

# Synthesis and evaluation of amide and thioureas derivatives as Carbonic Anhydrase (CAs) inhibitors

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## 1.1 Proton NMR, $^{13}\text{C}$ Spectra of the synthesized compounds

**Compound 20**

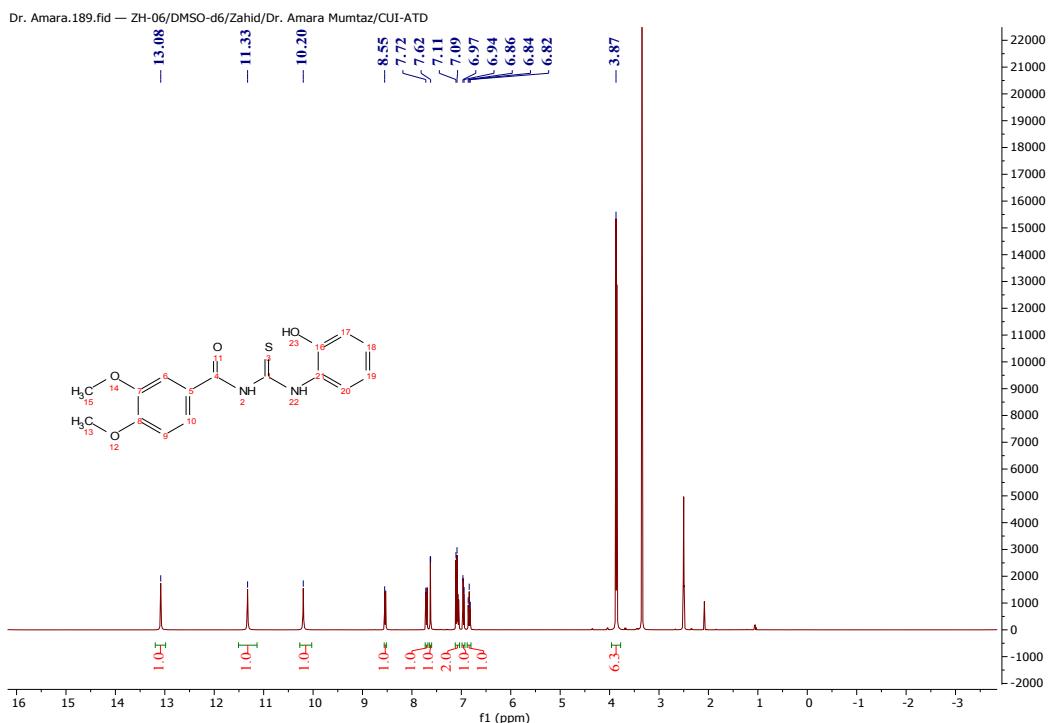


Figure S1:  $^1\text{H}$  NMR of compound 20

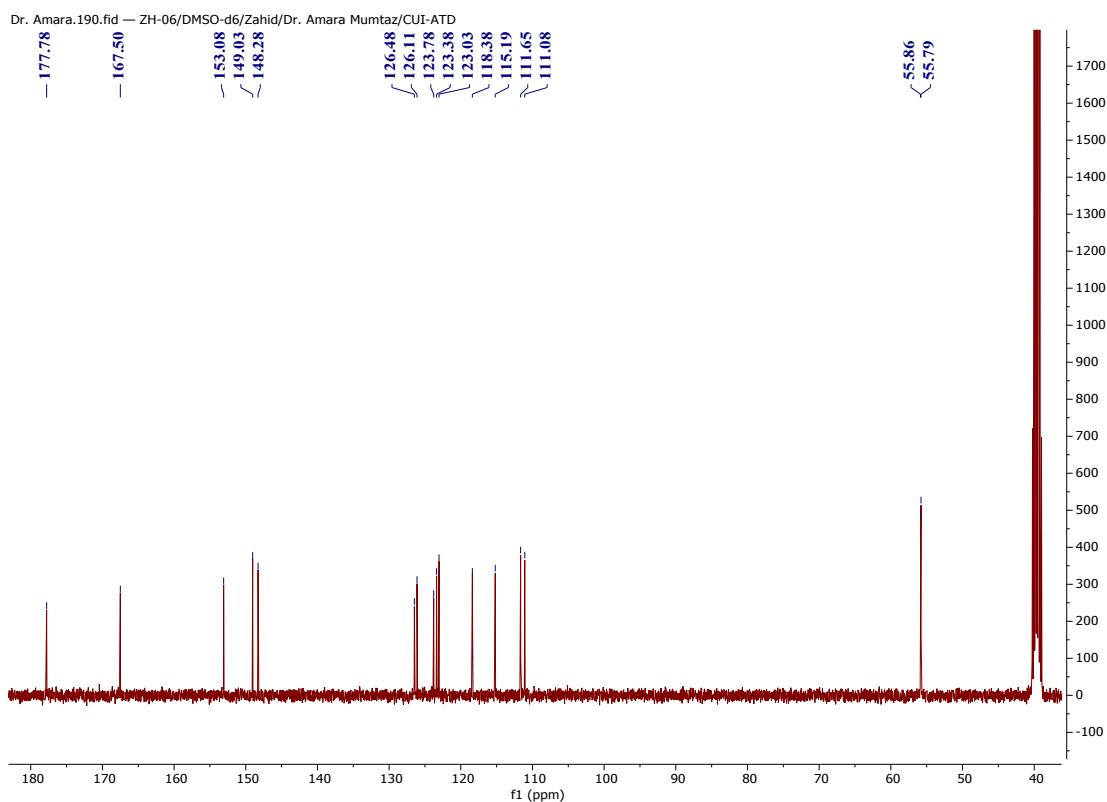


Figure S2:  $^{13}\text{C}$  NMR of compound 20

## Compound 22

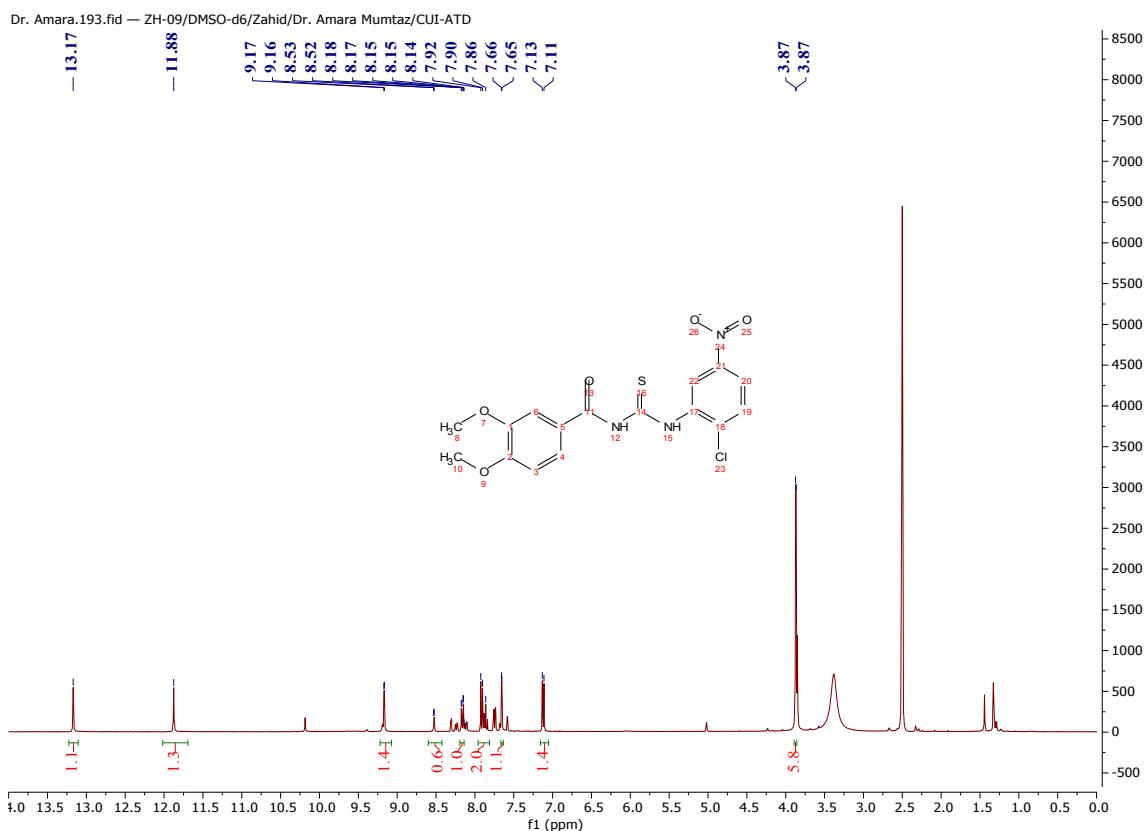


Figure S3:  $^1\text{H}$  NMR of compound 22

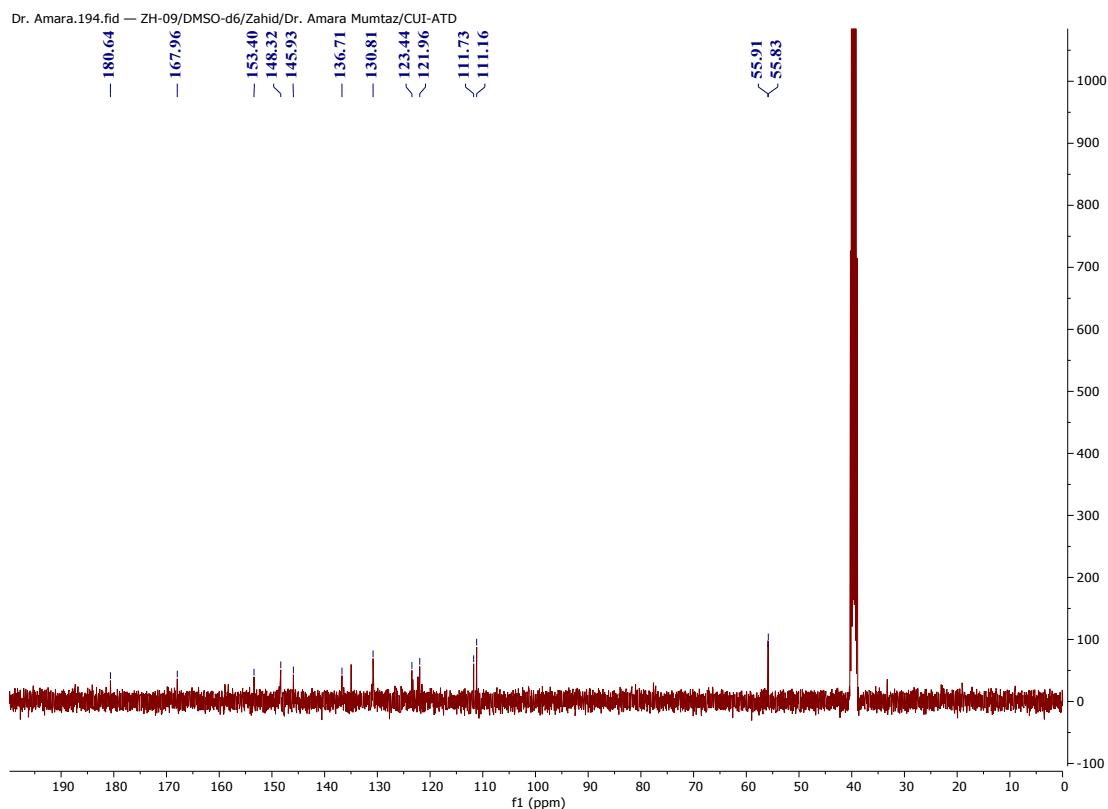


Figure S4:  $^{13}\text{C}$  NMR of compound 22

## Compound 23

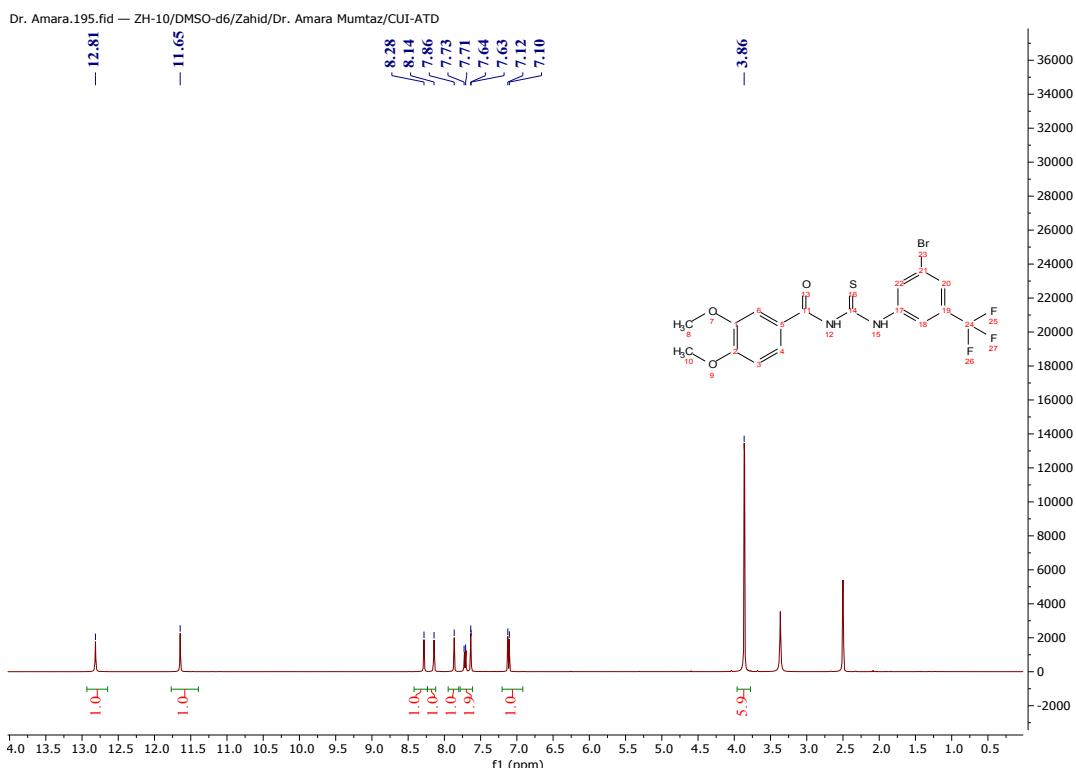


Figure S5: <sup>1</sup>H NMR of compound 23

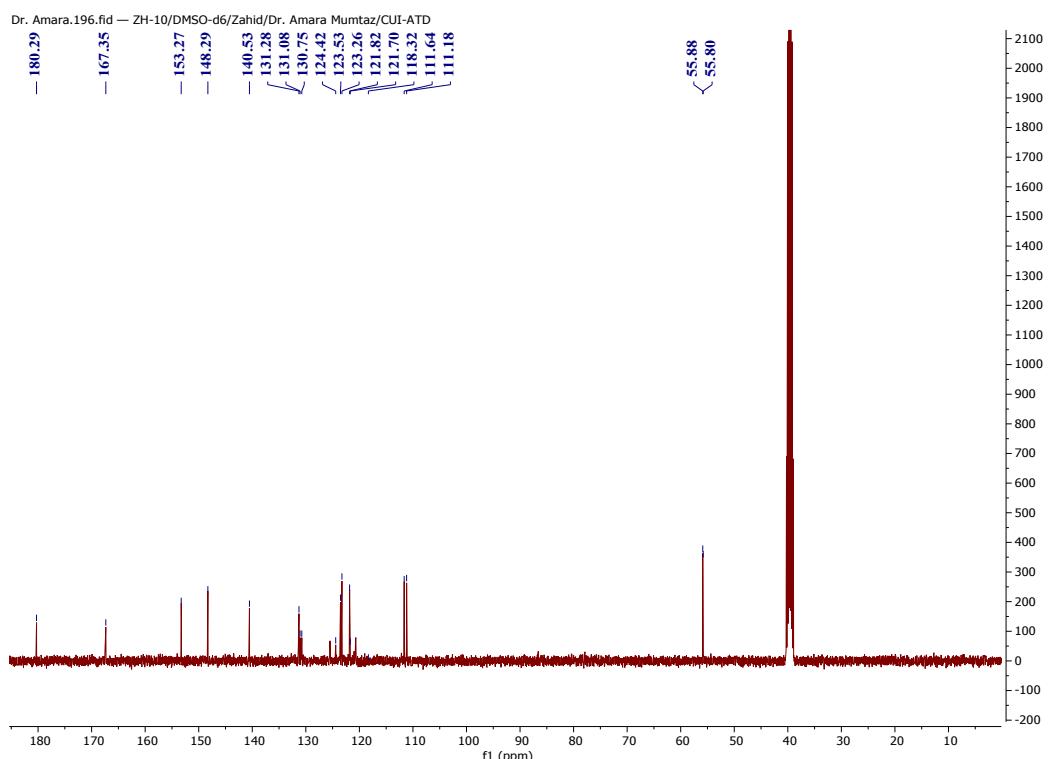


Figure S6: <sup>13</sup>C NMR of compound 23

## Compound 21

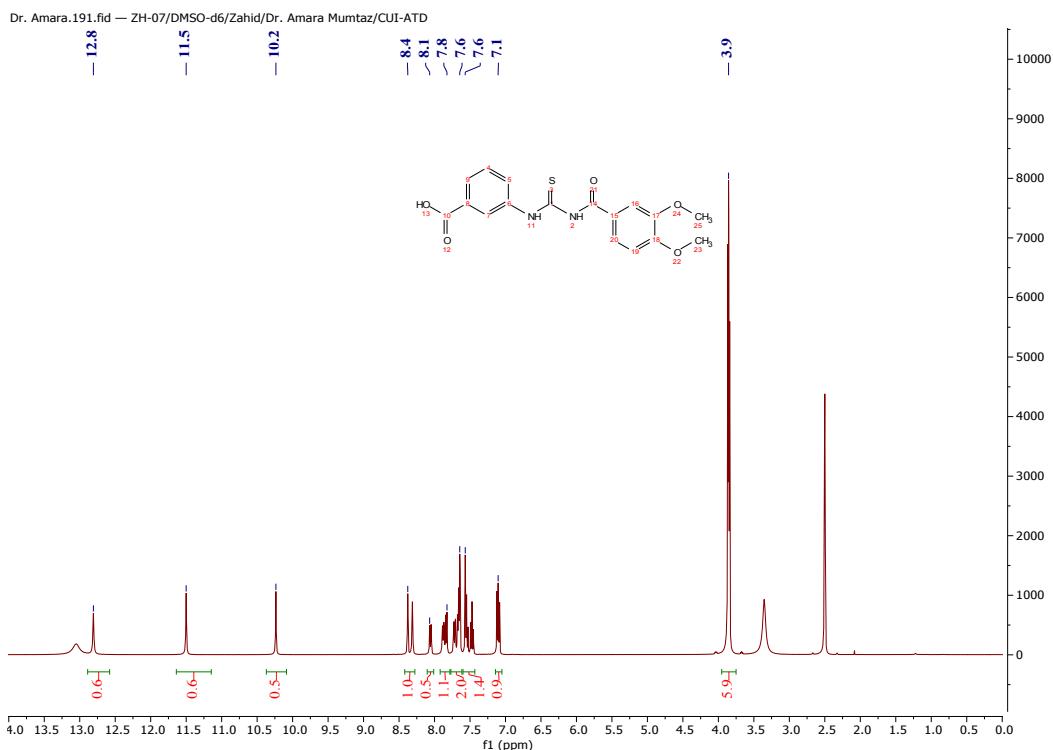


Figure S7:  $^1\text{H}$  NMR of compound 21

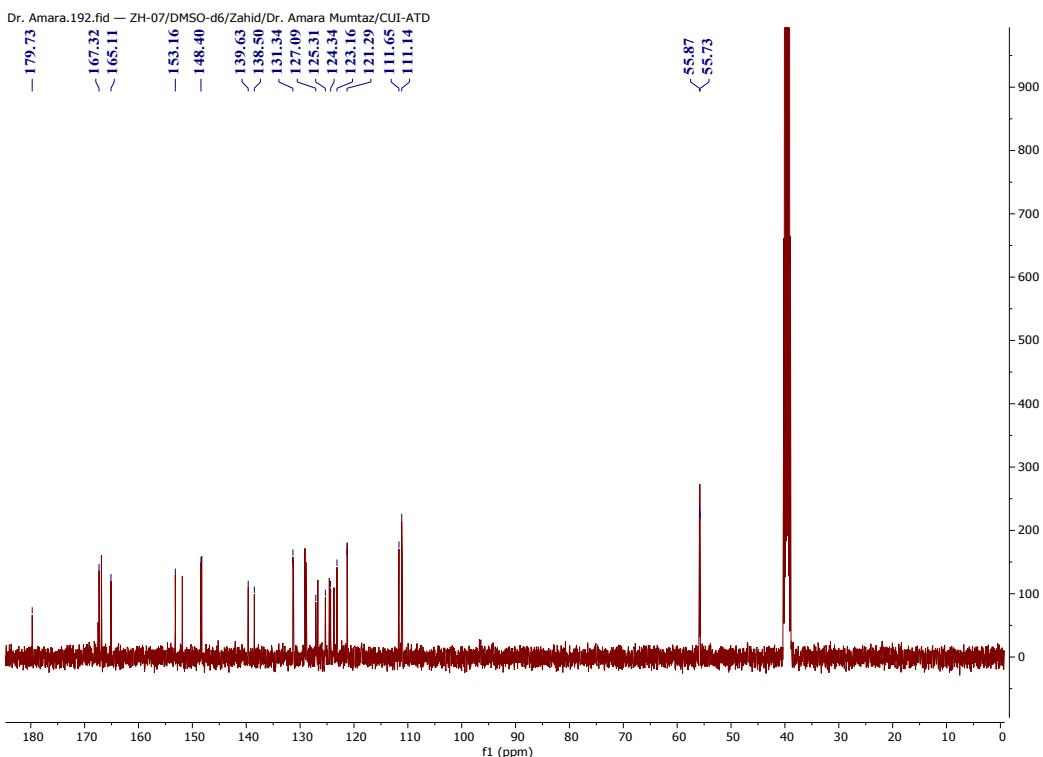


Figure S8:  $^{13}\text{C}$  NMR of compound 21

## Compound 18

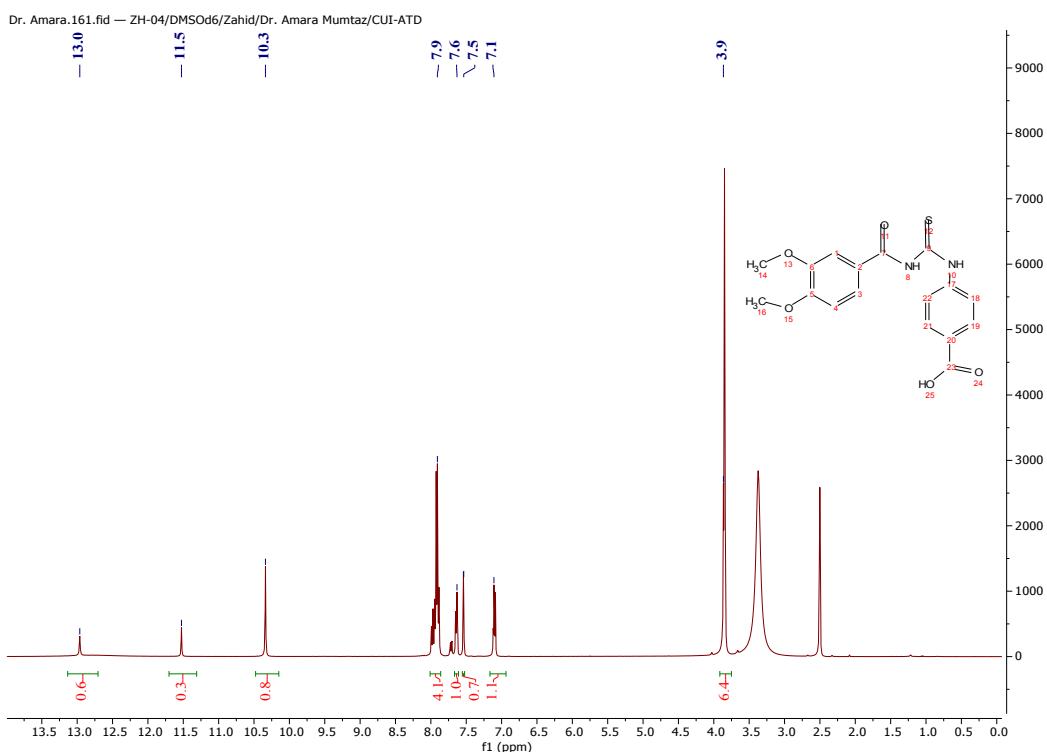


Figure S9:  $^1\text{H}$  NMR of compound 18

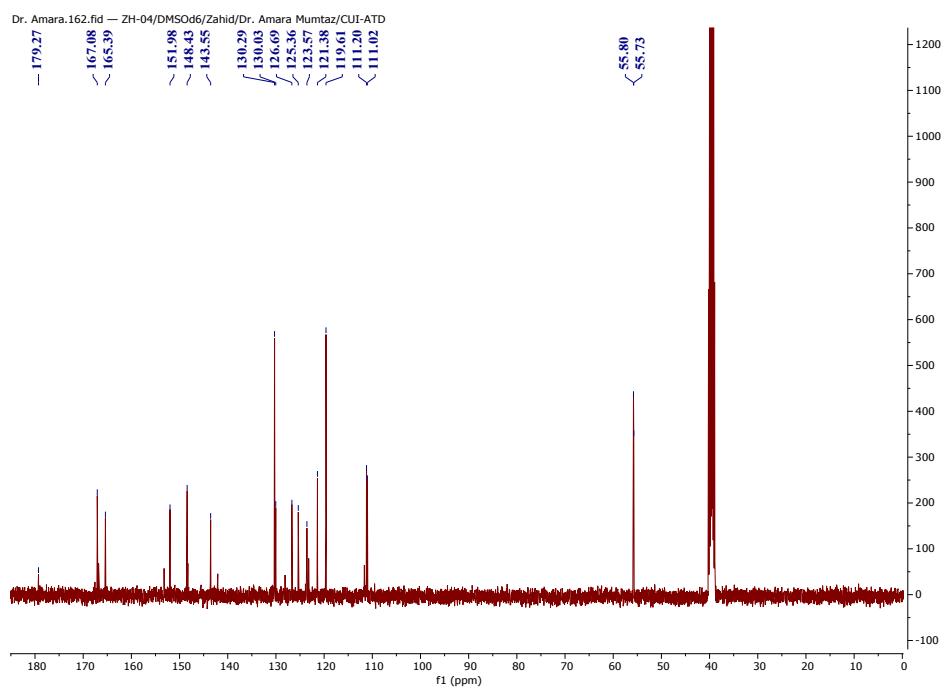


Figure S10:  $^{13}\text{C}$  NMR of compound 18

## Compound 17

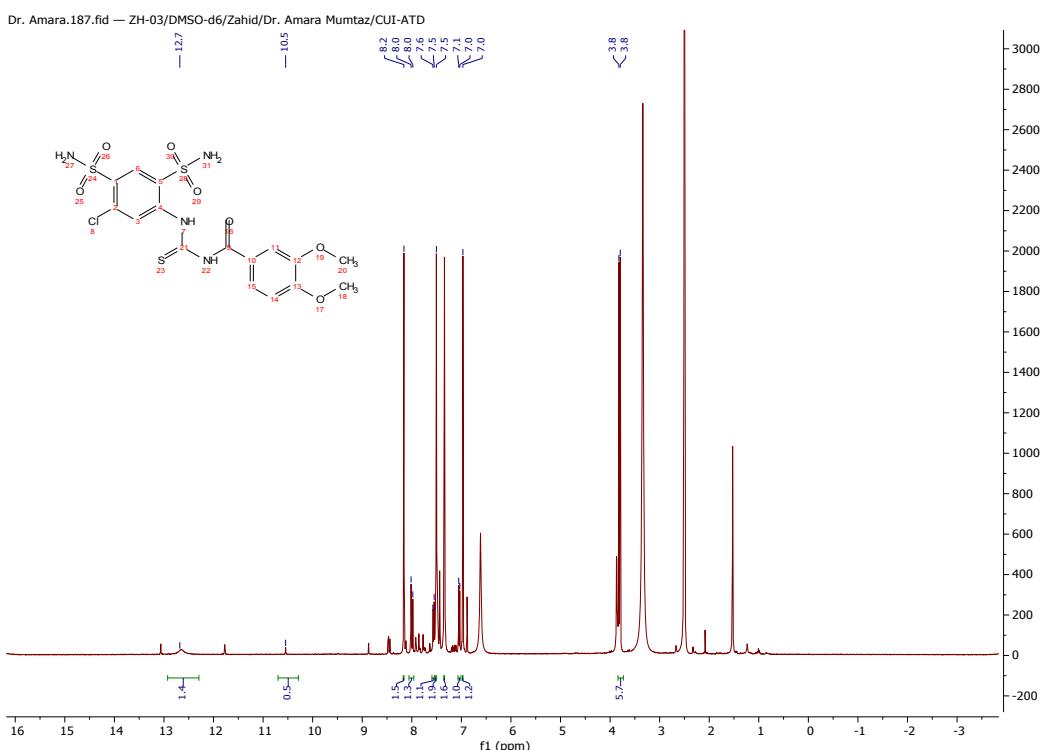


Figure S11:  $^1\text{H}$  NMR of compound 17

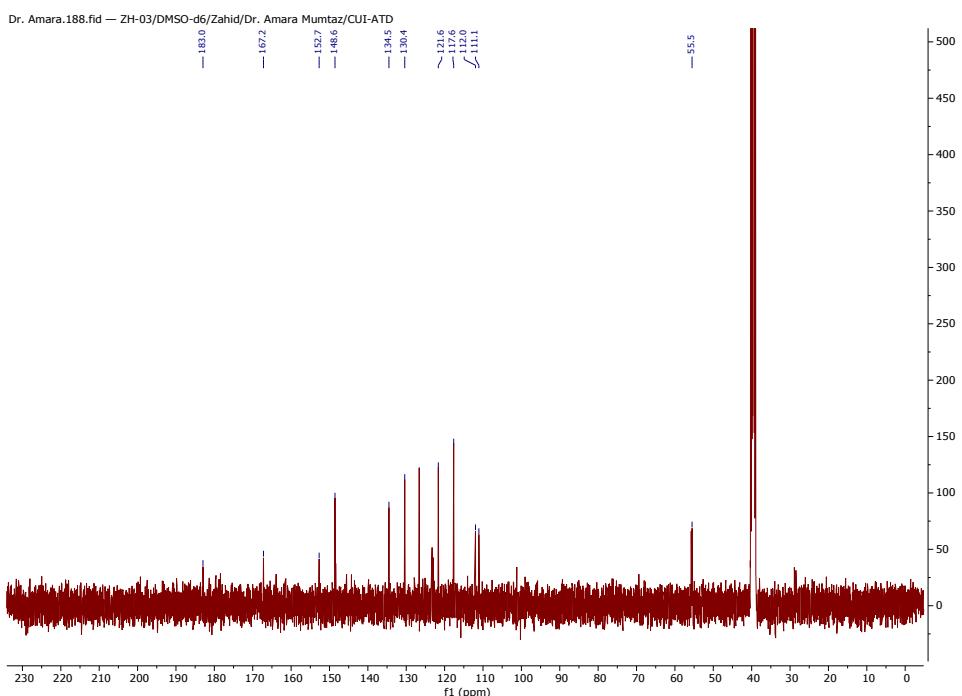


Figure S12:  $^{13}\text{C}$  NMR of compound 17

## Compound 12

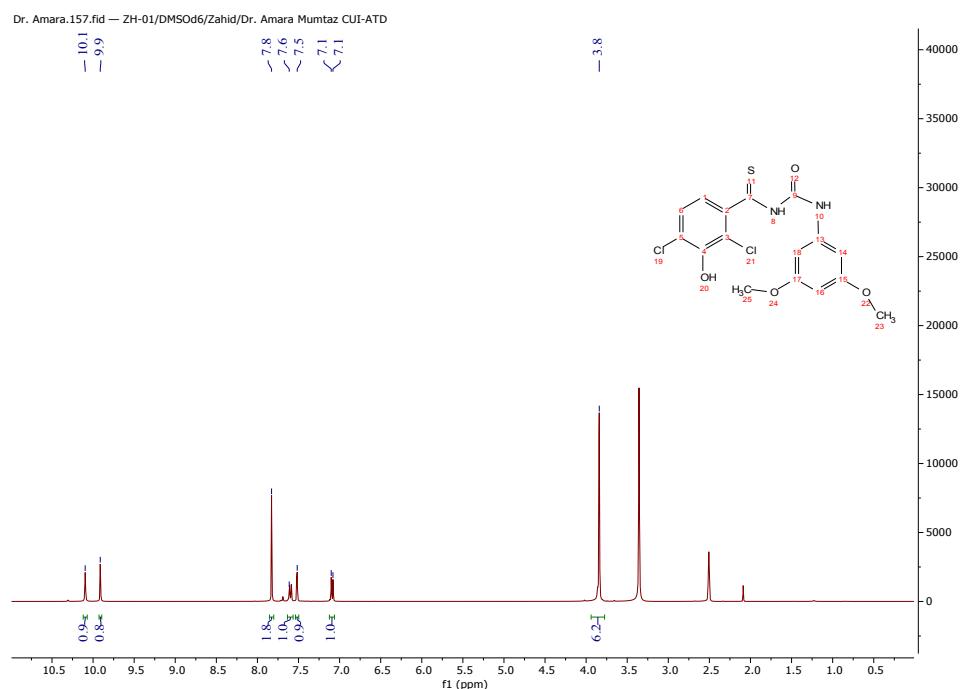


Figure S13:  $^1\text{H}$  NMR of compound 12

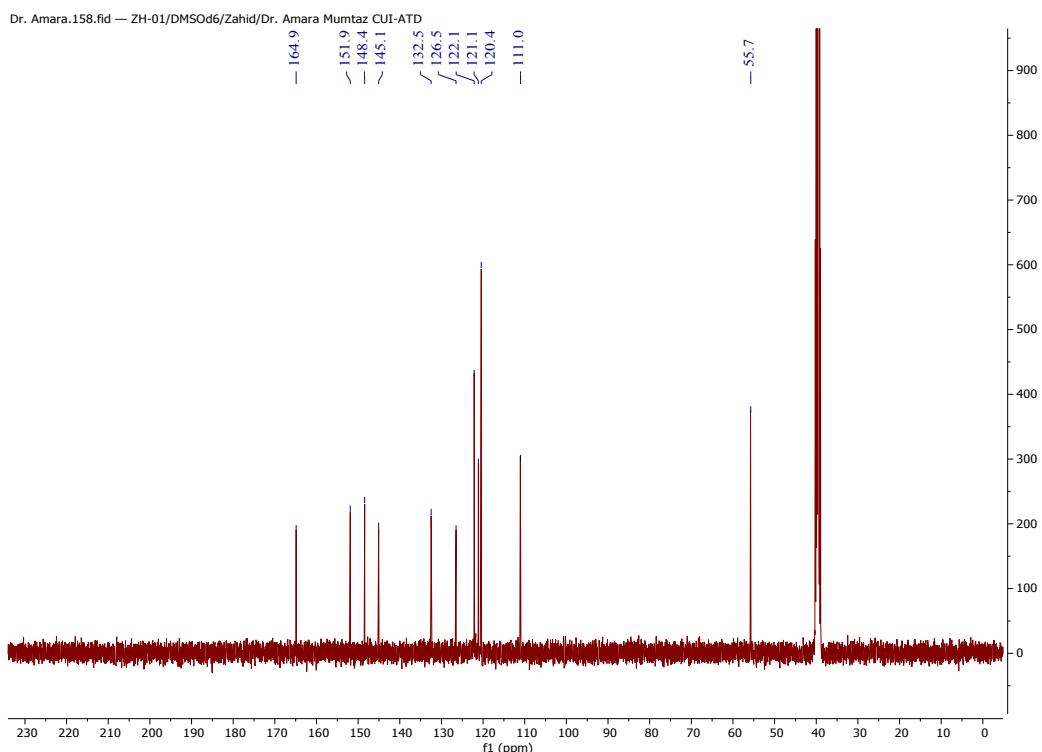


Figure S14:  $^{13}\text{C}$  NMR of compound 12

## Compound 09

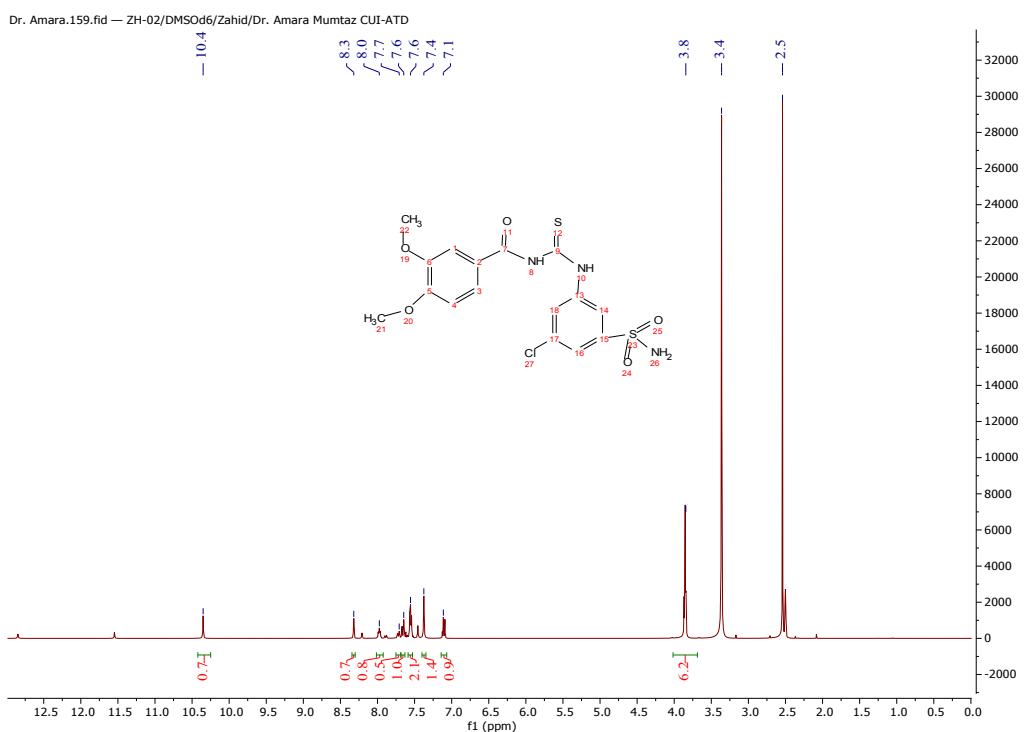


Figure S15:  $^1\text{H}$  NMR of compound 09

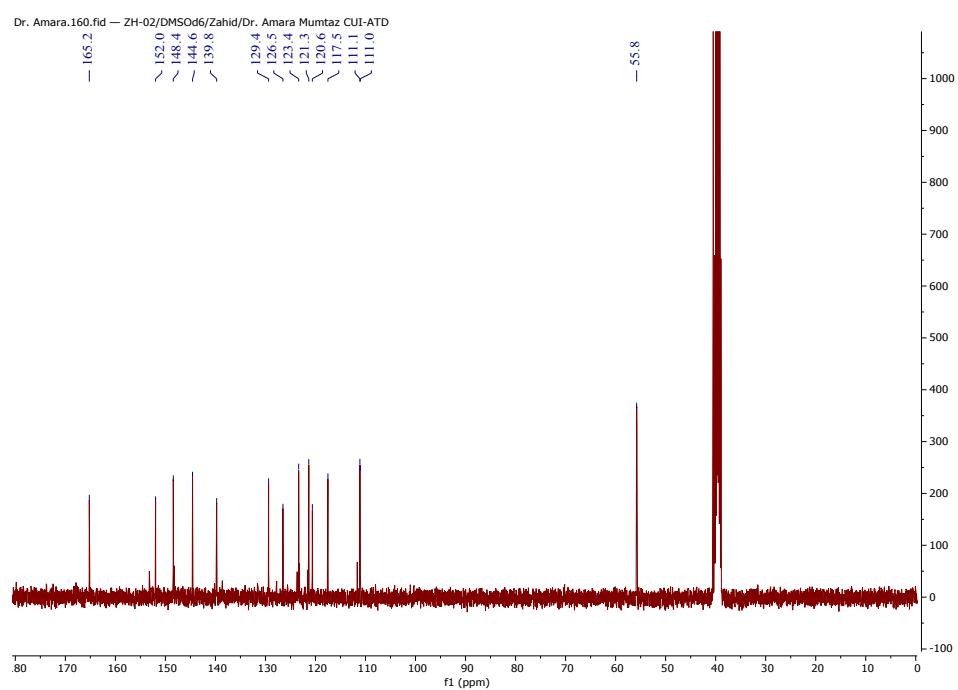


Figure S16:  $^{13}\text{C}$  NMR of compound 09

## Compound 24

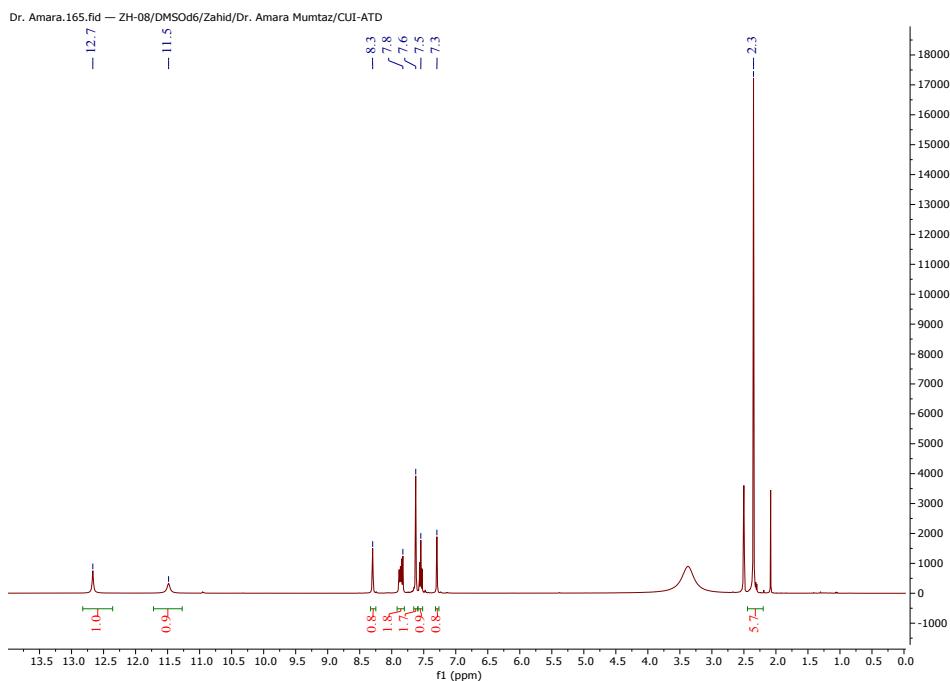


Figure S17: <sup>1</sup>H NMR of compound 24

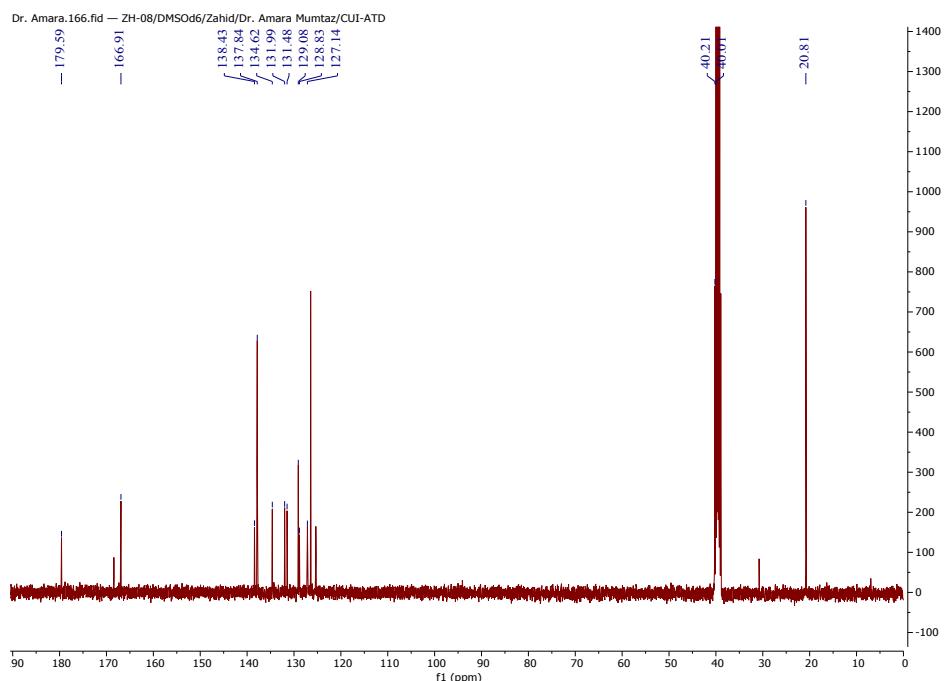


Figure S18: <sup>13</sup>C NMR of compound 24

## Compound 26

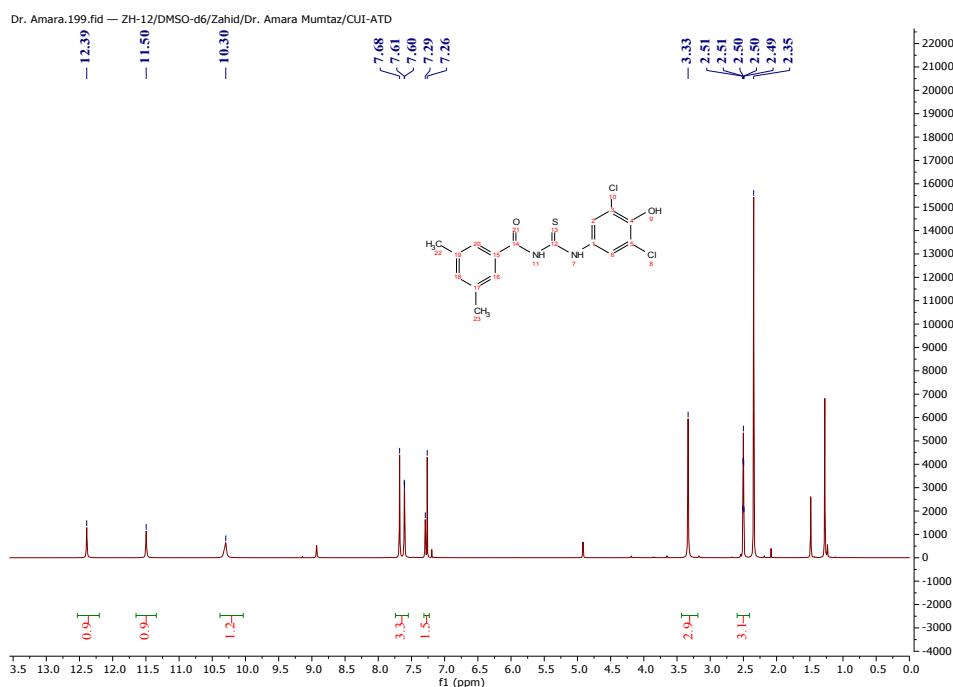


Figure S19:  $^1\text{H}$  NMR of compound 26

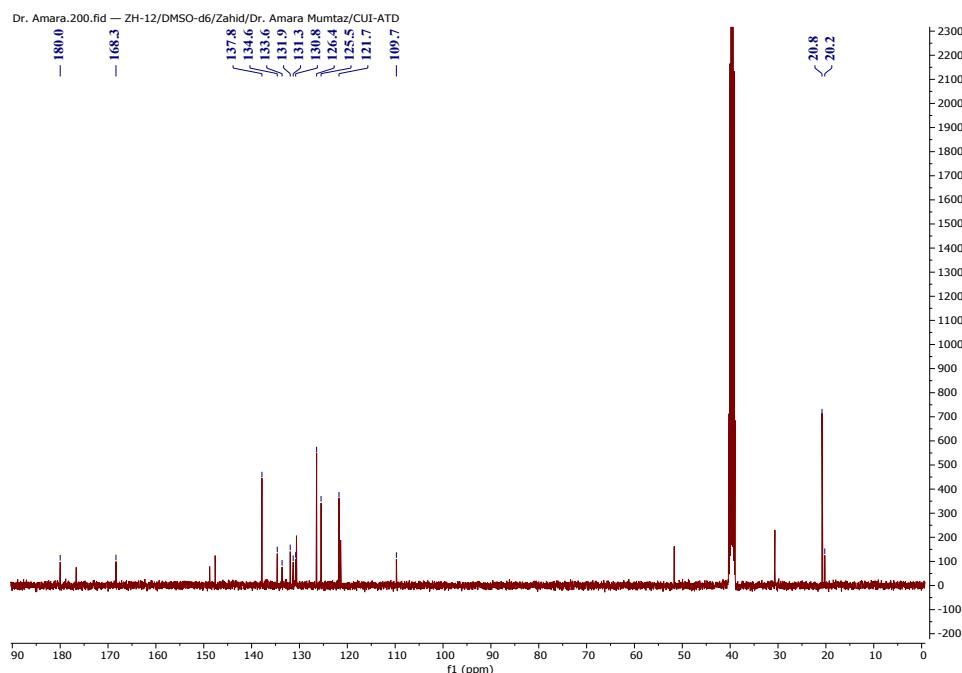


Figure S20:  $^{13}\text{C}$  NMR of compound 26

## Compound 10

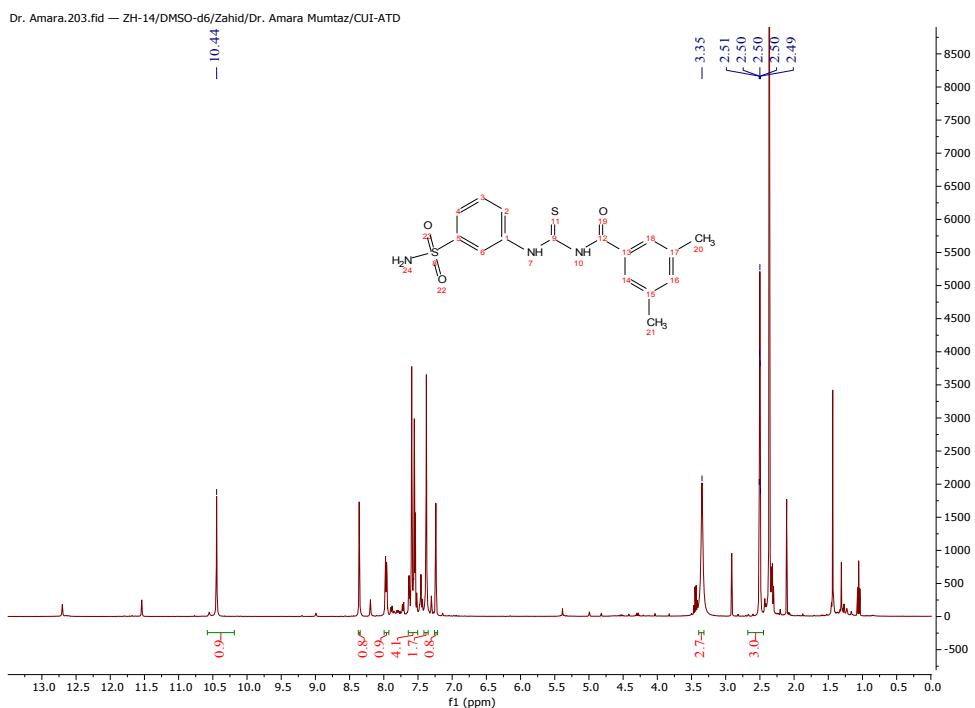


Figure S21:  $^1\text{H}$  NMR of compound 10

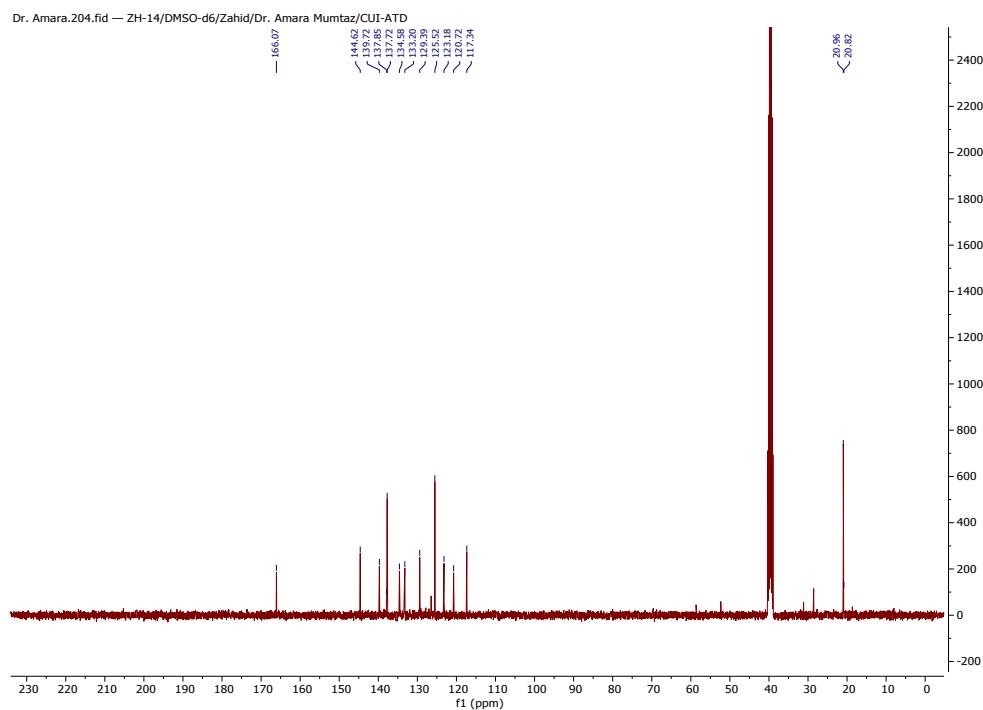


Figure S22:  $^{13}\text{C}$  NMR of compound 10

## Compound 11

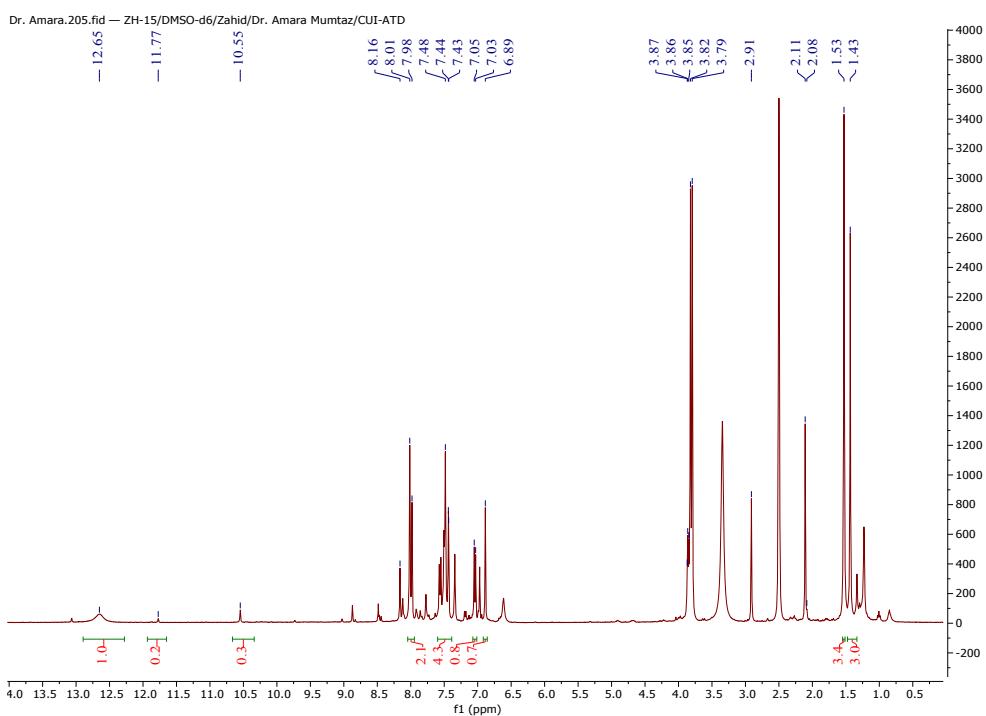


Figure S23:  $^1\text{H}$  NMR of compound 11

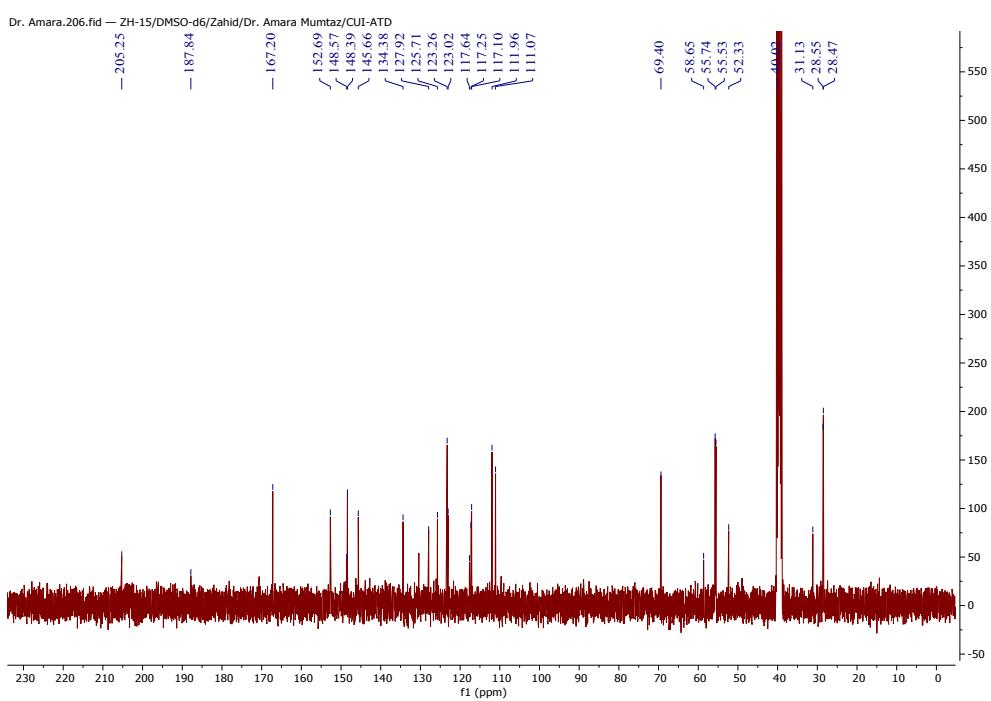


Figure S24:  $^{13}\text{C}$  NMR of compound 11

## Compound 14

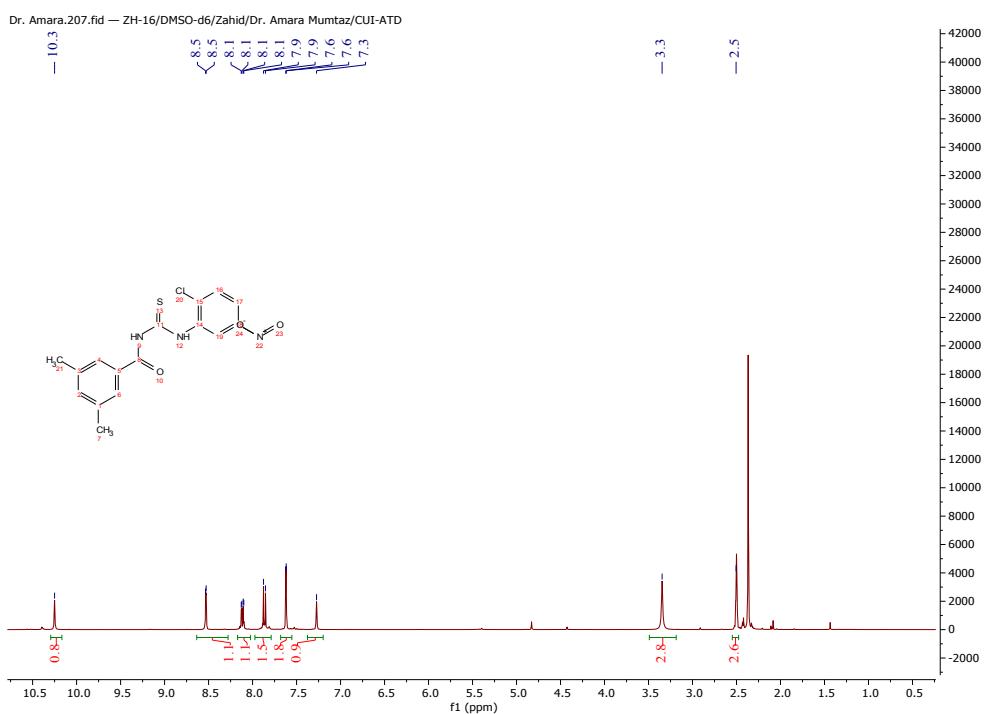


Figure S25:  $^1\text{H}$  NMR of compound 14

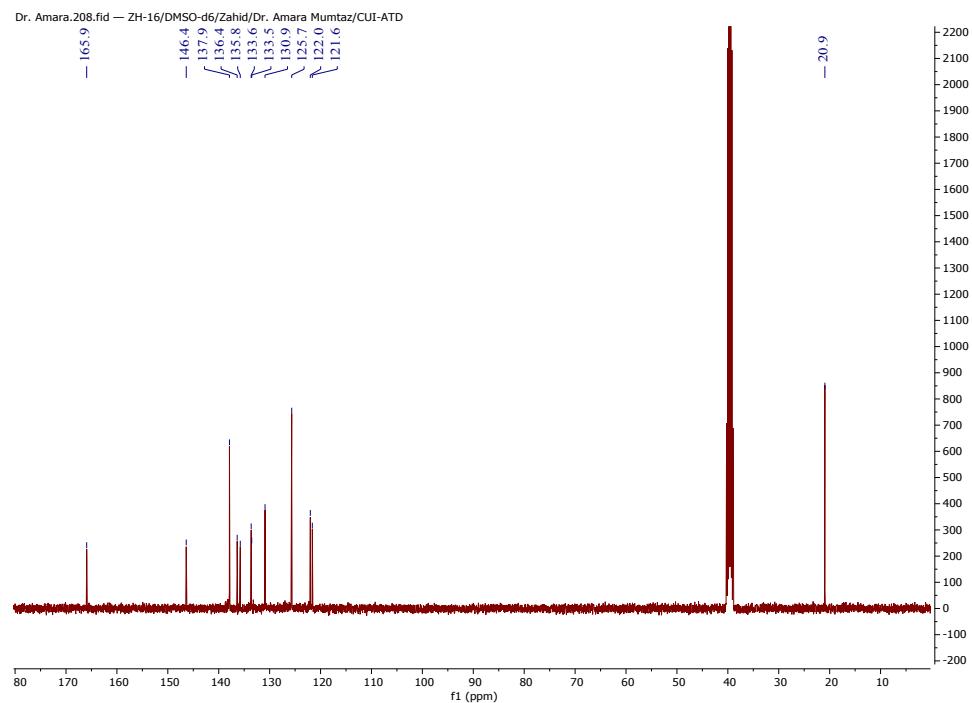


Figure S26:  $^{13}\text{C}$  NMR of compound 14

## Compound 15

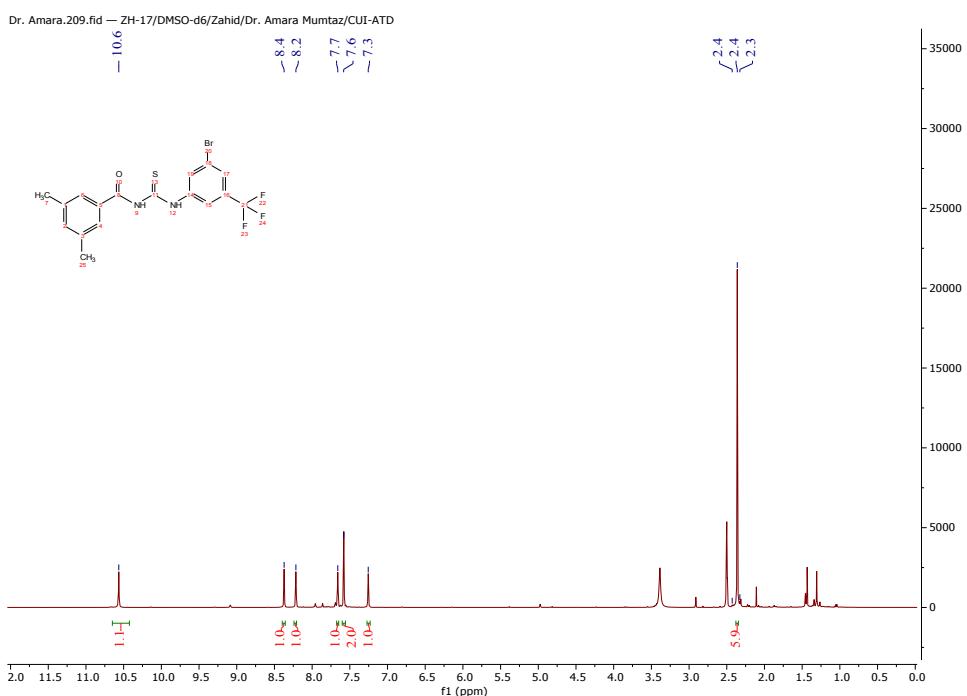


Figure S27:  $^1\text{H}$  NMR of compound 15

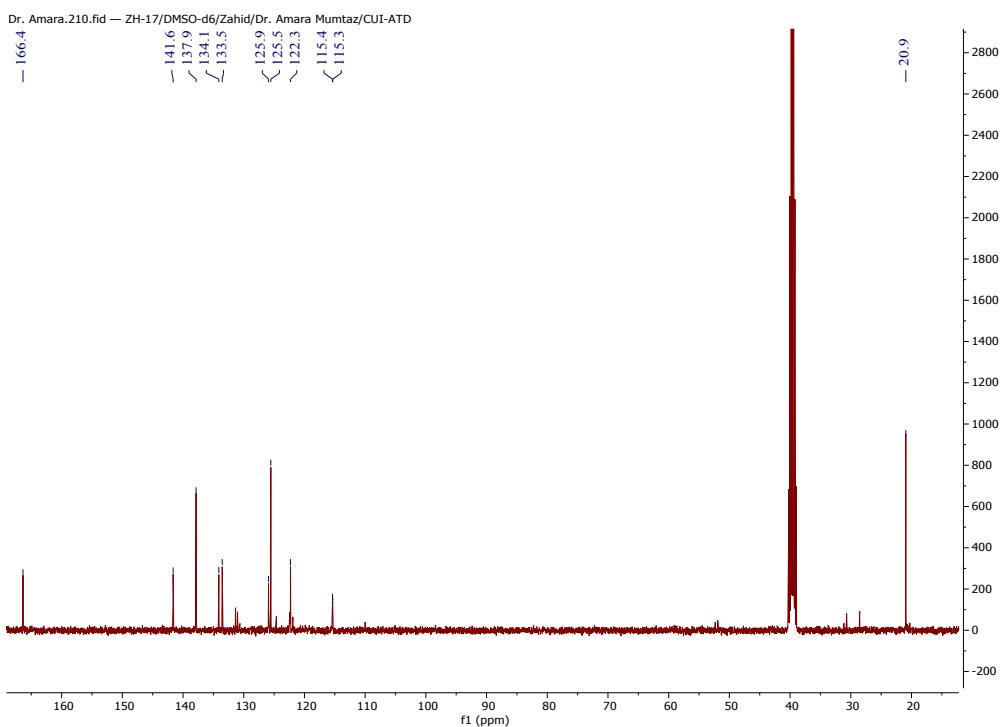


Figure S28:  $^{13}\text{C}$  NMR of compound 15

## Compound 25

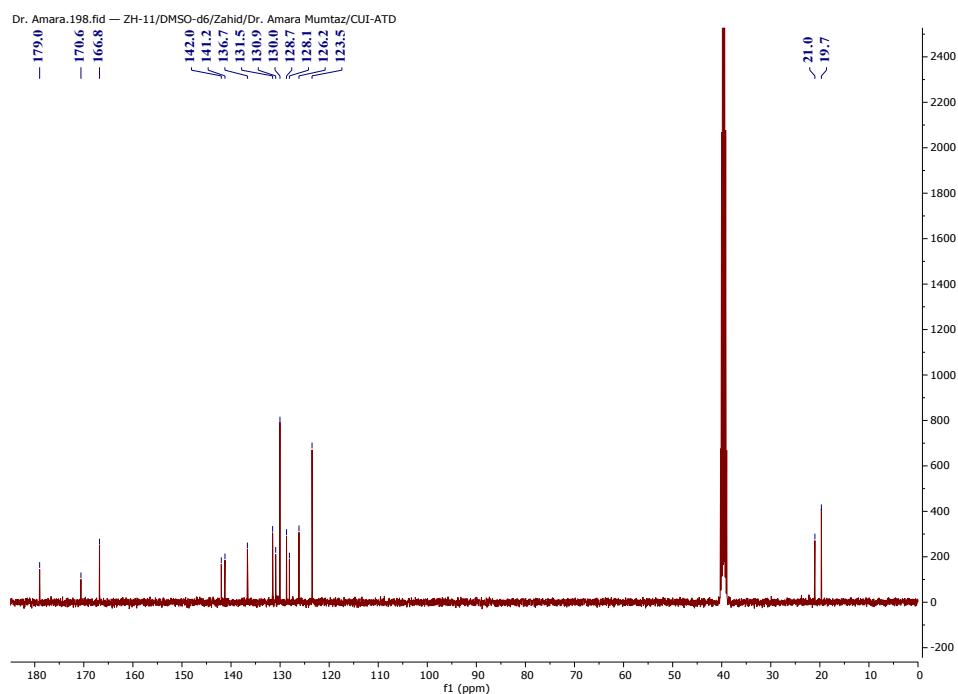
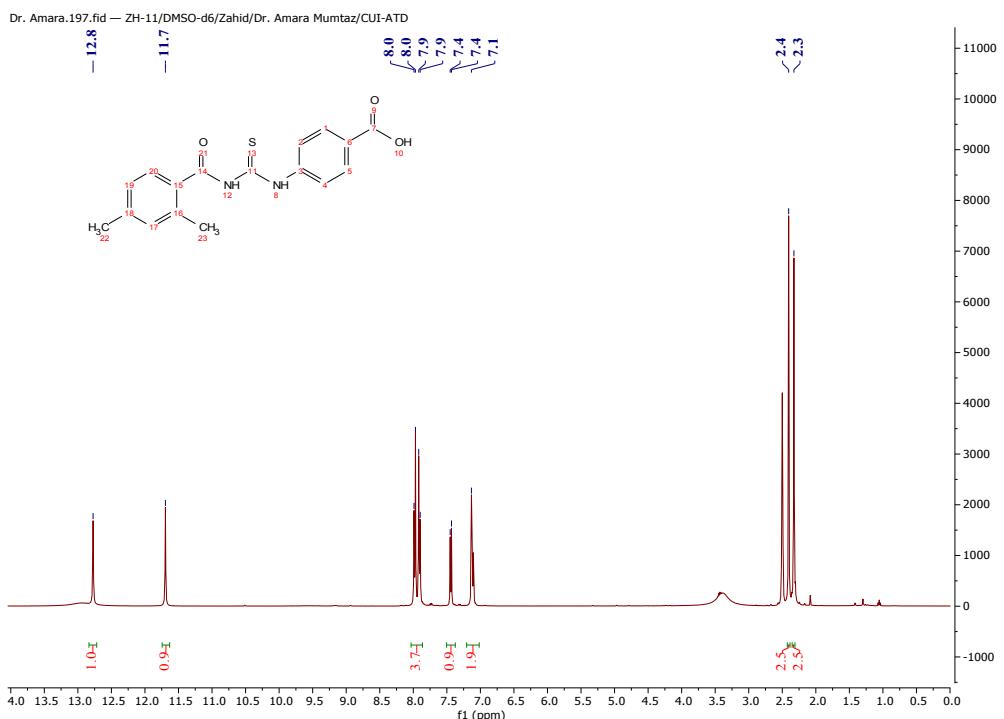


Figure S30: <sup>13</sup>C NMR of compound 25

## Compound 13

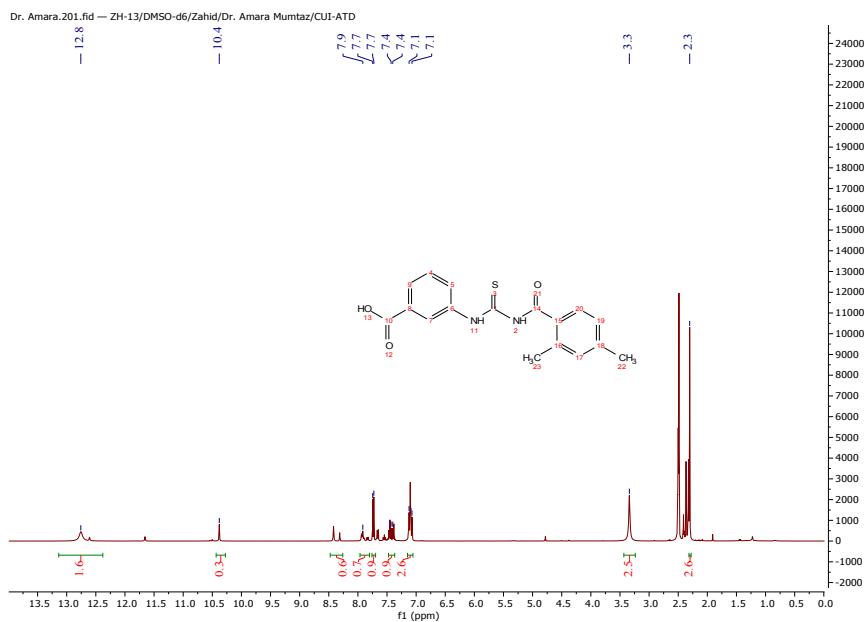


Figure S31:  $^1\text{H}$  NMR of compound 13

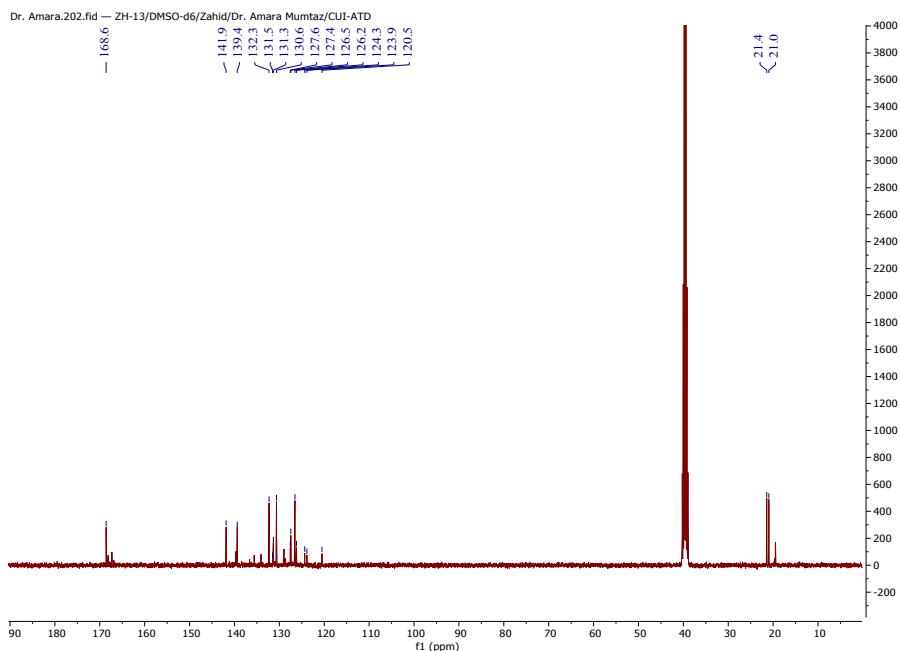


Figure S32:  $^{13}\text{C}$  NMR of compound 13

## 2 Supporting IR spectra of the synthesized compounds

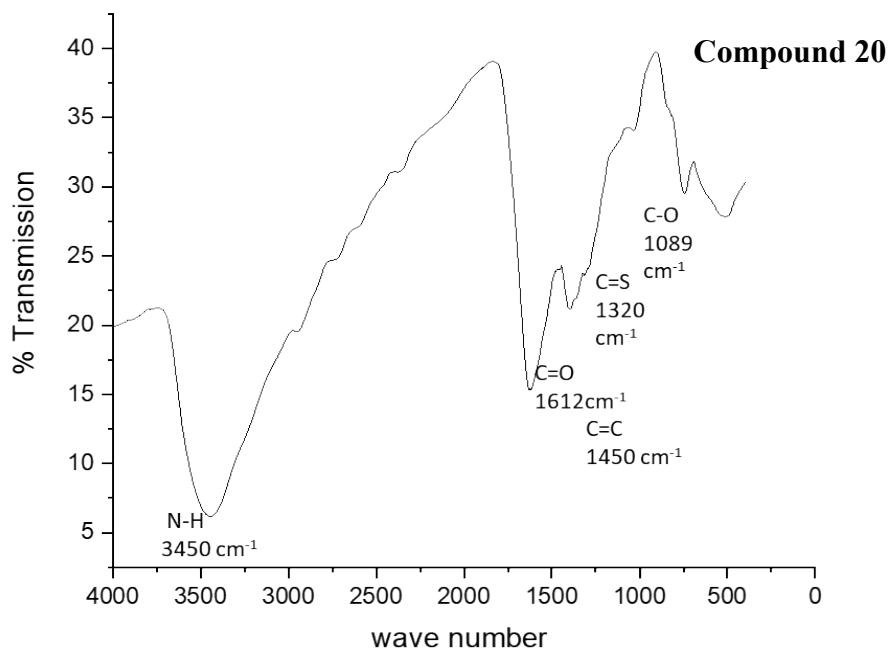


Figure S33: IR graph of Compound 20

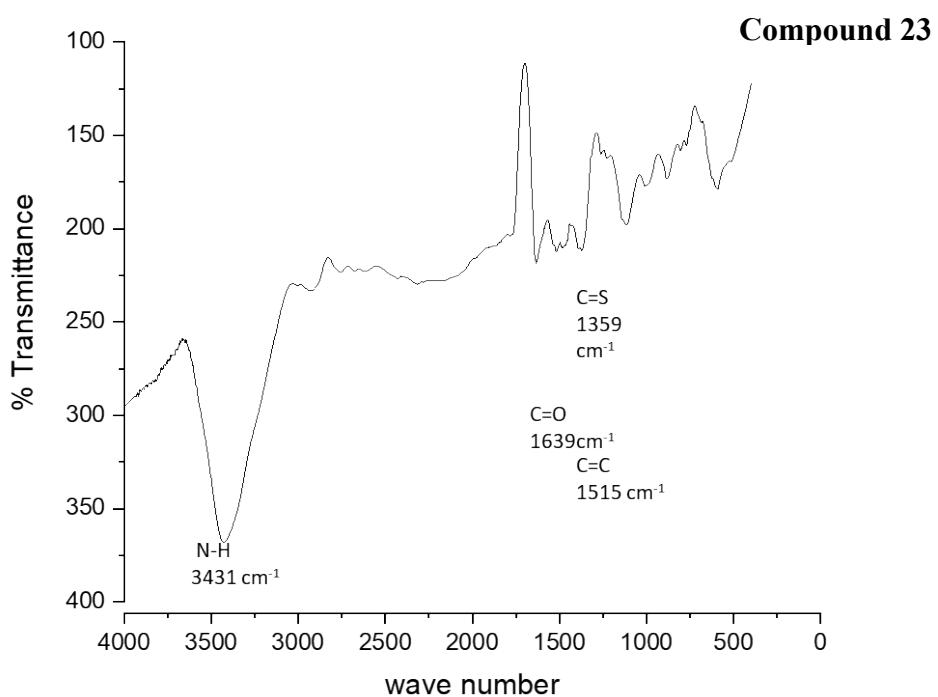


Figure S34: IR graph of Compound 23

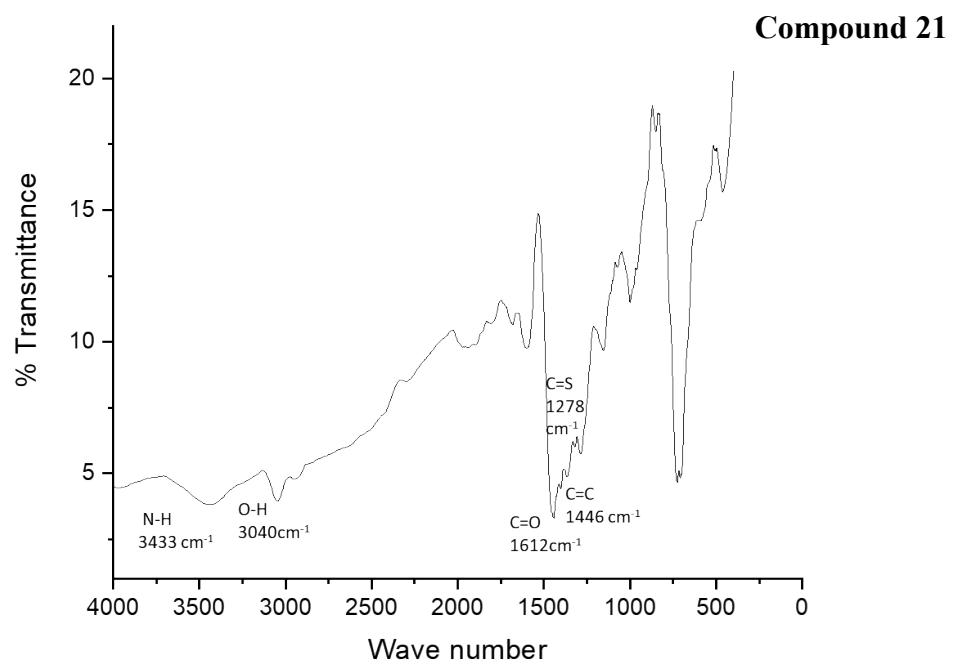


Figure S35: IR graph of Compound 21

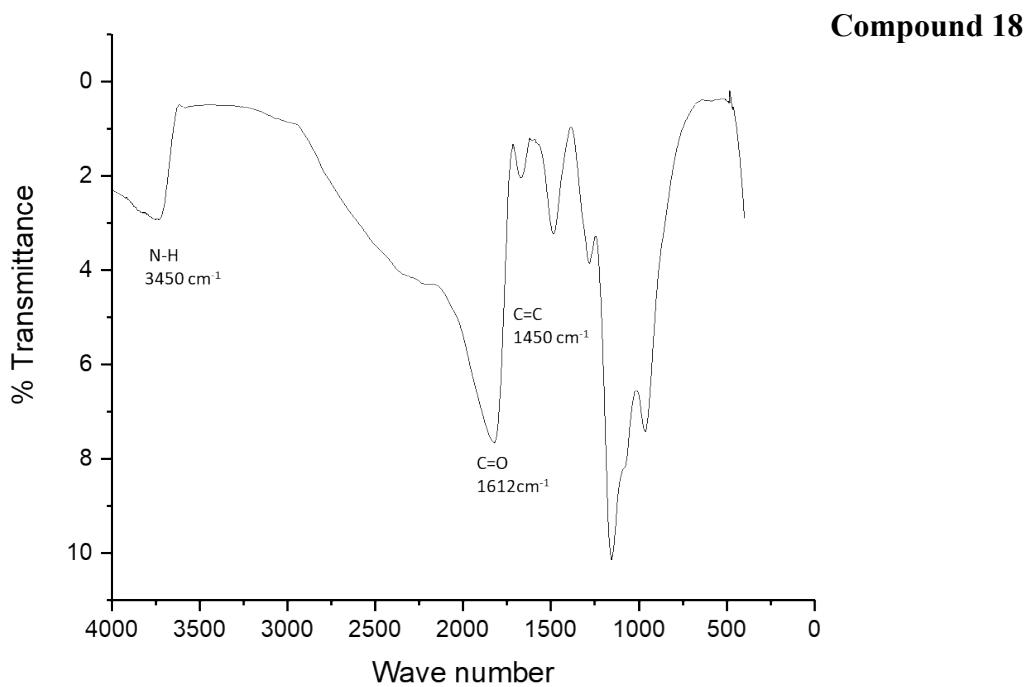


Figure S36: IR graph of Compound 18

**Compound 17**

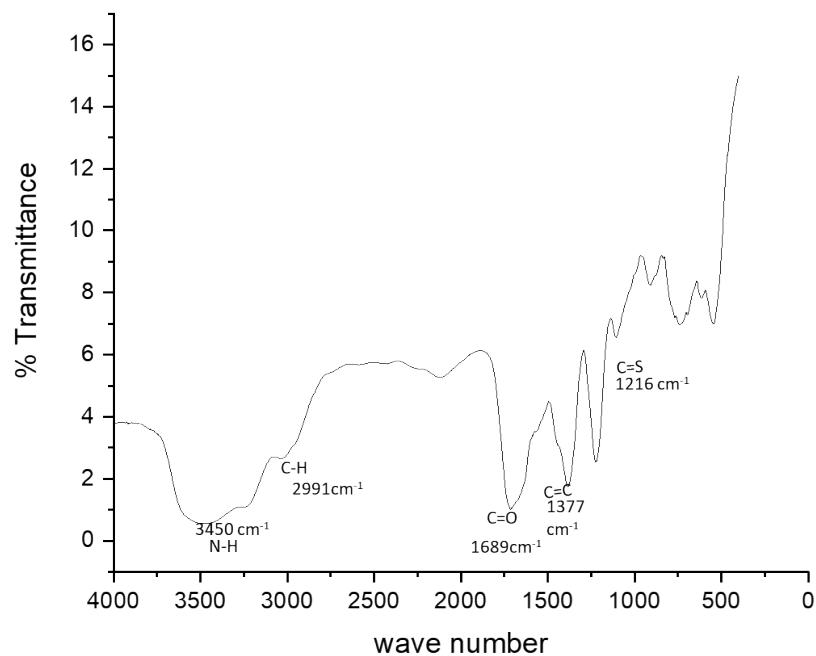


Figure S37: IR graph of Compound 17

**Compound 12**

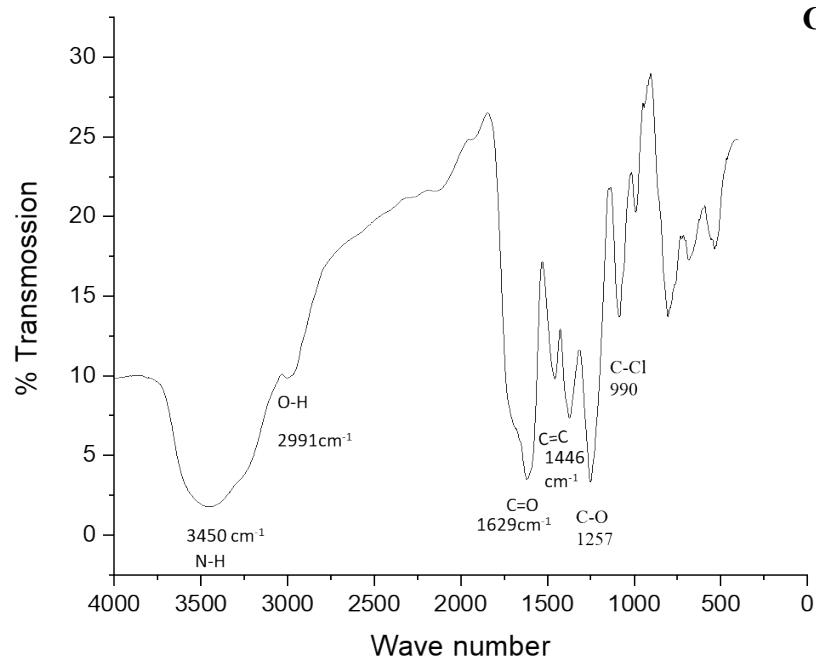


Figure S38: IR graph of Compound 12

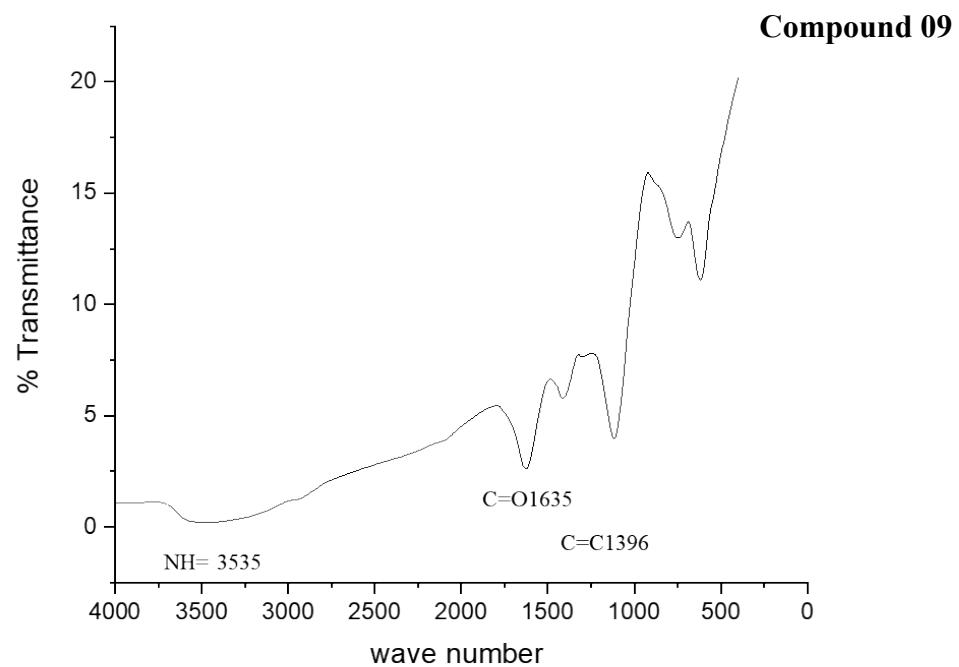


Figure S39: IR graph of Compound 20

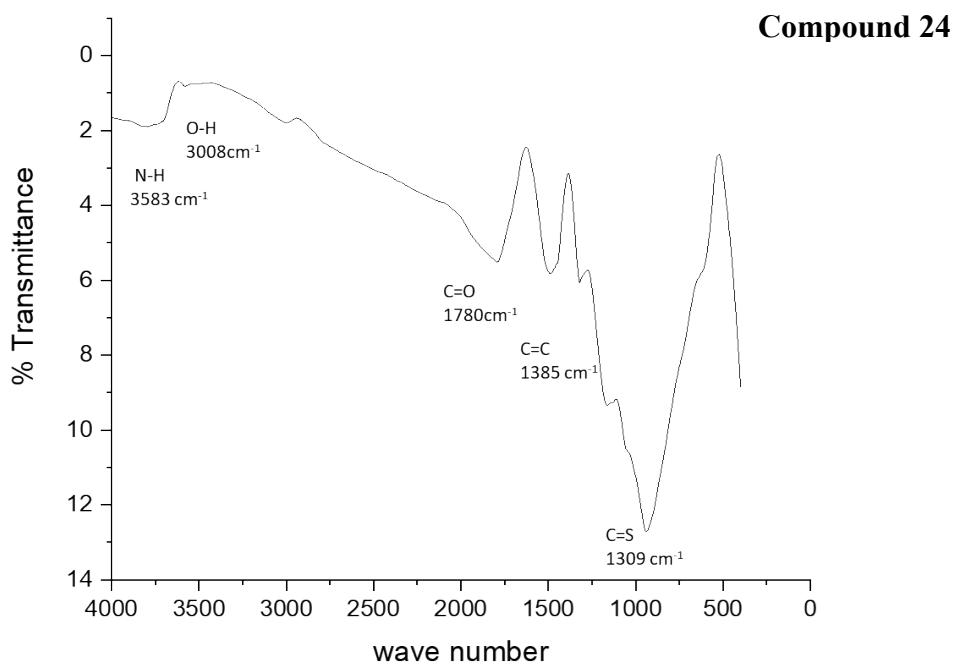


Figure S40: IR graph of Compound 24

**Compound 26**

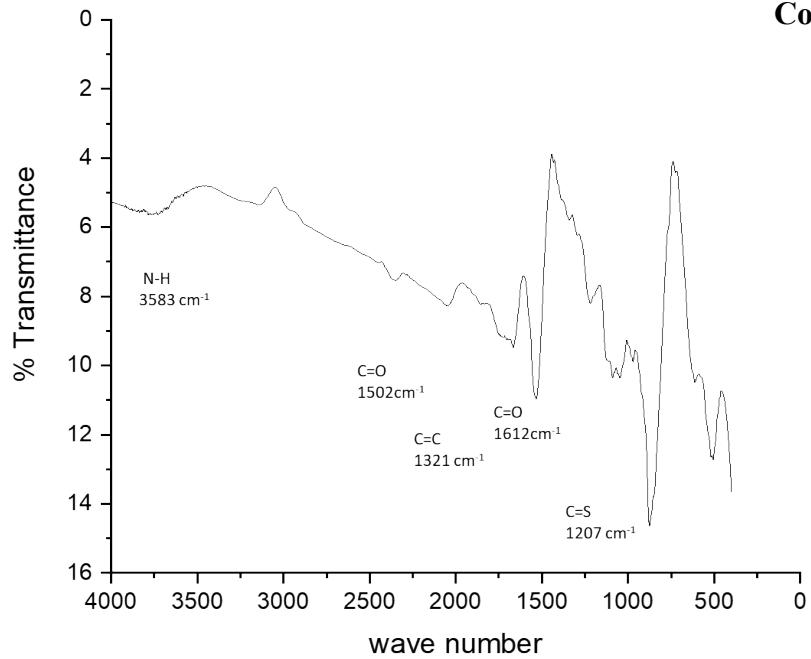


Figure S41: IR graph of Compound 26

**Compound 10**

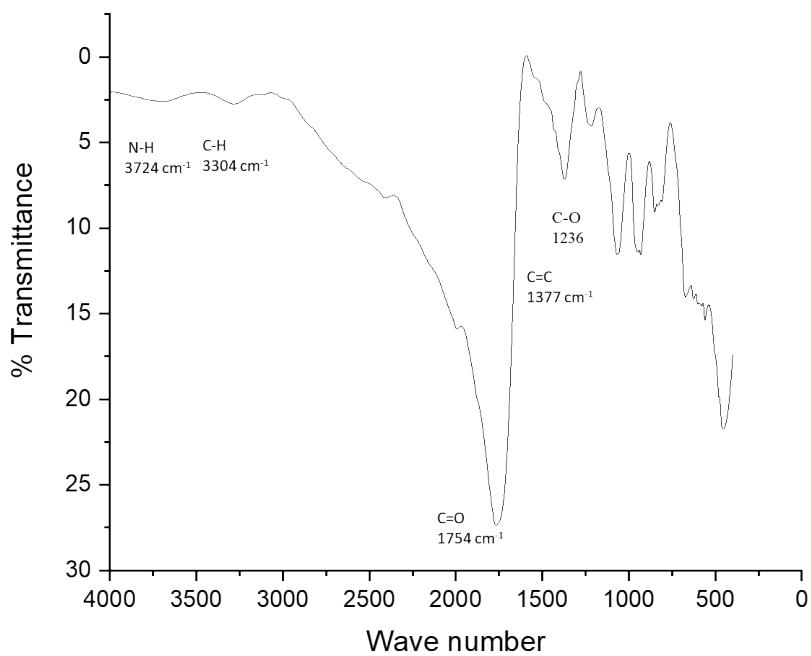


Figure S42: IR graph of Compound 10

**Compound 11**

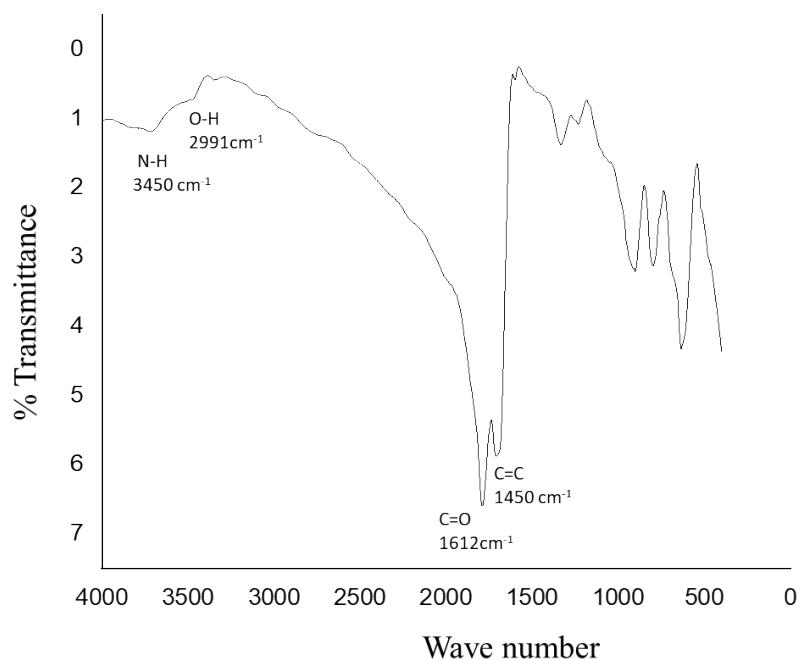


Figure S43: IR graph of Compound 11

**Compound 14**

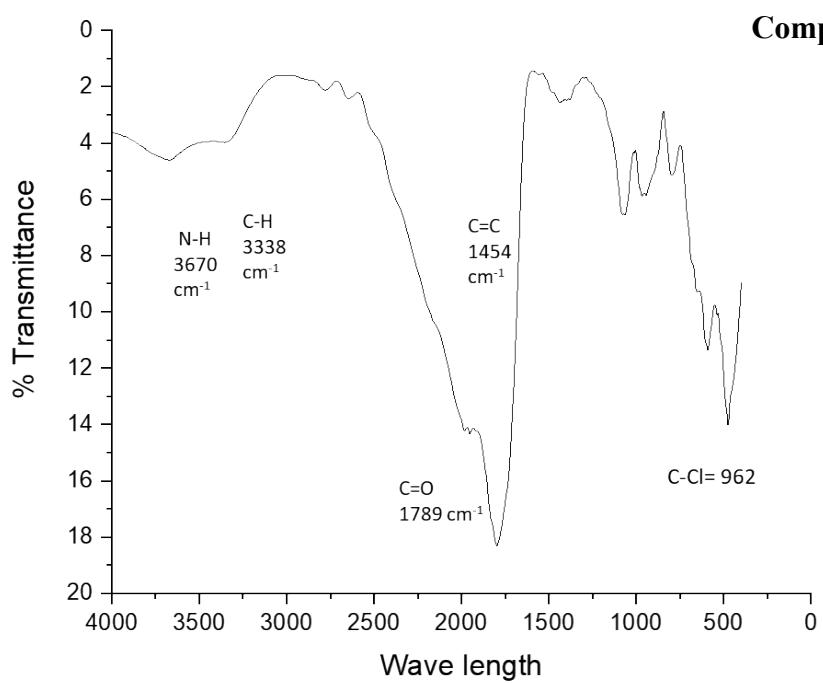


Figure S44: IR graph of Compound 14

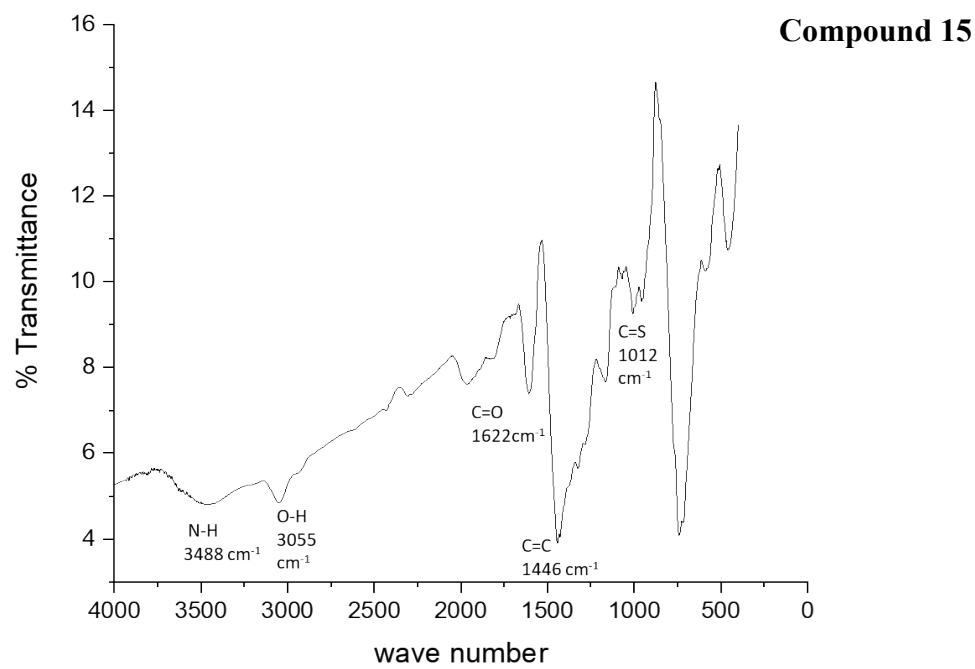


Figure S45: IR graph of Compound 15

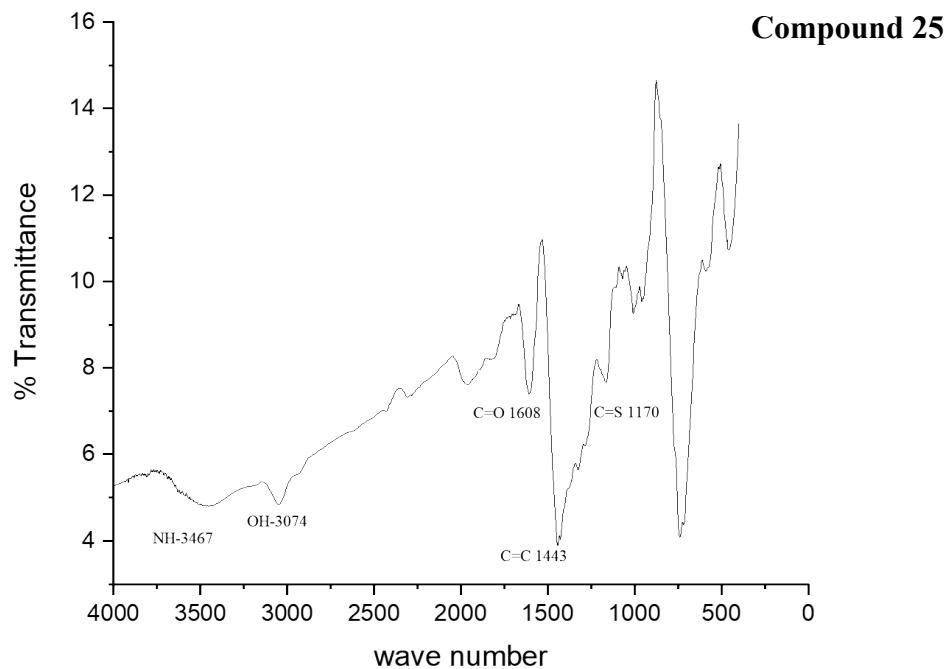


Figure S46: IR graph of Compound 25

**Compound 13**

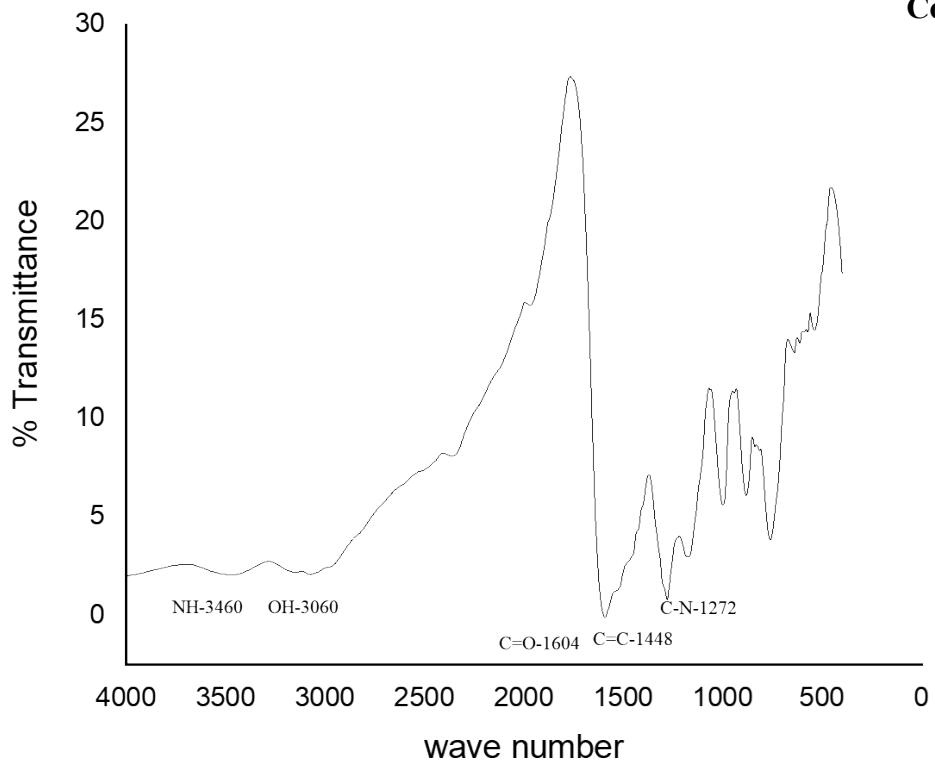
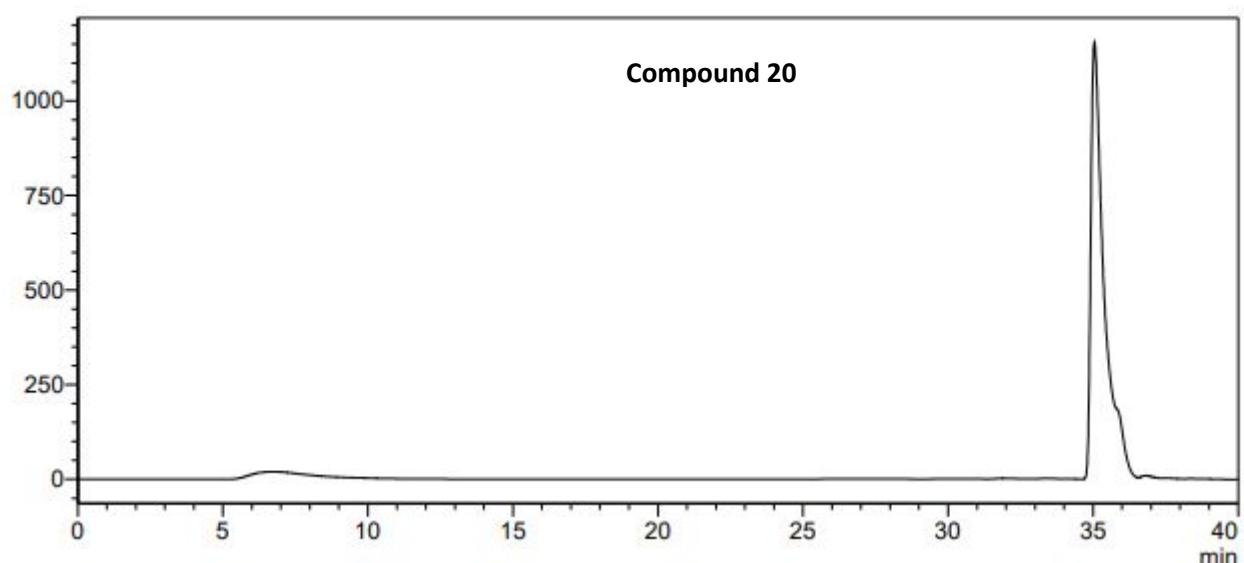


Figure S47: IR graph of Compound 20

# HPLC Chromatogram

mV



## <Chromatogram>

mV

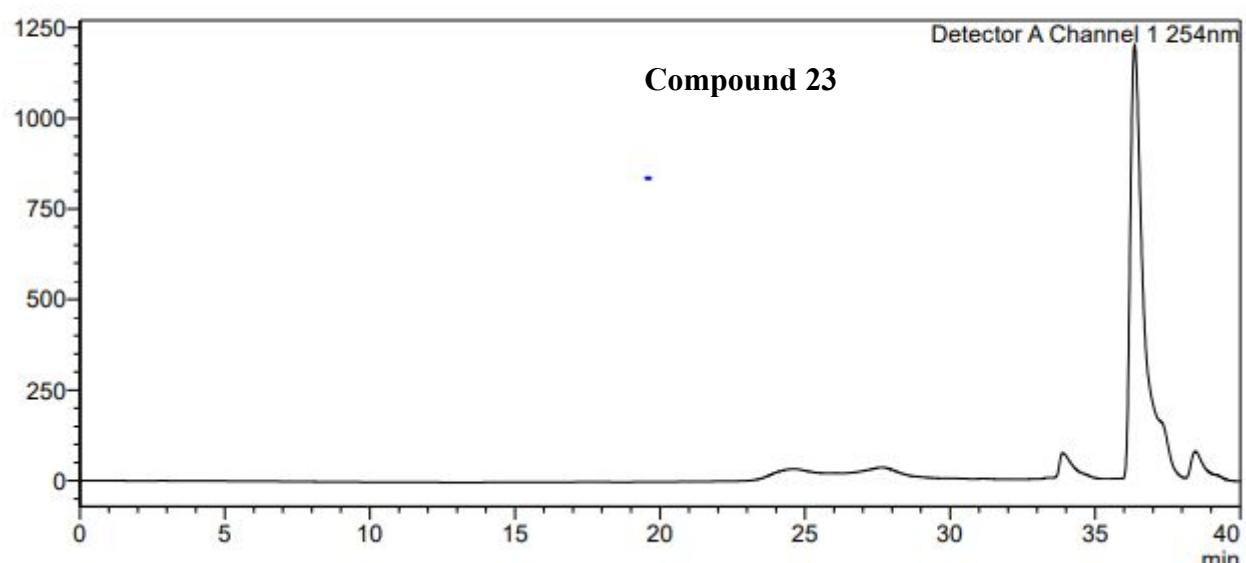


Figure S48: HPLC chromatogram of compound 20 and 23

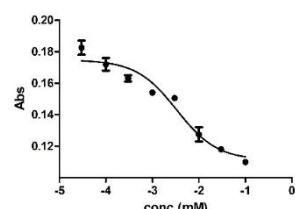
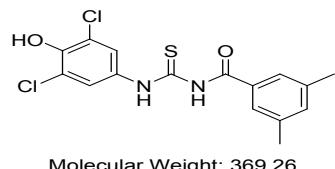
### 3 In-vitro studies of the synthesized compounds and their IC<sub>50</sub> values.

Table S1: IC<sub>50</sub> values and Graphs of hCA II

| h-Carbonic Anhydrase-II (hCA-II) |                              |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
|----------------------------------|------------------------------|--|--------------------|------------------|------|------|------|------|------|------|------|------|------|-------|------|------|------|-------|------|------|-----|------|-------------|
| Compounds                        | Structure                    | Graph  | IC50               |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 09                               | <br>Molecular Weight: 336.36 | <table border="1"> <caption>Data points for compound 09 graph</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.28</td></tr> <tr><td>-4.0</td><td>0.27</td></tr> <tr><td>-3.5</td><td>0.26</td></tr> <tr><td>-3.0</td><td>0.25</td></tr> <tr><td>-2.5</td><td>0.245</td></tr> <tr><td>-2.0</td><td>0.24</td></tr> <tr><td>-1.5</td><td>0.235</td></tr> <tr><td>-1.0</td><td>0.23</td></tr> <tr><td>0.0</td><td>0.24</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.5 | 0.28 | -4.0 | 0.27 | -3.5 | 0.26 | -3.0 | 0.25 | -2.5 | 0.245 | -2.0 | 0.24 | -1.5 | 0.235 | -1.0 | 0.23 | 0.0 | 0.24 | 0.18 ± 0.05 |
| concentration (mM)               | Absorbance (Abs)             |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.5                             | 0.28                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.0                             | 0.27                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.5                             | 0.26                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.0                             | 0.25                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.5                             | 0.245                        |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.0                             | 0.24                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.5                             | 0.235                        |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.0                             | 0.23                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 0.0                              | 0.24                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 10                               | <br>Molecular Weight: 304.36 | <table border="1"> <caption>Data points for compound 10 graph</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>1.4</td></tr> <tr><td>-4.0</td><td>1.3</td></tr> <tr><td>-3.5</td><td>1.1</td></tr> <tr><td>-3.0</td><td>1.0</td></tr> <tr><td>-2.5</td><td>0.7</td></tr> <tr><td>-2.0</td><td>0.6</td></tr> <tr><td>-1.5</td><td>0.55</td></tr> <tr><td>-1.0</td><td>0.5</td></tr> <tr><td>0.0</td><td>0.6</td></tr> </tbody> </table>           | concentration (mM) | Absorbance (Abs) | -4.5 | 1.4  | -4.0 | 1.3  | -3.5 | 1.1  | -3.0 | 1.0  | -2.5 | 0.7   | -2.0 | 0.6  | -1.5 | 0.55  | -1.0 | 0.5  | 0.0 | 0.6  | 0.93 ± 0.05 |
| concentration (mM)               | Absorbance (Abs)             |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.5                             | 1.4                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.0                             | 1.3                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.5                             | 1.1                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.0                             | 1.0                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.5                             | 0.7                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.0                             | 0.6                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.5                             | 0.55                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.0                             | 0.5                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 0.0                              | 0.6                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 11                               | <br>Molecular Weight: 244.66 | <table border="1"> <caption>Data points for compound 11 graph</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>1.5</td></tr> <tr><td>-4.0</td><td>1.5</td></tr> <tr><td>-3.5</td><td>1.4</td></tr> <tr><td>-3.0</td><td>1.3</td></tr> <tr><td>-2.5</td><td>0.9</td></tr> <tr><td>-2.0</td><td>0.6</td></tr> <tr><td>-1.5</td><td>0.4</td></tr> <tr><td>-1.0</td><td>0.3</td></tr> <tr><td>0.0</td><td>0.3</td></tr> </tbody> </table>            | concentration (mM) | Absorbance (Abs) | -4.5 | 1.5  | -4.0 | 1.5  | -3.5 | 1.4  | -3.0 | 1.3  | -2.5 | 0.9   | -2.0 | 0.6  | -1.5 | 0.4   | -1.0 | 0.3  | 0.0 | 0.3  | 1.18 ± 0.14 |
| concentration (mM)               | Absorbance (Abs)             |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.5                             | 1.5                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.0                             | 1.5                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.5                             | 1.4                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.0                             | 1.3                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.5                             | 0.9                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.0                             | 0.6                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.5                             | 0.4                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.0                             | 0.3                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 0.0                              | 0.3                          |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 14                               | <br>Molecular Weight: 304.73 | <table border="1"> <caption>Data points for compound 14 graph</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.22</td></tr> <tr><td>-4.0</td><td>0.21</td></tr> <tr><td>-3.5</td><td>0.19</td></tr> <tr><td>-3.0</td><td>0.17</td></tr> <tr><td>-2.5</td><td>0.14</td></tr> <tr><td>-2.0</td><td>0.12</td></tr> <tr><td>-1.5</td><td>0.11</td></tr> <tr><td>-1.0</td><td>0.11</td></tr> <tr><td>0.0</td><td>0.12</td></tr> </tbody> </table>   | concentration (mM) | Absorbance (Abs) | -4.5 | 0.22 | -4.0 | 0.21 | -3.5 | 0.19 | -3.0 | 0.17 | -2.5 | 0.14  | -2.0 | 0.12 | -1.5 | 0.11  | -1.0 | 0.11 | 0.0 | 0.12 | 6.49 ± 0.63 |
| concentration (mM)               | Absorbance (Abs)             |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.5                             | 0.22                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.0                             | 0.21                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.5                             | 0.19                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.0                             | 0.17                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.5                             | 0.14                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.0                             | 0.12                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.5                             | 0.11                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.0                             | 0.11                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 0.0                              | 0.12                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 15                               | <br>Molecular Weight: 372.19 | <table border="1"> <caption>Data points for compound 15 graph</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.22</td></tr> <tr><td>-4.0</td><td>0.21</td></tr> <tr><td>-3.5</td><td>0.19</td></tr> <tr><td>-3.0</td><td>0.17</td></tr> <tr><td>-2.5</td><td>0.15</td></tr> <tr><td>-2.0</td><td>0.14</td></tr> <tr><td>-1.5</td><td>0.13</td></tr> <tr><td>-1.0</td><td>0.13</td></tr> <tr><td>0.0</td><td>0.18</td></tr> </tbody> </table>   | concentration (mM) | Absorbance (Abs) | -4.5 | 0.22 | -4.0 | 0.21 | -3.5 | 0.19 | -3.0 | 0.17 | -2.5 | 0.15  | -2.0 | 0.14 | -1.5 | 0.13  | -1.0 | 0.13 | 0.0 | 0.18 | 1.93 ± 0.15 |
| concentration (mM)               | Absorbance (Abs)             |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.5                             | 0.22                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -4.0                             | 0.21                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.5                             | 0.19                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -3.0                             | 0.17                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.5                             | 0.15                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -2.0                             | 0.14                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.5                             | 0.13                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| -1.0                             | 0.13                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |
| 0.0                              | 0.18                         |  |                    |                  |      |      |      |      |      |      |      |      |      |       |      |      |      |       |      |      |     |      |             |

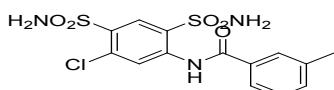
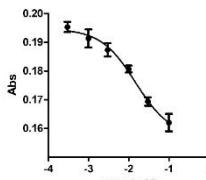
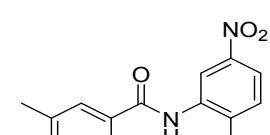
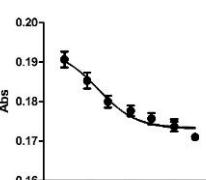
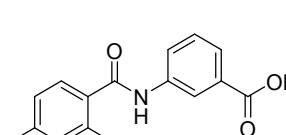
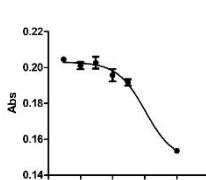
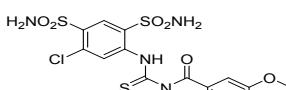
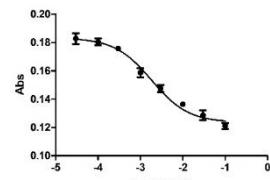
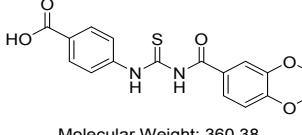
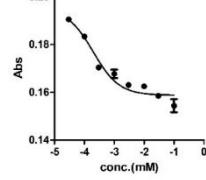
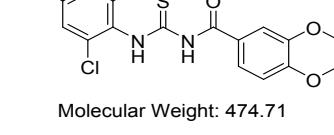
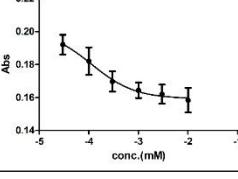
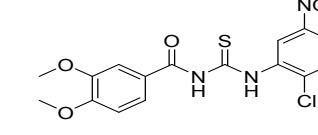
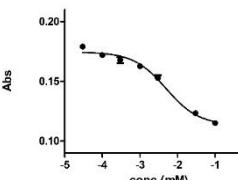
| 17                 | <p>Molecular Weight: 508.96</p> | <table border="1"> <caption>Data points estimated from graph for compound 17</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>1.20</td></tr> <tr><td>-3.5</td><td>1.10</td></tr> <tr><td>-3.0</td><td>1.05</td></tr> <tr><td>-2.5</td><td>0.95</td></tr> <tr><td>-2.0</td><td>0.85</td></tr> <tr><td>-1.5</td><td>0.75</td></tr> <tr><td>-1.0</td><td>0.65</td></tr> <tr><td>-0.5</td><td>0.55</td></tr> <tr><td>0.0</td><td>0.25</td></tr> </tbody> </table>          | concentration (mM) | Absorbance (Abs) | -4.0 | 1.20  | -3.5 | 1.10  | -3.0 | 1.05  | -2.5 | 0.95  | -2.0 | 0.85  | -1.5 | 0.75  | -1.0 | 0.65  | -0.5 | 0.55  | 0.0 | 0.25  | 11.51 ± 0.16 |
|--------------------|---------------------------------|--|--------------------|------------------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|-----|-------|--------------|
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 1.20                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 1.10                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 1.05                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 0.95                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 0.85                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 0.75                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 0.65                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 0.55                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 0.25                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 18                 | <p>Molecular Weight: 360.38</p> | <table border="1"> <caption>Data points estimated from graph for compound 18</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>0.180</td></tr> <tr><td>-3.5</td><td>0.165</td></tr> <tr><td>-3.0</td><td>0.160</td></tr> <tr><td>-2.5</td><td>0.155</td></tr> <tr><td>-2.0</td><td>0.145</td></tr> <tr><td>-1.5</td><td>0.135</td></tr> <tr><td>-1.0</td><td>0.130</td></tr> <tr><td>-0.5</td><td>0.125</td></tr> <tr><td>0.0</td><td>0.120</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.0 | 0.180 | -3.5 | 0.165 | -3.0 | 0.160 | -2.5 | 0.155 | -2.0 | 0.145 | -1.5 | 0.135 | -1.0 | 0.130 | -0.5 | 0.125 | 0.0 | 0.120 | 4.17 ± 0.29  |
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 0.180                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 0.165                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 0.160                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 0.155                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 0.145                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 0.135                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 0.130                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 0.125                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 0.120                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 19                 | <p>Molecular Weight: 474.71</p> | <table border="1"> <caption>Data points estimated from graph for compound 19</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>1.55</td></tr> <tr><td>-3.5</td><td>1.45</td></tr> <tr><td>-3.0</td><td>1.35</td></tr> <tr><td>-2.5</td><td>1.25</td></tr> <tr><td>-2.0</td><td>1.15</td></tr> <tr><td>-1.5</td><td>1.10</td></tr> <tr><td>-1.0</td><td>1.05</td></tr> <tr><td>-0.5</td><td>1.00</td></tr> <tr><td>0.0</td><td>1.00</td></tr> </tbody> </table>          | concentration (mM) | Absorbance (Abs) | -4.0 | 1.55  | -3.5 | 1.45  | -3.0 | 1.35  | -2.5 | 1.25  | -2.0 | 1.15  | -1.5 | 1.10  | -1.0 | 1.05  | -0.5 | 1.00  | 0.0 | 1.00  | 0.72 ± 0.06  |
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 1.55                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 1.45                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 1.35                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 1.25                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 1.15                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 1.10                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 1.05                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 1.00                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 1.00                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 22                 | <p>Molecular Weight: 395.81</p> | <table border="1"> <caption>Data points estimated from graph for compound 22</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>1.50</td></tr> <tr><td>-3.5</td><td>1.35</td></tr> <tr><td>-3.0</td><td>1.15</td></tr> <tr><td>-2.5</td><td>0.95</td></tr> <tr><td>-2.0</td><td>0.85</td></tr> <tr><td>-1.5</td><td>0.75</td></tr> <tr><td>-1.0</td><td>0.65</td></tr> <tr><td>-0.5</td><td>0.55</td></tr> <tr><td>0.0</td><td>0.55</td></tr> </tbody> </table>          | concentration (mM) | Absorbance (Abs) | -4.0 | 1.50  | -3.5 | 1.35  | -3.0 | 1.15  | -2.5 | 0.95  | -2.0 | 0.85  | -1.5 | 0.75  | -1.0 | 0.65  | -0.5 | 0.55  | 0.0 | 0.55  | 0.26 ± 0.03  |
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 1.50                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 1.35                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 1.15                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 0.95                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 0.85                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 0.75                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 0.65                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 0.55                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 0.55                            |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 24                 | <p>Molecular Weight: 300.38</p> | <table border="1"> <caption>Data points estimated from graph for compound 24</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>0.240</td></tr> <tr><td>-3.5</td><td>0.215</td></tr> <tr><td>-3.0</td><td>0.205</td></tr> <tr><td>-2.5</td><td>0.195</td></tr> <tr><td>-2.0</td><td>0.185</td></tr> <tr><td>-1.5</td><td>0.175</td></tr> <tr><td>-1.0</td><td>0.165</td></tr> <tr><td>-0.5</td><td>0.160</td></tr> <tr><td>0.0</td><td>0.160</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.0 | 0.240 | -3.5 | 0.215 | -3.0 | 0.205 | -2.5 | 0.195 | -2.0 | 0.185 | -1.5 | 0.175 | -1.0 | 0.165 | -0.5 | 0.160 | 0.0 | 0.160 | 1.24 ± 0.96  |
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 0.240                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 0.215                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 0.205                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 0.195                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 0.185                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 0.175                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 0.165                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 0.160                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 0.160                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 25                 | <p>Molecular Weight: 328.39</p> | <table border="1"> <caption>Data points estimated from graph for compound 25</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.0</td><td>0.180</td></tr> <tr><td>-3.5</td><td>0.170</td></tr> <tr><td>-3.0</td><td>0.160</td></tr> <tr><td>-2.5</td><td>0.150</td></tr> <tr><td>-2.0</td><td>0.140</td></tr> <tr><td>-1.5</td><td>0.130</td></tr> <tr><td>-1.0</td><td>0.120</td></tr> <tr><td>-0.5</td><td>0.120</td></tr> <tr><td>0.0</td><td>0.120</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.0 | 0.180 | -3.5 | 0.170 | -3.0 | 0.160 | -2.5 | 0.150 | -2.0 | 0.140 | -1.5 | 0.130 | -1.0 | 0.120 | -0.5 | 0.120 | 0.0 | 0.120 | 0.38 ± 0.01  |
| concentration (mM) | Absorbance (Abs)                |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -4.0               | 0.180                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.5               | 0.170                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -3.0               | 0.160                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.5               | 0.150                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -2.0               | 0.140                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.5               | 0.130                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -1.0               | 0.120                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| -0.5               | 0.120                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |
| 0.0                | 0.120                           |  |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |              |

26



4.45 ± 0.11

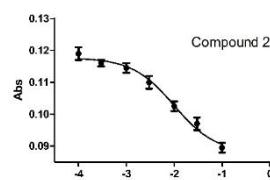
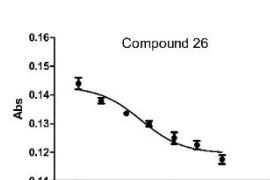
Table S2: IC<sub>50</sub> values and Graphs of hCA IX

| Human Carbonic Anhydrase IX (hCA-IX) |   |  |                  |
|--------------------------------------|---|--|------------------|
| Compounds                            | STRUCTURE   | GRAPH  | IC <sub>50</sub> |
| 10                                   | <br>Molecular Weight: 244.66   |    | 14.58 ± 1.07     |
| 11                                   | <br>Molecular Weight: 304.73   |    | 0.17 ± 0.05      |
| 13                                   | <br>Molecular Weight: 269.30   |    | 10.36 ± 1.45     |
| 17                                   | <br>Molecular Weight: 508.96 |  | 1.71 ± 0.65      |
| 18                                   | <br>Molecular Weight: 360.38 |  | 0.21 ± 0.09      |
| 19                                   | <br>Molecular Weight: 474.71 |  | 1.01 ± 0.05      |
| 22                                   | <br>Molecular Weight: 395.81 |  | 4.93 ± 1.83      |

| 24                 | <p>Molecular Weight: 300.38</p> | <table border="1"> <caption>Data points estimated from Graph for Compound 24</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.220</td></tr> <tr><td>-4.0</td><td>0.218</td></tr> <tr><td>-3.5</td><td>0.215</td></tr> <tr><td>-3.0</td><td>0.212</td></tr> <tr><td>-2.5</td><td>0.208</td></tr> <tr><td>-2.0</td><td>0.205</td></tr> <tr><td>-1.5</td><td>0.202</td></tr> <tr><td>-1.0</td><td>0.200</td></tr> <tr><td>-0.5</td><td>0.198</td></tr> <tr><td>0.0</td><td>0.165</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.5 | 0.220 | -4.0 | 0.218 | -3.5 | 0.215 | -3.0 | 0.212 | -2.5 | 0.208 | -2.0 | 0.205 | -1.5 | 0.202 | -1.0 | 0.200 | -0.5 | 0.198 | 0.0 | 0.165 | 1.25 ± 0.02 |
|--------------------|---------------------------------|---|--------------------|------------------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|-----|-------|-------------|
| concentration (mM) | Absorbance (Abs)                |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.5               | 0.220                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.0               | 0.218                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.5               | 0.215                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.0               | 0.212                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.5               | 0.208                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.0               | 0.205                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.5               | 0.202                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.0               | 0.200                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -0.5               | 0.198                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| 0.0                | 0.165                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| 25                 | <p>Molecular Weight: 328.39</p> | <table border="1"> <caption>Data points estimated from Graph for Compound 25</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.200</td></tr> <tr><td>-4.0</td><td>0.198</td></tr> <tr><td>-3.5</td><td>0.195</td></tr> <tr><td>-3.0</td><td>0.192</td></tr> <tr><td>-2.5</td><td>0.188</td></tr> <tr><td>-2.0</td><td>0.185</td></tr> <tr><td>-1.5</td><td>0.182</td></tr> <tr><td>-1.0</td><td>0.178</td></tr> <tr><td>-0.5</td><td>0.175</td></tr> <tr><td>0.0</td><td>0.140</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.5 | 0.200 | -4.0 | 0.198 | -3.5 | 0.195 | -3.0 | 0.192 | -2.5 | 0.188 | -2.0 | 0.185 | -1.5 | 0.182 | -1.0 | 0.178 | -0.5 | 0.175 | 0.0 | 0.140 | 9.76 ± 1.03 |
| concentration (mM) | Absorbance (Abs)                |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.5               | 0.200                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.0               | 0.198                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.5               | 0.195                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.0               | 0.192                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.5               | 0.188                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.0               | 0.185                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.5               | 0.182                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.0               | 0.178                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -0.5               | 0.175                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| 0.0                | 0.140                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| 26                 | <p>Molecular Weight: 369.26</p> | <table border="1"> <caption>Data points estimated from Graph for Compound 26</caption> <thead> <tr> <th>concentration (mM)</th> <th>Absorbance (Abs)</th> </tr> </thead> <tbody> <tr><td>-4.5</td><td>0.230</td></tr> <tr><td>-4.0</td><td>0.228</td></tr> <tr><td>-3.5</td><td>0.225</td></tr> <tr><td>-3.0</td><td>0.220</td></tr> <tr><td>-2.5</td><td>0.215</td></tr> <tr><td>-2.0</td><td>0.210</td></tr> <tr><td>-1.5</td><td>0.208</td></tr> <tr><td>-1.0</td><td>0.205</td></tr> <tr><td>-0.5</td><td>0.202</td></tr> <tr><td>0.0</td><td>0.200</td></tr> </tbody> </table> | concentration (mM) | Absorbance (Abs) | -4.5 | 0.230 | -4.0 | 0.228 | -3.5 | 0.225 | -3.0 | 0.220 | -2.5 | 0.215 | -2.0 | 0.210 | -1.5 | 0.208 | -1.0 | 0.205 | -0.5 | 0.202 | 0.0 | 0.200 | 1.28 ± 0.09 |
| concentration (mM) | Absorbance (Abs)                |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.5               | 0.230                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -4.0               | 0.228                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.5               | 0.225                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -3.0               | 0.220                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.5               | 0.215                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -2.0               | 0.210                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.5               | 0.208                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -1.0               | 0.205                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| -0.5               | 0.202                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |
| 0.0                | 0.200                           |   |                    |                  |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |      |       |     |       |             |

Table S3: IC<sub>50</sub> values and Graphs of hCA XII

| h-Carbonic Anhydrase-XII (hCA-XII) |                              |       |                  |
|------------------------------------|------------------------------|-------|------------------|
| Compounds                          | STRUCTURE                    | GRAPH | IC <sub>50</sub> |
| Compound 10                        |                              |       | 9.17 ± 0.85      |
| Compound 11                        |                              |       | 2.99 ± 0.32      |
| Compound 12                        |                              |       | 0.58 ± 0.06      |
| Compound 14                        |                              |       | 0.95 ± 0.08      |
| Compound 15                        |                              |       | 1.05 ± 0.11      |
| Compound 18                        | <br>Molecular Weight: 360.38 |       | 1.68 ± 0.15      |
| Compound 22                        |                              |       | 4.29 ± 0.24      |

|             |   |  |             |
|-------------|---|--|-------------|
| Compound 24 | <chem>O=C(NC(=S)Nc1ccccc1O)c2ccc(cc2)C</chem>         |  | 9.90 ± 0.66 |
| Compound 26 | <chem>O=C(NC(=S)Nc1ccc(Cl)c(Cl)c1O)c2ccc(cc2)C</chem> |  | 2.02 ± 0.28 |

#### 4 Cell Viability assay

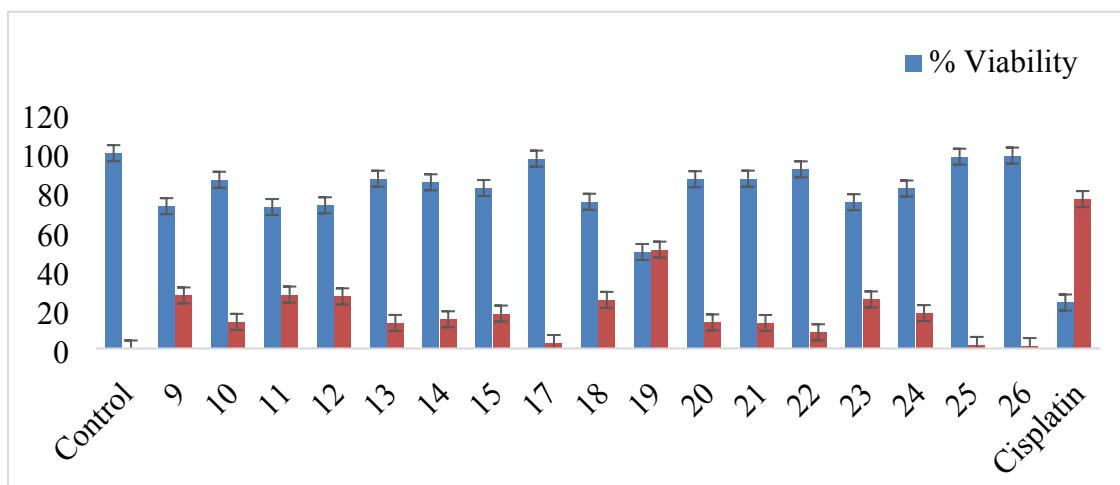


Figure S49: Analysis of cell viability/cytotoxicity using HEK-293 cells treated with compounds 1–17 at final concentration (100  $\mu$ M). Statistics indicating significant values \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. untreated group. Cisplatin (100  $\mu$ M) is used as a positive control.

## 5 Docking interaction of the synthesized compounds

2D interaction of the compounds **09** and **11**

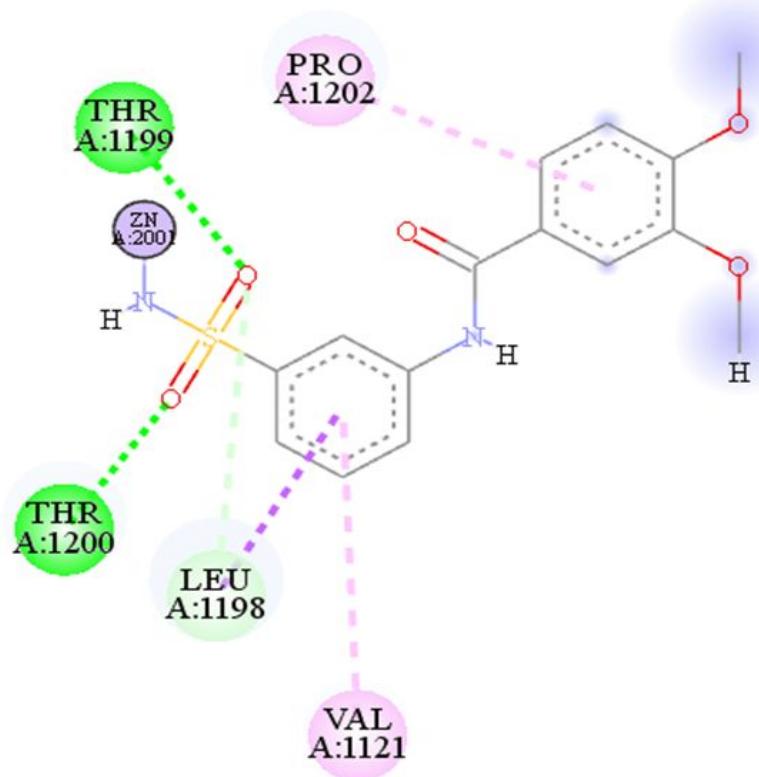


Figure S50: 2D interaction of Compound **09**

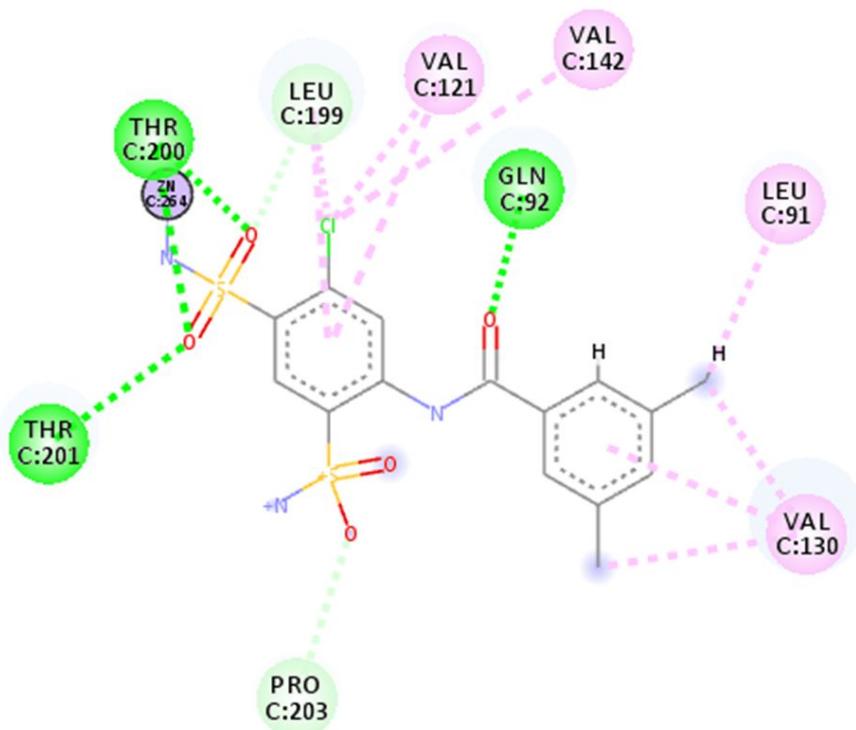


Figure S51: 2D interaction and geometrical graph of compound **11**

Interaction of compounds **12** and **18** against hCA XII

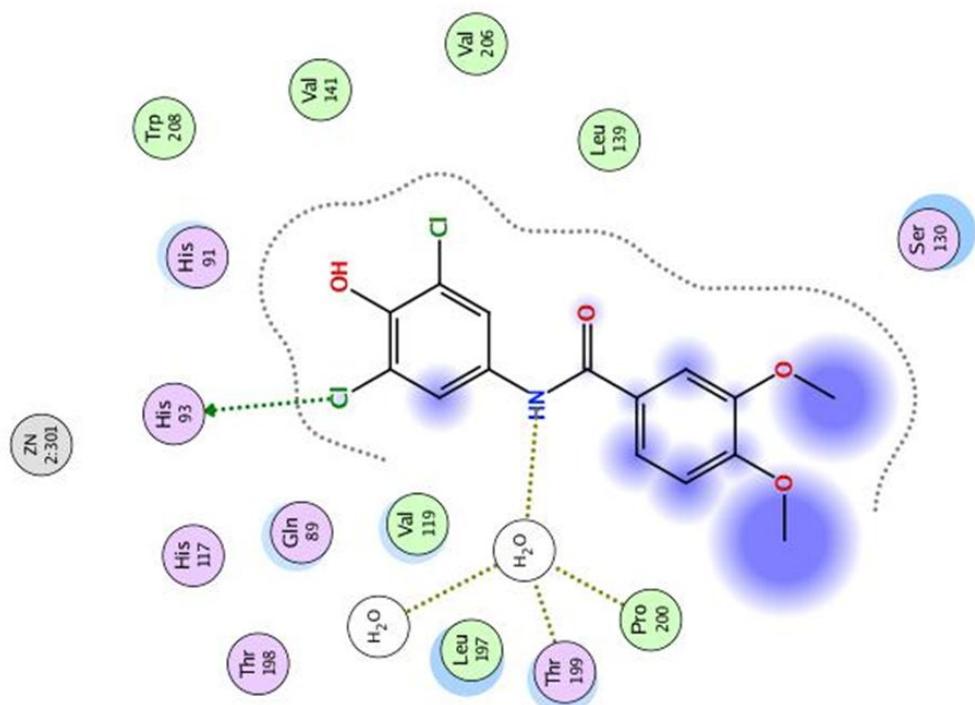


Figure S52: 2D interaction Compound 12

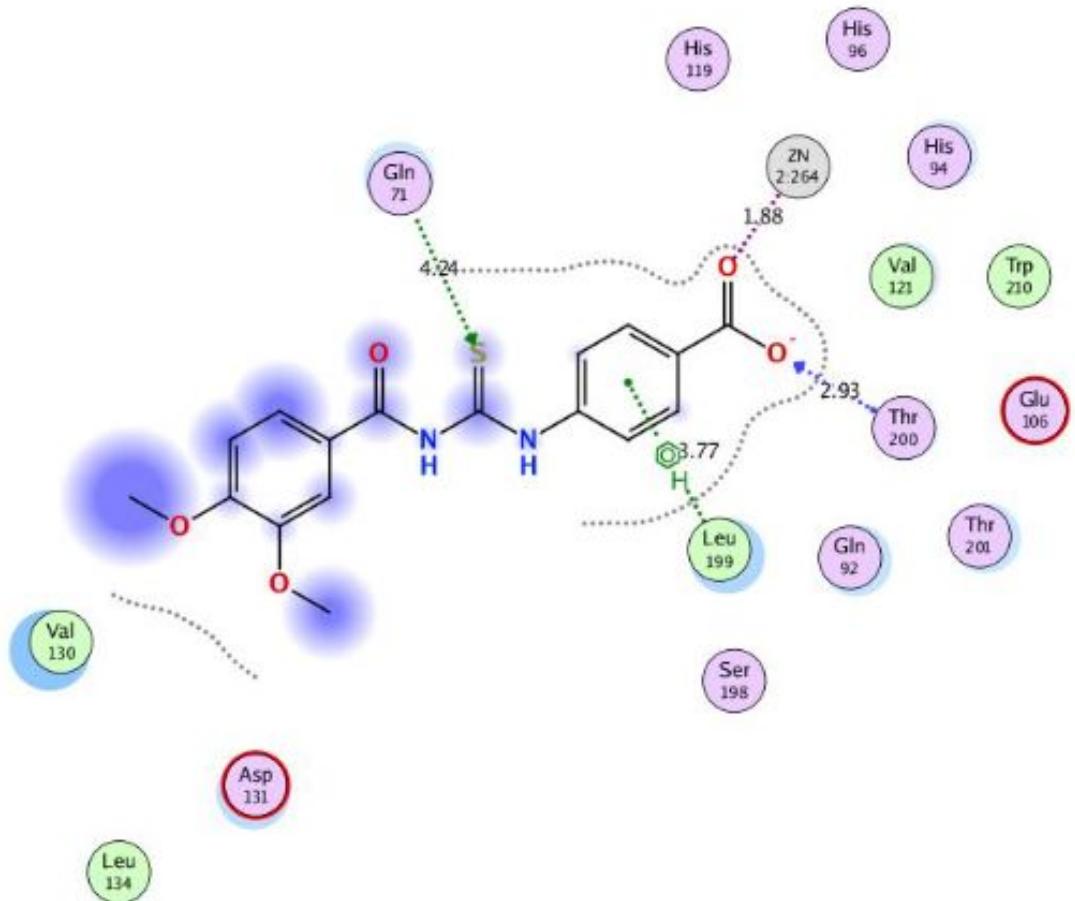


Figure S53: 2D interaction Compound 18