Bis((butylimidazolium tetrafluoroborate)thiadiazole Schiff base as a new hepatoprotective agent against breast cancer-induced angiogenesis: *in vivo*/*in vitro* studies and molecular docking

Norah F. Alqahtani[1], Mohammad Y. Alfaifi[2,3], Ali A Shati[2,3], Serag Eldin I. Elbehairi[2,3,4], Abdulrahman M. Saleh[5,6], Ebtesam S. Kotb[7], Waleed M. Serag[7], Reda F. M. Elshaarawy[7,8,*], Yasser A. Hassan[9,10]

[1] Department of Chemistry, College of Science, University of Jeddah, Jeddah 21589, Saudi Arabia.

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

[3] Tissue Culture and Cancer Biology Research Laboratory, King Khalid University, Abha, 9004, Saudi Arabia

[4] Cell Culture Lab, Egyptian Organization for Biological Products and Vaccines (VACSERA Holding Company), 51 Wezaret El-Zeraa St., Agouza, Giza, Egypt.

[5] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt

[6] Aweash El-Hagar Family Medicine Center, Epidemiological Surveillance Unit, MOHP, Mansoura 35711, Egypt

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

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

[9] Department of pharmaceutics, Faculty of Pharmacy, Delta University for Science and Technology, Gamasa, Egypt.

[10] Department of Pharmaceutics and Pharmaceutical Technology ,College of Pharmacy, Al-Kitab University, Kirkuk, Iraq.

***** Correspondence: WMS, waleed.ibrahim@suezuniv.edu.eg

Contents

1. Materials and Instrumentation

1.1. Materials

Chemicals were obtained from the following suppliers and used without further purification: *o*-vanillin, 1-butylimidazole (1-BuIm), and hydrazine monohydrate (Sigma–Aldrich), paraformaldehyde ($(CH_2O)_n$) (Roth), anhydrous zinc chloride ($ZnCl_2$) (GRÜSSING GmbH), thiosemicarbazide hydrochloride (**3**) (TCI), potassium thiocyanate (KSCN), anhydrous potassium carbonate (K_2CO_3), sodium bicarbonate ($NaHCO_3$), sodium sulphate anhydrous (Na_2SO_4), sodium hydroxide (NaOH) and 3% hydrogen peroxide (H_2O_2) (ADWIC).

1.2. Instrumentation

Melting points (uncorrected) were determined in open glass capillaries on a Gallenkamp melting point apparatus. Elemental analyses for C, H, N and S 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 discs or in the 4000-550 cm^{-1} region with 2 cm^{-1} resolution 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). NMR-spectra were obtained with a Bruker Avance DRX200 (200 MHz for ^1H) or Bruker Avance DRX500 (500 MHz for ^{13}C) spectrometer with calibration to the residual proton solvent signal in DMSO-d_6 (^1H NMR: 2.52 ppm, ^{13}C NMR: 39.5 ppm), $CDCl_3$ (^1H NMR: 7.26 ppm, ^{13}C NMR: 77.16 ppm) against TMS with $\delta = 0.00$ ppm. Multiplicities of the signals were specified s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The mass spectra of the synthesized salicyldehyde ionic liquids (Sal-ILs) were acquired in the linear mode for positive ions on a BRUKER Ultraflex MALDI-TOF instrument equipped with a 337 nm nitrogen laser pulsing at a repetition rate of 10 Hz.

2. Synthesis of vanillyl butyl imidazolium ionic liquids (VBIILs) (2a,b)

2.1. Synthesis of 5-chloromethyl-3-methoxysalicylaldehyde (1)

A mixture of 9 g (300 mmol) of para formaldehyde, 6g (44 mmol) of anhydrous zinc chloride and conc. hydrochloric acid (100 mL) into 500 mL two-necked RB flask was strongly stirred at room temperature under HCl atmosphere for 30 min, until complete dissolution of solids. After which a solution of 30 g (197.2 mmol) *o*-vanillin in 150 mL of benzene was added dropwise with continuous stirring under HCl atmosphere. Continue passing HCl gas over the vigorously stirred reaction mixture for further 4 h while the reaction temperature must kept below 20 °C. Then 100 mL and 100 g of crushed ice were simultaneously added to the black-red reaction mixture. A phase separation was observed from the mixture, and two layers were obtained. The benzene layer was separated by aspirating freed from resinous products, and thoroughly washed with deionized water until the pH of washing effluent reached 7. After drying over anhydrous sodium sulfate, the benzene was distilled off.

The obtained crude viscous brown-black oil was subjected to crystallization twice from petroleum ether 100-120 to furnish 5-chloromethyl-3-methoxysalicylaldehyde (**1**) (22.15 g, 56 %) as orange needles. FTIR (KBr, cm^{-1}): 3437 (m, br, $\nu_{(O-H)}$), 3086 (m, br, $\nu_{asym(C-H)}$, Ar), 3043 (m, br, $\nu_{sym(C-H)}$, Ar), 2970 (m, sh, $\nu_{asym(CH_2)}$), 2867 (m, sh, $\nu_{sym(CH_2)}$), 1645 (vs, sh, $\nu_{(C=O)}$), 1451, 1395 (s, sh, $\nu_{(C=C_{Ar}+\ C\text{-}H_{bend})}$), 1275 (s, sh, $\nu_{(Ar-O)}$), 689 (s, sh, $\nu_{(C-Cl)}$). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 11.20 (s, 1 H, Ar-O**H**), 9.94 (s, 1 H, Ar-**H**C=O), , 7.35-7.11 (m, 2 H, 2 x Ar-**H**), 4.62 (s, 2 H, C$\mathbf{H_2}$-Ar), 3.98 (s, 3 H, OC$\mathbf{H_3}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 196.56, 152.31, 149.14, 124.47, 120.58, 118.33, 118.27, 56.81, 56.72, and 46.21. EI-MS [$C_9H_9ClO_3$] Calcd.: 200.62 Found: 200.00.

2.2. Synthesis of VBIILs

A solution of 5-chloromethyl-3-methoxysalicylaldehyde (1.96 g, 9.8 mmol) in dry toluene (25 mL) was added dropwise, over 30 min, to a vigorously stirred solution of 1-BuIm (9.65 mmol) in dry toluene (50 mL) at room temperature under N_2 atmosphere. The resulting solution was further stirred under this inert atmosphere at 60 °C for 24 h. After cooling, the isolated product was intensively washed with dry toluene (5 x 15 mL), several with ether (5x10 mL), to remove the unreacted materials, and dried under vacuum to give the desired vanillyl ionic liquids (**2a**) which used for the following preparation without further purification. A sample of isolated product was characterized as follow;

5-(1-butylimidazolium)-vanillyl chloride (**2a**): Obtained as a pale yellow solid, yield (92 %), mp = 70-71 ºC. FTIR (KBr, cm^{-1}): 3434 (m, br, $\nu_{(O-H)}$), 3113 (m, sh, $\nu_{asym(C-H)}$, Im and Ar), 3051 (m, sh, $\nu_{sym(C-H)}$, Im and Ar), 2967 (m, sh, $\nu_{(CH_3)}$) 2872 (m, sh, $\nu_{(C-H)}$), 1644 (vs, sh, $\nu_{(C=O)}$), 1537, 1464, 1401 (s, sh, $\nu_{(C=C_{Ar}\ +\ C\text{-}H_{bend})}$), 1324 (m, sh, $\nu_{(C-H)}$), 1270 (s, sh, $\nu_{(Ar-O)}$), 1153 (s, sh, $\nu_{(H\text{-}C=C\ +\ H\text{-}C=N)_{bend}}$, Im), 743 (m, sh), 674 (m, sh), 535 (m, sh). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 11.45 (s, 1 H, Ar-O**H**), 9.95 (s, 1 H, Ar- **H**C=O), 7.72 (s, br, 3 H, Ar-**H** and Im-**H**), 7.48 (s, br, 1 H, Ar-**H**), 5.57 (s, 2 H, N(3)-C$\mathbf{H_2}$-Ar), 3.98 (s, 3 H, N(1)-C$\mathbf{H_3}$), 3.31 ($m_{(7)}$, 1 H, C**H**$(CH_3)_2$), 2.86 (s, 3 H, C(2)-C$\mathbf{H_3}$), 1.21 (d, J = 6.85 Hz, 6 H, CH(C$\mathbf{H_3}$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 197.76 (H**C**=O), 160.04 (**C**-OH), 144.69 (N(1)**C**(CH_3)N(3)), 138.90 (**C**-C=O), 133.76 (**C**H, Ar), 132.03 (**C**H, Ar), 124.61 (**C**, Ar), 123.42 (N(1)**C**HCHN(3)), 122.07 (N(1)**C**HCHN(3)), 120.69 (**C**, Ar), 52.02 (N(3)-**C**H_2-Ar), 36.39 (N(1)-**C**H_3), 26.97 (**C**H$(CH_3)_2$), 22.52 (CH(**C**$H_3)_2$), 11.49 (C(2)-**C**H_3). ESI MS: In positive mode peaks at m/z 273.16 (100%, $[C_{16}H_{21}N_2O_2]^+$) a.m.u. and in negative mode peak at m/z 34.97 (100%, $[Cl]^-$) a.m.u. HRMS $[C_{16}H_{21}N_2O_2]^+$ Calcd.: 273.1603 Found: 273.1597.

Anion metathesis

To a solution of **2a** (3.63 g, 11.75 mmol) in milli-Q water (50 mL) was added aqueous solution of $NaBF_4$ (1.37 g, 12.43 mmol) portion-wise with vigorous stirring while cooling in ice bath over 1 h. After the addition was completed, the reaction was stirred at room temperature for 24 h. The solid product was filtered, washed with milli-Q water (to remove $NaBF_4$ - solution and any water-soluble impurities) until it was neutral. The final product was dried under vacuum at 40 °C for 24 h. Samples of the isolated products are fully characterized below.

5-(1-butylimidazolium)-vanillyl tetrafluoroborate (**2a**): Obtained as of pale orange solid, Yield (91 %), mp: 68-69 °C. FTIR (KBr, cm^{-1}): 3467 (m, br, $\nu_{(O-H)}$), 3156 (m, sh, $\nu_{asym(C-H)}$, Im and Ar), 3095 (m, sh, $\nu_{sym(C-H)}$, Im and Ar), 2968 (m, sh, $\nu_{(CH_3)}$) 2874 (m, sh, $\nu_{(C-H)}$), 1645 (vs, sh, $\nu_{(C=O)}$), 1544, 1463, 1392 (s, sh, $\nu_{(C=C_{Ar} + C-H_{bend})}$), 1330 (m, sh, $\nu_{(C-H)}$), 1271 (s, sh, $\nu_{(Ar-O)}$), 1156 (s, sh, $\nu_{(H-C=C + H-C=N)_{bend}}$, Im), 1063 (vs, sh, $\nu_{(BF_4^-)str}$),749 (s, sh), 675 (m, sh), 534 (m, sh). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 11.24 (s, 1 H, Ar-O**H**), 10.03 (s, 1 H, Ar-**H**C=O), 7.74 (d, *J* = 2.10 Hz, 1 H, N(1)CHC**H**N(3)), 7.67 (d, *J* = 2.05 Hz, 2 H, 2 x Ar-**H**), 7.60 (d, *J* = 2.20 Hz, 1 H, N(1)C**H**CHN(3)), 5.40 (s, 2 H, N(3)-C**H$_2$**-Ar), 3.78 (s, 3 H, N(1)-C**H$_3$**), 3.31 ($m_{(7)}$, 1 H, C**H**$(CH_3)_2$), 2.66 (s, 3 H, C(2)-C**H$_3$**), 1.23 (d, *J* = 6.91 Hz, 6 H, CH(C**H$_3$**)$_2$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 197.23 (H**C**=O), 158.30 (**C**-OH), 144.93 (N(1)**C**(CH_3)N(3)), 137.59 (**C**-C=O), 133.90 (**C**H, Ar), 130.70 (**C**H, Ar), 126.33 (**C**, Ar), 122.92 (N(1)CH**C**HN(3)), 121.30 (N(1)**C**HCHN(3)), 121.09 (**C**, Ar), 50.31 (N(3)-**C**H_2-Ar), 35.16 (N(1)-**C**H_3), 26.31 (**C**H$(CH_3)_2$), 22.47 (CH(**C**$H_3)_2$), 9.80 (C(2)-**C**H_3). ^{19}F NMR (470 MHz, DMSO-d_6): -148.69 ppm (singlet). MALDI-TOF MS, *m/z*: 499.2 $[M{\cdot}DIT - BF_4^-]^+$ and 273.0 $[M - BF_4^-]^+$.

3. Synthesis of 2,5-diamino-1,3,4-thiadiazole

3.1. Synthesis of hydrazine-1,2-bis(carbothioamide) (3)

It is synthesized according to the method described in Adediji J. F. *et al*. [S1], in brief equimolar amounts of thiosemicabazide hydrochloride and potassium thiocynate were dissolved in water, refluxed for 3 h and allowed to cool. After cooling, white crystals were separated, filtered, dried in the oven for 1 h and recrystallized from water to give compound **3** as white crystals, m. p. 203ºC, Yield (53 %). FTIR (KBr, cm^{-1}): 3356 (s, sh, $\nu_{(N-H)asym}$), 3273 (s, sh, $\nu_{(N-H)symm}$), 3169 (s, sh, $\nu_{(S-H)str}$), 1610 (s, sh, $\nu_{(C=N)str}$), 1511(s, sh, $\nu_{(N-H)bend}$), 1466 (s, sh, $\nu_{(C=S)str}$), 1284 (m, sh, $\nu_{(C-N)str}$), 1044 (m, sh, $\nu_{(C-S-C)str}$), 826.348 (m, sh, $\nu_{(C-S-C)bend}$). EI-MS, *m/z* (%):165 (5.23) ($([C_2H_7N_5S_2]^+$, M), 150 (11.20), 135 (37.82), 127.85 (13.71), 115 (2.49), 107 (5.95), 95.85 (15.35), 91 (8.02), 79.90 (27.98), 63.90 (100), 57 (9.93), 55 (5.89).

3.2. Synthesis of 1,3,4-thiadiazole-2,5-diamine

It was synthesized according to reference [S1] with slight modification, in brief, bithiourea (3.01 g, 18.2 mmol) was added dropwise with stirring to aqueous hydrogen peroxide solution (40 mL, 3%) and then the mixture was heated at 50-60 ºC under reflux for 1 h with continuous stirring. The product was filtered and dried at 100 ºC then recrystallized from water to yield dark white crystals, Yield (3.02 g, 95.31%), mp: 240 °C. FTIR (KBr, cm^{-1}): 3438 (s, sh, $\nu_{(N-H)_{asym}}$), 3401 (s, sh, $\nu_{(N-H)_{sym}}$), 3272 (s, br, $\nu_{(S-H)}$), 1606 (s, sh, $\nu_{(C=N)}$, thiadiazole ring), 1565 (s, sh, $\nu_{(N-H)_{bend}}$), 1510 (vs, sh, $\nu_{(C-N)}$), 1310 (m, sh, $\nu_{(C-S-C)}$), 746 (w, sh, $\nu_{(C-S-C)_{bend}}$). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 6.16 (s, 4H, 2 x N**H$_2$**). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 160.04. EI-MS, *m/z*: 135 (1.67) ($[C_2H_4N_4S{\cdot}H_2O]^+$, M+$H_2O$), 133 (17.18), 117 (6.87), 116 (100) ($[C_2H_4N_4S]^+$, M), 101 (2.27), 76 (3.22), 74 (55.41), 72 (1.55), 62 (1.43), 61 (2.51), 60 (31.04), 57 (12.84), 47 (4.45), 43 (10.98), 42 (3.10).

Figures Captions

Figure S1: ESI-MS spectrum of TISSB1.

Figure S2: ESI-MS spectrum of TISSB2.

Figure S3: FTIR spectrum of 2,5-H_2N-TDA.

Figure S4: FTIR spectrum of VBIIL1.

Figure S5: FTIR spectrum of VBIIL2.

Figure S6: FTIR of spectrum TISSB1.

Figure S7: FTIR of spectrum TISSB2.

Figure S8: ^{1}H NMR spectrum (500 MHz) of VBIIL1.

Figure S9: ^{13}C NMR spectrum (126 MHz) of VBIIL1.

Figure S10: ^{1}H NMR spectrum (500 MHz) of VBIIL2.

Figure S11: ^{13}C NMR spectrum (126 MHz) of VBIIL2.

Figure S12: ^{1}H NMR spectrum (500 MHz) of TISSB1.

Figure S13: ^{13}C NMR spectrum (126 MHz) of TISSB1.

Figure S14: Impacts of serial doses of TISSBs on the viability of MCF-7 cell line, in comparison to a clinical drug 5-Fluorouracil (5-Fu)

Figure S15: Impacts of serial doses of TISSBs on the viability of HSF cell line

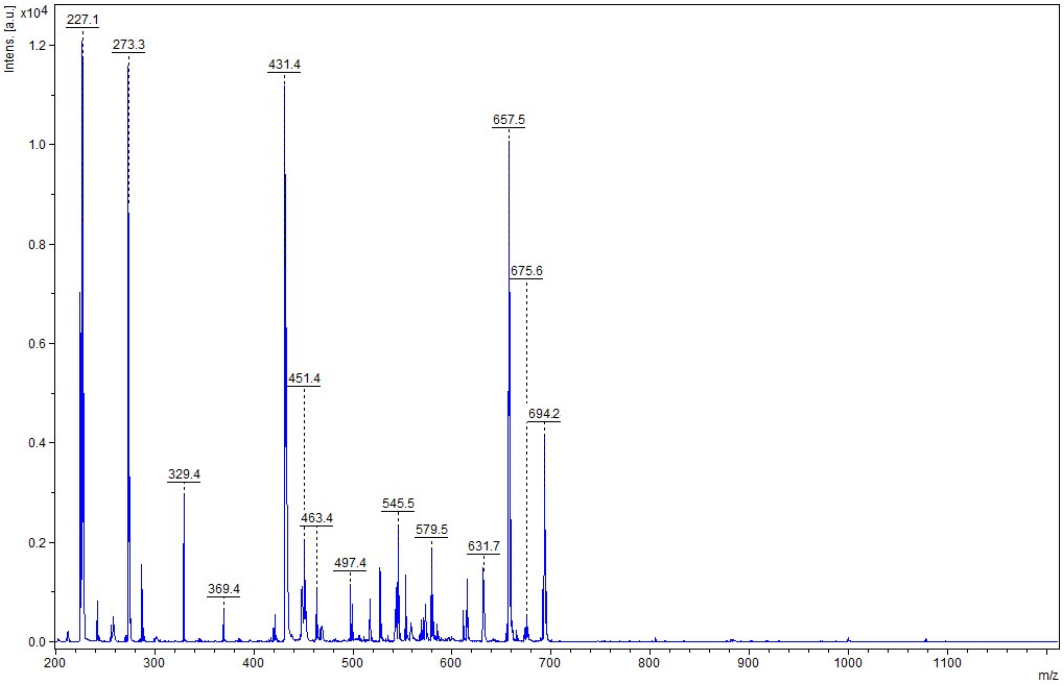

Figure S1: ESI-MS spectrum of TISSB1.

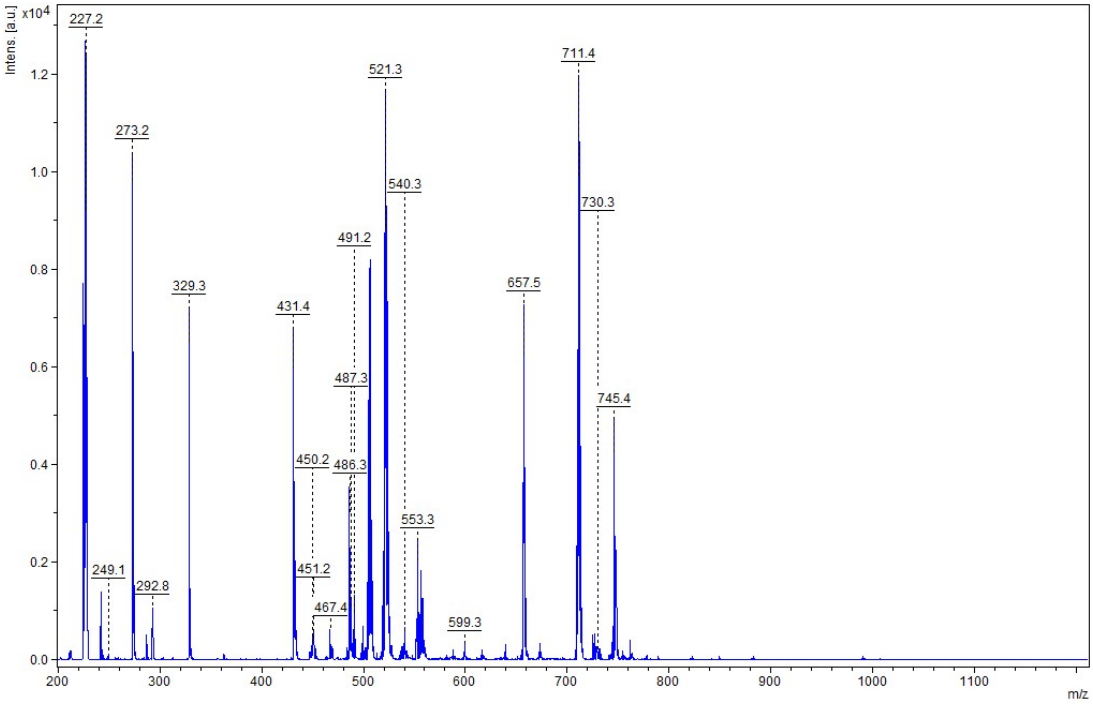

Figure S2: ESI-MS spectrum of TISSB1.

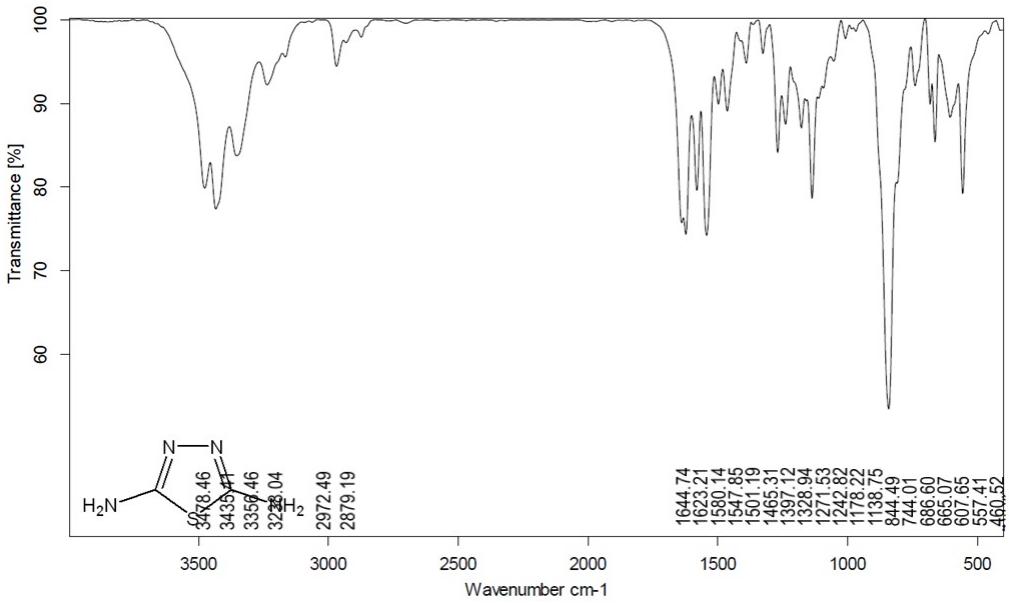

Figure S3: FTIR spectrum of 2,5-H_2N-TDA.

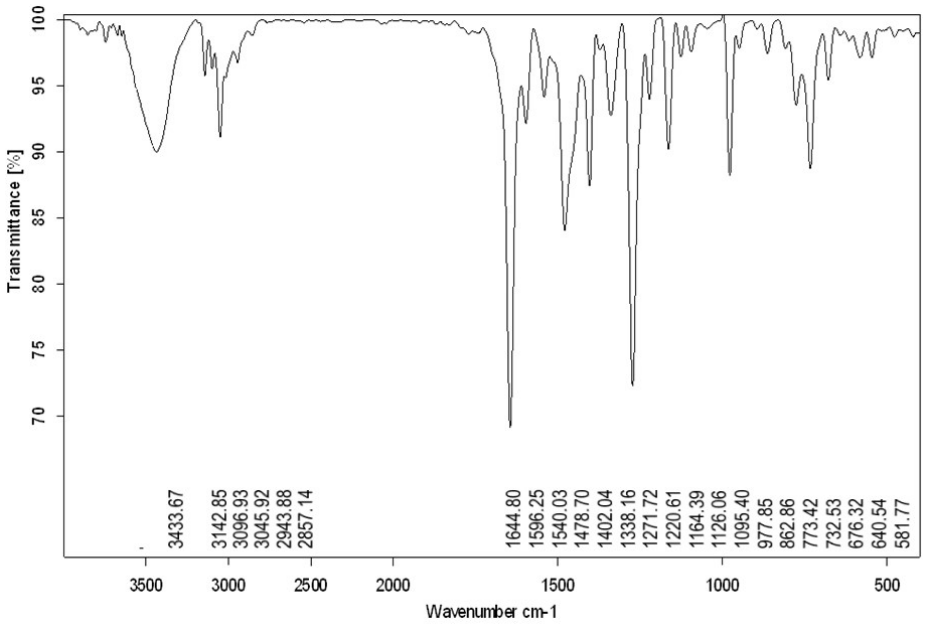

Figure S4: FTIR spectrum of VBIIL1.

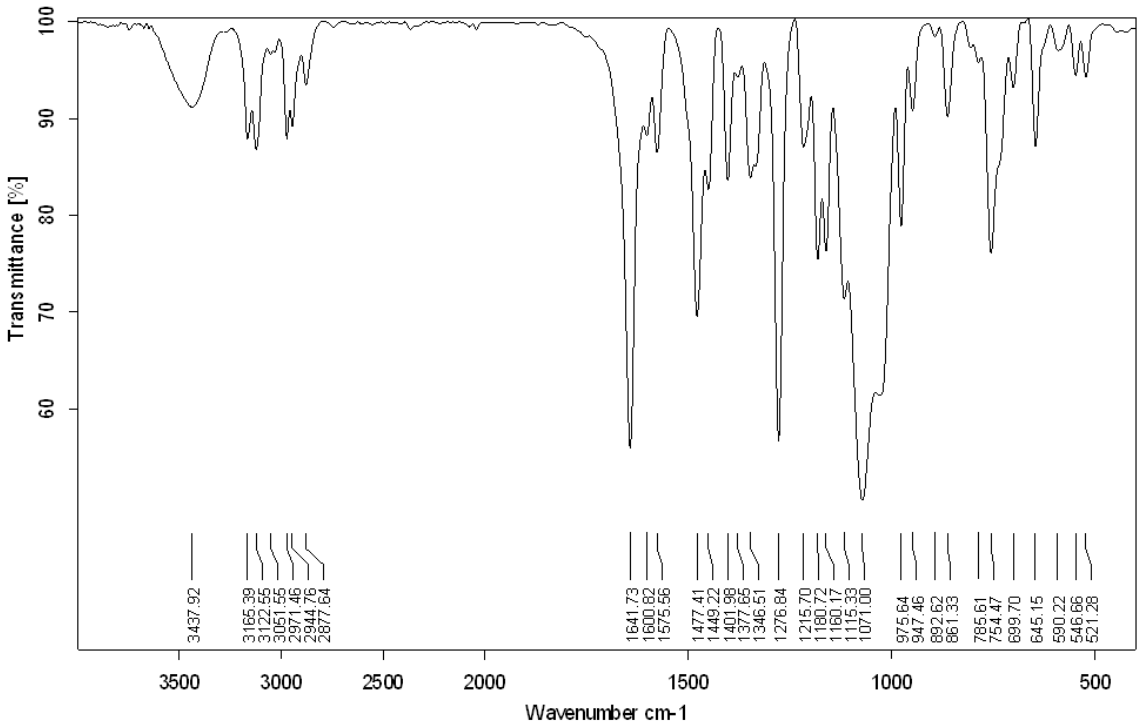

Figure S5: FTIR spectrum of VBIIL2.

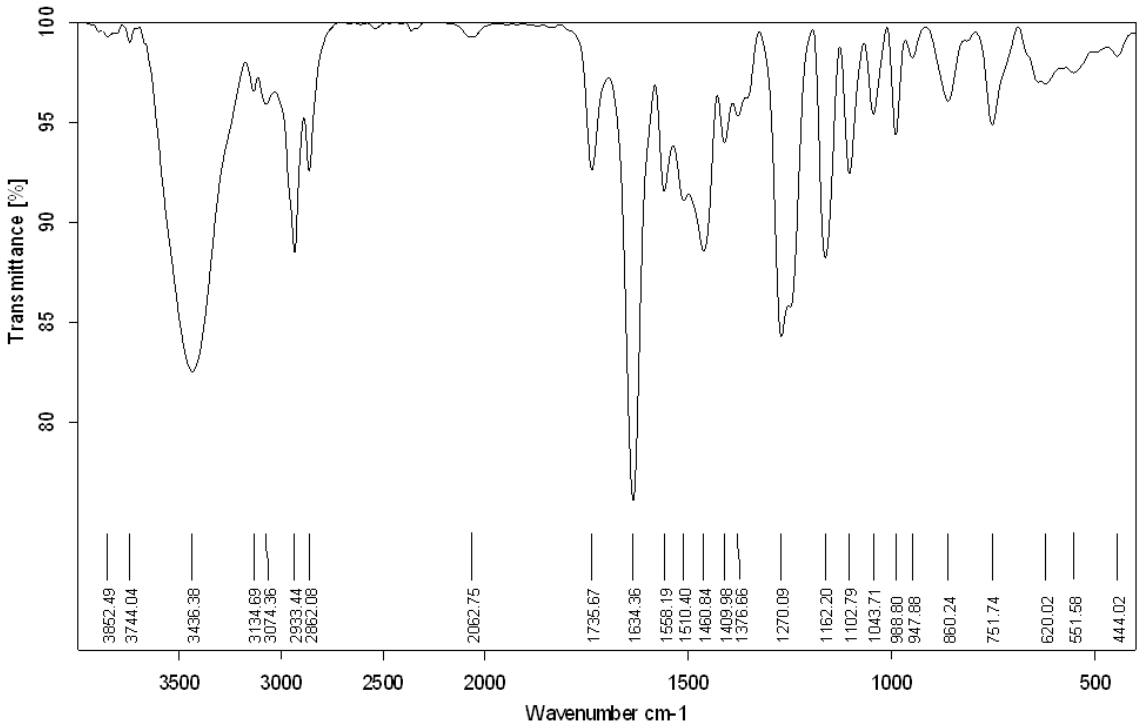

Figure S6: FTIR of spectrum TISSB1.

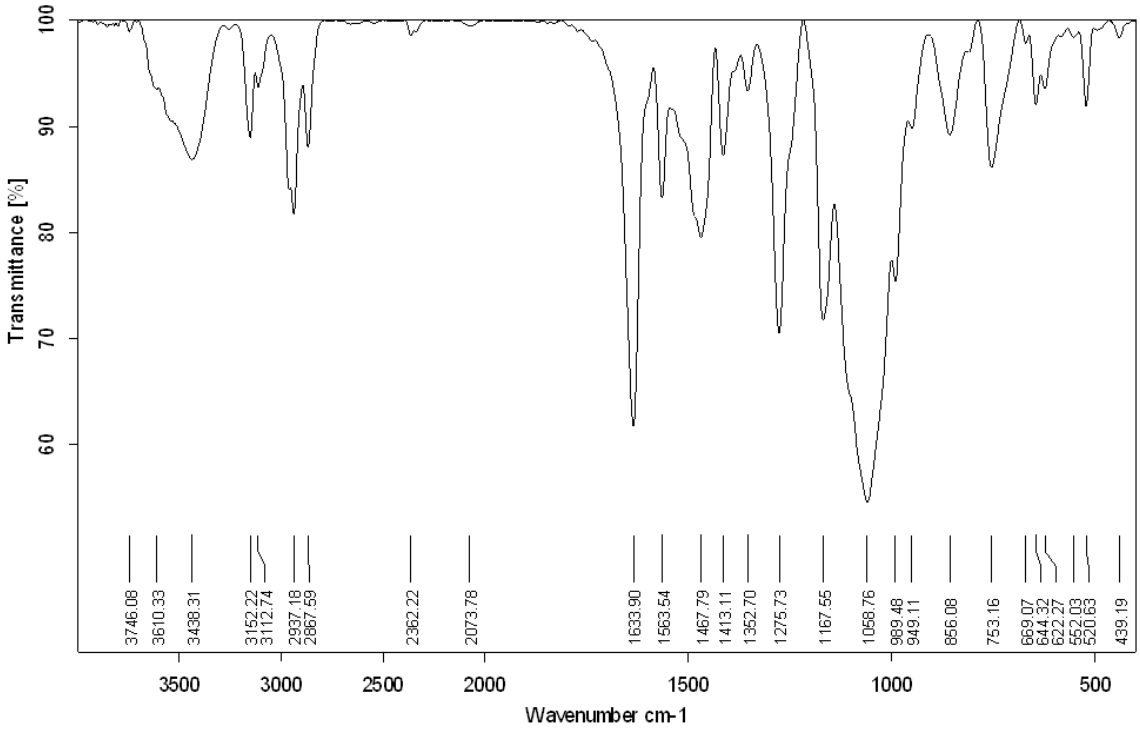

Figure S7: FTIR of spectrum TISSB2.

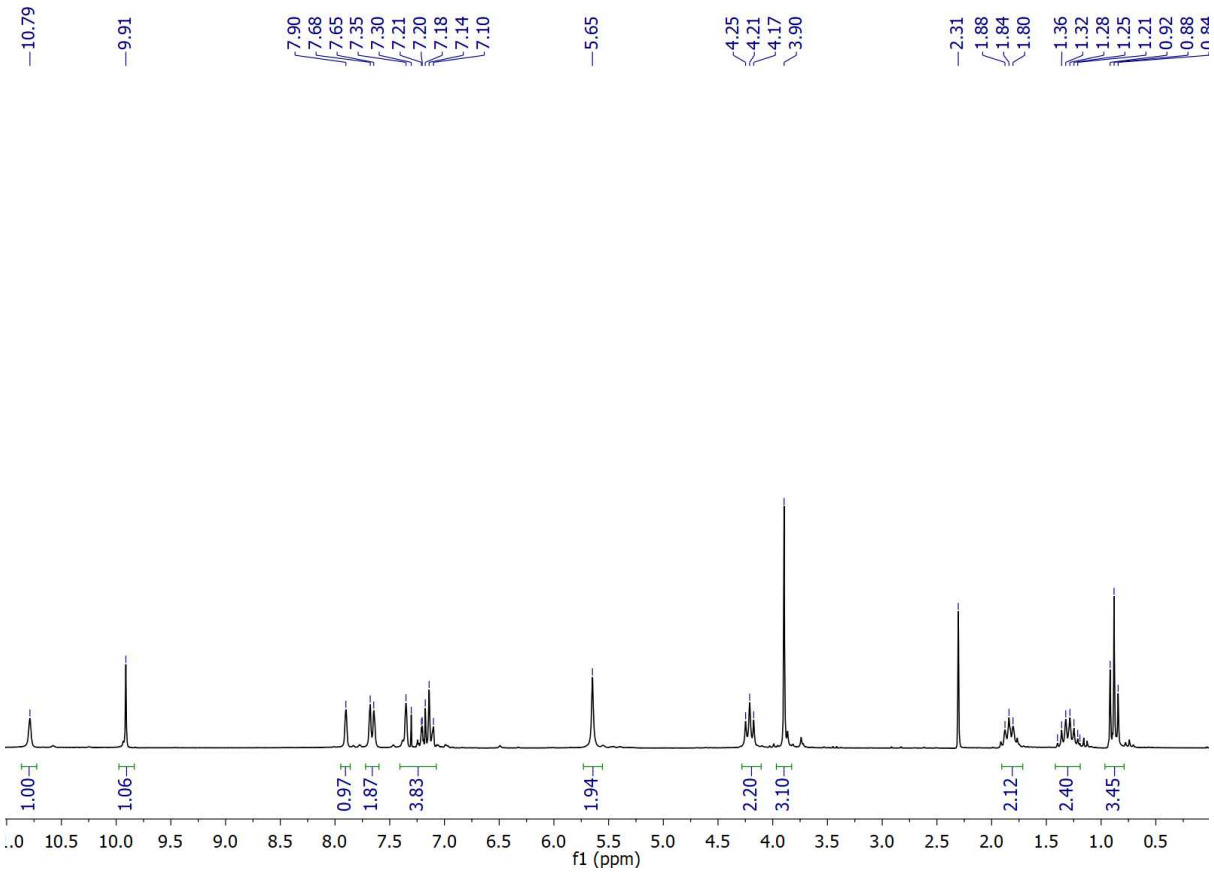

Figure S8: ^{1}H NMR spectrum (500 MHz) of VBIIL1.

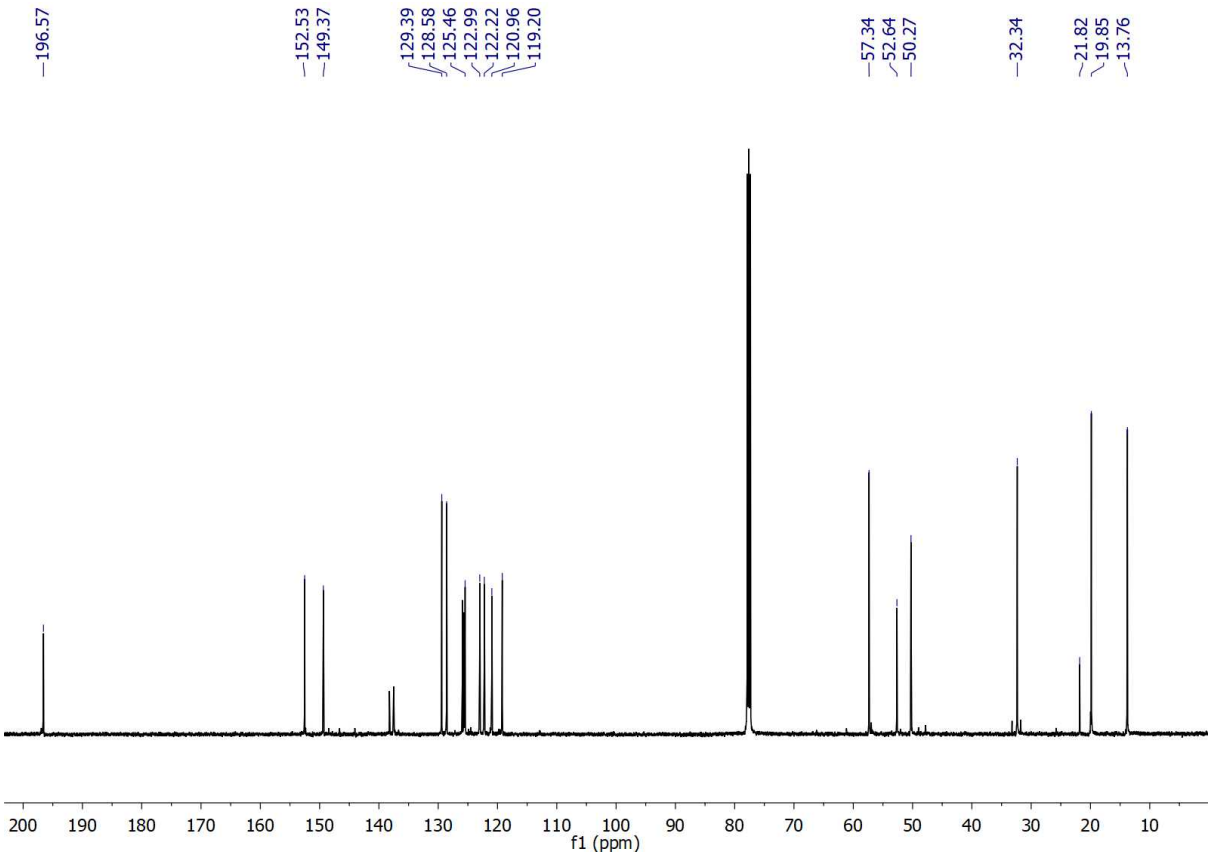

Figure S9: ^{13}C NMR spectrum (126 MHz) of VBIIL1.

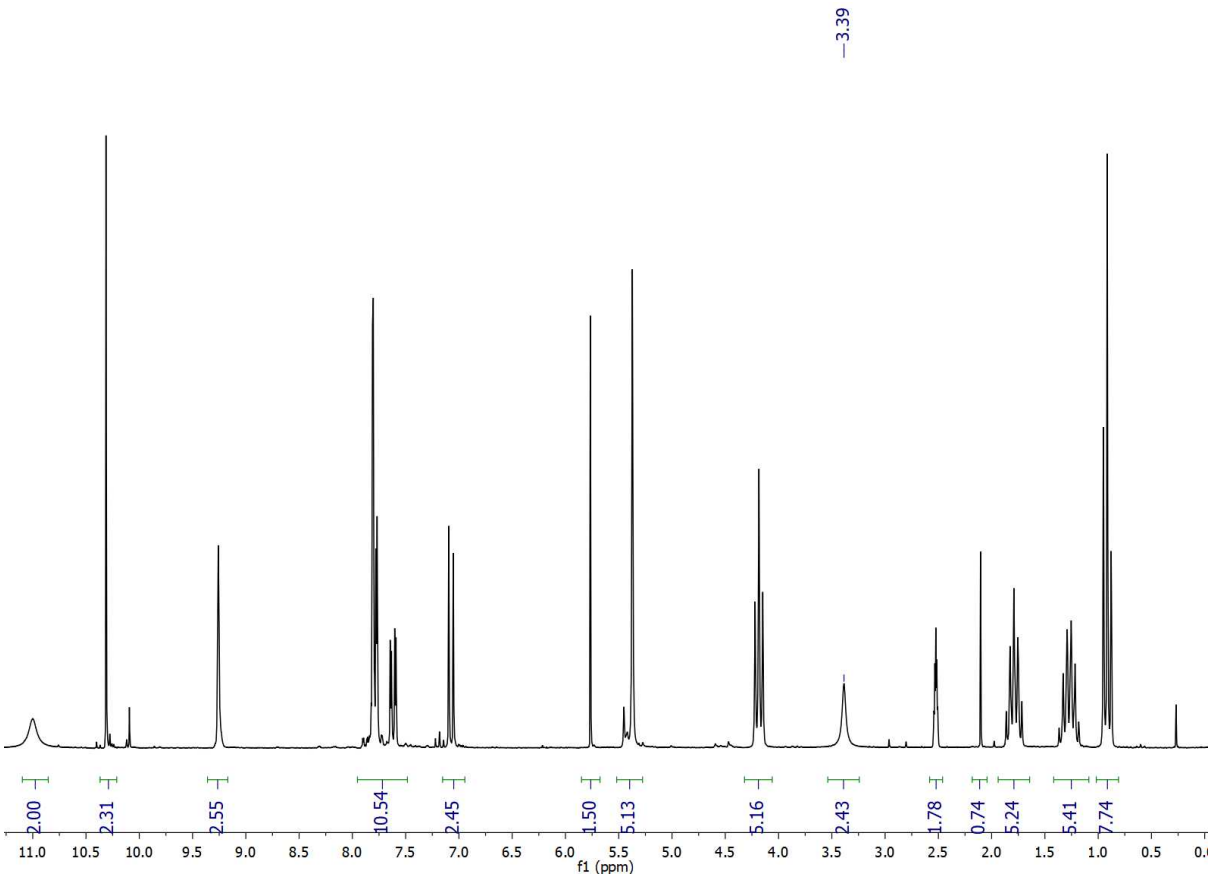

Figure S10: ^{1}H NMR spectrum (500 MHz) of VBIIL2.

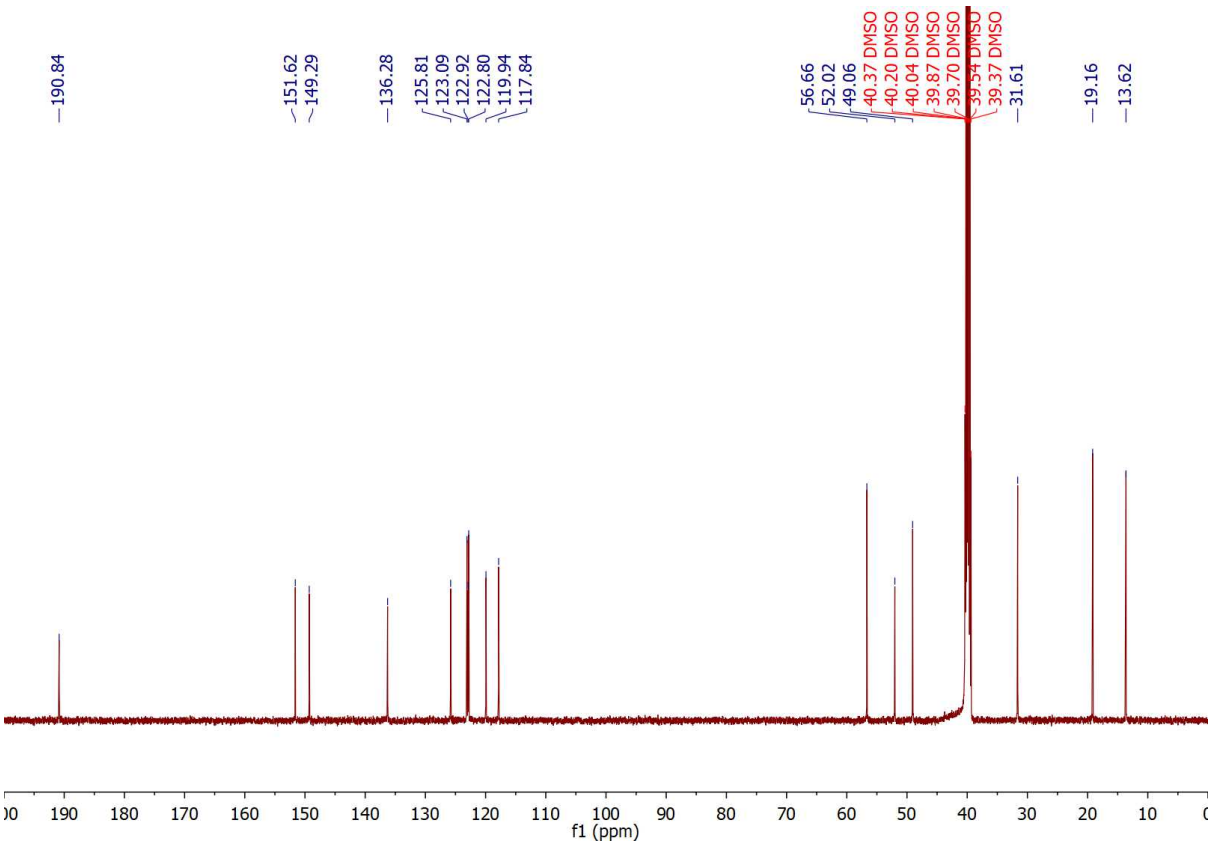

Figure S11: ^{13}C NMR spectrum (126 MHz) of VBIIL2.

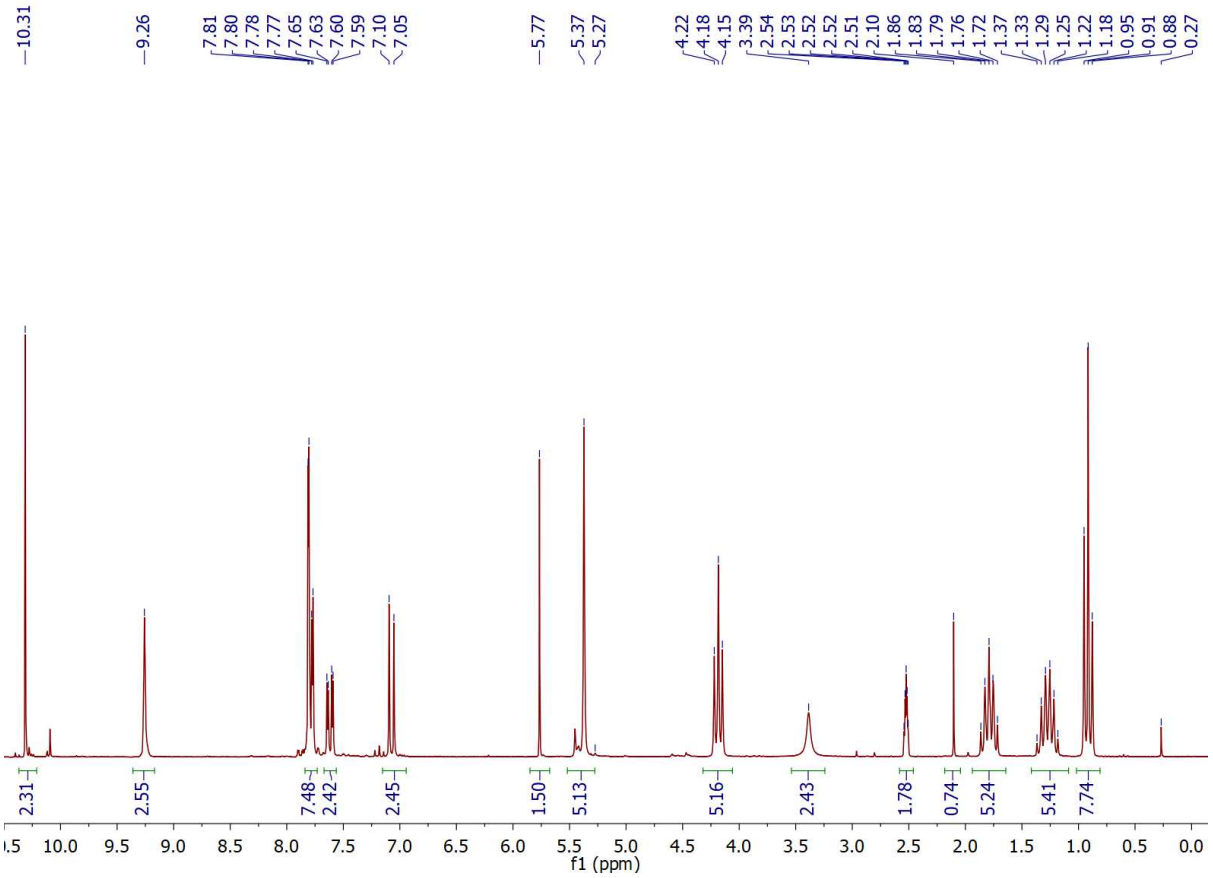

Figure S12: ^{1}H NMR spectrum (500 MHz) of TISSB1.

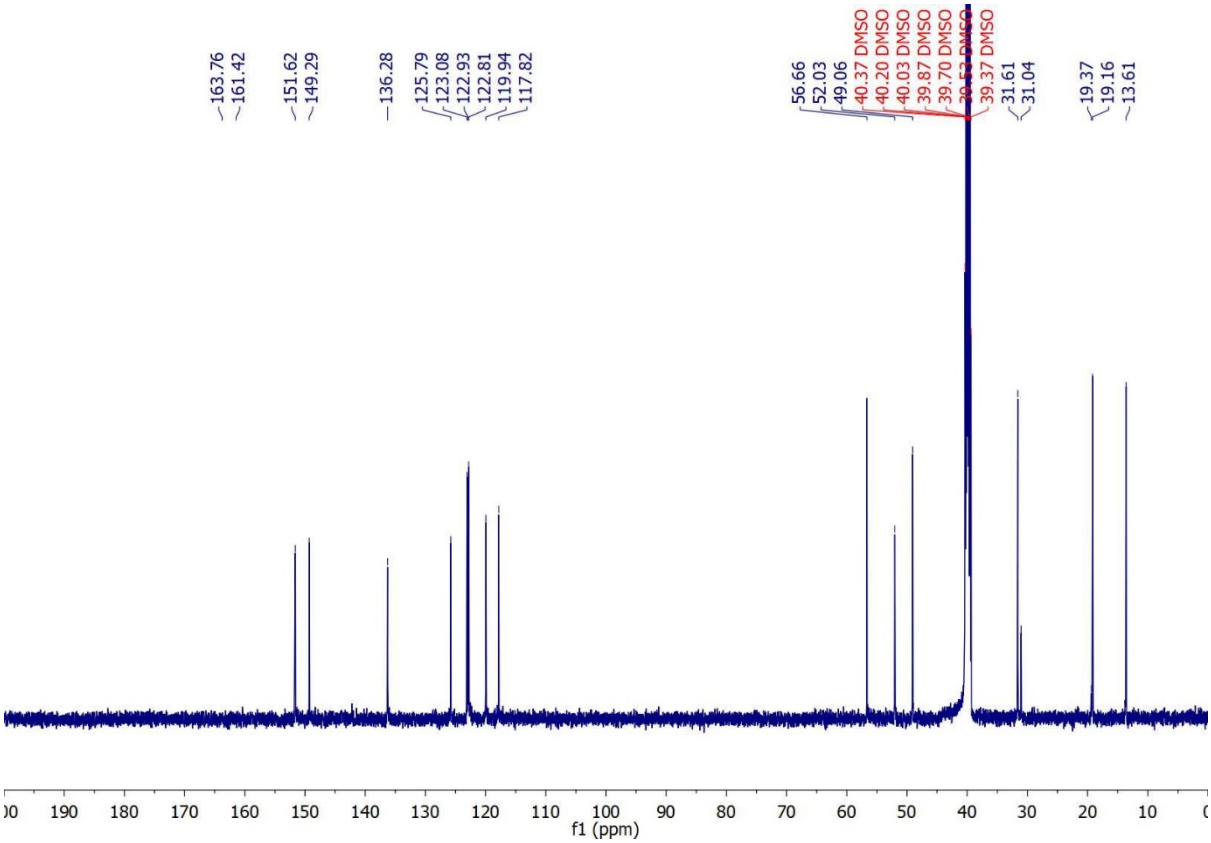

Figure S13: ^{13}C NMR spectrum (126 MHz) of TISSB1.

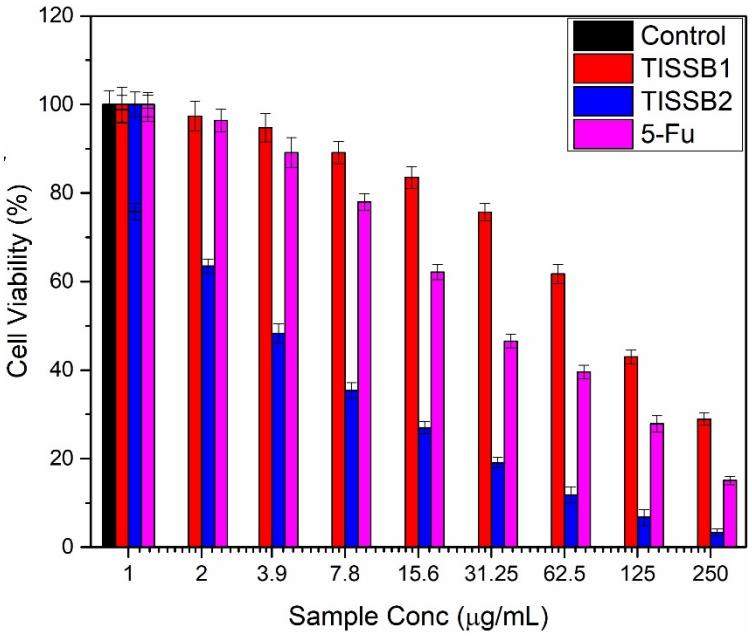

Figure S14: Impacts of serial doses of TISSBs on the viability of MCF-7 cell line, in comparison to a clinical drug 5-Fluorouracil (5-Fu)

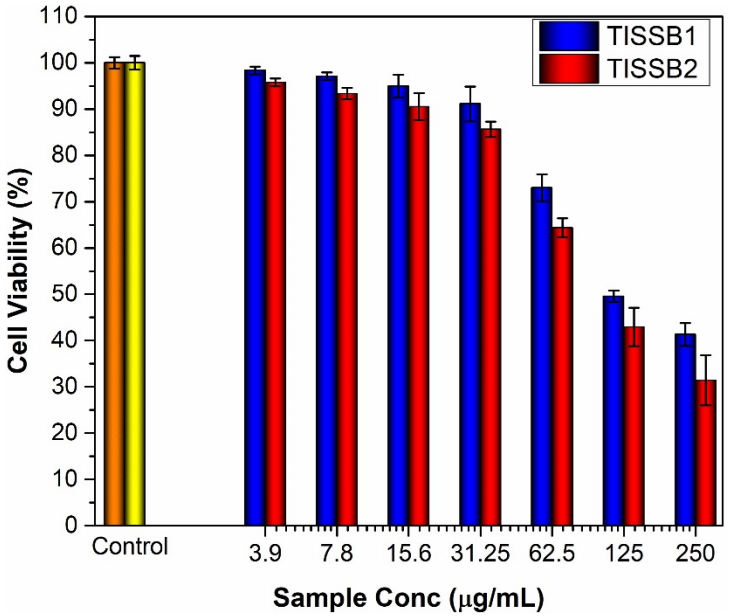

Figure S15: Impacts of serial doses of TISSBs on the viability of HSF cell line

6. Tables Captions

Table S1: Values of IC_{50} (µg/mL) of newly developed anticancer agents against MCF-7 and HSF cell lines, as compared to a clinical anticancer drug (5-FU)

Cells	IC_{50} (µg/mL) ± SD		
	TISSB1	**TISSB2**	**5-FU**
MCF-7	16.45±1.25	3.68 ±0.31	12.03 ± 1.97
HSF	123.71 ± 0.06	119.88 ± 2.32	78.23 ± 1.89
SI	7.52	5.40	6.50

$$SI = \frac{IC_{50}\ against\ normal\ cell\ line}{IC_{50}\ against\ cancer\ cell\ line}$$

References

[S1] J. F. Adediji, S.A. Amolegbe, S. Adewuyi, C.A. Akinremi, Y.C. Oyeniran, B.O. Afolayan, Ligation of Fe(III) and Mn(II) complexes by bithiourea and their biological activity, ISRN Inorganic Chemistry, (2013) 1-8.