# G-quadruplex DNA-binding quaternary alkaloids from *Tylophora atrofolliculata*

Cheng-Yu Chen[1], Li-Ping Bai[1], Zhuo-feng Ke[2], Yan Liu[3], Jing-Rong Wang[1*], Zhi-Hong Jiang[1*]

[1]*State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau, China*

[2] *School of Materials Science and Engineering, MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry, Sun Yat-sen University, Guangzhou 510275, PR China*

[3]*School of chemical engineering and light industry, Guangdong University of Technology Guangzhou 510006, PR China*

## Contents

**Figures S1 and S2** Fluorescent spectra

**Figure S3** Molecular modeling of alkaloids **6** and **7** binding with human telomeric G-quadruplex DNA (PDB entry 2JPZ)

**Figures S4-S23** $^{1}$H, $^{13}$C NMR, HSQC, HMBC and $^{1}$H-$^{1}$H COSY spectra of compounds **1**-**4**.

**Table S1** Percent fluorescence decrease of TO at the maximum emission wavelength of 530 nm by compound **6** or **7** displacement

**Tables S2** $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) spectral data of **6**.

* Corresponding authors. E-mail address: jrwang@must.edu.mo and zhjiang@must.edu.mo

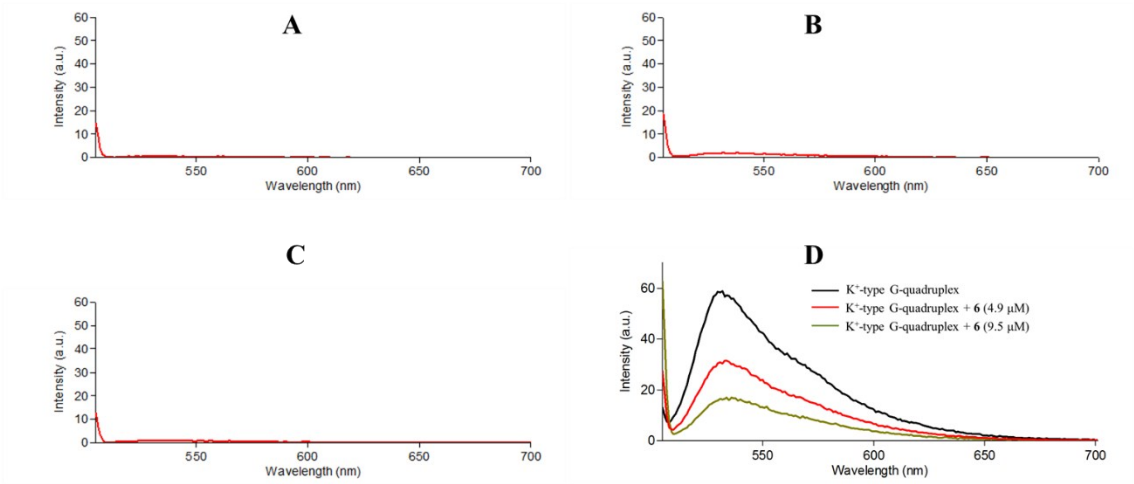



Figure S1 Fluorescent spectra. The spectra of 0.5 μM triazole orange (A), 9.5 μM compound **6** (B), 9.5 μM compound **6** with 0.25 μM DNA G-quadruplex (C) in 25 mM Tris-HCl buffer (pH 7.45) containing 100 mM KCl. The fluorescence spectra of 0.5 μM triazole orange with 0.25 μM d[$(TTAGGG)_4$TTA] (D). All the spectra were measured with the excitation wavelength at 501 nm.

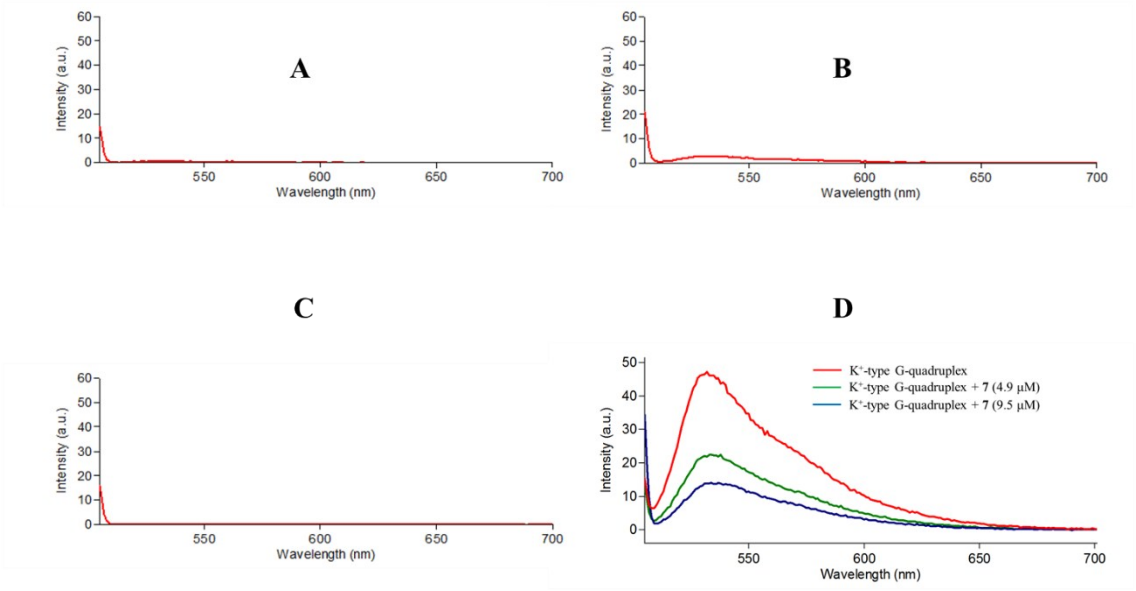



Figure S2 Fluorescent spectra. The spectra of 0.5 μM triazole orange (A), 9.5 μM compound **7** (B), 9.5 μM compound **7** with 0.25 μM DNA G-quadruplex (C) in 25 mM Tris-HCl buffer (pH 7.45) containing 100 mM KCl. The fluorescence spectra of 0.5 μM triazole orange with 0.25 μM d[(TTAGGG)$_4$TTA] (D). All the spectra were measured with the excitation wavelength at 501 nm.

Docking simulations

The receptor human telomeric DNA d[(TTAGGG)4TTA] G-quadruplex was obtained from the Protein Data Bank (PDB entry 2JPZ).[1] Gasteiger partial charges are assigned and non-polar hydrogen atoms are merged with AutoDock Tools. The whole 3-D space of the receptors is searched to obtain the most possible binding sites. The grid maps were calculated using a 120×120×120 points grid box with 0.375 Å grid-point spacing. All the docking simulations were performed with the AutoDock4.2 program.[2] The Lamarckian genetic algorithm was used for each compound. At least 80 docking runs

were performed, using a population size of 250 individuals with a maximum number of energy evaluations at $2.5 \times 10^8$ and a maximum number of generations at $2.7 \times 10^4$. To estimate the energetics of the unbound states of the receptor and the compounds, an extended model was used to evaluate their binding free energies.

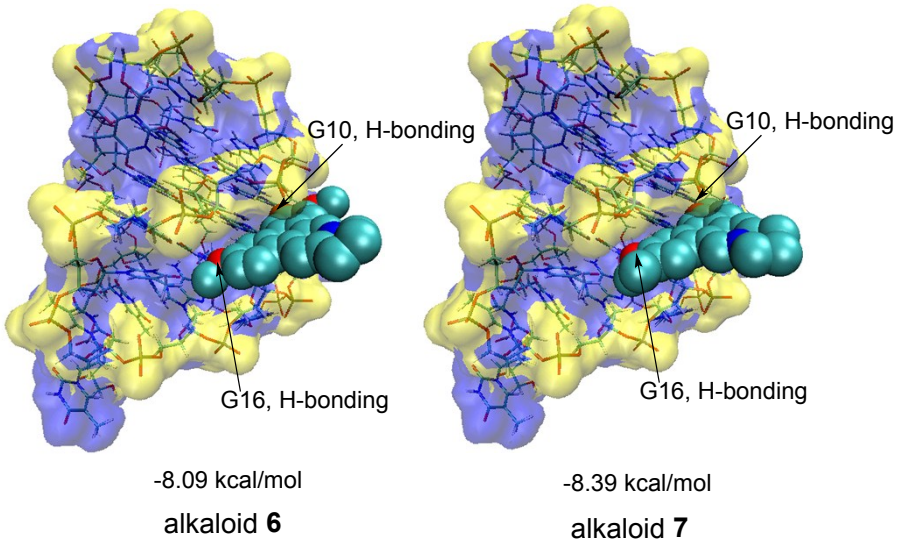



Figure S3. Molecular modeling of alkaloids **6** and **7** binding with human telomeric G-quadruplex DNA (PDB entry 2JPZ).

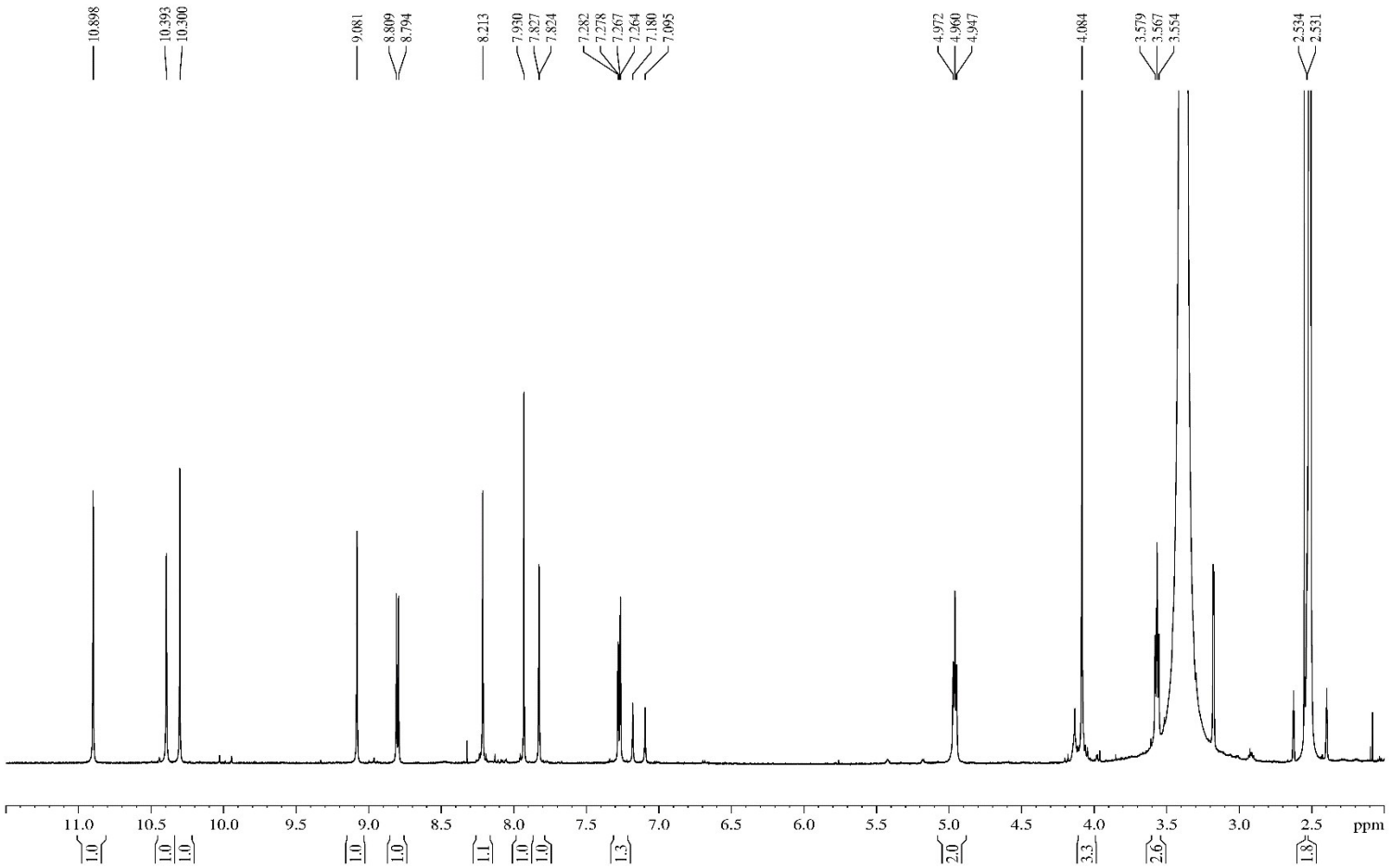



**Figure S4** $^{1}$H NMR spectrum of compound **1**.

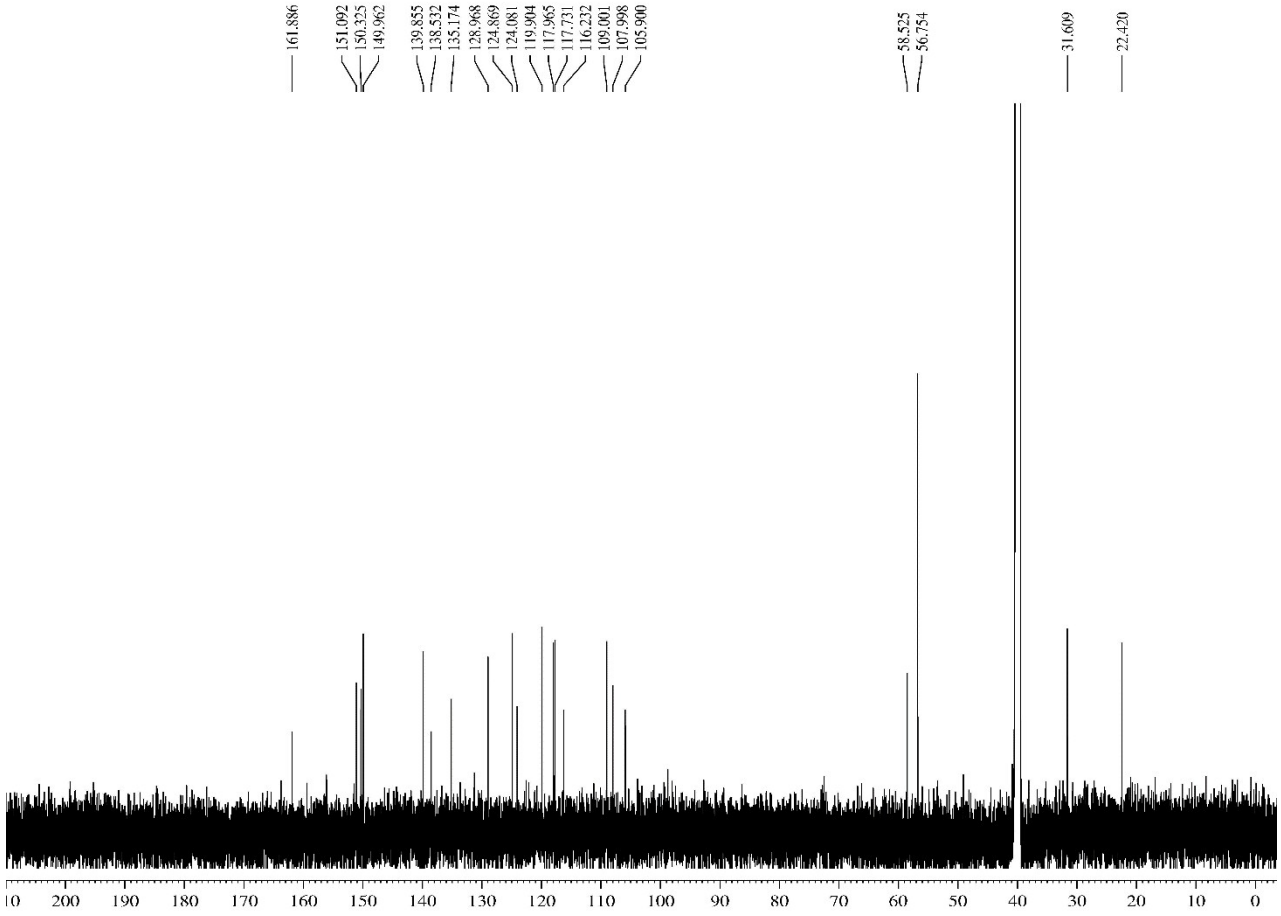



**Figure S5** $^{13}$C NMR spectrum of compound **1**.

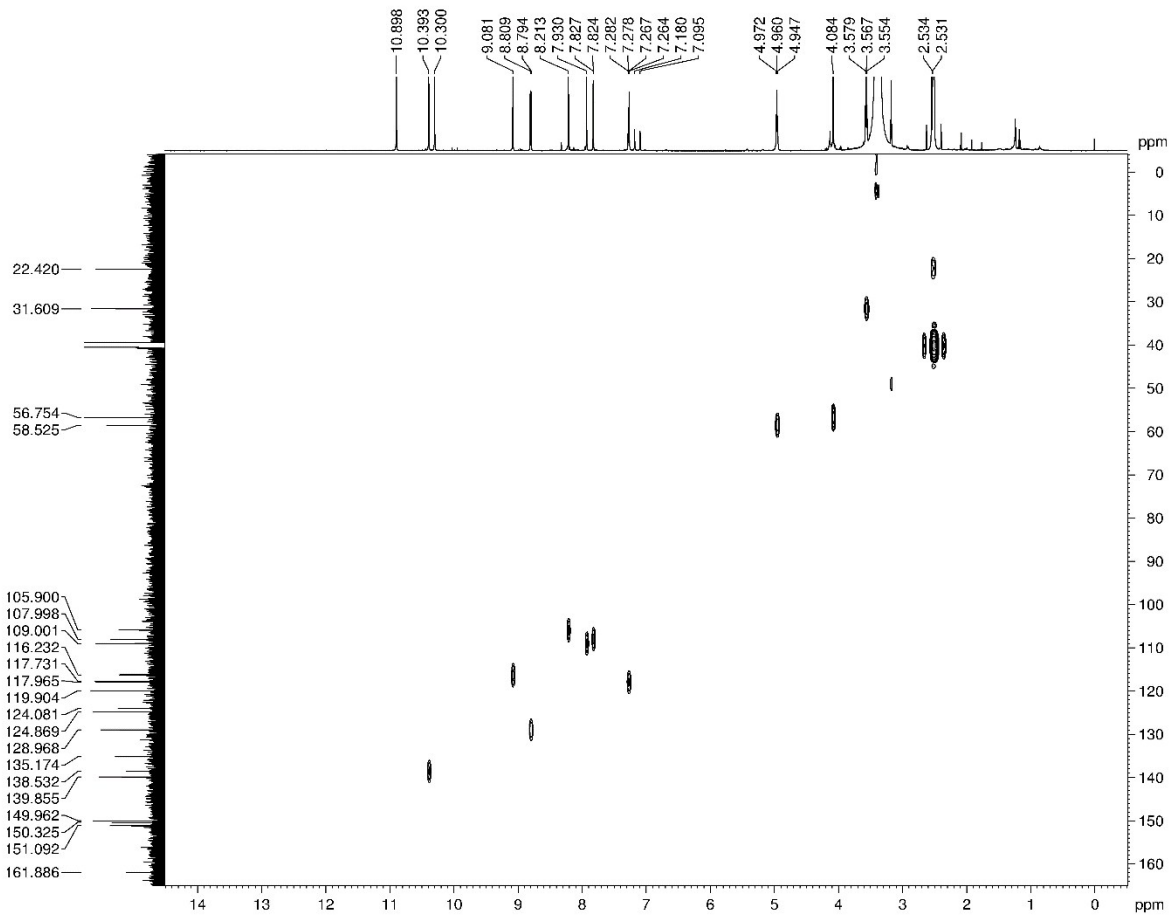



**Figure S6** HSQC spectrum of compound **1**.

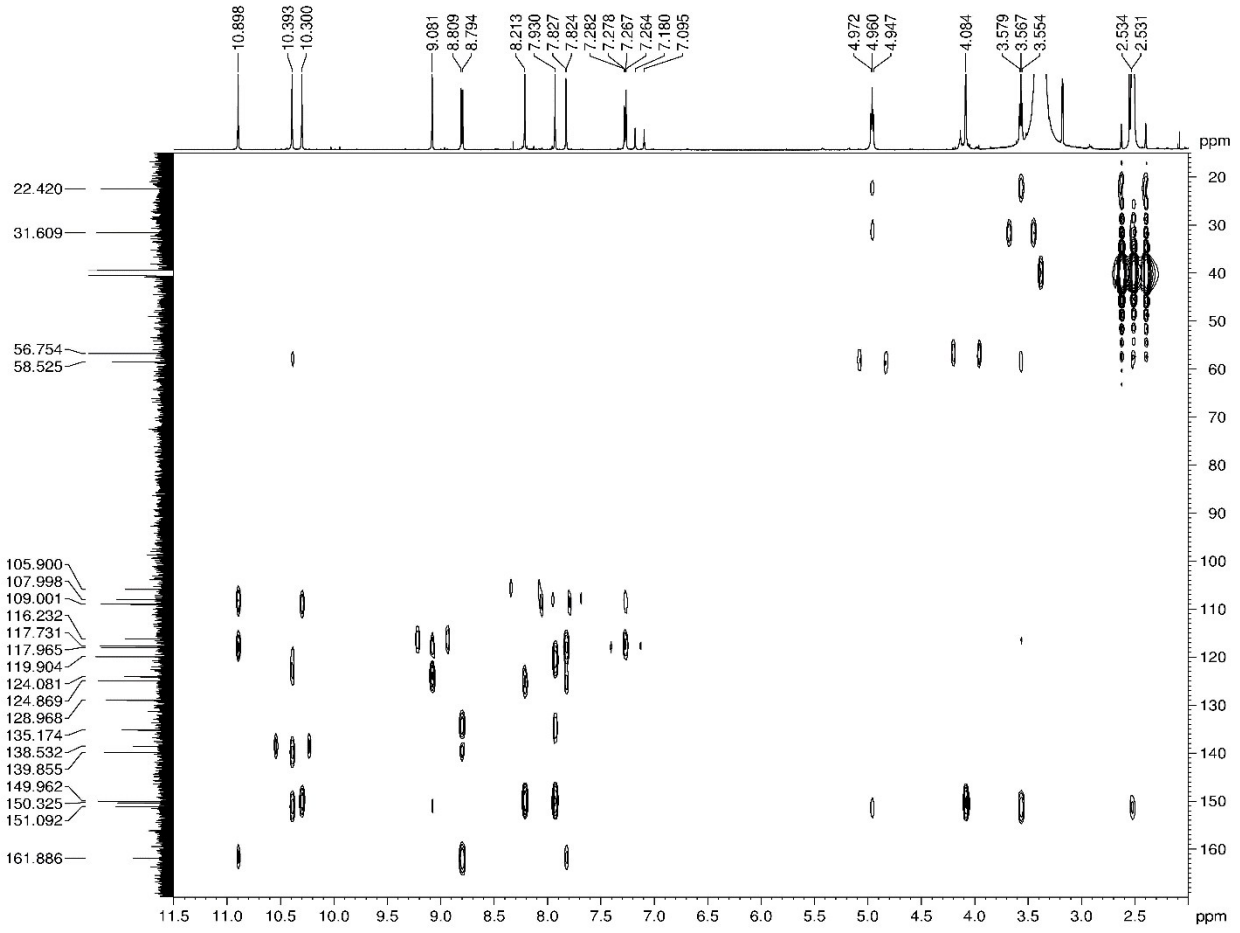



**Figure S7** HMBC spectrum of compound **1**.

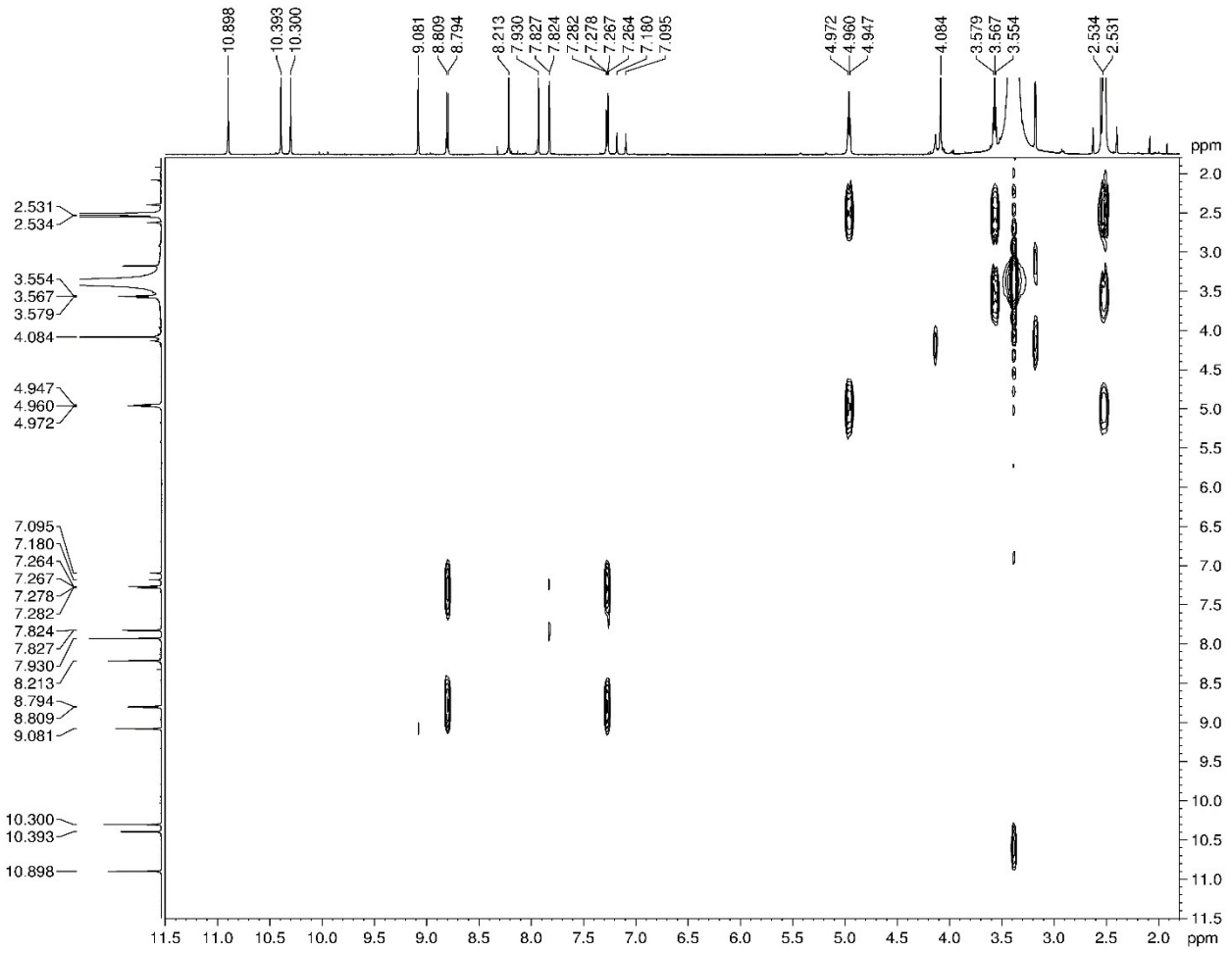



**Figure S8** $^{1}$H-$^{1}$H COSY spectrum of compound **1**.

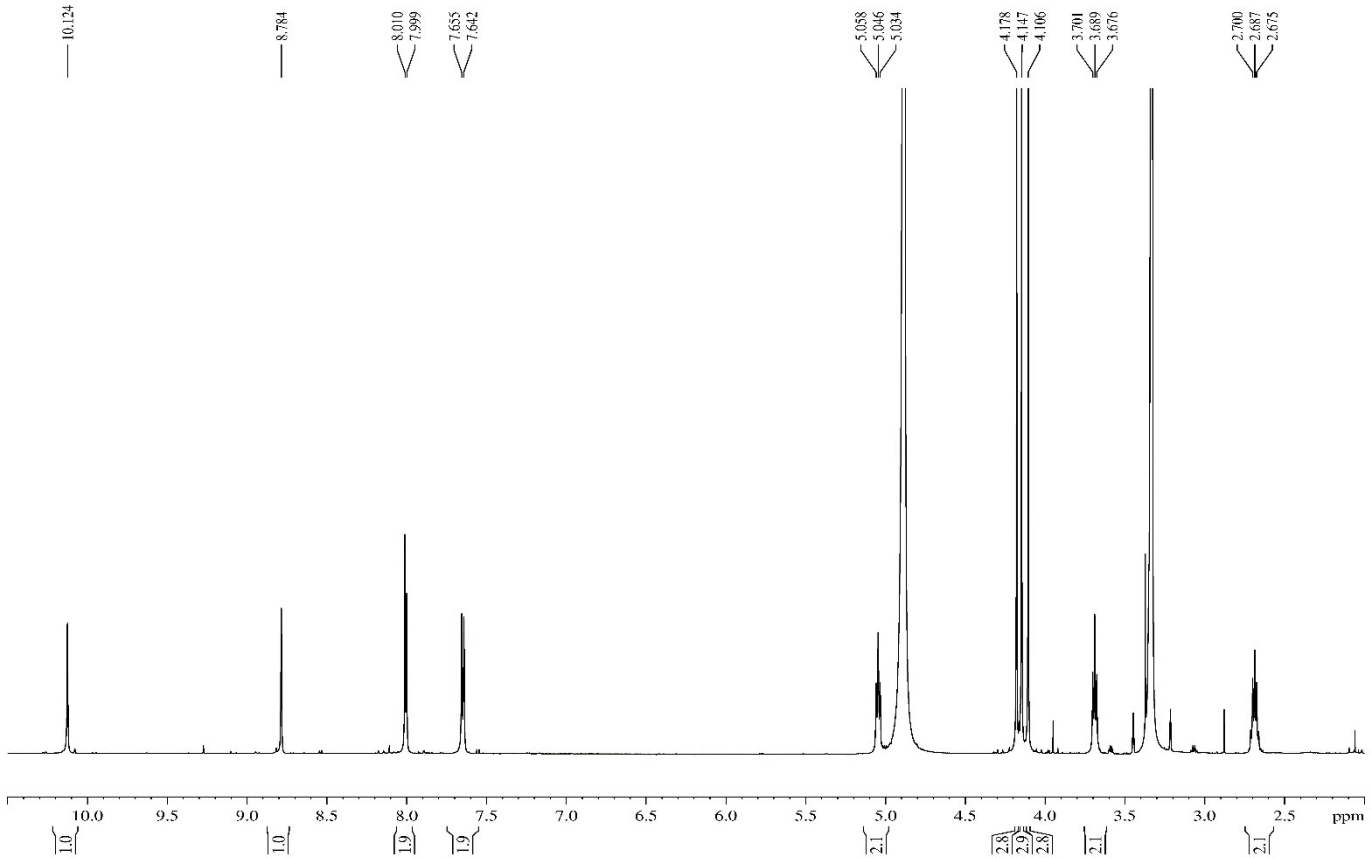



**Figure S9** $^{1}$H NMR spectrum of compound **2**.

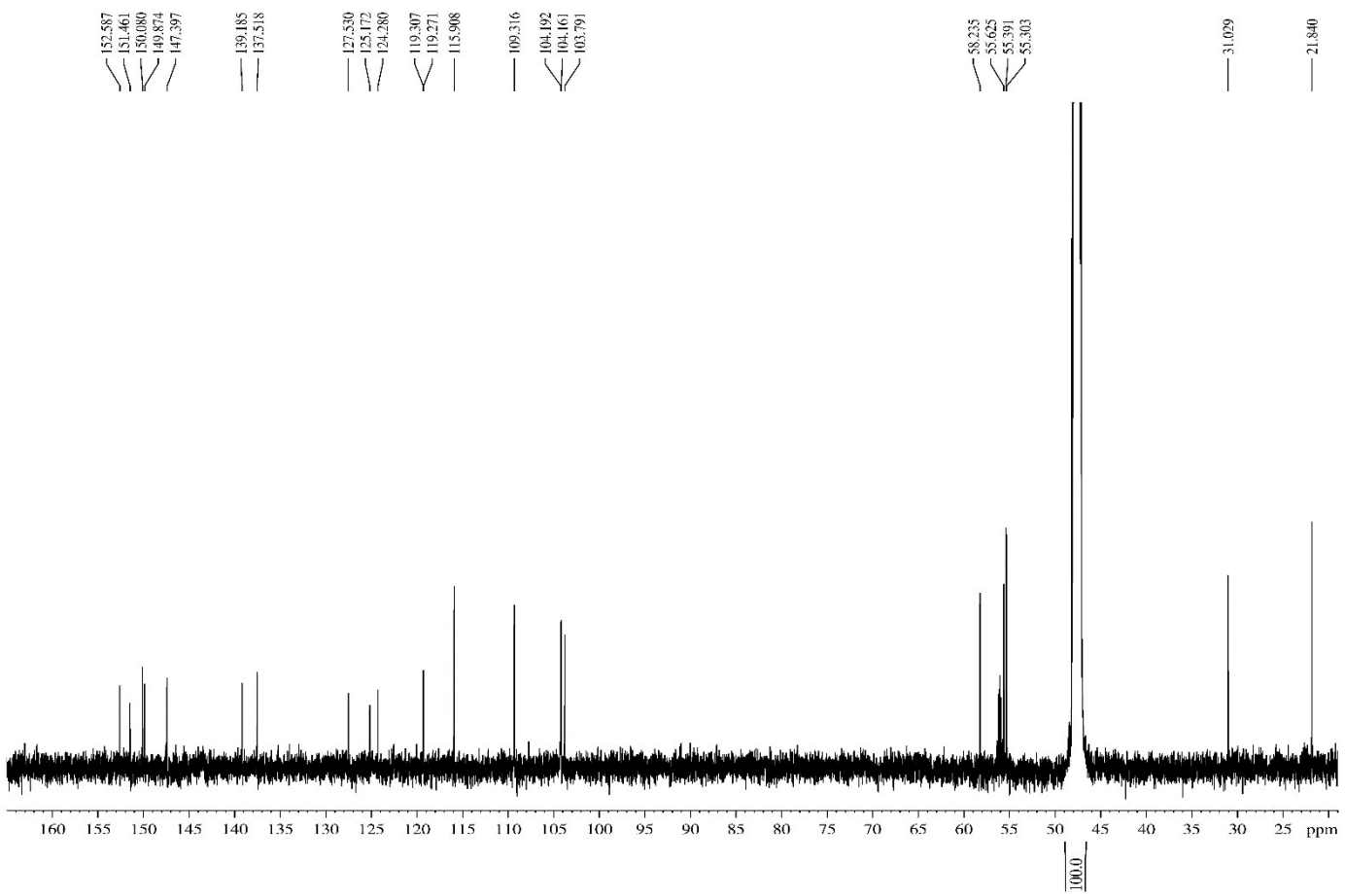



**Figure S10** $^{13}$C NMR spectrum of compound **2**.

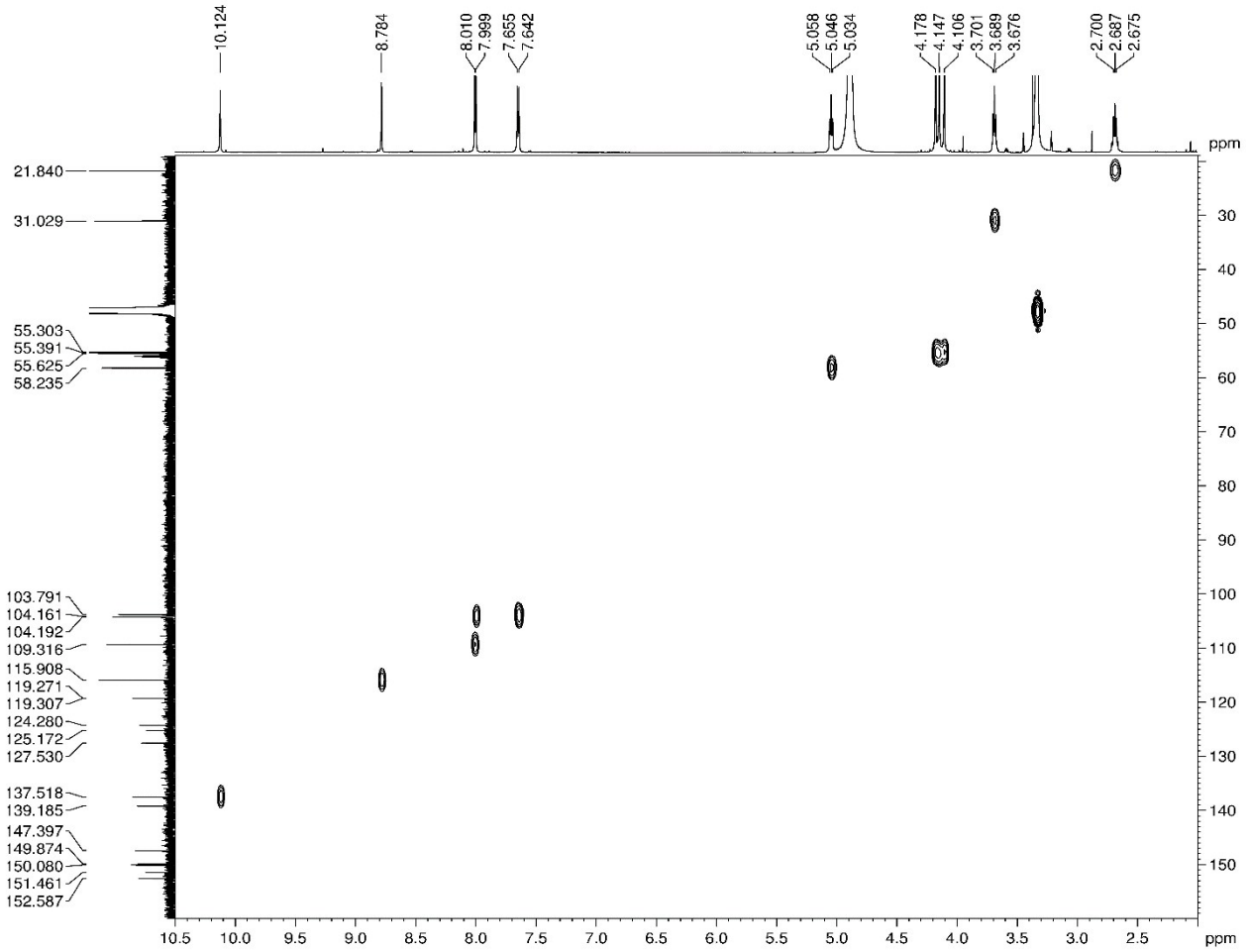



**Figure S11** HSQC spectrum of compound **2**.

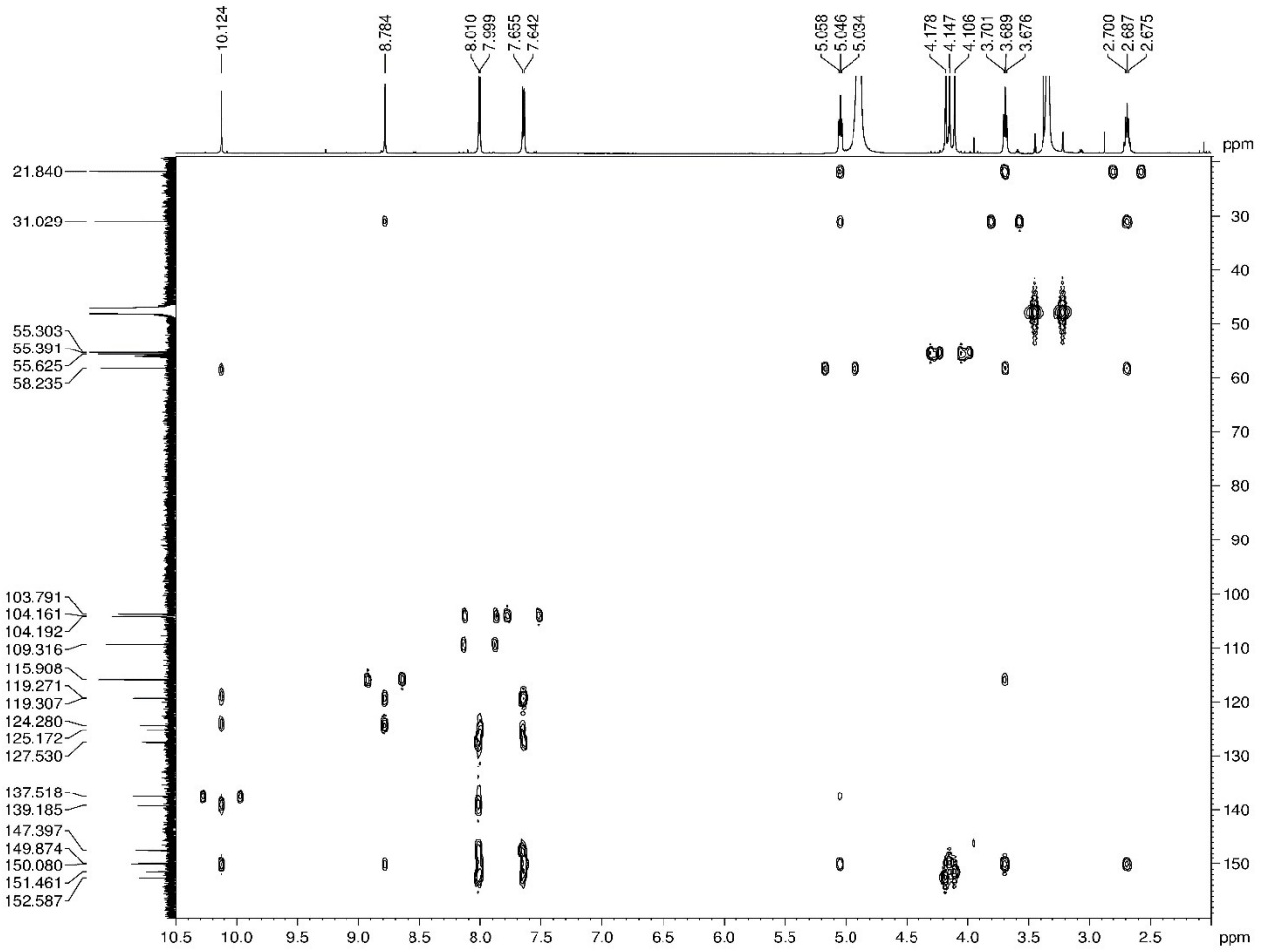
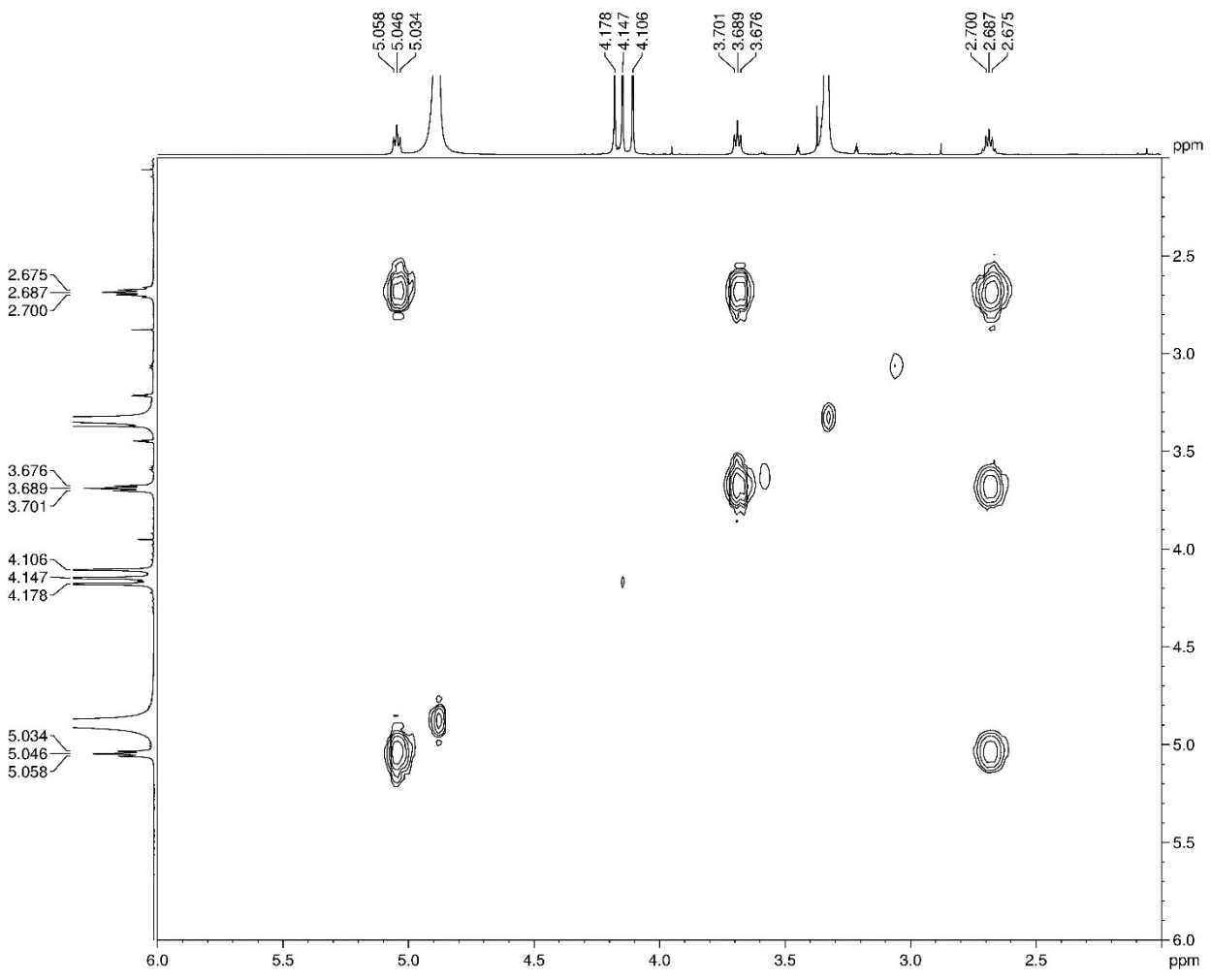



**Figure S12** HMBC spectrum of compound **2**.




81

82 **Figure S13** $^{1}$H- H COSY spectrum of compound **2**.

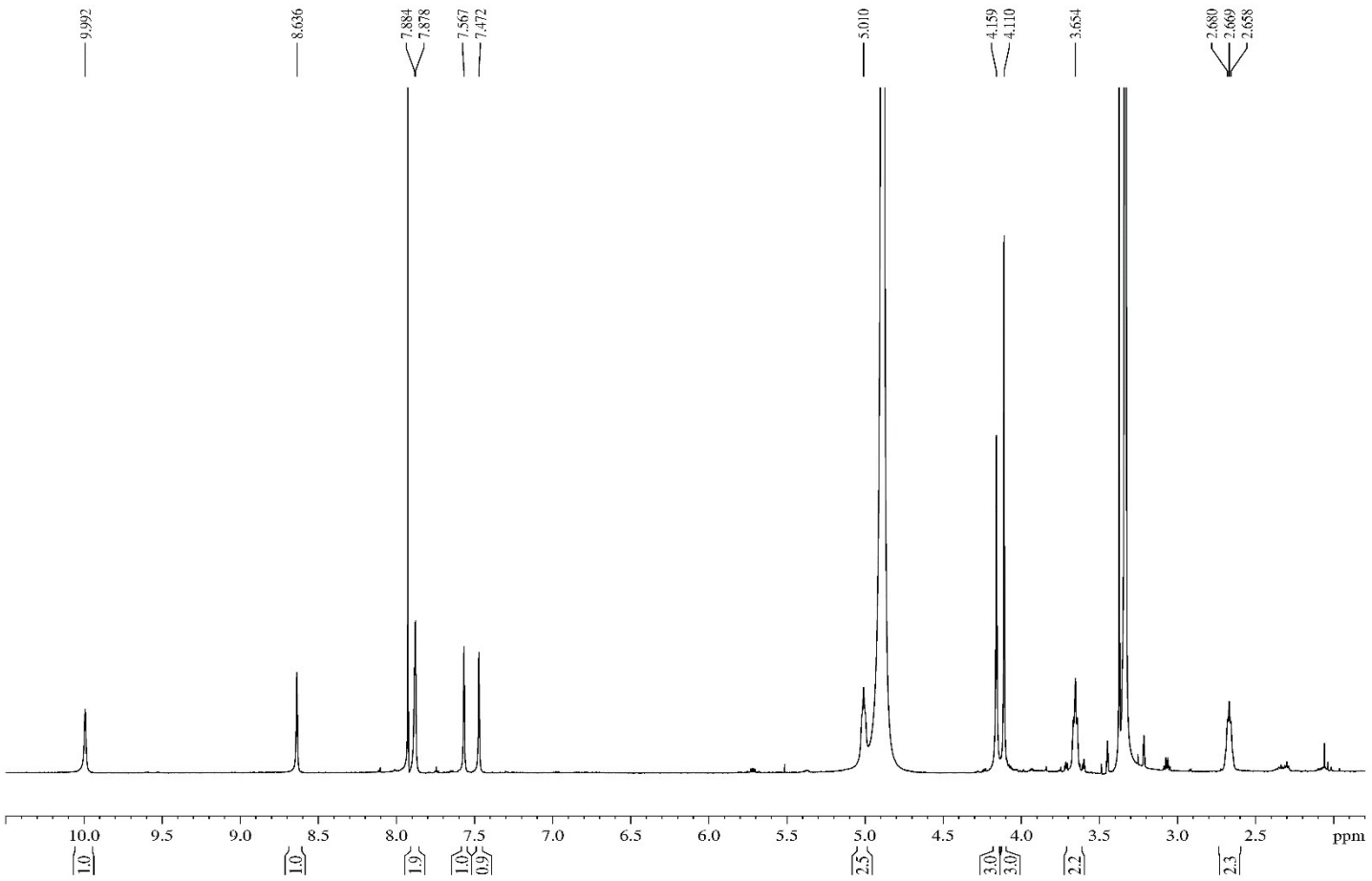
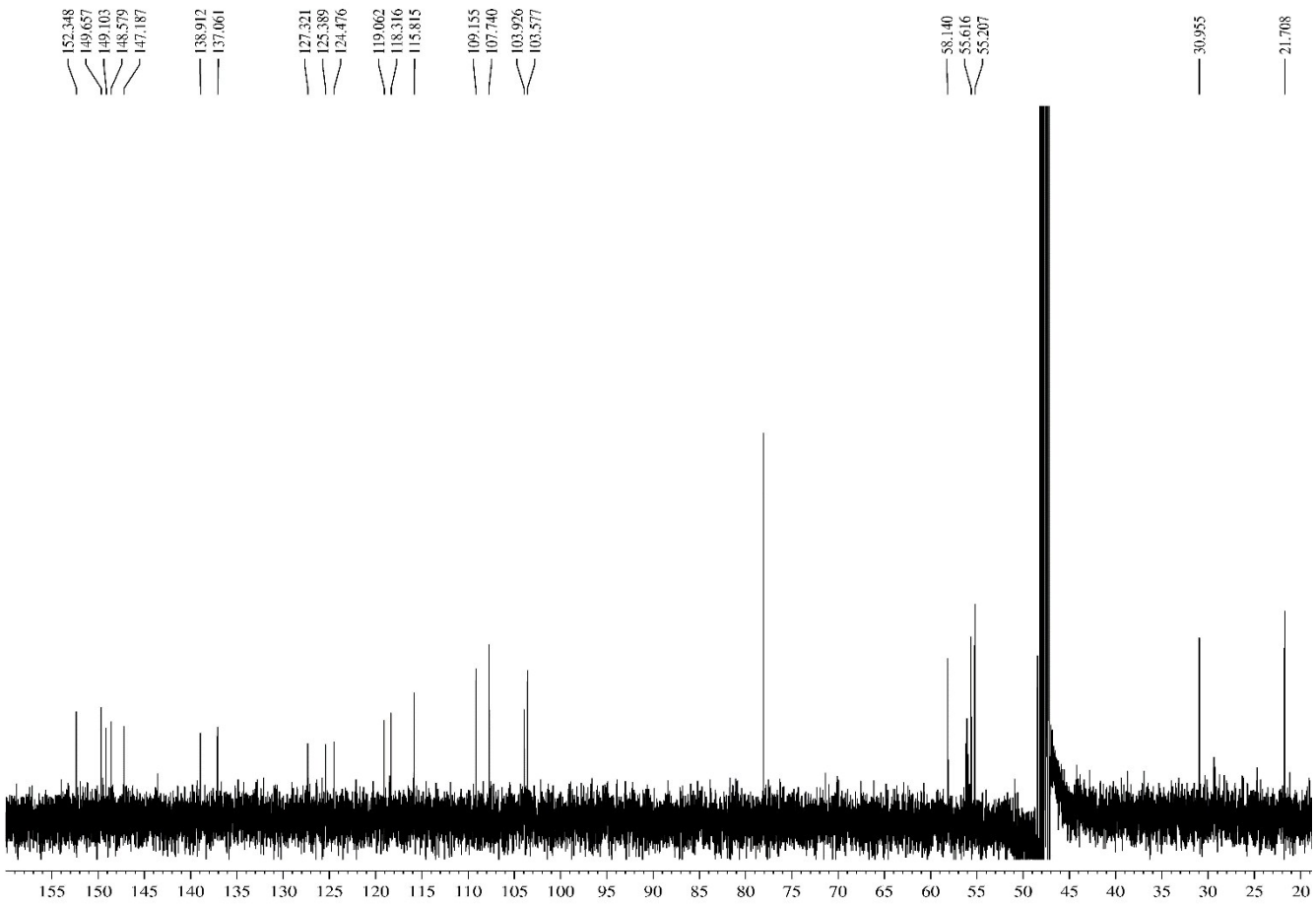



**Figure S14** $^{1}$H NMR spectrum of compound **3**.




86

87 **Figure S15** $^{13}$C NMR spectrum of compound **3**.

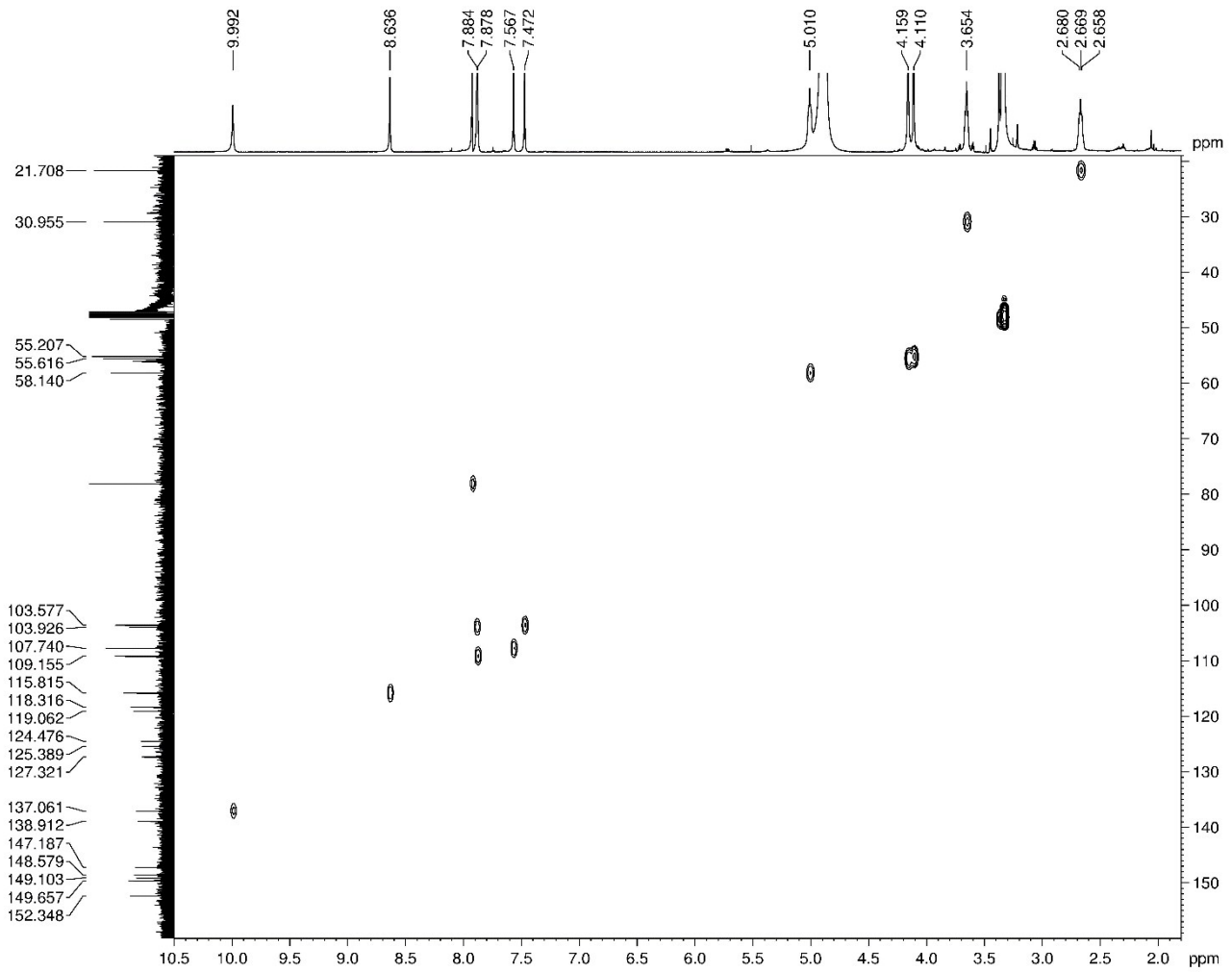



**Figure S16** HSQC spectrum of compound **3**.

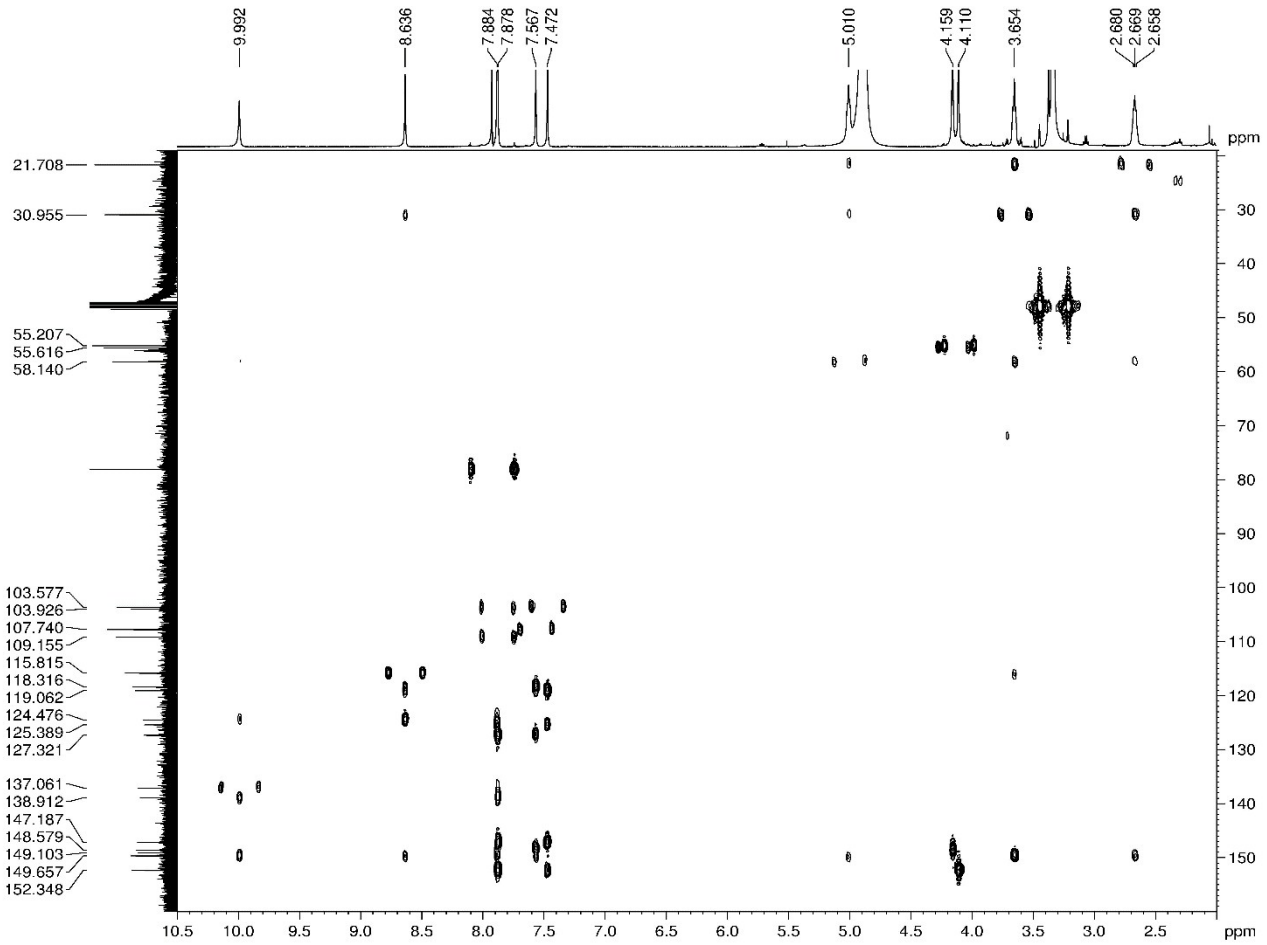
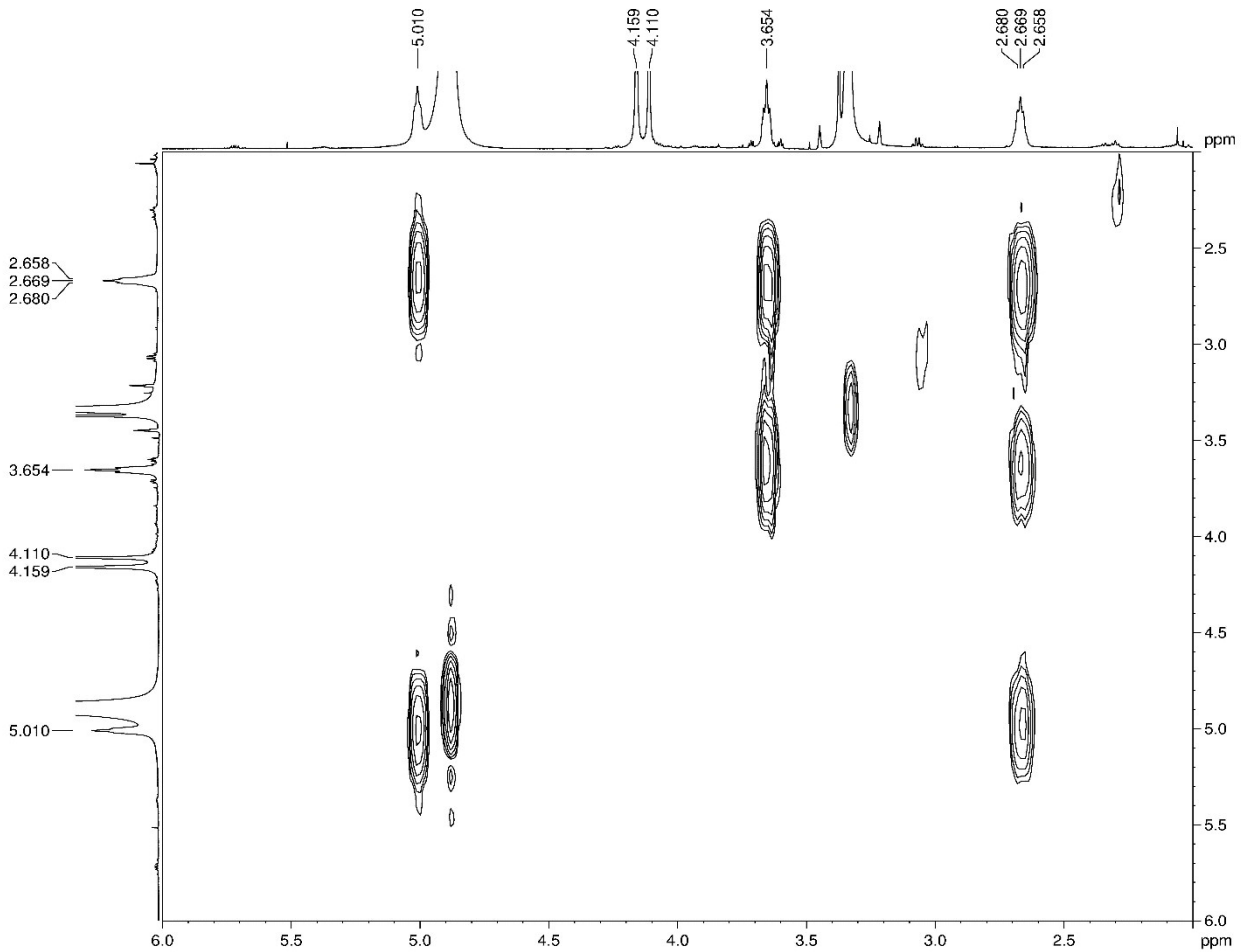



**Figure S17** HMBC spectrum of compound **3**.




92

93 **Figure S18** $^{1}$H-$^{1}$H COSY spectrum of compound **3**.

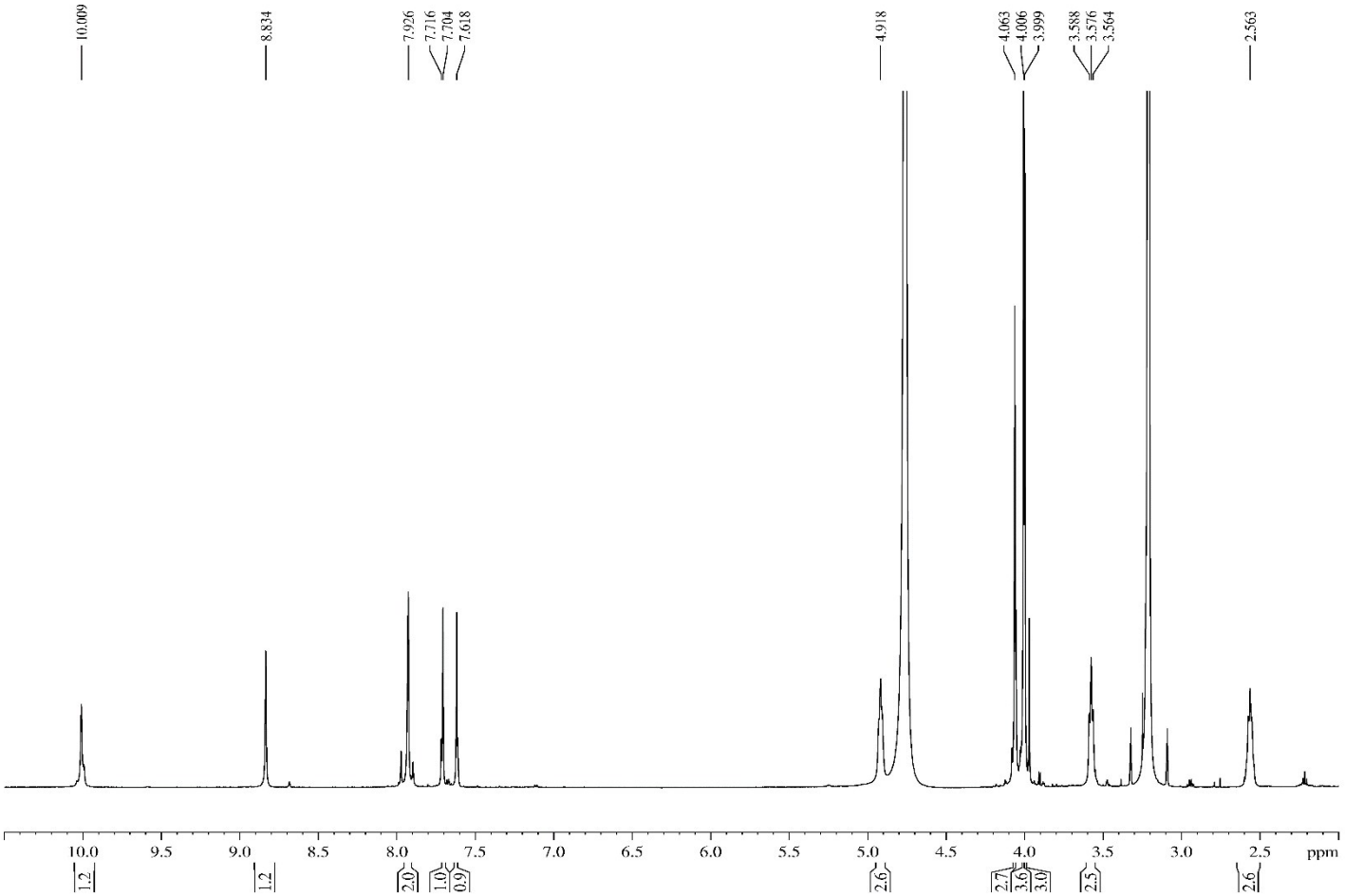



**Figure S19** $^{1}$H NMR spectrum of compound **4**.

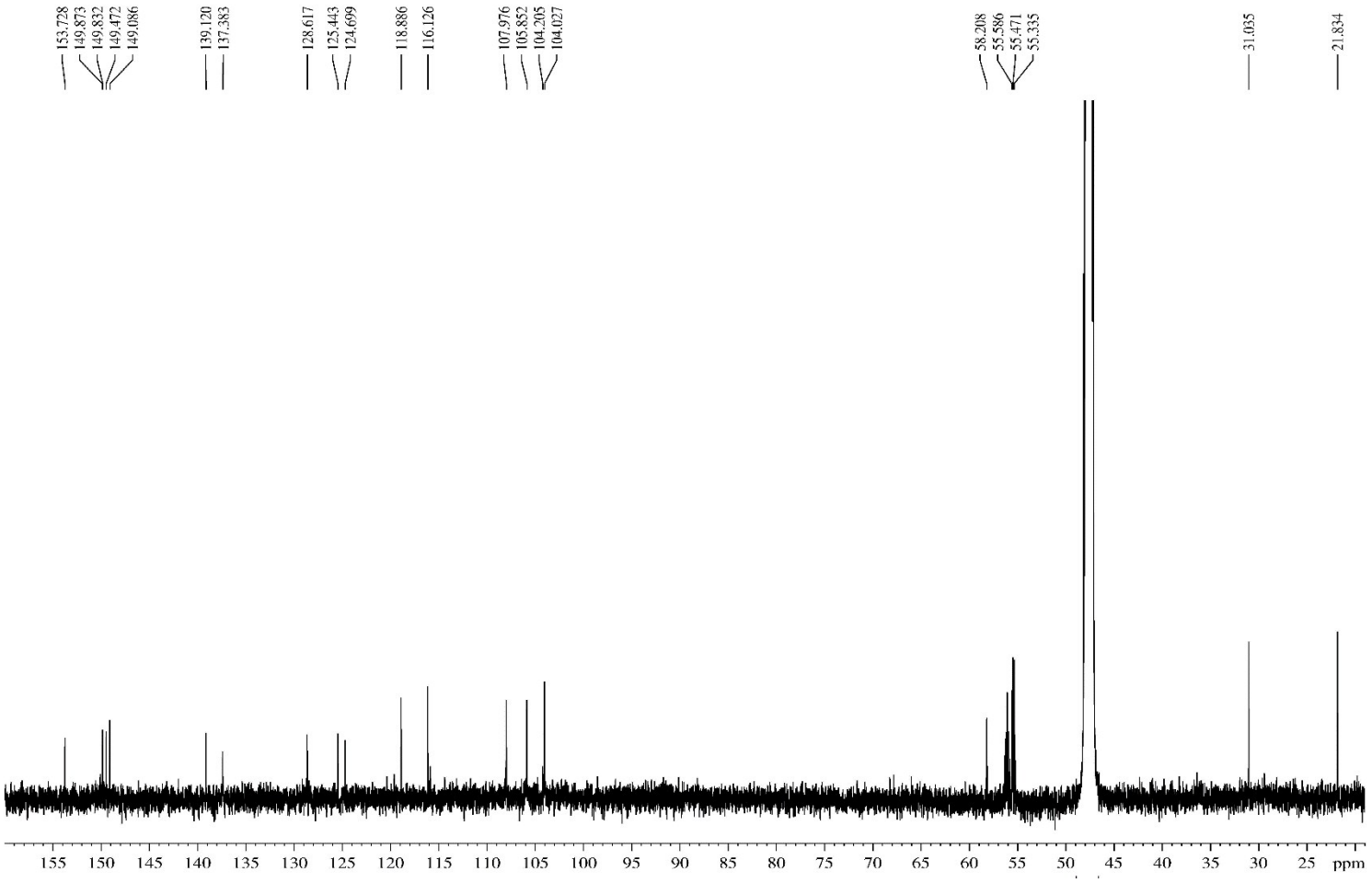
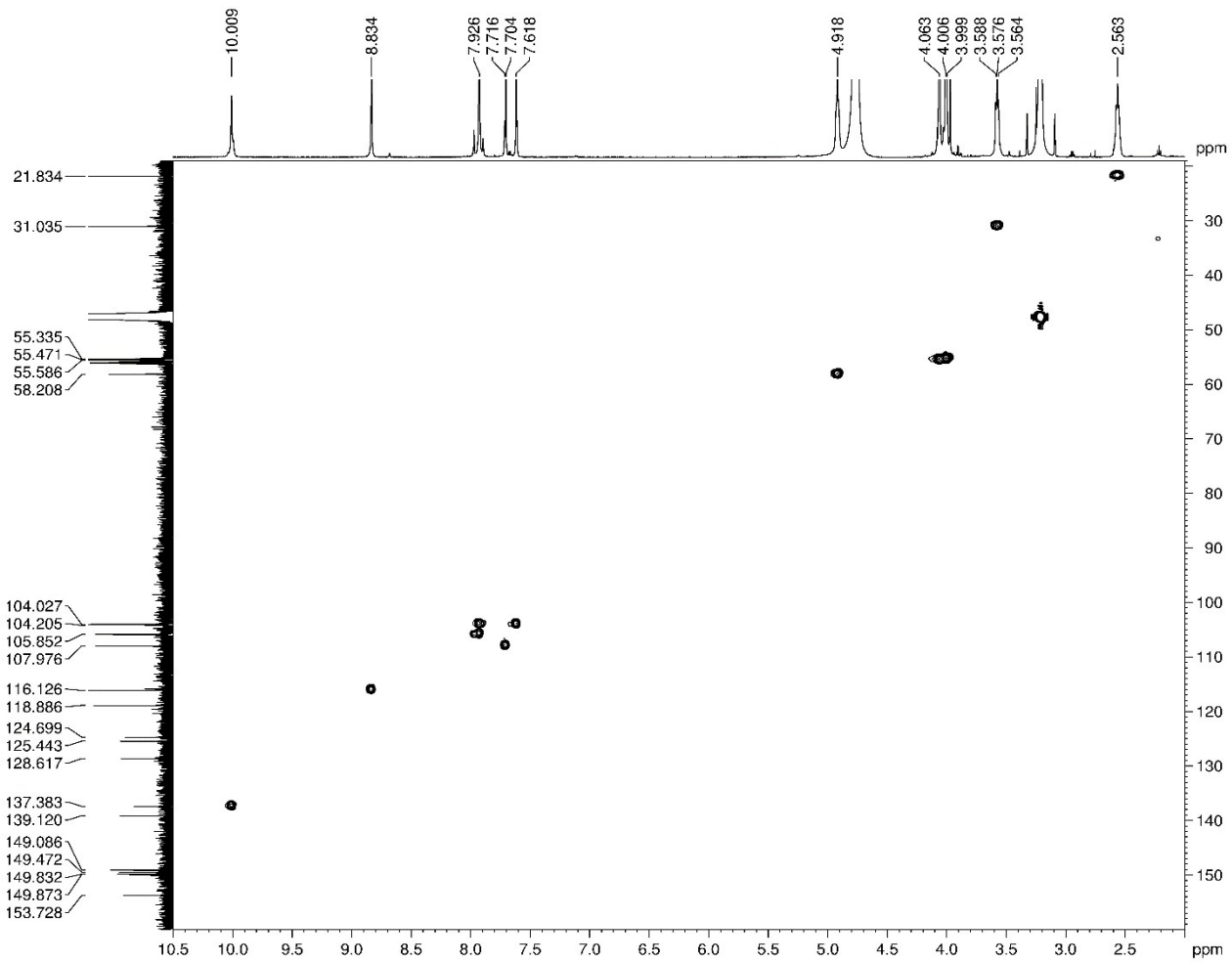
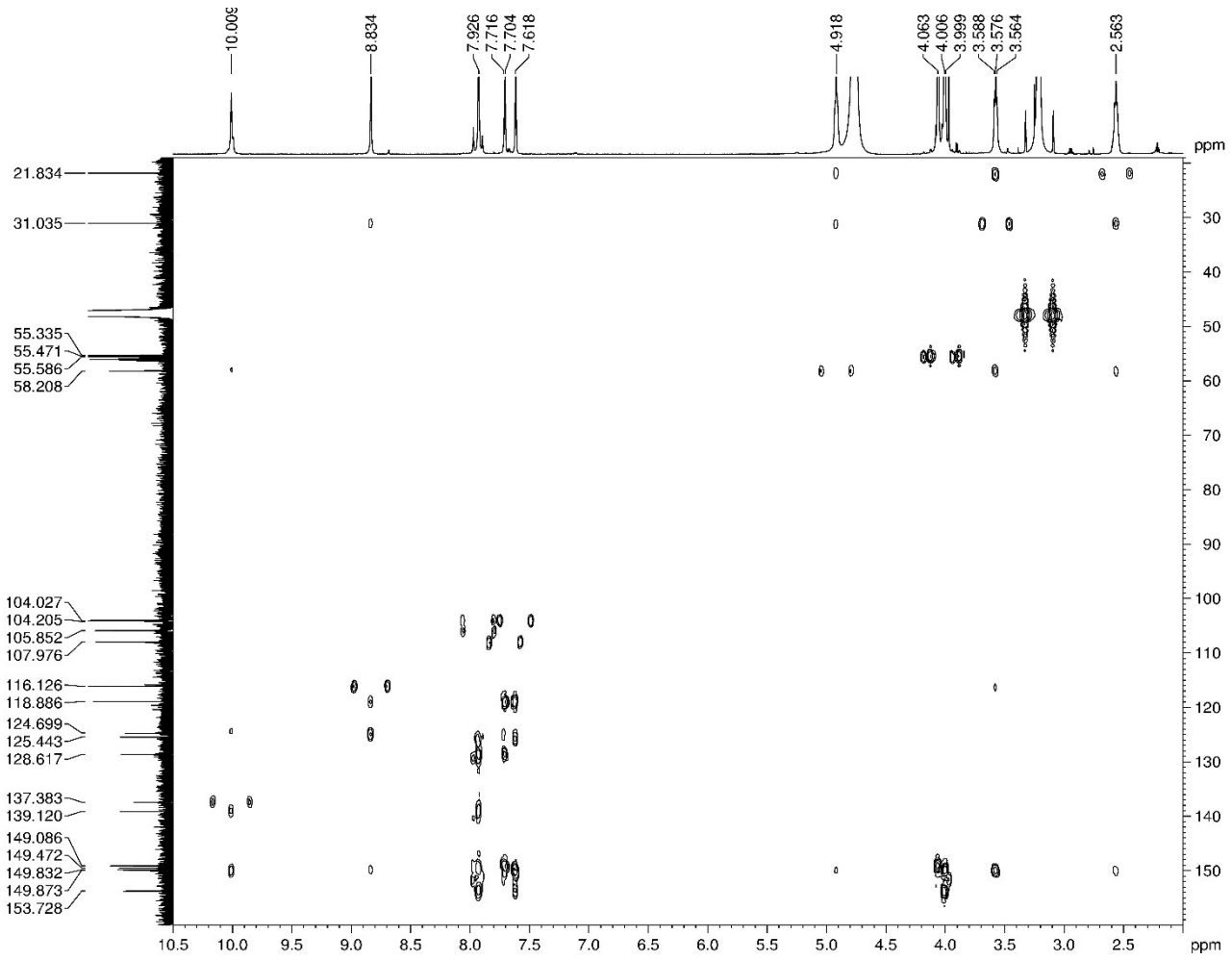



**Figure S20** $^{13}$C NMR spectrum of compound **4**.




98

99 **Figure S21** HSQC spectrum of compound **4**.




100

101 **Figure S22** HMBC spectrum of compound **4**.

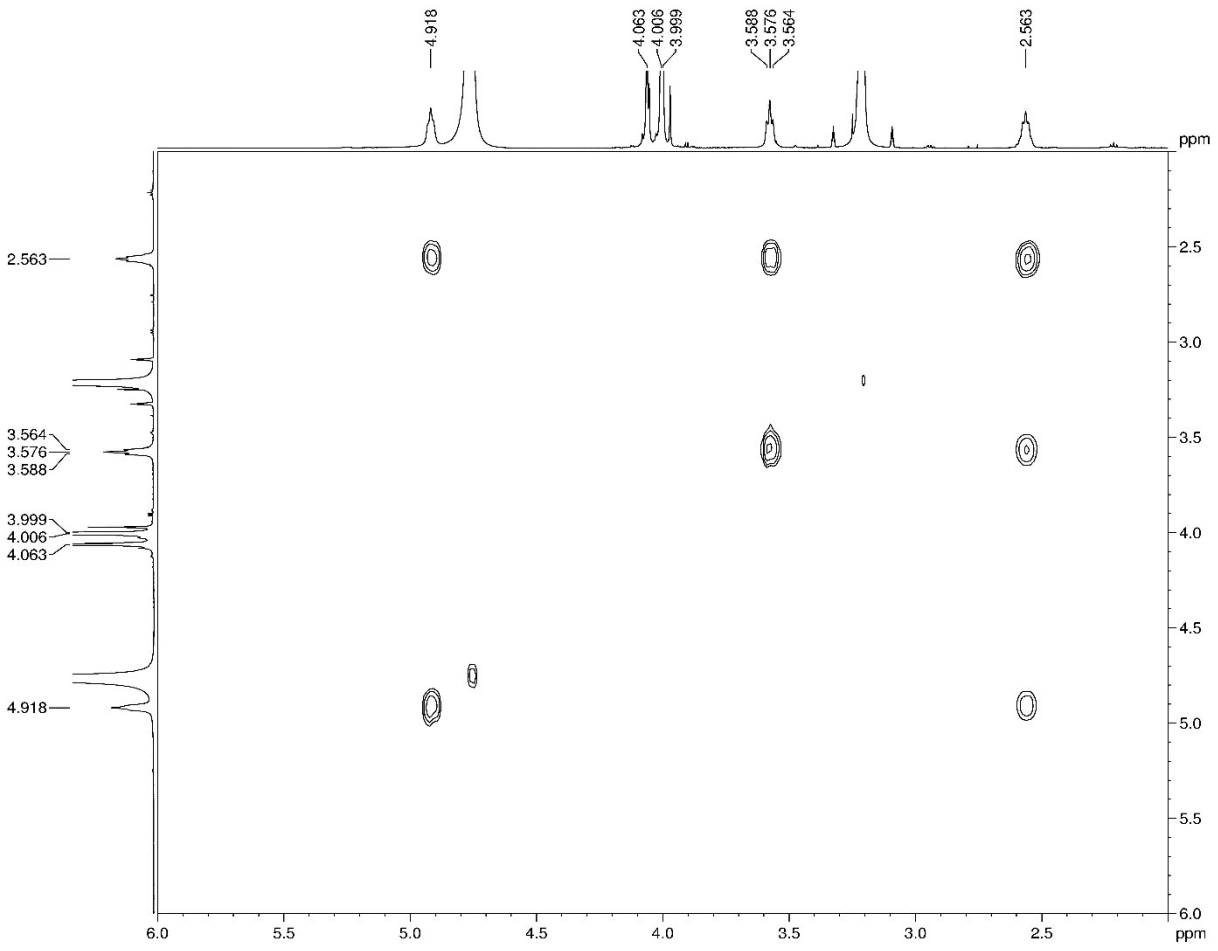



**Figure S23** $^{1}$H-$^{1}$H COSY spectrum of compound **4**.

Table S1 Percent fluorescence decrease of TO at the maximum emission wavelength of 530 nm by compound **6** or **7** displacement

| Concentration (μM) | Percent fluorescence decrease (%) | |
|---|---|---|
| | **6** | **7** |
| 4.9 | 47.6 | 53.5 |
| 9.5 | 72.8 | 71.1 |

**Table S2** $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) spectral data of **6** ($\delta$ in ppm, $J$ in Hz, measured in $CD_3OD$).

| position | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1 | 7.98, d, 6.6 | 126.9 |
| 2 | 6.86, d, 7.2 | 115.4 |
| 3 | | 161.8 |
| 4 | 6.67, s | 104.5 |
| 5 | 6.81, s | 107.6 |
| 6 | | 148.0 |
| 7 | | 148.6 |
| 8 | 7.12, s | 103.5 |
| 9 | 9.38, s | 136.3 |
| 11 | 4.77, s | 57.9 |
| 12 | 2.57, s | 21.3 |
| 13 | 3.45, s | 30.8 |
| 13a | | 149.5 |
| 14 | 8.18, s | 115.2 |
| 4a | | 133.3 |
| 4b | | 123.5 |
| 8a | | 118.6 |
| 8b | | 123.1 |
| 14a | | 138.7 |
| 14b | | 117.4 |
| $OCH_3$-3 | 3.86, s | 54.8 |
| $OCH_3$-7 | 3.98, s | 55.9 |

## References

1. (a) S. Alcaro, C. Musetti, S. Distinto, M. Casatti, G. Zagotto, A. Artese, L. Parrotta, F. Moraca, G. Costa, F. Ortuso, E. Maccioni and C. Sissi, *J. Med. Chem.*, 2013, 56, 843. (b) LP. Bai, J. Liu, L. Han, H.-M. Ho, R. Wang, and Z.-H. Jiang, *Anal. Bioanal. Chem.* **2014**, 406, 5455.

2. J. Dai, M. Carver, C. Punchihewa, R. Jones, D. Yang, *Nucleic Acids Res*. **2007**, 35, 4927.