Supporting Information

Neuroprotective constituents from the aerial parts of *Cannabis sativa*

L. subsp. Sativa

Jia Li^a, Guan Wang^{a,b}, Yu Qin^a, Xue Zhang^a, Hai-Feng Wang^a, Hong-Wei Liu^c, Ling-

Juan Zhu*,^a and Xin-Sheng Yao*, ^a

^a College of Traditional Chinese Materia, Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China.

^b State Key Laboratory of Biotherapy and Cancer Center and Department of Gastrointestinal Surgery, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu 610041, China.

^c State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Contents:

Figure S1. ¹ H NMR spectrum of cannabisativas A (1) in CDCl ₃	1
Figure S2. ¹³ C NMR and DEPT 135 spectra of cannabisativas A (1) in CDCl ₃	1
Figure S3. ¹ H- ¹ H COSY spectrum of cannabisativas A (1) in CDCl ₃	2
Figure S4. HSQC spectrum of cannabisativas A (1) in CDCl3	2
Figure S5. HMBC spectrum of cannabisativas A (1) in CDCl ₃	3
Figure S6. ROESY spectrum of cannabisativas A (1) in CDCl3	3
Figure S7. HRESIMS spectrum of cannabisativas A (1).	4
Figure S8. UV spectrum of cannabisativas A (1).	4
Figure S9. ¹ H NMR spectrum of cannabisativas B (2) in CDCl ₃	5
Figure S10. ¹³ C NMR and DEPT 135 spectra of cannabisativas B (2) in CDCl ₃	5
Figure S11. ¹ H- ¹ H COSY spectrum of cannabisativas B (2) in CDCl ₃	6
Figure S12. HSQC spectrum of cannabisativas B (2) in CDCl ₃	6
Figure S13. HMBC spectrum of cannabisativas B (2) in CDCl ₃	7
Figure S14. NOESY spectrum of cannabisativas B (2) in CDCl ₃	7
Figure S15. HRESIMS spectrum of cannabisativas B (2).	8
Figure S16. UV spectrum of cannabisativas B (2)	8
Figure S17. ¹ H NMR spectrum of cannabisativas C (3) in CDCl ₃	9
Figure S18. ¹³ C NMR and DEPT 135 spectra of cannabisativas C (3) in CDCl ₃	9
Figure S19. ¹ H- ¹ H COSY spectrum of cannabisativas C (3) in CDCl ₃	10
Figure S20. HSQC spectrum of cannabisativas C (3) in CDCl ₃	10
Figure S21. HMBC spectrum of cannabisativas C (3) in CDCl ₃	11
Figure S22. NOESY spectrum of cannabisativas C (3) in CDCl ₃	11
Figure S23. HRESIMS spectrum of cannabisativas C (3).	12
Figure S24. UV spectrum of cannabisativas C (3)	12
Figure S25. ¹ H NMR spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl ₃	13
Figure S26. ¹³ C NMR and DEPT 135 spectra of (7Z, 9Z)-cannabiphenolic acid A (4) in CD	Cl ₃ 13
Figure S27. ¹ H- ¹ H COSY spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl ₃	14
Figure S28. HSQC spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl ₃	14
Figure S29. HMBC spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl ₃	15
Figure S30. NOE difference spectrum ($\delta_{\rm H}$ 4.40) of (7Z, 9Z)-cannabiphenolic acid A (4) in	CDCl ₃ .
	15
Figure S31. NOE difference spectrum ($\delta_{\rm H}$ 6.58) of (7Z, 9Z)-cannabiphenolic acid A (4) in	CDCl ₃ .
Figure S32. HRESIMS spectrum of (7Z, 9Z)-cannabiphenolic acid A (4)	
Figure S33. UV spectrum of (7Z, 9Z)-cannabiphenolic acid A (4).	17
Figure S34. ¹ H NMR spectrum of (8S, 9Z)-cannabiphenolic acid B (5) in CDCl ₃	17
Figure S35. ¹³ C NMR and DEPT 135 spectra of (8 <i>S</i> , 9 <i>Z</i>)-cannabiphenolic acid B (5) in CD0	Cl318
Figure S36. ¹ H- ¹ H COSY spectrum of (8 <i>S</i> , 9 <i>Z</i>)-cannabiphenolic acid B (5) in CDCl ₃	
Figure S37. HSQC spectrum of (8 <i>S</i> , 9 <i>Z</i>)-cannabiphenolic acid B (5) in CDCl ₃	19
Figure S38. HMBC spectrum of (8S, 9Z)-cannabiphenolic acid B (5) in CDCl ₃	19
Figure S39. HRESIMS spectrum of (8 <i>S</i> , 9 <i>Z</i>)-cannabiphenolic acid B (5)	20
Figure S40. UV spectrum of (8S, 9Z)-cannabiphenolic acid B (5).	20

Figure S41. Results of DP4+ analysis of (7Z, 9Z)-cannabiphenolic acid A (4).	.21
Figure S42. ¹ H-NMR spectrum of (<i>R</i>)-AT Ester of (8 <i>S</i> , 9 <i>Z</i>)-cannabiphenolic acid B (5) in pyridin	ne-
l5	.22
Figure S43. ¹ H-NMR spectrum of (S)-AT Ester of (8S, 9Z)-cannabiphenolic acid B (5) in pyridin	ne-
<i>l</i> 5	.22
Table S1. Effects of compounds 1-5, 7, 8 and 12-16 against H_2O_2 -induced cytotoxicity in PC	12
ells	.23



Figure S1. ¹H NMR spectrum of cannabisativas A (1) in CDCl₃.



Figure S2. ¹³C NMR and DEPT 135 spectra of cannabisativas A (1) in CDCl₃.



Figure S3. ¹H-¹H COSY spectrum of cannabisativas A (1) in CDCl₃.



Figure S4. HSQC spectrum of cannabisativas A (1) in CDCl₃.



Figure S5. HMBC spectrum of cannabisativas A (1) in CDCl₃.



Figure S6. ROESY spectrum of cannabisativas A (1) in CDCl₃.

Single Mass Analysis Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron lons 40 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 20-20 H: 0-900 O: 0-80 23Na: 0-1 1 20170410-78 218 (1.765) 1: TOF MS ES+ 1.00e+005 301.1805 100-355.1885 283.1699 % 333,2071 259.1694 284.1729 302.1832 356.1917 352.1797 285.1765 387.1780^{393.1826} 315.1945327.1622 342.2070 369.1681 437.1926 260,1735 417.1573 430 m/z 0-380 390 400 410 420 Hipm 330 340 350 270 280 290 310 320 370 260 300 360 Minimum: -1.550.0 10.0 5.0 Maximum: Mass Calc. Mass 333.2071 333.2066 mDa 0.5 PPM 1.5 DBE 6.5 i-FIT Norm Conf(%) Formula 137.8 n/a n/a C20 H29 O4

Figure S7. HRESIMS spectrum of cannabisativas A (1).



Figure S8. UV spectrum of cannabisativas A (1).



Figure S9. ¹H NMR spectrum of cannabisativas B (2) in CDCl₃.



Figure S10. ¹³C NMR and DEPT 135 spectra of cannabisativas B (2) in CDCl₃.



Figure S11. ¹H-¹H COSY spectrum of cannabisativas B (2) in CDCl₃.



Figure S12. HSQC spectrum of cannabisativas B (2) in CDCl₃.



Figure S13. HMBC spectrum of cannabisativas B (2) in CDCl₃.



Figure S14. NOESY spectrum of cannabisativas B (2) in CDCl₃.

Single Mass Analysis Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron lons 76 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 22-30 H: 0-900 O: 0-80 23Na: 0-1 2 20170410-79 247 (1.993) 1: TOF MS ES+ 5.86e+004 329 2120 100-383.2202 311.2013 361.2383 % 560.3264 384.2234 271.1700 561.3285 207.1037 231.1383 399.1921 445.1911 657.4128 689.4390 544.3193 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 0- $\begin{array}{c}
-1.5 \\
10.0 \\
50.0
\end{array}$ Minimum: Maximum: 5.0 Mass Calc. Mass mDa 361.2383 361.2379 0.4 PPM DBE 6.5 i-FIT Norm Conf(%) Formula 135.4 n/a n/a C22 H33 04 1.1









Figure S17. ¹H NMR spectrum of cannabisativas C (3) in CDCl₃.



Figure S18. ¹³C NMR and DEPT 135 spectra of cannabisativas C (3) in CDCl₃.



Figure S19. ¹H-¹H COSY spectrum of cannabisativas C (3) in CDCl₃.



Figure S20. HSQC spectrum of cannabisativas C (3) in CDCl₃.



Figure S21. HMBC spectrum of cannabisativas C (3) in CDCl₃.



Figure S22. NOESY spectrum of cannabisativas C (3) in CDCl₃.







Figure S24. UV spectrum of cannabisativas C (3).



Figure S25. ¹H NMR spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.



Figure S26. ¹³C NMR and DEPT 135 spectra of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.



Figure S27. ¹H-¹H COSY spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.



Figure S28. HSQC spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.



Figure S29. HMBC spectrum of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.



Figure S30. NOE difference spectrum ($\delta_{\rm H}$ 4.40) of (7Z, 9Z)-cannabiphenolic acid A (4) in CDCl₃.







Figure S32. HRESIMS spectrum of (7Z, 9Z)-cannabiphenolic acid A (4).



Figure S33. UV spectrum of (7Z, 9Z)-cannabiphenolic acid A (4).



Figure S34. ¹H NMR spectrum of (8S, 9Z)-cannabiphenolic acid B (5) in CDCl₃.



Figure S35. ¹³C NMR and DEPT 135 spectra of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in CDCl₃.



Figure S36. ¹H-¹H COSY spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in CDCl₃.



Figure S37. HSQC spectrum of (8S, 9Z)-cannabiphenolic acid B (5) in CDCl₃.



Figure S38. HMBC spectrum of (8S, 9Z)-cannabiphenolic acid B (5) in CDCl₃.

Single Mass Analysis Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0 Element prediction: Off Number of isotope peaks used for i-FIT = 3 Monoisotopic Mass, Even Electron Ions 101 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass) Elements Used: C: 15-15 H: 0-199 N: 0-1 O: 0-50 Na: 0-1 S: 0-1 CS-EH-3245 2017050348 193 (1.556) 1: TOF MS ES-4.07e+005 231.1387 100-% 232.1416 249.1495.379.1912 541.2802 695.4305 980.49131109.7258 1263.9337 1442.9756 1540.9813 1937.0363 m/z 800 0 1600 1800 400 600 1000 1200 1400 200 Minimum: Maximum: $^{-1.5}_{50.0}$ 20.0 5.0 Mass 231.1387 Calc. Mass mDa 231.1385 0.2 PPM 0.9 DBE 6.5 i-FIT 337.4 Norm n/a Conf(%) Formula n/a C15 H19 O2





Figure S40. UV spectrum of (8S, 9Z)-cannabiphenolic acid B (5).

Funct	ional	Solv	ent?	Basi	s Set	Туре о	f Data
=PT1	T1PT91 PCM			6-311+G(d, p)		Unscaled Shifts	
		DP4+	d 0.00%	100.00%	-	-	-
Nuclei	sp2?	speriments	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
С		15. 5344	18.66921	18.64712			
С	x	123. 1251	130.8866	131. 57709			
С	x	153.6654	164. 20831	163. 5768			
С	x	115.4674	116.92795	120.40864			
С	x	137. 0233	150. 20307	146. 2386			
С	x	140. 5277	149.07616	152.03422			
С		67.9152	64. 42602	74.71416			
С	x	122. 3789	132.17665	130. 53072			
С	x	122. 6142	128.25533	130.47206			
С	x	141. 5551	153.91382	152.83204			
С		27.0115	34.46574	34.66421			
С		23.0881	25.20402	25.20637			
С		23.0881	24.9941	25.17819			
С	x	121.3131	124.09433	127.20956			
С	x	130.9557	137. 42589	137.22484			
Н		2.2514	2. 42538	2.44689			
Н		2.2514	2.41829	2.44163			
Н		2.2514	2.10521	2.13054			
Н	x	6.705	7.29012	7.03457			
Н		4. 3997	4.47124	4.65955			
Н		4.3997	5.08463	4.46226			
Н	x	6. 58275	7.16738	6.97964			
Н	x	5.9486	6.73015	6.48497			
Н	х	5.2775	5.78576	5.64706			
Н		2.8773	3.1381	3.08682			
Н		0.9977	1.06429	1.04998			
Н		0.9977	1.25974	1.23863			
Н		0.9977	0.93798	0.91876			
Н		1.0087	1.22845	1.21345			
Н		1.0087	1.00984	1.00926			
Н		1.0087	0.90695	0.88608			
Н	x	6. 75745	7, 293	7.07616			
Н	x	7.1087	7.3652	7.38118			

Figure S41. Results of DP4+ analysis of (7Z, 9Z)-cannabiphenolic acid A (4).



Figure S42. ¹H-NMR spectrum of (*R*)-AT Ester of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in pyridine-*d*₅.



Figure S43. ¹H-NMR spectrum of (S)-AT Ester of (8S, 9Z)-cannabiphenolic acid B (**5**) in pyridine- d_5 .

cells						
Compound	Compound	Cell viability of	Cell viability of H ₂ O ₂	Rescue effect of		
1	concentration	compound group	+ compound group	compound (%) ^b		
	(µM)	(%) ^a	(%) ^a	-		
1	1	94.51 ± 1.26	77.26 ± 0.98	12.61		
	10	118.36 ± 0.57	77.96 ± 1.05	13.63		
	50	124.53 ± 0.95	72.71 ± 0.08	5.98		
2	1	110.66 ± 0.09	74.72 ± 1.09	8.91		
	10	107.10 ± 0.37	75.67 ± 0.83	10.29		
	50	115.32 ± 0.85	75.09 ± 1.34	9.44		
3	1	121.55 ± 0.48	71.99 ± 1.03	4.05		
	10	118.46 ± 0.83	84.54 ± 1.30	22.19		
	50	97.11 ± 0.28	75.44 ± 1.10	9.03		
4	1	99.76 ± 1.31	84.19 ± 1.49	22.71		
	10	103.04 ± 0.57	80.90 ± 1.49	17.91		
	50	120.25 ± 1.02	66.43 ± 0.05	-3.31		
5	1	120.82 ± 0.94	70.36 ± 0.75	1.69		
	10	99.06 ± 0.16	68.08 ± 1.05	-1.60		
	50	94.24 ± 0.59	54.80 ± 0.41	-20.80		
7	0	100.00 ± 0.03	48.87 ± 0.03			
	3.125	96.44 ± 0.04	44.44 ± 0.02	-9.07		
	6.25	96.71 ± 0.06	40.96 ± 0.04	-16.20		
	12.5	93.87 ± 0.03	34.80 ± 0.01	-28.80		
8	0	100.00 ± 0.02	48.93 ± 0.04			
	3.125	98.66 ± 0.02	44.46 ± 0.25	-9.13		
	6.25	91.60 ± 0.03	27.95 ± 0.05	-42.87		
	12.5	89.17 ± 0.01	42.48 ± 0.49	-13.17		
12	0	100.00 ± 0.03	48.87 ± 0.03			
	6.25	98.11 ± 0.02	61.16 ± 0.04	25.15		
	12.5	99.62 ± 0.02	63.94 ± 0.04	30.83		
	25	101.85 ± 0.04	59.50 ± 0.03	21.74		
	50	98.41 ± 0.03	53.78 ± 0.02	10.05		
13	0	100.00 ± 0.04	48.87 ± 0.03			
	12.5	102.59 ± 0.07	57.63 ± 0.05	17.92		
	25	97.39 ± 0.01	58.71 ± 0.02	20.13		
	50	93.30 ± 0.03	58.09 ± 0.02	18.85		
14	100	92.04 ± 0.01	58.57 ± 0.01	19.83		
14	0	100.00 ± 0.04	48.95 ± 0.04	10.20		
	12.5	96.54 ± 0.03	57.93 ± 0.03	18.39		
	25	95.32 ± 0.03	60.22 ± 0.07	23.07		
	50	94.54 ± 0.03	54.50 ± 0.05	11.38		
15	100	102.00 ± 0.01	51.90 ± 0.04	6.07		
15	0	100.00 ± 0.02	48.93 ± 0.04	4.00		
	3.125	100.26 ± 0.01	51.32 ± 0.05	4.88		
	0.25	92.86 ± 0.02	54.50 ± 0.04	11.39		
16	12.5	92.09 ± 0.01	$5/./8 \pm 0.04$	18.08		
10	0	100.00 ± 0.02	51.50 ± 0.06	11 74		
	5.125	$9/.14 \pm 0.02$	45.45 ± 0.05	-11./4		
	0.25	98.09 ± 0.02	48.12 ± 0.05	-0.5/		
	12.5	93.54 ± 0.02	46.50 ± 0.31	-9.70		

Table S1. Effects of compounds 1-5, 7, 8 and 12-16 against H_2O_2 -induced cytotoxicity in PC 12

^a The cell viabilities are presented as mean \pm SD, which are determined by at least three independent experiments.

^b Rescue rate = [(cell viability)_{H2O2 + compound} - (cell viability)_{H2O2}]/[(cell viability)_{H2O2}] × 100%