***Supporting information***

# *In vitro* anti-inflammatory, *in silico* molecular docking model and molecular dynamic study of oleanane-type triterpenes from aerial parts of *Mussaenda recurvata*

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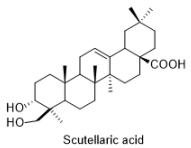
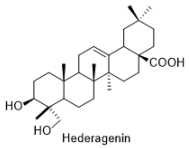
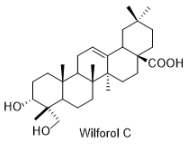
**Table S1.** NMR data of **1-4**

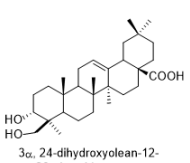
| N | **1 (DMSO-$d_6$)** | | **2 (DMSO-$d_6$)** | | **3 ($CD_3OD$)** | | **3 (DMSO-$d_6$)** | **4 (DMSO-$d_6$)** | |
|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_H$ (*J* in Hz) | $\delta_C$ |
| 1 | 1.49 (1H, m)<br>1.48 (1H, m) | 37.9 | 1.46 (1H, m)<br>1.45 (1H, m) | 37.7 | | 41.7 | | | 39.5 |
| 2 | 1.45 (1H, m)<br>1.44 (1H, m) | 26.5 | 1.47 (1H, m)<br>1.45 (1H, m) | 26.4 | | 27.6 | | | 26.6 |
| 3 | 3.44 (1H, m) | 70.3 | 3.44 (1H, m) | 70.4 | 3.57 (1H, dd, J = 12.0, 4.0) | 73.9 | 3.39 (1H, m) | 3.39 (1H, m) | 70.5 |
| 4 | | 41.8 | | 41.8 | | 44.2 | | | 42.7 |
| 5 | 1.10 (1H, m) | 46.4 | 1.12 (1H, m) | 46.5 | 1.18 (1H, d, J = 1.5) | 49.5 | | | 47.4 |
| 6 | 1.40 (1H, m)<br>1.22 (1H, m) | 17.5 | 1.42 (1H, m)<br>1.18 (1H, m) | 17.6 | 4.41 (1H, brs) | 68.6 | 4.29 (1H, m) | 4.24 (1H, m) | 66.2 |
| 7 | 1.45 (1H, m)<br>1.42 (1H, m) | 32.0 | 1.49 (1H, m)<br>1.48 (1H, m) | 32.1 | 1.52 (1H, dd, J = 4.5, 2.5)<br>1.78 (1H, m) | 41.2 | | | 39.5 |
| 8 | | 38.8 | | 37.8 | | 39.7 | | | 38.2 |
| 9 | 1.50 (1H, m) | 47.1 | 1.63 (1H, m) | 47.3 | | 49.3 | | | 47.7 |
| 10 | | 36.3 | | 36.3 | | 37.4 | | | 35.9 |
| 11 | 1.82 (1H, m)<br>1.78 (1H, m) | 22.9 | 1.86 (1H, m)<br>1.83 (1H, m) | 23.2 | | 24.5 | | | 23.6 |
| 12 | 5.16 (1H, brs) | 121.6 | 5.21 (1H, brs) | 122.3 | 5.30 (1H, t, J = 3.5) | 123.9 | 5.17 (1H, brs) | 5.25 (1H, brs) | 122.5 |
| 13 | | 143.9 | | 143.8 | | 144.6 | | | 142.8 |
| 14 | | 41.4 | | 41.2 | | 43.5 | | | 41.6 |
| 15 | 1.64 (1H, m)<br>0.97 (1H, m) | 27.2 | 1.50 (1H, m)<br>1.47 (1H, m) | 27.2 | | 28.9 | | | 27.9 |
| 16 | 1.90 (1H, m)<br>1.49 (1H, m) | 22.6 | 1.50 (1H, m)<br>1.47 (1H, m) | 27.3 | | 24.1 | | | 27.3 |
| 17 | | 45.5 | | 44.7 | | 47.7 | | | 44.8 |
| 18 | 2.74 (1H, dd, J = 14.0, 4.0) | 40.8 | 2.93 (1H, brs) | 43.3 | 2.89 (1H, dd, J = 13.5, 4.0) | 42.8 | 2.75 (1H, dd, J = 14.0, 4.0) | 2.93 (1H, brs) | 43.2 |
| 19 | 1.60 (1H, m)<br>1.07 (1H, m) | 45.7 | 3.12 (1H, brs) | 80.1 | | 47.3 | | 3.12 (1H, brs) | 80.1 |
| 20 | | 30.4 | | 34.9 | | 31.6 | | | 34.9 |
| 21 | 1.15 (1H, m)<br>1.13 (1H, m) | 33.3 | 0.91 (1H, m)<br>0.88 (1H, m) | 28.6 | | 35.0 | | | 28.5 |
| 22 | 1.60 (1H, m)<br>1.18 (1H, m) | 32.1 | 1.61 (1H, m)<br>1.14 (1H, m) | 32.3 | | 33.9 | | | 32.4 |
| 23 | 3.34 (1H, m)<br>3.08 (1H, dd, J = 10.0, 4.5) | 64.5 | 3.32 (1H, m)<br>3.07 (1H, d, J = 10.0) | 64.4 | 3.62 (1H, d, J = 11.0)<br>3.48 (1H, d, J = 11.0) | 66.8 | 3.38 (1H, m)<br>3.25 (1H, dd, J = 10.0, 3.5) | 3.40 (1H, m)<br>3.25 (1H, dd, J = 10.0, 3.5) | 64.1 |
| 24 | 0.53 (3H, s) | 12.6 | 0.54 (3H, s) | 12.5 | 1.09 (3H, s) | 14.1 | 0.91 (3H, s) | 0.91 (3H, s) | 13.7 |
| 25 | 0.87 (3H, s) | 15.5 | 0.87 (3H, s) | 15.3 | 1.35 (3H, s) | 17.7 | 0.97 (3H, s) | 0.95 (3H, s) | 17.4 |
| 26 | 0.72 (3H, s) | 16.9 | 0.68 (3H, s) | 16.8 | 1.11 (3H, s) | 18.8 | 1.22 (3H, s) | 1.22 (3H, s) | 16.6 |
| 27 | 1.10 (3H, s) | 25.6 | 1.25 (3H, s) | 25.6 | 1.16 (3H, s) | 26.5 | 1.05 (3H, s) | 1.21 (3H, s) | 24.1 |
| 28 | | 178.6 | | 179.1 | | 182.0 | | | 179.2 |
| 29 | 0.87 (3H, s) | 23.4 | 0.88 (3H, s) | 24.1 | 0.97 (3H, s) | 24.0 | 0.88 (3H, s) | 0.88 (3H, s) | 24.6 |
| 30 | 0.87 (3H, s) | 32.8 | 0.84 (3H, s) | 28.0 | 0.93 (3H, s) | 33.6 | 0.88 (3H, s) | 0.84 (3H, s) | 28.1 |
| 3-OH | 4.13 (1H, d, J = 4.5) | | | | | | | | |
| 19-OH | | | 4.48 (1H, d, J = 6.0) | | | | | 4.48 (1H, d, 6.0) | |
| 23-OH | 4.38 (1H, t, J = 4.5) | | | | | | | | |

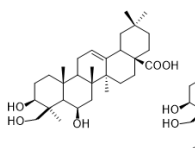
**Table S2.** NMR data of related compounds

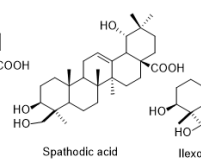

Scutellaric acid

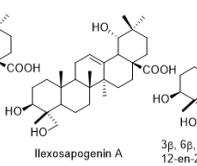

Hederagenin

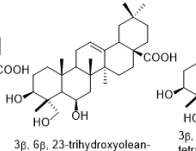

Wilforol C

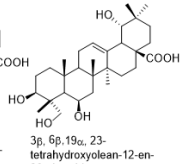

3α, 24-dihydroxyolean-12-en-28-oic acid


3β, 6β, 24-trihydroxyolean-12-en-28-oic acid


Spathodic acid


Ilexosapogenin A


3β, 6β, 23-trihydroxyolean-12-en-28-oic acid


3β, 6β,19α, 23-tetrahydroxyolean-12-en-28-oic acid

| No | Scutellaric acid (**1C**) | Hederagenin (**1D**) | | Wilforol C (**1A**) | | | | 3α, 24-dihydroxyolean-12-en-28-oic acid (**1B**) | | 3β, 6β, 24-trihydroxyolean-12-en-28-oic acid (**3A**) | | Spathodic acid (**2A**) | | Ilexosapogenin A (**3B**) | | | | 3β, 6β, 23-trihydroxyolean-12-en-28-oic acid (**3**) | | 3β, 6β,19α, 23-tetrahydroxyolean-12-en-28-oic acid (**4**) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [1] | [2] | | [3] | | [4] | | [5] [6] | | [7] | | [8] | | [9] | | [10] | | [11] | | [11] | |
| | DMSO-$d_6$ | Pyridine-$d_5$ | | Pyridine-$d_5$ | | Acetone-$d_6$ | | DMSO-$d_6$ +CD$Cl_3$ | DMSO-$d_6$ | Pyridine-$d_5$ | | DMSO-$d_6$ | | Pyridine-$d_5$ | | Pyridine-$d_5$ | | Pyridine-$d_5$ | | Pyridine-$d_5$ | |
| | $^1$H | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C | $^1$H | $^{13}$C |
| 3 | 3.55 | 4.23 | 73.5 | 3.94 | 75.7 | 3.88 | 76.5 | 3.77 | 68.8 | 4.29 | 72.6 | 4.23 | 78.4 | 4.30 | 73.3 | 4.31 | 73.3 | 4.04 | 73.3 | 4.04 | 73.4 |
| 5 | | | 48.7 | | 43.6 | | 43.7 | | | | 48.7 | | 48.7 | | 48.6 | | 48.4 | | 49.3 | | 49.7 |
| 6 | | | | | | | | | | 5.06 | 66.9 | | | | | | | 4.85 | 67.5 | 4.83 | 67.9 |
| 19 | | | | | | | | | | | | | 80.1 | 3.73 | 81.2 | 3.18 | 81.2 | | | 3.44 | 81.6 |
| $CH_2$-OH | 3.43<br>3.19 | 4.18<br>3.73 | 67.8 | 3.88<br>3.68 | 71.3 | 3.55<br>3.44 | 71.3 | 3.65<br>3.37 | 64.4 | 4.41<br>4.05 | 66.4 | 4.18<br>3.73 | 63.0 | 4.29<br>3.82 | 64.5 | 4.28<br>3.82 | 67.8 | 4.18<br>3.83 | 67.1 | 4.19<br>3.85 | 67.3 |
| $CH_3$ 24/23 | 0.68 | 1.05 | 13.3 | 0.79 | 18.2 | 0.69 | 18.3 | 0.89 | | 1.73 | 14.1 | 1.05 | 22.9 | 1.10<br>1.17 | 13.6 | 1.10<br>1.16 | 13.0 | 1.51 | 14.7 | 1.53 | 14.6 |
| 25 | 0.87 | 0.97 | 16.1 | 0.96 | 15.8 | 0.91 | 16.0 | 1.05 | | 1.67 | 16.8 | 0.97 | 15.3 | 1.19 | 15.8 | 1.19 | 15.8 | 1.47 | 17.4 | 1.48 | 17.3 |
| 26 | 1.10 | 1.04 | 17.7 | 1.07 | 17.5 | 0.97 | 17.7 | 0.76 | | 1.63 | 17.9 | 1.04 | 16.6 | 1.22 | 17.5 | 1.22 | 17.5 | 1.42 | 18.6 | 1.46 | 18.4 |
| 27 | 1.10 | 1.26 | 26.3 | 1.23 | 26.2 | 1.19 | 26.5 | 1.14 | | 1.25 | 25.6 | 1.26 | 24.5 | 1.29 | 24.8 | 1.29 | 24.8 | 1.06 | 26.2 | 1.42 | 24.8 |
| 29 | 0.87 | 0.95 | 33.4 | 0.92 | 33.3 | 0.82 | 33.5 | 0.89 | 33.1 | 0.91 | 32.6 | 0.95 | 28.1 | 1.72 | 28.9 | 1.71 | 28.9 | 0.80 | 33.2 | 0.99 | 28.8 |
| 30 | 0.88 | 1.02 | 23.9 | 1.00 | 23.8 | 0.94 | 24.0 | 0.89 | 23.6 | 0.99 | 23.1 | 1.02 | 24.0 | | 24.8 | | 24.8 | 0.72 | 23.7 | 0.92 | 25.0 |


[1] Kuo YH, Lin YL, Lee SM. 1988. Scutellaric Acid, a new triterpene from *Scutellaria rivularis*. Chem. Pharm. Bull. 36(9):3619–3622.

[2] Joshi BS, Singh KL, Roy R. 1999. Complete assignments of $^1$H and $^{13}$C NMR spectra of the pentacyclic triterpene hederagenin from *Nigella sativa* Linn. Magn Reson Chem. 37(4):295–298.

[3] Morota T. 1995. Triterpenes from *Tripterigium wilfordii*. Phytochemistry. 39(5):1153–1157.

[4] Liu J, Wu Q, Shu J, Zhang R, Liu L. 2020. A novel spermidine macrocyclic alkaloid from the roots of *Tripterygium wilfordii*. Chemistry of Natural Compounds. 56(3):496–499.

[5] Deepak M, Handa SS. 1998. 3α,24-Dihydroxy-urs-12-en-28-oic acid from *Verbena officinal.* Phytochemistry. 49(1):269–271.

[6] Fereda-Miranda R., Delgado G., Vivar AR. 1996. New triterpenoids from *Salvia nicolsoniana*. J Nat Prod. 49(2):225–230.

[7] Fang S, He Z, Fan G, Wu H, Xu J. 1996. Triterpenoids from *Adina rubella*. J Nat Prod. 59(3):304–307.

[8] Ngouela S, Nyasse B, Tsamo E, Sondengam BL, Connolly JD. 1990. Spathodic acid: A triterpene acid from the stem bark of *Spathodea campanulata*. Phytochemistry. 29(12):3959–3961.

[9] Amimoto K, Yoshikawa K, Arihara S. 1993. Triterpenoid saponins of aquifoliaceous plants. XII. Ilexosides XLVI-LI from the leaves of *Ilex rotunda* Thunb. Chem. Pharm. Bull. 41(1):77–80.

[10] Amimoto K, Yoshikawa K, Arihara S. 1992. Triterpenoid saponins of aquifoliaceous plants. VIII. Ilexosides XXIX-XXXII from the leaves of *Ilex rotunda* Thunb. Chem. Pharm. Bull. 40(12):3138–3141.

[11] Khan IA, Sticher O, Rali T. 1993. New Triterpenes from the leaves of *Timonius timon*. J Nat Prod. 56(12):2163–2165.

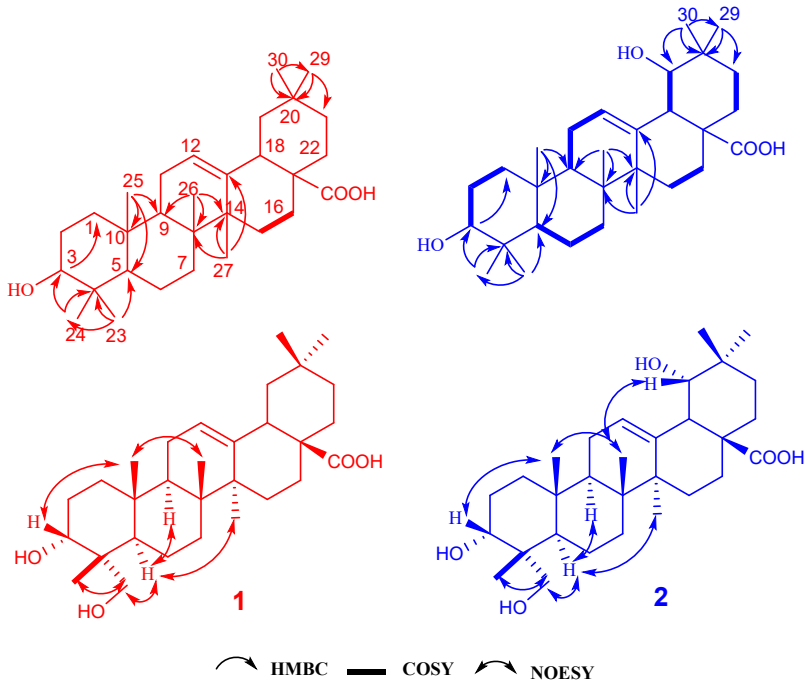



**Figure S1**. Selected HMBC and NOESY correlations of **1** and **2**

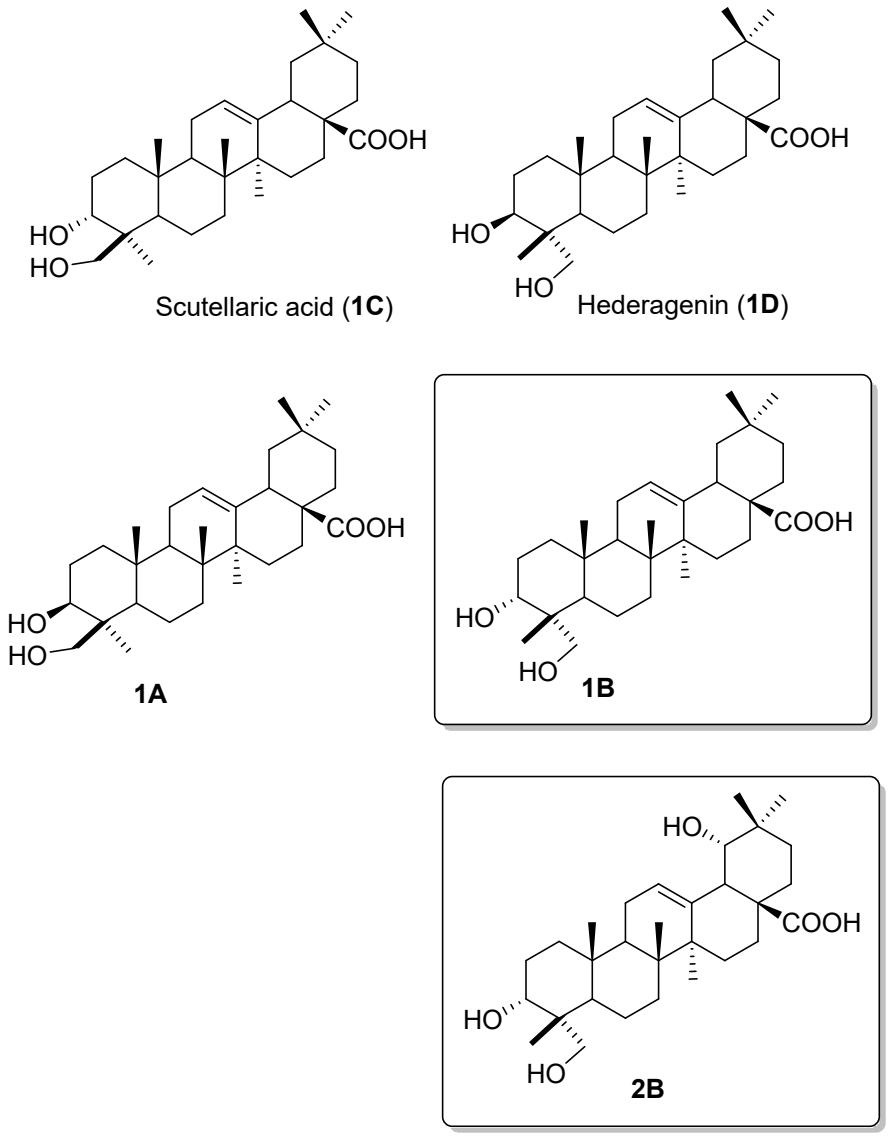
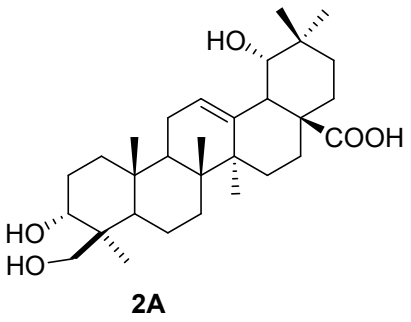
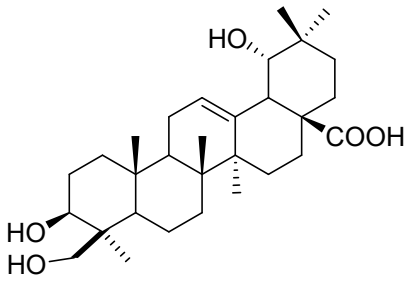


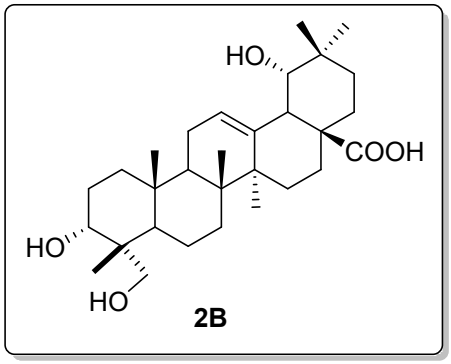

**Figure S2.** Four possible stereoisomers of **1**



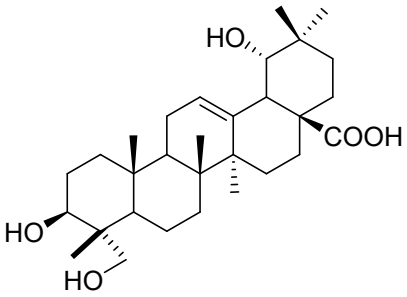

**2A**




Spathodic acid (**2C**)




**2B**




Ilexosapogenin A (**3B**)

**Figure S3.** Four possible stereoisomers of **2**

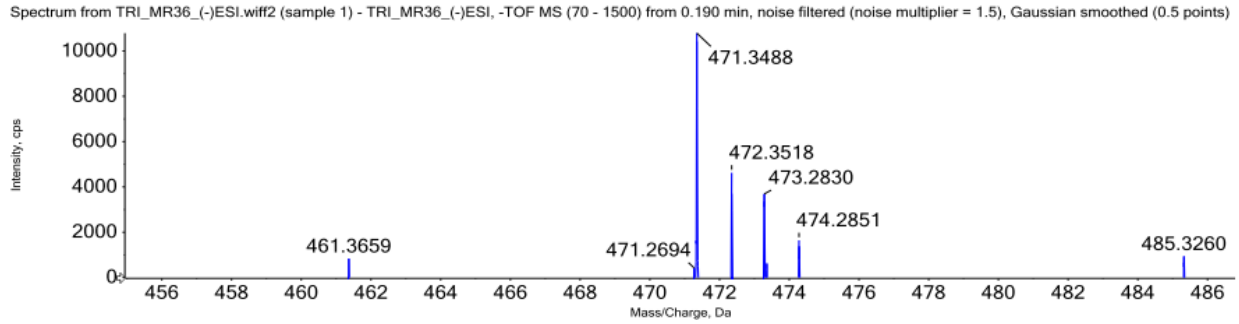



**Figure S4.** HRESIMS spectrum of **1.**

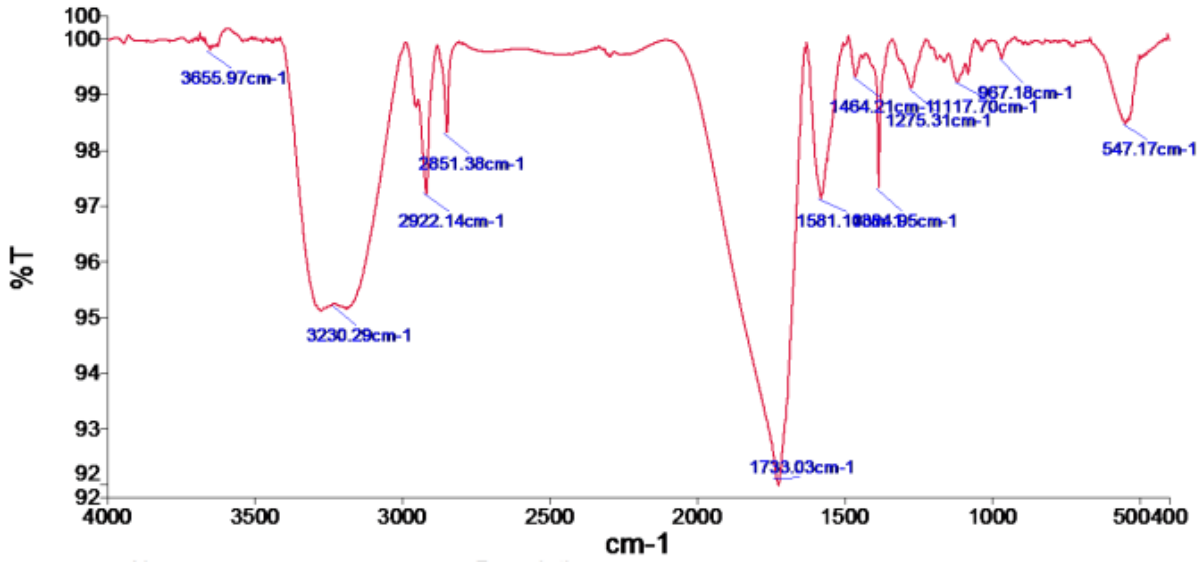



**Figure S5.** IR spectrum of **1**.

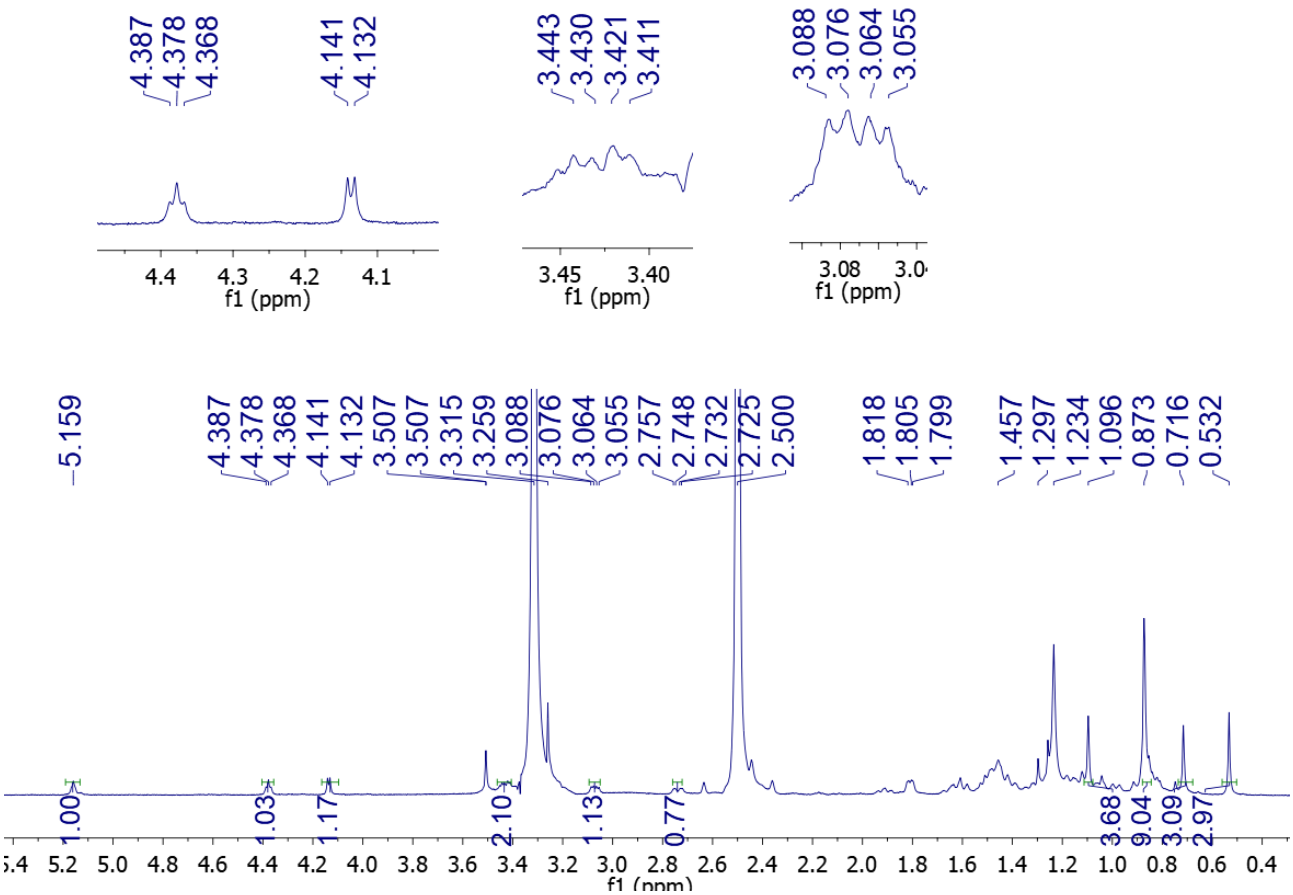



**Figure S6.** The $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) spectrum of **1.**

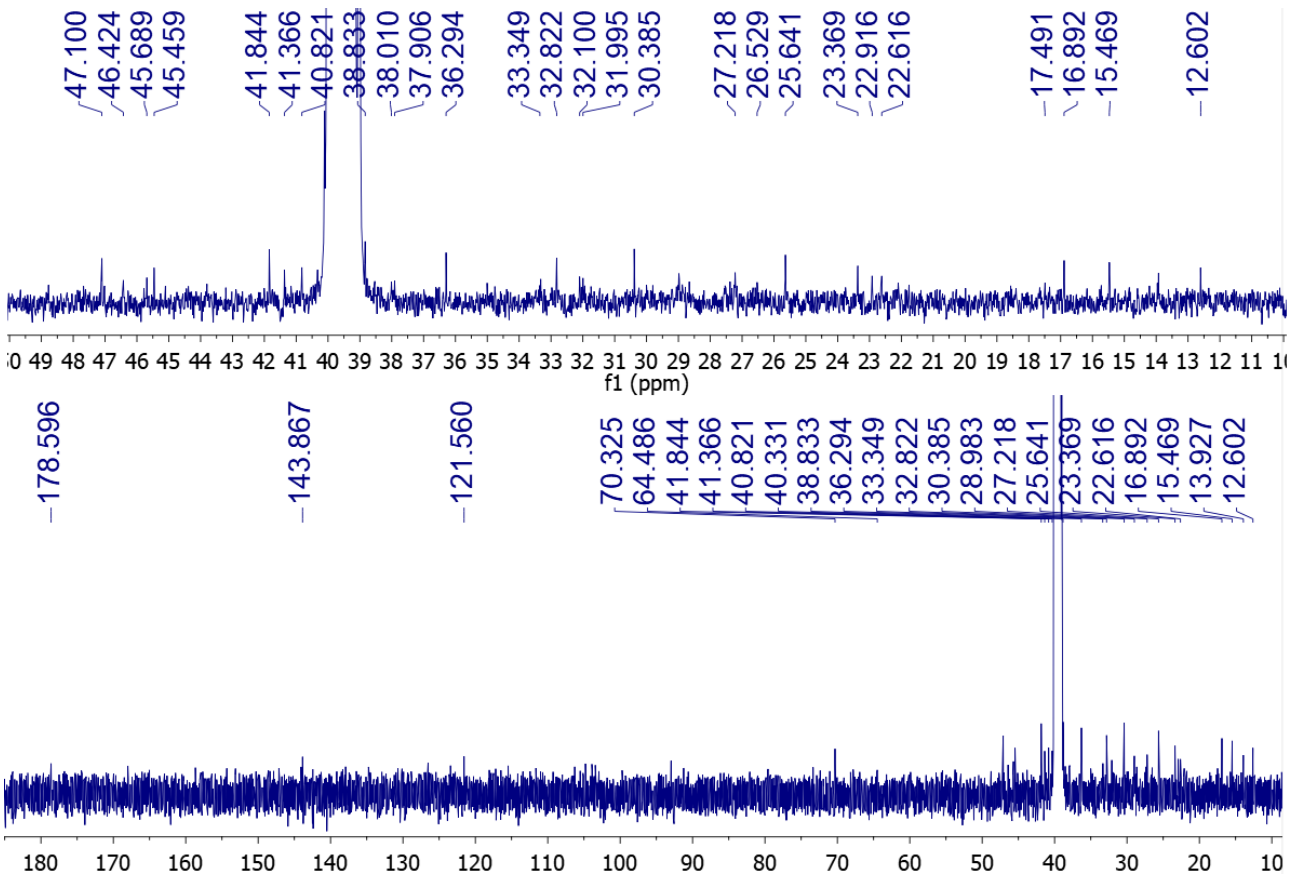



**Figure S7.** The $^{13}$C-NMR (DMSO-$d_6$, 500 MHz) spectrum of **1.**

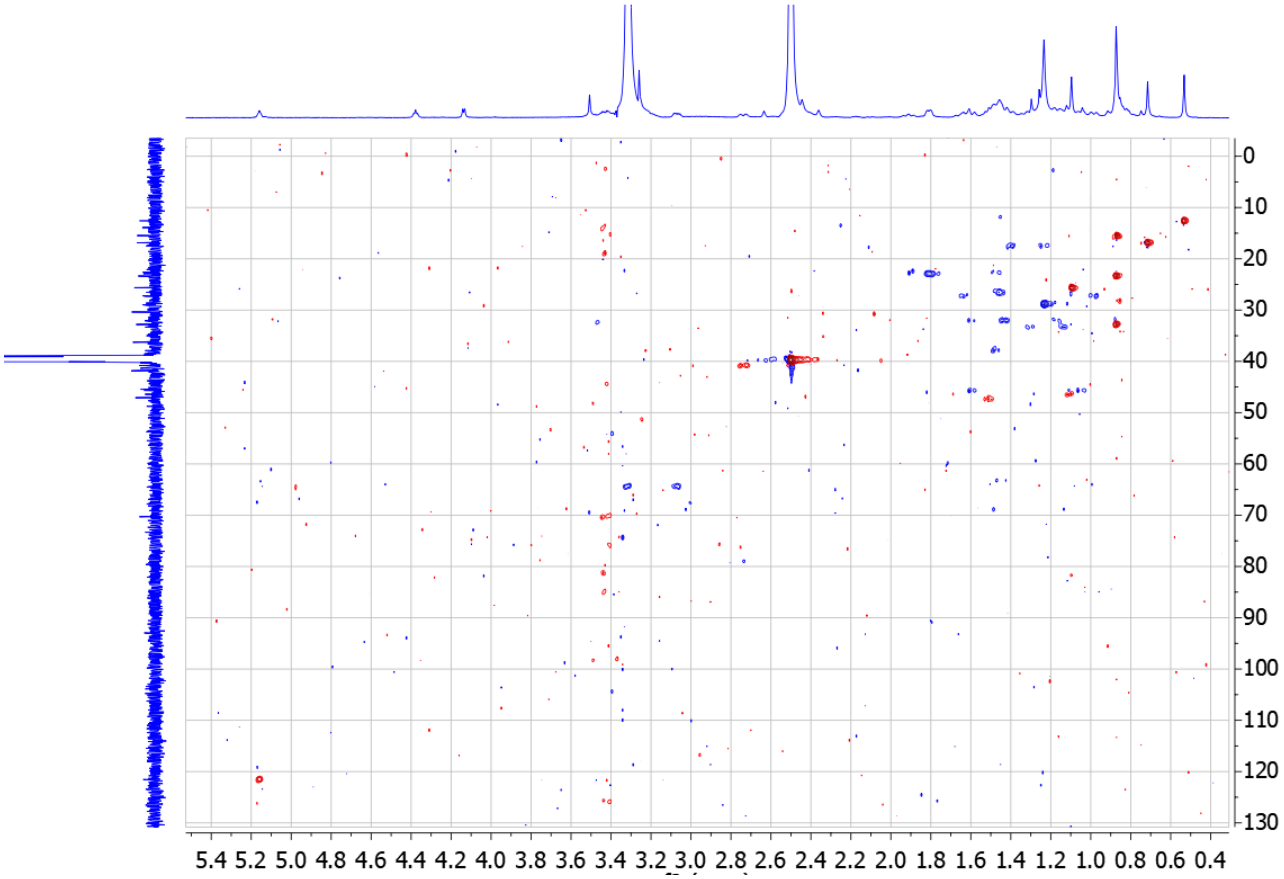



**Figure S8.** The HSQC (DMSO-$d_6$) spectrum of **1**

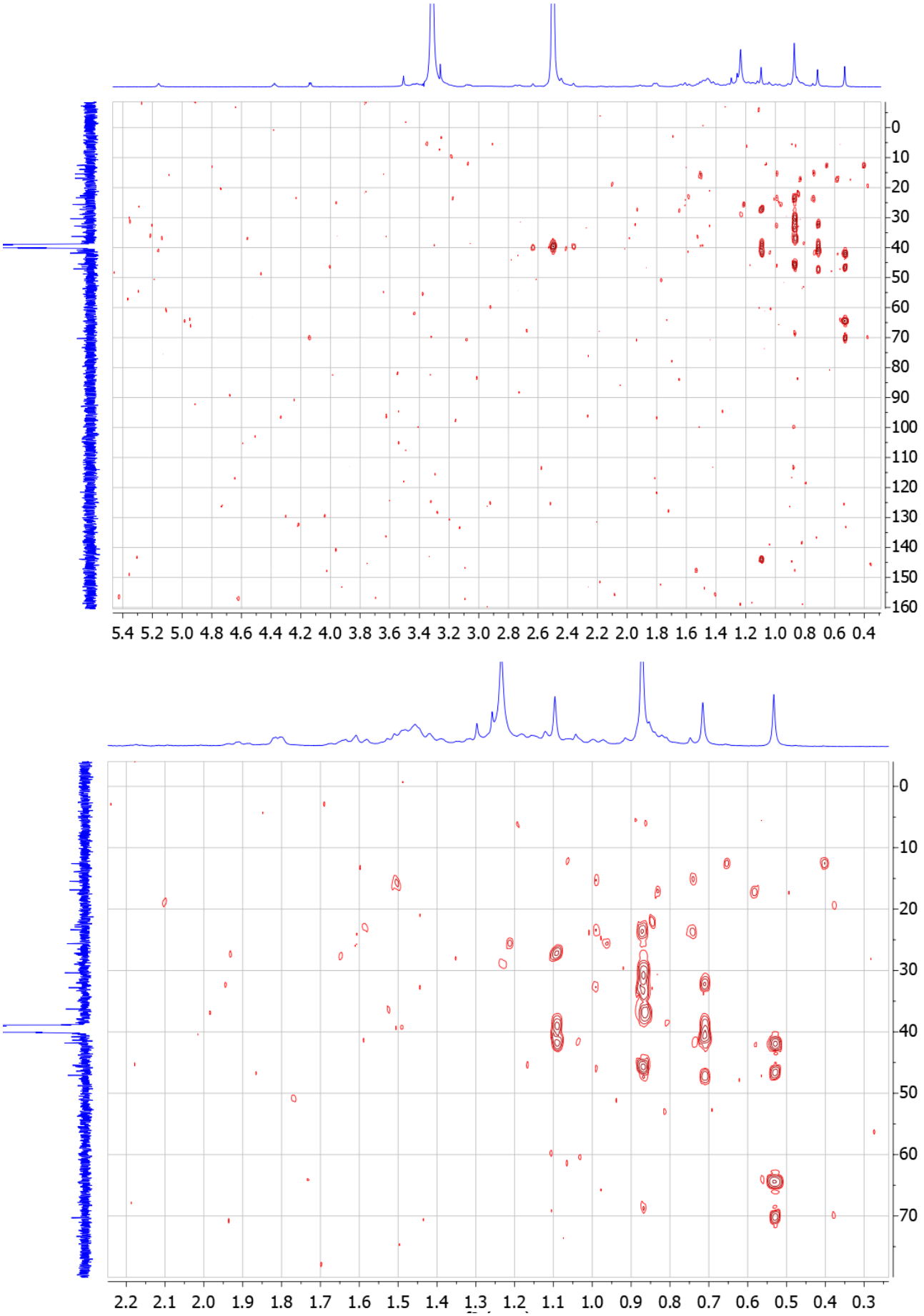



**Figure S9.** The HMBC (DMSO-$d_6$) spectrum of **1**.

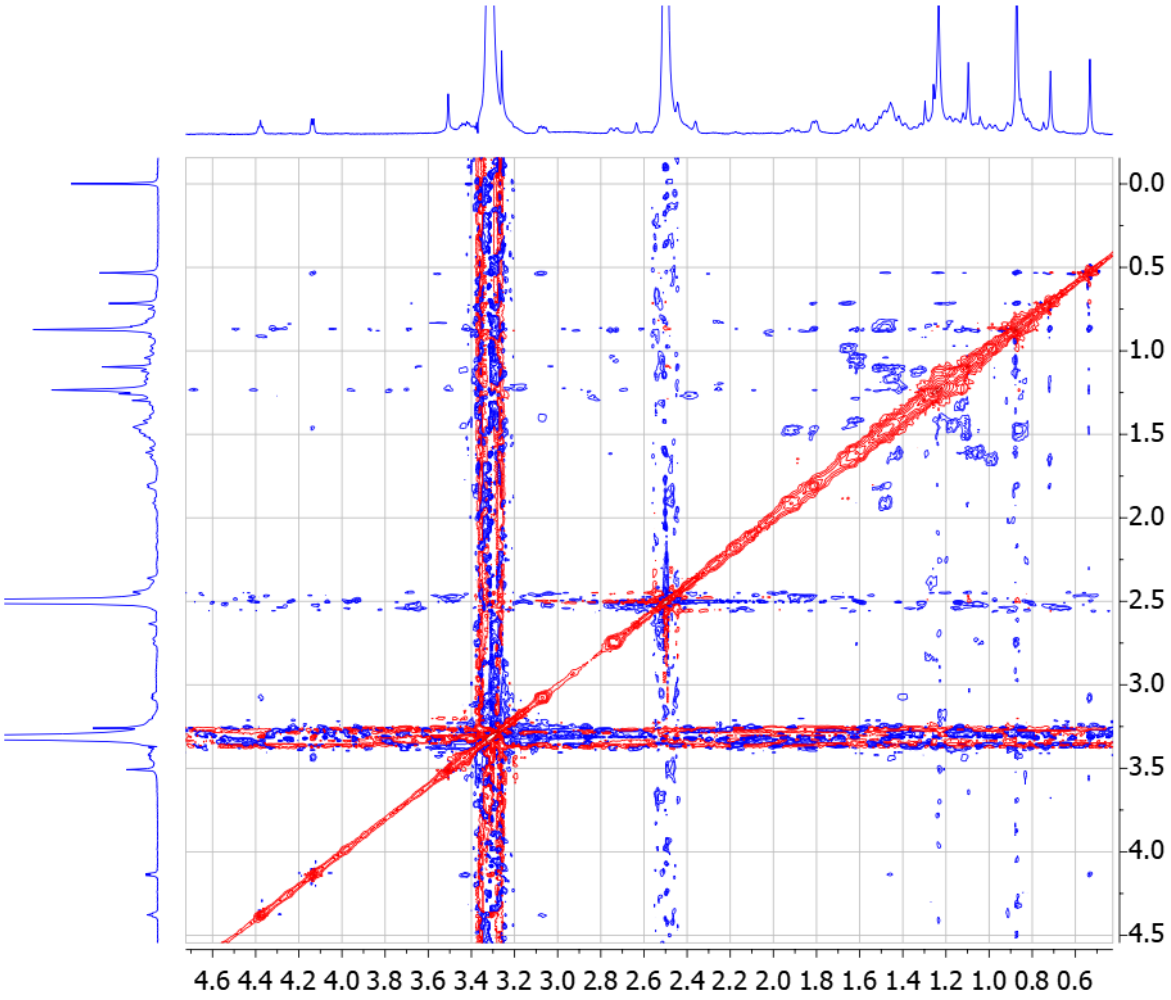



**Figure S10.** The NOESY (DMSO-$d_6$) spectrum of **1**.

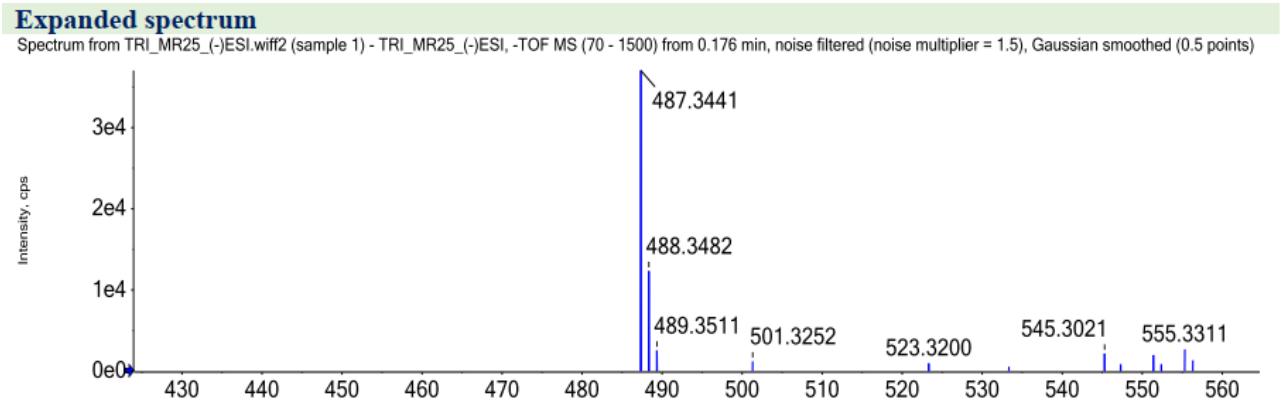



**Figure S11.** HRESIMS spectrum of **2.**

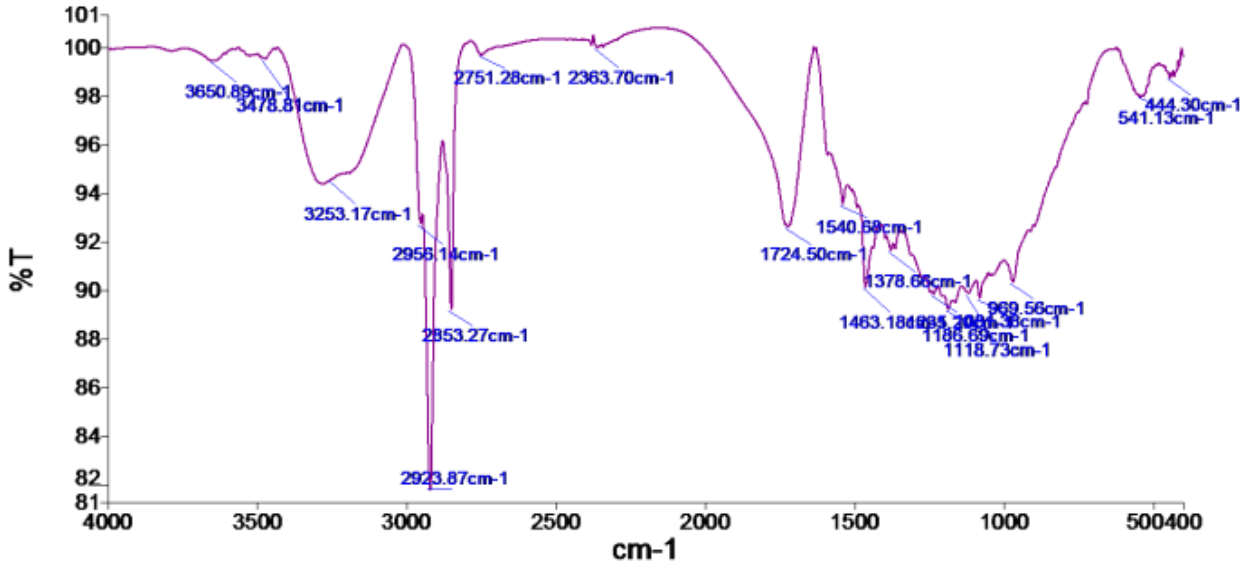



**Figure S12.** IR spectrum of **2**.

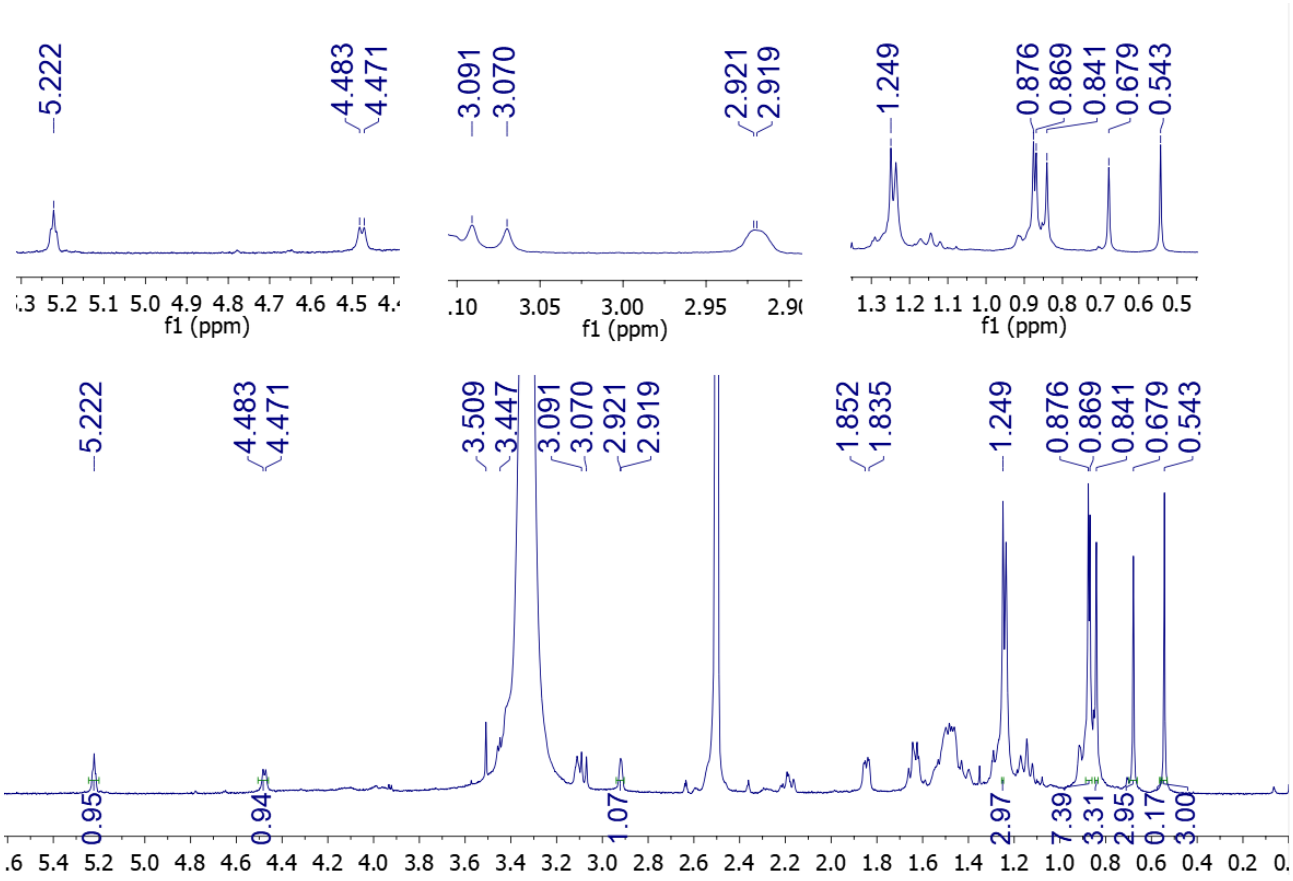



**Figure S13.** The $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) spectrum of **2.**

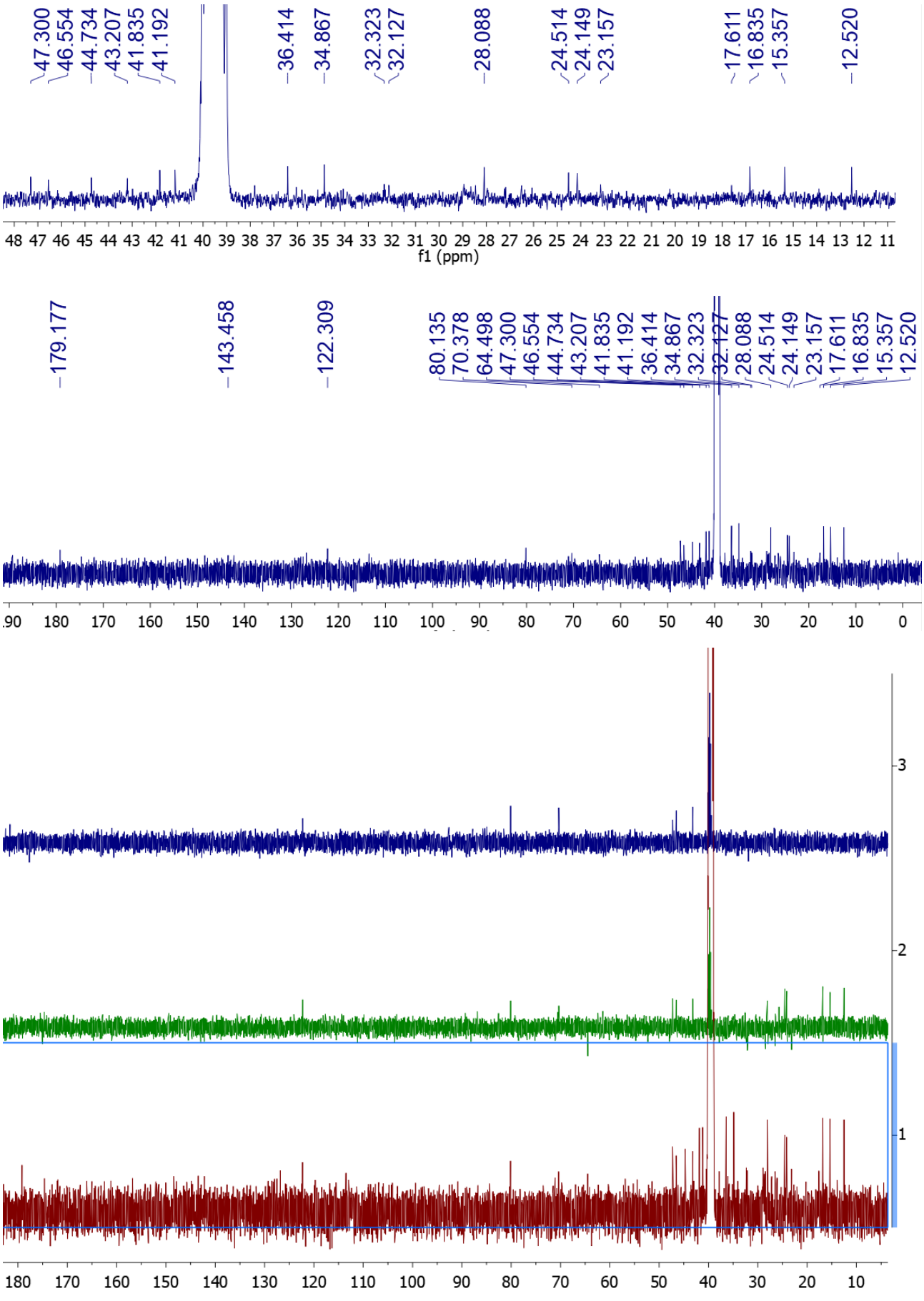



**Figure S14.** The $^{13}$C-NMR (DMSO-$d_6$, 500 MHz) spectrum of **2.**

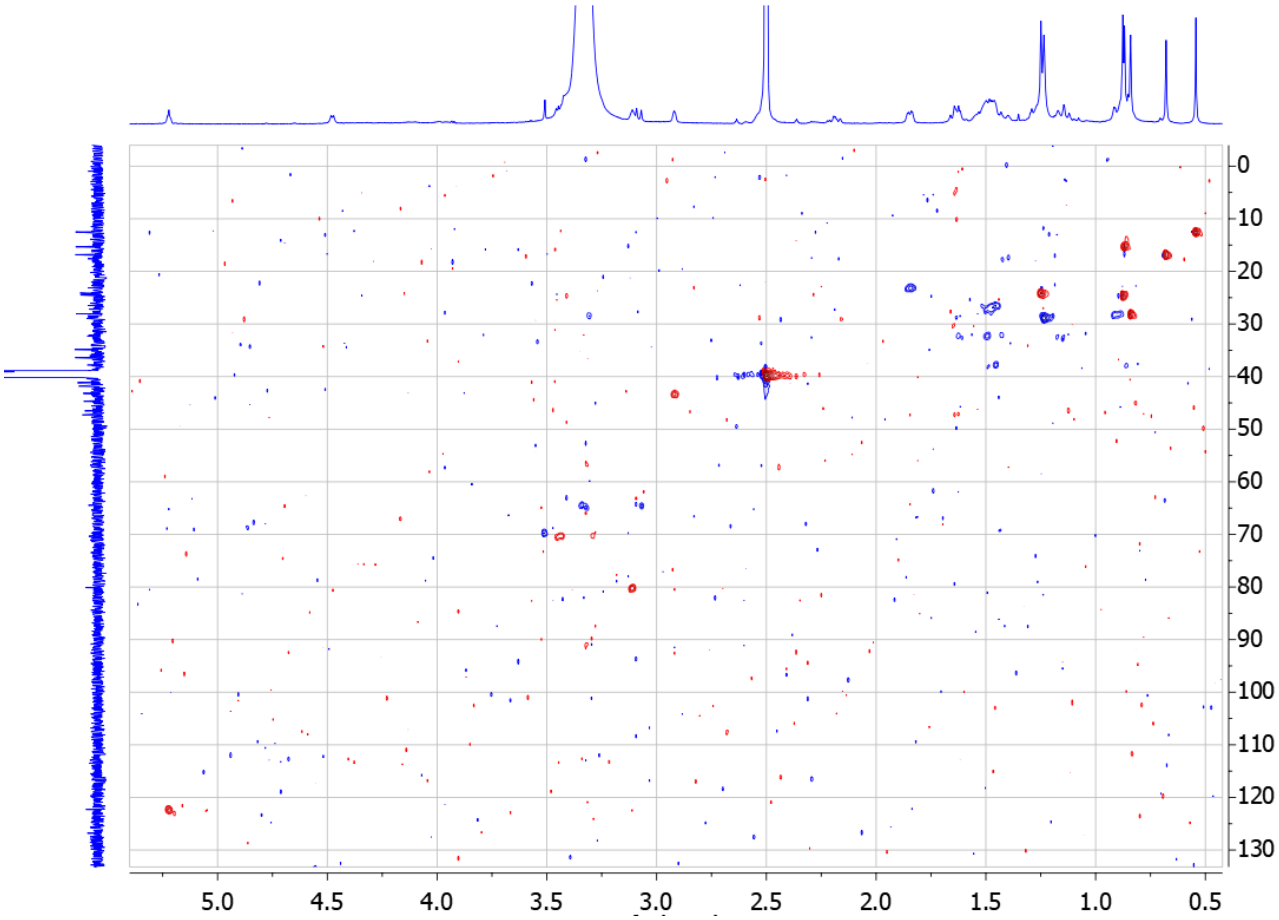



**Figure S15.** The HSQC (DMSO-$d_6$) spectrum of **2**

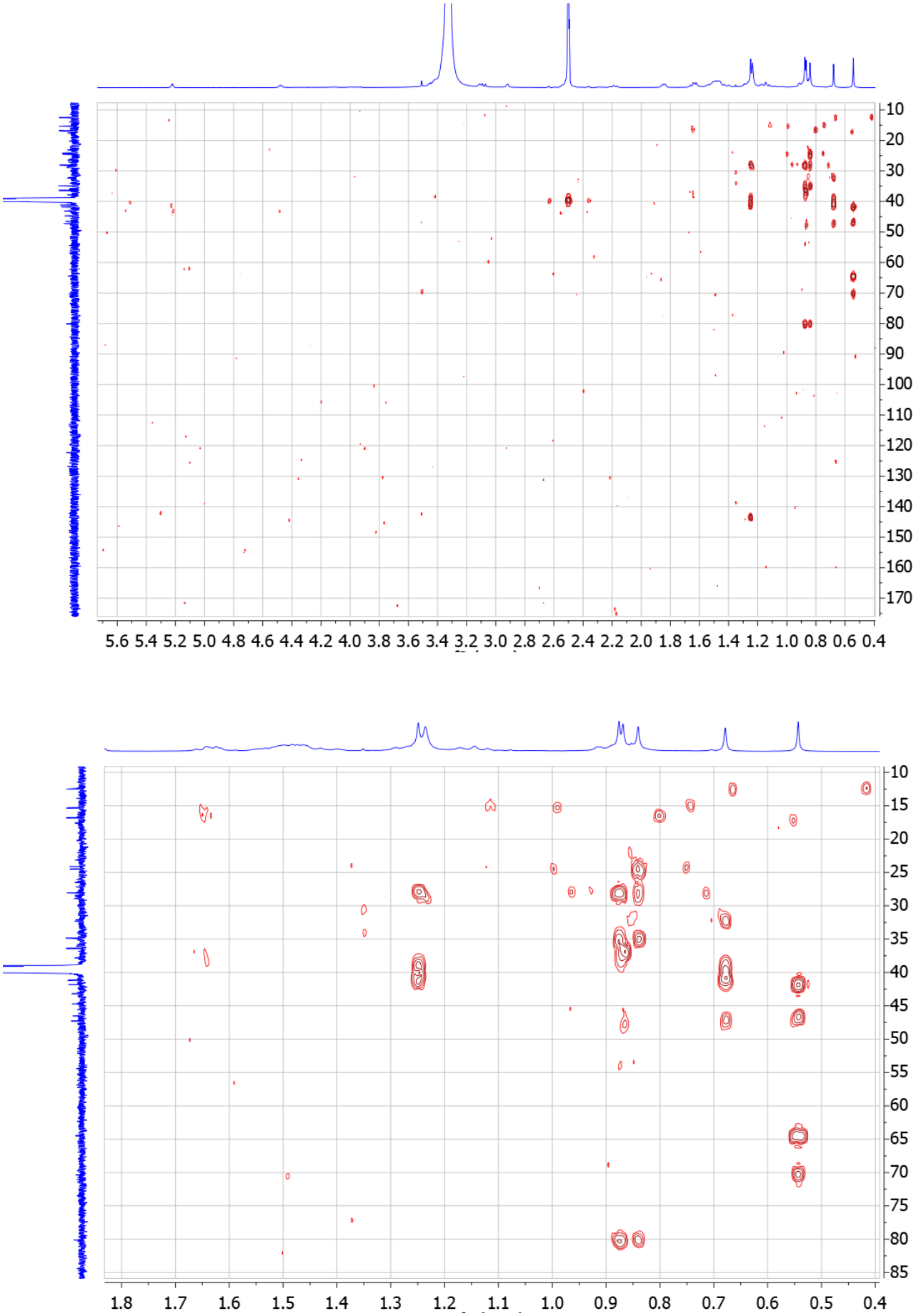



**Figure S16.** The HMBC (DMSO-$d_6$) spectrum of **2**.

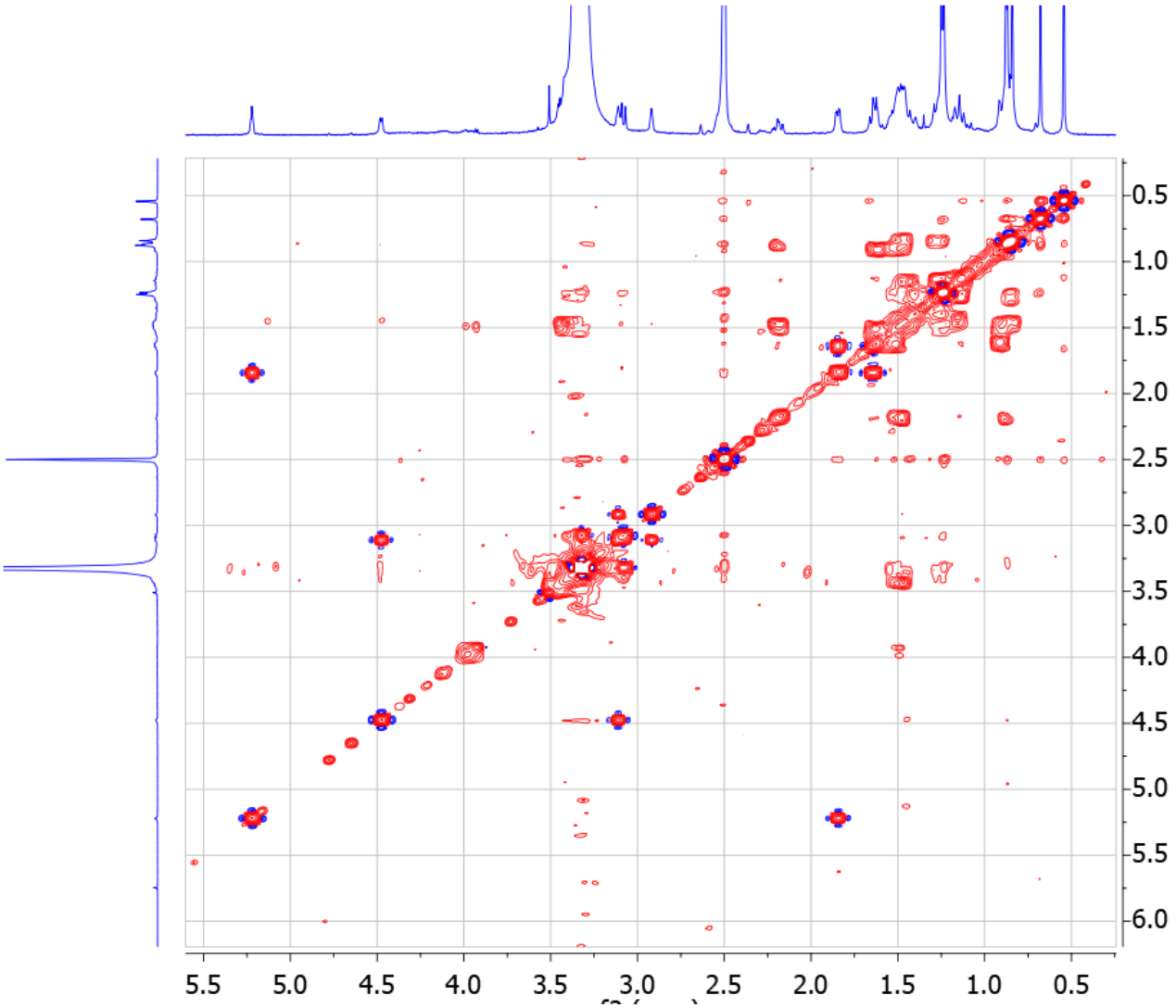



**Figure S17.** The COSY (DMSO-$d_6$) spectrum of **2**.

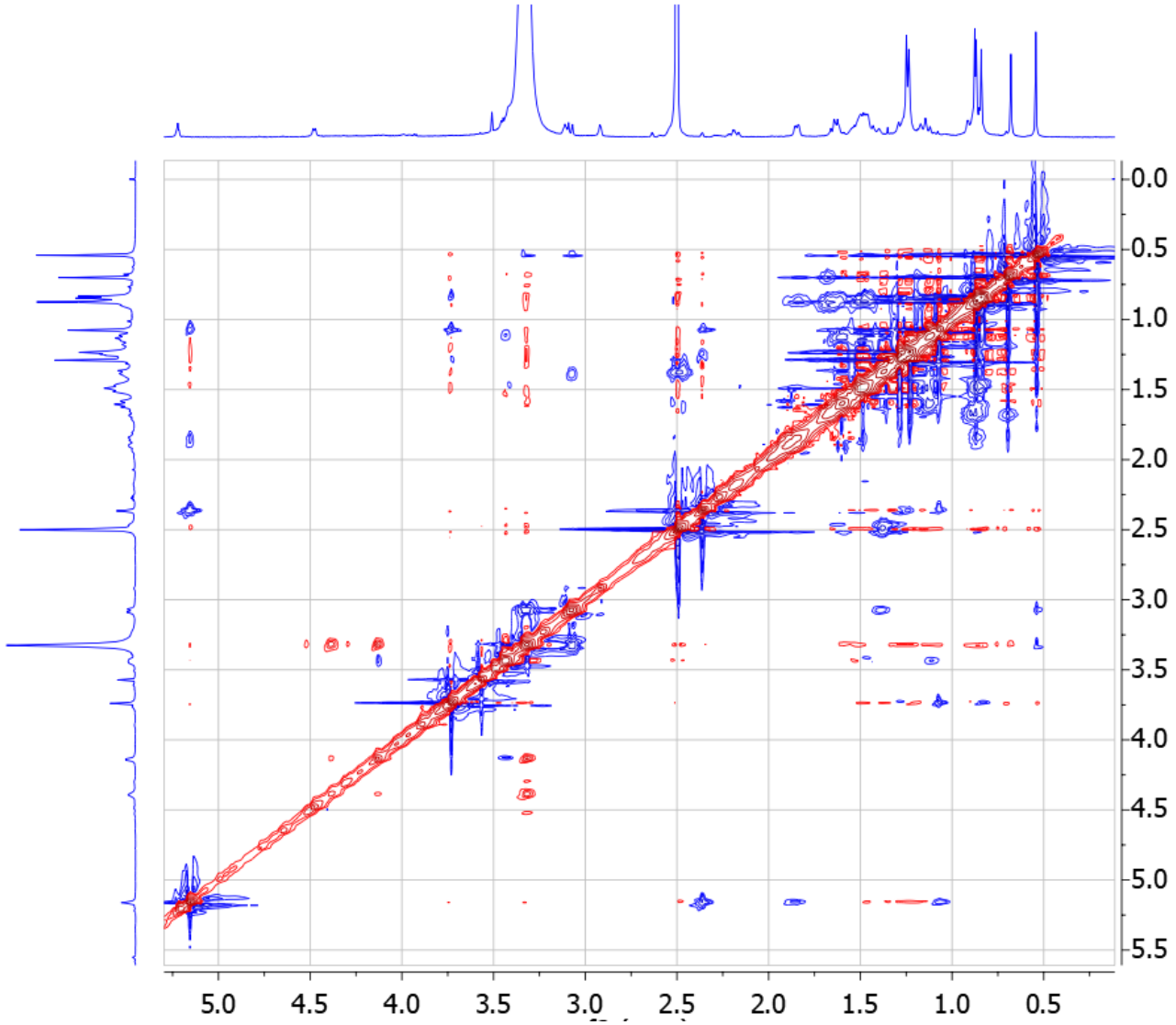



**Figure S18.** The NOESY (DMSO-$d_6$) spectrum of **2**.

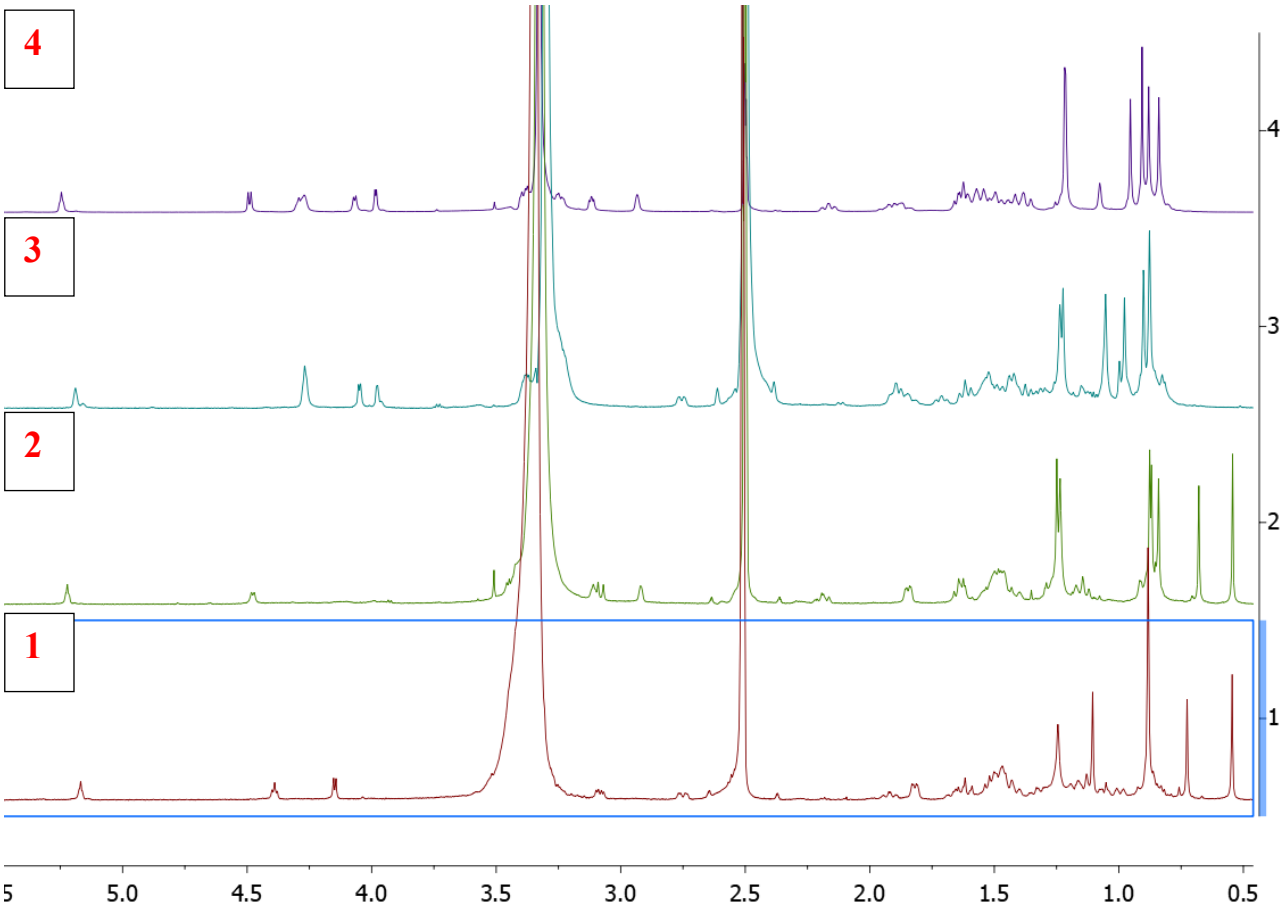



**Figure S19.** Comparison of $^{1}$H-NMR spectra of **1-4.**

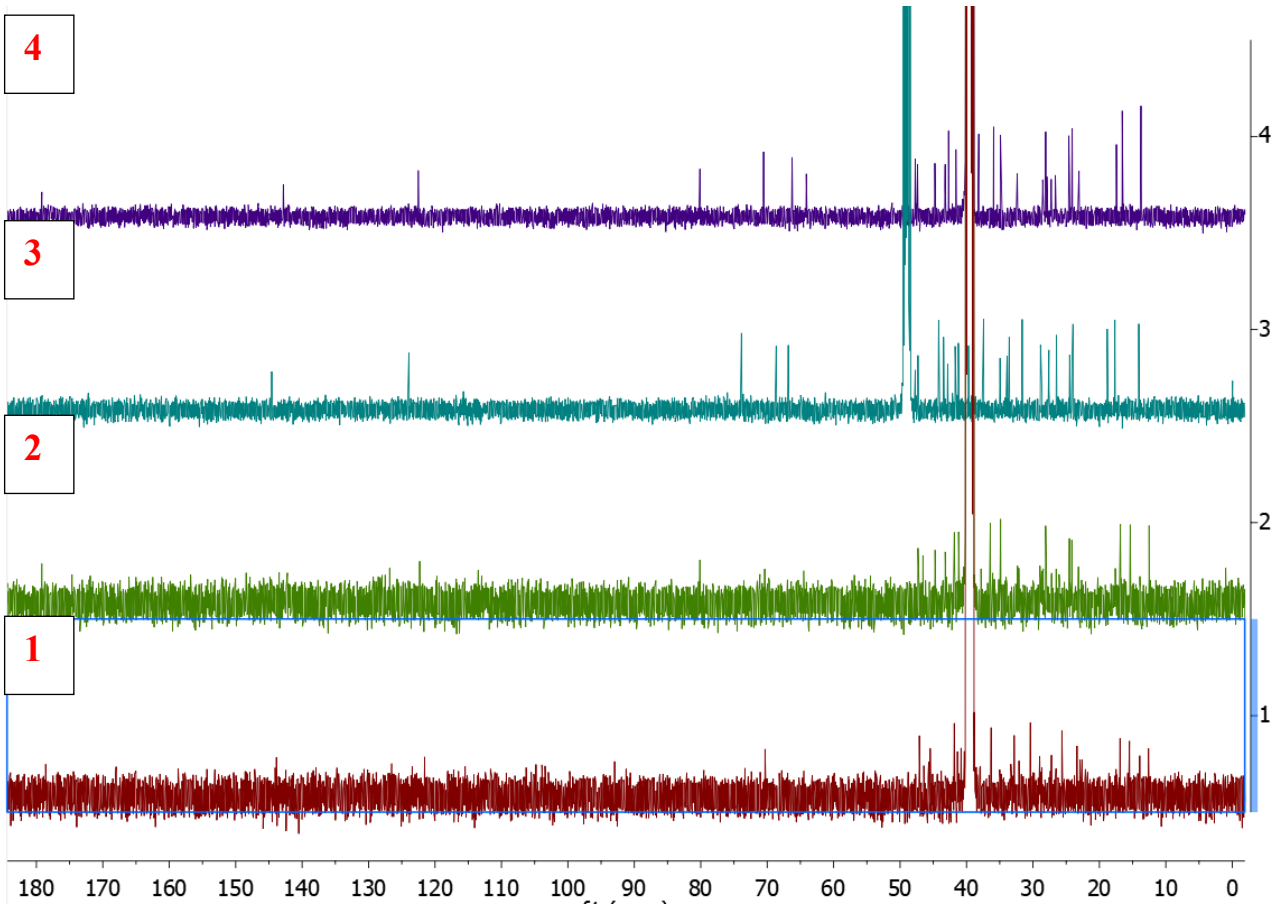



**Figure S20.** Comparison of $^{13}$C-NMR spectra of **1-4.**

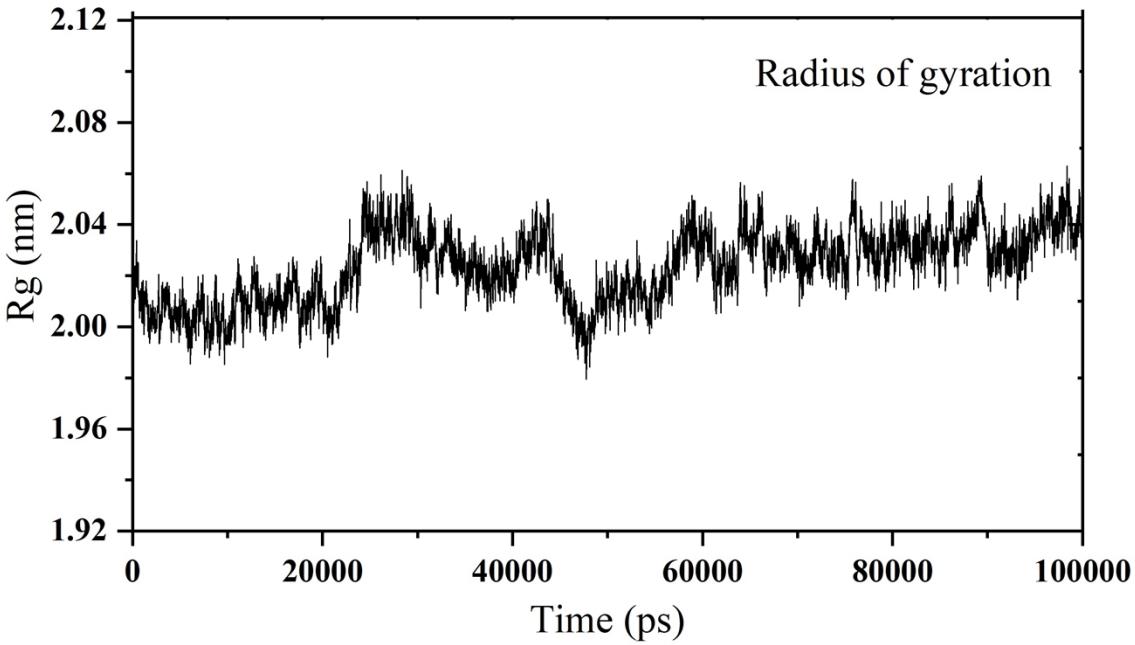
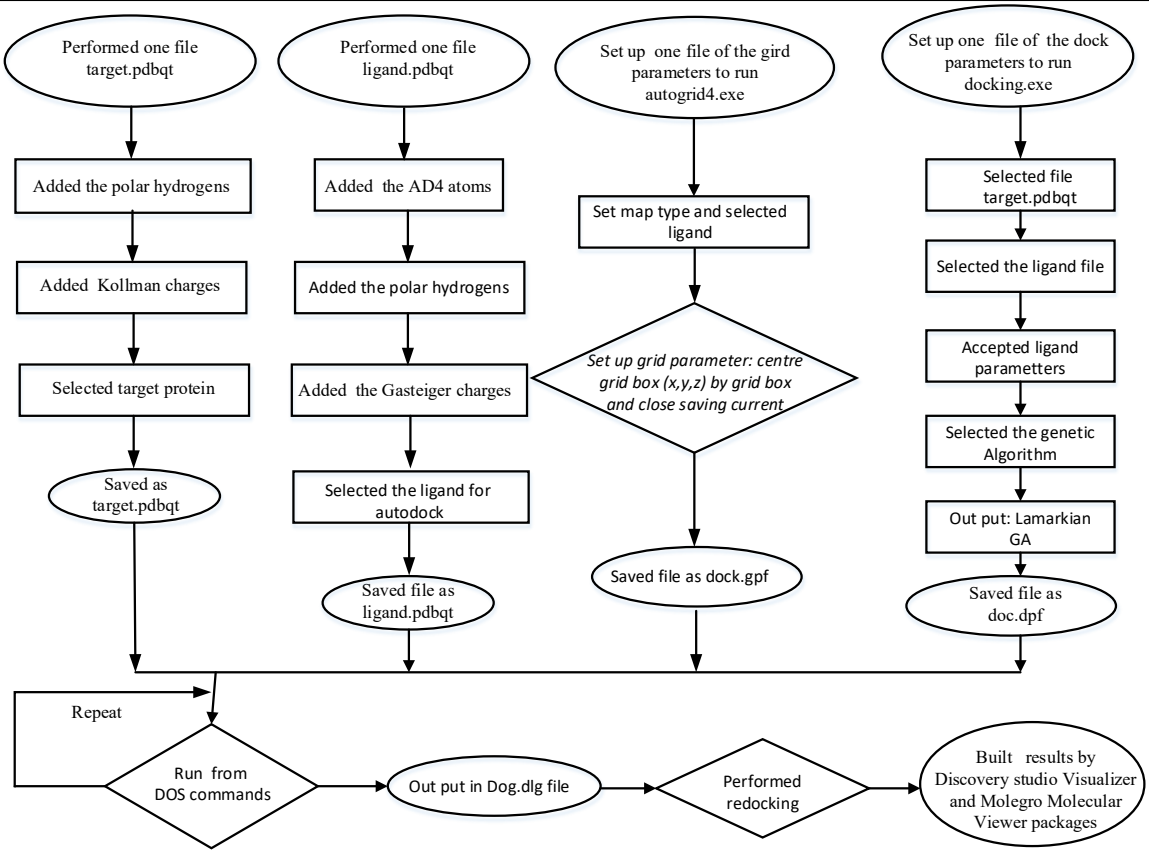



**Figure S21**. The radius of gyration of the protein-best pose was calculated using 100-ns MD simulations.

Table S3. Biological activity of tested compounds on the NO inhibitory activity

| Concentration (µg/mL) | 1 | | 2 | | 3 | | 4 | | **L-NMMA** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % NO inhibition | % Survival | % NO inhibition | % Survival | % NO inhibition | % Survival | % NO inhibition | % Survival | % NO inhibition | % Survival |
| 100 | 46.81 | 97.86 | 67.58 | 44.74 | 86.01 | 15.48 | 44.51 | 103.77 | 95.60 | 82.20 |
| 20 | 35.66 | 103.48 | 44.51 | 97.24 | 40.66 | 96.08 | 27.47 | 106.02 | 75.38 | 93.10 |
| 4 | 4.69 | | 36.26 | | 21.43 | | 12.09 | | 19.34 | |
| 0.8 | -8.83 | | 23.08 | | 6.59 | | 7.69 | | 12.77 | |
| $IC_{50}$ | >100 | - | 27.15±1.23 | - | 29.39±1.55 | - | >100 | - | 10.24±1.65 | - |




**Scheme S1**. Procedure docking of ligand to receptor based on autodock