

## SUPPLEMENTARY FILE

# Analysis of 1-aryl-3-[3-chloro-2-methylphenyl]thiourea hybrids as potent urease inhibitors: Synthesis, biochemical evaluation and computational approach

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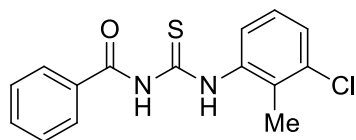
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## Experimental

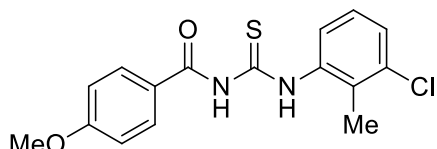
### Characterization data

#### 4a) *N*-((3-chloro-2-methylphenyl)carbamothioyl)benzamide



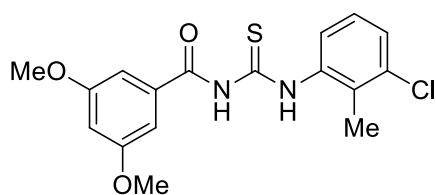
Off white powder, Yield=82%, M.P=134°C,  $R_f$  =0.25(n-Hexane: Ethyl acetate 1:1), FTIR( $\text{cm}^{-1}$ ): 1248 (C=S stretch), 3241 (N-H stretch), 2979.4 (Aromatic C-H, stretching), 1582 (C-C, stretching), 1138 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) 12.161 (s, 1H, ArCO-N-H), 11.815 (s, 1H, Ar-NH), 8.037-7.609 (m, 4H, Ar), 7.448-7.290 (m, 3H, Ar), 1.62 (s, 3H, CH<sub>3</sub>)  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ ) 181.06 (C=S), 167.60 (C=O), 139.25, 138.49, 134.23, 133.18, 131.41, 131.21, 131.08, 129.00, 128.44, 127.57, 127.12, 15.57 (CH<sub>3</sub>); Anal. Calcd. for C<sub>16</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>2</sub>S, C, 57.40; H, 4.52; N, 8.37; S, 9.58, found; C, 58.41; H, 4.63; N, 8.71; S, 9.62.

#### 4b) *N*-((3-chloro-2-methylphenyl)carbamothioyl)-4-methoxybenzamide



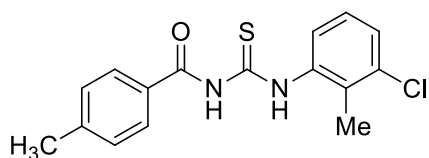
Off white crystalline powder, Yield: 85%, M.P=145°C,  $R_f$  = 0.28 (n-Hexane: Ethyl acetate 1:1), FTIR( $\text{cm}^{-1}$ ): 1238 (C=S stretch), 3256 (N-H stretch), 2978.8 (Aromatic C-H, stretching), 1572 (C-C, stretching), 1132 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) 12.350 (s, 1H, ArCO-N-H), 9.207 (s, 1H, Ar-NH), 7.916-6.983 (m, 7H, Ar), 3.914 (s, 3H, OCH<sub>3</sub>), 2.398 (s, 3H, CH<sub>3</sub>)  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 180.17 (C=S), 166.54 (C=O), 164.1, 137.7, 135.2, 132.5, 129.8, 127.0, 126.7, 125.4, 114.5, 144.0, 55.6 (OCH<sub>3</sub>), 15.23 (CH<sub>3</sub>); Anal. Calcd. for C<sub>15</sub>H<sub>13</sub>ClN<sub>2</sub>OS, C, 46.95; H, 3.15; N, 7.30; S, 8.36, found; C, 45.99; H, 3.44; N, 7.20; S, 8.23.

#### 4c) *N*-((3-chloro-2-methylphenyl)carbamothioyl)-3,5-dimethoxybenzamide



Colourless crystals, Yield: 83%, M.P=152°C,  $R_f$  = 0.31 (n-Hexane: Ethyl acetate 1:1), FTIR( $\text{cm}^{-1}$ ): 1236 (C=S stretch), 3255 (N-H stretch), 2949.6 (Aromatic C-H, stretching), 1572 (C-C, stretching), 1135 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) 12.020 (s, 1H, ArCO-N-H), 11.207 (s, 1H, Ar-NH), 8.516-6.983 (m, 6H, Ar), 3.614 (s, 6H, 2(OCH<sub>3</sub>)), 2.498 (s, 3H, CH<sub>3</sub>)  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) 181.17 (C=S), 169.0 (C=O), 161.8, 140.3, 137.4, 134.7, 125.7, 124.8, 124.5, 104.0, 103.8, 55.9 (OCH<sub>3</sub>), 15.23 (CH<sub>3</sub>); Anal. Calcd. for C<sub>17</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>3</sub>S, C, 55.96; H, 4.70; N, 7.68; S, 8.79, found; C, 56.16; H, 4.90; N, 7.65; S, 8.57.

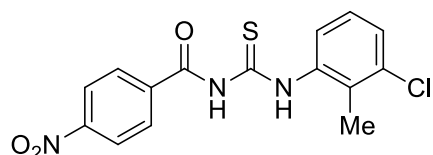
#### 4d) *N*-((3-chloro-2-methylphenyl)carbamothioyl)-4-methylbenzamide



Light yellow powder, Yield=86%, M.P=128°C,  $R_f$  =0.31 (n-Hexane: Ethyl acetate 1:1), FTIR( $\text{cm}^{-1}$ ): 1238 (C=S stretch), 3259 (N-H stretch), 2947 (Aromatic C-H, stretching), 1572 (C-C stretching), 1145 (C-N stretch),

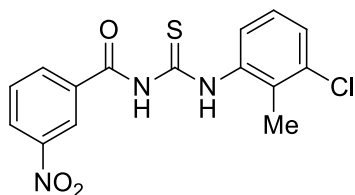
<sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>): δ (ppm) 11.99 (s, 1H, ArCO-N-H), 11.30 (s, 1H, Ar-NH), 7.816-6.98 (m, 7H, Ar), 2.61 (s, 3H, CH<sub>3</sub>), 2.398 (s, 3H, CH<sub>3</sub>), <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>): δ (ppm) 182.1 (C=S), 169.3 (C=O), 141.8, 140.3, 137.4, 134.7, 129.2, 127.4, 124.8, 124.4, 24.4, 6.1 Anal. Calcd. for C<sub>16</sub>H<sub>15</sub>ClN<sub>2</sub>OS, C, 60.28; H, 4.74; N, 8.79; S, 10.06, found; C, 61.08; H, 4.81; N, 8.77; S, 10.23

**4e) N-((3-chloro-2-methylphenyl)carbamothioyl)-4-nitrobenzamide**



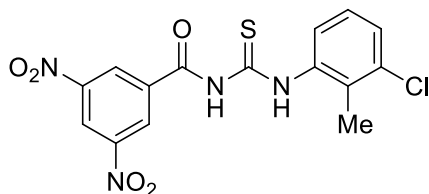
Light brown powder, Yield= 88%, M.P=122°C, R<sub>f</sub> = 0.26 (n-Hexane: Ethyl acetate 1:1), FTIR (cm<sup>-1</sup>): 1246 (C=S stretch), 3299 (N-H stretch), 2947.4 (Aromatic C-H, stretching), 1572 (C=C), 1175 (C-N stretch), <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>): δ (ppm) 12.00 (s, 1H, ArCO-N-H), 11.707 (s, 1H, Ar-NH), 8.37 (d, 2H, Ar-NO<sub>2</sub>), 8.21 (d, 2H, Ar-NO<sub>2</sub>), 6.22-6.76 (m, 3H, Ar), 2.35 (s, 3H, CH<sub>3</sub>), <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>): δ (ppm) 180.1 (C=S), 168.3 (C=O), 151.8, 140.3, 137.8, 134.7, 128.4, 127.5, 124.8, 124.5, 121.2, 6.7 (CH<sub>3</sub>) Anal. Calcd. for C<sub>15</sub>H<sub>12</sub>ClN<sub>3</sub>O<sub>3</sub>S, C, 51.51; H, 3.46; N, 12.01; S, 9.17, found; C, 51.61; H, 3.51; N, 12.61; S, 9.77.

**4f) N-((3-chloro-2-methylphenyl)carbamothioyl)-3-nitrobenzamide**



Light yellow, Yield=87%, M.P=132°C, R<sub>f</sub> = 0.29 (n-Hexane: Ethyl acetate 1:1), FTIR (cm<sup>-1</sup>): 1226 (C=S stretch), 3229 (N-H stretch), 2957.4 (Aromatic C-H, stretching), 1572 (C=C), 1155 (C-N stretch), <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>): δ (ppm) 12.20 (s, 1H, ArCO-N-H), 11.607 (s, 1H, Ar-NH), 8.37-7.44 (m, 4H, Ar-NO<sub>2</sub>), 7.32-6.76 (m, 3H, Ar), 2.45 (s, 3H, CH<sub>3</sub>), <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>): δ (ppm) 180.3 (C=S), 167.3 (C=O), 148.5, 140.3, 137.4, 135.2, 133.6, 139.8, 127.5, 124.5, 124.3, 122.4, 6.8 (CH<sub>3</sub>), Anal. Calcd. for C<sub>15</sub>H<sub>12</sub>ClN<sub>3</sub>O<sub>3</sub>S, C, 51.51; H, 3.46; N, 12.01; S, 9.17 found; C, 52.71; H, 3.66; N, 12.41; S, 9.67.

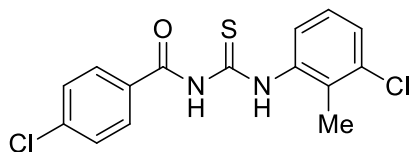
**4g) N-((3-chloro-2-methylphenyl)carbamothioyl)-3,5-dinitrobenzamide**



White crystals, Yield= 78%, M.P =124°C, R<sub>f</sub> = 0.27 (n-Hexane: Ethyl acetate 1:1), FTIR (cm<sup>-1</sup>): 1232 (C=S stretch), 3226 (N-H stretch), 2977.4 (Aromatic C-H, stretching), 1572 (C=C), 1115 (C-N stretch), <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>): δ (ppm) 12.70 (s, 1H, ArCO-N-H), 11.577 (s, 1H, Ar-NH), 8.27-7.47 (m, 3H, Ar-NO<sub>2</sub>), 7.32-6.36 (m, 3H, Ar), 2.46 (s, 3H, CH<sub>3</sub>), <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>): δ (ppm) 178.3 (C=S), 166.3 (C=O), 149.4, 140.3, 137.4, 136.0, 134.7, 128.5, 127.5, 124.8, 124.4, 122.1, 15.23 (CH<sub>3</sub>) Anal. Calcd. for C<sub>15</sub>H<sub>11</sub>ClN<sub>4</sub>O<sub>5</sub>S, C, 45.63; H, 2.81; N, 14.19; S, 8.12 found; C, 46.33; H, 2.91; N, 14.29; S, 8.16.

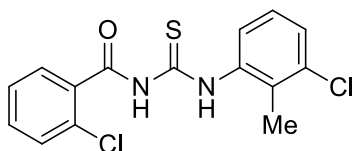
**4h) 4-chloro-N-((3-chloro-2-methylphenyl)carbamothioyl)benzamide**

Light yellow crystals, Yield=75%, M.P=160°C,  $R_f$  = 0.25 (n-Hexane: Ethyl acetate 1:1), FTIR ( $\text{cm}^{-1}$ ): 1242(C=S stretch), 3227 (N-H stretch), 2979.4 (Aromatic C-H, stretching), 1575(C-C stretching), 1115 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ );  $\delta$  (ppm) 11.90 (s, 1H, ArCO-N-H), 11.537 (s, 1H, Ar-NH), 8.37-7.57 (d, 2H, Ar-Cl), 7.52-6.96 (d, 2H, Ar-Cl), 6.72-6.53(m, 3H, Ar), 2.96 (s, 3H, CH<sub>3</sub>),  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ ):  $\delta$ (ppm) 185.3(C=S), 168.3(C=O), 140.3, 137.7, 137.4, 134.7, 132.3, 129.0, 128.9, 127.5, 124.8, 124.5, 15.24(CH<sub>3</sub>) Anal. Calcd. for C<sub>15</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>OS; C, 53.11; H, 3.57; N, 8.26; S, 9.45 found; C, 53.13; H, 3.77; N, 8.36; S, 9.85.



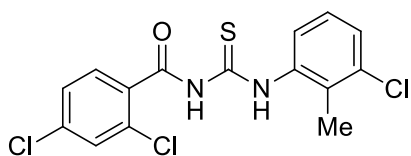
**4i) 2-chloro-N-((3-ethyl-2-methylphenyl)carbamothioyl)benzamide**

Light yellow precipitates, Yield=80%, M.P=150°C,  $R_f$  = 0.25 (n-Hexane : Ethyl acetate 1:1), FTIR ( $\text{cm}^{-1}$ ): 1222(C=S stretch), 3226 (N-H stretch), 2996.4 (Aromatic C-H, stretching), 1565 (C-C, Stretching), 1125 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ );  $\delta$  (ppm) 12.00(s, 1H, ArCO-N-H), 11.837 (s, 1H, Ar-NH), 8.37-7.57 (m, 4H, Ar-Cl), 7.52-6.96(m, 3H, Ar), 2.36 (s, 3H, CH<sub>3</sub>),  $^{13}\text{C-NMR}$  (75 MHz, DMSO- $d_6$ ):  $\delta$ (ppm) 181.3(C=S), 169.3(C=O), 140.3, 137.4, 134.7, 133.6, 132.4, 132.2, 129, 129.9, 127.9, 127, 124.8, 124.5, 15.25(CH<sub>3</sub>), Anal. Calcd. for C<sub>15</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>OS; C, 53.11; H, 3.57; N, 8.26; S, 9.45 found: C, 53.23; H, 3.71; N, 8.37; S, 10.12.

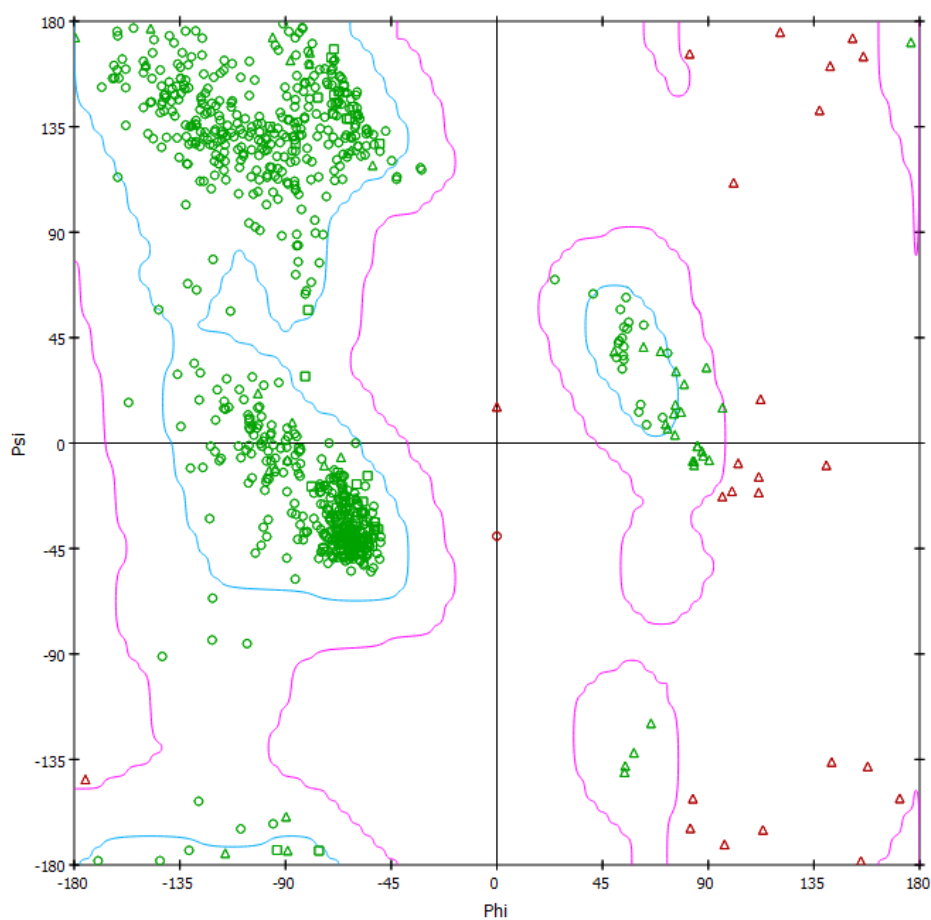


**4j) 2, 4-dichloro-N-((3-chloro-2-methylphenyl)carbamothioyl)benzamide**

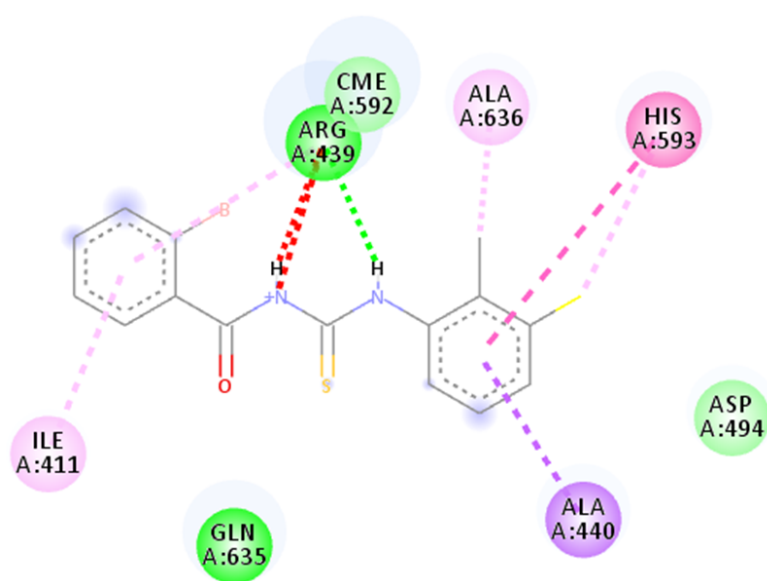
White precipitates, Yield = 82%, M.P =122°C,  $R_f$  =0.26, (n-Hexane: Ethyl acetate 1:1), FTIR y ( $\text{cm}^{-1}$ ) 1242 (C=S stretch), 3236 (N-H stretch), 2986.4 (Aromatic C-H, stretching), 1566 (C-C, Stretching), 1128 (C-N stretch),  $^1\text{H-NMR}$  (300 MHz, DMSO- $d_6$ );  $\delta$  (ppm) 12.10 (s, 1H, ArCO-N-H), 11.937 (s, 1H, Ar-NH), 8.47-7.67 (m, 3H, Ar-Cl), 7.53-6.76(m, 3H, Ar), 2.39 (s, 3H, CH<sub>3</sub>),  $^{13}\text{C-NMR}$  (75MHz, DMSO $d_6$ )  $\delta$ (ppm) 182.3 (C=S), 166.3 (C=O), 140.3, 139.1, 137.4, 134.7, 133.7, 133.7, 130.5, 130.4, 130.3, 127.5, 124.8, 124.5, 15.26 (CH<sub>3</sub>); Anal. Calcd. C, 48.21; H, 2.97; N, 7.50; S, 8.58 found: C, 48.23; H, 2.89; N, 7.67; S, 8.62.



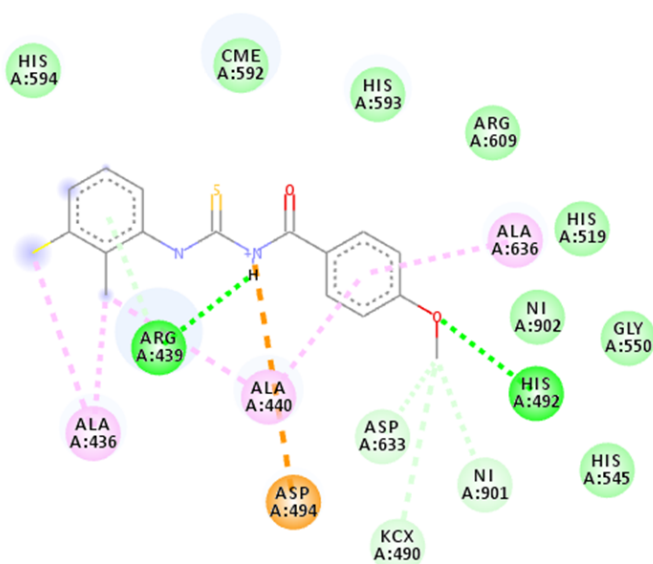
## Supplementary images



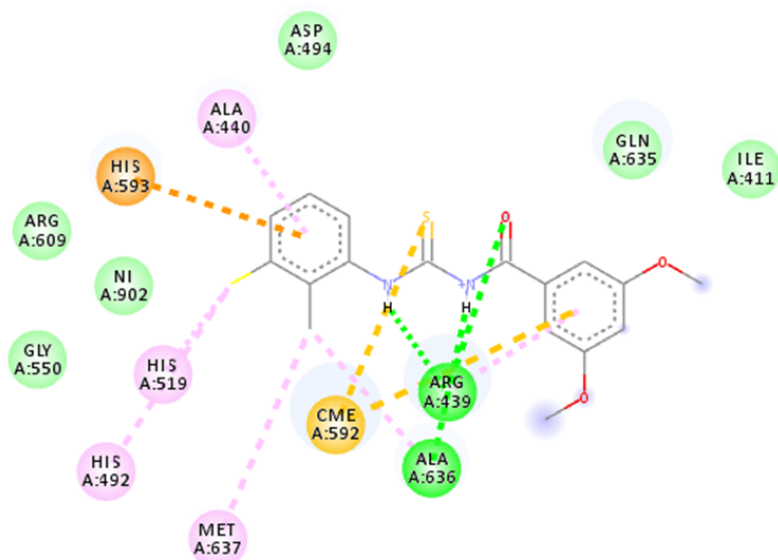
**Figure S1.** Ramachandran graph of jack bean urease



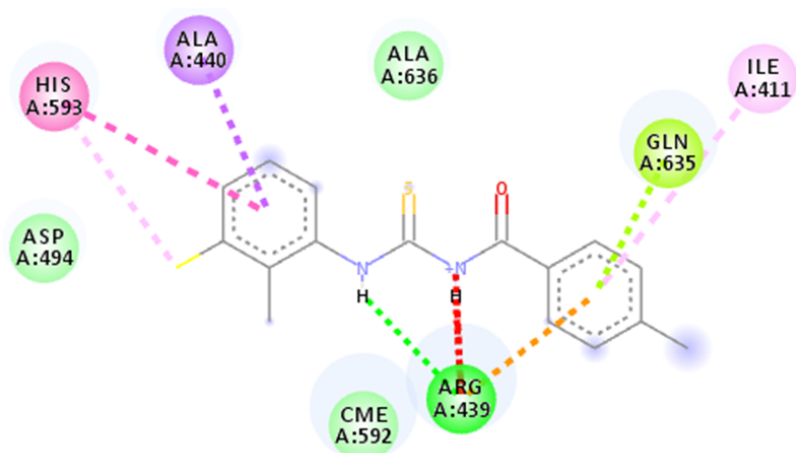
**Figure S2.** Docking complex 4a



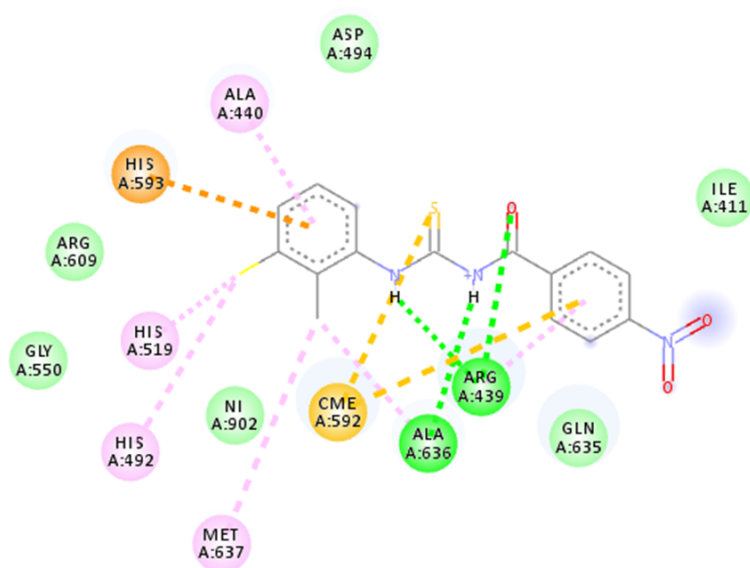
**Figure S3.** Docking complex **4b**



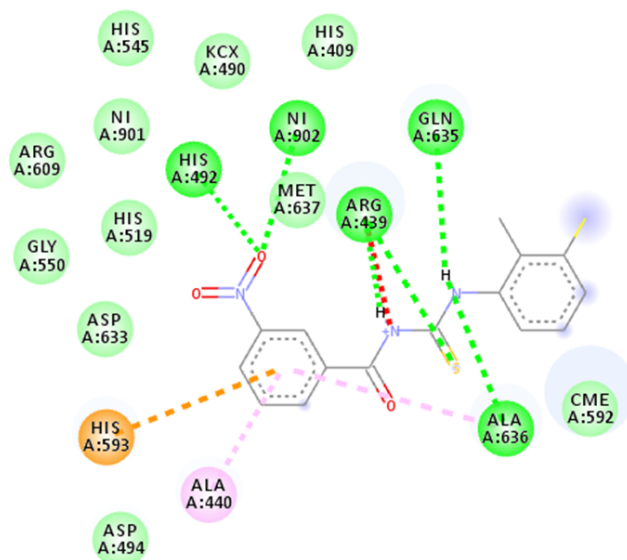
**Figure S4.** Docking complex **4c**



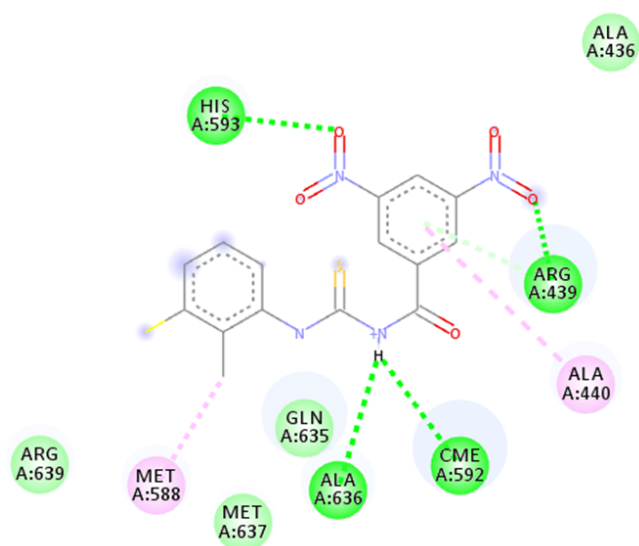
**Figure S5.** Docking complex **4d**



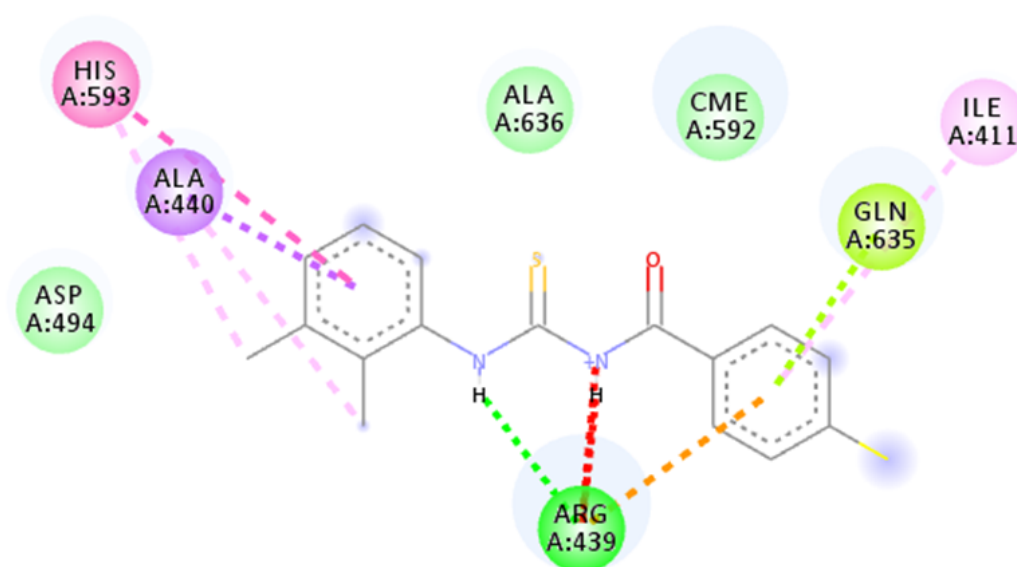
**Figure S6.** Docking complex 4e



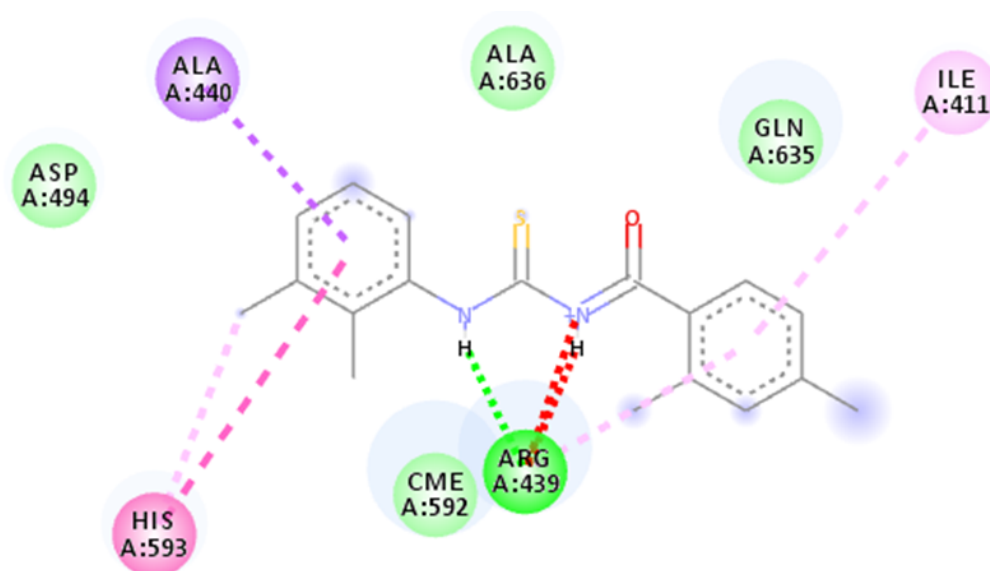
**Figure S7.** Docking complex 4f



**Figure S8.** Docking complex **4g**



**Figure S9.** Docking complex **4h**



**Figure S10.** Docking complex **4j**



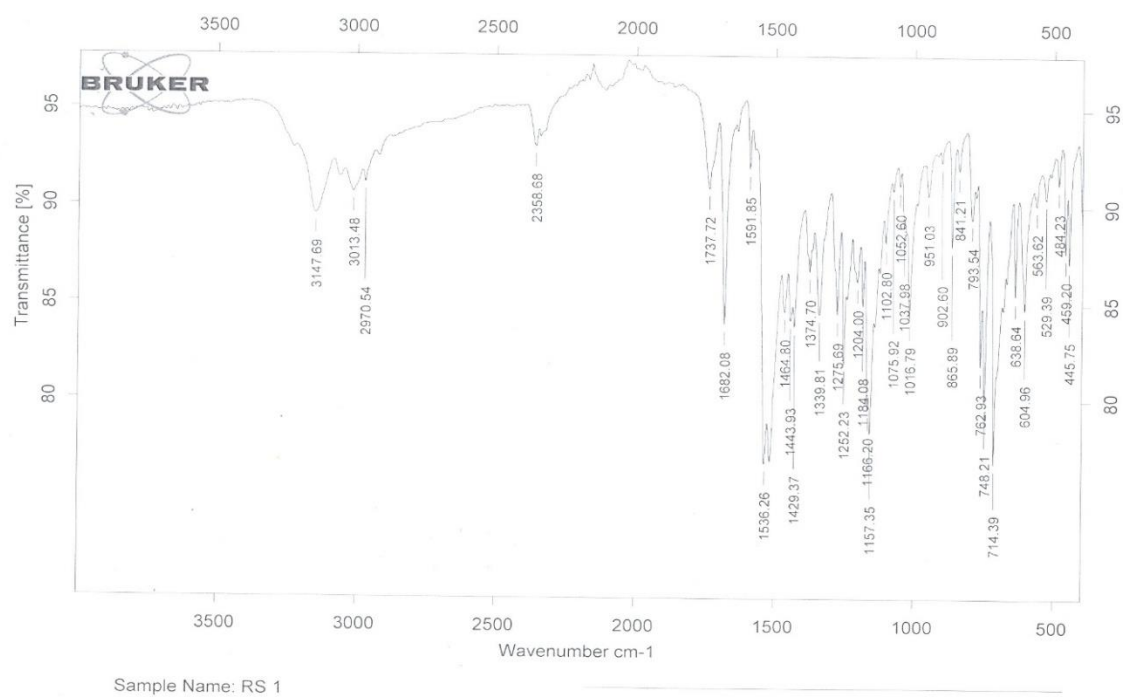


Figure S11 IR Spectrum of **4a**

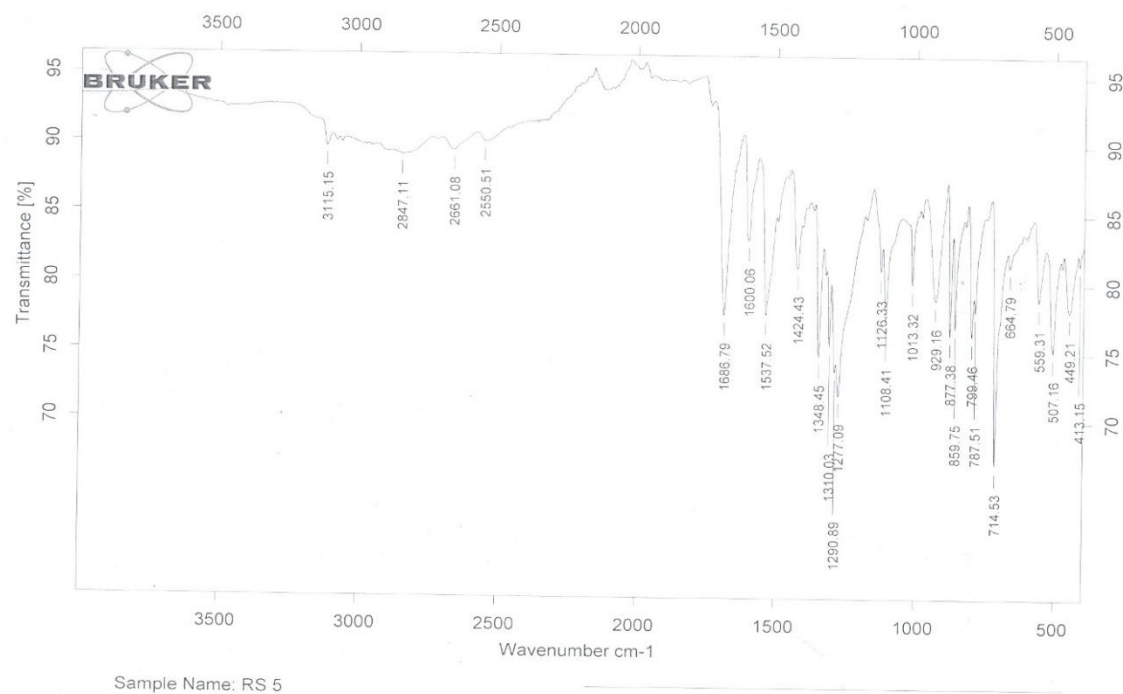
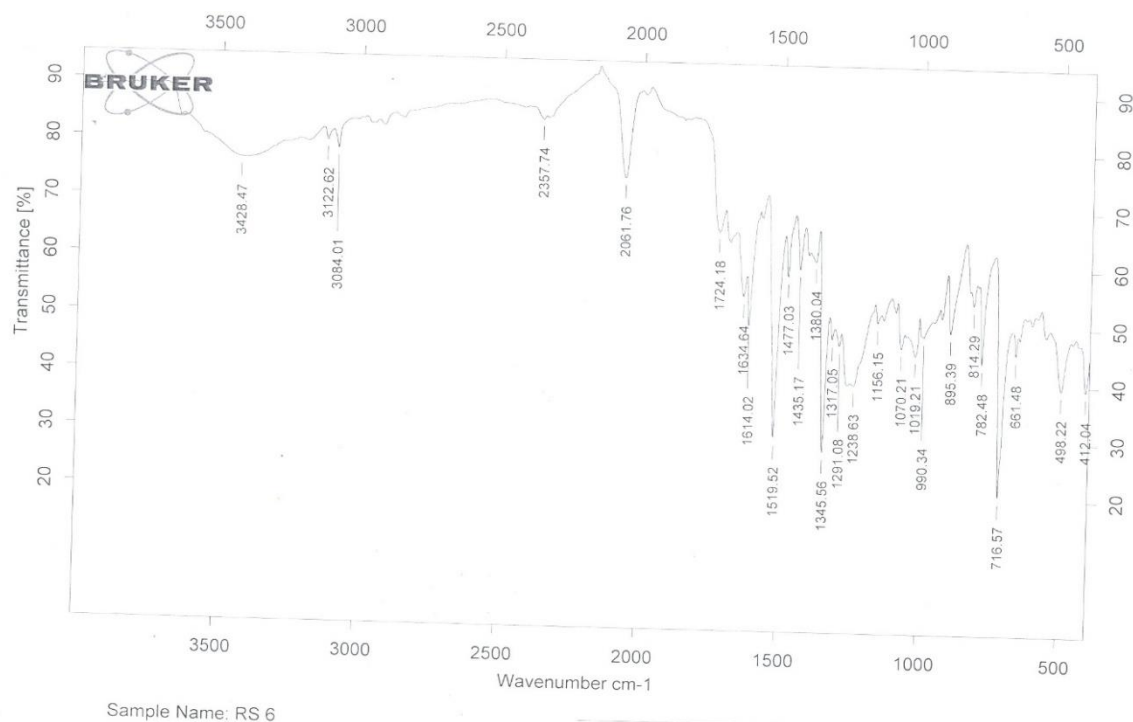
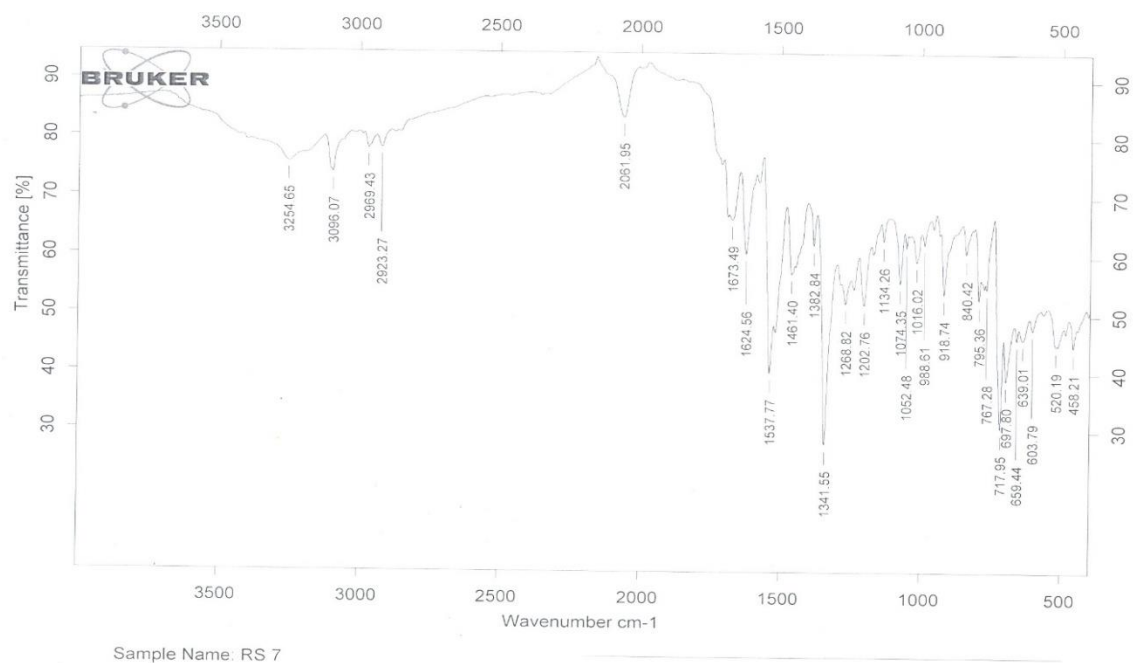


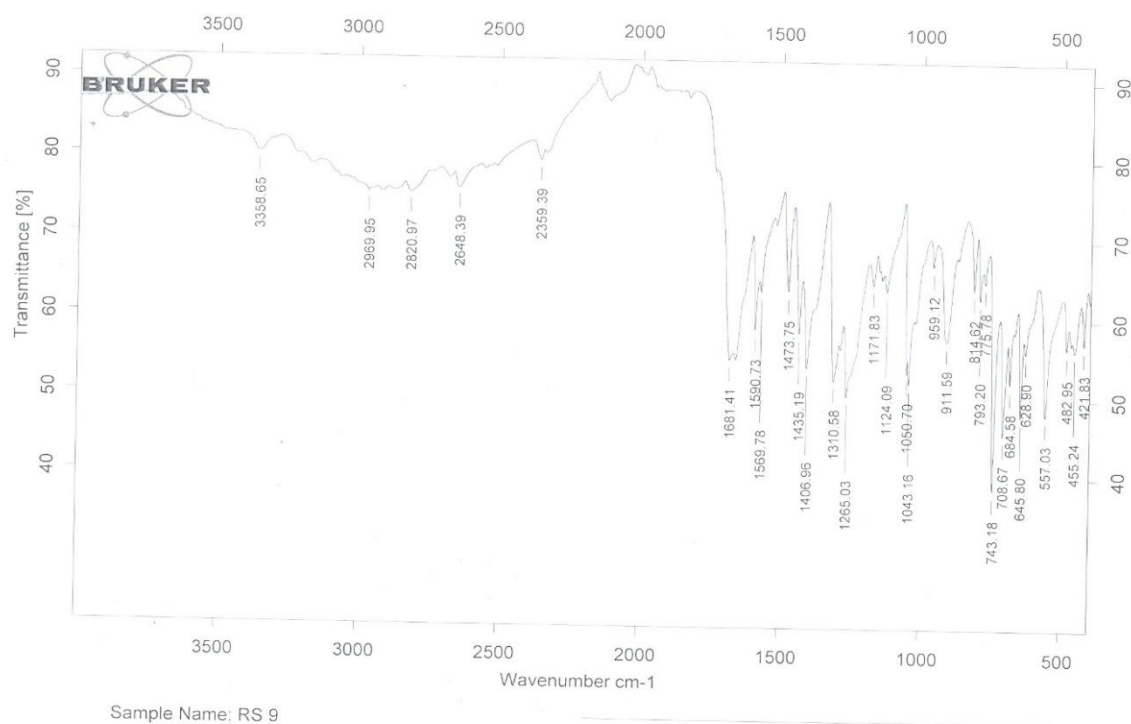
Figure S12. IR Spectrum of **4e**



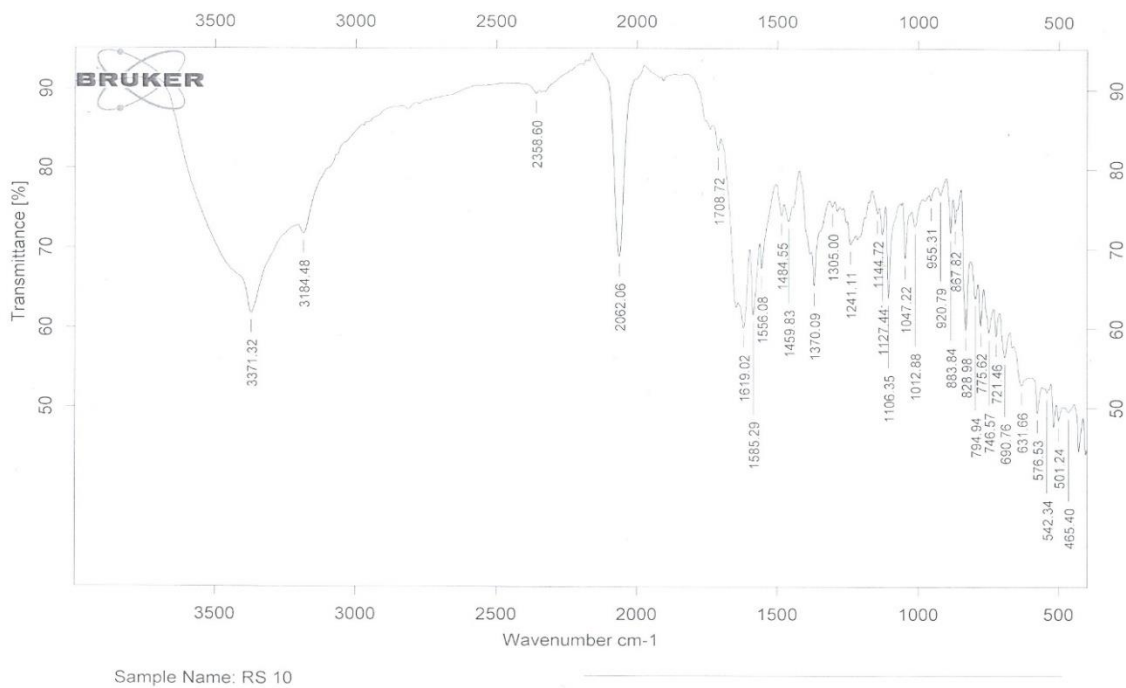
**Figure S13. IR Spectrum of 4f**



**Figure S14 IR Spectrum of 4g**



**Figure S15 IR Spectrum of 4i**



**Figure S16. IR Spectrum of 4j**

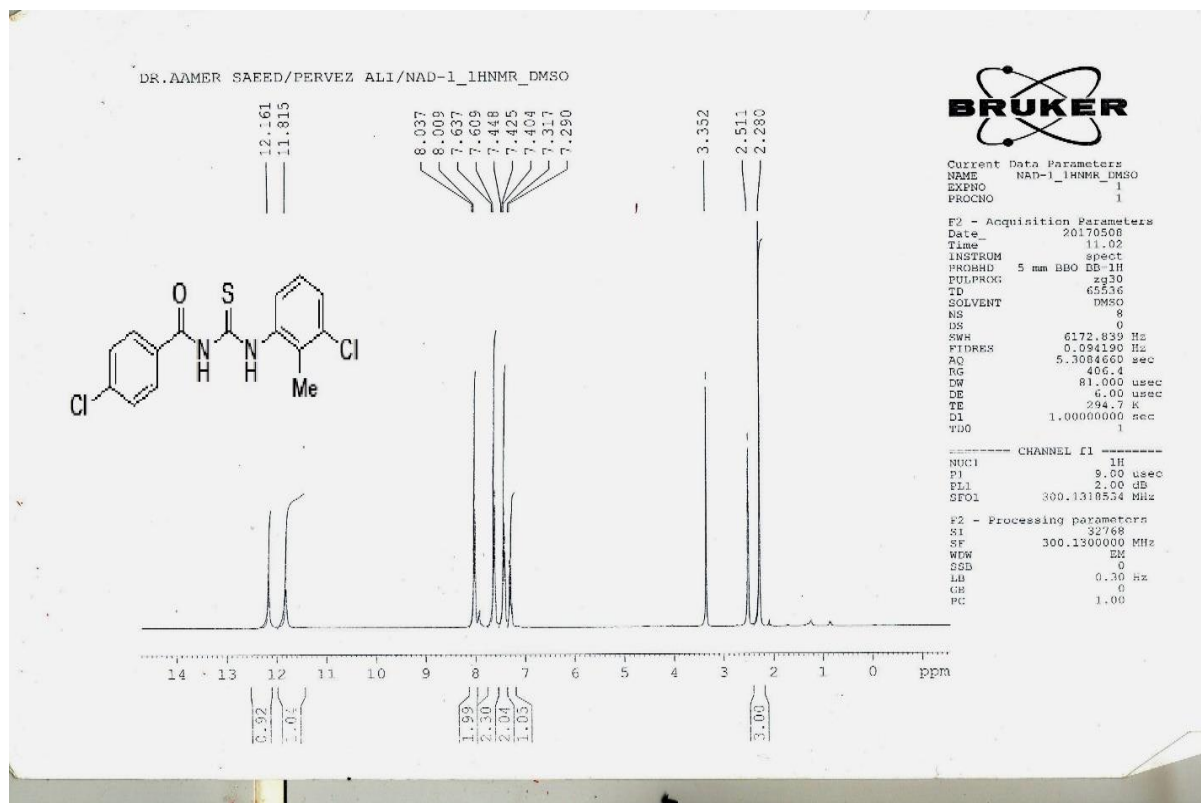


Figure S17.  $^1\text{H}$ -NMR spectrum of 4h

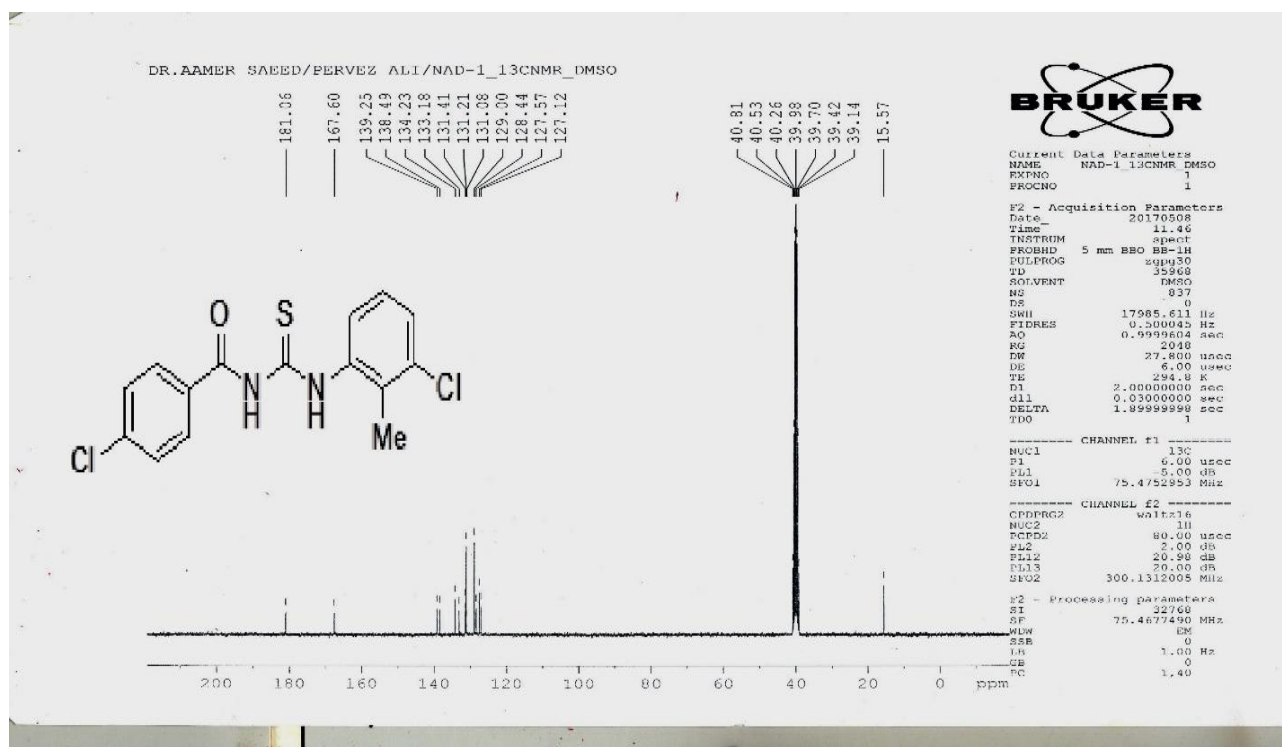


Figure S18  $^{13}\text{C}$ -NMR spectrum of 4h