Supplementary Materials: Chemical Constituents from the Roots and Rhizomes of *Asarum heterotropoides* var. *mandshuricum* and the In Vitro Anti-Inflammatory Activity

Yu Jing, Yi-Fan Zhang, Ming-Ying Shang, Guang-Xue Liu, Yao-Li Li, Xuan Wang and Shao-Qing Cai

1. Spectra of compounds 1–11

		Display	Report		
Analysis Info	北京大学	医药卫生分析中心 	Acquisition Date	5/29/2007 6:00:01 P	м
Method Sample Name	C.DATA_Samp	18/231_070321-30/18/ACDI_7/A_1	Operator Instrument	spect	
Comment MALDI C19H24O7 MW 365.15948 CAL; BLANK DHB 20070524					
Acquisition Re	esult:				
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	
;	387.14143	387.14033	11000-2.8300	CAL ;M+Na,-e	•



Figure S1. HRMALDIMS spectrum of compound 1.







Figure S3. ¹³C-NMR spectrum of compound 1 in CD₃OD.



Figure S4. DEPT NMR spectrum of compound 1 in CD₃OD.



Figure S5. ¹H–¹H COSY NMR spectrum of compound 1 in CD₃OD.



Figure S6. HMQC-NMR spectrum of compound 1 in CD₃OD.



Figure S7. HMBC-NMR spectrum of compound 1 in CD₃OD.



Pulse Sequence: NOESY Solvent: cd3od Temp. 25.0 C / 298.1 K File: jingyu-XX-66-noesy INOVA-500 "BNU500"

Relax. delay 1.000 sec Mixing 6.60.5 sec With the 466.5 Nz 20 With 4648.5 Nz 30 State 10 Sec Mirror 11 Continents 00550VC Hi.493.038718 HHz 00550VC Hi.493.038718 HHz 00550VC Hi.493.038718 HHz 00550VC Hi.493.038718 Sec Gauss apodization 0.030 sec fsize 4056 x 4856 Contal time 12 hr,45 min.3 sec





		Display	Report		
Analysis Info	北京大学	医药卫生分析中心	Acquisition Date	e 5/29/2007 7:22:21 F	'n
Analysis Name Method Sample Name	e C:\DATA_Samp	le\ESI_070521-30\MALDI_XX_	89_20070528\3 Operator Instrument	spect	
Comment	MALDI C20H2 CAL; DHB 20	4O6 MW 360.15729 070524		Spool.	
Acquisition R	esult:				14771
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	
	383.14651 🗆 🗆	383.14576	080000-1.9600	CAL ;M+Na,-e	•



Figure S9. HRMALDIMS spectrum of compound 2.

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Figure S11. ¹³C-NMR spectrum of compound 2 in CD₃OD.



Figure S12 DEPT NMR spectrum of compound 2 in CD₃OD.



Figure S13. ¹H–¹H COSY NMR spectrum of compound 2 in CD₃OD.



Figure S14. HMQC-NMR spectrum of compound 2 in CD₃OD.



Figure S15. HMBC-NMR spectrum of compound 2 in CD₃OD.

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Figure S16. NOESY NMR spectrum of compound 2 in CD₃OD.



Figure S17. HRESIMS spectrum of compound 3.



Figure S18. ¹H-NMR spectrum of compound 3 in CD₃OD.



Figure S19. ¹³C-NMR spectrum of compound 3 in CD₃OD.



Figure S20. ¹H–¹H COSY NMR spectrum of compound 3 in CD₃OD.



Figure S21. HMQC-NMR spectrum of compound 3 in CD₃OD.



Figure S22. HMBC-NMR spectrum of compound 3 in CD₃OD.







Figure S23. NOESY NMR spectrum of compound 3 in CD₃OD.



Figure S24. HRESIMS spectrum of compound 4.



Figure S25. ¹H-NMR spectrum of compound 4 in CD₃OD.



Figure S26. ¹³C-NMR spectrum of compound 4 in CD₃OD.



Figure S27. DEPT NMR spectrum of compound 4 in CD₃OD.



Figure S28.¹H–¹H COSY NMR spectrum of compound 4 in CD₃OD.



Figure S29. HMQC-NMR spectrum of compound 4 in CD₃OD.



Figure S30. HMBC-NMR spectrum of compound 4 in CD₃OD.

			Display F	Report		
Analysis Info	北京大学	医药卫生分析中	中心		Acquisition Date	4/2/2007 3:51:12 PM
Analysis Name	C:\DATA_Sam	ple/ESI_070401-10/ESI	_XX_70_200704	02\10		
Method					Operator	
Sample Name					Instrument	spect
Comment	ESI_NEG-XX7	70 2006/04/02				
Acquisition Res	ult:					
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	Descripti	ion
	551.17702		0.0006		-H;+e	



Figure S31. HRESIMS spectrum of compound 5.



Figure S32. ¹H-NMR spectrum of compound 5 in CD₃OD.



Figure S33. ¹³C-NMR spectrum of compound 5 in CD₃OD.



Figure S34. DEPT NMR spectrum of compound 5 in CD₃OD.



Figure S35.¹H–¹H COSY NMR spectrum of compound 5 in CD₃OD.



Figure S36. HMQC-NMR spectrum of compound 5 in CD₃OD.



Figure S37. HMBC-NMR spectrum of compound 5 in CD₃OD.





Figure S38. HRESIMS spectrum of compound 6.



Figure S39. ¹H-NMR spectrum of compound 6 in CD₃OD.



Figure S40. ¹³C-NMR spectrum of compound 6 in CD₃OD.



Figure S41. DEPT NMR spectrum of compound 6 in CD₃OD.



Figure S42. ¹H–¹H COSY NMR spectrum of compound 6 in CD₃OD.



Figure S43. HMQC-NMR spectrum of compound 6 in CD₃OD.



Figure S44. HMBC-NMR spectrum of compound 6 in CD₃OD.

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File: PROTON Pulse Sequence: NOESY Solvent: cd3od Temp. 25.0 C / 298.1 K INOVA-500 "BMU500"

Rotr-Soc DB0500 Relax. dely 1.000 sec Mixing 0.600 sec Vidth 4200.8 Hz 20 Vist 4200.8 Hz 2 x 255 increments 055FWF H. 459.903718 MHz DATA PROCESSING Gauss apodization 0.113 sec Gauss apodization 0.228 sec FT uss 4095 Total time 12 hr, 52 min, 30 sec



Figure S45. NOESY NMR spectrum of compound 6 in CD₃OD.

		Display	Report			
Analysis Info	北京大学医药卫生	 上分析中心		Acquisition Date	10/22/2007 11:08:33	3 AM
Analysis Name	C:\DATA_Sample\ESI_0710	020-30\ESI_XX_65_2007	71022\2			
Method						
Sample Name				Instrument	spect	
Acquisition Resu	M/Z(509) = 186.08732;286 M/Z(Tune) = 323.0695;322.	5.10872;441.16697; 509. 0481;623.0323;622.028	25407; 611.26126 9;923.0131;922.00	;633.24321;804.489 1980	926;826.47121	
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	Description	
	411.141	43	000-0.0005 0		M+Na,-e	•
	799.2930	63000799.290150		100 -4.36 00	2M+Na ;-e	•



Figure S46. HRESIMS spectrum of compound 7.



Figure S47. ¹H-NMR spectrum of compound 7 in CDCl₃.



Figure S48. ¹³C-NMR spectrum of compound 7 in CDCl₃.

		Display	Report			
Analysis Info	北京大学医药卫生	生分析中心		Acquisition Date	10/22/2007 2:52:25	PM
Analysis Name	C:\DATA_Sample\ESI_071	020-30\ESI_XX_63_200	71022\3			
Method		Operator				
Sample Name				Instrument	spect	
Acquisition Res	M/2(509) = 186.08732;28 M/2(Tune) = 323.0695;322 ult:	.0481;623.0323;622.028	9;923.0131;922.00	5;633.24321;804.48 0980	926;826.47121	
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	Description	
	153.127	739			M+H , -e	•
	305.24	751			2М+Н ;-е	•







Figure S50. ¹H-NMR spectrum of compound 8 in CD₃OD.



Figure S51. ¹³C-NMR spectrum of compound 8 in CD₃OD.



Figure S52.1H-1H COSY NMR spectrum of compound 8 in CD3OD.



Figure S53. HMQC-NMR spectrum of compound 8 in CD₃OD.



Figure S54. HMBC-NMR spectrum of compound 8 in CD₃OD.

XX-63 File: PROTON

Pulse Sequence: NOESY Solvent: CD30D Temp. 25.0 C / 298.1 K INOVA-500 "BMU500"

Rotray de La 1.000 sec Mixing 0.600 sec Mixing 0.600 sec Vidth 3310.3 Hz 20 Midth 3310.3 Hz 2 x 256 increments 055EVF H, 1493.4038716 Miz 0ATA PROCESSIMO Gauss apodization 0.071 sec F size 4055 x 4086 Total time 16 hr, 25 min, 26 sec



Figure S55. NOESY NMR spectrum of compound 8 in CD₃OD.

		Display	Report			
Analysis Info	北京大学医药卫生	主分析中心		Acquisition Date	10/22/2007 11:45:38	B AM
Analysis Name	C:\DATA_Sample\ESI_071					
Method						
Sample Name				Instrument	spect	
Assulation Dec	M/Z(509) = 186.08732;286 M/Z(Tune) = 323.0695;322.	3.10872;441.16697; 509. 0481;623.0323;622.028	25407; 611.26126 9;923.0131;922.00	;633.24321;804.489 980	926;826.47121	
Acquisition Rest	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	Description	
	153.127	39		- 1.52 00	М+Н,-е	
	305.247	51000305.247400	0001	100-0 3500	2M+H :-e	



Figure S56. HRESIMS spectrum of compound 9.



Figure S57. ¹H-NMR spectrum of compound 9 in CD₃OD.



Figure S58. ¹³C-NMR spectrum of compound 9 in CD₃OD.



Figure S59. ¹H–¹H COSY NMR spectrum of compound 9 in CD₃OD.



Figure S60. HMQC-NMR spectrum of compound 9 in CD₃OD.



Figure S61. HMBC-NMR spectrum of compound 9 in CD₃OD.





		Display	Report			
Analysis Info	北京大学医药卫生	生分析中心		Acquisition Date	10/22/2007 4:17:33	РМ
Analysis Name	C:\DATA_Sample\ESI_071	020-30\ESI XX35 2007	1022\2	-		
Method				Operator		
Sample Name				Instrument	spect	
Acquisition Res	M/Z(509) = 186.08732;280 M/Z(Tune) = 323.0695;322	6.10872;441.16697; 509 .0481;623.0323;622.028	25407; 611.26126 9;923.0131;922.00	3;633.24321;804.48 9980	926;826.47121	
	Exact Mass	Measured Mass	Error (m/e)	Error (ppm)	Description	
	282.20	637	000060	000 2.02 60	М+Н,-е	•
	304.188	32 304.18839			M+Na, -e	·







Figure S64. ¹H-NMR spectrum of compound 10 in CD₃OD (25 °C).



Figure S65. ¹H-NMR spectrum of compound 10 in CD₃OD (–25 °C).



Figure S66. ¹H-NMR spectrum of compound 10 in CD₃OD (–45 $^{\circ}$ C).



Figure S67. ¹³C-NMR spectrum of compound 10 in CD₃OD.



Figure S68. DEPT NMR spectrum of compound 10 in CD₃OD.



Figure S69.1H-1H COSY NMR spectrum of compound 10 in CD3OD.



Figure S70. HMQC-NMR spectrum of compound 10 in CD₃OD.









Figure S72. ¹H-NMR spectrum of compound 11 in CD₃OD.



Figure S73. ¹³C-NMR spectrum of compound 11 in CD₃OD.



Figure S74. DEPT NMR spectrum of compound 11 in CD₃OD.



Figure S75. ¹H–¹H COSY NMR spectrum of compound 11 in CD₃OD.



Figure S76. HMQC-NMR spectrum of compound 11 in CD₃OD.

2. Extraction and Isolation of Known Compounds 13-47

The air-dried and powdered roots and rhizomes of A. heterotropoides var. mandshuricum (36 kg) were extracted three times (2 h, 1.5 h, 1.5 h for each) under reflux with 95% ethanol and then three times (2 h, 1.5 h, 1.5 h for each) with 50% ethanol successively. The combined extracts were concentrated under reduced pressure to give a dark brown residue (9.2 kg), then 7.8 kg of it was suspended in H₂O (16.5 L) and partitioned sequentially with petroleum ether (Pet.) (60–90 °C °C) (4 \times 5 L), CHCl₃ (4 \times 5 L), EtOAc (4 \times 5 L), and *n*-BuOH (4 \times 5 L), respectively. The CHCl₃ layer (231 g) was fractionated on silica gel CC eluting with a gradient of petroleum ether–EtOAc (10:1 to 0:1) to obtain 16 fractions C1–C16. Fraction C5 and C6 were left to stand overnight and colorless columnar crystal as precipitate were collected, the colorless crystal was purified by recrystallized from EtOAc to afford compound 14 (10 g). Fraction C7 produced two different colorless needle crystals when standing overnight, and then the two needle crystals were further recrystallized from EtOAc to obtain compounds 15 (1 g) and 16 (0.8 g). The residue of fraction C7 was subjected to silica gel column chromatography eluting with a gradient of petroleum ether-EtOAc (100:15, 100:30, 0:100) to yield seven subfractions, C7-1–C7-7. C7-7 was further separated by silica gel CC and purified on Sephadex LH-20 eluting with CHCl3-MeOH (6:4) to afford compound 45 (17 mg). Fraction C10 was then subjected to silica gel CC eluting with a gradient of CHCl3-MeOH (1:40 to 1:30) to provide three subfractions, C10-1–C10-3. C10-1 was further separated by silica gel CC and purified on Sephadex LH-20 eluting with CHCl3-MeOH (1:1) to afford compound 13 (12 mg). C10-3 was chromatographed over silica gel with petroleum CHCl3-MeOH (1:30) and further separated by Sephadex LH-20 (CHCl3-MeOH, 1:1) to obtain compound 41 (31 mg). Fraction C11 was subjected to silica gel CC eluting with a gradient of CHCl3-MeOH (1:30 to 1:35) to yield four subfractions, C11-1-C11-4. C11-1 was subjected to chromatography on silica gel CC eluting with petroleum ether-Me₂CO (4:1) to produce seven subfractions, C11-1-I-C11-1-7. C11-1-3 was further separated by silica gel column chromatography using CHCl3-EtOAc (9:1) to yield four subfractions, C11-1-3-1-C11-1-3-4. C11-1-3-3

was then separated by Sephadex LH-20 (CHCl3-MeOH, 1:1) and semi-preparative HPLC eluted with a gradient of MeOH-H2O to yield compound 21 (21 mg). C11-1-4 was separated on silica gel CC eluting with CHCl3-EtOAc (9:1) and further purified by Sephadex LH-20 (CHCl3-MeOH, 6:4) to afford compound 18 (33 mg). C11-1-5 was chromatographed over silica gel with petroleum ether-Me₂CO (2.5:1) as eluent, and subfraction was then separated by Sephadex LH-20 (CHCl₃–MeOH, 6:4) and semi-preparative HPLC eluted with a gradient of MeOH-H2O to yield compound 17 (6.3 mg). C11-3 yielded a colorless needle crystal, collected by filtering, and further purified by washing with CHCl₃ to afford compound **39** (33 mg). The residue of C11-3 was subjected to silica gel CC eluting with a gradient of CHCl3-MeOH to yield three subfractions, C11-3-1-C11-3-3. C11-3-2 was subjected to silica gel CC eluting with petroleum ether-Me₂CO (4.5:1) to yield compound 46 (6 mg). C11-3-3 was also chromatographed on silica gel with petroleum ether-Me₂CO (4.5:1) as eluent to obtain compounds 34 (7.2 mg) and 35 (11mg). Fraction C12 was chromatographed on silica gel with a gradient of CHCl3-MeOH to give four fractions, C12-1-C12-4. C12-3 was further divided into five subfractions, C12-3-1–C12-3-5, with repeated silica gel CC eluting with CHCl3–MeOH (80:1 to 15:1). Chromatography of C12-3-1 on silica gel with CHCl3-EtOAc (5:1, 4:1) and petroleum ether-Me2CO (2:1) as eluent successively and purified by semi-preparative HPLC eluting with a gradient of MeOH-H₂O to yield compound 25 (7.8 mg). C12-3-3 was chromatographed over silica gel with CHCl₃-EtOAc (4:1, 1:1) as eluent, and the subfraction was then separated by Sephadex LH-20 (CHCl3-MeOH, 6:4) and semi-preparative HPLC eluted with a gradient of MeOH-H2O to yield compound 44 (21 mg).C12-4 was separated on silica gel CC eluting with CHCl3-MeOH (70:1 to 15:1) and further purified by Sephadex LH-20 eluting with (CHCl3-MeOH, 1:1) to afford compound 47 (28 mg). Fraction C13 was chromatographed with a gradient of CHCl3-MeOH (35:1, 20:1, 5:1) as eluent to give six subfractions, C13-1–C13-6. C13-6 was then subjected to silica gel CC with petroleum ether–Me₂CO (30:1) to obtain five subfractions, C13-6-1–C13-6-5, and subfraction C13-6-4 was further separated by Sephadex LH-20 (petroleum ether-CHCl3-MeOH, 2:1:1) and semi-preparative HPLC with a gradient of MeOH–H₂O as the mobile phase to yield compounds 20 (2.7 mg) and 23 (34 mg). Fraction C14 was subjected to silica gel CC eluting with a gradient of CHCl3-Me2CO (25:1 to 10:1) to yield five subfractions, C14-1–C14-5. C14-4 was chromatographed over silica gel with CHCl3–Me₂CO (10:1 to 5:1) and further separated by Sephadex LH-20 (petroleum ether-CHCl3-MeOH, 2:1:1) to obtain compound 22 (18 mg). C14-5 was subjected to silica gel CC eluting with CHCl3-Me2CO (10:1 to 5:1) to give seven subfractions, C14-5-1–C14-5-7. C14-5-5 was purified by Sephadex LH-20 eluting with petroleum ether-CHCl3-MeOH (2:1:1) and semi-preparative HPLC with a gradient of MeOH-H2O as eluent to afford compounds 38 (6.8 mg) and 19 (9.8 mg). C14-5-7 was further purified by Sephadex LH-20 (petroleum ether-CHCl3-MeOH, 2:1:1) to obtain compound 24 (21 mg). Fraction C15 was subjected to silica gel CC eluting with a gradient of CHCl3-MeOH (25:1 to 10:1) and further separated by chromatographed over silica gel with CHCl3-Me2CO (5:1) and Sephadex LH-20 (CHCl3-MeOH, 6:4) to obtain compound 37 (69 mg).

The EtOAc layer (50 g) was fractionated on silica gel CC eluting with a gradient of CHCl₃–MeOH (10:1 to 0:1) to yield 10 fractions, E1–E10. Fraction E3 was separated by silica gel CC with CHCl₃–MeOH–H₂O (16:1:0.1) to give compound **36** (2.5 mg). Fraction E5 was subjected to silica gel CC eluting with CHCl₃–MeOH–H₂O (15:1:0.1, 10:1:0.1) to yield E5-1–E5-4, and E5-4 was separated on Sephadex LH-20 eluting with MeOH to yield compound **33** (8 mg). E5-5 was further purified by semi-preparative HPLC with MeOH-H₂O as eluent to obtain compound **40** (8.2 mg). Fraction E7 yielded a yellow powder when standing overnight, and then the yellow powder was further purified to obtain compound **26** (62 mg). The residue of Fraction E7 was chromatographed on silica gel CC eluting with CHCl₃–MeOH–H₂O (9:1:0.1, 8:1:0.1, 5:1:0.1), and separated by semi-preparative HPLC with a gradient of MeOH–H₂O as eluent to afford compounds **27** (6.7 mg), **28** (7 mg), and **29** (41 mg). Fraction E8 was separated on silica gel CC eluting with CHCl₃–MeOH–H₂O (8:1:0.1, 5:1:0.1) to give nine subfractions, E8-1–E8-9, and then E8-7 was further fractionated on silica gel CC (CH₂Cl₂–isopropanol–H₂O, 4:1:0.05 to 2:1:0.05) and purified by Sephadex LH-20 with MeOH as eluent to yield compound **32** (13 mg).

The *n*-BuOH layer (400 g) was fractionated on silica gel CC eluting with a gradient of CHCl₃– MeOH (7:1 to 0:1) to yield 14 fractions, B1–B14. Fraction B5 yielded a white powder, collected by filtering, which was then purified by washing with MeOH to obtain compound 43 (120mg). Fraction B6 was further separated on silica gel CC eluting with CHCl₃–MeOH–H₂O (5:1:0.1 to 2:1:0.1) to give nine subfractions, B6-1–B6-9. B6-7 was further divided by silica gel CC using CHCl₃–MeOH–H₂O (5:1:0.1), and purified by Sephadex LH-20 eluting with MeOH to afford compound **42** (14 mg). Fraction B12 was separated on repeated silica gel CC eluting with CHCl₃–MeOH–H₂O (2:1:0.1, 1:1:0.1) and further purified by silica gel CC (CH₂Cl₂–isopropanol–H₂O, 4:1:0.1) and semi-preparative HPLC with MeOH–H₂O as eluent to yield compounds **30** (7 mg) and **31** (6 mg), respectively.

3. Anti-Inflammatory Activity Assay In Vitro

Plate	Compound	OD Value ^b	I.R. (%)	Plate	Compound	OD Value ^b	I.R. (%)
	control	0.643 ± 0.064			control	0.559 ± 0.044	
	model	0.923 ± 0.032			model	0.930 ± 0.079	
	Ginkgolide B ^c	0.712 ± 0.015 ***	75.4		Ginkgolide B ^c	0.649 ± 0.028 **	75.7
	Pet. extract d	0.861 ± 0.085	22.1	2#	15	0.850 ± 0.020	21.6
1#	CHCl3 extract ^d	0.708 ± 0.139 **	76.8	3"	19	0.427 ± 0.024 ***	135.6 ^e
1"	EtOAc extract ^d	0.847 ± 0.099	27.1		27	0.832 ± 0.042	26.4
	<i>n</i> -BuOH extrac t ^d	0.867 ± 0.076	20.0		29	0.788 ± 0.044 *	38.3
	14	$0.808 \pm 0.069 ***$	41.1		41	0.806 ± 0.013 *	33.4
	17	$0.845 \pm 0.029 **$	27.9		control	0.710 ± 0.033	
	18	0.819 ± 0.023 **	37.1		model	1.046 ± 0.115	
	control	0.619 ± 0.034			Ginkgolide B ^c	$0.774 \pm 0.025 **$	80.9
	Model	0.861 ± 0.053			2	0.915 ± 0.051	39.0
	Ginkgolide B ^c	0.706 ± 0.015 **	64.1		4	0.811 ± 0.014 **	69.9
	1	0.763 ± 0.048 *	40.5	4#	22	0.879 ± 0.052 *	49.7
	5	0.799 ± 0.055	25.6		32	0.903 ± 0.041 *	42.6
	7	0.721 ± 0.075 **	57.9		33	0.897 ± 0.010 *	44.4
0#	8	0.726 ± 0.038 **	55.8		40	0.802 ± 0.055 **	72.6
Ζ"	23	0.795 ± 0.024	27.3		43	0.821 ± 0.096 **	66.9
	24	0.777 ± 0.053 *	34.7		45	0.855 ± 0.042 **	56.9
	25	0.737 ± 0.067 *	51.2				
	30	0.773 ± 0.064 *	36.4				
	42	0.786 ± 0.014 *	31.0				
	44	0.796 ± 0.142	26.9				
	46	0.693 ± 0.039 **	69.4				

Table S1. Anti-inflammatory activity assay in vitro ^a.

^a All samples were assigned to five different 96-well plates, 1[#]–4[#]; The Student's *t*-test for unpaired observations between model (stimulated by PAF alone) and control (cultured in medium alone) or tested samples was carried out to identify statistical differences; * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001; *p* < 0.05 were considered as significantly different; All the compounds were tested at 10⁻⁵ mol/L. ^b OD values were expressed as mean ± SD (for control and sample, *n* = 3; for model, *n* = 4). ^c Ginkgolide B, positive control. ^d Pet. extract, CHCl₃ extract, EtOAc extract, and n-BuOH extract represent the extract described in *"Extraction and Isolation"* (tested at 10 µg/mL). ^e The tests were repeated several times, and the results were reproducible.