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Anti-AIDS Agents—XXVI.† Structure–Activity Correlations of Gomisin-G-Related Anti-HIV Lignans From *Kadsura interior* and of Related Synthetic Analogues

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Abstract—Bioactivity-directed fractionation of an ethanolic extract of the stems of *Kadsura interior* led to the isolation and identification of 12 known lignans (**1–12**). Seven of these compounds (**1**, **6**, **8–12**) were active as anti-HIV agents. Gomisin-G (**11**) exhibited the most potent anti-HIV activity with EC_{50} and therapeutic index (TI) values of 0.006 μg/mL and 300, respectively. Schisantherin-D (**6**), kadsuranin (**8**), and schisandrin-C (**10**) showed good activity with EC_{50} values of 0.5, 0.8, and 1.2 μg/mL, and TI values of 110, 56, and 33.3, respectively. Ten related synthetic biphenyl compounds, five variously substituted bismethylenedioxy, dimethoxy, and dimethoxycarbonyl isomers (**18–22**) and five brominated derivatives (**23–27**) also were evaluated for inhibitory activity against HIV-1 replication in acutely infected H9 cells. The total syntheses of two new isomers (**21** and **22**) are reported for the first time. The anti-HIV data indicated that the relative position and types of substituents on the phenolic hydroxy groups of either the natural lignans or the synthetic biphenyl compounds rather than the numbers of bromine(s) on the aromatic rings are of primary importance. In the cyclooctane ring of the natural lignans, the position and substitution of hydroxy groups are also important to enhanced anti-HIV activity. © 1997 Elsevier Science Ltd.

Introduction

The stems of *Kadsura interior* A. C. Smith (Schizandraceae) are the main botanical components of the Chinese medicinal herb Ji-Xue-Teng.[2,3] An extract of these plant stems is used to prepare the Chinese medicine Ji-Xue-Teng Gao, which is prescribed for the treatment of menstrual irregularities, blood deficiencies, and other feminine disorders.[4] In the course of our continuing search for natural products as anti-AIDS agents, an ethanolic extract of the stems of *K. interior* was tested for inhibition of HIV replication and showed significant activity. Bioactivity-directed fractionation of this extract led to the isolation and identification of 12 lignans (**1–12**). Seven of these compounds (**1**, **6**, **8–12**) were active in an HIV growth inhibition assay with therapeutic index (TI) values >5. In particular, gomisin-G (**11**) exhibited the most potent anti-HIV activity with EC_{50} and TI values of 0.006 μg/mL and 300, respectively. The active natural lignans all contain a cyclooctane ring linking a biphenyl ring system, which is substituted with methoxy and/or methylenedioxy groups. Since the biphenyl substitution pattern seems important to the anti-HIV activity, structure–activity correlations with related synthetic biphenyl compounds that also contain bismethylenedioxy and methoxy groups were performed. To determine the optimal pattern(s) of biphenyl substituion, five isomeric biphenyl compounds were evaluated including three known compounds (**18–20**)[5] and two newly synthesized isomers, 2,2′-dimethoxycarbonyl-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxybiphenyl (**21**) and 2,2′-dimethoxy-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxycarbonyl biphenyl (**22**). The parent structure **18**, which is the antihepatitic drug DDB (2,2′-dimethoxycarbonyl-4,4′-dimethoxy-5,6,5′,6′-bismethylenedioxybiphenyl) and its mono- (**24**) and di-(**23**) bromo derivatives were previously identified as potent anti-HIV agents.[5]

Chemistry

In a previous paper,[6] we reported the isolation and characterization of interiotherin A (**1**) and interiotherin B (**2**) from *K. interior*. Further silica-gel chromato-

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†For Part 25, see ref 1.

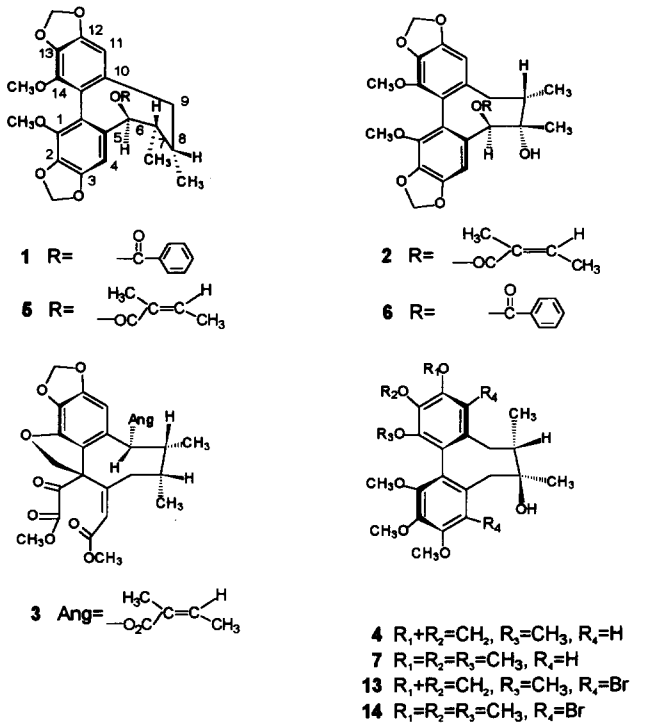


Figure 1. Structures of lignans **1–7** and **13**, **14**.

graphy of the active ethyl ether fraction yielded 10 known compounds, heterolitin-F (**3**), which was isolated previously from *Kadsura heterclita*,[7] gomisin-A (**4**), schisantherin-D (**6**), gomisin-J (**9**), schizandrin (**7**), gomisin-G (**11**), and (+)-deoxyschizandrin (**12**), which were isolated from *Schizandra chinensis*,[8–11] and angeloylgomisin-R (**5**), kadsuranin (**8**), and schisandrin-C (**10**), which were isolated from *Kadsura longipedunculata*.[12] All compounds (Figs 1 and 2) were identified by comparison of their CD, MS, UV, IR, and ^{1}H and ^{13}C NMR spectral data with those described in the literature.

In the related synthetic biphenyl compounds, a series of isomers (see Fig. 3) is possible by changing the relative position of the three groups on each phenyl ring. During the total synthesis of **18** from gallic acid, two isomers, 6,6′-dimethoxy-4,5,4′,5′-bismethylenedioxy-2,2′-dimethoxycarbonyl biphenyl (**19**) and 4,6′-dimethoxy-5,6,4′,5′-bismethylenedioxy-2,2′-dimethoxycarbonyl biphenyl (**20**), were both obtained in an Ullmann reaction.[13] Here, two new biphenyl isomers, 5,5′-dimethoxy-3,4,3′,4′-bismethylenedioxy-2,2-dimethoxycarbonyl biphenyl (**21**) and 2,2′-dimethoxy-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxycarbonyl biphenyl (**22**), were synthesized according to the route shown in Scheme 1.

The starting material 2,3,4-trihydroxybenzoic acid was first esterified to give methyl 2,3,4-trihydroxybenzoate (**28**). The monomethylation of the 4-OH group in **28** was achieved by Scheline's method.[14] The IR spectra of the product showed an absorption peak for the ester carbonyl group at 1660 cm^{-1}, indicating the presence of

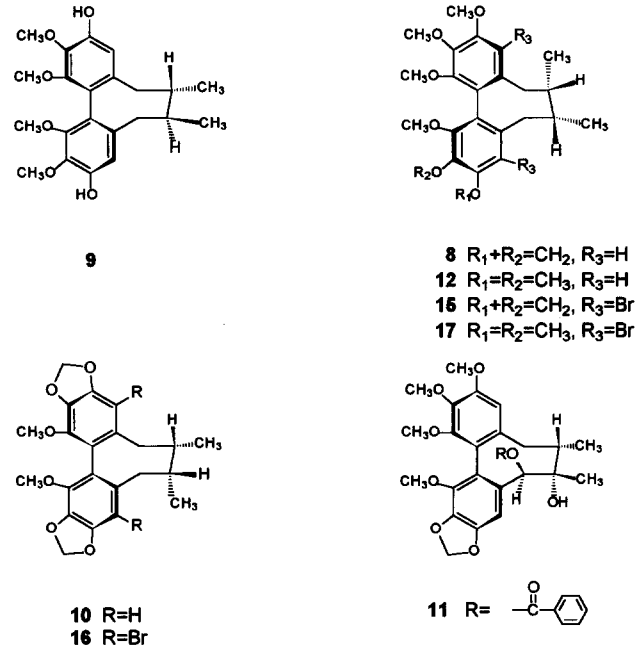


Figure 2. Structures of lignans **8–12** and **15–17**.

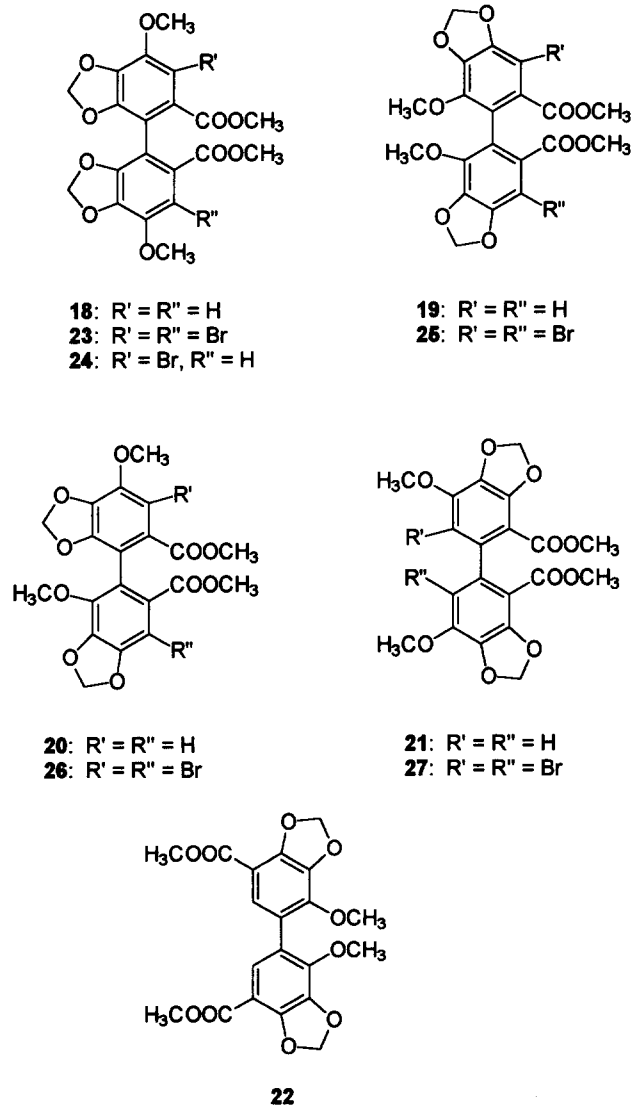
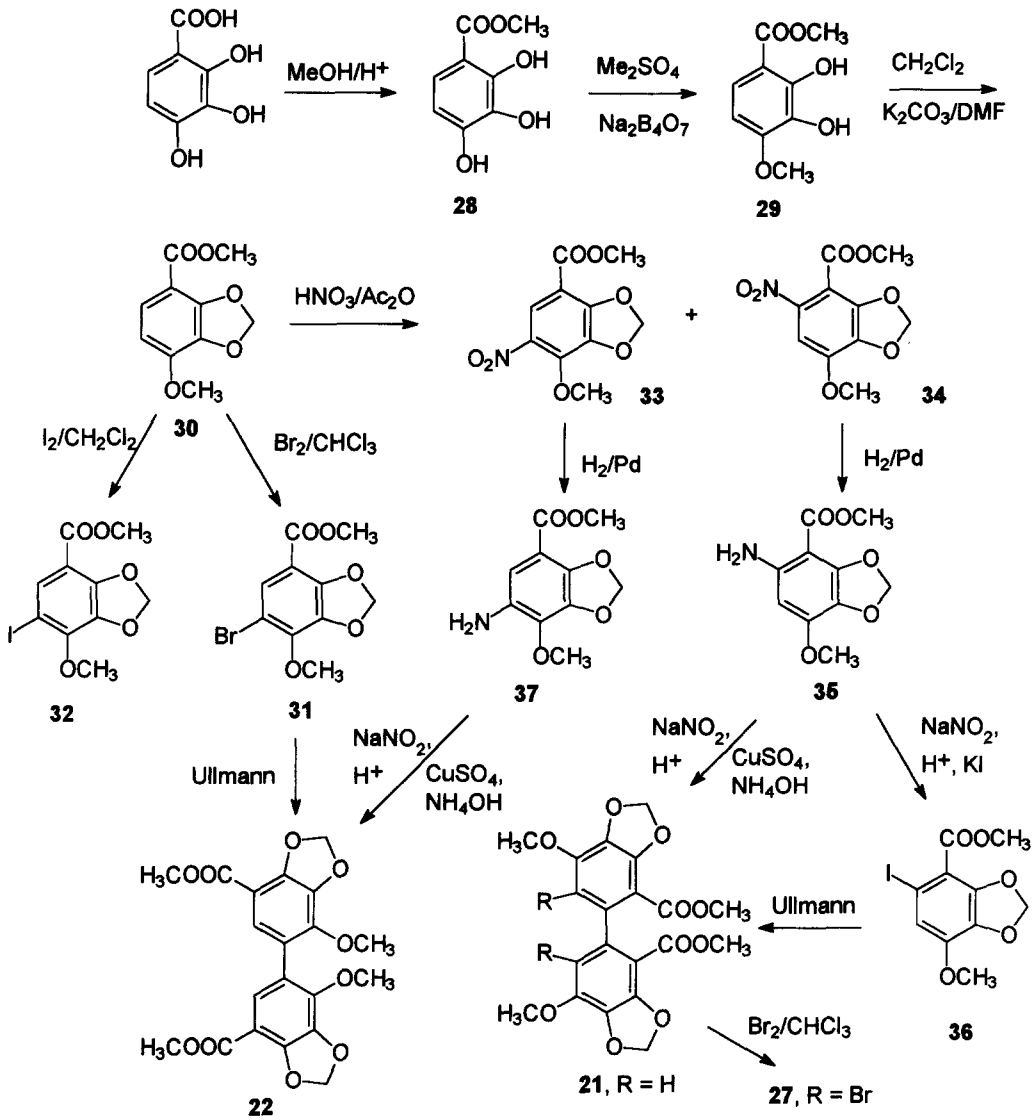


Figure 3. Structures of synthetic biphenyl compounds **18–27**.



Scheme 1.

internal hydrogen bonding. In the ^{1}H NMR spectra, a signal was observed at δ10.98 ppm, ascribable to the chelated OH group. These spectroscopic data indicated that monomethylation occurred at the 4-OH group, not at the 2-position. Thus, the structure of the monomethyl ether of compound **28** was assigned as methyl-2,3-dihydroxy-4-methoxybenzoate (**29**). Methylenation of compound **29** with CH_2Cl_2 in DMF afforded methyl-2,3-methylenedioxy-4-methoxybenzoate **30** in good yield (91%). The IR absorption bands of the ester carbonyl group in **30** shifted from 1660 to 1710 cm^{-1}. Bromination of **30** with dioxane–dibromide reagent[15] in Et_2O yielded methyl-2,3-methylene-dioxy-4-methoxy-5-bromobenzoate (**31**) in good yield (>80%). Compound **31** was also obtained by treating **30** with dry bromine in $CHCl_3$. The position of the bromine atom introduced was determined unequivocally on the basis of a two-dimensional NOE spectrum. A strong NOE response was observed between the aromatic proton and the methyl protons of the methoxycarbonyl group. Therefore, bromination must have taken place at the 5-position, which is in accordance with the expected orientation for electrophilic aromatic substitution. Ullmann reaction of compound **31** with active copper powder in DMF gave only a very small amount of the biphenyl derivative **22**. This compound gave a molecular ion at *m/z* 418 as the base peak in its MS spectrum. The IR and ^{1}H NMR spectra agreed well with the assigned structure. The new compound **22** was named δ-DDB.

In order to improve the yield of the desired biphenyl **22**, we also attempted to prepare the iodo derivative (**32**) of **30**, which might be more reactive than the bromide (**31**) in the Ullmann reaction. Iodination of compound **30** was effected using silver trifluoroacetate–iodine[16] or iodine–copper (II) acetate–acetic acid.[17] However, when the iodo compound **32** was subjected to the Ullmann reaction, the main product was the dehalogenated derivative **30**. We ascribed the poor yield of the biphenyl **22** to the fact that the halogen atoms in compounds **31** and **32** might be deactivated by the *meta*-

oriented electronegative ester group. Therefore, we postulated that a derivative including a halogen atom at the *ortho* position of the ester carbonyl group would be more reactive.[18] Nitration of compound **30** with concentrated HNO_3 in Ac_2O afforded two nitro derivatives, **33** and **34**, in a ratio of 1:10. The nitro group in compound **34** was determined to be at C-6 from ^1H NMR data. The signal of the aromatic proton (δ 7.48 ppm) is identical to the value calculated (δ 7.40 ppm) for an aromatic proton in the 5-position. Further confirmation was obtained by a NOESY NMR spectrum. An NOE response was clearly observed between the aromatic proton (δ 7.48 ppm) and the methoxy proton (δ 4.04 ppm), confirming the presence of the nitro group at the 6-position. Accordingly, the minor product **33** was identified as the 5-nitro compound. The chemical shift of its aryl proton is at δ 8.11 ppm, consistent with a proton at the 6-position. Compounds **33** and **34** were reduced separately with titanium chloride[19] or Pd–C hydrogenation to obtain amino derivatives **37** and **35**, respectively, which showed IR absorption bands at 1665 cm^{-1} (chelated ester CO).

Diazotization of **35** with $NaNO_2$ in the present of H_2SO_4 easily afforded the diazonium salt. Subsequent treatment of the resulting diazonium salt with KI and Cu powder[20] afforded the iodo compound **36**. In its ^1H NMR spectrum, the aromatic proton resonated at δ 7.11 ppm, whereas in the spectrum of **32**, the corresponding proton occurred at δ 7.88 ppm; the difference of the chemical shift (0.77 ppm) between the two signals must be the result of the anisotropic effect caused by the adjacent methoxycarbonyl group. When the iodo compound **36** was subjected to the Ullmann reaction, compound **21**, named ω-DDB, was formed in a 44% yield. The reactivity of the iodine atom in **36** was apparently increased by the *ortho*-located ester group.[21] Another convenient coupling method is the Gombery–Bachmann reaction. Compound **21** was obtained directly from **35** via an aryldiazenium salt intermediate in the presence of alkali.[22] The yield was 48%. Meanwhile, compound **22** (δ-DDB) was also obtained directly from **37** by the same reaction in a 40% yield.

Five variously substituted biphenyl isomers (**18–22**) were now obtained via different pathways. Bromination of **18** to give the di- (**23**) and mono-(**24**) brominated derivatives was previously reported.[5] Dibromination of three additional biphenyl compounds and five of the natural lignans was readily accomplished at room temperature with a molar ratio of bromine to biphenyl of 2:1. The structures of the dibrominated derivatives, **13–17** and **25–27** (see Figs 1–3), prepared from **4**, **7**, **8**, **10**, **12**, and **19–21**, respectively, were identified by their ^1H NMR, MS, IR and EA data. However, compound **22** could not be brominated by this method due to the effects of other functional groups in the biphenyl system.

Results and Discussion

All compounds were evaluated for their inhibitory activity against HIV-1 replication in acutely infected H9 cells. The anti-HIV activities of the natural lignans (**1–12**) and their brominated derivatives (**13–17**) are shown in Table 1. Among these compounds, gomisin-G (**11**) showed the most potent anti-HIV activity with an EC_{50} value of 0.006 μg/mL and a therapeutic index (TI) of 300. Schisantherin-D (**6**), kadsuranin (**8**), and schisandrin-C (**10**) showed good activity with EC_{50} values of 0.5, 0.8, and 1.2 μg/mL, and TI values of 110, 56, and 33.3, respectively. Compounds **1**, **9**, and **12** also were active, but not so potent as the former compounds.

Comparison of the anti-HIV activities of compounds **1–12** suggested that 6-benzoyl and 7-hydroxy substituents might enhance anti-HIV activity. Interiotherin-A (**1**), interiotherin-B (**2**), and schisantherin-D (**6**) are structurally similar to each other differing only in the substituents on C-6 and C-7. Compound **2** showed no anti-HIV activity and has an angeloyl group at C-6 rather than the benzoyl group found in the active compound **6**. While compounds **1** and **6** both contain a benzoyl group, **1** does not contain the C-7 hydroxy group found in **6** and is also less active. Finally, gomisin-A (**4**) has no C-6 benzoyl group and was inactive, while gomisin-G (**11**) with a C-6 benzoyl group was extremely active. These results suggested that both benzoyl and hydroxy groups at C-6 and C-7, respectively, are important for enhanced anti-HIV activity.

Compounds **8**, **9**, **10**, and **12** have no substituents on C-6 and C-7, but do have different substituents on the aromatic rings. While these four compounds had similar EC_{50} values, compounds **9** and **12**, which do not have a methylenedioxy group, had much lower TI values than compounds **8** and **10**, which contain one or more methylenedioxy groups on the aromatic rings. Compound **10**, which has methylenedioxy groups on both aromatic rings, was less active than compound **8**, which has dimethoxy substitution instead of the methylenedioxy group on one ring. Similarly, compound **11**, which has the same aromatic substituents as compound **8**, was significantly more active than compound **6**, which has the same aromatic and cyclooctane substituents as compound **10**. These results showed that 2,3-methylenedioxy and 12,13-dimethoxy substitutions on the aromatic rings also are important to enhanced anti-HIV activity.

Based on the above SAR results, compound **5** was expected to be and was inactive. Heteroclitin-F (**3**), which is a C-2,3-*seco*-type lignan, also showed no anti-HIV activity.

Ten biphenyl compounds that are related in structure to the natural lignans **1–12** were also studied. The five parent isomers (**18–22**) each contained two methoxy, two methoxycarbonyl, and two methylenedioxy groups at different positions on the phenyl rings. Five brominated derivatives (**23–27**) were also evaluated.

Table 1. Anti-HIV activities of lignans (**1–12**) and brominated derivatives (**13–17**)

Compound	IC_{50} (μg/mL)	EC_{50} (μg/mL)	Therapeutic index
1	50	3	13
2	50	20	2.5
3	>100	35	2.9
4	50	12	2.3
5	40	11	3.6
6	55	0.5	110
7	9	–	–
8	45	0.8	56
9	9	1.5	6
10	40	1.2	33.3
11	1.8	0.006	300
12	9	1	9
13	9	–	–
14	25	–	–
15	20	4	5
16	>100	–	–
17	25	20	1.3

Table 2. Anti-HIV activities of DDB (**18**), its isomers (**19–22**) and brominated analogues (**23–27**)

Compound	IC_{50} (μg/mL)	EC_{50} (μg/mL)	Therapeutic index
18	>100	5	>20
19	>100	65	>1.2
20	>100	7.5	>13
21	>100	70	>1.4
22	1.8	No inhibition	–
23	>100	0.23	>480
24	100	0.52	190
25	>100	90	>1.1
26	>100	2.1	>48
27	45	No inhibition	–

[a]IC_{50} refers to the concentration of drug that causes 50% reduction in total cell number. Drugs that have IC_{50} values >100 μg/mL cannot be tested at higher concentrations for a more exact IC_{50} value due to the effect of the solvent, DMSO.
[b]Therapeutic Index (TI) is a ratio of the IC_{50} value/EC_{50} value. Therefore, when the IC_{50} value is >100 μg/mL (refer to a, above), the TI value must also be reported as greater than.

As shown from the data in Table 2, the relative order of potency was **23** > **24** > **26** >**18** > **20**. The other five compounds (**19**, **21**, **22**, **25**, **27**) had no or significantly less anti-HIV activity. Comparison of the anti-HIV activities of **18–22** and **23–27** suggested that, like the natural lignans, the relative position and types of substituents on the phenolic hydroxy groups are most important. The 2- (or 2′)-methoxycarbonyl and 4- (or 4′)-methoxy groups on the biphenyl ring as found in **18** and **20** composed an essential anti-HIV structural feature. Activity could then be enhanced greatly by a bromine introduced at the 3- (or 3′)-position as found in **23**, **24** and **26**. Isomers (**19**, **21**, **22**, **25**, **27**) with different substituent patterns (the methyl-carboxylate groups at C-2 and C-2′ or the methoxy groups at C-4 and C-4′ replaced by other groups) showed greatly reduced or no anti-HIV activity, regardless of the presence of bromine in the molecule. With the natural lignans, bromination of **8** and **12** gave the less active analogues **15** and **17**.

In summary, the natural lignan gomisin-G (**11**) was identified as the most active anti-HIV principle of *K. interior*. In the related synthetic biphenyl compounds, the di- (**23**) and mono-(**24**) brominated derivatives of **18** had impressive anti-HIV activity with EC_{50} and TI values of 0.52 μg/mL and 190 and 0.23 μg/mL and >480, respectively.[5] Preliminary mechanism of action studies with these two compounds have shown template-primer HIV-1 reverse transcriptase (RT) inhibition.[5] This type of inhibitory activity has also been observed for other non-nucleoside HIV RT inhibitors.[23,24] Potent inhibitory effects were also found with **23** and **24** against HIV-1 RT-associated DNA polymerase and RNase H activities under certain conditions.[5,25] Further studies on the mechanism of action and further structural modification of the lead compounds are in progress.

Experimental

General experimental procedures

Table 3 lists the elemental analyses.

Melting points were determined on a Kofler or Fluka 51 micromelting point apparatus and are uncorrected. The IR spectra were recorded as KBr pellets on a Perkin–Elmer 783 or 1320 spectrophotometer. The UV spectra were measured on a Shimadzu UV-250 spectrophotometer in absolute MeOH. For the synthetic biphenyl derivatives, mass spectra were recorded on a Fisons Trio-1000 mass spectrometer and elemental analyses were determined on a Fisons CHNES1108 elemental analyzer. All other mass spectra were determined on a Varian MAT-711 mass spectrometer for eims and HP 5989A mass spectrometer for hrms. ^1H and ^{13}C NMR spectra were measured on a Bruker AC-300 spectrometer with TMS as internal standard and $CDCl_3$ as solvent, unless otherwise specified. Optical rotations were measured with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-500N data processor. Analytical TLC was performed on silica-gel plates (Yantai Institute of Chemical Technology) with petroleum ether:EtOAc (4:1). Silica gel H (200–300 mesh, Qing Dao) was used for column chromatography. Spots on the plate were observed under UV light and visualized by spraying with 10% H_2SO_4 followed by heating.

Plant material

The stem bark of *Kadsura interior* A. C. Smith was collected in Feng-qing county, Yunnan province, People's Republic of China in August 1992. A voucher specimen is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of

Table 3. Elemental analyses

		Calculated			Found		
		C	H	N	C	H	N
21	$C_{20}H_{18}O_{10}$	57.42	4.34		57.21	4.59	
22	$C_{20}H_{18}O_{10}$	57.42	4.34		57.32	4.34	
25	$C_{20}H_{16}O_{10}Br_2$	41.82	2.81		41.84	2.94	
26	$C_{20}H_{16}O_{10}Br_2$	41.82	2.81		41.78	2.91	
27	$C_{20}H_{16}O_{10}Br_2 \cdot \frac{1}{2}H_2O$	41.12	2.92		41.27	2.79	
28	$C_8H_8O_5$	52.16	4.38		51.99	4.34	
29	$C_9H_{10}O_5$	54.54	5.09		54.51	5.09	
30	$C_{10}H_{10}O_5$	57.13	4.80		57.01	4.80	
31	$C_{10}H_9O_5Br$	41.67	3.15		41.65	3.13	
33	$C_{10}H_9O_7N$	47.05	3.56	5.49	47.21	3.59	5.52
34	$C_{10}H_9O_7N$	47.05	3.56	5.49	47.22	3.57	5.42
35	$C_{10}H_{11}O_5N$	53.32	4.93	6.22	53.49	5.03	5.84
36	$C_{10}H_9O_5I$	35.72	3.70		35.86	2.79	
37	$C_{10}H_{11}O_5N \cdot \frac{1}{2}H_2O$	47.61	5.60		47.30	5.43	

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Extraction and isolation

The stem bark of *K. interior* was air-dried, ground, and extracted with 95% EtOH. The EtOH extract was evaporated in vacuo to yield a semisolid (1034 g). H_2O (2500 mL) was added to the residue, and this solution was extracted with Et_2O five times. This Et_2O solution was concentrated to yield 215 g of residue. The residue was chromatographed on silica gel (1250 g), employing petroleum ether containing increasing amounts of EtOAc. The fractions eluted with petroleum ether: EtOAc (95:5) gave schisandrin-C (**10**, 156 mg). The fractions eluted with petroleum ether:EtOAc (90:10) gave interiotherin-A (**1**, 219 mg), angeloylgomisin-R (**5**, 141 mg), and kadsuranin (**8**, 981 mg). The fractions eluted with petroleum ether:EtOAc (80:20) were subjected to repeated column chromatography with the same solvent to yield interiotherin-B (**2**, 686 mg); the subsequent fraction was purified by preparative TLC with petroleum ether:acetone (90:10) to yield (+)-deoxyschizandrin (**12**, 866 mg). Similarly, the same fractions were further purified separately by chromatography with petroleum ether:EtOAc (70:30) as eluent to afford schisantherin-D (**6**, 142 mg), gomisin-J (**9**, 75 mg), heteroclitin-F (**3**, 30 mg), gomisin-G (**11**, 1.3 g), gomisin-A (**4**, 984 mg), schizandrin (**7**, 2.5 g).

Synthetic methods

Methyl 2,3,4-trihydroxybenzoate (28). A 6 g (94 mmol) amount of 2,3,4,-trihydroxybenzoic acid in 200 mL of MeOH was refluxed in the presence of molecular sieves and 2 mL of H_2SO_4 for five days. After the usual workup, 16.92 g of methyl ester **28** was obtained in a 98% yield: mp 150–152 °C (recrystallized from EtOAc); IR cm^{-1} 3345 (br, s, OH), 1645 (chelated ester C=O) and 1260 (ester C–O); ^1H NMR δ 3.92 (s, 3H, OCH_3), 5.46 and 5.79 (each s, 1H, OH exchangeable with D_2O), 10.98 (s, 1H, chelated OH), 6.51 and 7.35 (each d, J = 9 Hz, 1H, ArH). Anal. ($C_8H_8O_5$) C, H.

Methyl-2,3-dihydroxy-4-methoxybenzoate (29). Compound **28** (18.4 g, 0.1 mol) in 5% aqueous borax solution was treated with 47 mL of Me_2SO_4 and 100 mL of 20% NaOH solution at 30 °C. The mixture was stirred for 5 h at room temperature. Then the mixture was cooled in an ice-bath and neutralized to pH 2 with HCl (37%). A precipate occurred and was filtered and washed with water until the filtrate was neutral. The dried product **29** weighed 16.19 g (yield 86%) and was recrystallized from dilute aqueous EtOH as needles: mp 100–101 °C; IR cm^{-1} 3330 and 3420 (OH), 1660 (chelated ester C=O) and 1255 (ester C–O); ^1H NMR δ 3.94 and 3.95 (each s, 3H, OCH_3), 5.51 (s, 1H, OH), 6.51 and 7.42 (each d, J = 9 Hz, 1H, ArH), 10.84 (s, 1H, chelated OH). Anal. ($C_9H_{10}O_5$) C, H.

Methyl-2,3-methylenedioxy-4-methoxybenzoate (30). Compound **29** (16.12 g, 0.081 mol) was dissolved in 500 mL of DMF. Then 100 mL of CH_2Cl_2 and 26 g (0.464 mol) of K_2CO_3 were added. The mixture was heated to 105 °C with vigorous stirring for 6 h. The K_2CO_3 was filtered and the DMF was removed in vacuo. The residue was poured into ice-water and let stand overnight. The precipitated product **30** was filtered and washed with water until neutral. The dried gray-white solid weighed 15.60 g (yield 91%) and was recrystallized from MeOH as brilliant long needles: mp 122–124 °C; IR cm^{-1} 1710 (ester C=O); ^1H NMR δ 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, $ArOCH_3$), 6.11 (s, 2H, OCH_2O), 6.57 and 7.45 (each d, J = 9 Hz, 1H, ArH). Anal. ($C_{10}H_{10}O_5$) C, H.

Methyl-2,3-methylenedioxy-4-methoxy-5-bromobenzoate (31). Compound **30** (5.56 g, 25.6 mmol) was dissolved in anhydrous $CHCl_3$. Bromine (4.5 g, 28.5 mmol) was added dropwise over 2 h at 5–8 °C. Then the mixture was maintained for an additional 2 h at

the same temperature. After the usual workup, 6.9 g of white solid **31** was obtained (88% yield): mp 165–166 °C (crystallized from EtOAc); IR cm^{-1} 1710 (s, C=O), 1425, 1400 (s, C–O); MS *m/z* (%) 288 (M^+, 100), 290 (M + 2, 96); ^1H NMR δ 3.91 (s, 3H, OCH_3), 4.14 (s, 3H, $ArOCH_3$), 6.10 (s, 2H, OCH_2O), 7.65 (s, 1H, 6-ArH); Anal. ($C_{10}H_9O_5Br$) C, H.

Methyl-2,3-methylenedioxy-4-methoxy-5-iodobenzoate (32). A mixture of dry silver trifluoroacetate (442 mg, 2 mmol) and compound **30** (420 mg, 2 mmol) was placed in a reaction flask. Then a solution of iodine (506 mg, 2 mmol) in anhydrous CH_2Cl_2 (8 mL) was added under reflux over a period of 2 h. To this reaction mixture was added more silver trifluoroacetate (40 mg) and iodine (59 mg) and reflux was continued until the starting material had disappeared on TLC. The mixture was then filtered and the filtrate was concentrated. The resulting yellowish solid was crystallized from DMF as short needles (190 mg, yield 28%): mp 162–164 °C; IR cm^{-1} 1706 (ester C=O), 1390 (C–O); ^1H NMR δ 3.90 (s, 3H, OCH_3), 4.13 (s, 3H, $ArOCH_3$), 6.10 (s, 2H, OCH_2O), 7.88 (s, 1H, ArH). Anal. ($C_{10}H_9O_5I$) C, H; I calcd 37.90; found 37.80.

Methyl-2,3-methylenedioxy-4-methoxy-5- and -6-nitrobenzoate (33 and 34). To a stirred suspension of compound **30** (442 mg, 2 mmol) in Ac_2O (5 mL), concentrated HNO_3 (1 mL) was added dropwise over a period of 20 min. The reaction temperature was maintained at 10–22 °C. The reaction mixture was stirred for an additional 30 min and then was poured into ice–water. A yellowish solid (437 mg) was collected. Total yield was 86%. It was separated by silica-gel column chromatography to afford the 5-nitro compound **33** and 6-nitro compound **34** (ratio 1:10). For **33**: mp 162–164 °C; ^1H NMR δ 3.93 (s, 3H, OCH_3), 4.19 (s, 3H, $ArOCH_3$), 6.23 (s, 2H, OCH_2O), 8.11 (s, 1H, 6-ArH). Anal. ($C_{10}H_9NO_7$) C, H, N. For **34**: mp 145–147 °C (yellowish needles from EtOAc); IR cm^{-1} 1735 (s, C=O); MS *m/z* (%) 255 (M^+, 100); ^1H NMR (CD_3COCD_3) δ 3.86 (s, 3H, OCH_3), 4.04 (s, 3H, $ArOCH_3$), 6.31 (s, 2H, OCH_2O), 7.48 (s, 1H, 5-ArH). Anal. ($C_{10}H_9NO_7$) C, H, N.

Methyl-2,3-methylenedioxy-4-methoxy-6- and -5-aminobenzoate (35 and 37). A solution of **34** (225 mg, 1 mmol) in EtOH (10 mL) and EtOAc (5 mL) was placed in a reaction bottle and 10% Pd–C catalyst (54 mg) was added. Hydrogen was added under vigorous stirring at rt and 1 atm pressure for 4 h. Then the catalyst was filtered and solvent was removed in vacuo. The amino compound **35** (148 mg) was thus obtained in a 93% yield: mp 163–164 °C; IR cm^{-1} 3450, 3320 (NH), 1665 (chelated ester C=O); MS *m/z* (%) 225 (M^+, 100); ^1H NMR (CD_3COCD_3) δ 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, $ArOCH_3$), 5.88 (s, 3H, OCH_2O), 6.02 (s, 1H, 5-ArH), 6.21 (br. S, 1H, NH exchangeable with D_2O), 10.72 (s, 1H, chelated NH). Anal. ($C_{10}H_{11}O_5N$) C, H, N.

According to the above procedure, **37** was obtained from **33** in an 84% yield: mp 139–140 °C; ^1H NMR δ 3.67 (br s, 2H, NH_2), 3.89 (s, 3H, OCH_3), 4.09 (s, 3H, $ArOCH_3$), 5.99 (s, 2H, OCH_2O), 6.78 (s, 1H, 6-ArH). Anal. ($C_{10}H_{11}O_5N.1/2H_2O$) C, H.

Methyl-2,3-methylenedioxy-4-methoxy-6-iodobenzoate (36). A suspension of compound **35** (400 mg, 1.78 mmol) in 20% H_2SO_4 (2 mL) was cooled to 0–5 °C and then a solution of $NaNO_2$ (127 mg, 1.78 mmol) in H_2O (4 mL) was added. After the diazonium salt was formed, a cooled solution of KI (332 mg, 2 mmol) in H_2O (2 mL) was added with evolution of a gas. Copper powder (340 mg) was added and the reaction mixture was stirred for 20 min, then heated on a water bath to 80–90 °C for 1 h. Nitrogen evolved vigorously. When the reaction subsided, the mixture was extracted with $CHCl_3$. A deep-red oil (2.43 g) was obtained and was purified by silical-gel column chromatography (eluent hexane:chloroform, 4:1) giving 150 mg of **36** (25% yield): mp 146–147 °C (white solid); IR cm^{-1} 1705 (C=O), 1285 and 1265 (s, C–O); MS *m/z* (%) 336 (M^+, 100); ^1H NMR δ 3.93 (s, 6H, 2 × OCH_3), 6.07 (s, 2H, OCH_2O), 7.11 (s, 1H, ArH). Anal. ($C_{10}H_9O_5I$) C, H.

2,2′-Dimethoxycarbonyl-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxybiphenyl (ω-DDB) (21). Method A: a mixture of compound **36** (1 g, 2.85 mmol), activated copper powder (1 g) and anhydrous DMF (3.5 mL) was heated to reflux for 2.5 h under vigorous stirring. After cooling to 100 °C, it was poured into ice–water and the precipitate was collected and chromatographed on Al_2O_3. Elution with hexane then hexane–chloroform afforded ω-DDB (**21**, 280 mg, 44% yield); mp 253–255 °C; IR cm^{-1} 1730 (C=O), 1175 and 1150 (s, C–O); MS *m/z* (%) 418 (M^+, 100); ^1H NMR δ 3.68 (s, 6H, 2 × OCH_3), 3.92 (s, 6H, 2 × OCH_3), 6.13 (s, 4H, 2 × OCH_2O), 6.36 (s, 2H, 2 × ArH). Anal. ($C_{20}H_{18}O_{10}$) C, H.

Method B: copper(II) sulfate (1.25 g) was dissolved in 5 mL of water containing 2.5 mL NH_4OH (conc.). The mixture was cooled in an ice-bath to below 10 °C and solution of 0.4 g hydroxylamine chloride in 2 mL water and 1 mL NaOH (6 N) was added. The diazonium salt of compound **35** (225 mg), prepared according to the above method for **36**, was slowly added into the copper(II) solution and remained at below 10 °C with vigorous stirring. The blue color faded gradually. The mixture was heated to 80–90 °C for a few minutes, then cooled to rt and neutralized with HCl to pH 2–3. After the usual workup, 86 mg of pure compound **21** was obtained (41% yield).

2,2′-Dimethoxy-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxycarbonyl biphenyl (δ-DDB) (22). Method A: (Ullmann reaction) compound **22** was obtained from **31** in a 10% yield using method A given above for **21**; mp 195–200 °C; IR cm^{-1} 1700 (s, ester C=O), 1430, 1270 and 1210 (s, C–O); MS *m/z* (%) 418 (M^+, 100);

^{1}H NMR δ 3.90 and 4.00 (each s, 3H, OCH_3), 6.13 (s, 4H, OCH_2O), 7.30 (s, 2H, ArH). Anal. ($C_{20}H_{18}O_{10}$), C, H.

Method B: compound **22** was obtained in a 40% yield from **37** using method B as given for **21**.

Bromination of lignans and biphenyl derivatives

A natural lignan or biphenyl compound in $CHCl_3$ was dropped slowly into two molar equivalents of bromine at 0–5 °C. The mixture was stirred at rt for 3 h and poured with stirring into ice–water containing sodium bisulfite until the orange–red color faded completely. The organic phase was separated and washed successively with aqueous $NaHCO_3$, water and brine, then dried over Na_2SO_4. The organic solvent was removed in vacuo. The crude product was purified by crystallization.

5,12-Dibromo-gomisin A (13). Obtained in 85% yield from **4**. Purified by preparative TLC with hexane:Et_2O (2:1) to give a semisolid; ^{1}H NMR δ 0.91 (d, J = 7.0 Hz, 3H, 9-CH_3), 1.36 (s, 3H, 8-CH_3), 1.93 (m, 1H), 2.75 (m, 2H), 2.99 (m, 2H), 3.44 (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 3.93 (s, 3H), 3.97 (s, 3H), 6.09 (d, 2H); HRMS calcd for $C_{23}H_{26}O_7Br_2$ 572.0042, $C_{23}H_{26}O_7BrBr^*$ 574.0021, $C_{23}H_{26}O_7Br_2^*$ 576.0001; found 572.0066, 573.9981, 576.0039, respectively.

5,12-Dibromo-schizandrin (14). Obtained in 90% yield from **7**. Purified by preparative TLC with hexane:Et_2O (2:1) to give a semisolid; ^{1}H NMR δ 0.91 (d, J = 6.5 Hz, 3H), 1.36 (s, 3H), 1.87 (m, 2H), 2.17 (m, 1H), 2.87–3.00 (m, 2H), 3.48 (s, 3H), 3.53 (s, 3H), 3.88 (s, 3H), 3.94 (s, 3H), 3.96 (s, 3H), 3.97 (s, 3H); HRMS calcd for $C_{24}H_{30}O_7Br_2$ 588.0354, $C_{24}H_{30}O_7BrBr^*$ 590.0334, $C_{24}H_{30}O_7Br_2^*$ 592.0334; found 588.0313, 590.0325, 592.0333, respectively.

5,12-Dibromo-kadsuranin (15). Obtained in 84% yield from **8**. Purified by preparative TLC with hexane:Et_2O (2:1) to give a semisolid; ^{1}H NMR δ 0.86 (d, J = 7.0 Hz, 3H), 1.07 (d, J = 7.0 Hz, 3H), 1.79 (m, 1H), 1.98 (m, 1H), 2.27–2.55 (m, 4H), 3.48 (s, 3H), 3.84 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 3.97 (s, 3H), 6.08 (d, J = 2.0 Hz, 2H); HRMS calcd for $C_{23}H_{26}O_6Br_2$ 556.0092, $C_{23}H_{26}O_6BrBr^*$ 558.0072; found 556.0115, 558.0093, respectively.

5,12-Dibromo-schisandrin-C (16). Obtained in 90% yield from **10**. Crystals from $CHCl_3$; mp 205–206 °C; ^{1}H NMR δ 0.85 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 7.0 Hz, 3H), 1.74 (m, 1H), 1.92 (m, 1H), 2.27–2.56 (m, 4H), 3.75 (s, 3H), 3.77 (s, 3H), 6.06 (m, 4H); HRMS calcd for $C_{22}H_{22}O_6Br_2$ 539.9779, $C_{22}H_{22}O_6BrBr^*$ 541.9759, $C_{22}H_{22}O_6Br_2^*$ 543.9739; found 539.9768, 541.9810, 543.9737, respectively.

5,12-Dibromo-deoxyschizandrin (17). Obtained in 84% yield from **12**. Purified by preparative TLC with hexane:Et_2O (2:1) to give a semisolid; ^{1}H NMR δ 0.88 (d, J = 7.0 Hz, 3H), 1.07 (d, J = 7.0 Hz, 3H), 1.83 (m, 1H), 1.99 (m, 1H), 2.28–2.58 (m, 4H), 3.51 (s, 3H), 3.62 (s, 3H), 3.93 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 3.97 (s, 3H); HRMS calcd for $C_{24}H_{30}O_6Br_2$ 572.0405, $C_{24}H_{30}O_6BrBr^*$ 574.0385, $C_{24}H_{30}O_6Br^*_2$ 576.0365; found 572.0398, 574.0426, 576.0403, respectively.

2,2′-Dimethoxycarbonyl-3,3′-dibromo-4,5,4′,5′-bismethylenedioxy-6,6′-dimethoxybiphenyl (25). Obtained in 87% yield from **19**. Crystals from $CHCl_3$ and MeOH (2:1); mp 181–182 °C; MS m/z (%) 574 (M^+, 48), 576 (M + 2, 100), 578 (M + 4, 51); ^{1}H NMR δ 3.67 (s, 6H, 2,2′-OCH_3), 3.90 (s, 6H, 6,6′-OCH_3), 6.12 (s, 4H, 2 × OCH_2O), Anal. ($C_{20}H_{16}O_{10}Br_2$) C, H.

2,2′-Dimethoxycarbonyl-3,3′-dibromo-4,6′-dimethoxy-5,6,4′,5′-bismethylenedioxybiphenyl (26). Obtained in 82% yield from **20**. Crystals from $CHCl_3$ and MeOH (2:1); mp 166–169 °C; MS m/z (%) 574 (M^+, 45), 576 (M + 2, 90), 578 (M + 4, 45), 59 (100); ^{1}H NMR δ 3.68 and 3.69 (each s, 3H, 2,2′-OCH_3), 3.91 (s, 3H, 4-OCH_3), 4.09 (s, 3H, 6′-OCH_3), 5.98–6.08 (m, 4H, 2 × OCH_2O). Anal. ($C_{20}H_{16}O_{10}Br_2$) C, H.

2,2′-Dimethoxycarbonyl-3,4,3′,4′-bismethylenedioxy-5,5′-dimethoxy-6,6′-dibromobiphenyl (27). Obtained in 98% yield from **21**; mp 187–188 °C (crystals from EtOAc); MS m/z (%) 574 (M^+, 50), 576 (M + 2, 100), 578 (M + 4, 50); ^{1}H NMR δ 3.69 (s, 6H, 2,2′-OCH_3), 4.14 (s, 6H, 5,5′-OCH_3), 6.10 and 6.13 (each s, 4H, 2 × OCH_2O). Anal. ($C_{20}H_{16}O_{10}Br_2 \cdot 0.5H_2O$) C, H.

HIV growth inhibition assay

The H9 T-cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37 °C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1–0.01 IU/cell) for 1 h at 37 °C and 5% CO_2. The cells then were washed thoroughly to remove unabsorbed virions and resuspended at 4×10^5 cells/mL in complete medium. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a four-day incubation at 37 °C, cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24 antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated-on-96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 'captured' onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cut-off for the p24 ELISA

assay is 12.5 pg/mL. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

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