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EVALUATION OF TEA POLYPHENOLS AS ANTI-HIV AGENTS[1]

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Abstract: Thirty-eight tea polyphenols were evaluated for their inhibitory effect against HIV replication in H9 lymphocyte cells. 8-*C*-Ascorbyl (-)-epigallocatechin (**13**) and theasinensin-D (**32**) demonstrated relatively potent anti-HIV activity with EC_{50} values of 4 and 8 μg/mL and therapeutic indexes of 9.5 and 5, respectively.

Many research approaches are currently aimed at developing new therapeutic agents to arrest the replication of the human immunodeficiency virus (HIV), including inhibitions of HIV-1 reverse transcriptase (RT),[2,3] protease,[4-6] membrane fusion[7,8] and integrase[9,10] as mechanism(s) of action. We are continually screening plant-derived natural products as anti-HIV agents to find potential new lead compounds with a novel structure and/or mechanism(s) of action. As part of our screening, we have examined the inhibitory effect of tea polyphenols against HIV-1 replication in acutely infected H9 lymphocytic cells. We have thus far isolated and characterized seventy-one tea polyphenols from green,[11,12] oolong,[13-15] and black teas.[16-18] Among these, 36 typical tea polyphenols, along with two related compounds, were selected for anti-HIV evaluation.

The tea polyphenols (**1–38**) evaluated for inhibitory effects against HIV-1 replication in H9 lymphocytes are shown in Figure 1. They were categorized structurally into 6 groups, including flavan-3-ols (**1–14**), proanthocyanidins (**15–21**), assamicains (**22–24**), oolonghomobisflavans (**25–27**), theasinensins (**28–34**), and theaflavins (**35–38**). The anti-HIV data for these compounds are shown in Table 1. Among these, 8-*C*-ascorbyl (-)-epigallocatechin (**13**) demonstrated relatively potent anti-HIV activity (EC_{50} 4 μg/mL) with

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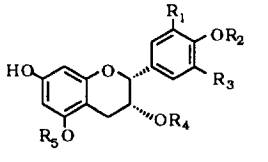
an adequate therapeutic index (T.I.) value (9.5). Introduction of a galloyl group at the C-3 hydroxyl of **13** gave **14** and decreased anti-HIV activity (EC_{50} 10 μg/mL), although the toxicity against H9 cells shown by **14** (IC_{50} 40 μg/mL) was similar to that (IC_{50} 38 μg/mL) found in **13**. An inseparable mixture of (-)-epigallocatechin 3,3'- (**11**) and 3,4'-di-*O*-gallate (**12**) (whose galloyl groups at the C-3' or -4' hydroxyl showed ready migration in solution) showed moderate anti-HIV activity (EC_{50} 6.5 μg/mL; T.I. 6.2). In contrast, (-)-epigallocatechin 3,5-di-*O*-gallate (**10**) inhibited HIV-1 replication by 50% at a similar concentration (EC_{50} 5.5 μg/mL), but exhibited a lower T.I. value (3.6) than that of the 11:12 mixture. This observation suggested that the location(s) of the galloyl group(s) are important for retaining anti-HIV activity. Theasinensin D (**32**) also showed moderate anti-HIV activity (EC_{50} 8 μg/mL; T.I. 5), whereas theasinensin A (**28**), which differs structurally only in the stereochemistry at the biphenyl bond, exhibited no anti-HIV activity. This result suggested that biphenyl atropisomerism is also important to the anti-HIV activity. Marginal anti-HIV activity (EC_{50} 5.5 μg/mL; T.I. 4.5) was observed with (-)-epitheaflagalin 3-*O*-gallate (**35**). The other tea polyphenols exhibited anti-HIV activity only at toxic concentrations or no anti-HIV activity.

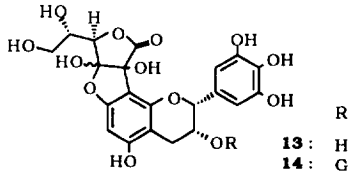
It should be noted that (-)-epicatechin 3-*O*-gallate (**4**) and (-)-epigallocatechin 3-*O*-gallate (**8**), which were reported previously as potent inhibitors of HIV-reverse transcriptase (RT),[19] did not demonstrate an inhibitory effect against HIV-1 replication in H9 lymphocytic cells. Previously, we have screened various tannins and related compounds and concluded that RT inhibition does not correlate with inhibition of HIV replication.[20] The absence of anti-HIV activities with **4** and **8** was consistent with our previous results.

Figure 1

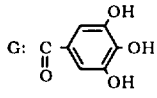
Flavan-3-ols (1 - 14)

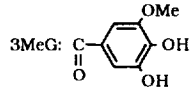


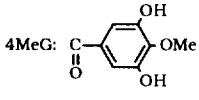
	R_1	R_2	R_3	R_4	R_5
1:	H	H	H	H	H
2:	H	H	H	G	H
3:	H	H	OH	H	H
4:	H	H	OH	G	H
5:	H	H	OH	3MeG	H
6:	H	H	OH	4MeG	H
7:	OH	H	OH	H	H
8:	OH	H	OH	G	H
9:	OH	H	OH	*p*-Cou	H
10:	OH	H	OH	G	G
11:	OH	H	OG	G	H
12:	OH	G	OH	G	H



13: H
14: G







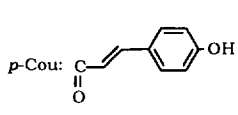
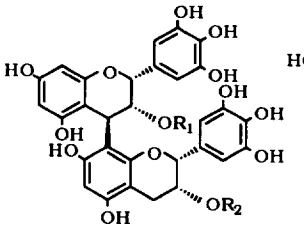
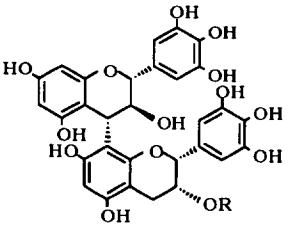


Figure 1 -continued

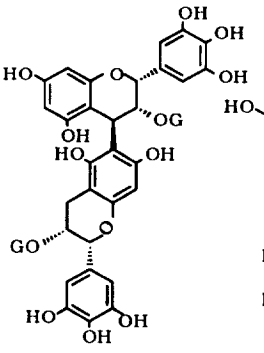
Proanthocyanidins (15 - 21)



	R_1	R_2
15 :	H	H
16 :	H	G
17 :	G	G



	R
18 :	H
19 :	G



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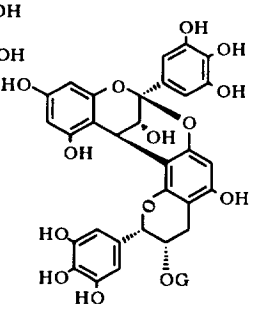
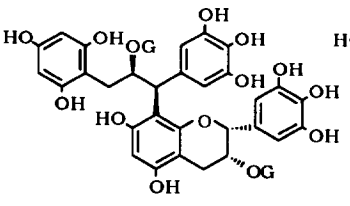
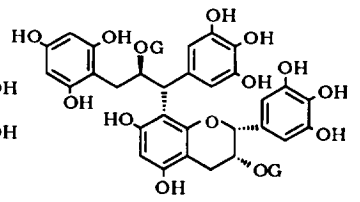


21

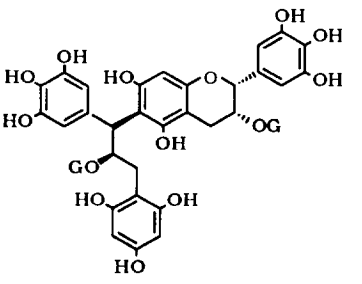
Assamicains (22 - 24)



22

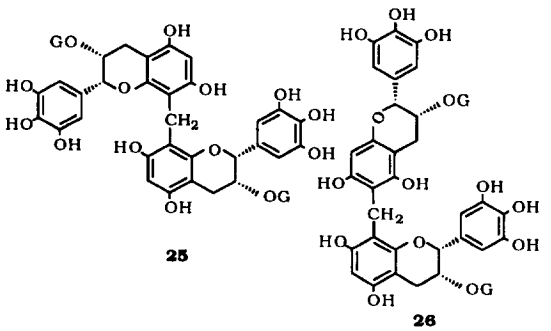


23



24

Oolonghomobisflavans (25 - 27)



25

26

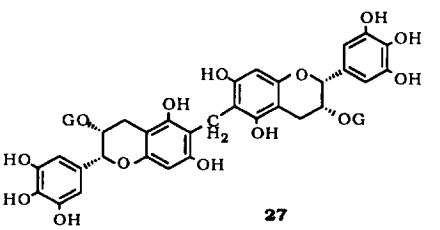


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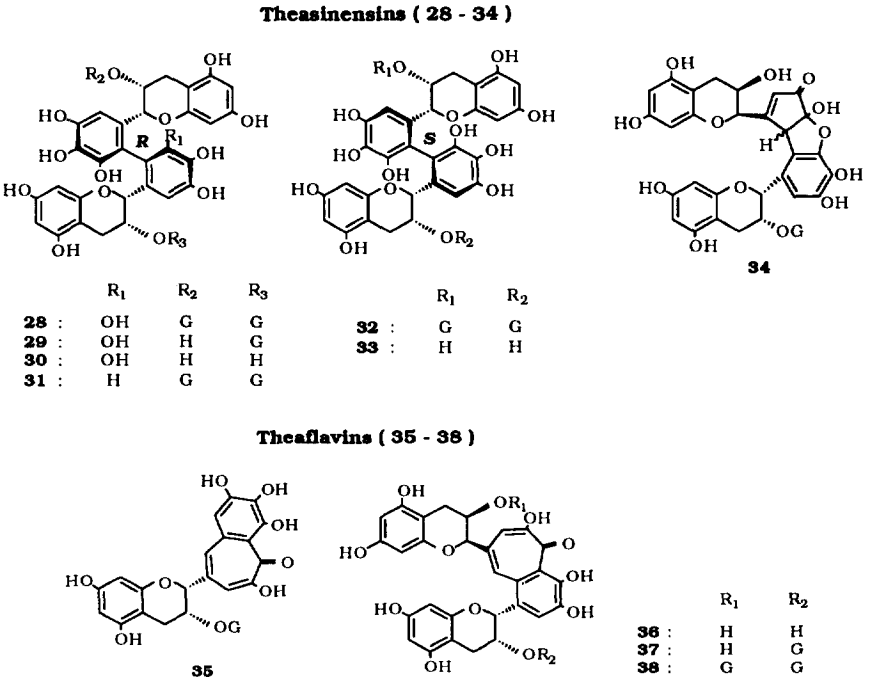
Figure 1 -continued



	R_1	R_2	R_3
28 :	OH	G	G
29 :	OH	H	G
30 :	OH	H	H
31 :	H	G	G

	R_1	R_2
32 :	G	G
33 :	H	H

	R_1	R_2
36 :	H	H
37 :	H	G
38 :	G	G

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Table 1. Inhibition of HIV-1 Replication in H9 Lymphocytic Cells by Tea Polyphenols[21]

Compounds	IC_{50} (μg/ml)[a]	EC_{50} (μg/ml)[b]	Therapeutic Index[c]
1	100	70	1.4
2	50	d	-
3	9.8	9.8	1.0
4	>10	d	-
5	50	d	-
6	50	d	-
7	1.8	d	-
8	8	7	1.1
9	35	8	4.4
10	20	5.5	3.6
11 and 12	40	6.5	6.2
13	38	4	9.5
14	40	10	4
15	18	15	1.2
16	28	15	1.9
17	40	d	-
18	18	12	1.5
19	9	d	-
20	30	15	2
21	40	d	-
22	8	d	-
23	9	d	-
24	35	14	2.5
25	45	d	-
26	40	25	1.6
27	35	9	3.9
28	18	19	0.95
29	9.5	d	-
30	9.5	6	1.6
31	50	d	-
32	40	8	5
33	20	10	2
34	48	25	1.9
35	25	5.5	4.5
36	48	d	-
37	48	d	-
38	48	d	-

[a] Concentration which inhibits uninfected H9 cell growth by 50%.
[b] Concentration which inhibits viral replication by 50%.
[c] Therapeutic index = IC_{50}/EC_{50}
[d] No Suppression

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