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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and metal ion binding activity of methyl 12-amino-13-nitro-7-oxo dehydrodeisopropylabietate derivatives

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Abstract Starting from dehydrobaietic acid with the analogous structure of carnosic acid, the intermediate methyl 12-bromo-13-nitro-7-oxo dehydrodeisopropylabietate was synthesized through methylation, bromination, carbonylation, and nitration. Subsequently, the coupling of methyl 12-bromo-13-nitro-7-oxo dehydrodeisopropylabietate with aliphatic or aromatic primary amine by Ullmann condensation reaction produced a series of methyl 12-imino-13-nitro-7-oxo dehydrodeisopropylabietate derivatives. In order to probe their antioxidant effects through metal ion chelation mechanism, the metal ion binding abilities on Cu^{2+} and Fe^{2+} of these compounds were studied using fluorescence quenching method. The results indicated that each compound showed obvious chelation activity with the binding constants (K_A) of the 10^2 L mol^{-1} order of magnitude, which implied its potential pharmacology application as antioxidant by the inhibition of Feton reaction through chelation with Cu^{2+} and Fe^{2+}.

Keywords Dehydrodeisopropylabietate · Ion chelation activity · Antioxidant

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Introduction

Dehydroabietic acid (DDA) is one of the isomerides in the renewable rosin. Previous studies have demonstrated that DDA derivatives shows the bioactivity of antiulcer (Wada *et al*., 1985), against schistosomiasis (Steck, 1981), antimicrobial (Feio *et al*., 1999), and gastroprotective (Beatriz and Guillermo, 2005). With the special skeleton, DDA was also used to synthesize other bioactivity compound such as 3-oxosapriparaquinone (Matsumoto and Takeda, 1996), steroidal hormones (Matsumoto and Imai, 1988), 12-deoxyroyleanone, cryptoquinone, and 11, 14-dihydroxy-8,11,13-abietatrien-7-one (Matsushita *et al*., 2005) as the substance. It is noteworthy that DDA possesses a basic skeleton and an aromatic moiety similar to carnosic acid (Fig. 1), so it has been modified to some antioxidant derivatives with excellent free radical scavenging activity (Esteves and Narender, 2001; Gigante *et al*., 2003; Justino *et al*., 2006).

For antioxidants, three main mechanisms were proposed based on the recent antioxidant theory (Wright *et al*., 2001; Leopoldini *et al*., 2004). The H-atom transfer mechanism, through which a free radical R• removes a hydrogen atom from the antioxidant (ArOH):

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$$

And the one-electron transfer mechanism, in which one of the antioxidant can give an electron to the free radical:

$$R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet +}$$

In the above two mechanisms, the radicals could be stable so that the chain radical reactions were prevented or delayed arising from both reactions. Another antioxidant mechanism is based on the ability of some of these compounds to chelate transition metals ions (especially

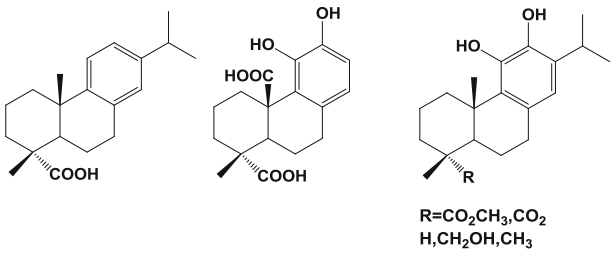

Fig. 1 Molecular structure of DDA, carnosic acid, and catechol derived from abietic acid

iron and copper), which gives rise to stable complexes that entrap metals (Jovanovic *et al*., 1998; Leopoldini *et al*., 2006). Metals ions in human body could catalyze biomolecular oxidations especially the conversion of less reactive species such as H_2O_2 or lipid peroxides into more reactive ones such as hydroxyl radical (OH•) or peroxyl/alkoxyl radicals through Feton reaction or Haber–Weiss reaction. So the role of iron and other transition metals in catalyzing free radical reactions, and the safe sequestration of such metals in non-redox-active forms can be regarded as a component of the antioxidant defense network (Halliwell and Gutteridge, 1990; Halliwell, 2009).

During the last few years, some studies about antioxidant has been made by our group (Pan *et al*., 2007a, b, 2008, 2009). In our opinion, most of the antioxidants reported play their protective role through H-atom transfer mechanism and one-electron transfer mechanism, such as DDA derivatives with analogous structure of carnosic acid (Esteves and Narender, 2001; Gigante *et al*., 2003). However, antioxidants through transition metals chelate ions mechanism was not exhaustively studied in recently years, especially DDA derivatives with transition metals ions chelation activity has not been reported as we know. In this article, to further explore the antioxidant activity of DDA derivatives based on the mechanism of chelate transition metals, we report a facile synthesis of 12-amine-13-nitro-7-oxo dehydrodeisopropylabietate derivatives (Scheme 1) as well as its chelation activity on Cu^{2+} and Fe^{2+} using fluorescence quenching method.

Results and discussion

Starting from DDA according to the literature procedure (Esteves *et al*., 1999; Wada *et al*., 1985; Fonseca *et al*., 2004), methyl dehydrodeisopropylabietate (compound **2**) was synthesized through methylation by freshly distilled dimethyl sulfite in acetone. Then bromination was conducted with NBS (N-bromosuccinimide) in dark place to obtain methyl 12-bromo dehydrodeisopropylabietate (compound **3**). Based on this, methyl 12-bromo 7-oxo dehydrodeisopropylabietate (compound **4**) was prepared through carbonylation from compound **3** using CrO_3 at low temperature. Subsequently, nitration was completed in the condition of mixed acid (HNO_3/H_2SO_4) in cold traps and methyl 12-imino-13-nitro-7-oxo dehydrodeisopropylabietate (compound **5**) was prepared. Finally, the coupling of methyl 12-bromo-13-nitro-7-oxo dehydrodeisopropylabietate with aliphatic or aromatic primary amine by Ullmann condensation reaction produced a series of methyl 12-imino-13-nitro-7-oxo dehydrodeisopropylabietate derivatives. The melting point and yields of all compounds **6** are given in Table 1. Using the aliphatic primary amine as the substrates, the reaction produced good yields (80.1–92.3%) and the work-up after the completion of this reaction is very simple. The mixture was added to ice water and a lot of solid was precipitated, filtered, washed, and then dried. The solid was recrystallized from ethanol and crystals could be obtained. However, the products of methyl 12-bromo-13-nitro-7-oxo dehydrodeisopropylabietate with aromatic primary amine could not be obtained through crystallization directly, so the products should be purified by silica gel column chromatography using petroleum ether/ethyl acetate (10:1, V/V) as the eluent with the yield of 75.1–79.5%.

Scheme 1 Synthetic route of 7-oxo-12-amine-13-nitro dehydrodeisopropylabietate derivatives

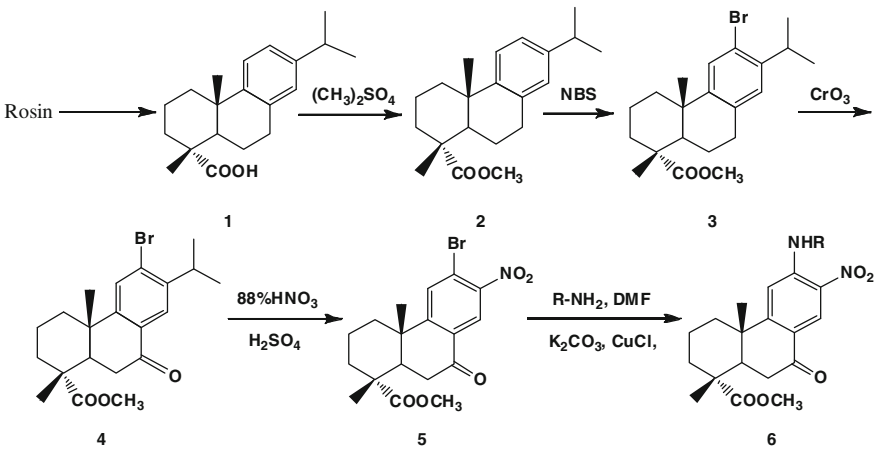

Table 1 Physicochemical property of compounds **6a–6k**

Compounds	R-moiety	Molecular weight	m.p. (°C)	Yield (%)
6a	CH_3-	359.40	191.8–192.6	82.4
6b	C_2H_5-	373.42	190.4–191.9	83.9
6c	$CH_3CH_2CH_2-$	387.45	182.4–183.6	84.2
6d	$(CH_3)_2$ CH−	387.45	174.1–174.8	87.2
6e	$CH_3(CH_2)_3-$	401.48	134.6–135.5	80.1
6f	$(CH_3)_3$ C−	401.48	234.8–235.3	92.3
6g	OH CH_2CH_2-	389.42	138.0–138.7	89.7
6h	$CH_2CH_2NH_2-$	388.44	185.4–186.1	83.9
6i	C_6H_5-	421.47	172.8–173.0	75.1
6j	*p*-CH_3–C_6H_4-	435.49	149.7–150.1	78.3
6k	*p*-CH_3O–C_6H_4-	451.49	152.4–153.0	79.5

The crystal structure of compound **6b** has been determined by X-ray diffraction and is shown in Fig. 2. Rings A (atoms C9–C14) and rings B (atoms C5–C10) demonstrate a trans ring junction, with two methyl groups in the axial positions of the six-membered rings. The torsion angles display classical chair and half-chair skeletons for rings A and B, respectively. The crystal structure is stabilized by weak intermolecular C–H···O and N–H···O interactions.

We then explored the metals ion chelation ability with Cu^{2+} and Fe^{2+} in DMF/H_2O solutions of the pure compounds **6a–6k** as described by Bermejo *et al*., 2008 with a little modification. Take **6h** for example, the fluorescence quenching effect chelated with Cu^{2+} and Fe^{2+} are shown in Fig. 3, the excitation peak of **6h** can be recorded at 337 nm that are generally ascribed to $\pi \rightarrow \pi^*$ electronic transitions from the conjugated aromatic system. The increase of the metal concentration caused the decrease in the peak amplitude and this great variation in the fluorescence spectrum suggests the formation of the **6h**–Cu^{2+} and **6h**–Fe^{2+} complex. With these fluorescence quenching effect conditions in hand, the binding constants (K_A) were estimated from the fluorescence quenching spectral data using Stern–Volmer curves involving plots of the inverse of Cu^{2+}/Fe^{2+} concentrations against the inverse of changes in their respective fluorescence spectrum (Bermejo *et al*., 2008; Xu and Wang, 2006). The following equation was used to calculate the binding constants from these plots:

$$F_0/F = 1 + K_q\tau_0 c(Q) = 1 + K_A c(Q)$$

where F_0 is the fluorescence intensity of compounds **6** without any metal ion, F is the fluorescence intensity of mixture of compounds **6** with the increase of the metal concentration of the metal ion, *c(Q)* is the concentration of Cu^{2+}/Fe^{2+}, and K_A is the binding constants.

The binding constants (K_A) of methyl 12-imino-13-nitro-7-oxo dehydrodeisopropylabietate derivatives are shown in Table 2. All the derivatives showed metals ion chelation ability with the K_A of the 10^2 L mol^{-1} order of magnitude. The K_A (Fe^{2+}) were bigger than K_A (Cu^{2+}) indicating that the compounds showed more activity metals ion chelation ability with Fe^{2+} than Cu^{2+} except for **6f**. Compound **6f** has the strongest chelation activity with Cu^{2+}, while **6h** has the strongest chelation activity with Fe^{2+}, respectively. However, from the factors of electronic effect, steric hindrance, and chain length of substituting group, respectively, it is difficult to find a relationship between the chelation ability and the structure compounds **6**. This could be due to the chelation activity of compounds **6** which were not determined by single factor but by the synergistic effect of all these factors.

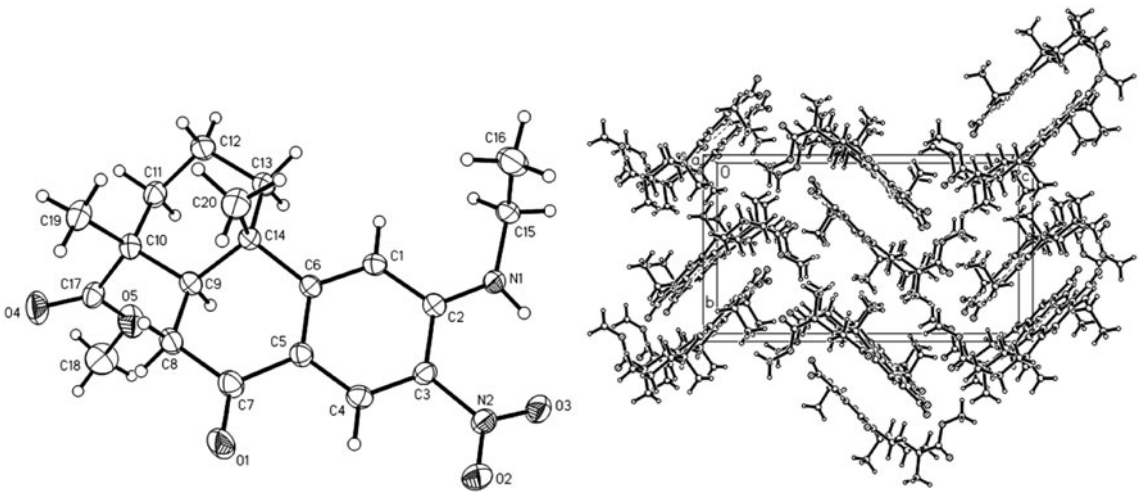

Fig. 2 X-ray crystal structure and packing diagram of compound **6b**

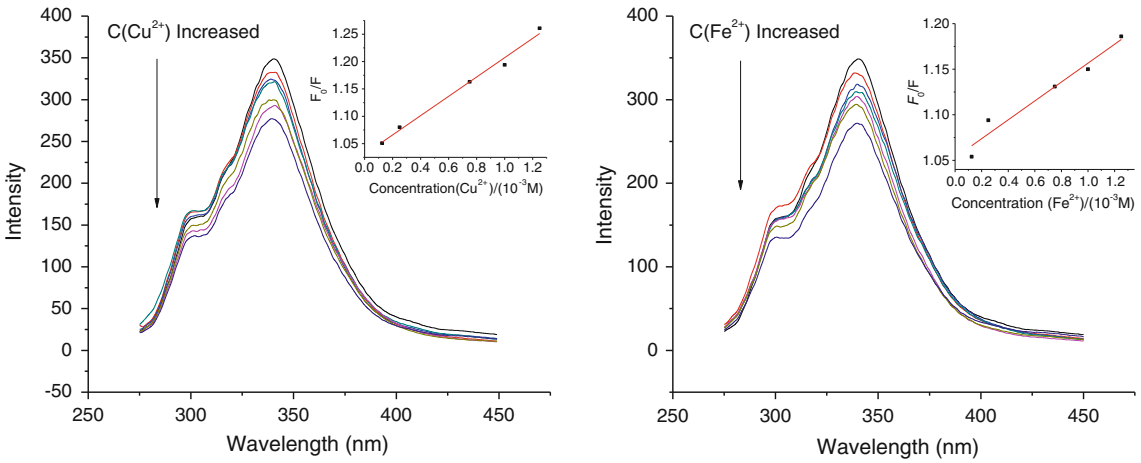

Fig. 3 Fluorescence quenching effect of compound **6f** and the Stern–Volmer curves chelated with Cu^{2+} and Fe^{2+}

Table 2 Fluorescence parameter and chelation to Cu^{2+}/Fe^{2+} of compounds **6a–6k**

Compound	λ_{ex} (nm)	λ_{em} (nm)	K_A (L mol^{-1})/(Cu^{2+})	K_A (L mol^{-1})/(Fe^{2+})
6a	279	330	107.0	186.0
6b	283	331	81.3	138.3
6c	280	331	61.7	135.7
6d	263	338	102.9	168.3
6e	284	322	137.2	181.7
6f	264	340	175.6	103.6
6g	280	330	70.3	108.0
6h	261	337	97.6	305.9
6i	286	330	60.7	106.9
6j	283	316	60.7	122.7
6k	285	317	89.2	165.0

Experimental

Reagents and apparatus

All chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade. The ^{1}H-NMR and ^{13}C-NMR spectra were carried out by the instrument NMR (BRUKER AVANCE 500, BRUKER company, Switzerland), and the mass spectral studies were done using BRUKER ESQUIRE HCT instrument (BRUKER DALTON company, USA). Elemental analyses were determined in the apparatus Carlo Erba model 1106 (Carlo Erba company, Italy). Melting points were determined on a WRS-IA apparatus (Shanghai Precision & Scientific Instrument Co,. Ltd) without correction. Fluorescence analysis was done using RF-5301PC (SHIMADZU Scientific Instruments, Inc., Japan).

Synthesis

(a) General procedure for the preparation of **6a–6h**: compound **5** was synthesized through 4 steps starting from DDA, which were prepared according to the literature procedure (Esteves *et al*., 1999; Wada *et al*., 1985; Fonseca *et al*., 2004). The compound **5** (2.5 mmol), potassium carbonate (1 mmol), cuprous chloride (2 mmol) were added to 15 mL DMF. After stirring for 5–10 min, alicyclic amine (2.5 mmol) was added drop-wise. The resultant solution was stirred for 3–4 h at 100°C (monitored by TLC), and then plenty of ice water was added, a lot of orange–yellow solid was precipitated, filtered, washed with water, and then dried. The solid was recrystallized with ethanol and pale orange–yellowish crystals were obtained.

(b) General procedure for the preparation of **6i–6k**: compound **5** (2.5 mmol), potassium carbonate (1 mmol), cuprous chloride (2 mmol) were added to 15 mL DMF. After stirring for 5–10 min, aromatic amine (2.5 mmol) was added drop-wise. The resultant solution was stirred for 3–4 h at 100°C (monitored by TLC), then plenty of ice water was added. A lot of orange–yellow solid was precipitated, filtered, and washed with water. Subsequently, the solid obtained was purified by silica gel column chromatography with petroleum ether/ethyl acetate (20:1, V/V) as the eluent. Yellow powders of **6i–6k** were obtained.

Spectra data for representative compounds

*Methyl 12-methylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6a**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.31 (3H, s, 4-CH_3), 1.37 (3H, s, 10-CH_3), 1.71–1.77 (2H, m, 2-H_2), 1.80–1.88 (3H, m, 1-H_2, 3-H_α), 2.33–2.38 (2H, m, 3-H_β, 6-H_α), 2.67–2.69 (2H, m, 6-H_β, 5-H), 3.11(3H, d, $J = 5.1$, N-CH_3), 3.68 (3H, s, –OCH_3), 6.70 (1H, s, 11-H), 8.36 (1H, d, $J = 5.1$, N–H), 8.91 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 16.63, 18.07, 23.43, 29.83, 36.51, 36.99, 37.42, 38.27, 42.96, 46.77, 52.28, 106.65, 120.19, 128.31, 131.26, 148.64, 162.33, 177.49, 194.40; EI-MS(*m/z*): 361.22 $[M + H]^+$; Anal. Calcd. for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77%. Found: C, 63.48; H, 6.54; N, 7.92%.

*Methyl 12-ethylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6b**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.30 (3H, s, 4-CH_3) 1.36 (3H, s, 10-CH_3), 1.36 (3H, s, 10-CH_3), 1.43 (3H, t, $J = 6.85$, N–CH_2CH_3), 1.71–1.77 (2H, m, 2-H_2,), 1.80–1.83 (3H, m, 1-H_2, 3-H_α,), 2.30–2.38 (2H, m, 3-H_β, 6-H_α), 2.64–2.71 (2H, m, 6-H_β, 5-H), 3.43 (2H, q, $J = 6.85$, N–CH_2), 3.68 (3H, s, $COOCH_3$), 6.70 (1H, s, 11-H), 8.26 (1H, s, N–H), 8.9 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 14.13, 16.78, 17.94, 23.14, 36.50, 36.99, 37.41, 37.91, 38.21, 42.95, 47.04, 52.26, 107.03 (C-11), 120.41, 128.26, 131.23, 148.04, 162.53, 177.83, 195.02. EI-MS(*m/z*): 375.21 $[M + H]^+$; Anal. Calcd. for $C_{20}H_{26}N_2O_5$: C, 64.15; H, 7.00; N, 7.48%. Found: C, 64.02; H, 7.25; N, 7.36%.

*Methyl 12-propylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6c**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.11 (3H, t, $J = 7.4$ Hz, –$NCH_2CH_2CH_3$), 1.30 (3H, s, 4-CH_3), 1.37 (3H, s, 10-CH_3), 1.69–1.77 (2H, m, 2-H_2), 1.80–1.85 (5H, m, –$NHCH_2CH_2CH_3$, 2-H_2, 3-H_α), 2.30–2.38 (2H, m, 3-H_β, 6-H_α), 2.64–2.72 (2H, m, 6-H_β, 5-H), 3.35 (2H, q, $J = 12.3$, –NCH_2–), 3.69 (3H, s, –OCH_3), 6.70 (1H, s, 11-H), 8.35 (1H, s, N–H), 8.9 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 11.55, 16.61, 18.80, 23.13, 23.42, 36.51, 37.42, 38.21, 42.94, 45.07, 46.82, 52.28, 107.08, 120.03, 128.42, 131.08, 147.94, 162.13, 177.51, 194.88. EI-MS(*m/z*): 389.22 $[M + H]^+$; Anal. Calcd. for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21%. Found: C, 64.73; H, 7.12; N, 7.56%.

*Methyl 12-isopropylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6d**)*

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.29 (3H, s, 4-CH_3), 1.35 (3H, s, 10-CH_3), 1.38 (6H, d, $J = 12.6$, –$CH(CH_3)_2$), 1.70–1.76 (2H, m, 2-H_2), 1.83–1.87 (3H, m, 1-H_2, 3-H_α), 2.28–2.36 (2H, m, 3-H_β, 6-H_α), 2.63–2.68 (2H, m, 6-H_β, 5-H), 3.91 (1H, q, $J = 12.6$, –$CH(CH_3)_2$), 3.68 (3H, s, $COOCH_3$), 6.71 (1H, s, 11-H), 8.27 (1H, s, N–H), 8.88 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Mz) δ: 16.47, 18.01, 22.27, 23.32, 36.37, 36.88, 37.28, 38.03, 42.78, 44.25, 46.63, 52.16, 107.35, 119.63, 128.47, 130.80, 146.91, 161.86, 177.39, 194.70. EI-MS(*m/z*): 389.21 $[M + H]^+$; Anal. Calcd. for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21%. Found: C, 65.16; H, 7.40; N, 6.95%.

*Methyl 12-butylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6e**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.02 (3H, t, $J = 7.25$, –$NH(CH_2)_3CH_3$), 1.29 (3H, s, 4-CH_3,), 1.49 (3H, s, 10-CH_3), 1.49–1.54 (2H, m, –CH_2CH_3), 1.73–1.84 (7H, m, –$CH_2CH_2CH_3$, 1-H_2, 2-H_2, 3-H_α), 2.28–2.36 (2H, m, 3-H_β, 6-H_α), 3.37 (2H, dd, $J = 7.25$, –$NHCH_2$–), 3.67 (3H, s, –OCH_3), 6.69 (1H, s, 11-H), 8.33 (1H, s, N–H), 8.87 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Mz) δ: 16.63, 16.51, 17.9, 20.09, 23.32, 30.72, 36.40, 36.85, 37.31, 38.07, 42.8, 46.66, 52.2, 106.97, 119.80, 128.26, 130.86, 147.83, 162.05, 177.37, 194.79. EI-MS(*m/z*): 403.21 $[M + H]^+$; Anal. Calcd. for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96%. Found: C, 65.31; H, 7.71; N, 7.10%.

*Methyl 12-tert-butylamino-13-nitro-7-oxo dehydrodeisopropylabietate (**6f**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.28 (3H, s, 4-CH_3), 1.34 (3H, s, 10-CH_3), 1.54 (9H, s, –$C(CH_3)_3$), 1.49–1.54 (2H, m, –CH_2CH_3), 1.70–1.75 (2H, m, 1-H_2), 1.78–1.84 (3H, m, 2-H_2, 3-H_α), 2.24–2.26 (1H, m, 3-H_β,), 2.30–2.35 (1H, m,6-H_α), 2.61–2.68 (2H, m, 6-H_β, 5-H), 3.66 (3H, s, –$COOCH_3$), 6.95 (1H, s, 11-H), 8.61 (1H, s, N–H), 8.88 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 16.59, 18.16, 23.49, 29.68, 36.48, 37.13, 37.42, 38.11, 42.93, 46.76, 52.16, 52.29, 109.24, 119.48, 128.71, 131.66, 147.29, 161.02, 177.50, 194.85. EI-MS(*m/z*): 403.22 $[M + H]^+$; Anal. Calcd. for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96%. Found: C, 65.35; H, 7.61; N, 7.16%.

*Methyl 12-(2-hydroxyethyl)-amino-13-nitro-7-oxo dehydrodeisopropylabietate (**6g**)*

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.29 (3H, s, 4-CH_3), 1.35 (3H, s, 10-CH_3), 1.77–1.79 (2H, m, 2-H_2,), 1.82–1.83 (3H, m, 1-H_2, 3-H_α), 2.32 (2H, dd, $J = 4.68$, 3-H_β, 6-H_α), 2.51 (1H, s, –OH), 2.64–2.71 (2H, m, 6-H_β, 5-H_α), 3.57 (2H, q, $J = 5.10$, –$NHCH_2$–), 3.99 (2H, q, $J = 5.10$, –$NHCH_2CH_2$–), 3.68 (3H, s, $COOCH_3$), 6.75 (1H, s, 11-H), 8.55 (1H, s, N–H), 8.77 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$,

125 Mz) δ: 16.46, 17.9, 23.21, 36.33, 36.80, 37.27, 38.08, 42.71, 45.09, 46.61, 52.19, 60.59, 107.27, 119.26, 128.47, 130.97, 147.91, 162.16, 177.63, 195.10. EI-MS(*m/z*): 391.16 $[M + H]^+$; Anal. Calcd. for $C_{20}H_{26}N_2O_6$: C, 61.53; H, 6.71; N, 7.18%. Found: C, 61.28; H, 6.94; N, 7.30%.

*Methyl 12-(2-aminoethyl)-amino-13-nitro-7-oxo dehydrodeisopropylabietate (**6h**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.22 (6H, t, $J = 7.04$, $-CH_2CH_3$),1.30 (3H, s, 4-CH_3), 1.36 (3H, s, 10-CH_3), 1.69–1.76 (2H, m, 1-H_2), 1.84–1.88 (3H, m, 2-H_2, 3-H_α), 2.29–2.37 (2H, m, 3-H_β, 6-H_α), 2.64–2.72 (2H, m,6-H_β, 5-H), 3.31 (4H, q, $J = 7.04$, $-CH_2CH_3$), 3.68 (3H, s, $COOCH_3$), 6.90 (1H, s, 11-H), 8.36 (1H, s, 14-H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 12.46, 16.53, 18.12, 23.54, 36.49, 37.03, 37.40, 38.04, 43.38, 45.91, 48.78, 52.27, 113.18, 121.54, 127.14, 139.09, 147.56, 159.19, 177.52, 194.99. EI-MS(*m/z*): 391.22 $[M + H]^+$; Anal. Calcd. for $C_{20}H_{27}N_3O_5$: C, 61.68; H, 6.99; N, 10.79%. Found: C, 61.96; H, 6.79; N, 10.71%.

*Methyl 12-phenyl-amino-13-nitro-7-oxo dehydrodeisopropylabietate (**6i**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.22 (3H, s, 4-CH_3,) 1.33 (3H, s, 10-CH_3), 1.51–1.58 (1H, m, 1-H_α), 1.71–1.79 (4H, m, 1-H_β, 2-H_2, 3-H_α,), 1.99 (1H, d, $J = 12.5$, 3-H_β), 2.35 (H, m, 6-H_α), 2.67 (2H, m, 6-H_β, 5-H), 3.68 (3H, s, $COOCH_3$), 7.12 (1H, s, 11-H), 7.33–7.35 (3H, m, 3′-H, 4′-H and 5′-H), 7.49–7.52 (2H, m, 2′-H and 6′-H), 8.94 (1H, s, 14-H), 9.81 (1H, s, N–H). ^{13}C NMR ($CDCl_3$, 125 Mz) δ: 16.46, 17.87, 23.19, 36.35, 36.53, 37.32, 38.05, 42.80, 46.62, 52.39, 109.41, 121.75, 124.57, 126.56, 128.10, 130.06, 131.91, 137.83, 145.63, 161.54, 177.45, 194.39. EI-MS(*m/z*): 423.19 $[M + H]^+$; Anal. Calcd. for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63%. Found: C, 68.41; H, 6.32; N, 6.33%.

*Methyl 12-(p-methylphenyl)-amino-13-nitro-7-oxo dehydrodeisopropylabietate (**6j**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.22 (3H, s, 4-CH_3) 1.33 (3H, s, 10-CH_3), 1.51–1.55 (1H, m, 1-H_α), 1.72–1.80 (4H, m, 1-H_β, 2-H_2, 3-H_α,), 1.99 (1H, d, $J = 12.7$, 3-H_β), 2.31–2.39 (H, m, 6-H_α), 2.44 (3H, s, ph-CH_3), 2.63–2.71 (2H, m, 6-H_β, 5-H), 3.68 (3H, s, $COOCH_3$), 7.07 (1H, s, 11-H), 7.19–7.21 (2H, d, $J = 8.15$, 3′-H and 5′-H), 7.29–7.30 (2H, m, $J = 8.15$, 2′-H and 6′-H), 8.93 (1H, s, 14-H), 9.75 (1H, s, N–H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 16.58, 17.98, 21.06, 23.27, 36.46, 36.62, 37.42, 38.14, 42.9, 46.72, 52.30, 109.44, 121.57, 124.78, 128.15, 130.53, 131.42, 135.03, 136.57, 146.36, 161.72, 177.51, 194.88. EI-MS(*m/z*): 437.22 $[M + H]^+$; Anal. Calcd. for $C_{24}H_{26}N_2O_5$: C, 68.79; H, 6.47; N, 6.42%. Found: C, 68.60; H, 6.57; N, 6.51%.

*Methyl 12-(p–methoxyphenyl)-amino-13-nitro-7-oxo dehydrodeisopropylabietate (**6k**)*

^{1}H-NMR ($CDCl_3$, 500 Hz) δ: 1.18 (3H, s, 4-CH_3) 1.30 (3H, s, 10-CH_3), 1.43–1.50 (1H, m, 1-H_α), 1.60–1.76 (4H, m, 1-H_β, 2-H_2, 3-H_α,), 1.93 (1H, d, $J = 12.58$, 3-H_β), 2.27–2.36 (H, m, 6-H_α), 2.59–2.68 (2H, m, 6-H_β, 5-H), 3.65 (3H, s, $COOCH_3$), 3.87 (3H, s, ph-OCH_3), 6.90 (1H, s, 11-H), 6.99–7.01 (2H, d, $J = 8.83$, 3′-H and 5′-H), 7.19–7.12 (2H, d, $J = 8.83$, 2′-H and 6′-H), 8.90 (1H, s, 14-H), 9.67 (1H, s, N–H). ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 16.56, 17.59, 23.23, 36.45, 36.59, 37.41, 38.10, 42.87, 46.72, 52.31, 52.55, 109.27, 115.17, 121.37, 126.95, 128.17, 130.28, 131.49, 147.05, 158.37, 161.75, 177.52, 194.92. EI-MS(*m/z*): 453.20 $[M + H]^+$; Anal. Calcd. for $C_{25}H_{28}N_2O_6$: C, 66.36; H, 6.24; N, 6.19%. Found: C, 66.51; H, 6.43; N, 5.85%.

Metals ion chelation ability

The metals ion chelation ability of the title compound was evaluated according to the method of Bermejo *et al*., 2008 and Perez *et al*., 2009 with little modification. Different volume of $CuCl_2$/$FeCl_2$ solution [10^{-3} mol/L, DMF/H_2O (1:1, V/V)] was added to the title compound solution (10^{-3} mol/L, DMF) and then distilled to the appropriate concentration with DMF. The mixture was mixed thoroughly and the fluorescence intensity of different solution was measured with slit of 5 nm.

Conclusions

Compounds **6** were synthesized through coupling of methyl 12-bromo-13-nitro-7-oxo dehydrodeisopropylabietate with aliphatic or aromatic primary amine by Ullmann condensation reaction. The fluorescence quenching results indicated that each compound showed obvious chelation activity with the K_A of the 10^2 L mol^{-1} order of magnitude. Compound **6f** has the strongest chelation activity with Cu^{2+} while **6h** has the strongest chelation activity with Fe^{2+}, respectively. It is difficult to find a relationship between the chelation abilities and the structures of compounds **6**. This could be due to the chelation activity of compound 6 which was not determined by single factor of electronic effect, steric hindrance or chain length of substituting group, but by the synergistic effect of all these factors. Further experiments were needed to elucidate their metal chelation mechanism of compounds **6**. However, the

results imply its potential pharmacology application as antioxidant by the inhibition of Feton reaction or Haber–Weiss reaction through chelation with Cu^{2+} and Fe^{2+}.

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