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SUPPLEMENTARY MATERIAL TO

In vitro antioxidant activity of nicotinic acid hydrazides: Experimental and theoretical study

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1. STRUCTURAL CHARACTERIZATION OF COMPOUNDS NCA-1- NCA-7

1.1. (NcA-1) 1-[(2-Hydroxyphenyl) methyl]-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a slightly yellow powder was obtained with a yield of 72% and a melting point of 48.9 °C.

Elem. anal.: Measured on $C_{14}H_{13}N_5O_2S$ (M_w = 315.35 gmol^{-1}): C, 53.32; H, 4.16; N, 22.21; O, 10.15; S, 10.17%. Found: C, 53.36; H, 4.14; N, 22.28; O, 10.08; S, 10.17 %. IR(KBr, cm^{-1}) ν_{max}: 3054.48 (N-H stretching vibration of amide group), 1686.08 (C=O stretching vibration of amide group), 1489.52 (C=S vibration), 1583.23 (C=N vibration).^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 6.84–6.91 (m, 2H, H-C13, H-C11); 7.25 (t, 1H, J = 8.0 Hz, H-C12); 7.58 (s, 1H, H-C3); 8.08 (d, 1H, J = 8.0 Hz, H-C14); 8.26 (d, 1H, J = 8.0 Hz, H-C4), 8.45 (s, 1H, H-C2); 8.78 (d, 1H, J = 4.0 Hz, H-C8); 9.08 (s, 1H, H-C1), 9.99 (brs, 1H, H-N3); 10.35 (brs,1H, H-N2); 10.78 (s, 1H, H-C10(OH)), 11.84 (s, 1H, H-N4). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm, TMS): 116.58 (C11); 119.69 (C9); 120.66 (C13); 124.11 (C3); 127.49 (C14); 129.16 (C5); 131.81 (C12); 135.74 (C4); 141.03 (C8); 148.97 (C2); 152.85 (C1); 157.07 (C10); 164.78 (C=O); 179.43 (C=S), $[M-H]^-$, m/z 314.28.

1.2. (NcA-2) 1-(2-Pyridinylmethylene)-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a slightly yellow powder was obtained with a yield of 52% and a melting point of 207.6 °C.

Elem. anal.: Measured on $C_{13}H_{12}N_6OS$ (M_w = 300.34 gmol^{-1}): C, 51.99; H, 4.03; N, 27.98; O, 5.33; S, 10.67%. Found: C, 52.00; H, 4.02; N, 27.91; O, 5.43; S, 10.64%. IR (KBr, cm^{-1}) ν_{max}: 3145.36 (N-H stretching vibration of amide group), 1669.80 (C=O stretching vibration of amide group), 1470.97 (C=S vibration), 1590.27 (C=N vibration). ^{1}H NMR (400 MHz, DMSO-

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d_6) δ(ppm)): 7.38–7.42 (m, 1H, H-C8); 7.58–7.59 (m, 1H, H-C12); 7.86–7.90 (m, 1H, H-C3), 8.08–8.44 (m, 3H, H-C11, C10, H-C4); 8.56–8.60 (m, 1H, H-C13); 8.74–8.78 (m, 1H, H-C2); 9.10 (s, 1H, H-C1), 10.86 (s, 1H, H-N3), 11.80 (s, 1H, H-N2), 12.14 (s, 1H, H-N4). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 121.04 (C10); 124.19 (C3); 124.81 (C12); 129.10 (C5); 135.80 (C4); 137.15 (C11); 144.00 (C1); 148.93 (C2); 149.80 (C13); 152.90 (C8); 153.59 (C9); 164.80 (C=O); 179.94 (C=S), $[M-H]^-$, m/z 299.17.

1.3. (NcA-3) 1-[1-(2-Pyridinyl)ethylidene]-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a yellow powder was obtained with a yield of 84% and a melting point of 200.5 °C.

Elem. anal.: Measured on $C_{14}H_{14}N_6OS$ (M_w = 314.37 gmol^{-1}): C, 53.49; H, 4.49; N, 26.73; O, 5.09; S, 10.20%. Found: C, 53.45; H, 4.54; N, 26.74; O, 5.08; S, 10.20%. IR (KBr, cm^{-1}) ν_{max}: 3181.03 (N-H stretching vibration of amide group), 1636.07 (C=O stretching vibration of amide group), 1466.72 (C=S vibration), 1596.43 (C=N vibration).^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 2.45 (d, 3H, J = 24.0 Hz, H-C9); 7.42 (t, 1H, J = 4.0 Hz, H-C3), 7.58 (t, 1H, J = 4.0 Hz, H-C13); 7.83 (t, 1H, J = 8.0 Hz, H-C12); 8.28 (d, 1H, J = 8.0 Hz, H-C11); 8.59–8.61 (m, 2H, H-C4,H-C14); 8.79 (d, 1H, J = 4.0 Hz, H-C2); 9.10 (s, 1H, H-C1); 10.48 (s, 1H-N4); 10.86 (s, 1H, H-N3); 10.88 (s, 1H, H-N2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 12.71 (C9); 121.70 (C12); 124.14 (C3); 124.61 (C10); 129.15 (C5); 135.76 (C4); 136.78 (C11); 148.96 (C1, C2); 150.11 (C14); 152.87 (C13); 154.93 (C8); 164.71 (C=O); 180.62 (C=S), $[M-H]^-$, m/z 313.28.

1.4. (NcA-4) 1-(8-Quinolylmethylene)-5-(pyridine-3-carbonyl) Dyhidrazide Thiocarbonyl Acid

After synthesis, a brown powder was obtained with a yield of 73% and a melting point of 191.8 °C.

Elem. anal.: Measured on $C_{17}H_{14}N_6OS$ (M_w = 350.40 gmol^{-1}): C, 58.27; H, 4.03; N, 23.98; O, 4.57; S, 9.15%. Found: C, 58.21; H, 4.11; N, 23.96; O, 4.60; S, 9.12%.

IR (KBr, cm^{-1}) ν_{max}: 3167.12 (N-H stretching vibration of amide group), 1667.82 (C=O stretching vibration of amide group), 1473.60 (C=S vibration), 1592.34 (C=N vibration). ^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 7.54–7.63 (m, 2H, H-C3, H-C14), 7.70 (t, 1H, J = 8.0 Hz, H-C11); 8.08 (d, 1H, J = 4.0 Hz, H-C4); 8.27 (d, 1H, J = 8.0 Hz, H-N10); 8.44 (d, 1H, J = 8.0 Hz, H-C12); 8.70–8.81 (m, 2H, H-C8, H-C13); 8.99 (s, 1H, H-C2); 9.06 (s, 1H, H-C15); 9.42 (s, 1H, H-C1); 10.56 (s, 1H, H-N3); 10.83 (s, 1H, H-N2) ; 12.15 (s, 1H, H-N4).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 122.31 (C11); 124.15 (C14); 126.89 (C3, C12a); 128.46 (C10); 129.16 (C12); 130.53 (C5); 131.38 (C9); 135.76 (C4); 137.12 (C13); 140.74 (C8); 145.89 (C15a); 148.97 (C2); 150.92 (C1); 152.87 (C15); 164.82 (C=O); 179.76 (C=S), $[M-H]^-$, m/z 349.17.

1.5. (NcA-5) 1-(2-Quinolylmethylene)-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a slightly yellow powder was obtained with a yield of 53% and a melting point of 174.7 °C.

Elem. anal.: Measure on $C_{17}H_{14}N_6OS$ (M_w = 350.40 gmol^{-1}):C, 58.27; H, 4.03; N, 23.98; O, 4.57; S, 9.15%. Found: C, 58.20; H, 4.10; N, 23.93; O, 4.65; S, 9.12%. IR (KBr, cm^{-1}) ν_{max}: 3191.56 (N-H stretching vibration of amide group), 1668.0 (C=O stretching vibration of amide group), 1501.50 (C=S vibration), 1596.50 (C=N vibration). ^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 7.44–7.66 (m, 2H, H-C3, H-C13); 7.80 (t, 1H, J = 8.0 Hz, H-C8); 8.02–8.06 (m, 2H,

H-C10, H-C14); 8.30–8.31 (m, 2H, H-C12, H-C15); 8.42 (d, 1H, J = 6.0 Hz, H-C4), 8.61 (dd, 1H, H-C11); 8.79 (d, 1H, J = 4.0 Hz, H-C2), 9.12 (s, 1H, H-C1); 10.75 (s, 1H, H-N3), 10.91 (s, 1H, H-N2); 12.30 (s, 1H, H-N4). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 118.77 (C10); 124.18 (C3); 127.74 (C13); 128.39 (C12, C15); 129.31 (C5, C14); 130.45 (C11a); 135.75 (C4); 136.79 (C11); 144.14 (C8); 147.84 (C2); 148.96 (C1); 152.94 (C9, C15a); 164.81 (C=O), 179.95 (C=S), $[M-H]^-$, m/z 349.32.

1.6. (NcA-6) 1-[(8-Hydroxy)-2-quinolylmethylene]-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a brown powder was obtained with a yield of 81% and a melting point of 210 °C.

Elem. anal.: Measured on $C_{17}H_{14}N_6O_2S$ (M_w = 366.40 gmol^{-1}): C, 55.73; H, 3.85; N, 22.94; O, 8.73; S, 8.75%. Found: C, 55.80; H, 3.78; N, 22.99; O, 8.68; S, 8.75%. IR (KBr, cm^{-1}) ν_{max}: 3200.65 (N-H stretching vibration of amide group), 1658.31 (C=O stretching vibration of amide group), 1455.72 (C=S vibration), 1590.32 (C=N vibration).

^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 7.13 (d, 1H, J = 8.0 Hz, H-C14); 7.42–7.46 (m, 2H, H-C3, H-C8); 7.58–7.62 (m, 1H, H-C13); 8.33–8.41 (m, 3H, H-C4, H-C10, H-C12); 8.57 (d, 1H, J = 8.0 Hz, H-C11); 8.80 (d, 1H, J = 4.0 Hz, H-C2); 9.12 (s, 1H, H-C1) 9.90 (s, 1H, H-C15(OH)), 10.72 (s, 1H, H-N2), 10.91 (s, 1H, H-N3); 12.35 (s, 1H, H-N4).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 112.62 (C14); 118.23 (C12); 119.09 (C10); 124.18 (C3); 128.70 (C13); 129.09 (C5); 129.34 (C11a); 135.76 (C4); 136.64 (C11); 138.68 (C15a); 144.00 (C8); 148.96 (C2); 152.09 (C1); 152.93 (C9), 153.93 (C15); 164.83 (C=O); 179.95 (C=S), $[M-H]^-$, m/z 365.37, $[M\text{-}OH]^-$, m/z 349.30.

1.7. (NcA-7) 1-(Phenyl-methylen)-5-(pyridine-3-carbonyl) Dihydrazide Thiocarbonyl Acid

After synthesis, a white powder was obtained with a yield of 64% and a melting point of 191 ºC.

Elem. anal.: Measured on $C_{14}H_{13}N_5OS$ (M_w = 299.35 gmol^{-1}): C, 56.17; H, 4.38; N, 23.40; O, 5.34; S, 10.71%Found: C, 56.20; H, 4.47; N, 23.63; O, 5.35; S, 10.35%. IR (KBr, cm^{-1}) ν_{max}: 3211.24 (N-H stretching vibration of amide group), 1660.20 (C=O stretching vibration of amide group), 1449.19 (C=S vibration), 1600.27 (C=N vibration).

^{1}H NMR (400 MHz, DMSO-d_6, δ(ppm)): 7.44 (s, 3H, H-C11, H-C12, H-C13); 7.58 (t, 1H, J = 4.0 Hz, H-C3); 7.91 (s, 1H, H-C10); 7.92 (s, 1H, H-C14); 8.13 (s, 1H, H-C4); 8.27 (d, 1H, J = 8.0 Hz, H-C8); 8.78 (s, 1H, H-C2); 9.09 (s, 1H, H-C1); 10.44 (s, 1H, H-N3); 10.81 (s, 1H, H-N2); 11.93 (s, 1H, H-N4).

^{13}C NMR (100 MHz, DMSO-d_6, δ ppm, TMS): 124.15 (C3); 128.07 (C11, C13); 129.14 (C10, C14, C5); 130.54 (C12); 134.49 (C9); 135.76 (C4); 143.88 (C8); 148.96 (C2); 152.86 (C1); 164.80 (C=O); 179.69 (C=S), $[M-H]^-$, m/z 298.17.

IR spectrometry showed characteristic peaks of amide bond, thione form, and carbonyl group. Stretching vibrations of the amide group are presented around 3054–3211 cm^{-1}. Characteristic strong absorption bands between 1583 and 1600 cm^{-1} are attributed to the azomethine ν(C=N) stretching vibration. The stretching band of amide group ν(C=O) shows absorption between 1636 and 1686 cm^{-1}. Group characteristics for thiocarbohydrazide are shown between 1449 and 1501 cm^{-1} as the form ν(C=S).

^{1}H NMR spectroscopy showed characteristic peaks of nicotinic amides for N-H hydrogens according to δ 10.48–12.30 for N4 atom, δ 9.99–10.87 for N3 atom and δ 10.35–11.8 for N2 atom. Near them is a singlet for O-H atom on δ 9.88–10.78 for NcA-1 and NcA-6. Also, one of the characteristic peaks is azomethine (-CH=N), which could be seen between δ 7.40 and 8.79

with the exception of NcA-3. NcA-3 has a methyl group (-CH_3) connected to azomethine moiety, giving a singlet on δ 2.45. Aromatic rings from nicotinic acid, phenol, and quinoline nuclei are shown between δ 6.87 and 9.42.

^{13}C NMR spectrum showed characteristic peaks for all seven compounds. Thione carbon (C=S) is present between 179.43 and 180.62 ppm, carbonyl carbon (C=O) is shown at 164.71–164.83 ppm, and azomethine carbon (C=N) between 140.74 and 154.93 ppm. NcA-3 has a characteristic methyl group at 12.71 ppm. The carbon atom attached to the O-H group at NcA-1 and NcA-6 is from 127.49 to 153.93 ppm. Also, the carbon atoms resulting from phenyl and quinoline groups are shown between 112.62 and 157.07 ppm.

The MS analysis was used for the identification of analytes present in the synthesized nicotinamides. A mass spectrum was obtained for each sample. Mass spectra have shown that masses of the obtained analytes correspond to deprotonated $[M-H]^-$ ions of nicotinamide derivatives, and those ions were the most intense in the obtained spectra.

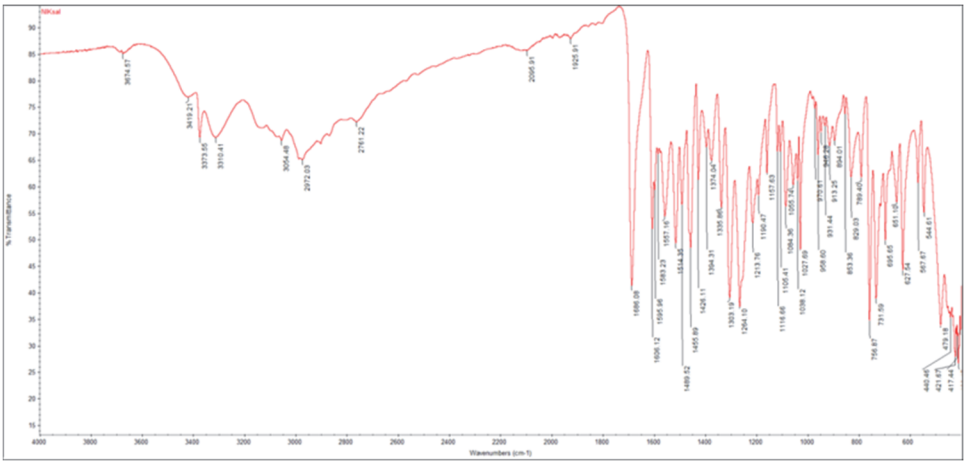

Fig. S1. FTIR spectrum of NcA-1

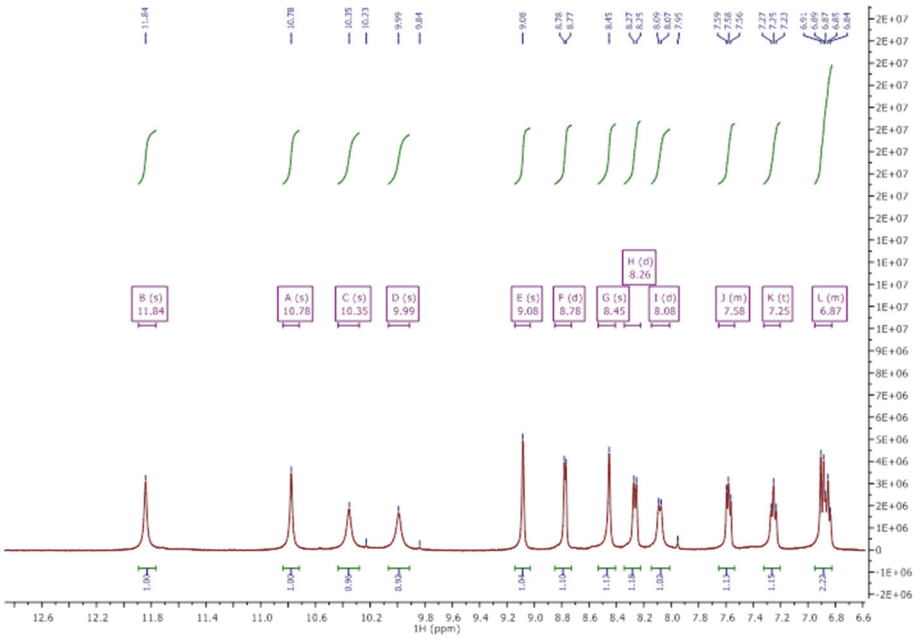

Fig. S2. ^{1}H spectrum of NcA-1

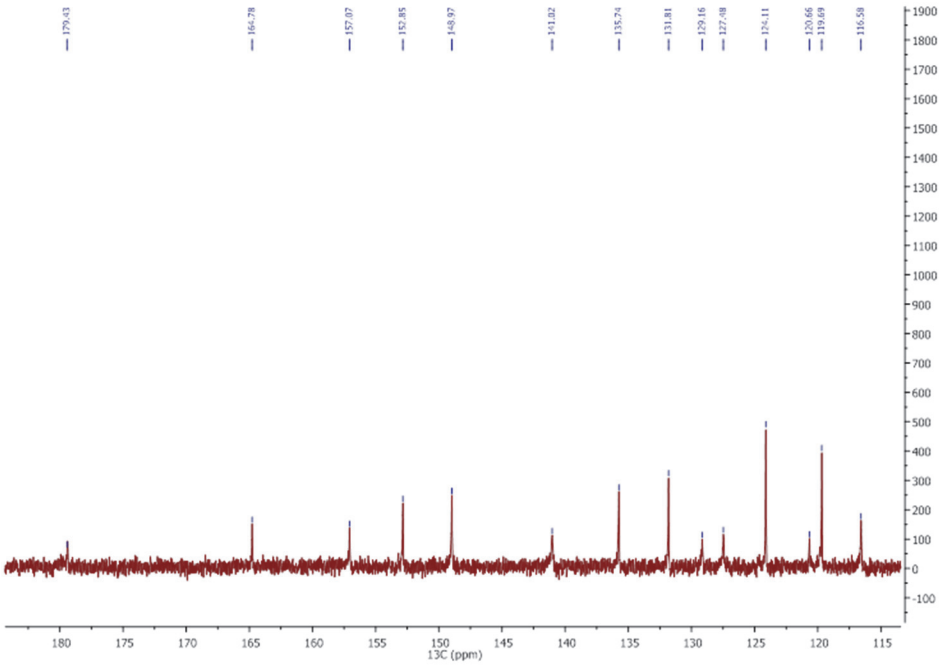

Fig. S3. ^{13}C spectrum of NcA-1

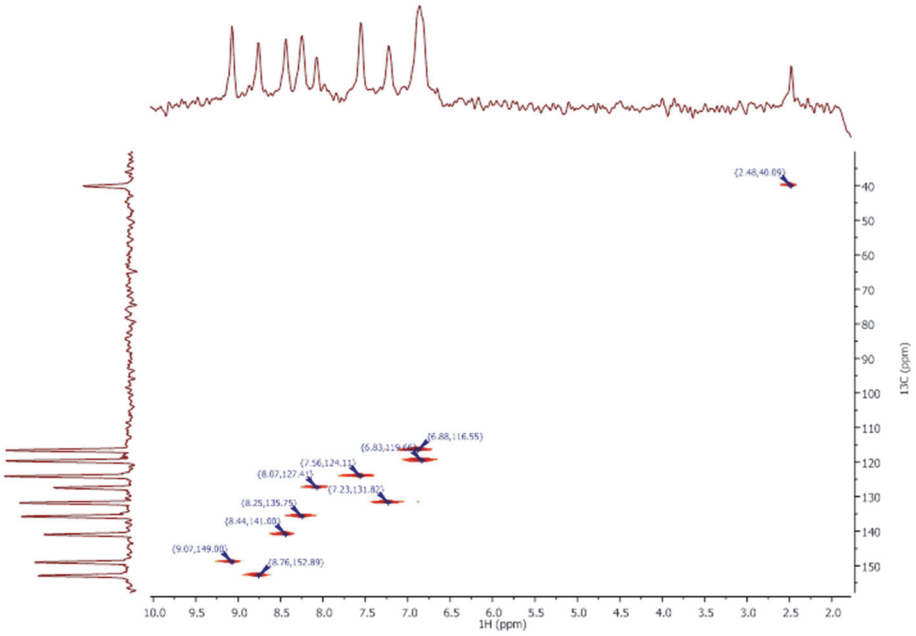

Fig. S4. HSQC spectrum of NcA-1

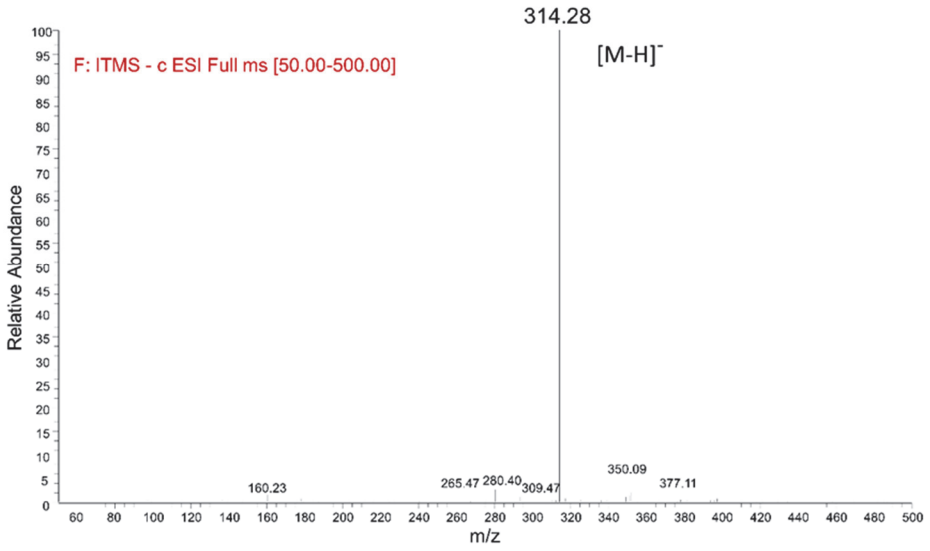

Fig. S5. MS spectrum of compound NcA-1

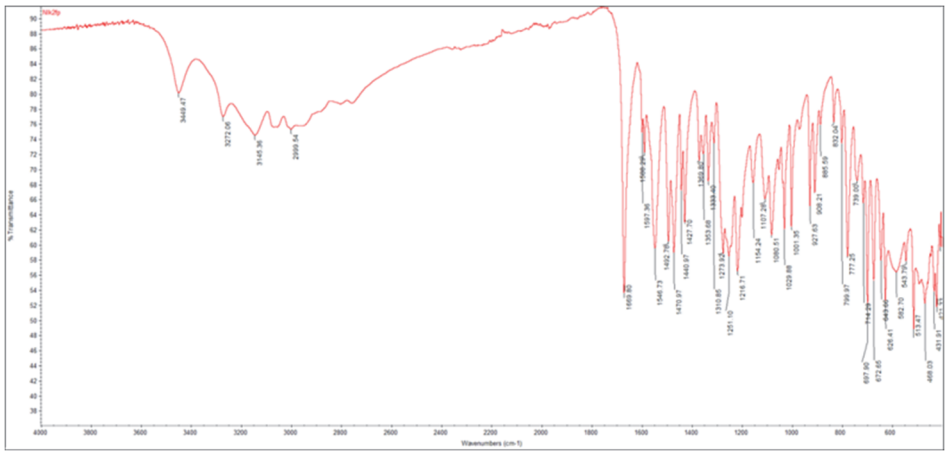

Fig. S6. FTIR spectrum of NcA-2

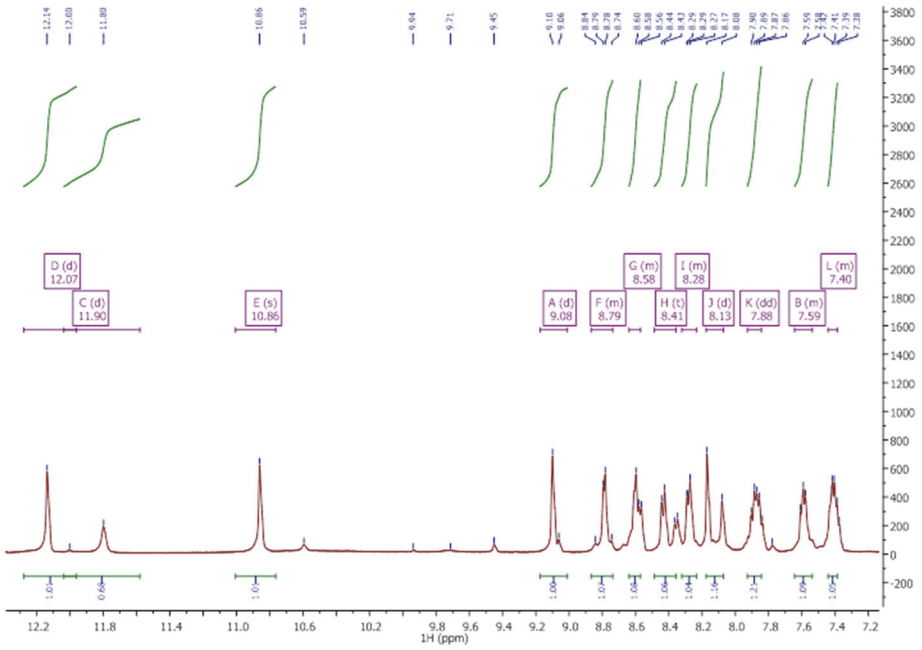

Fig. S7. ^{1}H spectrum of NcA-2

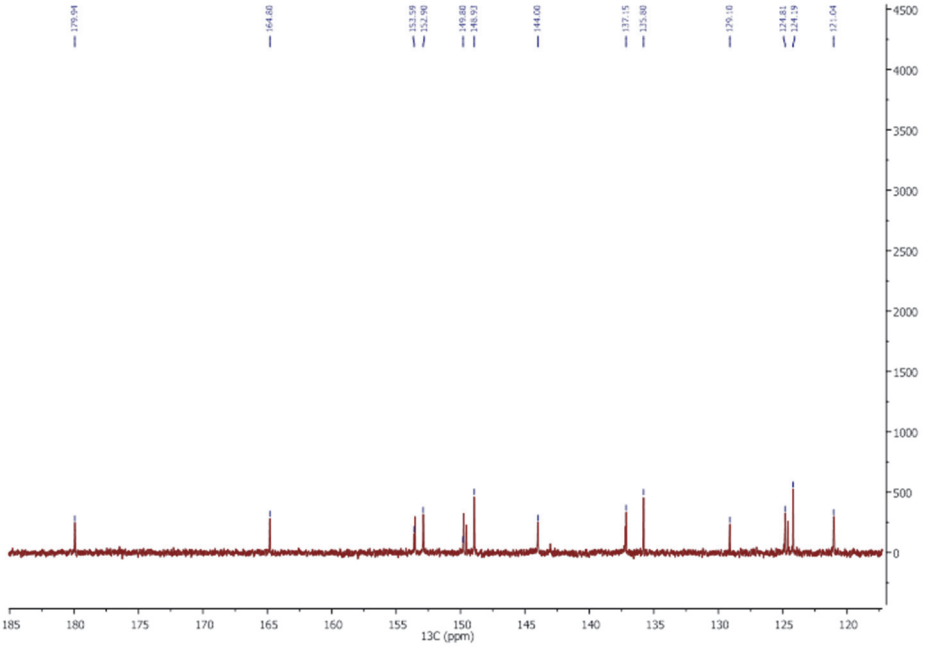

Fig. S8. ^{13}C spectrum of NcA-2

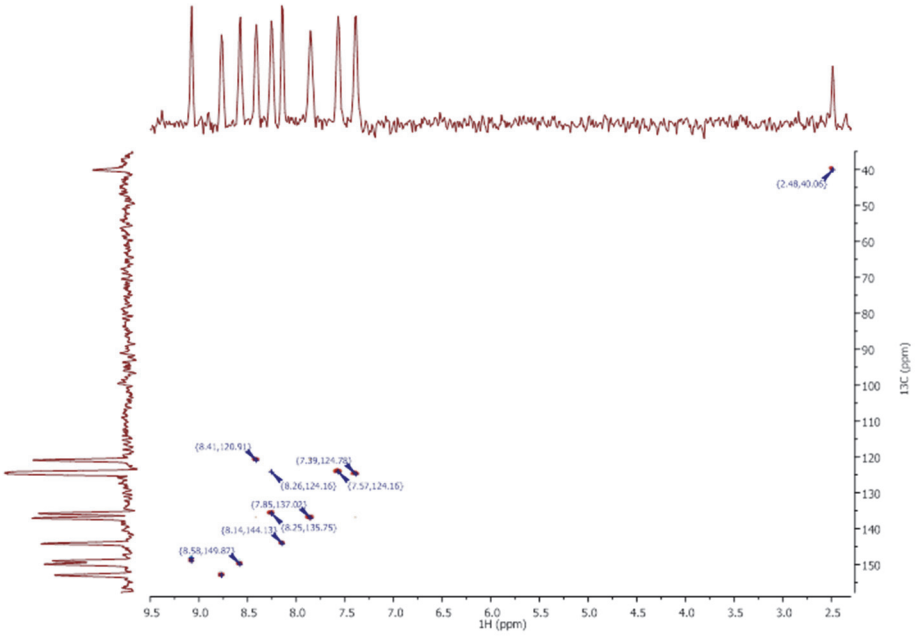

Fig. S9. HSQC spectrum of NcA-2

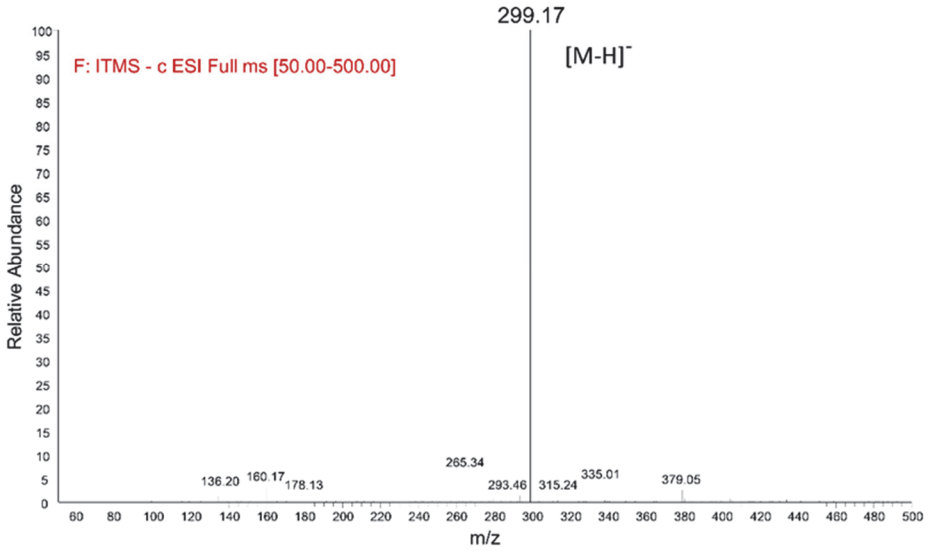

Fig. S10. MS spectrum of compound NcA-2

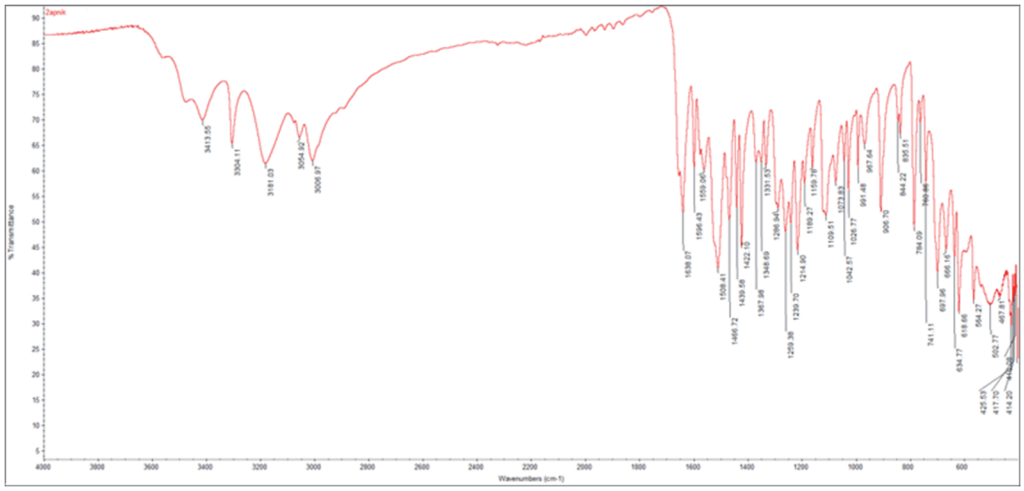

Fig. S11. FTIR spectrum of NcA-3

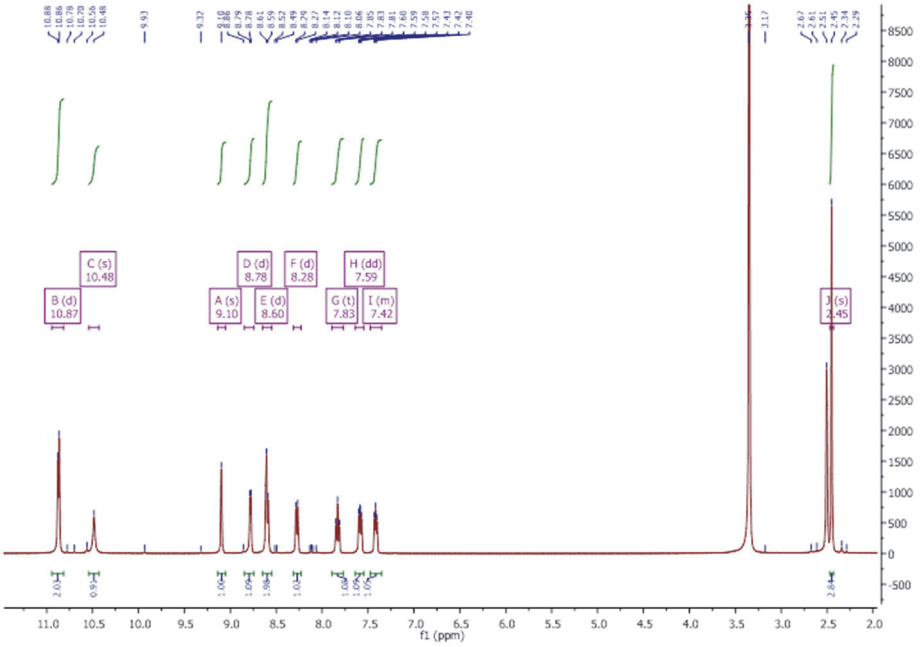

Fig. S12. ^{1}H spectrum of NcA-3

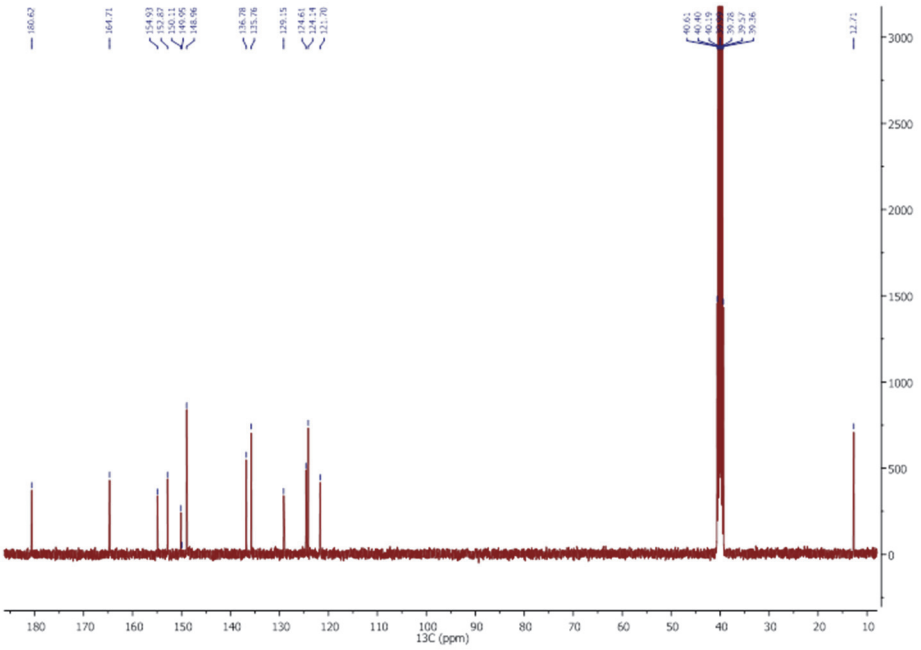

Fig. S13. ^{13}C spectrum of NcA-3

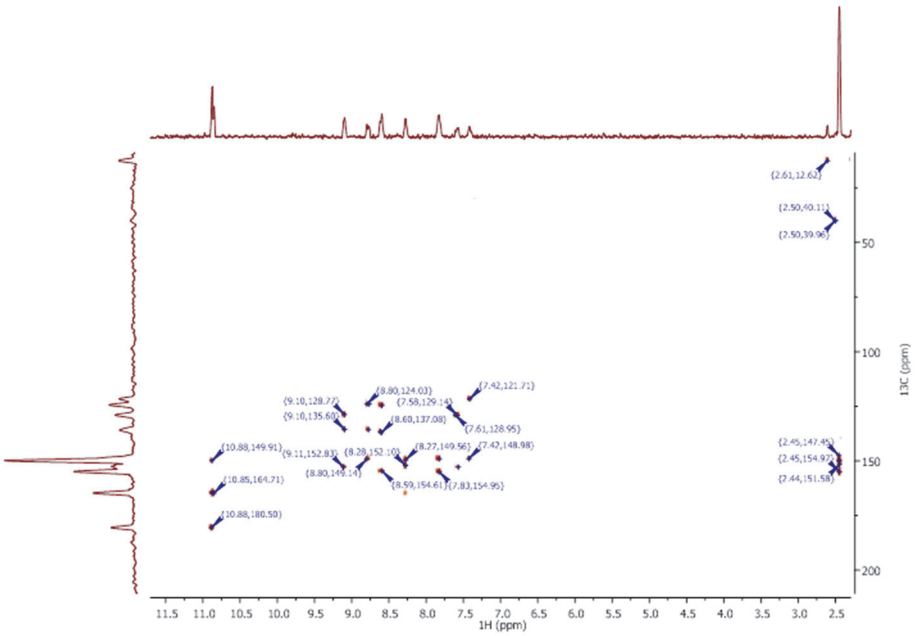

Fig. S14. HSQC spectrum of NcA-3

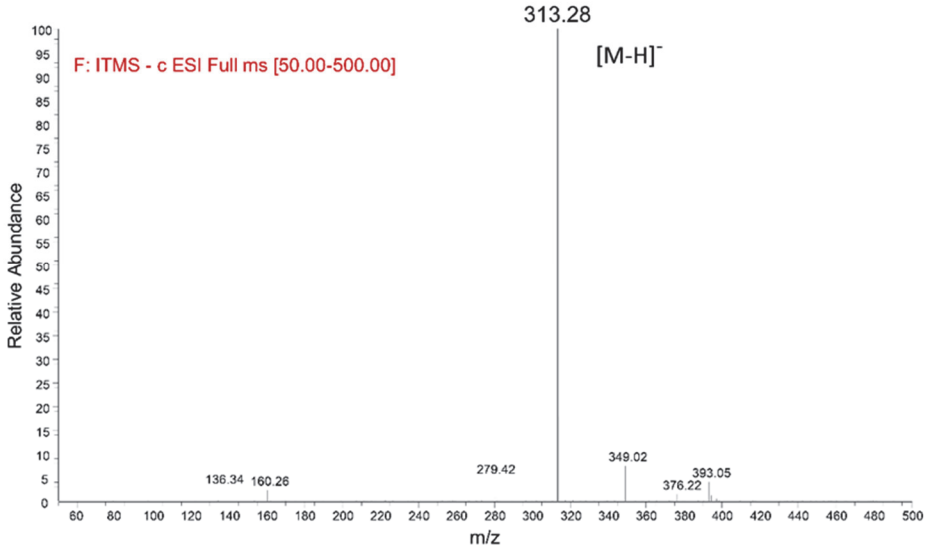

Fig. S15. MS spectrum of compound NcA-3

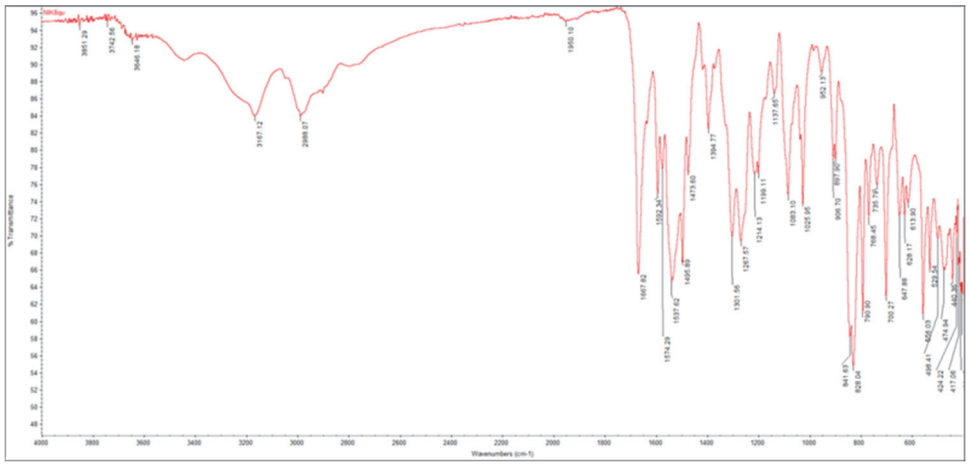

Fig. S16. FTIR spectrum of NcA-4

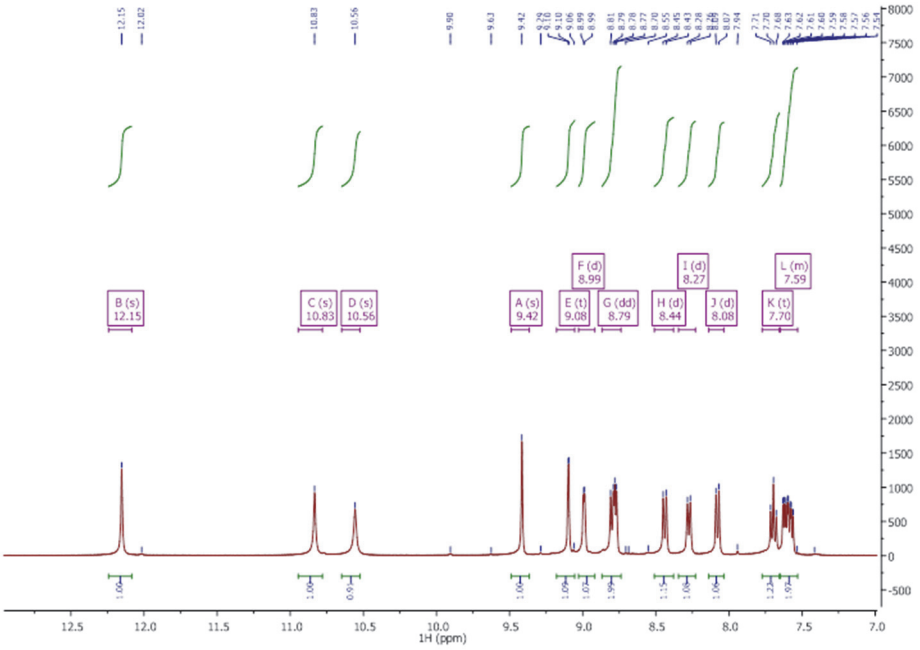

Fig. S17. ^{1}H spectrum of NcA-4

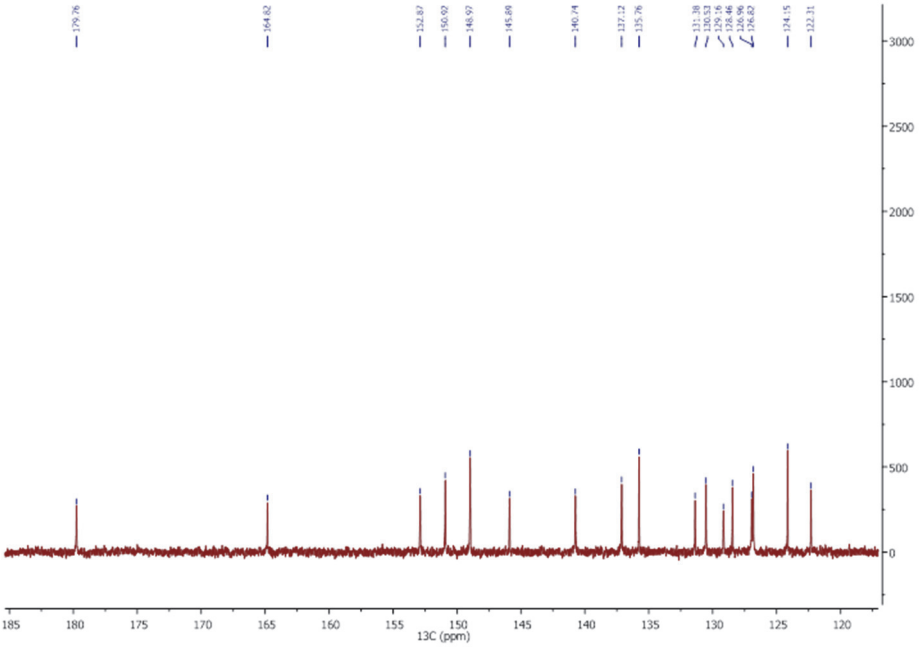

Fig. S18. ^{13}C spectrum of NcA-4

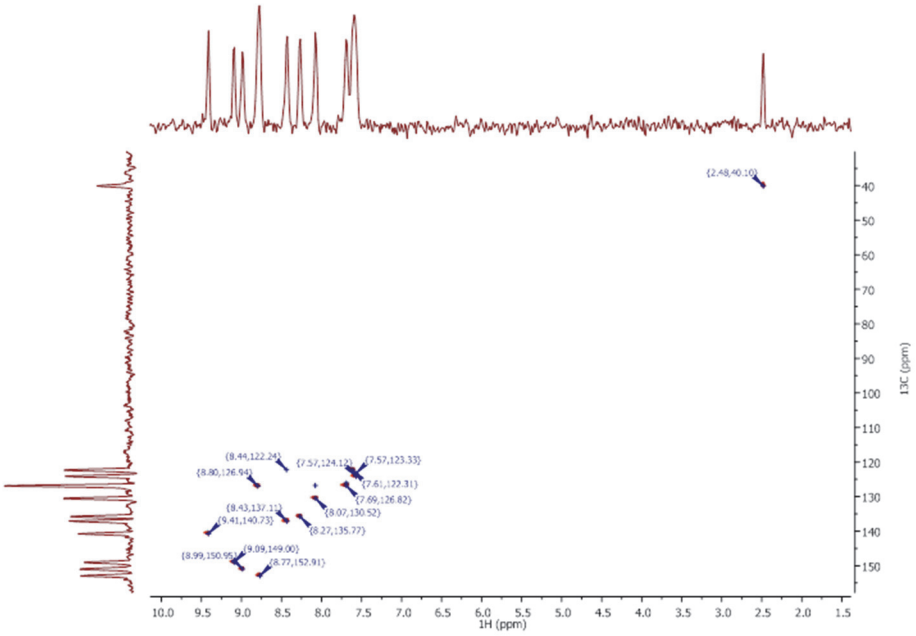

Fig. S19. HSQC spectrum of NcA-4

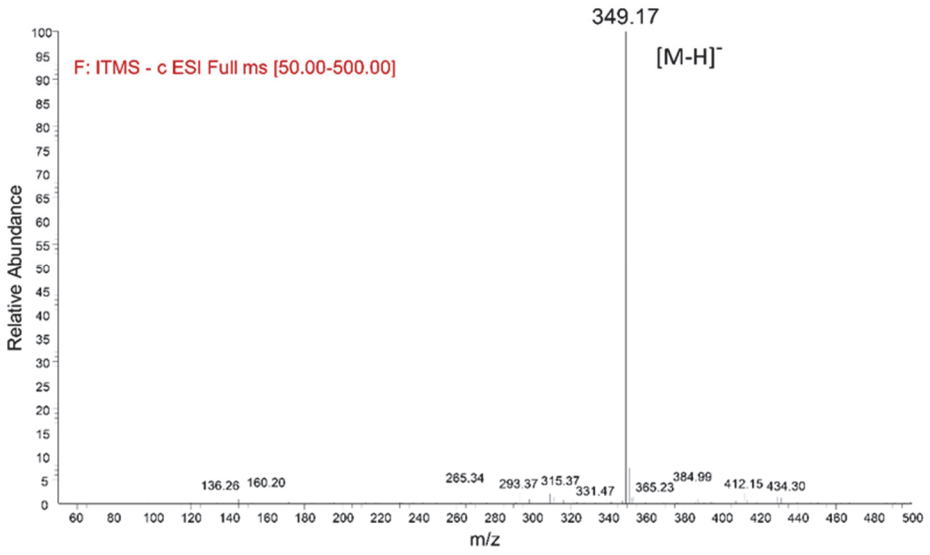

Fig. S20. MS spectrum of compound NcA-4

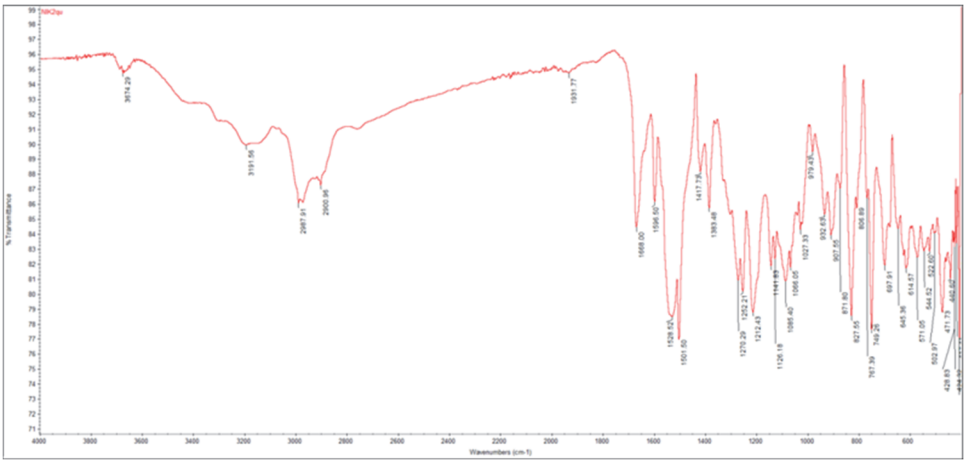

Fig. S21. FTIR spectrum of NcA-5

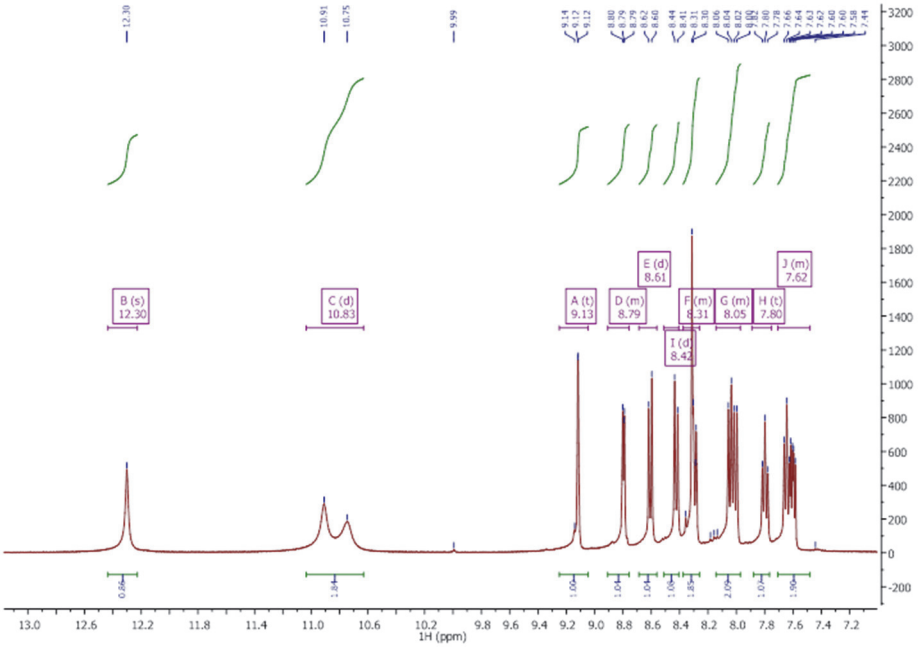

Fig. S22. ^{1}H spectrum of compound NcA-5

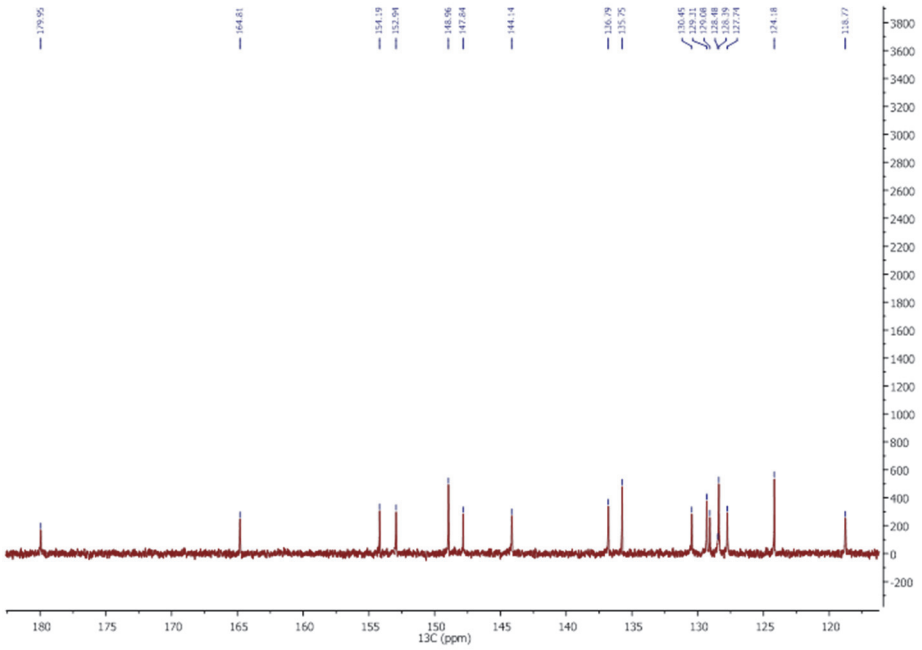

Fig. S23. ^{13}C spectrum of NcA-5

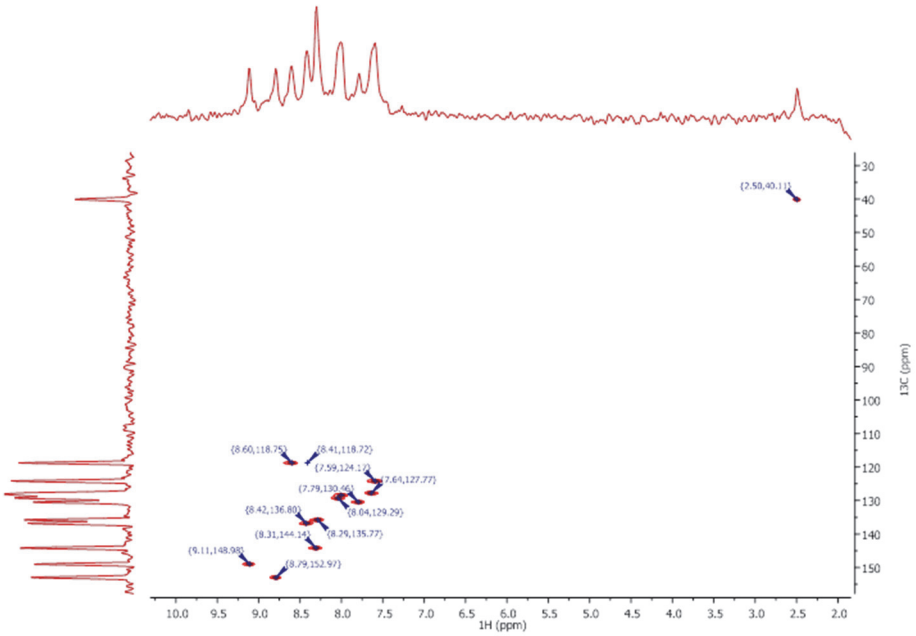

Fig. S24. HSQC spectrum of NcA-5

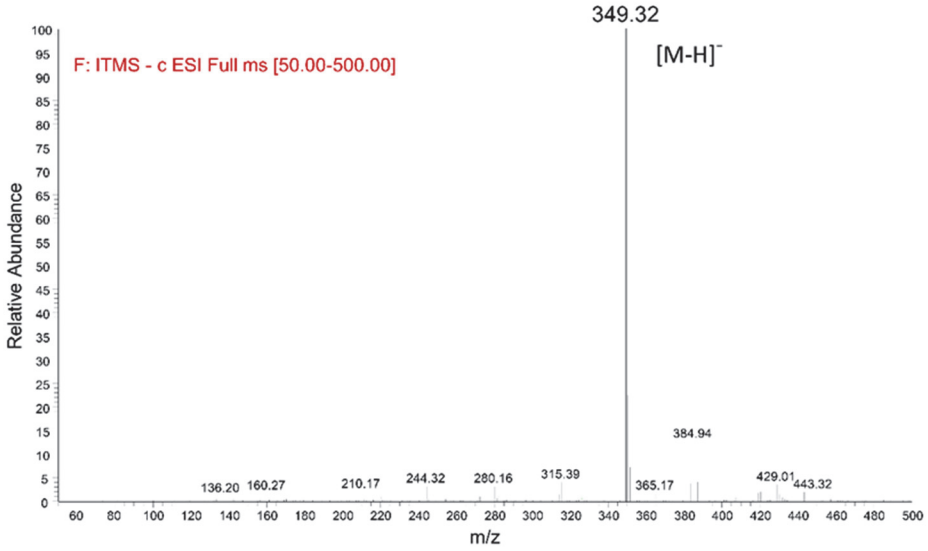

Fig. S25. MS spectrum of compound NcA-5

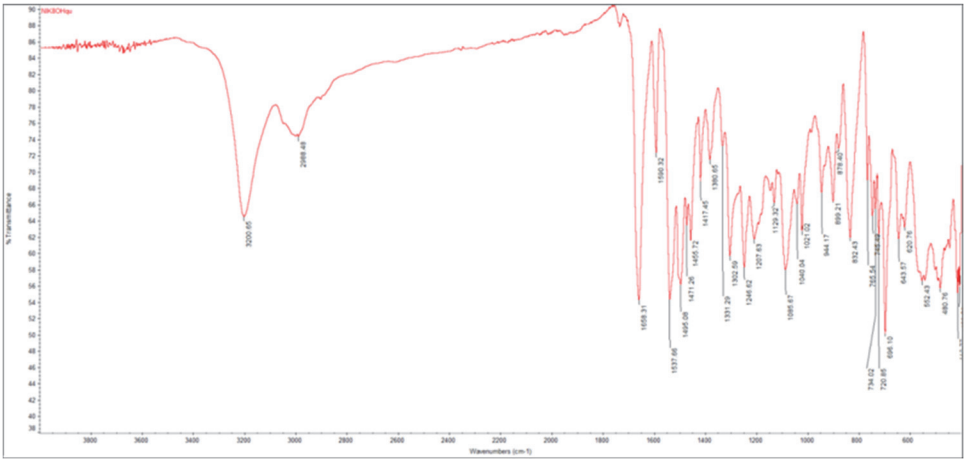

Fig. S26. FTIR spectrum of NcA-6

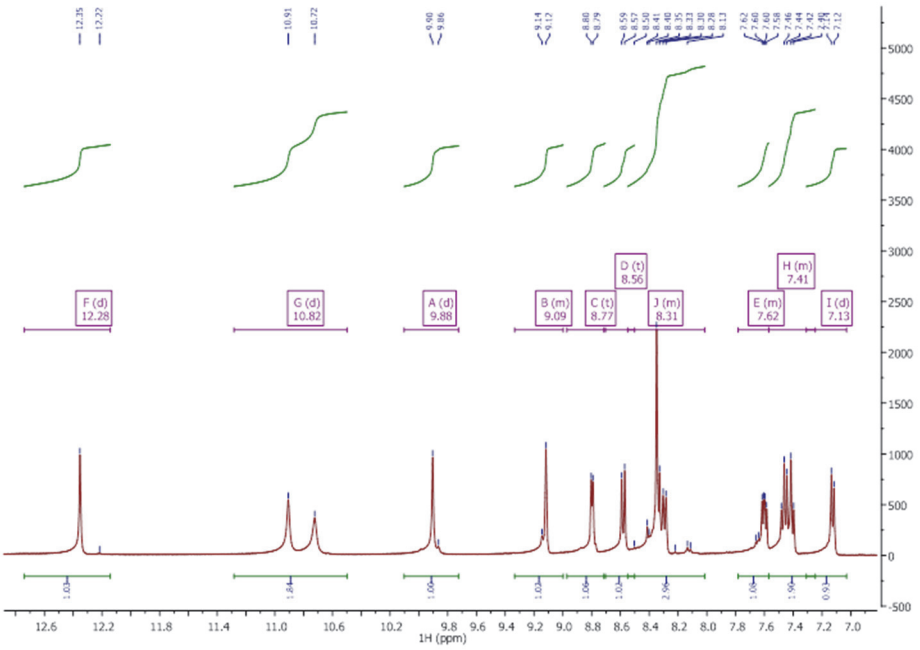

Fig. S27. ^{1}H spectrum of NcA-6

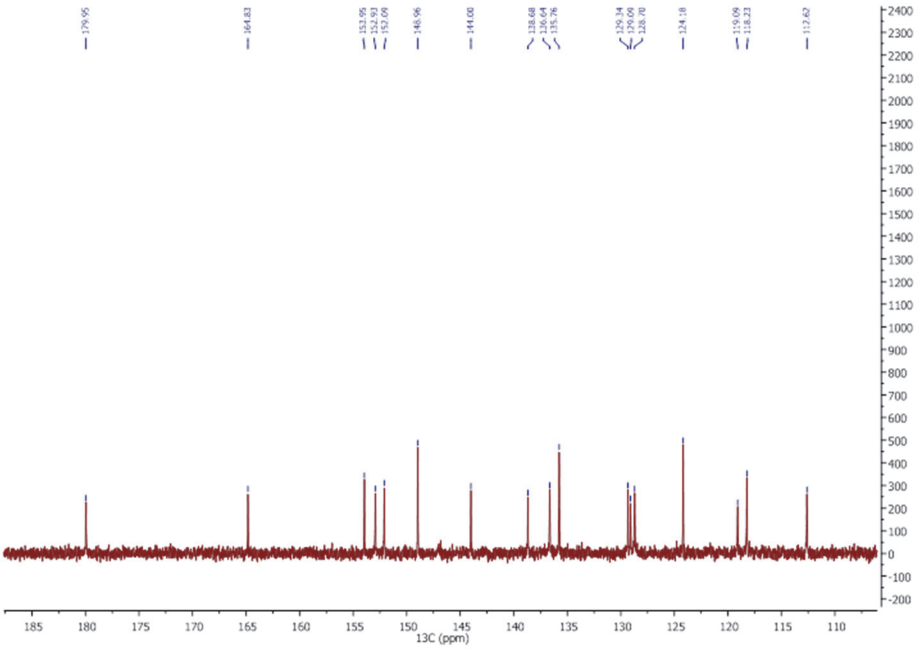

Fig. S28. ^{13}C spectrum of NcA-6

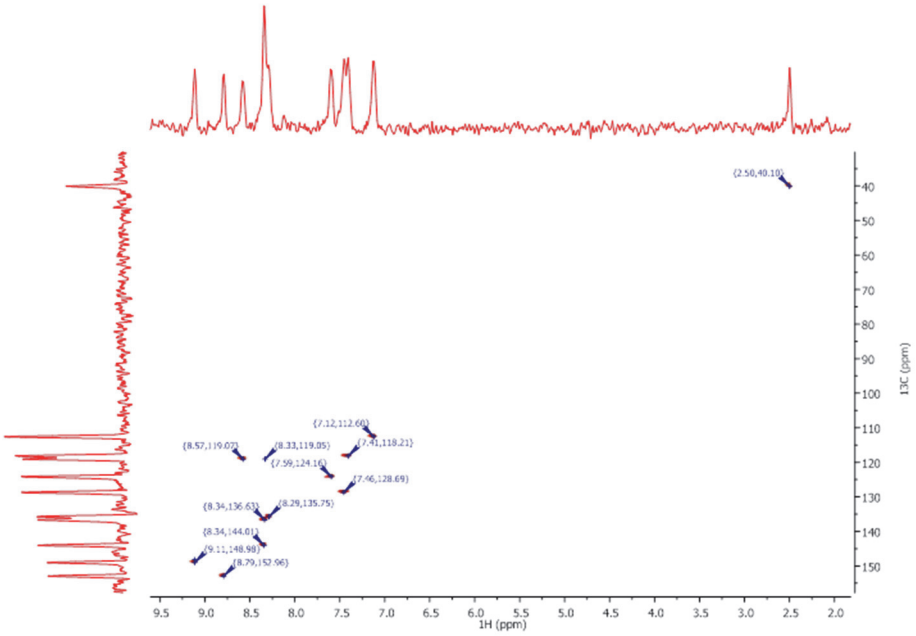

Fig. S29. HSQC spectrum of NcA-6

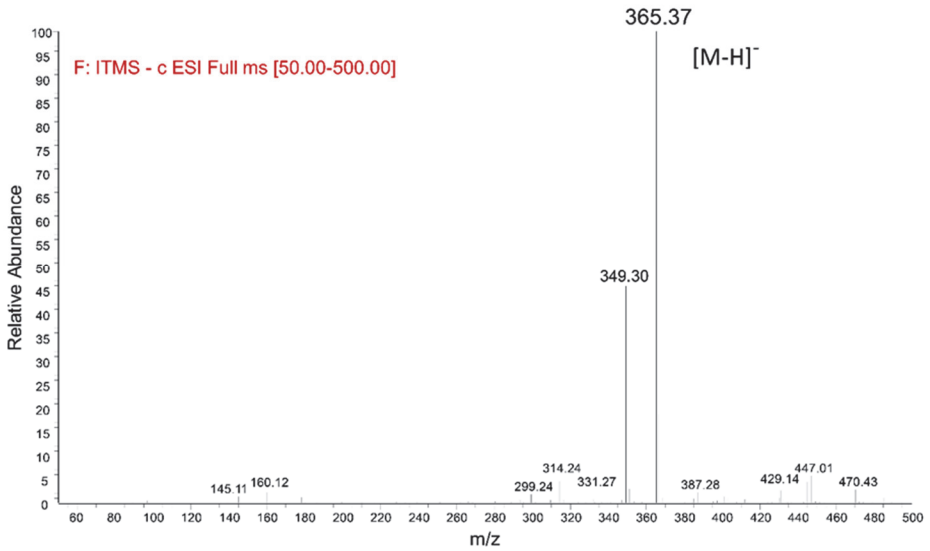

Fig. S30. MS spectrum of compound NcA-6

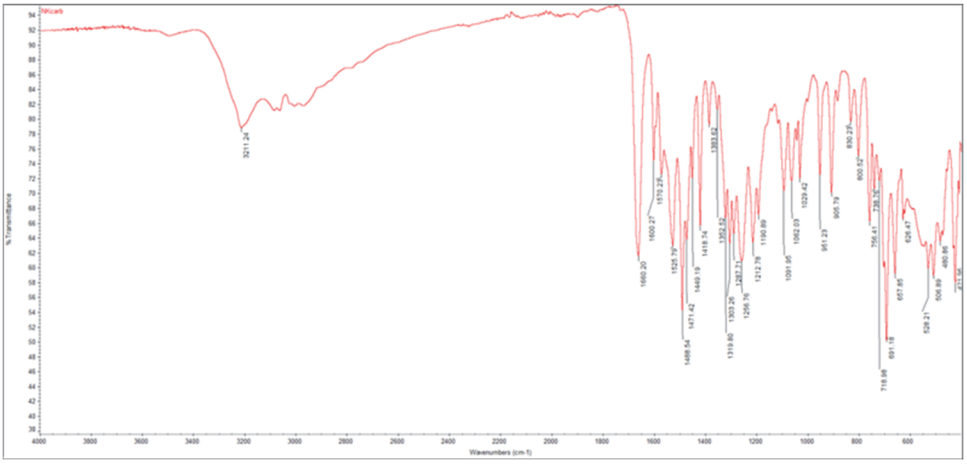

Fig. S31. FTIR spectrum of NcA-7

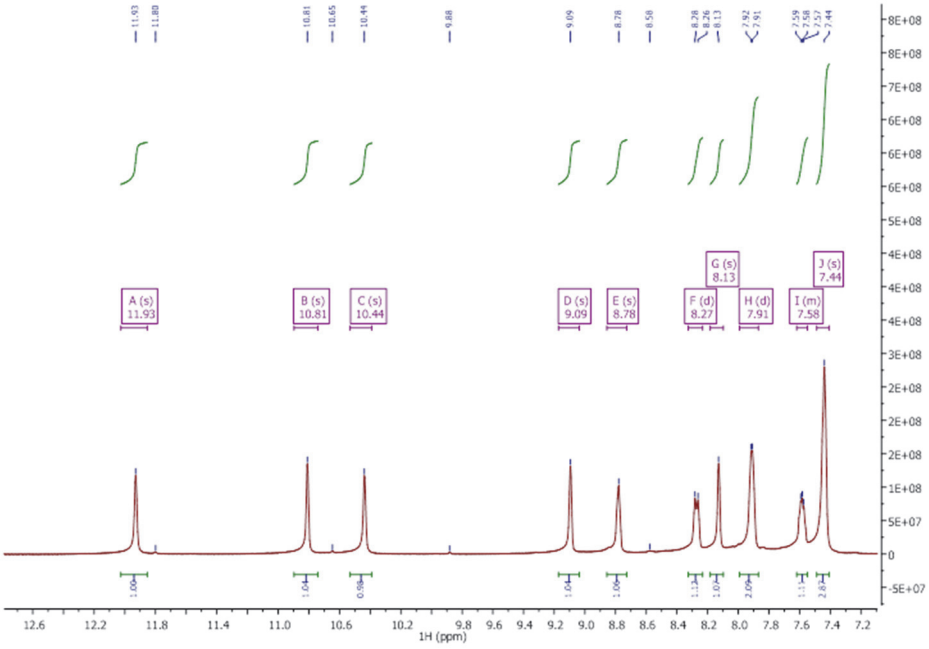

Fig. S32. ^{1}H spectrum of NcA-7

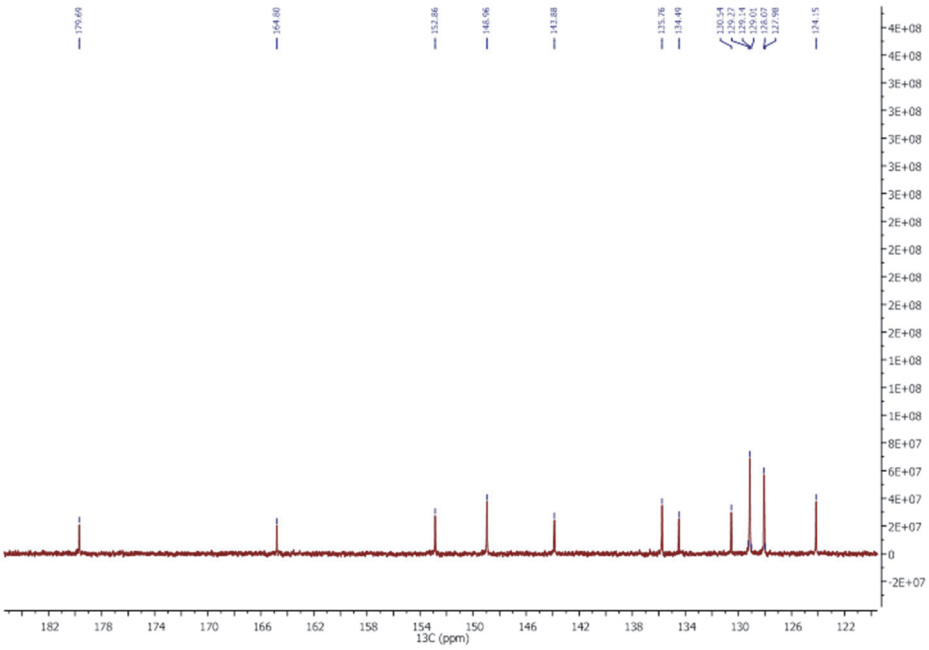

Fig. S33. ^{13}C spectrum of NcA-7

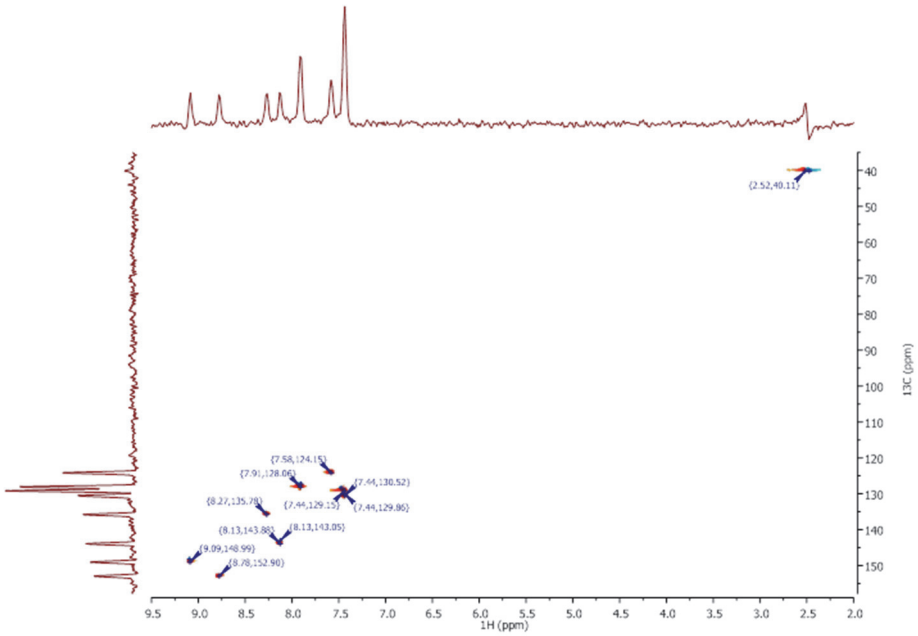

Fig. S34. HSQC spectrum of NcA-7

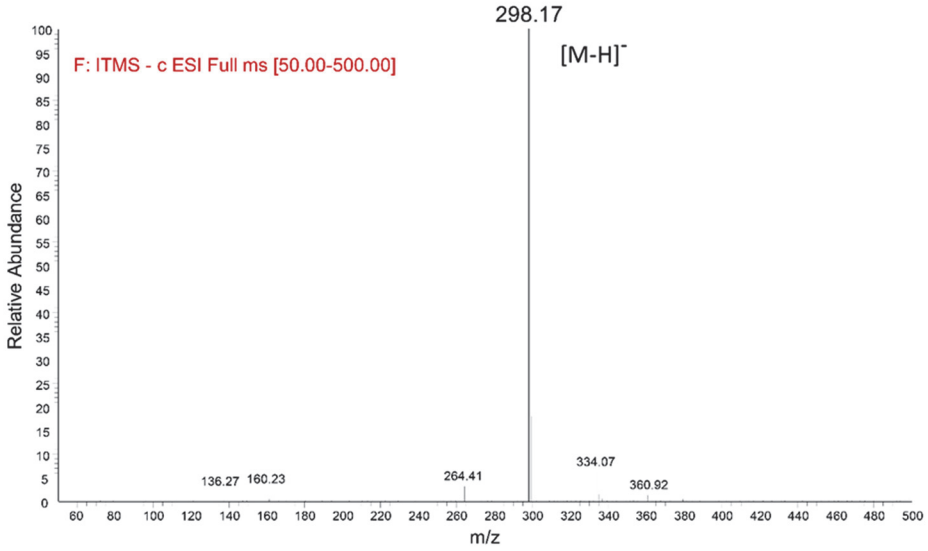

Fig. S35. MS spectrum of compound NcA-7

2. ANTIOXIDANT CAPACITY OF NCAS

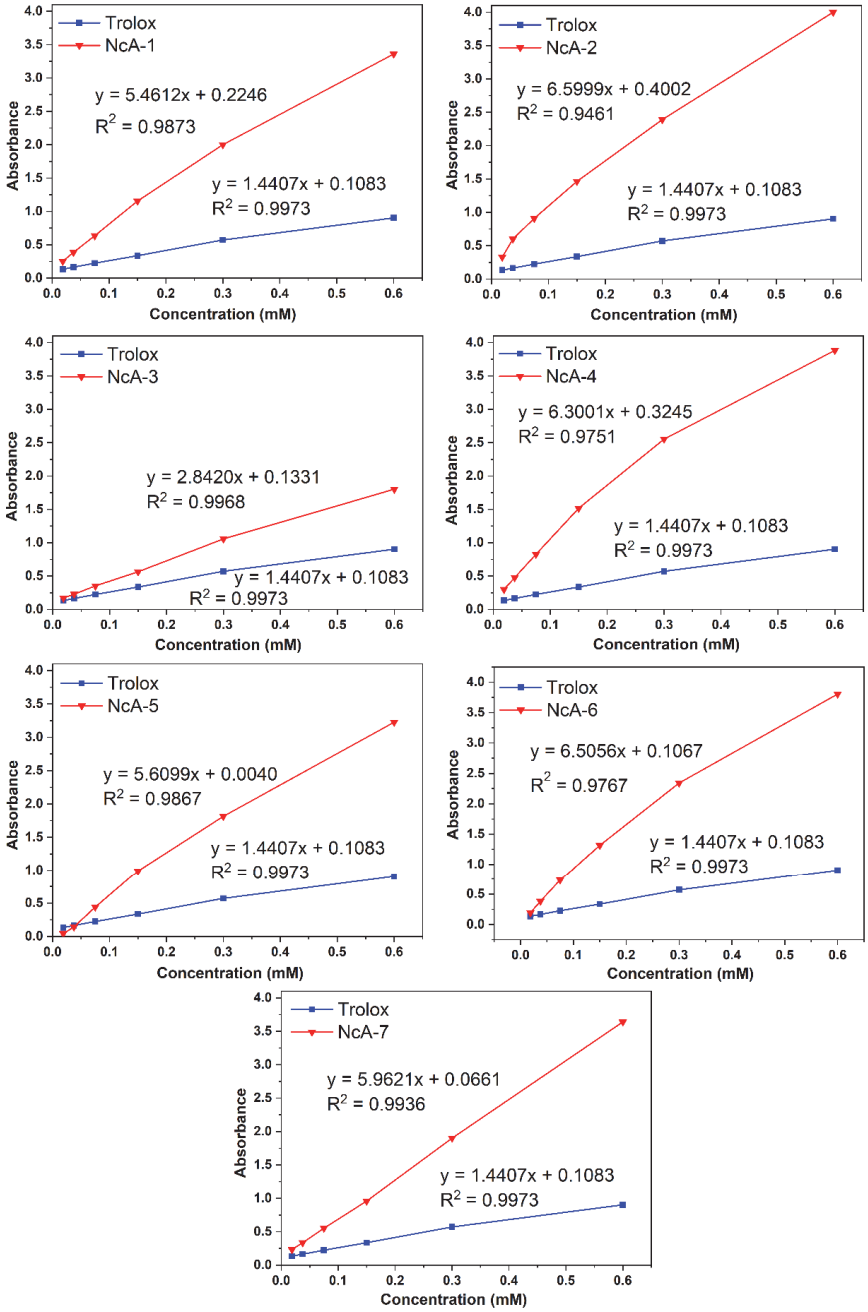

Fig. S36. Calibration curves of 7 NcAs and a standard (Trolox) for the determination of molar absorptivity and TEAC

3. IRON CHELATING ABILITY OF NCA-6

a) b)

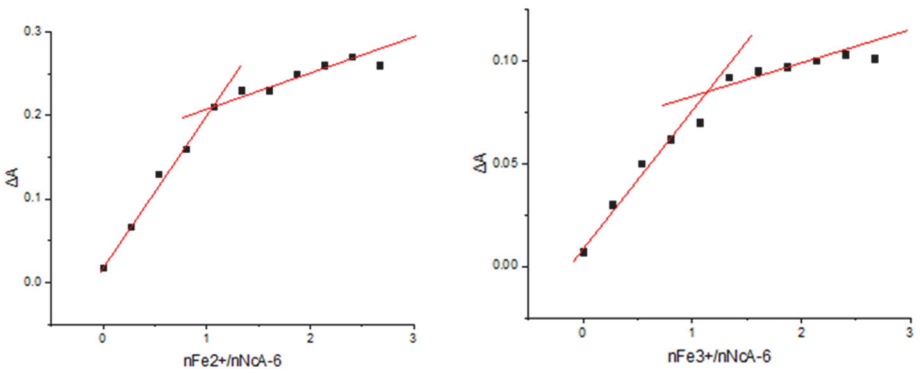

Fig. S37. The mole ratio plot for the formation of complexes between the NcA-6 and a) Fe^{2+}; b) Fe^{3+}