

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

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1.1 Proton NMR, ¹³C Spectra of the synthesized compounds

Compound 20

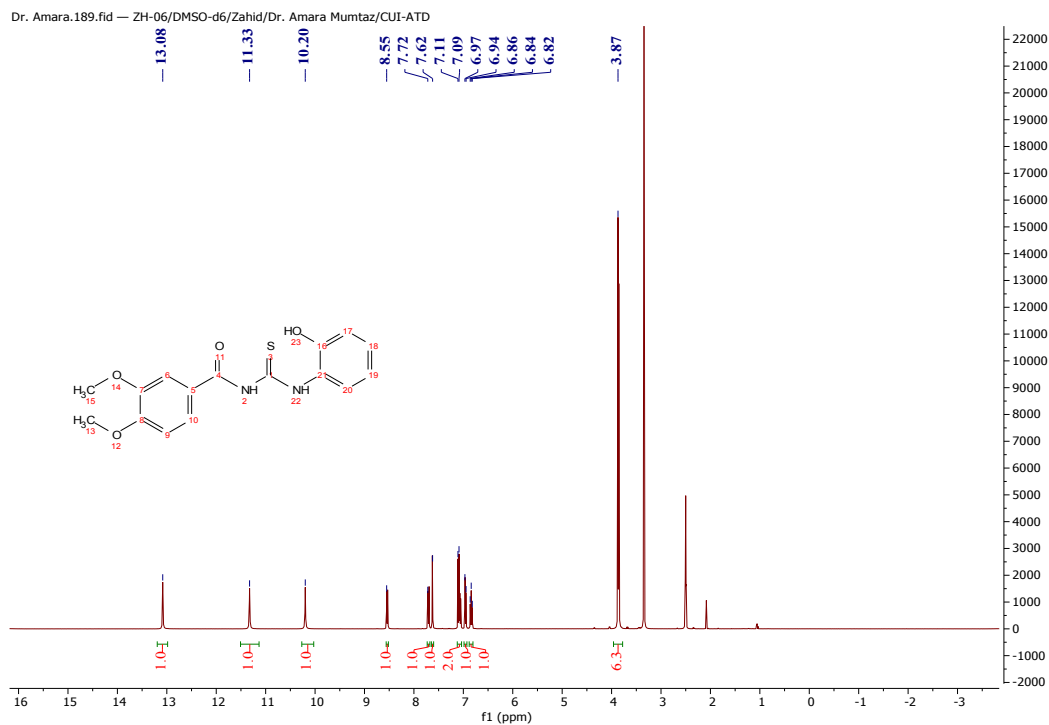


Figure S1: ¹H NMR of compound 20

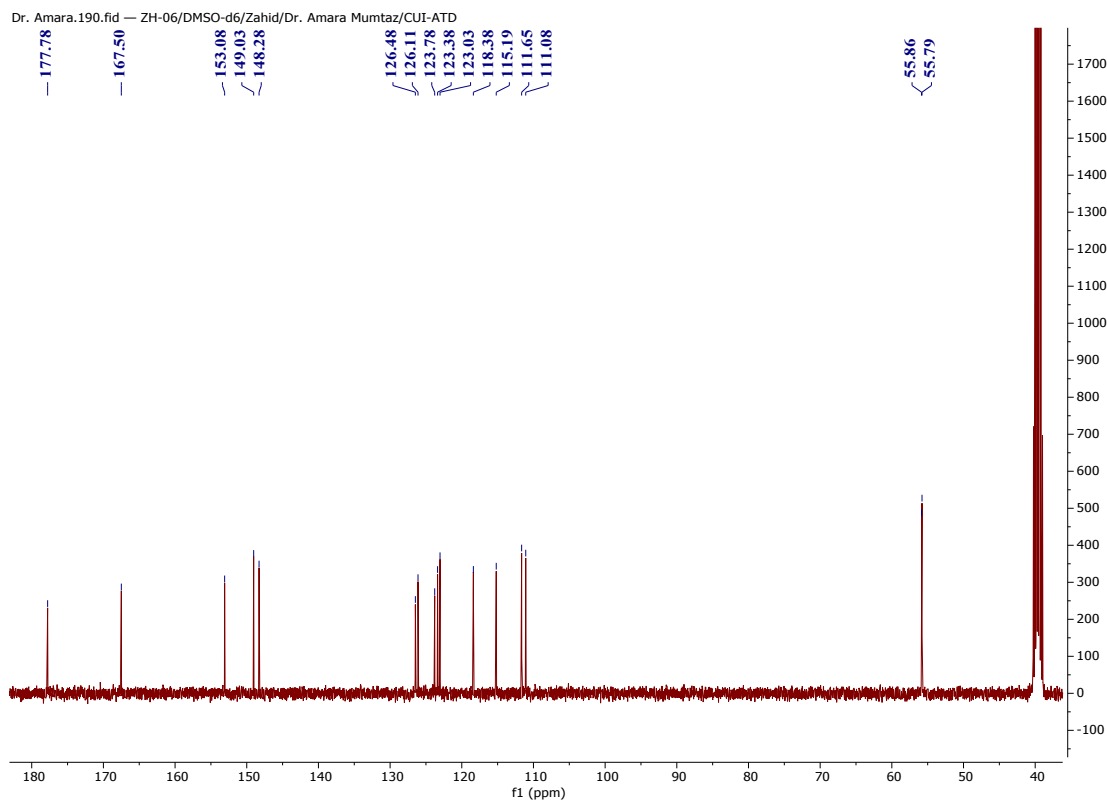


Figure S2: ¹³C NMR of compound 20

Compound 22

Dr. Amara.193.fid — ZH-09/DMSO-d6/Zahid/Dr. Amara Mumtaz/CUI-ATD

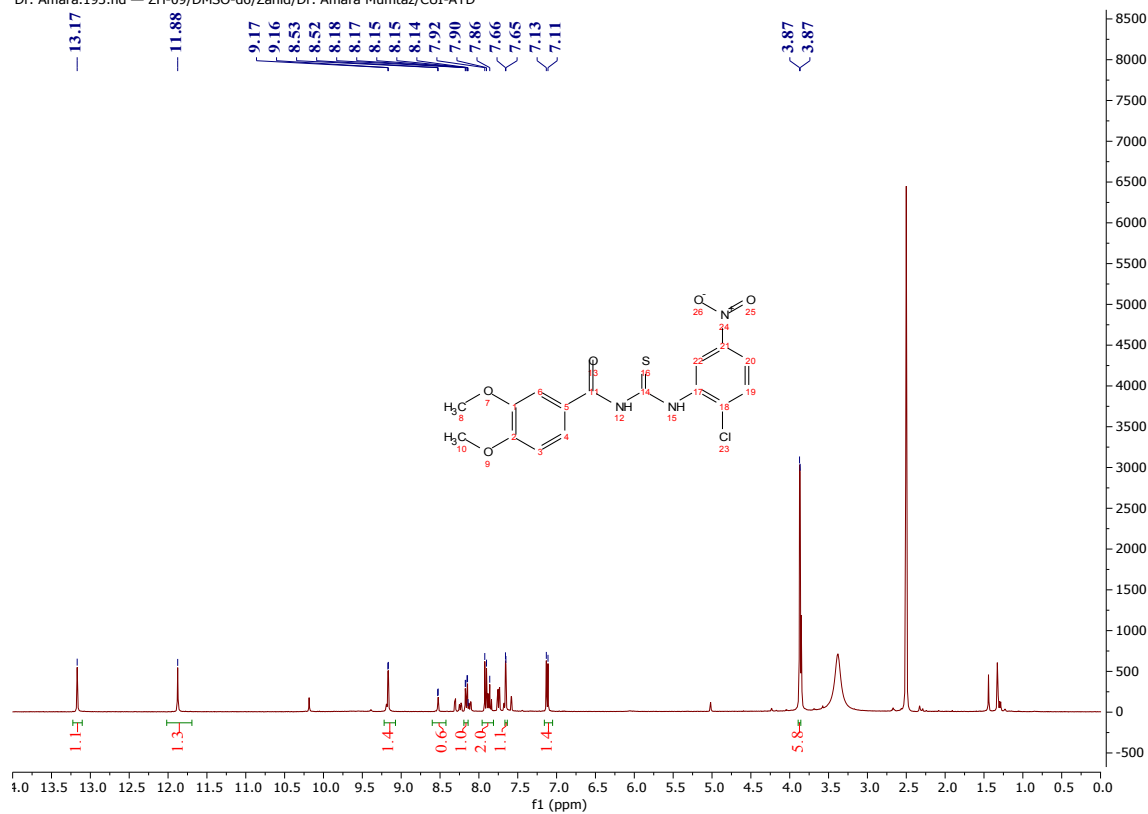


Figure S3: ¹H NMR of compound 22

Dr. Amara.194.fid — ZH-09/DMSO-d6/Zahid/Dr. Amara Mumtaz/CUI-ATD

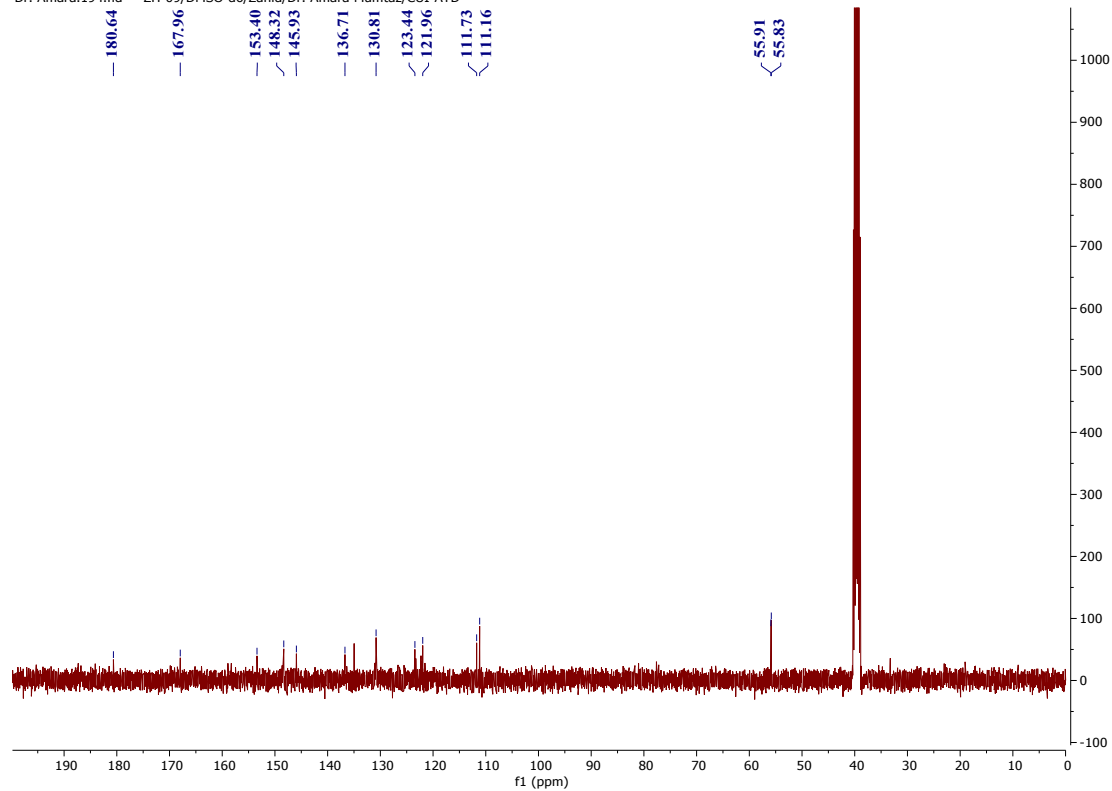


Figure S4: ¹³C NMR of compound 22

Compound 23

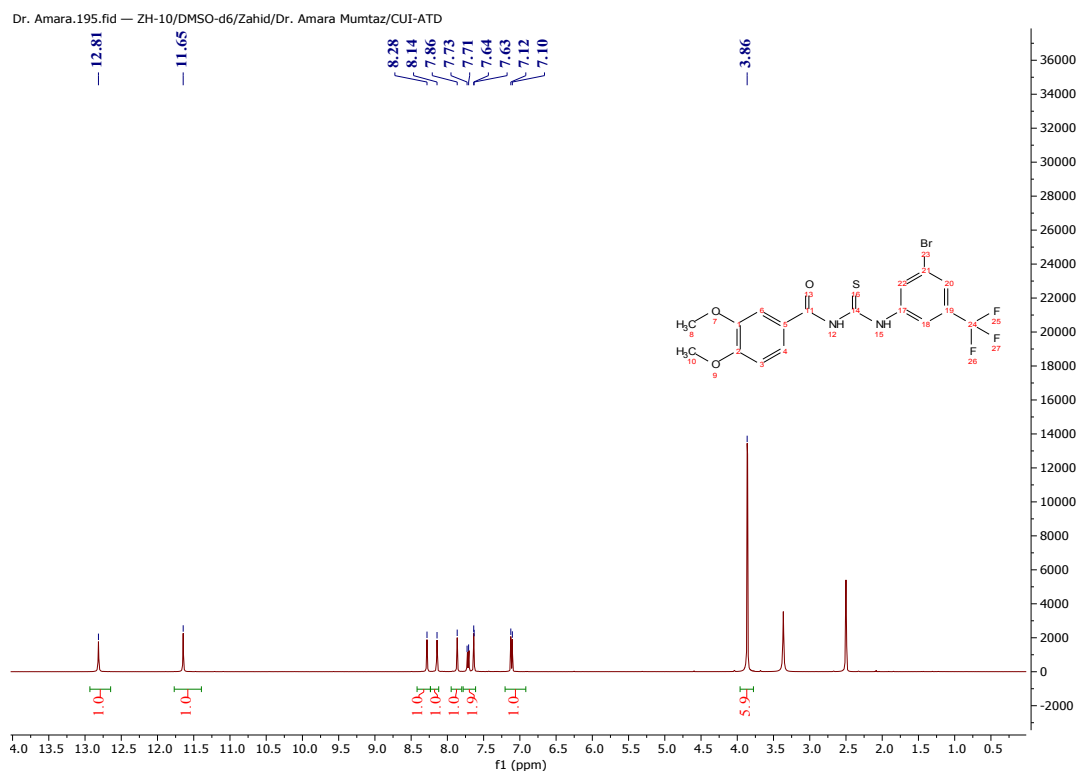


Figure S5: ^1H NMR of compound 23

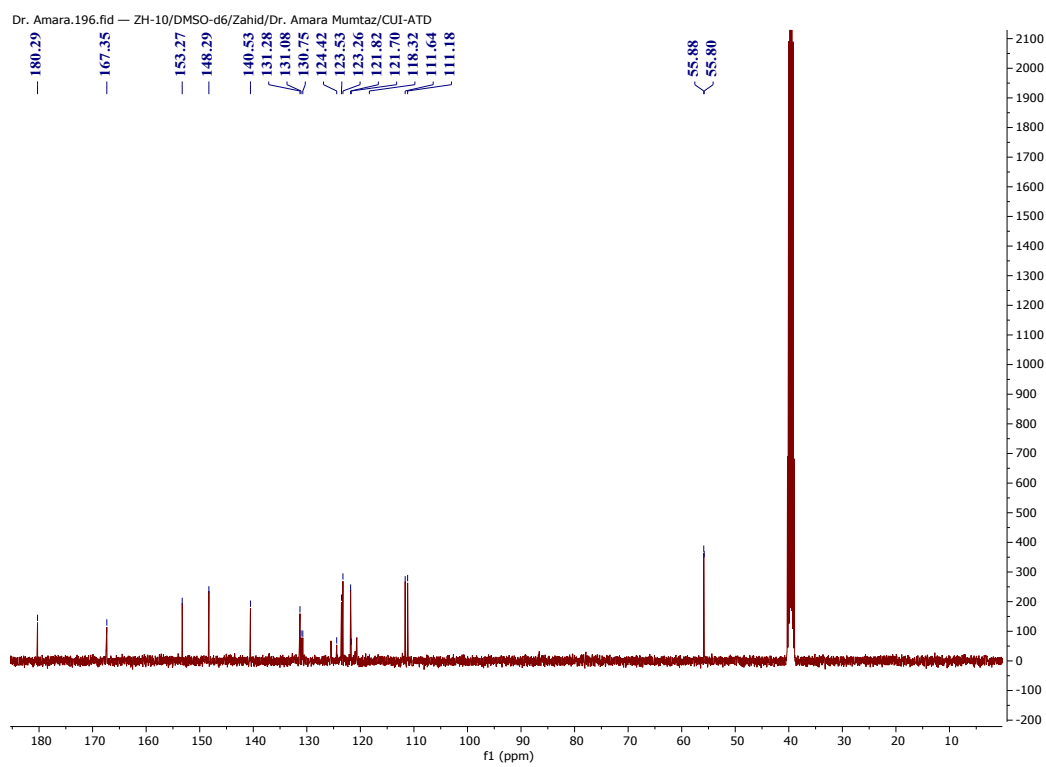


Figure S6: ^{13}C NMR of compound 23

Compound 21

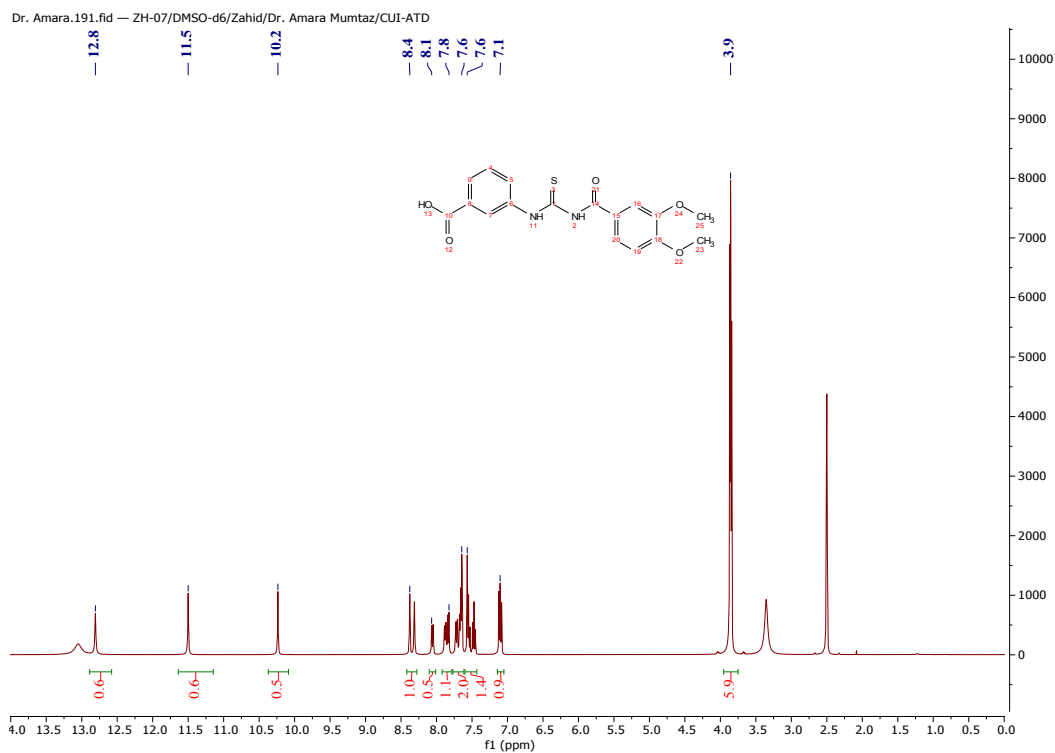


Figure S7: ^1H NMR of compound 21

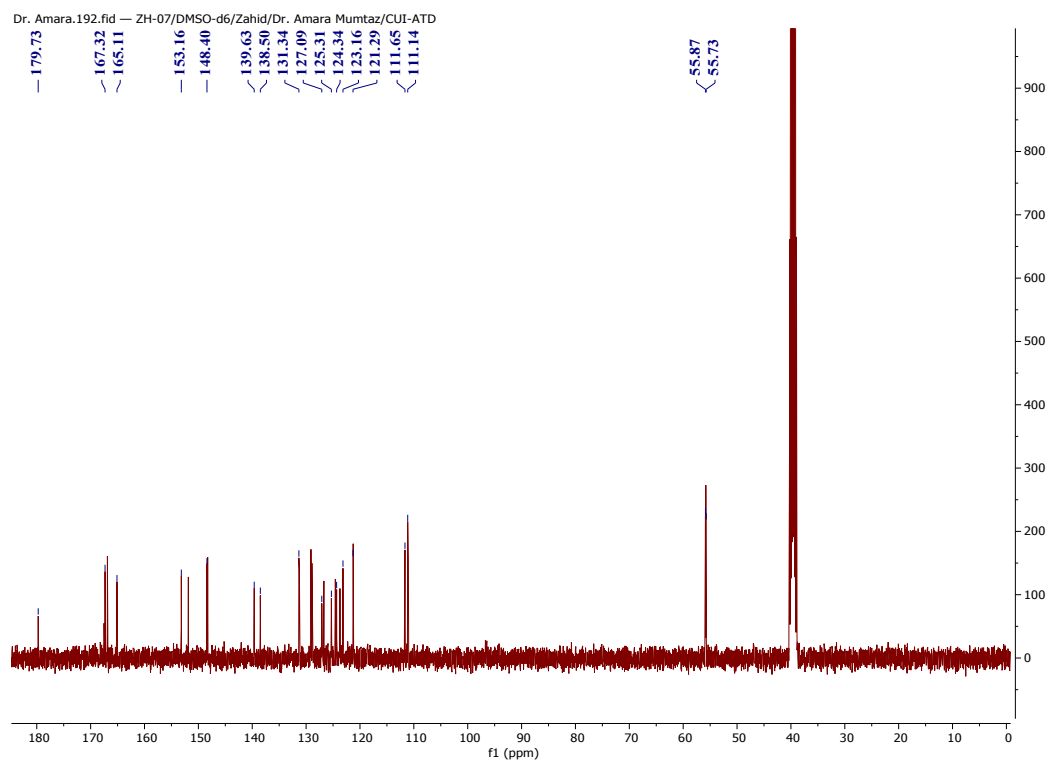


Figure S8: ^{13}C NMR of compound 21

Compound 18

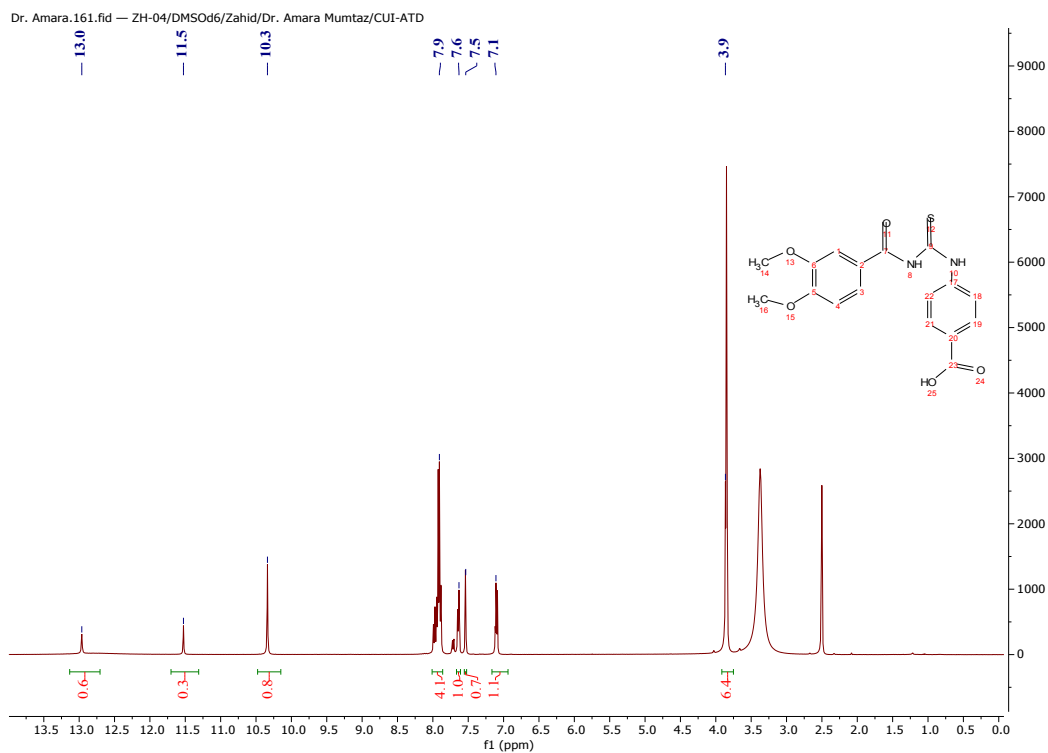


Figure S9: ^1H NMR of compound 18

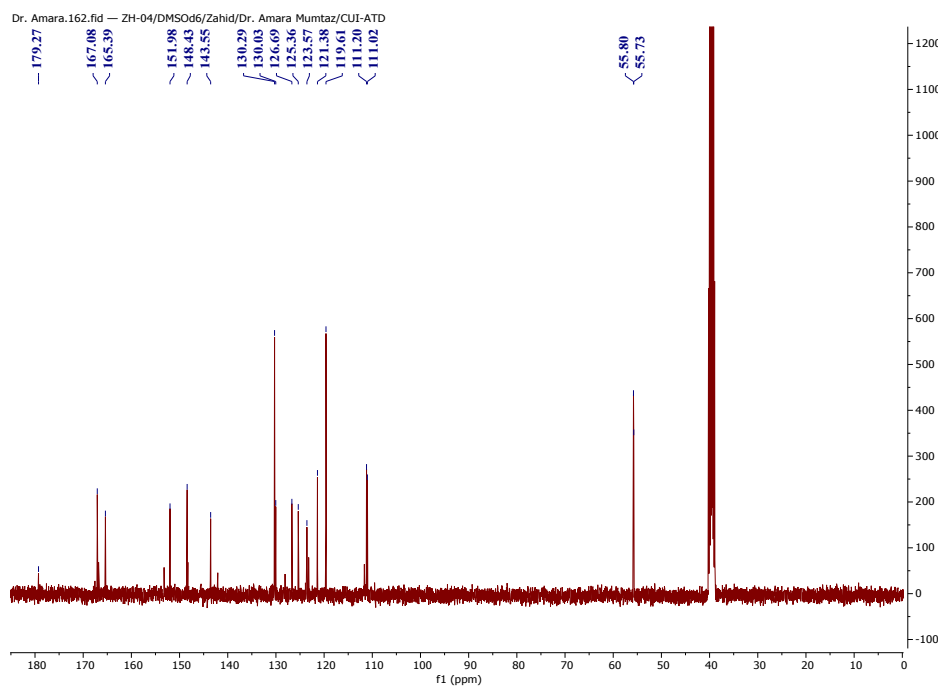


Figure S10: ^{13}C NMR of compound 18

Compound 17

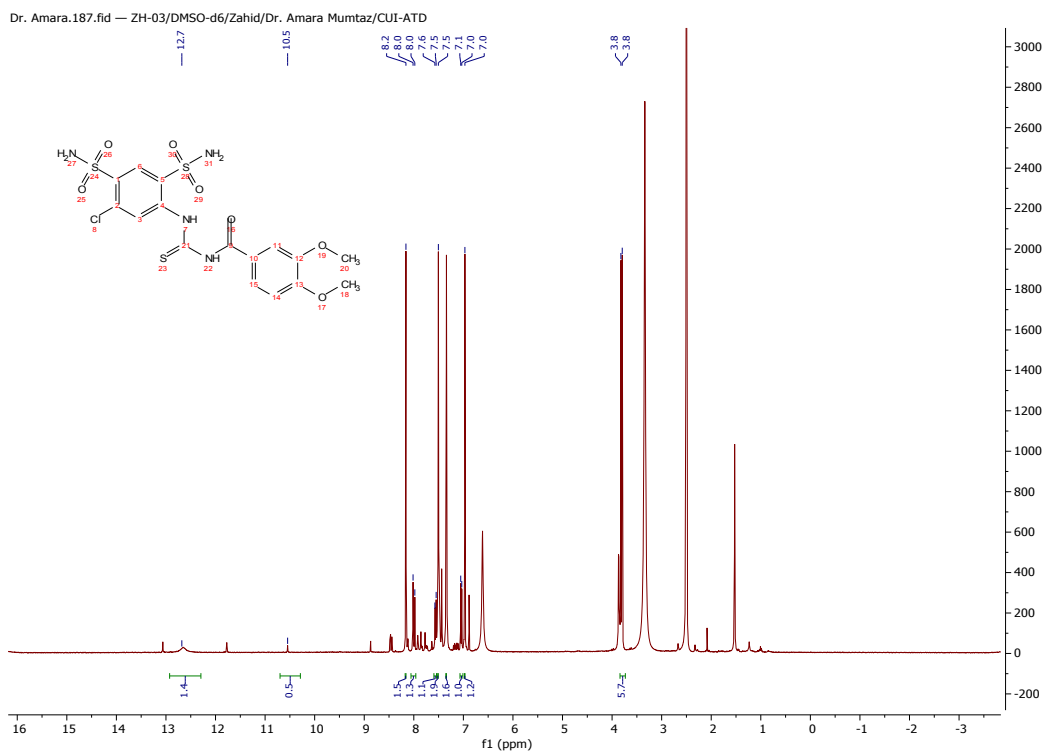


Figure S11: ^1H NMR of compound 17

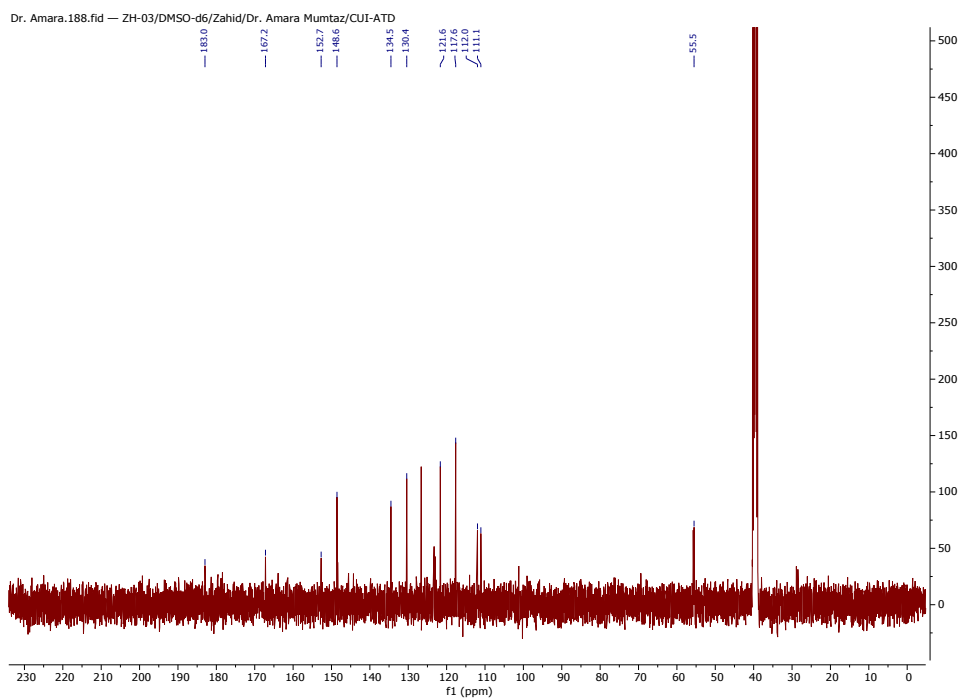


Figure S12: ^{13}C NMR of compound 17

Compound 12

Dr. Amara.157.fid — ZH-01/DMSOd6/Zahid/Dr. Amara Mumtaz CUI-ATD

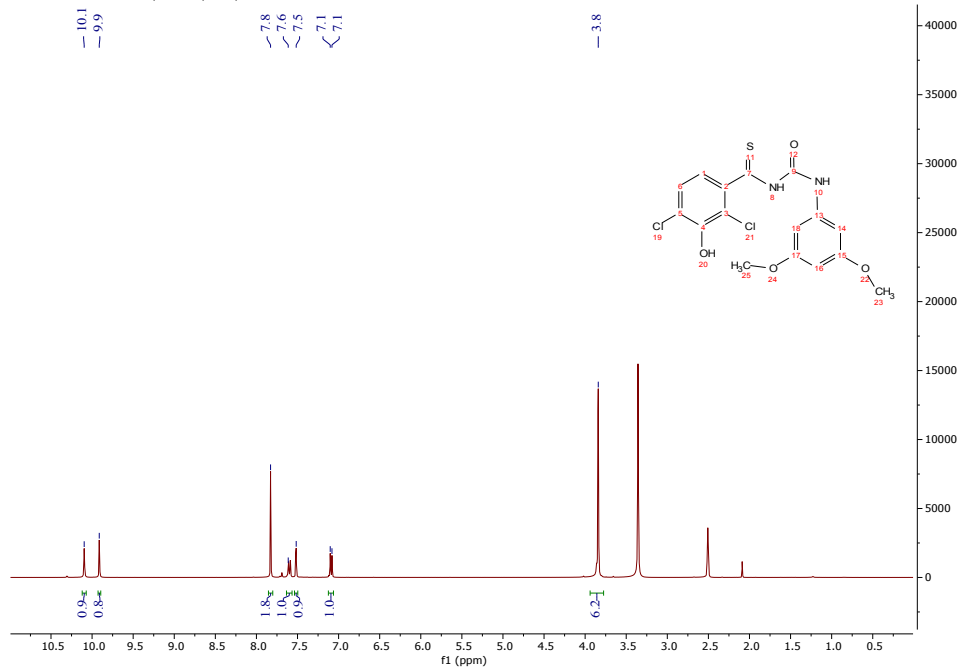


Figure S13: ^1H NMR of compound 12

Dr. Amara.158.fid — ZH-01/DMSOd6/Zahid/Dr. Amara Mumtaz CUI-ATD

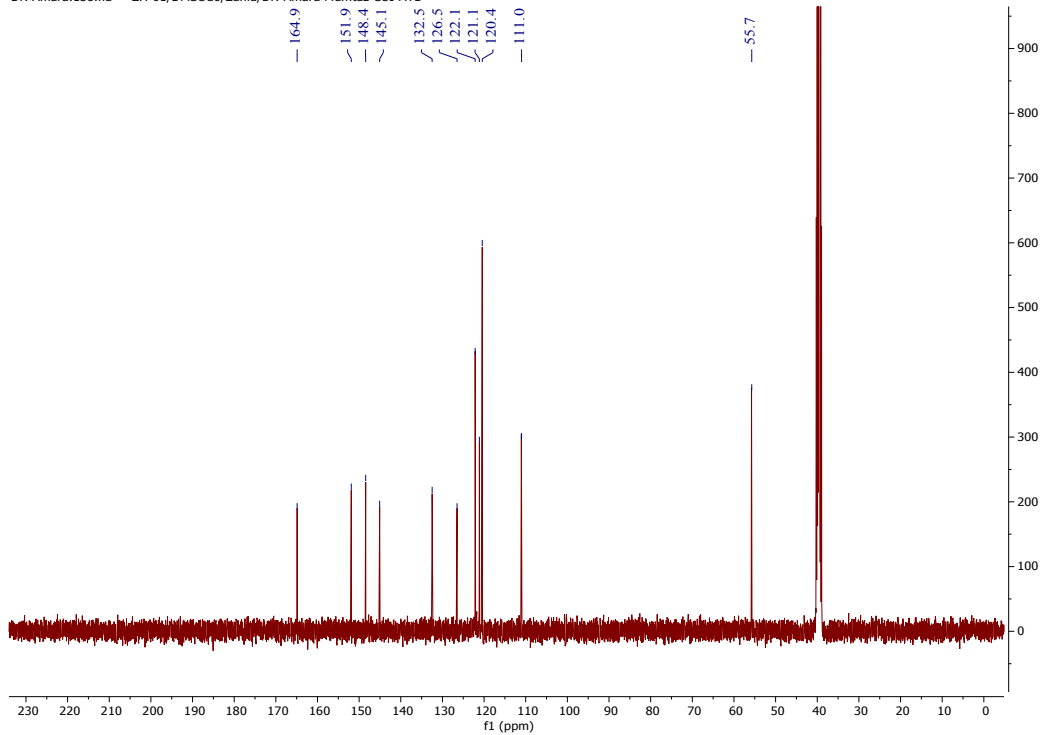


Figure S14: ^{13}C NMR of compound 12

Compound 09

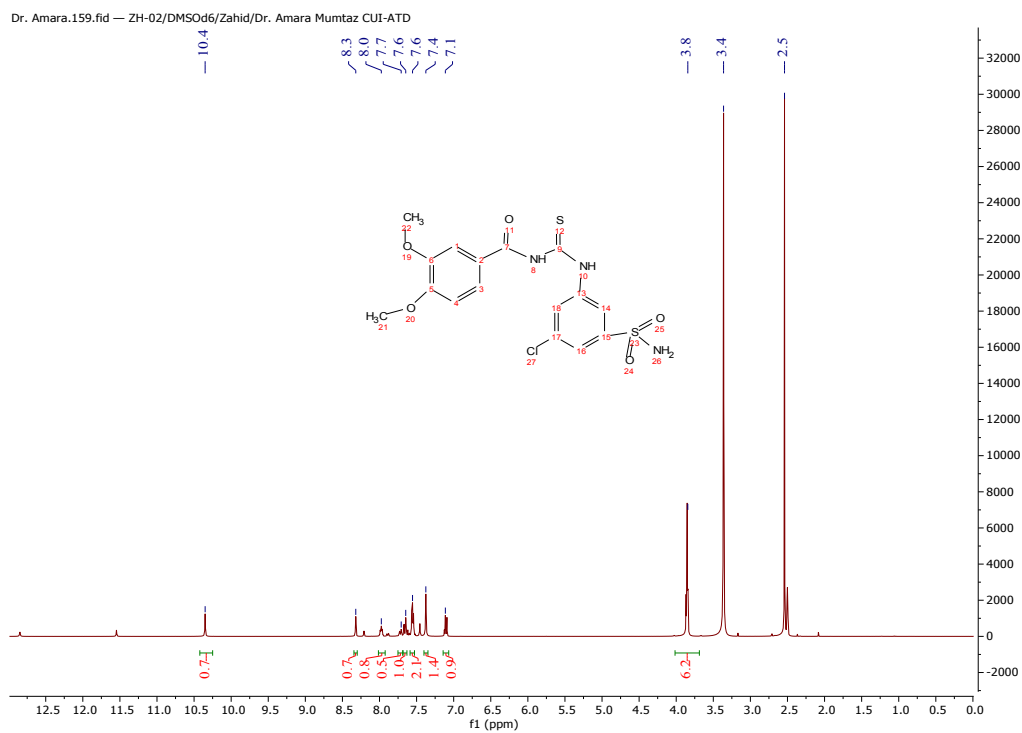


Figure S15: ¹H NMR of compound 09

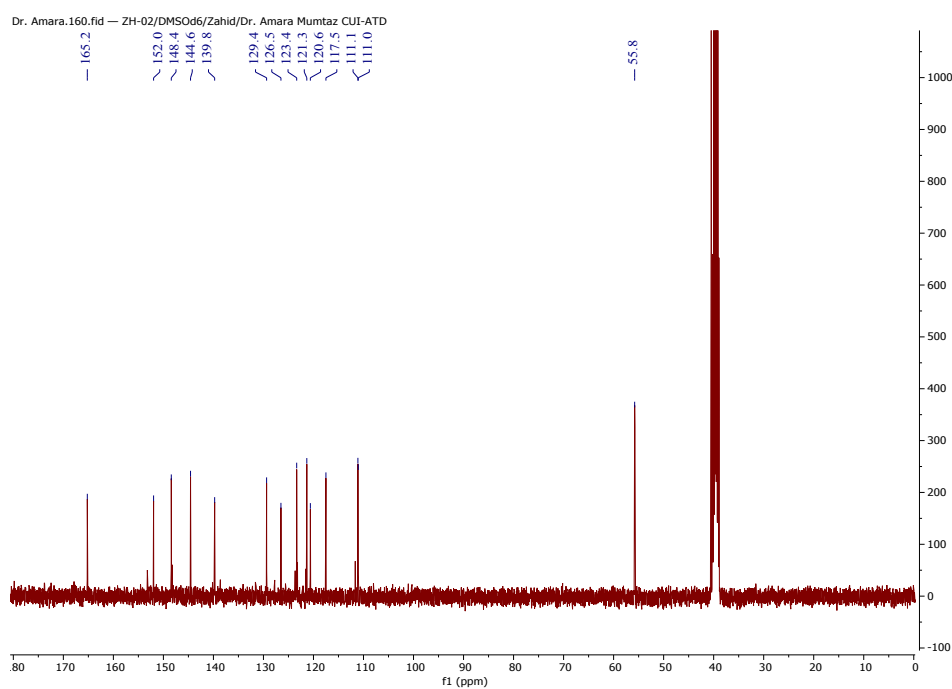


Figure S16: ¹³C NMR of compound 09

Compound 24

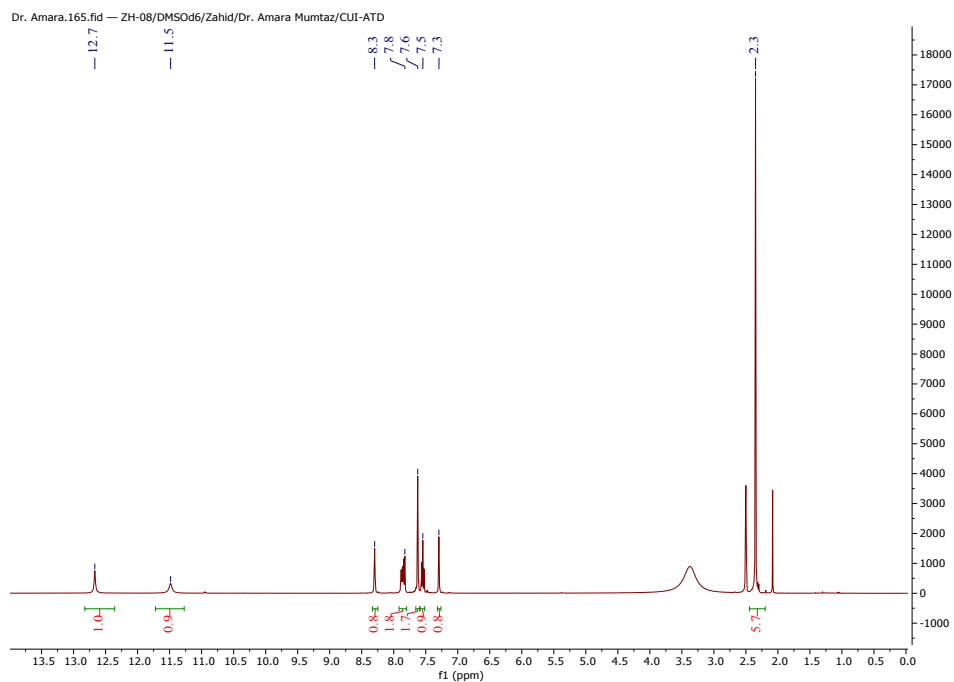


Figure S17: ^1H NMR of compound 24

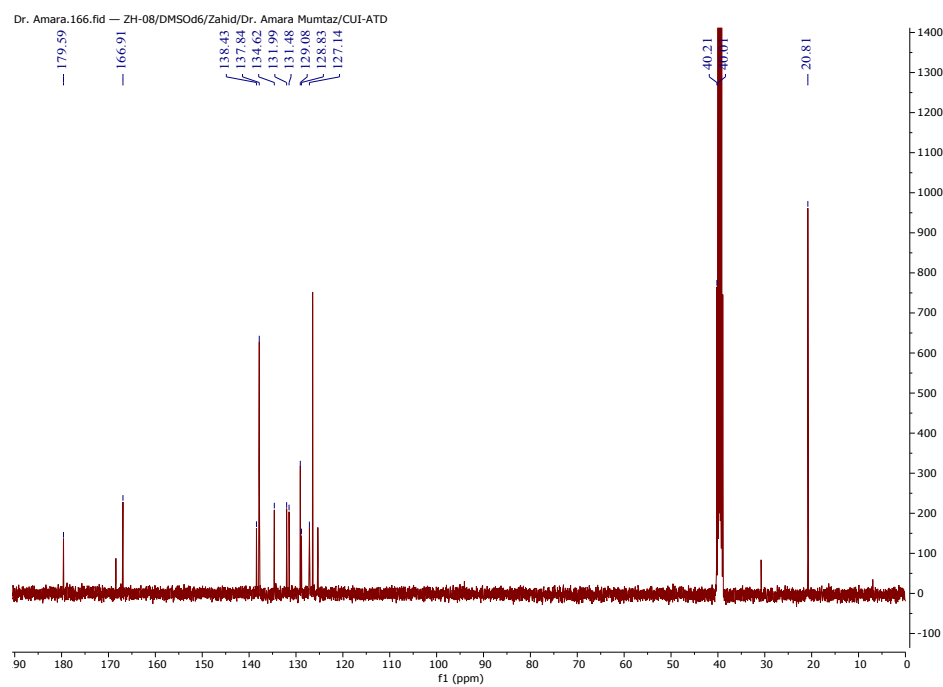


Figure S18: ^{13}C NMR of compound 24

Compound 26

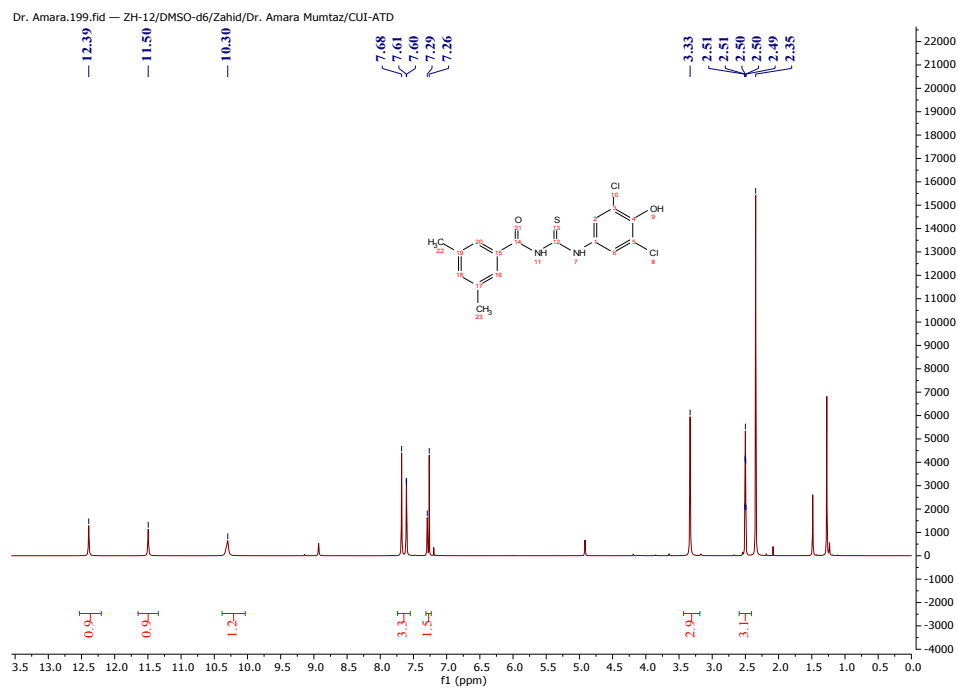


Figure S19: ^1H NMR of compound 26

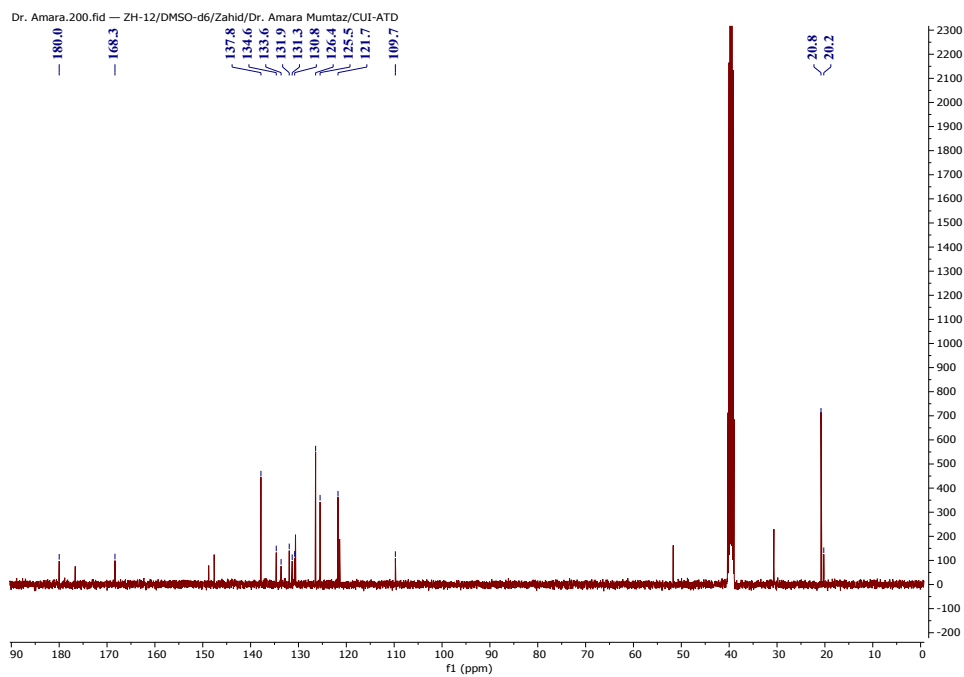


Figure S20: ^{13}C NMR of compound 26

Compound 10

Dr. Amara.203.fid — ZH-14/DMSO-d6/Zahid/Dr. Amara Mumtaz/CUI-ATD

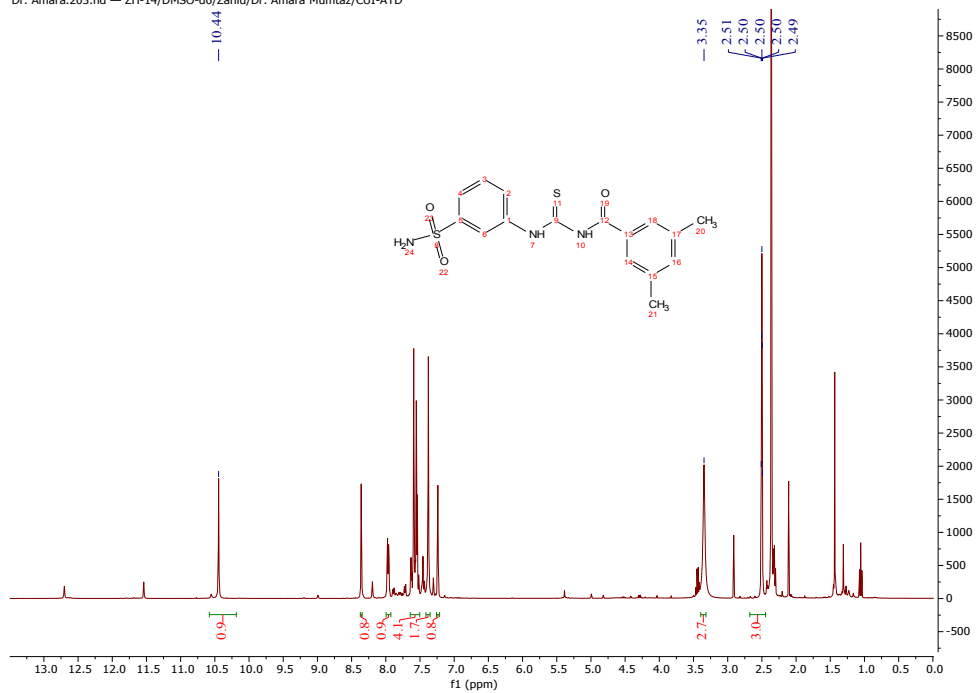


Figure S21: ¹H NMR of compound 10

Dr. Amara.204.fid — ZH-14/DMSO-d6/Zahid/Dr. Amara Mumtaz/CUI-ATD

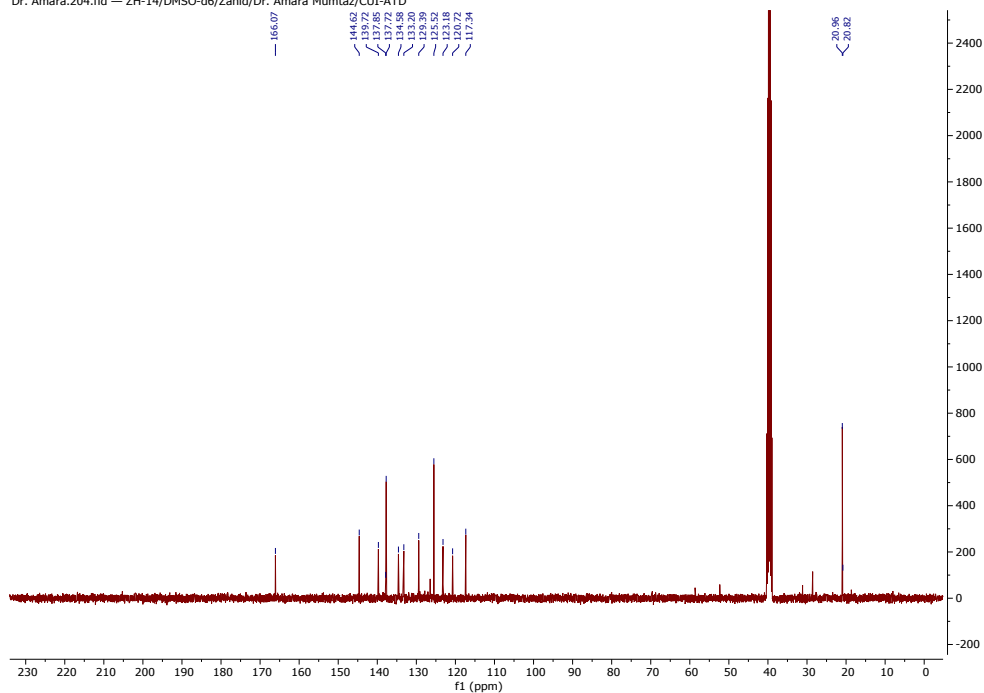


Figure S22: ¹³C NMR of compound 10

Compound 11

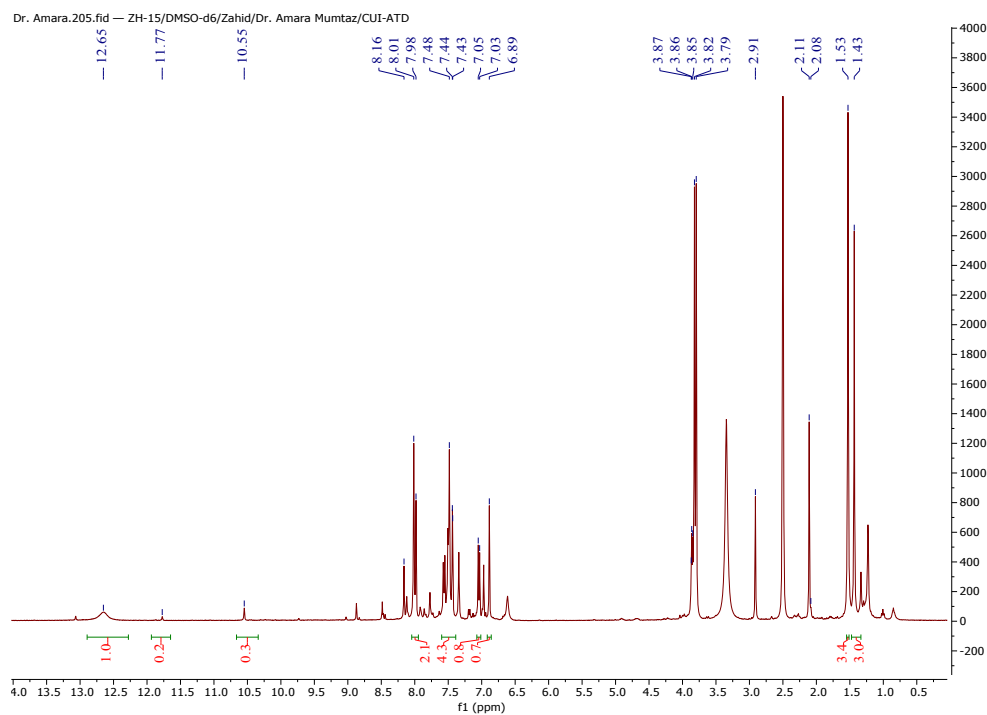


Figure S23: ^1H NMR of compound 11

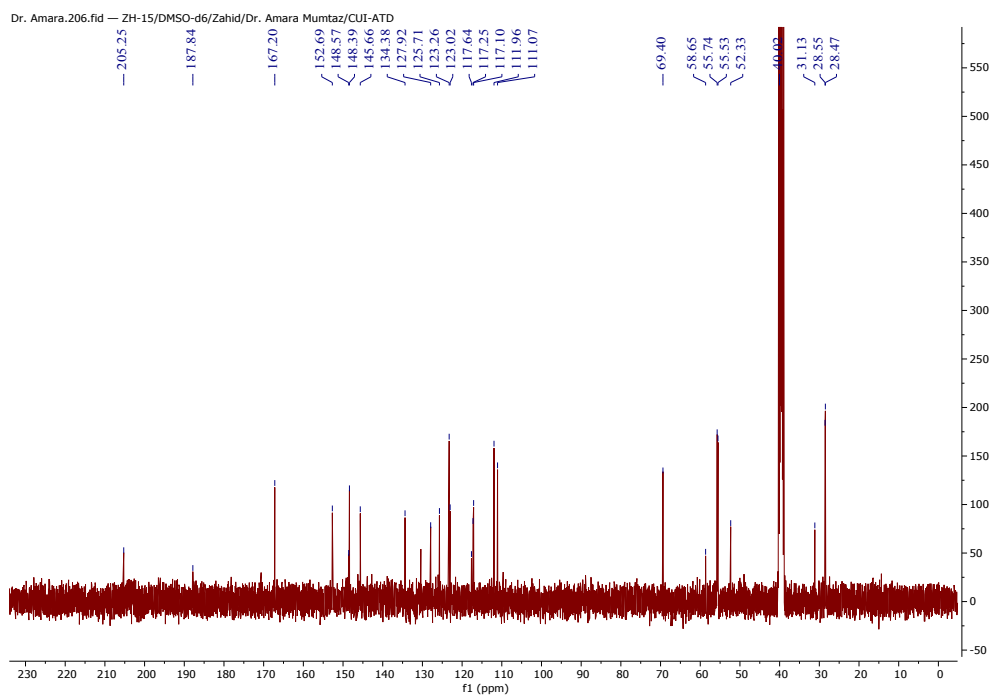


Figure S24: ^{13}C NMR of compound 11

Compound 14

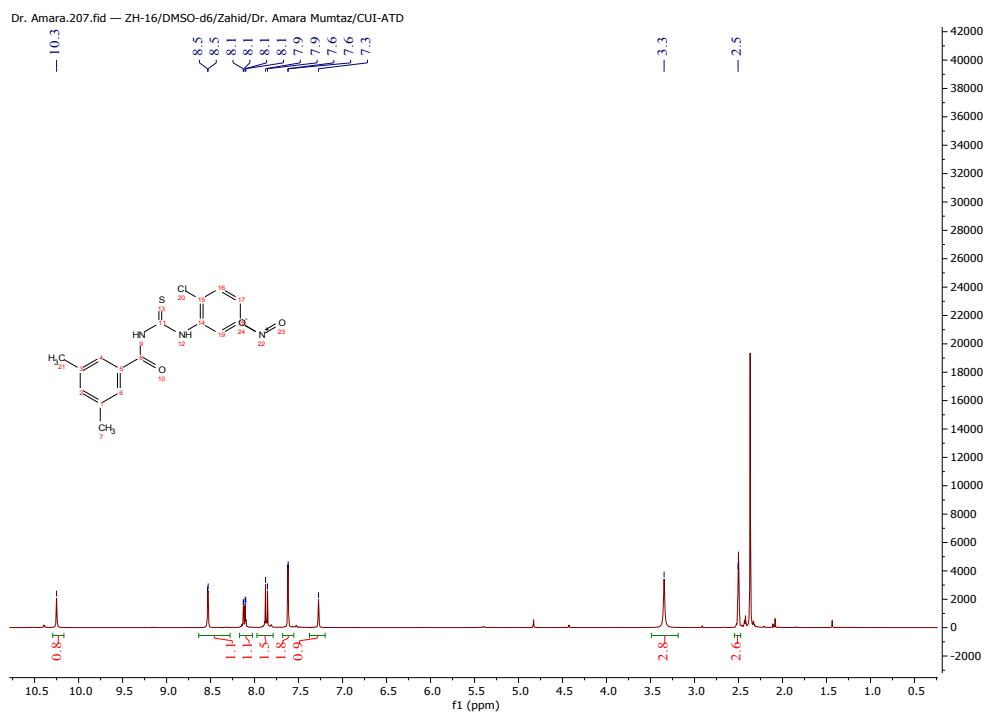


Figure S25: ^1H NMR of compound 14

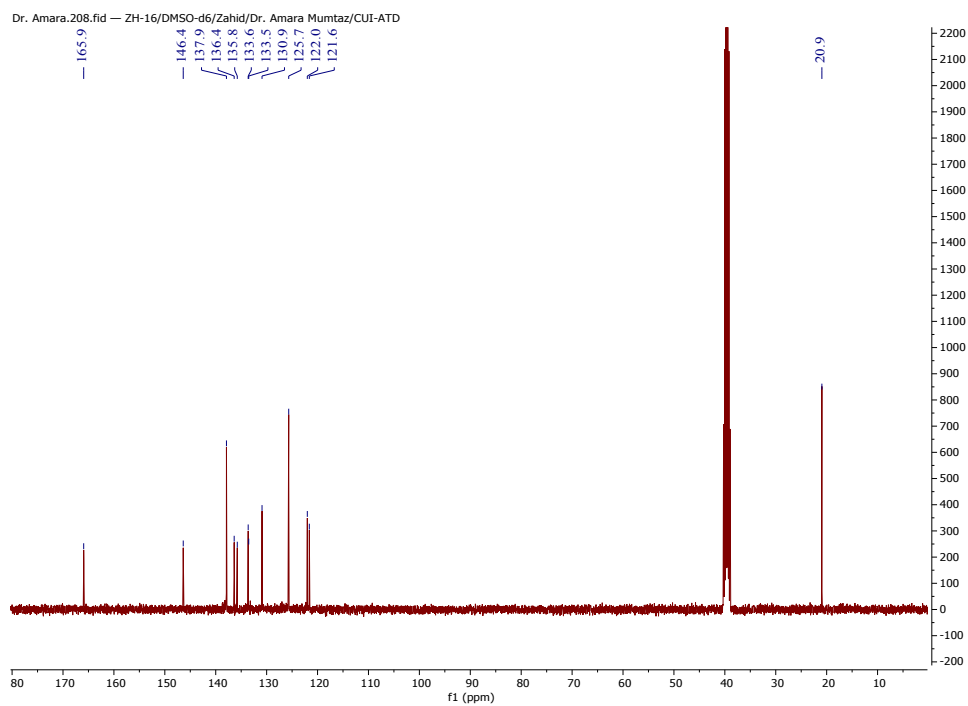


Figure S26: ^{13}C NMR of compound 14

Compound 15

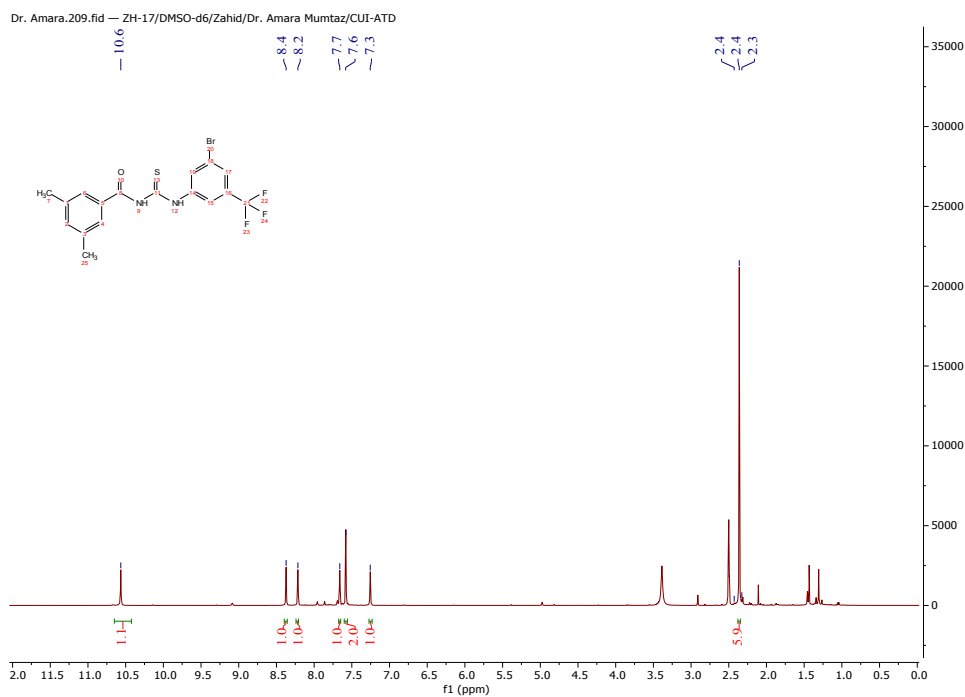


Figure S27: ¹H NMR of compound 15

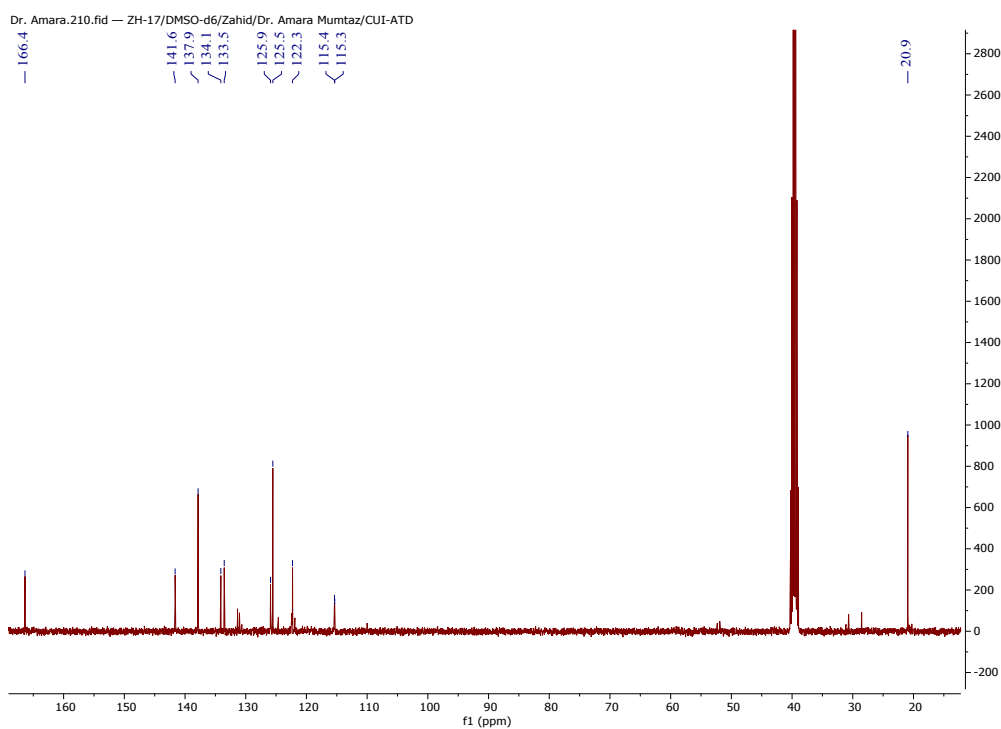


Figure S28: ¹³C NMR of compound 15

Compound 25

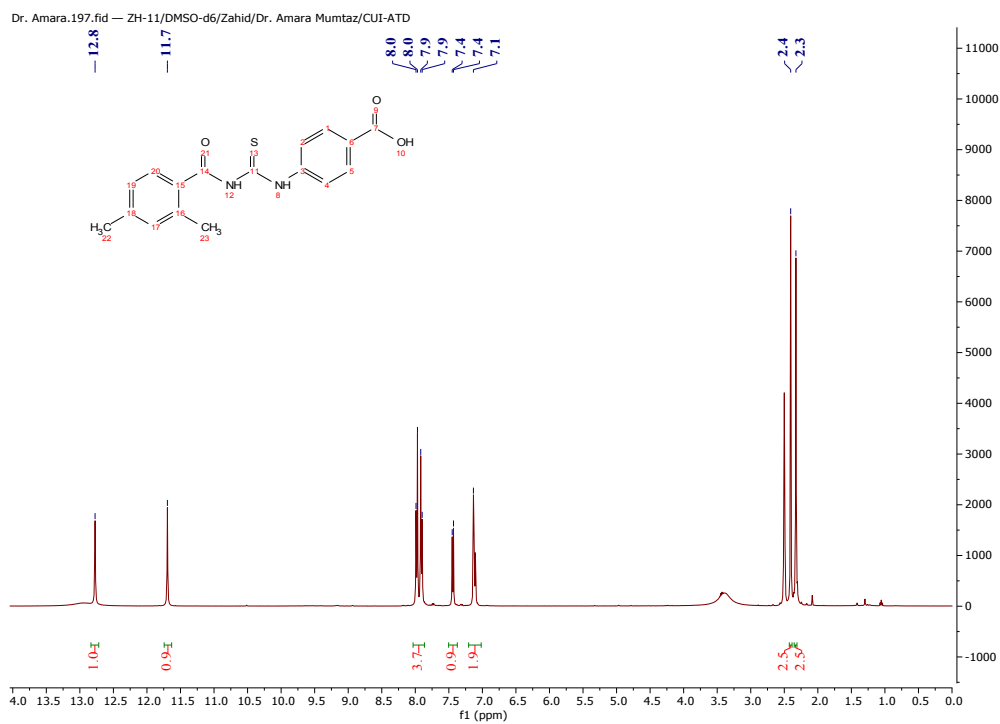


Figure S29: ^1H NMR of compound 25

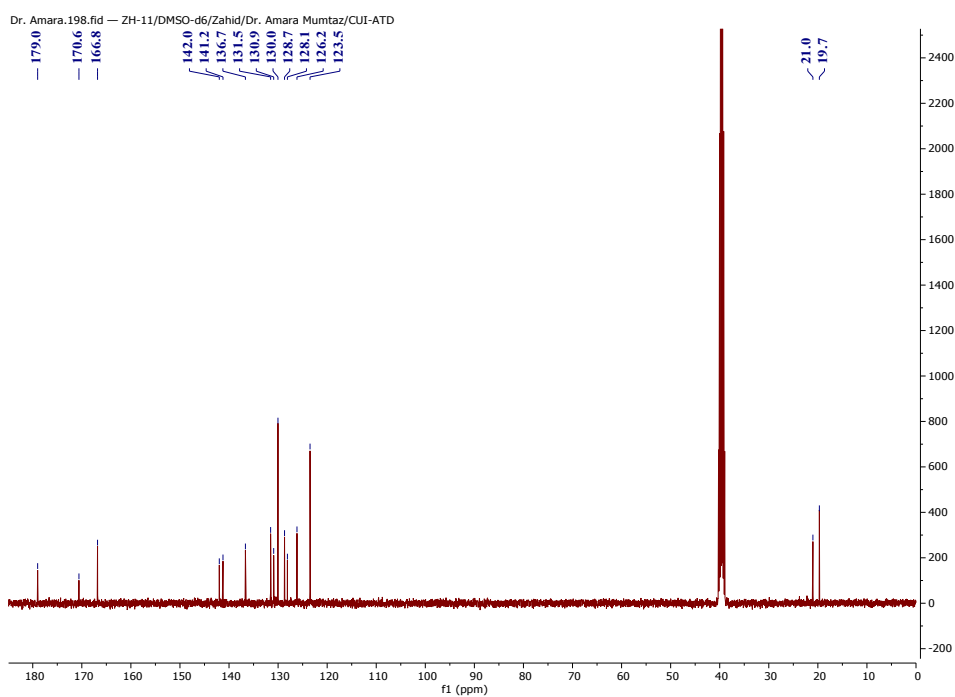


Figure S30: ^{13}C NMR of compound 25

Compound 13

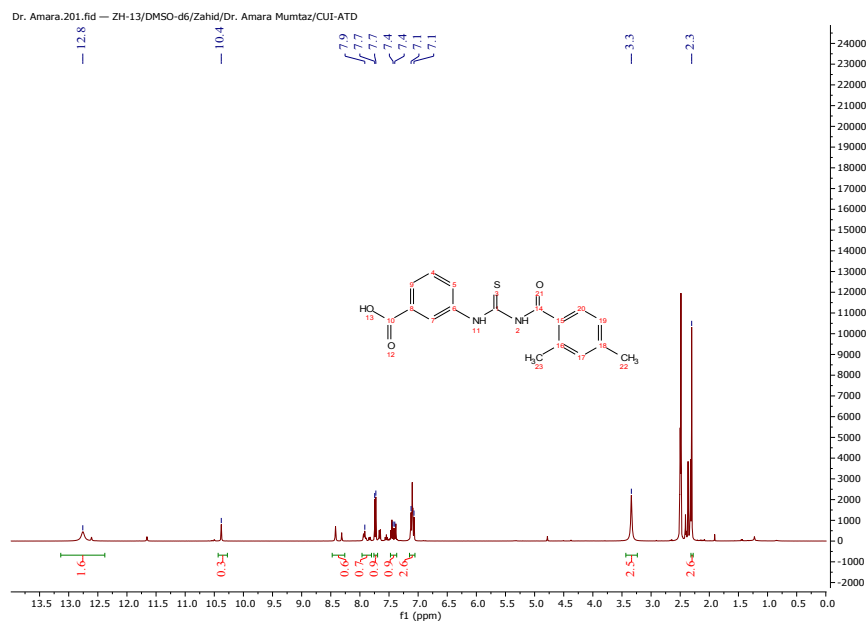


Figure S31: ^1H NMR of compound 13

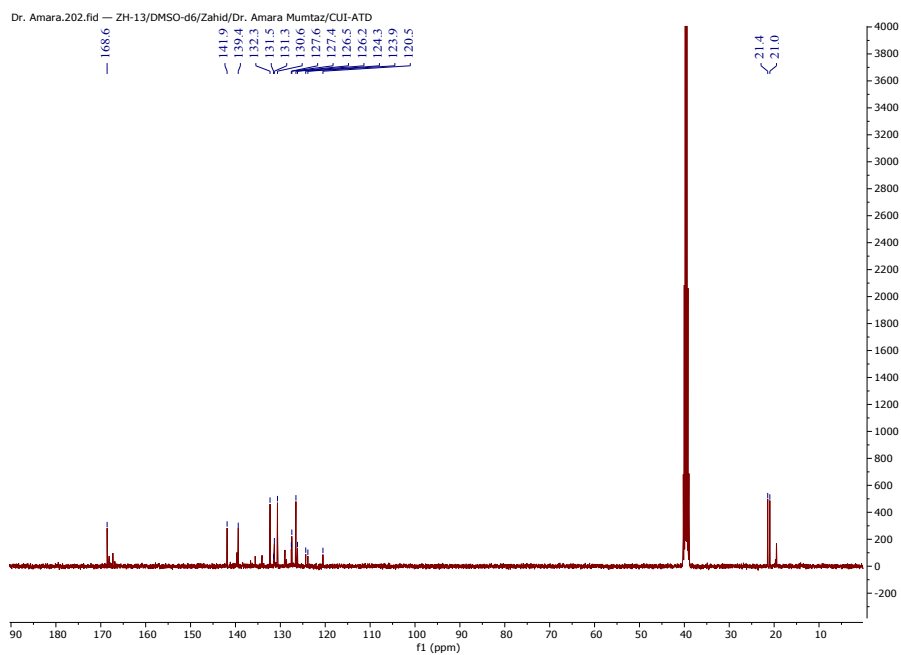


Figure S32: ^{13}C NMR of compound 13

2 Supporting IR spectra of the synthesized compounds

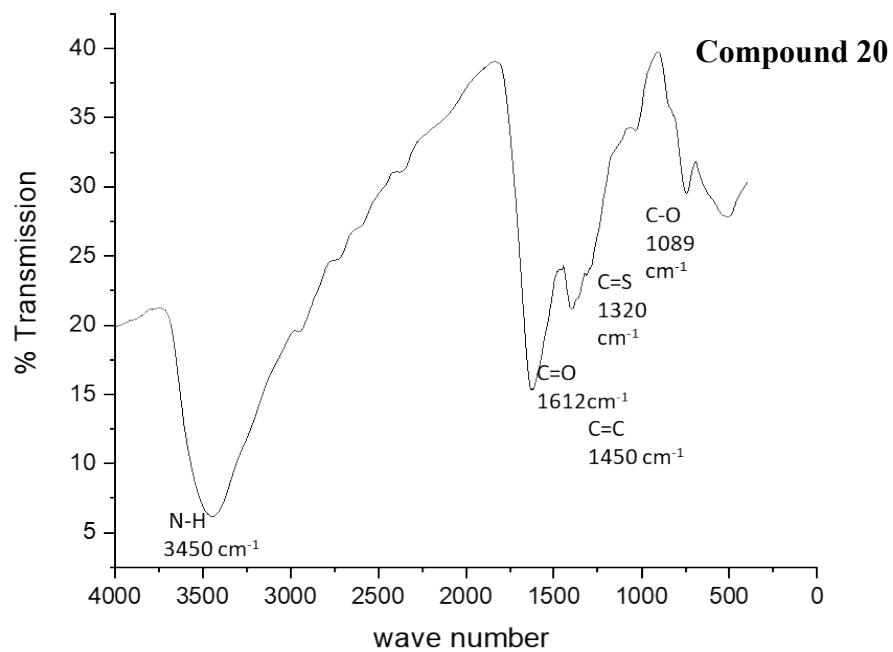


Figure S33: IR graph of Compound 20

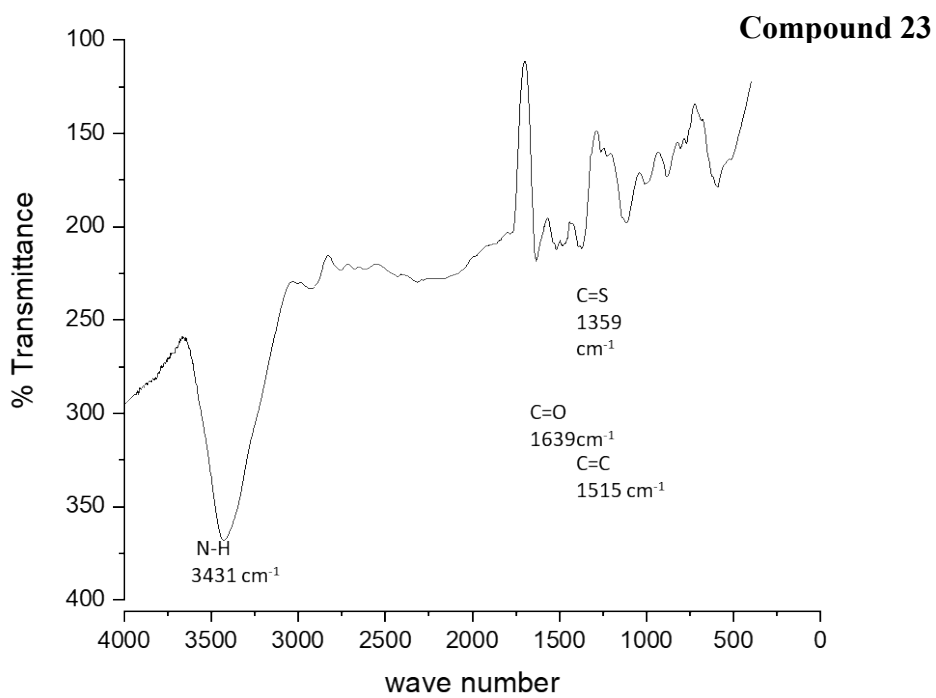


Figure S34: IR graph of Compound 23

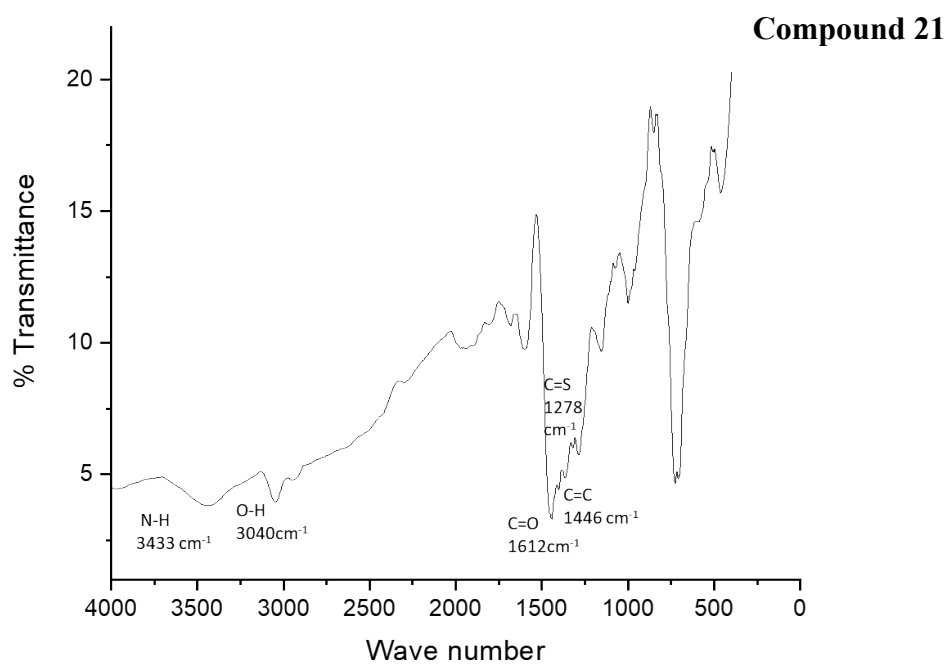


Figure S35: IR graph of Compound 21

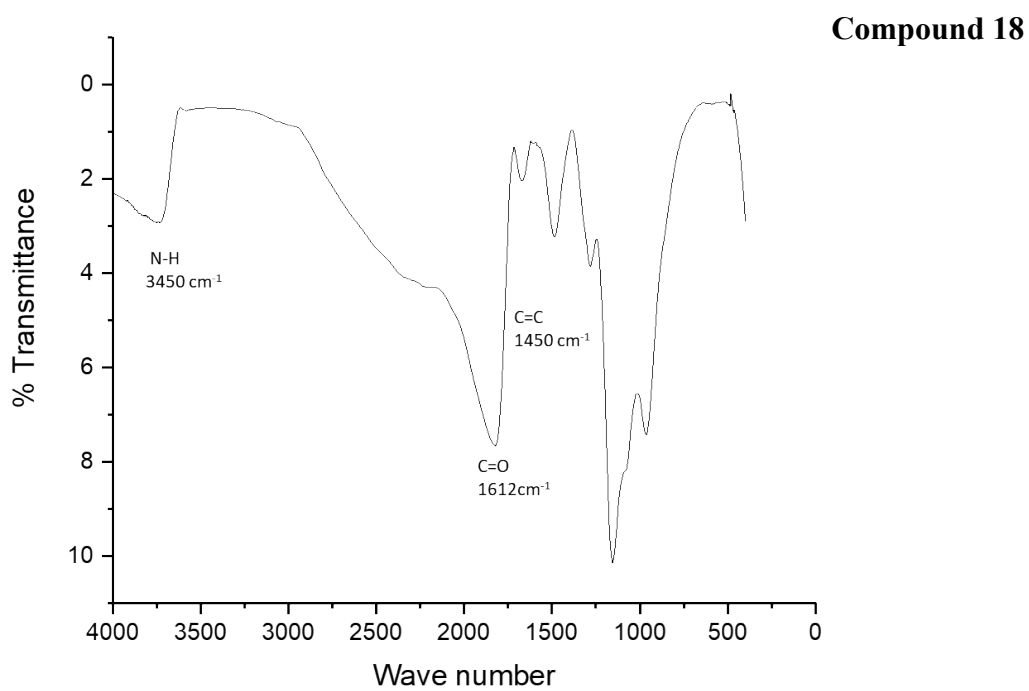


Figure S36: IR graph of Compound 18

Compound 17

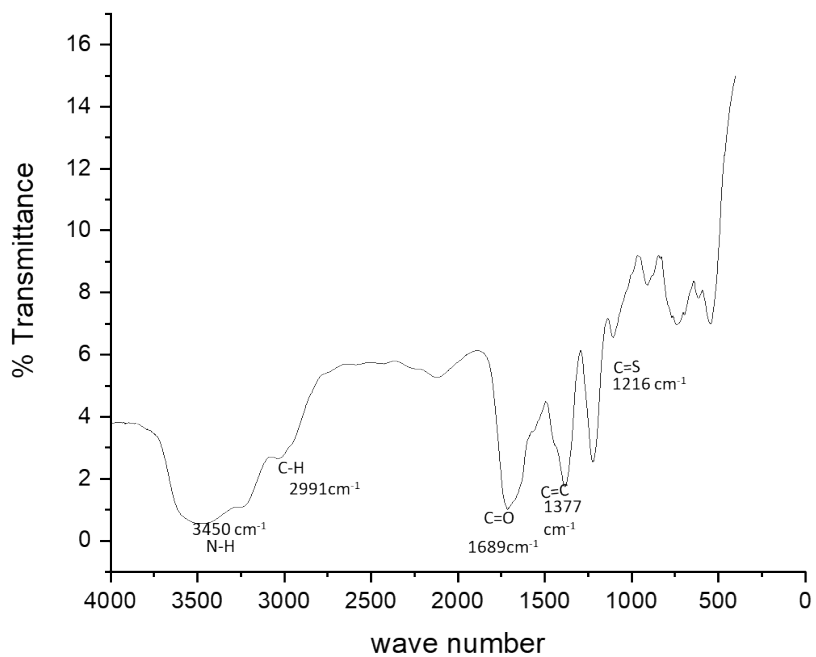


Figure S37: IR graph of Compound 17

Compound 12

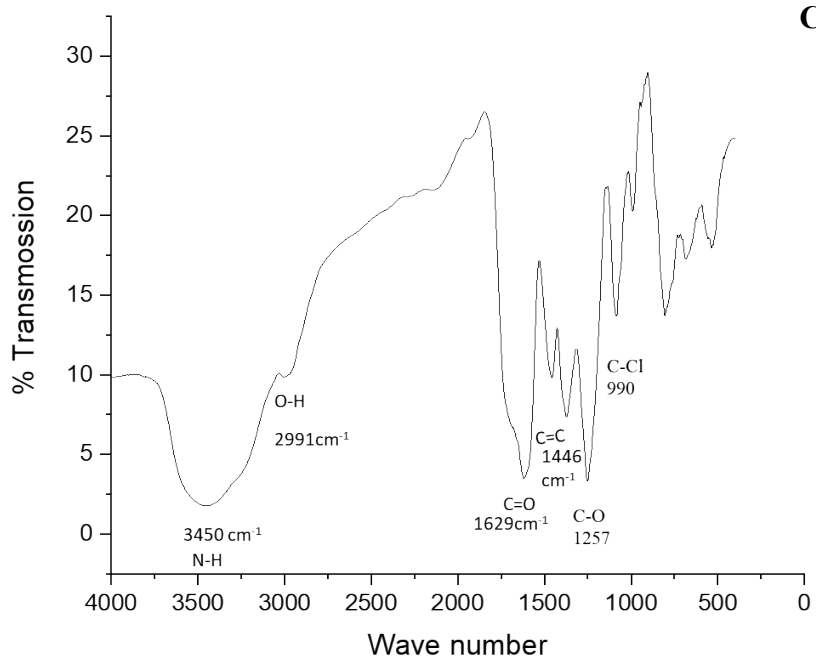


Figure S38: IR graph of Compound 12

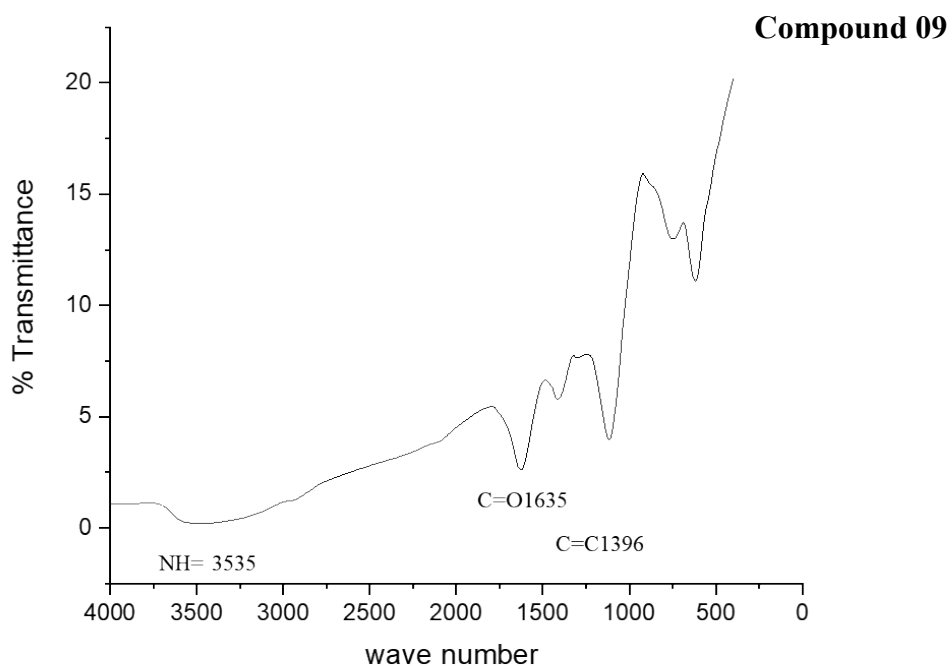


Figure S39: IR graph of Compound 20

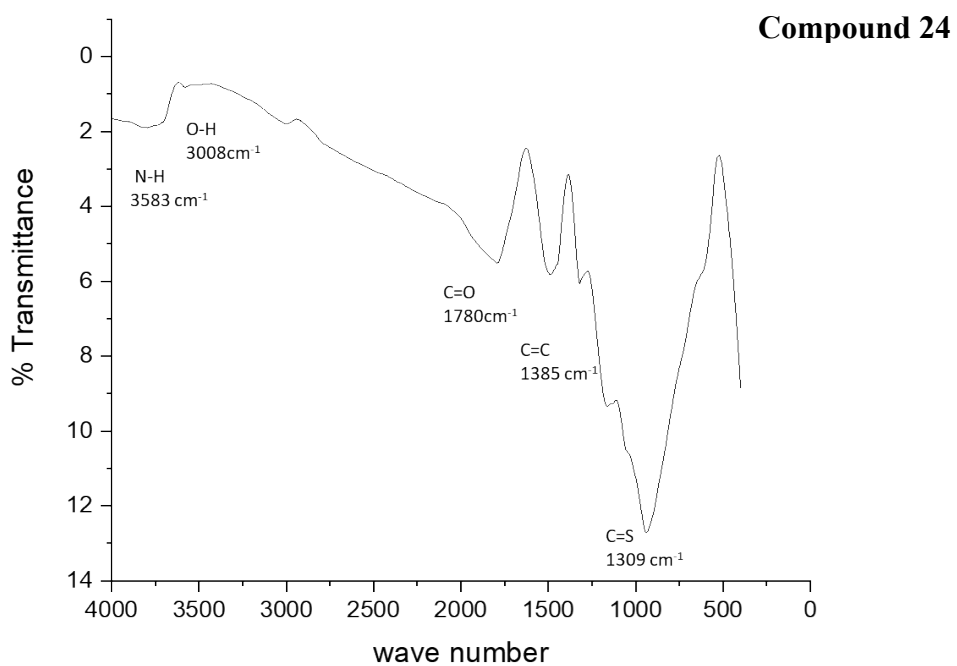


Figure S40: IR graph of Compound 24

Compound 26

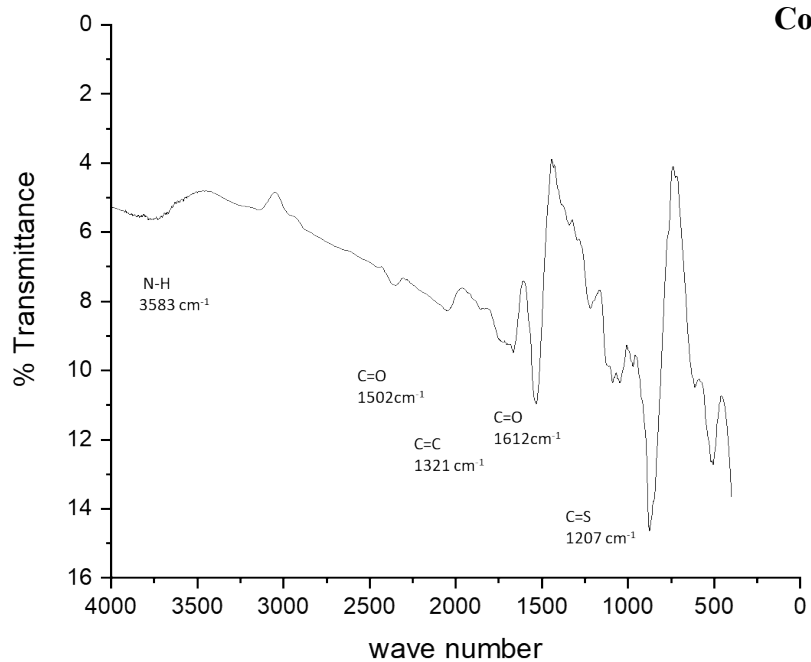


Figure S41: IR graph of Compound 26

Compound 10

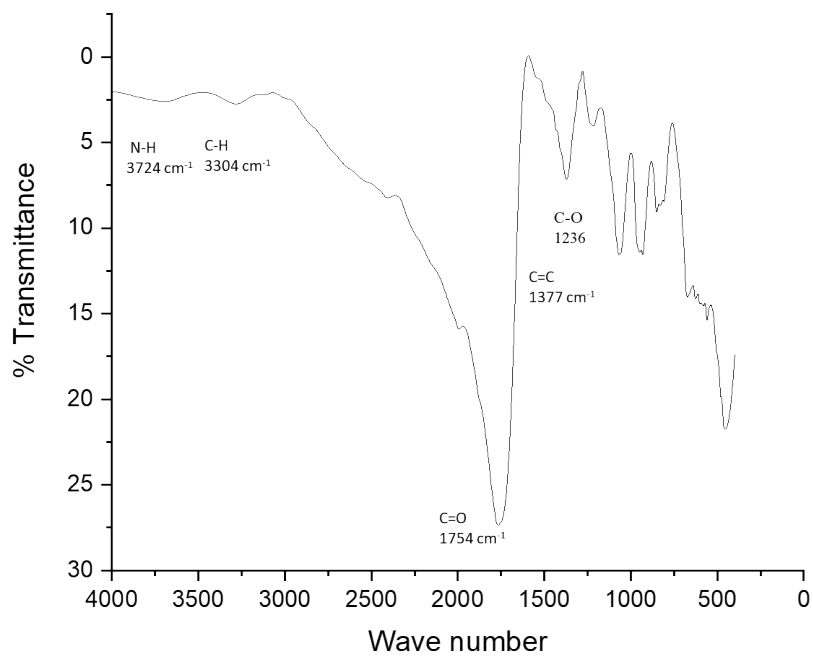


Figure S42: IR graph of Compound 10

Compound 11

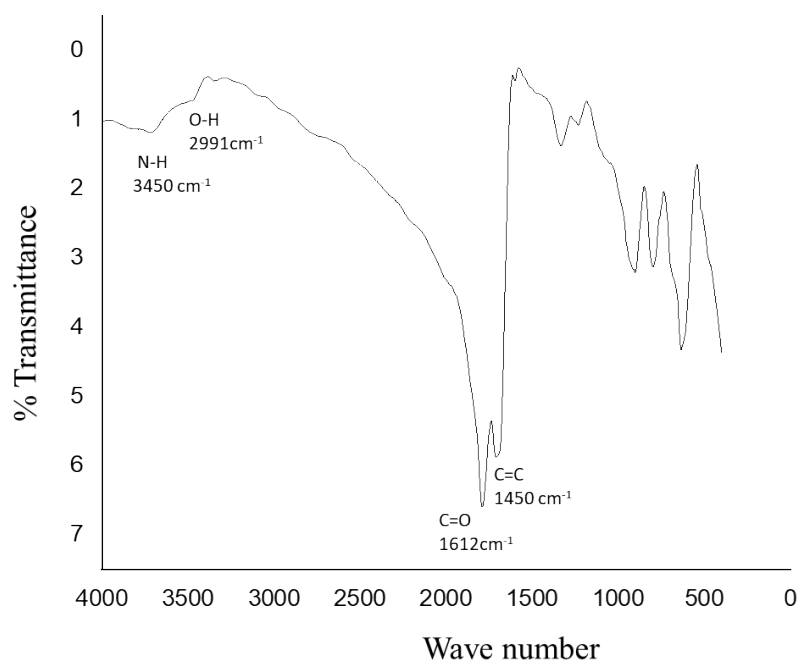


Figure S43: IR graph of Compound 11

Compound 14

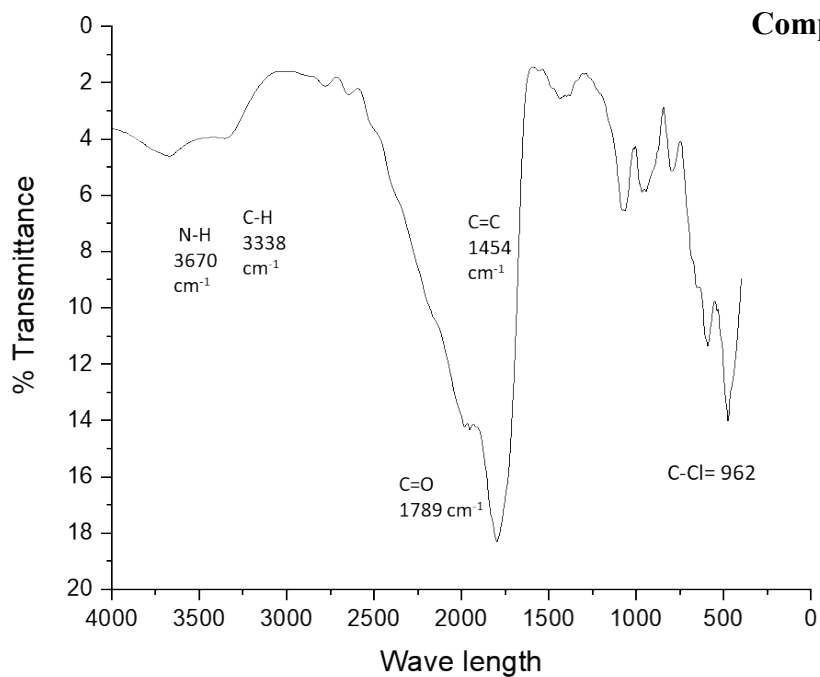


Figure S44: IR graph of Compound 14

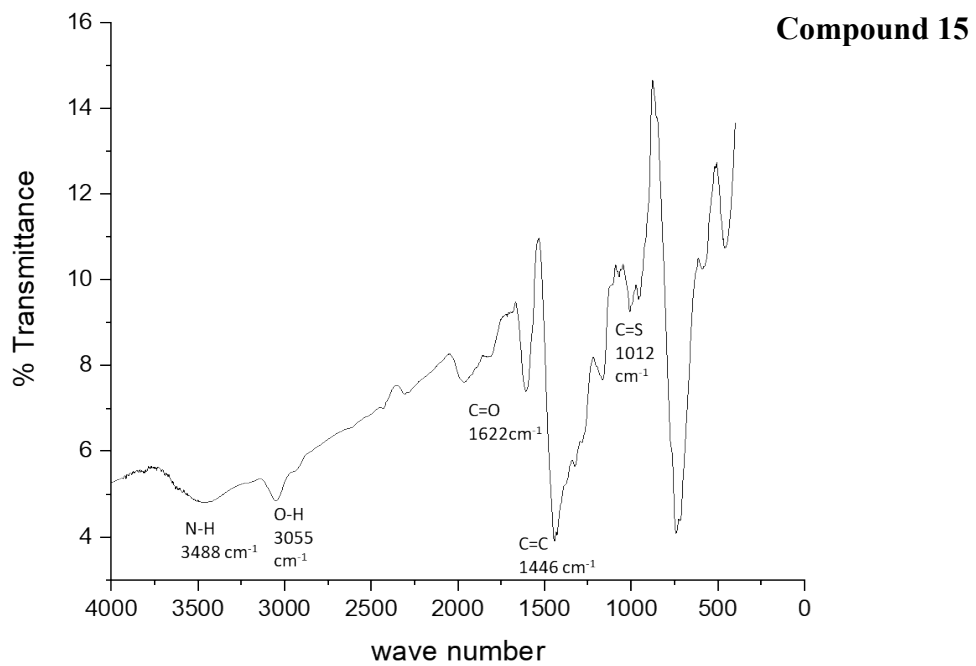


Figure S45: IR graph of Compound 15

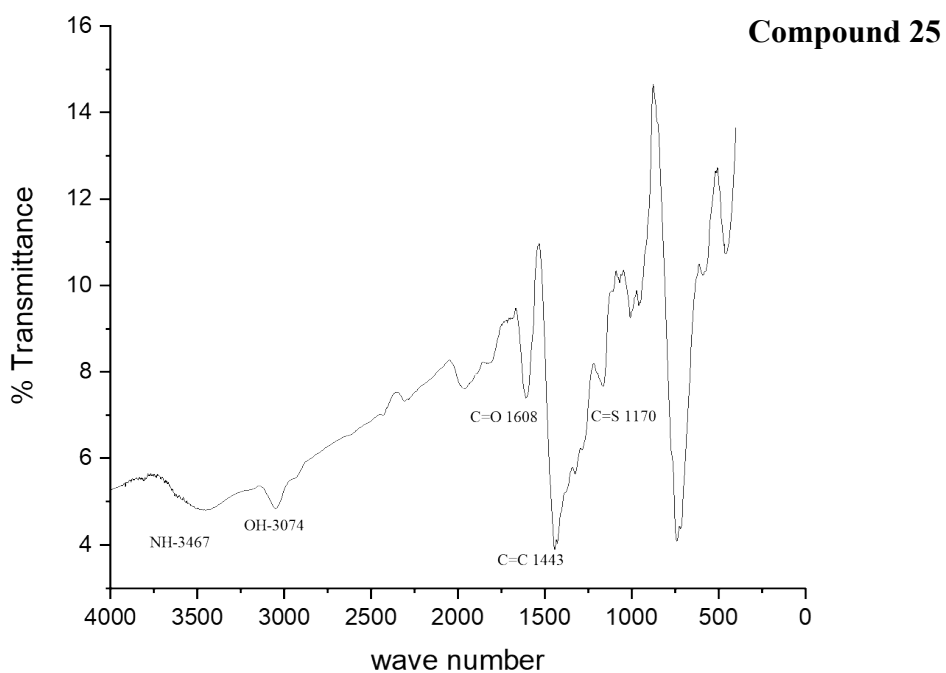


Figure S46: IR graph of Compound 25

Compound 13

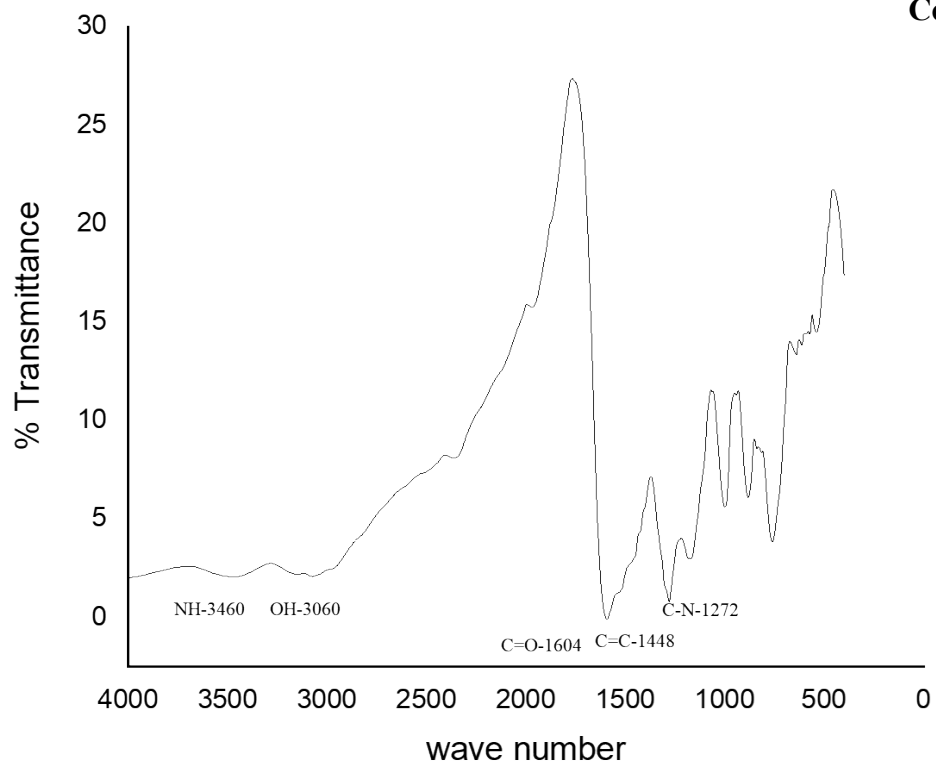
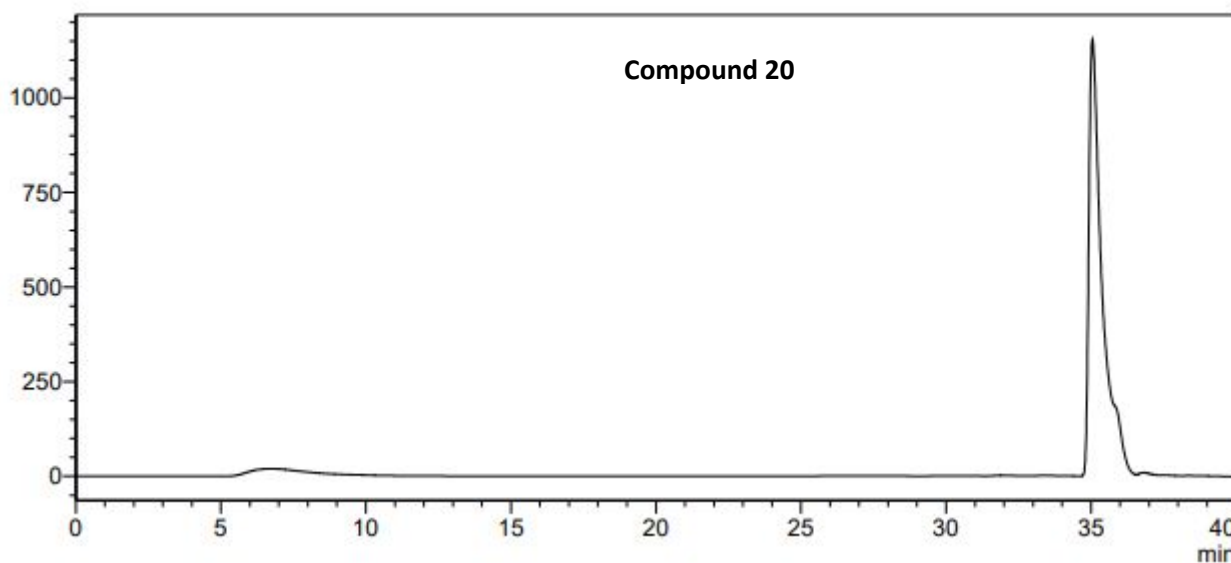


Figure S47: IR graph of Compound 20

HPLC Chromatogram

mV



<Chromatogram>

mV

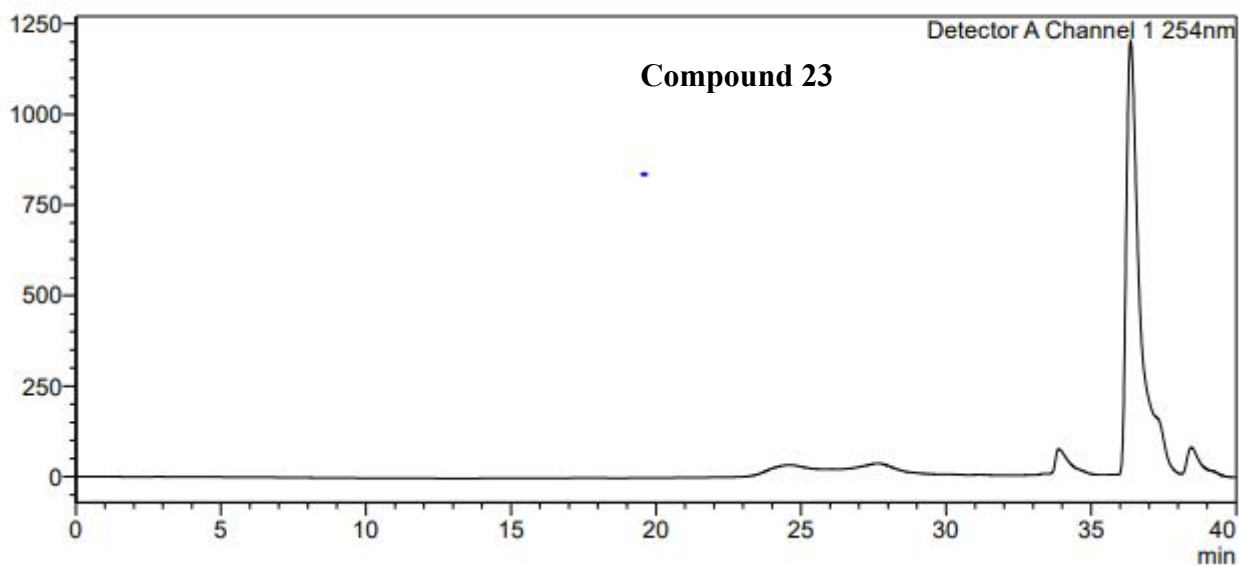
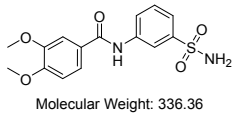
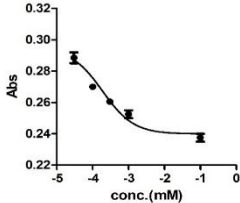
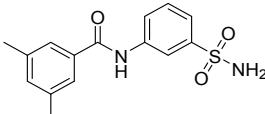
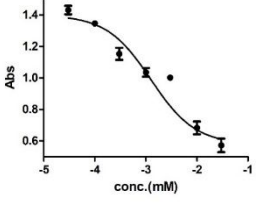
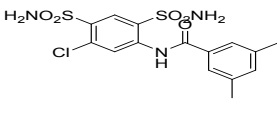
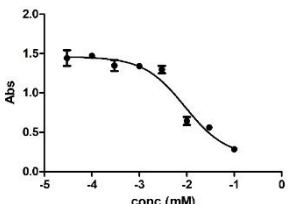
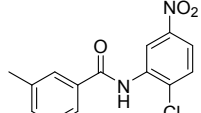
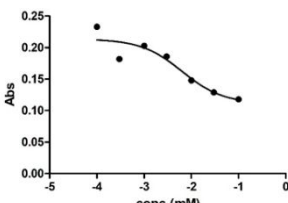
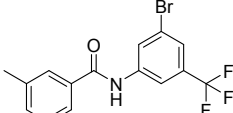
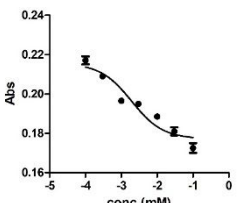
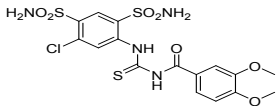
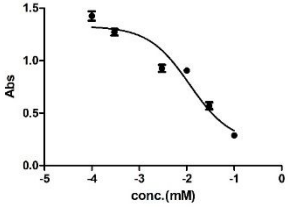
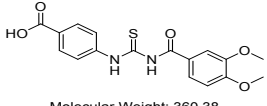
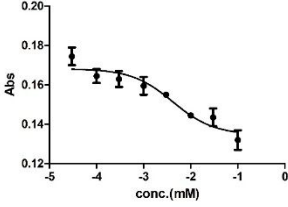
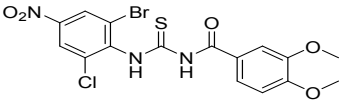
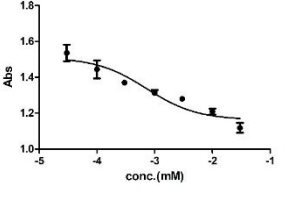
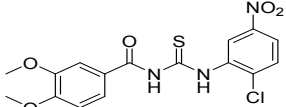
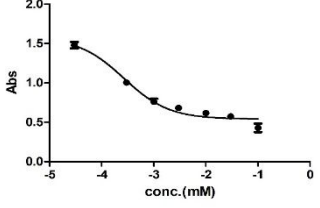
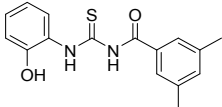
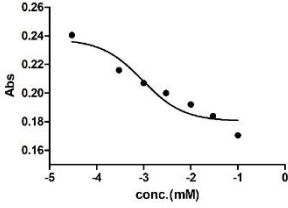
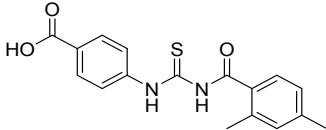
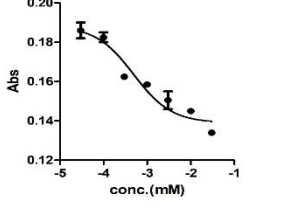


Figure S48: HPLC chromatogram of compound 20 and 23

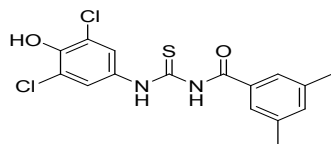
3 *In-vitro* studies of the synthesized compounds and their IC₅₀ values.

Table S1: IC₅₀ values and Graphs of hCA II

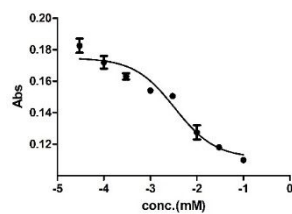
h-Carbonic Anhydrase-II (hCA-II)			
Compounds	Structure	Graph	IC ₅₀
09	 <p>Molecular Weight: 336.36</p>		0.18 ± 0.05
10	 <p>Molecular Weight: 304.36</p>		0.93 ± 0.05
11	 <p>Molecular Weight: 244.66</p>		1.18 ± 0.14
14	 <p>Molecular Weight: 304.73</p>		6.49 ± 0.63
15	 <p>Molecular Weight: 372.19</p>		1.93 ± 0.15

17	 <p>Molecular Weight: 508.96</p>		11.51 ± 0.16
18	 <p>Molecular Weight: 360.38</p>		4.17 ± 0.29
19	 <p>Molecular Weight: 474.71</p>		0.72 ± 0.06
22	 <p>Molecular Weight: 395.81</p>		0.26 ± 0.03
24	 <p>Molecular Weight: 300.38</p>		1.24 ± 0.96
25	 <p>Molecular Weight: 328.39</p>		0.38 ± 0.01

26

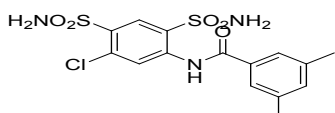
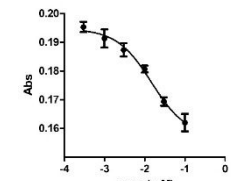
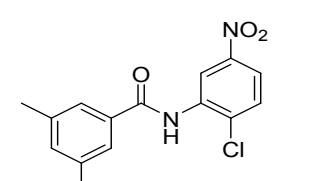
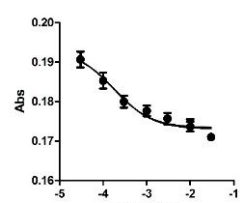
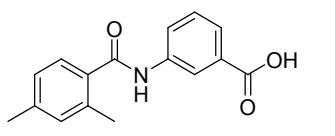
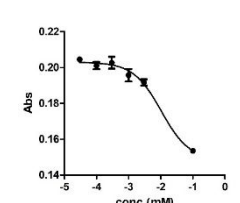
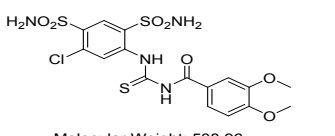
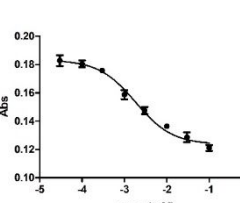
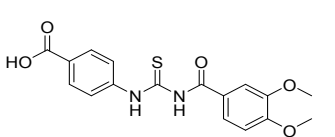
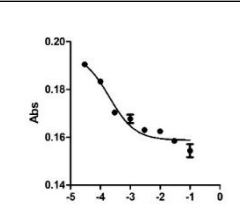
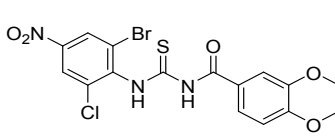
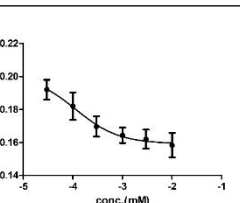
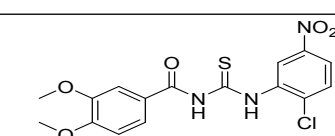
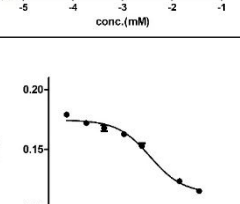


Molecular Weight: 369.26



4.45 ± 0.11

Table S2: IC₅₀ vales and Graphs of hCA IX

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

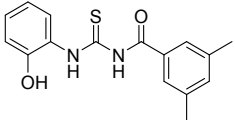
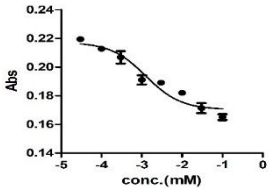
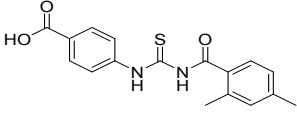
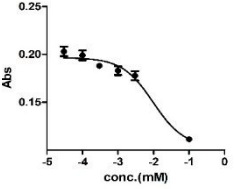
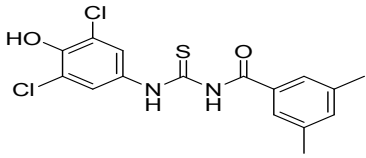
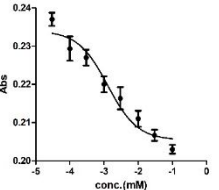
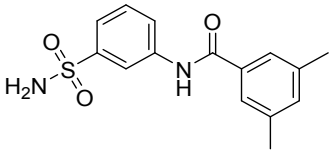
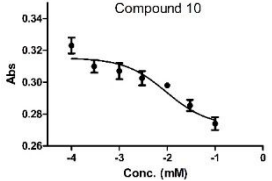
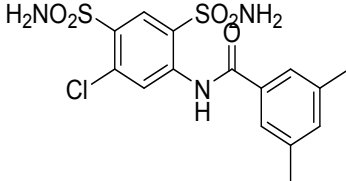
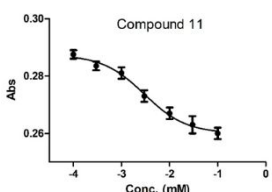
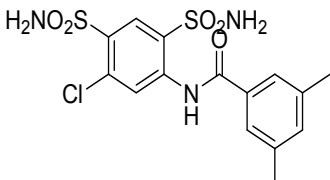
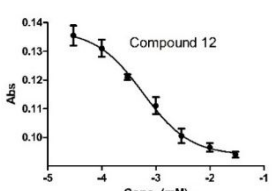
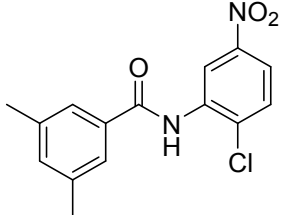
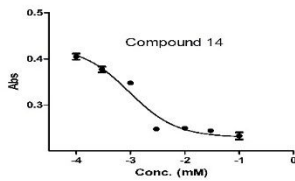
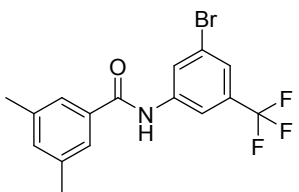
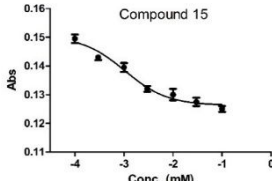
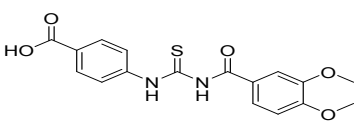
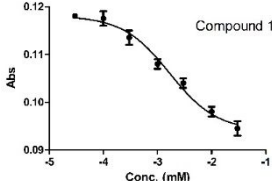
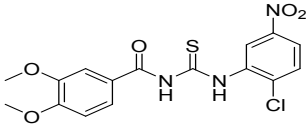
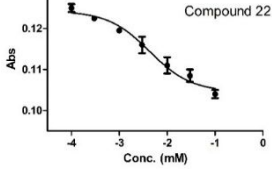
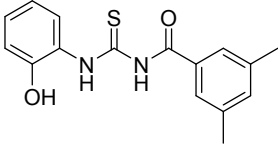
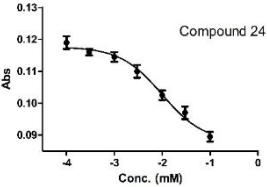
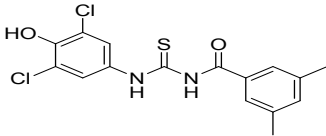
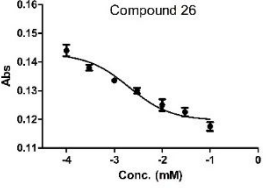
24	 <p>Molecular Weight: 300.38</p>		1.25 ± 0.02
25	 <p>Molecular Weight: 328.39</p>		9.76 ± 1.03
26	 <p>Molecular Weight: 369.26</p>		1.28 ± 0.09

Table S3: IC₅₀ vales and Graphs of hCA XII

h-Carbonic Anhydrase-XII (hCA-XII)			
Compounds	STRUCTURE	GRAPH	IC ₅₀
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	 Molecular Weight: 360.38		1.68 ± 0.15
Compound 22			4.29 ± 0.24

Compound 24			9.90 ± 0.66
Compound 26			2.02 ± 0.28

4 Cell Viability assay

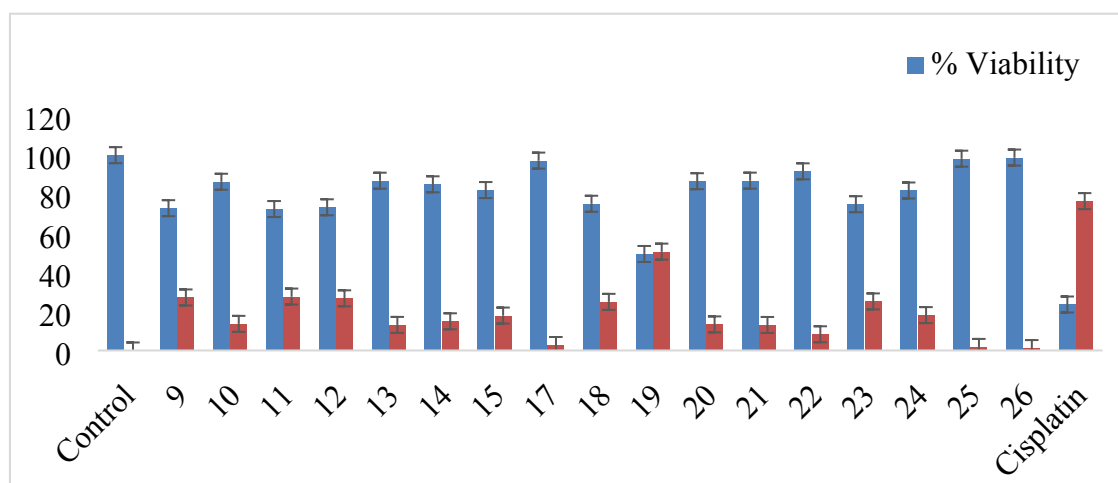


Figure S49: Analysis of cell viability/cytotoxicity using HEK-293 cells treated with compounds 1 –17 at final concentration (100 μ M). Statistics indicating significant values * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. untreated group. Cisplatin (100 μ M) is used as a positive control.

5 Docking interaction of the synthesized compounds

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

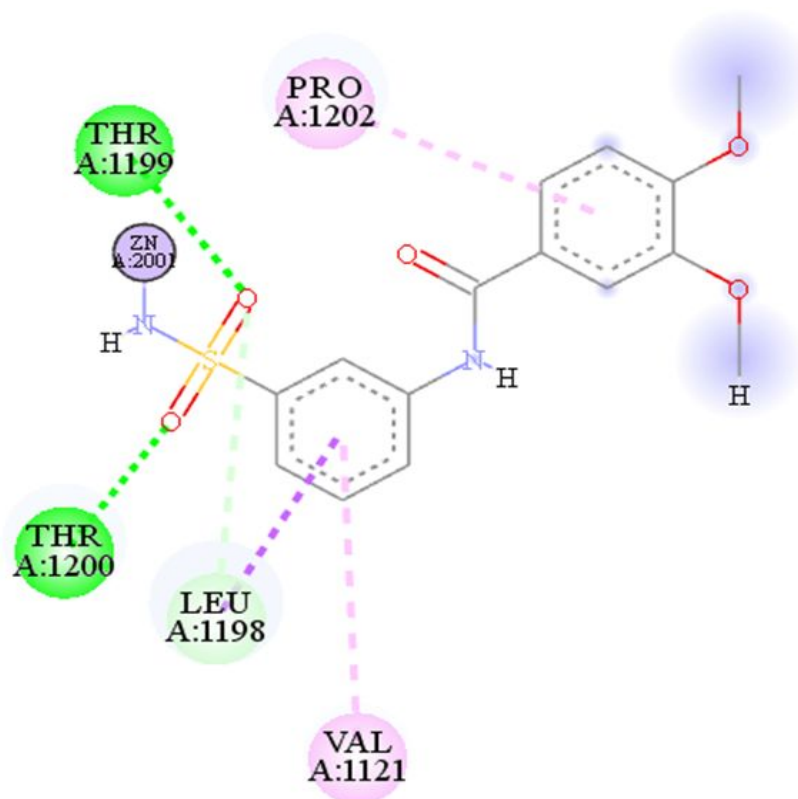


Figure S50: 2D interaction of Compound **09**

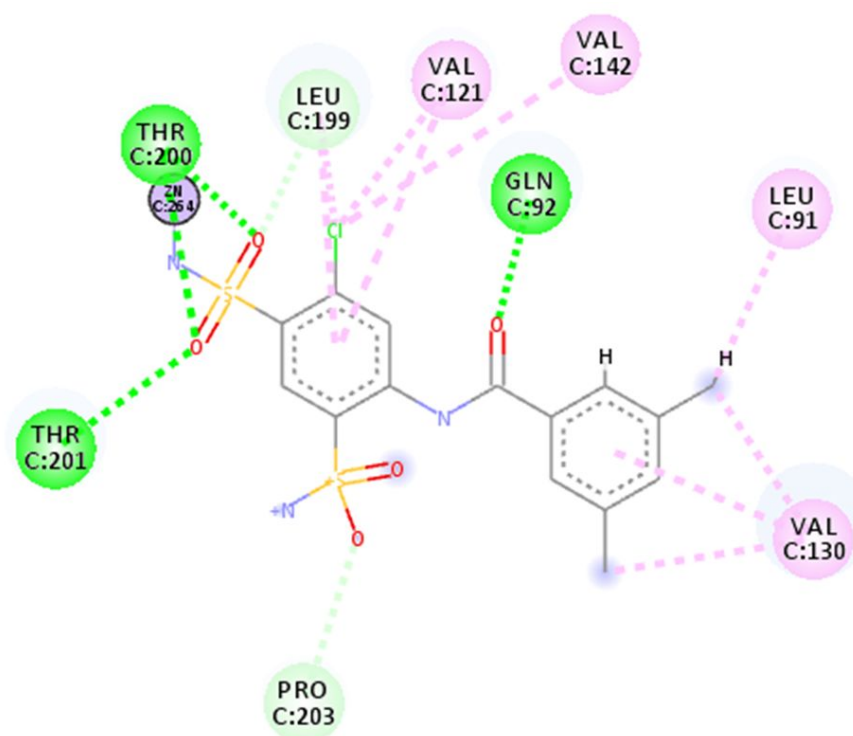


Figure S51: 2D interaction and geometrical graph of compound **11**

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

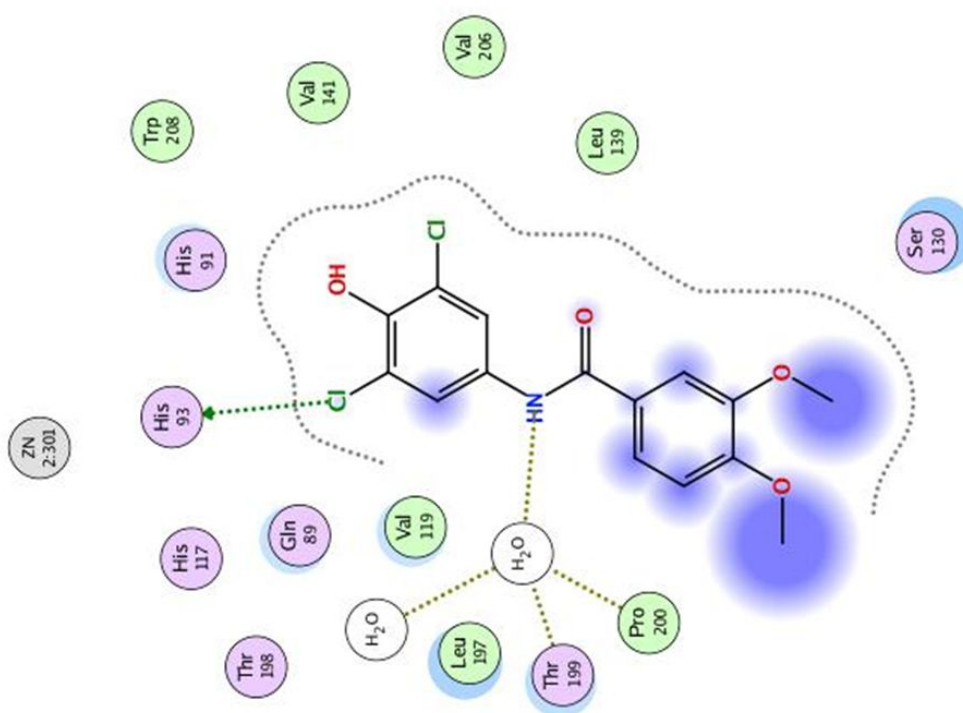


Figure S52: 2D interaction Compound 12

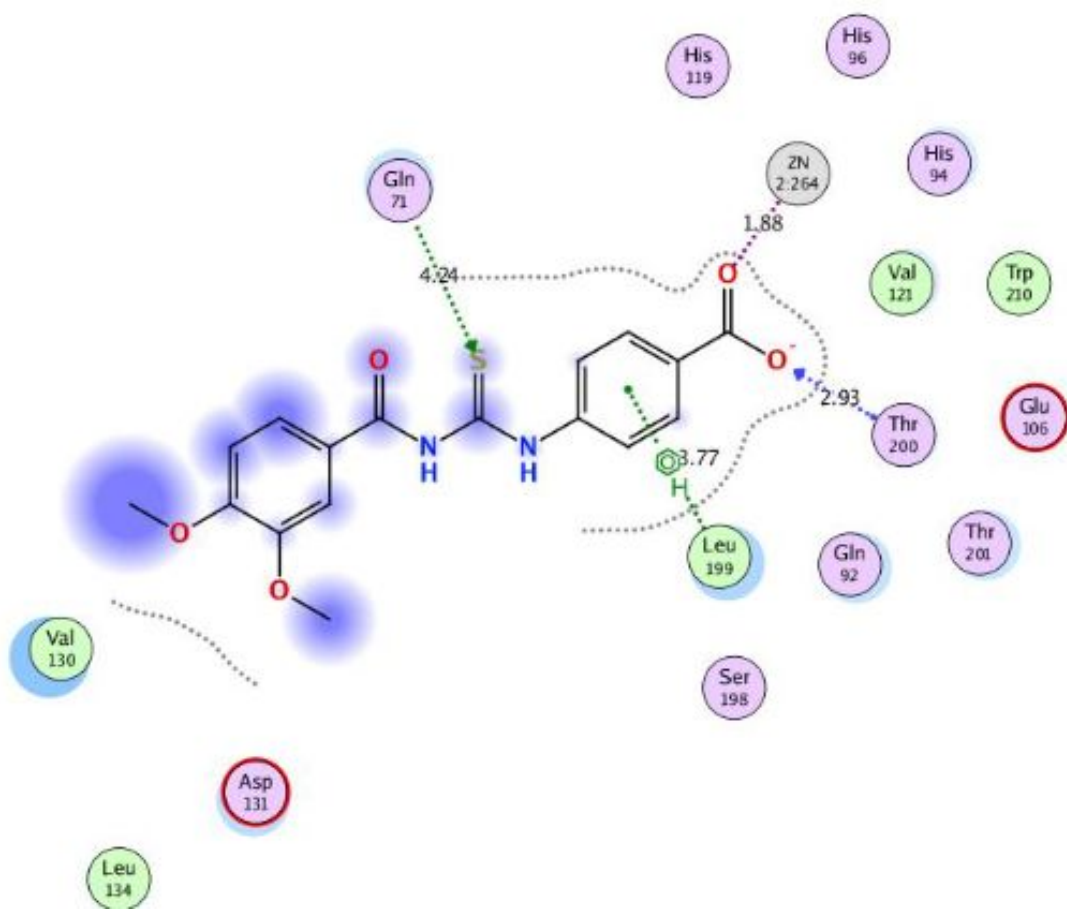


Figure S53: 2D interaction Compound 18