# Synthesis, biological evaluation and molecular dynamic of carbothioamides derivatives as carbonic anhydrase II and 15-Lipoxygenase inhibitors

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**Supporting Information**

**Contents**

1. Experimental data

2. Results and Discussion

3. Figure S1: $^{1}$H-NMR spectrum of (3c)

4. Figure S2; Expanded of $^{C13}$NMR spectrum of (3c)

5. Figure S3: $^{1}$H-NMR spectrum of (3f)

6. Figure S4: $^{C13}$-NMR spectrum of (3f)

7. Figure S5: $^{1}$H-NMR spectrum of (3h)

8. Figure S6: $C^{13}$-NMR spectrum of (3h)

9. Figure S7: $^{1}$H-NMR spectrum of (3j)

10. Figure S8: $C^{13}$-NMR spectrum of (3h)

**Experimental**

*Spectral characterization of compounds (3a-j)*

***(E)-1-((2,3,6,7-tetramethoxynaphthalen-1-yl)methylene)thiosemicarbazide* (3a)**

Yield:82%: M.P 240°C $R_f$:0.56; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3373 ($NH_2$), 3230 (N-H), 3125 ($sp^2CH$), 2960 ($sp^3CH$), 1607 (C=N), 1577 (Ar-C=C), 1080 (C=S), 1051 (C-O) $^1$HNMR; (300MHz, DMSO-$d_6$): δ 11.2 (s, 1H, NH), 7.9(s, 1H, CH=N), 7.6 (s, 2H, -$NH_2$); 6.84-7.40 (m 3H, Ar-H), 3.74 (s, 3H, OMe). $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ (ppm): 180.2, 151.9, 150.5, 148.3, 145.8, 138.6, 124.7, 123.3, 122.6, 108.4, 107.4, 107.1, 60.7, 56.8, Anal. Calcd. for $C_{16}H_{19}N_3O_4S$: C, 55.01; H, 5.49; N, 12.04; S, 9.18 found: C, 54.97; H, 5.41; N, 11.99S, 9.17 HRMS Caled for $C_{16}H_{19}N_3O_4S$ +H : 349.1096. Found 349.1093

***(E)-1-(4-(methyl(prop-2-ynyl)amino)benzylidene)thiosemicarbazide* (3b)**

Yield: 85%: M.P 245°C $R_f$: 0.65; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3420 ($NH_2$), 3256 (N-H), 3133 ($sp^2$-CH), 2970 ($sp^3CH$), 2180 (C≡C), 1608 (C=N), 1580 (Ar-C=C), 1092 (C=S), $^1$H NMR (300 MHz, DMSO-$d_6$): δ (ppm): 11.3 (s, 1H, NH), 8.12(s, 1H, CH=N), 7.67-7.3 (m, 4H, Ar-H), 7.1 (s, 2H, -$NH_2$); 6.62 (s, 2H,); 4.20 (s, 1H), 1.8 (s, 3H, -$CH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ (ppm): 184.4, 151.7, 145.8, 129.5, 129.4, 123.1, 112.65, 77.9, 77.4, 40.6, 39.6. Anal. Calcd. for $C_{12}H_{14}N_4S$: C, 58.53; H, 5.71; N, 22.71;S, 13.02.found: C, 58.49; H, 5.68; N, 22.697 S, 13.04. HRMS Caled for $C_{12}H_{14}N_4S$ +H : 246.0939. Found 246.0941

***(E)-1-(2-fluorobenzylidene) thiosemicarbazide* (3c)**

Yield:75%: M.P 235°C $R_f$: 0.52; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3412 ($NH_2$), 3250 (N-H), 3120 ($sp^2CH$), 1608 (C=N), 1597 (Ar-C=C), 1085 (C=S), $^1$H NMR (300 MHz, DMSO-$d_6$): δ (ppm): 11.5 (s, 1H, NH), 8.05 (s, 1H, CH=N), 8.27-7.01 (m, ,4H Ar-H), 7.42 (s, 2H, -$NH_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ (ppm):.178.5, 162.9, 159.6, 135.2, 132.2, 127.2, 125.1,

122.1Anal. Calcd.for $C_8H_8FN_3S$: C, 48.71; H, 4.10; N, 21.29; S, 16.26 found: C, 48.67; H, 4.09; N, 21.26 S, 16.24. HRMS Caled for $C_8H_8FN_3S$ +H : 197.0423. Found 197.0420

***(E)-1-(2-chloro-5-nitrobenzylidene) thiosemicarbazide*** **(3d)**

Yield:75%: M.P 248°C $R_f$: 0.46; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3415 ($NH_2$), 3250 (N-H), 3126 ($sp^2CH$), 1605 (C=N), 1597 (Ar-C=C), 1080 (C=S), $^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 10.82 (s, 1H, NH), 7.63 (s, 1H, CH=N), 6.69- 7.94 (m, 4H Ar-H) 7.52 (s, 1H -$NH_2$), $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 179.4, 145.9, 144.6, 139.1, 135.5, 131.2, 124.7, 122.1. Anal.Calcd.for $C_8H_7ClN_4O_2S$: C, 37.12; H, 2.73; N, 21.65; S, 12.40 found: C, 37.09; H, 2.69; N, 21.57 S, 12.42. HRMS Caled for $C_8H_7ClN_4O_2S$ +H : 257.9978. Found 257.9975

***(E)-1-(3-hydroxy-4-methoxybenzylidene) thiosemicarbazide*** **(3e)**

Yield:80%: M.P 228°C $R_f$:0.57; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3454 (OH), 3374 ($NH_2$), 3240 (N-H), 3125 ($sp^2CH$), 2970 ($sp^3CH$),1601 (C=N), 1589 (Ar-C=C), 1070 (C=S), 1053(C-O) $^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 11.52 (s, 1H, NH), 8.31 (s, 1H, CH=N), 7.52 (s, 1H -$NH_2$), 6.71-6.92 (m, 4H Ar-H), 5.71 (s, 1H -OH), 3.80 (s, 3H- -$OCH_3$). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 179.4, 148.9, 147.6, 145.8, 129.6, 120.4, 115.3, 115.2, 56.8. Anal.Calcd. for $C_9H_{11}N_3O_2S$: C, 47.98; H, 4.93; N, 18.63; S, 14.23. found: C, 47.95; H, 4.91; N, 18.60 S, 14.22

HRMS Caled for $C_9H_{11}N_3O_2S$ +H : 225.0572. Found 225.0569

***(E)-1-((5-bromothiophen-2-yl) methylene) thiosemicarbazide*** **(3f)**

Yield:75%: M.P 243 °C $R_f$: 0.42; Petroleum ether: ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3417 ($NH_2$), 3264 (N-H), 3126($sp^2CH$), 1609(C=N), 1587 (Ar-C=C), 1080 (C=S),$^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 12.88 (s, 1H, NH), 8.36 (s, 1H, CH=N), 7.94 (s, 2H -$NH_2$), 7.08 – 6.70 (m, 2H Ar-H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 178.3, 149.3,148.0, 147.0, 143.2, 117.7. Anal. Calcd. for $C_6H_6BrN_3S_2$: C, 27.01; H, 2.28; N, 15.89; S, 24.28 found: C, 26.97; H, 2.27; N, 15.84.S, 24.26 found 262 (Channar, et al., 2017). HRMS Caled for $C_6H_6BrN_3S_2$ +H : 262.9187. Found 262.9184

***(E)-1-(2-chloro-6-fluorobenzylidene)thiosemicarbazide*** **(3g)**

Yield:75%: M.P 240°C $R_f$:0.42; Petroleum ether: ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3418 ($NH_2$), 3267 (N-H), 3126 ($sp^2CH$), 1608 (C=N), 1589 (Ar-C=C), 1079 (C=S), $^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 10.36 (s, 1H NH), 8.12 (s, 1H, CH=N), 7.79 (s, 1H), 6.66 – 7.07 (m, 4H Ar-H), 6.92 (s, 2H -$NH_2$). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 178.2, 162.08, 144.7, 137.6, 131.2, 126.8, 125.9, 116.5 Anal. Calcd. for $C_8H_7ClFN_3S$: C, 41.45; H, 3.05; N, 18.13; found: C, 41.42; H, 3.01; N, 18.09. HRMS Caled for $C_8H_7ClFN_3S$ +H : 231.0033. Found 231.0030

***(E)-1-(3-fluorobenzylidene)thiosemicarbazide*** **(3h)**

Yield: 64%: M.P 214°C $R_f$: 0.56; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3479 (NH2), 3249 (N-H), 1605 (C=N), 1595 (Ar-C=C), 1115 (C=S), $^1H$ NMR (300 MHz, **Acetone**): δ (ppm): 10.71 (s, 1H, NH), 8.17 (s, 2H, -$NH_2$); 7.92 (s, 1H, CH=N), 7.38-7.72 (m, 4H, Ar-H), $^{13}C$ NMR **$^{13}$C-NMR (75 MHz, Acetone)**: δ (ppm): 179.6., 140.9, 136.5, 134,3, 130.3, 129.6, 126.3, 126.2. Anal. Calcd. for $C_8H_8FN_3S$: C, 48.71; H, 4.09; N, 21.29;S, 16.26 found: C, 48.67; H, 4.06; N, 21.24, S, 16.25 HRMS Caled for $C_8H_8FN_3S$ +H : 197.0423. Found 197.0419

**(*E)-1-(4-chloro-3-nitrobenzylidene)thiosemicarbazide* (3i)**

Yield:90%: M.P 233°CRf: 0.70; IR; (KBr, $cm^{-1}$): 3435 ($NH_2$), 3240 (N-H), 1595 (C=N), 1565(Ar-C=C), 1085 (C=S), $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 10.11 (s, 1H, NH), 8.43 (s, 1H, CH=N), 6.64-7.97 (m, 4H, ArH) 7.1 (s, 2H, -$NH_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 182.1, 147.1, 145.5, 137.7, 133.3, 130.5, 125.9, 124.8 Anal. Calcd. for $C_8H_7ClN_4O_2S$: C, 37.14; H, 2.74; N, 21.64;S, 12.40. found: C, 37.12; H, 2.70; N, 21.60 S, 12.38. HRMS Caled for $C_8H_7ClN_4O_2S$ +H : 257.9978. Found 257.9975

***(E)-2-(thiophen-2-ylmethylene)hydrazinecarbothioamide*** **(3j)**

Yield:75%: M.P 232°C $R_f$: 0.64; Petroleum ether : ethyl acetate (6:4) IR; (KBr, $cm^{-1}$): 3414 ($NH_2$), 3260 (N-H), 3125 ($sp^2CH$), 2980 ($sp^3CH$), 1603 (C=N), 1587 (Ar-C=C), 1090 (C=S), $^1H$ NMR (300 MHz, DMSO-$d_6$): δ (ppm): 11.42 (s, 1H, NH), 8.20 (s, 1H, CH=N), 7.96 (s, 1H), 7.80 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H), 7.61 (t, *J* = 7.4 Hz, H). 7.96 (s, 2H $NH_2$), $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ (ppm): 178.20, 148.02, 139.75, 133.80 130.20, 123.75..Anal.Calcd.for $C_6H_7N_3S_2$: C, 38.90; H,

3.81; N, 22.68; S, 34.61 found: C, C, 38.92; H, 3.77; N, 22.62; S, 34.60 HRMS Caled for $C_6H_7N_3S_2$ +H : 185.0181. Found 185.0078

**Biochemical Assays**

**Carbonic anhydrase assay**

This method is based on the spectrophotometric determination of hydrolytic product of substrate *p*-nitrophenyl acetate substrate. Assay volume was kept at 100 µL, comprised of 60 µL of Tris-sulfate buffer (50 mM, 0.1 mM $ZnCl_2$ at pH 7.6), 10 µL of test compound with concentration of 50 µM and 10 µL (50 U) of *b*-CA II. The reaction mixture was incubated at 25 $^oC$ for 10 min and absorbance was measured at 405 nm using FLUOstar Omega, Germany. Reaction was initiated by addition of 20 µL of *p*-nitrophenyl acetate (6mM) as substrate and incubated for further 30 min. Absorbance was measured at 405 nm as after read and percentage of inhibition was calculated for test compounds. All experiments were performed in triplicate and dose response curves were determined to calculate for test compounds that exhibited percentage of inhibition more that 50.

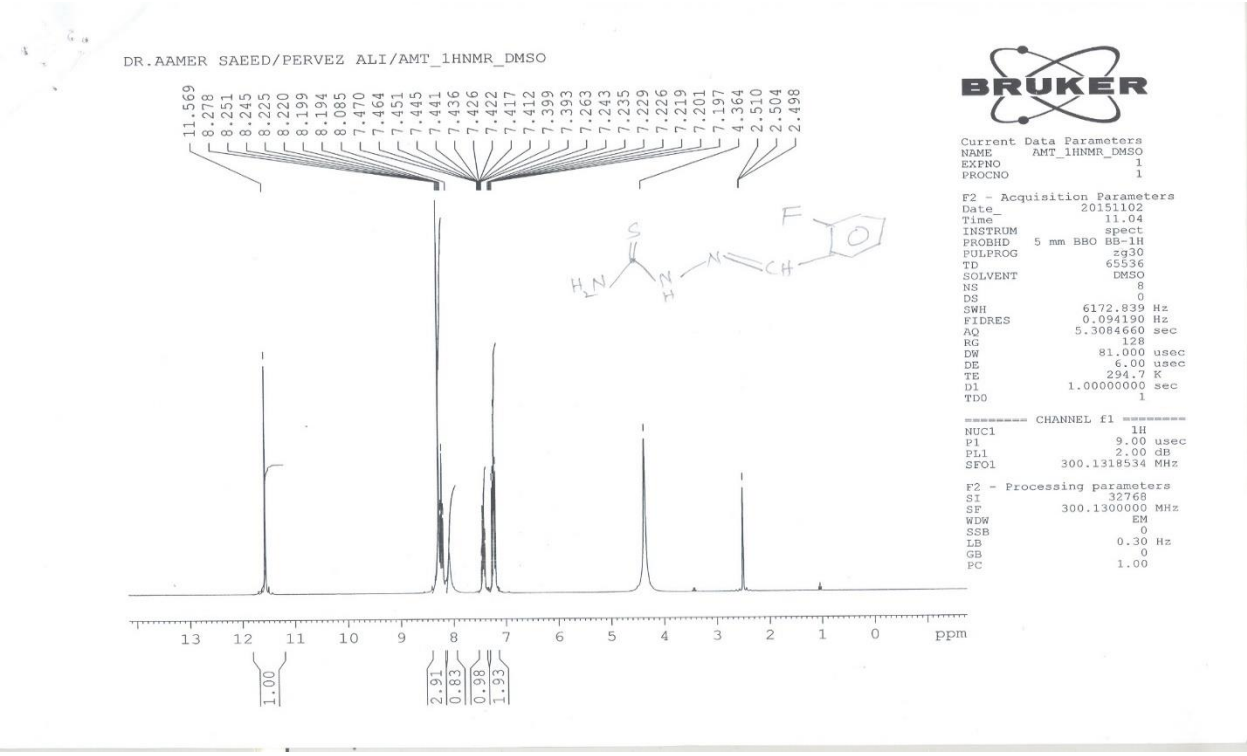

Figure S1: $^{1}$H-NMR spectrum of **(3c)**

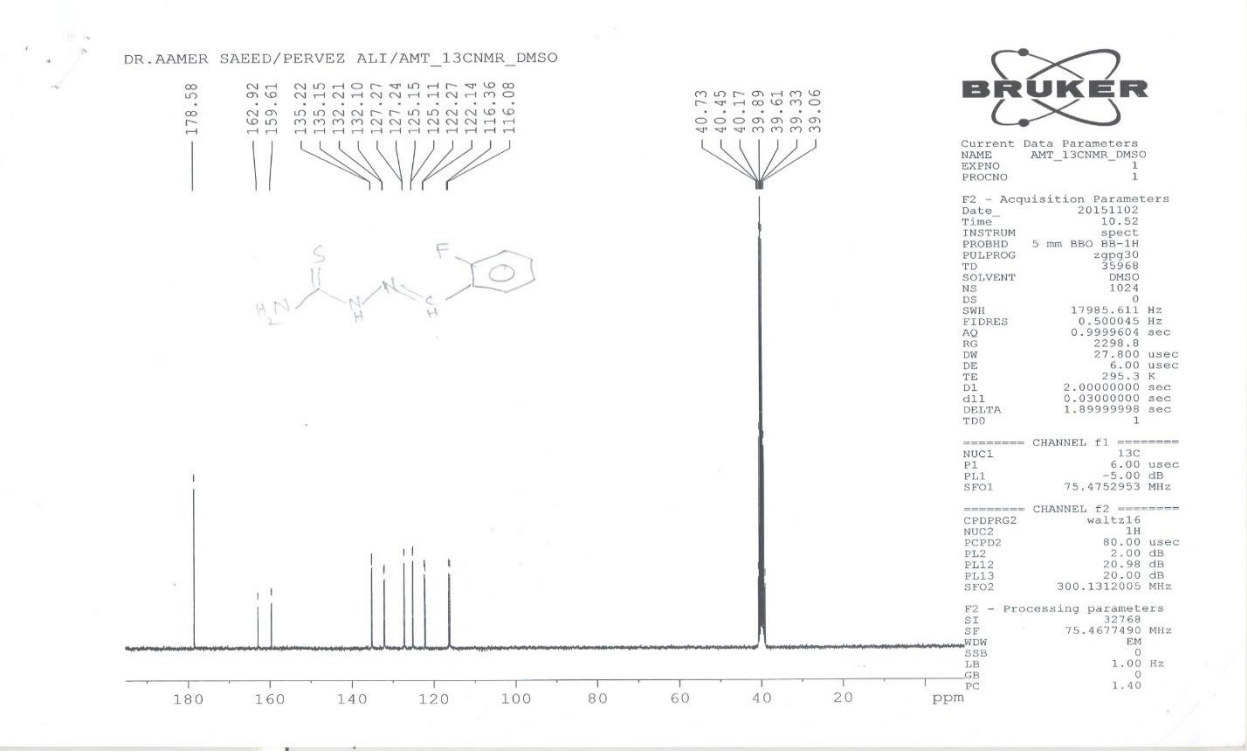


Figure S2: $^{C13}$-NMR spectrum of **(3c)**

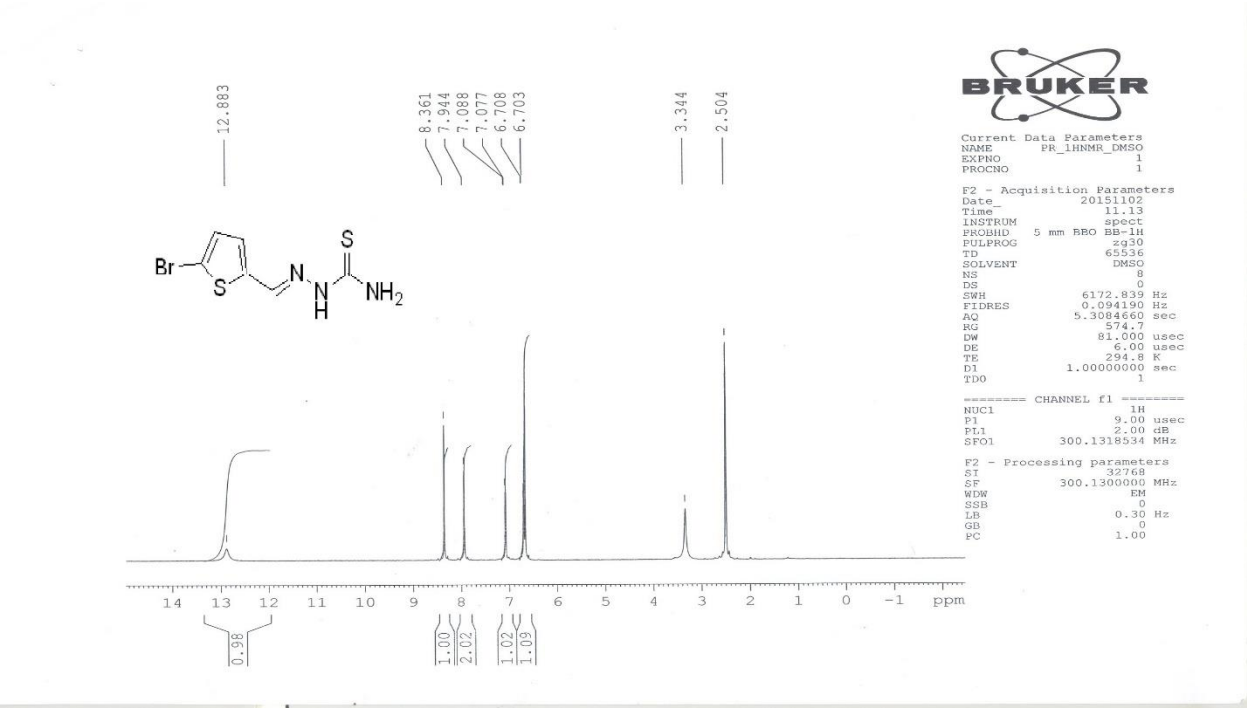

Figure S3: $^{1}$H-NMR spectrum of **(3f)**

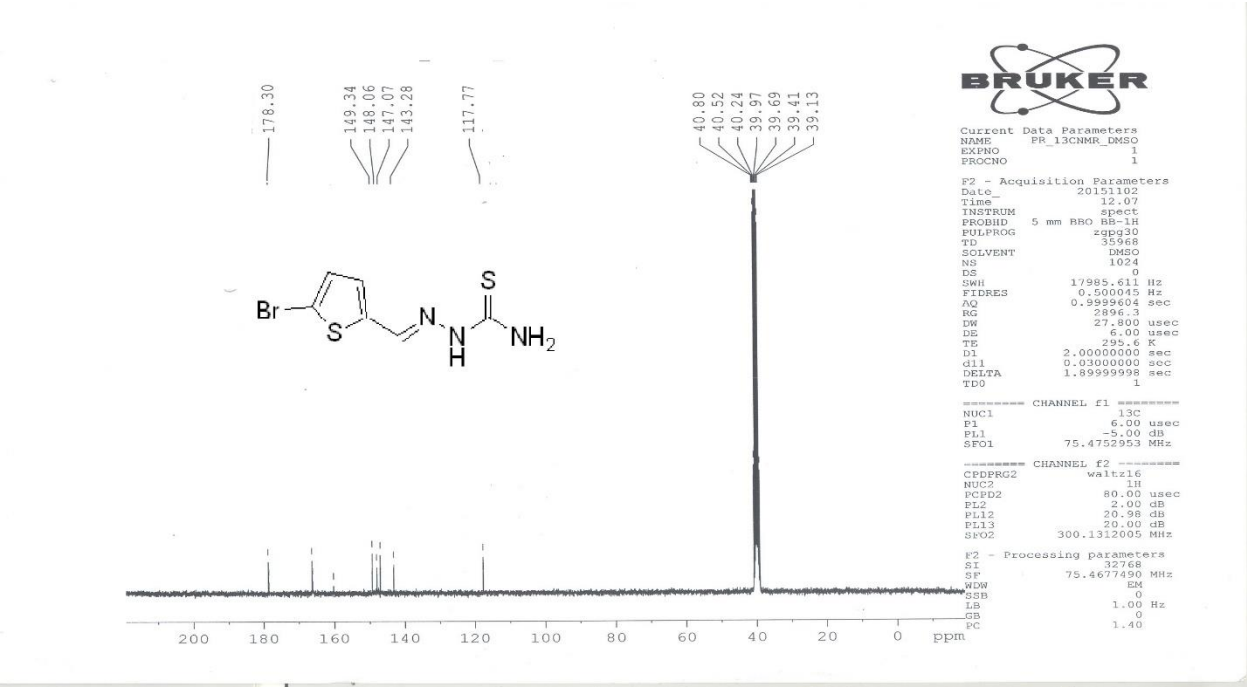


Figure S4: $^{C13}$-NMR spectrum of **(3f)**

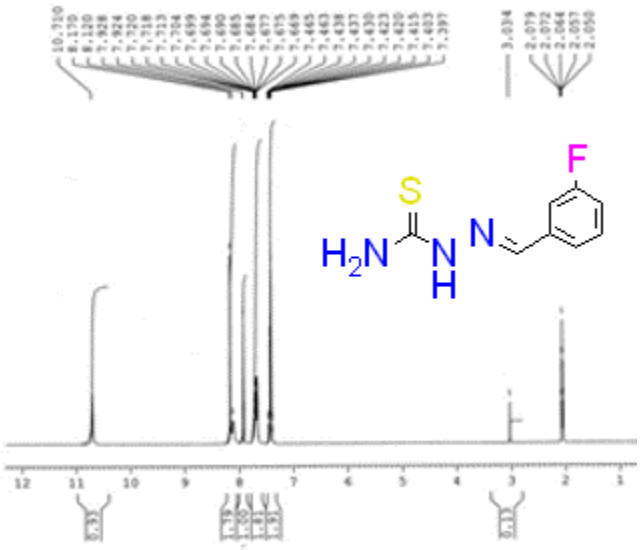


Figure S5: $^{1}$H-NMR spectrum of **(3h)**

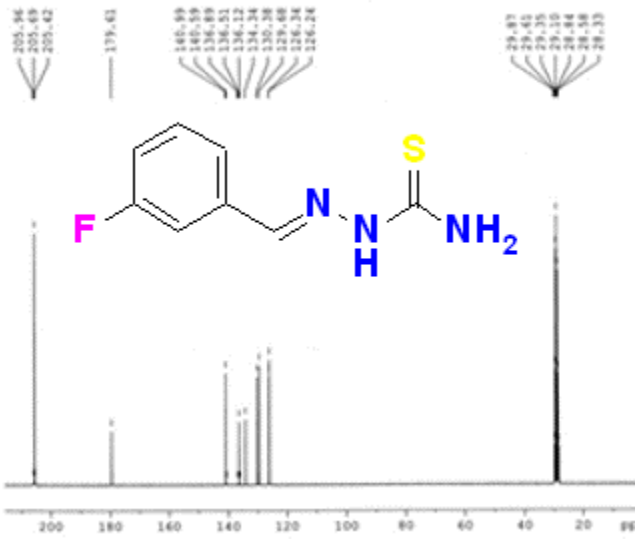


Figure S6: $C^{13}$-NMR spectrum of **(3h)**

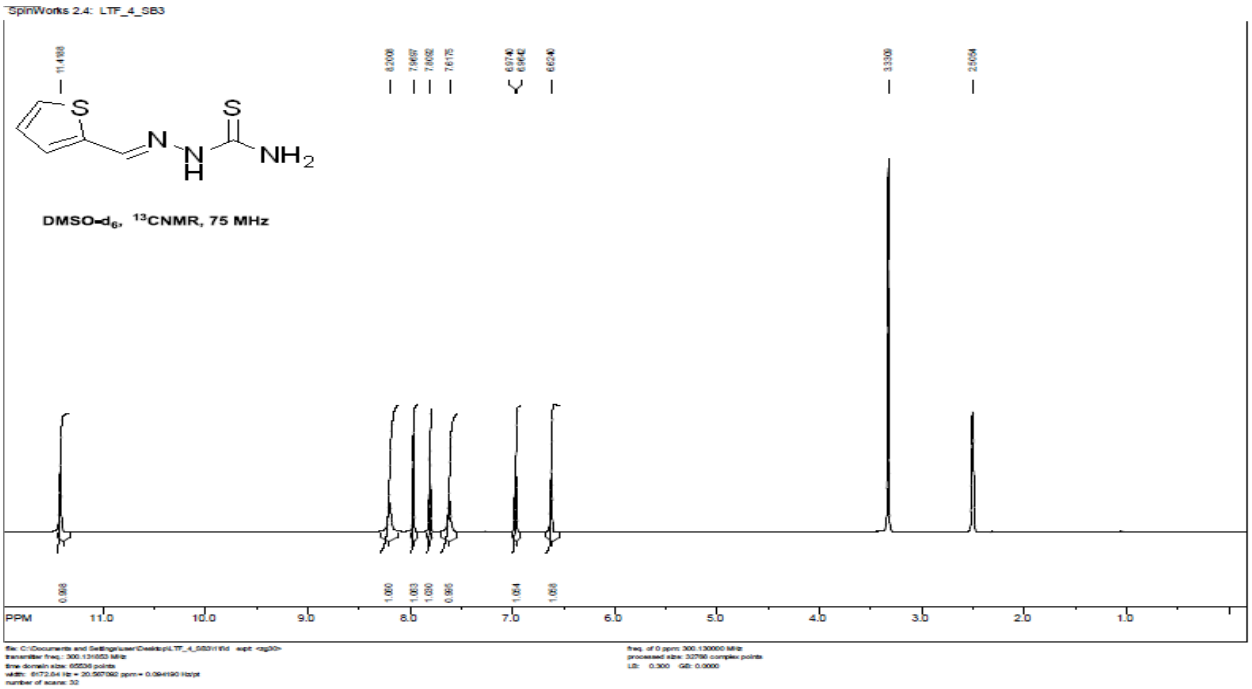


Figure S7: $^{1}$H-NMR spectrum of **(3j)**

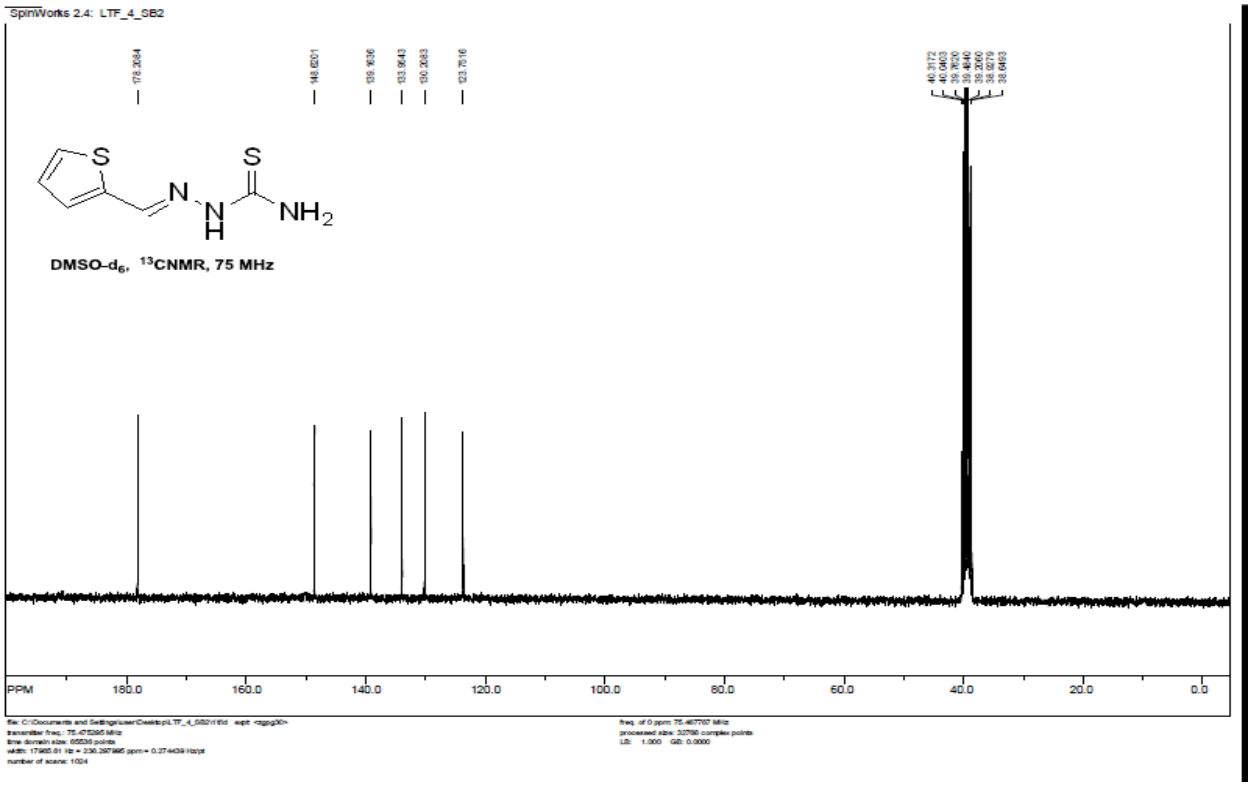


Figure S8: $C^{13}$-NMR spectrum of **(3h)**