Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 662–667
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, crystal structure and antimicrobial activity of deoxybenzoin derivatives from genistein

Huan-Qiu Li, Jia-Yu Xue, Lei Shi, Shan-Ying Gui, Hai-Liang Zhu*

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

Received 13 March 2007; received in revised form 11 May 2007; accepted 24 May 2007
Available online 8 June 2007

Abstract

A series of deoxybenzoin derivatives from genistein were synthesized and their structures were elucidated by ^{1}H NMR, mass spectral data and micro analyses. The structures of **2**, **7** and **10** were determined by single-crystal X-ray analysis. These obtained compounds were evaluated for their assayed antibacterial (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence* and *Staphylococcus aureus*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) activities by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method. Most compounds have displayed comparable antibacterial activity against bacterial. On the basis of the biological results, structure–activity relationships are discussed.

Keywords: Deoxybenzoin derivatives; Metal complexes; Antibacterial activity; Structure–activity relationships

1. Introduction

The rapid development of pathogen resistance to most of the known antibiotics is becoming a serious health problem [1–3]. Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed towards the design of new agents [4–7]. Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, GEN, **1**) is the most abundant isoflavone which has demonstrated potent biological activity such as anticarcinogenic [8], anti-inflammatory [9], antiviral [10], anti-protozoal [11] and estrogen receptor [12].

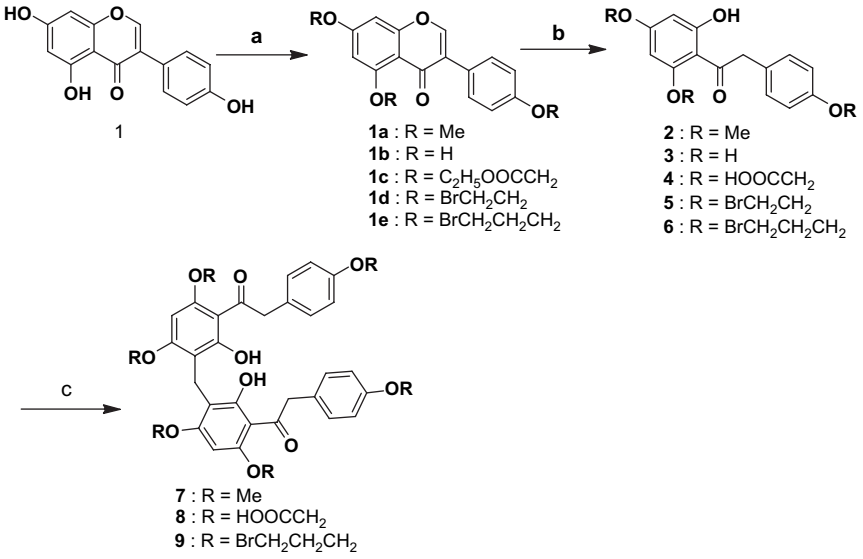
As important derivatives of isoflavones, deoxybenzoins also exhibited estrogenicity [13] and antimicrobial activities [14]. During recent years coordination compounds of biologically active ligands [15,16] have received much attention, and our interest in this area is to design and synthesize diverse biologically active genistein derivatives [17,18]. In this paper, genistein was first used as the starting material to synthesize deoxybenzoins in high yield through a convenient ring-breaking method. For further analytical and biological investigations, novel dimeric deoxybenzoin derivatives and transition metal complexes were also prepared to study their in vitro antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence* and *Staphylococcus aureus*.

2. Chemistry

Compounds **2–14** were synthesized for antimicrobial activity screening, and the synthetic methods adopted for preparing compounds **2–14** are illustrated in Schemes 1 and 2. The hydroxy groups on the aromatic rings of genistein were first protected by means of nucleophilic displacement (step a). Treating the obtained compounds **1a–1e** with 10% aq NaOH and acidified by 30% aq HCl for 17–19 h gave deoxybenzoins **2–6** (step b). This two-step ring-cleavage reaction was carried out as a one-pot reaction so that it was a convenient method to obtain deoxybenzoins and their derivatives

* Corresponding author. Tel.: +86 25 8359 2572; fax: +86 25 8359 2672.
E-mail address: zhuhl@nju.edu.cn (H.-L. Zhu).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.05.013



Scheme 1. Syntheses of compounds **2**–**9**. Reagents: (a) 1a – dimethyl sulfate/NaOH/H_2O, 1c – benzyl bromide/K_2CO_3, 1d – 1,2-dibromoethane/K_2CO_3, 1e – 1,3-dibromopropane/K_2CO_3; (b) 10% aq NaOH; (c) formaldehyde/HCl.

in high yield. Thus, starting from the obtained deoxybenzoins **2**–**6**, novel dimeric derivatives **7**–**9** (Scheme 1) were prepared by Friedel–Crafts reaction (step c), while working with formaldehyde in a strong acid media [19].

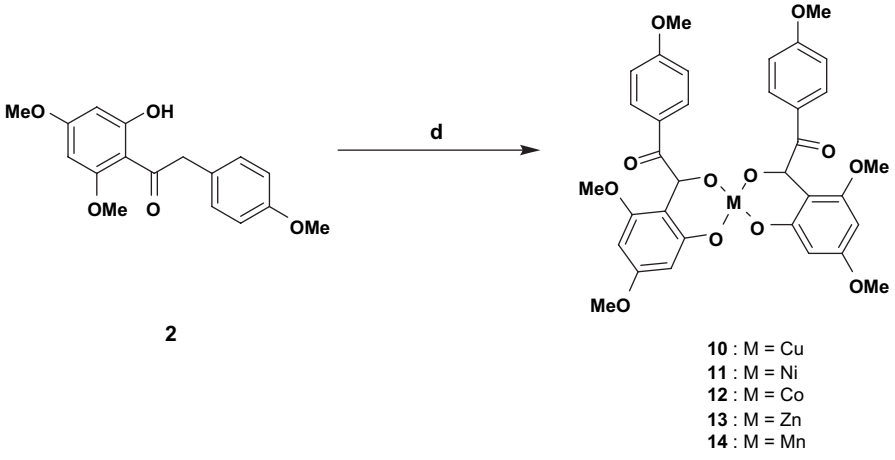
The deoxybenzoin ligand **2**, on refluxing with copper acetate, nickel acetate, cobalt acetate, zinc acetate and manganese acetate in acetonitrile solution, in (2:1) molar ratio, yielded corresponding complexes **10**–**14**, respectively. The products formed were filtered, washed with warm water, ethanol and dried under reduced pressure in a vacuum desiccator containing anhydrous $CaCl_2$. On the basis of elemental analysis, ligand stoichiometry to the metal of 2:1 has been proposed for these complexes. The proposed structure (Scheme 2) for these complexes has the support of IR and UV spectra. The complexes along with their characteristics are recorded in Table 1, their UV spectra in Table 2 and their IR spectra in Table 3.

3. Results and discussion

3.1. Crystal structures of compounds 2, 7 and 10

The crystal structure of **2** is illustrated in Fig. 1, **2** crystallizes in the monoclinic, space group $P2_1/n$ with unit cell parameters: $a = 4.8350(10)$ Å, $b = 11.429(2)$ Å, $c = 27.415(6)$ Å, $\beta = 90.63(3)^\circ$, $V = 1514.8(5)$ Å^3, $Z = 4$. There is a strong intramolecular O–H···O hydrogen bond between O4 and O2 (O4—H4A···O2, 2.465(3) Å, 148.5°) in the structure.

The crystal structure of **7** is illustrated in Fig. 2, **7** crystallizes in the triclinic, space group $\overline{P}1$ with the following crystallographic parameters: $a = 10.107(2)$ Å, $b = 11.350(2)$ Å, $c = 14.090(3)$ Å, $\alpha = 73.16(3)^\circ$, $\beta = 81.07(3)^\circ$, $\gamma = 80.16(3)^\circ$, $V = 1514.6(5)$ Å^3, $Z = 2$. There exists both intra and intermolecular hydrogen interactions in the crystal structure. In each molecule, there are two intramolecular O—H···O hydrogen



Scheme 2. Syntheses of complexes **10**–**14**.

Table 1
Data of color and elemental analysis of the complexes

Complex	Color	Molecular formula	Data of mature (data of theory) (%)		
			C	H	N
10	Green	$Cu(C_{17}H_{16}O_6)_2CNCH_3$	58.65 (58.53)	4.79 (4.67)	1.91 (1.80)
11	Purple	$Ni(C_{17}H_{16}O_6)_2$	59.07 (59.01)	4.67 (4.58)	0
12	Red	$Co(C_{17}H_{16}O_6)_2$	59.05 (58.86)	4.66 (4.61)	0
13	White	$Zn(C_{17}H_{16}O_6)_2$	58.50 (58.38)	4.62 (4.55)	0
14	Brown	$Mn(C_{17}H_{16}O_6)_2$	59.39 (59.33)	4.69 (4.53)	0

bonds (O3—H3···O2, 2.482(4) Å, 147.4° and O15—H15···O1, 2.458(4) Å, 149.1°). And these hydrogen bonds result in significant elongations of the O(2)=C(27) bond (1.239(4) Å) and O(1)=C(15) bond (1.250(5) Å) compared to normal C=O bond. The intermolecular interaction is O3—H3···O10#1 (3.102(4) Å and 122.8°), with symmetry code #1: $-x+1, -y, -z+1$. In the crystal structure, the molecules are linked by intermolecular hydrogen bonds forming a three-dimensional network.

The crystal structure of Cu complex **10** is illustrated in Fig. 3, **10** also belongs to tricilinic, space group $\overline{P}1$ with the following crystallographic parameters: $a = 8.549$ Å, $b = 14.736$ Å, $c = 15.200$ Å, $\alpha = 64.12°$, $\beta = 85.75°$, $\gamma = 88.87°$, $V = 1717.9$ Å^3, $Z = 2$. The molecular structure of compound **10** consists of a mononuclear [$Cu(C_{17}H_{16}O_6)_2CNCH_3$] molecule and an uncoordinated acetonitrile molecule. The Cu atom is in a square-planar geometry and is four-coordinated by four O atoms from two deoxybenzoin ligands. The distances between Cu—O4 (1.922(5) Å), Cu—O5 (1.914(5) Å), Cu—O6 (1.936(5) Å) and Cu—O7 (1.901(5) Å) are comparable with the values found in most copper(II) complexes [20]. The four coordinating atoms around the central metal atom are approximately coplanar, giving a square-planar geometry with an average deviation of 0.0549 Å, with the Cu atom 0.037(3) Å above this plane.

An unexpected detail in structure of **10** was that both methylenes of deoxybenzoins were oxidized to two carbonyl groups before ligation to the Cu ion. When the hydroxyl group of **2** was protected, the methylene group in the deoxybenzoins was easily oxygenized in the air. It could be deduced that complex **10** was formed and then oxidized prior to crystallization.

3.2. In vitro antibacterial assay

All the synthesized compounds were screened for their antibacterial (*B. subtilis*, *E. coli*, *P. fluorescence* and *S. aureus*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) activities by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide) method. The MICs (minimum inhibitory concentrations) of the compounds against four bacterias are presented in Table 4. Also included is the activity of reference compounds kanamycin (Nanjing Zhuyan Biotechnology Co. Ltd, Amresco 060D0504, Nanjing 210002, China) and penicillin (North China Pharmaceutical Co. Ltd, D0211107, Hebei 050015, China). All synthesized compounds were found to be inactive against the tested fungi strains. Compounds **3–9** showed antimicrobial activity against *B. subtilis* and *E. coli* at the values of 12.5–50 μg/ml. Compound **9** displayed potent acitivity with an MIC value of 6.25 μg/ml against *B. subtilis*, which was comparable to the positive control penicillin. Dimeric **7–9** were all found to be more active than other deoxybenzoins, implying the dimeric derivative may be a new therapy agent of antibiotics.

Table 2
UV spectra of the ligand and the complexes (nm)

Compound	**10**	**11**	**12**	**13**	**14**
Bands (nm)	326	373	380	374	382

Complexes **10–14** also showed antimicrobial activity against *S. aureus* with MIC values at 10.5–50 μg/ml, but they were completely inactive against *B. subtilis* and *E. coli*. Complex $Ni(\mathbf{2})_2$ (**11**) exhibited potent activity against *S. aureus* with an MIC value of 10.5 μg/ml, while $Co(\mathbf{2})_2$ (**12**) and $Zn(\mathbf{3})_2$ (**13**) showed moderate activities against *S. aureus* (22.5 and 18.5 μg/ml). The activities of these compounds were also compared with the two antibiotics kanamycin and penicillin, and some of them exhibited similar antibacterial activities with commercial antibiotics. Compounds **1–14** were also tested against *A. niger*, *C. albicans* and *T. rubrum* and they had no antifungal activity.

According to structure–activity relationships (SAR), it can be concluded that dimeric deoxybenzoin derivatives from genistein (**7–9**) are generally more active than genistein and deoxybenzoins **2–6** against selected microorganisms. Additionally, the organic-metal complexes **10–14** of deoxybenzoins have potential antimicrobial activity and deserve further investigation.

4. Experimental protocols

4.1. General

1,2-Dibromoethane, 1,3-dibromopropane, triethylamine, mercaptoethanol, the metal salts copper acetate, nickel acetate, cobalt acetate, zinc acetate and manganese acetate were

Table 3
IR spectra of the ligand and the complexes (cm^{-1})

Compound	$\nu_{C=O}$	ν_{C-O}
10	1625	1149
11	1611	1152
12	1618	1146
13	1637	1178
14	1608	1148

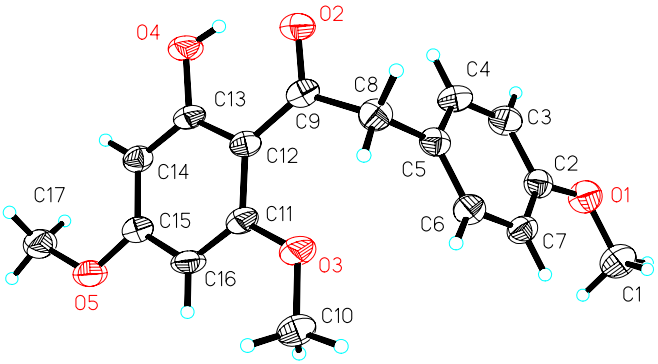


Fig. 1. Crystal structure of **2**.

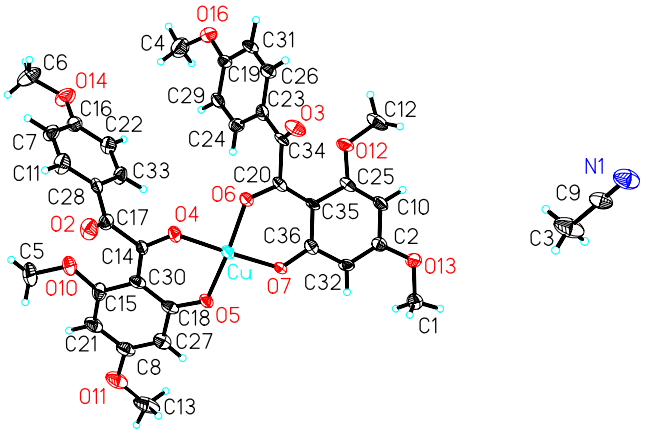


Fig. 3. Crystal structure of **10**.

purchased from Shanghai Chemical Reagent Company (Shanghai, China), and were used as received without purification. Genistein (>96%) was purchased from Shanxi Huike Botanical Development Co. Ltd. Reactions and the resulted products were monitored by thin-layer chromatography (TLC), and were run on the silica gel coated aluminum sheets (silica gel 60 GF_{254}, E. Merck, Germany) and visualized in UV light (254 nm). Sonication was performed in a Kunshan KQ 500E ultrasonic cleaner (Jiangsu, China) with irradiation delivered at 40 kHz and 500 W. All the NMR spectra were recorded on a Bruker DRX 500 or DPX 300 model spectrometer in DMSO-d_6. Chemical shifts (δ) for ^{1}H NMR spectra were reported in parts per million to residual solvent protons. Melting points were measured on a Boetius micro melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass Spectrometer.

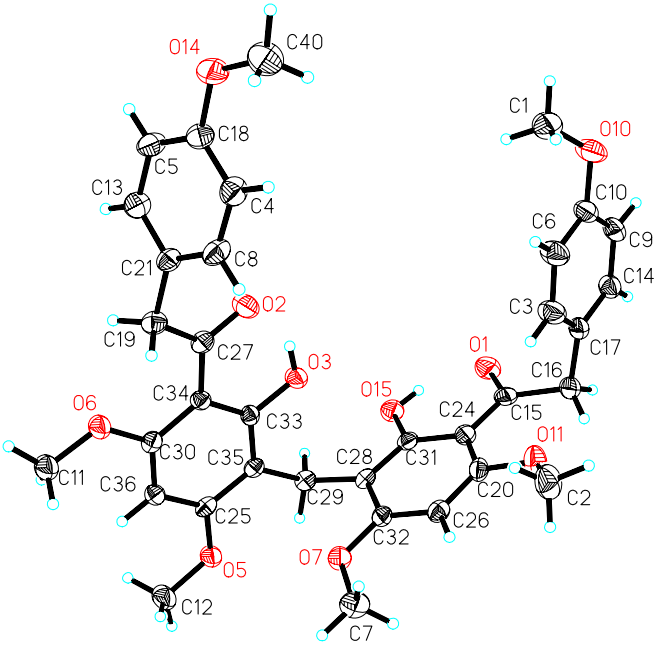


Fig. 2. Crystal structure of **7**.

4.2. Syntheses

1-(2-Hydroxy-4,6-dimethoxyphenyl)-2-(4-methoxyphenyl)ethanone (**2**) genistein (8.1 g, 30 mmol) was dissolved in 100 ml of 15% NaOH, then dimethyl sulfate (13.5 ml, 100 mmol) was carefully added portionwise over 30 min after cooling in an ice bath, and the resulting solution was stirred at 25 °C for 6 h and then filtered. Without further purifying, 50 ml 10% NaOH solution was added, the reaction mixture was stirred for 17–19 h at 80 °C, filtered on sand. The filtrate is neutralized with a HCl solution (30%) to pH 7 and then extracted with AcOEt (3 × 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate, obtained **2** as colorless crystal. M.p. 147–149 °C; yield: 85%; ^{1}H NMR (500 MHz, DMSO-d_6): 3.7 (s, 3H); 3.8 (s, 3H); 3.9 (s, 3H); 4.2 (s, 2H); 6.1 (d, $J = 2.0$, 1H); 6.2 (d, $J = 2.0$, 1H); 6.8 (d, $J = 8.5$, 2H); 7.1 (d, $J = 8.5$, 2H); 13.4 (s, 1H). MS (ESI) $C_{17}H_{18}O_5$ $[M+H]^+$ 303.3. Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.55; H, 5.96%. Found: C, 67.43; H, 5.88%.

Table 4
Antimicrobial activity of the synthesized compounds

Compound	Minimum inhibitory concentration (μg/ml)			
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*
1	>50	>50	>50	>50
2	>50	>50	>50	>50
3	40.5	42.5	>50	>50
4	45.5	48.5	>50	>50
5	30.5	36.5	>50	>50
6	25.0	25.0	>50	>50
7	31.5	38.5	>50	>50
8	12.5	25.0	>50	>50
9	6.25	17.5	>50	>50
10	>50	>50	>50	40.5
11	>50	>50	>50	10.5
12	>50	>50	>50	22.5
13	>50	>50	>50	18.5
14	>50	>50	>50	>50
Ketoconazole	>50	>50	>50	>50
Kanamycin	1	0.45	3.9	1
Penicillin	7.8	>50	>50	2

*4.2.1. 2-(4-Hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl) ethanone (**3**)*

Genistein (2.7 g, 10 mmol) was dissolved in 40 ml of 10% NaOH, under nitrogen, the reaction mixture was stirred for 17–19 h at 60 °C, filtered on sand. The filtrate is neutralized with a HCl solution (30%) to pH 7 and then extracted with AcOEt (3 × 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate, obtained **3** as yellow powder. M.p. 157–159 °C; yield: 65%; ^{1}H NMR (300 MHz, DMSO-d_6): 4.2 (s, 2H); 5.8 (s, 2H); 6.6 (d, $J = 8.4$, 2H); 7.0 (d, $J = 8.4$, 2H); 9.1 (s, 1H); 10.3 (s, 1H); 12.2 (s, 2H). MS (ESI) $C_{14}H_{12}O_5$ $[M + H]^+$ 261.1. Anal. Calcd for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65%. Found: C, 64.84; H, 4.34%.

*4.2.2. 2,2′-(4-(2-(4-(Carboxymethoxy)phenyl)acetyl)-5-hydroxy-1,3-phenylene)bis(oxy)diacetic acid (**4**)*

Genistein (2.7 g, 10 mmol) was added to a mixture containing potassium carbonate (2.0 g, 15 mmol) and anhydrous DMF (15 ml), and this solution was stirred at 60 °C. Then ethyl bromoacetate (5.0 g, 30 mmol) was carefully added to the above solution in 15 min, and the resulting solution was stirred at 60 °C for 4.5 h and cooled, and 30 ml of water was added and filtered. Without further purifying, 50 ml 10% NaOH solution was added, the reaction mixture was stirred for 17–19 h at 80 °C, filtered on sand. The filtrate is neutralized with a HCl solution (30%) to pH 7 and then extracted with AcOEt (3 × 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate, obtained **4** as white powder. M.p. 236–238 °C; yield: 50%; ^{1}H NMR (300 MHz, DMSO-d_6): 4.3 (s, 2H); 4.6 (s, 2H); 4.6 (s, 2H); 4.8 (s, 2H); 6.0 (d, $J = 2.0$, 1H); 6.1 (d, $J = 2.0$, 1H); 6.6 (d, $J = 8.5$, 2H); 7.0 (d, $J = 8.5$, 2H); 12.8 (s, 2H); 13.1 (s, 1H). MS (ESI) $C_{20}H_{18}O_{11}$ $[M + H]^+$ 435.1. Anal. Calcd for $C_{20}H_{18}O_{11}$: C, 55.30; H, 4.18%. Found: C, 55.64; H, 4.34%.

*4.2.3. 1-(2,4-Bis(2-bromoethoxy)-6-hydroxyphenyl) -2-(4-(2-bromoethoxy)phenyl)ethanone (**5**)*

Genistein (2.7 g, 10 mmol) was added to a mixture containing potassium carbonate (2.0 g, 15 mmol) and anhydrous DMF (15 ml), and this solution was stirred at 60 °C. Then dibromoethane (9.4 g, 50 mmol) was carefully added to the above solution in 15 min, and the resulting solution was stirred at 60 °C for 4.5 h and cooled, and 30 ml of water was added and filtered. Without further purifying, 50 ml 10% NaOH solution was added, the reaction mixture was stirred for 17–19 h at 80 °C, filtered on sand. The filtrate was neutralized with a HCl solution (30%) to pH 7 and then extracted with AcOEt (3 × 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate, obtained **5** as white powder. M.p. 186–188 °C; yield: 70%; ^{1}H NMR (300 MHz, DMSO-d_6): 3.7–3.9 (overlapped multiplets, 6H); 4.1 (s, 2H); 4.3–4.4 (overlapped multiplets, 6H); 6.1 (d, $J = 2.0$, 1H); 6.1 (d, $J = 2.0$, 1H); 6.8 (d, $J = 8.5$, 2H); 7.1 (d, $J = 8.5$, 2H); 12.4 (s, 1H). MS (ESI) $C_{20}H_{21}Br_3O_5$ $[M + H]^+$ 582.1. Anal. Calcd for $C_{20}H_{21}Br_3O_5$: C, 41.34; H, 3.64; Br, 41.25%. Found: C, 41.56; H, 3.35; Br, 41.41%.

*4.2.4. 1-(2,4-Bis(3-bromopropoxy)-6-hydroxyphenyl)-2-(4-(3-bromopropoxy)phenyl)ethanone (**6**)*

Genistein (2.7 g, 10 mmol) was added to a mixture containing potassium carbonate (2.0 g, 15 mmol) and anhydrous DMF (15 ml), and this solution was stirred at 60 °C. Then dibromopropane (5.1 g, 25 mmol) was carefully added to the above solution in 15 min, and the resulting solution was stirred at 60 °C for 4.5 h and cooled, and 30 ml of water was added and filtered. Without further purifying, 50 ml 10% NaOH solution was added, the reaction mixture was stirred for 17–19 h at 80 °C, filtered on sand. The filtrate neutralized with HCl solution (30%) to pH 7 and then extracted with AcOEt (3 × 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate, obtained **6** as white powder. M.p. 142–146 °C; yield: 67%; ^{1}H NMR (300 MHz, DMSO-d_6): 2.1–2.2 (overlapped multiplets, 6H); 3.5–3.7 (overlapped multiplets, 6H); 4.1 (s, 2H); 4.5–4.6 (overlapped multiplets, 6H); 6.2 (d, $J = 2.0$, 1H); 6.2 (d, $J = 2.0$, 1H); 6.8 (d, $J = 8.5$, 2H); 7.1 (d, $J = 8.5$, 2H); 13.1 (s, 1H). MS (ESI) $C_{23}H_{27}Br_3O_5$ $[M + H]^+$ 624.1. Anal. Calcd for $C_{23}H_{27}Br_3O_5$: C, 44.33; H, 4.37; Br, 38.47%. Found: C, 44.15; H, 4.54; Br, 38.19%.

*4.2.5. 1,1′-[3,3′-Methylenebis(2-hydroxy-4,6-dimethoxy-3,1-phenylene)]bis[2-(4-methoxyphenyl)ethanone] (**7**)*

Deoxybenzoin **2** (0.2 g, 0.8 mmol) was added to a mixture containing HCl (3 ml) and ethanol (20 ml), and this solution was stirred at 60 °C for 15 min and then HCHO (3 ml) was carefully added to the above solution in 15 min, and the resulting solution was refluxed for 12 h and then filtered, gave a white solid, recrystallization of the solid from 15 ml acetone gave compound **7** as clear crystals. M.p. 151–153 °C; yield: 78%; ^{1}H NMR (300 MHz, DMSO-d_6): 3.6 (s, 2H); 3.7 (s, 3H); 3.8 (s, 3H); 3.9 (s, 3H); 4.1 (s, 2H); 6.1 (s, 1H); 6.8 (d, $J = 8.4$, 2H); 7.1 (d, $J = 8.4$, 2H); 13.6 (s, 1H). MS (ESI) $C_{35}H_{36}O_{10}$ $[M + H]^+$ 517.6. Anal. Calcd for $C_{35}H_{36}O_{10}$: C, 41.34; H, 3.64%. Found: C, 41.13; H, 3.47%.

Compounds **7**–**9** were prepared in analogy.

*4.2.6. 2,2′-(4-(6-(Carboxymethoxy)-3-(2-(4-(carboxymethoxy)phenyl)acetyl)-4-(carboxyperoxy)-2-hydroxybenzyl)-6-(2-(4-(carboxymethoxy)phenyl)acetyl)-5-hydroxy-1,3-phenylene)bis(oxy)diacetic acid (**8**)*

M.p. 212–214 °C; yield: 70%; ^{1}H NMR (300 MHz, DMSO-d_6): 3.8 (s, 2H); 4.3 (s, 2H); 4.6 (s, 2H); 4.7 (s, 2H); 4.8 (s, 2H); 6.0 (d, $J = 2.0$, 1H); 6.1 (d, $J = 2.0$, 1H); 6.7 (d, $J = 8.5$, 2H); 7.1 (d, $J = 8.5$, 2H); 11.0 (s, 1H); 12.8 (s, 2H); 13.2 (s, 1H). MS (ESI) $C_{41}H_{36}O_{24}$ $[M + H]^+$ 805.2. Anal. Calcd for $C_{41}H_{36}O_{24}$: C, 46.34; H, 7.64%. Found: C, 46.51; H, 7.48%.

*4.2.7. 1-(3-(4,6-Bis(3-bromopropoxy)-3-(2-(4-(3-bromopropoxy)phenyl)acetyl)-2-hydroxybenzyl)-4-(4-bromobutoxy)-6-(3-bromopropoxy)-2-hydroxyphenyl)-2-(4-(3-bromopropoxy)phenyl)ethanone (**9**)*

M.p. 115–117 °C; yield: 70%; ^{1}H NMR (300 MHz, DMSO-d_6): 2.1–2.2 (overlapped multiplets, 6H); 3.6–3.7

(overlapped multiplets, 6H); 3.8 (s, 2H); 4.1 (s, 2H); 4.6–4.7 (overlapped multiplets, 6H); 6.2 (d, $J = 2.0$, 1H); 6.3 (d, $J = 2.0$, 1H); 6.8 (d, $J = 8.5$, 2H); 7.1 (d, $J = 8.5$, 2H); 13.1 (s, 1H). MS (ESI) $C_{48}H_{56}Br_6O_{10}$ $[M + H]^+$ 1273.1. Anal. Calcd for $C_{48}H_{56}$ Br_6O_{10}: C, 41.34; H, 3.64%. Found: C, 41.87; H, 3.79%.

Complexes **10**–**14** along with their characteristics and UV, IR spectra are recorded in Table 2.

4.3. Crystallographic data

Crystallographic data (excluding structure factors) for compounds **2**, **7** and **10** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 614278-614280. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.4. Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against *B. subtilis*, *E. coli*, *P. fluorescence* and *S. aureus* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 ml), the antifungal activity of the compounds was tested against *A. niger*, *C. albicans* and *T. rubrum* using RPMI-1640 medium (RPMI-1640 (GIBCO BRL) 10 g, $NaHCO_3$ 2.0 g, 0.165 mol/L morpholinepropanesulfonic acid (MOPS) (Sigma) (34.5 g), triple distilled water 900 ml, buffered to pH 7.0 with 1 mol/L NaOH (25 °C), metered volume to 1000 ml, filtered sterilization, conservation at 4 °C). The MICs of the test compounds were determined by a colorimetric method using the dye MTT [21]. A stock solution of the synthesized compound (50 μg/ml) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (MH medium for antibacterial activity and RPMI-1640 medium for antifungal activity). A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/ml and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h and 48 h for bacterial and fungi, respectively. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (Phosphate Buffered Saline 0.01 mol/L, pH 7.4, $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 ml) containing 2 mg of MTT/ml was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

Acknowledgments

The work was supported by National Natural Science Foundation of China (Project: 30672516), and by Analytical Test Fund in Nanjing University for Dr. Hai-Liang Zhu.

References

[1] D.T.W. Chu, J.J. Plattner, L. Katz, J. Med. Chem. 39 (1996) 3853–3874.
[2] K.M. Overbye, J.F. Barrett, Drug Discov. Today 10 (2005) 45–52.
[3] C. Walsh, Nature 406 (2000) 775–781.
[4] J. Travis, Science 264 (1994) 360–362.
[5] L. Otvos Jr., J.D. Wade, F. Lin, B.A. Condie, J. Hanrieder, R. Hoffmann, J. Med. Chem. 48 (2005) 5349.
[6] H. Xin, K.A. Reynolds, Antimicrob. Agents Chemother. 46 (2002) 1310–1318.
[7] Y. Cui, Y. Dang, Y. Yang, S. Zhang, R. Ji, Eur. J. Med. Chem. 40 (2005) 209–214.
[8] Z.M. Shao, J. Wu, Z.Z. Shen, S.H. Barsky, Cancer Res. 58 (21) (1998) 4851–4857.
[9] L. Sun, N. Tran, F. Tang, J. Med. Chem. 41 (1998) 2588–2593.
[10] V.K. Pandey, S. Tusi, Z. Tusi, R. Raghubir, M. Dixit, M.N. Joshi, S.K. Bajpai, Indian J. Chem. B 43 (1) (2004) 180–183.
[11] A. Baliani, G.J. Bueno, M.L. Stewart, V. Yardley, R. Brun, M.P. Barrett, I.H. Gilbert, J. Med. Chem. 48 (2005) 5570–5579.
[12] B.R. Henke, T.G. Consler, N. Go, R.L. Hale, D.R. Hohman, S.A. Jones, A.T. Lu, L.B. Moore, J.T. Moore, L.A. Orband-Miller, R.G. Robinett, J. Shearin, P.K. Spearing, E.L. Stewart, P.S. Turnbull, S.L. Weaver, S.P. Williams, G.B. Wisely, M.H. Lambert, J. Med. Chem. 45 (2002) 5492–5505.
[13] N. Fokialakis, G. Lambrinidis, D.J. Mitsiou, N. Aligiannis, S. Mitakou, A.L. Skaltsounis, H. Pratsinis, E. Mikros, M.N. Alexis, Chem. Biol. 11 (2004) 397–406.
[14] A.D. Richard, F. Daneel, Phytochemistry 60 (1998) 205–211.
[15] S. Renner, G. Schneider, Chem. Med. Chem. 1 (2006) 181–185.
[16] W.T.M. Mooij, M.J. Hartshorn, L.J. Tickle, A.J. Sharff, M.L. Verdonk, H. Jhoti, Chem. Med. Chem. 1 (2006) 827–838.
[17] L. Shi, H.-M. Ge, S.-H. Tan, H.-Q. Li, Y.-C. Song, H.-L. Zhu, R.-X. Tan, Eur. J. Med. Chem. 42 (2007) 558–564.
[18] H.-Q. Li, H.-M. Ge, Y.-X. Chen, C. Xu, L. Shi, H. Ding, H.-L. Zhu, R.-X. Tan, Chem. Biodivers. 3 (2006) 463–471.
[19] N. Haddad, Y. Xu, J.A. Baron, K.Y. Nathan, Tetrahedron Lett. 43 (2002) 1135–1138.
[20] R.J. Butcher, G.M. Mockler, O. McKern, Acta Cryst. E59 (2003) 1104–1106.
[21] J. Meletiadis, J.F. Meis, J.W. Mouton, J.P. Donnelly, P.E. Verweij, J. Clin. Microbiol. 38 (2000) 2949–2954.