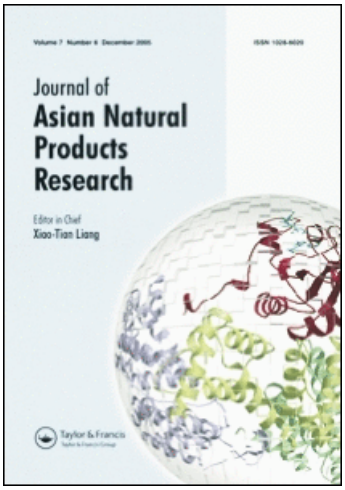

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Studies on the alkaloid constituents of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang and their acute toxicity in mice

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Six alkaloids (**1**–**6**) have been isolated from the fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang, two of which are new compounds, identified as 2-undecyl-4(1*H*)-quinolone (**4**) and 1-methyl-2-undecanone-10′-4(1*H*)-quinolone (**5**); the known compounds were identified as rutaecarpine (**1**), evodiamine (**2**), 1-methyl-2-undecyl-4(1*H*)-quinoline (**3**) and 2-undecanone-10′-4(1*H*)-quinolone (**6**). Compounds **1–5** were evaluated for their acute toxicity.

Keywords: *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang; 2-Undecyl-4(1*H*)-quinolone; 1-Methyl-2-undecanone-10′-4(1*H*)-quinolone

1. Introduction

The medicinal evodiae fruits [1] [fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang, *E. rutaecarpa* (Juss) Benth, and *E. rutaecarpa* (Juss) Benth. var. *officinalis* (Dode) Huang], a traditional Chinese drug, have been used in the treatment of headache, abdominal pain, migraines, chill limbs, postpartam hemorrhage, amenorrhage, dysentery, nausea and hyperbaropathy. Pharmacological studies showed that the medicinal evodiae fruits have analgesic, sedative, antiseptic, antianxia and antihypersensitive actions [2]. To elucidate the biologically active principles from the drug, further studies have been undertaken.

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2. Results and discussion

An ethanolic extract of the fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang was separated as described in the Experimental section to yield compounds **1**–**6**. Compounds **1**–**3** and **6** were identified as rutaecarpine, evodiamine, 1-methyl-2-undecyl-4(1*H*)-quinoline and 2-Undecanone-10′-4(1*H*)-quinolone (**6**) (figure 1), respectively, by means of 1D and 2D NMR spectroscopic techniques, including ^{1}H, ^{13}C NMR, $^{1}H—^{1}H$ COSY, DEPT, HMQC and HMBC.

Compound **4** was isolated as an amorphous powder, with a molecular formula of $C_{20}H_{29}NO$ determined by HR-EI-MS. The UV spectrum (213, 235, 315, 327 nm) shows the characteristic absorption of *N*-demethylquinolone skeleton [3]. Its IR spectrum exhibits strong absorption of N–H (3058 cm^{-1}) and —CO—CH═CH— (1637 cm^{-1}). The ^{1}H NMR spectrum of **4** show signals due to the quinolone skeleton, *i.e.*, N—H at δ 13.02, conjugated olefinic proton at δ 6.30 (1H, s, H-3), aromatic protons at δ 7.32 (1H, dd, $J = 7.7$, 8.4 Hz, H-6), 7.59 (1H, dd, $J = 7.7$,8.4 Hz, H-7), 7.88 (1H, d, $J = 8.4$ Hz, H-8), and 8.37 (1H, d, $J = 8.4$ Hz, H-5). The EI-MS exhibits an M^{+} peak at *m/z* 299 and fragment ion peaks arising from McLafferty rearrangement at *m/z* 159, from displacement rearrangement at *m/z* 172, and elimination of the ketene at *m/z* 130 (figure 2). These suggest that compound **4** is an *N*-demethylquinolone derivative substituted at C_2. The substituted group at C_2 of **4** is an alkyl

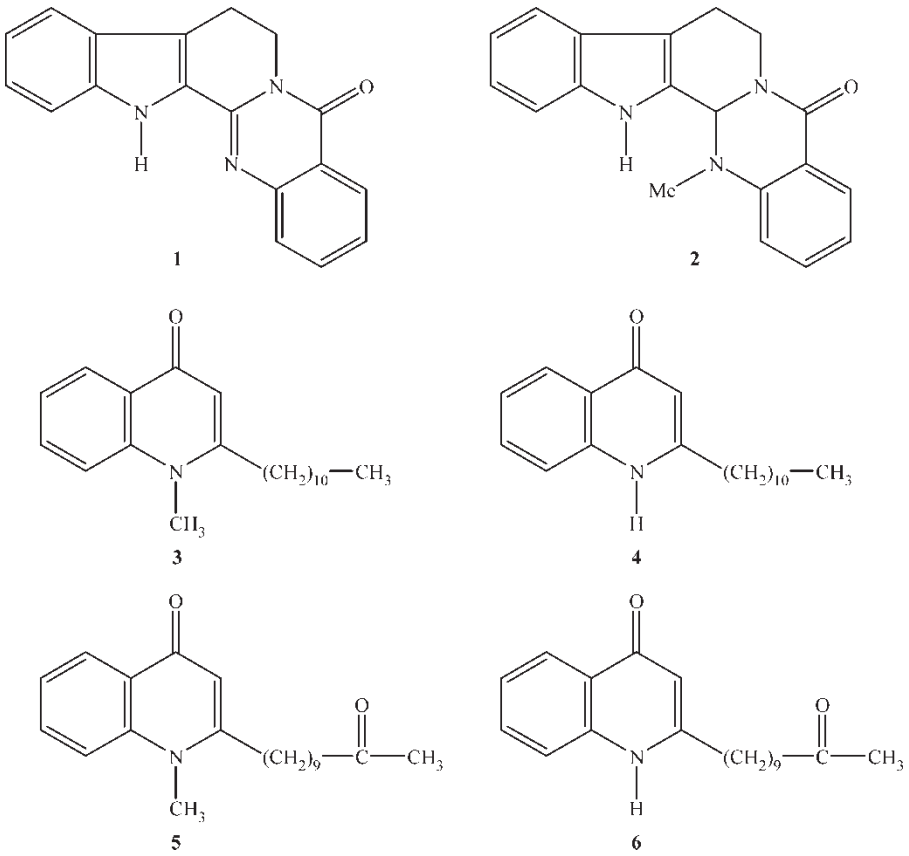

Figure 1. Structures of alkaloids **1**–**6**.

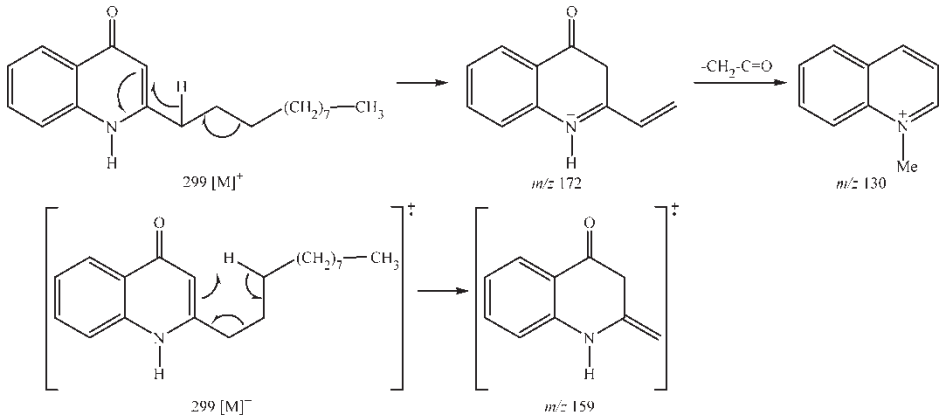

Figure 2. Characteristic mass fragmentation ions of **4**.

side-chain having eleven carbons, as shown from its ^{13}C NMR and DEPT spectrum (see Experimental section) and HR-EIMS *m/z* 144 (C_9H_6NO) [M^+—$(CH_2)_{10}$—CH_3]. From these results, the structure of compound **4** was established as 2-undecyl-4(1*H*)-quinolone. It is a previously unknown compound.

Compound **5** was isolated as an amorphous powder, with a molecular formula of $C_{21}H_{29}NO_2$ determined by HR-EIMS. The IR, UV, ^1H NMR and EI-MS spectra exhibit signals due to an *N*-methylquinolone skeleton [3,4]. The ^1H NMR spectrum also exhibits a conjugated olefinic proton at δ 6.05 (1H, s, H-3), which is shifted upfield ($\Delta\delta$ 0.25) compared with that of **4**, and aromatic protons at δ 7.23 (1H, ddd, $J = 1.5$, 7.2, 8.0 Hz, H-6), 7.52 (1H, ddd, $J = 1.5$, 7.2, 8.0 Hz, H-7), 7.38 (1H, d, $J = 1.5$, 8.0 Hz, H-8), 8.28 (1H, dd, $J = 1.5$, 8.0 Hz, H-5). These suggested that **5** was an *N*-methylquinolone derivative substituted at C_2, having an alkyl ketone side-chain as deduced from its ^{13}C NMR and DEPT spectra data. In the HMBC spectrum of **5**, the signals at δ 2.03 (3H, s) and 2.32 (2H, t, $J = 7.2$, 7.5 Hz) correlate with the ketone group at δ 209.3. Therefore, the methyl group was located at the terminal position in side-chain. In addition, the EIMS spectrum shows an M^+ peak at *m/z* 327 ($C_{21}H_{29}NO_2$ from its HR-EIMS) and fragment ion peak at *m/z* 158 ($C_{10}H_8NO$) arising from elimination of side-chain (figure 3). This suggested eleven carbons in the side-chain, having a —$(CH_2)_9$—CO—CH_3 structure.

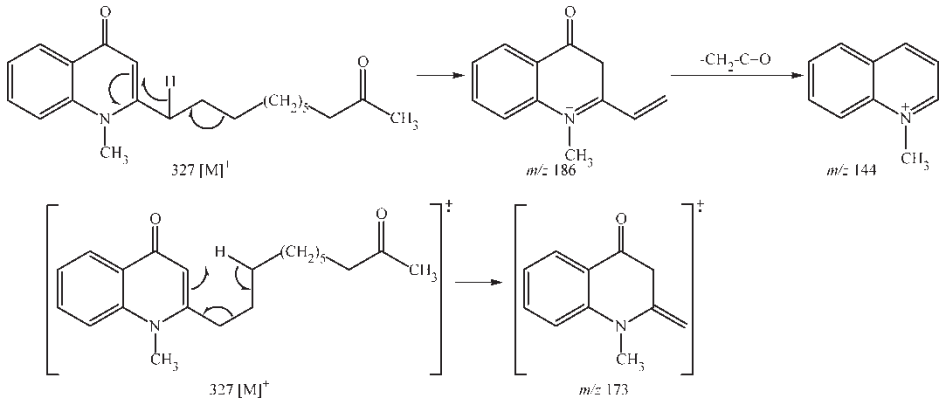

Figure 3. Characteristic mass fragmentation ions of **5**.

From these results, **5** was established as 1-methyl-2-undecanone-10′-4(1*H*)-quinolone. It is also a previously unknown compound.

Table 1. Bio-assay results of acute toxicities of the alkaloids in mice.

Compounds	***1***	***2***	***3***	***4***	***5***	***6***	*Total quinolones*[a]
LD_{50} (mg kg^{-1})	65.0000	77.7938	64.8709	36.0624	47.5742	–[b]	14.0160

[a] Total quinolones = Fr. 4 (see Experimental section).
[b] Not assayed because of small amount.

The two main types of alkaloids in the fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang are indoles (*i.e.* rutaecarpine, evodiamine, *etc*.) and quinolones [1-methyl-2-undecyl-4(1*H*)-quinoline, 2-undecanone-10′-4(1*H*)-quinolone, *etc*.]. table 1 shows the acute toxicities of the tested five components and a quinolone mixture, which are (from low to high) evodiamine (**2**) < rutaecarpine (**1**) ≤ 1-methyl-2-undecyl-4(1*H*)-quinoline (**3**) < 1-methyl-2-undecanone-10′-4(1*H*)-quinolone (**5**) < 2-undecyl-4(1*H*)-quinolone (**4**) < total quinolone mixture. No death or side effects, except a decrease in body weight, were observed. Rutaecarpine is a demethylevodiamine with an additional double bond at C_3-N; the acute toxicity is stronger than evodiamine. The acute toxicity of the total quinolone mixture was stronger than 1-methyl-2-undecyl-4(1*H*)-quinoline (**3**), 2-undecyl-4(1*H*)-quinolone (**4**) and 1-methyl-2-undecanone-10′-4(1*H*)-quinolone (**5**), but the other quinolones have not been isolated from the total quinolone mixture. The *N*-demethyl compound **4** had about twice the acute toxicity of *N*-methyl compound **4** (*i.e.* **3**) (table 1).

3. Experimental

3.1 General experimental procedures

All melting points were determined on an X-4 microscopic apparatus and are uncorrected. IR spectra were taken with a Perkin–Elmer 683 spectrophotometer. UV spectra were measured with a Varian Cary 300 ultraviolet–visible spectrophotometer. NMR data were recorded on a Varian INOVA-500 Spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, including COSY, HMQC, HMBC, and a Varian VXR-300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, and a ARX-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C; chemical shifts are given in δ relative to TMS as internal standard. EIMS and HR-EIMS were recorded on VG ZAB-HS and APEX II (Bruker) instruments, respectively.

3.2 Plant material

Fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang were collected at Leye country, Guangxi Zhuang Autonomous Region, China and identified by Professor Chen Dao-feng. A voucher specimen (No. 20000901) of the plant has been deposited at the State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University (Beijing, China).

3.3 Extraction and isolation

Powdered fruits of *Evodia rutaecarpa* (Juss) Benth var. *bodinaieri* (Dode) Huang (11 kg) were refluxed with 95% EtOH to afford an ethanolic extract. The extract was then suspended in H_2O and partitioned successively with cyclohexane, $CHCl_3$ and n-BuOH to afford the corresponding extracts, 250 g (yield 2.27%), 381 g (3.46%) and 843 g (7.66%). The $CHCl_3$ extract was subjected to column chromatography over silica gel, eluting with cyclohexane and cyclohexane—$CHCl_3$, to yield four fractions (1–4). Fraction 3 (cyclohexane—$CHCl_3$ = 5:1) was subjected to column chromatography on silica gel, eluting with cyclohexane—$CHCl_3$ (19:1), to yield compounds **1** and **2**. Fraction 4 was subjected to column chromatography on neutral aluminium oxide, eluting with cyclohexane—$CHCl_3$ (1:1), to give compounds **3**, **4** and a mixture fraction. The latter was further purified by silica gel column chromatography to afford **5** and **6**.

3.3.1 Rutaecarpine (1). Colorless needles (EtOAc), mp 260–261°C, $C_{18}H_{13}N_3O$. UV (EtOH) λ_{max} (nm) (log ϵ): 362 (3.46), 345 (3.55), 331 (3.48), 289 (2.91), 276 (2.88), 240 (sh), 234 (sh), 213 (3.54). IR (KBr) ν_{max} (cm^{-1}): 3339, 1647, 1597, 1544, 1487, 1466, 1325, 1227, 766, 758, 727. EI-MS: *m/z* 287 $[M]^+$ (100), 258 (6.1), 144 (19.1), 129 (13.6), 115 (3.1), 77 (3.5). ^1H and ^{13}C NMR data were identified as reported in the literature [5].

3.3.2 Evodiamine (2). Colorless pellet (EtOH), mp 278–282°C, $C_{19}H_{17}N_3O$. UV (EtOH) λ_{max} (nm) (log ϵ): 203 (4.46), 225 (4.71), 268 (4.06). IR (KBr) ν_{max} (cm^{-1}): 3218, 2909, 2540, 1626, 1603, 1508, 1446, 746, 734. EI-MS: *m/z* 303 $[M]^+$(100), 288 $[M—CH_3]^+$ (9.9), 274 (11.3), 169 (68.0), 161 (23.2), 134 (75.6). ^1H and ^{13}C NMR data were identified as reported [5].

3.3.3 1-Methyl-2-undecyl-4(1*H*)-quinoline (3). Colorless needles (EtOAc-diethyl ether), mp 68–69°C, $C_{21}H_{31}NO$. UV (MeOH) λ_{max} (nm) (log ϵ): 334 (4.21), 322 (4.20), 239 (4.51), 214 (4.49). IR (KBr) ν_{max} (cm^{-1}): 3435, 3007, 2953, 1727, 1635, 1593, 1568, 1465, 1337, 1304, 774, 763. EI-MS: *m/z* 313 $[M]^+$ (16.2), 298 $[M—CH_3]^+$ (6.1), 284 (9.8), 270 (7.9), 256 (6.2), 242 (5.4), 228 (6.1), 200 (9.2), 186 (82.5), 173 (100), 144 (13.0), 91 (4.7), 77 (5.3). ^1H and ^{13}C NMR data were identified as reported [6].

3.3.4 2-Undecyl-4(1*H*)-quinolone (4). A white amorphous powder, $C_{20}H_{29}NO$. UV (MeOH) λ_{max} (nm) (log ε): 213 (4.43), 235 (4.44), 315 (4.03), 327 (4.01). IR (KBr) ν_{max} (cm^{-1}): 3248, 3058, 2921, 1739, 1637, 1591, 1548, 1497, 1468, 1352, 1318, 779, 758, 750. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm): 13.02 (1H, br s, H–*N*), 8.37 (1H, d, J = 8.4 Hz, 5-H), 7.88 (1H, d, J = 8.4*Hz*, H-8), 7.59 (1H, dd, J = 7.7, 8.4 Hz, H-7), 7.32 (1H, dd, J = 7.7, 8.4 Hz, H-6), 6.30 (1H, s, H-3), 2.73 (2H, t, J = 7.8 Hz,H-1′), 1.73 (2H, m, H-2′), 1.16–1.25 (16H, m, H-3′–10′), 0.83 (3H, t, J = 4.8 Hz,H-11′). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.7 (C_4), 155.8 (C_2), 140.8 (C_{8a}), 131.7 (C_7), 125.1 (C_5), 124.8 (C_{4a}), 123.6 (C_6), 118.8 (C_8), 107.9 (C_3), 34.3 ($C_{1'}$), 31.9 ($C_{9'}$), 29.2–29.6 ($C_{3'}$–$C_{8'}$), 22.6 ($C_{10'}$), 14.0 ($C_{11'}$). EI-MS *m/z*: 299 $[M]^+$ (8.7), 284 (3.1), 270 (5.9), 256 (5.1), 242 (3.7), 228 (5.9), 214 (6.4), 186 (10.0), 172 (70.9), 159 (100.0). HR-EIMS: *m/z* 299.2259 $[M]^+$ (calcd for $C_{20}H_{29}NO$, 299.2249), 284.1863 ($C_{19}H_{26}NO$), 186.1006 ($C_{13}H_{14}O$), 172.0746 ($C_{11}H_{10}NO$), 159.0729 ($C_{10}H_9NO$).

3.3.5 1-Methyl-2-undecanone-10′-4(1*H*)-quinolone (5). A white amorphous powder, $C_{21}H_{29}NO_2$. UV (MeOH) λ_{max} (nm) (log ε): 216, 239, 321, 334. IR (KBr) ν_{max} (cm^{-1}): 3464, 2925, 2849, 1752, 1701, 1596, 1550, 1497, 1465, 1365, 1159, 868, 763, 678. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.28 (1H, dd, $J = 1.5$, 8.0 Hz, H-5), 7.52 (1H, ddd, $J = 1.5$, 7.2, 8.0 Hz, H-7), 7.38 (1H, d, $J = 1.5$, 8.0 Hz, H-8), 7.23 (1H, ddd, $J = 1.5$, 7.2, 8.0 Hz, H-6), 6.05 (1H, s, H-3), 3.59 (3H, s, *N*–Me), 2.56 (2H, t, $J = 6.9$, 8.4 Hz, H-1′), 2.32 (2H, t, $J = 7.2$, 7.5 Hz, H-9′), 2.03 (3H, s, H-11′), 1.53 (2H, t, $J = 6.6$Hz, H-2′), 1.45 (2H, t, $J = 6.6$ Hz, H-8′), 1.55–0.98 (10H, m, H-3′–7′). ^{13}C NMR (75 MHz, $CDCl_3$) δ(ppm): 209.3 ($C_{10'}$), 177.2 (C_4), 154.5 (C_2), 141.5 (C_{8a}), 131.6 (C_7), 125.9 (C_5), 125.9 (C_{4a}), 122.8 (C_6), 115.2 (C_8), 110.4 (C_3), 43.3 ($C_{9'}$), 34.2 ($C_{1'}$), 33.8 (*N*–Me), 29.2–29.5 ($C_{3'}$–$C_{8'}$), 23.4 ($C_{11'}$). EI-MS: *m/z* 327 $[M]^+$ (9.0), 284 (20.6), 270 (25.0), 256 (4.4), 242 (2.9), 228 (3.1), 186 (50.6), 173 (100.0), 144 (14.4), 43 (7.7). HR-EIMS: *m/z* 327.2192 $[M]^+$ (calcd for $C_{21}H_{29}NO_2$ 327.2198), 284.1977 ($C_{19}H_{26}NO$), 270.1797 ($C_{18}H_{24}NO$), 186.0954 ($C_{12}H_{12}NO$), 173.0809 ($C_{11}H_{11}NO$), 144.0852 ($C_{11}H_{12}$).

3.3.6 2-Undecanone-10′-4(1*H*)-quinolone (6). A white amorphous powder, $C_{20}H_{27}NO_2$. UV (MeOH) λ_{max} (nm) (log ϵ): 212 (4.29), 235 (4.31), 315 (3.90), 327 (3.87). IR (KBr) ν_{max} (cm^{-1}): 3409, 3058, 2918, 1706, 1591, 1548, 1497, 1467, 1632, 1352, 1318, 835, 779, 759. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 12.81 (1H, br s, *N*–H), 8.35 (1H, d, $J = 8.0$ Hz,H-5), 7.83 (1H, d, $J = 8.0$ Hz, H-8), 7.58 (1H, dd, $J = 7.2$,8.0 Hz, H-7), 7.32 (1H, dd, $J = 7.2$,8.0 Hz, H-6), 6.26 (1H, s, H-3), 2.71 (2H, t, $J = 7.2$,7.5 Hz, H-1′), 2.36 (2H, t, $J = 7.2$,7.2 Hz, H-9′), 3.00 (3H, s, H-11′), 1.70 (2H, t, $J = 6.3$ Hz, H-2′), 1.49 (2H, t, $J = 6.6$ Hz,H-8′), 1.24–1.15 (10H, m, H-3′–7′). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 209.3 ($C_{10'}$), 178.6 (C_4), 155.5 (C_2), 140.7 (C_{8a}), 131.7 (C_7), 125.1 (C_5), 124.8 (C_{4a}), 123.5 (C_6), 118.7 (C_8), 107.9 (C_3), 43.7 ($C_{9'}$), 34.3 ($C_{1'}$), 29.8–29.0 ($C_{3'}$–$C_{8'}$), 23.7 ($C_{11'}$). EI-MS: *m/z* 313 $[M]^+$ (6.3), 270 $[M—C_2H_3O]^+$ (13.4), 256 (17.2), 242 (3.2), 228 (3.3), 214 (4.1), 200 (1.7), 186 (7.3), 172 (49.9), 159 (100.0), 130 (8.5), 43 $[C_2H_3O]^+$ (7.8).

3.4 Animals and methods

Kunming species mice (hemisexual, 20.845–20.030 g) were supplied from the Institute of laboratory animal, Chinese Academy of Medical Sciences (certification: (95) 01-3001). A total of 80 mice were divided into 4 groups of 20. Samples were dissolved with 3% Tween-80 in physiological saline and administered by vein in tail. Volume of administration was 0.1 ml per 10 g body weight. Just after 7 days from tested compound treatment, a medial lethal dose (LD_{50}) was calculated by sequential analysis.

3.5 Statistics

Data were analyzed using the EXCEL soft ware and sequential analysis for parameters.

$$\log LD_{50} = \sum nx / \sum n$$

$$S_{\log LD50} = d \cdot \sqrt{\sum \left[p(1-p)/n - 1 \right]}$$

$$95\%\ \text{confidence} = LD_{50}\ \ 1.96s$$

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