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Novel imidazolium-thiohydantoin hybrids and their Mn(III) complexes for antimicrobial and anti-liver cancer applications

Lamia A. Ismail,[a] R. Zakaria,[a] Eman M. Hassan,[a] Mohammad Y. Alfaifi,[b] Ali A. Shati,[b] Serag Eldin I. Elbehairi,[b,c] A.A.El-Bindary[d], Reda F.M. Elshaarawy[e,f,*]

[a] Department of Chemistry, Faculty of Science, Port Said University, 42526 Port Said, Egypt

[b] Biology Department, Faculty of Science, King Khalid University, 9004 Abha, Saudi Arabia

[c] Cell Culture Lab, Egyptian Organization for Biological Products and Vaccines (VACSERA Holding Company), Giza 12311, Egypt.

[d] Chemistry Department, Faculty of Science, Damietta University, Damietta, 34517, Egypt

[e] Department of Chemistry, Faculty of Science, Suez University, 43533 Suez, Egypt.

[f] Institut für Anorganische Chemie und Strukturchemie, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany.

Contents:

1- Synthesis and characterization of the key starting materials

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1.1 *Materials*

1.2 *Instrumentation*

1.3 *5-chloromethyl-2-hydroxybenzaldehyde* (**1**).

1.1 Materials

Chemicals were obtained from the following suppliers and used without further purification: 2-*tert*-butylphenol, anhydrous $MgCl_2$ and $Mn(CH_3COO)_2 \cdot 4H_2O$ (Sigma–Aldrich), paraformaldehyde ($(CH_2O)_n$) (Roth), 1-ethylimidazole (1-Et-Im) (Alfa Aesar), triethylamine (Et_3N) and anhydrous $ZnCl_2$ (GRÜSSING GmbH).

1.2 Instrumentation

Melting points were measured using a BÜCHI Melting point B-540 apparatus; all melting points were measured in open glass capillaries and are uncorrected. Elemental analyses for C, H, N, were performed with a Perkin–Elmer 263 elemental analyzer. FT-IR spectra were recorded on a BRUKER Tensor-37 FT-IR spectrophotometer in the range 400–4000 cm^{-1} as KBr disc in the 4000-550 cm^{-1} region with 2 cm^{-1} resolution or with an ATR (attenuated total reflection) unit (Platinum ATR-QL, diamond). For signal intensities the following abbreviations were used: br (broad), sh (sharp), w (weak), m (medium), s (strong), vs (very strong). UV/Vis spectra were measured at 25 °C in ethanol (10^{-5} mol/L) on a Shimadzu UV-2450 spectrophotometer using quartz cuvettes (1 cm). NMR-spectra were obtained with a Bruker Avance DRX200 (200 MHz for ^{1}H) or Bruker Avance DRX500 (125, 202 and 470 MHz for ^{13}C, ^{31}P and ^{19}F respectively) spectrometer with calibration to the residual proton solvent signal in DMSO-d_6 (^{1}H NMR: 2.52 ppm, ^{13}C NMR: 39.5 ppm), $CDCl_3$ (^{1}H NMR: 7.26 ppm, ^{13}C NMR: 77.16 ppm) against TMS ($\delta = 0.00$ ppm) for ^{1}H and ^{13}C, 85% phosphoric acid ($\delta = 0.00$ ppm) for ^{31}P and $CFCl_3$ ($\delta = 0.00$ ppm) for ^{19}F NMR. Multiplicities of the signals were specified s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The mass spectra of the synthesized saldach-bis(imidazolium) salts and their metal complexes were acquired in the linear mode for positive ions on a UHR-QTOF maXis 4G (Bruker Daltonics) and BRUKER Ultraflex MALDI-TOF instrument equipped with a 337 nm nitrogen laser pulsing at a repetition rate of 10 Hz. The 2+ charge assignment of ions in HR-ESI-MS was confirmed by the m/z = 0.5 difference between the isotope peaks (x, x+1, x+2). The MALDI matrix material (1,8-dihydroxy-9(10H)-anthracenone (dithranol, DIT, $^{12}C_{14}H_{10}O_3$, M = 226.077 g/mol) was dissolved in chloroform at a concentration of 10 mg/mL. MALDI probes were prepared by mixing compound solution (1 mg/mL in CH_2Cl_2) with the matrix solution (1:10, v/v) in a 0.5 mL Eppendorf® micro tube. Finally 0.5 µL of this mixture was deposited on the sample plate, dried at room temperature and then analyzed. Peaks with chlorine showed the isotope ratio $^{35/37}Cl$ = 75.8:24.2. Manganese (^{55}Mn 54.938 Da, 100%) or iron (^{56}Fe 55.934 Da, 91.2%) are either isotope pure or with a predominant isotope (^{54}Fe 53.939, 5.8%; ^{57}Fe 56.935 Da,

2.1%). For the mass spectral assignment: Peaks are based on ^{12}C with 12.0000 Da,^{35}Cl with 34.968 Da, ^{55}Mn 54.938 Da, ^{56}Fe 55.934. dithranol, DIT, $^{12}C_{14}H_{10}O_3$, M = 226.077 g/mol $C_{38}H_{52}N_6O_2$ = [**4** – 2 anions]$^{2+}$ = 624.45 $C_{38}H_{50}N_6O_2$ = [**4** – $2H^+$ – 2 anions]^0 = 622.44 Me_2Im = $C_5H_8N_2$ = 96.078 Me_2ImH = $C_5H_9N_2$ = 97.086 $C_{38}H_{50}N_6O_2FeCl$ = 713.3. The molar conductances of 10^{-3} mol/L solution of various salts have been measured at ambient temperature with a digital conductivity meter (S30 SevenEasy™ conductivity, Mettler-Toledo Electronics, LLC, Polaris Parkway, Columbus). The overall accuracy of the conductance measurements was found to be ± 0.2%. Magnetic measurements of target complexes were carried out at room temperature using a Vibrating Sample Magnetometer (VSM), (Model PAR 155).

1.2 Synthesis of 3-tert-butylsalicylaldehyde (**1**):

To a stirred mixture of dry anhydrous magnesium dichloride (9.52 g, 100 mmol) and dry paraformaldehyde (4.50 g, 150 mmol) in dry ACN (200 ml) was added dry triethylamine (26.1 ml, 185 mmol) dropwise and the mixture was stirred at room temperature for 15 min under nitrogen atmosphere. 2-*tert*-butylpylphenol (6.80 g, 50.0 mmol) was then added dropwise, resulting an opaque, light pink mixture. This solution was heated at gentle reflux temperature under nitrogen for ca. 3 h, during which time the color of the reaction mixture changes from light pink to orange. The solution was allowed to cool to room temperature then 200 mL of 1 N HCl was added followed by stirring for 30 min. The product was extracted with diethyl ether (5 x 75 ml portions) and the ether fractions collected together and washed with 1 N HCl (2 x 100 mL) and saturated NaCl(aq) (3 x 100 ml portions). The ether layer was dried over anhydrous $MgSO_4$ followed by filtration. Volatiles were removed under reduced pressure to yield the corresponding salicylaldehyde, usually contaminated with the starting phenol. The crude product which was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (90 : 10) mixture as the eluent to give pure 3-isopropylsalicylaldehyde (6.84 g, 83 %) as a pale yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 11.45 (s, 1 H, Ar-O**H**), 9.89 (s, 1 H, Ar-**H**C=O), 7.50 (dd, J_2 = 7.51 Hz, J_1 = 1.61 Hz, 1 H, Ar-**H**), 7.41 (dd, , J_2 = 7.72 Hz, J_1 = 1.68 Hz, 1 H, Ar-**H**), 7.01 (t, J_2 = 7.61 Hz, J_1 = 7.61 Hz, 1 H, Ar-**H**), 1.30 (s, 9H, C(C**H**$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 196.45 (H**C**=O), 160.03 (**C**-OH), 136.79 (**C**, Ar), 133.21 (**C**H, Ar), 130.86 (**C**H, Ar), 120.34 (**C**, Ar), 119.51 (**C**H, Ar) , 26.31 (**C**(CH$_3$)$_3$), 21.98 (C(**C**H$_3$)$_3$).

1.3 Synthesis of 3-tert-butyl-5-chloromethyl-2-hydroxybenzaldehyde (2):

They were synthesized from the corresponding salicylaldehydes according to the modified chloromethylation procedure [S1]. In a typical synthesis, (2.5 g, 15.2 mmol) of 3-*tert*-butyl-salicylaldehyde was treated with para-formaldehyde (1.0 g, 33.3 mmol) and zinc chloride (0.2

g, 1.46 mmol) in 11 ml of concentrated hydrochloric acid. The mixture was vigorously stirred under HCl_g atmosphere for 72 h at 313 K. The reaction mixture was extracted several times with diethyl ether (3x15 mL). Then the collected ether fractions were washed by 2x10 mL 5% aqueous $NaHCO_3$ solution, 2x10 mL brine, 5x10 mL milli-Q water and dried over anhydrous $MgSO_4$. After filtration and removal of the volatiles under reduced pressure, the obtained product was characterized and used in the next step without further purification. It is obtained as faint yellow crystals (3.00 g, 93%). FT-IR (KBr, cm^{-1}): 3510 (m, br, $\nu_{(O-H)}$), 3075 (m, br, $\nu_{asym(C-H)}$, Ar), 3030 (m, br, $\nu_{sym(C-H)}$, Ar), 2971 (m, sh, $\nu_{(CH_3)}$), 2869 (m, sh, $\nu_{(CH_2)}$), 1647 (vs, sh, $\nu_{(C=O)}$), 1446, 1385 (s, sh, $\nu_{(C=C_{Ar} + C-H_{bend})}$), 1320 (m, sh, $\nu_{(CH_2)}$), 1266 (s, sh, $\nu_{(Ar-O)}$), 690 (s, sh, $\nu_{(C–Cl)}$). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 11.47 (s, 1 H, Ar-O**H**), 9.92 (s, 1 H, Ar-**H**C=O), 7.49 (dd, J_2 = 10.37, J_1 = 2.37 Hz, 2 H, 2 x Ar-**H**), 4.64 (s, 2 H, C**H$_2$**-Ar), 1.30 (s, 9 H, C (C**H$_3$**)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 196.93 (H**C**=O), 159.80 (**C**-OH), 138.42 (**C**, Ar), 134.42 (**C**H, Ar), 131.59 (**C**H, Ar), 129.21 (**C**, Ar), 120.29 (**C**, Ar), 46.19 (**C**H_2-Ar), 26.86 (**C**$(CH_3)_3$), 22.58 (C(**C**H_3)$_3$).

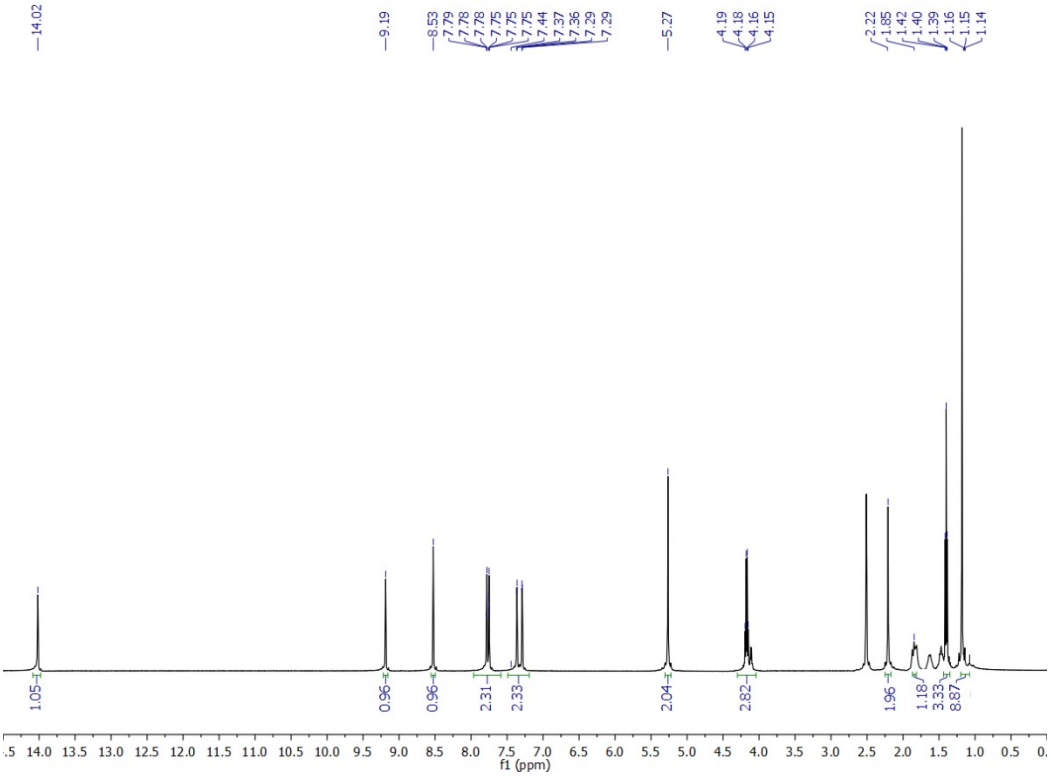

Figure S1: [1]HNMR (DMSO-d_6, 200 MHz) spectrum of $IMTH_1$ (**5a**).

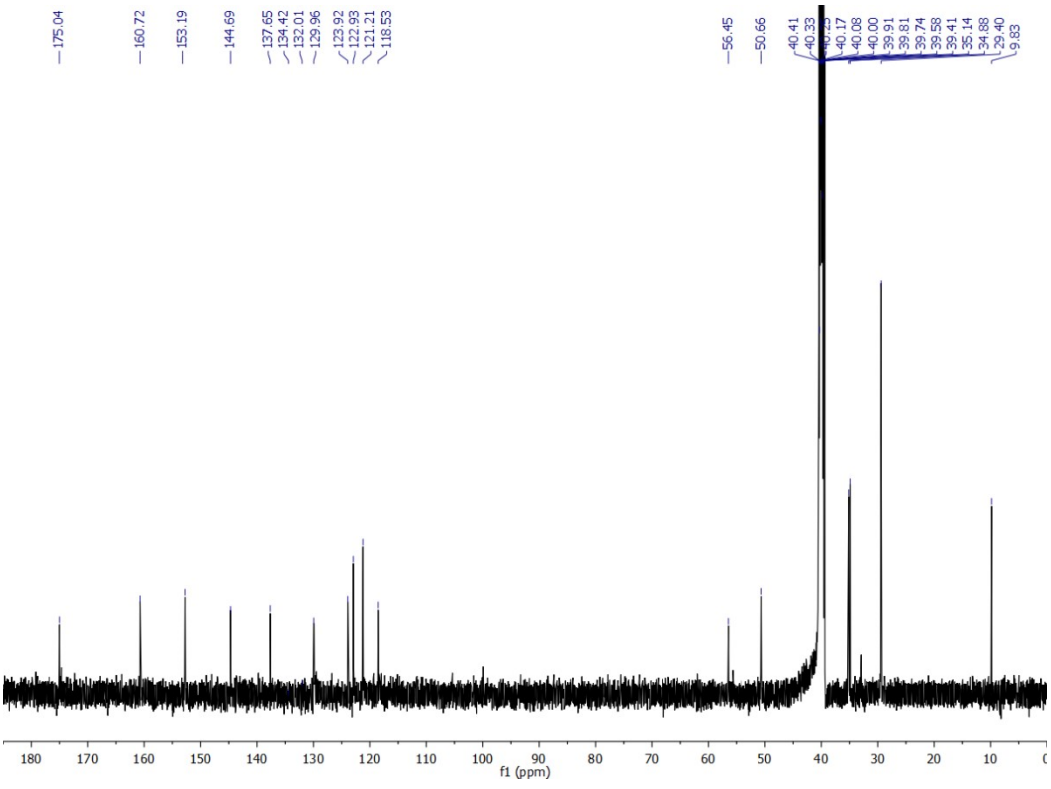

Figure S2: [13]CNMR (DMSO-d_6, 125 MHz) spectrum of $IMTH_1$ (**5a**).

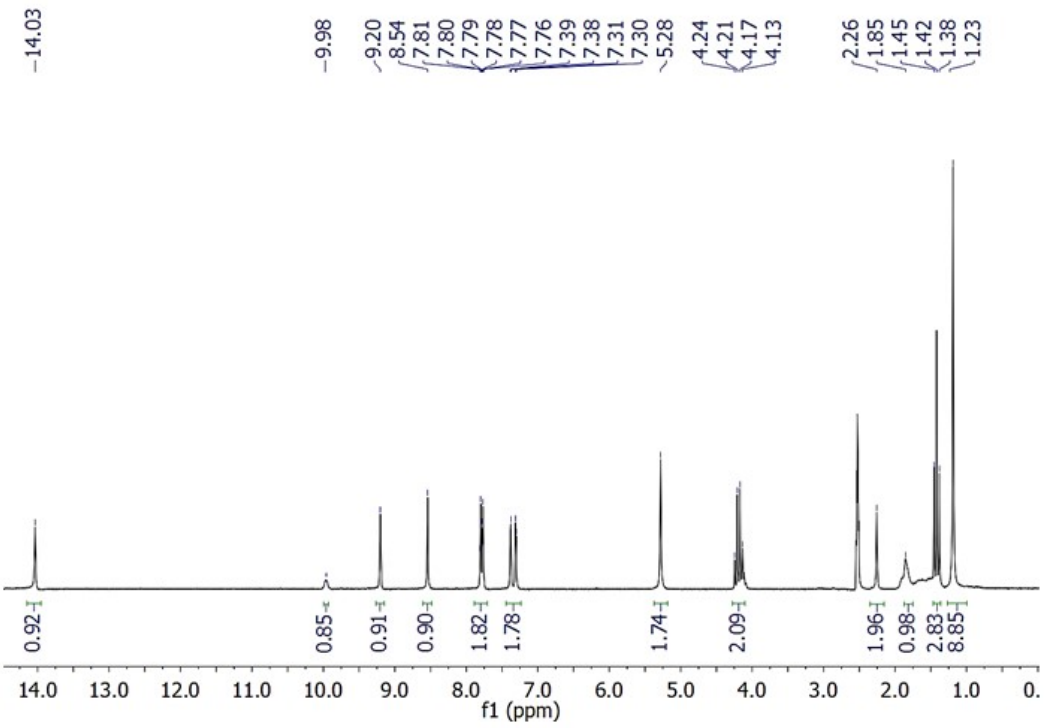

Figure S3: [1]HNMR (DMSO-d_6, 200 MHz) spectrum of $IMTH_2$ (**5b**).

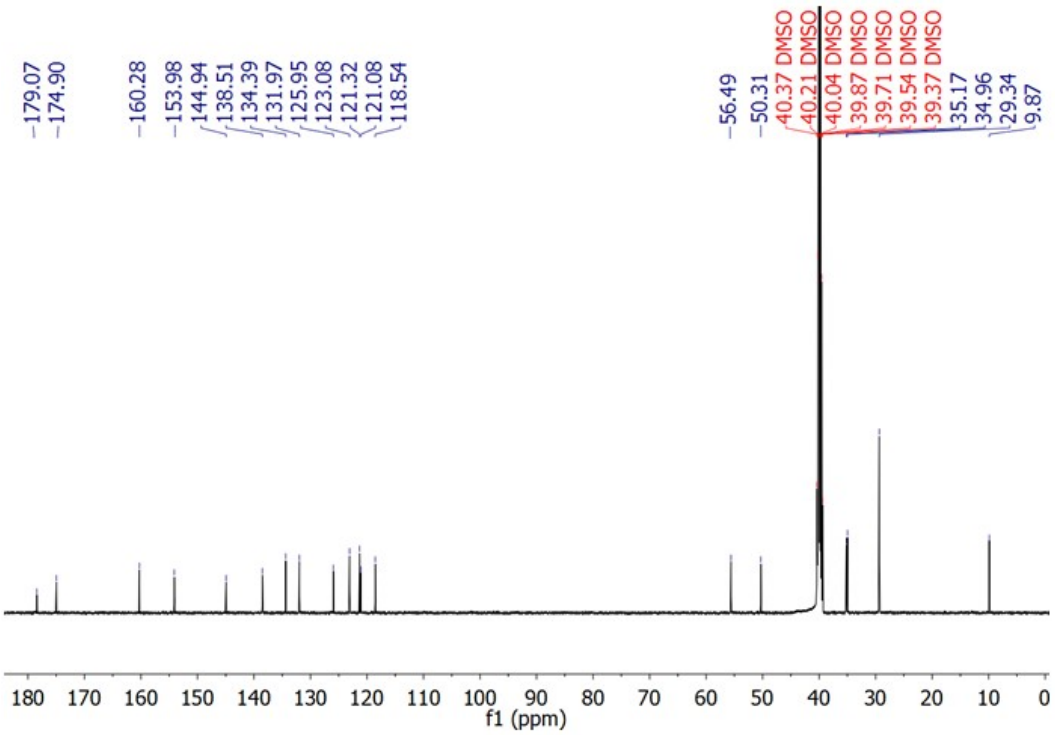

Figure S4: [13]CNMR (DMSO-d_6, 125 MHz) spectrum of $IMTH_2$ (**5b**).

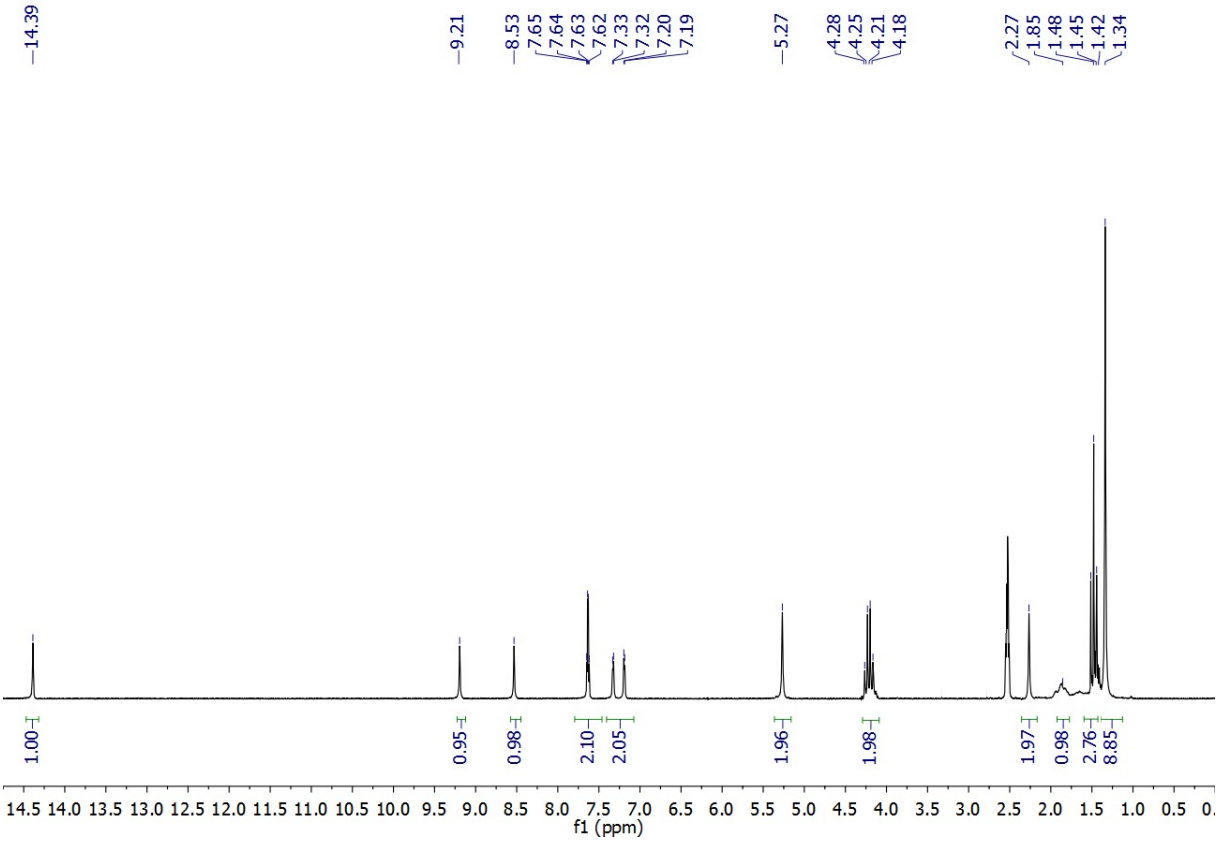

Figure S5: ^{1}HNMR (DMSO-d_6, 200 MHz) spectrum of IMTH$_3$ (**5c**).

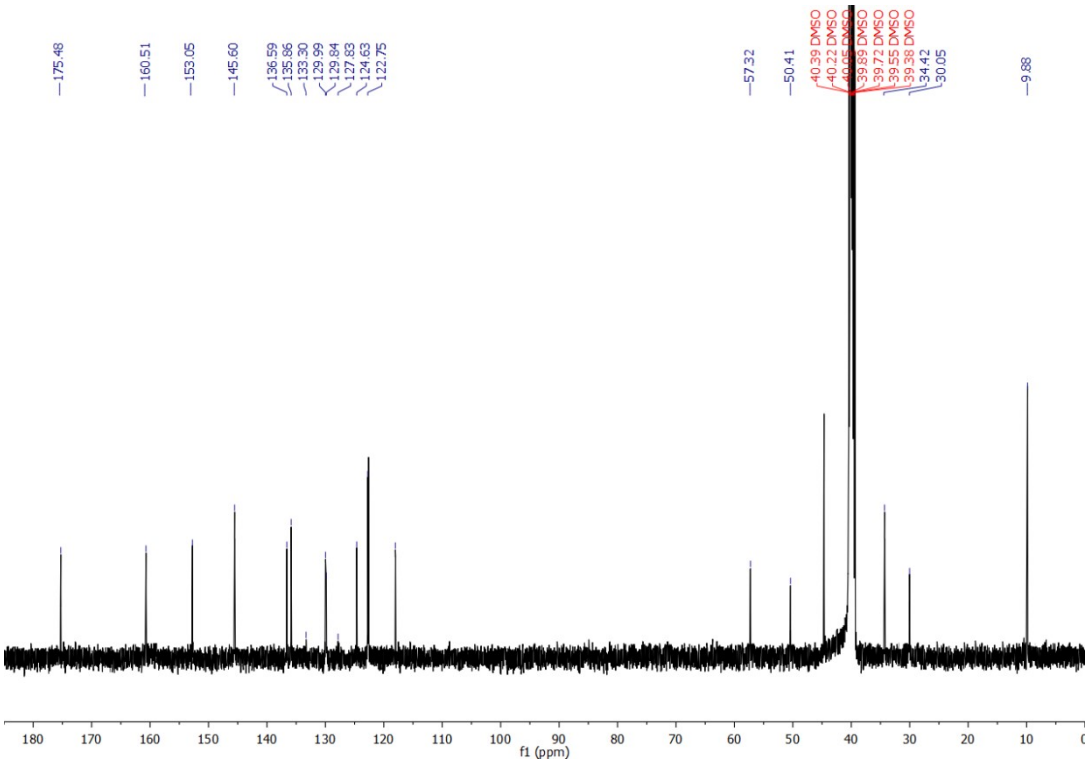

Figure S6: ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum of IMTH$_3$ (**5c**).

[S1] Reda F.M. Elshaarawy, C. Janiak, *Eur. J. Med. Chem.*, 2014, **75**, 31-42.