

Synthesis, molecular simulation, DFT and kinetic study of imidazotriazole based thiazolidinone as dual inhibitor of acetylcholinesterase and butyrylcholinesterase enzymes

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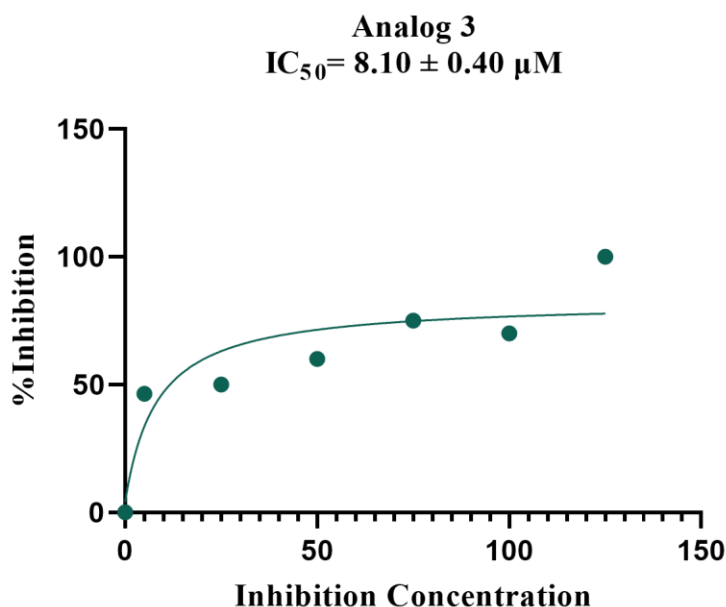
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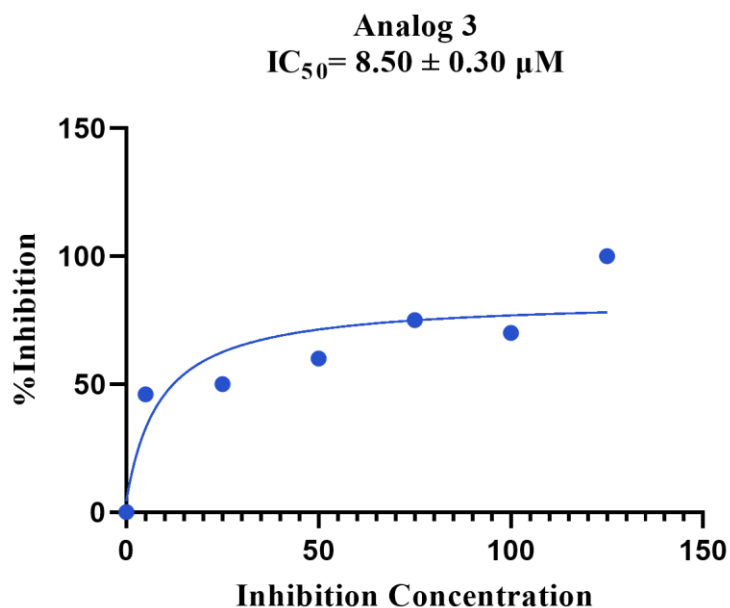
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Supplementary Information

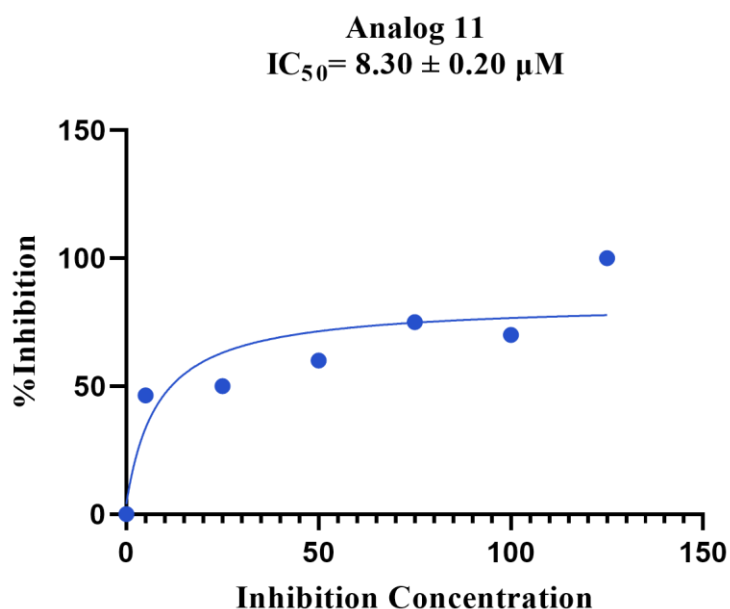
Graph-S1: Percentage inhibition of analog 3 vs. inhibitor concentration which illustrates the drug-dose relationship against AChE



Graph-S2: Percentage inhibition of analog 3 vs. inhibitor concentration which illustrates the drug-dose relationship against BChE



Graph-S3: Percentage inhibition of analog 11 vs. inhibitor concentration which illustrates the drug-dose relationship against AChE



Graph-S4: Percentage inhibition of analog 11 vs. inhibitor concentration which illustrates the drug-dose relationship against BChE

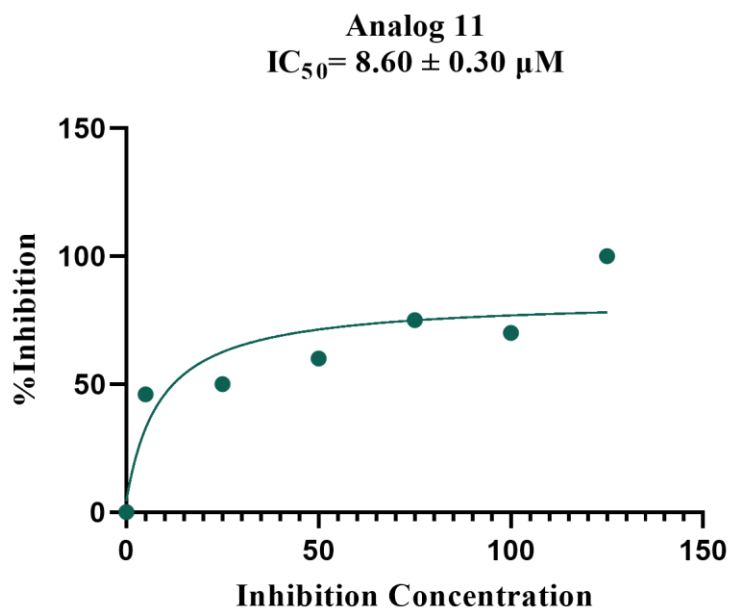


Table-S1: ADMET predictions for analog 10 and its toxilological profile

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-2.892	Numeric (log mol/L)
Absorption	Caco2 permeability	1.26	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	89.584	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.735	Numeric (log Kp)
Absorption	P-glycoprotein substrate	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	Yes	Categorical (Yes/No)
Distribution	VDss (human)	-0.117	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0.263	Numeric (Fu)
Distribution	BBB permeability	-1.613	Numeric (log BB)

Distribution	CNS permeability	-2.311	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	No	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitor	Yes	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitor	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitor	Yes	Categorical (Yes/No)
Excretion	Total Clearance	0.353	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	Yes	Categorical (Yes/No)
Toxicity	AMES toxicity	Yes	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.488	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.24	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	0.912	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	Yes	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)
Toxicity	<i>T.Pyriformis</i> toxicity	0.285	Numeric (log ug/L)
Toxicity	Minnow toxicity	1.204	Numeric (log mM)

S.1 Spectral Analysis

1. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-fluorophenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.96 (s, 1H, NH-imidazole), 7.73 (d, *J* = 8.46 Hz, 1H, Ar-H), 7.41 (d, *J* = 8.40 Hz, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.25 (d, *J* = 8.70 Hz, 2H, Ar-H), 7.18 (d, *J* = 6.82 Hz, 2H, Ar-H), 7.12 (d, *J* = 7.26 Hz, 1H, H-imidazole), 7.08 (d, *J* = 8.99 Hz, 1H, H-imidazole), 6.42 (s, 1H, H-thiazolidinone), 3.95 (s, 2H, H-thiazolidinone), 3.82 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.1, 161.2, 153.1, 152.7, 152.4, 135.8, 134.6, 130.3, 130.1, 128.4, 127.1, 123.2, 120.1, 115.4, 115.3, 114.3, 111.2, 72.6, 55.6, 33.2; HREI MS:*m/z* calcd for C₂₀H₁₆FN₅O₂S [M]⁺ 409.44 Found 409.40.

2. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(3,5-difluorophenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.95 (s, 1H, NH-imidazole), 7.76 (d, *J* = 8.46 Hz, 1H, Ar-H), 7.43 (d, *J* = 7.38 Hz, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.18 (d, *J* = 7.15 Hz, 1H, H-imidazole), 7.04 (s, 2H, Ar-H), 7.00 (s, 1H, Ar-H), 6.88 (d, *J* = 7.20 Hz, 1H, H-imidazole), 6.41 (s, 1H, H-thiazolidinone), 3.94 (s, 2H, H-thiazolidinone), 3.81 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.3, 162.7, 162.3, 153.8, 152.7, 152.5, 142.3, 135.2, 128.7, 127.6, 123.3, 120.8, 114.7, 111.6, 111.3, 111.2, 103.8, 72.8, 55.8, 33.8; HREI MS:*m/z* calcd for C₂₀H₁₅F₂N₅O₂S [M]⁺ 427.43 Found 427.39.

3. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(2,4-difluorophenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.94 (s, 1H, NH-imidazole), 7.77 (d, *J* = 8.76 Hz, 1H, Ar-H), 7.44 (d, *J* = 7.38 Hz, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.23 (d, *J* = 6.87 Hz, 1H, Ar-H), 7.13 (d, *J* = 7.20 Hz, 1H, H-imidazole), 7.04 (d, *J* = 7.25 Hz, 1H, H-imidazole), 7.02 (d, *J* = 7.26 Hz, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.49 (s, 1H, H-thiazolidinone), 3.95 (s, 2H, H-thiazolidinone), 3.85 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.2, 162.6, 159.4,

153.3, 152.6, 152.5, 135.2, 131.6, 128.5, 127.6, 123.3, 120.2, 114.2, 111.4, 111.2, 104.4, 100.1, 66.2, 55.3, 33.4; HREI MS: m/z calcd for $C_{20}H_{15}F_2N_5O_2S$ $[M]^+$ 427.43 Found 427.39.

4. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-hydroxyphenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.91 (s, 1H, NH-imidazole), 9.21 (s, 1H, H-OH) 7.87 (d, J = 8.76 Hz, 1H, Ar-H), 7.64 (d, J = 7.98 Hz, 2H, Ar-H), 7.44 (d, J = 6.87 Hz, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.13 (d, J = 6.75 Hz, 1H, H-imidazole), 7.05 (d, J = 7.26 Hz, 1H, H-imidazole), 7.02 (d, J = 7.25 Hz, 2H, Ar-H), 6.43 (s, 1H, H-thiazolidinone), 3.94 (s, 2H, H-thiazolidinone), 3.83 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO- d_6): δ 171.4, 156.6, 153.2, 152.3, 152.1, 135.1, 131.4, 130.2, 130.1, 128.4, 127.2, 123.3, 120.1, 115.6, 115.4, 114.2, 111.3, 72.6, 55.5, 33.4; HREI MS: m/z calcd for $C_{20}H_{17}N_5O_3S$ $[M]^+$ 407.45 Found 407.41.

5. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-nitrophenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.92 (s, 1H, NH-imidazole), 8.18 (d, J = 7.40 Hz, 2H, Ar-H), 7.75 (d, J = 8.46 Hz, 1H, Ar-H), 7.58 (d, J = 8.28 Hz, 2H, Ar-H), 7.43 (d, J = 7.20 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.12 (d, J = 7.02 Hz, 1H, H-imidazole), 7.02 (d, J = 7.86 Hz, 1H, H-imidazole), 6.88 (s, 1H, H-thiazolidinone), 3.94 (s, 2H, H-thiazolidinone), 3.81 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO- d_6): δ 171.1, 153.2, 152.4, 152.3, 144.2, 141.4, 135.1, 129.3, 129.2, 128.6, 127.2, 123.6, 123.3, 123.2, 120.2, 114.2, 111.4, 72.8, 55.8, 33.8; HREI MS: m/z calcd for $C_{20}H_{16}N_6O_4S$ $[M]^+$ 436.45 Found 436.41.

6. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-chlorophenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.74 (s, 1H, NH-imidazole), 7.75 (d, J = 6.75 Hz, 1H, Ar-H), 7.58 (d, J = 7.56 Hz, 1H, Ar-H), 7.43 (d, J = 7.57 Hz, 2H, Ar-H), 7.33 (s, 1H, Ar-H), 7.20 (d, J = 7.38 Hz, 2H, Ar-H), 7.14 (d, J = 7.08 Hz, 1H, H-imidazole), 7.03 (d, J = 7.26 Hz, 1H, H-imidazole), 6.44 (s, 1H, H-thiazolidinone), 3.93 (s, 2H, H-thiazolidinone), 3.84 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO- d_6): δ 171.4, 153.2, 152.4, 152.3, 137.2, 135.1, 131.5,

129.3, 129.0, 128.7, 127.6, 127.4, 123.5, 123.2, 120.2, 114.5, 111.5, 72.3, 55.4, 33.5; HREI MS: m/z calcd for $C_{20}H_{16}ClN_5O_2S$ $[M]^+$ 425.89 Found 425.85.

7. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(3,4-dichlorophenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.90 (s, 1H, NH-imidazole), 7.75 (d, J = 8.94 Hz, 1H, Ar-H), 7.63 (d, J = 8.16 Hz, 1H, Ar-H), 7.42 (d, J = 8.16 Hz, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.12 (d, J = 8.58 Hz, 1H, H-imidazole), 7.09 (d, J = 7.86 Hz, 1H, Ar-H), 7.06 (d, J = 7.86 Hz, 1H, H-imidazole), 6.45 (s, 1H, H-thiazolidinone), 3.96 (s, 2H, H-thiazolidinone), 3.84 (s, 3H, CH₃); ^{13}C NMR (150 MHz, DMSO- d_6): δ 171.2, 153.4, 152.5, 152.2, 138.6, 135.4, 131.6, 131.4, 130.2, 129.5, 128.4, 128.3, 127.2, 123.2, 120.4, 114.2, 111.3, 72.6, 55.1, 33.2; HREI MS: m/z calcd for $C_{20}H_{15}Cl_2N_5O_2S$ $[M]^+$ 460.33 Found 460.29.

8. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-chloro-2-nitrophenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.93 (s, 1H, NH-imidazole), 8.24 (s, 1H, Ar-H), 7.77 (d, J = 8.46 Hz, 1H, Ar-H), 7.74 (d, J = 7.75 Hz, 1H, Ar-H), 7.46 (d, J = 6.80 Hz, 2H, Ar-H), 7.33 (s, 1H, Ar-H), 7.14 (d, J = 6.50 Hz, 1H, H-imidazole), 7.05 (d, J = 7.28 Hz, 1H, H-imidazole), 6.41 (s, 1H, H-thiazolidinone), 3.94 (s, 2H, H-thiazolidinone), 3.87 (s, 3H, CH₃); ^{13}C NMR (150 MHz, DMSO- d_6): δ 171.2, 153.4, 152.5, 152.4, 150.2, 135.1, 134.5, 132.1, 130.2, 130.1, 128.6, 127.3, 124.2, 123.5, 120.4, 114.3, 111.2, 68.2, 55.4, 33.5; HREI MS: m/z calcd for $C_{20}H_{15}ClN_6O_4S$ $[M]^+$ 470.89 Found 470.85.

9. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(3-fluoro-5-hydroxyphenyl)thiazolidin-4-one

1H NMR (600MHz, DMSO- d_6): δ 12.92 (s, 1H, NH-imidazole), 9.42 (s, 1H, H-OH), 7.74 (d, J = 7.56 Hz, 1H, Ar-H), 7.41 (d, J = 7.50 Hz, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.12 (d, J = 6.54 Hz, 1H, H-imidazole), 7.04 (d, J = 7.74 Hz, 1H, H-imidazole), 7.02 (s, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 6.73 (s, 1H, Ar-H), 6.44 (s, 2H, H-thiazolidinone), 3.97 (s, 2H, H-thiazolidinone),

3.86 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.3, 164.7, 160.7, 153.8, 152.7, 152.5, 142.3, 135.8, 128.4, 127.5, 123.3, 120.5, 114.7, 111.6, 110.3, 108.3, 100.8, 73.1, 55.7, 33.8; HREI MS:*m/z* calcd for C₂₀H₁₆FN₅O₃S [M]⁺ 425.44 Found 425.40.

10. 3-(4-(7H-imidazo[2,1-*c*][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-(trifluoromethyl)phenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.87 (s, 1H, NH-imidazole), 7.79 (d, *J* = 8.34 Hz, 1H, Ar-H), 7.56 (d, *J* = 7.26 Hz, 2H, Ar-H), 7.42 (d, *J* = 6.91 Hz, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.22 (d, *J* = 8.70 Hz, 2H, Ar-H), 7.14 (d, *J* = 8.99 Hz, 1H, H-imidazole), 7.03 (d, *J* = 7.20 Hz, 1H, H-imidazole), 6.42 (s, 1H, H-thiazolidinone), 3.94 (s, 2H, H-thiazolidinone), 3.81 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.2, 153.4, 152.5, 152.4, 142.5, 135.1, 129.5, 129.3, 129.2, 128.7, 128.6, 127.3, 124.3, 124.2, 123.5, 120.3, 114.3, 111.2, 72.6, 55.7, 33.3; HREI MS:*m/z* calcd for C₂₁H₁₆F₃N₅O₂S [M]⁺ 459.45 Found 459.41.

11. 3-(4-(7H-imidazo[2,1-*c*][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-fluoro-2-hydroxyphenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.95 (s, 1H, NH-imidazole), 9.75 (s, 1H, H-OH), 7.74 (d, *J* = 7.02 Hz, 1H, Ar-H), 7.44 (d, *J* = 7.26 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.12 (d, *J* = 7.50 Hz, 1H, H-imidazole), 7.08 (d, *J* = 8.99 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.58 Hz, 1H, H-imidazole), 7.01 (s, 1H, Ar-H), 6.85 (d, *J* = 7.26 Hz, 1H, Ar-H), 6.43 (s, 1H, H-thiazolidinone), 3.96 (s, 2H, H-thiazolidinone), 3.87 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 171.7, 162.6, 156.2, 153.2, 152.4, 152.2, 135.3, 131.2, 128.5, 127.2, 123.3, 120.4, 120.1, 115.5, 115.4, 111.9, 104.2, 72.8, 55.8, 33.8; HREI MS:*m/z* calcd for C₂₀H₁₆FN₅O₃S [M]⁺ 425.44 Found 425.40.

12. 3-(4-(7H-imidazo[2,1-*c*][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-fluoro-3-hydroxyphenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.93 (s, 1H, NH-imidazole), 9.03 (s, 1H, H-OH), 7.76 (d, *J* = 7.08 Hz, 1H, Ar-H), 7.45 (d, *J* = 6.75 Hz, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.13 (d, *J* = 7.14 Hz, 1H, H-imidazole), 7.05 (d, *J* = 7.98 Hz, 1H, Ar-H), 7.03 (d, *J* = 8.10 Hz, 1H, H-imidazole), 6.93 (d, *J* = 7.08 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 6.42 (s, 1H, H-

thiazolidinone), 3.95 (s, 2H, H-thiazolidinone), 3.85 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-d₆): δ 171.2, 153.4, 152.2, 152.1, 152.0, 143.6, 135.7, 131.6, 128.3, 127.2, 123.3, 120.7, 120.6, 115.5, 115.2, 114.8, 104.3, 72.6, 53.3, 33.7; HREI MS:*m/z* calcd for C₂₀H₁₆FN₅O₃S [M]⁺ 425.44 Found 425.40.

13. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-fluoro-3-nitrophenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.99 (s, 1H, NH-imidazole), 8.18 (s, 1H, Ar-H), 7.73 (d, *J* = 7.08 Hz, 1H, Ar-H), 7.63 (d, *J* = 8.28 Hz, 1H, Ar-H), 7.47 (d, *J* = 6.78 Hz, 2H, Ar-H), 7.34 (s, 1H, Ar-H), 7.17 (d, *J* = 7.02 Hz, 1H, H-imidazole), 7.08 (d, *J* = 7.14 Hz, 1H, H-imidazole), 6.41 (s, 1H, H-thiazolidinone), 3.96 (s, 2H, H-thiazolidinone), 3.83 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-d₆): δ 171.5, 153.6, 153.2, 152.4, 152.3, 136.5, 135.1, 131.3, 129.6, 128.7, 127.6, 126.4, 123.5, 123.2, 120.2, 114.5, 111.5, 71.3, 55.4, 33.5; HREI MS:*m/z* calcd for C₂₀H₁₅FN₆O₄S [M]⁺ 454.44 Found 454.40.

14. 3-(4-(7H-imidazo[2,1-c][1,2,4]triazol-3-yl)-2-methoxyphenyl)-2-(4-methoxyphenyl)thiazolidin-4-one

¹H NMR (600MHz, DMSO-*d*₆): δ 12.94 (s, 1H, NH-imidazole), 7.80 (d, *J* = 7.68 Hz, 2H, Ar-H), 7.63 (d, *J* = 8.28 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.99 Hz, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.14 (d, *J* = 8.22 Hz, 1H, H-imidazole), 7.08 (d, *J* = 7.25 Hz, 1H, H-imidazole), 6.90 (d, *J* = 6.69 Hz, 2H, Ar-H), 6.43 (s, 1H, H-thiazolidinone), 3.95 (s, 2H, H-thiazolidinone), 3.85 (s, 3H, CH₃), 3.80 (s, 3H, CH₃); ¹³C NMR (150 MHz, DMSO-d₆): δ 171.4, 160.7, 153.8, 152.7, 152.5, 135.8, 128.4, 127.5, 123.3, 120.5, 127.3, 114.7, 111.6, 114.3, 110.3, 108.3, 100.8, 71.1, 55.7, 55.5, 33.3; HREI MS:*m/z* calcd for C₂₁H₁₉N₅O₃S [M]⁺ 421.48 Found 421.44.

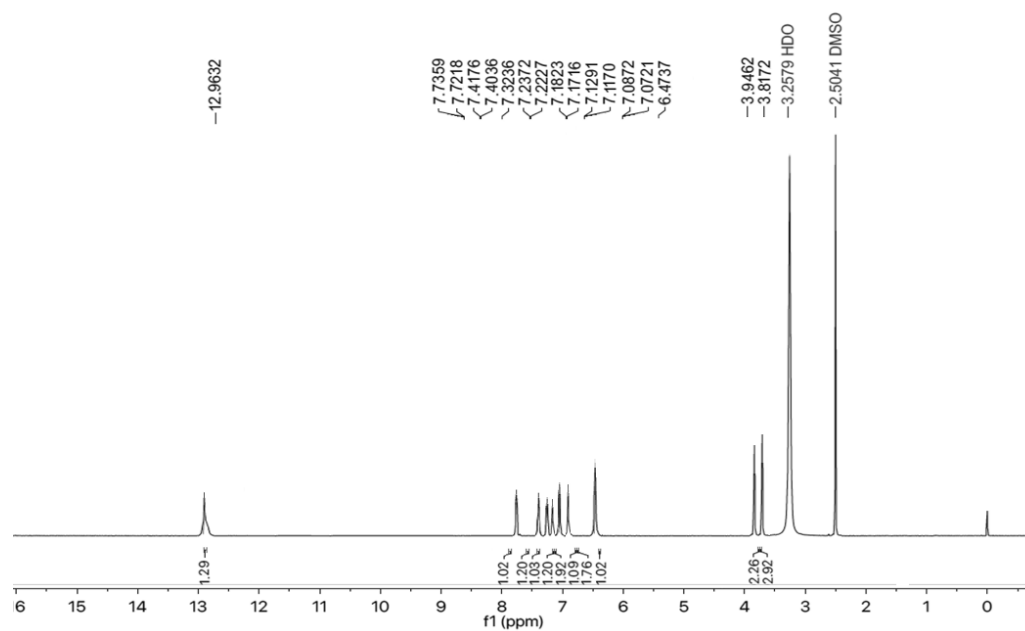


Figure-S.1 Proton spectral analysis of compound-1

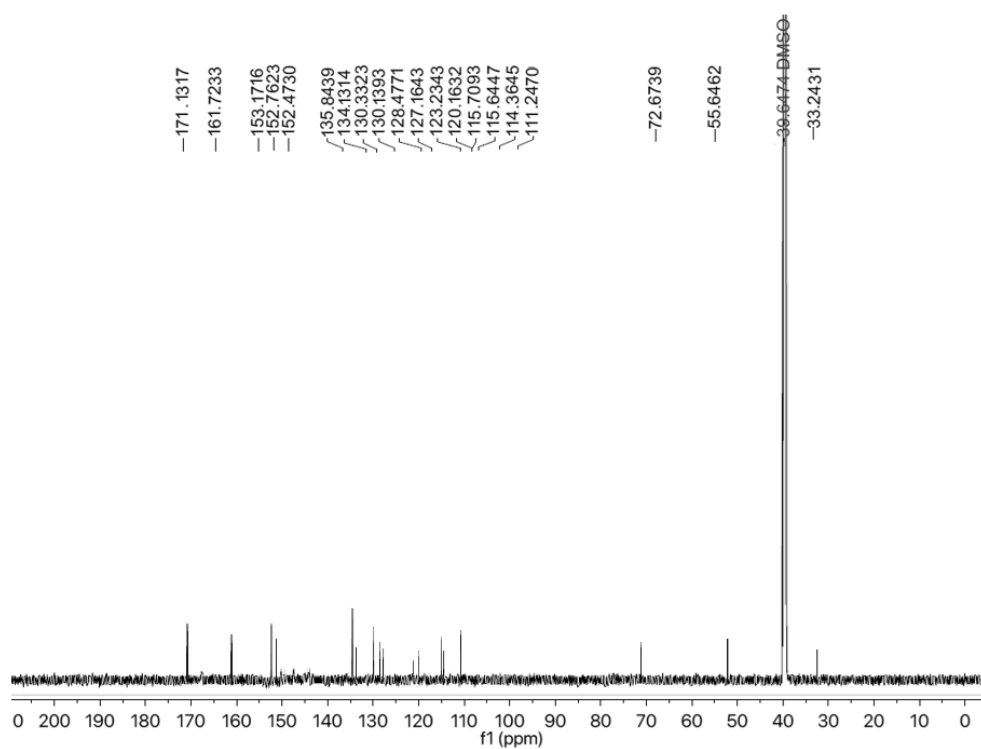


Figure-S.2 Carbon spectral analysis of compound-1

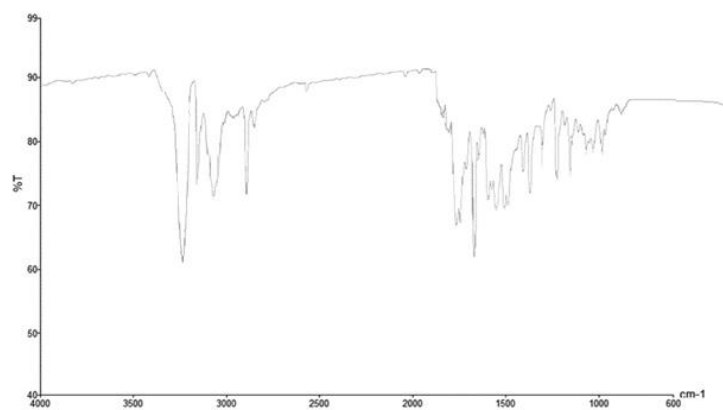


Figure-S.3 IR analysis of compound-1

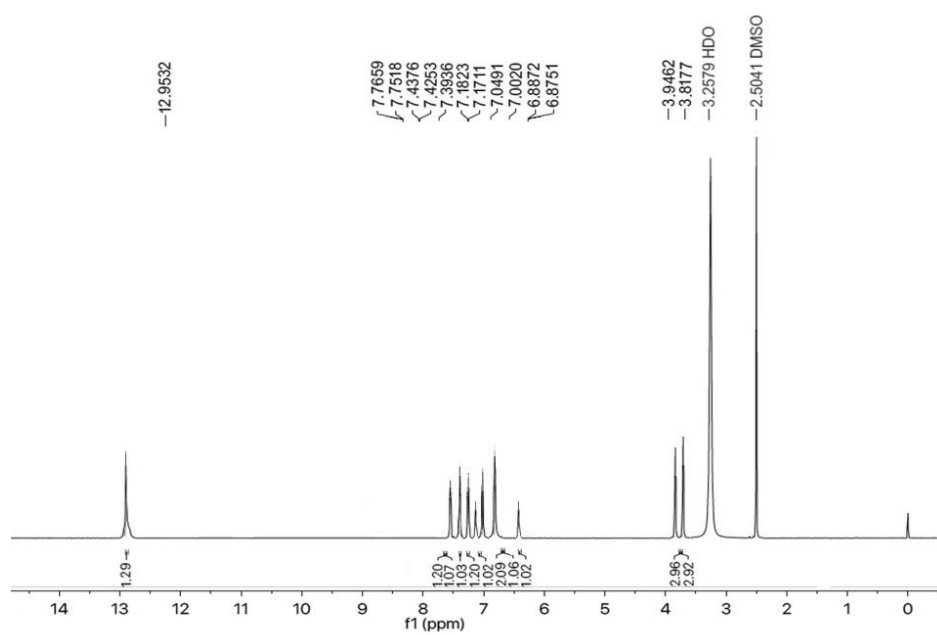


Figure-S.4 Proton spectral analysis of compound-2

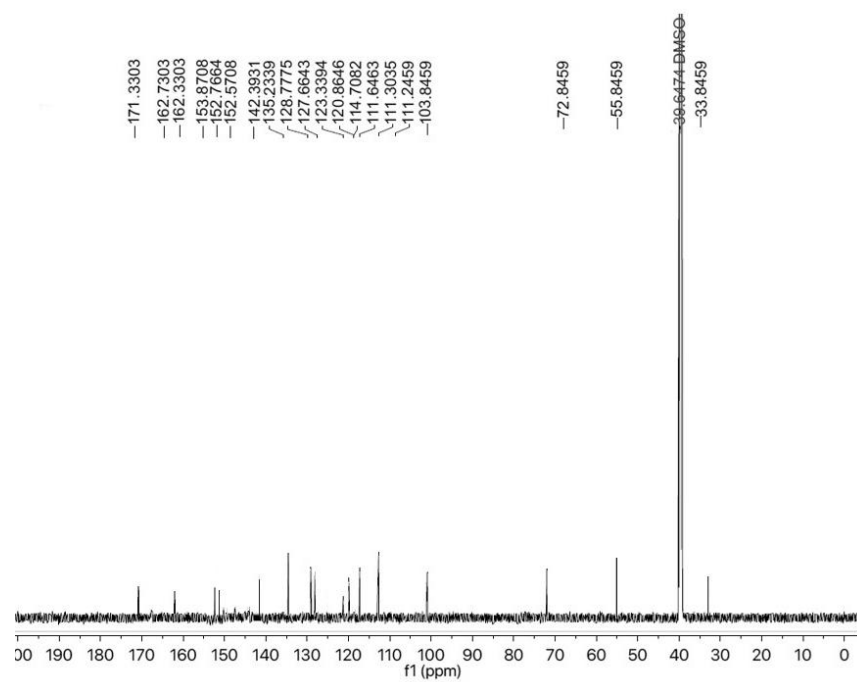


Figure-S.5 Carbon spectral analysis of compound-2

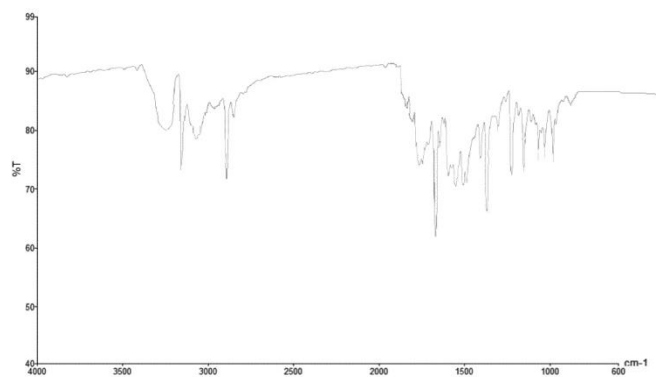


Figure-S.6 IR analysis of compound-2

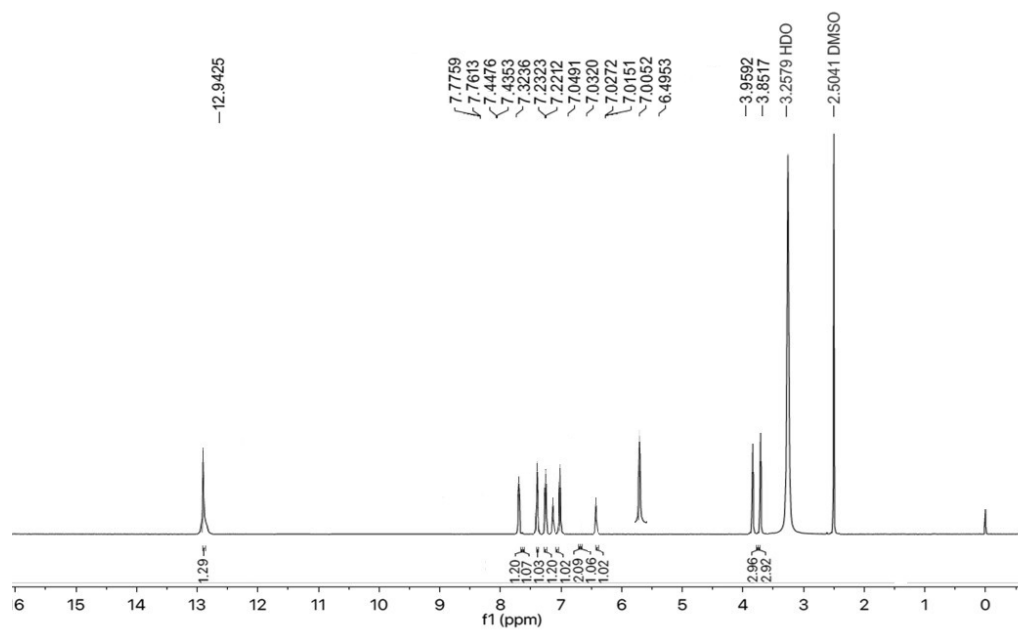


Figure-S.7 Proton spectral analysis of compound-3

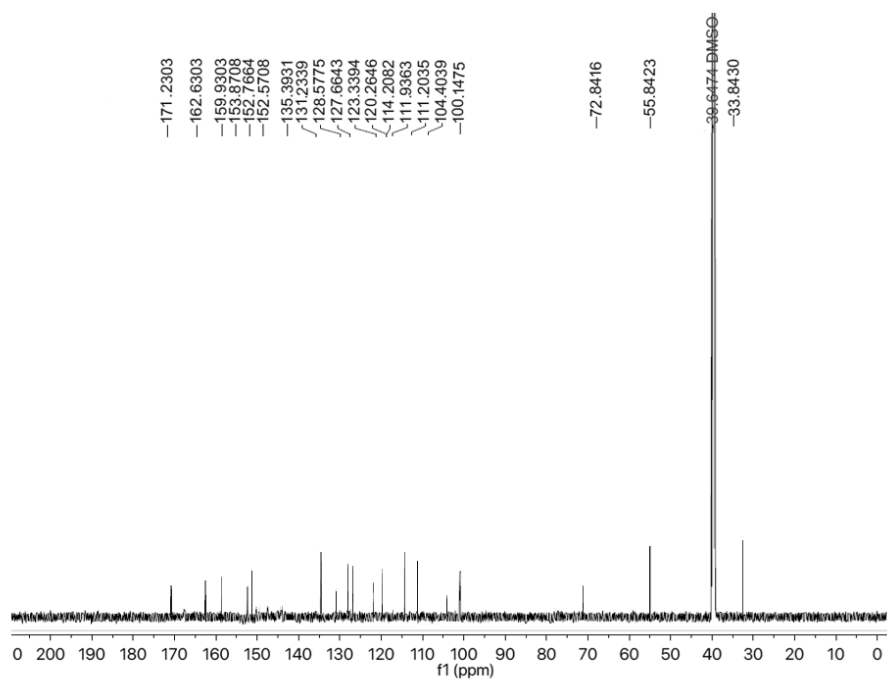


Figure-S.8 Carbon spectral analysis of compound-3

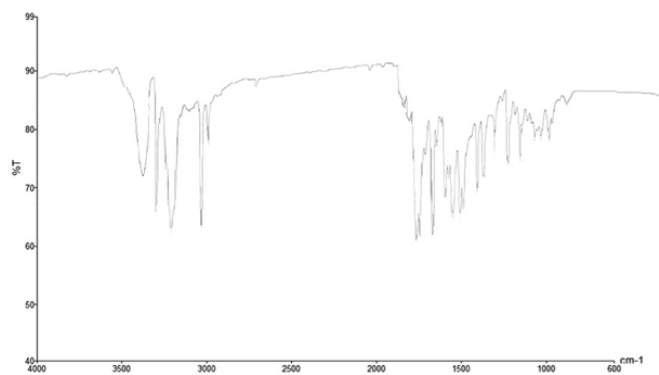


Figure-S.9 IR analysis of compound-3

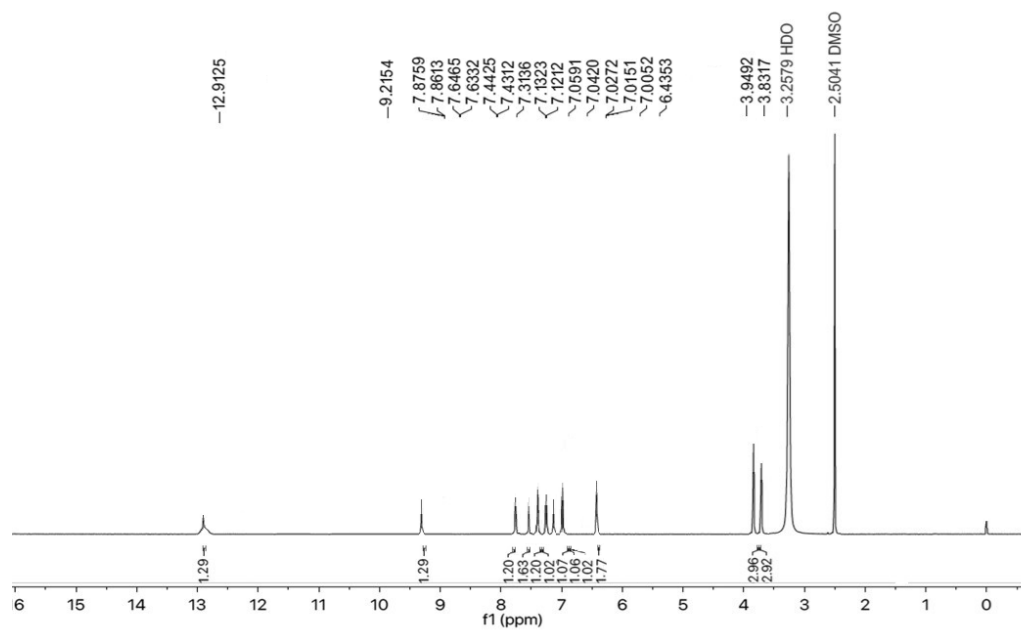


Figure-S.10 Proton spectral analysis of compound-4

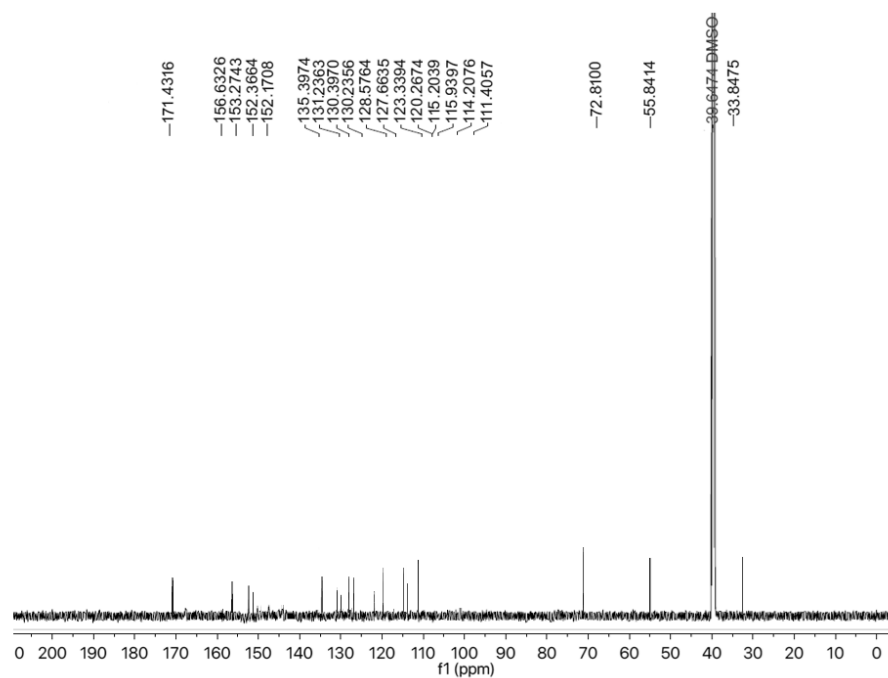


Figure-S.11 Carbon spectral analysis of compound-4

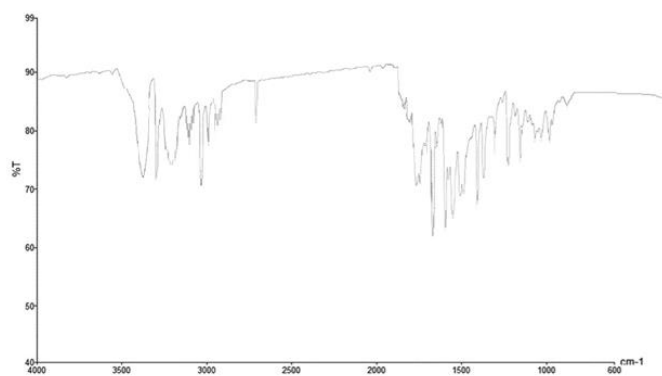


Figure-S.12 IR analysis of compound-4

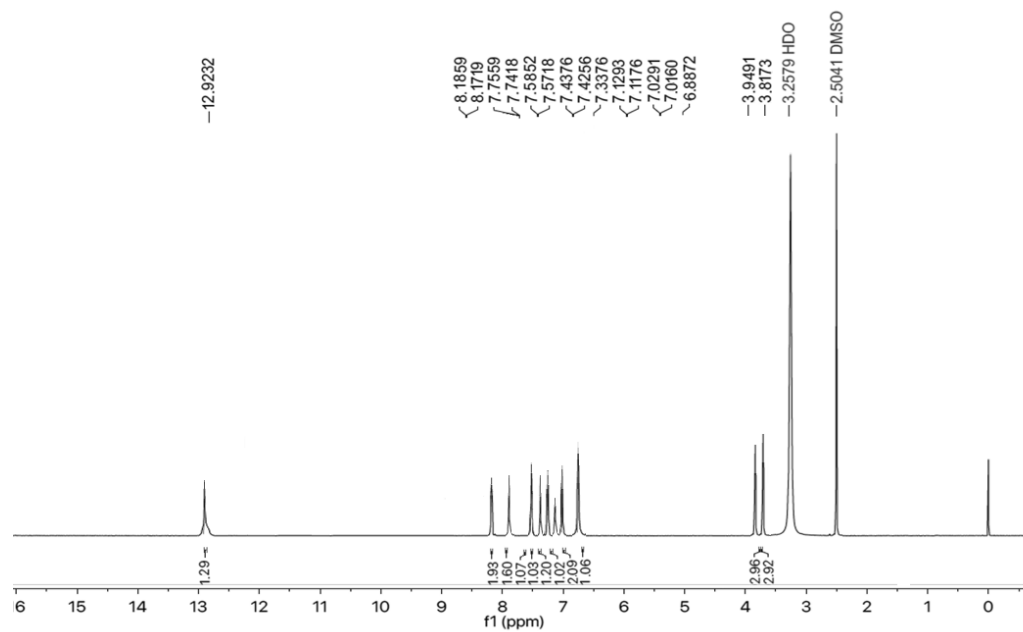


Figure-S.13 Proton spectral analysis of compound-5

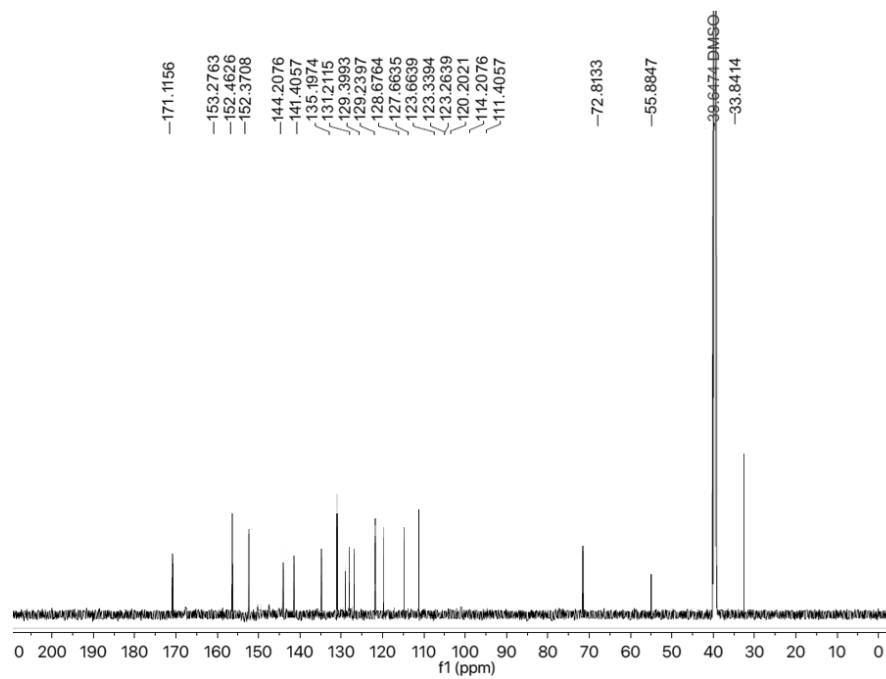


Figure-S.14 Carbon spectral analysis of compound-5

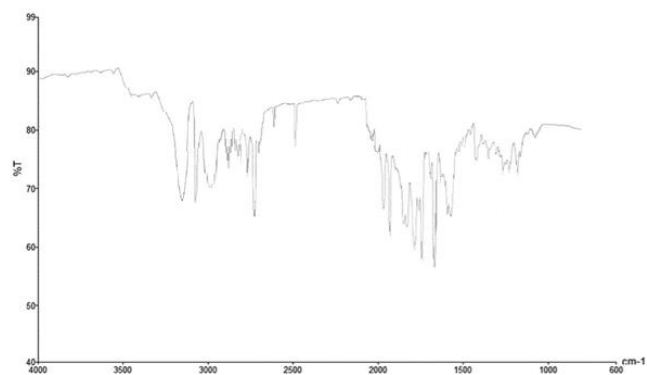


Figure-S.15 IR analysis of compound-5

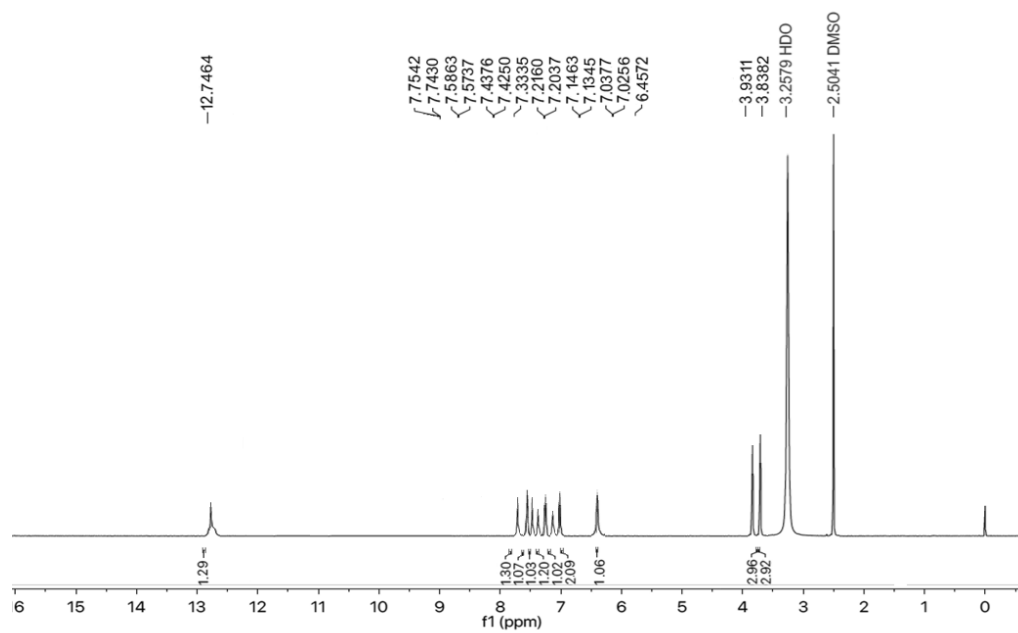


Figure-S.16 Proton spectral analysis of compound-6

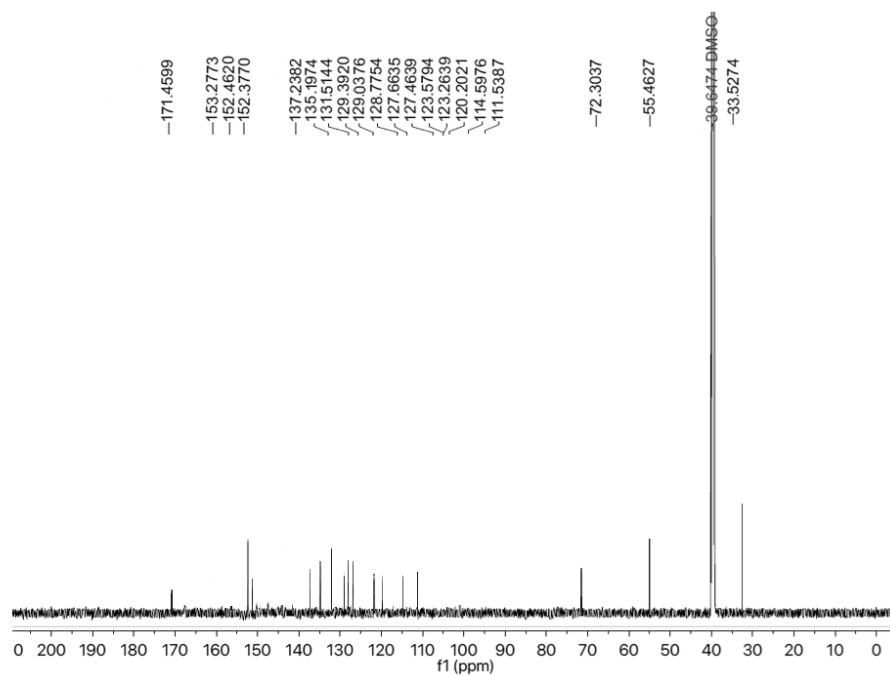


Figure-S.17 Carbon spectral analysis of compound-6

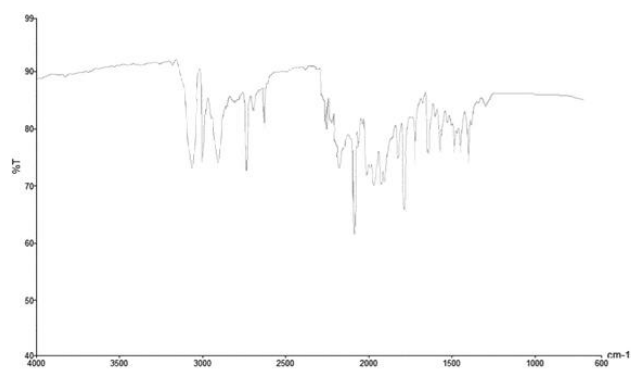


Figure-S.18 IR analysis of compound-6

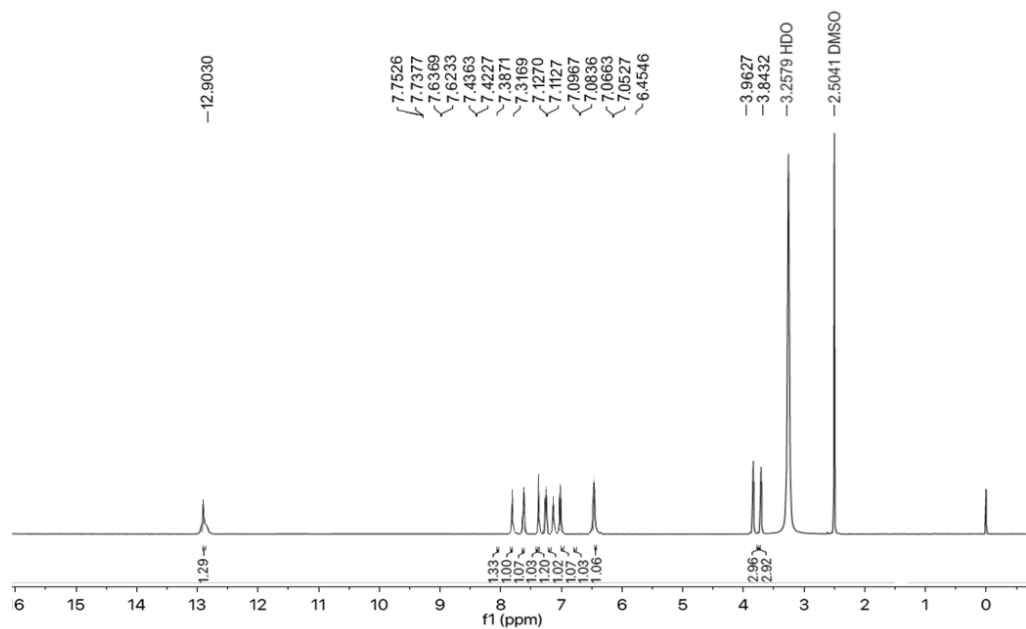


Figure-S.19 Proton spectral analysis of compound-7

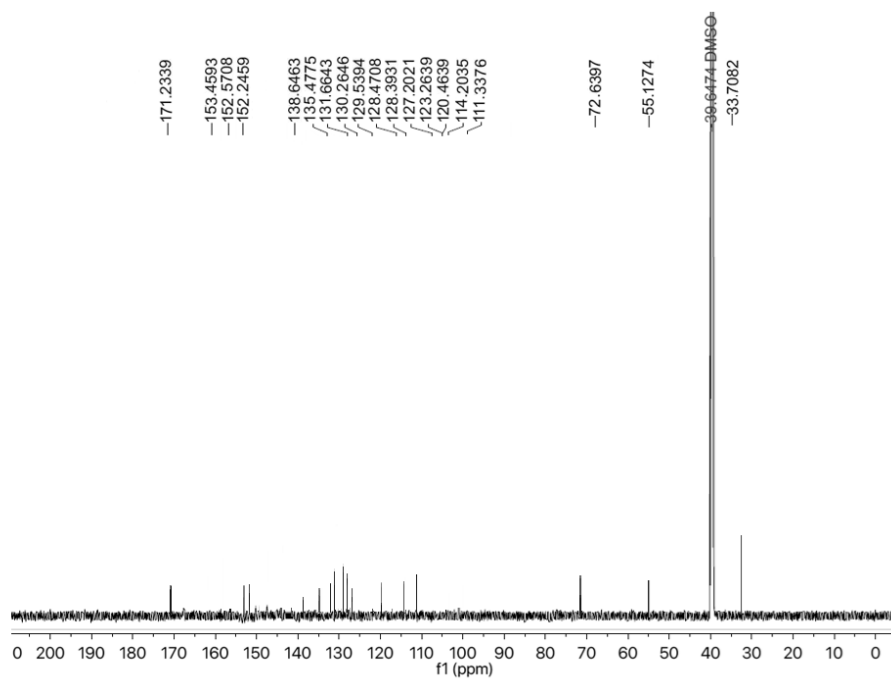


Figure-S.20 Carbon spectral analysis of compound-7

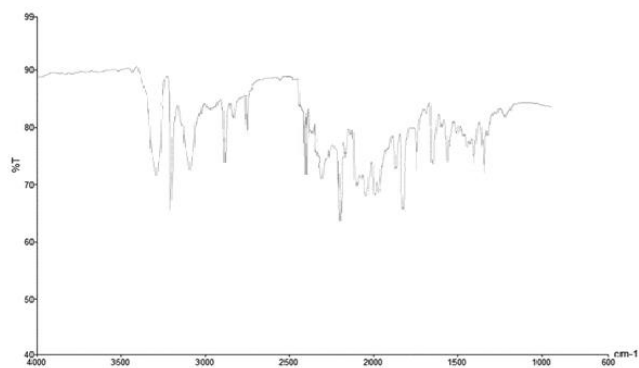


Figure-S.21 IR analysis of compound-7

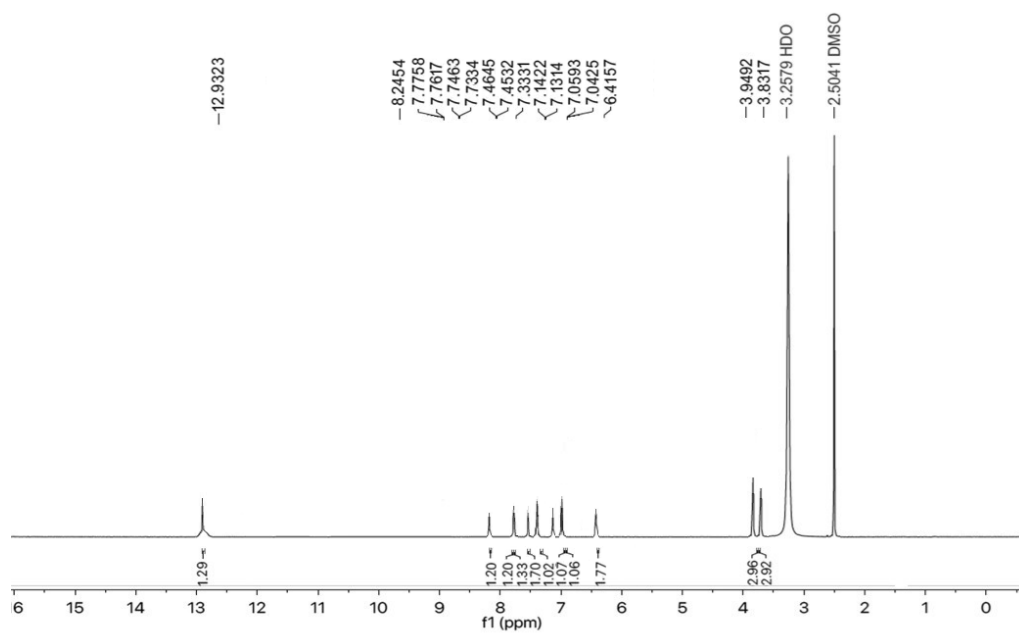


Figure-S.22 Proton spectral analysis of compound-8

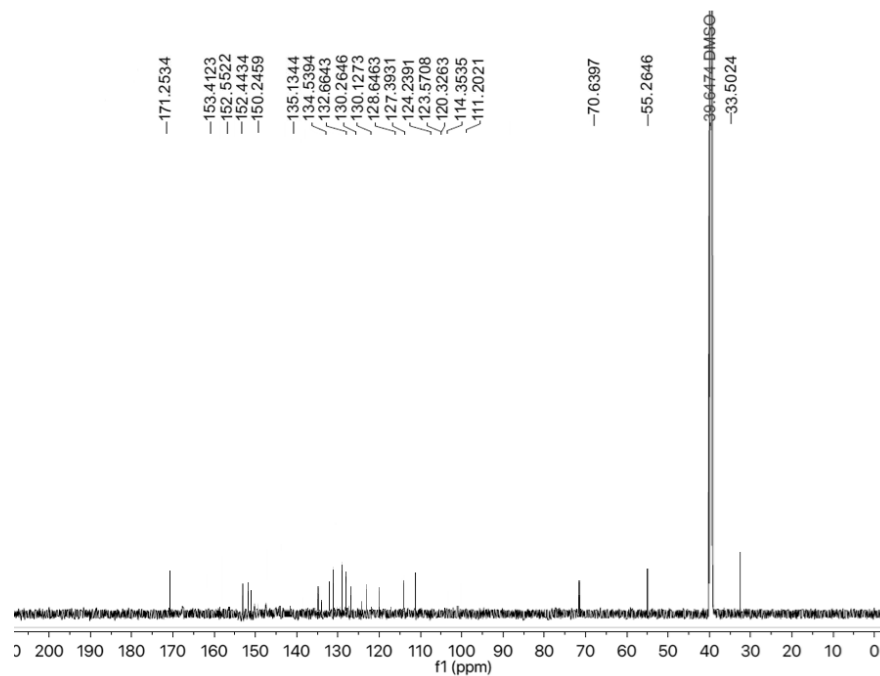


Figure-S.23 Carbon spectral analysis of compound-8

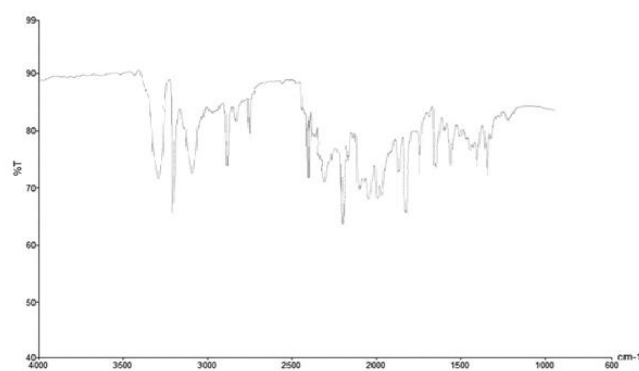


Figure-S.24 IR analysis of compound-8

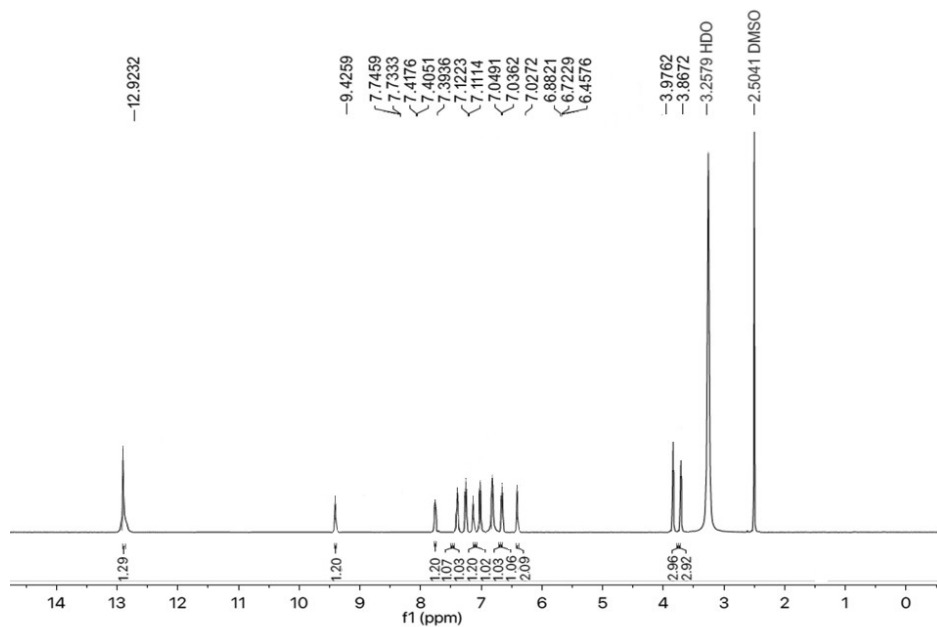


Figure-S.25 Proton spectral analysis of compound-9

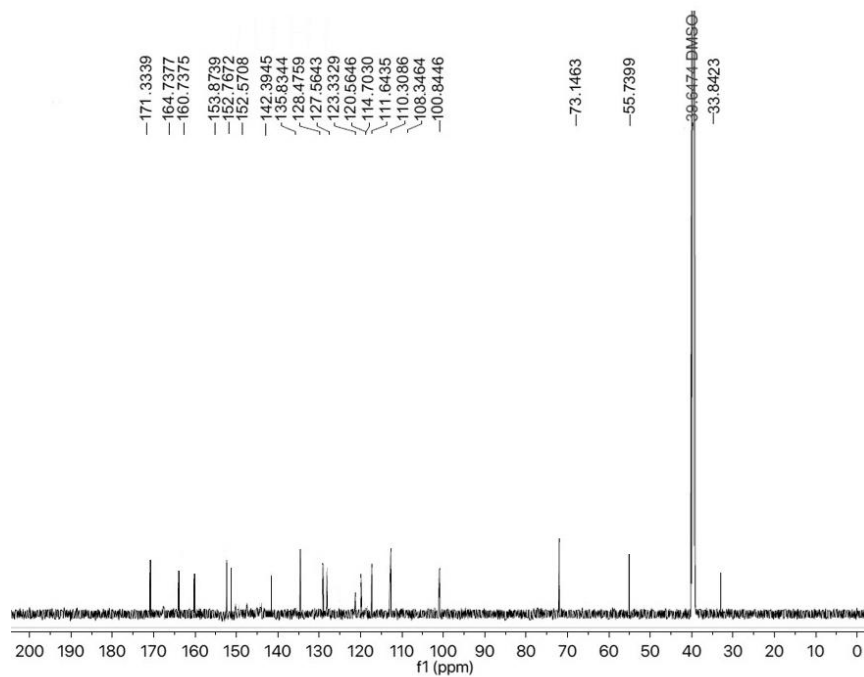


Figure-S.26 Carbon spectral analysis of compound-9

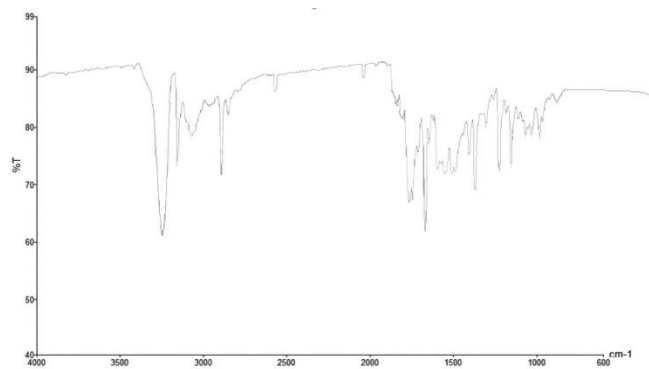


Figure-S.27 IR analysis of compound-9

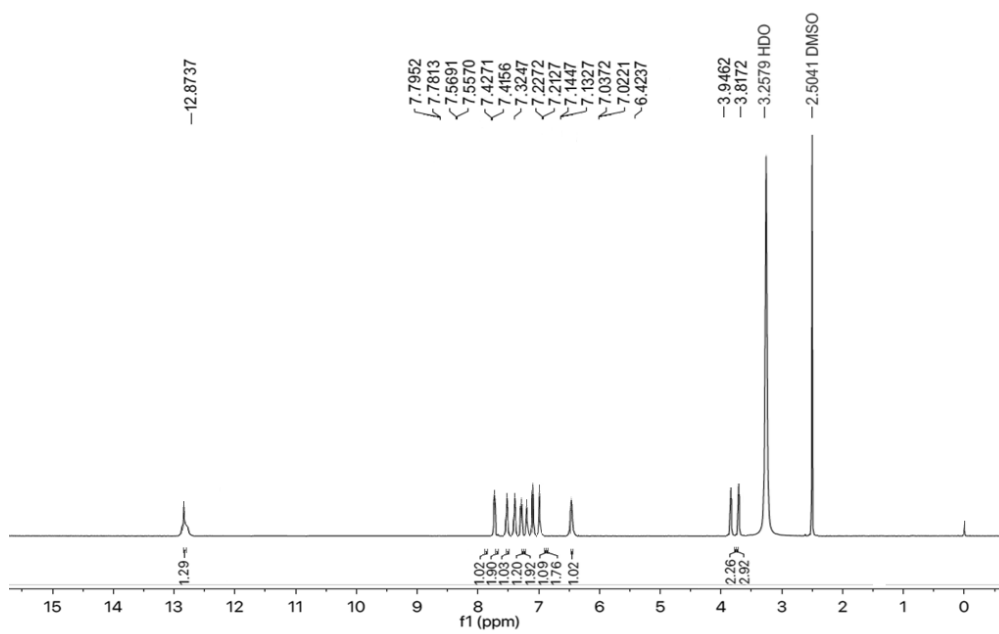


Figure-S.28 Proton spectral analysis of compound-10

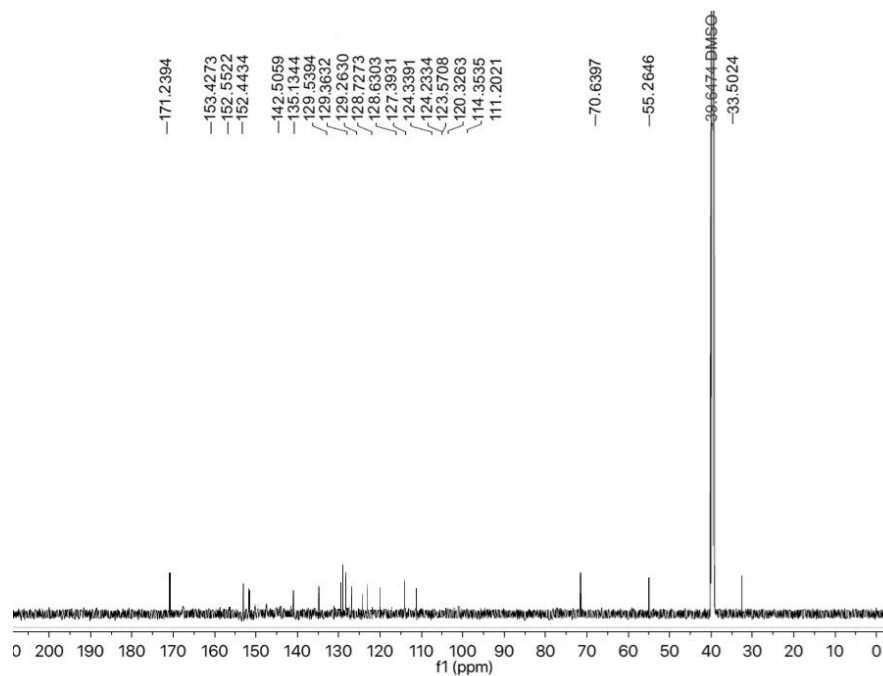


Figure-S.29 Carbon spectral analysis of compound-10

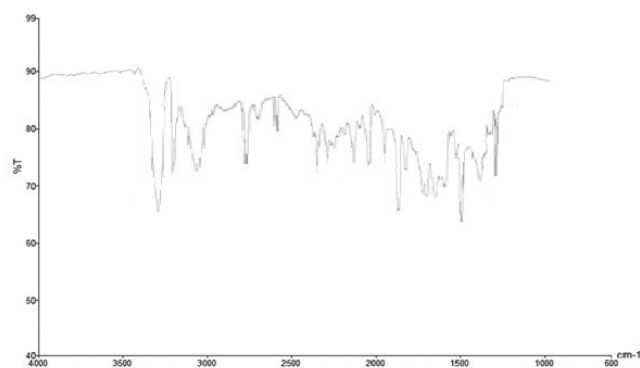


Figure-S.30 IR analysis of compound-10

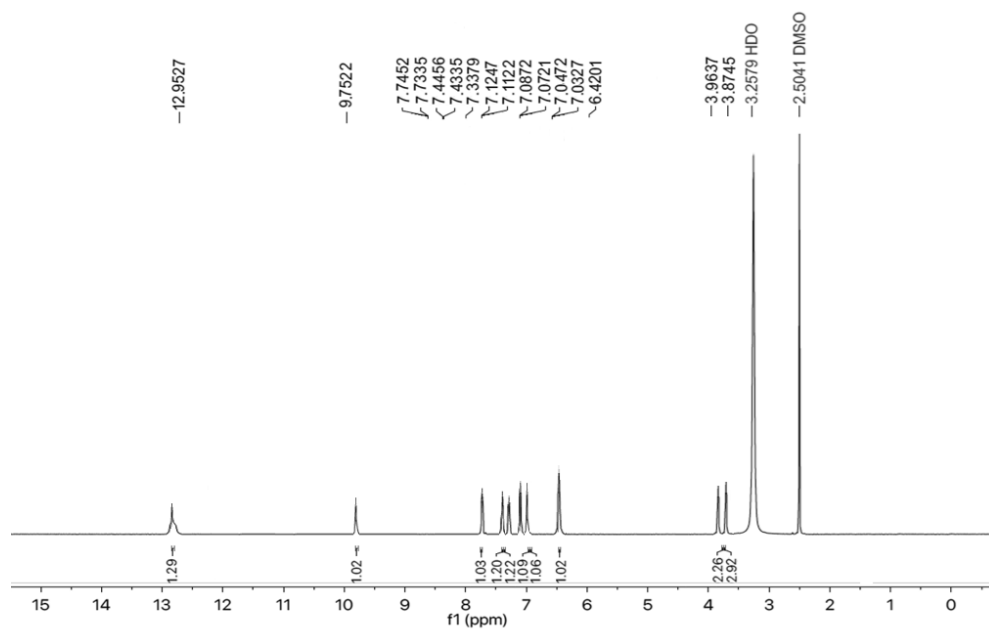


Figure-S.31 Proton spectral analysis of compound-11

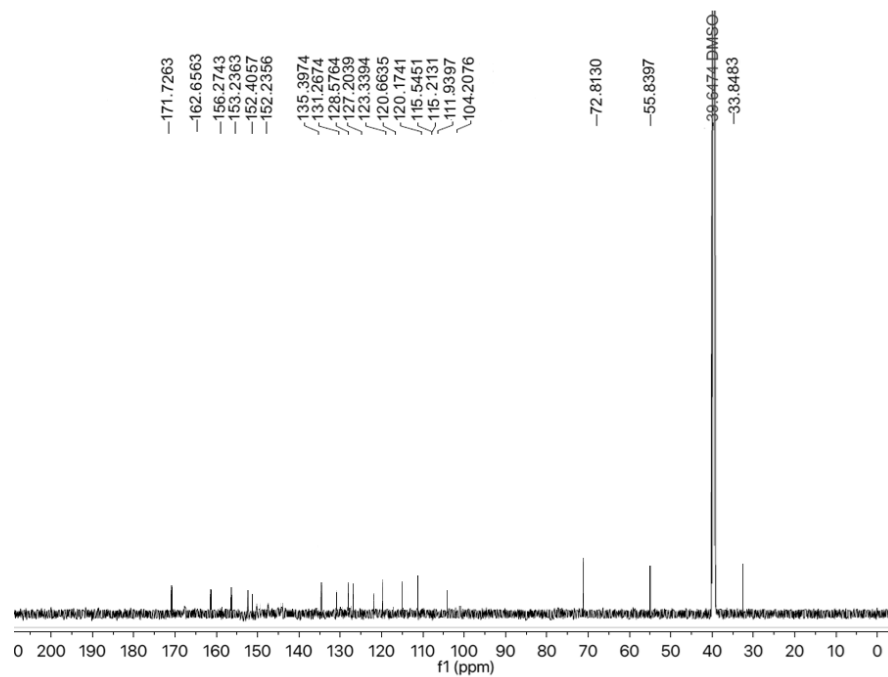


Figure-S.32 Carbon spectral analysis of compound-11



Figure-S.33 IR analysis of compound-11

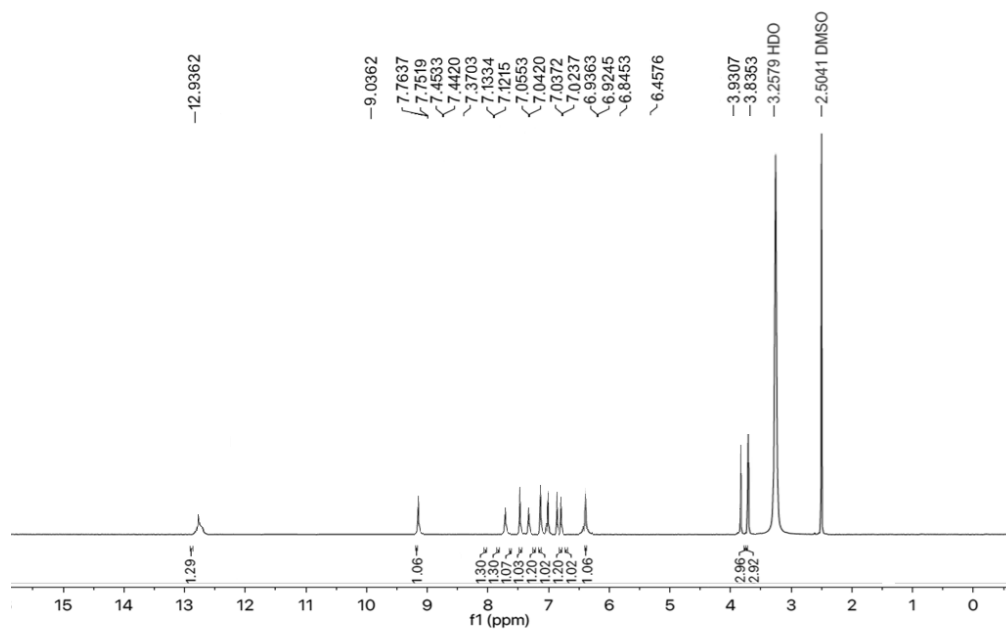


Figure-S.34 Proton spectral analysis of compound-12

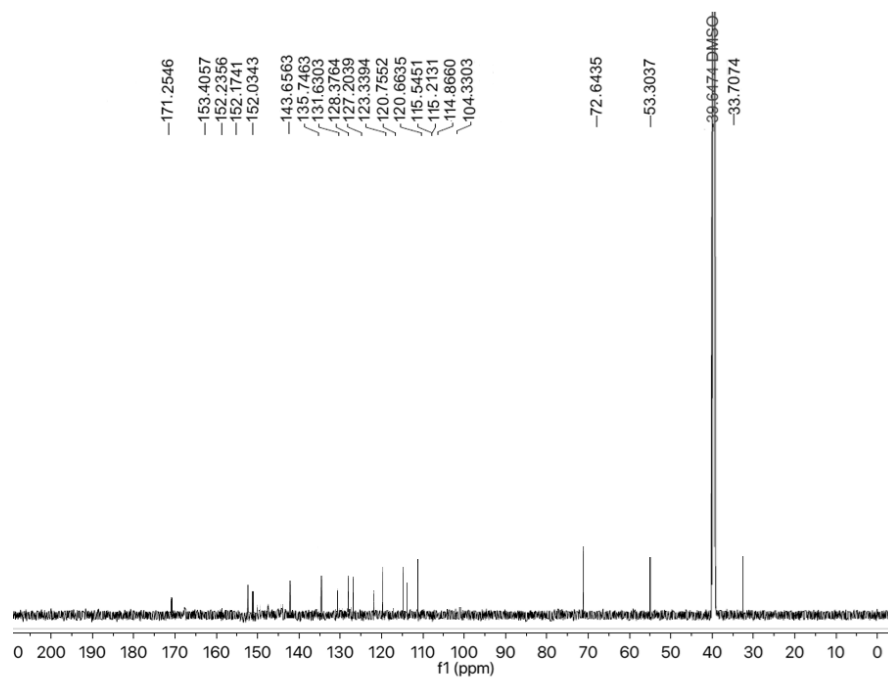


Figure-S.35 Carbon spectral analysis of compound-12

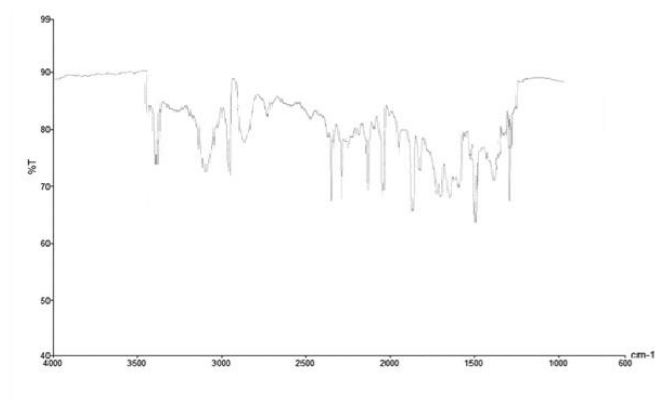


Figure-S.36 IR analysis of compound-12

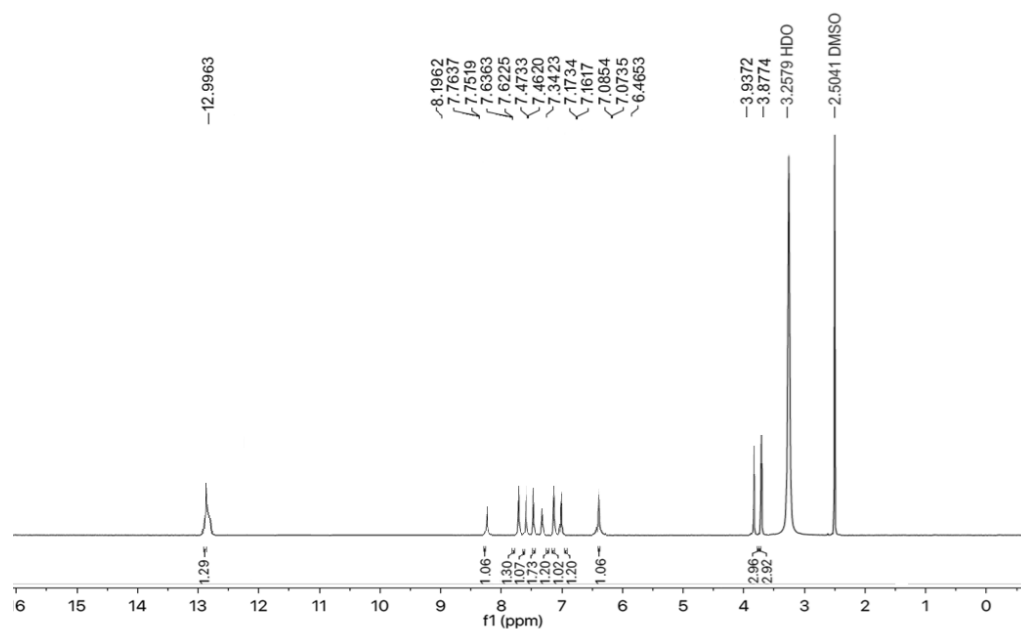


Figure-S.37 Proton spectral analysis of compound-13

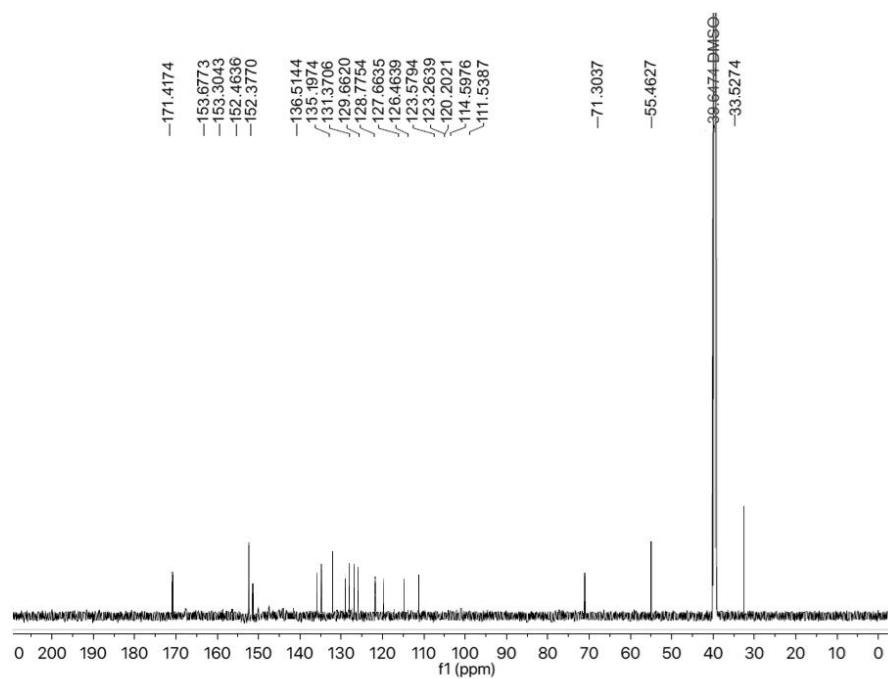


Figure-S.38 Carbon spectral analysis of compound-13

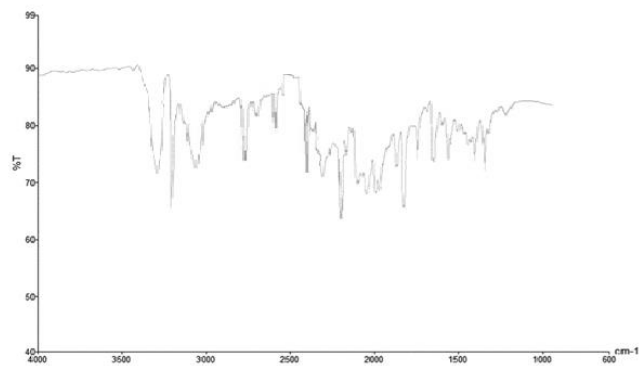


Figure-S.39 IR analysis of compound-13

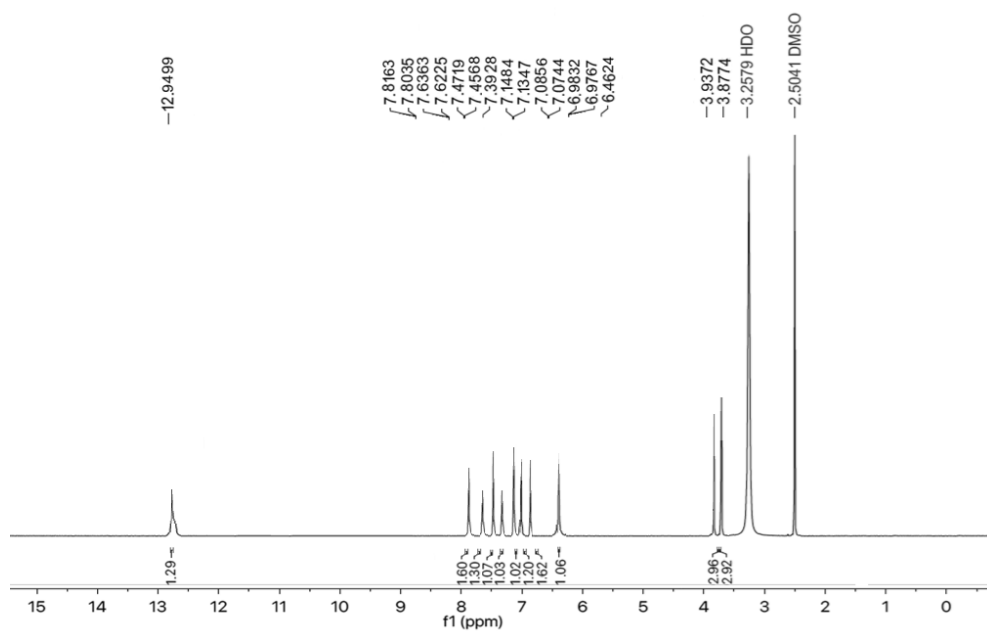


Figure-S.40 Proton spectral analysis of compound-14

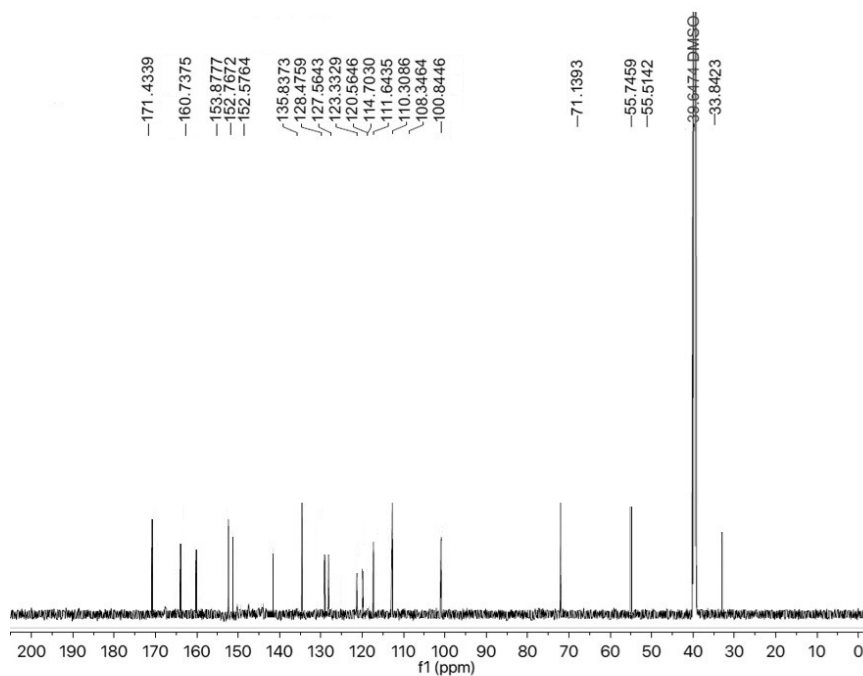


Figure-S.41 Carbon spectral analysis of compound-14

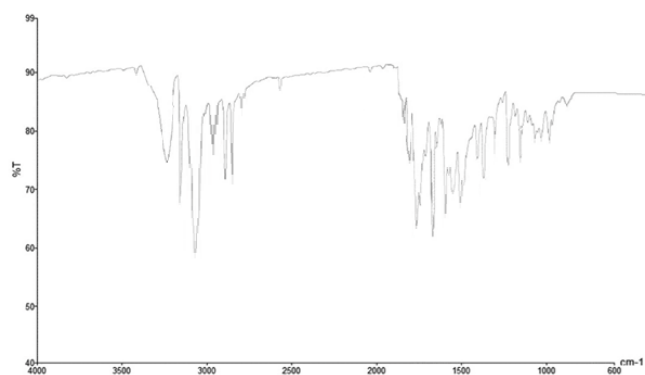


Figure-S.42 IR analysis of compound-14

S.2 AChE and BuChE assay protocols

With a little bit modification, inhibitory potential against AChE and BuChE was investigated according to the reported protocol [44]. The total volume of the reaction mixture was kept at 100 μ L, containing 60 μ L of Na_2HPO_4 buffer with a concentration of 50 mM and a pH of 7.7. Added test

compound (well-1) with volume of 10 μ L and a concentration of 0.5mM followed by the addition of an enzyme of 10 μ L (0.005 unit well-1). Pre-incubation of substances at 37°C for 10 min was achieved. Reaction was started by addition of 10 μ L of 0.5mM well-1 substrate (acetylthiocholine iodide/ butyrylthiocholine chloride) followed by addition of 10 μ L DTNB (0.5mM well-1). Absorbance at 405nm was measured after 15 min of incubation at 37°C using the 96-well plate reader Synergy HT, BioTek, US. All experiments were done with their respective controls in triplicate. Standard drug used was Donepezil. The % inhibition was computed using the following equation

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Control EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) was used for the calculation of IC₅₀ values.

S.3 Molecular docking protocol

The crystal structure was retrieved from the protein data bank (PDB) and further, the structure was optimized by removing the water molecules, hetero atoms, and cofactors. Hydrogen bonds, missing atoms, and charges were computed. The synthesized pyrazolone derived thiazolidinone based chalcone scaffolds used in these docking studies was prepared and optimized using built and Ligand Preparation module implemented in Discovery Studio 2018 (Dassault Systemes BIOVIA, USA). For the purpose of docking; Gold docking tool was used, Ligand preparation includes generating various tautomer's, assigning bond orders and stereochemistry. Additionally, receptor grid was generated around the AChE and BuChE active site by choosing centroid of complexed ligand (Montbretin A). The active site was defined with a radius of 12 Å around the Montbretin A binding site. Docking calculations were accomplished using Chem PLP scoring function [45].

S.3 DFT assay protocol

The geometric parameters and energies were computed by density functional theory at the B3LYP/CEP-31G level of theory, using the GAUSSIAN 98W package of the programs [46], on geometries that were optimized at CEP-31G basis set. The high basis set was chosen to detect the energies at a highly accurate level. The atomic charges were computed using the natural atomic orbital populations. The B3LYP is the key word for the hybrid functional [47], which is a linear combination of the gradient functionals proposed by Becke [48] and Lee, Yang and Parr [49], together with the Hartree-Fock local exchange function [50]. UV spectra were recorded in Rigol,

Ultra- 3000 series in Enzymology and Fungal Biotechnology Lab, Faculty of Science, Zagazig University.

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