Synthesis and Antiviral Activities of Some 4,4′- and 2,2′-Dihydroxytriphenylmethanes

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We synthesized some 4,4′- and 2,2′-dihydroxytriphenylmethane derivatives 3a—e and 4a—c by condensation of phenol 1 and aromatic aldehyde 2 in moderate to good yields (30—83%). Most of them showed significant antiviral activity against herpes simplex virus type 1 (anti-HSV-1 activity) in a plaque reduction assay. The most potent antiviral activity (EC_{50}=0.79 μg/ml) was observed in the 4,4′-dihydroxytriphenylmethane derivative 3b. This compound 3b showed lower cytotoxicity (CC_{50}=30.2 μg/ml), compared to that of the prototype 3a.

Key words dihydroxytriphenylmethane; antiviral activity; herpes simplex virus type 1 (HSV-1); plaque reduction assay; condensation; triarylmethane

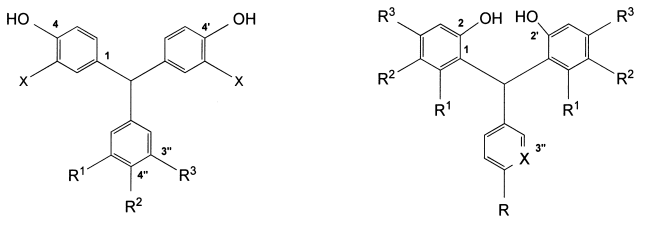
In connection with our search for antiviral compounds by using HSV-1 (herpes simplex virus type 1), we have already reported that some 2,2′- and 4,4′-dihydroxytriphenylmethanes show significant antiviral (Anti-HSV-1) activity in a plaque reduction assay.[1,2] Our finding of anti-HSV-1 activity in this new class of dihydroxytriphenylmethane derivatives led us to further molecular modification of the substituents in the phenyl rings of the dihydroxytriphenylmethane template. In our previous studies, we observed that the introduction of halogen atoms in the phenyl rings of triphenylmethanes generally enhances cytotoxicity resulting in low selectivity indexes.[2] To single out more promising anti-HSV-1 leads, we further investigated the synthesis and evaluation of antiviral activity in some related compounds. Here, we describe the synthesis and antiviral activity of 4,4′- and 2,2′-dihydroxytriphenylmethane derivatives **3a**—**e** and **4a**—**c**.

Results and Discussion

To synthesize the target 4,4′-dihydroxytriphenylmethane derivatives **3a**—**e** (Fig. 1), we employed various acid-catalyzed condensation methods using phenol **1** and aromatic aldehyde **2** as starting materials. To obtain improved yields for the preparation of the target molecules we employed various Brønsted and Lewis acids as a catalyst, including trifluoroacetic acid (TFA) [Method A], conc. H_2SO_4 [Method B], and phosphotungstic acid hydrate (PW_{12}) [Method C], as listed in Table 1. Other examples such as boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) [Method D] and trifluoromethanesulfonic acid (TFSA) [Method E] are also examined (see Experimental). As mentioned in a previous paper, in the synthesis of halogenated 4,4′-dihydroxytriphenylmethane derivatives,[2] the method using TFA as the catalyst [Method A] was conventional and gave fairly good yield for the preparation of **3d** by the reaction of phenol and 3,4,5-trimethoxybenzaldehyde (66%)(entry 5 in Table 1). This procedure with TFA for other derivatives **3a**—**c**, and **3e**, however, gave lower yields (<26%) (see Experimental).

The procedure with conc. H_2SO_4 [Method B] for the preparation of the target compounds **3b**, **3c**, and **3e** was more effective than other procedures, and the yields of the target products **3** were in the range of 30—83%. The yield of **3a** (15%) by this procedure (see Experimental) was lower than that (26%) of Method A (entry 1 in Table 1). We previously reported the yield of 37% by Method D for the synthesis of **3a**.[3] Regarding this compound, we further examined a few other methods in this study, and Method C was found to be an improved procedure giving an excellent result (entry 2 in Table 1).

On the other hand, a new successful method for the prepa-



3a: X=H, R^1,R^3=H, R^2=OMe
3b: X=H, R^1=H, R^2,R^3=OCH_2O
3c: X=H, R^1,R^3=H, R^2=OPh
3d: X=H, R^1,R^2,R^3=OMe
3e: X=Br, R^1,R^3=H, R^2=CF_3

4a: X=C, R^1,R^3=OMe, R^2=H, R=$CH(CH_3)_2$
4b: X=C, R^1=H, R^2,R^3=OCH_2O, R=OMe
4c: X=N, R^1=H, R^2,R^3=OCH_2O, R=H

Fig. 1

Table 1. Reactions of Phenol with Aldehyde

Entry	Product	Method	Acid	Ratio of ArOH : ArCHO : Acid	Conditions	Yield %
1	**3a**	A	CF_3COOH	2 : 1 : 10	r.t., 1 d	26
2	**3a**	C	$H_3PO_4 \cdot 12WO_3 \cdot xH_2O$	20 : 1 : 0.006	60 °C, 19 h, N_2	82
3	**3b**	B	H_2SO_4	2 : 1 : 2	r.t., 39 h, AcOH	41
4	**3c**	B	H_2SO_4	2.35 : 1 : 2.5	r.t., 20 h, AcOH	83
5	**3d**	A	CF_3COOH	2 : 1 : 10	r.t., 3 d	66
6	**3e**	B	H_2SO_4	2 : 1 : 2	r.t., 5 h, 55 °C, 20 min, AcOH	30

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Table 2. Physical Data for Compounds **3b**—**e**

Compound	mp (°C) (Recryst solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	Formula, HR-MS *m/z* Calcd (Found)	IR (cm^{-1}) (KBr)
3b	94—96 (*i*-PrOH–H_2O)	$C_{20}H_{16}O_4 \cdot C_3H_8O$	72.61 (72.45	6.36 6.33	0.00 0.00)	$C_{20}H_{16}O_4$ (M^+) 320.1049 (320.1049)	3405 (OH) 1250 (C–O)
3c	136—137 (Benzene–*n*-hexane)	$C_{25}H_{20}O_3$	81.50 (81.69	5.47 5.67	0.00 0.00)	$C_{25}H_{20}O_3$ (M^+) 368.1407 (368.1412)	3250 (OH) 1240 (C–O)
3d	178—179 (Benzene)	$C_{22}H_{22}O_5$	72.12 (72.10	6.05 6.08	0.00 0.00)	$C_{22}H_{22}O_5$ (M^+) 366.1467 (366.1471)	3455 (OH) 1215 (C–O) 1125 (C–O)
3e	47—48	$C_{20}H_{13}Br_2F_3O_2$	47.84 (47.68	2.61 2.70	0.00 0.00)	$C_{20}H_{13}Br_2F_3O_2$ (M^+) 501.9215 (501.9211)	3500 (OH) 1325 (CF_3) 1165 (C–O)

Tabel 3. ^{13}C- and ^1H-NMR Data of 4,4′-Dihydroxytriphenylmethanes **3b**—**e** (δ ppm, *J* Hz)[a)]

Position	**3b** ^{13}C	**3b** ^1H	**3c** ^{13}C	**3c** ^1H	**3d** ^{13}C	**3d** ^1H	**3e** ^{13}C	**3e** ^1H
1,1′	136.55		16.66		136.71		136.35	
2,2′	130.34	6.91 dm (8.6)	130.43	6.96 dm (8.6)	131.27	6.90 d (8.5)	132.39	7.15 d (2.1)
3,3′	115.17	6.71 dm (8.6)	115.17	6.74 dm (8.6)	115.96	6.70 d (8.5)	110.46	
4,4′	153.86		153.95		156.76		151.22	
5,5′	115.17	6.71 dm (8.6)	115.17	6.74 dm (8.6)	115.96	6.70 d (8.5)	116.20	6.96 d (8.2)
6,6′	130.34	6.91 dm (8.6)	130.43	6.96 dm (8.6)	131.27	6.90 d (8.5)	129.97	6.91 ddd (8.2, 2.1, 0.6)
OH		4.5 br s		4.76 br s				5.49 s
–CH<	54.77	5.29 s	54.54	5.39 s	56.61	5.31 s	54.40	5.41 s
1″	138.62		139.39		142.75		147.12	
2″	109.80	6.56 dm (8.6)	130.48	7.04 dm (8.55)	108.03	6.36 s	129.49	7.19 dd (7.9, 0.6)
3″	147.51		118.61	6.91 dm (8.55)	154.18		125.54 q (4.1)	7.56 d (7.9)
4″	145.77		155.54		137.41		129.14 q (32.1)	
5″	107.95	6.68 d (8.2)	118.61	6.91 dm (8.55)	154.18		125.54 q (4.1)	
6″	122.32	6.52 dd (8.2, 1.5)	130.48	7.04 dm (8.55)	108.03	6.36 s	129.49	6.36 s
–OCH_2O–	100.82	5.87 s						
–OC_6H_5			118.83 *o*- 123.15 *p*- 129.68 *m*- 157.30 –O<u>C</u>	7.00 dm (8.6) *o*- 7.07 tm (7.3) *p*- 7.31 dd (8.6, 7.3) *m*-				
CF_3							124.11 q (272.1)	
OCH_3					56.50 on 3″ 61.13 on 4″	3.67 s on 3″ 3.74 s on 4″		

a) **3b**, **c** and **e** were measured in $CDCl_3$. **3d** was measured in DMSO-d_6.

ration of triphenylmethane derivatives from benzaldehydes and benzene under superacid conditions has already been reported.[4)] Our trials of this method for the preparation of **3a** using TFSA [Method E] unfortunately resulted in the cleavage of ether functionality at C-4″, probably after the intended condensation, and the yield of desired **3a** was very low (4%). An unexpected demethylated product, 4,4′,4″-trihydroxytriphenylmethane **3f**, was also formed in 22% yield (see Experimental). In the Method D (see Experimental), though the formation of **3f** was also detected by TLC monitoring, the target compound **3a** could be obtained in a moderate yield (47%).

Phosphotungstic acid hydrate (PW_{12}), which is a strong acid, showed an effective catalytic activity in dehydration among heteropoly acids (HPAs).[5,6)] By the procedure with PW_{12} [Method C], the target compound **3a** was obtained in excellent yield (82%) (entry 2 in Table 1).[7)]

The favorable results in the above experiments for the synthesis of target compounds **3a**—**e** are summarized in Table 1, together with entry 1 by Method A. The physical and NMR data for new compounds **3b**—**e** synthesized in this study are summarized in Tables 2 and 3, the data of which are satisfactory for each structure of the compounds. We also synthesized 2,2′-dihydroxytriphenylmethanes **4a**—**c** according to the procedure reported previously.[3)]

The antiviral activities of compounds **3a**—**e** and **4a**—**c** were estimated by the plaque reduction assay.[8)] The obtained 50% effective concentration (EC_{50}) values and 50% cytotoxic concentration (CC_{50}) values for the compounds synthesized above are summarized in Table 4. In our previous study on halogenated triphenylmethane derivatives, we observed a general tendency of enhancement of cytotoxicity and no sig-

Table 4. Anti-HSV-1 Activity (EC_{50}) and Cytotoxicity (CC_{50}) of 4,4′- and 2,2′-Dihydroxytriphenylmethanes **3a**—**e** and **4a**—**c**

Compound	EC_{50} (μg/ml)	CC_{50} (μg/ml)
3a	1.7 (1.8)[a]	11.8
3b	0.79	30.2
3c	3.3	10.2
3d	4.65	41.0
3e	3.5	18.5
4a	ND	18.0
4b	6.6	19.6
4c	2.7	>50

a) The value in parentheses is from the reference 1. In comparison, we also reexamined antiviral activity of this compound under the same conditions and obtained reproducible results. The value of 1.7 is in excellent agreement with the value which we previously obtained.[2)]

nificant potentiation effect regarding anti-HSV-1 activities.[2)] The additionally synthesized brominated compound **3e** showed a similar property in its antiviral activity compared to those of the halogenated derivatives reported previously.[2)]

The most active compound in this study was compound **3b** which has a methylenedioxy group among C-3″ and C-4″ in a phenyl ring of a triphenylmethane skeleton, and this showed comparatively lower cytotoxicity. As shown in Table 4, compound **3b** showed potent antiviral activity (EC_{50}= 0.79 μg/ml) and considerably low cytotoxicity (CC_{50}= 30.2 μg/ml) in the assay with Vero cells (see Experimental). Thus, the selectivity index (CC_{50}/EC_{50}) had a value of 38.2, which is larger than that of prototype **3a** (CC_{50}/EC_{50}=*ca.* 15) described in our previous paper.[1,2)] In structural comparison of **3a** to **3d**, the introduction of two methoxy groups into a phenyl ring resulted in a slight reduction of the anti-HSV-1 activity, as well as decreased cytotoxicity. We also observed that the modification of 2,2′-dihydroxytriphenylmethane derivatives **4b** to **4c**, in which a phenyl ring is substituted to a pyridine ring, increases its activity and decreases its cytotoxicity.

In this study, we emphasize that the 4,4′-dihydroxytriphenylmethane compound **3b** showed higher significant anti-HSV-1 activity and less cytotoxicity, resulting in a better selectivity index (CC_{50}/EC_{50}=38.2) than the prototype compound **3a**. The transformation of a methoxy group into a methylenedioxy group in a phenyl ring may influence the substituted phenyl ring or the total molecular shape and contribute to the profile of this compound, the factor of which is ambiguous at this moment. As a result, this may show antiviral activity. Further synthetic studies and the mode of antiviral action of this class of compounds are under investigation.

Experimental

Physical and spectroscopic data were obtained by the same apparatus described previously.[2)] Routine monitoring of reactions was carried out using precoated Kieselgel 60F_{254} plates (E. Merck). Centrifugal or flash column chromatography was performed on silica gel of Able-Biott or Fuji Silysia FL40D, respectively, with a UV detector.

General Procedures for the Condensation of Phenol and Aldehyde. Method A To a melted mixture of phenol (2 eq) and aldehyde (1 eq) was added TFA (10—32 eq). After stirring for an appropriate period at an appropriate temperature, TFA was removed under reduced pressure. The residue was purified by chromatography. By this procedure, compound **3a** was obtained in 26% yield (see entry 1 in Table 1).

Method B To a solution of phenol (2 or 2.35 eq) and aldehyde (1 eq) in AcOH was added conc. H_2SO_4 (2 or 2.5 eq), and this mixture was stirred for an appropriate period at an appropriate temperature. The reaction mixture was poured into ice-water and extracted from ether. The organic layer was washed with brine and dried over $MgSO_4$. After evaporation, the residue was purified by chromatography.

Method C: Preparation of 3a[3)] A mixture of phenol (1.90 g, 20 mmol), 4-methoxybenzaldehyde (138 mg, 1 mmol), and phosphotungstic acid hydrate (18 mg, 6 μmol) was stirred at 60 °C for 19 h under N_2 atmosphere. CH_2Cl_2 (4 ml) was added, and the mixture was shaken and filtrated to separate the insoluble catalyst. After evaporation, centrifugal chromatography (SiO_2: 1% EtOH in CH_2Cl_2) gave **3a** (250 mg, 0.820 mmol) as an orange semisolid in 82% yield.

Method D: Preparation of 3a A stirring solution of phenol (952 mg, 10 mmol) and 4-methyoxybenzaldehyde (138 mg, 1 mmol) in absolute ether (50 ml) was accomplished by a flow of dry nitrogen for 20 min at 0 °C, then $BF_3 \cdot OEt_2$ (47%, 2.16 ml, 4 mmol) was injected. In a nitrogen stream, the reaction mixture was further stirred for 32 h at 0 °C, then kept at ambient temperature for 8 h. The resulting mixture was diluted with AcOEt (20 ml) and immediately washed with 0.1 M aqueous NaOH (25 ml). The organic layer was washed with brine and dried over $MgSO_4$. After evaporation of the solvent, centrifugal chromatography (SiO_2: 60% AcOEt in *n*-hexane) afforded **3a** (143 mg, 0.467 mmol) in 47% yield as an orange semisolid.

Method E: Preparation of 3a To an ice-cooled mixture of phenol (476 mg, 5 mmol) and 4-methoxybenzaldehyde (138 mg, 1 mmol) was added TFSA (9.0 ml, 100 mmol) dropwise under vigorous stirring. After the addition of TFSA, the cooling bath was removed. Then, the reaction mixture was stirred at room temperature for 17 h. The resulting mixture was poured into a large excess of ice and water (400 ml) and extracted with AcOEt (200 ml×3). The organic extract was washed with brine, dried over $MgSO_4$, and evaporated. Purification of the residue by centrifugal choromatography (SiO_2: 40% AcOEt in *n*-hexane) afforded **3a** (12 mg, 0.039 mmol: 4% yield) as an orange semisolid compound, and 4,4′,4″-trihydroxytriphenylmethane (**3f**, 66 mg, 0.224 mmol: 22%) as an orange solid. **3f**: HR positive ion FAB-MS: Calcd for $C_{19}H_{16}O_3$ (M^+): 292.1099. Found: 292.1110. ^{1}H-NMR (CD_3OD) δ: 5.26 (1H, s, >CH–), 6.69 (6H, dm, J=8.5 Hz, H3,5), 6.87 (6H, dm, J=8.5 Hz, H2,6). ^{13}C-NMR (CD_3OD) δ_C: 55.69 (>CH–), 115.80 (C3,5), 131.21 (C2,6), 137.45 (C1), 156.34 (C4).

4,4′-Dihydroxy-4″-methoxytriphenylmethane (3a) Compound **3a** was prepared according to Methods A—E from phenol and 4-methoxybenzaldehyde. Methods A and C are listed in Table 1. [Method B] The reaction with phenol (951 mg, 10 mmol) and 4-methoxybenzaldehyde (688 mg, 5 mmol) using conc. H_2SO_4 (98%, 544 μl, 10 mmol) in AcOH (2.30 ml, 40 mmol) was carried out at room temperature for 20 h, and purification of the reaction mixture by flash chromatography (SiO_2: 2% EtOH in CH_2Cl_2) gave **3a** (226 mg, 0.739 mmol: 15%) as an orange semisolid. In both Method A and B, the demethylated compound **3f** was detected by TLC analysis and the isolation was disregarded. Methods C, D, and E are written above in General Procedures.

4,4′-Dihydroxy-3″,4″-methylenedioxytriphenylmethane (3b) [Method A]: The reaction of phenol (380 mg, 4 mmol) and 3,4-methylenedioxybenzaldehyde (303 mg, 2 mmol), using TFA (5.0 ml, 64 mmol) at room temperature for 2 d, followed by purification by centrifugal chromatography (SiO_2: 2% EtOH in CH_2Cl_2), gave the compound **3b** (72 mg, 0.22 mmol: 11%) as an orange semisolid.

[Method B]: The reaction of phenol (760 mg, 8 mmol) and 3,4-methylenedioxybenzaldehyde (606 mg, 4 mmol) using conc. H_2SO_4 (98%, 436 μl, 8 mmol) in AcOH (2.30 ml, 40 mmol) at room temperature for 39 h was carried out. Subsequent purification by centrifugal chromatography (SiO_2: 40% AcOEt in *n*-hexane) gave **3b** (260 mg, 1.64 mmol: 41%) as an orange semisolid (see entry 3 in Table 1). Recrystallization from water/iso-PrOH (4/1) gave pale reddish crystals.

4,4′-Dihydroxy-4″-phenoxytriphenylmethane (3c) [Method A]: After the reaction of phenol (380 mg, 4 mmol), 4-phenoxybenzaldehyde (404 mg, 2 mmol), and TFA (5.0 ml, 64 mmol) at room temperature for 4 d, purification by centrifugal chromatography (SiO_2: 2% EtOH in CH_2Cl_2) gave **3c** (10 mg, 0.027 mmol: 1%) as an orange semisolid.

[Method B]: This was shown in entry 4 in Table 1. Thus, to a solution of phenol (475 mg, 5 mmol) and 4-phenoxybenzaldehyde (445 mg, 2.2 mmol) in AcOH (1 ml, 17 mmol) was added conc. H_2SO_4 (98%, 0.30 ml, 5.5 mmol), followed by stirring for 20 h at room temperature. The reaction mixture was poured into ice-water (10 ml) and extracted from ether (30 ml×3). The organic layer was washed with brine and dried over $MgSO_4$. Subsequent evaporation flash chromatography (SiO_2: 2% EtOH in CH_2Cl_2) gave **3c** (0.676 g, 1.837 mmol) as an orange semisolid in 83% yield. Recrystallization from benzene–*n*-hexane afforded a pale orange powder.

4,4′-Dihydroxy-3″,4″,5″-trimethoxytriphenylmethane (3d) [Method

A]: To a melted mixture of phenol (570 mg, 6 mmol) and 3,4,5-trimethoxybenzaldehyde (601 mg, 3 mmol) was added TFA (2.36 ml, 30 mmol). After stirring for 3 d at room temperature, TFA was removed under reduced pressure. The residue was purified by flash chromatography (SiO_2: 50% AcOEt in *n*-hexane) to yield **3d** (726 mg, 1.98 mmol) as a pale yellow amorphous substance in 66% yield. Recrystallization from benzene afforded a white powder.

3,3′-Dibromo-4″-trifluoromethyl-4,4′-dihydroxytriphenylmethane (3e) [Method A]: The reaction of 2-bromophenol (706 mg, 4 mmol) and 4-trifluoromethylbenzaldehyde (355 mg, 2 mmol), using TFA (2.0 ml, 25 mmol) at 70 °C for 7 d, was carried out. Subsequent purification by centrifugal chromatography (SiO_2: 60% CH_2Cl_2 in *n*-hexane) gave **3e** (121 mg, 0.241 mmol: 12%) as pale yellow flakes, together with isomeric 2,4′-dihydroxytriphenylmethane derivative **3′e** (83.5 mg, 0.166 mmol) as a colorless semisolid in 8% yield.

[Method B]: After the reaction of 2-bromophenol (706 mg, 4 mmol) and 4-trifluoromethylbenzaldehyde (355 mg, 2 mmol), using conc. H_2SO_4 (98%, 218 μl, 4 mmol) in AcOH (920 μl, 16 mmol) under the conditions shown in Table 1 (entry 6), purification by centrifugal chromatography (SiO_2: 50% AcOEt in *n*-hexane) gave **3e** (301 mg, 0.600 mmol: 30%) as pale yellow flakes.

3,3′-Dibromo-4″-trifluoromethyl-2,4′-dihydroxytriphenylmethane (3′e) HR positive ion FAB-MS: Calcd for $C_{20}H_{13}Br_2F_3O_2$ (M^+): 501.9215. Found: 501.9214. ^{1}H-NMR ($CDCl_3$) δ: 5.46 (1H, br s, OH), 5.63 (1H, br s, OH),5.85 (1H, s, >CH–), 6.74—6.78 (2H, m, H6, 5), 6.93 (1H, dd, J=8.4, 1.5 Hz, H6′), 6.96 (1H, d, J=8.4 Hz, H5′), 7.17 (1H, d, J=2.1 Hz, H2′), 7.20 (2H, d, J=8.2 Hz, H2″), 7.37—7.41 (1H, m, H4), 7.55 (2H, d, J=8.2 Hz, H3″). ^{13}C-NMR ($CDCl_3$) δ: 49.42 (>CH–), 110.43 (C3′), 110.93 (C3), 116.08 (C5′), 121.48 (C5), 124.19 (CF_3, q, J=271.04 Hz), 125.41 (C3″, q, J=4.14 Hz), 129.01 (C4″, q, J=32.07 Hz), 129.50 (C2″), 129.73 (C6), 130.08 (C6′), 130.67 (C4), 130.71 (C1), 132.48 (C2′), 135.73 (C1′), 146.66 (C1″), 149.81 (C2), 151.15 (C4′).

The physical and spectroscopic (^{1}H- and ^{13}C-NMR) data on **3b**—**e** are summarized in Tables 1—3. These data on **3a** are taken from a previous report,[3] and the preparation of 2,2′-dihydroxytriphenylmethane derivatives **4a**—**c** for antiviral activity assay has already been reported.[3]

Antiviral Activity Assay The antiviral activities of the compounds were measured by the plaque reduction assay[8] described in previous paper.[1,2] Calculated EC_{50} values for the tested compounds are summarized in Table 4.

Cytotoxicity Assay The antiviral activities of the compounds were examined as described below. Confluent monolayers of Vero cells were seeded in 96-well plastic plates at 5×10^4 cells per well. After 1 d, the cells were refed with 100 μl of DMEM containing 5% fetal calf serum and various concentrations of the target compounds. After 69 h incubation, 10 μl of Alamar-Blue reagent was added to each culture, then the plates were reincubated for 4 h. The optical density of each culture at 570 nm was determined by spectrophotometer using a reference wavelength of 630 nm.[9] Calculated cytotoxicity (CC_{50}) values for the tested compounds are summarized in Table 4.

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References and Notes

1) Sumoto K., Mibu N., Yokomizo K., Uyeda M., *Chem. Pharm. Bull.*, **50**, 298—300 (2002).
2) Mibu N., Yokomizo K., Uyeda M., Sumoto K., *Chem. Pharm. Bull.*, **51**, 1325—1327 (2003).
3) Mibu N., Sumoto K., *Chem. Pharm. Bull.*, **48**, 1810—1813 (2000).
4) Olah G. A., Rasul G., York C., Prakash G. K. S., *J. Am. Chem. Soc.*, **117**, 11211—11214 (1995).
5) Aoshima A., Yamamatsu S., Yamaguchi T., *Nippon Kagaku Kaishi*, **1987**, 976—983 (1987).
6) Ohtsuka R., Morioka Y., Kobayashi J., *Bull. Chem. Soc. Jpn.*, **63**, 2071—2075 (1990).
7) Application of Method A (TFA) and Method B (conc. H_2SO_4) for this compound, however, afforded a complex mixture including 2,2′- and 2,4′-dihydroxytriphenylmethane derivatives, and unknown polymeric compounds. This complex composition could be detected by TLC analysis.
8) Schinazi R. F., Peters J., Williams C., Chance D., Nahmias A., *Antimicrob. Agents Chemother.*, **22**, 499—507 (1982).
9) Lancaster M. V., Fields R. D., U.S. Patent No. 5501959 (1996).