SUPPLEMENTARY MATERIAL

A new isoflavone with anti-inflammatory effect from the seeds of *Millettia pachycarpa*

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ABSTRACT

A new isoflavone, milletenol A (**1**), along with four known flavonoids (**2**-**5**) were isolated from the seeds of *Millettia pachycarpa*. The structure of **1** was established by extensive spectroscopic methods while known compounds were identified by comparisons with literature data. Compound **1** and **2** showed significant anti-inflammatory activities against nitric oxide production in LPS-induced RAW264.7 macrophages. The state of $CuSO_4$-stimulated inflammation was effectively alleviated by compound **1** in zebrafish. However, no significant cytotoxicity against human breast cancer cells was observed among all isolates.

KEYWORDS

Millettia pachycarpa; isoflavone; cytotoxicity; anti-inflammatory activity

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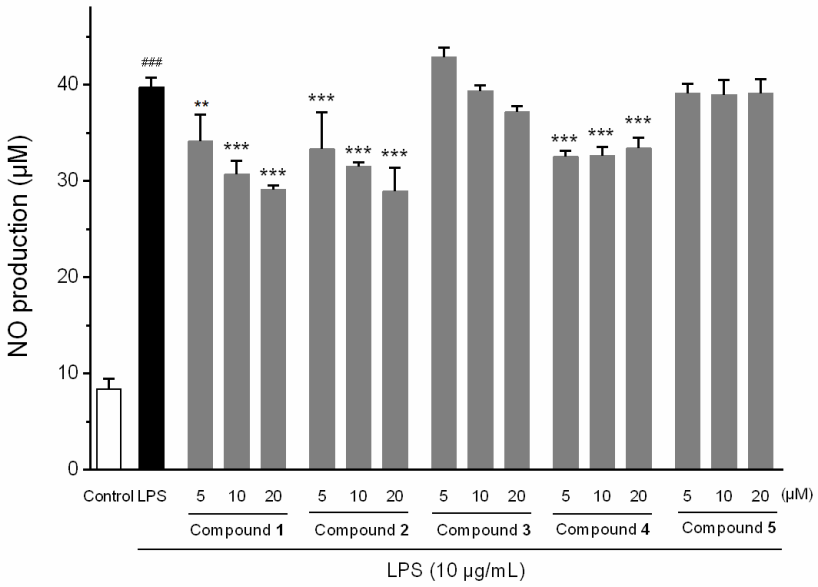

Fig. S1. Effect of compounds **1**-**5** on NO production in LPS-induced RAW264.7 macrophages. Cells were treated with indicated concentrations of compounds in the absence or presence of LPS (10 μg/mL) for 24 h. NO levels were measured using the Griess reaction. Data were expressed as mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus LPS group.

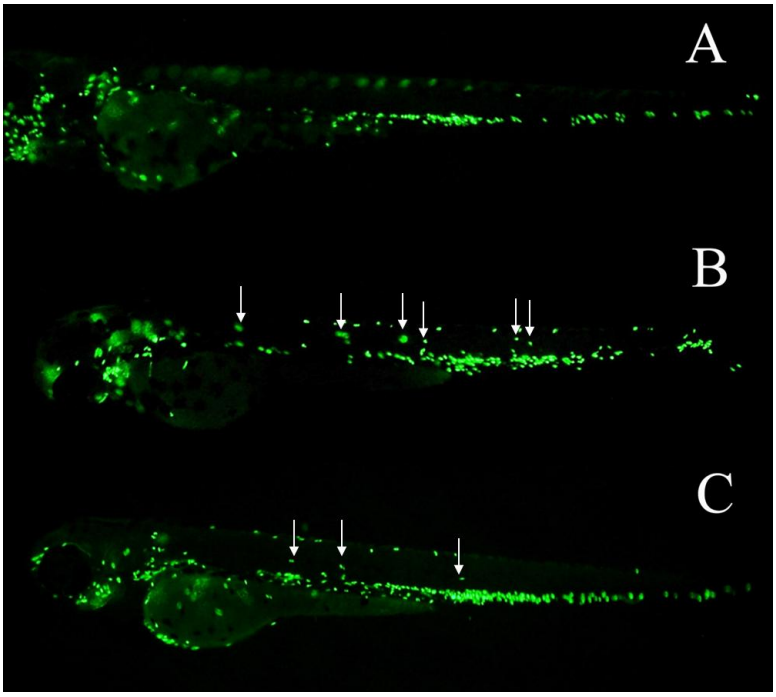

Fig. S2. Chemically induced inflammation (ChIn) assay in zebrafish. (A) DMSO-treated *Tg* (*mpo: EGFP*) larvae showed the normal distribution of labeled cells, mostly localized in the ventral trunk and tail. (B) In

CuSO4-treated siblings, neutrophils became localized preferentially to a few clusters along the horizontal midline of the trunk and tail (white arrows). (C) Larvae exposed to compound **1** exhibited a decreasing number of neutrophil along the horizontal midline of the trunk and tail. Compound **1** was added to the incubation medium 30 min prior to addition of CuSO4 and was tested for inhibition of neutrophil migration using ChIn assays.

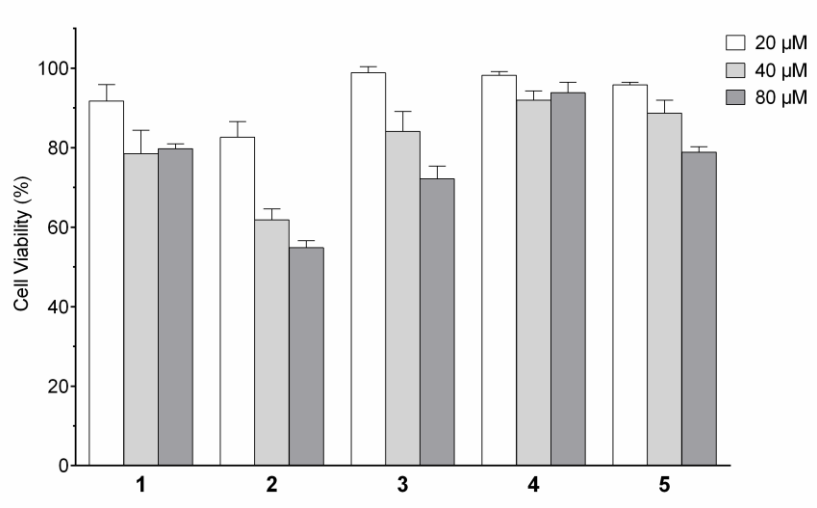

Fig. S3. Cytotoxicity of compounds **1**-**5** on MCF-7 cells. Cells were treated with different concentrations of isolates (20, 40 and 80 μM) for 48 h, and cell viability was determined by MTT assay.

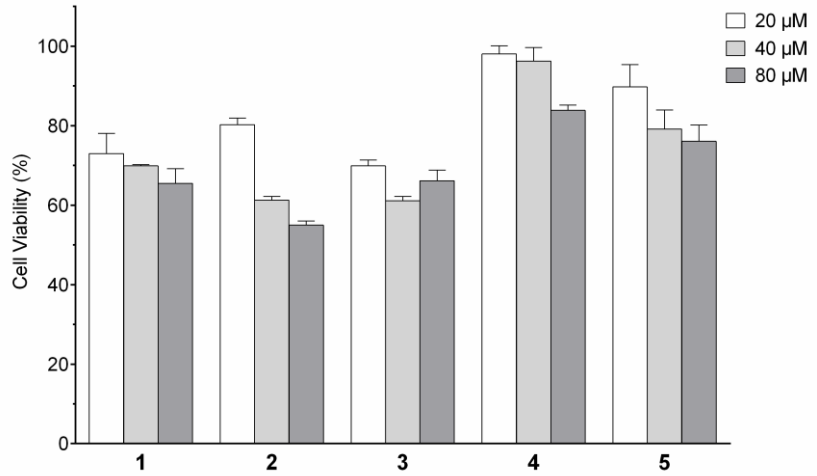

Fig. S4. Cytotoxicity of compounds **1**-**5** on MDA-MB-231 cells. Cells were treated with different concentrations of isolates (20, 40 and 80 μM) for 48 h, and cell viability was determined by MTT assay.

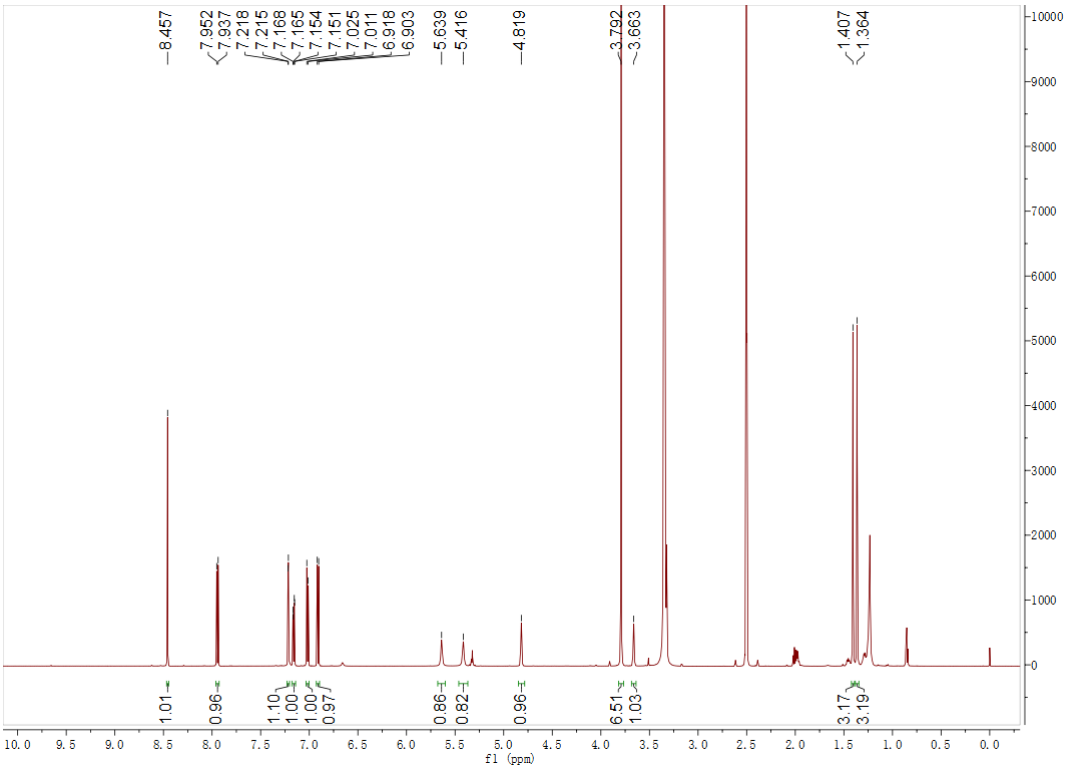

Fig. S5. ^{1}H NMR spectrum of compound **1** (DMSO-d_6, 600 MHz).

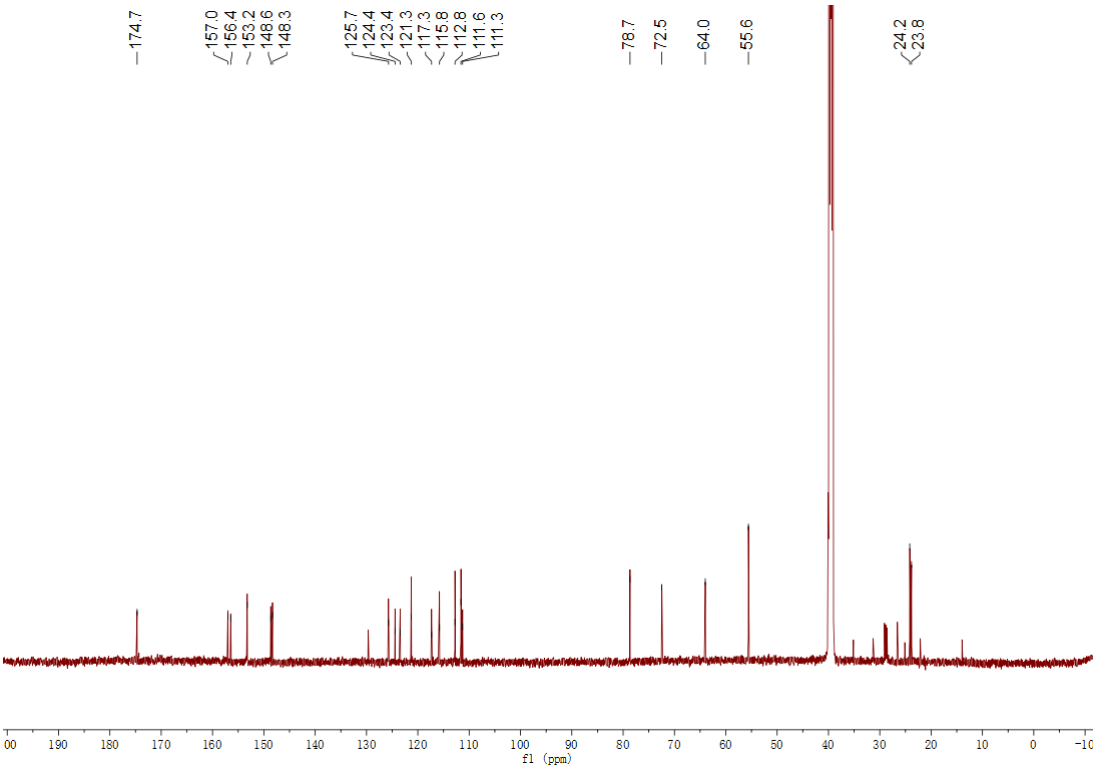

Fig. S6. ^{13}C NMR spectrum of compound **1** (DMSO-d_6, 150 MHz).

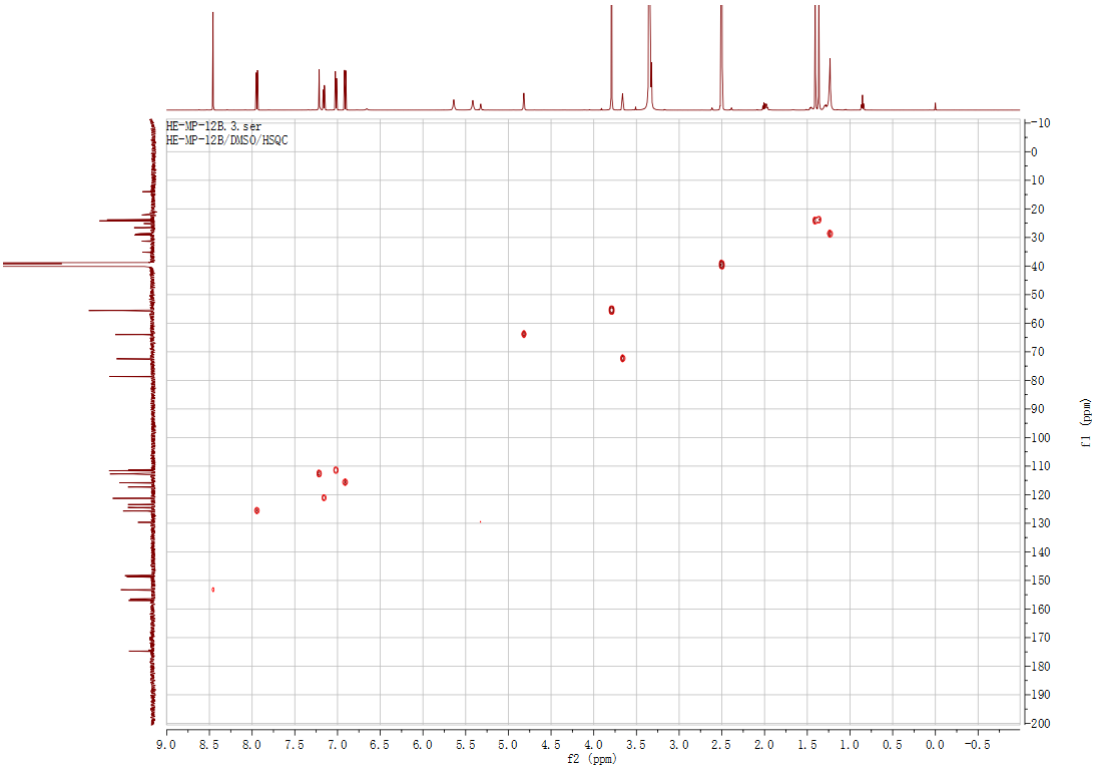

Fig. S7. HSQC spectrum of compound **1** (DMSO-d_6, 600 MHz).

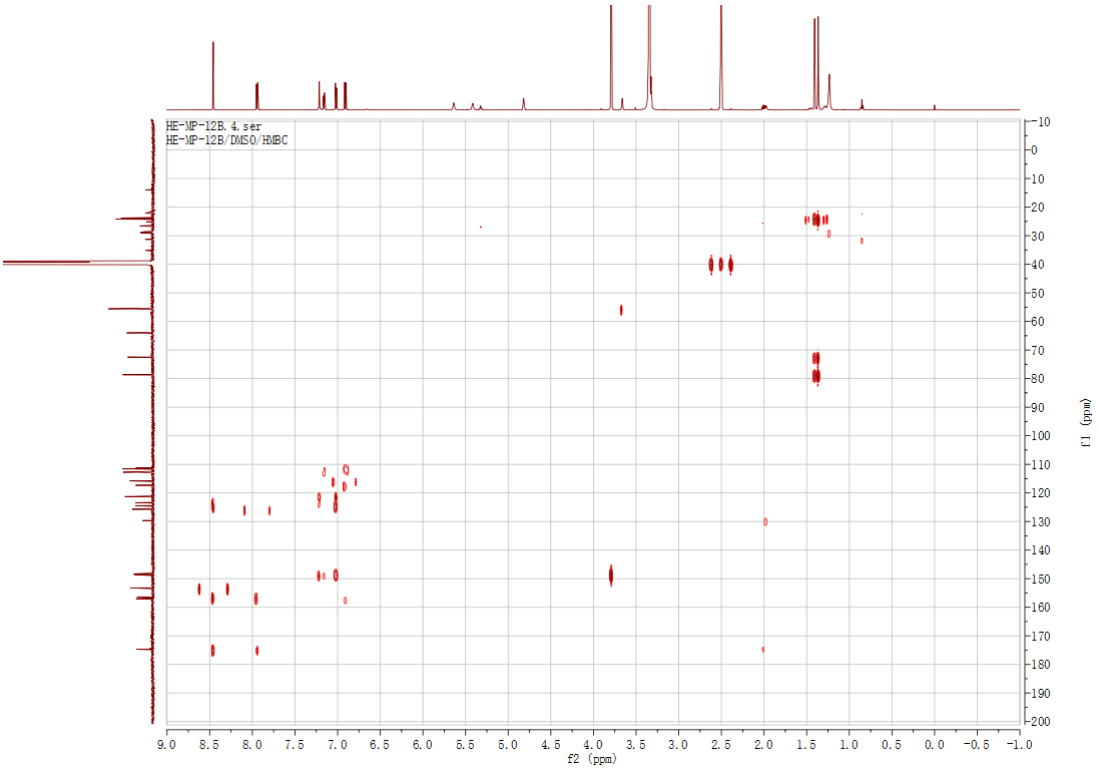

Fig. S8. HMBC spectrum of compound **1** (DMSO-d_6, 600 MHz).

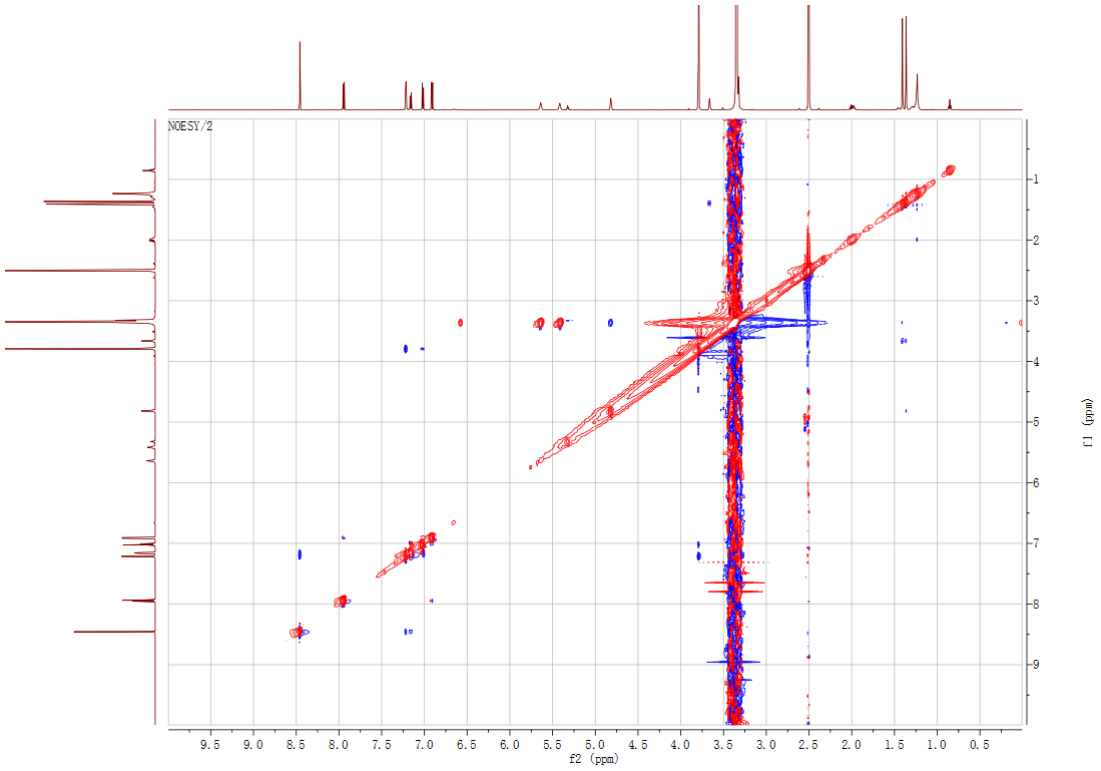

Fig. S9. NOESY spectrum of compound **1** (DMSO-d_6, 600 MHz).

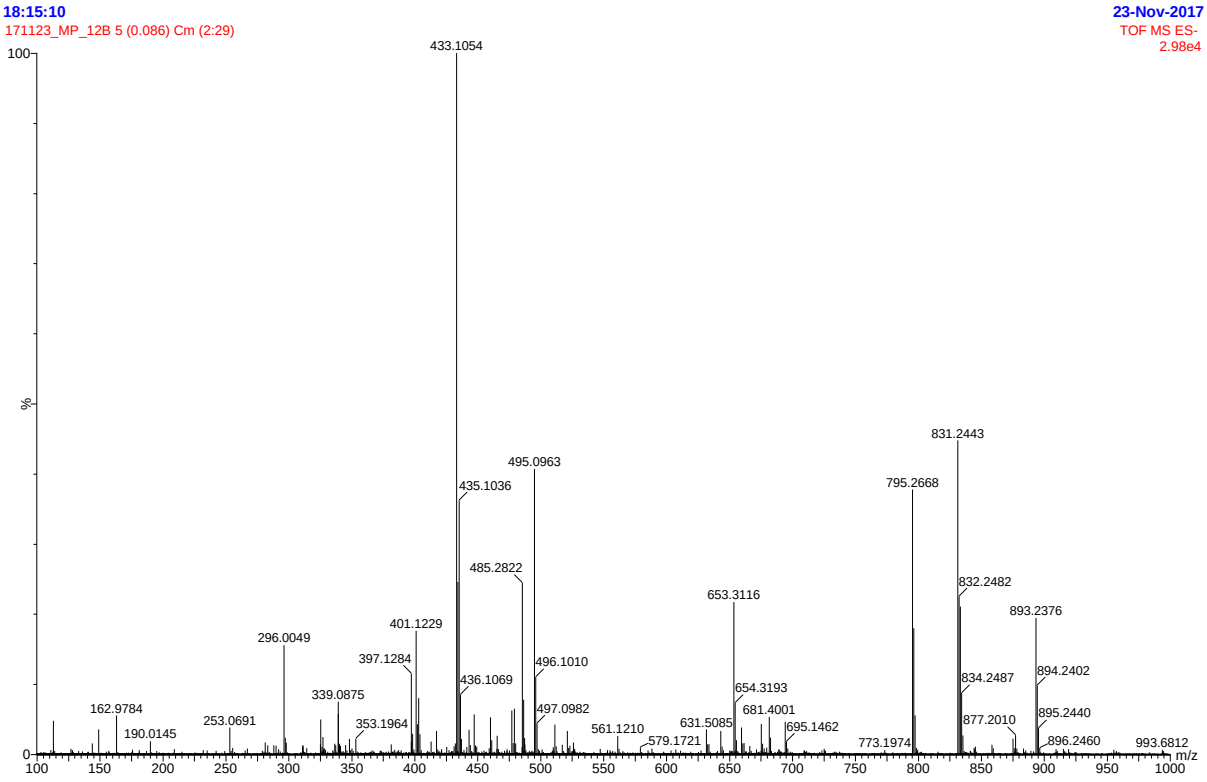

Fig. S10. HR-ESI-MS spectrum of compound **1**.