Supplementary Information

Pyrazolyl-Tetrazoles and Imidazolyl-Pyrazoles as Potential Anticoagulants and their Integrated Multiplex Analysis Virtual Screening

André L. P. G. Lourenço,[a,b] Percilene F. Vegi,[c] Jéssica V. Faria,[c] Gustavo S. P. Pinto,[c] Maurício S. dos Santos, *,[d] Plínio C. Sathler,[e] Max S. Saito,[a] Marcos Santana,[f] Tatiana P. P. Dutra,[g] Carlos R. Rodrigues,[b] Robson Q. Monteiro,[g] Alice M. R. Bernardino[c] and Helena C. Castro[a,f]

[a]Programa de Pós-Graduação em Patologia, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, 24033-900 Niterói-RJ, Brazil

[b]Laboratório de Modelagem Molecular e QSAR (ModMolQSAR), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, 21941-170 Rio de Janeiro-RJ, Brazil

[c]Programa de Pós-Graduação em Química, Laboratório de Síntese de Heterociclos (LASINTHET), Instituto de Química, Universidade Federal Fluminense, 24020-150 Niterói-RJ, Brazil

[d]Programa de Pós-Graduação Multicêntrico em Química de Minas Gerais, Laboratório de Síntese Orgânica (LABSINTO), Instituto de Física e Química, Universidade Federal de Itajubá, 37500-903 Itajubá-MG, Brazil

[e]Laboratório de Tecnologia Farmacêutica, Instituto de Biologia, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro-RJ, Brazil

[f]Programa de Pós-Graduação em Ciências e Biotecnologia, Instituto de Biologia, Universidade Federal Fluminense, 24020-140 Niterói-RJ, Brazil

[g]Laboratório de Trombose e Câncer, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro-RJ, Brazil

*e-mail: mauriciosantos@unifei.edu.br

Table S1. HOMO and LUMO energy values for both series **1a**-**g** and **5a**-**g** compared to known serine protease inhibitors

Compound	E_{HOMO} / eV	E_{LUMO} / eV
1a	–4.23	5.10
1b	–4.38	5.01
1c	–4.36	5.04
1d	–4.22	5.35
1e	–4.12	5.58
1f	–4.13	5.48
1g	–3.97	5.84
5a	–11.80	–1.25
5b	–11.87	–1.49
5c	–11.54	–1.46
5d	–11.42	–1.34
5e	–11.57	–1.28
5f	–11.30	–1.35
5g	–10.74	–1.05
Benzamidine	–13.21	–1.71
Dabigatran	–10.6	–0.27
Apixaban	–8.15	2.15
Rivaroxaban	–8.17	1.75
Edoxaban	–8.76	2.09

E_{HOMO} and E_{LUMO}: HOMO and LUMO energy values.

Table S2. Comparison of the docking complexes of all 4,5-dihydro-1*H*-imidazolyl pyrazoles with factor Xa (PDB No. 2Y7X) and thrombin (PDB No. 1PPB) including the parameters, number of clusters, conformations on lowest energy cluster, binding energy, electrostatic contacts, hydrogen bonds and hydrophobic interactions

Compound	Coagulation factor Xa (PDB No. 2JKH)						Coagulation factor IIa (PDB No. 3RM2)					
	NC	NP	BE / (kcal mol^{-1})	Electrostatic	H bond	Hydrophobic	NC	NP	BE / (kcal mol^{-1})	Electrostatic	H bond	Hydrophobic
5a	1	50	–4.71	3	1	5	1	50	–5.03	5	1	13
5b	1	50	–5.56	3	1	5	2	48	–5.62	7	1	13
5c	1	50	–5.55	3	1	8	2	49	–5.48	5	1	9
5d	2	49	–5.18	2	1	7	3	32	–4.74	4	1	10
5e	2	49	–4.4	1	1	7	2	49	–4.64	5	1	10
5f	2	30	–4.95	0	1	9	4	25	–4.38	6	1	6
5g	2	46	–4.19	1	1	8	2	45	–4.21	6	1	6

NC: number of clusters; NP: conformations on lowest energy cluster; BE: binding energy.

Table S3. Evaluation of novel pyrazole derivatives through known pharmacokinetics filters in comparison with commercial anticoagulants

Compound	Lipinski	Veber	Muegge	Ghose	Lead likeness	Bioavailability
1a	yes	yes	yes	yes	yes	yes
1b	yes	yes	yes	yes	yes	yes
1c	yes	yes	yes	yes	yes	yes
1d	yes	yes	yes	yes	yes	yes
1e	yes	yes	yes	yes	yes	yes
1f	yes	yes	yes	yes	yes	yes
1g	yes	yes	yes	yes	yes	yes
5a	yes	yes	yes	yes	yes	yes
5b	yes	yes	yes	yes	yes	yes
5c	yes	yes	yes	yes	yes	yes
5d	yes	yes	yes	yes	yes	yes
5e	yes	yes	yes	yes	yes	yes
5f	yes	yes	yes	yes	yes	yes
5g	yes	yes	yes	yes	yes	yes
Dabigatran	yes	no	no	no	no	yes
Apixaban	yes	yes	yes	yes	no	yes
Rivaroxaban	yes	yes	yes	yes	yes	yes
Edoxaban	no	yes	yes	no	no	yes
Betrixaban	yes	yes	yes	no	no	yes
Benzamidine	yes	yes	no	no	yes	yes
Argatroban	no	no	no	no	no	no

Table S4. Quantitative evaluation of the anticoagulant potential of novel 4,5-dihydro-1*H*-imidazolyl pyrazoles defined as the concentration necessary to double the normal activated partial thromboplastin time, prothrombin time and thrombin time

Compound	APTT CT_{X2} / µM	PT_{50} CT_{X2} / µM	TT_{50} CT_{X2} / µM
5a	> 500	> 500	> 500
5b	> 500	191.3 ± 4.98	> 500
5c	> 500	163.1 ± 4.93	> 500
5d	> 500	> 500	> 500
5e	> 500	> 500	> 500
5f	> 500	> 500	> 500
5g	> 500	> 500	> 500

APTT CT_{X2}, PT_{50} CT_{X2} and TT_{50} CT_{X2}: concentration necessary to double the normal activated partial thromboplastin time, prothrombin time and thrombin time, respectively.

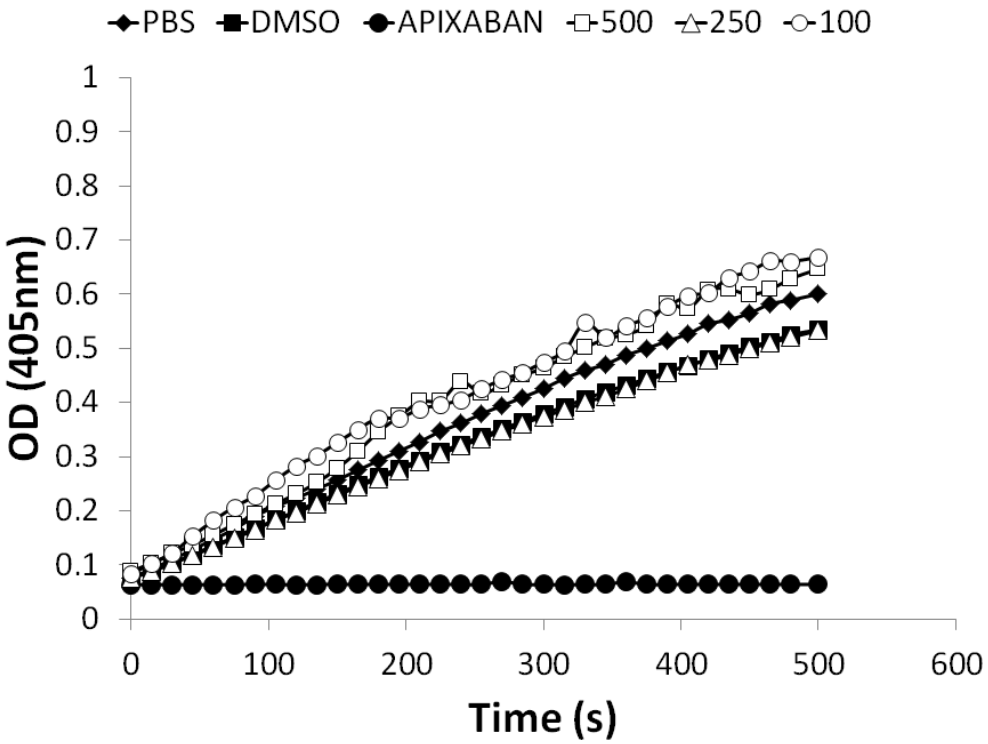

Figure S1. Kinetics of human FXa against the chromogenic substrate S-2765 in the presence of several doses of compound **5b**.

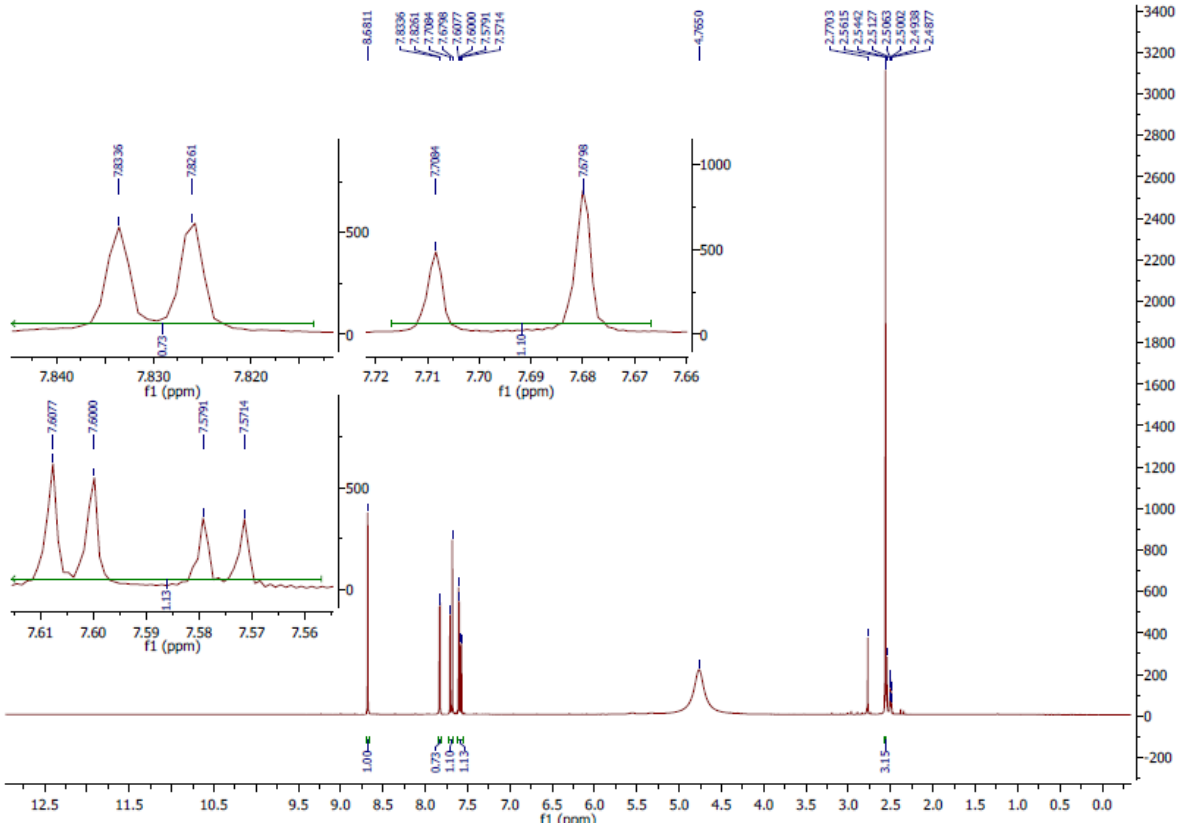

Figure S2. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **1a**.

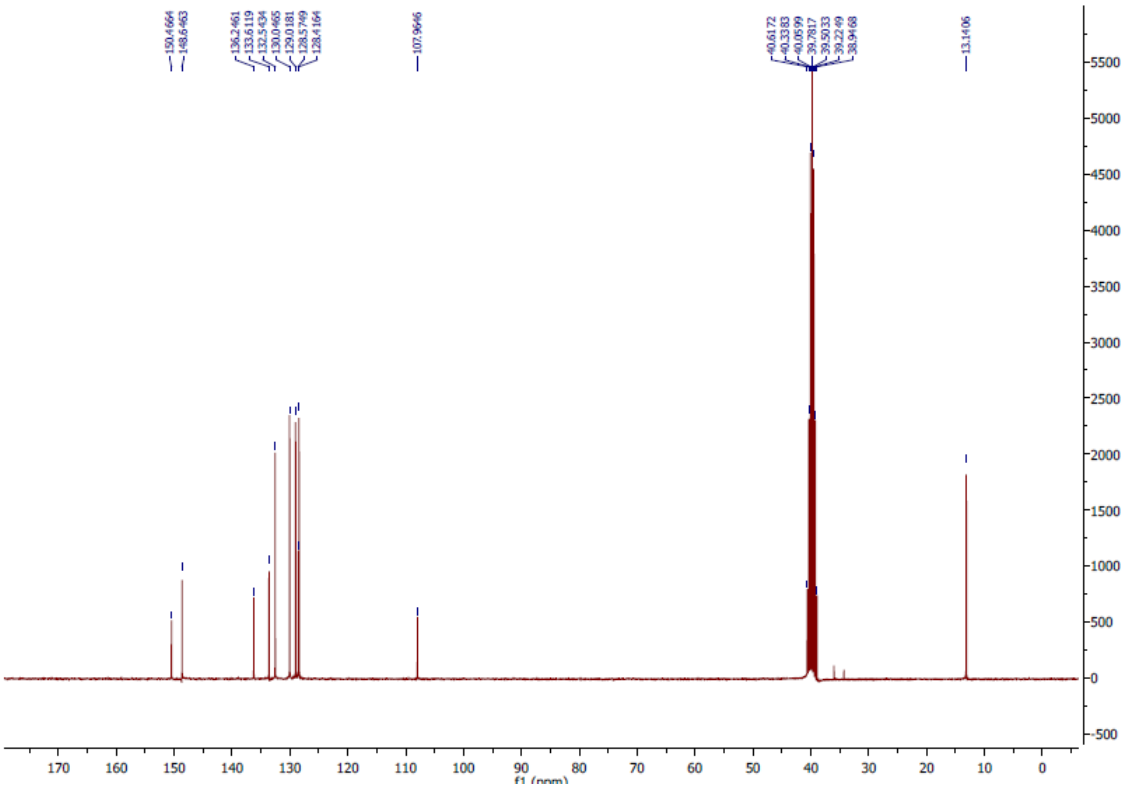

Figure S3. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of compound **1a**.

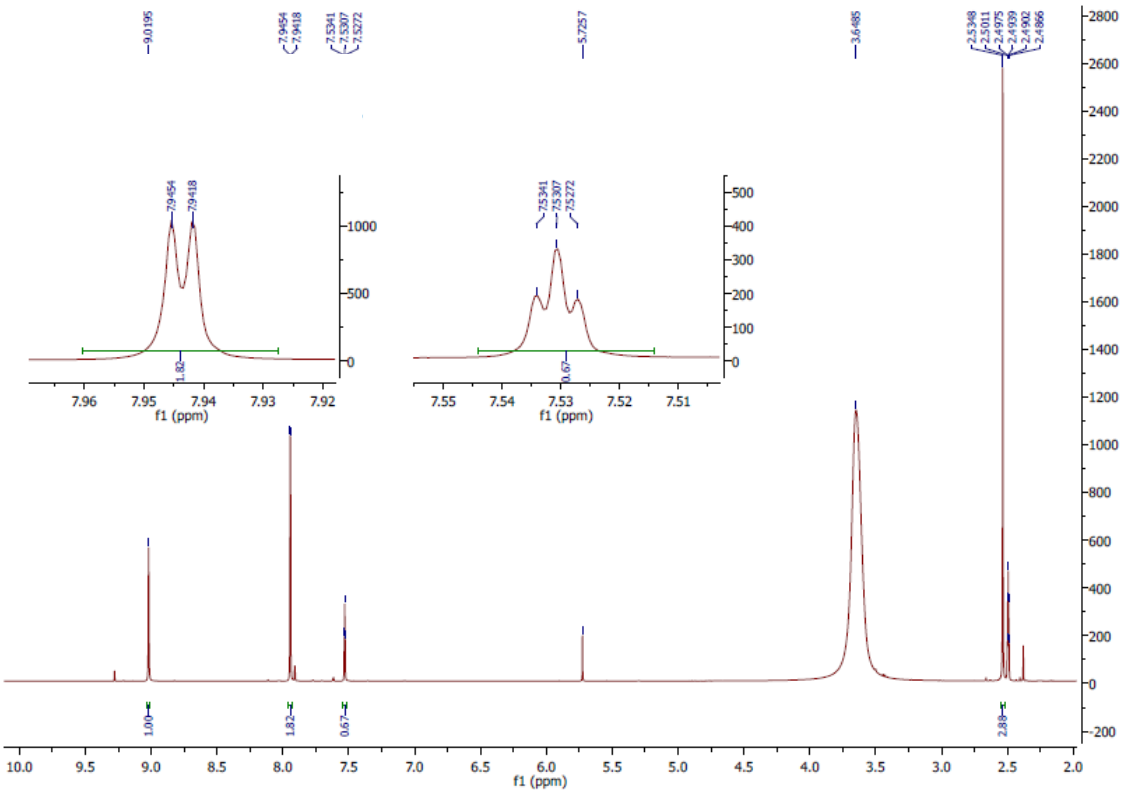

Figure S4. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1b**.

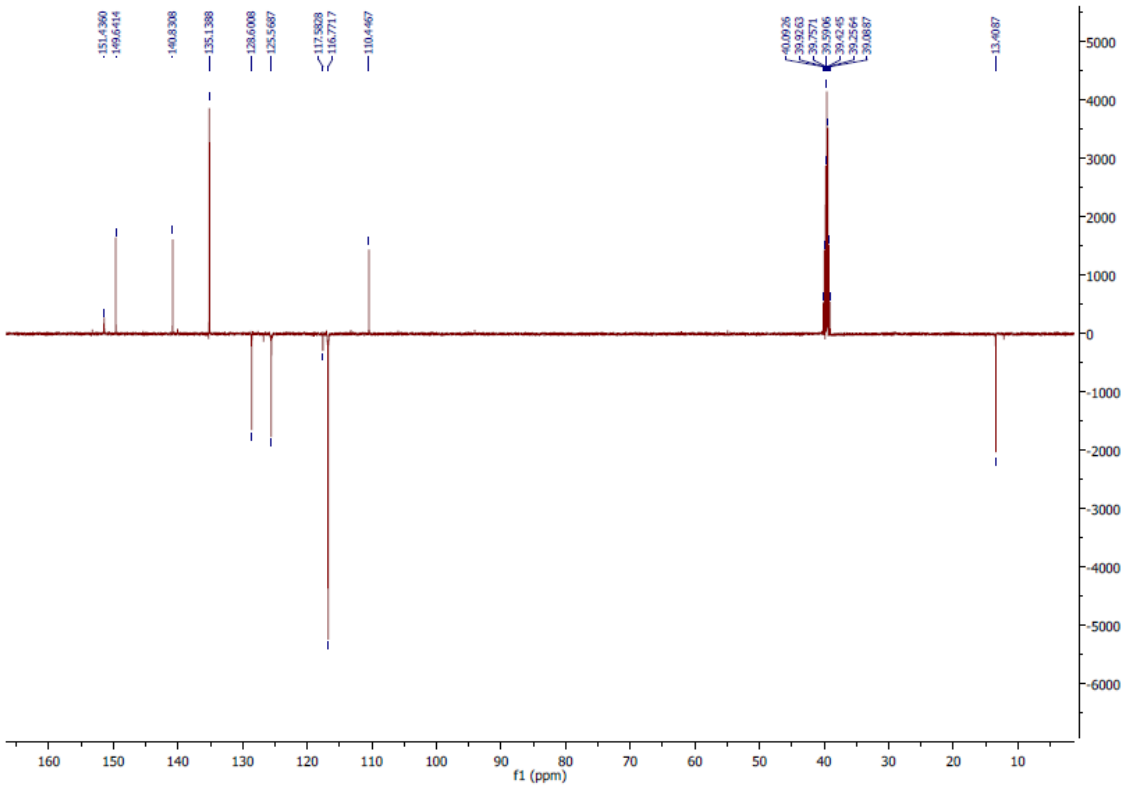

Figure S5. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1b**.

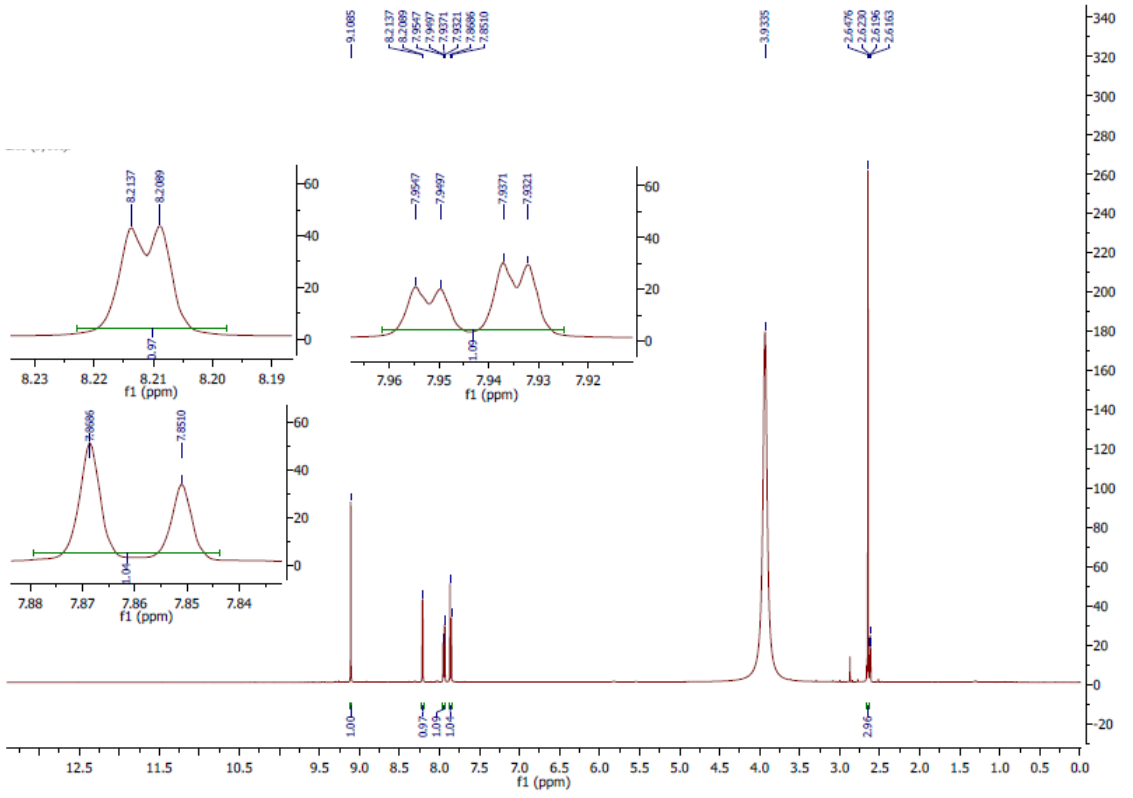

Figure S6. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1c**.

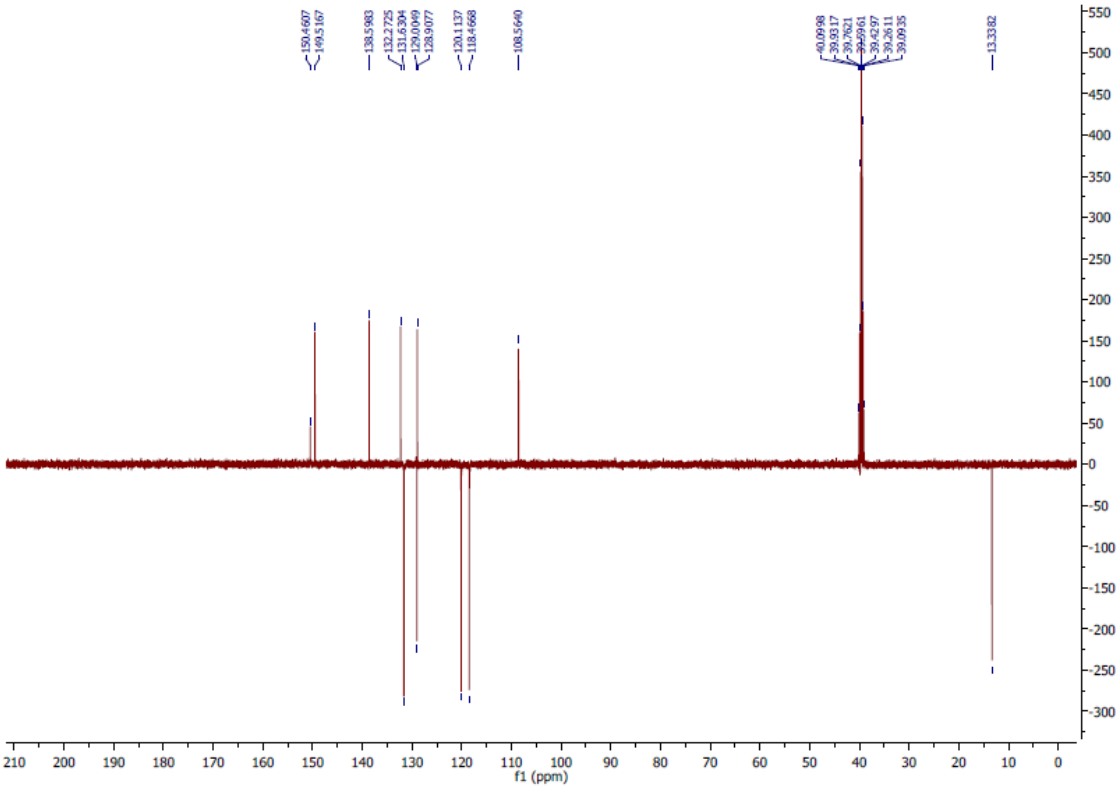

Figure S7. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1c**.

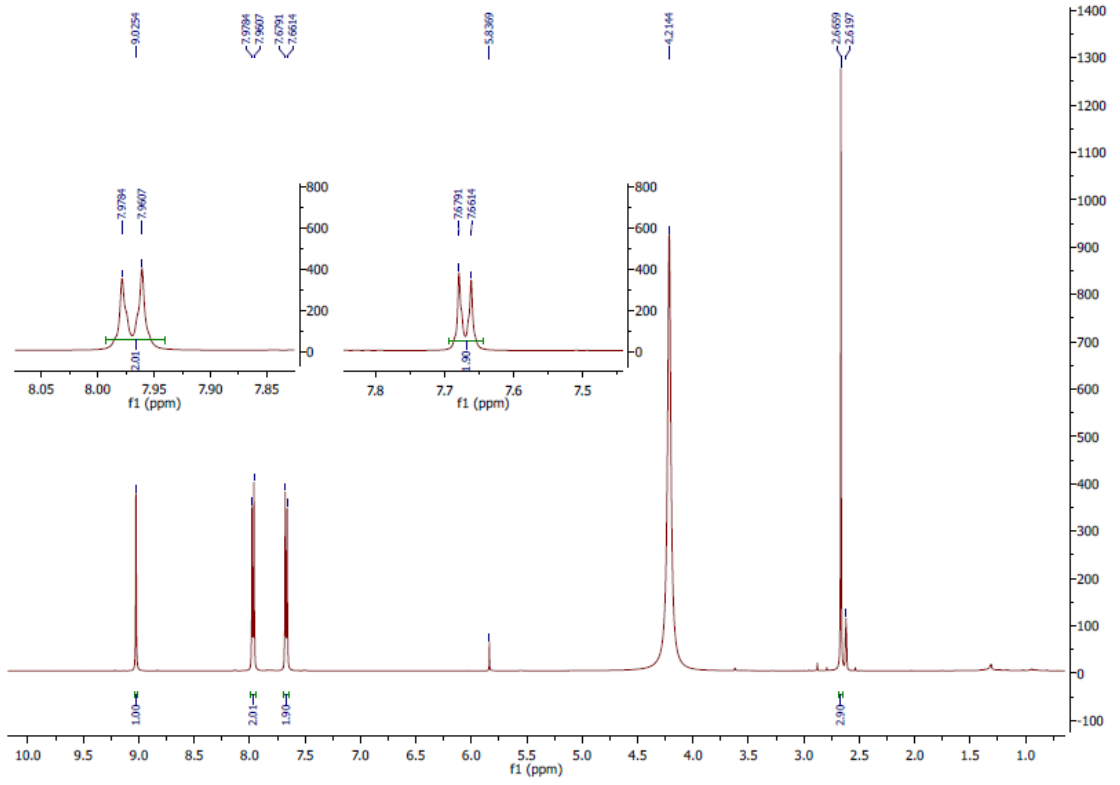

Figure S8. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1d**.

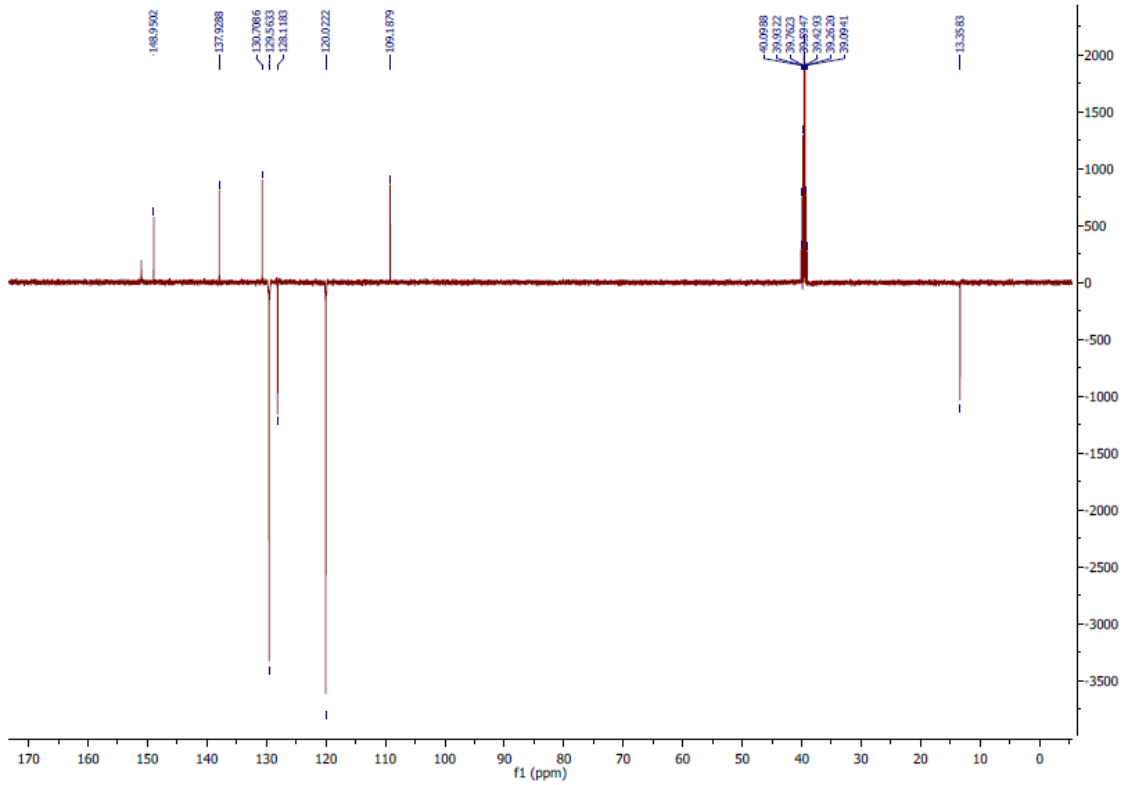

Figure S9. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1d**.

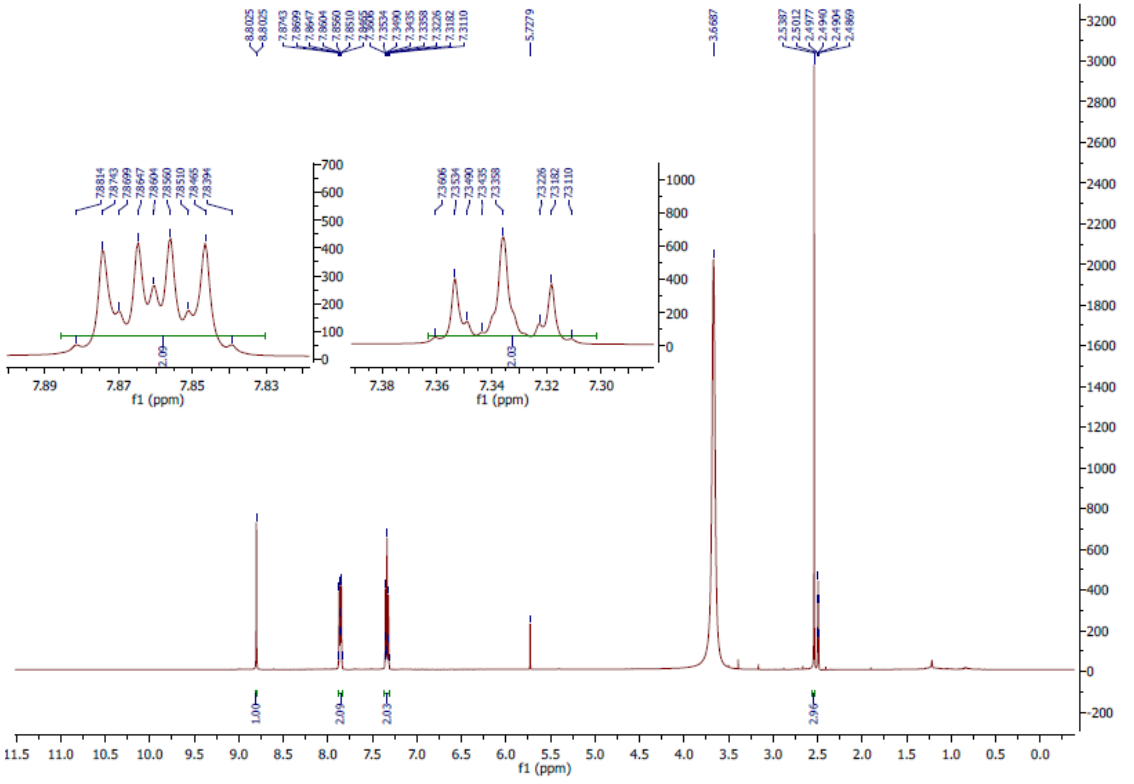

Figure S10. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1e**.

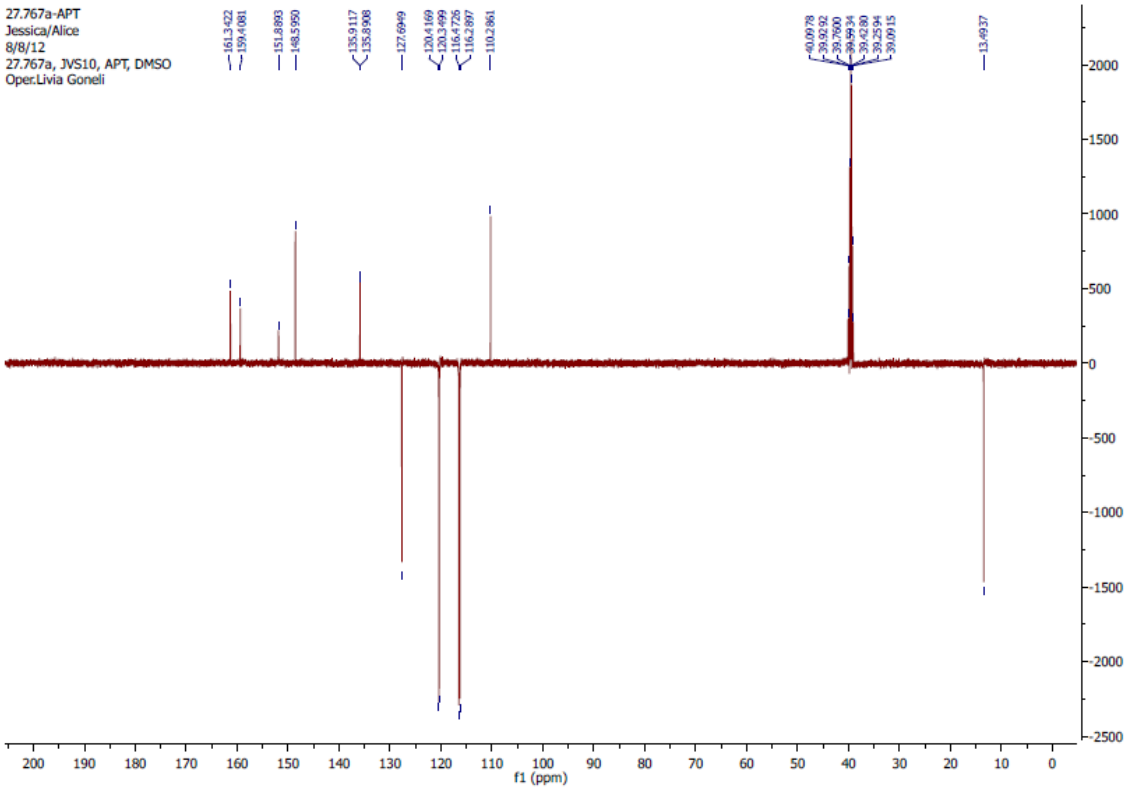

Figure S11. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1e**.

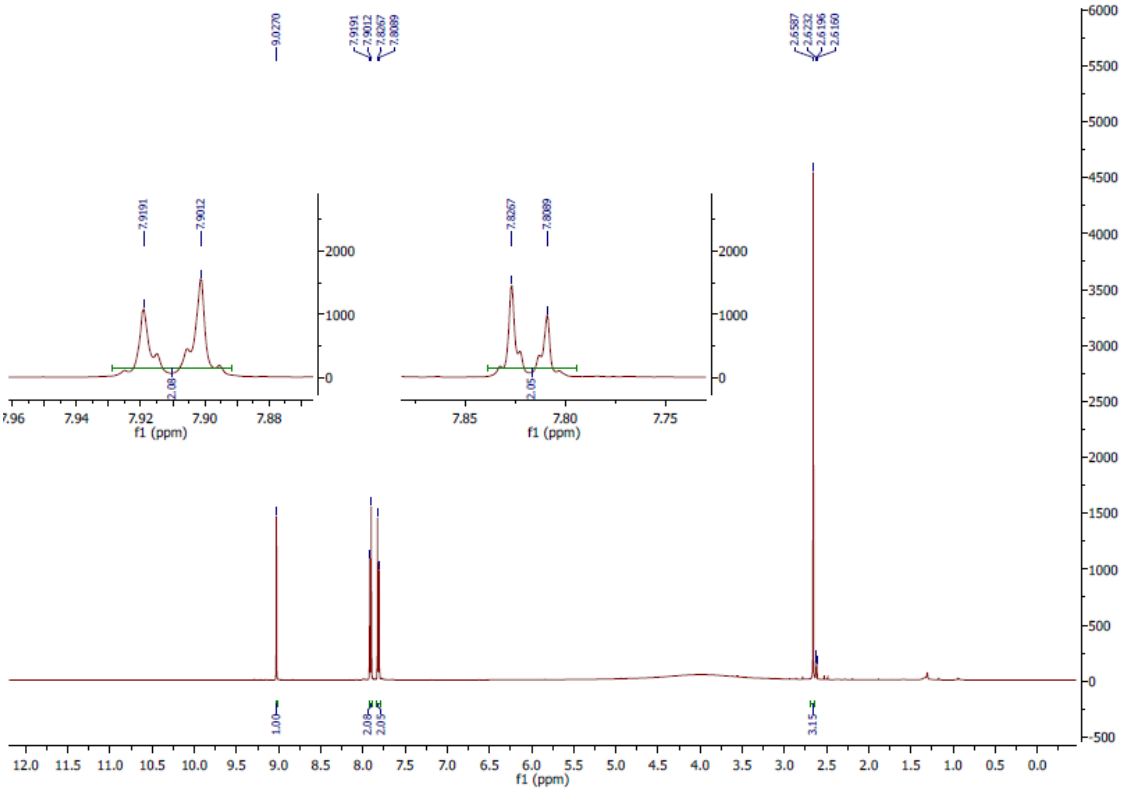

Figure S12. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1f**.

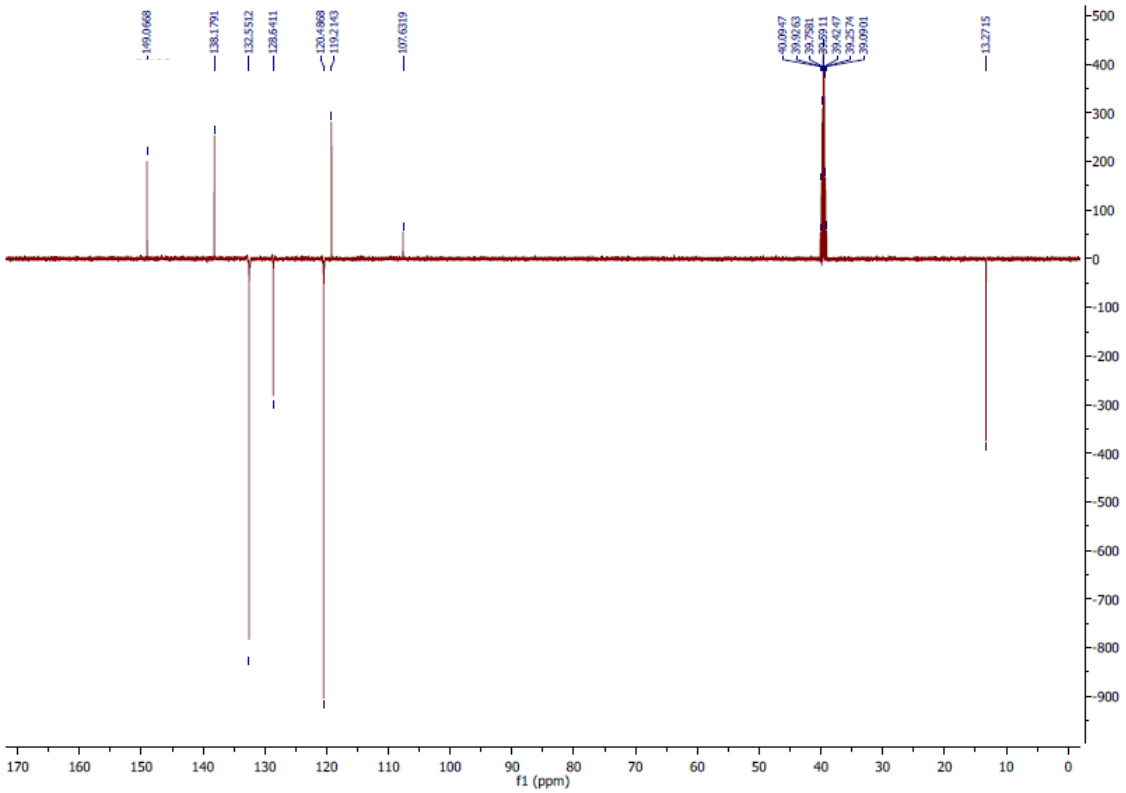

Figure S13. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1f**.

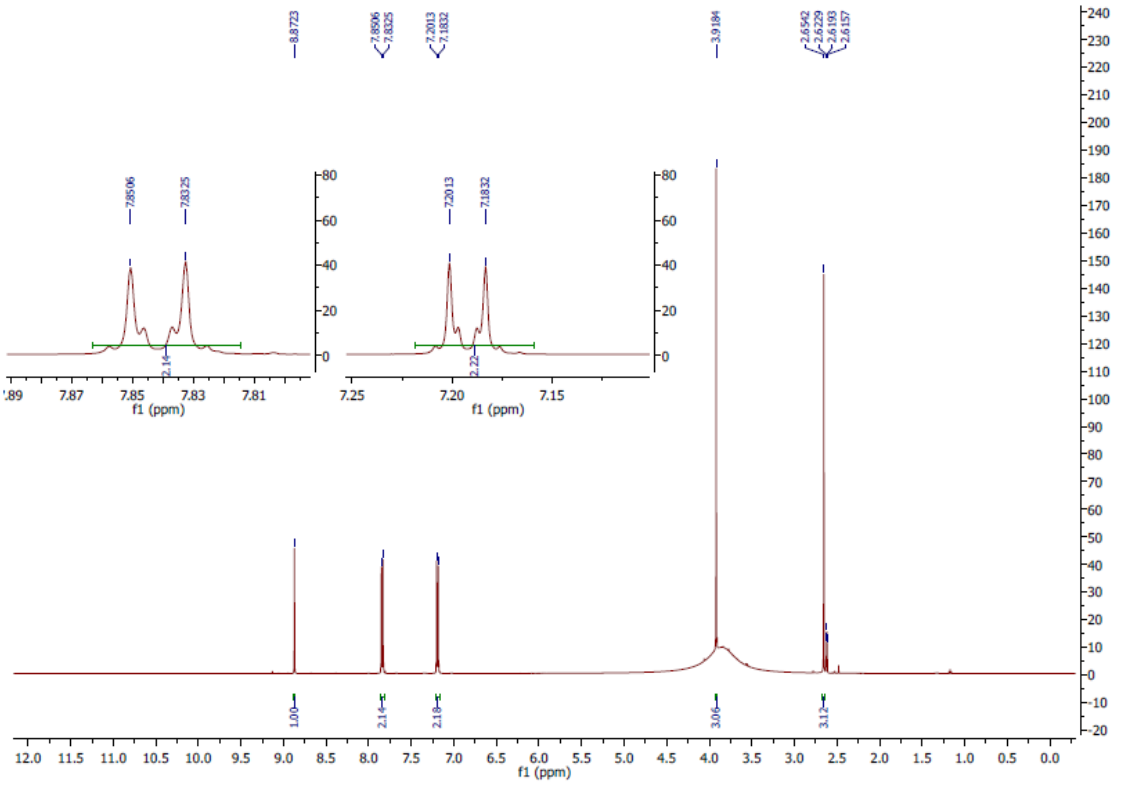

Figure S14. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1g**.

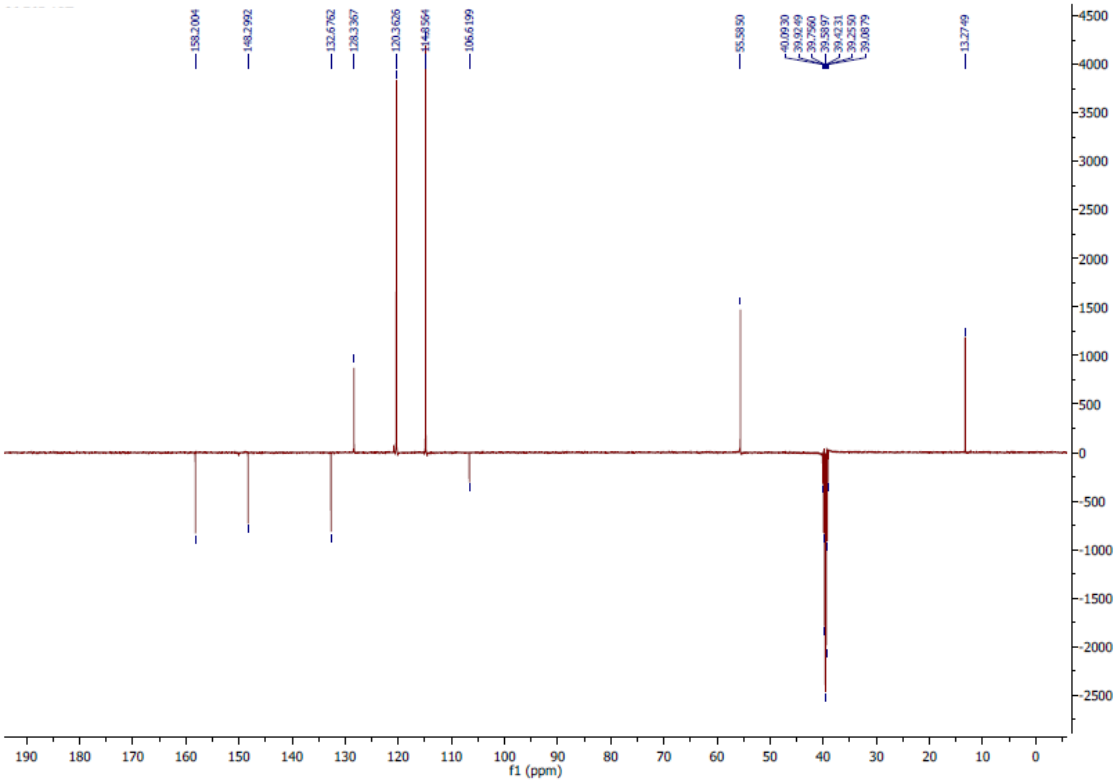

Figure S15. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **1g**.

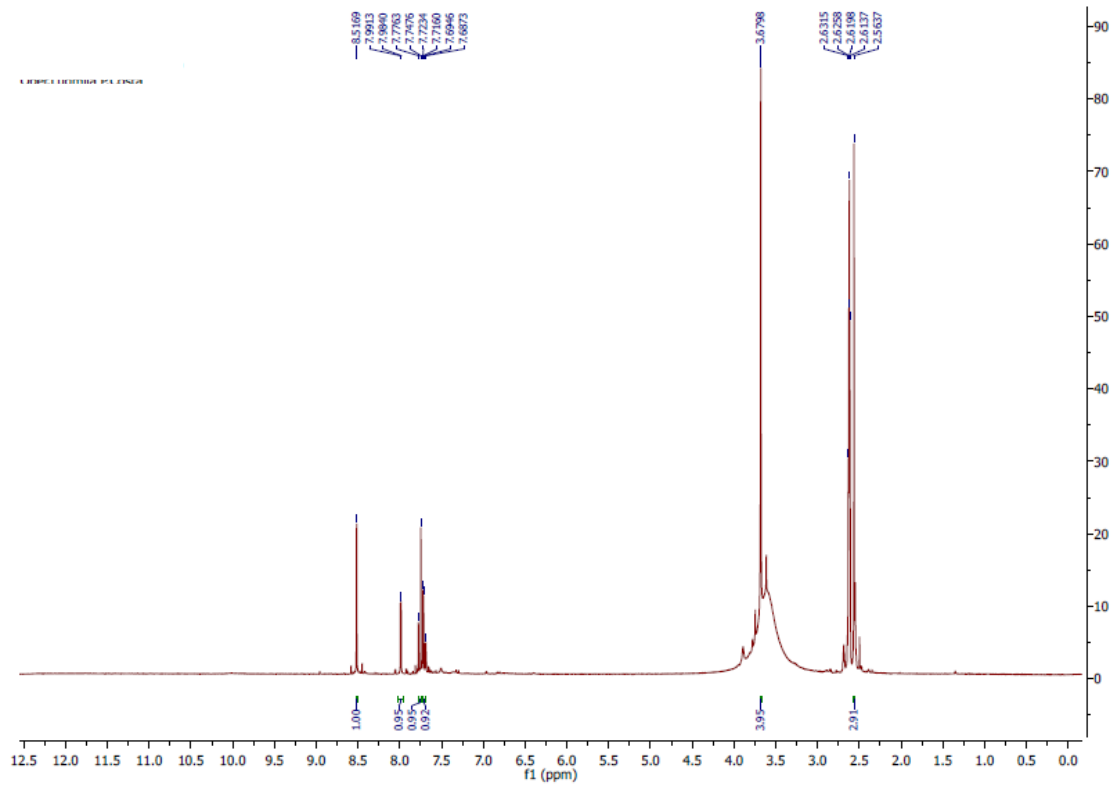

Figure S16. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **5a**.

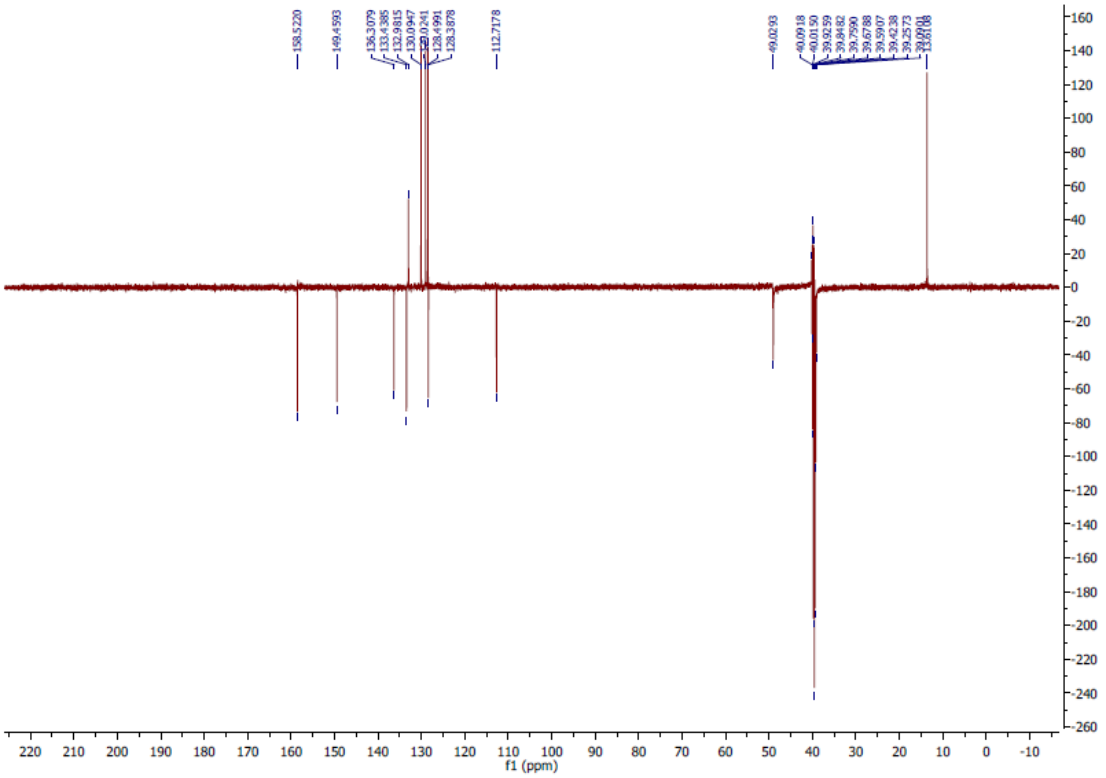

Figure S17. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of compound **5a**.

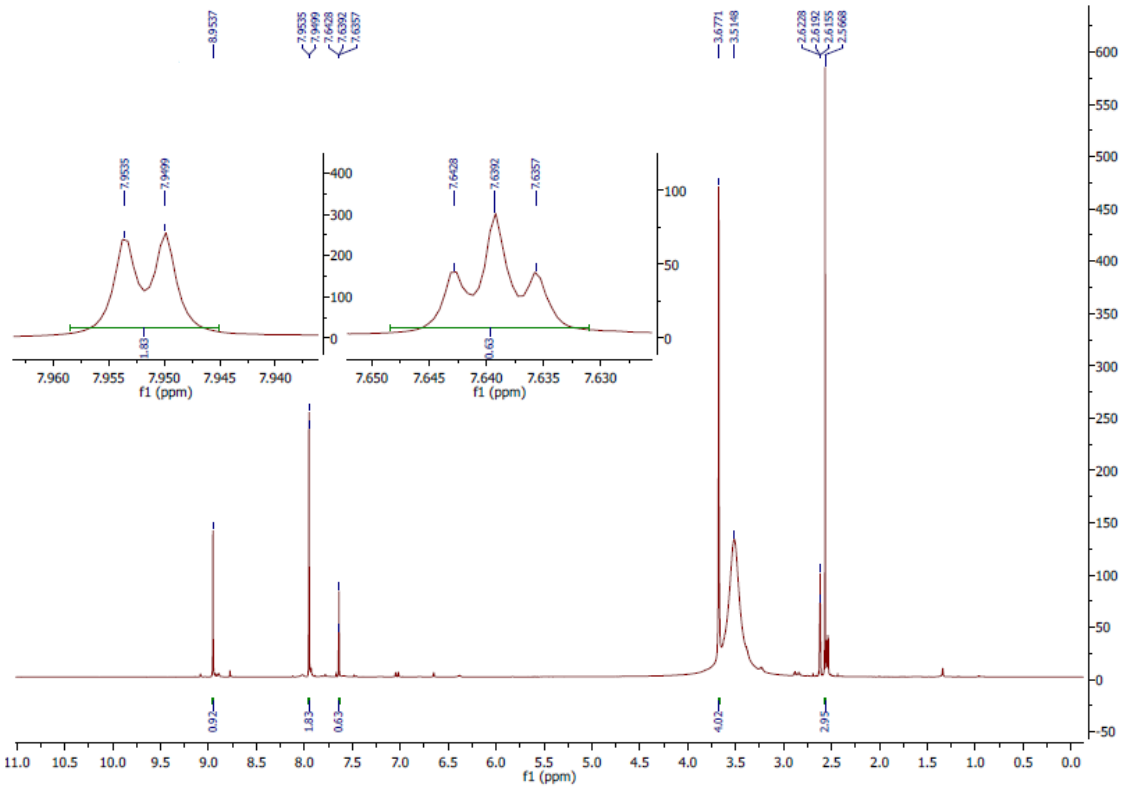

Figure S18. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **5b**.

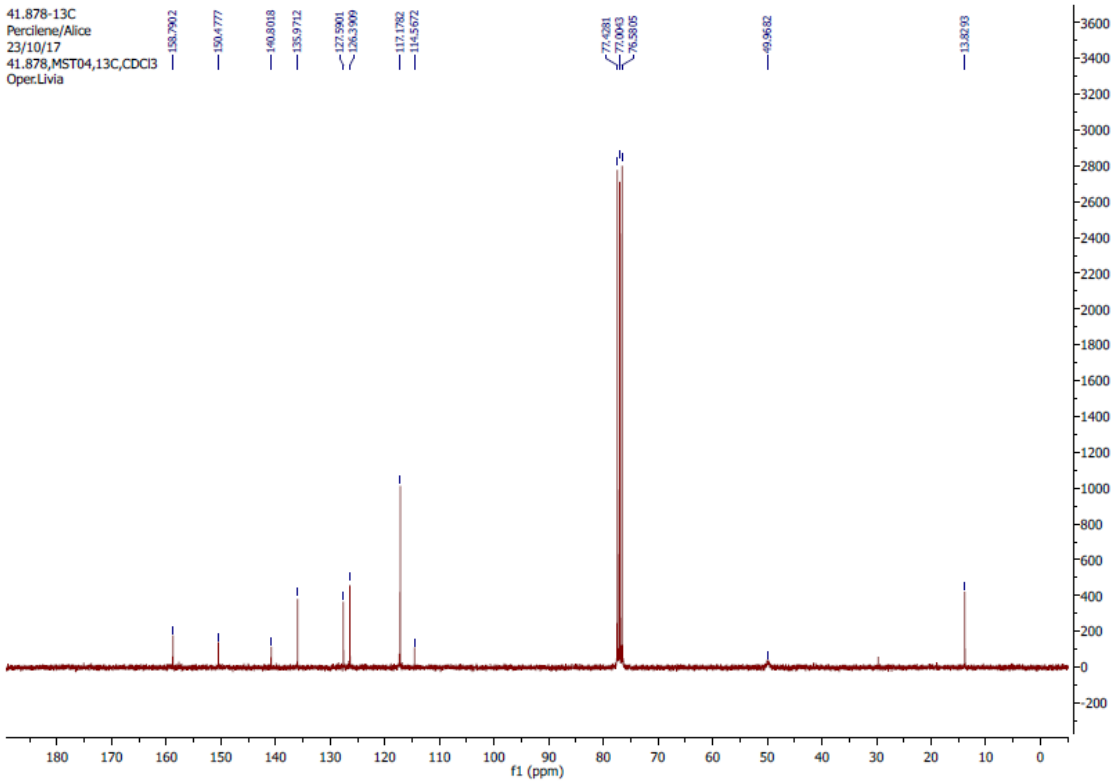

Figure S19. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **5b**.

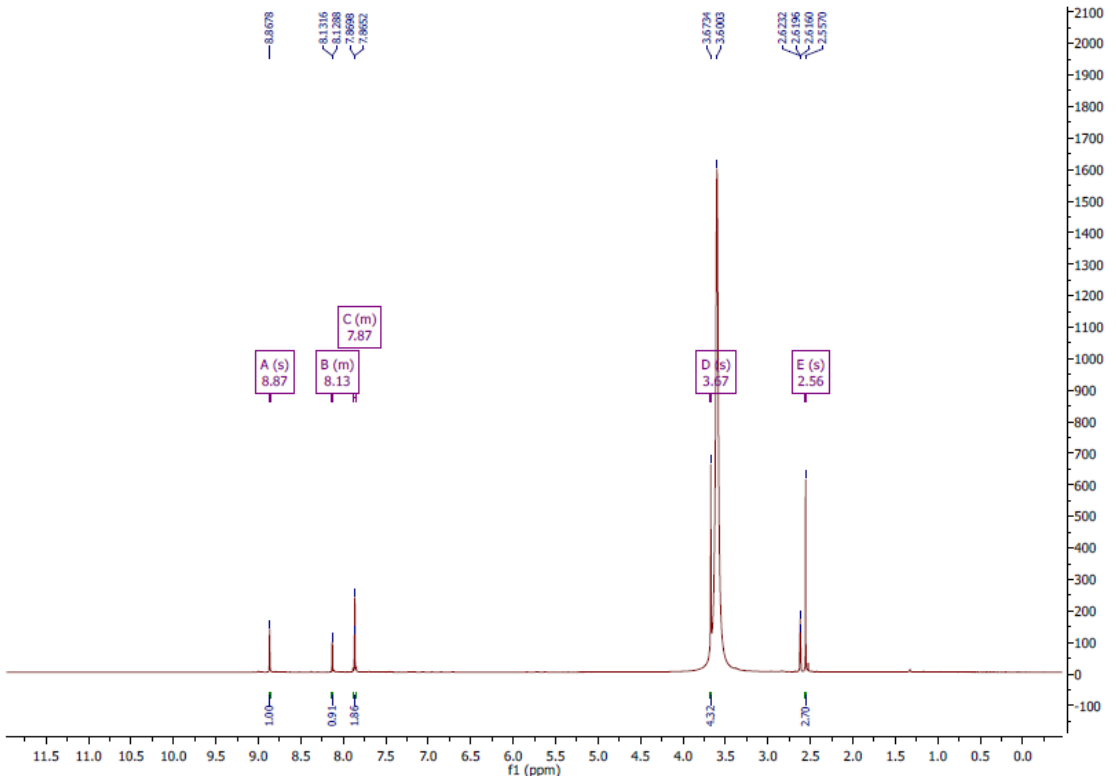

Figure S20. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **5c**.

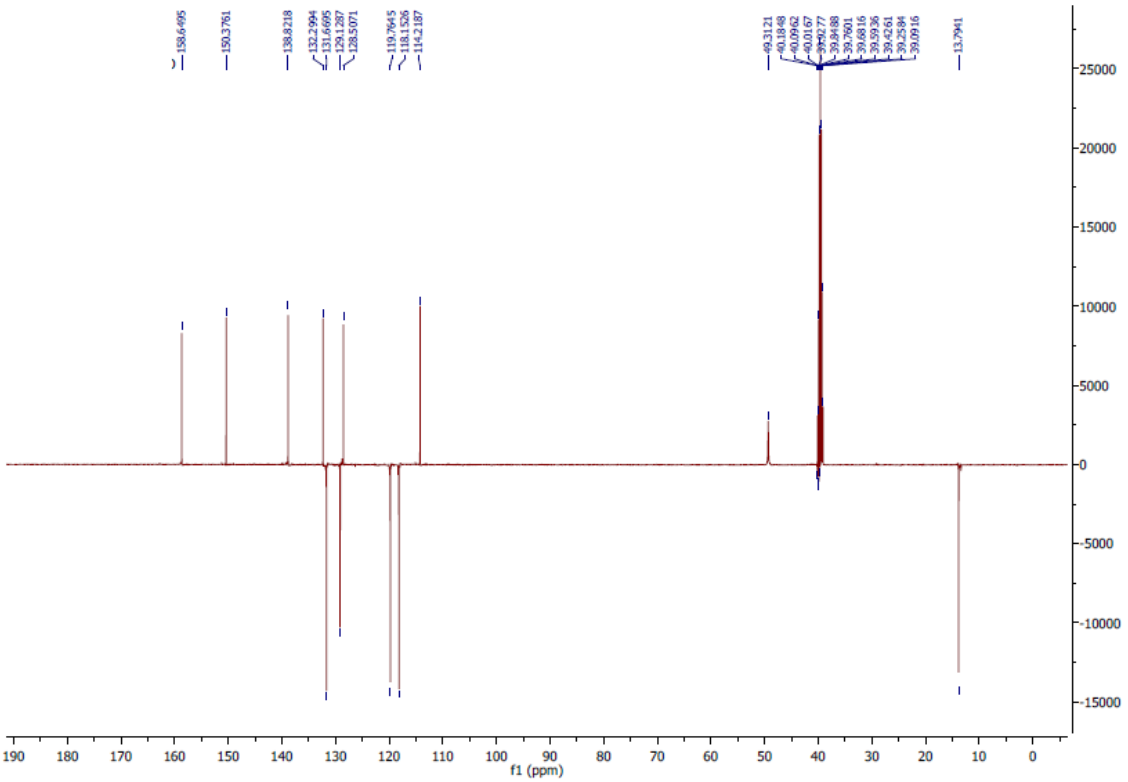

Figure S21. ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of compound **5c**.

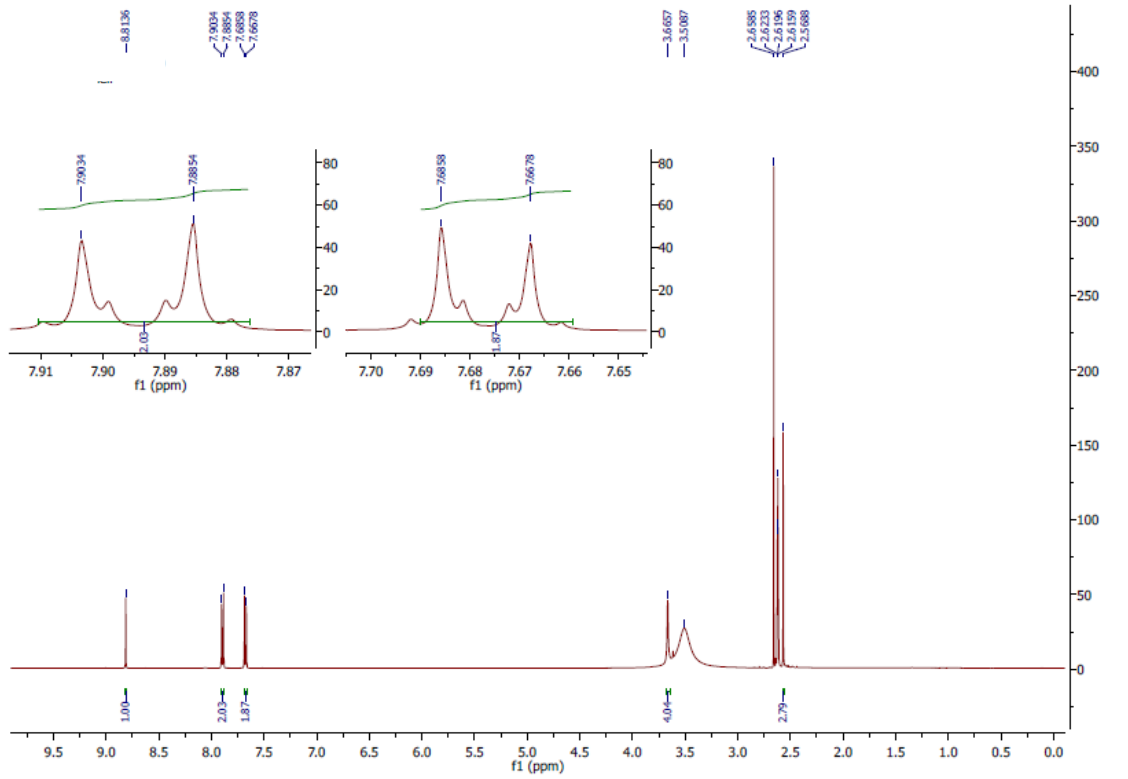

Figure S22. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **5d**.

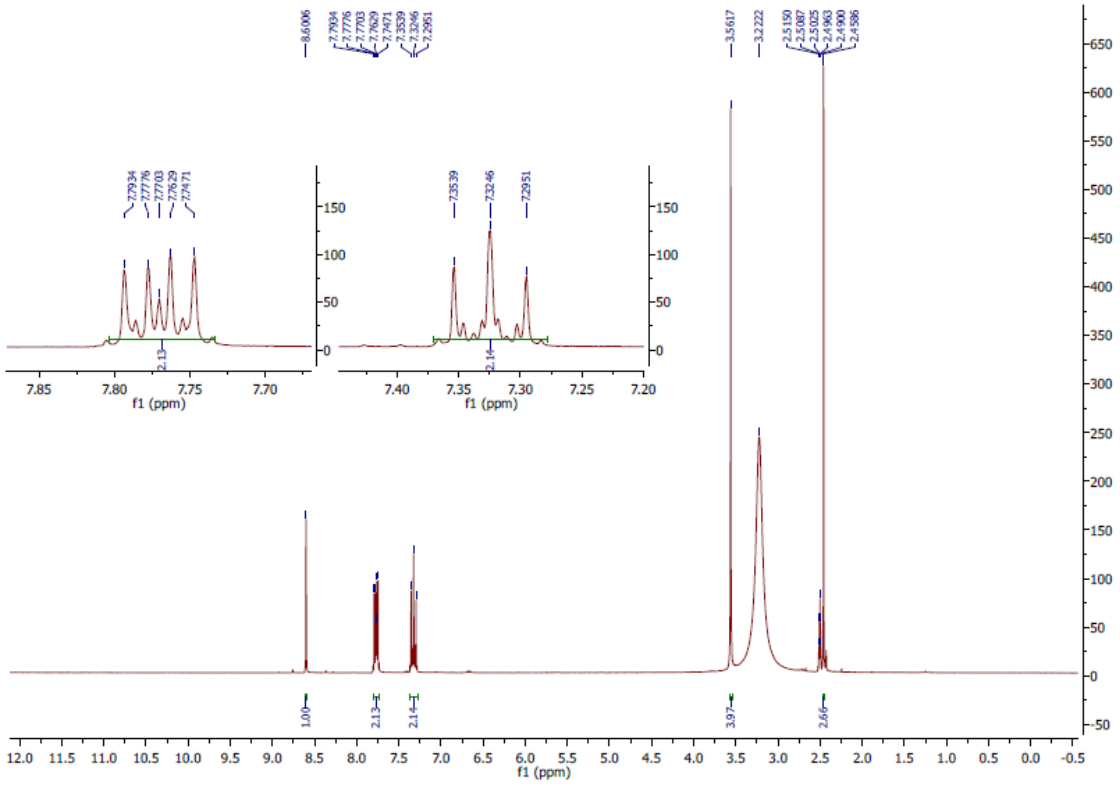

Figure S23. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **5e**.

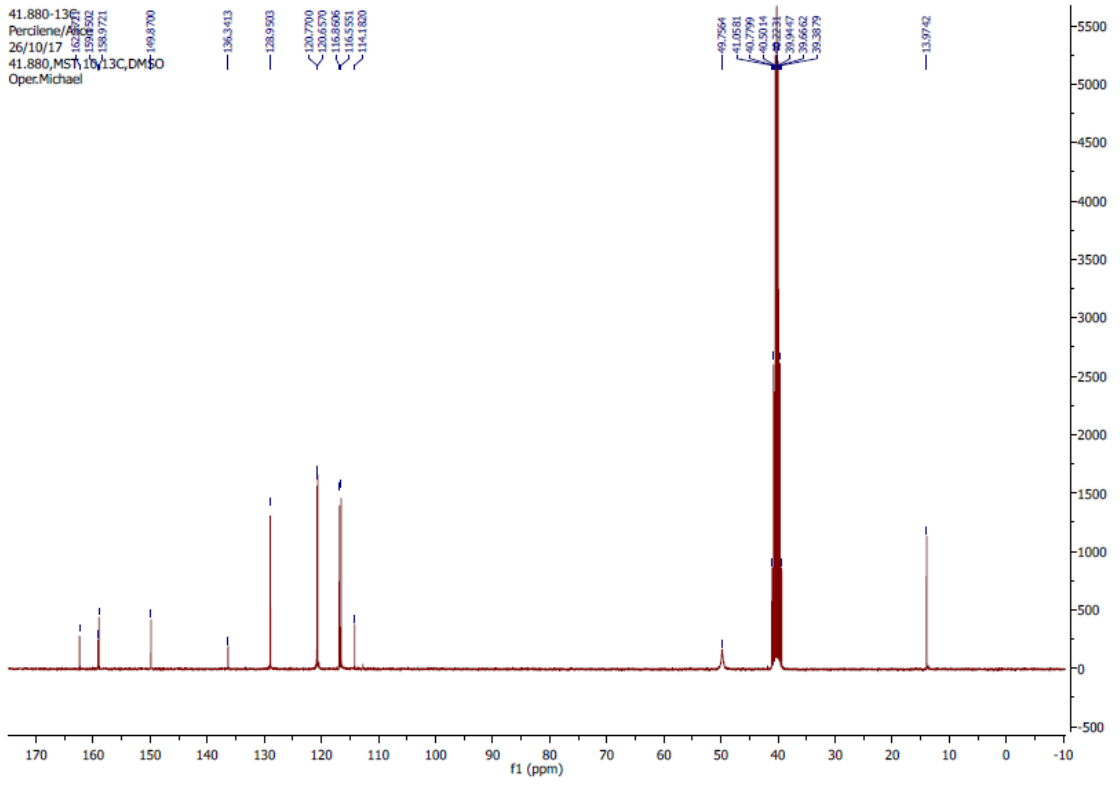

Figure S24. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of compound **5e**.

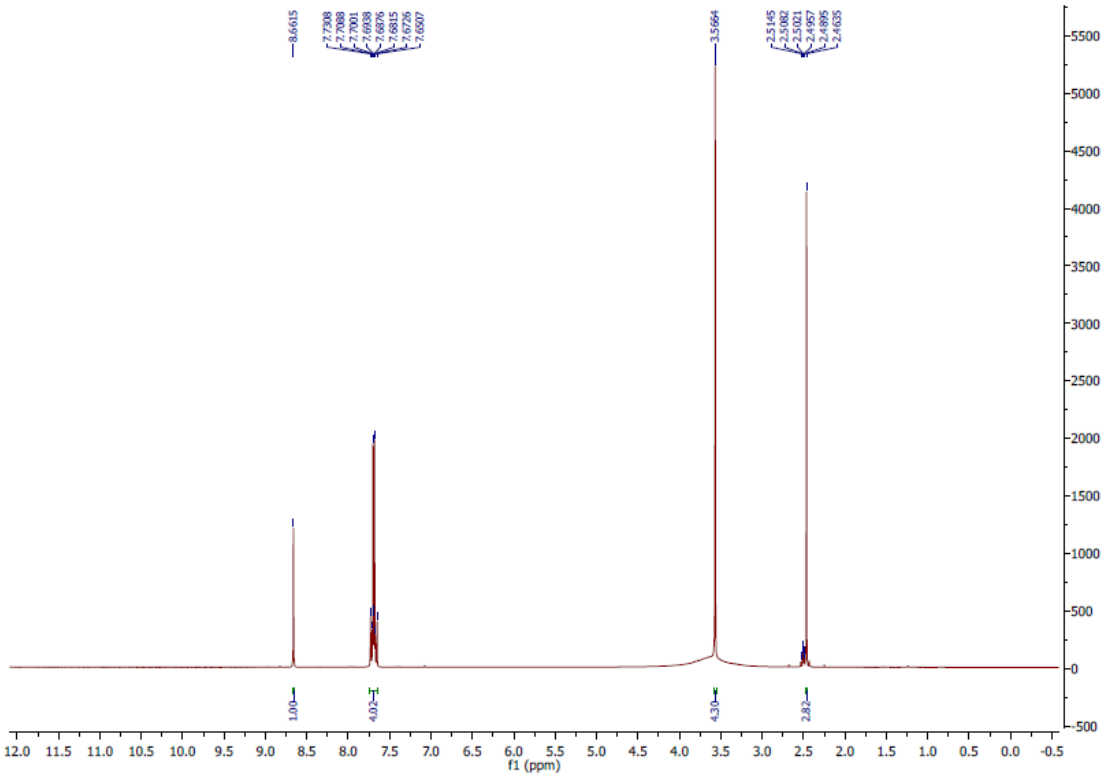

Figure S25. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **5f**.

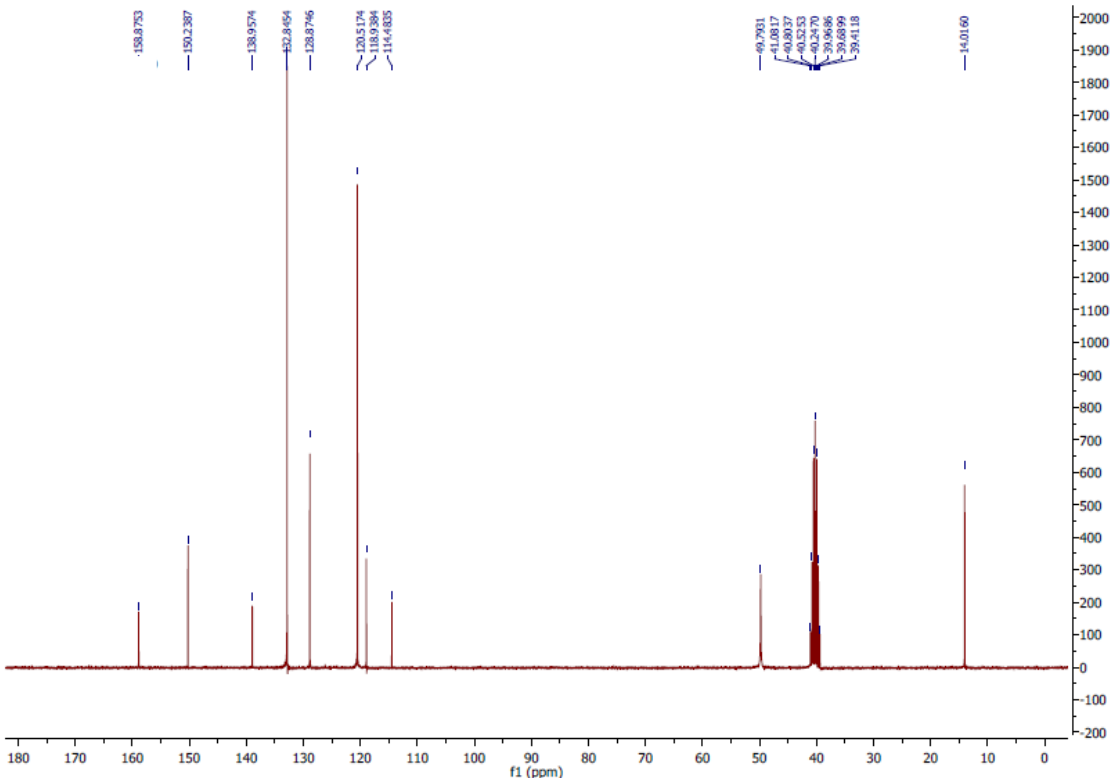

Figure S26. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of compound **5f**.

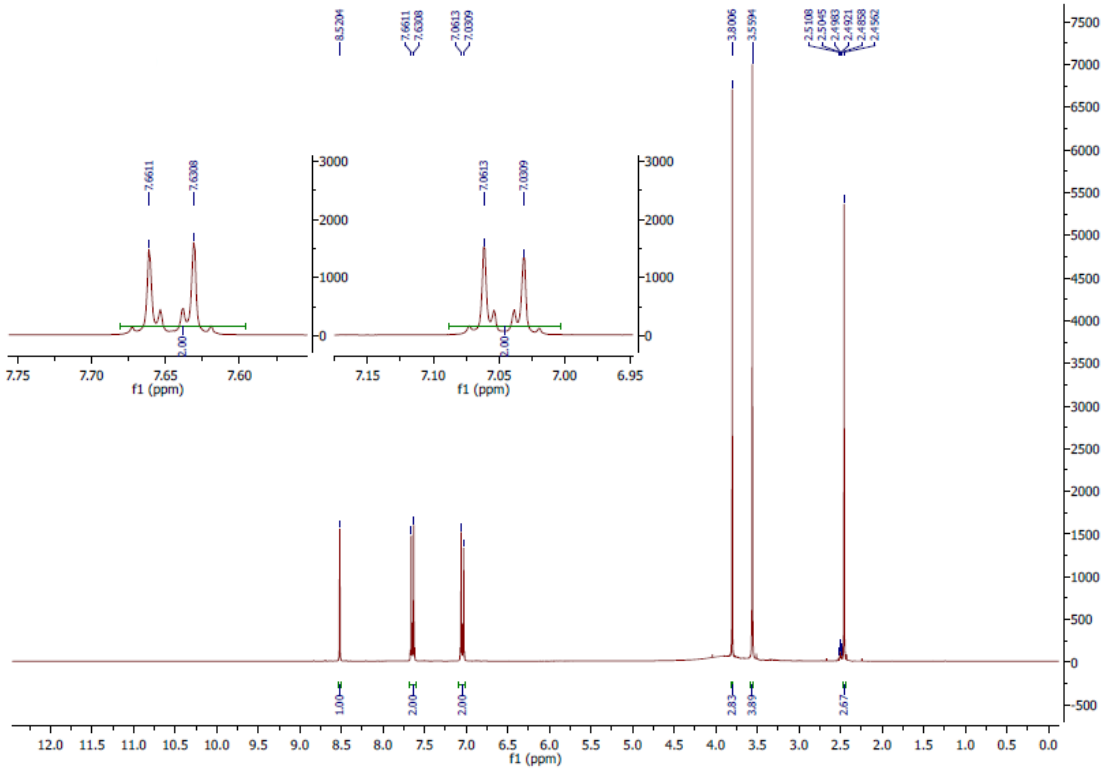

Figure S27. [1]H NMR spectrum (300 MHz, DMSO-d_6) of compound **5g**.

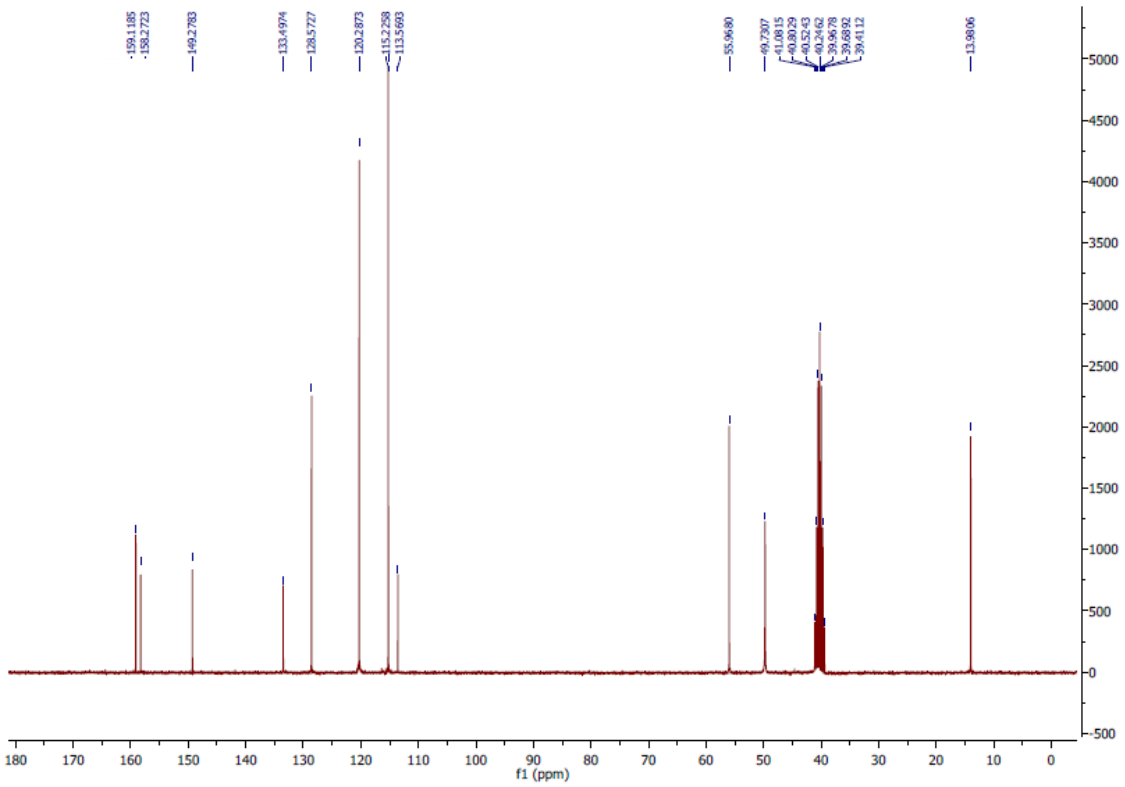

Figure S28. [13]C NMR spectrum (75 MHz, DMSO-d_6) of compound **5g**.