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# Adenanthin, a natural *ent-kaurane* diterpenoid isolated from the herb *Isodon adenantha* inhibits adipogenesis and the development of obesity by regulation of ROS

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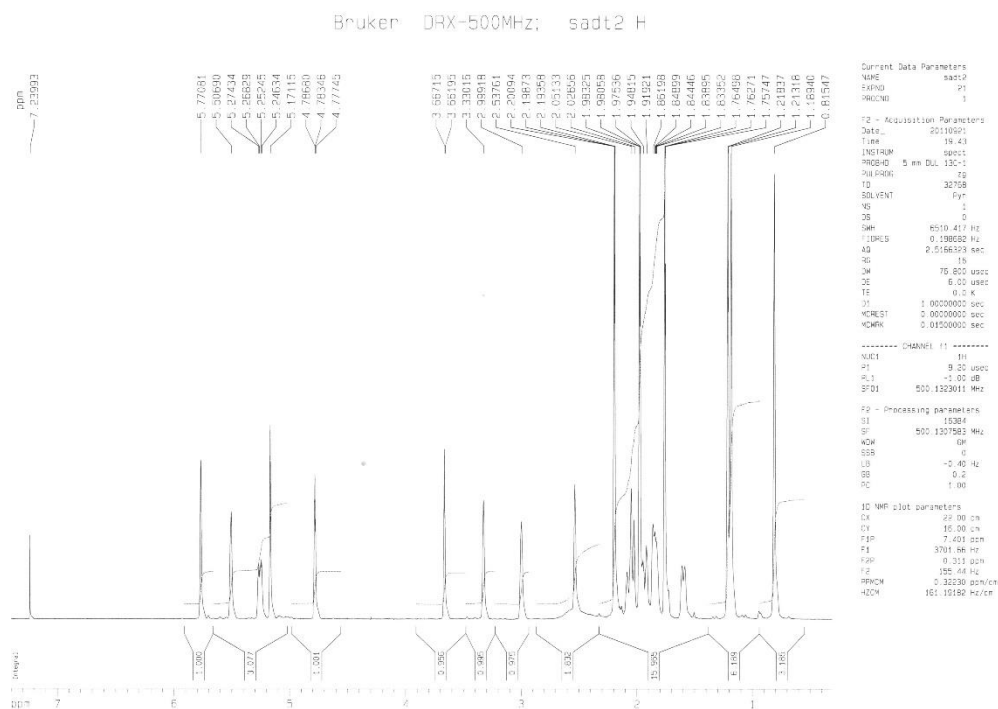
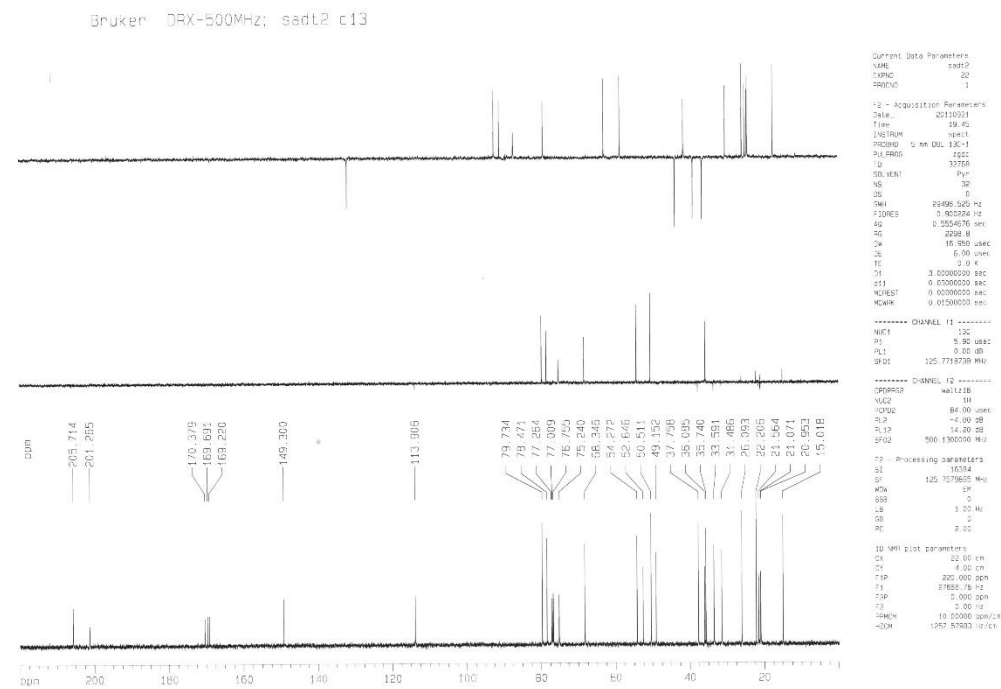
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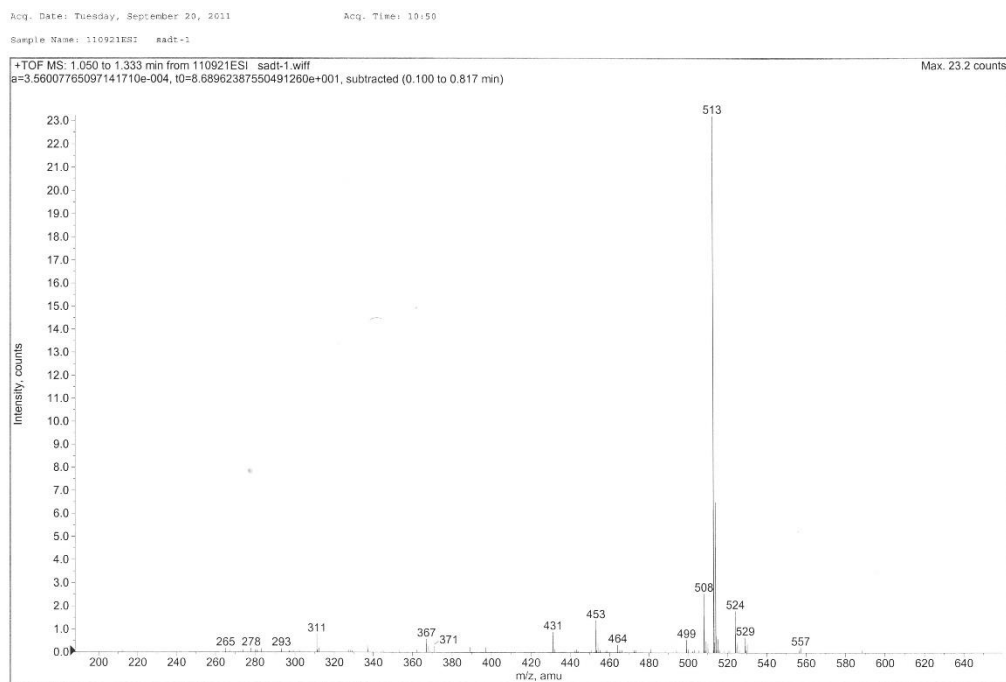
## Supporting data

1. The NMR spectra of adenanthin
2. The MS spectra of adenanthin
3. The HRESIMS spectra of adenanthin
4. Supplementary table1 (Table S1)
5. Supplementary figure1 (Figure S1)
6. Supplementary figure2 (Figure S2)

1. Copies of  $^1\text{H}$  NMR spectra of Adenanthin

Copies of  $^{13}\text{C}$  NMR spectra of Adenanthin

## 2. Copies of MS spectra of Adenanthin



## 3. The HRESIMS spectra of adenanthin

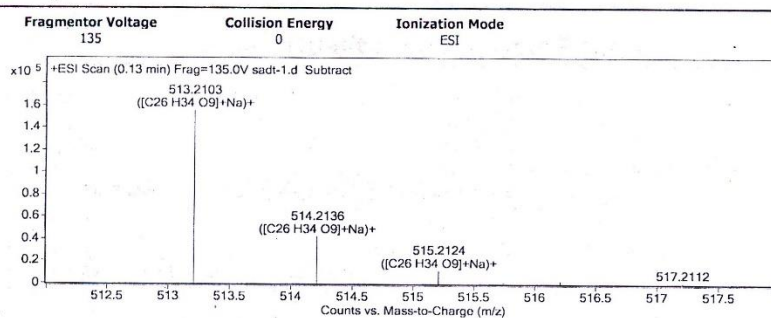
## Qualitative Analysis Report

<b>Data Filename</b>	sadt-1.d	<b>Sample Name</b>	sadt-1
<b>Sample Type</b>	Sample	<b>Position</b>	P1-A1
<b>Instrument Name</b>	Instrument 1	<b>User Name</b>	
<b>Acq Method</b>	s.m	<b>Acquired Time</b>	12/24/2018 11:55:22 AM
<b>IRM Calibration Status</b>	Success	<b>DA Method</b>	Default.m
<b>Comment</b>			

<b>Sample Group</b>		<b>Info.</b>	
<b>Acquisition SW</b>	6200 series TOF/6500 series		
<b>Version</b>	Q-TOF B.05.01 (B5125.2)		

## User Spectra



## Peak List

m/z	z	Abund	Formula	Ion
508.2549	1	87311.02		
513.2103	1	155241.19	C <sub>26</sub> H <sub>34</sub> O <sub>9</sub>	(M+Na)+
514.2136	1	42244.24	C <sub>26</sub> H <sub>34</sub> O <sub>9</sub>	(M+Na)+
529.1846	1	142186.94		
530.1876	1	37896.65		
1003.4331	1	271884.41		
1004.4363	1	151853.25		
1005.4386	1	50421.89		
1019.4071	1	41739.49		
1020.4083	1	24297.93		

## Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

## Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C <sub>26</sub> H <sub>34</sub> O <sub>9</sub>	490.2203	513.2095	513.2103	-0.80	-1.56	10.0000

--- End Of Report ---

## 4. Supplementary table1 (Table S1)

Table S1. Effect of Adenanthin on Serum ALT、AST and Creatinine in Mice

Serum concentration	ALT U/L	AST U/L	Creatinine $\mu$ M
Normal	8.87 $\pm$ 1.23	14.86 $\pm$ 1.49	81.73 $\pm$ 1.89
HFD (2% DMSO)	13.15 $\pm$ 1.74	13.45 $\pm$ 1.30	88.57 $\pm$ 4.29
HFD+Ade (2.5 mg/kg)	11.03 $\pm$ 1.06	11.67 $\pm$ 1.90	84.82 $\pm$ 6.56
HFD+Ade (5 mg/kg)	14.71 $\pm$ 2.86	16.05 $\pm$ 1.36	78.17 $\pm$ 2.07
HFD+Ade (10 mg/kg)	16.75 $\pm$ 3.39	14.40 $\pm$ 1.48	91.89 $\pm$ 6.89
HFD+Orlistat (60 mg/kg)	15.76 $\pm$ 2.79	20.46 $\pm$ 2.14	80.87 $\pm$ 3.91

Mice were treated with vehicle (2% DMSO), adenanthin (2.5, 5 or 10 mg/kg) or orlistat (60mg/kg) once a day for continuous ten courses. Each course included continuous 2- day injection and 1- day break. The mice were fasted 4 h before serum collected. Error bars represent SE, n=8. ALT: alamine amino transferase; AST: aspartate amino transferase; CK: creatine kinase; Normal: normal diet; HFD: high-fat diet; Ade: Adenanthin.

## 5. Supplementary figure1 (Figure S1)

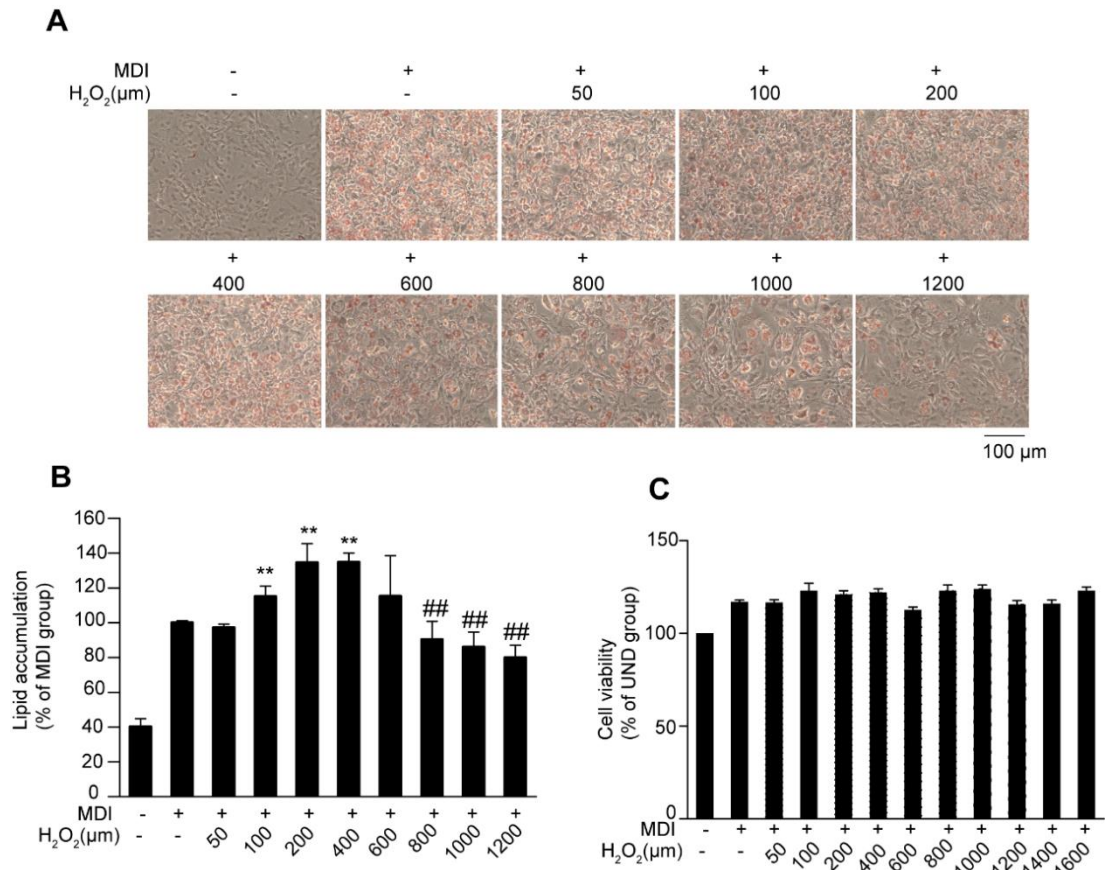


Figure S1. Effect of H<sub>2</sub>O<sub>2</sub> on 3T3-L1 adipogenesis. (A) Lipid accumulation of 3T3-L1 adipocytes treated with various doses of H<sub>2</sub>O<sub>2</sub> were visualized by Oil Red O staining, Scale bars, 100  $\mu$ m. (B) Quantification of accumulated lipid content by measuring absorbance at 492 nm. (C) Effect of H<sub>2</sub>O<sub>2</sub> on 3T3-L1 cell viability after 7 days of differentiation. Data are presented as mean  $\pm$  SD of three independent experiments; \*\*p<0.01 versus MDI group; ##p<0.01 versus MDI group.

## 6. Supplementary figure2 (Figure S2)

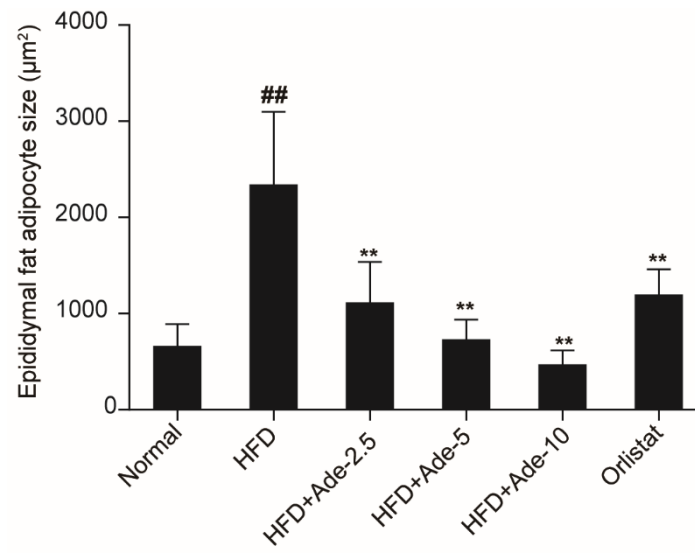


Figure S2. The epididymal fat adipocyte size quantification of cells from Figure 5d. The data are presented as mean  $\pm$  SD; \*\* $p < 0.01$  versus HFD-induced group; ## $p < 0.01$  versus normal diet group.