sample which was able to suppress HIV replication by 50%; and Therapeutic Index (TI), the ratio of IC_{50} to EC_{50}.

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