

Article

Inflammatory and Cytotoxic Activities of Abietane Terpenoids from *Nepeta bracteata* Benth.

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Abstract: *Nepeta bracteata* Benth. is used clinically to treat tracheal inflammation, coughs, asthma, colds, fevers, adverse urination, and other symptoms, along with functions in clearing heat and removing dampness. However, there have been few studies characterizing the material basis of its efficacy. Therefore, the aim of this study was to screen for compounds with anti-inflammatory activities in *N. bracteata* Benth. Using silica gel, ODS C18, and Sephadex LH-20 column chromatography, as well as semipreparative HPLC, 10 compounds were separated from *N. bracteata* Benth. extract, including four new diterpenoids (1–4), one amide alkaloid (5), and five known diterpenoids (6–10). The structures of all the isolates were elucidated by HR-ESI-MS, NMR, and CD analyses. Using lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, we investigated the anti-inflammatory activities of compounds 1–10. It is worth noting that all were able to inhibit nitric oxide (NO) production with IC₅₀ values < 50 μM and little effect on RAW 264.7 macrophage viability. Compounds 2 and 4 displayed remarkable inhibition with IC₅₀ values of 19.2 and 18.8 μM, respectively. Meanwhile, screening on HCT-8 cells demonstrated that compounds 2 and 4 also had moderate cytotoxic activities with IC₅₀ values of 36.3 and 41.4 μM, respectively, which is related to their anti-inflammatory effects.

Keywords: terpenoids; *Nepeta bracteata* Benth.; anti-inflammatory



Citation: Zhang, M.; Chen, M.; Hou, Y.; Fan, C.; Wei, H.; Shi, L.; Ma, G.; Zhang, J. Inflammatory and Cytotoxic Activities of Abietane Terpenoids from *Nepeta bracteata* Benth.. *Molecules* **2021**, *26*, 5603. <https://doi.org/10.3390/molecules26185603>

Academic Editors: Luisa Tesoriere and Alessandro Attanzio

Received: 10 August 2021

Accepted: 8 September 2021

Published: 15 September 2021

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1. Introduction

Inflammation, a common clinical pathological process, is closely related to many diseases such as arthritis, psychosis, cardiovascular and cerebrovascular diseases, and cancer [1–4]. Current anti-inflammatory drugs, such as glucocorticoids, insulin, and the tyrosinase inhibitor kojic acid, are associated with significant side effects [5,6]. Therefore, finding effective anti-inflammatory drugs with fewer side effects is of great importance. Natural products are an important source for new drug discovery; thus, finding and discovering active components from medicinal plants is a hot topic in pharmaceutical chemistry. *Nepeta bracteata* Benth. belongs to the genus *Nepeta* of the Lamiaceae family and is mainly distributed in Pakistan, Nepal, Iran, and other countries. It is a folk medicine used by Xinjiang Uyghurs and a medicinal material imported for use in the Xinjiang Uyghur hospital, with the Uyghur name “Zufa” [7]. Clinically, it is used to treat tracheal inflammation, coughs, asthma, colds, fevers, adverse urination, and other symptoms [8,9], along with functions in clearing heat and removing dampness. While modern pharmacology has shown that its extract has significant anti-inflammatory activity, no related research has been conducted to characterize its chemical components [10–12]. With the aim of screening for anti-inflammatory active compounds in *N. bracteata* Benth., a 95% ethanol extract

was investigated, and 10 compounds—four new diterpenoids, nepetabrates A–D (1–4), one amide alkaloid, 6-methyl-1,4-oxazocane-5,8-dione (5), and five known diterpenoids, angustanoic acid F (6) [13], 7a-hydroxycallitrisic acid (7) [14], 1-phenanthrenecarboxylic acid (8) [15], angustanoic acid G (9) [16], and jiadifenoic acid K (10) [17]—were obtained (drawn in Figure 1). The structures of the isolates were characterized using comprehensive spectroscopic data analyses. Moreover, the anti-inflammatory activities of the isolated compounds were investigated. In this paper, we describe the structural elucidation of the isolated compounds, as well as their potential anti-inflammatory and cytotoxic effects.

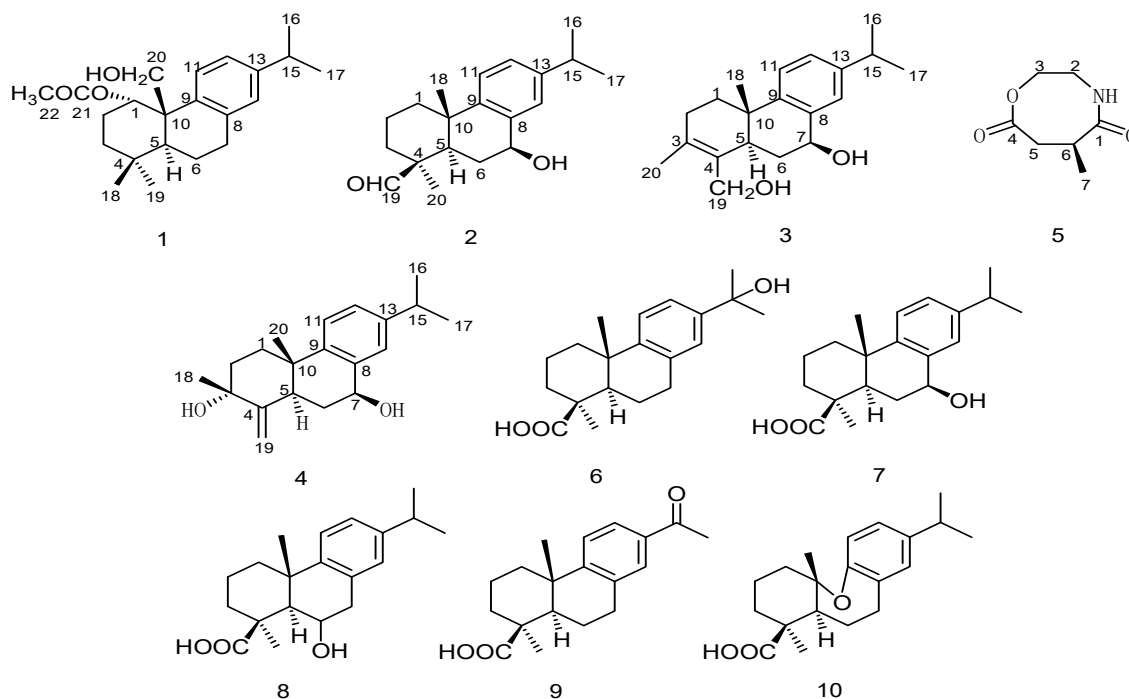


Figure 1. Structures of compounds 1–10.

2. Results

2.1. Structure Elucidation

Compound 1 was isolated as a white powder. The molecular formula $C_{22}H_{32}O_3$ was established from the HR-ESI-MS spectrum with a positive-ion peak at m/z $[M + Na]^+$ 367.2215 (calculated 367.2249). The IR spectrum of 1 showed absorptions of hydroxyl (3339 cm^{-1}) and ester carbonyl (1759 cm^{-1}) groups. In the $^1\text{H-NMR}$ spectrum (Table 1), compound 1 showed peaks for three aromatic protons at δ_{H} 7.14 (1H, d, $J = 8.4$ Hz), 7.17 (1H, d, $J = 8.4$ Hz), and 7.22 (1H, d, $J = 1.8$ Hz), which suggests the presence of a benzene moiety. Four methyl protons at δ_{H} 1.25 (3H, d, $J = 7.2$ Hz), 0.97 (3H, d, $J = 7.2$ Hz), 1.15 (3H, s), and 1.07 (1H, s) indicate the basic diterpenoid skeleton. The downfield methyl signal at δ_{H} 2.07 (3H, s) is evidence for the existence of an acetoxy group. Meanwhile, three oxygenated protons at δ_{H} 4.82 (1H, m), 4.00 (1H, d, $J = 6.6$ Hz), and 4.32 (1H, dd, $J = 6.6, 2.4$ Hz) suggest the presence of $-\text{OCH}_2-$ and $-\text{OCH}-$ groups in the structure, which is in accordance with the $^{13}\text{C-NMR}$ spectrum showing signals at δ_{C} 68.5, 67.3. The $^{13}\text{C-NMR}$ spectrum (Table 1) revealed six aromatic carbon signals at δ_{C} 135.9, 147.1, 124.9, 126.9, 146.8, and 127.9. Aside from these aromatic carbons, the $^{13}\text{C-NMR}$ also showed five methyl signals at δ_{C} 24.2, 13.9, 24.8, 28.7, and 19.4, five methylene signals at δ_{C} 36.2, 37.9, 19.4, 27.4, and 67.3, three methine signals at δ_{C} 68.5, 45.3, and 33.7, and two quartus carbon signals at δ_{C} 36.8 and 38.6. The proton signals were assigned to the corresponding carbons through direct ^1H and ^{13}C correlations in the HSQC spectrum. From the $^1\text{H}-^1\text{H}$ COSY analysis, four substructures (drawn with bold bonds in Figure 2) were established as $\text{H}_2-1/\text{H}_2-2/\text{H}_2-3$,

H-5/H₂-6/H₂-7, H-11/H-12, and H-15/H₃-16/H₃-17, suggesting that compound **1** has an abietane diterpene skeleton [16]. In the HMBC spectrum (Figure 2), the correlations from δ_H 4.82 (1H, m, H-1) to δ_C 171.6 indicate that the acetoxy group is attached to C-1. Meanwhile, the HMBC correlations from δ_H 4.00 (1H, d, $J = 6.6$ Hz) and 4.32 (1H, dd, $J = 6.6, 2.4$ Hz) to C-10 (δ_C 38.6) and C-9 (δ_C 147.1) indicate that the angular methyl group at C-10 was further oxidized to a hydroxymethyl unit. Thus, the planar structure of compound **1** was fully elucidated. In the NOESY spectrum, the enhancement between H-1 and H₂-20, H-5, and H₃-19 suggests the α -orientation of the acetoxy group at C-1. Considering the identical biosynthetic relationship of abietane diterpenoids, the absolute configuration of **1** can be inferred as 1*S*, 5*S*, 10*S*. The ECD spectra were calculated using density functional theory (DFT) at the APFD/6-311 + g (2d, p) level to further support the deduction (Figure 3). As a result, the structure of compound **1** was determined as shown and given the trivial name nepetabrate A.

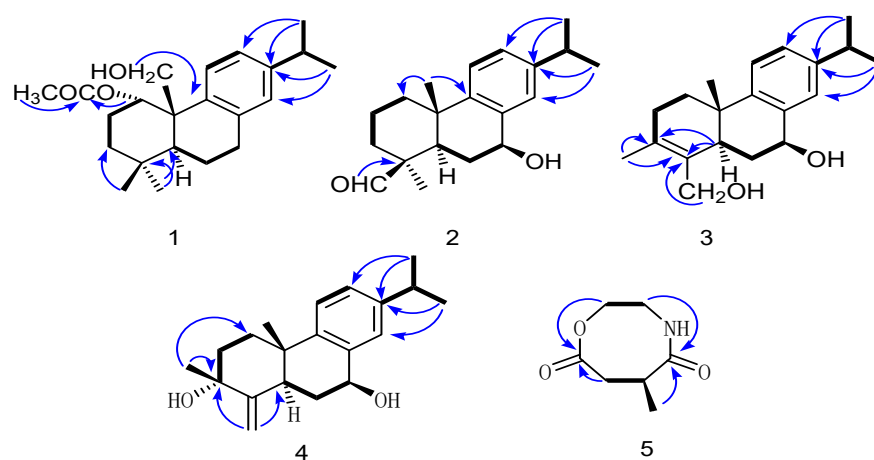


Figure 2. Key ^1H - ^1H COSY (in bolds) and HMBC (arrows) correlations of compounds 1–5.

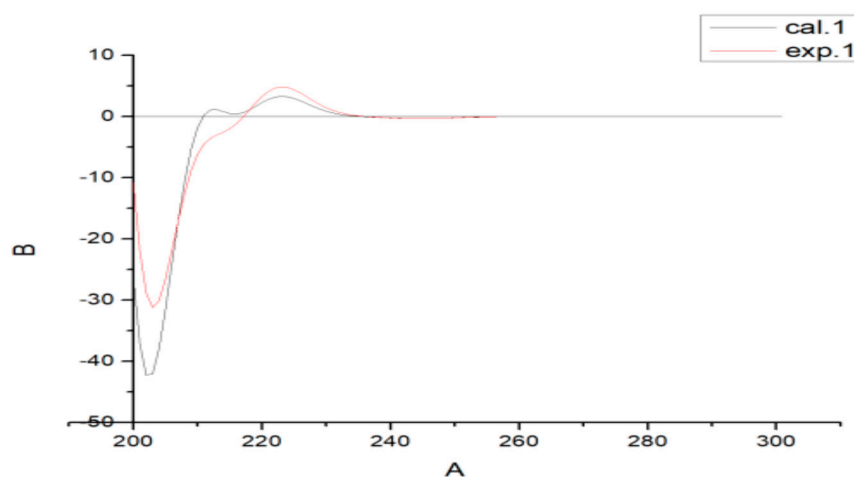


Figure 3. Experimental and calculated ECD spectra of **1**.

Table 1. NMR spectral data of 1–5 (600 MHz for ¹H-NMR and 150 MHz for ¹³C-NMR).

No.	1 ^a		2 ^a		3 ^a		4 ^a		5 ^a	
	δ_C , Type	δ_H (J in Hz)	δ_C , Type	δ_H (J in Hz)	δ_C , Type	δ_H (J in Hz)	δ_C , Type	δ_H (J in Hz)	δ_C , Type	δ_H (J in Hz)
1	68.5, CH	4.82, m	36.2, CH ₂	2.38, m; 1.63, m	34.4, CH ₂	2.33, m; 1.62, m	39.1, CH ₂	1.90, m; 1.65, m	177.4, C	–
2	36.2, CH ₂	1.82, m; 1.21, m	29.9, CH ₂	2.89, m; 1.27, m	30.5, CH ₂	2.76, m; 1.23, m	30.5, CH ₂	1.86, m; 1.29, m	41.9, CH ₂	3.71, m; 2.87, m
3	37.9, CH ₂	2.33, d (12.6); 5566771.43, m	21.1, CH ₂	1.89, m; 1.33, m	126.5, C	–	71.5, C	–	60.9, CH ₂	3.79, m; 2.97, m
4	36.8, C	–	55.5, C	–	136.2, C	–	153.7, C	–	181.5, C	–
5	45.3, CH	1.87, m	43.0, CH	1.87, m	37.7, CH	2.32, d (6.0)	36.9, CH	2.31, m	36.6, CH ₂	2.99, m; 2.30, m
6	19.4, CH ₂	1.46, m; 1.72, m	27.2, CH ₂	1.95, m; 1.85, m	32.8, CH ₂	1.75, m; 1.63, m	33.7, CH ₂	2.01, m; 1.03, m	34.9, CH	2.91, m
7	27.4, CH ₂	2.13, m; 2.01, m	71.9, CH	3.85, dd (8.4, 4.2)	69.5, CH	4.83, t (9.0)	68.6, CH	4.86, t (4.8)	16.9, CH ₃	1.37, d (7.2)
8	135.9, C	–	134.6, C	–	136.4, C	–	135.9, C	–	–	–
9	147.1, C	–	146.5, C	–	148.0, C	–	146.9, C	–	–	–
10	38.6, C	–	38.2, C	–	36.5, C	–	43.0, C	–	–	–
11	124.9, CH	7.14, d (8.4)	124.7, CH	7.17, d (9.0)	124.3, CH	7.28, d (7.8)	125.7, CH	7.25, s	–	–
12	126.9, CH	7.17, d (8.4)	127.2, CH	6.91, d (9.0)	126.6, CH	7.16, dd (7.8, 2.4)	126.8, CH	7.15, dd (8.4, 1.8)	–	–
13	146.8, C	–	145.7, C	–	144.5, C	–	144.7, C	–	–	–
14	127.9, CH	7.22, d (1.8)	129.0, CH	7.72, d (3.6)	128.2, CH	7.72, d (2.4)	128.3, CH	7.22, d (8.4)	–	–
15	33.7, CH	2.89, m	33.7, CH	2.83, m	24.4, CH	1.95, m	33.7, CH	1.24, m	–	–
16	24.2, CH ₃	1.25, d (7.2)	13.9, CH ₃	1.21, s	19.4, CH ₃	0.85, s	24.2, CH ₃	1.26, dd (6.6, 2.4)	–	–
17	13.9, CH ₃	0.97, t (7.2)	13.9, CH ₃	1.21, s	21.5, CH ₃	0.85, s	24.0, CH ₃	1.26, dd (6.6, 2.4)	–	–
18	24.8, CH ₃	1.15, s	25.4, CH ₃	1.22, d (1.8)	33.7, CH ₃	1.79, s	21.0, CH ₃	0.88, s	–	–
19	28.7, CH ₃	1.07, s	206.6, CH	9.43, s	61.9, CH ₂	(12.0); 5566774.69, d (12.0)	107.1, CH ₂	5.12, s; 5566774.74, s	–	–
20	67.3, CH ₂	4.00, d (6.6); 5566774.32, dd (6.6, 2.4)	23.7, CH ₃	1.16, s	13.9, CH ₃	1.65, s	28.1, CH ₃	1.45, s	–	–
21	171.6, C	–	–	–	–	–	–	–	–	–
22	21.2, CH ₃	2.07, s	–	–	–	–	–	–	–	–

^a Spectral data were recorded in CDCl₃.

Compound **2** was obtained as a white powder with its molecular formula assigned as $C_{20}H_{28}O_2$ according to the positive HR-ESI-MS peak at m/z $[M + Na]^+$ 323.2013 (calculated 323.2089), exhibiting seven degrees of unsaturation. Through the 1H - and ^{13}C -NMR spectra, we inferred that the basic mother nucleus of compound **2** was an abietane diterpene, which was further confirmed by the 1H - 1H COSY and HMBC spectra (Figure 2). In fact, the NMR data of compound **2** were similar to those reported for 1-phenanthrenecarboxylic acid, except for an additional aldehyde signal at δ_H 9.43 (s), δ_C 206.6, and oxygenated methine carbon at δ_C 71.9 [15]. The presence of the aldehyde group was due to the oxidation of a methyl group at C-19, as supported by the correlations between H-19 (δ_H 9.43) and C-4 (δ_C 55.5) in the HMBC spectrum. In addition, one hydroxyl group placed at C-7 led to the downfield chemical shift of C-7 (δ_C 71.9), as confirmed by HMBC correlations. The relative configuration of compound **2** was established by analysis of its NOESY data. The key NOE correlations between H-5 and H-7 and between H₃-18 and H-19 supported the β -orientations of both the aldehyde and the hydroxyl groups. Combined with the experimental and calculated CD curves (Supplementary Materials, Figure S31), the absolute configuration of compound **2** was identified as established and given the trivial name nepetabrate B.

Compound **3**, purified as a white powder, has a molecular formula of $C_{20}H_{28}O_2$, deduced from the HR-ESI-MS quasimolecular ion at m/z 323.2021 $[M + Na]^+$ (calculated 323.2089). The 1H - and ^{13}C -NMR spectroscopic data (Table 1) of **3** were similar to those of compound **2**, except for the additional hydroxymethyl group at δ_C 61.9 and double bond at δ_C 126.5 and 136.2, indicating that compound **3** is an analogue of compound **2**. In the HMBC spectrum, the correlations from H-5 (δ_H 2.32) to C-3 (δ_C 126.5) and C-4 (δ_C 136.2), from H₃-20 (δ_H 1.65) to C-4 (δ_C 136.2), and from H₂-19 to C-3 (δ_C 126.5) (Figure 2) implied a double bond at C-3/C-4, as well as substitutions of its methyl and hydroxymethyl groups. Compound **3** is the product of methyl migration and dehydrogenation of compound **2**, which is not common in abietane diterpenes. The similar NOESY spectra of compounds **2** and **3** suggest their identical relative configurations. Together with the experimental and calculated CD curves (Supplementary Materials, Figure S32), compound **3** was elucidated as nepetabrate C.

Compound **4** was obtained as a white powder with a molecular formula of $C_{20}H_{28}O_2$ based on the positive-ion HR-ESI-MS peak at m/z 323.2023 $[M + Na]^+$ (calculated 323.2089). The 1H - and ^{13}C -NMR spectroscopic data (Table 1) of **4** were quite similar to those of **3**, except for the appearance of olefin protons at δ_H 5.12 and 4.74 and the disappearance of two oxygenated protons in **4**. Further analysis of the NMR data of compound **4** revealed the presence of an outer ring double bond at C-4/19, which was confirmed by the HMBC correlations from H-19 (δ_H 4.78, 4.69) to C-3 (δ_C 71.5) and C-5 (δ_C 36.9). Furthermore, the quartet carbon of C-3 (δ_C 71.5), together with the molecular formula above, implies the presence of hydroxyl substitution in the structure. The HMBC correlations from H₃-18 (δ_H 0.88) to δ_C 71.5 reveal the location of CH₃-18 at C-3. The key NOESY enhancements between H-5 and H-7 and between CH₃-18 and CH₃-20 revealed the opposing configurations of 3-OH and 7-OH. The absolute configuration was determined by DFT calculations of the ECD spectra (Supplementary Materials, Figure S33), which confirmed the 3*R*, 5*R*, 7*S*, and 10*S* configurations. Thus, compound **4** was assigned as nepetabrate D.

Compound **5** was obtained as a yellow amorphous powder, with its molecular formula assigned as $C_7H_{11}NO_3$ on the basis of its positive HR-ESI-MS (m/z 180.0723 $[M + Na]^+$ (calculated 180.0739), implying three degrees of unsaturation. The IR spectrum of **5** showed carbonyl (1724 cm^{-1}) and methyl ($2938, 2924\text{ cm}^{-1}$) groups. In the 1H -NMR spectrum (Table 1), compound **5** showed four downfield chemical signals at δ_H 3.79, 2.97, 2.99, and 2.39, one methyl proton at δ_H 1.37 (3H, d, $J = 7.2\text{ Hz}$), and one methine proton at δ_H 2.91 (1H, m). The ^{13}C -NMR spectrum (Table 1) revealed the presence of two carbonyl groups at δ_C 177.4 and 181.5, three methylene carbons at δ_C 41.9, 60.9, and 36.6, one methine carbon at δ_C 34.9, and one methyl carbon at δ_C 16.9. Analysis of the 1H - 1H COSY correlations revealed the presence of two partial structures of $-(CH_2)_2-$ and $-CH_2-(CH)CH_3-$, as shown

in Figure 2. In the HMBC spectrum, the correlations from δ_{H} 3.79, 2.97 (2H, m, H-3) and δ_{H} 2.99 (1H, H-5a), 2.39 (1H, dd, $J = 4.2, 4.2$ Hz, H-5b) to δ_{C} 181.5 (C-4), δ_{H} 3.71, 2.87 (2H, m, H-2), and δ_{H} 1.37 (3H, d, $J = 7.2$ Hz, H₃-7) to δ_{C} 177.4 (C-1) demonstrate that the two fragments were connected through ester carbonyl carbons. Further analysis of the carbon signal of C-2 (δ_{C} 41.9) and the molecular formula above confirmed an amide unit between C-2 and C-6. The absolute configuration of C-6 was assigned as *S* on the basis of a comparison of its experimental and calculated CD curves. From all the above data, compound 5 was established as shown and named 6-methyl-1,4-oxazocane-5,8-dione.

2.2. Bioactive Activity

The anti-inflammatory activities of compounds 1–10 were evaluated in RAW 264.7 macrophages with aspirin as the positive control [18,19]. As shown in Table 2, in the RAW 264.7 macrophage viability test, all compounds showed mild or inactive effects for RAW 264.7 macrophages. On the contrary, compounds 1–10 displayed different degrees of anti-inflammatory activity against RAW 264.7 macrophages, with IC₅₀ values ranging from 18.0 to 46.3 μM . Among the abietane diterpenes, compounds 2 and 4 displayed the most anti-inflammatory activity with IC₅₀ values of 19.2 and 18.8 μM , respectively. Compound 5, as an amide alkaloid, also showed a remarkable inhibition effect with an IC₅₀ value of 18.0 μM , as compared with the IC₅₀ values of 15.9 for the positive control, aspirin. Cytotoxic testing on HCT-8 cells showed that compounds 2 and 4 had moderate activity with IC₅₀ values of 36.3 and 41.4 μM , respectively, while the other compounds were inactive or mildly active when compared with the positive control, adriamycin.

Table 2. Anti-inflammatory and cytotoxic activities of the isolated compounds.

Compounds	Cell Survival Rate (%)		IC ₅₀ (μM)	
	RAW 264.7 Macrophages	RAW 264.7 Macrophages	RAW 264.7 Macrophages	HCT-8
1	97.93 \pm 0.26		38.2 \pm 1.15 ^a	>50
2	88.26 \pm 0.32		19.2 \pm 1.25	36.3 \pm 1.10
3	99.84 \pm 0.21		22.3 \pm 1.26	>50
4	92.21 \pm 0.19		18.8 \pm 0.75	41.4 \pm 0.91
5	99.21 \pm 0.15		18.0 \pm 1.13	>50
6	99.89 \pm 0.13		36.2 \pm 1.21	>50
7	98.75 \pm 0.45		37.1 \pm 0.81	>50
8	99.12 \pm 0.28		37.5 \pm 0.92	>50
9	98.26 \pm 0.18		42.3 \pm 0.56	>50
10	98.75 \pm 0.20		46.3 \pm 1.02	>50
Aspirin ^b			15.9 \pm 0.38	
Adriamycin				1.78 \pm 0.14
DMEM (PBS) ^c	100.00			

^a Values are means \pm SD of triplicate experiments. ^b Positive control substance. ^c Negative control substance.

3. Discussion

Although *Nepeta bracteata* Benth. is widely used clinically and has promising curative effects, there are few studies about this medicinal plant. Previous research found that *N. bracteata* Benth. has certain free-radical-scavenging ability and an anti-inflammatory effect in vitro, and its alcohol extract displayed certain DPPH free-radical-scavenging ability, providing a theoretical basis for further research [20,21]. Therefore, we first investigated the active substances of *N. bracteata* Benth. and obtained nine abietane diterpenoids including four new ones, nepetabrates A–D (1–4), and one new amide alkaloid (5). Compared with previous studies, we explored the anti-inflammatory activities of compounds 1–10 on RAW 264.7 macrophages cells. The cell viability of the RAW 264.7 macrophages experiment displayed that all the isolated compounds had mild toxicity to cells at 50 μM . The anti-inflammatory activity test showed that all the abietane diterpenoids displayed different degrees of inhibition effect. Among them, compounds 2 and 4 displayed the greatest anti-inflammatory activities with IC₅₀ values 19.2 and 18.8 μM , as well as moderate cytotoxic

activities with IC_{50} values of 36.3 and 41.4 μM , further proving the correlation between inflammation and cancer. Moreover, the results also showed that compound 5 had significant anti-inflammatory activity but had no significant advantage over diterpenes. Accordingly, we believe that diterpenes represent the material basis for the plant to exert its clinical anti-inflammatory effect, which deserves further study.

4. Materials and Methods

4.1. General Experimental Procedures

Optical rotation data were measured using a Perkin-Elmer 341 digital polarimeter (PerkinElmer, Norwalk, OH, USA). UV (1.0 mg of sample was dissolved in 3 mL of chromatographic grade methanol for each sample) and IR (1.0 mg of sample was pressed in KBr for each sample) spectral data were recorded on Shimadzu UV2550 and FTIR-8400S spectrometers (Shimadzu, Kyoto, Japan). CD spectra were obtained using a JASCO J-815 spectropolarimeter. NMR spectra were obtained using a Bruker AV III 600 NMR spectrometer with chemical shift values presented as δ values using TMS as the internal standard (samples dissolved in an appropriate amount of deuterated chloroform). HR-ESI-MS was performed using an LTQ-Orbitrap XL spectrometer (Thermo Fisher Scientific, Boston, MA, USA); samples were dissolved in chromatographic methanol and treated through a membrane, single pump. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Plant, Qingdao, China). Semi-preparative HPLC was performed using an HPLC PUMP K-501, LC3000 high-performance liquid chromatograph (Beijing Tong Heng Innovation Technology Co., Ltd, Beijing, China), and Kromasil 100-5C18, 250 \times 10 mm, E108850. Precoated silica gel GF₂₅₄ plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development, Yantai, China) were used for TLC. All solvents used (petroleum ether, ethyl acetate, dichloromethane, methanol (analytical grade and chromatographic grade), and deuterated chloroform) were of analytical grade (Beijing Chemical Plant, Beijing, China).

4.2. Plant Material

Nepeta bracteata Benth. was purchased from Xinjiang Uygur hospital (Urumqi, China) and identified as *Nepeta bracteata* Benth. by Professor Leiling Shi. A voucher specimen (M20191025) was deposited at the Medical Laboratory of Xinjiang Institute of Chinese and Ethnic Medicine (Urumqi, China).

4.3. Isolation and Purification of Compounds 1–10

The aerial part of *Nepeta bracteata* Benth. (6.0 kg) was soaked in ethanol at room temperature (3 \times 40 L, 3 h each time) and extracted three times under reflux. Removal of the ethanol under reduced pressure yielded the ethanol extract (437.0 g). The ethanol extract was dissolved in water and successively extracted with petroleum ether (3 \times 1000 mL), dichloromethane (3 \times 1000 mL), and ethyl acetate (3 \times 1000 mL). The petroleum fraction (134.8 g) was subjected to CC (12.0 cm \times 40.0 cm, 300.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 1:0 to 0:1; i.e., 1:0, 100:1, 50:1, 25:1, 8:1, 5:1, 1:1, and 0:1, *v/v*) to yield fractions A–H. Fr.F was subjected to CC (5.0 cm \times 15.0 cm, 70.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 20:1 to 1:1; i.e., 20:1, 10:1, 3:1, and 1:1, *v/v*) to yield four fractions (Fr.F 1–4). Fr.F 3 was subjected to CC (3.0 cm \times 20.0 cm, 65.0 g) over a silica gel (200–300 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 10:1 to 3:1; i.e., 10:1, 5:1, and 3:1, *v/v*) to yield three fractions (Fr.F 3-1–3). Fr.F 3-2 was purified using semi-preparative HPLC with MeOH/H₂O (90:10, *v/v*) as the mobile phase to yield compound 1 (9.0 mg, t_R = 42.4 min). Fr.F 3-3 was purified using semi-preparative HPLC of MeOH/H₂O (85:15, *v/v*) as the mobile phase to yield compounds 2 (5.2 mg, t_R = 27.0 min) and 3 (9.2 mg, t_R = 28.6 min). Fr.H was subjected to CC (4.0 cm \times 15.0 cm, 30.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 15:1 to 1:1; i.e., 15:1, 5:1, 3:1 and 1:1, *v/v*) to yield four fractions (Fr.H 1–4). Fr.F 3 was

subjected to CC (3.0 cm × 20.0 cm, 70.0 g) over a silica gel (200–300 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 4:1 to 1:1; i.e., 4:1, 2:1, and 1:1, *v/v*) to yield three fractions (Fr.H 3-1–3). Fr.F 3-3 was purified by semi-preparative HPLC of MeOH/H₂O (85:15, *v/v*) as the mobile phase to yield compound **4** (3.2 mg, *t_R* = 25.6 min). The dichloromethane fraction (67.4 g) was subjected to CC (8.0 cm × 40.0 cm, 240.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of CH₂Cl₂/MeOH (from 1:0 to 0:1; i.e., 1:0, 100:1, 50:1, 30:1, 20:1, 5:1, 1:1, and 0:1, *v/v*) to yield fractions I–O. Fr.I was subjected to CC (8.0 cm × 15.0 cm, 105.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 15:1 to 1:2; i.e., 15:1, 8:1, 4:1, 2:1, 1:1, and 1:2, *v/v*) to yield six fractions (Fr.I 1–6). Fr.I 3 was isolated through ODS MPLC elution with MeOH/H₂O (50:50, 70:30, 90:10, and 100:0, *v/v*), and purified using semi-preparative HPLC to give compounds **6** (2.0 mg, *t_R* = 23.2 min), **7** (2.3 mg, *t_R* = 26.4 min), and **8** (1.4 mg, *t_R* = 12.7 min). Fr.J was subjected to CC (6.0 cm × 20.0 cm, 70.0 g) over a silica gel (100–200 mesh), eluting with a stepwise gradient of petroleum ether/EtOAc (from 15:1 to 1:2; i.e., 15:1, 8:1, 4:1, 2:1, 1:1, and 1:2 *v/v*) to yield six fractions (Fr.J 1–6). Fr.J 2 was purified by semi-preparative HPLC of MeOH/H₂O (85:15, *v/v*) as the mobile phase to yield compounds **9** (3.6 mg, *t_R* = 15.9 min) and **10** (1.4 mg, *t_R* = 19.3 min). Fr.J 6 was purified by semi-preparative HPLC of MeOH/H₂O (38:62, *v/v*) as the mobile phase to yield compound **5** (3.6 mg, *t_R* = 9.5 min).

4.4. Characterization of Compounds 1–5

Nepetabrate A (**1**), white powder (MeOH); UV (MeOH) λ_{\max} (log ϵ) 291 (3.52) nm; IR (film) ν_{\max} 3339, 2962, 2871, 1759, 1467, 1213, 1144 cm⁻¹; ¹H- and ¹³C-NMR data (CDCl₃), see Table 1; HR-ESI-MS *m/z* 367.2215 [M + Na]⁺ (calculated 367.2249, C₂₂H₃₂O₃).

Nepetabrate B (**2**), white powder (MeOH); UV (MeOH) λ_{\max} (log ϵ) 294 (3.76) nm; IR (film) ν_{\max} 3338, 2994, 2936, 2872, 1460, 1221, 1099 cm⁻¹; ¹H- and ¹³C-NMR data (CDCl₃), see Table 1; HR-ESI-MS *m/z* 323.2013 [M + Na]⁺ (calculated 323.2089, C₂₀H₂₈O₂).

Nepetabrate C (**3**), white powder (MeOH); UV (MeOH) λ_{\max} (log ϵ) 292 (3.93) nm; IR (film) ν_{\max} 3342, 2958, 2875, 1456, 1230, 1138 cm⁻¹; ¹H- and ¹³C-NMR data (CDCl₃), see Table 1; HR-ESI-MS *m/z* 323.2021 [M + Na]⁺ (calculated 323.2089, C₂₀H₂₈O₂).

Nepetabrate D (**4**), white powder (MeOH); UV (MeOH) λ_{\max} (log ϵ) 294 (3.53) nm; IR (film) ν_{\max} 3351, 2940, 2866, 1445, 1227, 1115 cm⁻¹; ¹H- and ¹³C-NMR data (CDCl₃), see Table 1; HR-ESI-MS *m/z* 323.2023 [M + Na]⁺ (calculated 323.2089, C₂₀H₂₈O₂).

6-Methyl-1,4-oxazocane-5,8-dione (**5**), yellow amorphous powder (MeOH); UV (MeOH) λ_{\max} (log ϵ) 291 (3.76) nm; IR (film) ν_{\max} 3350, 2938, 2874, 1724 cm⁻¹; ¹H- and ¹³C-NMR data (CDCl₃), see Table 1; HR-ESI-MS *m/z* 180.0723 [M + Na]⁺ (calculated 180.0739, C₇H₁₁NO₃).

4.5. RAW 264.7 Macrophage Viability Test

The MTT colorimetric method was used to detect the effect of compounds **1–10** on the viability of RAW 264.7 macrophages. The RAW 264.7 macrophages in the logarithmic growth phase were digested with trypsin to prepare a single-cell suspension, which was seeded in a 96-well plate at a density of 1 × 10⁴ cells per well and cultured in a 5% CO₂ incubator for 24 h at 37 °C, before discarding the supernatant. The blank control group was cultured with 10% FBS-containing DMEM, and the drug group was treated with aqueous solutions of compounds **1–10**, with six replicate wells for each concentration. Incubation was continued in 5% CO₂ at 37 °C. After 24 h of incubation, 10 μ L of 5 mg/mL MTT was added to each well. The culture solution was removed after culturing for 4 h. Then, 100 μ L of DMSO was added to each well, before shaking for 10 min to achieve complete dissolution. The optical density (OD) was measured at 492 nm using a microplate reader to calculate cell viability.

4.6. Anti-Inflammation Assay

The anti-inflammatory activity of the isolated compounds was evaluated in lipopolysaccharide-stimulated RAW 264.7 macrophages using the MTT colorimetric method. The

RAW 264.7 macrophages were seeded in 96-well plates at a density of 1×10^4 cells per well for 24 h, followed by treatment with different extracts of identical purity for another 24 h. The compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted appropriately just before cell treatments. Cells were incubated with the extract at indicated concentrations, with DMSO not exceeding 0.1% in all experiments. The cells were cultured in DMEM with 10% FBS and antibiotics (100 U/mL penicillin and 100 μ g/mL streptomycin) at 37 °C with 5% CO₂. NO release was measured as an indicator of the nitrite concentration.

4.7. Cytotoxicity Test

The cytotoxic activities of compounds 1–10 against HCT-8 cells were tested using the MTT colorimetric method. HCT-8 cells were cultivated on DMEM medium at 37 °C and 5% CO₂. After diluting the DMEM medium, cells were seeded into 96-well sterile microplates (6×10^4 cells/well) and cultured with a series of various concentrations of tested compounds or adriamycin (positive control) for 24 h at 37 °C. After incubation, all compounds were tested at five concentrations (10–100 μ M) for 1 h. Following this, the supernatant was removed, and all components were dissolved in 100% DMSO, at such an amount that there was a final DMSO concentration of 0.1% added to each well. The absorbance was measured using a microplate reader at a wavelength of 570 nm. Data are displayed as the means \pm SD ($n = 3$). The cell growth assay was repeated three times, and the IC₅₀ values were calculated using Microsoft Excel software.

5. Conclusions

Nine abietane diterpenoids, including four new ones and one new amide alkaloid, were obtained from the ethnic medicine *Nepeta bracteata* Benth. for the first time, which clarified the active substances of *N. bracteata* Benth. and laid the foundation for its further clinical application. Furthermore, the anti-inflammatory and cytotoxic activities of all isolates were tested. Compounds 2 and 4 displayed potential biological activities with IC₅₀ values of 19.2 and 18.8 μ M in the anti-inflammation assay and IC₅₀ values of 36.3 and 41.4 μ M in the cytotoxicity test, respectively. Both compounds are active molecules with potential research value.

Supplementary Materials: Supplementary data associated with this article can be found in the online version.

Author Contributions: L.S., G.M. and J.Z. conceptualized and designed the experiments; M.Z. performed the experiments; M.C. and Y.H. helped with data analysis; C.F. and H.W. helped with structure elucidation. All authors have read and agreed to the published version of the manuscript.

Funding: The work was financially supported by the sub-project of the National Key R&D Program (2019YFC1712303), the Special Regional Collaborative Innovation Project of Xinjiang Uygur Autonomous Region (2020E01011), and the Major Science and Technology projects of Xinjiang Uygur Autonomous Region (202107638).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data of the NMR and cellular anti-inflammatory and toxic activity presented in this study are available in supporting information.

Acknowledgments: The authors especially thank Xiaoling Ma for her guidance and help in the process of revising the article.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds 1–10 are available from the authors.

References

1. Alexander, A.; Maurya, J.; Swarna; Sahu, P.; Sahu, D.; Deshmukh, H. Various aspects of inflammation and the herbal drugs that are used for the treatment of inflammation: An overview. *J. Pharm. Res.* **2011**, *4*, 1598–1600.
2. Broz, P.; Dixit, V.M. Inflammasomes: Mechanism of assembly, regulation and signalling. *Nat. Rev. Immunol.* **2016**, *16*, 407–420. [[CrossRef](#)]
3. Lee, K.H.; Kang, T.B. The Molecular Links between Cell Death and Inflammasome. *Cells* **2019**, *8*, 1057. [[CrossRef](#)] [[PubMed](#)]
4. Mei, Y.; Li, L.; Wang, X.; Zhang, M.; Xu, Y. AGEs induces apoptosis and autophagy via reactive oxygen species in human periodontal ligament cells. *J. Cell Biochem.* **2019**, *121*, 1–16. [[CrossRef](#)] [[PubMed](#)]
5. Marcondes-De-Mello, L.D.F.; Serafim-Costa, M.S.; Alves-E-Silva, M.M.; Oliveira, N.R.; Chaves, V.E. Effect of glucocorticoids on glyceroneogenesis in adipose tissue: A systematic review. *Biochimie* **2019**, *168*, 201–219. [[CrossRef](#)] [[PubMed](#)]
6. Burnett, C.L.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W. Final report of the safety assessment of kojic acid as used in cosmetics. *Int. J. Toxicol.* **2010**, *29*, 187s–213s. [[CrossRef](#)] [[PubMed](#)]
7. Liu, Y.M.; Yikemu, S.W. *Uighur Medical Records*; Urumqi Xinjiang People's Publishing House: Urumchi, China, 1999; Volume I, pp. 426–429.
8. Xu, F.; Zhao, J.; He, J. Pharmacognostical identification of Uyghur medicine hard pointed divine herb and its adulterant *Schizonepeta tenuifolia*. *China Pharm.* **2012**, *23*, 3321–3323.
9. Zhang, M.; Ma, X.P.; Ma, D.H. Effect of Uighur Medicine *Schizonepeta bracteata* total polysaccharide on cytokines in asthmatic rats. *J. Immunol.* **2012**, *28*, 222–226.
10. Kalpoutzakis, E.; Aligiannis, N.; Mentis, A.; Mentis, S.; Charvala, C. Composition of the essential oil of two *Nepeta* species and in vitro evaluation of their activity against *Helicobacter pylori*. *Planta Med.* **2001**, *67*, 880–883. [[CrossRef](#)] [[PubMed](#)]
11. Milica, G.L.; Miloa, S.; Jelena, V. Antifungalna aktivnost etarskog ulja. *J. Serb. Chem. Soc.* **2008**, *73*, 961–965.
12. Miceli, N.; Taviano, M.F.; Giuffrida, D.; Trovato, A.; Tzakou, O.; Galati, E.M. Anti-inflammatory activity of extract and fractions from *Nepeta sibthorpii* Benth. *J. Ethnopharmacol.* **2005**, *97*, 261–266. [[CrossRef](#)] [[PubMed](#)]
13. Sy, L.; Brown, G.D. Abietane Diterpenes from *Illicium angustisepalum*. *J. Nat. Prod.* **1998**, *61*, 907–912. [[CrossRef](#)] [[PubMed](#)]
14. Yang, X.W.; Feng, L.; Li, S.M.; Liu, X.H.; Li, Y.L.; Wu, L.; Shen, Y.H.; Tian, J.M.; Zhang, X.; Liu, X.R. Isolation, structure, and bioactivities of abiesadines A–Y, 25 new diterpenes from *Abies georgei* Orr. *Bioorgan Med. Chem.* **2010**, *18*, 744–754. [[CrossRef](#)] [[PubMed](#)]
15. Fitzgerald, L.J.; Gerkin, R.E. Phenanthrene-9-carboxylic acid. *Acta Cryst.* **1997**, *53*, 1265–1267. [[CrossRef](#)] [[PubMed](#)]
16. Zhang, G.J.; Li, Y.H.; Jiang, J.D.; Yu, S.S.; Qu, J.; Ma, S.G.; Liu, Y.B.; Yu, D.Q. Anti-Coxsackie virus B diterpenes from the roots of *Illicium jiadifengpi*. *Tetrahedron* **2013**, *69*, 1017–1023. [[CrossRef](#)]
17. Zhang, G.J.; Li, Y.H.; Jiang, J.D.; Yu, S.S.; Wang, X.J.; Zhuang, P.Y.; Zhang, Y.; Qu, J.; Ma, S.G.; Li, Y. Diterpenes and sesquiterpenes with anti-Coxsackie virus B3 activity from the stems of *Illicium jiadifengpi*. *Tetrahedron* **2014**, *70*, 4494–4499. [[CrossRef](#)]
18. Shackelford, R.E.; Alford, P.B.; Xue, Y.; Thai, S.F.; Pizzo, S. Aspirin Inhibits Tumor Necrosis Factor- α Gene Expression in Murine Tissue Macrophages. *Mol. Pharm.* **1997**, *52*, 421–429. [[CrossRef](#)] [[PubMed](#)]
19. Grosser, N.; Schroder, H. Aspirin Protect Endothelial Cells from Oxidant Damage Via the Nitric Oxide-cGMP Pathway. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1345–1351. [[CrossRef](#)] [[PubMed](#)]
20. Jing, W.; Li, F.S.; Pang, N.N.; Tian, G.; Jiang, M.; Zhang, H.P.; Ding, J.B. Inhibition of Asthma in OVA Sensitized Mice Model by a Traditional Uygur Herb *Nepeta bracteata* Benth. *Evid. -Based Complementary Altern. Med.* **2016**, *2016*, 1–8. [[CrossRef](#)] [[PubMed](#)]
21. Hajiheydari, M.R.; Yarmohammadi, M.E.; Izadi, P.; Jafari, F.; Naseri, M. Effect of *Nepeta bracteata* Benth. On allergic rhinitis symptoms: A randomized double-blind clinical trial. *J. Res. Med. Sci.* **2017**, *22*, 1–6.