

# Chemical and Pharmacological Prospection of the *Ascidian Cystodytes dellechiaiei*

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**Figure S1.** Evaluation of the Toll-Like Receptor (TLR) and Dectin 1b activation with 5 and 30  $\mu\text{g/mL}$  of total extract (ext) and HRX-SPE fractions (B–E) in TLR-2 (A), TLR-4 (B) and Dectin (C) NF- $\kappa\text{B}$ /SEAP reporter cell lines. Pam2CSK4 (PAM) and LPS were used as positive controls for TLR-2 and TLR-4 respectively, while zymosan was used as positive control on dectin assay. Asterisks indicate significant differences from the cells treated only with vehicle (control, Ctrl) at a 95% ( $P < 0.05$ ) confidence level, as determined using two-way ANOVA analysis.

**Figure S2.**  $^1\text{H}$  NMR Bioactive Fraction B (600 MHz, MeOD).

**Figure S3.**  $^1\text{H}$  NMR Compound 1 (600 MHz, MeOD).

**Figure S4.** Mass spectra (ESI positive and negative modes) of compound 1.

**Figure S5.**  $^1\text{H}$  NMR Compound 2 (600 MHz, MeOD).

**Figure S6.** HSQC NMR Compound 2 (600 MHz, MeOD).

**Figure S7.**  $^{13}\text{C}$  NMR Compound 2 (600 MHz, MeOD).

**Figure S8.**  $^1\text{H}$  NMR Compound 2 (600 MHz, DMSO- $d_6$ ).

**Figure S9.** HSQC NMR Compound 2 (600 MHz, DMSO- $d_6$ ).

**Figure S10.**  $^{13}\text{C}$  NMR Compound 2 (600 MHz, DMSO- $d_6$ ).

**Figure S11.** Mass spectra (ESI positive and negative modes) of compound 2.

**Figure S12.**  $^1\text{H}$  NMR of fraction containing compounds 3 (600 MHz, MeOD).

**Figure S13.**  $^1\text{H}$ - $^1\text{H}$  COSY spectra of fraction containing compounds 3 (400 MHz, MeOD).

**Figure S14.** HSCQ NMR spectra of fraction containing compounds 3 (600 MHz, MeOD).

**Figure S15.** HMBC NMR spectra of fraction containing compounds 3 (600 MHz, MeOD).

**Figure S16.**  $^{13}\text{C}$  NMR spectra of fraction containing compounds 3 (600 MHz, MeOD).

**Figure S17.** LC-MS/MS (ESI negative mode) of fraction containing compounds 3.

**Figure S18.** MS/MS (ESI negative mode) of the main peak at  $m/z$  738.55.

**Figure S19.** MS/MS (ESI negative mode) of the main peak at  $m/z$  752.57.

**Figure S20.** MS/MS (ESI negative mode) of the main peak at  $m/z$  780.60.

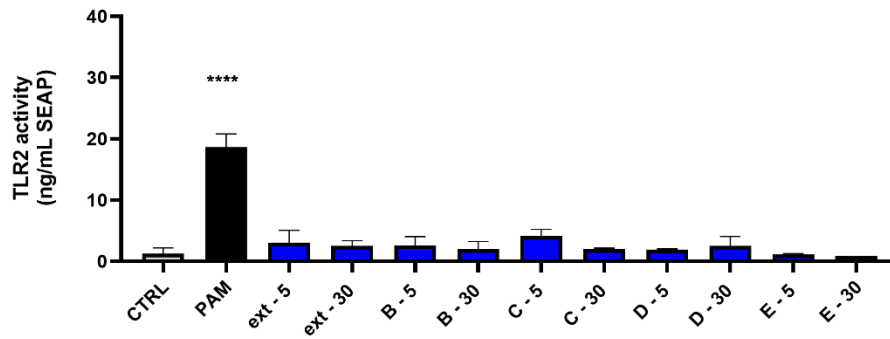
**Figure S21.** MS/MS (ESI negative mode) of the main peak at  $m/z$  794.61.

**Figure S22.** MS/MS (ESI negative mode) of the main peak at  $m/z$  808.63.

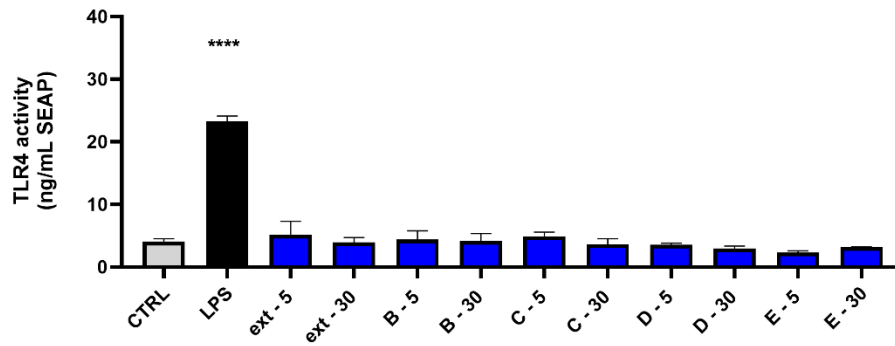
**Figure S23.** MS/MS (ESI negative mode) of the main peak at  $m/z$  822.65.

**Figure S 1.** Evaluation of the Toll-Like Receptor (TLR) and Dectin 1b activation with 5 and 30  $\mu\text{g/mL}$  of total extract (ext) and HRX-SPE fractions (B–E) in TLR-2 (**A**), TLR-4 (**B**) and Dectin (**C**) NF-kB/SEAP reporter cell lines. Pam2CSK4 (PAM) and LPS were used as positive controls for TLR-2 and TLR-4 respectively, while zymosan was used as positive control on dectin assay. Asterisks indicate significant differences from the cells treated only with vehicle (control, Ctrl) at a 95% ( $P < 0.05$ ) confidence level, as determined using two-way ANOVA analysis.

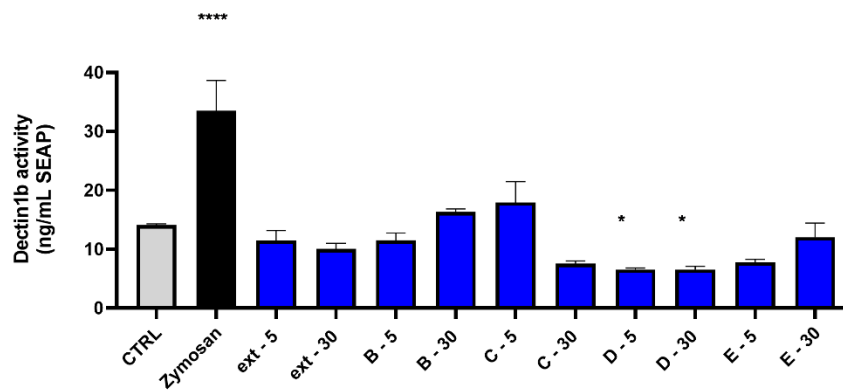
**A**



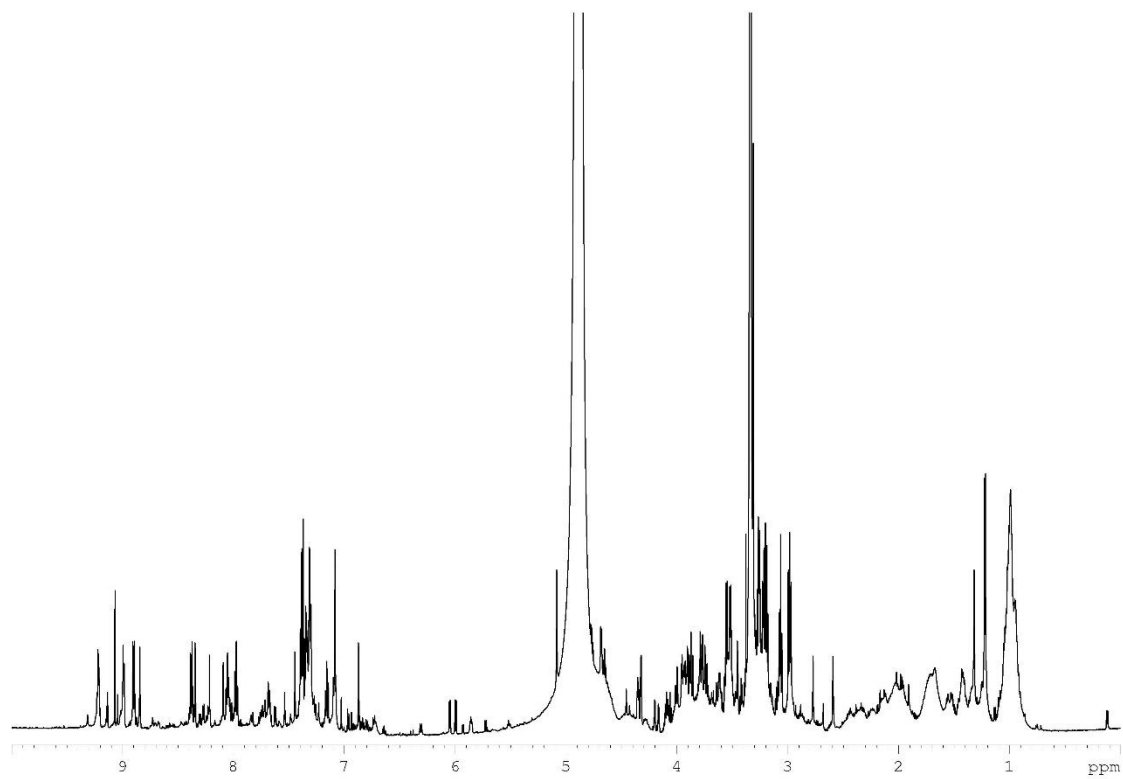
**B**



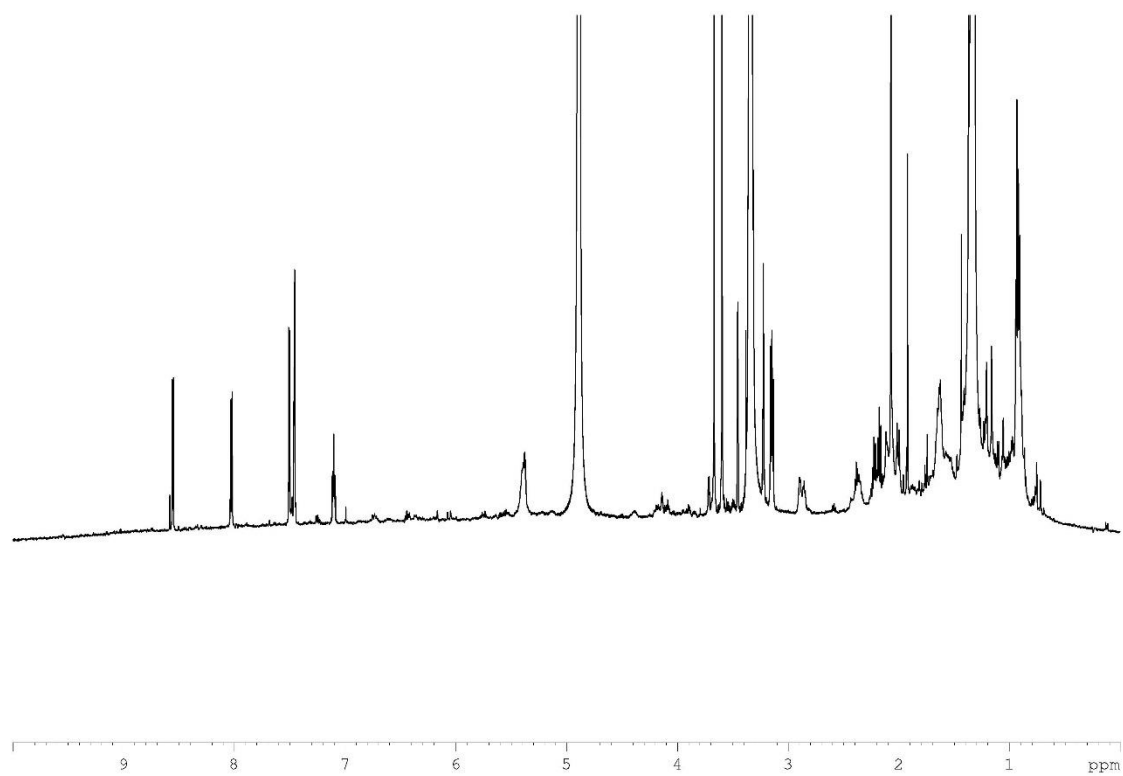
**C**



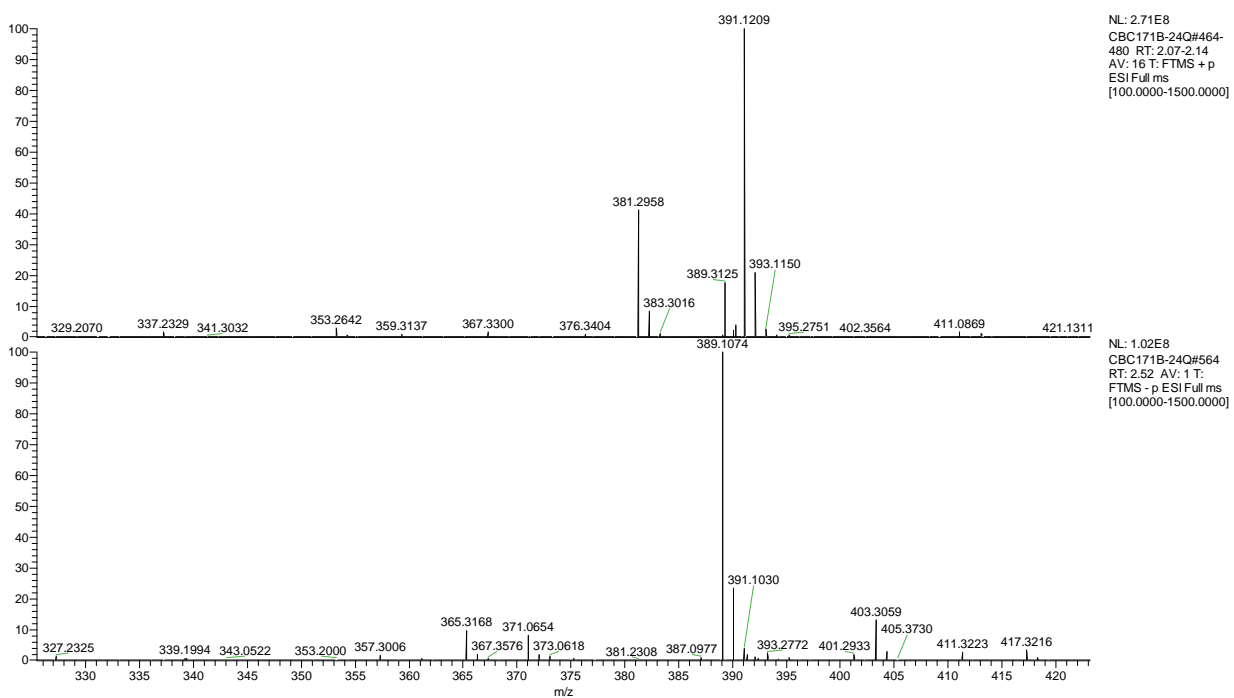
**Figure S 2.**  $^1\text{H}$  NMR Bioactive Fraction B (600 MHz, MeOD).



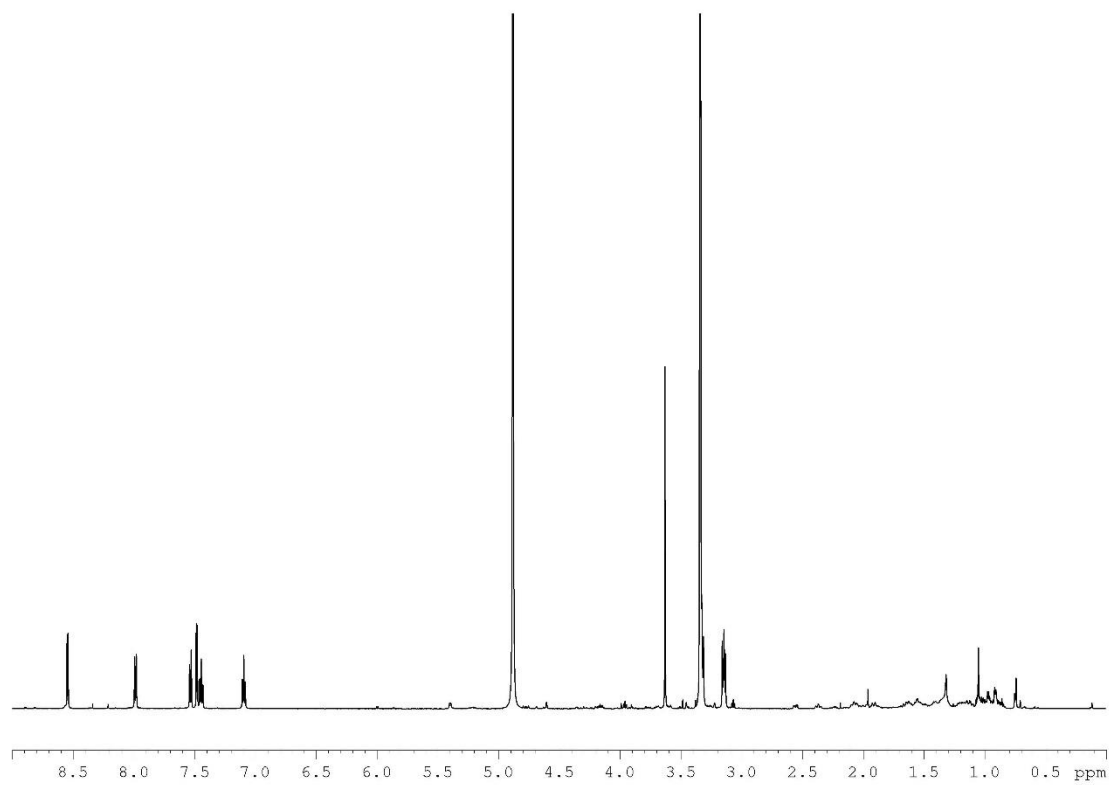
**Figure S 3.**  $^1\text{H}$  NMR Compound **1**(600 MHz, MeOD).



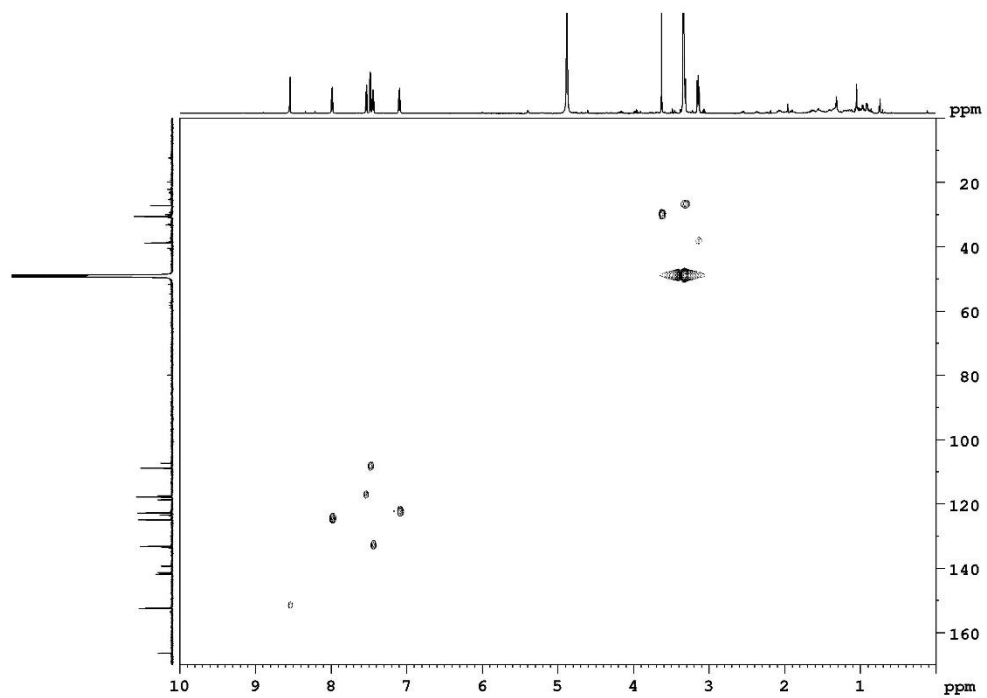
**Figure S 4.** Mass spectra (ESI positive and negative modes) of compound **1**.



**Figure S 5.**  $^1\text{H}$  NMR Compound **2** (600 MHz, MeOD).

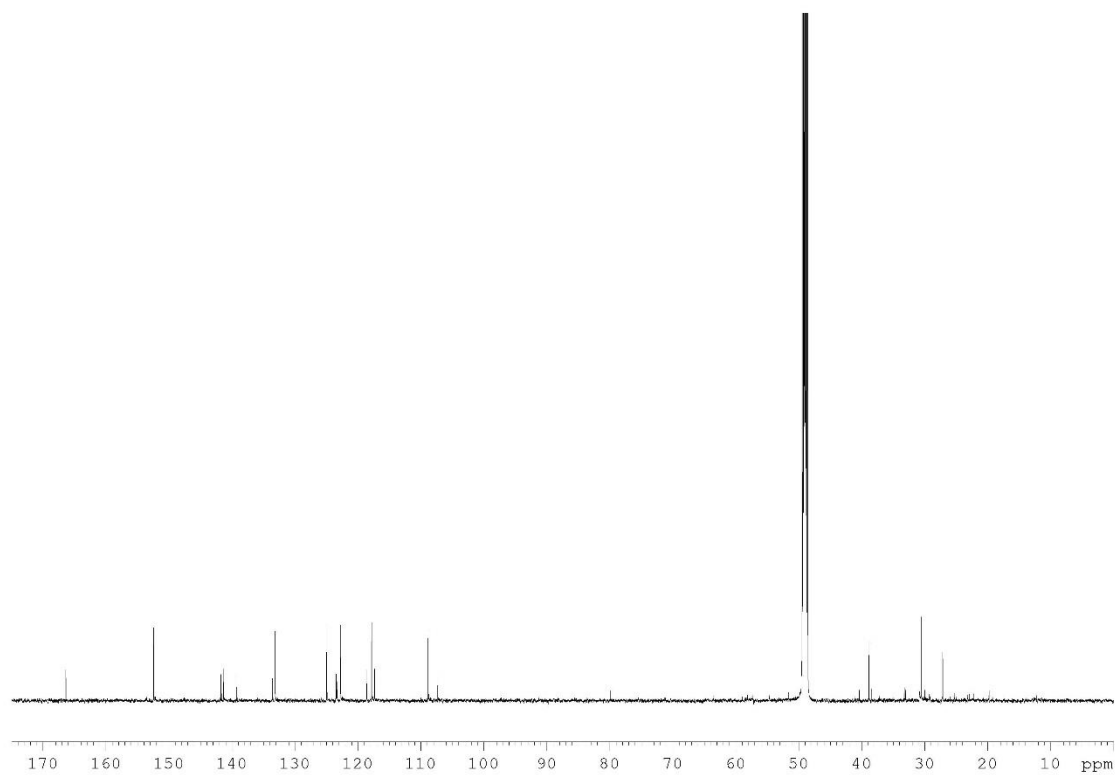


**Figure S 6.** HSQC NMR Compound 2 (600 MHz, MeOD).

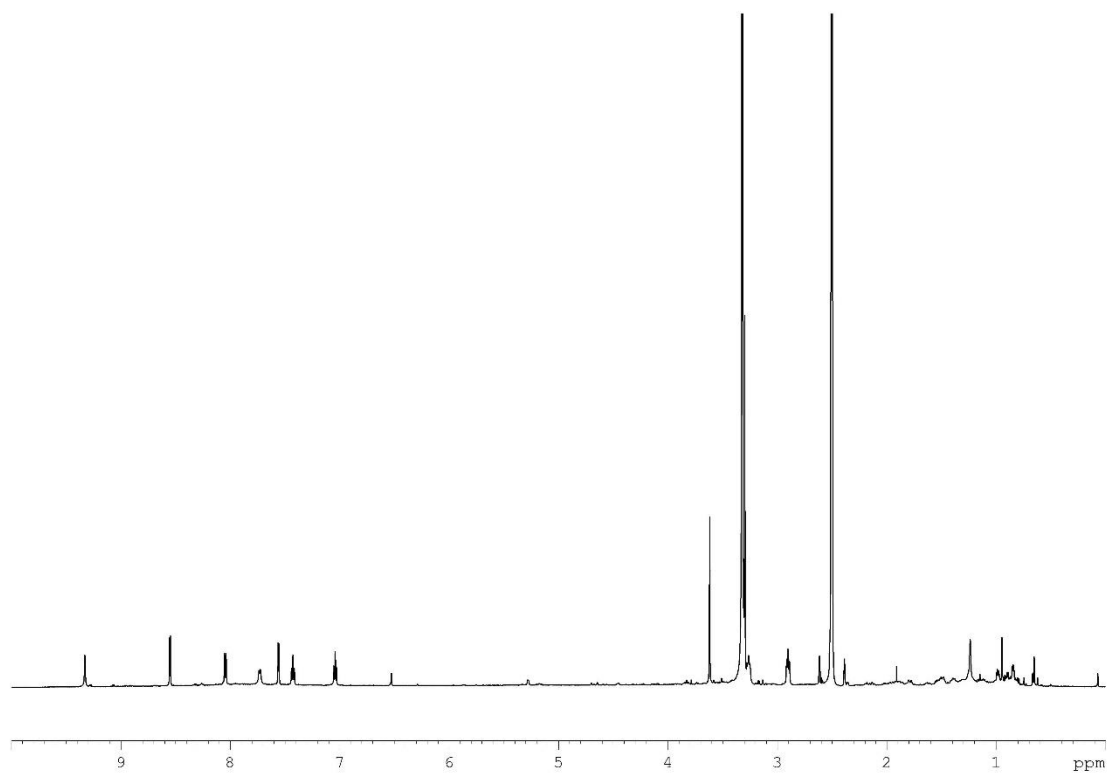




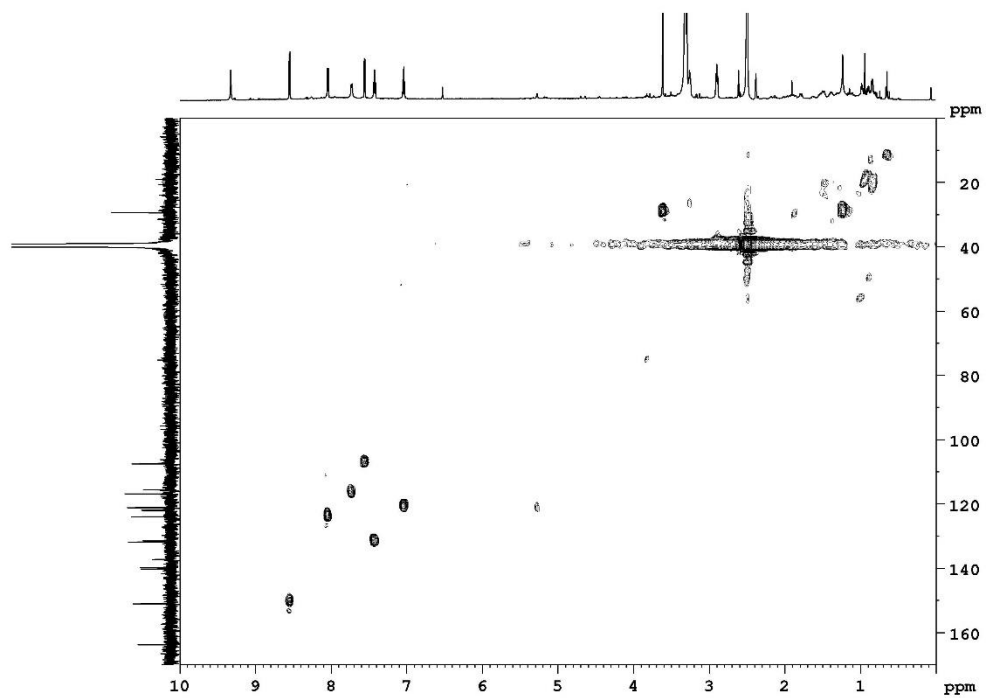
**Figure S 7.**  $^{13}\text{C}$  NMR Compound **2** (600 MHz, MeOD).



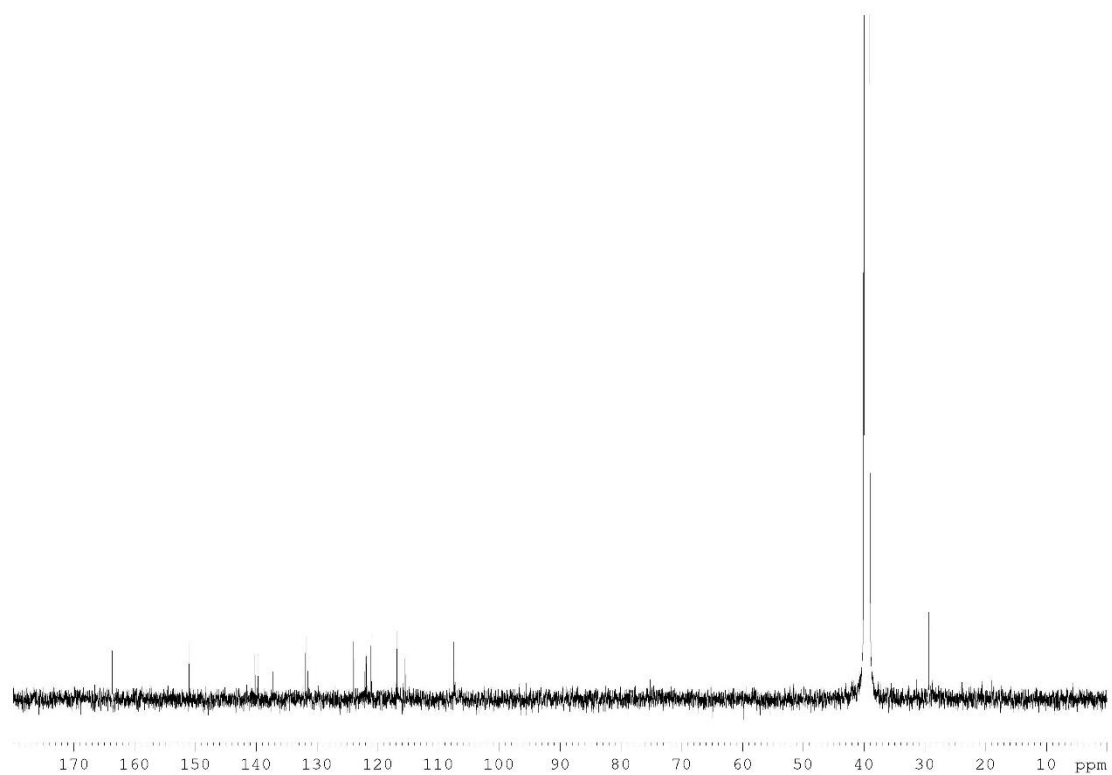
**Figure S 8.**  $^1\text{H}$  NMR Compound **2** (600 MHz,  $\text{DMSO-d}_6$ ).



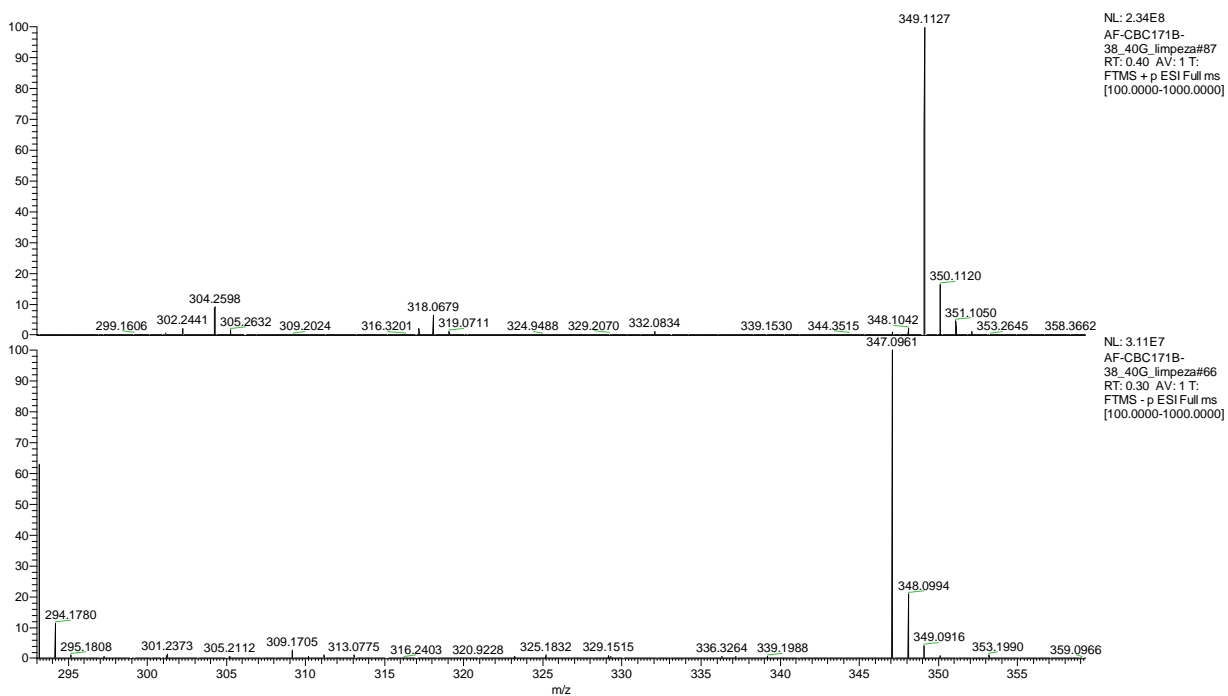
**Figure S 9.** HSQC NMR Compound 2 (600 MHz, DMSO-d<sub>6</sub>).



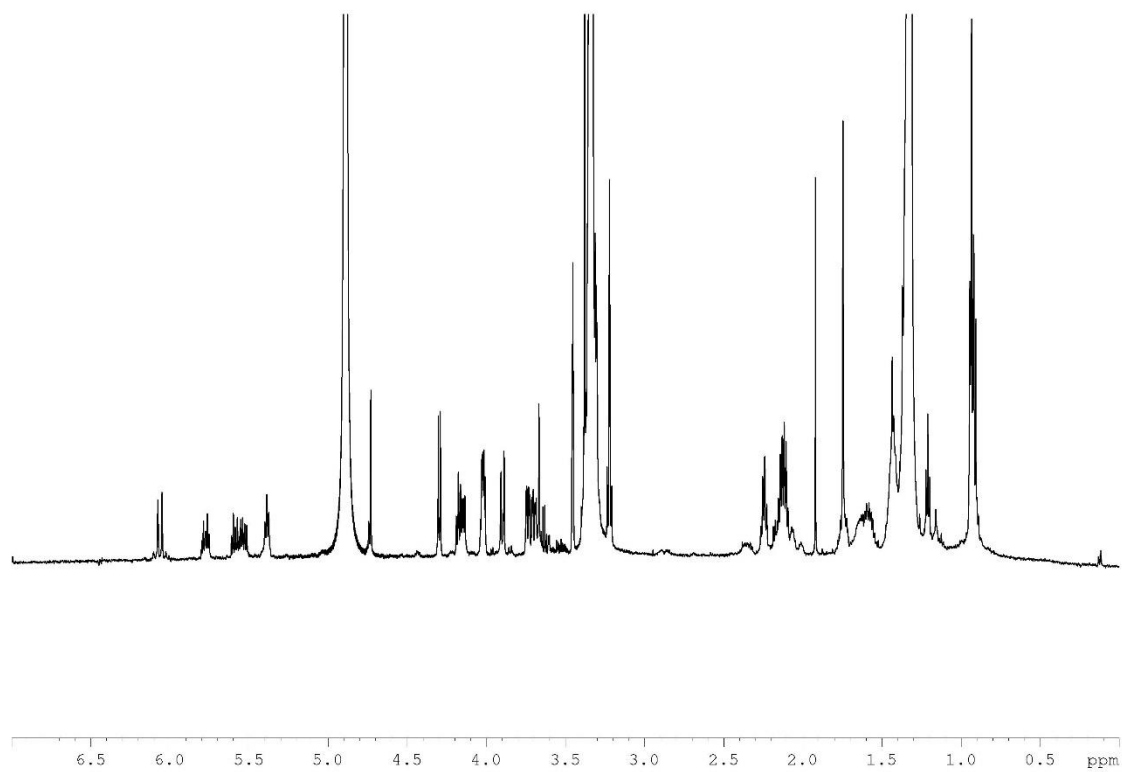
**Figure S 10.**  $^{13}\text{C}$  NMR Compound **2** (600 MHz,  $\text{DMSO-d}_6$ ).



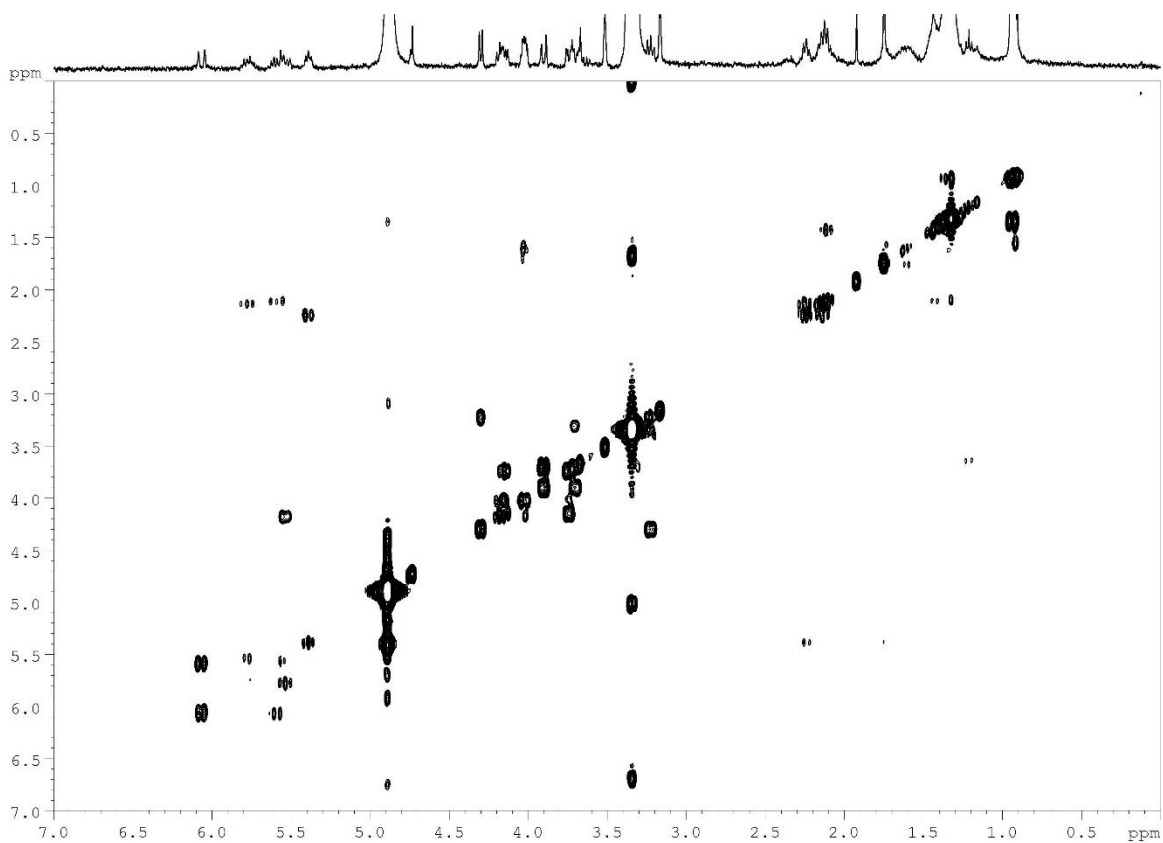
**Figure S 11.** Mass spectra (ESI positive and negative modes) of compound **2**.



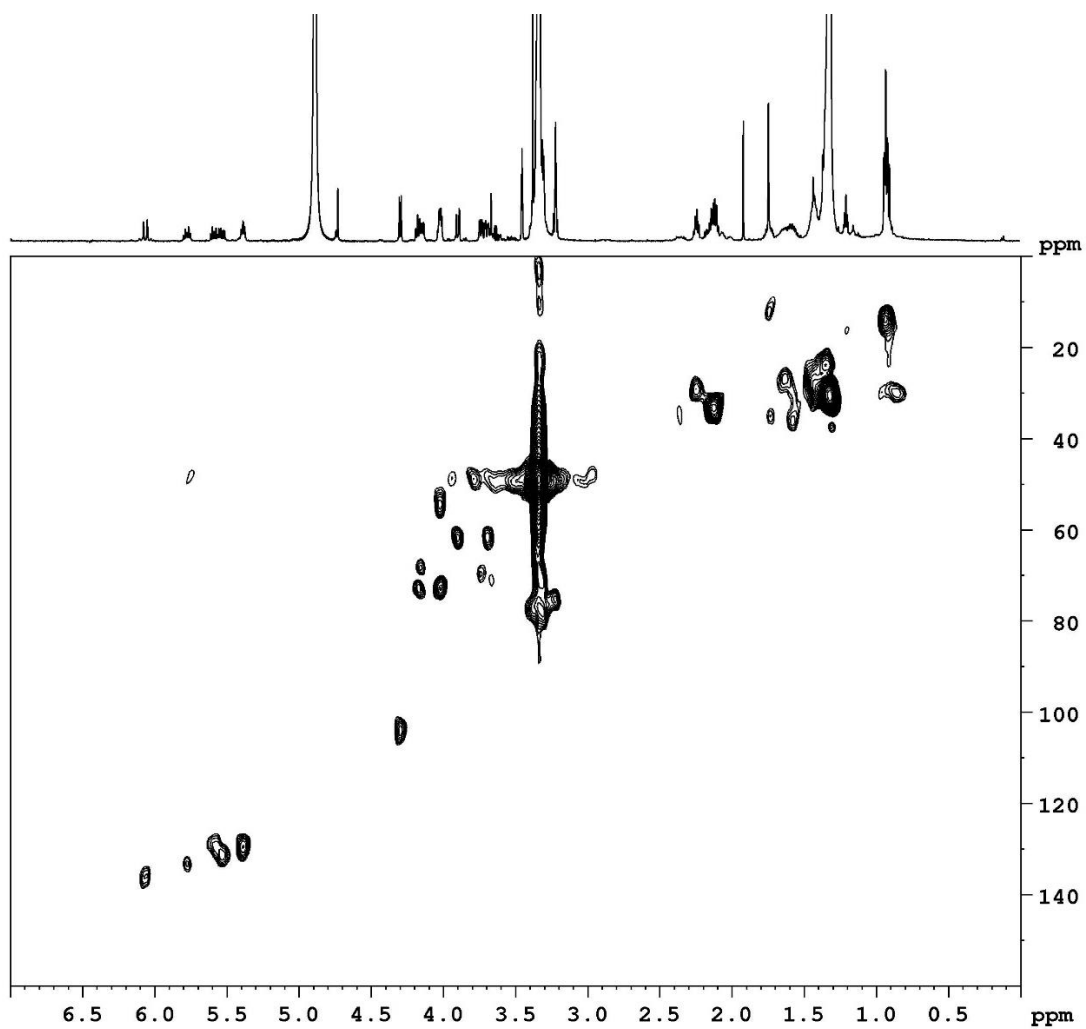
**Figure S 12.**  $^1\text{H}$  NMR of fraction containing compounds **3** (600 MHz, MeOD).



**Figure S 13.**  $^1\text{H}$ - $^1\text{H}$  COSY spectra of fraction containing compounds **3** (400 MHz, MeOD).

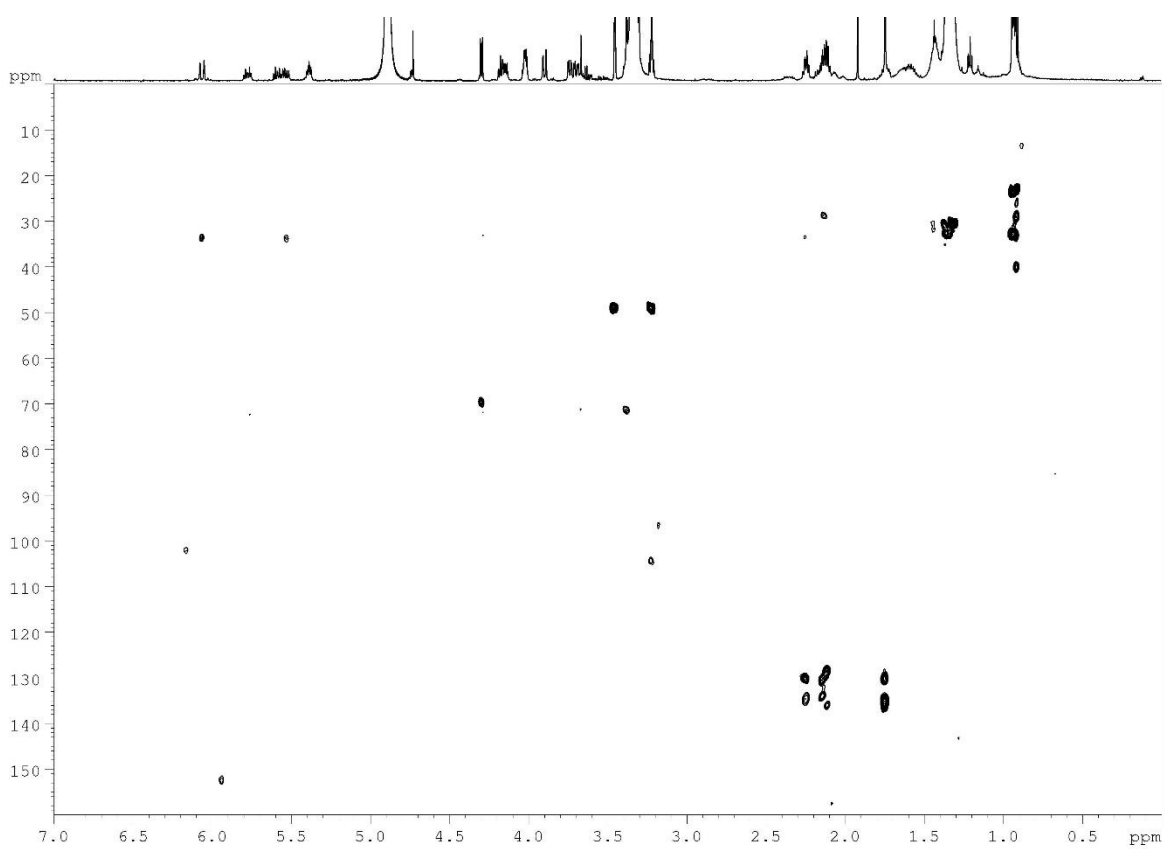


**Figure S 14.** HSCQ NMR spectra of fraction containing compounds **3** (600 MHz, MeOD).

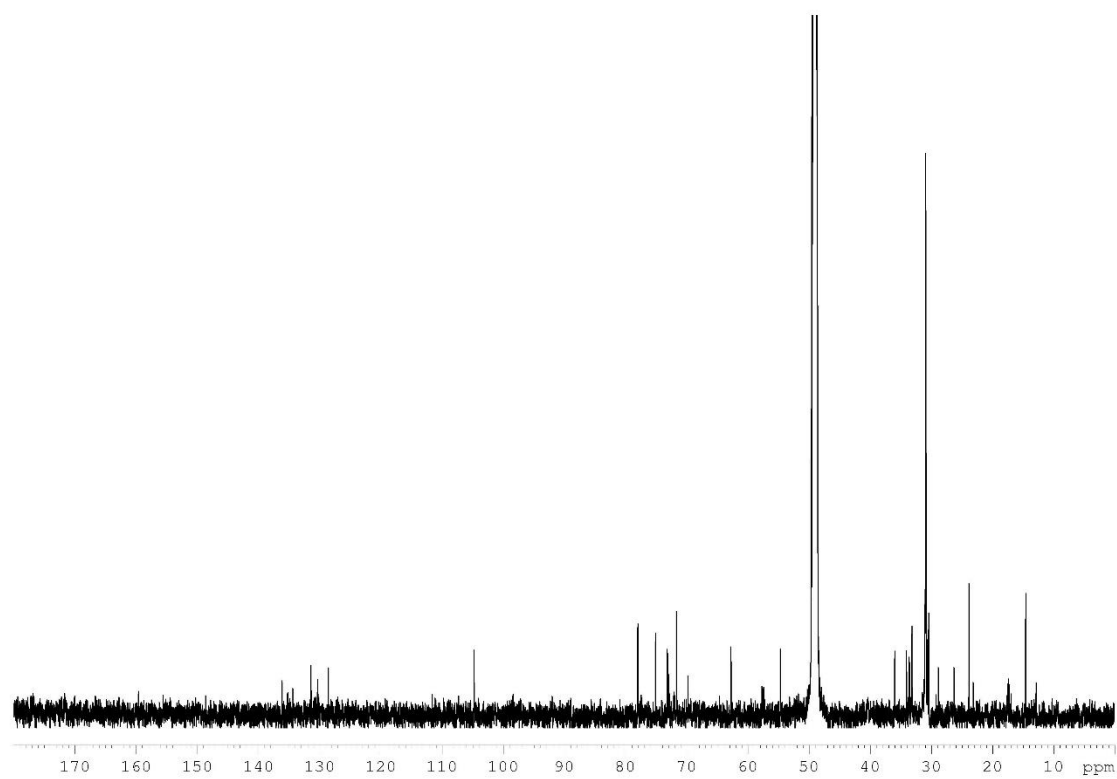




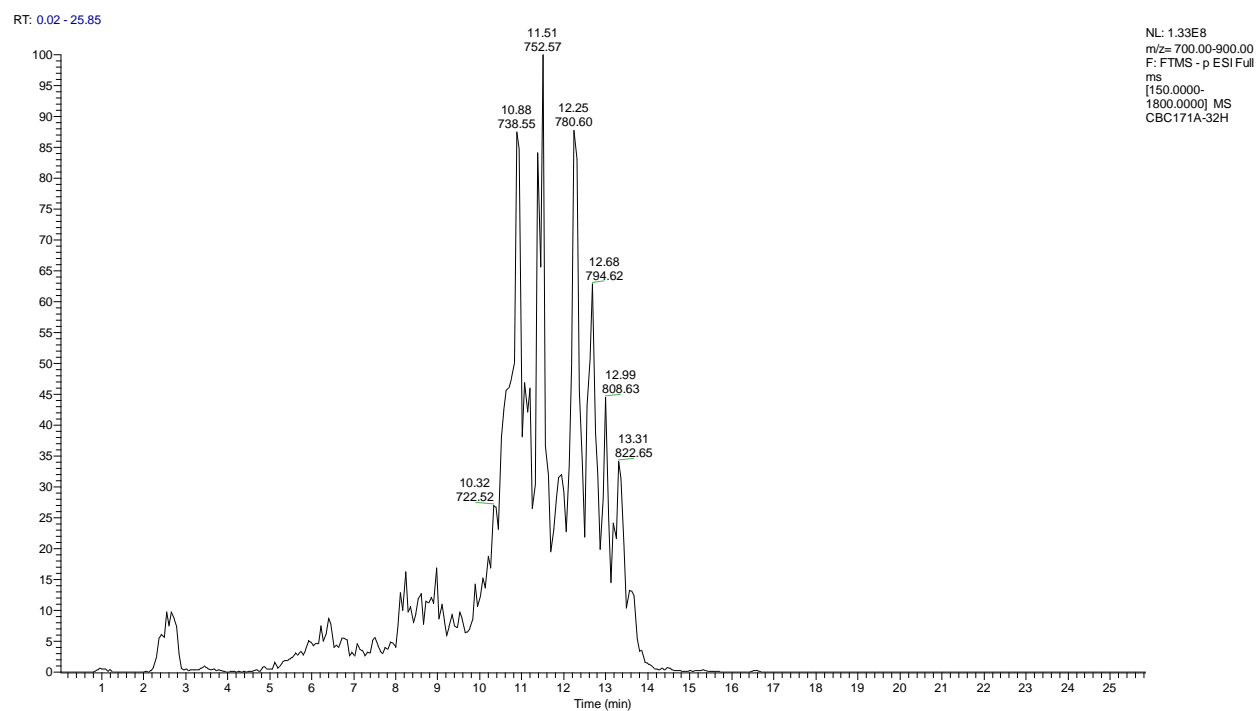
**Figure S 15.** HMBC NMR spectra of fraction containing compounds **3** (600 MHz, MeOD).



**Figure S 16.**  $^{13}\text{C}$  NMR spectra of fraction containing compounds **3** (600 MHz, MeOD).

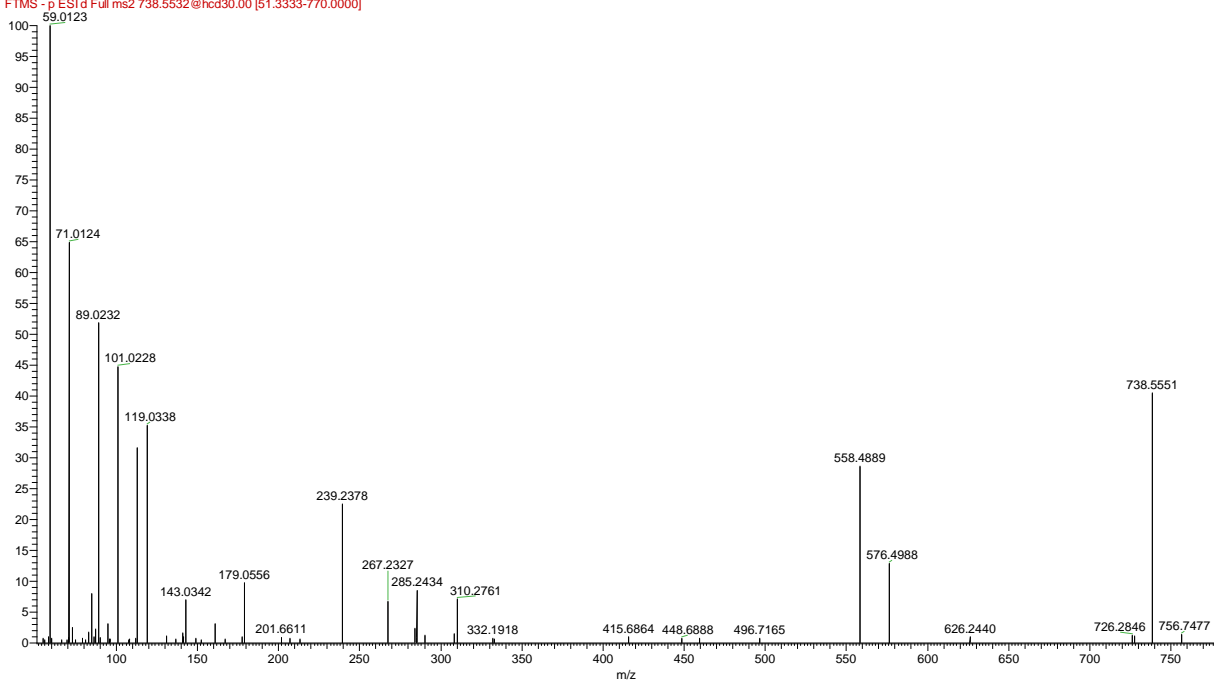


**Figure S 17.** LC-MS/MS (ESI negative mode) of fraction containing compounds **3**.



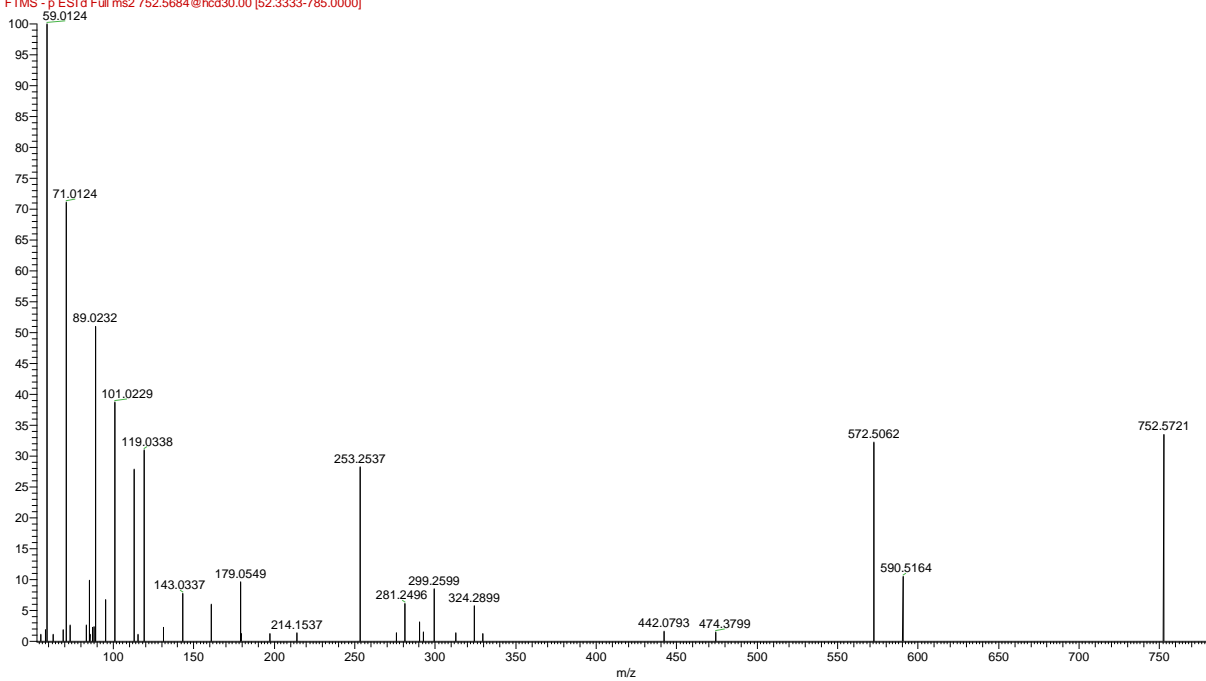
**Figure S 18.** MS/MS (ESI negative mode) of the main peak at  $m/z$  738.55.

CBC171A-32H #3893-3956 RT: 10.82-10.95 AV: 2 NL: 1.02E6  
F: FTMS - p ESI d Full ms2 738.5532@hcd30.00 [51.3333-770.0000]



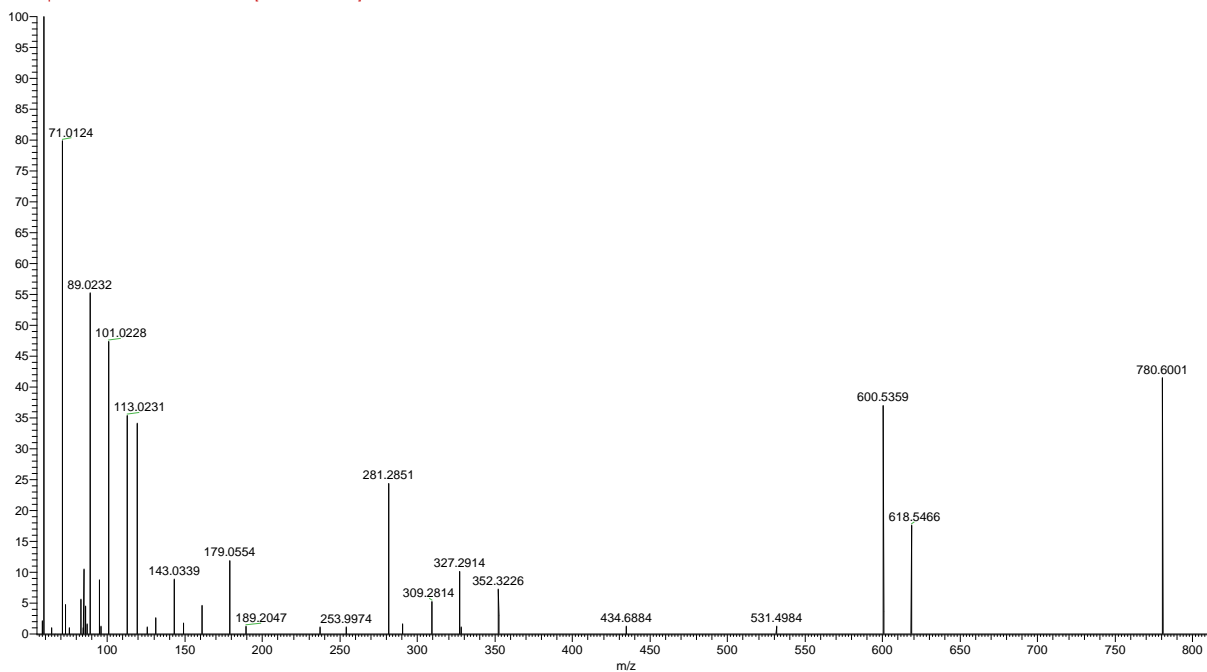
**Figure S19.** MS/MS (ESI negative mode) of the main peak at  $m/z$  752.57.

CBC171A-32H#4080 RT: 11.45 AV: 1 NL: 1.71E6  
F: FTMS - p ESI d Full ms2 752.5684@hcd30.00 [52.3333-785.0000]



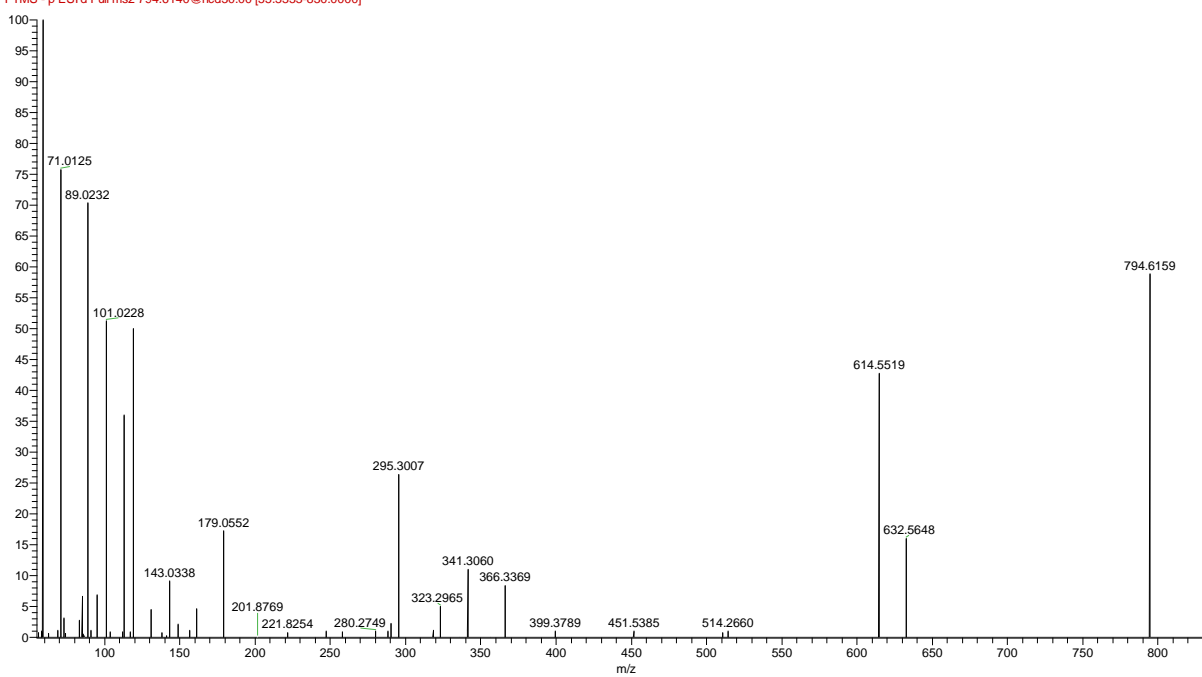
**Figure S 20.** MS/MS (ESI negative mode) of the main peak at  $m/z$  780.60.

CBC171A-32H #4373 RT: 12.25 AV: 1 NL: 1.62E6  
F: FTMS - p ESI d Full ms2 780.6002@hcd30.00 [54.3333-815.0000]



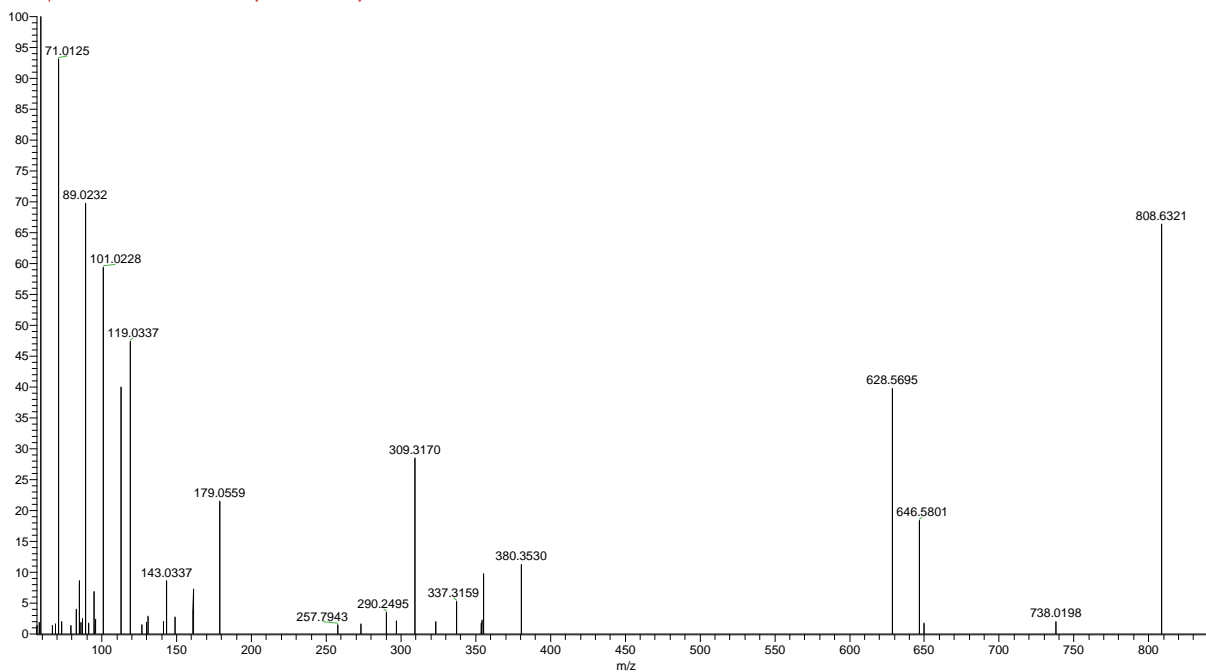
**Figure S 21.** MS/MS (ESI negative mode) of the main peak at  $m/z$  794.61.

CBC171A-32H #4490-4589 RT: 12.51-12.75 AV: 3 NL: 5.33E5  
F: FTMS - p ESI d Full ms2 794.6140@hcd30.00 [55.3333-830.0000]



**Figure S 22.** MS/MS (ESI negative mode) of the main peak at  $m/z$  808.63.

CBC171A-32H #4632 RT: 13.00 AV: 1 NL: 4.55E5  
F: FTMS - p ESI d Full ms2 808.6088@hcd30.00 [56.3333-845.0000]





**Figure S 23.** MS/MS (ESI negative mode) of the main peak at  $m/z$  822.65.

CBC171A-32H #4776 RT: 13.31 AV: 1 NL: 3.72E5  
F: FTMS - p ESI d Full ms2 822.6472@hcd30.00 [57.0000-855.0000]

