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NEW HEXAHYDROXYDIPHENYL DERIVATIVES AS POTENT INHIBITORS OF HIV REPLICATION IN H9 LYMPHOCYTES[1]

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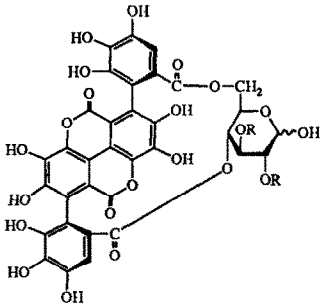
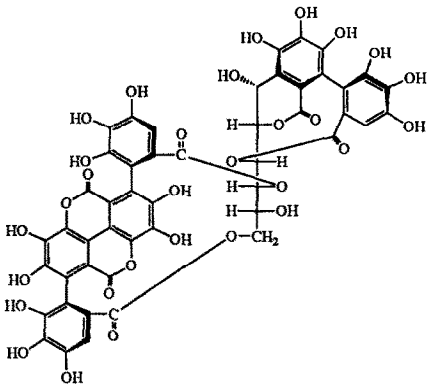
Abstract: A series of hexahydroxydiphenyl derivatives of ellagic acid have been synthesized as simple analogs of ellagitannins and evaluated for their inhibitory activity against HIV replication in H9 lymphocyte cells. Compound **10** was found to be a potent inhibitor of HIV replication in infected H9 lymphocytes with little cytotoxicity.

In the course of our search for natural products as anti-HIV agents, we previously found that galloylquinic acids, especially tetragalloylquinic acids, were potent HIV inhibitors.[2] Our further investigation of other classes of tannins revealed that punicalin (**1**), punicalagin (**2**) and punicacortein-C (**3**) are the most potent inhibitors of HIV replication.[3, 4] Since **1**-**3** all contain a gallagyl (tetraphenoyl) group, it suggests that this structure may be important for the HIV inhibition. In addition, the gallagyl group is



1 : R = H

2 : R =



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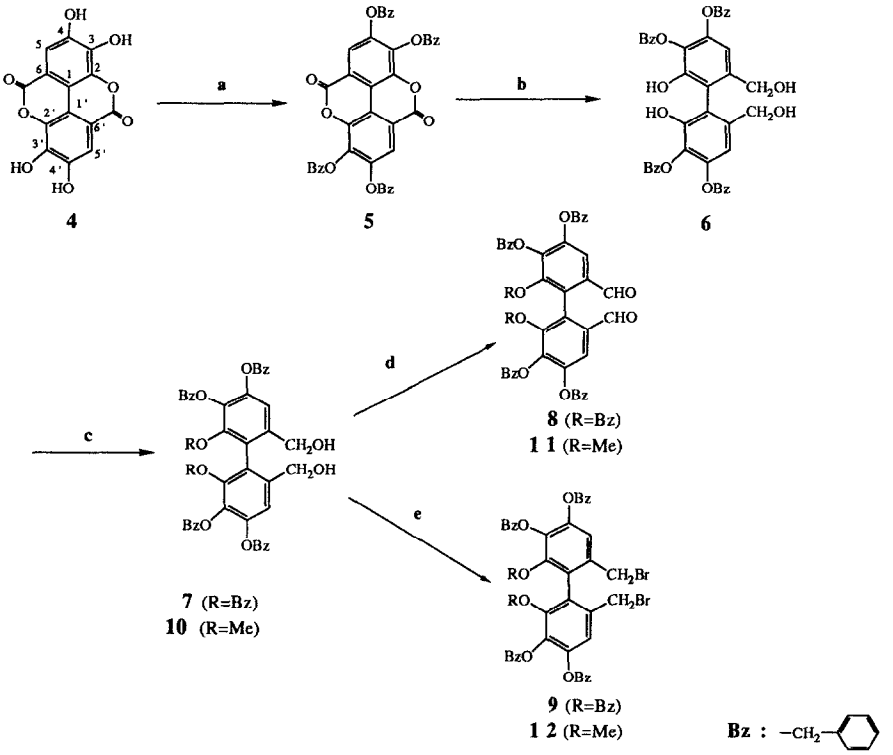
considered to be formed biosynthetically from a hexahydroxydiphenoyl (HHDP) group and two galloyl groups.[5] Therefore, the HHDP group is regarded as a basic skeleton for gallagyl group. Based on these observations, we have prepared derivatives of HHDP group from ellagic acid, and have evaluated their inhibitory effect on HIV replication.

The biphenyl derivatives (**6**-**12**) were synthesized from the commercially available ellagic acid (**4**). As shown in Scheme I, compound **4** was benzylated according to a procedure reported by Schmidt, *et al* [6] to give tetrabenzylellagic acid (**5**). Reduction of **5** with $LiAlH_4$ yielded the tetraol **6**. Further benzylation of **6** afforded the hexabenzyl compound **7**. Methylation of **6** with Me_2SO_4/K_2CO_3 furnished the dimethyoxytetrabenzyl derivative **10**.[7] Treatment of **7** and **10** with MnO_2 and $SOBr_2$ led to the formation of **8**, **11** and **9**, **12**, respectively.



Scheme 1 a : $PhCH_2Cl$, K_2CO_3, KI, $PhCOCH_3$, reflux. b : $LiAlH_4$, dioxane, reflux
c : Me_2SO_4 or $PhCH_2Br$, K_2CO_3, acetone, reflux. d : MnO_2, benzene, R.T.
e : $SOBr_2$, benzene, ice-cooling.

TABLE I. Biological Evaluation[8] of the Hexahydroxydiphenyl Derivatives (6-16)

Cmpd. No.	$IC_{50}(\mu M)^a$	$EC_{50}(\mu M)^b$
6	13	6
7	>120	>120
8	70	45
9	>100	>100
10	>140	10
11	4	2
12	30	13
AZT	2000	0.04

[a]Concentration which inhibits uninfected cell growth by 50%.
[b]Concentration which inhibits virus replication by 50%.

As illustrated in Table I, only one hexahydroxydiphenyl derivative (**10**) demonstrated potent inhibitory activity against HIV in acutely infected H9 lymphocyte cells (EC_{50}=10μM) combined with relatively low toxicity with IC_{50} (concentration which inhibits uninfected cell growth by 50%) of >140 μM. All other derivatives did not inhibit HIV or did so only at toxic concentrations. The two CH_2OH groups at C-6 and C-6' appear to be essential for the selective HIV inhibition. Replacement of these two groups with CHO as seen in **11** greatly increased the toxicity and only slightly enhanced the anti-HIV activity. When the two groups were replaced by CH_2Br as found in **12**, toxicity increased with no change in the anti-HIV activity.

The OMe groups at C-2 and C-2' were also essential for retaining the selective antiviral activity. When they were both replaced by OH as in the case of **6**, toxicity was greatly increased. The compound became inactive when the OMe groups were replaced by OBz as observed in **7**. In **8** and **9**, OBz replaced the OMe groups of **11** and **12**, respectively. Both **8** and **9** are much less active than **11** and **12**.

Previously,[3] it was determined that tannins can inhibit HIV, at least in part, by interfering with virus-cell interactions. Compound **10** was incubated with virions before infection, during the one hour infection step, or added to the infected cells immediately after infection. Compound **10** was found to inhibit only when present after infection (data not shown). This indicates that the mode of inhibition by **10** is different from that of the tannins. Investigation on the detailed mechanism of action of **10** and related tannins as potent anti-HIV agents is in progress.

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References and Notes

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4. Since the absolute configuration at C-1 of the C-glucosidic ellagitannins has recently been revised by Nonaka *et al* (Nonaka, G.; Sakai, T.; Tanaka, T.; Mihashi, K.; Nishioka, I. *Chem Pharm. Bull.* **1990**, *38*, 2151), the configuration at C-1 in **3** is revised accordingly.
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7. 2,2'-Dimethoxy-3,3',4,4'-tetrabenzyloxy-1,1'-diphenyl-6,6'-dimethanol (**10**): colorless needles (from EtOH): mp 136-137 °C; ^{1}H-NMR ($CDCl_3$) δ 7.47~7.24 (20H in total, aromatic-H), 6.96 (2H, s, H-5, H-5'), 5.17, 5.11 (2H each, d, *J*=12 Hz, $PhCH_2O$), 5.07 (4H, s, $PhCH_2O$), 4.13 (4H, s, CH_2OH), 3.63 (6H, s, OMe), and 2.77 (2H, OH). *Anal.* Calcd for $C_{44}H_{42}O_8 \cdot 1/2\ H_2O$: C 74.66 ; H, 6.12. Found : C, 75.04 ; H, 6.13.
8. HIV Inhibition Assay : The HIV inhibition was measured as described previously.[1-3] Briefly, H9 lymphocytes (3.5 x 10^6 cells/ml) were incubated in the presence or absence of HIV-1 (IIIB strain, 0.01-0.1 $TCID_{50}$/cell) for 1 hour at 37°C. Cells were washed thoroughly and resuspended at a final concentration of 2 x 10^5 cells/ml in the presence or absence of compound. After incubation for 3 days at 37° C, the cell density of uninfected cultures was determined by cell count to assess toxicity of the drug. A p24 antigen capture assay[2] was used to determine the level of virus released into the medium of HIV infected cultures.