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Cytotoxicity and molecular docking analysis of new sesquiterpenoids from the leaves of *Datura stramonium* L.

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Abstract

Four new sesquiterpenoids, dstramoniums A-D (**1-4**), and one new natural product (**5**), together with three known compounds (**6–8**), were isolated from the leaves of *Datura stramonium* L. The structures of new compounds were elucidated by extensive spectroscopic analysis and comparison with the literature. Among them, the absolute configuration of **5** was determined by X-ray diffraction analysis. The cytotoxicity of isolates against LN229 cells was assessed and compounds **2**, and **4** displayed strong cytotoxic activity with IC_{50} values lower than 10 μM. Later on, the computational screening of isolates targeting Polo-like kinase-1(PLK-1) was performed by molecular docking in order to validate the isolates *in vitro* results.

Keywords

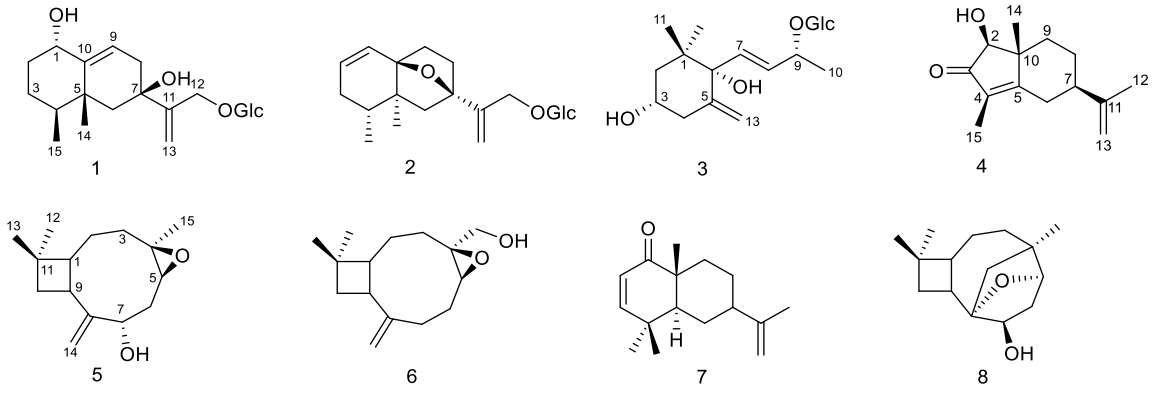
Datura stramonium L.; sesquiterpenoids; cytotoxicity; molecular docking

Introduction

Datura stramonium L. (genus *Datura*, family *Solanaceae*) has long been known globally as a popular folklore medicinal herb and distributed over tropical and warm temperate regions of the earth. It was reported that the *D. stramonium* L has been used traditionally in medicine and ceremonial rituals among the indigenous peoples of the Indian subcontinent, and the different extracts from the leaves were used for the treatment of various diseases, such as asthma, sinus infections, and rheumatism, and to relieve pain. Pharmacological studies have shown that the plant has antiasthmatic, anti-cancer, antibacterial, antioxidant, and anti-inflammatory activities [1-4]. The different pharmacology activities of this plant are due to various secondary metabolites, such as alkaloids, tannins, steroids, flavonoids, and sesquiterpenoids [5]. However, there are few studies related to these plant components, therefore, further phytochemistry investigations were required.

As part of our continuing studies on the novel and bioactive secondary metabolites from the leaves of *Datura stramonium* L., resulting in the isolation of eight new steroids [6]. In this study, eight multifarious sesquiterpenoids (**1-8**), including four new compounds (**1-4**), one new natural product (**5**), and three related known ones (**6-8**) were isolated and characterized from *D. stramonium* L. leaves for the first time (**Fig. 1**). Herein, the isolation, structural elucidation, cytotoxic activities on LN229 cell are described in this manuscript. Furthermore, the possible mechanism of anticancer of bioactive compounds was investigated by molecular docking analysis.

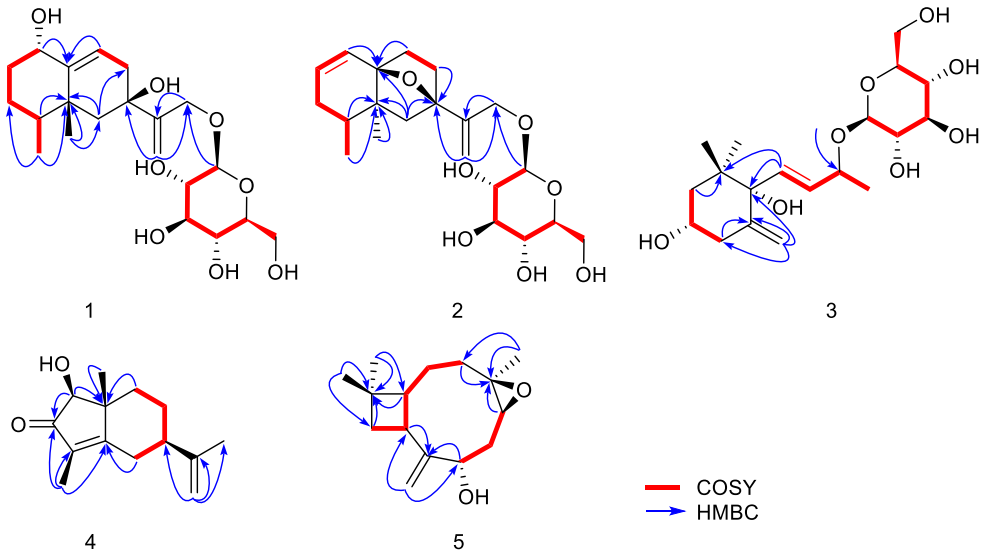
Fig. 1 Chemical structures of compounds (**1-8**).



Results and Discussion

Dstramonium A (**1**) was obtained as a white amorphous powder. Its molecular formula $C_{21}H_{34}O_8$ of **1** was deduced by the ion peak at m/z 459.2223 $[M+HCOO]^-$ (calcd. for 459.2236), in the HR-ESI-MS. The ^{1}H-NMR spectrum of compound **1** showed two methyl proton signals [δ_H 1.10 (3H, s), and 0.88 (3H, d, J = 6.7 Hz)], a β-D-glucopyranoside terminal proton [δ_H 4.35 (1H, d, J = 8.1 Hz)], three-terminal olefinic protons [δ_H 5.57 (1H, br. s), 5.37 (1H, s), and 5.22 (1H, s)]. Additionally, analysis of the ^{13}C NMR (**Table 1**) and DEPT spectra exhibited 21 carbon resonances attributed to three methyl groups, a group of glucose (six carbons), five methylenes (one oxygenated), two methines (one oxygenated), and four quaternary carbons. All groups of **1** have been assigned HSQC, ^{1}H-^{1}H COSY, and HMBC spectra (**Fig. 2**). The coupling constants of H-1 of **1** suggested the hydroxyl group at C-1 to be in an axial orientation. The β orientations of Me-14 and Me-15 were confirmed by the NOESY spectra (**Fig. 3**). The hydroxy group at C-7 of **1** to be in the equatorial orientation by comparing with integrifonol A [7], Thus, **1** was elucidated to be 1α,7β-dihydroxy-14β,15β-dimethyl-eudesman-11(12)-ene-12-O-β-glucopyranoside.

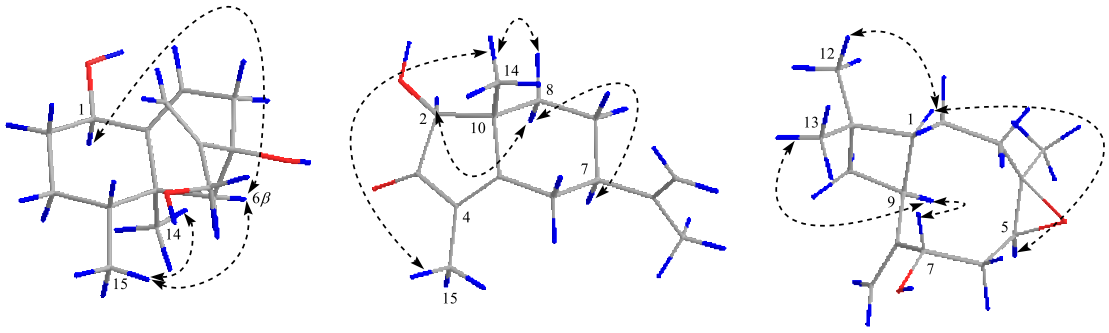
Fig. 2 Key HMBC (bule arrows) and ^{1}H-^{1}H COSY (bold red lines) of compounds **1-5.**



Dstramonium B (**2**) was isolated as a colorless oil. Its molecular formula was deduced to be $C_{21}H_{32}O_7$ by HR-ESI-MS. The ^{1}H NMR spectrum of **2** showed two methyl groups [δ_H 0.89 (3H, s) and 0.87 (3H, s)], four olefinic protons [δ_H 6.05 (1H, ddd, J = 10.0, 5.4,

1.7 Hz), 5.64 (1H, ddd, J = 10.0, 2.2, 1.4 Hz), 5.29 (1H, d, J = 1.5 Hz), and 5.16 (1H, s)] and a typical β-glucose terminal proton [δ_H 4.32 (1H, d, J = 7.8 Hz)]. The ^{1}H-^{1}H COSY spectrum of **2** identifies H-1/H-2/H-3/H-4 and H-8/H-9 fragments (**Fig. 2**). These data coupled with the correlations from H-2/H-9 to C-10, H-4/H-6 to C-5, H-6/H-8/H-13 to H-7, and H-1' to H-12 established the planar structure by the HMBC (**Fig. 2**), which was the similar as the known compound 7β,l0β-epoxy-4βH-eremophila-1,11(12)-diene [8], expect the presence of signals for β-D-glucose. Thus, compound **2** was elucidated to be 7β,l0β-epoxy-4βH-eremophila-1,11(13)-diene-12-O-β-glucopyranoside, and the structure of **2** was assigned as shown in **Fig. 1**.

Fig. 3 Key NOESY correlations of **1**, **4** and **5.**



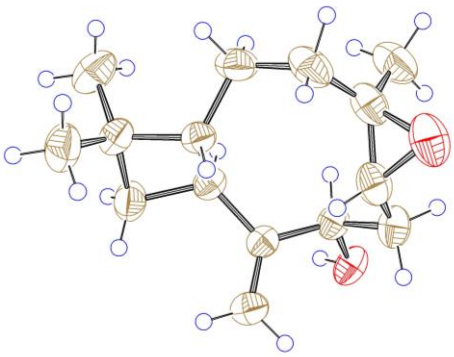
1 **4** **5**

Dstramonium C (**3**) was isolated as a white amorphous powder with a molecular formula of $C_{19}H_{32}O_8$ (m/z 433.2066 $[M+HCOO]^-$, calcd. for 433.2079). The chemical shift values of the 1D NMR of **3** were similar to those of the known compound alangionoside A [9], the major difference being the absence of signals for a 13-Me and the presence of signals for another doubled bond (δ_C 150.9, and 113.7) in **3**. Close analysis of the 2D NMR spectrum of **3** established the structure as megastigman-5(13),7-ene-3,6,9-triol 9-O-β-glucopyranoside. The absolute configuration of **3** was most likely the same as that elucidated as dendranthemoside A [10]. The absolute configuration of C-6 was elucidated as *S* by a positive Cotton effect at 240 nm in the CD spectrum [11]. The absolute configuration of C-3 and C-9 have been determined as 3*S* and 9*R* by compared with the ^{13}C-NMR chemical shift alignment [9]. Finally, the structure of **3** was elucidated to be (3*S*,6*S*,9*R*)-megastigman-5(13),7-ene-3,6,9-triol 9-

O-*β*-glucopyranoside (**Fig. 1**).

Dstramonium D (**4**) was obtained as a colorless oil. Its HR-ESI-MS showed $[M+H]^+$ at 221.1541, molecular formula of $C_{14}H_{21}O_2$. The 1D NMR data (**Table 1**) of **4** were extremely similar to those of the (2*S*,7*R*,10*R*)-2-hydroxy-1-nor-3-oxoeudesm-4,11(13)-dien-12-oic acid [12], the obvious difference being the absence of signal for a carboxyl unit and the presence of signals for another methyl group in the 1D NMR spectra. The plane structure of **4** was also judged by elucidation of the 2D NMR spectra (**Fig. 2**). The *α* orientations of H-2 and H-7, with the *β* orientations of Me-14 and Me-15, were confirmed by the NOESY spectra (**Fig. 3**). Hence, the structure of **4** was identified as (2*S*,7*R*,10*R*)-2-hydroxy-1-nor-3-oxoeudesm-4,11(13)-dien. Moreover, compound **4**, which represented an irregular type of sesquiterpene with 14 carbons, is rare in nature.

Fig. 4 X-ray ORTEP drawing of compound **5.**



(1*R*,4*R*,5*R*,7*R*,9*S*)-4,5-epoxy-8(14)-caryophyllen-7-ol **(5)** was purified as white crystals with a molecular formula of $C_{15}H_{24}O_2$ (m/z 237.1844 $[M+H]^+$, calcd. for 237.1849). The ^{1}H NMR spectrum of **5** showed diagnostic signals for three methyl groups [δ_H 1.32 (3H, s), 1.03 (3H, s) and 1.02 (3H, s)], two oxygenated methines [δ_H 4.05 (1H, dd, J = 9.5, 6.5 Hz) and 2.67 (1H, dd, J = 9.4, 5.5 Hz)], and one pair of terminal double bond protons [δ_H 5.42 (1H, s), and 5.29 (1H, s)]. The ^{13}C-NMR and DEPT spectra of **5** displayed 15 carbon signals, including three methyl carbons (δ_C 30.2, 22.9, and 16.8), two oxygenated carbons (δ_C 73.9, and 61.2), two quaternary carbons (δ_C 61.0, 33.8), along with a terminal double bond (δ_C 158.9, and 111.7). The structure of **5** was consistent with the known (1*R*,4*R*,5*R*,7*R*,9*S*)-4,5-epoxy-8(14)-caryophyllen-7-ol [13],

and this is the first time to be isolated from plants. NOE correlations of H-1/Me-12 and H-1/H-5 indicated the α-orientations of H-5. H-7 was also determined to be β-orientation by the key NOE correlations of 13-Me/H-9 and H-9/H-7. At the same time, the absolute configurations of **5** were unambiguously determined by X-ray diffraction analysis (**Fig. 4**).

By comparing the NMR data with those reported in the literature, the three known compounds **6**-**8** were identified as 14-hydroxycaryophyllene 4,5-oxide (**6**) [14], (4*R*,5*R*,7*R*,10*R*)-4-hydroxy-eudesma-2,11-dien-1-one (**7**) [15], and (1*S*,9*R*,11*R*)-1,9-epoxycaryolan-11-ol (**8**) [16].

Table 1 ^{1}H-NMR data of compounds **1-5** (δ in ppm, J in Hz)

NO.	1[a]	2[a]	3[a]	4[b]	5[a]
1	4.21 br. s	5.64 ddd (10.0, 2.2, 1.4)	-	-	1.57 m
2	1.85 m; 1.59 m	6.05 ddd (10.0, 5.4, 1.7)	1.88 t (12.0); 1.43 m	3.85 s	1.55 m
3	1.77 m; 1.27 m	2.00 m; 1.78 m	3.74 m	-	2.09 m; 0.88 m
4	1.58 m	1.84 dd (7.1, 4.6)	2.52 t (12.0); 2.42 dd (12.0, 4.6)	-	-
5	-	-	-	-	2.67 dd (9.4, 5.5)
6	1.87 m; 1.73 m	1.76 m; 1.60 d (11.9)	-	2.78 brd (13.5); 2.25 t (13.5)	2.06 m; 1.74 m
7	-	-	5.99 d (15.7)	2.08 tt (12.4, 3.1)	4.05 dd (9.5, 6.5)
8	2.55 dd (17.3, 4.0); 2.15 dd (17.3, 3.1)	1.87 m	5.94 dd (15.7, 6.5)	1.81 m 1.67 dd (12.4, 3.0)	-
9	5.57 br. s	2.14 m; 1.56 m	4.42 t (6.5)	2.14 dt (13.1, 3.0); 1.47 td (13.1, 3.0)	2.50 dd (18.1, 8.4)
10	-	-	1.32 d (6.5)	-	1.90 t (10.6); 1.80 m
11	-	-	0.88 s	-	-
12	4.51 d (13.3); 4.34 d (13.3)	4.46 d (13.5); 4.20 d (13.5)	0.88 s	1.80 s	1.02 s
13	5.37 s; 5.22 s	5.30 d (1.5); 5.16 s	4.97 s; 4.92 s	4.81 s	1.03 s
14	1.10 s	0.89 s	-	1.12 s	1.32 s
15	0.88 d (6.7)	0.87 d (6.7)	-	1.71 s	5.42 s; 5.29 s
1'	4.34 d (8.1)	4.32 d (7.8)	4.32 d (7.9)		
2'	3.22 t (8.1)	3.22 dd (9.1, 7.8)	3.17 m		
3'	3.35 m	3.35 m	3.32 m		
4'	3.29 m	3.27 m	3.19 m		
5'	3.28 m	3.26 m	3.18 m		
6'	3.87 br. d (12.1) 3.67 dd (12.1, 5.2)	3.87 dd (12.1, 1.8) 3.66 dd (12.1, 5.5)	3.83 br. d (11.6) 3.56 dd (11.6, 5.5)		

"m" means overlapped or multiplet with other signals; [a]Measured in MeOD, [b]Measured in $CDCl_3$

Table 2 ^{13}C-NMR data of compounds **1-5** (δ in ppm)

NO.	1[a]	2[a]	3[a]	4[b]	5[a]
1	75.7	126.9	39.8	-	57.6
2	35.3	135.9	45.9	82.8	28.4
3	26.9	32.6	68.2	207.9	40.9
4	44.7	36.6	42.4	129.8	61.0
5	40.2	45.3	150.9	175.9	61.2
6	48.4	51.6	78.4	30.3	38.5
7	73.5	89.0	133.4	45.6	73.9
8	39.2	37.3	134.6	27.3	158.9
9	122.7	33.9	78.7	38.2	46.2
10	145.2	87.4	21.7	45.6	40.1
11	150.5	147.6	25.0	148.5	33.8
12	70.2	69.4	25.2	20.6	22.9
13	113.9	112.0	113.7	109.7	30.2
14	21.0	15.2	-	20.3	16.8
15	16.1	14.8	-	7.7	111.7
1'	103.8	103.5	102.7		
2'	75.2	75.2	75.3		
3'	78.2	78.2	78.3		
4'	71.7	71.7	71.9		
5'	78.0	78.0	78.1		
6'	62.8	62.8	63.0		

[a]Measured in MeOD, [b]Measured in $CDCl_3$

Cytotoxic activity

The isolated compounds were evaluated for their cytotoxic against LN229 with the CCK8 method, and the results as shown in **Table 3**. Doxorubicin was used as the positive control. As a result, compounds **2**, and **4** displayed strong cytotoxic activity against LN229 with IC_{50} values lower than 10 μM, compounds **1**, **5**, **6**, and **8** exhibited no cytotoxicity against LN229 with IC_{50} values of >50.0 μM, and others had moderate inhibitory activity.

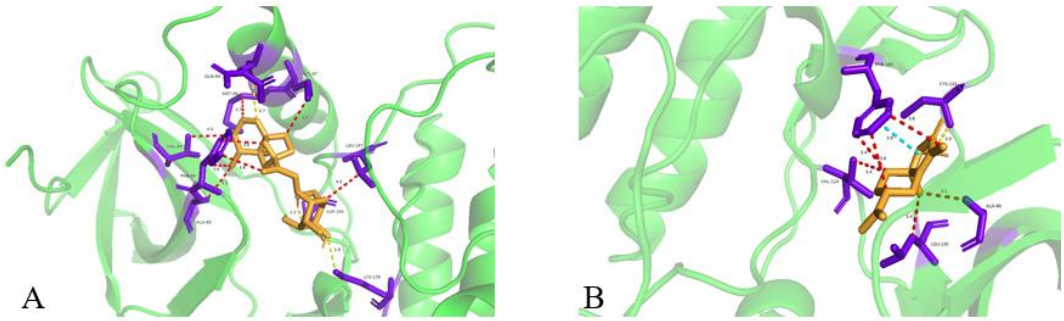
Table 3. Cytotoxic activities (IC_{50}, μM) of all tested compounds on LN229 cell line.

Compounds	IC_{50} mean ± SD (μM, n=3)	Compounds	IC_{50} mean ± SD (μM, n=3)
1	>50	**5**	8.03 ± 0.49
2	>50	**6**	>50
3	9.91 ± 0.62	**7**	11.49 ± 0.29
4	13.83 ± 0.60	**8**	>100
Doxorubicin	1.80 ± 0.01		

Molecular docking studies

Cancer is the uncontrolled growth of cells. It was noted that the PLK-1 plays a key role in the progress of the cell cycle, and overexpression/dysfunction of PLK-1 is directly related to cancer transformation [17]. In this study, the computational screening of isolates targeting the PLK-1 kinase domain was performed by molecular docking in order to validate and investigate the *in vitro* mechanism of inhibition of cell proliferation in LN229 cells by isolates treatment. The study results have shown that the isolated compounds **2** and **4** were optioned for molecular docking studies, and had well affinities with PLK-1 proteins (**Fig. 5**)The free binding energies (FBE, kcal/mol) of compounds **2**-PLK-1 and **4**-PLK-1 interaction were estimated to be -7.7 kcal/mol and -8.0 kcal/mol, respectively.

Fig. 5 Molecular docking simulations of compounds **2** (A) and **4** (B) with PLK-1 (2OWB). (The hydrogen bonds interactions shown by yellow dashed lines, the hydrophobic Interactions shown by red dashed lines, the π-Stacking shown by cyan dashed lines.)



Conclusion

In conclusion, four new sesquiterpenoids dstramoniums A-D (**1-4**), one new natural product (**5**), and three known analogs (**6-8**), were obtained from the leaves of *Datura stramonium*. The absolute configuration of **5** was unambiguously determined by X-ray

diffraction analyses. In vitro bioassays, compounds **2**, and **4** displayed strong cytotoxic activity against LN229 with IC_{50} values lower than 10 μM. Molecular docking analysis revealed that the **2**, and **4** inhibitors bind with PLK-1 mainly by hydrogen bonding interaction, hydrophobic force, and π-stacking. This work provided a deep insight into this plant's sesquiterpenoids, which act as potent PLK-1 inhibitors, however, further biological research is needed to confirm its use as an anti-cancer drug.

Experimental

General experimental procedures. The NMR spectral data were performed on a Bruker AVANCE NEO 600 spectrometer. The HR-ESI-MS spectra were carried out on an Orbitrap Fusion Lumos Tribrid spectrometer and AB SCIEX TripleTOF™ 5600. Optical rotations were recorded on a JASCO P-2000 polarimeter. Semi-preparative HPLC (Shimadzu Corporation) was performed with a YMC J'sphere ODS-H8O (5 μm, 10×250 mm), furnished with a RID-20A detector. Silica gel (200–300 mesh, and 80~100 mesh, Qingdao Haiyang Chemical Co. Ltd., China) was used for column chromatography. The LN229 cells were from the Procell Life Science&Technology Co., Ltd.

Plant material. *Datura stramonium* was collected from Dali, Yunnan, China, in September 2020 and identified by Prof. Rui-Feng Fan (Heilongjiang University of Chinese Medicine). A voucher specimen (No. 20200915) has been deposited in the Herbarium of Heilongjiang University of Chinese Medicine.

Extraction and isolation. To retain the original plant chemical composition as much as possible, dry leaves (5 kg) of *D. stramonium* L. were extracted with methanol at 25 °C and then sequentially eluted with H_2O, 30% EtOH, and 95% EtOH on HPD-BJQH macroporous resin. The 95% EtOH elution (75.0 g) was subjected to silica gel chromatography with CH_2Cl_2/MeOH (1:0 to 0:1, v/v), and eight fractions (Fr.A-Fr.H) were combined and concentrated in vacuo based on their TLCs. Fr. C (10 g) was subjected to ODS column chromatography with MeOH/H_2O (3:7 to 1:0) to afford sub-fractions C_1-C_{12}. Sub-fraction C_{10} (300 mg) was purified by semi-preparative HPLC

with MeOH/H_2O (72-28, 3 mL/min) to **4** (0.8 mg, t_R = 8.3 min), **5** (12.0 mg, t_R = 13.1 min), **6** (2.1 mg, t_R = 11.3 min), **7** (1.9 mg, t_R = 9.8 min), **8** (1.2 mg, t_R = 14.3 min). Fr. E (6.0 g) was purified with MeOH/H_2O (3:7 to 1:0, gradient) by ODS column chromatography to afford sub-fractions (E_1-E_8). Sub-fraction E_6 (450 mg) was purified by semi-preparative HPLC with MeOH/H_2O (69-31, 3 mL/min) to afford **1** (1.5 mg, t_R = 12.5 min), **2** (1.3 mg, t_R = 15.9 min), **3** (1.9 mg, t_R = 13.6 min).

Identification of new compounds

Dstramonium A (1): white amorphous powder; $[\alpha]_D^{20}$ +10.3 (c 0.08, MeOH); HRESIMS m/z 459.2223 $[M+HCOO]^-$ (calcd. for $C_{22}H_{35}O_{10}$, 459.2236); ^1H and ^{13}C-NMR data (CD_3OD, 600, and 150 MHz), see **Table1 and Table 2.**

Dstramonium B (2): colorless oil; $[\alpha]_D^{20}$ +90.7 (c 0.01, MeOH); HRESIMS m/z 441.2118 $[M+HCOO]^-$ (calcd. for $C_{22}H_{33}O_9$, 441.2130); ^1H and ^{13}C-NMR data (CD_3OD, 600, and 150 MHz), see **Table1 and Table 2.**

Dstramonium C (3): white amorphous powder; $[\alpha]_D^{20}$ -30.1 (c 0.09, MeOH); HRESIMS m/z 433.2066 $[M+HCOO]^-$ (calcd. for $C_{19}H_{32}O_8$, 433.2079); ^1H and ^{13}C-NMR data (CD_3OD, 600, and 150 MHz), see **Table1 and Table 2.**

Dstramonium D (4): colorless oil; $[\alpha]_D^{20}$ -14.0 (c 0.10, MeOH); HRESIMS m/z 221.1541 $[M+H]^+$ (calcd. for $C_{14}H_{21}O_2$, 221.1536); ^1H and ^{13}C-NMR data ($CDCl_3$, 600, and 150 MHz), see **Table1 and Table 2.**

(1*R*,4*R*,5*R*,7*R*,9*S*)-4,5-epoxy-8(14)-caryophyllen-7-ol (5): white crystal; $[\alpha]_D^{20}$-124.7 (c 0.10, MeOH); HRESIMS m/z 237.1844 $[M+H]^+$ (calcd. for $C_{15}H_{25}O_2$ 237.1849); ^1H and ^{13}C-NMR data (CD_3OD, 600, and 150 MHz), see **Table1 and Table 2.**

X-ray crystallographic analysis. (1*R*,4*R*,5*R*,7*R*,9*S*)-4,5-epoxy-8(14)-caryophyllen-7-ol (**5**) was crystallized from MeOH at 4 °C. The crystallographic data were collected on a Bruker APEX-II CCD diffractometer using CuKα radiation (λ = 1.54178 Å). Empirical formula: $C_{15}H_{24}O_2$ (M =236.34 g/mol): trigonal, a = 24.6660(7) Å, c =

6.0868(2) Å, V = 3207.1(2) Å3, Z = 9, T = 193.15 K, μ(CuKα) = 0.553 mm-1, D_{calc} = 1.101 g/cm^3, 40957 reflections measured (4.136° ≤ 2θ ≤ 138.284°), -29 ≤ h ≤ 27, -29 ≤ k ≤ 29, -7 ≤ l ≤ 7, 7916 unique (R_{int} = 0.0719, R_{sigma} = 0.0431) which were used in all calculations. The final R_1 was 0.0401 (I > 2σ(I)) and wR_2 was 0.1100 (all data). Flack parameter: -0.1 (2).

Enzyme hydrolysis and sugar analysis. Compounds **1–3** were hydrolyzed to determine monosaccharides by D-glucosidase. Compounds **1-3** (1.0 mg) and D-glucosidase (2 mg) were dissolved in water (4 mL) at 37 °C for 24 h. The reaction mixture was extracted with EtOAc (3 times), and the aqueous layer was dried by vacuum evaporation to obtain the monosaccharide., The monosaccharide was treated as described in [18]. Finally, the n-hexane layer was injected into GC for chromatographic detection. By comparing the retention time with the standard substance, the monosaccharides were determined to be D-glucose.

Cytotoxicity assays. The logarithmic phase human brain glioblastoma LN229 cell line was seeded onto 96-well culture plates at the concentration of 5 ×10^4 cells/mL in DMEM, supplemented with an incubator at 37 °C and 5% CO_2. The cells were plated overnight and then the supernatant was removed. Compounds of different concentrations (3.125, 6.25, 12.5, 25, 50, 100 μg/mL) were added into each well and culture for 24 h. Then add the CCK8 reagent, and the absorbance at 450 nm was measured with a microplate reader. The IC_{50} values were calculated using GraphPad Prism software.

Molecular docking studies. Molecular docking simulations were performed using the hybrid Lamarckian Genetic Algorithm (LGA). Three dimensional (3D) crystal structure of PLK-1 (PDB ID: 2OWB) was obtained from the RCSB Protein Data Bank at a resolution of 2.10 Å. The standard 3D structures (PDB format) of compounds **2** and **4** were constructed as ligands, and the energy was minimized by Chem3D Pro 14.0. We prepared proteins for molecular docking by adding hydrogen atoms and deleting any other nonprotein atoms/molecules, including nonessential water molecules, and used

AutoDock Tools 1.5.7 to process proteins and ligands and convert them into the PDBQT format. All of the other parameters used were the default settings of AutoDock Vina. The most favorable result of binding free energy was selected as the final composite structure. Pymol 2.4.0 was applicable to visualize the interactions between ligands and receptors.

Associated content

*Supporting Information

Supplementary data related to this article can be found in Supporting Information.

Notes

The authors declare no competing financial interest.

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