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Discovery of Novel 2′,3′,4′-Trihydroxy-2-phenylacetophenone Derivatives as Anti-Gram-Positive Antibacterial Agents

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A number of 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives were synthesized and examined for growth inhibition of several kinds of bacteria. 2′,3′,4′-Trihydroxy-2-phenylacetophenone itself exhibited no antibacterial activity, but some of its derivatives showed various antibacterial activities depending on functional groups introduced on the 2-phenyl ring. Eighteen out of 24 compounds synthesized in this study appeared to possess antibacterial activities against at least two Gram-positive strains of *Bacillus subtilis* and *Staphylococcus aureus*, 2-(biphenyl-4-yl)-2′,3′,4′-trihydroxyacetophenone being the most active with LC_{50} of 5.8 μM and 5.6 μM respectively. However, none of the synthesized compounds exhibited inhibitory effects on Gram-negative strains, such as *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella enterica*, suggesting that anti-Gram-positive specificity of the antibacterial compounds.

Key words: antibacterial agent; 2-phenylacetophenone; Gram-positive; *Staphylococcus aureus*

Over the past few decades, antibiotics have a history of success in controlling morbidity due to infectious diseases. However, as a consequence of frequent and excessive use of antibiotics, antibiotic-resistant bacteria have emerged and now cause severe clinical problems.[1,2] One of the most serious problems is provoked by methicillin-resistant *Staphylococcus aureus* (MRSA), a Gram-positive pathogen responsible for difficult-to-treat infections in humans, since it is resistant to a large group of β-lactam antibiotics. Accordingly, there is urgent and increasing need to discover and develop novel classes of antibacterial agents to make possible control of such antibiotic-resistant and infectious bacteria as MRSA. To date, numbers of natural and synthetic compounds have been investigated for their possible antibacterial activities.[3,4] 2-Phenylacetophenone (1,2-diphenylethanone, or deoxybenzoin) derivatives are a class of potential candidates for novel antibacterial agents, because previous reports suggest that they might have certain chemical, medical, and biological activities, including estrogenicity, protein tyrosine phosphatase inhibitory activity, and antimicrobial activity.[5–7] As this class of compounds came to receive increasing attention, we were prompted to synthesize various derivatives to test for the antibacterial activity. Here we report the synthesis of 24 novel 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives with a series of substitutions on the 2-phenyl ring and the evaluation of their antibacterial activity, yielding 18 candidates for new antibacterial agents specific for Gram-positive bacteria.

Materials and Methods

General procedure for synthesis of 2′,3′,4′-trihydroxy-2-phenylacetophenones. Pyrogallol (**y** in Fig. 1, 3.9 mmol) and one of the phenylacetic acid derivatives (**a–x** in Fig. 1, 3.7 mmol) were added to $BF_3 \cdot OEt_2$ (8.8 mmol). The mixture was heated once at 120 °C for 10 min, cooled to room temperature, and diluted with cold water. The resulting mixture was extracted with Et_2O. The organic layer was washed with brine and sat. aq. $NaHCO_3$, and dried over anhydrous $MgSO_4$. After complete evaporation of the diethyl ether, the residual solid containing the product was subjected to reverse-liquid chromatography using an ODS column eluted with AcCN–H_2O (70:30, v/v). The elluent was evaporated, and the product was crystallized in EtOH. After filtration, the product crystals were dried under reduced pressure to obtain purified compounds **1–24** (Fig. 1). The FAB-MS spectrum of the synthesized compounds was recorded on a JEOL JMS-700 spectrometer. ^{1}H (500 MHz)- and ^{13}C (125 MHz)-NMR were performed in DMSO-d_6 (containing < 0.003% water) with TMS as internal standard at 25 °C on a JEOL ECA-500 spectrometer.

Compound **1**, 2′,3′,4′-trihydroxy-2-phenylacetophenone, was obtained as pale violet crystals. M.p. 121–122 °C. Yield: 56%. ^{1}H-NMR (DMSO-d_6) δ: 4.29 (2H, s, CH_2), 6.42 (1H, d, J = 8.6 Hz, 5′-H), 7.23 (2H, t, J = 7.4 Hz, 4-H), 7.29 (2H, d, J = 7.4 Hz, 2,6-H), 7.31 (2H, t, J = 7.4 Hz, 3,5-H), 7.51 (1H, d, J = 8.6 Hz, 6′-H), 12.45 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 44.00 (CH_2), 107.80 (5′-C), 112.47 (1′-C), 123.03 (6′-C), 126.57 (4-C), 128.36 (3,5-C), 129.54 (2,6-C), 132.35 (3′-C), 135.29 (1-C), 152.54 (2′-C), 152.63 (4′-C), 202.87 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{13}O_4$ 245.0814, found 245.0805.

Compound **2**, 2′,3′,4′-trihydroxy-2-(2-methylphenyl) acetophenone, was obtained as beige crystals. M.p. 120–121 °C. Yield: 61%. ^{1}H-NMR (DMSO-d_6) δ: 2.18 (3H, s, 2-Me), 4.36 (2H, s, CH_2), 6.45 (1H, d, J = 9.2 Hz, 5′-H), 7.14 (2H, t, J = 8.0 Hz, 4,5-H), 7.15 (1H, d, J = 8.0 Hz, 6-H), 7.16 (1H, d, J = 8.0 Hz, 3-H), 7.52 (1H, d, J = 9.2 Hz, 6′-H), 8.64 (1H, s, 4′-OH), 10.11 (1H, s, 3′-OH), 12.44 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 19.30 (2-Me), 42.06 (CH_2), 107.76 (5′-C), 112.78 (1′-C), 122.55 (6′-C), 125.68 (5-C), 126.72 (4-C), 129.80 (3-C), 130.48 (6-C), 132.34 (3′-C), 134.12 (1-C), 136.80 (2-C), 152.27 (2′-C), 152.52 (4′-C), 202.76 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{15}H_{15}O_4$ 259.0970, found 259.0956.

Compound **3**, 2′,3′,4′-trihydroxy-2-(3-methylphenyl) acetophenone, was obtained as violet crystals. M.p. 127–128 °C. Yield: 56%. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (3H, s, 3-Me), 4.23 (2H, s, CH_2), 6.42 (1H, d, J = 9.2 Hz, 5′-H), 7.04–7.09 (3H, m, 2,4,6-H), 7.19 (1H, t, J = 7.7 Hz, 5-H), 7.50 (1H, d, J = 9.2 Hz, 6′-H), 8.63 (1H, s, 4′-OH), 10.12 (1H, s, 3′-OH), 12.53 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 20.95 (3-Me), 43.92 (CH_2), 107.78 (5′-C), 112.46 (1′-C), 123.03 (6′-C), 126.54 (6-C), 127.19 (4-C), 128.25 (5-C), 130.01 (2-C), 132.33 (3′-C), 135.16 (1-C), 137.40 (3-C), 152.56 (2′-C), 152.61 (4′-C), 202.90 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{15}H_{15}O_4$ 259.0970, found 259.0954.

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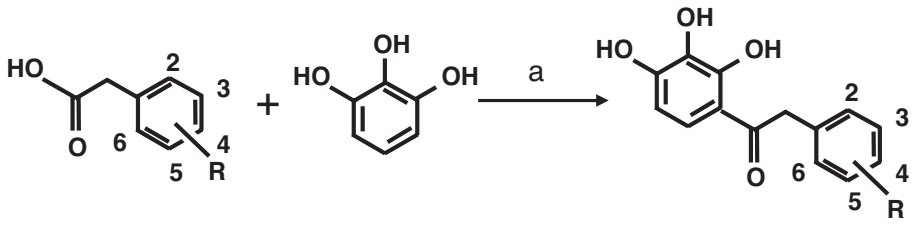

Phenylacetic acid derivatives		Pyrogallol	Product					
			Compound	R				
				C2	C3	C4	C5	C6
a	phenylacetic acid		**1**	H	H	H	H	H
b	*o*-tolylacetic acid		**2**	CH_3	H	H	H	H
c	*m*-tolylacetic acid		**3**	H	CH_3	H	H	H
d	*p*-tolylacetic acid		**4**	H	H	CH_3	H	H
e	mesitylacetic acid		**5**	CH_3	H	CH_3	H	CH_3
f	4-isopropylphenylacetic acid		**6**	H	H	$CH(CH_3)_2$	H	H
g	2-hydroxyphenylacetic acid		**7**	OH	H	H	H	H
h	3-hydroxyphenylacetic acid		**8**	H	OH	H	H	H
i	4-methoxyphenylacetic acid		**9**	H	H	OCH_3	H	H
j	4-ethoxyphenylacetic acid		**10**	H	H	OCH_2CH_3	H	H
k	2-fluorophenylacetic acid		**11**	F	H	H	H	H
l	3-fluorophenylacetic acid	**y** pyrogallol	**12**	H	F	H	H	H
m	4-fluorophenylacetic acid		**13**	H	H	F	H	H
n	2-chlorophenylacetic acid		**14**	Cl	H	H	H	H
o	3-chlorophenylacetic acid		**15**	H	Cl	H	H	H
p	4-chlorophenylacetic acid		**16**	H	H	Cl	H	H
q	2,4-dichlorophenylacetic acid		**17**	Cl	H	Cl	H	H
r	2-bromophenylacetic acid		**18**	Br	H	H	H	H
s	3-bromophenylacetic acid		**19**	H	Br	H	H	H
t	4-bromophenylacetic acid		**20**	H	H	Br	H	H
u	3-iodophenylacetic acid		**21**	H	I	H	H	H
v	1-naphthaleneacetic acid		**22**	CH=CH-CH=CH		H	H	H
w	2-naphthaleneacetic acid		**23**	H	CH=CH-CH=CH		H	H
x	4-biphenylacetic acid		**24**	H	H	Ph	H	H

Fig. 1. Molecular Structure of the 2′,3′,4′-Trihydroxy-2-phenylacetophenone Derivatives Synthesized in This Study.
Schematic protocol for the synthesis of compounds **1–24** is shown at the top. Reagent, (a) $BF_3 \cdot OEt_2$. Compounds **1–24** with indicated functional groups on the 2-phenyl ring were produced from the respective phenylacetic acid derivatives (**a–x**) and pyrogallol (**y**).

Compound **4**, 2′,3′,4′-trihydroxy-2-(4-methylphenyl) acetophenone, was obtained as pale violet crystals. M.p. 125–126 °C. Yield: 55%. ^{1}H-NMR (DMSO-d_6) δ: 2.26 (3H, s, 4-Me), 4.22 (2H, s, CH_2), 6.41 (1H, d, J = 9.2 Hz, 5′-H), 7.11 (2H, d, J = 8.0 Hz, 3,5-H), 7.17 (2H, d, J = 8.0 Hz, 2,6-H), 7.49 (1H, d, J = 9.2 Hz, 6′-H), 8.61 (1H, s, 4′-OH), 10.10 (1H, s, 3′-OH), 12.52 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 20.59 (4-Me), 43.62 (CH_2), 107.74 (5′-C), 112.39 (1′-C), 123.00 (6′-C), 128.92 (3,5-C), 129.29 (2,6-C), 132.16 (1-C), 132.31 (3′-C), 135.58 (4-C), 152.56 (2′,4′-C), 203.02 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{15}H_{15}O_4$ 259.0970, found 259.0970.

Compound **5**, 2′,3′,4′-trihydroxy-2-(2,4,6-trimethylphenyl) acetophenone, was obtained as gray crystals. M.p. 166–167 °C. Yield: 73%. ^{1}H-NMR (DMSO-d_6) δ: 2.11 (6H, s, 2,6-Me), 2.22 (3H, s, 4-Me), 4.35 (2H, s, CH_2), 6.46 (1H, d, J = 8.6 Hz, 5′-H), 6.83 (2H, s, 3,5-H), 7.62 (1H, d, J = 8.6 Hz, 6′-H), 8.63 (1H, s, 4′-OH), 10.09 (1H, s, 3′-OH), 12.41 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 19.87 (2,6-Me), 20.50 (4-Me), 38.07 (CH_2), 107.73 (5′-C), 112.94 (1′-C), 122.22 (6′-C), 128.19 (3,5-C), 129.73 (1-C), 132.34 (3′-C), 135.08 (4-C), 136.71 (2,6-C), 152.06 (2′-C), 152.45 (4′-C), 202.65 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{17}H_{18}O_4$ 287.1283, found 287.1289.

Compound **6**, 2′,3′,4′-trihydroxy-2-(4-isopropylphenyl) acetophenone, was obtained as beige crystals. M.p. 130–131 °C. Yield: 67%. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (6H, d, J = 6.9 Hz, 4-isopropy-diMe), 2.84 (1H, tt, J = 13.7, 6.9 Hz, 4-isopropy-H), 4.22 (2H, s, CH_2), 6.42 (1H, d, J = 8.6 Hz, 5′-H), 7.17 (1H, d, J = 8.0 Hz, 3,5-H), 7.20 (1H, d, J = 8.0 Hz, 2,6-H), 7.50 (1H, d, J = 8.6 Hz, 6′-H), 8.62 (1H, s, 4′-OH), 10.11 (1H, s, 3′-OH), 12.53 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 23.86 (4-isopropy-diMe), 33.02 (4-isopropy-C), 43.58 (CH_2), 107.77 (5′-C), 112.41 (1′-C), 123.01 (6′-C), 126.26 (3,5-C), 129.34 (2,6-C), 132.34 (3′-C), 132.53 (1-C), 146.60 (4-C), 152.56 (4′-C), 152.59 (2′-C), 203.02 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{17}H_{18}O_4$ 287.1283, found 287.1283.

Compound **7**, 2′,3′,4′-trihydroxy-2-(2-hydroxyphenyl) acetophenone, was obtained as reddish-brown crystals. M.p. 159–161 °C. Yield: 8%. ^{1}H-NMR (DMSO-d_6) δ: 4.19 (2H, s, CH_2), 6.41 (1H, d, J = 9.2 Hz, 5′-H), 6.74 (1H, t, J = 8.0 Hz, 5-H), 6.80 (1H, d, J = 8.0 Hz, 3-H), 7.07 (1H, t, J = 8.0 Hz, 4-H), 7.08 (1H, d, J = 8.0 Hz, 6-H), 7.49 (1H, d, J = 9.2 Hz, 6′-H), 8.60 (1H, s, 4′-OH), 9.45 (1H, s, 2-OH), 10.03 (1H, s, 3′-OH), 12.54 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 38.80 (CH_2), 107.61 (5′-C), 112.66 (1′-C), 114.88 (3-C), 118.82 (5-C), 121.85 (1-C), 122.47 (6′-C), 127.82 (4-C), 131.09 (6-C), 132.28 (3′-C), 152.30 (2′-C), 152.33 (4′-C), 155.04 (2-C), 203.12 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{13}O_5$ 261.0763, found 261.0758.

Compound **8**, 2′,3′,4′-trihydroxy-2-(3-hydroxyphenyl) acetophenone, was obtained as violet crystals. M.p. 147–148 °C. Yield: 51%. ^{1}H-NMR (DMSO-d_6) δ: 4.16 (2H, s, CH_2), 6.41 (1H, d, J = 8.6 Hz, 5′-H), 6.62 (1H, dd, J = 8.0, 1.7 Hz, 4-H), 6.68 (1H, d, J = 1.7 Hz, 2-H), 6.70 (1H, d, J = 8.0 Hz, 6-H), 7.09 (1H, t, J = 8.0 Hz, 5-H), 7.47 (1H, d, J = 8.6 Hz, 6′-H), 8.62 (1H, s, 4′-OH), 9.33 (1H, s, 3-OH), 10.12 (1H, s, 3′-OH). ^{13}C-NMR (DMSO-d_6) δ: 44.04 (CH_2), 107.76 (5′-C), 112.45 (1′-C), 113.58 (4-C), 116.16 (2-C), 120.00 (6-C), 123.12 (6′-C), 129.32 (5-C), 132.33 (3′-C), 136.56 (1-C), 152.62 (2′,4′-C), 157.30 (3-C), 202.94 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{13}O_5$ 261.0763, found 261.0738.

Compound **9**, 2′,3′,4′-trihydroxy-2-(4-methoxyphenyl) acetophenone, was obtained as pale violet crystals. M.p. 131–132 °C. Yield: 42%. ^{1}H-NMR (DMSO-d_6) δ: 3.72 (3H, s, 4-OMe), 4.20 (2H, s, CH_2), 6.41 (1H, d, J = 9.2 Hz, 5′-H), 6.87 (2H, d, J = 8.6 Hz, 3,5-H), 7.20 (2H, d, J = 8.6 Hz, 2,6-H), 7.50 (1H, d, J = 9.2 Hz, 6′-H), 8.64 (1H, s, 4′-OH), 10.12 (1H, s, 3′-OH), 12.54 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.12 (CH_2), 55.01 (4-OMe), 107.76 (5′-C), 112.36 (1′-C), 113.81 (3,5-C), 123.01 (6′-C), 127.07 (1-C), 130.51 (2,6-C), 132.35 (3′-C), 152.57 (2′-C), 152.58 (4′-C), 158.00 (4-C), 203.23 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{15}H_{15}O_5$ 275.0919, found 275.0945.

Compound **10**, 2-(4-ethoxyphenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 152–153 °C. Yield: 71%. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, t, J = 6.9 Hz, 4-ethoxy-CH_3), 3.98 (2H, q, J = 6.9 Hz, 4-ethoxy-CH_2), 4.19 (2H, s, CH_2), 6.41 (1H, d, J = 8.6 Hz, 5′-H), 6.85 (2H, d, J = 8.6 Hz, 3,5-H), 7.18 (2H, d, J = 8.6 Hz, 2,6-H), 7.49 (1H, d, J = 8.6 Hz, 6′-H), 8.61 (1H, s, 4′-OH), 10.10 (1H, s, 3′-OH), 12.54 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 14.62 (4-ethoxy-CH_3), 43.09 (CH_2), 62.87 (4-ethoxy-CH_2), 107.73 (5′-C), 112.35 (1′-C), 114.27 (3,5-C), 122.97 (6′-C), 126.91 (1-C), 130.44 (2,6-C), 132.32 (3′-C), 152.53 (2′-C), 152.56 (4′-C), 157.24 (4-C), 203.19 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{16}H_{17}O_5$ 289.1076, found 289.1079.

Compound **11**, 2-(2-fluorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 119–120 °C. Yield: 39%. ^{1}H-NMR (DMSO-d_6) δ: 4.41 (2H, s, CH_2), 6.46 (1H, d, J = 9.2 Hz, 5′-H), 7.15–7.20 (2H, m, 3,5-H), 7.31–7.35 (2H, m, 4,6-H), 7.50 (1H, d, J = 9.2 Hz, 6′-H), 8.66 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.25 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 38.05 (CH_2), 107.85 (5′-C), 112.50 (1′-C), 114.95 (d, J = 21.5 Hz, 3-C), 122.33 (d, J = 15.5 Hz, 1-C), 122.46 (6′-C), 124.23 (d, J = 3.6 Hz, 5-C), 128.92 (d, J = 8.3 Hz, 4-C), 132.34 (d, J = 3.5 Hz, 6-C), 132.36 (3′-C), 152.15 (2′-C), 152.63 (4′-C), 160.76 (d, J = 243.2 Hz, 2-C), 201.10 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{12}FO_4$ 263.0720, found 263.0701.

Compound **12**, 2-(3-fluorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as pale violet crystals. M.p. 137–138 °C. Yield: 47%. ^{1}H-NMR (DMSO-d_6) δ: 4.16 (2H, s, CH_2), 6.41 (1H, d, J = 8.6 Hz, 5′-H), 7.06–7.09 (1H, m, 4-H), 7.11–7.15 (2H, m, 2,6-H), 7.33–7.37 (1H, m, 5-H), 7.50 (1H, d, J = 8.6 Hz, 6′-H), 8.65 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.37 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.53 (CH_2), 107.84 (5′-C), 112.50 (1′-C), 113.35 (d, J = 21.5 Hz, 4-C), 116.50 (d, J = 20.3 Hz, 2-C), 122.86 (6′-C), 125.83 (d, J = 3.6 Hz, 6-C), 130.05 (d, J = 8.4 Hz, 5-C), 132.36 (3′-C), 137.97 (d, J = 7.2 Hz, 1-C), 152.41 (2′-C), 152.67 (4′-C), 162.02 (d, J = 242.0 Hz, 3-C), 202.09 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{12}FO_4$ 263.0720, found 263.0720.

Compound **13**, 2-(4-fluorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 145–146 °C. Yield: 64%. ^{1}H-NMR (DMSO-d_6) δ: 4.31 (2H, s, CH_2), 6.43 (1H, d, J = 8.6 Hz, 5′-H), 7.12–7.16 (2H, m, 3,5-H), 7.30–7.33 (2H, m, 2,6-H), 7.50 (1H, d, J = 8.6 Hz, 6′-H), 8.64 (1H, s, 4′-OH), 10.13 (1H, s, 3′-OH), 12.44 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 42.99 (CH_2), 107.79 (5′-C), 112.44 (1′-C), 114.98 (d, J = 20.3 Hz, 3,5-C), 122.82 (6′-C), 131.32 (1-C), 131.50 (d, J = 7.2 Hz, 2,6-C), 132.34 (3′-C), 152.43 (2′-C), 152.61 (4′-C), 161.07 (d, J = 240.9 Hz, 4-C), 202.60 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{12}FO_4$ 263.0720, found 263.0704.

Compound **14**, 2-(2-chlorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as pale violet crystals. M.p. 133–134 °C. Yield: 50%. ^{1}H-NMR (DMSO-d_6) δ: 4.51 (2H, s, CH_2), 6.46 (1H, d, J = 8.6 Hz, 5′-H), 7.30–7.32 (2H, m, 4,5-H), 7.38–7.40 (1H, m, 3-H), 7.44–7.46 (1H, m, 6-H), 7.52 (1H, d, J = 8.6 Hz, 6′-H), 8.66 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.23 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 42.36 (CH_2), 107.86 (5′-C), 112.66 (1′-C), 122.30 (6′-C), 127.04 (4-C), 128.69 (5-C), 128.93 (6-C), 132.37 (3′-C), 132.58 (3-C), 133.56 (2-C), 133.83 (1-C), 152.08 (2′-C), 152.60 (4′-C), 201.02 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}ClO_4$ 278.0346, found 278.0360.

Compound **15**, 2-(3-chlorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 138–139 °C. Yield: 51%. ^{1}H-NMR (DMSO-d_6) δ: 4.35 (2H, s, CH_2), 6.44 (1H, d, J = 9.2 Hz, 5′-H), 7.25 (1H, d, J = 7.4 Hz, 6-H), 7.31 (1H, dt, J = 7.4, 1.7 Hz, 4-H), 7.35 (1H, t, J = 7.4 Hz, 5-H), 7.37 (1H, t, J = 1.7 Hz, 2-H), 7.50 (1H, d, J = 9.2 Hz, 6′-H), 8.66 (1H, s, 4′-OH), 10.15 (1H, s, 3′-OH), 12.36 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.37 (CH_2), 107.85 (5′-C), 112.50 (1′-C), 122.80 (6′-C), 126.53 (5-C), 128.47 (6-C), 129.59 (2-C), 130.03 (4-C), 132.37 (3′-C), 132.78 (3-C), 137.68 (1-C), 152.38 (2′-C), 152.69 (4′-C), 202.03 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}ClO_4$ 278.0346, found 278.0345.

Compound **16**, 2-(4-chlorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as reddish-brown crystals. M.p. 147–148 °C. Yield: 60%. ^{1}H-NMR (DMSO-d_6) δ: 4.33 (2H, s, CH_2), 6.44 (1H, d, J = 8.6 Hz, 5′-H), 7.31 (2H, d, J = 8.6 Hz, 2,6-H), 7.38 (2H, d, J = 8.6 Hz, 3,5-H), 7.49 (1H, d, J = 8.6 Hz, 6′-H), 8.65 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.40 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.18 (CH_2), 107.83 (5′-C), 112.47 (1′-C), 122.84 (6′-C), 128.19 (3,5-C), 131.30 (4-C), 131.53 (2,6-C), 132.35 (3′-C), 134.24 (1-C), 152.41 (2′-C), 152.66 (4′-C), 202.30 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}ClO_4$ 278.0346, found 278.0331.

Compound **17**, 2-(2,4-dichlorophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as beige crystals. M.p. 182 °C decompose. Yield: 20%. ^{1}H-NMR (DMSO-d_6) δ: 4.52 (2H, s, CH_2), 6.46 (1H, d, J = 8.6 Hz, 5′-H), 7.44–7.42 (2H, m, 5,6-H), 7.51 (1H, d, J = 8.6 Hz, 6′-H), 7.62 (1H, d, J = 1.7 Hz, 3-H), 8.67 (1H, s, 4′-OH), 10.16 (1H, s, 3′-OH), 12.13 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 41.90 (CH_2), 107.90 (5′-C), 112.62 (1′-C), 122.28 (6′-C), 127.17 (5-C), 128.39 (3-C), 132.31 (4-C), 132.36 (3′-C), 132.82 (2-C), 133.83 (6-C), 134.86 (1-C), 152.01 (2′-C), 152.66 (4′-C), 200.46 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{10}Cl_2O_4$ 311.9956, found 311.9978.

Compound **18**, 2-(2-bromophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 131–132 °C. Yield: 40%. ^{1}H-NMR (DMSO-d_6) δ: 4.52 (2H, s, CH_2), 6.46 (1H, d, J = 8.6 Hz, 5′-H), 7.23 (1H, t, J = 7.6 Hz, 5-H), 7.34–7.40 (2H, m, 3,4-H), 7.53 (1H, d, J = 8.6 Hz, 6′-H), 7.62 (1H, d, J = 7.6 Hz, 6-H), 8.65 (1H, s, 4′-OH), 10.13 (1H, s, 3′-OH), 12.22 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 44.79 (CH_2), 107.85 (5′-C), 112.72 (1′-C), 122.24 (6′-C), 124.81 (1-C), 127.57 (4-C), 128.86 (5-C), 132.16 (6-C), 132.36 (3′-C), 132.62 (3-C), 135.38 (2-C), 152.05 (2′-C), 152.58 (4′-C), 200.95 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}BrO_4$ 321.9841, found 321.9828.

Compound **19**, 2-(3-bromophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as beige crystals. M.p. 134–135 °C. Yield: 42%. ^{1}H-NMR (DMSO-d_6) δ: 4.35 (2H, s, CH_2), 6.44 (1H, d, J = 9.2 Hz, 5′-H), 7.28–7.29 (2H, m, 4,6-H), 7.43–7.46 (1H, m, 5-H), 7.49 (1H, d, J = 9.2 Hz, 6′-H), 7.51 (1H, s, 2-H), 8.65 (1H, s, 4′-OH), 10.15 (1H, s, 3′-OH), 12.36 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.32 (CH_2), 107.85 (5′-C), 112.50 (1′-C), 121.42 (1-C), 122.80 (6′-C), 128.86 (6-C), 129.41 (4-C), 130.33 (5-C), 132.37 (3′-C), 132.44 (2-C), 137.96 (3-C), 152.38 (2′-C), 152.69 (4′-C), 202.04 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}BrO_4$ 321.9841, found 321.9833.

Compound **20**, 2-(4-bromophenyl)-2′,3′,4′-trihydroxyacetophenone, was obtained as reddish-brown crystals. M.p. 140–141 °C. Yield: 64%. ^{1}H-NMR (DMSO-d_6) δ: 4.31 (2H, s, CH_2), 6.43 (1H, d, J = 9.2 Hz, 5′-H), 7.25 (2H, d, J = 8.6 Hz, 2,6-H), 7.49 (1H, d, J = 9.2 Hz, 6′-H), 7.51 (2H, d, J = 8.6 Hz, 3,5-H), 8.65 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.39 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.26 (CH_2), 107.82 (5′-C), 112.47 (1′-C), 119.79 (1-C), 122.84 (6′-C), 131.11 (3,5-C), 131.93 (2,6-C), 132.35 (3′-C), 134.68 (4-C), 152.41 (2′-C), 152.66 (4′-C), 202.23 (C=O). FAB-HRMS m/z (M^+) calcd. for $C_{14}H_{11}BrO_4$ 321.9841, found 321.9817.

Compound **21**, 2′,3′,4′-trihydroxy-2-(3-iodophenyl) acetophenone, was obtained as violet crystals. M.p. 147–148 °C. Yield: 51%. ^{1}H-NMR (DMSO-d_6) δ: 4.29 (2H, s, CH_2), 6.41 (1H, d, J = 8.6 Hz, 5′-H), 7.12 (1H, t, J = 7.4 Hz, 5-H), 7.30 (1H, d, J = 7.4 Hz, 6-H), 7.47 (1H, d, J = 8.6 Hz, 6′-H), 7.61 (1H, d, J = 7.4 Hz, 4-H), 7.68 (1H, s, 2-H). ^{13}C-NMR (DMSO-d_6) δ: 43.15 (CH_2), 94.62 (3-C), 107.94 (5′-C), 112.20 (1′-C), 122.90 (6′-C), 129.18 (6-C), 130.38 (5-C), 132.40 (3′-C), 135.22 (4-C), 137.98 (1-C), 138.18 (2-C), 152.21 (2′-C), 153.37

(4′-C), 201.83 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{14}H_{12}IO_4$ 370.9780, found 370.9769.

Compound **22**, 2′,3′,4′-trihydroxy-2-(1-naphthyl) acetophenone, was obtained as violet crystals. M.p. 178–179 °C. Yield: 46%. ^{1}H-NMR (DMSO-d_6) δ: 4.83 (2H, s, CH_2), 6.49 (1H, d, J = 9.2 Hz, 5′-H), 7.43 (1H, d, J = 8.0 Hz, 2-H), 7.47 (1H, t, J = 8.0 Hz, 3-H), 7.49–7.52 (2H, m, 6,7-H), 7.67 (1H, d, J = 9.2 Hz, 6′-H), 7.85 (1H, d, J = 8.0 Hz, 4-H), 7.88 (1H, d, J = 8.6 Hz, 8-H), 7.94 (1H, d, J = 8.6 Hz, 5-H), 8.65 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.37 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 41.59 (CH_2), 107.85 (5′-C), 112.72 (1′-C), 122.68 (6′-C), 124.24 (8-C), 125.47 (2-C), 125.61 (6-C), 126.08 (7-C), 127.25 (4-C), 128.31 (3,5-C), 128.40 (1-C), 132.10 (8a-C), 132.38 (3′-C), 133.31 (4a-C), 152.30 (4′-C), 152.62 (2′-C), 202.78 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{18}H_{15}O_4$ 295.0970, found 295.0971.

Compound **23**, 2′,3′,4′-trihydroxy-2-(2-naphthyl) acetophenone, was obtained as pale violet crystals. M.p. 144–145 °C. Yield: 69%. ^{1}H-NMR (DMSO-d_6) δ: 4.48 (2H, s, CH_2), 6.44 (1H, d, J = 8.6 Hz, 5′-H), 7.44 (1H, d, J = 8.0 Hz, 3-H), 7.47–7.50 (2H, m, 6,7-H), 7.57 (1H, d, J = 8.6 Hz, 6′-H), 7.81 (1H, s, 1-H), 7.88 (2H, m, 5,8-H), 7.86 (1H, d, J = 8.0 Hz, 4-H), 8.64 (1H, s, 4′-OH), 10.14 (1H, s, 3′-OH), 12.50 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 44.14 (CH_2), 107.82 (5′-C), 112.54 (1′-C), 122.99 (6′-C), 125.65 (6-C), 126.10 (7-C), 127.38 (5-C), 127.46 (8-C), 127.68 (4-C), 127.88 (1-C), 128.00 (3-C), 131.78 (4a-C), 132.35 (8a-C), 132.98 (2-C), 133.01 (3′-C), 152.52 (4′-C), 152.64 (2′-C), 202.78 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{18}H_{15}O_4$ 295.0970, found 295.0971.

Compound **24**, 2-(biphenyl-4-yl)-2′,3′,4′-trihydroxyacetophenone, was obtained as violet crystals. M.p. 203–204 °C. Yield: 61%. ^{1}H-NMR (DMSO-d_6) δ: 4.34 (2H, s, CH_2), 6.45 (1H, d, J = 8.6 Hz, 5′-H), 7.35 (1H, t, J = 7.4 Hz, 4-phenyl-4-H), 7.38 (2H, d, J = 8.0 Hz, 2,6-H), 7.45 (2H, t, J = 7.4 Hz, 4-phenyl-3,5-H), 7.54 (1H, d, J = 8.6 Hz, 6′-H), 7.61 (2H, d, J = 8.0 Hz, 3,5-H), 7.64 (2H, d, J = 7.4 Hz, 4-phenyl-2,6-H), 8.69 (1H, s, 4′-OH), 10.13 (1H, s, 3′-OH), 12.51 (1H, s, 2′-OH). ^{13}C-NMR (DMSO-d_6) δ: 43.63 (CH_2), 107.83 (5′-C), 112.48 (1′-C), 123.00 (6′-C), 126.54 (4-phenyl-2,6-C), 126.64 (3,5-C), 127.29 (4-phenyl-4-C), 128.87 (4-Phenyl-3,5-C), 130.08 (2,6-C), 132.36 (3′-C), 134.52 (1-C), 138.48 (4-C), 139.89 (4-phenyl-1-C), 152.54 (4′-C), 152.66 (2′-C), 202.74 (C=O). FAB-HRMS m/z $(M + H)^+$ calcd. for $C_{20}H_{17}O_4$ 321.1127, found 321.1138.

Bacterial and yeast strains, cell-line, and bioassay. Bacterial strains were grown in LB medium with shaking at 37 °C. The yeast strain *Saccharomyces cerevisiae* Hansen BY4742 (Invitrogen, Carlsbad, CA) was grown in YPD medium with shaking at 30 °C. LC_{50} values were determined basically according to the microtiter plate method previously described.[8] Briefly, cells of the bacteria were inoculated into LB medium in the wells of a 96-well microtiter plate, each of which contained one of the serially diluted 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives (ranging from 0 to 250 μM), and were allowed to grow. After 3 h, the optical density for the cells at 630 nm was measured, and the LC_{50} of each compound was calculated from a plot of the optical density values against the concentrations of the compound. In the case of yeast, the same procedure as for the bacterial assay was employed, except that YPD was used as the culture medium. Mouse hepatoma Hepa-1c1c7 cells[9] obtained from the American Type Culture Collection were maintained in Eagle's medium (Nissui Pharmaceuticals, Tokyo) supplemented with 5% (v/v) fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in an atmosphere containing 5% CO_2. The cells were treated with diluted 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives for 24 h, and then cell survival was monitored by counting after trypan blue exclusion.

Results and Discussion

The 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives were synthesized by condensation of pyrogallol (**y** in Fig. 1) with the corresponding phenylacetic acid derivatives (**a–x** in Fig. 1).[10,11] Compounds **1–24** (Fig. 1) were synthesized successfully, and their chemical identity and purity were confirmed by spectral data (^{1}H- and ^{13}C-NMR, and mass), as summarized above in "Materials and Methods." The spectral data for all the newly synthesized compounds were in full agreement with the proposed structures.

The 24 compounds synthesized in this study were screened for the antibacterial activity against various strains of bacteria, including *Bacillus subtilis* 168, *Staphylococcus aureus* KSA-1, *Escherichia coli* ATCC43888, *Proteus mirabilis* Pm1, *Pseudomonas aeruginosa* T8, and *Salmonella enterica* KK1; the first two were Gram-positive and the last four Gram-negative. The activities were expressed as LC_{50} of the compounds against the six bacterial strains listed in Table 1. Also listed are the activities of reference compounds, including penicillin G, chloramphenicol, rifampicin, and mitomycin C. Eighteen of the 24 compounds were found to be active against both Gram-positive strains of *B. subtilis* and *S. aureus*, while they had no inhibitory effect on the four Gram-negative strains even at the highest concentration used in this study (250 μM), suggesting possible selective toxicity and specific inhibitory effects against Gram-positive bacteria.

2′,3′,4′-Trihydroxy-2-phenylacetophenone (compound **1**) itself did not show any antibacterial activity, but some functional groups introduced onto its 2-phenyl ring appeared to be able to enhance the activity. The introduction of 3-methyl, 4-methyl, 2,4,6-trimethyl (compounds **3–5**) and of 4-isopropyl (compound **6**) was able to confer the antibacterial activities, although 2-methyl (compound **2**) was not effective. On the other hand, 2-hydroxy, 3-hydroxy, and 4-methoxy abolished the activity completely (compounds **7–9**), and 4-ethoxy (compound **10**) resulted in moderate activity, suggesting that oxygen atoms attached on the 2-phenyl ring cause negative effects on the antibacterial activity. The introduction of halogens, including chloro, bromo, and iodo (compounds **14–21**), generally increased the antibacterial activity. Especially, 2,4-dichloro, 2-bromo, and 3-bromo (compounds **17–19**) were effective at increasing the activity against *S. aureus*. However, 3- and 4-fluoro (compounds **12** and **13**) were not as effective as the other halogen groups, and even 2-fluoro (compound **11**) abolished the activity. Replacing the entire 2-phenyl group with the naphthyl group (compounds **22** and **23**) also enhanced activity. Finally, 2-(biphenyl-4-yl)-2′,3′,4′-trihydroxyacetophenone (compound **24**) exhibited the highest activities against both *B. subtilis* and *S. aureus*, with LC_{50} calculated at 5.8 μM and 5.6 μM respectively, almost comparable to the positive control chloramphenicol.

Our results suggest that *S. aureus* is more susceptible than *B. subtilis* to these antibacterial 2′,3′,4′-trihydroxy-2-phenylacetophenone derivatives, implying that these compounds deserve further investigation as potential antimicrobial agents to control clinical problems with *S. aureus*. To clarify this possibility, however, we still need to test other strains, especially those including MRSA. Only recently, it was found that *B. subtilis* cells treated with compound **19** became more susceptible to osmotic stress (data not shown), suggesting that this compound disturbs the integrity of the cell surface. Further study is required to determine the mode of action of the active compounds, and would be worthwhile in the effort to design more effective antibacterial agents.

Two of the most effective compounds, **19** and **24**, were applied to yeast strain BY4742 and mouse hepatoma Hepa-1c1c7 cells, but neither of them exhibited detectable toxicity at 10 μM (data not shown),

Table 1. Antibacterial Activities of the Synthesized Compounds and Antibiotics Expressed as LC_{50} (μM)

Compound	*B. subtilis* 168[a]	*S. aureus* KSA-1[b]	*E. coli* ATCC43888[c]	*P. mirabilis* Pm1[b]	*P. aeruginosa* T8[b]	*S. enterica* KK1[b]
1	—[d]	—	—	—	—	—
2	—	—	—	—	—	—
3	120	18	—	—	—	—
4	98	14	—	—	—	—
5	28	28	—	—	—	—
6	11	5.8	—	—	—	—
7	—	—	—	—	—	—
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	110	33	—	—	—	—
11	—	—	—	—	—	—
12	100	43	—	—	—	—
13	94	32	—	—	—	—
14	55	24	—	—	—	—
15	14	11	—	—	—	—
16	12	11	—	—	—	—
17	10	5.8	—	—	—	—
18	42	7.7	—	—	—	—
19	18	6.1	—	—	—	—
20	15	11	—	—	—	—
21	23	12	—	—	—	—
22	12	12	—	—	—	—
23	14	11	—	—	—	—
24	5.8	5.6	—	—	—	—
Penicillin G	190	180	230	170	—	210
Chloramphenicol	8.4	9.8	15	40	—	13
Rifampicin	ND[e]	ND	20	19	57	51
Mitomycin C	1.3	9.4	110	2.6	14	25

Values are means from three independent measurements.
[a]Obtained from Génétique Microbienne, Institut National de la Recherche Agronomique, Jouy-en-Josas, France
[b]Obtained from the Bacterial Culture Collection of the Research Center for Food Safety and Security, Kobe University, Kobe, Japan
[c]Obtained from American Type Culture Collection, Georgetown University, Washington, DC
[d]—, no antibacterial activity was observed (higher than 250 μM)
[e]ND, not determined (lower than 0.98 μM)

suggesting that both compounds might not be sufficiently toxic to eukaryotic cells, at least at the lower concentrations to exert antibacterial activity. In addition, both chemicals can be regarded as harmless to yeast, because no growth inhibition was seen even at 250 μM, the highest concentration used under the assay conditions (data not shown). However, to Hepa-1c1c7 cells, both compounds appeared to be toxic at concentrations higher than 100 μM (data not shown). The possible dose-dependent toxicity to mammalian cells will be also investigated in the future.

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