Electronic supplementary materials *Mendeleev Commun.*, 2023, **33**, 331–333

Antibacterial screening of new bis(pyrazolo[1,5-*a*]pyrimidine) hybrids linked to different spacers

Ahmed E. M. Mekky and Sherif M. H. Sanad

Contents

1. Experimental

1.1. Materials

All solvents were acquired from commercial sources and used as received unless otherwise stated. All other chemicals were acquired from Merck or Aldrich and used without further purification. The melting points were measured on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded on a Smart iTR, which is an ultra-high-performance, versatile Attenuated Total Reflectance (ATR) sampling accessory on the Nicolet iS10 FT-IR spectrometer. NMR spectra were recorded on Bruker Avance III 400 MHz spectrophotometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using TMS as an internal standard and DMSO-d_6 as solvent and chemical shifts were expressed as δ ppm units. Elemental analyses were carried out on a EuroVector instrument C, H, N, S analyzer EA3000 Series.

1.2. Table S1. Optimization of the synthesis of the target bis(pyrazolo[1,5-*a*]pyrimidine) **4a**.

Entry[a,b]	Solvent	Base[e]	Temp. (°C)	Time (h)	Yield (%)[f]
1	DCM[c]	Piperidine	40	8	17
2	DCM	Diethylamine	40	8	12
3	Ethanol	Piperidine	80	6	62
4	Ethanol	Diethylamine	80	6	57
5	Ethanol	Triethylamine	80	6	50
6	Dioxane	Piperidine	100	6	64
7	Dioxane	Diethylamine	100	6	54
8	Dioxane	Triethylamine	100	6	49
9	DMF[d]	Diethylamine	150	6	77
10	DMF	Triethylamine	150	6	72
11	DMF	Piperidine	150	4	73
12	**DMF**	**Piperidine**	**150**	**5**	**83**
13	DMF	Piperidine	150	6	80

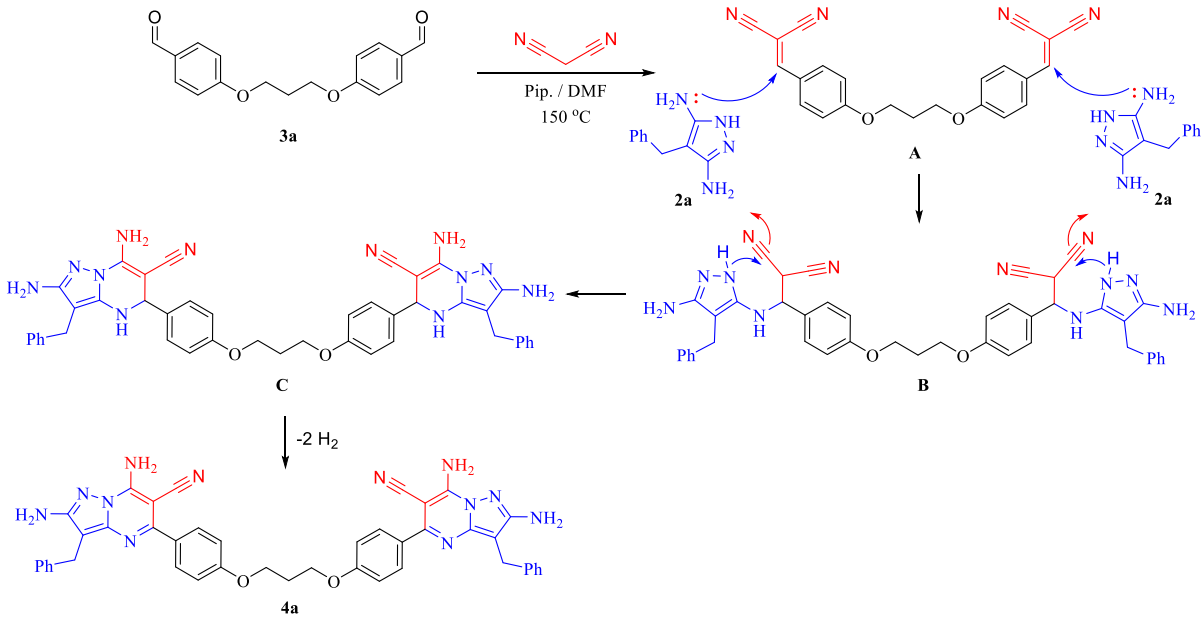
[a]All reactions were followed up by TLC analyses; [b]All reaction were conducted using 1 mmol of bis(benzaldehyde) **3a**; [c]Dichloromethane; [d]*N*,*N*-Dimethylformamide; [e]All reactions were first conducted using 1.0 mL of the corresponding amines; [f]The final yield of bis(pyrazolo[1,5-*a*]pyrimidine) **4a**.

1.3. Table S2. Optimizing the yield of piperidine-mediated synthesis of bis(pyrazolo[1,5-*a*]pyrimidine) **4a**.

Entry[a,b]	Volume[c]	Time (h)	Yield (%)[d]
1	0.5	6	57
2	0.6	6	60
3	0.7	4	80
4	**0.7**	**5**	**89**
5	0.7	6	87
6	0.8	5	86
7	0.9	5	84
8	1.0	5	83
9	1.1	5	80

[a]All reactions were followed up by TLC analyses; [b]All reaction were conducted using 1 mmol of bis(benzaldehyde) **3a** in DMF; [c]Volume used of piperidine; [d]The final yield of bis(pyrazolo[1,5-*a*]pyrimidine) **4a**.

1.4. Scheme S1. Detailed mechanism for the formation of bis(pyrazolo[1,5-*a*]pyrimidine) **4a**.



Initially, propane-linked bis(benzaldehyde) **3a** reacted with malononitrile to yield the corresponding bis(α,β-unsaturated nitrile) **A**. Next, aza-Michael addition reaction was conducted by the addition of one of the two amino functions in two molecules of **2a** and the olefinic double bonds in **A** to give the adduct **B**. The previous intermediate underwent successive cyclization and auto-oxidation to give the isolable bis(pyrazolo[1,5-*a*]pyrimidine) **4a** through the intermediate **C**.[S1]

1.5. The procedures and spectral data

1.5.1. General procedure for the synthesis of bis(pyrazolo[1,5-*a*]pyrimidine) 4-6, 8 and 10.

A ternary mixture of the corresponding 1*H*-pyrazole-3,5-diamines **2a**-**2c** (1 mmol), bis(aldehydes) **3**, **7** or **9** (1 mmol), and malononitrile (2 mmol) in *N*,*N*-dimethylformamide (10 mL) in the presence of piperidine (0.7 mL) was heated at 150 °C for 5 h. The mixture was evaporated to its half volume at reduced pressure and then cooled, and ethanol (5 mL) was added. The product so formed was collected by filtration, washed with cold ethanol, dried and recrystallized from the appropriate solvent.

1.5.2. 5,5'-((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-benzylpyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (4a).

Pale brown solid (dioxane / ethanol mixture, 89%); m.p. 212-214 ºC; IR (υ cm^{-1}): 3408, 3312, 3204 (NH_2), 2200 (CN); ^{1}H-NMR (DMSO-d_6): δ 2.12 (br s, 2H, OCH_2CH_2), 3.89 (s, 4H, 2 CH_2), 4.25 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.71 (s, 4H, 2 NH_2), 7.05 (d, *J* = 8.8 Hz, 4H, ArH), 7.13 (t, *J* = 7.2 Hz, 2H, ArH), 7.22 (t, *J* = 7.2 Hz, 4H, ArH), 7.34 (d, *J* = 7.2 Hz, 4H, ArH), 7.78 (d, *J* = 8.8 Hz, 4H, ArH), 7.95 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 26.7, 28.2, 66.3, 71.0, 96.2, 114.1, 117.2, 125.6, 128.1, 128.3, 130.2, 130.6, 142.5, 146.4, 149.6, 158.6, 159.8, 161.2; Anal. calcd. for $C_{43}H_{36}N_{12}O_2$ (752.8): C, 68.60; H, 4.82; N, 22.33; found: C, 68.74; H, 4.99; N, 22.15%.

1.5.3. 5,5'-((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-chlorobenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (4b).

Pale brown solid (dioxane, 87%); m.p. 226-228 ºC; IR (υ cm^{-1}): 3423, 3317, 3209 (NH_2), 2201 (CN); ^{1}H-NMR (DMSO-d_6): δ 2.15 (br s, 2H, OCH_2CH_2), 3.90 (s, 4H, 2 CH_2), 4.24 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.73 (s, 4H, 2 NH_2), 7.06 (d, *J* = 8.8 Hz, 4H, ArH), 7.17 (d, *J* = 8.4 Hz, 4H, ArH), 7.34 (d, *J* = 8.4 Hz, 4H, ArH), 7.77 (d, *J* = 8.8 Hz, 4H, ArH), 7.99 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 26.6, 28.3, 66.4, 70.9, 96.8, 114.2, 117.2, 128.1, 129.5, 130.1, 130.6, 130.7, 138.2, 146.0, 149.5, 158.7, 159.7, 161.0; Anal. calcd. for $C_{43}H_{34}Cl_2N1_2O_2$ (821.7): C, 62.85; H, 4.17; N, 20.45; found: C, 62.99; H, 4.06; N, 20.32%.

1.5.4. 5,5'-((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-methoxybenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (4c).

Pale brown solid (dioxane / ethanol mixture, 92%); m.p. 220-222 ºC; IR (υ cm^{-1}): 3402, 3300, 3214 (NH_2), 2200 (CN); ^{1}H-NMR (DMSO-d_6): δ 2.15 (br s, 2H, OCH_2CH_2), 3.68 (s, 6H, 2 *p*-OCH_3), 3.85 (s, 4H, 2 CH_2), 4.23 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.66 (s, 4H, 2 NH_2), 6.78 (d, *J* = 8.8 Hz, 4H, ArH), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.30 (d, *J* = 8.8 Hz, 4H, ArH), 7.80 (d, *J* = 8.8 Hz, 4H, ArH), 8.01 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 25.9, 28.2, 55.0, 66.4, 71.1, 96.7, 113.5, 114.0, 117.1, 129.8, 130.2, 130.7, 131.4, 146.0, 149.5, 157.3, 158.5, 160.1, 161.0; Anal. calcd. for $C_{45}H_{40}N_{12}O_4$ (812.9): C, 66.49; H, 4.96; N, 20.68; found: C, 66.33; H, 5.08; N, 20.79%.

1.5.5. 5,5'-((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-benzylpyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (5a).

Pale brown solid (dioxane / ethanol mixture, 85%); m.p. 204 ºC; IR (υ cm^{-1}): 3425, 3302, 3211 (NH_2), 2202 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.92 (br s, 4H, 2 OCH_2CH_2), 3.89 (s, 4H, 2 CH_2), 4.13 (br s, 4H, 2 OCH_2), 5.72 (s, 4H, 2 NH_2), 7.06 (d, *J* = 8.8 Hz, 4H, ArH), 7.12 (t, *J* = 7.2 Hz, 2H, ArH), 7.22 (t, *J* = 7.2 Hz, 4H, ArH), 7.32 (d, *J* = 7.2 Hz, 4H, ArH), 7.79 (d, *J* = 8.8 Hz, 4H, ArH), 7.95 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 25.2, 26.7, 67.5, 70.9, 96.4, 114.0, 117.3, 125.6, 128.2, 128.3, 130.2, 130.6, 142.5, 146.5, 149.7, 158.5, 160.0, 161.1; Anal. calcd. for $C_{44}H_{38}N_{12}O_2$ (766.8): C, 68.91; H, 4.99; N, 21.92; found: C, 69.07; H, 4.88; N, 21.74%.

1.5.6. 5,5'-((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-chlorobenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (5b).

Pale brown solid (dioxane, 89%); m.p. 216-219 ºC; IR (υ cm^{-1}): 3422, 3333, 3216 (NH_2), 2199 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.90 (br s, 4H, 2 OCH_2CH_2), 3.90 (s, 4H, 2 CH_2), 4.14 (br s, 4H, 2 OCH_2), 5.70 (s, 4H, 2 NH_2), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.16 (d, *J* = 8.4 Hz, 4H, ArH), 7.33 (d, *J* = 8.4 Hz, 4H, ArH), 7.78 (d, *J* = 8.8 Hz, 4H, ArH), 8.00 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 25.1, 26.6, 67.4, 70.8, 97.0, 114.2, 117.1, 128.2, 129.4, 130.2, 130.5, 130.6, 138.3, 146.1, 149.6, 158.5, 159.9, 160.9; Anal. calcd. for $C_{44}H_{36}Cl_2N_{12}O_2$ (835.7): C, 63.23; H, 4.34; N, 20.11; found: C, 63.23; H, 4.34; N, 20.11%.

1.5.7. 5,5'-((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-methoxybenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (5c).

Pale brown solid (dioxane, 90%); m.p. 204 ºC; IR (υ cm^{-1}): 3445, 3327, 3225 (NH_2), 2199 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.92 (br s, 4H, 2 OCH_2CH_2), 3.67 (s, 6H, 2 *p*-OCH_3), 3.83 (s, 4H, 2 CH_2), 4.14 (br s, 4H, 2 OCH_2), 5.68 (s, 4H, 2 NH_2), 6.77 (d, *J* = 8.8 Hz, 4H, ArH), 7.08 (d, *J* = 8.8 Hz, 4H, ArH), 7.30 (d, *J* = 8.8 Hz, 4H, ArH), 7.78 (d, *J* = 8.8 Hz, 4H, ArH), 7.95 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 25.1, 25.9, 54.9, 67.4, 70.9, 96.9, 113.6, 114.3, 117.2, 129.7, 130.2, 130.8, 131.4, 146.1, 149.6, 157.5, 158.4, 160.3, 160.9; Anal. calcd. for $C_{46}H_{42}N_{12}O_4$ (826.9): C, 66.81; H, 5.12; N, 20.33; found: C, 66.98; H, 5.00; N, 20.47%.

1.5.8. 5,5'-((Pentane-1,5-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-benzylpyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (6a).

Pale brown solid (dioxane / ethanol mixture, 88%); m.p. 192-193 ºC; IR (υ cm^{-1}): 3423, 3315, 3200 (NH_2), 2201 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.60 (br s, 2H, $OCH_2CH_2CH_2$), 1.78 (br s, 4H, 2 OCH_2CH_2), 3.89 (s, 4H, 2 CH_2), 4.05 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.69 (s, 4H, 2 NH_2), 7.05 (d, *J* = 8.8 Hz, 4H, ArH), 7.11 (t, *J* = 7.2 Hz, 2H, ArH), 7.22 (t, *J* = 7.2 Hz, 4H, ArH), 7.33 (d, *J* = 7.2 Hz, 4H, ArH), 7.76 (d, *J* = 8.8 Hz, 4H, ArH), 7.98 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 22.0, 26.8, 28.2, 67.9, 71.0, 96.3, 114.2, 117.2, 125.7, 128.1, 128.2, 130.3, 130.6, 142.4, 146.3, 149.5, 158.4, 159.8, 160.9; Anal. calcd. for $C_{45}H_{40}N_{12}O_2$ (780.9): C, 69.21; H, 5.16; N, 21.52; found: C, 69.02; H, 5.30; N, 21.69%.

1.5.9. 5,5'-((Pentane-1,5-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-chlorobenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (6b).

Pale brown solid (dioxane, 86%); m.p. 200-203 ºC; IR (υ cm^{-1}): 3420, 3331, 3199 (NH_2), 2202 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.58 (br s, 2H, $OCH_2CH_2CH_2$), 1.78 (m, 4H, 2 OCH_2CH_2), 3.89 (s, 4H, 2 CH_2), 4.04 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.67 (s, 4H, 2 NH_2), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.16 (d, *J* = 8.4 Hz, 4H, ArH), 7.34 (d, *J* = 8.4 Hz, 4H, ArH), 7.78 (d, *J* = 8.8 Hz, 4H, ArH), 7.97 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 22.1, 26.7, 28.1, 67.8, 71.0, 96.9, 114.1, 117.4, 128.2, 129.3, 130.1, 130.6, 130.7, 138.3, 145.9, 149.4, 158.5, 159.7, 161.0; Anal. calcd. for $C_{45}H_{38}Cl_2N_{12}O_2$ (849.7): C, 63.60; H, 4.51; N, 19.78; found: C, 63.42; H, 4.69; N, 19.97%.

1.5.10. 5,5'-((Pentane-1,5-diylbis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-methoxybenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (6c).

Pale brown solid (dioxane / ethanol mixture, 90%); m.p. 192-193 ºC; IR (υ cm^{-1}): 3440, 3329, 3212 (NH_2), 2200 (CN); ^{1}H-NMR (DMSO-d_6): δ 1.60 (br s, 2H, $OCH_2CH_2CH_2$), 1.80 (br s, 4H, 2 OCH_2CH_2), 3.68 (s, 6H, 2 *p*-OCH_3), 3.84 (s, 4H, 2 CH_2), 4.03 (t, *J* = 6.4 Hz, 4H, 2 OCH_2), 5.66 (s, 4H, 2 NH_2), 6.76 (d, *J* = 8.8 Hz, 4H, ArH), 7.06 (d, *J* = 8.8 Hz, 4H, ArH), 7.29 (d, *J* = 8.8 Hz, 4H, ArH), 7.79 (d, *J* = 8.8 Hz, 4H, ArH), 8.02 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 22.1, 25.8, 28.2, 55.0, 67.9, 70.8, 96.8, 113.7, 114.2, 117.1, 129.6, 130.1, 130.7, 131.5, 145.9, 149.4, 157.5, 158.3, 160.1, 160.8; Anal. calcd. for $C_{47}H_{44}N_{12}O_4$ (840.9): C, 67.13; H, 5.27; N, 19.99; found: C, 66.92; H, 5.09; N, 20.11%.

1.5.11. 5,5'-(((1,4-Phenylenebis(methylene))bis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-benzylpyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (8a).

Pale yellow solid (dioxane / ethanol mixture, 82%); m.p. 180-181 ºC; IR (υ cm^{-1}): 3441, 3356, 3214 (NH_2), 2202 (CN); ^{1}H-NMR (DMSO-d_6): δ 3.90 (s, 4H, 2 CH_2), 5.12 (s, 4H, 2 OCH_2), 5.74 (s, 4H, 2 NH_2), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.14 (t, *J* = 7.2 Hz, 2H, ArH), 7.23 (t, *J* = 7.2 Hz, 4H, ArH), 7.35 (d, *J* = 7.2 Hz, 4H, ArH), 7.49 (s, 4H, ArH), 7.81 (d, *J* = 8.8 Hz, 4H, ArH), 8.11 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 26.8, 69.0, 75.9, 97.0, 114.9, 115.9, 125.7, 127.8, 128.2, 128.4, 129.0, 133.2, 136.7, 142.4, 145.6, 147.3, 157.3, 157.5, 159.7; Anal. calcd. for $C_{48}H_{38}N_{12}O_2$ (814.9): C, 70.75; H, 4.70; N, 20.63; found: C, 70.58; H, 4.49; N, 20.72%.

1.5.12. 5,5'-(((1,4-Phenylenebis(methylene))bis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-chlorobenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (8b).

Pale yellow solid (dioxane, 85%); m.p. 192 ºC; IR (υ cm^{-1}): 3450, 3312, 3226 (NH_2), 2203 (CN); ^{1}H-NMR (DMSO-d_6): δ 3.91 (s, 4H, 2 CH_2), 5.14 (s, 4H, 2 OCH_2), 5.76 (s, 4H, 2 NH_2), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.18 (d, *J* = 8.4 Hz, 4H, ArH), 7.36 (d, *J* = 8.4 Hz, 4H, ArH), 7.48 (s, 4H, ArH), 7.80 (d, *J* = 8.8 Hz, 4H, ArH), 8.06 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 26.7, 69.2, 76.0, 97.1, 115.0, 116.0, 127.7, 128.2, 128.9, 129.5, 130.5, 133.3, 136.6, 138.2, 144.5, 144.7, 157.1, 157.4, 159.6; Anal. calcd. for $C_{48}H_{36}Cl_2N_{12}O_2$ (883.8): C, 65.23; H, 4.11; N, 19.02; found: C, 65.06; H, 3.98; N, 19.17%.

1.5.13. 5,5'-(((1,4-Phenylenebis(methylene))bis(oxy))bis(4,1-phenylene))bis(2,7-diamino-3-(4-methoxybenzyl)pyrazolo[1,5-*a*]pyrimidine-6-carbonitrile) (8c).

Pale yellow solid (dioxane, 86%); m.p. 186-188 ºC; IR (υ cm^{-1}): 3444, 3353, 3219 (NH_2), 2203 (CN); ^{1}H-NMR (DMSO-d_6): δ 3.70 (s, 6H, 2 *p*-OCH_3), 3.87 (s, 4H, 2 CH_2), 5.13 (s, 4H, 2 OCH_2), 5.69 (s, 4H, 2 NH_2), 6.79 (d, *J* = 8.8 Hz, 4H, ArH), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.32 (d, *J* = 8.8 Hz, 4H, ArH), 7.50 (s, 4H, ArH), 7.81 (d, *J* = 8.8 Hz, 4H, ArH), 8.14 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 26.0, 55.1, 69.2, 76.1, 97.1, 113.6, 115.0, 117.1, 127.8, 128.9, 129.7, 131.5, 133.3, 136.7, 145.7, 146.2, 157.2, 157.3, 157.4, 159.5; Anal. calcd. for $C_{50}H_{42}N_{12}O_4$ (874.9): C, 68.64; H, 4.84; N, 19.21; found: C, 68.49; H, 5.01; N, 19.03%.

1.5.14. Diethyl 3,4-bis((4-(2,7-diamino-3-benzyl-6-cyanopyrazolo[1,5-*a*]pyrimidin-5-yl)phenoxy)methyl)thieno[2,3-*b*]thiophene-2,5-dicarboxylate (10a).

Pale yellow solid (DMF / ethanol mixture, 83%); m.p. 162-164 ºC; IR (υ cm^{-1}): 3436, 3327, 3209 (NH_2), 2204 (CN), 1712 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.31 (t, *J* = 7.2 Hz, 6H, 2 OCH_2CH_3), 3.90 (s, 4H, 2 CH_2), 4.32 (q, *J* = 7.2 Hz, 4H, 2 OCH_2CH_3), 5.59 (s, 4H, 2 OCH_2), 5.71 (s, 4H, 2 NH_2), 7.07 (d, *J* = 8.8 Hz, 4H, ArH), 7.12 (t, *J* = 7.2 Hz, 2H, ArH), 7.22 (t, *J* = 7.2 Hz, 4H, ArH), 7.33 (d, *J* = 7.2 Hz, 4H, ArH), 7.78 (d, *J* = 8.8 Hz, 4H, ArH), 8.04 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 14.2, 26.8, 60.7, 61.9, 76.3, 97.4, 115.1, 116.5, 125.6, 127.9, 128.1, 128.3, 129.1, 130.2, 133.6, 136.6, 142.5, 145.1, 145.7, 146.2, 156.0, 157.2, 157.3, 164.2; Anal. calcd. for $C_{54}H_{44}N_{12}O_6S_2$ (1021.1): C, 63.52; H, 4.34; N, 16.46; found: C, 63.39; H, 4.58; N, 16.27%.

1.5.15. Diethyl 3,4-bis((4-(2,7-diamino-3-(4-chlorobenzyl)-6-cyanopyrazolo[1,5-*a*]pyrimidin-5-yl)phenoxy)methyl)thieno[2,3-*b*]thiophene-2,5-dicarboxylate (10b).

Pale yellow solid (DMF, 80%); m.p. 180-183 ºC; IR (υ cm^{-1}): 3419, 3320, 3219 (NH_2), 2203 (CN), 1710 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.32 (t, *J* = 7.2 Hz, 6H, 2 OCH_2CH_3), 3.90 (s, 4H, 2 CH_2), 4.33 (q, *J* = 7.2 Hz, 4H, 2 OCH_2CH_3), 5.60 (s, 4H, 2 OCH_2), 5.70 (s, 4H, 2 NH_2), 7.08 (d, *J* = 8.8 Hz, 4H, ArH), 7.18 (d, *J* = 8.4 Hz, 4H, ArH), 7.35 (d, *J* = 8.4 Hz, 4H, ArH), 7.81 (d, *J* = 8.8 Hz, 4H, ArH), 8.12 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 14.2, 26.8, 60.7, 61.8, 76.4, 97.2, 115.2, 116.6, 127.9, 128.3, 129.0, 129.6, 130.2, 130.6, 133.5, 136.5, 138.1, 145.0, 145.5, 146.2, 157.0, 157.1, 157.3, 164.3; Anal. calcd. for $C_{54}H_{42}Cl_2N_{12}O_6S_2$ (1090.0): C, 59.50; H, 3.88; N, 15.42; found: C, 59.23; H, 4.04; N, 15.30%.

1.5.16. Diethyl 3,4-bis((4-(2,7-diamino-6-cyano-3-(4-methoxybenzyl)pyrazolo[1,5-*a*]pyrimidin-5-yl)phenoxy)methyl)thieno[2,3-*b*]thiophene-2,5-dicarboxylate (10c).

Pale yellow solid (DMF, 84%); m.p. 176-178 ^{o}C; IR (υ cm^{-1}): 3428, 3317, 3212 (NH_2), 2203 (CN), 1713 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.33 (t, J = 7.2 Hz, 6H, 2 OCH_2CH_3), 3.69 (s, 6H, 2 *p*-OCH_3), 3.86 (s, 4H, 2 CH_2), 4.33 (q, J = 7.2 Hz, 4H, 2 OCH_2CH_3), 5.60 (s, 4H, 2 OCH_2), 5.69 (s, 4H, 2 NH_2), 6.78 (d, J = 8.8 Hz, 4H, ArH), 7.08 (d, J = 8.8 Hz, 4H, ArH), 7.33 (d, J = 8.8 Hz, 4H, ArH), 7.81 (d, J = 8.8 Hz, 4H, ArH), 8.12 (br s, 4H, 2 NH_2); ^{13}C-NMR (DMSO-d_6): δ 14.2, 26.0, 55.0, 60.6, 61.9, 76.4, 97.5, 113.6, 115.2, 116.4, 127.8, 129.2, 129.7, 130.1, 131.5, 133.7, 136.4, 145.2, 145.7, 146.3, 155.9, 157.2, 157.3, 157.4, 164.3; Anal. calcd. for $C_{56}H_{48}N_{12}O_8S_2$ (1081.2): C, 62.21; H, 4.48; N, 15.55; found: C, 62.00; H, 4.63; N, 15.44%.

1.6. Minimum inhibitory concentration (MIC) determination.

The inhibitory activity against *Staphylococcus aureus* (ATCC:6538), *Enterococcus faecalis* (ATCC:29212), *Escherichia coli* (ATCC:9637), and *Pseudomonas aeruginosa* (ATCC:27953) bacterial strains was estimated. MIC values were determined using microbroth serial dilution method[S2,S3] in a sterile 96-well microtiter plate after overnight incubation of tested bacteria at 37 °C. This assay was performed in triplicates for consistency in accordance with guidelines provided by CLSI (2012).[S4] Ciprofloxacin (100 µg susceptibility disc) was used as a standard drug. The concentration of the tested hybrids as well as Ciprofloxacin used in the study ranged from 250 to 0.9 µg/mL. The sterile Muller-Hinton broth (MHB) was enriched with 2% NaCl before the tested antimicrobial agents were inserted into the well at concentration gradient in a serial dilution. Then the diluted bacterial suspension at final inoculum of 10^6 CFU/mL was added. The tested compound in MHB was used as negative control to ensure medium sterility, while the inoculum in MHB served as positive control to ensure the adequacy of the broth for bacterial growth. To facilitate the observation of the growth of bacteria in each well, 20 µL of 2,3,5-triphenyltetrazolium chloride (TTC) at 2 mg/mL was added into each well.

1.7. Minimum bactericidal concentration (MBC) determination.

The tested hybrids were screened against *Staphylococcus aureus* (ATCC:6538), *Enterococcus faecalis* (ATCC:29212), *Escherichia coli* (ATCC:9637), and *Pseudomonas aeruginosa* (ATCC:27953) bacterial strains to estimate their MBC values.[S5] Each of the tested strains was cultured in sterile broth medium for 24 h at 37 ^{o}C. The assay was performed in 2 mL

microcentrifuge tubes with concentrations ranging from 250 to 0.9 μg/mL of the tested derivatives. To each concentration of the tested derivatives, 0.1 mL of the cultured bacterial strain was added and then, allowed to incubate for 24 h at 37 ºC. 10 μL sample was collected post incubation and seeded onto the agar plates and left to incubate for 24 h at 37 ºC. All the results were carried out in duplicate and the average values were determined.

2. References

S1. S. M. H. Sanad and A. E. M. Mekky, *Chem. Biodiversity*, 2022, **19**, e202100500.

S2. A. E. M. Mekky, S. M. H. Sanad and A. M. Abdelfattah, *Mendeleev Commun.*, 2022, **32**, 612.

S3. H. Mohammad, P. N. Reddy, D. Monteleone, A. S. Mayhoub, M. Cushman and M. N. Seleem, *Eur. J. Med. Chem.*, 2015, **94**, 306.

S4. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 7th edn., Approved Standard M07-A9, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, 2012.

S5 A. Kamal, A. Rahim, S. Riyaz, Y. Poornachandra, M. Balakrishna, C. G. Kumar, S. M. A. Hussaini, B. Sridhar and P. K. Machiraju, *Org. Biomol. Chem*., 2015, **13**, 1347.

3. ^{1}H- and ^{13}C-NMR copies of all new hybrids

Figure S1. ^{1}H-NMR spectrum of compound **4a**.

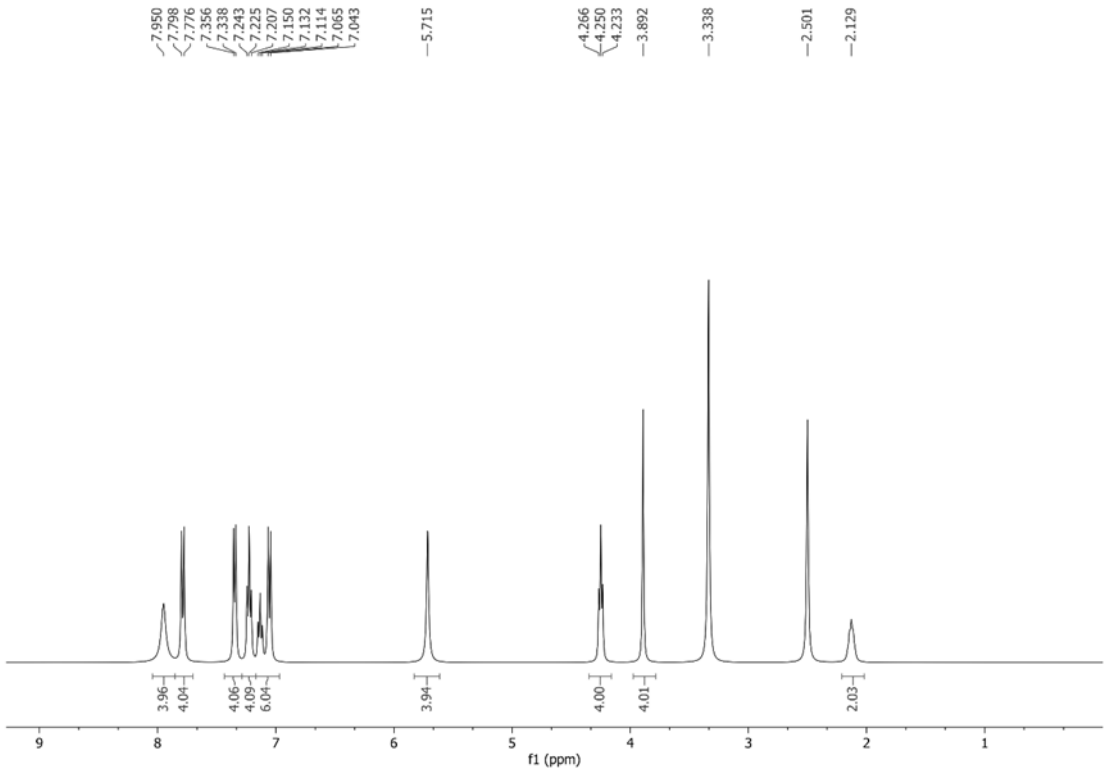


Figure S2. ^{13}C-NMR spectrum of compound **4a**.

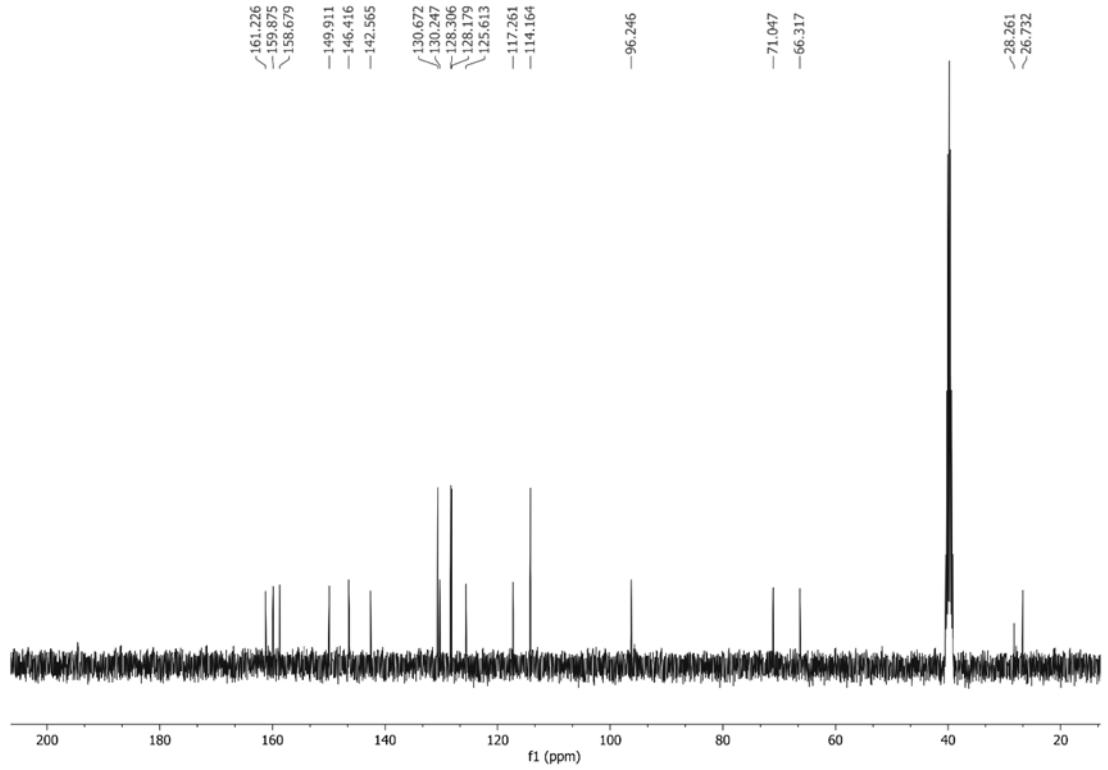


Figure S3. ^{1}H-NMR spectrum of compound **4b**.

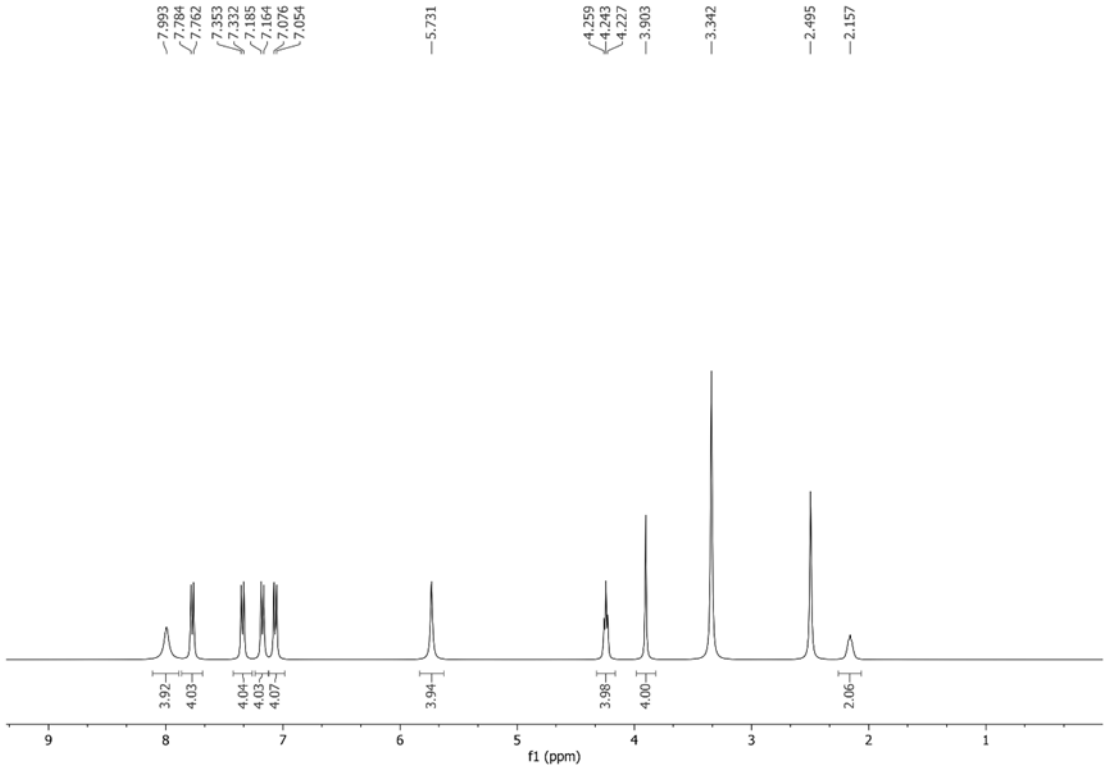


Figure S4. ^{13}C-NMR spectrum of compound **4b**.

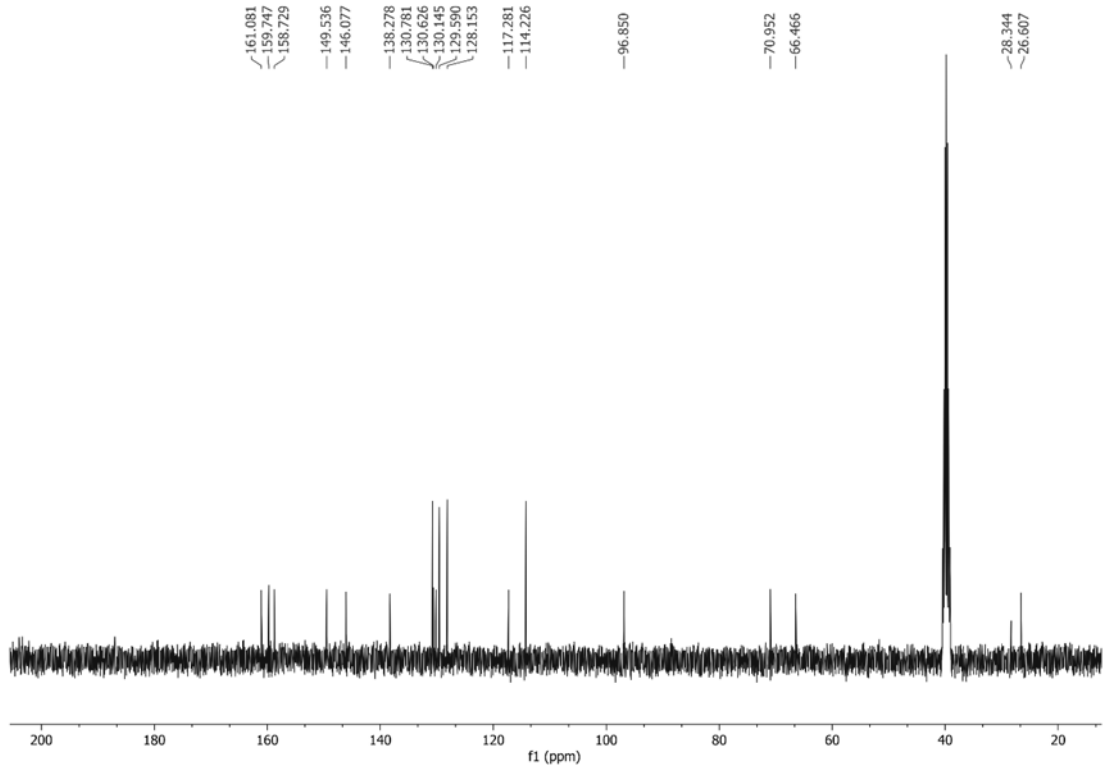


Figure S5. [1]H-NMR spectrum of compound **4c**.

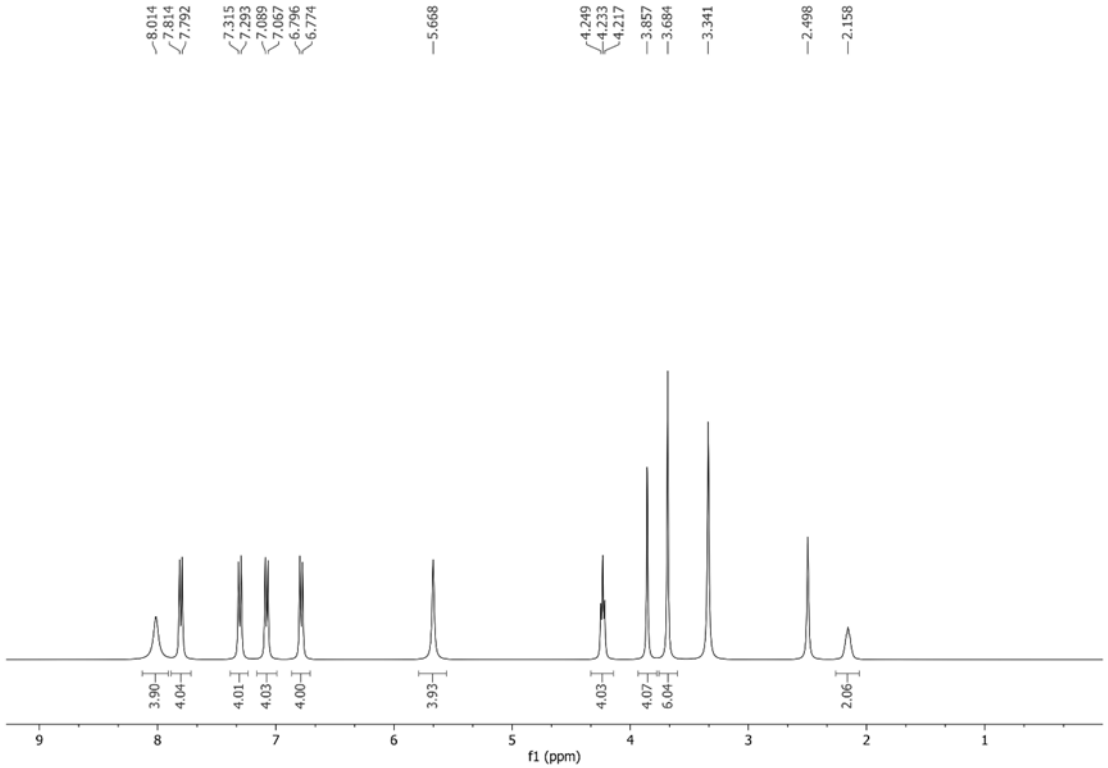


Figure S6. [13]C-NMR spectrum of compound **4c**.

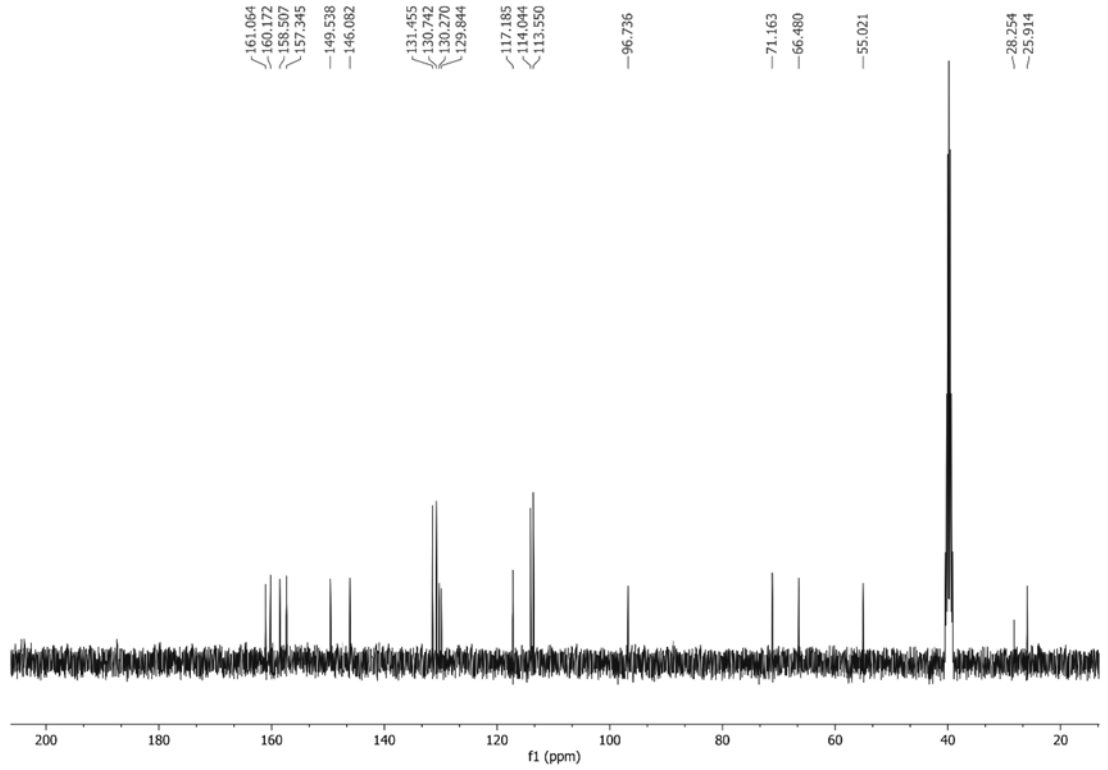


Figure S7. ^{1}H-NMR spectrum of compound **5a**.

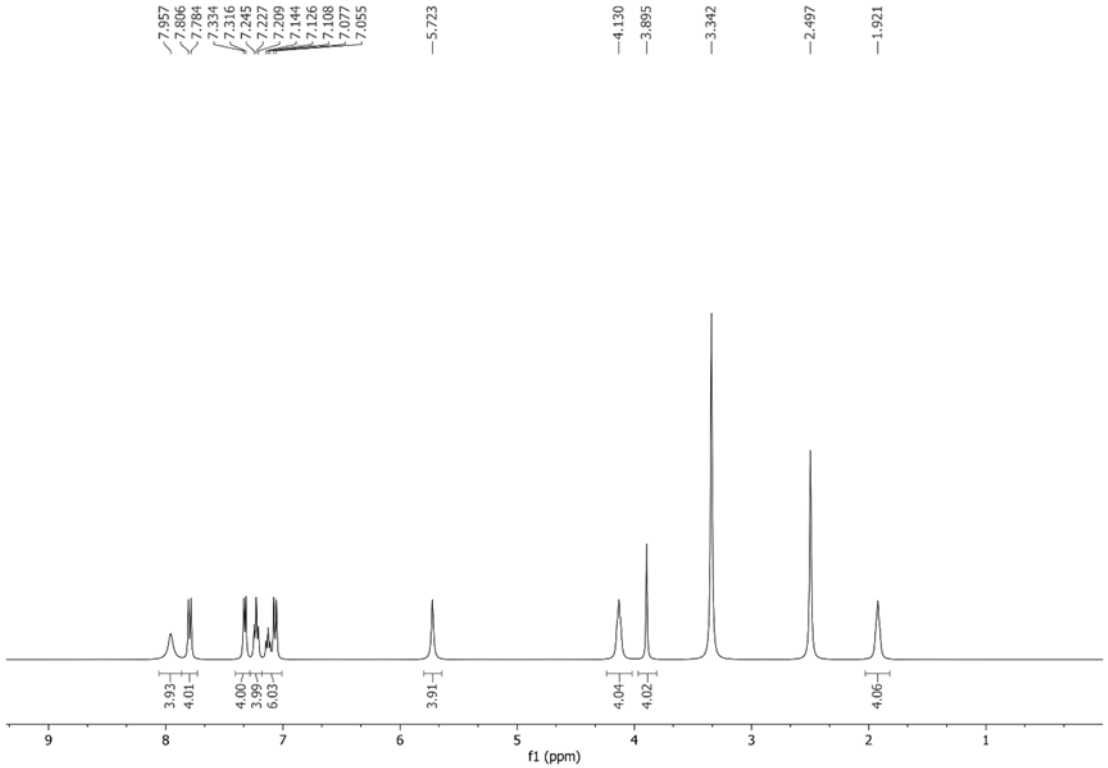


Figure S8. ^{13}C-NMR spectrum of compound **5a**.

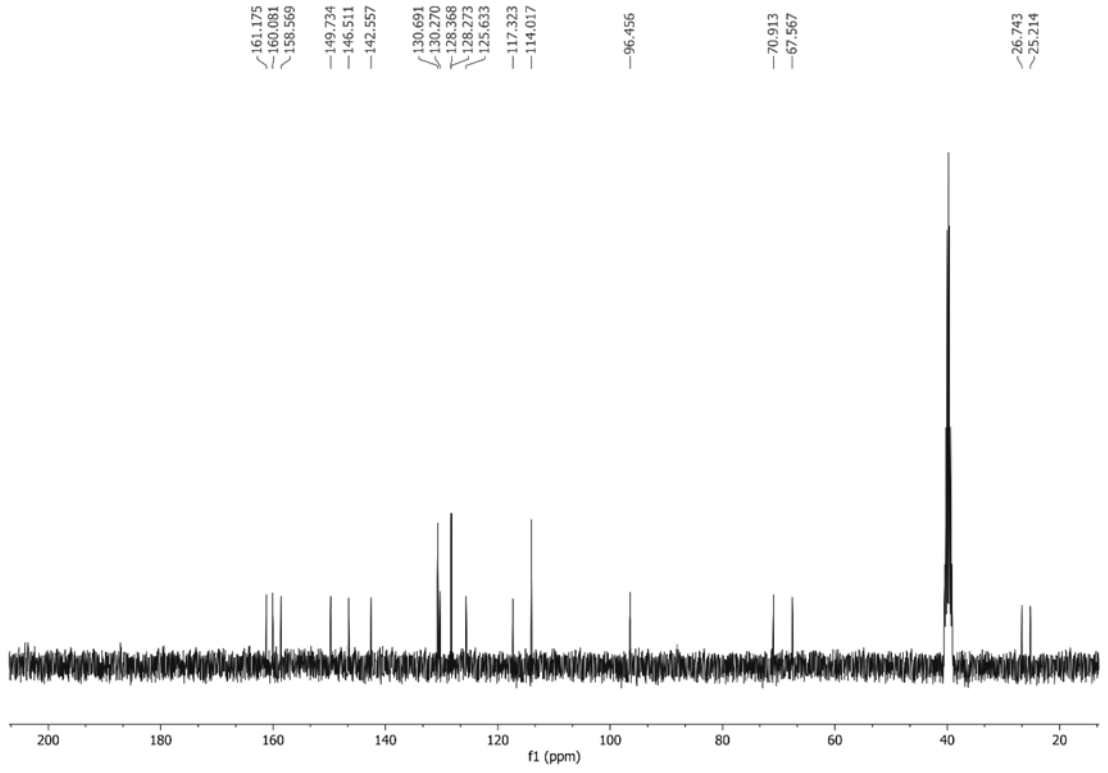


Figure S9. ^{1}H-NMR spectrum of compound **5b**.

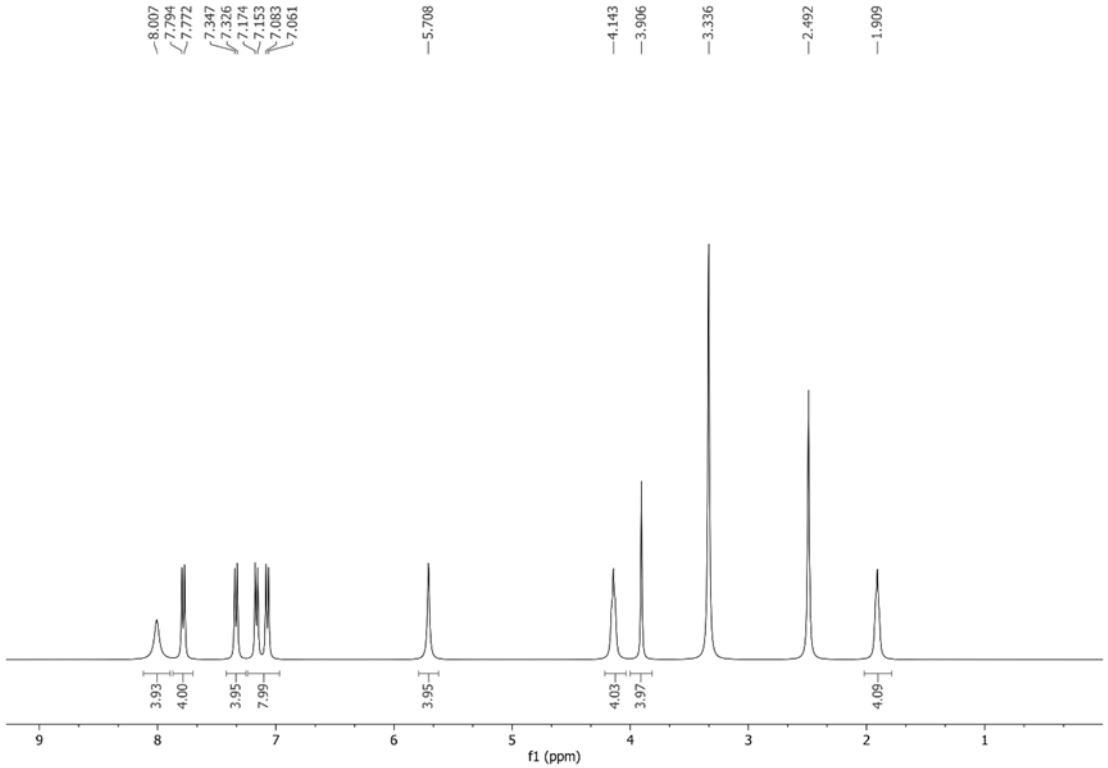


Figure S10. ^{13}C-NMR spectrum of compound **5b**.

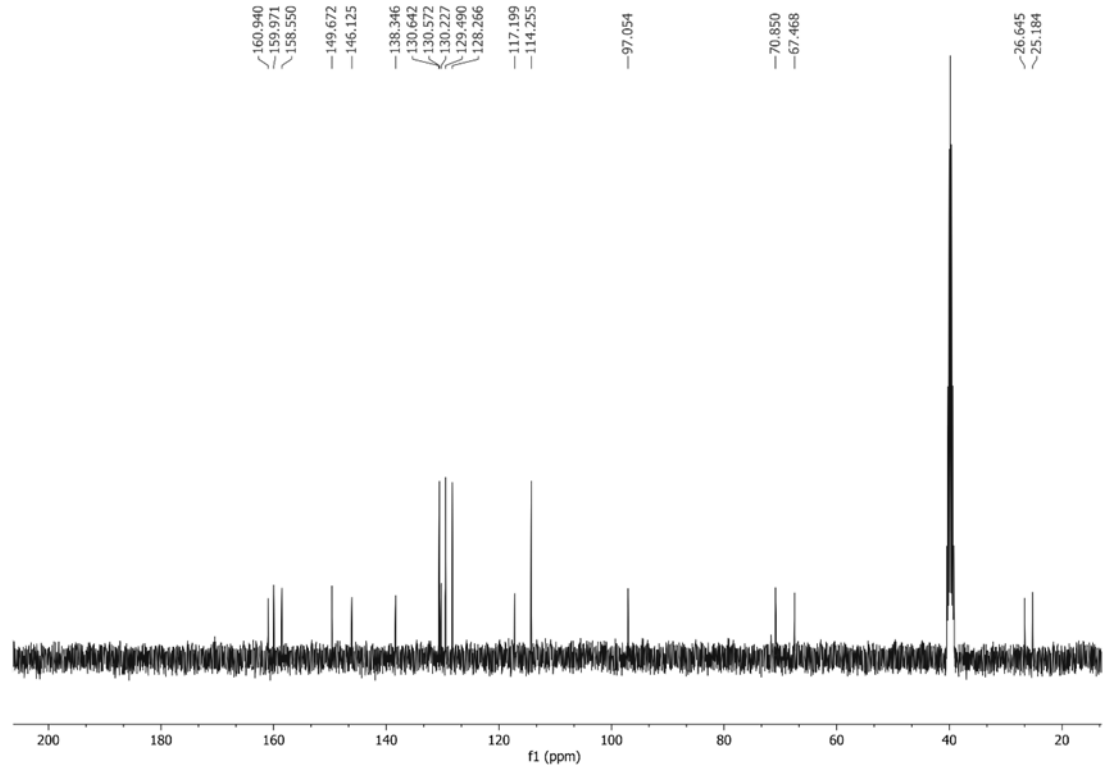


Figure S11. ^{1}H-NMR spectrum of compound **5c**.

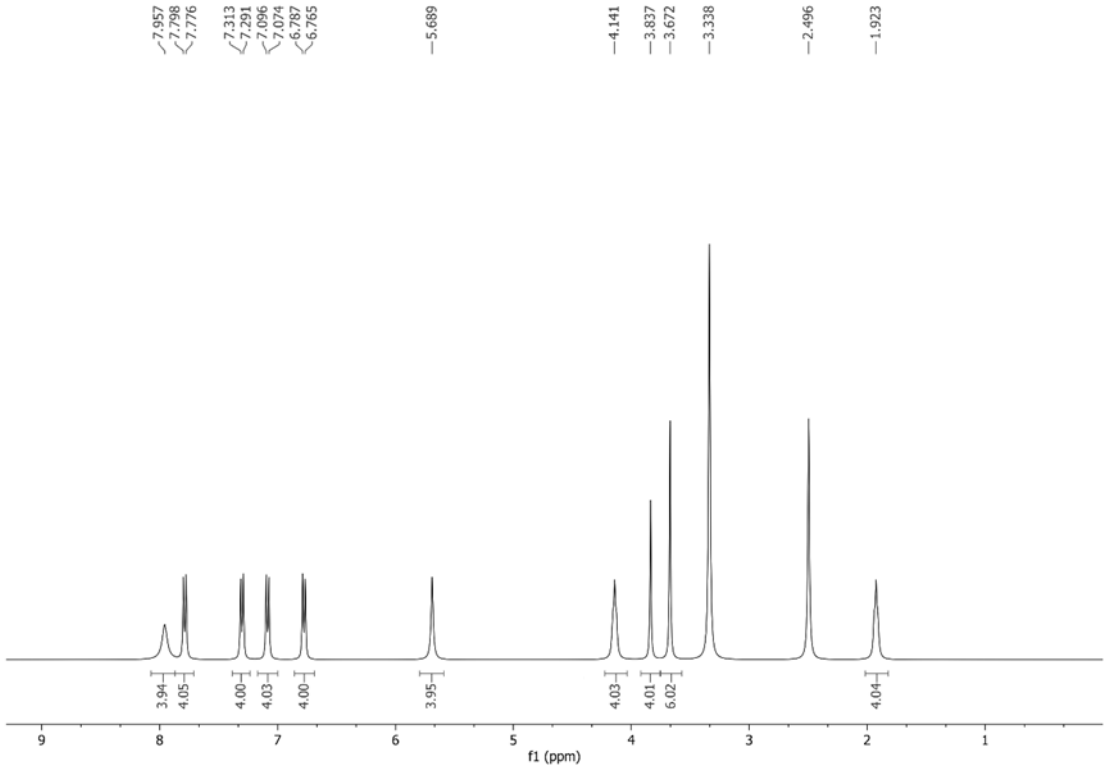


Figure S12. ^{13}C-NMR spectrum of compound **5c**.

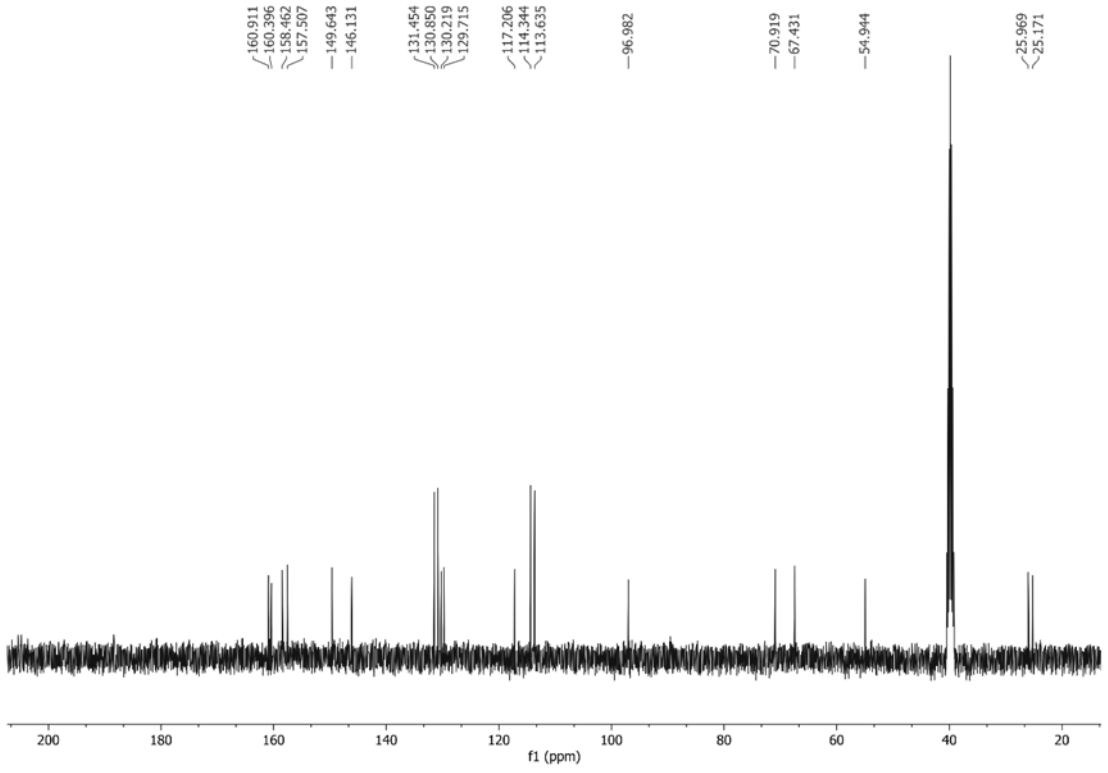


Figure S13. ^{1}H-NMR spectrum of compound **6a**.

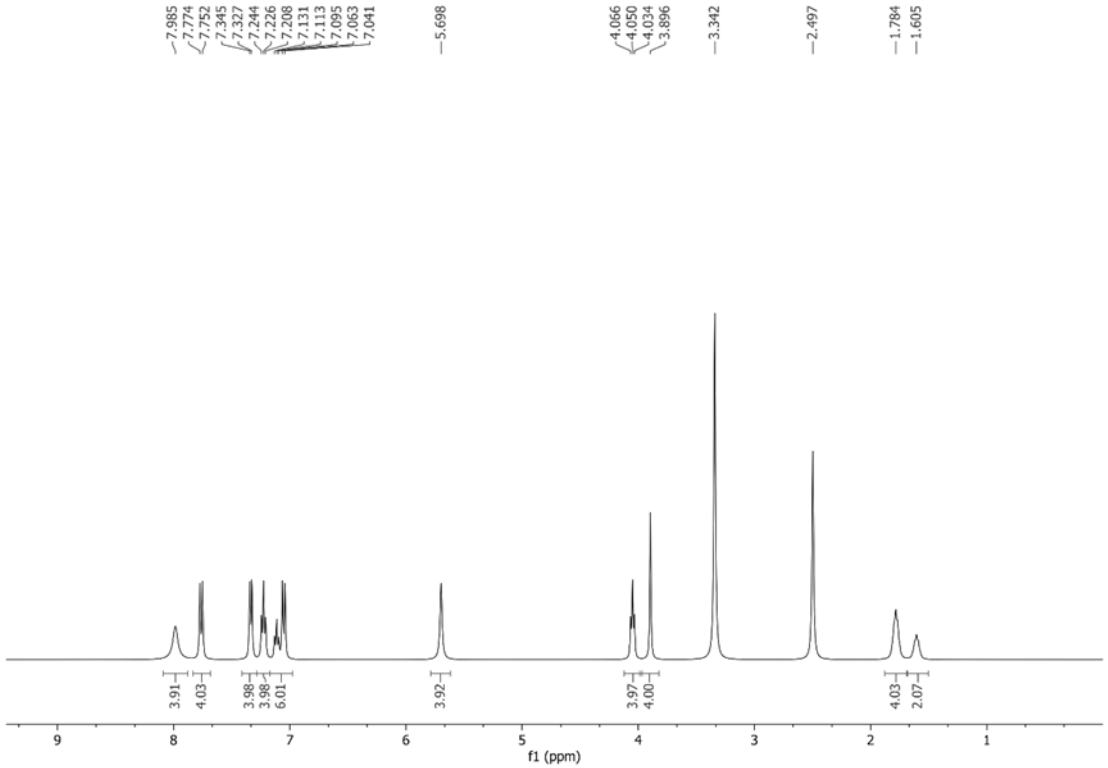


Figure S14. ^{13}C-NMR spectrum of compound **6a**.

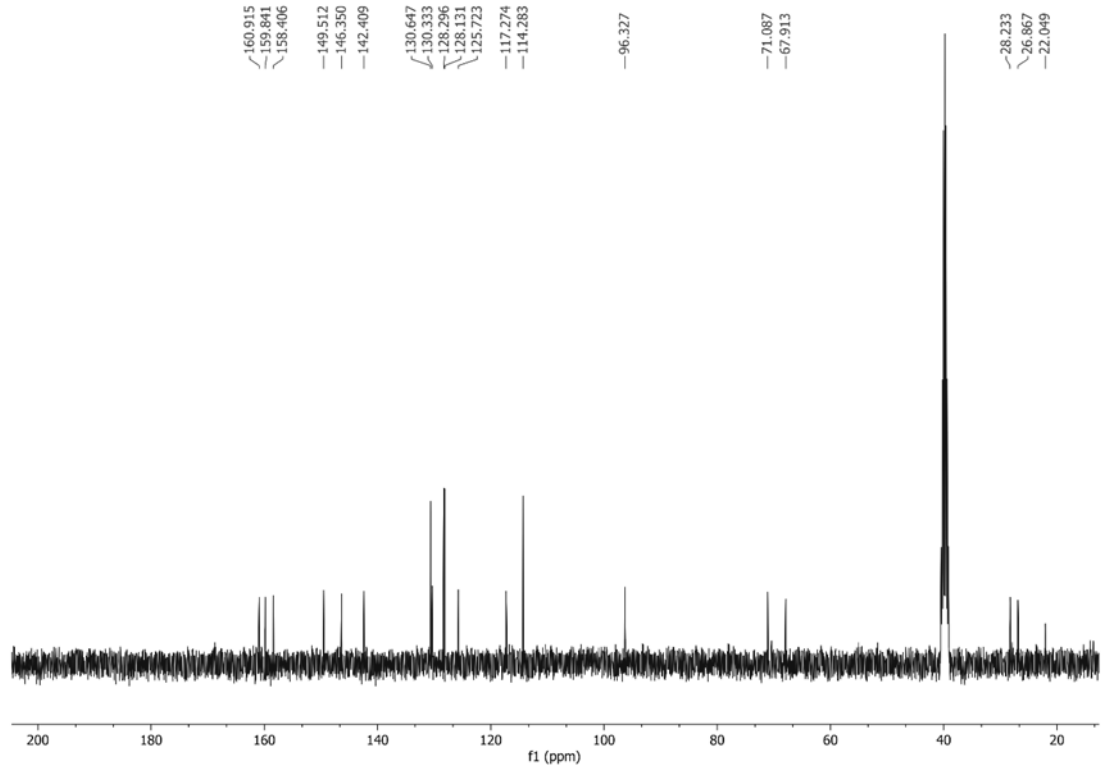


Figure S15. ^{1}H-NMR spectrum of compound **6b**.

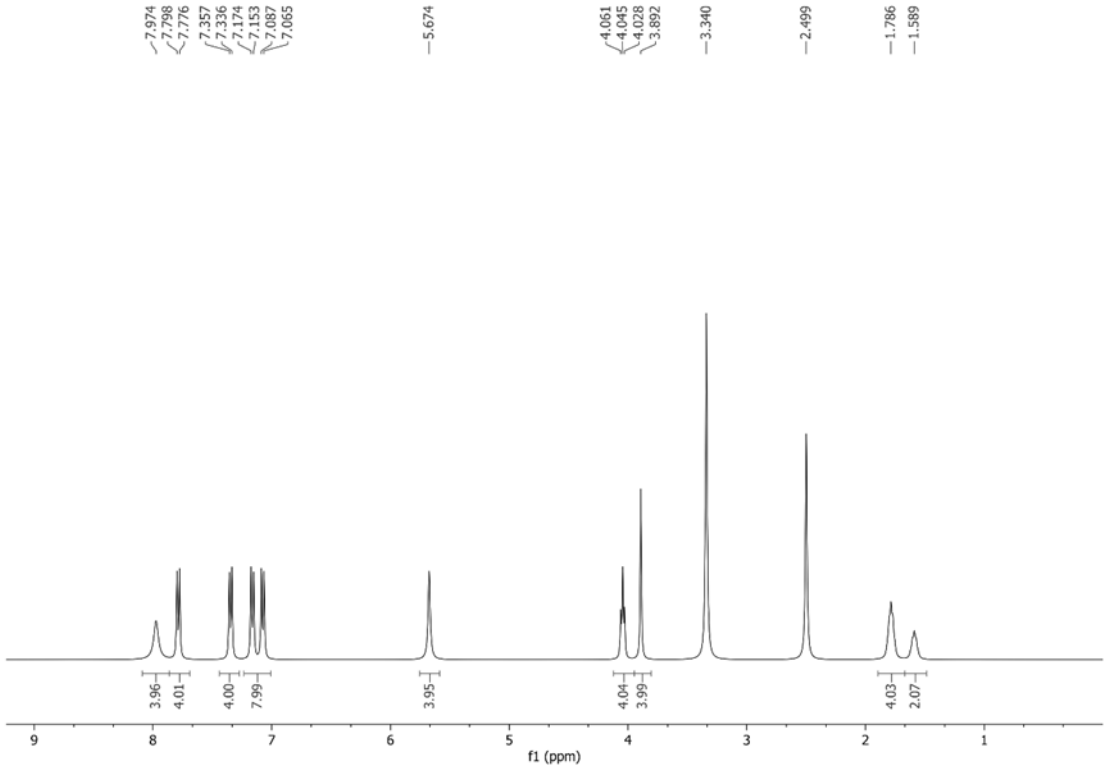


Figure S16. ^{13}C-NMR spectrum of compound **6b**.

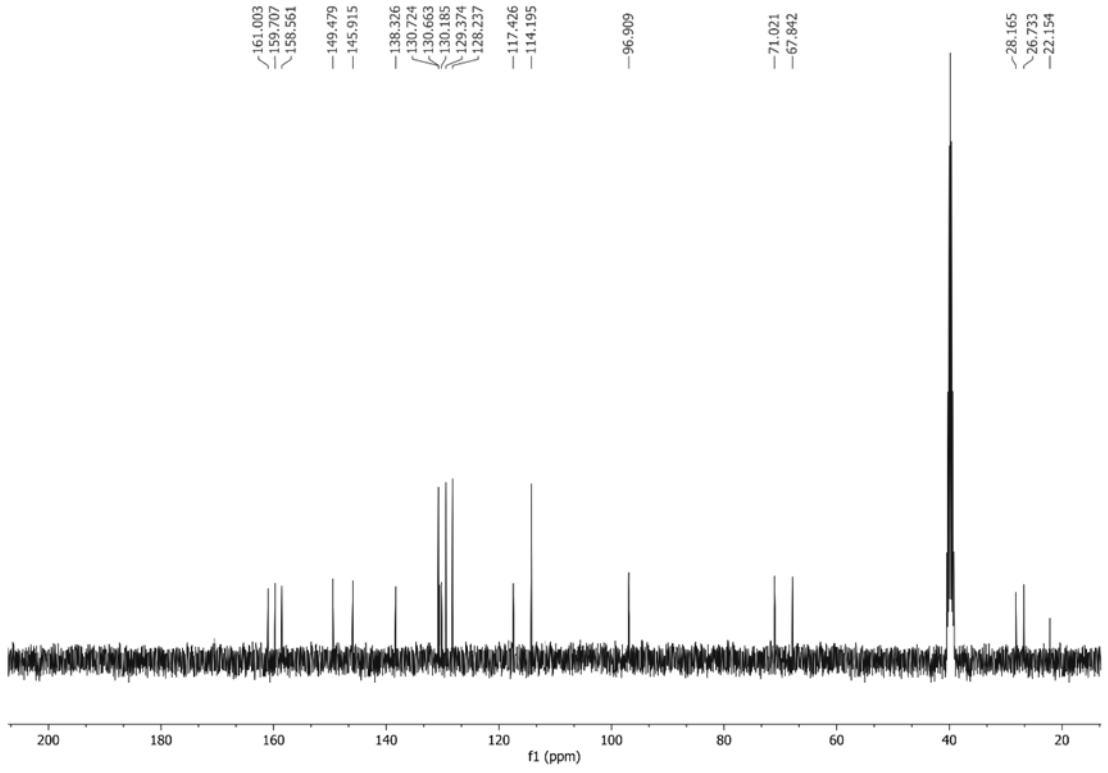


Figure S17. ^{1}H-NMR spectrum of compound **6c**.

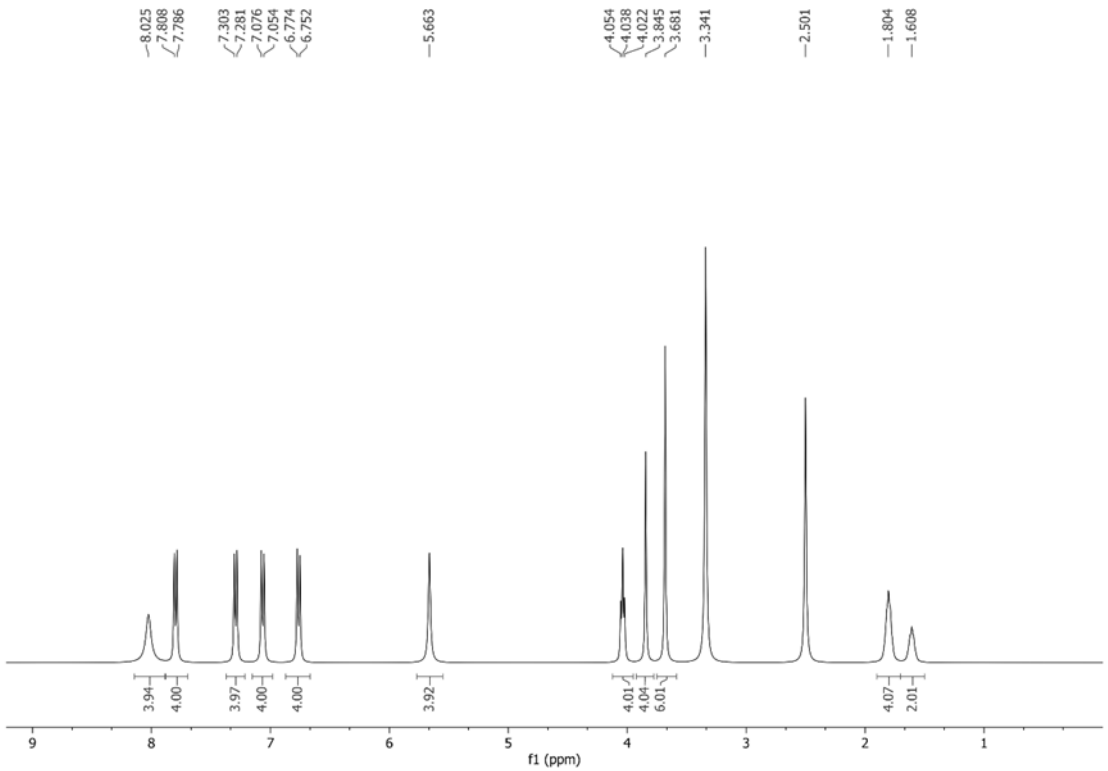


Figure S18. ^{13}C-NMR spectrum of compound **6c**.

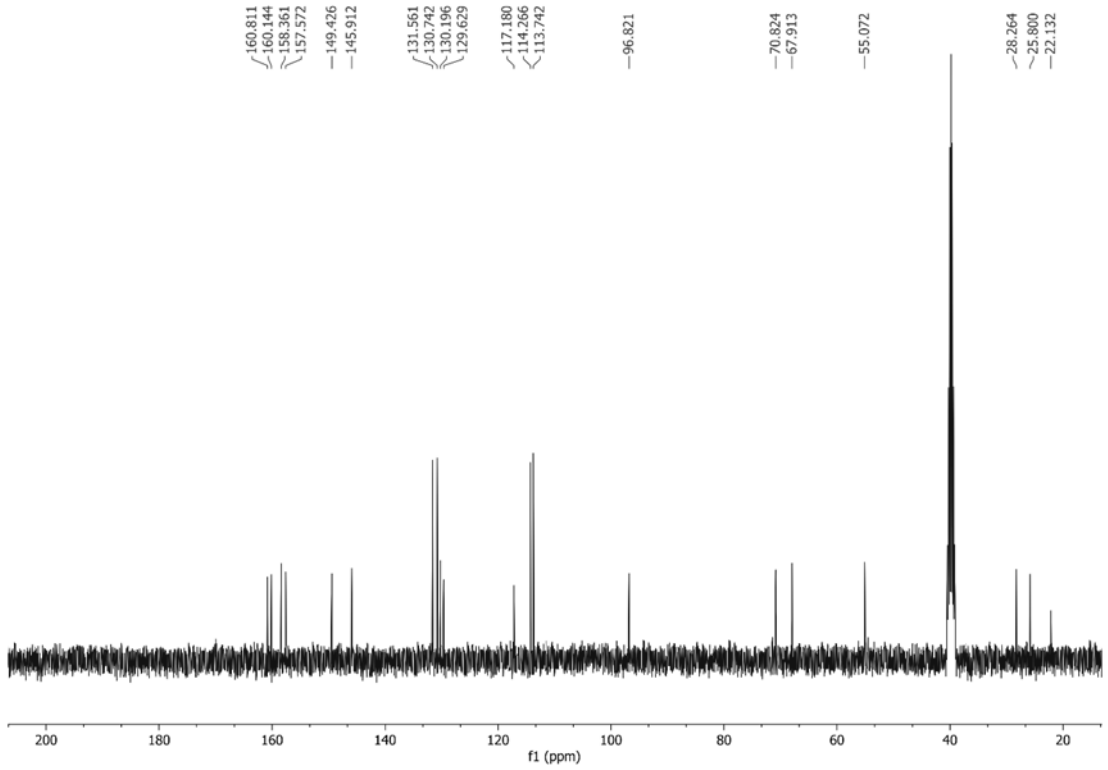


Figure S19. ^{1}H-NMR spectrum of compound **8a**.

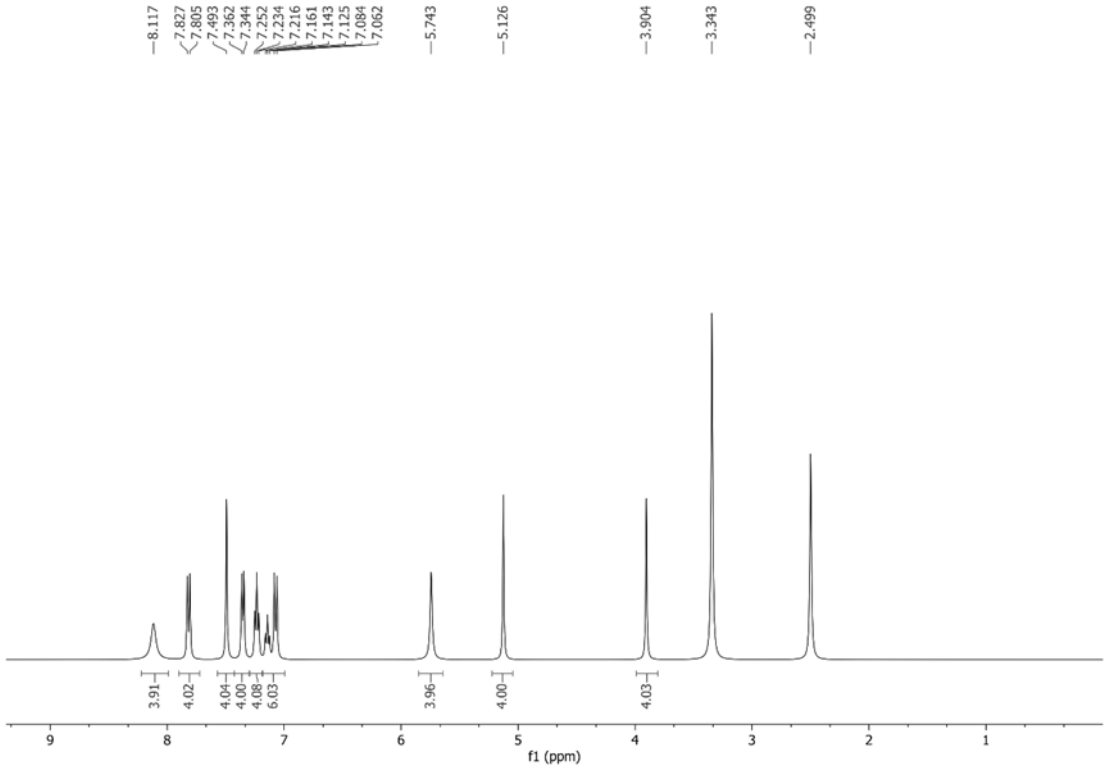


Figure S20. ^{13}C-NMR spectrum of compound **8a**.

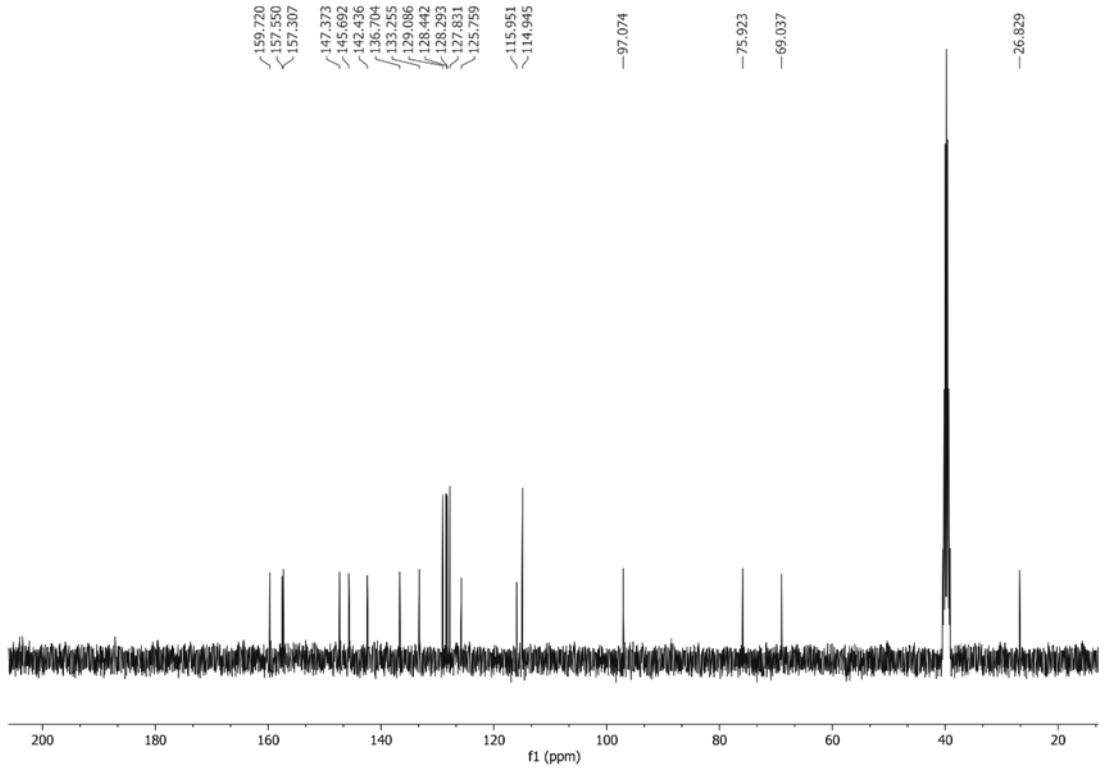


Figure S21. ^{1}H-NMR spectrum of compound **8b**.

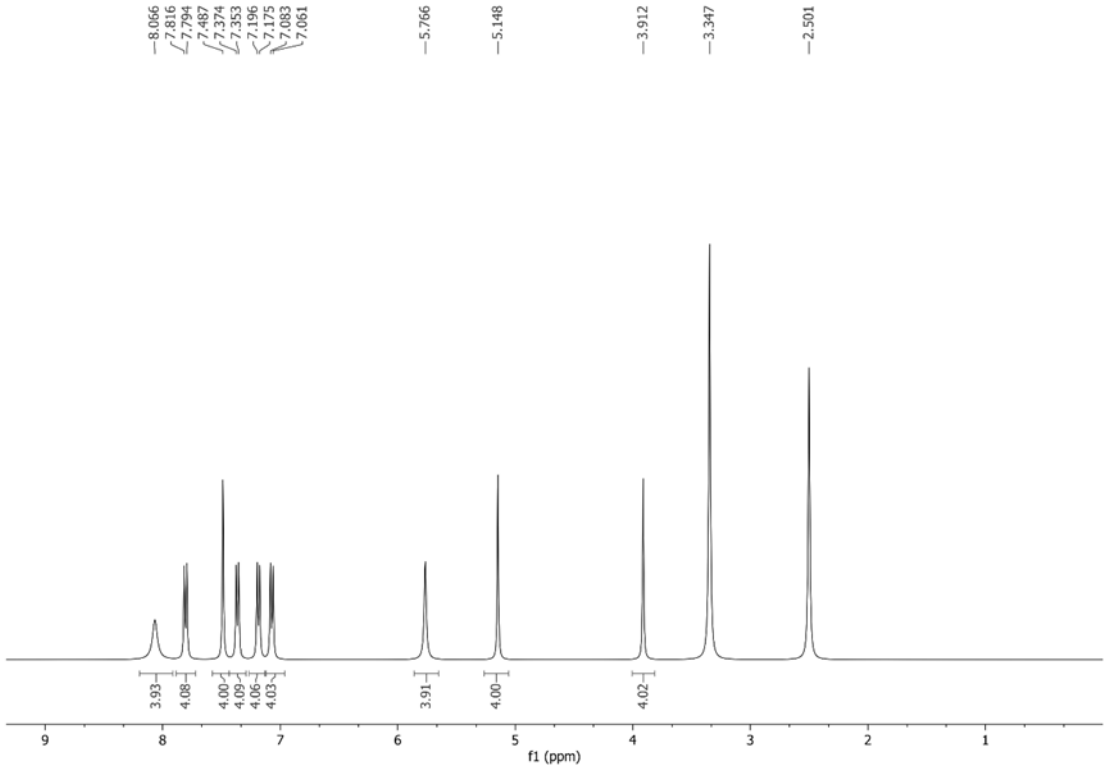


Figure S22. ^{13}C-NMR spectrum of compound **8b**.

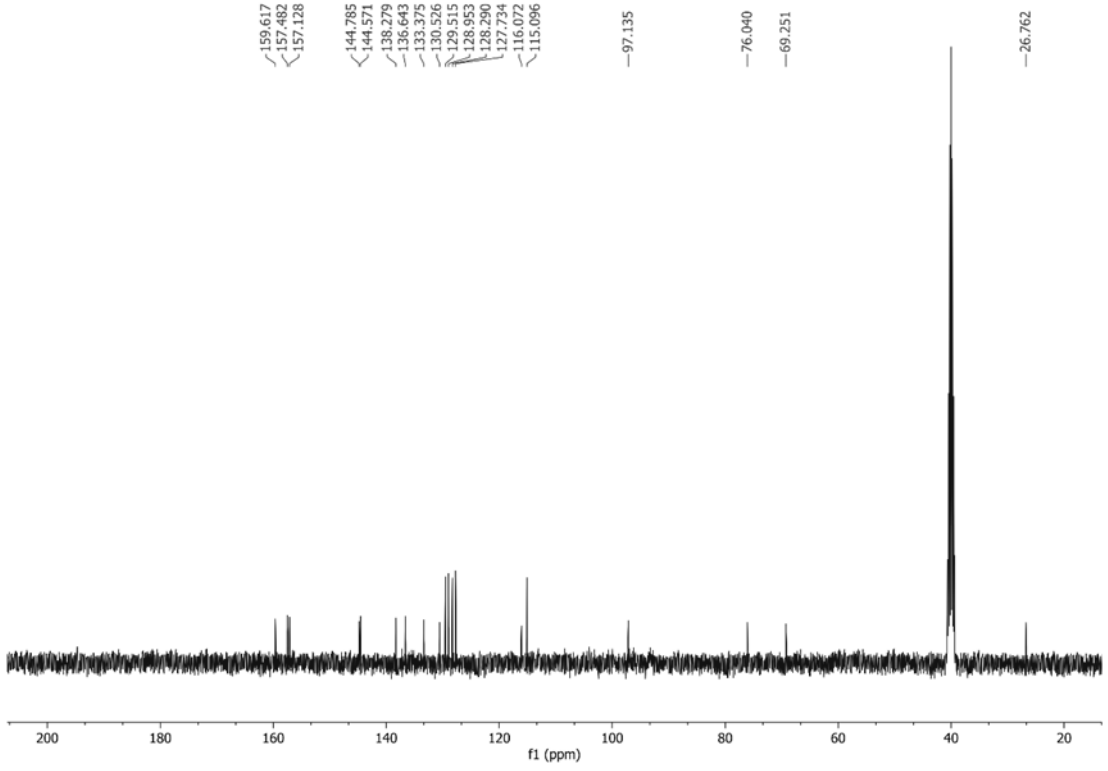


Figure S23. ^{1}H-NMR spectrum of compound **8c**.

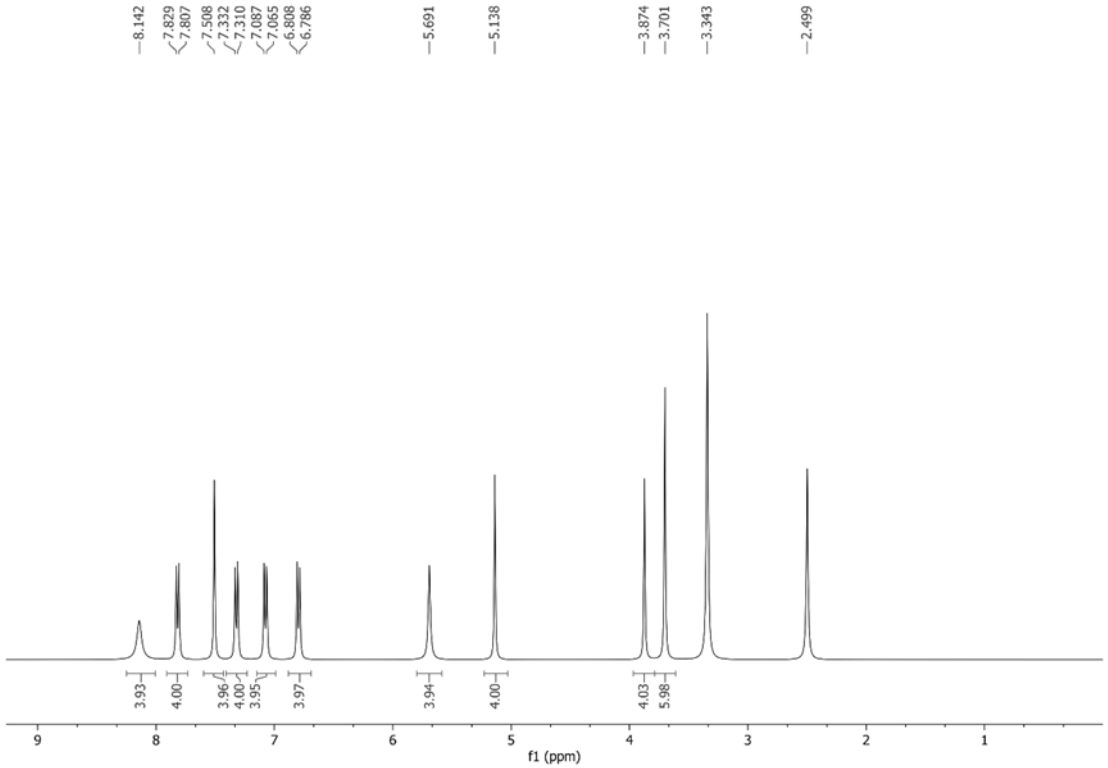


Figure S24. ^{13}C-NMR spectrum of compound **8c**.

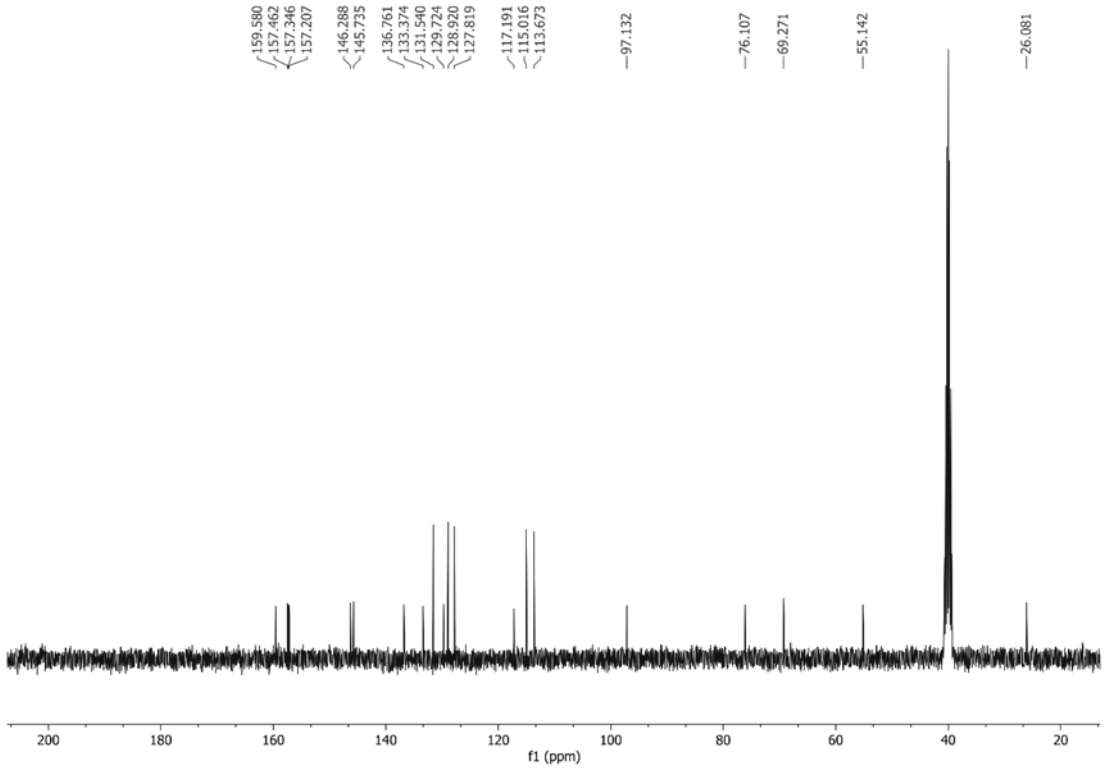


Figure S25. ^{1}H-NMR spectrum of compound **10a**.

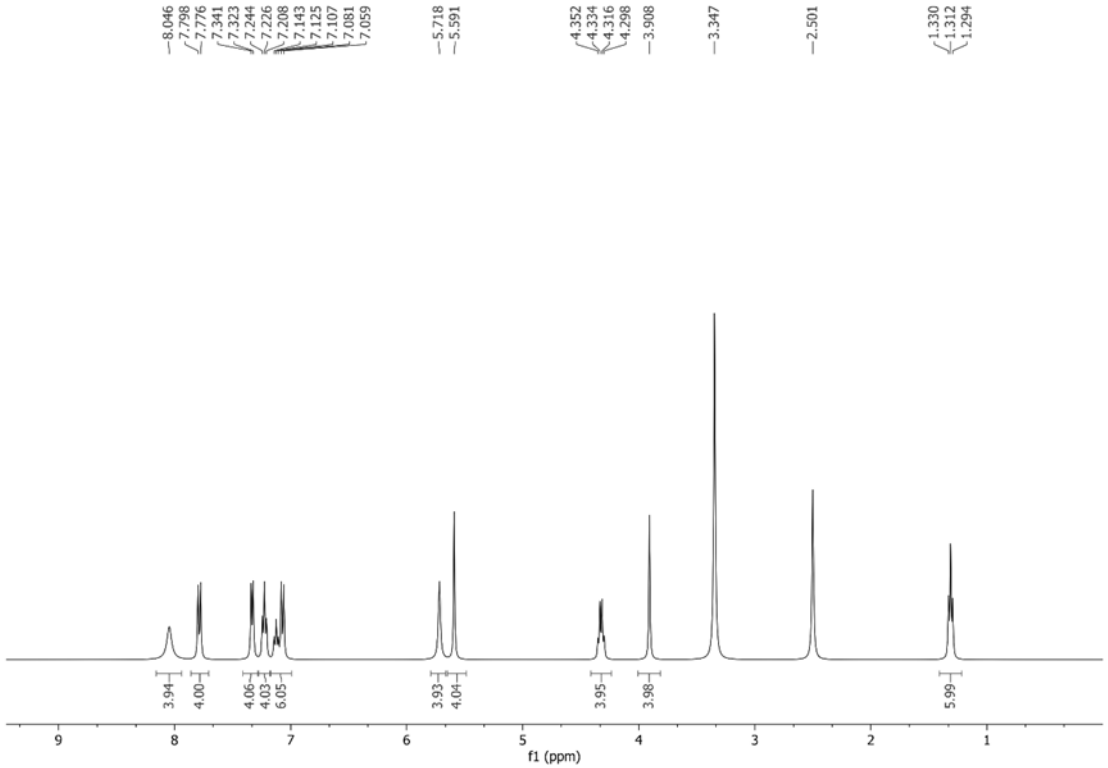


Figure S26. ^{13}C-NMR spectrum of compound **10a**.

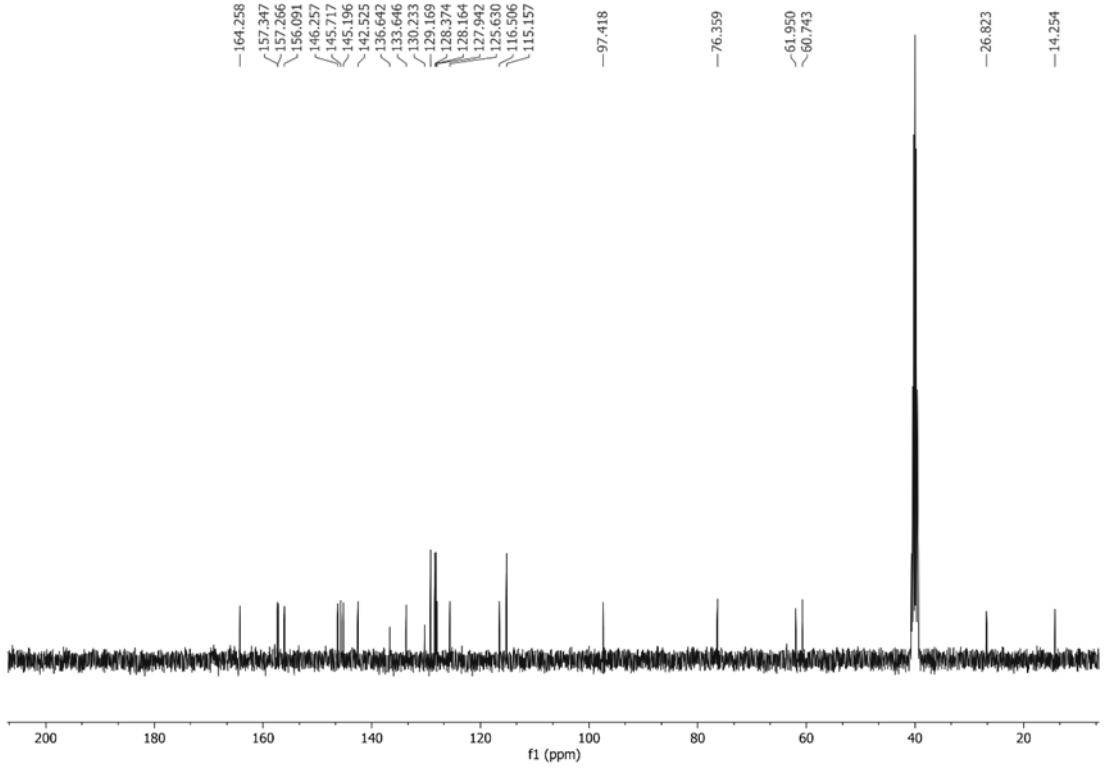


Figure S27. ^{1}H-NMR spectrum of compound **10b**.

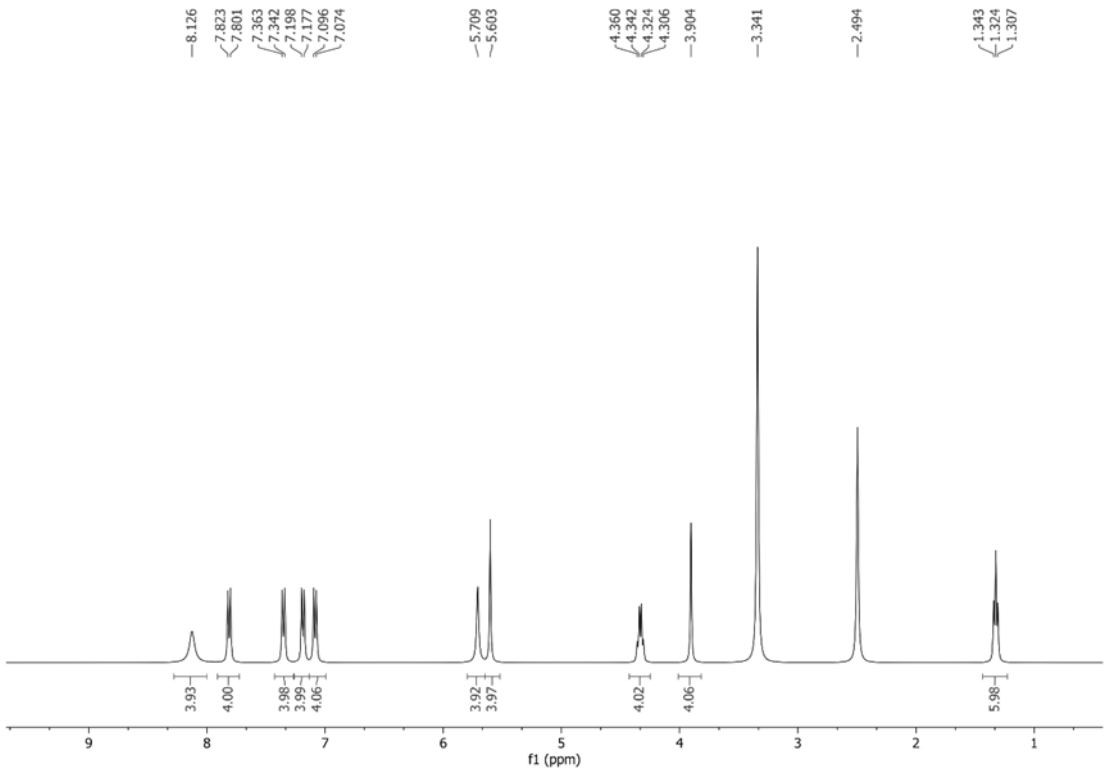


Figure S28. ^{13}C-NMR spectrum of compound **10b**.

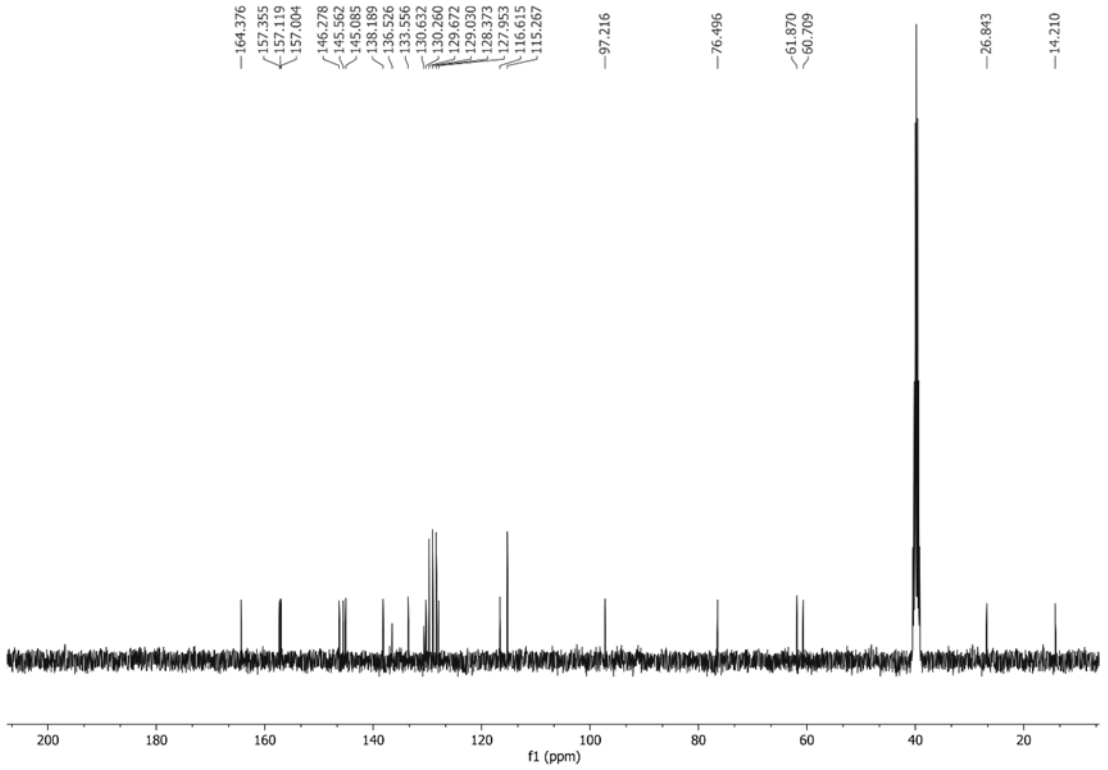


Figure S29. ^{1}H-NMR spectrum of compound **10c**.

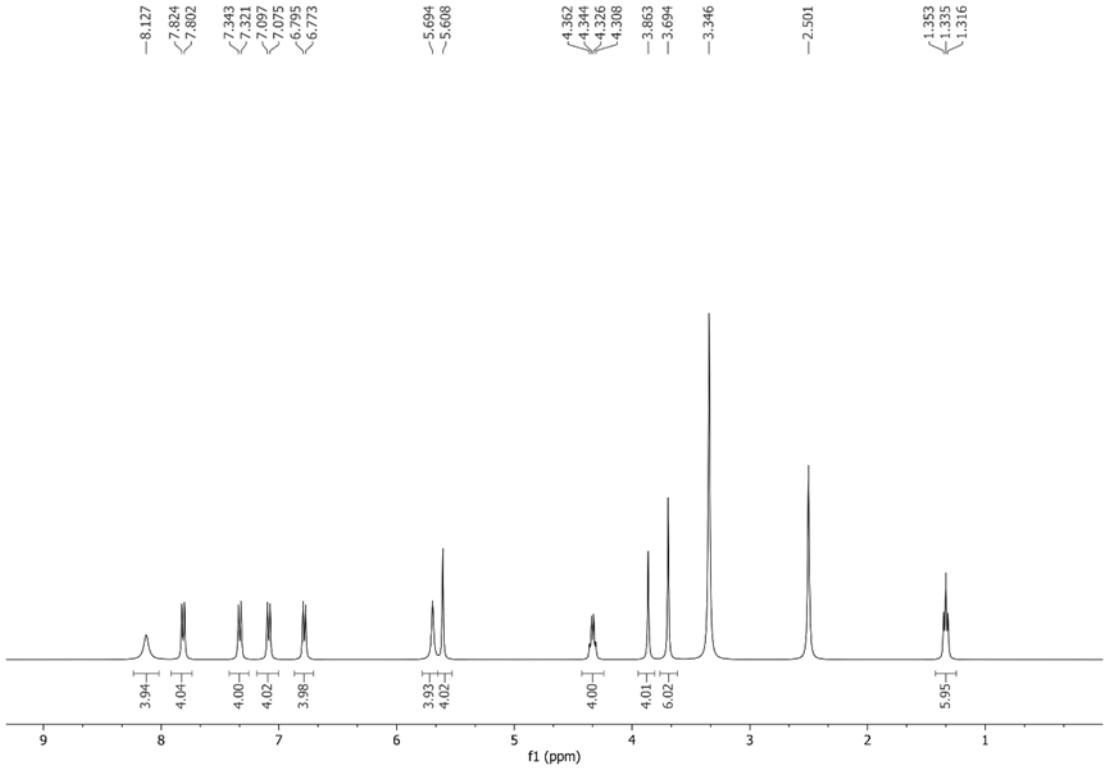


Figure S30. ^{13}C-NMR spectrum of compound **10c**.