New Rotenoids from Roots of *Mirabilis jalapa*

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Four new rotenoids named mirabijalone A–D[1]) (**1**–**4**), together with 9-*O*-methyl-4-hydroxyboeravinone B (**5**), boeravinone C (**6**) and F (**7**), and 1,2,3,4-tetrahydro-1-methylisoquinoline-7,8-diol (**8**), were isolated from the roots of *Mirabilis jalapa*. The structures of these compounds were determined on the basis of their HR-EI-MS, IR, UV, ^{1}H- and ^{13}C-NMR (DEPT), and 2D NMR (HMQC, HMBC, NOESY) data. Among them, 1,2,3,4-tetrahydro-1-methylisoquinoline-7,8-diol (**8**) showed a 48% inhibition against HIV-1 reverse transcriptase at 210 μg/ml.

1. Introduction. – Many natural products from the plant kingdom [1] and crude extracts from traditional Chinese folk herbs possess activity against HIV [2]. In the course of our preliminary screening of Chinese folk herbs for anti-HIV agents, it was found that the AcOEt fraction of the roots of *Mirabilis jalapa* L. showed potent inhibitory activity against HIV *in vitro* ($EC_{50} = 1.9$ μg/ml, $CC_{50} > 250$ μg/ml, $TI > 211$) [2]. *M. jalapa* is a plant belonging to the family *Nyctaginaceae*, widely used as a traditional folk herb to treat acute arthritis, anesthesia, inflammation, and so on [3]. However, until now, chemical investigation of *M. jalapa* has been limited to the isolation and structure elucidation of fatty acids [4], terpenoids and steroids [5], D-glucan [6], and phenolic compounds [7]. To isolate an effective compound against HIV, *M. jalapa* collected at Kunming in Yunnan Province was chemically investigated. This paper describes the isolation and structure identification of four new rotenoids from the AcOEt fraction of the roots of *M. jalapa.*

2. Results and Discussion. – The AcOEt fraction of the EtOH extract from the roots of *M. jalapa* showed activity against HIV and was repeatedly chromatographed on silica gel, *Sephadex LH-20*, *MCI CHP-20P*, *FUJI* gel (*ODS-Q_3*), and *RP-18* gel to afford mirabijalone A–D[1]) (**1**–**4**), 9-*O*-methyl-4-hydroxyboeravinone B (**5**), boeravinone C (**6**), and F (**7**), and the known isoquinoline-diol **8**.

Mirabijalone A (**1**) crystallized as yellow needles (Me_2CO). The HR-EI-MS showed a molecular-ion peak at *m*/*z* 358.1051, in accordance with the molecular formula $C_{19}H_{18}O_7$ (calc. 358.1053) (*Fig. 1*). Its UV, IR (see *Exper. Part*), and ^{1}H- and ^{13}C-NMR data (see *Tables 1* and *2*) were very similar to those of boeravinone C (**6**) [8], which indicated that **1** has the same skeleton as **6**. Thus the structure of **1** was

[1]) For systematic names, see *Exper. Part*.

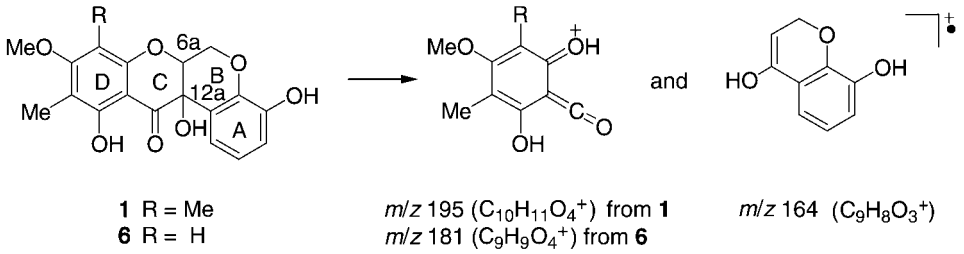


Fig. 1. *Structure and mass-spectral fragmentation of rotenoids* **1** *and* **6**

Table 1. *^{1}H-NMR* (400 MHz) *Chemical Shifts and Assignments for Compounds* **1**, **2**, *and* **4**. δ Values in ppm with reference to the signal of C_5D_5N; coupling constants J in Hz.

	1	**2**	**4**
H–C(1)	8.29 (*dd*, *J* = 8, 1.5)	8.81 (*d*, *J* = 8)	8.75 (*d*, *J* = 8.8)
H–C(2)	7.07 (*t*, *J* = 8)	7.16 (*t*, *J* = 8)	6.70 (*dd*, *J* = 8.8, 2.4)
H–C(3)	7.28 (*dd*, *J* = 8, 1.5)	7.30 (*dd*, *J* = 8, 1.2)	
H–C(4)			6.86 (*d*, *J* = 2.4)
H_α–C(6)	4.96 (*dd*, *J* = 8.5, 3.5)	6.83(*s*)	6.33 (*s*)
H_β–C(6)	4.93 (*dd*, *J* = 11.5, 8.5)		
H–C(6a)	4.73 (*dd*, *J* = 11.5, 3.5)		
H–C(8)			6.15 (*s*)
Me–C(8)	2.13 (*s*)[a])	2.48 (*s*)[a])	
MeO–C(9)	3.60 (*s*)		3.40 (*s*)
Me–C(10)	2.18 (*s*)[a])	2.43 (*s*)[a])	1.86 (*s*)

[a]) Data may be interchanged.

Table 2. *^{13}C-NMR* (100.6 MHz) *Chemical Shifts and Assignments for Compounds* **1**, **2**, **4**, *and* **7**). δ Values in ppm with reference to the signal of C_5D_5N.

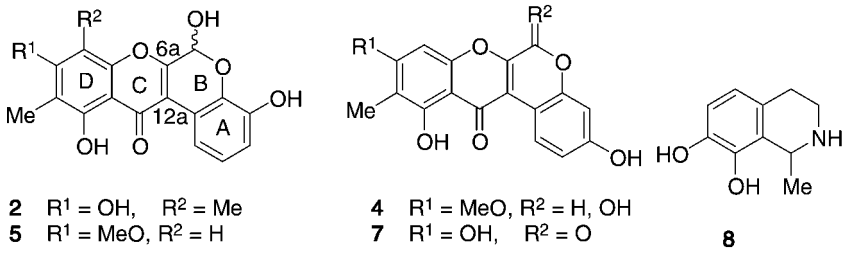
	1	**2**	**4**	**7**
C(1a)	121.9	119.3	108.9	121.0
C(1)	122.3	118.5	129.5	129.2
C(2)	121.5	123.1	110.6	114.7
C(3)	117.3	117.2	155.3	155.4
C(4)	147.6	148.5	105.5	103.6
C(4a)	144.4	138.7	151.5	142.1
C(6)	62.4	90.2	89.7	165.7
C(6a)	77.0	158.7	155.3	155.8
C(7a)	157.3	153.1	156.2	152.1
C(8)	109.2	103.1	89.9	93.9
Me–C(8)	8.3[a])	9.1[a])		
C(9)	165.4	162.0	163.6	161.4
*Me*O–C(9)	60.1		55.9	
C(10)	111.7	109.0	109.2	109.5
Me–C(10)	8.5[a])	8.9[a])	7.5	8.1
C(11)	161.0	158.7	160.0	160.5
C(11a)	104.9	106.0	105.4	106.0
C(12)	196.6	182.5	180.7	181.3
C(12a)	66.7	109.9	108.9	107.8

[a]) Data may be interchanged.

determined to be 6a,12a-dihydro-4,11,12a-trihydroxy-9-methoxy-8,10-dimethyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one.

Characteristic signals in the ^{1}H-NMR spectrum of **1** were observed at δ 7.07 (H–C(2)), 7.28 (H–C(3)), and 8.29 (H–C(1)), with coupling constants typical for the presence of three vicinal aromatic protons. The signals at δ 2.13, 2.18, and 3.60 were assigned to two Me groups and a MeO group at an aromatic moiety, respectively. Furthermore, the ^{1}H-NMR spectrum showed signals with a complex splitting pattern in the 4.73–4.96 ppm region, which was ascribed to an $OCHCH_2O$ group (H–C(6a) and H–C(6)). The B/C ring junction was considered to be *trans* from the chemical-shift value of H–C(1) at δ 8.29 in (D_5)pyridine, which is known to be strongly deshielded in *trans*-substituted compounds [9]. Moreover, this observation was supported by its optical-rotation value ($\alpha = -203.88$) as compared with those of gliricidol (*cis:* $\alpha = +230$) [10] and **6** (*trans:* $\alpha = -459.9$) [8]. Nineteen signals in the ^{13}C-NMR (DEPT) spectrum of **1** were recognized (11 C, 4 CH, 1 CH_2, 3 Me), including a keto C-atom and one MeO group. The EI-MS of **1** gave a molecular ion at *m*/*z* 358, suggesting an increase of 14 mass units compared to that of boeravinone C (**6**). A base peak at *m*/*z* 195 originated from a typical *retro-Diels–Alder* fragmentation of 6a,12a-saturated rotenoids [11], in accord with the proposed structure and the assignment of the two Me and a MeO groups to the D ring (see *Fig. 1*). The presence of one further Me signal at δ 2.13 and the lack of the aromatic-proton signal at δ 6.60 (H–C(8)) in the ^{1}H-NMR were the main differences between **1** and **6** [8]. On the other hand, **1** did not show the signal at δ 90.1 (*d*, C(8)) of **6**. Instead a quaternary C-atom at δ 109.2 appeared in the ^{13}C-NMR of **1**, suggesting that the additional Me group should be located at C(8).

Mirabijalone B (**2**) crystallized as pale yellow needles (Me_2CO). The HR-EI-MS showed a molecular-ion peak at *m*/*z* 342.0754, in accordance with the molecular formula $C_{18}H_{14}O_7$ (calc. 342.0740). Its ^{1}H- and ^{13}C-NMR spectral data were very similar to those of 9-*O*-methyl-4-hydroxyboeravinone B (**5**) [7], which indicated that **2** and **5** have similar skeletons. Compound **2** was deduced to be 4,6,9,11-tetrahydroxy-8,10-dimethyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one (*Fig. 2*).



2 R^1 = OH, R^2 = Me
5 R^1 = MeO, R^2 = H

4 R^1 = MeO, R^2 = H, OH
7 R^1 = OH, R^2 = O

8

Fig. 2. *Structure of isolated compounds* **2**, **4**, **5**, **7** *and* **8**

Comparison of the ^{1}H- and ^{13}C-NMR data of **2** and **5** showed that the absence of the signal at δ 3.37 (MeO) in **5** [7] and the presence of a further Me signal at δ 2.48 in the ^{1}H-NMR of **2** were the main differences, and the C(8) signal due to a methine group (δ 90.1) in **5** and a quaternary C-atom (δ 103.1) in **2** in the ^{13}C-NMR showed that the additional Me group was located at C(8).

Mirabijalone C (**3**) was a pale yellow amorphous powder. The HR-FAB-MS (neg. mode) showed a molecular-ion peak at *m*/*z* 505.1431, in accordance with the formula $C_{24}H_{25}O_{12}^-$ (calc. 505.1424). On acidic hydrolysis of **3**, glucose was detected by PC (paper chromatography) comparison with an authentic sample. From the spectral data (*Table 3*), the structure of compound **3** was determined to be 2′,5-dihydroxy-2-(hydroxymethyl)-7-methoxy-6-methylisoflavone 3′-β-D-glucopyranoside (*Fig. 3*).

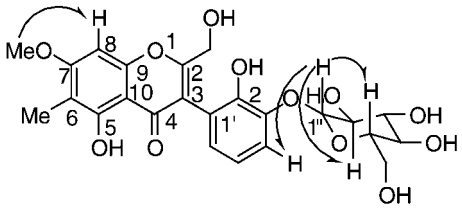


Fig. 3. *Structure and NOE correlations of* **3**

Table 3. *^{1}H- and ^{13}C-NMR* (125.8 and 500.1 MHz, resp.) *Chemical Shifts and Assignments, Homonuclear ^{1}H,^{1}H and Heteronuclear ^{1}H,^{13}C Long-Range Correlations for Compound* **3**[1]). δ Values in ppm with reference to the signal of C_5D_5N; *J* in Hz.

	δ (H)	δ (C)	^{1}H,^{1}H COSY	HMBC (H → C)
C(2)		165.9		
HOCH_2–C(2)	4.91 (*s*)	60.5		118.4, 165.9
C(3)		118.4		
C(4)		181.3		
C(5)		159.2		
C(6)		108.5		
Me–C(6)	2.23 (*s*)	7.7		108.5, 159.2, 163.8
C(7)		163.8		
*Me*O–C(7)	3.72 (*s*)	56.1		163.8
H–C(8)	6.44 (*s*)	89.9		105.7, 108.5, 156.5, 163.8
C(9)		156.5		
C(10)		105.7		
C(1′)		120.2		
C(2′)		148.4		
C(3′)		147.1		
H–C(4′)	7.56 (*dd*, *J* = 7.8, 1.3)	119.1	6.91	148.4, 127.4
H–C(5′)	6.91 (*t*, *J* = 7.8)	119.4	7.24, 7.56	120.2, 147.1
H–C(6′)	7.24 (*dd*, *J* = 7.8, 1.3)	127.4	6.91	118.4, 119.1, 148.4
H–C(1″)	5.34 (*d*, *J* = 7.6)	105.5	4.18	147.1
H–C(2″)	4.18 (*m*)	74.9	4.23, 5.34	71.2
H–C(3″)	4.23 (*m*)	79.0	4.28	74.9, 105.5
H–C(4′)	4.28 (*m*)	71.2	3.99	62.2, 74.9
H–C(5″)	3.99 (*m*)	78.4	4.28, 4.41	79.0
2 H–C(6″)	4.52 (*m*), 4.41 (*m*)	62.2	3.99	71.2

The ^{1}H-NMR spectrum of **3** showed characteristic signals assignable to three vicinal and an isolated aromatic proton (δ 7.56, 7.24, and 6.91, and δ 6.44, resp.) together with signals due to a Me (δ 2.23) and a MeO group (δ 3.72) at an aromatic moiety. The ^{1}H,^{1}H COSY, HMQC, and HMBC of compound **3** also suggested the presence of three vicinal aromatic protons.

One anomeric proton at δ 5.34 (*d*, *J* = 7.6 Hz) was observed, indicating a β-D-linkage of the sugar moiety. The ^{13}C-NMR (DEPT) signals at δ 105.5, 74.9, 79.0, 71.2, 78.4, and 62.2 suggested the presence of a β-D-glucopyranosyl group. This was also confirmed by a fragment *m*/*z* 343 ($[M - H - 162]^-$) in the FAB-MS. 2D-NMR Spectroscopy including HMBC and NOESY of **3** established the connectivity of partial structures and substituents. Thus, HMBC data allowed to correlate the proton signal at δ 2.23 (Me) with the C-signals at δ 108.5 (C(6)), 159.2 (C(5)), and 163.8 (C(7)), suggesting Me substitution at C(6). The proton signal at δ 3.72 (MeO) was correlated with a C-signal at δ 163.8 (C(7)), in accord with MeO substitution at C(7). The proton signal at δ 4.91 (CH_2OH) was correlated with C-signals at 165.9 (C(2)) and 118.4 (C(3)), consistent with CH_2OH substitution at C(2). The anomeric proton signal at δ 5.34 was correlated with the C-signal at δ 147.1 (C(3′)),

which suggested that the β-D-glucopyranosyl group was located at C(3′). The β-D-configuration was also confirmed by the NOESY spectrum (see *Fig. 3*).

Mirabijalone D (**4**) was an amorphous yellow powder. The HR-EI-MS showed a molecular-ion peak at *m/z* 342.0748, in accordance with the molecular formula $C_{18}H_{14}O_7$ (calc. 342.0740), which was 14 mass units higher than that of boeravinone F (**7**) [12]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **7** showed that **4** and **7** have similar skeletons. From the spectral data, the structure of **4** was deduced to be 3,6,11-trihydroxy-9-methoxy-10-methyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one (*Fig. 2*).

The ^{1}H-NMR spectrum of **4** showed four aromatic-proton signals at δ 6.15 (*s*), 6.70 (*dd*, *J* = 2.4, 8.8 Hz), 6.86 (*d*, *J* = 2.4 Hz), and 8.75 (*d*, *J* = 8.8 Hz), which were assigned to an isolated proton and three protons in 1-, 2-, and 4-positions, respectively, a MeO signal at δ 3.40 and a Me signal at δ 1.86. The presence of an additional MeO group and a methine group in **4** as compared to **7** were observed, the latter suggesting that **4** was a reduced derivative of **7**.

Comparison of the chemical properties with reported data allowed us to identify compounds **5**–**8** (*Figs. 1* and *2*) as 9-*O*-methyl-4-hydroxyboeravinone B [7], boeravinone C [8][13], boeravinone F [12], and 1,2,3,4-tetrahydromethylisoquinoline-7,8-diol [14], respectively. Compound **7** has been reported only as a minor component in a mixture, and no ^{13}C-NMR spectral data were given [12]; it is, thus, for the first time here obtained pure, from the roots of *M. jalapa*.

The inhibition percentages of **1**–**8** at 210 μg/ml to reverse transcriptase of HIV-1 were assayed. Only compound **8** showed 48% inhibition percentage against HIV-1 reverse transcriptase. The structure of this compound is simple. This is the first report of its inhibitory activity against HIV-1-reverse transcriptase.

Seven rotenoids were isolated from the roots of *M. jalapa*, four of them were fully unsaturated rotenoids and two were 12a-hydroxyrotenoids. All these compounds had a Me group at C(10), in contrast to most known natural rotenoids, which contain an isoprenoid-derived substituent, usually at C(8) and only occasionally at C(10). But two of the rotenoids had a Me group at C(8). The presented results show that further studies of the distribution of rotenoids in other *Nyctaginaceae* plants and of their biological activities are well worthwhile.

Experimental Part

General. Column chromatography (CC): *Qingdao* silica gel (200–300 mesh), *MCI* gel *CHP-20P*, and *FUJI* (*ODS-Q$_3$*) gel (*Mitsubishi Chemical Co.*). TLC: *Qingdao* precoated plates, silica *GF254* and *Merck RP-18 F$_{254}$* plates, eluents: *A*, MeOH/$CHCl_3$ 5:95, 10:90, and 20:80, *B*, H_2O/MeOH 2:8 and 3:7. M.p.: *XRC-1* apparatus. UV Spectra: *UV-210A Spectrometer Company* apparatus; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS* spectrometer: in cm^{-1}. NMR Spectra: *Bruker AM-400* or *DRX-500* spectrometer, C_5D_5N solns.; δ values (with ref. to the signal of C_5D_5N) with $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. MS: *Autospec 3000* spectrometer in *m/z* (rel. %).

Plant Material. The roots of *Mirabilis jalapa* (FAMILIC) L. were collected in Kunming, Yunnan, P.R. China, in October 1999. The plant identity was established by Dr. *Peng Hua*. A voucher specimen (No. 201009-2) was deposited in the herbarium of Kunming Institute of Botany, Kunming, P.R. China.

Extraction and Isolation. The air-dried roots (50 kg) were extracted thrice with 95% EtOH/H_2O at r.t. The solvent was evaporated at $< 50°$ to give a deep-brown waxy residue, which was suspended in H_2O and extracted with AcOEt (3 × 2000 ml) and BuOH (3 × 2000 ml). The AcOEt extract (380 g) was fractionated by CC (silica

gel (6000 g, 200–300 mesh), $CHCl_3$/MeOH 99:1, 95:5, 90:10, and 80:20) to afford several fractions. A 6-g amount of the fraction (43 g) obtained from $CHCl_3$/MeOH 99:1 was rechromatographed (silica gel (200–300 mesh), petroleum ether/Me_2CO 80:20) to afford four fractions. The 1st fraction (700 mg) was purified by repeated CC (silica gel, $CHCl_3$/MeOH 99:1 and 98:2; then *Sephadex-LH-20*, MeOH): pure **1** (10 mg). The 2nd fraction (1200 mg) was purified by CC (silica gel, $CHCl_3$/MeOH 98:2) and recrystallized (Me_2CO): pure **6** (600 mg). The 3rd fraction (300 mg) was purified by CC (silica gel, $CHCl_3$/MeOH 95:5): pure **5** (5 mg). The 4th fraction (2100 mg) was purified by CC (silica gel, petroleum ether/Me_2CO 70:30) and recrystallized (Me_2CO): pure **2** (250 mg), **3** (5 mg), and **7** (3 mg). The initial $CHCl_3$/MeOH 80:20 fraction was purified by repeated CC (silica gel (200–300 mesh), $CHCl_3$/MeOH 90:10 → 80:20; and *MCI* gel *CHP-20P*; *RP-18* gel F_{254}; MeOH/H_2O 70:30): pure **4** (25 mg) and **8** (28 mg).

Acid Hydrolysis. Compound **3** (5 mg) was dissolved in MeOH (1.0 ml) and 2M HCl (1.0 ml) and hydrolyzed by refluxing on a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted twofold with dist. H_2O, and partitioned between H_2O and AcOEt. The aq. layer was neutralized and evaporated to give a residue. Glucose was identified in the residue by PC (BuOH/AcOH/H_2O 5:1:5, upper layer) comparison with an authentic sample.

Mirabijalone A (*=6a,12a-Dihydro-4,11,12a-trihydroxy-9-methoxy-8,10-dimethyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one*; **1**). Yellow needles (Me_2CO). M.p. 240–243.5°. $[\alpha]_D^{16} = -203.88$ ($c = 0.31$, MeOH). UV (MeOH): 204.5 (4.59), 210 (4.50), 285.5 (4.25), 362 (3.56). IR (KBr): 3373, 2923, 1638, 1590, 1473, 1280, 1253, 1196, 1130. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 358 (60, M^+), 195 (100), 166 (42).

Mirabijalone B (*=4,6,9,11-Tetrahydroxy-8,10-dimethyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one*; **2**). Pale yellow needles (Me_2CO). M.p. > 330°. $[\alpha]_D^{26} = +7.5$ ($c = 0.4$, C_5H_5N). UV (MeOH): 205 (4.34), 217.5 (4.46), 274 (4.52), 307.5 (3.88). IR: 3393, 1653, 1622, 1473, 1197, 1139, 1118, 1015. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 342 (80, M^+), 313 (100).

Mirabijalone C (*=2′,5-Dihydroxy-2-(hydroxymethyl)-7-methoxy-6-methylisoflavone 3′-β-*D*-Glucopyranoside = 3-[3-(β-*D*-Glucopyranosyloxy)-2-hydroxyphenyl]-5-hydroxy-2-(hydroxymethyl)-7-methoxy-6-methyl-4*H*-1-benzopyran-4-one*; **3**). Yellow solid. M.p. 167–172°. UV (MeOH): 211 (4.51), 262.5 (4.32), 275.5 (4.14), 313 (3.81). IR (KBr): 3650–3200, 1662, 1578, 1485, 1345, 1301, 1278, 1221, 1129. ^{1}H- and ^{13}C-NMR: *Table 3*. FAB-MS (neg. mode): 505 (100, $M - H]^-$), 488, (21, $[M - OH]^-$), 325 (43).

Mirabijalone D (*=3,6,11-Trihydroxy-9-methoxy-10-methyl[1]benzopyrano[3,4-*b*][1]benzopyran-12(6*H*)-one*; **4**). Yellow solid. M.p. > 310°. IR: 3600–3200, 1718, 1652, 1591, 1509, 1448, 1279, 1201, 1161, 1131, 1117. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 342 (68, M^+), 326 (20), 313 (88), 269 (11), 64 (39), 55 (37).

Boeavinone F (*=3,9,11-Trihydroxy-10-methyl[1]benzopyrano[3,4-*b*][1]benzopyran-6,12-dione*; **7**). Yellow crystals. IR (KBr): 3407, 1709, 1645, 1625, 1586, 1438, 1289, 1260, 1207, 1121, 1086. ^{13}C-NMR: *Table 2*. EI-MS (70 eV): 326 (100, M^+), 297 (9).

Inhibition of HIV-1-RT Activity. The inhibition of recombinant-HIV-1-RT activity was performed with a commercially available ELISA kit (*Boehringer Mannheim*, Germany) according to the instructions of the manufacturer. Five serial dilutions of samples in DMSO (6 µl) in duplicate were added to the reaction mixture. The final DMSO concentration used was 10%. The highest concentration of compounds was 210 µg/ml. Compound-free samples containing an equivalent volume of DMSO were used for control assays. *Foscarnet* was used as a positive control compound. It inhibited 100% of the HIV-1-RT activity at 100 µg/ml. The absorption at 450 nm/490 nm ($A_{450/490}$) was read in an ELISA reader (Elx800, *Bio-Tek Instrument Inc.*, USA) and then the inhibitory percentage of the compounds calculated.

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