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

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4,4′-Dihydroxytriphenylmethanes were synthesized using Brønsted acid or Lewis acid in yields of 24—86% as target compounds for developing antiviral agents. Most of the 4,4′-dihydroxytriphenylmethanes showed significant activity against herpes simplex virus type 1 (anti-HSV-1 activity) in a plaque reduction assay. Higher cytotoxicity was observed generally in halogenated 4,4′-dihydroxytriphenylmethanes (2a—d) than in non-halogenated derivatives. The non-halogenated derivative, 4,4′,4″-trihydroxy-3″-methoxytriphenylmethane (3), showed remarkable antiviral activity with an EC_{50} value of 1.8 μg/ml.

Key words triphenylmethane; antiviral activity; herpes simplex virus type 1 (HSV-1); plaque reduction assay; halogenated compound; condensation

Discovering a new class of compounds with potent antiviral activity is important not only in the development of potential therapeutic agents against various viral diseases, but also in the study of the mechanisms of antiviral effects. Recently, we reported the synthesis and antiviral activity of compounds relating to triphenylmethane-type bisphenol derivatives.[1,2] In our previous study, we found that compound (**1a**) belongs to the class of 4,4′-dihydroxytriphenylmethanes possessing notable antiviral activity.[2] As a further extension of our studies, we synthesized some 4,4′-dihydroxytriphenylmethane derivatives of **1a** as the lead compound, and evaluated their antiviral activities using the plaque reduction assay.[3] We report here the synthesis of target compounds and the observation of a wide range of antiviral activity of these compounds.

Results and Discussion

The target 4,4′-dihydroxytriphenylmethane derivatives (**1**—**3**) (Fig. 1) with a substituted aryl group were synthesized from phenol or a halogenated phenol and the selected aromatic aldehyde with Brønsted acid or Lewis acid by a slightly modified procedure reported previously.[1,4,5] As mentioned before,[1,5] the reactions proceed in a C-*para* regiospecific manner *via* the bisarylation of aldehyde. These methods employed various acids, including trifluoroacetic acid (TFA) [Method A], conc. H_2SO_4 [Method B], boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) [Method C], and polyphosphoric acid (PPA) [Method D], as listed in Table 1. Using TFA as the catalyst for the preparation of the target compounds is conventional and usually gives good to moderate results.[6] The physical data for compounds (**1**—**3**) are summarized in Table 2. NMR-spectroscopic analyses confirmed the symmetrical structure of the target compounds (**1b**, **2a**—**d**) (Table 3). Full assignments of NMR signals for all products were confirmed based on ^1H–^1H shift correlation spectroscopy (^1H–^1H COSY), ^1H-detected heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra. The 4,4′,4″-trihydroxy-3″-methoxytriphenylmethane derivative **3** was synthesized with TFA [Method A] in 40% yield (entry 8 in Table 1).

The antiviral activities of the compounds were estimated using plaque reduction assays as described in the Experimental section. Calculated EC_{50} values for the tested compounds are summarized in Table 4. Frequently, introducing halogen

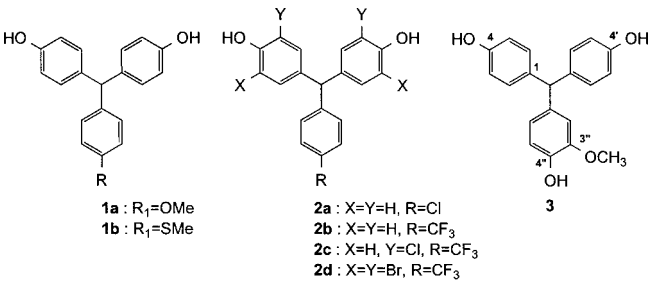

Fig. 1

Table 1. Reactions of Phenol with Aldehyde

Entry	Product	Method	Acid	Ratio of ArOH : ArCHO : acid	Conditions	Yield (%)
1	**1b**	A	CF_3COOH	2 : 1 : 1	rt, 2 d	33
2	**2a**	A	CF_3COOH	2 : 1 : 32	rt, 3 d	86
3		B	H_2SO_4	2 : 1 : 2	rt, AcOH, 1 h	28
4	**2b**	C	$BF_3 \cdot OEt_2$	4 : 1 : 1	Reflux, Et_2O, 1 d	24
5		B	H_2SO_4	2 : 1 : 2	rt, 1 d, then 70 °C, 6 h, AcOH	36
6	**2c**	B	H_2SO_4	2 : 1 : 2	rt, 2 d, then 65 °C, 1 d, AcOH	68
7	**2d**	D	PPA[a)]	2 : 1 : 33	85 °C, 1 d	50
8	**3**	A	CF_3COOH	2 : 1 : 10	rt, 2 d	40

a) Molar ratio was calculated as H_3PO_4.

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Table 2. Physical Data for Compounds (**1b**, **2a**—**d**, and **3**)

Compound	mp (°C) (Recryst solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	Formula, HR-MS *m/z* Calcd (Found)	IR (cm^{-1}) (KBr)
1b	62—64	$C_{20}H_{18}O_2S \cdot 0.4H_2O$	72.87 (72.89	5.75 5.94	0.00 0.00)	$C_{20}H_{18}O_2S$ (M^+) 322.1028 (322.1026)	3400 (OH) 1510 (C–O)
2a	Oil	$C_{19}H_{15}ClO_2 \cdot 0.7H_2O$	70.57 (70.64	5.11 5.33	0.00 0.00)	$C_{19}H_{15}ClO_2$ (M^+) 310.0761 (310.0768)	3385 (OH)
2b	65—69 (Benzene)	$C_{20}H_{15}F_3O_2 \cdot 0.3H_2O$	68.69 (68.53	4.50 4.55	0.00 0.00)	$C_{20}H_{15}F_3O_2$ (M^+) 344.1024 (344.1023)	3640 (OH) 1325 (CF_3) 1160 (CF_3)
2c	36—38 (*n*-Heptane)	$C_{20}H_{13}Cl_2F_3O_2$	58.13 (58.38	3.17 3.46	0.00 0.00)	$C_{20}H_{13}Cl_2F_3O_2$ (M^+) 412.0245 (412.0248)	3525 (OH) 1325 (CF_3) 1165 (CF_3)
2d	157—160 (Cyclohexane)	$C_{20}H_{11}Br_4F_3O_2$	36.40 (36.65	1.68 1.79	0.00 0.00)	$C_{20}H_{11}Br_4F_3O_2$ (M^+) 659.7405 (659.7416)	3475 (OH) 1325 (CF_3) 1165 (CF_3)
3	184—187	$C_{20}H_{18}O_4 \cdot H_2O$	70.58 (70.52	5.92 5.96	0.00 0.00)	$C_{20}H_{18}O_4$ (M^+) 322.1205 (322.1201)	3400 (OH) 1510 (C–O)

Tabel 3. ^{13}C- and ^1H-NMR Data of 4,4′-Dihydroxytriphenylmethanes **1b**, **2a**—**d**, and **3** (δ ppm, *J* Hz)[a]

Position	**1b**		**2a**		**2b**		**2c**		**2d**		**3**	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1, 1′	136.24		135.95		135.61		135.95		136.59		135.09	
2, 2′	130.36	6.89 dm (8.5)	130.38	6.92 dm (8.2)	130.44	6.93 dm (8.2)	129.47	7.01 d (2.1)	132.47	7.13 d (0.6)	129.63	6.84—6.88 m
3, 3′	115.21	6.70 dm (8.5)	115.25	6.74 dm (8.2)	115.34	6.75 dm (8.2)	120.11		110.31		114.79	6.63—6.68 m
4, 4′	153.95		154.18		154.19		150.28		148.66		155.30	
5, 5′	115.21	6.70 dm (8.5)	115.25	6.74 dm (8.2)	115.34	6.75 dm (8.2)	116.38	6.96 d (8.5)	110.31		114.79	6.63—6.68 m
6, 6′	130.36	6.89 dm (8.5)	130.38	6.92 dm (8.2)	130.44	6.93 dm (8.2)	129.21	6.87 dd (8.5, 2.1)	132.47	7.13 d (0.6)	129.63	6.84—6.88 m
OH		6.1 br s		5.28 br s		4.92 br s		5.53 br s		5.89 s		3.37 br s
–CH<	54.62	5.31 s	54.57	5.37 s	55.03	5.45 s	54.59	5.41 s	53.85	5.36 s	53.94	5.22 s
1″	141.72		143.13		148.63		147.13		145.63		135.86	
2″	126.74	6.97 dm (8.2)	130.61	7.01 dm (8.2)	129.59	7.22 d (7.9)	129.50	7.19 d (8.0)	129.41	7.18 d (8.5)	113.35	6.63—6.68 m
3″	129.75	7.13 dm (8.2)	128.36	7.22 dm (8.2)	125.19	7.51 d (7.9)	125.52	7.55 d (8.0)	125.87 (4.1)	7.60 d (8.5)	147.18	
4″	135.68		131.99		128.55 (32.1)		129.14 (33.1)		129.68 (33.1)		144.62	
5″	129.75	7.13 dm (8.2)	128.36	7.22 dm (8.2)	125.19	7.51 d (7.9)	125.52	7.55 d (8.0)	125.87 (4.1)	7.60 d (8.5)	115.06	6.63—6.68 m
6″	126.74	6.97 dm (8.2)	130.61	7.01 dm (8.2)	129.59	7.22 d (7.9)	129.50	7.19 d (8.0)	129.41	7.18 d (8.5)	121.14	6.41—6.45 m
CF_3					122.06 (282.4)		124.13 (271.1)		123.97 (272.0)			
SCH_3	15.96	2.41 s										
OCH_3											55.56	3.64 d (2.7)

a) **1b** and **2** were measured in $CDCl_3$. **3** was measured in DMSO-d_6.

atoms to the prototype of an antiviral compound enhances antiviral activity.[7,8] In the case of halogenated 4,4′-dihydroxytriphenylmethane-type derivatives (**2a**—**d**) however, no significant potentiating effect regarding anti-HSV-1 activity was observed (see, compounds **2a**—**d** in Table 4). The derivative **2a** showed significant antiviral activity. This compound had a 50% cytotoxic concentration (CC_{50}) of 5—10 μg/ml in additional experiments.[9] The selectivity index (CC_{50}/EC_{50}) was in the range of *ca.* 3—6, which is, unfortunately, lower than that of the prototype **1a** (*ca.* 15 of CC_{50}/EC_{50}) described in our previous paper. In our assay, compound **3**, which has no halogen atom, showed low cytotoxicity (CC_{50} of >20 μg/ml) and about a half magnitude of antiviral activity (EC_{50} value of 1.8 μg/ml) compared to acyclovir.[10] The selectivity index of **3** was roughly estimated at more than 11.

It is noteworthy that the assay of the title 4,4′-dihydroxy-

Table 4. Anti-HSV-1 Activity (EC_{50}) of 4,4′-Dihydroxytriphenylmethanes (**1**—**3**)

Compound	EC_{50} (μg/ml)
1a	1.7 (1.8)[a]
1b	3.4
2a	1.8
2b	4.2
2c	4.4
2d	5.2
3	1.8

a) The value in parentheses is from ref. 2. In comparison, we also reexamined the antiviral activity of this compound under the same conditions and obtained reproducible results.

triphenylmethane derivatives described here revealed a wide range of significant anti-HSV-1 activity. In addition, there are a great many combinations of starting phenols and aromatic aldehydes to provide a considerable number of target compounds. Considering the results described in this paper, together with the information in our previous report on 2,2′-dihydroxytriphenylmethanes,[2] further study is warranted.

Experimental

Melting points were determined using a micro melting point apparatus (Yanagimoto MP-S3) without correction. IR spectra were measured with a Shimadzu FTIR-8100 IR spectrophotometer. Low- and high-resolution mass spectra (LR-MS and HR-MS) were taken with a JEOL JMS HX-110 double-focusing model equipped with a FAB ion source interfaced with a JEOL JMA-DA 7000 data system. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM A-500. Chemical shifts were expressed in δ ppm downfield from an internal tetramethylsilane (TMS) signal for ^{1}H-NMR and the carbon signal of the corresponding solvent [$CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.5 ppm)] for ^{13}C-NMR. Microanalyses were performed with a Yanaco MT-6 CHN corder. Routine monitoring of reactions was carried out using precoated Kieselgel 60F_{254} plates (E. Merck). Centrifugal chromatography was performed on silica gel (Able-Biott) with a UV detector. Commercially available starting materials were used without further purification.

4,4′-Dihydroxy-4″-methylthiotriphenylmethane (1b) (Method A) To a melted mixture of phenol (6 mmol) and 4-(methylthio)benzaldehyde (3 mmol) was added TFA (3 mmol). After stirring for 2 d at room temperature, TFA was removed under reduced pressure. The residue was purified by chromatography (CH_2Cl_2–EtOH as solvent) to yield **1b** as bright reddish crystals. The compounds **2a** and 4,4′,4″-trihydroxy-3″-methoxytriphenylmethane (**3**) were prepared using substantially the same procedure changing only the molar ratio of phenol : aldehyde : TFA (see Table 1).

4″-Chloro-4,4′-dihydroxytriphenylmethane (2a) (Method B) To a solution of phenol (4 mmol) and 4-chlorobenzaldehyde (2 mmol) in AcOH (0.9 ml) was added conc. H_2SO_4 (4 mmol) and the mixture was stirred for 1 h. The reaction mixture was poured into ice-water (10 ml) and extracted with ether (30 ml×3). The organic layer was washed with brine and dried (Na_2SO_4). After evaporation, centrifugal chromatography (CH_2Cl_2 as solvent) gave **2a** as pale yellow oil. The compounds **2b** and 3,3′-dichloro-4″-trifluotomethyl-4,4′-dihydroxytriphenylmethane (**2c**) were also prepared using this method (see Table 1).

4″-Trifluoromethyl-4,4′-dihydroxytriphenylmethane (2b) (Method C) A solution of phenol (20 mmol) and 4-(trifluoromethyl)benzaldehyde (5 mmol) in absolute ether (40 ml) was degassed for 20 min, and then $BF_3 \cdot OEt_2$ (5 mmol) was injected. After refluxing for 1 d, the resulting solution was diluted with ether (100 ml) and immediately washed with 0.1 M aqueous NaOH (50 ml). The organic layer was washed with water and dried ($MgSO_4$), then evaporated under reduced pressure. Purification by chromatography (*n*-hexane–EtOAc as solvent) afforded **2b**. A yellow powder was obtained by dissolving in benzene and keeping the preparation at room temperature.

3,3′-Dibromo-4″-trifluotomethyl-4,4′-dihydroxytriphenylmethane (2d) (Method D) A mixture of PPA (66 mmol), 2,6-dibromophenol (4 mmol), and 4-(trifluoromethyl)benzaldehyde (2 mmol) was heated at 85 °C for 1 d. Ice-water (40 ml) was added to the resulting mixture, which was then extracted with ether (40 ml×3), washed with brine (5 ml), and dried over $MgSO_4$. After evaporation of the solvent, centrifugal chromatography (*n*-hexane–AcOEt) gave **2d**. Recrystallization from cyclohexane gave pale yellow crystals.

The reaction conditions, yields, physical data, and spectroscopic (^{1}H- and ^{13}C-NMR) data on **1**—**3** are summarized in Table 1—3.

Antiviral Activity Assay The antiviral activities of the compounds were measured by plaque reduction assays[3] as described below. Confluent monolayers of Vero cells (5×10^5 cells) in 6-well plastic plates were infected with 100 PFU of HSV-1 (KOS). After a 1 h adsorption period at 37 °C, the cultures were overlaid with 2 ml of DULBECCO's modified Eagle's minimum essential medium (DMEM) containing 2% heat-inactivated fetal calf serum and various concentrations of the target compounds. The cultures infected with HSV-1 were incubated in a CO_2 incubator, fixed with formalin and stained with crystal violet in methanol at 3 d after infection. After washes with water and drying, the plaques were enumerated. Calculated EC_{50} values for the tested compounds are summarized in Table 4. Roughly estimated cytotoxicity (CC_{50}) values obtained from the dose for inhibition of Vero cell culture are recorded in the Notes.[9]

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References and Notes

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6) The yields shown in Table 1 (method A) may not be crucial. Some other reaction conditions employed in the preparation of **2a** gave lower yields. Alteration of the molar ratio of reactants including TFA [phenol : aldehyde : TFA＝4 : 1 : 89] gave a 52% yield, and we could not isolate the target **2a** using $TiCl_2(OiPr)_2$ or Lewatit K2621, because of complex reactions including polymerization.
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9) The other CC_{50} values estimated are 10—20, 5—10, and 10—20 μg/ml for **2b**, **2c**, and **2d**, respectively.
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