

## Neuroprotective effects of ethanol extract of *Polyscias fruticosa* (EPPF) against glutamate-mediated neuronal toxicity in HT22 cells

Baskar Selvaraj<sup>1,8</sup>, Tam Tie Le<sup>1,8,9</sup>, Dae Won Kim<sup>2</sup>, Bo Hyun Jung<sup>3</sup>, Ki Yeun Yoo<sup>3</sup>, Hong Ryul Ahn<sup>1</sup>, Phuong Thien Thong<sup>4</sup>, Thi Thu Thuy Tran<sup>5,6</sup>, Ae Nim Pae<sup>7</sup>, Sang Hoon Jung<sup>1,8,\*</sup>, Jae Wook Lee<sup>1,8,\*</sup>

<sup>1</sup>Natural Product Research Center, Institute of Natural Product, Korea Institute of Science and Technology, Gangneung, 25451, Republic of Korea.

<sup>2</sup>Department of Biochemistry and Molecular Biology, Research Institute of Oral Science, College of Dentistry, Gangneung Wonju National University, Gangneung, 25457, Republic of Korea.

<sup>3</sup>Department of Anatomy, College of Dentistry, Gangneung Wonju National University, Gangneung, 25457, Republic of Korea.

<sup>4</sup>Division of Biotechnology, Vietnam Korea Institute of Science and Technology, Hoa Lac High-tech Park, km29 Thang Long Boulevard, Hanoi, Vietnam

<sup>5</sup>Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

<sup>6</sup>Department of Chemistry, Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

<sup>7</sup>Center of Brain Disorders, Brain Science Institute, Korea Institute of Science and Technology, Seoul, 02792, Republic of Korea

<sup>8</sup>Division of Bio-medical Science & Technology, University of Science and Technology, Daejeon, 34113, Republic of Korea.

<sup>9</sup>These authors equally contributed in this work

\* Correspondence: shjung@kist.re.kr; Tel.: (82-33-650-3653, S. H. Jung) jwlee5@kist.re.kr; Tel.: (82-33-650-3514, J.W. Lee)

## List of Figures

**Figure S1.** Cell cycle analysis of apoptotic cells using PI. .

**Figure S2.** Protective effects of novel compounds in EEPF.

**Figure S3.** EIMS spectrum of new compound **1**

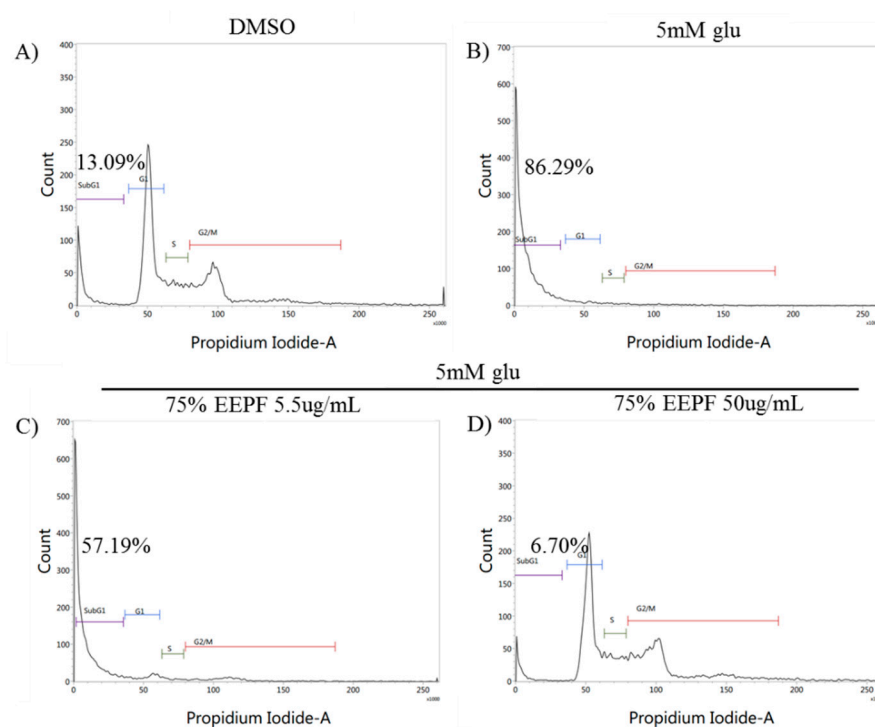
**Figure S4.**  $^1\text{H}$ -NMR spectrum of new compound **1** in  $\text{CH}_3\text{OH}-d_4$  at 500 MHz

**Figure S5.**  $^{13}\text{C}$ -NMR spectrum of new compound **1** in  $\text{CH}_3\text{OH}-d_4$  at 125 MHz

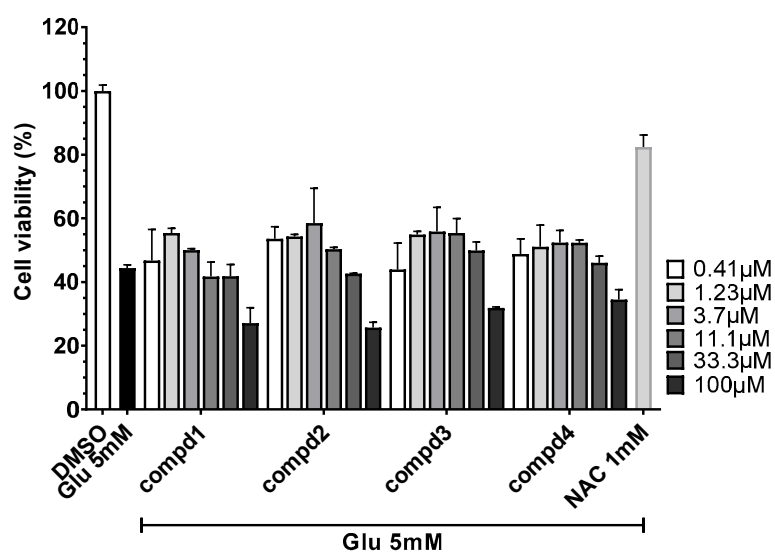
**Figure S6.** HSQC spectrum of new compound **1** in  $\text{CH}_3\text{OH}-d_4$

**Figure S7.** COSY spectrum of new compound **1** in  $\text{CH}_3\text{OH}-d_4$

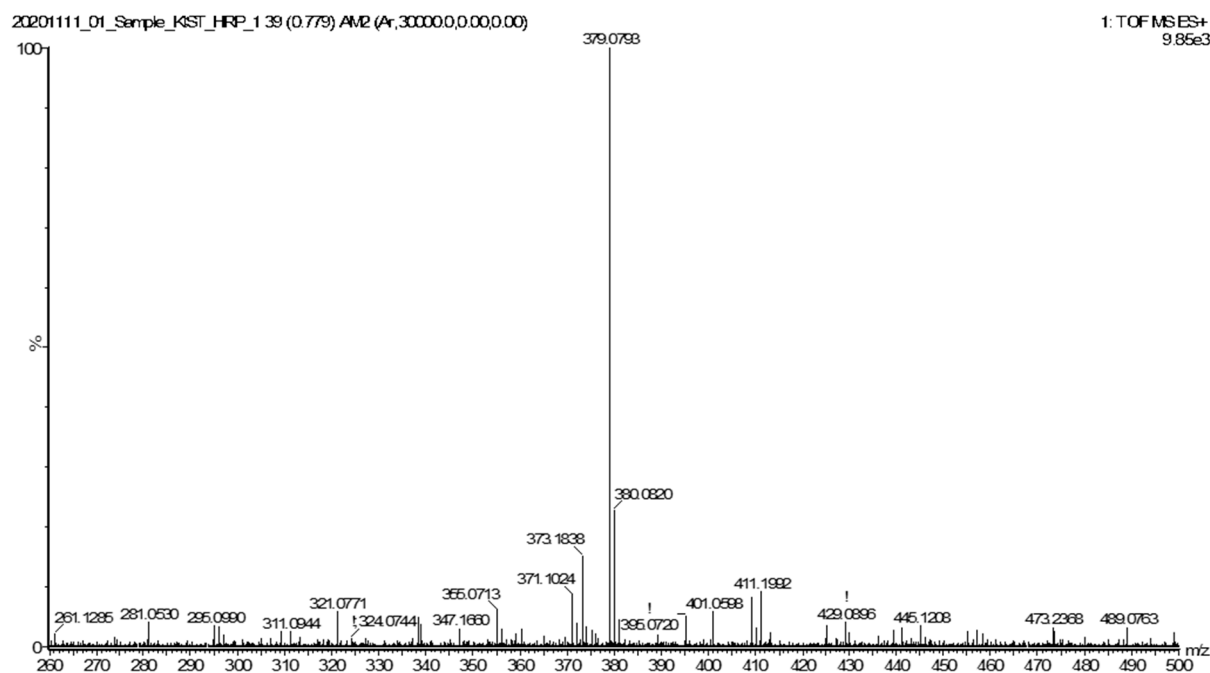
**Figure S8.** HMBC spectrum of new compound **1** in  $\text{CH}_3\text{OH}-d_4$



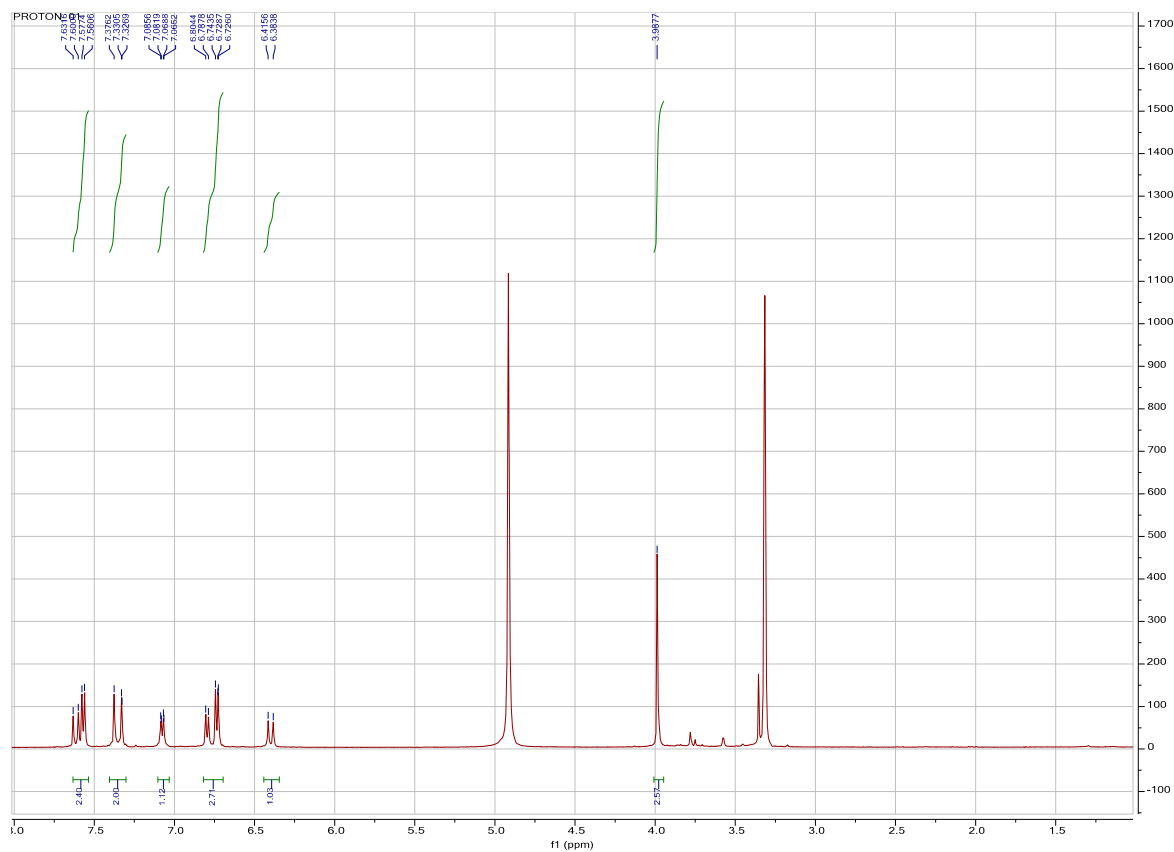
**Figure S1.** DNA contents of apoptotic cells in sub-G1 phase was determined by flow cytometry using PI (propidium iodide). The 5 mM glutamate has increased sub-G1 phase to 86.29%. However, the cotreatment of 50  $\mu\text{g}/\text{ml}$  of EEPF restored ratio of sub-G1 phase to 6.70%.



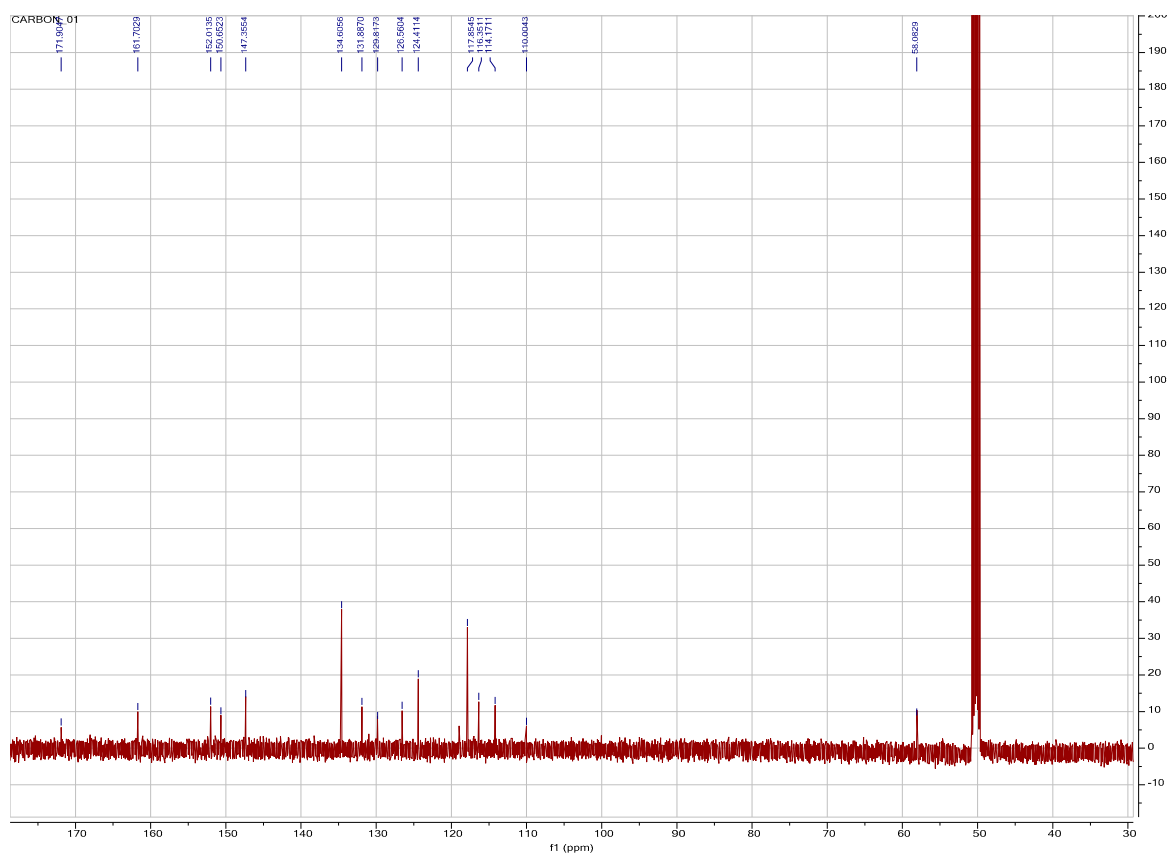
**Figure S2.** Protective effects of novel compounds in EEPF. The HT22 cells were treated with compounds, and 2 hr after 5 mM glutamate was treated and after 12 hrs the cell viability was measured by EZ-Cytox reagent. Data bar graphs are presented as means  $\pm$  SD, n=3.



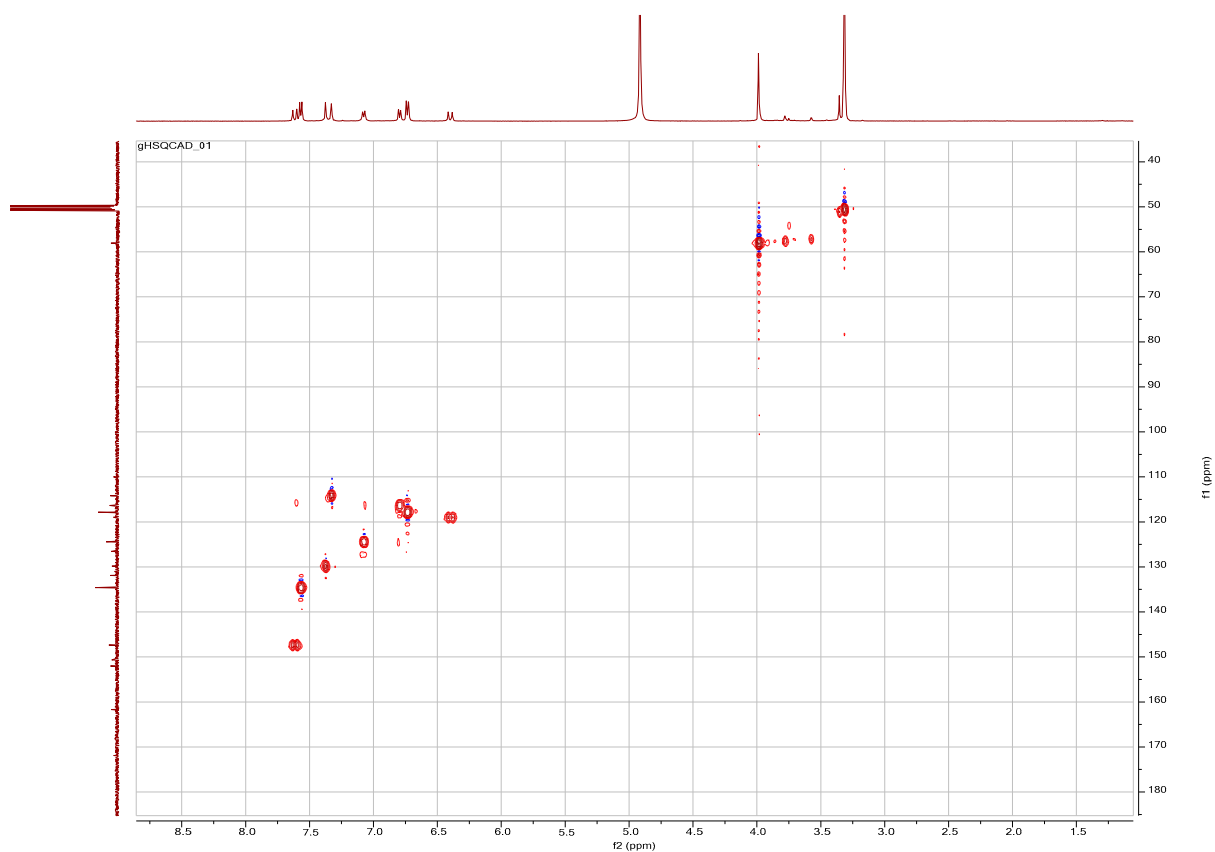
**Figure S3.** HRESI-MS spectrum of new compound 1



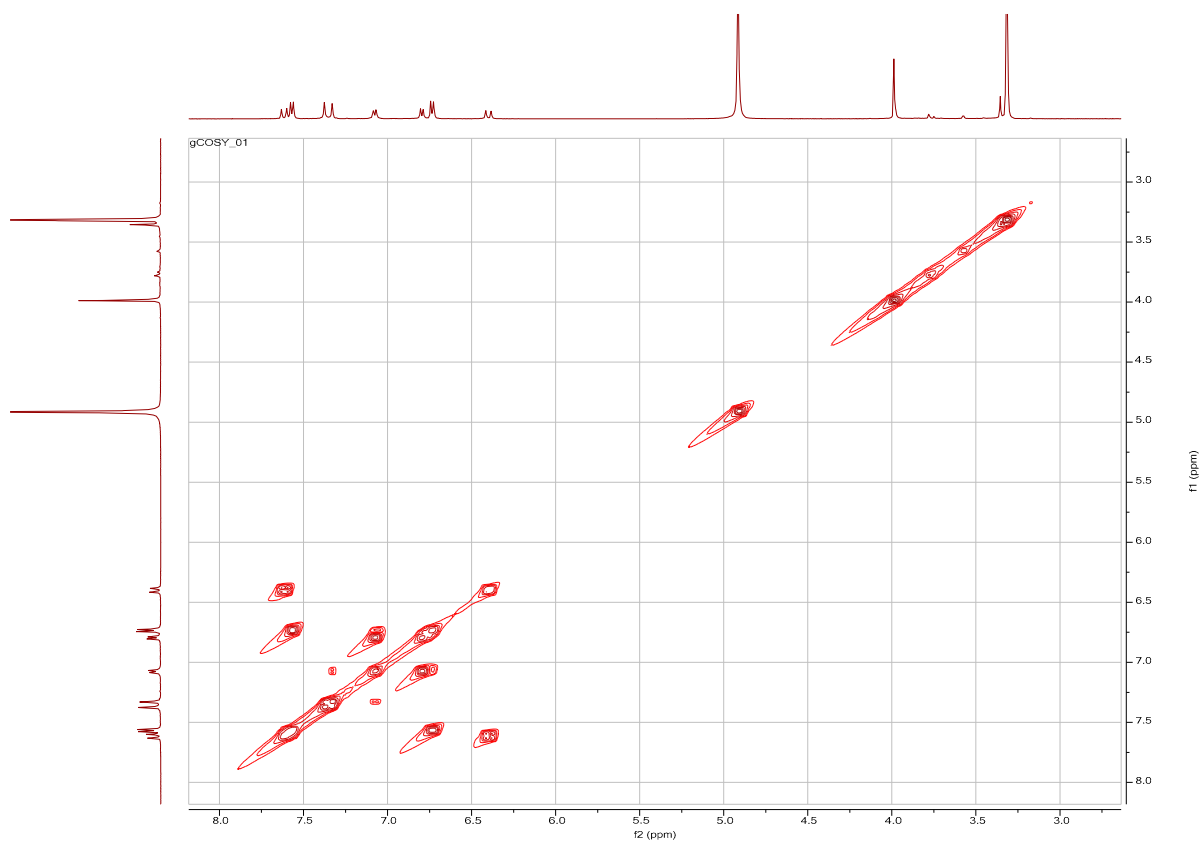
**Figure S4.** <sup>1</sup>H-NMR spectrum of new compound 1 in CH<sub>3</sub>OH-*d*<sub>4</sub> at 500 MHz



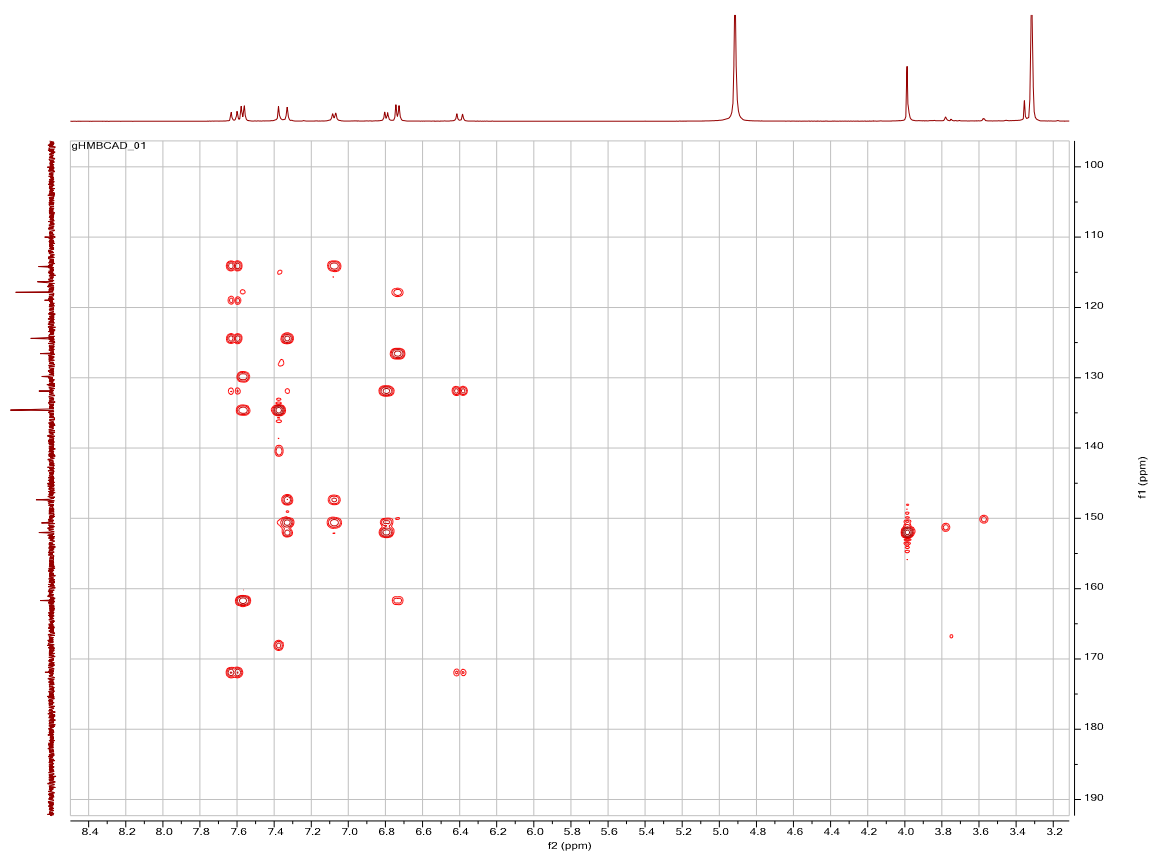
**Figure S5.**  $^{13}\text{C}$ -NMR spectrum of new compound 1 in  $\text{CH}_3\text{OH}-d_4$  at 125 MHz



**Figure S6.** HSQC spectrum of new compound 1 in CH<sub>3</sub>OH-*d*<sub>4</sub>



**Figure S7.** COSY spectrum of new compound 1 in CH<sub>3</sub>OH-*d*<sub>4</sub>



**Figure S8.** HMBC spectrum of new compound 1 in  $\text{CH}_3\text{OH}-d_4$