# Organoselenium compounds as novel adjuvants of chemotherapy drugs: a promising approach to fight cancer drug resistance.

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## Supplementary Material

### Contents:

**Table S1.:** Chemotherapeutic agents tested: stock solutions and final concentrations.

| Reference drug | Mechanism of action | Concentration of stock solution | Final concentration in the combination assay |
|---|---|---|---|
| **Topotecan (*Topo*)** | *Topoisomerase-I-inhibitor* | 1.0 mg/mL | 0.024 μM |
| **Doxorubicin (*Dox*)** | *Topoisomerase-II-inhibitor, ROS species generation* | 2.0 mg/mL | 17.242 μM |
| **Vincristine (*Vin*)** | *Inhibition of microtubule formation* | 1.0 mg/mL | 36.363 μM |
| **Cisplatin (*Cis*)** | *Alkylating-like agent (interstrand and intrastrand cross-links with purine bases at 1,2-positions within DNA strands)* | 0.5 mg/mL | 33.333 μM |
| **Cyclophosphamide (*Cpm*)** | *Alkylating agent (interstrand and intrastrand cross-links at N-7 position within DNA strands)* | 25 mg/mL | 250.000 μM |
| **5-fluorouracil (*5-FU*)** | *Pyrimidine-antagonist* | 50.0 mg/mL | 0.192 μM |
| **Methotrexate (*Mtx*)** | *Purine-antagonist* | 10.0 mg/mL | 11.000 μM |
| **Verapamil (*Ver*)** | *Efflux pump inhibitor ($Ca^{2+}$-channel blocker)* | 2.5 mg/mL | 200.000 μM |

**Table S2.:** Selenocompounds tested in combination assay: stock solutions and final concentrations.

| Tested compounds | Concentration of stock solution | Final concentration in the combination assay |
|---|---|---|
| **1** | 10 mM | 25.0 μM |
| **2** | | 25.0 μM |
| **3** | | 50.0 μM |
| **4** | | 100.0 μM |
| **5** | | 100.0 μM |
| **6** | | 100.0 μM |
| **7** | | 100.0 μM |
| **8** | | 100.0 μM |
| **9** | | 10.0 μM |
| **10** | | 10.0 μM |
| **11** | | 10.0 μM |
| **12** | | 100.0 μM |
| **13** | | 100.0 μM |
| **14** | | 100.0 μM |
| **15** | | 100.0 μM |

**Table S3.** Interactions between selenocompounds and topotecan (*Top*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Topo* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 0.006 : 12.500 | 1.19 | 0.07 | Slight antagonism |
| 2 | 0.006 : 12.500 | 0.78 | 0.10 | **Moderate synergism** |
| 3 | 0.0015 : 12.5000 | 1.76 | 0.28 | Antagonism |
| 4 | 0.003 : 50.000 | 0.72 | 0.11 | **Moderate synergism** |
| 5 | 0.0015 : 25.0000 | 0.95 | 0.12 | *Additive effect* |
| 6 | 0.006 : 100.000 | 1.84 | 0.26 | Antagonism |
| 7 | 0.006 : 50.000 | 1.26 | 0.08 | Moderate antagonism |
| 8 | 0.0015 : 50.0000 | 1.24 | 0.10 | Moderate antagonism |
| 9 | 0.024 : 1.250 | 0.49 | 0.21 | **Synergism** |
| 10 | 0.0015 : 1.2500 | 0.51 | 0.13 | **Synergism** |
| 11 | 0.0015 : 2.5000 | 0.77 | 0.09 | **Moderate synergism** |
| 12 | 0.0015 : 25.000 | 0.41 | 0.06 | **Synergism** |
| 13 | 0.003 : 100.000 | 0.68 | 0.08 | **Synergism** |
| 14 | 0.0015 : 100.0000 | 0.74 | 0.06 | **Moderate synergism** |
| 15 | 0.003 : 50.000 | 1.22 | 0.06 | Moderate antagonism |

**Table S4.** Interactions between selenocompounds and doxorubicin (*Dox*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Doxo* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 1 : 5 | 0.64 | 0.20 | **Synergism** |
| 2 | 1 : 50 | 0.42 | 0.08 | **Synergism** |
| 3 | 1 : 12.5 | 0.53 | 0.09 | **Synergism** |
| 4 | 8 : 50 | 1.03 | 0.09 | *Additive effect* |
| 5 | 2 : 100 | 0.61 | 0.09 | **Synergism** |
| 6 | 4 : 25 | 0.88 | 0.06 | **Slight synergism** |
| 7 | 2 : 50 | 1.48 | 0.31 | Antagonism |
| 8 | 8 : 50 | 1.15 | 0.02 | Slight antagonism |
| 9 | 1 : 2.5 | 0.81 | 0.14 | **Moderate synergism** |
| 10 | 1 : 2.5 | 0.83 | 0.07 | **Moderate synergism** |
| 11 | 2 : 2.5 | 1.04 | 0.13 | *Additive effect* |
| 12 | 1 : 100 | 1.04 | 0.04 | *Additive effect* |
| 13 | 2 : 25 | 0.47 | 0.22 | **Synergism** |
| 14 | 0.5 : 25 | 2.63 | 0.54 | Antagonism |
| 15 | 1 :100 | 0.97 | 0.08 | *Additive effect* |

**Table S5.** Interactions between selenocompounds and vincristine (Vin) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Vin* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 2.273 : 12.500 | 0.57 | 0.15 | **Synergism** |
| 2 | 9.091 : 12.500 | 0.20 | 0.01 | **Strong synergism** |
| 3 | 18.182 : 12.500 | 0.46 | 0.15 | **Synergism** |
| 4 | 9.091 : 6.250 | 0.27 | 0.07 | **Strong synergism** |
| 5 | 4.545 : 50.000 | 0.51 | 0.09 | **Synergism** |
| 6 | 2.273 : 100.000 | 1.26 | 0.14 | Moderate antagonism |
| 7 | 4.545 : 25.000 | 0.58 | 0.11 | **Synergism** |
| 8 | 4.545 : 25.000 | 0.80 | 0.06 | **Moderate synergism** |
| 9 | 1.136 : 1.250 | 0.39 | 0.01 | **Synergism** |
| 10 | 1.136 : 1.250 | 0.78 | 0.13 | **Moderate synergism** |
| 11 | 4.545 : 2.500 | 0.56 | 0.13 | **Synergism** |
| 12 | 2.273 : 100.000 | 0.68 | 0.10 | **Synergism** |
| 13 | 2.273 : 100.000 | 1.16 | 0.04 | Slight antagonism |
| 14 | 2.273 : 50.000 | 0.70 | 0.13 | **Moderate synergism** |
| 15 | 9.091 : 50.000 | 0.36 | 0.08 | **Synergism** |

**Table S6.** Interactions between selenocompounds and cisplatin (*Cis*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Cis* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 4.16 : 25.00 | 1.26 | 0.11 | Moderate antagonism |
| 2 | 4.16 : 100.00 | 0.96 | 0.04 | *Additive effect* |
| 3 | 1.04 : 6.25 | 1.31 | 0.07 | Moderate antagonism |
| 4 | 1.04 : 12.50 | 4.09 | 0.25 | Strong antagonism |
| 5 | 4.16 : 100.00 | 1.01 | 0.08 | *Additive effect* |
| 6 | 8.33 : 100.00 | 0.99 | 0.10 | *Additive effect* |
| 7 | 33.33 : 25.00 | 1.56 | 0.19 | Antagonism |
| 8 | 8.33 : 100.00 | 2.25 | 0.16 | Antagonism |
| 9 | 4.16 : 1.25 | 2.17 | 0.28 | Antagonism |
| 10 | 2.08 : 2.50 | 1.03 | 0.03 | *Additive effect* |
| 11 | 8.33 : 1.25 | 1.21 | 0.17 | Slight antagonism |
| 12 | 8.33 : 25.00 | 1.42 | 0.17 | Moderate antagonism |
| 13 | 8.33 : 50.00 | 1.28 | 0.22 | Moderate antagonism |
| 14 | 2.08 : 100.00 | 1.39 | 0.14 | Moderate antagonism |
| 15 | 33.33 : 50.00 | 1.10 | 0.08 | *Additive effect* |

**Table S7.** Interactions between selenocompounds and cyclophosphamide (*Cpm*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Cpm* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 125 : 6.25 | 1.40 | 0.16 | Moderate antagonism |
| 2 | 125 : 12.5 | 0.67 | 0.17 | **Synergism** |
| 3 | 125 : 12.5 | 0.61 | 0.16 | **Synergism** |
| 4 | 125 : 12.5 | 1.08 | 0.06 | *Additive effect* |
| 5 | 62.5 : 50 | 0.64 | 0.11 | **Synergism** |
| 6 | 62.5 : 50 | 1.39 | 0.16 | Moderate antagonism |
| 7 | 125 : 25 | 0.71 | 0.11 | Moderate synergism |
| 8 | 125 : 100 | 0.63 | 0.02 | **Synergism** |
| 9 | 125 : 2.5 | 0.75 | 0.10 | Moderate synergism |
| 10 | 125 : 2.5 | 1.43 | 0.14 | Moderate antagonism |
| 11 | 125 : 1.25 | 1.31 | 0.11 | Moderate antagonism |
| 12 | 125 : 25 | 1.55 | 0.08 | Antagonism |
| 13 | 125 : 100 | 1.58 | 0.39 | Antagonism |
| 14 | 62.5 : 100 | 1.39 | 0.25 | Moderate antagonism |
| 15 | 125 : 100 | 1.85 | 0.20 | Antagonism |

**Table S8.** Interactions between selenocompounds and methotrexate (*Met*) against multidrug resistant (MDR) mouse T-lymphoma cells

| Compound | Ratio [μM] (*Met* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 0.6875:50 | 2.03 | 0.46 | Antagonism |
| 2 | 11:12.5 | 0.36 | 0.05 | **Synergism** |
| 3 | 2.75:25 | 0.71 | 0.02 | **Moderate synergism** |
| 4 | 1.375:25 | 0.71 | 0.16 | **Moderate synergism** |
| 5 | 0.6875:100 | 0.48 | 0.09 | **Synergism** |
| 6 | 5.5:100 | 1.40 | 0.16 | **Slight synergism** |
| 7 | 1.375:100 | 1.36 | 0.21 | Moderate antagonism |
| 8 | 1.375:100 | 0.68 | 0.16 | **Synergism** |
| 9 | 1.375:1.25 | 3.24 | 0.42 | Antagonism |
| 10 | 11:2.5 | 2.41 | 0.33 | Antagonism |
| 11 | 0.3438:1.25 | 2.26 | 0.39 | Antagonism |
| 12 | 5.5:12.5 | 1.34 | 0.13 | Moderate antagonism |
| 13 | 0.6875:12.5 | 0.59 | 0.05 | **Synergism** |
| 14 | 1.375:12.5 | 0.39 | 0.14 | **Synergism** |
| 15 | 1.375:25 | 1.33 | 0.09 | Moderate antagonism |

**Table S9.** Interactions between selenocompounds and 5-fluorouracil (*5-FU*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*5-FU* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 0.012 : 6.250 | 0.97 | 0.05 | *Additive effect* |
| 2 | 0.024 : 100.000 | 0.77 | 0.03 | **Moderate synergism** |
| 3 | 0.024 : 25.000 | 1.48 | 0.22 | Antagonism |
| 4 | 0.096 : 50.000 | 0.48 | 0.05 | **Synergism** |
| 5 | 0.024 : 100.000 | 0.42 | 0.14 | **Synergism** |
| 6 | 0.012 : 100.000 | 0.68 | 0.20 | **Synergism** |
| 7 | 0.012 : 100.000 | 0.83 | 0.07 | **Moderate synergism** |
| 8 | 0.024 : 50.000 | 1.34 | 0.11 | Moderate antagonism |
| 9 | 0.096 : 1.250 | 2.81 | 0.29 | Antagonism |
| 10 | 0.048 : 1.250 | 2.54 | 0.48 | Antagonism |
| 11 | 0.096 : 1.250 | 2.01 | 0.41 | Antagonism |
| 12 | 0.012 : 100.000 | 0.46 | 0.18 | **Synergism** |
| 13 | 0.012 : 100.000 | 0.42 | 0.07 | **Synergism** |
| 14 | 0.024 : 25.000 | 0.69 | 0.14 | **Synergism** |
| 15 | 0.012 : 100.000 | 1.06 | 0.05 | *Additive effect* |

**Table S10.** Interactions between selenocompounds and verapamil (*Ver*) against multidrug resistant mouse T-lymphoma cells

| Compound | Ratio [μM] (*Ver* : Se) | CI | SD ± | Type of interaction |
|---|---|---|---|---|
| 1 | 12.5 : 12.5 | 1.67 | 0.28 | Antagonism |
| 2 | 25 : 25 | 0.67 | 0.09 | **Synergism** |
| 3 | 12.5 : 25 | 0.57 | 0.19 | **Synergism** |
| 4 | 25 : 25 | 1.05 | 0.03 | *Additive effect* |
| 5 | 6.25 : 25 | 1.98 | 0.38 | Antagonism |
| 6 | 50 : 100 | 1.89 | 0.26 | Antagonism |
| 7 | 12.5 : 50.0 | 1.01 | 0.07 | *Additive effect* |
| 8 | 6.25 : 12.50 | 2.30 | 0.54 | Antagonism |
| 9 | 25.0 : 12.5 | 1.41 | 0.13 | Moderate antagonism |
| 10 | 50 : 5 | 1.56 | 0.29 | Antagonism |
| 11 | 25 : 12.5 | 1.73 | 0.30 | Antagonism |
| 12 | 50 : 50 | 1.18 | 0.04 | Slight antagonism |
| 13 | 12.5 : 50 | 1.43 | 0.12 | Moderate antagonism |
| 14 | 12.5 : 50 | 1.91 | 0.36 | Antagonism |
| 15 | 25 : 100 | 1.68 | 0.14 | Antagonism |

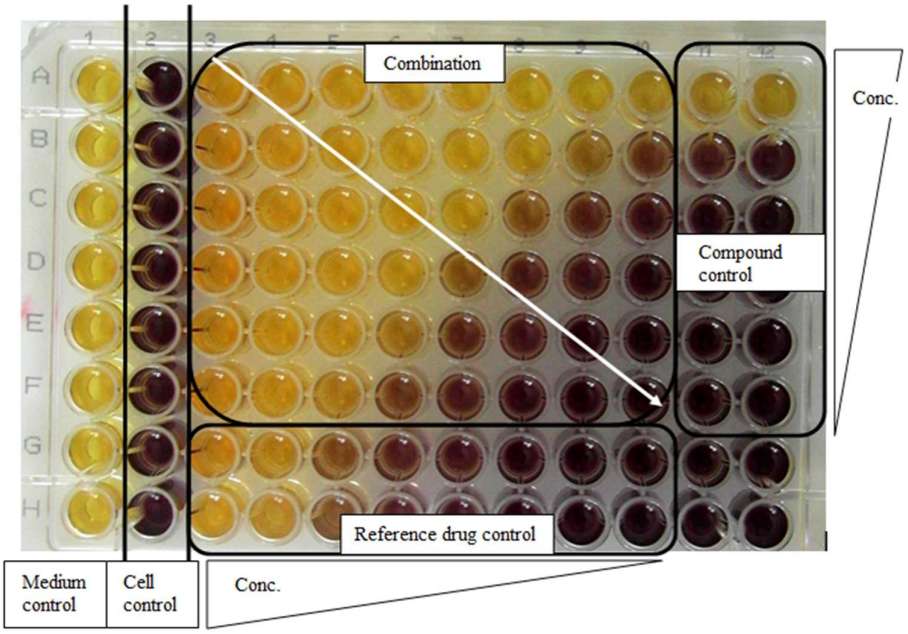


**Figure 1.** Arrangement of 96-well microtiter plates for checkerboard combination assay. The dilutions of the chemotherapeutic drugs (or verapamil) were made in a horizontal direction in 100 µL, and the dilutions of the selenocompounds vertically in the microtiter plate in 50 µL volume.

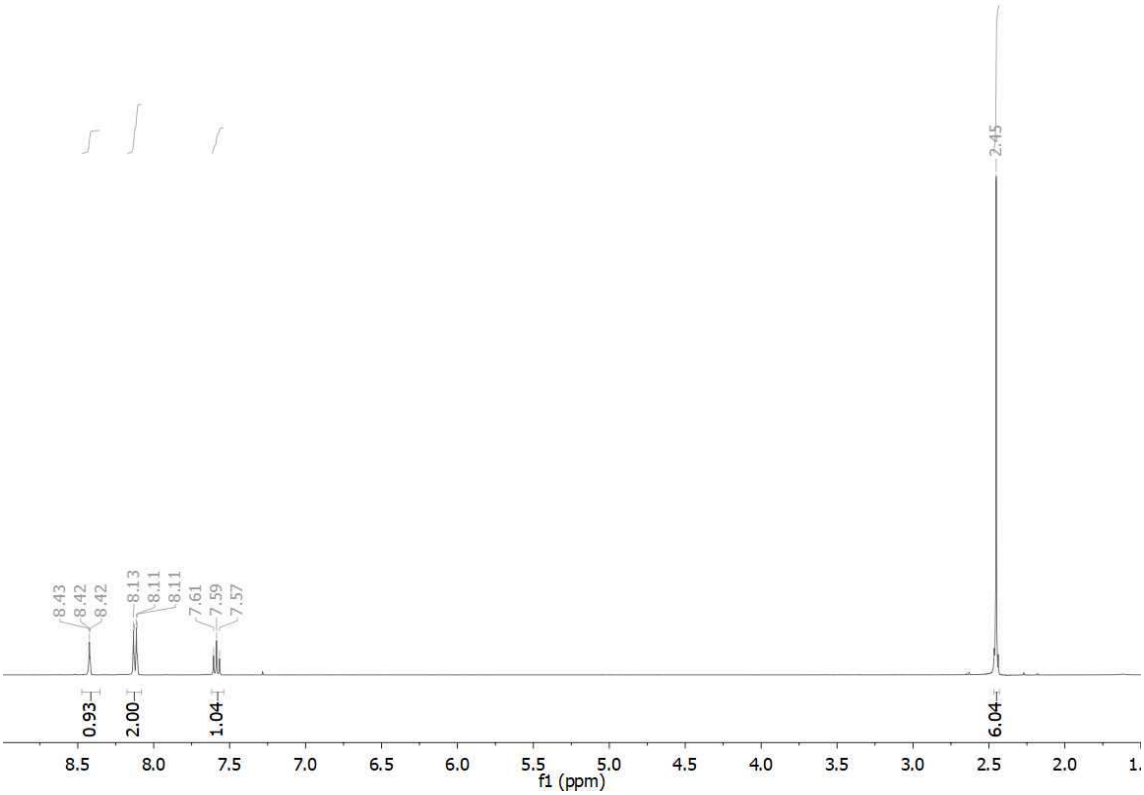


**Figure S2.** $^{1}$H-NMR of compound 4 in $CDCl_3$.

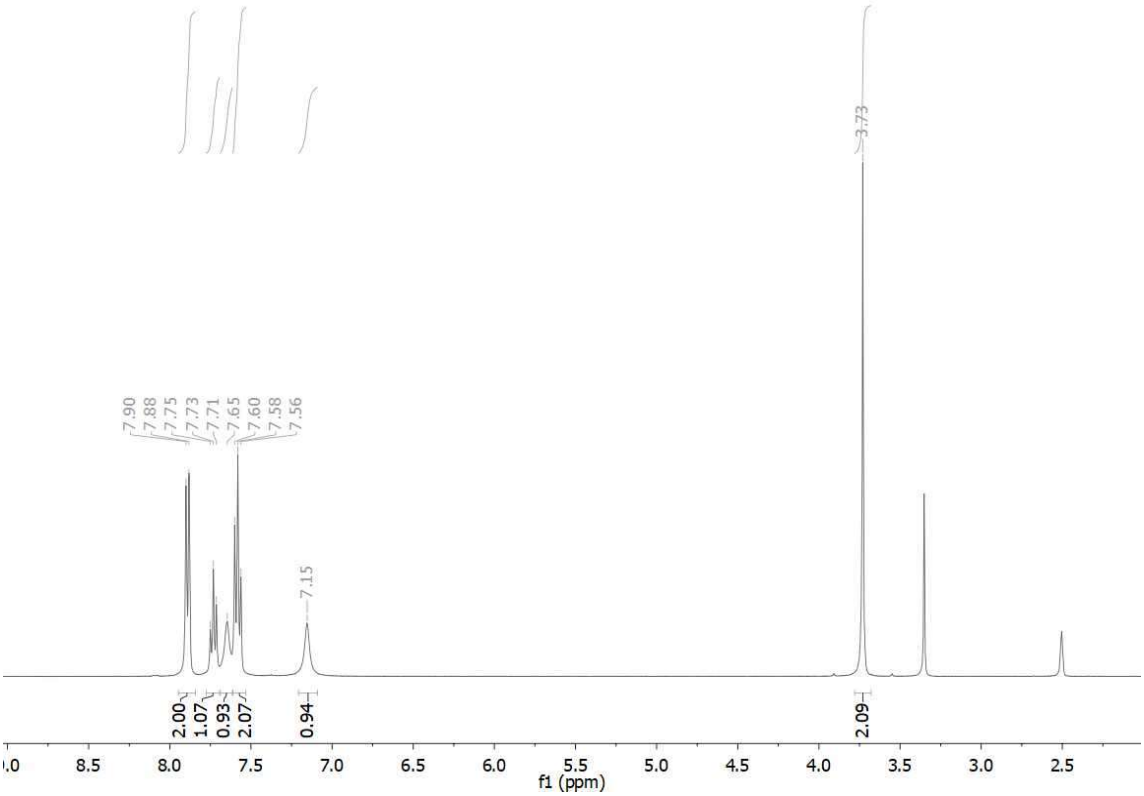


**Figure S3.** $^{1}$H-NMR of compound 6 in DMSO-$d_6$.

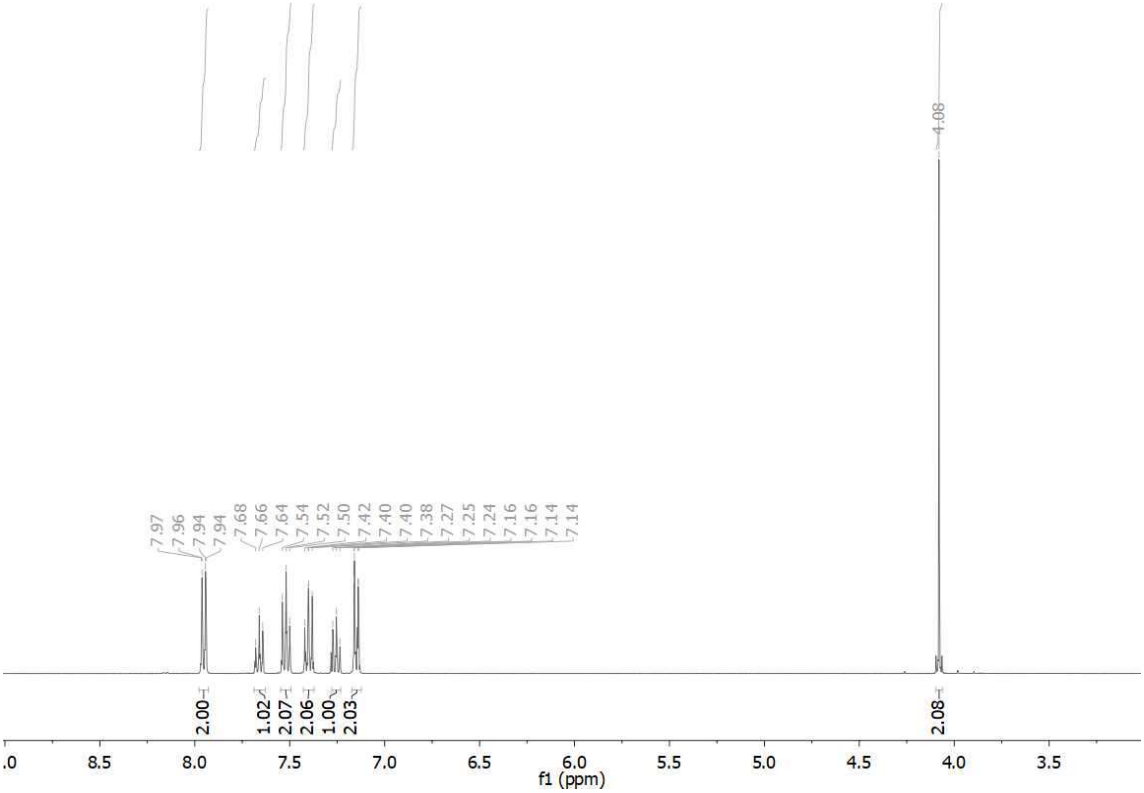


**Figure S4.** $^{1}$H-NMR of compound 8 in $CDCl_3$.

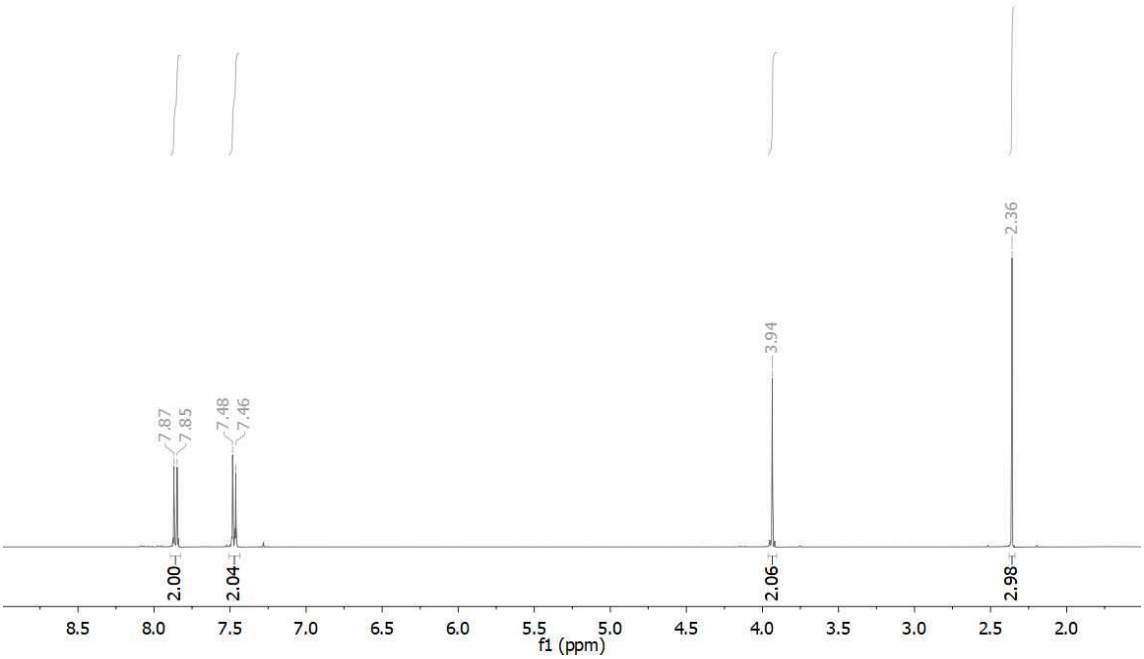


**Figure S5.** $^{1}$H-NMR of compound 9 in $CDCl_3$.

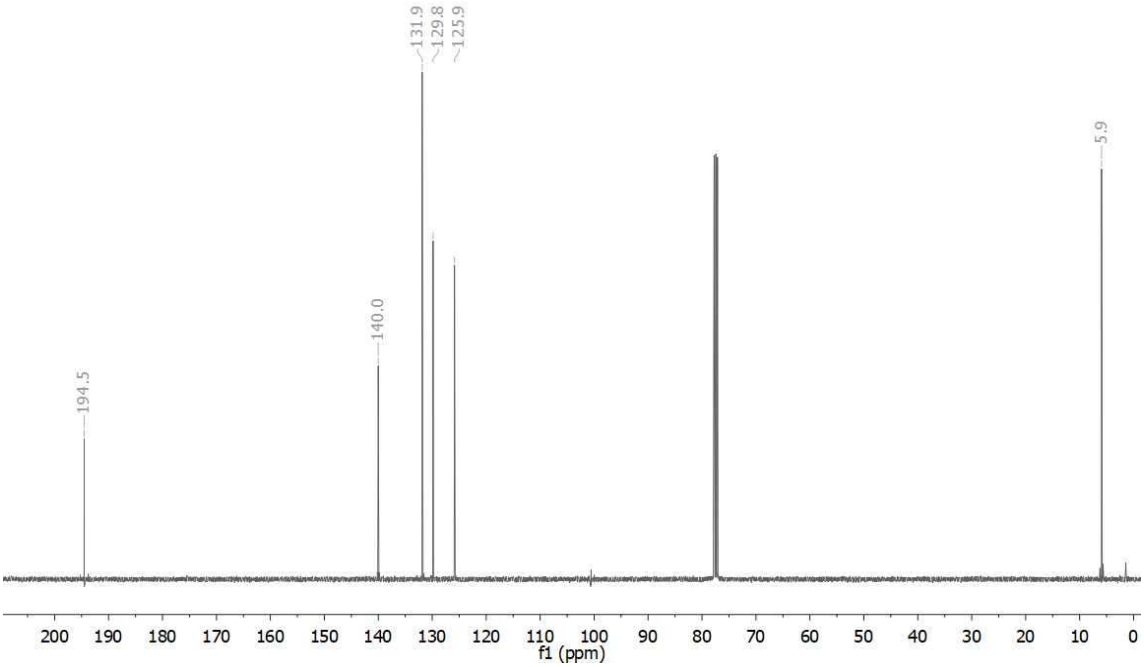


**Figure S6.** $^{13}$C-NMR of compound 4 in $CDCl_3$.

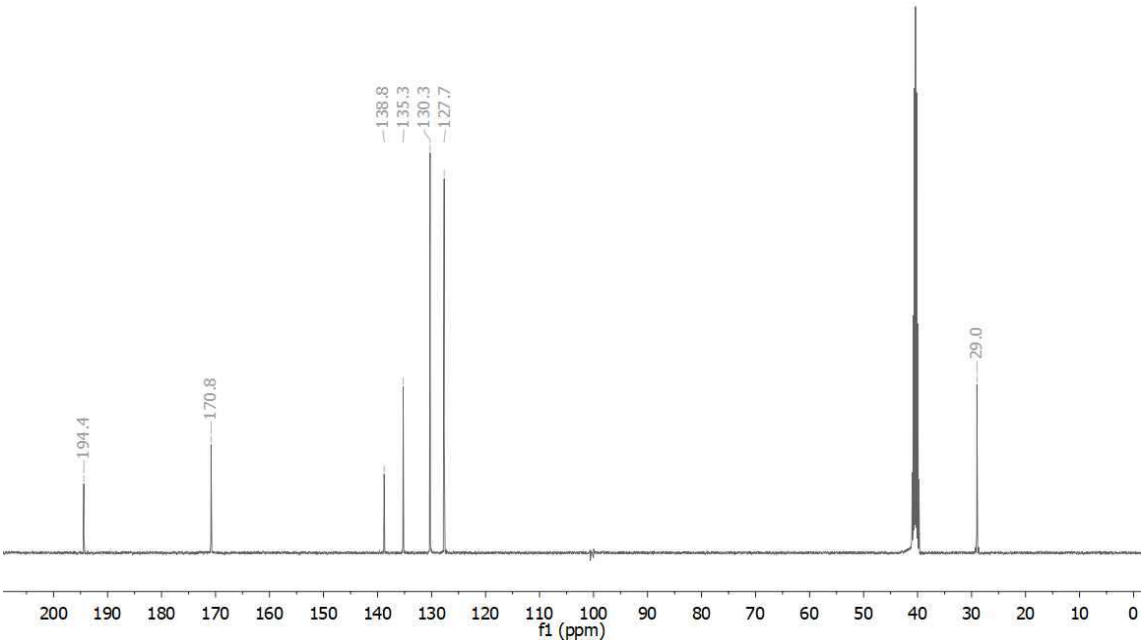


**Figure S7.** $^{13}$C-NMR of compound 6 in DMSO-$d_6$.

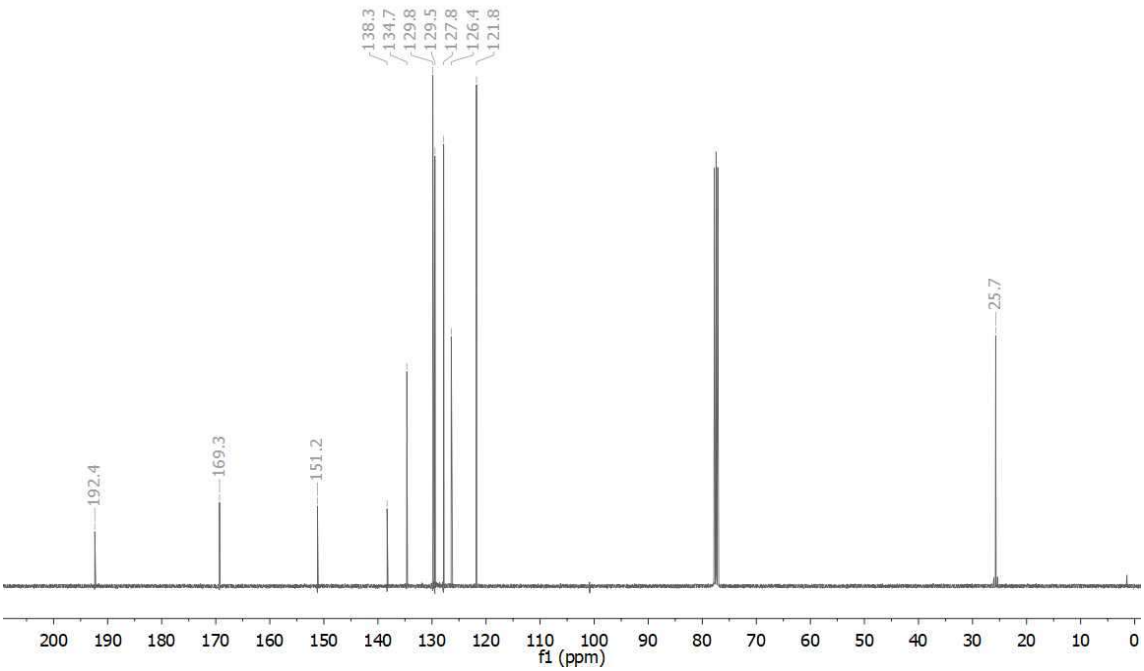


**Figure S8.** $^{13}$C-NMR of compound 8 in $CDCl_3$.

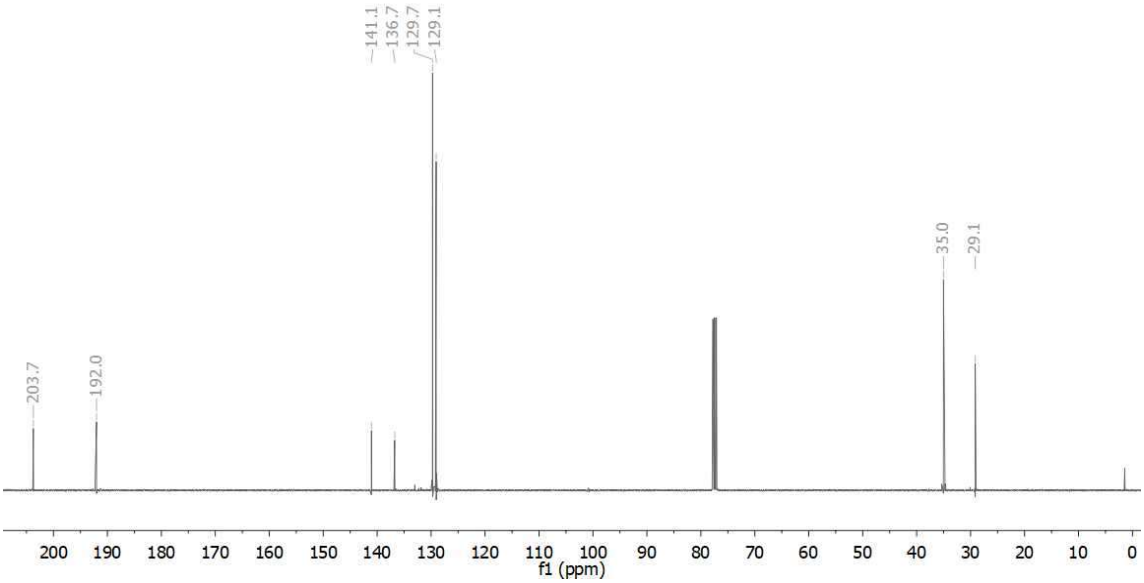


**Figure S9.** $^{13}$C-NMR of compound 9 in $CDCl_3$.