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HIV AND REVERSE TRANSCRIPTASE INHIBITION BY TANNINS [1]

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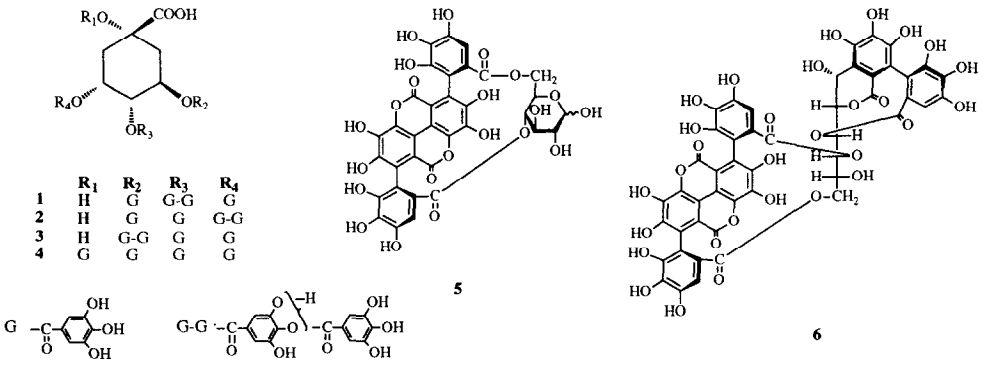
Abstract : Further evaluation of tannins as anti-HIV agents indicates that these compounds inhibited HIV replication only slightly in the absence of toxicity (therapeutic index ≤ 5). In addition, no correlation was found between inhibition of reverse transcriptase and of HIV in cell culture.

Since the acquired immunodeficiency syndrome is caused by HIV-1, [2, 3] the development of therapy against HIV infection has been based on the screening of compounds for anti-HIV efficacy in cell culture. The first drug (AZT) approved by the FDA for AIDS therapy was discovered as a result of screening efforts.[4] Another nucleoside, ddI, has also been approved by the FDA. Continued efforts at anti-HIV drug discovery are necessary due to the toxicity of AZT to bone marrow cells *in vivo*[5] as well as the appearance of AZT and ddI resistant strains after prolonged AZT therapy.[6, 7] Since reverse transcriptase (RT) plays an essential role in the replication of HIV, RT is an attractive target in the development of anti-AIDS drugs.



Previously, we reported the anti-HIV activity and reverse transcriptase inhibition shown by several tannins,[1, 8, 9] such as tetragalloylquinic acids (**1** - **4**), punicalin (**5**), and punicacortein-C (**6**). Since these were the first identified anti-HIV tannins, it is important to evaluate other classes of tannins as HIV inhibitors. In an effort to extend these observations, we have evaluated 42 additional tannins,[1] including gallo- (**7** - **17**), ellagi- (**18** - **35**), condensed (**36** - **45**), and complex (**46**, **47**) tannins, and a related compound (**48**) for RT inhibition and for inhibition of HIV in cell culture assays in an effort to detect potential anti-HIV natural products.

The IC_{50} and EC_{50} values for 42 tannins and related compounds are shown in Table 1. The IC_{50} and EC_{50} concentrations were very close to one another in each class of tannins.

Gallotannins showed inhibitory activity only at toxic concentrations. Ellagitannins and condensed tannins inhibited HIV replication (EC_{50} 12 - 50 μM) only slightly in the absence of toxicity. Among the tannins tested, complex tannins showed a potent inhibitory effect against HIV replication ($EC_{50} \approx 4$ μM). However, the IC_{50} values indicated that these compounds were somewhat toxic.

Table 1. HIV Inhibition by Tannins

Compound	IC_{50}	EC_{50}	therapeutic index	Compound	IC_{50}	EC_{50}	therapeutic index
7	80	100	0.8	**28**	40	21	1.9
8	38	50	0.8	**29**	39	12	3.3
9	19	50	0.4	**30**	21	15	1.4
10	19	15	1.3	**31**	15	15	1.0
11	45	18	2.5	**32**	15	16	0.9
12	80	>100	<0.8	**33**	15	35	0.4
13	45	25	1.8	**34**	28	18	1.6
14	25	18	1.4	**35**	40	40	1.0
15	40	30	1.3	**36**	45	45	1.0
16	45	50	0.9	**37**	>25	>25	ND
17	40	45	0.9	**38**	>25	>25	ND
18	15	20	0.8	**39**	>25	14	1.8
19	62	19	3.3	**40**	>25	>25	ND
20	15	15	1.0	**41**	>25	25	>1
21	32	21	1.5	**42**	>25	17	>1.5
22	40	28	1.4	**43**	>25	21	>1.2
23	15	15	1.0	**44**	>25	21	>1.2
24	34	19	1.8	**45**	>25	15	>1.7
25	14	12	1.2	**46**	15	4	3.8
26	14	14	1.0	**47**	15	4	3.8
27	70	50	1.4	**48**	>25	>25	ND

ND = not determined

The measurement of the inhibitory activity of these 42 tannins against HIV RT was also carried out in order to investigate the correlation between HIV inhibition and reverse transcriptase inhibition. The measurements of HIV RT were made over a range of concentrations (30 - 60 μM) for individual compounds. Table 2 shows the inhibition of HIV replication and the reverse transcriptase inhibition tested at 20 μg/ml.

Table 2. HIV and HIV-RT Inhibition (%) at 20 μg/ml by Tannins

Compound	HIV	HIV-RT	Compound	HIV	HIV-RT
7	0	18	**28**	50	13
8	40	14	**29**	50	0
9	20	16	**30**	38	34
10	60	2	**31**	90	40
11	65	0	**32**	90	45
12	0	0	**33**	35	27
13	65	0	**34**	47	17
14	90	0	**35**	23	18
15	60	31	**36**	40	22
16	40	16	**37**	40	5
17	30	25	**38**	40	21
18	75	8	**39**	90	0
19	60	66	**40**	30	18
20	80	33	**41**	68	18
21	50	3	**42**	47	13
22	45	27	**43**	42	18
23	90	57	**44**	90	13
24	60	0	**45**	73	19
25	92	15	**46**	88	24
26	80	23	**47**	89	23
27	33	38	**48**	35	18

Only mild HIV RT inhibition was detected. The percentage of inhibition detected by the two assays at similar concentrations was not correlated (R=0.147, p=0.35). For example, compounds **25** and **44** inhibited HIV replication by ≈90% at 20 μg/ml, but reverse transcriptase was inhibited by only ≈15% at this same concentration.

This evaluation of a variety of tannins, including gallo-, ellagi-, condensed, and complex tannins and related compounds as inhibitors of HIV replication and HIV reverse transcriptase showed that none of the compounds displays substantial anti-HIV activity, as displayed by their low therapeutic indices. Despite previous observations of significant reverse transcriptase inhibition by tannins,[1, 8, 9] we have found in this study that reverse transcriptase inhibition does not correlate with inhibition of HIV replication. In addition, previous studies[1, 9] suggest that anti-HIV activity exhibited by tannins is due to a mechanism other than reverse transcriptase inhibition, probably inhibition of virus-cell interactions.

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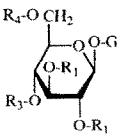
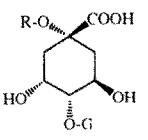
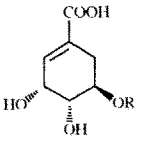


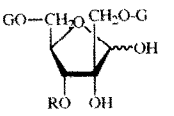
	R_1	R_2	R_3	R_4
7	H	H	H	H
8	H	H	H	G
9	G	H	H	G
10	G	G	G	G
11	G	G	G	G



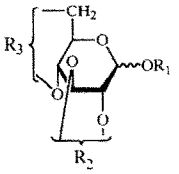
	R
12	H
13	G



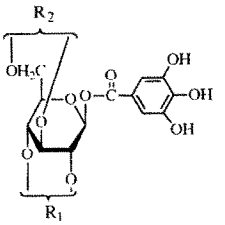
	R
14	G
15	G-G



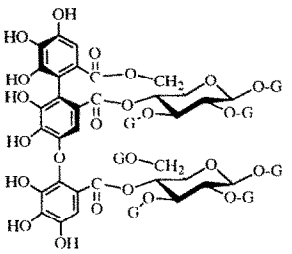
	R
16	H
17	G



	R_1	R_2	R_3
18	H	*S*-HHDP	H, H
19	G(β)	H, H	*S*-HHDP
20	H	*S* HHDP	*S*-HHDP
21	G(β)	*S*-HHDP	*S*-HHDP
22	G(β)	G, G	*S*-HHDP



	R_1	R_2
23	H, H	*R*-HHDP
24	G, G	*R*-HHDP
25	DHHDP	*R*-HHDP
26	Ela	*R*-HHDP
27	DHHDP	H, H
28	DHHDP	G, G



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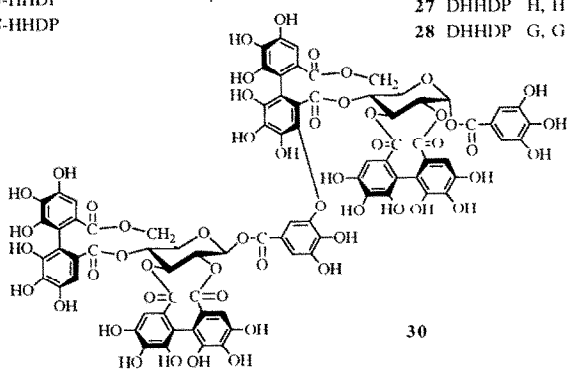

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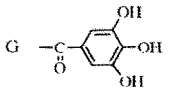
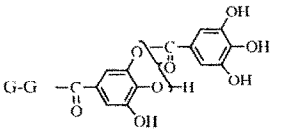
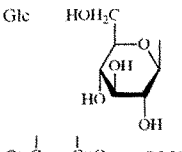
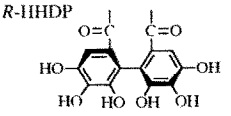
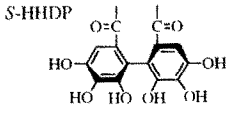
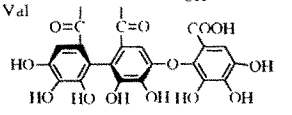


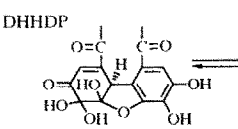


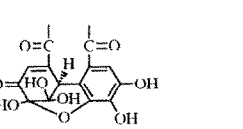


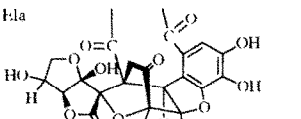


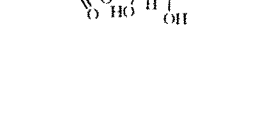


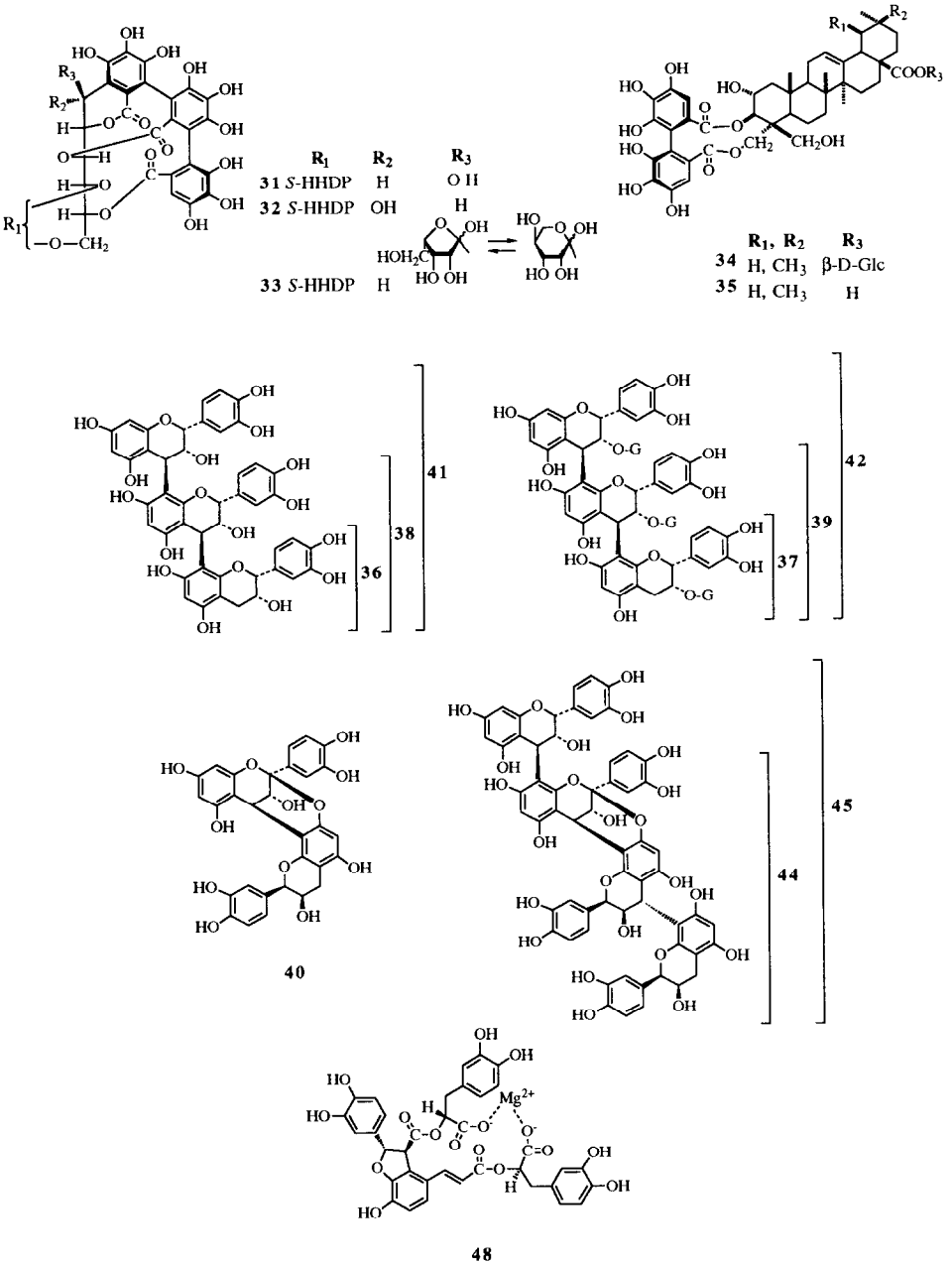


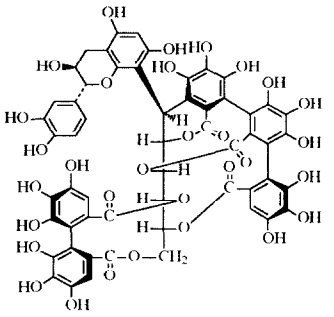









R1 R2 R3
31 S-HHDP H OH
32 S-HHDP OH H
33 S-HHDP H
R1, R2 R3
34 H, CH3 β-D-Glc
35 H, CH3 H
36
37
38
39
40
41
42
44
45
48
Mg2+



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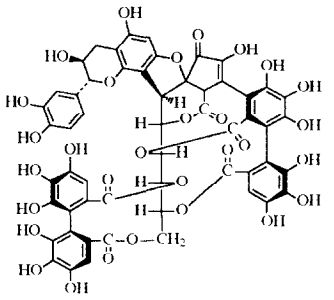

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References and Notes

1. Anti-AIDS Agents 8. For part 7, see Lee, K. H.; Kashiwada, Y.; Nonaka, G.; Nishioka, I.; Nishizawa, M.; Yamagishi, T.; Bodner, A. J.; Kilkuskie, R. E.; Cheng, Y.-C. "Tannins and Related Compounds as Anti-HIV Agents" in "Natural Products as Anti-viral Agents," *Amer. Chem. Soc. Symposium Series.* in press.
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11. HIV inhibition assay was conducted as described previously.[1, 9]
12. The HIV RT assay was performed according to the method described previously.[10]