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Novel *neo*-clerodane diterpenoids from *Teucrium quadrifarium* and their anti-ferroptosis effect

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Abstract

Teucrifarides A–D (**1–4**), four previously unreported *neo*-clerodane-type diterpenoids, combined with sixteen known analogs (5–20), were purified from *Teucrium quadrifarium*. The absolute form of compounds **1–4** were determined via spectroscopic and ECD calculation methods, together with X-ray crystallography experiments. Among them, compound **1** possessed a 5,20-epoxy ring featuring a unique cage-like 12-oxatricyclo [5.3.2.0^{1,6}]undecane skeleton. Meanwhile, **2** incorporated a 6,20-epoxy ring with a novel 12-oxatricyclo [6.2.2.0^{2,7}]undecane skeleton. Compounds **1** and **12** exhibited significant inhibitory effects against HT-22 cells ferroptosis induced by RSL3, with EC₅₀ values of 11.8 ± 1.0 μM, and 4.52 ± 1.24 μM, respectively. Moreover, ROS accumulation in HT22 cells treated with compound **1** was also observed.

Keywords *Teucrium quadrifarium*, Lamiaceae, *Neo*-clerodane, Ferroptosis inhibitory activity, ROS

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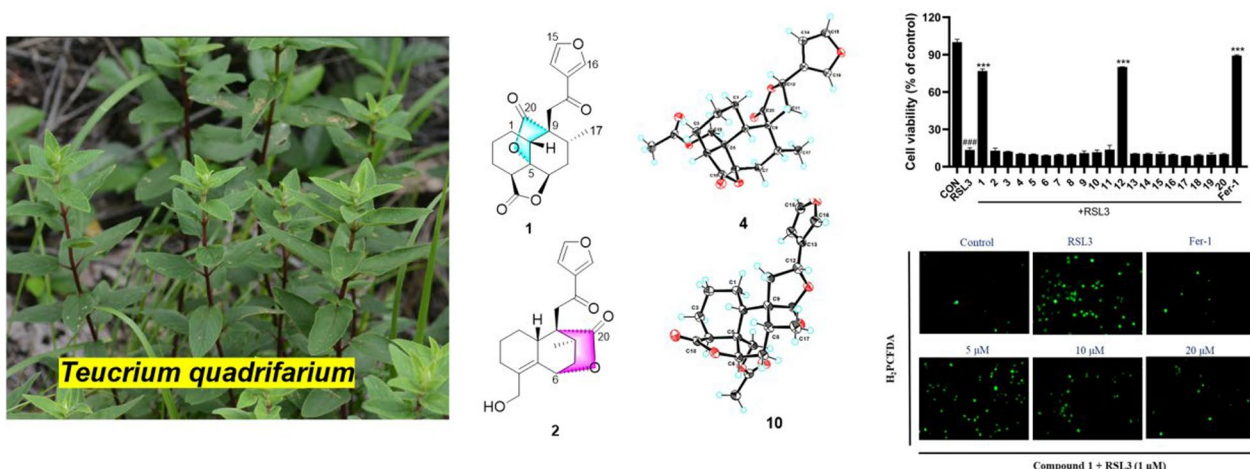
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Graphical Abstract



1 Introduction

The genus *Teucrium* (Lamiaceae) comprises shrubs and herbs, and the majority predominantly distributed in southwest regions of China [1]. The phytochemical research on this genus revealed that diterpenes, flavonoids, and triterpenes were the main metabolites of this plant [2]. Diterpenoids are well known as one of the primary secondary metabolites within the Lamiaceae family, and several complex diterpenoid scaffolds have been reported from the genus *Teucrium* [3–5]. Especially, abietane and clerodane exhibit interesting biological activities, including anti-ferroptosis [6], anti-inflammatory [7], anti-neuroinflammation [8], antifeedant [9], and cytotoxic activities [10], which have drawn the interest of pharmacologist.

Teucrium quadrifarium, a plant of the genus *Teucrium*, is a folk medicine to treat wind heat syndrome, and nosotoxicosis [11, 12]. However, to date, the phytochemical investigation of *T. quadrifarium* has yielded only a few natural compounds, such as flavonoids [13] and *neo*-clerodane diterpenoids [14]. As part of our efforts to obtain structurally intriguing and biologically significant clerodane-diterpenoids, a phytochemical study of *T. quadrifarium* was undertaken. As a result, twenty clerodane diterpenoids, including four previously undescribed *neo*-clerodane diterpenoids (1–4) were obtained. Herein, the structural elucidation, and anti-ferroptosis effects of these isolates were reported.

2 Results and discussion

Teucrifaride A (1) possesses the molecular formula of $C_{19}H_{20}O_6$, based on the HR-ESI-MS ion at m/z 367.1149 $[M+Na]^+$ (calcd 367.1152). In the ^1H NMR spectrum

(Table 1), one methyl (δ_H 1.03) and three olefinic protons (δ_H 6.75, 7.46, 8.09) were observed. Moreover, in the ^{13}C NMR (Table 2) spectrum, 19 carbon resonances were assigned to one methyl, five methylenes, seven methines, and six non-protonated carbons, respectively. Moreover, a characteristic furan ring [δ_H/δ_C 6.75 (d, $J=2.4$ Hz)/108.4 (C-14), 7.46 (t, $J=1.8$ Hz)/144.7 (C-15), 8.09 (s)/147.6 (C-16), and δ_C 127.8 (C-13)] was deduced based on the 1D NMR data. The ^1H - ^1H COSY correlations of H_2 -1/ H_2 -2/ H_2 -3/ H -4, H -10/ H_2 -1, and H -6/ H_2 -7/ H -8, H -8/ H_3 -17, combined with the HMBs of H -4 (δ_H 3.12) with C-5, C-18, of H -6 (δ_H 4.50) with C-5, C-18, of H -10 (δ_H 2.90) with C-6, C-8, C-11, C-20, of H_3 -17 (δ_H 1.03) with C-9, and of H -11, H -13, and H -16 with C-12 (δ_C 191.8) suggested that compound 1 was a characteristic *neo*-clerodane diterpenoid analogue, with one γ -lactonic ring unit [15]. In addition, an additional γ -lactonic ring fragment constructed via C-5–C-20 according to the key HMBC correlations of H -10, H -8, and H -11 with C-20, combined with the downfield characteristic at C-5 (δ_C 83.9). Thus, as shown in Fig. 1, the planner structure of 1 was constructed.

The NOESY data was used for analyzing the relative configuration of 1 (Fig. 2). The signals of CH_3 -17/ H -6/ H -4, assigned these groups were as α -orientation. On the contrary, the correlations of H -8/ H -10/ H -11 demonstrated these protons were β -orientation. Ultimately, the structure of 1 was fixed to be 4*R*,5*R*,6*S*,8*S*,9*S*, and 10*S*, through ECD calculation method (Fig. 3).

Teucrifaride B (2) with a molecular formula $C_{19}H_{22}O_5$ in line with its HR-ESI-MS ion at m/z 353.1346

Table 1 ^1H NMR (600 MHz) data of compounds **1–4** in CDCl_3 (δ in ppm, J in Hz)

Position	1 $\delta_{\text{H mult.}}$ (J in Hz)	2 $\delta_{\text{H mult.}}$ (J in Hz)	3 $\delta_{\text{H mult.}}$ (J in Hz)	4 $\delta_{\text{H mult.}}$ (J in Hz)
1 α	1.59 m	0.94 m	1.31 m	1.56 m
1 β	0.99 m	1.65 m	1.99 m	1.89 ^a
2 α	1.74 m	1.58 m	1.60 m	1.53 m
2 β	1.23 dt (13.8, 3.0)	1.83 m	1.97 m	1.89 ^a
3 α	2.23 ^a	2.15 m	2.18 m	1.72 m
3 β	1.62 m	2.25 m	2.34 m	2.03 m
4	3.12 d (6.6)	–	–	2.86 dd (3.0, 7.2)
6	4.50 t (7.8)	5.41 d (4.8)	4.99 m	4.48 t (3.0)
7	2.08 ddd (15.0, 7.8, 2.4)	1.72 dt (13.8, 4.2)	1.41 td (12.6, 4.2)	2.06 m
7 β	2.23 ^a	2.23 m	2.36 m	2.37 m
8	2.46 m	2.91 m	2.22 m	1.92 m
10	2.90 dd (12.6, 4.8)	3.04 m	3.23 m	1.76 dd (12.0, 1.8)
11a	3.31 d (18.6)	3.24 q	1.97 m	2.32 dd (13.8, 7.2)
11b	2.86 d (18.6)	–	2.51 dd (14.4, 9.6)	2.44dd (13.8, 9.6)
12	–	–	5.26 t (8.4)	5.33 dd (10.2, 7.8)
14	6.75 d (2.4)	6.76 dd (1.8, 1.2)	6.09 s	6.38 d (1.2)
15	7.46 t (1.8)	7.44 t (1.8)	–	7.44 t (1.8)
16	8.09 s	8.14 t (1.2)	5.89 s	7.45 s
17	1.03 d (7.8)	0.91 d (7.2)	1.37 d (7.8)	1.06 d (6.6)
18a	–	4.10 d (8.0)	–	–
18b	–	4.18 d (8.0)	–	–
19a	–	–	–	4.45 d (12.6)
19b	–	–	–	4.86 d (12.6)
1' α	–	–	3.78 m	–
1' β	–	–	3.99 m	–
2'	–	–	1.26 t (7.2)	2.09 s

^a Overlapped signal

$[\text{M}+\text{Na}]^+$ (calcd 353.1359). The NMR data of **2** closely resembled those of **1**, indicated these two compounds share the almost same *neo*-clerodane skeleton. The main difference was a methylene [δ_{H} 4.14 (H-18), δ_{C} 61.6 (C-18)] in **2** replace of a carbonyl carbon δ_{C} 173.3 (C-18) in **1**. Besides, an unusual δ -lactone ring was formed via C-6–C-7–C-8–C-9–C-20 based on the HMBC correlations of H-6, H-8, H-10, and H-11 with C-20, combined with the signals of H-6/H₂–7/H-8/CH₃–17 in the COSY spectrum (Fig. 1). Therefore, the planner structure of **2** was elucidated. Furthermore, the NOESY correlations (Fig. 2) of H-10/H-8/H-11, and H-8/H-7 β /H-6, manifested that H-10, H-8, H-6, and H-11 were β -orientation. Similarly, CH₃–17 was α -oriented deduced by the NOESY signals of CH₃–17/H-7 α . Subsequently, the structure of **2** was determined to be 6R, 8S, 9S, 10R, according to the calculated ECD curve was the same with that of experimental ECD curves in the range of 200–400 nm (Fig. 3).

Teucrifaride C (**3**) was yield as a white powder. The HR-ESI-MS data (m/z 411.1408 $[\text{M}+\text{Na}]^+$; calcd for 411.1414) suggested a molecular of $\text{C}_{21}\text{H}_{24}\text{O}_7$. By comparison of 1D NMR data, the structure of **3** is similar to the structure of teucvidin (**5**) [16], except for an ethoxy group [δ_{H} (1.26, t, $J=7.2$ Hz), 3.78(1H, m), 3.99 (1H, m); δ_{C} 15.2, 67.4] and an α,β -unsaturated- γ -lactone fragment (δ_{C} 169.1, 163.3, 119.0, 101.9) in **3**. Furthermore, the ethoxy unit was fixed at C-16 due to the ^1H - ^1H COSY correlations of H₃–2'/H₂–1' (Fig. 1), together with the HMBC correlations (Fig. 1) from H₂–1' (δ_{H} 3.78, 3.99) to C-16. Besides, the HMBC correlations from H-16 to C-15, C-13, C-14, and from H-12 to C-13, C-14, C-16, further constructed an *a,b*-unsaturated- γ -lactone fragment at C-12. Furthermore, the signals of H-10/H-6/H₃–17 in the NOESY spectrum revealed that they were α -oriented. In contrast, the NOESY correlations of H-8/H-11b/H-16, and H-12/H-14 were deserved indicating that H-8, H-16 were β -oriented (Fig. 1). The test ECD

Table 2 ^{13}C NMR (150 MHz) data of compounds **1–4** in CDCl_3

Position	1	2	3	4
1	21.9	24.9	23.4	21.3
2	20.9	21.8	21.4	24.2
3	20.1	26.7	20.2	19.5
4	40.8	133.5	128.3	45.4
5	83.9	133.2	161.6	46.0
6	76.2	73.5	76.0	77.7
7	30.9	32.9	35.9	29.2
8	31.5	31.7	38.3	34.4
9	51.2	50.2	51.4	49.9
10	41.0	41.4	36.3	46.9
11	37.9	36.7	37.5	41.8
12	191.8	192.8	72.0	72.0
13	127.8	128.1	163.3	124.8
14	108.4	108.5	119.0	108.1
15	144.7	144.5	169.1	144.4
16	147.6	147.5	101.9	140.0
17	18.1	19.9	14.1	16.3
18	173.3	61.6	172.5	177.2
19				59.3
20	177.3	176.1	177.1	177.7
1'			67.4	170.5
2'			15.2	21.0

curve of **3** was well matched with that of calculated ECD spectra (Fig. 3), and the structure of **3** was assigned as Fig. 4.

Teucrifaride D (**4**), a colorless acicular crystal, with the molecular formula of $\text{C}_{22}\text{H}_{26}\text{O}_7$ based on the HR-ESI-MS ion at m/z of 425.1559 $[\text{M} + \text{Na}]^+$ ($\text{C}_{22}\text{H}_{26}\text{O}_7\text{Na}$, calcd for 425.1571) and ^{13}C NMR data. After careful analysis of 1D NMR data of **4** (Tables 1 and 2), we deduced that **4** possesses the same structure as that of teucvisin B (**10**) [17]. Moreover, the NOESY correlations of H-10/H-7 β /H-8/H-11/H-14, combined with the opposite signals of H-6/H-7 α /CH₃-17, further suggested H-12 was α -oriented. Finally, the structure of **4** was undoubtedly confirmed to be 4*S*,5*R*,6*R*,8*R*,9*R*,10*S*,12*R* through the signal-crystal X-ray diffraction experiment (Fig. 5).

Sixteen known diterpenoids were determined to be teucvidin (**5**) [16], 12-epiteucvidin (**6**) [16], salvifaricin (**7**) [18], 19-acetyl-teuspinin (**8**) [19], isoteuflin (**9**) [20], teucvisin B (**10**) [17], crotocaudin (**11**) [21], isosalviperuln (**12**) [22], de-*O*-acetylsalvigenolide (**13**) [23], salvileucalin A (**14**) [18], 6*a*-acetoxyteuscordin (**15**) [24], montanin D (**16**) [25], teumicropodin (**17**) [26], 6*a*-hydroxy-teuscordin (**18**) [27], teucvin (**19**) [28] and 12-epiteuflin (**20**) [16] through comparison of the NMR data with those of literatures.

Considering that clerodane-diterpenoids were reported a remarkable anti-ferroptosis effect [6, 29], we evaluated the ferroptosis inhibitory effect of all isolates in HT22 cells. Firstly, their cytotoxicity was assayed, and none of them exhibited cytotoxicity on HT22 cells (Fig. 6A) at 40 μM . Then, the anti-ferroptosis effect of the isolates on HT22 cells deduced by RSL3 and erastin as depicted in Fig. 6B and 6C. Notably, of them, compounds **1** and **12** indicated obvious inhibitory effect against RSL3-induced ferroptosis, and potent ferroptosis inhibition on erastin-induced model, respectively. And the EC_{50} values of **1** and **12** were $11.8 \pm 1.0 \mu\text{M}$, and $4.52 \pm 1.24 \mu\text{M}$, respectively, for above two different induced-models (Fig. 6D).

The increase of intracellular reactive oxygen species (ROS) was characteristic biomarkers of ferroptosis. To further explore the ferroptosis inhibition of new compound **1** against RSL3-reduced HT22 cells, the intracellular ROS levels by fluorescence microscopy was detected. As shown in Fig. 7, at the concentrations of 5, 10, and 20 μM , compound **1** can prominently inhibit intracellular ROS accumulation in a dose-dependent manner. The above results preliminarily uncovered that new compound **1** exerts anti-ferroptosis activity should be related to target mitochondria. However, to date, there is no clear mechanism for ferroptosis. Thus, compound **1** should be further researched for its anti-ferroptosis effective mechanism in the future.

3 Conclusion

In conclusion, twenty *neo*-clerodane isoates were isolated from *T. quadrifarium*, including four new (**1–4**) and sixteen known ones (**5–20**). Among them, fifteen compounds were isolated from this plant for the first time, and the results enriches the chemical components of this plant. Additionally, new compound **1** exhibited a significant ferroptosis inhibitory effect against RSL3-induced HT22 cells.

4 Experimental section

4.1 General experimental procedures

XtaLAB Pro diffractometer was used to determine X-ray crystallography. HR-ESI-MS, IR, UV, NMR spectra were determined as described previously [30].

4.2 Plant material

The whole plants of *T. quadrifarium* were collected in Pingtang County, Guizhou Province, China, in August, 2021, and identified by Prof. Ni Zhang. A specimen voucher (No. TZC20210806) is stored in the Natural Products Research Center of Guizhou Province.

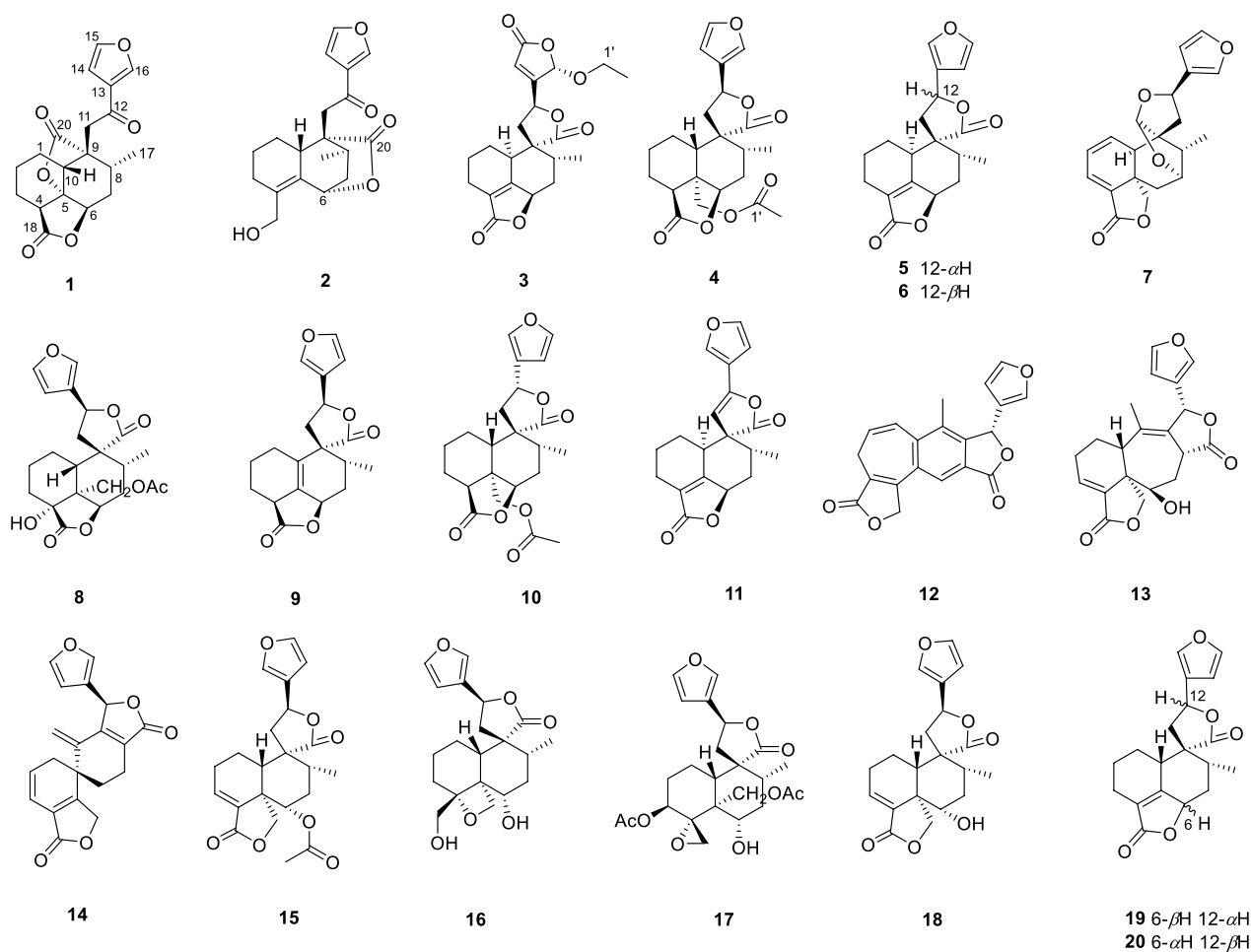


Fig. 1 Key ^1H - ^1H COSY and HMBC correlations of compounds 1–4

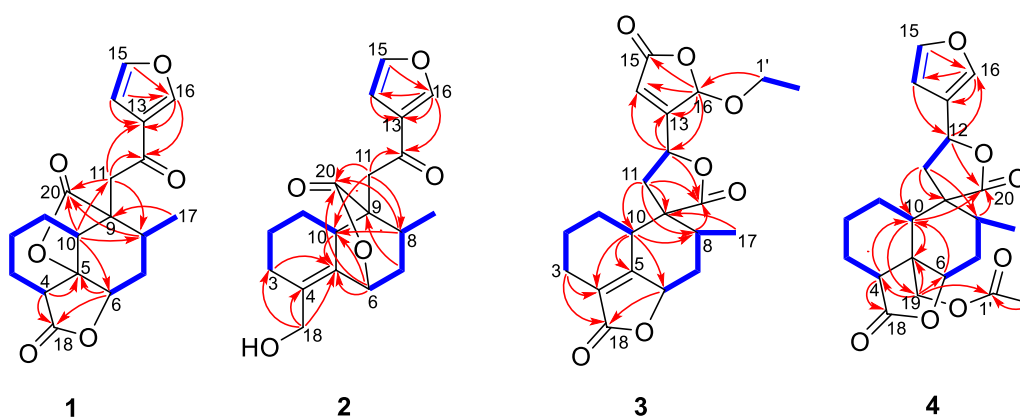


Fig. 2 Key NOESY correlations of compounds 1–4

4.3 Extraction and isolation

The air-dried material (20.0 kg) was smashed and extracted with 95% aqueous EtOH (3×70 L, each 2 h) under reflux. The crude EtOH extract was obtained

by evaporation under vacuum conditions. The crude samples were suspended in H_2O and then leached with petroleum ether (PE), and ethyl acetate (EA), respectively. The PE part (1047.4 g) and EA part (819.9 g) were

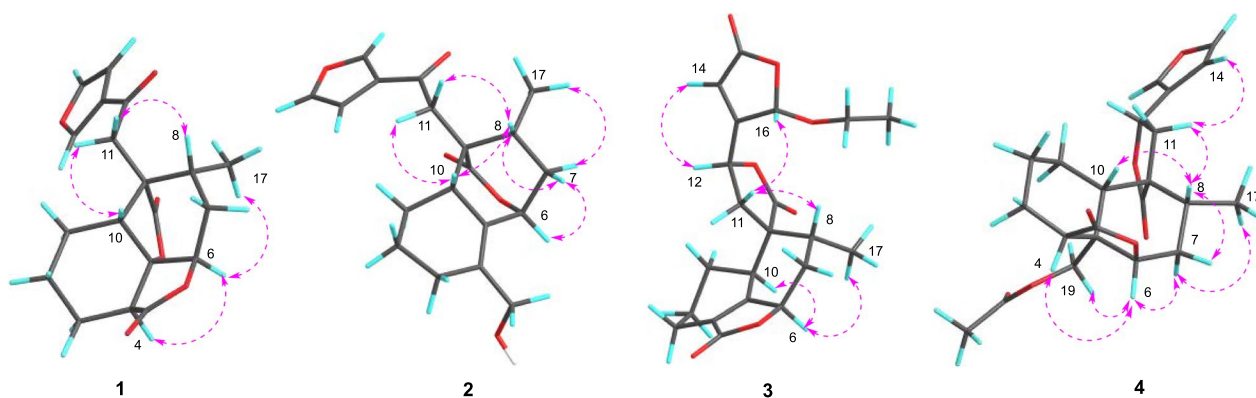


Fig. 3 Experimental and Calculated ECD curves of **1–3**

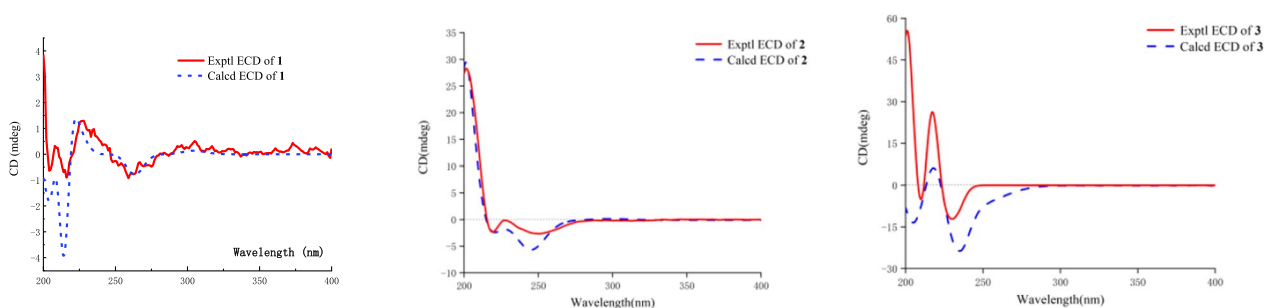


Fig. 4 The chemical structures of compounds **1–20**

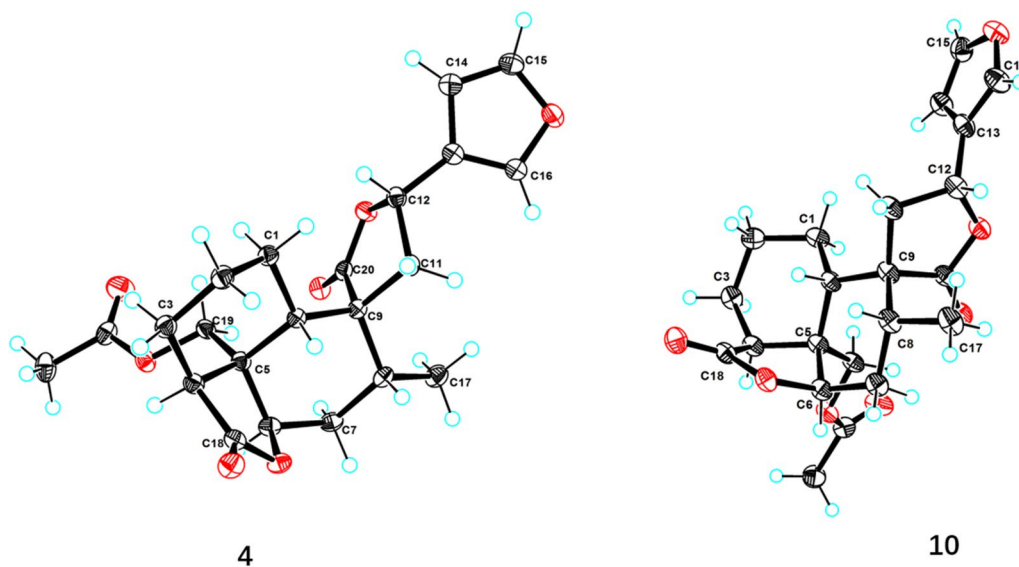


Fig. 5 X-ray ORTEP drawing of compounds **4** and **10**

evaporated to dryness. The PE layer was purified with silica gel chromatography, employing stepwise gradient elution with PE/EA mixture ranging from.

100:0 to 0:100, to afford seven fractions (Fr. A–G), according to TLC plates. Fr. D (74.5 g) was isolated on CC (MeOH/H₂O, from 50:50 to 95:5) to yield eight fractions (Fr. D1–D8). Fr. D5 (13.0 g) was further

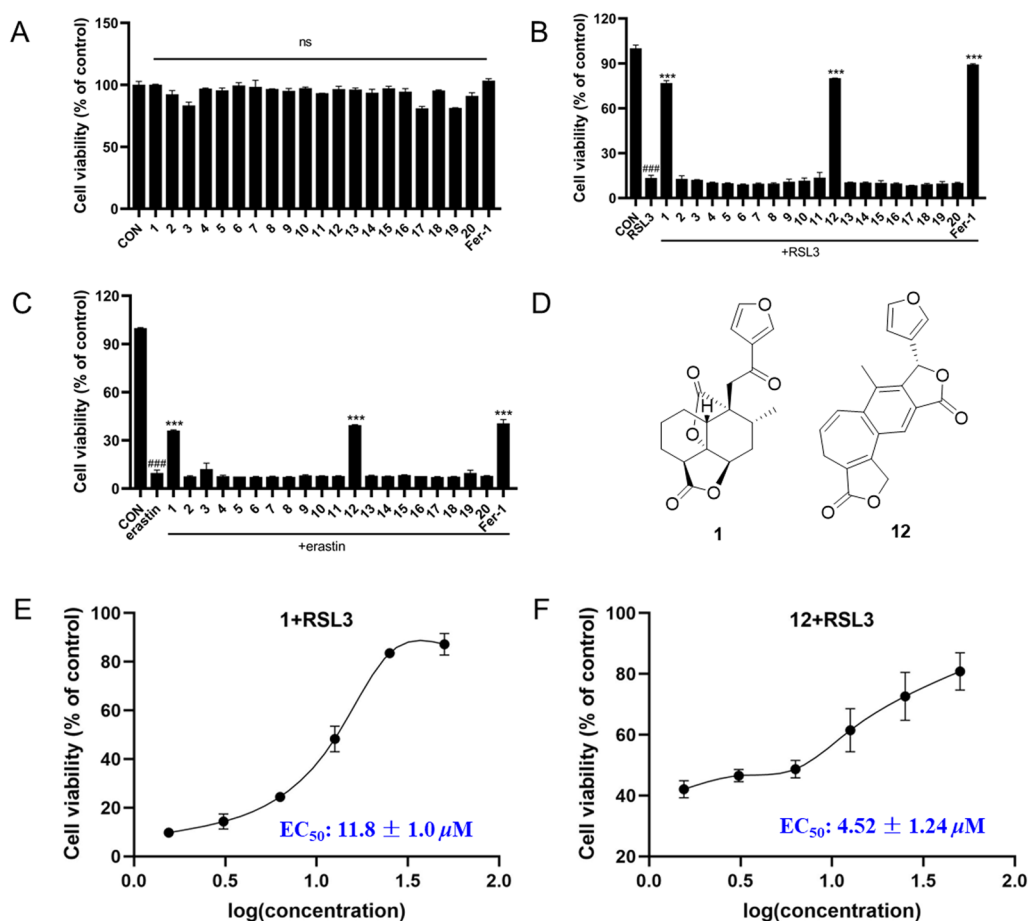


Fig. 6 Effects of compounds **1–20** on RSL3-induced HT22 cells ferroptosis. **A** Cell viability of HT22 cells treated with 40 μM isolated compounds and 1 μM Fer-1. **B** Cell viability of HT22 cells co-treated with 1 μM RSL3 and 40 μM isolated compounds and 1 μM Fer-1 for 24 h determined by an MTT assay. **C** Cell viability of HT22 cells co-treated with 1 μM erastin and 40 μM isolated compounds and 1 μM Fer-1 for 24 h determined by an MTT assay. **D** Chemical structures of **1** and **12**. Dose–response relationship of compounds **1** (**E**), and **12** (**F**) inhibiting RSL3-induced ferroptosis in the HT22 cells. Data represent the means $\pm$ SEM from triplicate experiments. ### $P < 0.0001$ compared with control cell; *** $P < 0.0001$ compared with cells treated with RSL3

purified via Sephadex LH-20 ($CH_2Cl_2/MeOH$, 1:1) to yield compound **5** (32.3 mg), **6** (18.4 mg). Compound **1** ($MeOH/H_2O$, 57:43, t_R 19.5 min, 9.3 mg), and **9** ($MeCN/H_2O$, 49:51, t_R 26.0 min, 9.1 mg) were obtained from Fr. D6 via semi-preparative HPLC. And compounds **4** ($MeCN/H_2O$, 45:55, t_R 53.0 min, 5.6 mg), **10** ($MeCN/H_2O$, 45:55, t_R 47.0 min, 3.6 mg) isolated from Fr. D6 by semi-preparative HPLC. Fr. D4 was isolated through Sephadex LH-20 ($CH_2Cl_2/MeOH$, 1:1) and semi-preparative HPLC to give compounds **11** ($MeOH/H_2O$, 64:36, t_R 27.5 min, 9.6 mg), and **12** ($MeOH/H_2O$, 48:52, t_R 31.0 min, 1.9 mg). Fr. D3 was repeatedly separated on CC, and subsequently purified to have **7** (13.2 mg) and **8** (6.3 mg) via Sephadex LH-20 ($CH_2Cl_2/MeOH$, 1:1). Fr. F (87.5 g) was chromatographed on CC ($MeOH/H_2O$, from 30:70 to 95:5) to give eight fractions (Fr. F1–Fr. F8). Fr. F2 (17.2 g) further afforded six fractions (Fr. F2a–Fr. F2f)

after purified by silica gel ($CH_2Cl_2/MeOH$, from 100:1 to 0:100). Compounds **13** ($MeCN/H_2O$, 35:65, t_R 27.0 min, 2.8 mg), **14** ($MeCN/H_2O$, 35:65, t_R 40.0 min, 3.3 mg), and **15** ($MeOH/H_2O$, 48:52, t_R 48.0 min, 17.9 mg) were obtained from Fr. F2c, and compounds **16** ($MeCN/H_2O$, 40:60, t_R 36.0 min, 17.8 mg), **17** ($MeOH/H_2O$, 48:52, t_R 39.0 min, 12.5 mg), **18** ($MeCN/H_2O$, 48:52, t_R 26.0 min, 9.8 mg) were yield from Fr.F2d, by semi-preparative HPLC.

The EA layer was purified with silica gel chromatography and afforded eight fractions (Fr. EA–Fr. EH) according to TLC plates. Fr. EB (26.8 g) was further chromatographed on CC (MCI, $MeOH/H_2O$, from 30:70 to 95:5) to give nine fractions (Fr. EB1–EB9). Compounds **2** ($MeOH/H_2O$, 56:44, t_R 27.0 min, 12.5 mg), **3** ($MeCN/H_2O$, 45:55, t_R 37.0 min, 7.0 mg), **19** ($MeOH/H_2O$, 40:60, t_R 48.0 min, 2.5 mg), and **20**

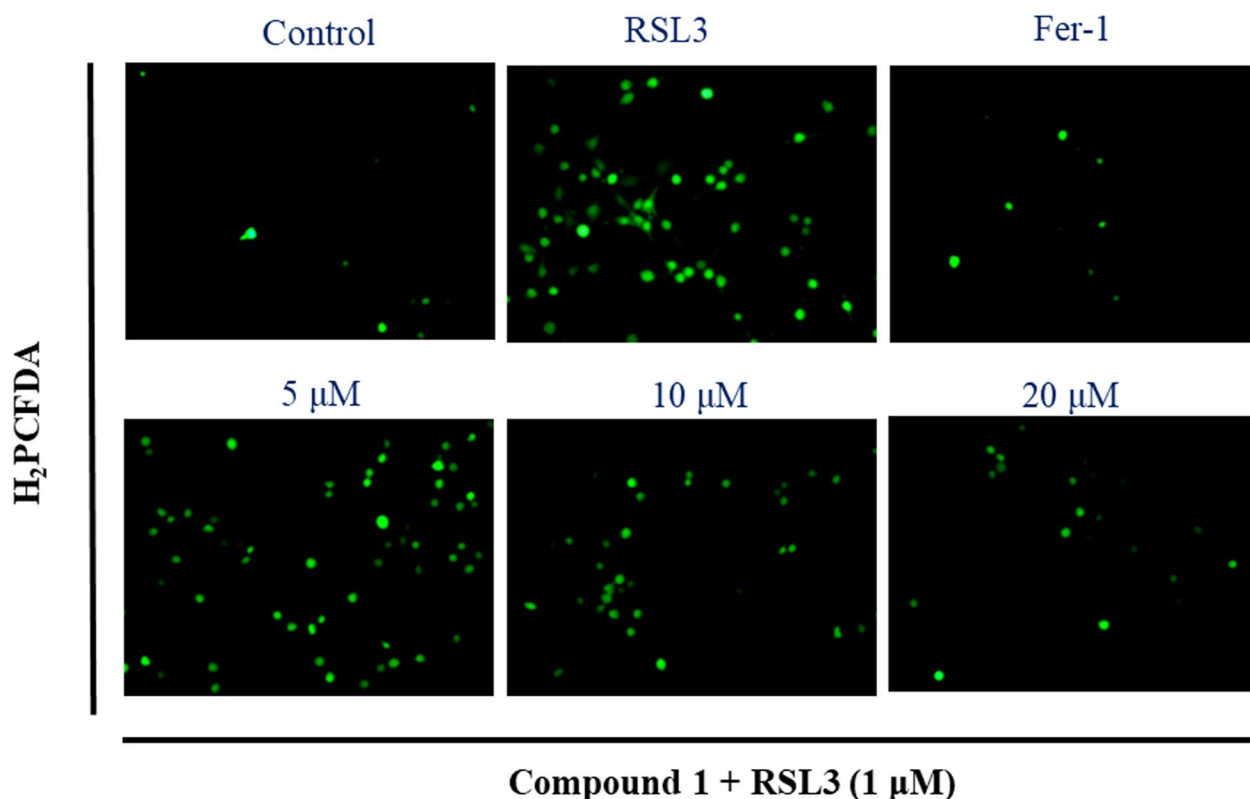


Fig. 7 The intracellular ROS levels was detected by fluorescence microscopy: HT22 cells induced by RSL3 and treated with an indicated concentrations of compound **1** (5, 10, and 20 μ M) or Fer-1 (1 μ M) for 4 h under a fluorescence microscope after H₂PCFDA staining (20x)

(MeOH/H₂O, 40:60, t_R 57.0 min, 4.2 mg) were purified from Fr. EB5 through semi-preparative HPLC.

Teucrifaride A (**1**): Yellow oil; UV (MeOH) λ_{max} (log ϵ): 200 nm: (2.87); $[\alpha]_D^{25} +14.21$ (0.02, MeOH); IR (KBr) ν_{max} : 3444, 2935, 1786, 1678, 1118, 1010 cm^{-1} ; 1D NMR data: see Tables 1 and 2. HR-ESI-MS $[M+Na]^+$: m/z 367.1149 (calcd for C₁₉H₂₀O₆Na⁺, 367.1152).

Teucrifaride B (**2**): Yellow oil; UV (MeOH) λ_{max} (log ϵ): 200 (2.87) nm, 254 (2.00) nm; $[\alpha]_D^{20} +34.62$ (0.03, MeOH); IR (KBr) ν_{max} : 2933, 2866, 1734, 1676, 1558, 1508, 1155 cm^{-1} ; 1D NMR data: see Tables 1 and 2; HR-ESI-MS $[M+Na]^+$: m/z 353.1346 (calcd for C₁₉H₂₂O₅Na⁺, 353.1359).

Teucrifaride C (**3**): White and amorphous powder; UV (MeOH) λ_{max} (log ϵ): 206 nm: (3.28); $[\alpha]_D^{20} -54.17$ (0.02, MeOH); IR (KBr) ν_{max} : 3525, 2939, 1689, 1180 cm^{-1} ; 1D NMR data: see Tables 1 and 2; HR-ESI-MS $[M+Na]^+$: m/z 411.1408 (calcd for C₂₁H₂₄O₇Na⁺, 411.1414).

Teucrifaride D (**4**): Colorless acicular crystal; mp 151–152 °C; UV (MeOH) λ_{max} (log ϵ): 208 nm: (3.68); $[\alpha]_D^{20} + 27.03$ (0.01, MeOH); IR (KBr) ν_{max} : 3155, 2970, 2939, 1774, 1751 cm^{-1} ; 1D NMR data: see Tables 1

and 2; HR-ESI-MS $[M+Na]^+$: m/z 425.1559 (calcd for C₂₂H₂₆O₇Na⁺, 425.1571).

4.4 Single-crystal X-ray diffraction analysis of **4** and **10**

A suitable crystal of compounds **4** and **10** were chosen and subjected to X-ray diffraction analysis on a XtaLAB AFC12 single X-ray diffractometer. The crystal was maintained at 100.00 (10) K for **4**, and at 99.99 (10) K for **10**, during data collection. The structural analysis was initiated using the Olex2 software, where the crystal structures were determined by the SHELXT program with intrinsic phasing. Subsequently, the structures were refined using the SHELXL refinement package, employing least squares minimization techniques [30]. The crystallographic data (CCDC.2383165 for **4** and CCDC.2383166 for **10**) can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

4.5 ECD calculations

ECD calculation methods were the same with previous reports [30].

4.6 Cell viability assay

HT22 cells were grown in a culture medium consisting of DMEM (from HyCyte) supplemented with 1% penicillin/streptomycin (ECOTOP) and 10% fetal bovine serum (HyCyte). The culture was maintained in a 5% CO₂ environment at a temperature of 37 °C within an incubator. HT22 cells were plated at a density of 3000 cells per well in 96-well plates and allowed to attach for 24 h. Subsequently, RSL3 (1 μM) or erastin (1 μM) along with different concentrations of compounds were introduced. After 24 h, MTT reagent (10 μL, 5 mg/mL) was introduced to each well and incubated for an additional 4 h. Following this, the formazan crystals were solubilized using 150 μL of DMSO. The plates were softly shaken for 15 min at ambient temperature, and then tested the absorbance at 490 nm using a microplate reader.

4.7 Intracellular reactive oxygen species detection

HT22 cells were cultured at 300,000 cells/well in 6-well plates for 24 h. RSL3 (1 μM) and three different concentrations (5, 10, 20 μM) of compound **1** and Fer-1 (1 μM) were added to plates. After a 4 h reaction, the supernatant medium was aspirated off and H₂PCFDA (1 mL, 10 μM) was added in an incubator and incubated for 40 min (37 °C), then washed twice with serum-free medium and photographed with a fluorescence microscope under dark conditions.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s13659-024-00489-1>.

Additional file 1.

Author contributions

Huan Wang and Han-Fei Liu: Writing-original draft, methodology, investigation. Xiao-Qiao Yang: Methodology, investigation, formal analysis. Yu-Qiong Liao: Formal analysis, investigation. Fen-Cong Pan: Formal analysis, investigation. Jin-Yu Li: Supervision, Project administration, Writing-original draft. Hua-Yong Lou: Design experiment, Writing – review & editing, Project administration, Funding acquisition. Wei-Dong Pan: Writing-review, editing, project administration. All authors read and approved the final submission.

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Data availability

The data that support the findings of this study are openly available in the Science Data Bank at <https://doi.org/https://doi.org/>

Declarations

Competing interests

There is no competing interest.

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References

1. Flora of China Editorial Committee of Chinese Academy of Sciences. Flora of China. Beijing: Science Press; 1990.
2. Zhang K, Wang XL, Li YK, Li J, Xu KP, Tan GS. Chemical constituents and pharmacological activities of genus *Teucrium*. Central South Pharm. 2016;14(7):735–41.
3. Aydoğan F, Boga M, Khan SI, Zulfiqar F, Khan IA, Ali Z. Phytochemical investigation of *Teucrium pruinatum* and biological potential assessment of the isolated diterpenoids. Biochem Syst Ecol. 2022;105: 104545.
4. Kurimoto S, Wakabayashi K, Sasaki YF, Nakamura T, Kubota T. Teuchamaedol A, a new neo-clerodane diterpenoid from the aerial parts of *Teucrium chamaedrys*. Tetrahedron Lett. 2022;100: 153890.
5. Lv HW, Luo JG, Zhu MD, Zhao HJ, Kong LY. neo-Clerodane diterpenoids from the aerial parts of *Teucrium fruticosum* cultivated in China. Phytochemistry. 2015;119:26–31.
6. Tan Q, Fang Y, Peng X, Zhou H, Xu J, Gu Q. A new ferroptosis inhibitor, isolated from *Ajuga nipponensis*, protects neuronal cells via activating NRF2-antioxidant response elements (AREs) pathway. Bioorg Chem. 2021;115: 105177.
7. Li S, Xu D, Jia J, Zou W, Liu J, Wang Y, Zhang K, Zheng X, Ma YY, Zhang X, Zhao DG. Structure and anti-inflammatory activity of neo-clerodane diterpenoids from *Scutellaria barbata*. Phytochemistry. 2023;213: 113771.
8. Peng X, Tan Q, Zhang Z, Wu D, Xu J, Zhou H, Gu Q. Discovery of neo-clerodane diterpenoids from *Ajuga campylantha* as neuroprotective agents against ferroptosis and neuroinflammation. J Nat Prod. 2023;86(8):2006–21.
9. Krishna Kumari GN, Aravind S, Balachandran J, Ganesh MR, Soundarya Devi S, Rajan SS, Malathi R, Ravikumar K. Antifeedant neo-clerodanes from *Teucrium tomentosum* Heyne (Labiatae). Phytochemistry. 2003. [https://doi.org/10.1016/S0031-9422\(03\)00510-7](https://doi.org/10.1016/S0031-9422(03)00510-7).
10. Kheawchaum S, Mahidol C, Thongnest S, Boonsombat J, Batsomboon P, Sitthimonchai S, Ruchirawat S, Prawat H. Ent-abietane diterpenoid lactone glycosides and a phenolic glycoside from *Phlogacanthus pulcherrimus* T Anderson with cytotoxic and cancer chemopreventive activities. Phytochemistry. 2022;201:113261.
11. Hunan Academy of Chinese Medicine. Pharmaceutical Records of Hunan. Changsha: Hunan People's Publishing House; 1979.
12. National Institutes for Food and Drug Control. Yunnan Institute for Drug Control. In: Annals of Chinese national medicine. Beijing: People's Medical Publishing House; 1984.
13. Xie N, Ming ZD, Zhao SX, Feng R. Flavones from *Teucrium quadrifarium*. J China Pharm Univ. 1991;22(4):200–2.
14. Zhu YY, Li GY. Studies on the diterpenoids of *Teucrium quadrifarium* Buch-Ham. Acta Pharm Sin B. 1993;28(9):679–83.
15. Aydoğan F, Ali Z, Zulfiqar F, Karaalp C, Khan IA, Bedir E. neo-clerodanes from *Teucrium divaricatum* subsp. *divaricatum* and their biological activity assessment. Phytochem Lett. 2023;54:45–9.
16. Simoes F, Rodríguez B, Bruno M, Piozzi F, Savona G, Arnold NA. neo-Clerodane diterpenoids from *Teucrium kotschyianum*. Phytochemistry. 1989;28(10):2763–8.
17. Lv HW, Luo JG, Meng DZ, Shan SM, Kong LY. Teucvisins A-E, five new neo-clerodane diterpenes from *Teucrium viscidum*. Chem Pharm Bull. 2014;62(5):472–6.
18. Aoyagi Y, Yamazaki A, Nakatsugawa C, Fukaya H, Takeya K, Kawachi S, Izumi H. Salvileucalin B, a novel diterpenoid with an unprecedented rearranged neoclerodane skeleton from *Salvia leucantha* Cav. Org Lett. 2008;10(20):4429–32.
19. Piozzi F, Savona G, Paternostro M, Rodríguez BP. New clerodane diterpenoids from *Teucrium spinosum* L. Heterocycles. 1980;14:193.

20. Bruno M, Piozzi F, Savona G, De La Torre MC, Rodríguez B. *neo*-Clerodane diterpenoids from *Teucrium canadense*. *Phytochemistry*. 1989;28(12):3539–41.
21. Li W, Wang RM, Pan YH, Zhao YY, Yuan FY, Huang D, Tang GH, Bi HC, Yin S. Crotonpenoids A and B, two highly modified clerodane diterpenoids with a tricyclo[7.2.1.0^{2,7}]dodecane core from *Croton yanhuii*: isolation, structural elucidation, and biomimetic semisynthesis. *Org Lett*. 2020;22(11):4435–9.
22. Xu G, Peng L, Niu X, Zhao Q, Li R, Sun H. Novel diterpenoids from *Salvia dugesii*. *Helv Chim Acta*. 2004;87(4):949–55.
23. Jiang YJ, Su J, Shi X, Wu XD, Chen XQ, He J, Shao LD, Li XN, Peng LY, Li RT, Zhao QS. *neo*-Clerodanes from the aerial parts of *Salvia leucantha*. *Tetrahedron*. 2016;72(35):5507–14.
24. Papanov GY, Malakov PY. New furanoid diterpenes from *Teucrium scordium* L. *Zeitschrift für Naturforschung B*. 1981;36(1):112–3.
25. Ye D, Shu LP, Qiang Z, Xun L, Li SD. Clerodane diterpenoids from *Kinostemon alborubrum*. *Helv Chim Acta*. 2002;85(8):2547–52.
26. Maria C, Rodríguez B, Bruno M, Savona G, Piozzi F, Servettaz O. *neo*-clerodane diterpenoids from *Teucrium micropodioides*. *Phytochemistry*. 1988;27(1):213–6.
27. Gacs-Baitz E, Kajtar M, Papanov G, Malakov P. Carbon-13 NMR spectra of some furanoid diterpenes from *Teucrium species*. *Heterocycles*. 1982;19:539–50.
28. Mbawambo ZH, Foubert K, Chacha M, Kapingu MC, Magadula JJ, Moshi MM, Lemiere F, Goubitz K, Fraanje J, Peschar R, Vlietinck A, Apers S, Pieters L. New furanoditerpenoids from *Croton jatrophoides*. *Planta Med*. 2009;75(3):262–7.
29. Shi HW, Jiang XF, Cao LD, Peng X, Tan QY, Teng XF, Gu Q, He L. Chemical constituents of *Ajuga forrestii* and their anti-ferroptosis activity. *Fitoterapia*. 2023;166: 105461.
30. Zhao X, Zheng ZP, Chen C, Wang H, Liu HF, Li JY, Sun C, Lou HY, Pan WD. New clerodane diterpenoids from *Callicarpa pseudorubella* and their antitumor proliferative activity. *Fitoterapia*. 2024;174: 105878.

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