Supporting Information

Solvent and Catalyst-Free Mechanochemical Synthesis of Indenyl-Derived Thiazolone imine, Thiazolidin-4-one and Thiazolidin-4-ol Hybrids: Anticancer Evaluation and Molecular Modeling Studies

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1. Experimental Section:

All the chemicals, catalyst were purchased from Sigma-Aldrich and used without any further purification. Solvents were purchased from commercial supplier and distilled prior to use. The progress of reactions was monitored by thin layer chromatography with TLC Silica gel 60 F254 purchased from Merck. Column chromatography was performed on silica gel (60–120 mesh). The IR spectra were recorded on PerkinElmer Frontier FT-IR spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker Ultra shield, Avance II model NMR spectrometer. Chemical shifts of ^{1}H NMR and ^{13}C NMR are reported in parts per million (ppm) from tetramethyl silane (TMS) as an internal standard in $CDCl_3$/DMSO-d_6 as a solvent. Mass spectra were recorded on AB SCIEX QTRAP 3200 model LC-MS spectrophotometer.

2. Biological study magnifying anticancer activity:

Cancer is the most frequent life threatening disease when certain body cells proliferate out of control and invade other body areas. The possibility that tumors would acquire resistance to chemotherapeutic drugs (such as doxorubicin) remains a major hurdle to the successful treatment of many malignancies. Drug resistance in cancer cells has been linked to a number of causes, including altered tumor microenvironment, high rate of drug metabolism in resistant cells, genetic and epigenetic changes in cancer cells and impaired drug delivery to tumors.

According to the literature, thiozole core have garnered plenty of interest in the field of drug discovery due to their diverse and extensive spectrum of biological actions. Based on the above mentioned promising aspects, this strategy of this work aimed to synthesize the bioactive indane based thiazole derivatives and examined the synthesized compounds as anticancer agents using the SRB assay and the results showed have promising activity.

3. General procedure for the synthesis of compounds (4a–h)

An equimolar mixture of 5-aminoindane (1.0 mmol) and substituted phenyl isothiocyanate (1.0 mmol) was added in mortar and pestle (Table 2). All the contents were grinded for 10-12 min, to generate thiocarbamide in situ. To this reaction mixture, substituted 2-bromoacetophenone (1.0 mmol) was added and the progress of the reaction was monitored through TLC. After TLC indicated that the reaction was completed upon the disappearance of starting materials and the formation of the product, work up has been done, the product was extracted in 3×5 ml of ethyl

acetate, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (60–120 mesh) using EtOAc/n-hexane (5:95) gradients to give pure product (**4a-h**) and characterized using ^1H, ^{13}C NMR and mass spectrometry.

General procedure for the synthesis of compounds (6a-e)

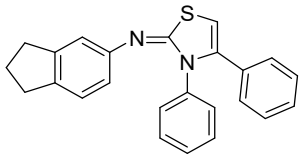
An equimolar mixture of 5-aminoindane (1.0 mmol) and substituted phenyl isothiocyanate (1.0 mmol) was added in mortar and pestle (Table 3). All the contents were grinded for 10-12 min, to generate thiocarbamide in situ. To this reaction mixture, ethylbromoacetate (1.0 mmol) was slowly added and the progress of the reaction was monitored through TLC. After TLC indicated that the reaction was completed upon the disappearance of starting materials and the formation of the product, work up has been done, the product was extracted in 3×5 ml of ethyl acetate, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (60–120 mesh) using EtOAc/n-hexane (5:95) gradients to give pure product **(6a–e)** and characterized using ^1H, ^{13}C NMR and mass spectrometry.

General procedure for the synthesis of compounds (8a–e)

An equimolar mixture of 5-aminoindane (1.0 mmol) and substituted phenyl isothiocyanate (1.0 mmol) was added in mortar and pestle (Table 4). All the contents were grinded for 10-12 min, to generate thiocarbamide in situ. To this reaction mixture, dropwise addition of 3-bromo-1,1,1-trifluoropropan-2-one (1.2 mmol) was done and the after 15-20 min check the progress of the reaction which was monitored through TLC. After TLC indicated that the reaction was completed upon the disappearance of starting materials and the formation of the product, work up has been done, the product was extracted in 3×5 ml of ethyl acetate, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (60–120 mesh) using EtOAc/n-hexane (5:95) gradients to give pure product (**8a-e**) and characterized using ^1H, ^{13}C NMR and mass spectrometry.

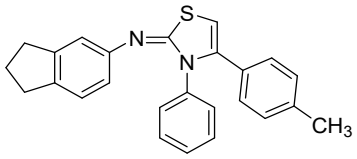
4. Physical and spectroscopic data:

***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3,4-diphenylthiazol-2(3*H*)-imine (4a):**



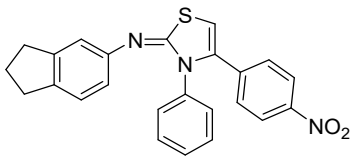
Light yellow solid; Yield: 74%, mp: 114-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.03 (m, 13H), 6.01 (s, Hz, 1H), 2.84 (m, 4H), 2.04 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.54, 145.24, 144.19, 140.59, 137.44, 135.41, 131.51, 129.40, 128.32, 126.52, 124.99, 123.58, 122.07, 119.59, 117.99, 97.40, 32.82, 25.40; LC-MS: *m/z* $(M)^+$ 369.1.

***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-phenyl-4-(*p*-tolyl)thiazol-2(3*H*)-imine (4b):**



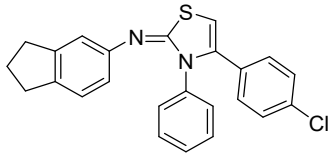
Brown solid; Yield: 80%, mp: 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.37 (m, 1H), 7.32–7.27 (m, 2H), 7.22 (d, *J* = 20.2 Hz, 1H), 7.12 (dt, *J* = 12.9, 6.3 Hz, 2H), 7.00 (dd, *J* = 18.2, 5.9 Hz, 6H), 5.88 (s, 1H), 2.95–2.81 (m, 4H), 2.27 (s, 3H), 2.12–2.00 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.22, 160.06, 145.06, 143.85, 140.38, 138.09, 129.37, 128.85, 128.09, 126.61, 124.84, 123.13, 121.81, 117.53, 96.23, 29.70, 25.38, 21.21; LC-MS: *m/z* $(M+1)^+$ 383.1.

***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-4-(4-nitrophenyl)-3-phenylthiazol-2(3*H*)-imine (4c):**



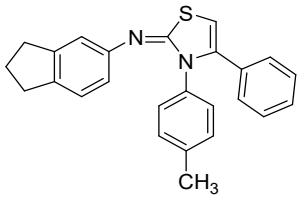
Yellow solid, Yield: 88%, mp: 130-132 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (m, 2H), 7.88–7.81 (m, 2H), 7.60 (d, 1H), 7.34–7.24 (m, 2H), 7.18 (m, 2H), 7.11 (d, *J* = 7.9 Hz, 1H), 7.05–6.99 (m, 2H), 6.88 (s, 1H), 2.85–2.68 (m, 4H), 1.96 (m, 7.5, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.46, 151.78, 149.90, 147.48, 144.83, 144.02, 142.27, 139.62, 138.55, 137.54, 129.31, 128.87, 127.33, 126.51, 125.34, 124.79, 124.19, 123.57, 121.95, 119.69, 117.76, 93.38, 32.22, 25.48; LC-MS: *m/z* $(M+1)^+$ 414.1.

***(E)*-4-(4-chlorophenyl)-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-phenylthiazol-2(3*H*)-imine (4d):**



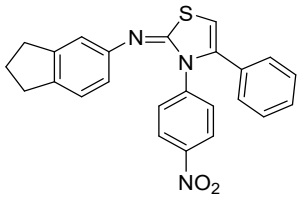
Yellow solid, Yield: 75%, mp: 62-64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 14.5, 6.8 Hz, 2H), 7.24 (dd, *J* = 6.8, 2.1 Hz, 2H), 7.18 (dd, *J* = 5.7, 3.6 Hz, 2H), 7.14 (dd, *J* = 7.7, 2.9 Hz, 1H), 7.12 (d, *J* = 2.1 Hz, 1H), 7.09–7.06 (m, 1H), 7.05–6.95 (m, 3H), 6.94–6.86 (m, 1H), 5.97 (s, 1H), 2.85 (m, 4H), 2.04 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.51, 152.08, 145.27, 144.16, 138.84, 135.56, 134.06, 133.44, 129.40, 128.97, 128.76, 128.27, 128.20, 127.65, 126.55, 126.31, 126.19, 125.13, 124.94, 124.72, 124.42, 123.25, 121.67, 119.01, 117.41, 98.46, 97.94, 32.55, 25.44; LC-MS: *m/z* $(M+1)^+$ 403.0.

***(E)-N*-(2,3-dihydro-1*H*-inden-5-yl)-4-phenyl-3-(*p*-tolyl)thiazol-2(3*H*)-imine (4e):**



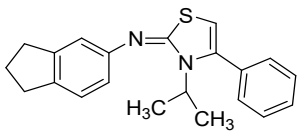
Yellow solid, Yield: 78%, mp: 136-138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (t, *J* = 11.0 Hz, 1H), 7.39 (d, *J* = 7.4 Hz, 1H), 7.20 (q, *J* = 5.9 Hz, 3H), 7.12 (t, *J* = 6.1 Hz, 6H), 6.95 (s, 1H), 2.91–2.81 (m, 4H), 2.33–2.28 (m, 3H), 2.04 (dd, *J* = 15.6, 7.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.03, 149.62, 145.13, 143.91, 140.41, 139.02, 137.38, 135.92, 132.62, 131.92, 130.07, 129.65, 128.64, 128.33, 128.22, 126.71, 124.92, 124.61, 121.66, 119.33, 117.71, 96.99, 29.82, 25.50, 21.07; LC-MS: *m/z* $(M+1)^+$ 403.0.

***(E)-N*-(2,3-dihydro-1*H*-inden-5-yl)-3-(4-nitrophenyl)-4-phenylthiazol-2(3*H*)-imine (4f):**



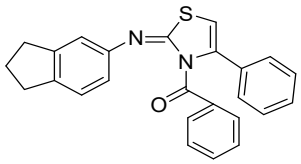
Yellow solid, Yield: 84%, mp: 180-182 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 3H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.36–7.35 (m, 1H), 7.23–7.19 (m, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.13–7.09 (m, 4H), 6.99–6.96 (m, 1H), 6.09 (s, 1H), 2.89–2.81 (m, 4H), 2.09–2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.59, 147.23, 145.41, 135.37, 128.73, 128.52, 128.40, 126.60, 125.64, 124.77, 122.15, 97.36, 32.71, 25.50; LC-MS: *m/z* $(M+1)^+$ 414.1.

***(E)-N*-(2,3-dihydro-1*H*-inden-5-yl)-3-isopropyl-4-phenylthiazol-2(3*H*)-imine (4g):**



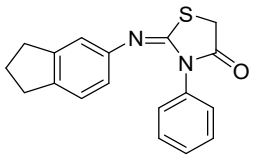
Red semi solid, Yield: 70%, mp: 62-64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 22.9, 7.1 Hz, 1H), 7.68–7.34 (m, 5H), 7.23–6.93 (m, 3H), 4.93 (s, 1H), 4.08 (s, 1H), 2.98–2.76 (m, 4H), 2.14–2.04 (m, 2H), 1.25 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.54, 130.20, 128.97, 128.02, 124.86, 124.57, 121.36, 119.13, 110.44, 50.82, 31.57, 25.81, 22.84; LC-MS: *m/z* $(M+1)^+$ 335.1.

(E)-(2-((2,3-dihydro-1H-inden-5-yl)imino)-4-phenylthiazol-3(2H)-yl)(phenyl)methanone **(4h):**



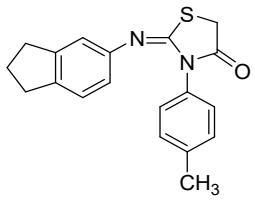
Yellow solid, Yield: 72%, mp: 48-50 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (s, 2H), 8.11 (d, *J* = 8.5 Hz, 1H), 7.88 – 7.80 (m, 3H), 7.60 (t, *J* = 7.4 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 4H), 6.69 (s, 1H), 4.67 (q, *J* = 7.1 Hz, 4H), 1.46 (t, *J* = 7.1 Hz, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.30, 162.63, 133.03, 128.85, 127.62, 107.24, 69.29, 29.59, 13.63; LC-MS: *m/z* $(M+1)^+$ 397.1.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-phenylthiazolidin-4-one **(6a):**



Brown solid, Yield: 74%, mp: 78-80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (t, J = 7.7 Hz, 1H), 7.45 – 7.29 (m, 3H), 7.21 (s, 1H), 7.18 – 7.09 (m, 2H), 6.92 (d, *J* = 9.5 Hz, 1H), 6.79 (s, 1H), 6.69 (d, *J* = 9.8 Hz, 1H), 3.97 (s, 2H), 3.01 – 2.85 (m, 4H), 2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.75, 155.43, 154.20, 148.30, 146.27, 145.75, 145.35, 140.50, 134.84, 132.64, 129.31, 129.09, 128.89, 127.99, 125.70, 125.20, 124.68, 123.89, 120.94, 118.47, 116.86, 32.91, 32.35, 25.49; LC-MS: *m/z* $(M+1)^+$ 309.0.

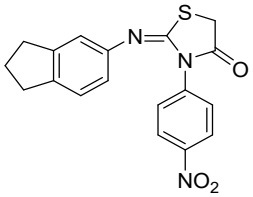
(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-(p-tolyl)thiazolidin-4-one **(6b):**



Light yellow solid, Yield: 80%, mp: 90-92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.07 (m, 5H), 6.84 – 6.75 (m, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 3.94 (s, 2H), 2.98 – 2.85 (m, 4H), 2.35 (d, *J* = 35.5 Hz, 3H), 2.13 – 2.04 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.79, 155.18, 146.39, 145.29, 140.42, 138.96,

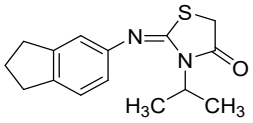
134.06, 132.71, 130.07, 129.67, 127.69, 125.71, 124.63, 123.90, 120.75, 118.52, 116.88, 32.92, 32.35, 25.49, 21.29; LC-MS: *m/z* $(M+1)^+$ 323.0.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-(4-nitrophenyl)thiazolidin-4-one **(6c):**



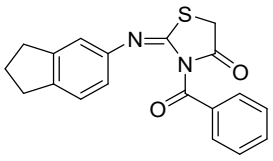
Yellow solid, Yield: 88%, mp: 102-104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 9.0 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.18 – 7.08 (m, 5H), 4.22 (s, 2H), 2.90 (dd, J = 14.1, 7.1 Hz, 4H), 2.09 – 2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.12, 158.38, 155.12, 145.19, 144.12, 133.44, 130.36, 126.63, 125.66, 124.61, 122.28, 118.90, 117.04, 33.67, 32.76, 25.76; LC-MS: *m/z* $(M+1)^+$ 354.0.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-isopropylthiazolidin-4-one **(6d):**



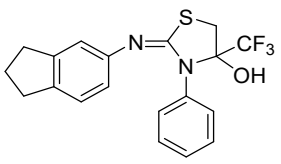
Red semi-solid, Yield: 75%, mp: 78-80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 2.9 Hz, 1H), 6.99 (dd, *J* = 7.9, 2.1 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 1H), 3.95 (s, 2H), 2.93 (dt, *J* = 10.3, 7.3 Hz, 4H), 2.14 – 2.05 (m, 2H), 1.12 (d, *J* = 6.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.85, 150.11, 146.90, 145.35, 133.29, 125.81, 124.91, 124.01, 111.03, 54.08, 32.98, 29.80, 25.55, 23.38; LC-MS: *m/z* $(M+1)^+$ 275.0.

(E)-3-benzoyl-2-((2,3-dihydro-1H-inden-5-yl)imino)thiazolidin-4-one **(6e):**



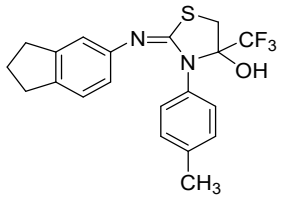
Yellow solid, Yield: 78%, mp: 46-48 °C; ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.36, 162.87, 145.12, 140.51, 136.08, 133.10, 131.58, 128.90, 127.75, 127.08, 124.44, 118.60, 116.92, 32.37, 29.68, 25.61; LC-MS: *m/z* $(M+1)^+$ 338.1.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-phenyl-4-(trifluoromethyl)thiazolidin-4-ol **(8a):**



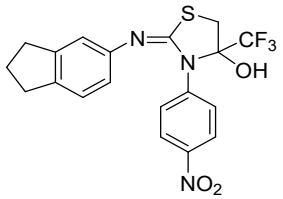
Yellow solid, Yield: 86%, mp: 50-52 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (d, *J* = 16.7 Hz, 1H), 8.18 (d, *J* = 27.1 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.45 – 7.28 (m, 3H), 7.28 – 7.21 (m, 1H), 7.21 – 7.06 (m, 3H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 3.81 (d, *J* = 12.6 Hz, 1H), 3.49 (d, *J* = 12.6 Hz, 1H), 2.88 – 2.78 (m, 4H), 2.02 (dt, J = 14.7, 5.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.19, 151.19, 144.43, 140.59, 137.87, 136.27, 130.96, 129.40, 128.82, 124.15, 122.63, 121.64, 120.61, 119.37, 117.47, 114.98, 32.34, 25.74, 22.57; LC-MS: *m/z* $(M+1)^+$ 379.0.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-(p-tolyl)-4-(trifluoromethyl)thiazolidin-4-ol **(8b):**



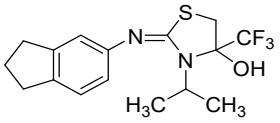
Yellow solid, Yield: 88%, mp: 104-106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.2 Hz, 1H), 7.27 – 7.20 (m, 3H), 7.17 (d, *J* = 1.8 Hz, 1H), 7.10 – 7.02 (m, 2H), 6.62 (dd, *J* = 15.3, 4.2 Hz, 2H), 6.49 (dd, *J* = 7.8, 2.1 Hz, 1H), 3.79 (d, *J* = 12.7 Hz, 1H), 3.47 (d, *J* = 12.7 Hz, 1H), 2.90 – 2.75 (m, 4H), 2.32 (s, 2H), 2.01 (ddd, *J* = 14.6, 13.0, 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.07, 148.69, 144.92, 143.87, 138.76, 137.59, 136.35, 135.78, 132.41, 130.68, 129.88, 128.77, 126.66, 124.85, 121.46, 119.39, 117.45, 34.83, 32.18, 25.72, 21.17; LC-MS: *m/z* $(M+1)^+$ 393.0.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-(4-nitrophenyl)-4-(trifluoromethyl)thiazolidin-4-ol (8c):



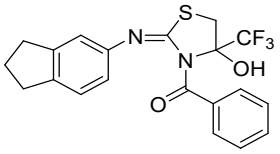
Pale yellow solid, Yield: 94%, mp: 112-114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.21 (d, *J* = 45.6 Hz, 2H), 8.19 (d, *J* = 9.2 Hz, 2H), 7.84 (d, *J* = 9.3 Hz, 2H), 7.34 (s, 1H), 7.19 (dd, *J* = 17.6, 7.2 Hz, 2H), 3.91 (d, *J* = 12.8 Hz, 1H), 3.58 (d, *J* = 12.8 Hz, 1H), 2.89 – 2.81 (m, 4H), 2.06 – 1.98 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.78, 146.94, 144.63, 142.65, 141.22, 137.42, 124.78, 124.63, 122.63, 121.98, 120.56, 32.89, 32.37, 25.73; ^{19}F NMR (377 MHz, $CDCl_3$) δ -110.42 (m, 3F), -89.57 (q, J = 31.9 Hz); LC-MS: *m/z* $(M+1)^+$ 424.0.

(E)-2-((2,3-dihydro-1H-inden-5-yl)imino)-3-isopropyl-4-(trifluoromethyl)thiazolidin-4-ol (8d):



Brown solid, Yield: 80%, mp: 52-54 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 2H), 7.13 (d, J = 8.5 Hz, 3H), 6.95 (dd, J = 7.9, 2.2 Hz, 1H), 6.61 (m, 1H), 3.39 (d, J = 11.5 Hz, 1H), 3.30 (d, J = 11.5 Hz, 1H), 2.86 (td, J = 7.4, 3.3 Hz, 4H), 2.11 – 2.02 (m, 2H), 1.13 (d, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.33, 145.75, 140.64, 136.25, 124.94, 120.53, 118.84, 42.37, 33.06, 32.42, 29.83, 23.29; LC-MS: *m/z* $(M+1)^+$ 345.0.

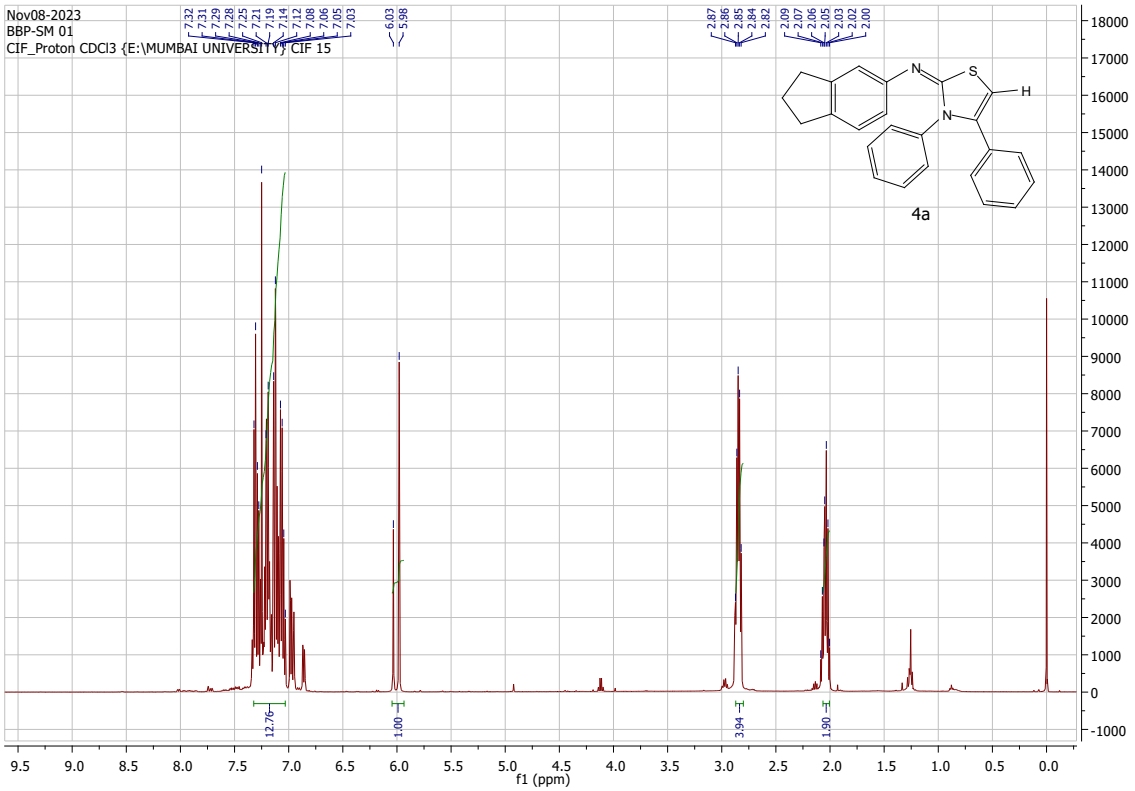
(E)-(2-((2,3-dihydro-1H-inden-5-yl)imino)-4-hydroxy-4-(trifluoromethyl)thiazolidin-3-yl)(phenyl)-methanone (8e):

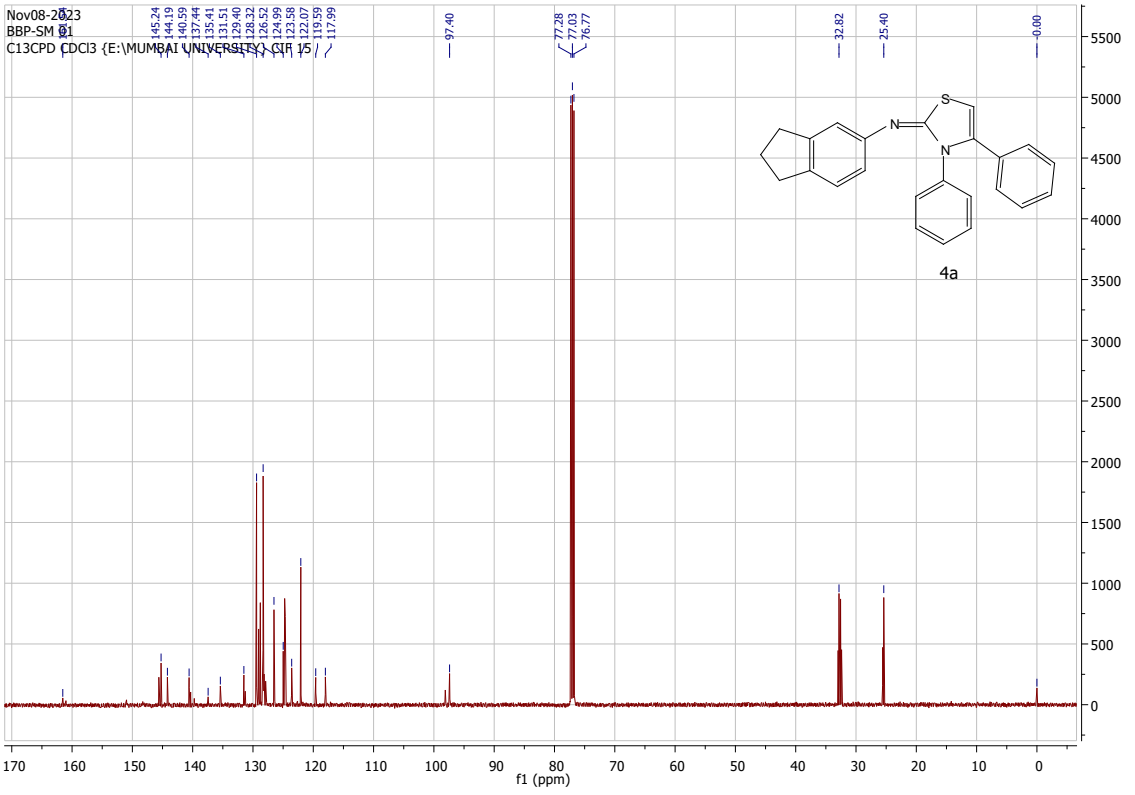


Off white solid, Yield: 75%, mp: 83-85 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (ddd, J = 26.1, 8.4, 1.2 Hz, 3H), 7.68 – 7.47 (m, 6H), 7.37 (dd, J = 8.0, 2.3 Hz, 1H), 2.97 – 2.88 (m, 4H), 2.15 – 2.07 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.51, 178.62, 167.02, 162.68, 145.26, 143.42, 135.76, 133.79, 133.24, 131.79, 129.29, 127.60, 124.63, 122.57, 120.59, 69.58, 33.04, 32.66, 29.80, 25.68, 13.84; LC-MS: *m/z* $(M+1)^+$ 409.0.

5. Copies of ^{1}H, ^{13}C NMR and LC-MS spectra

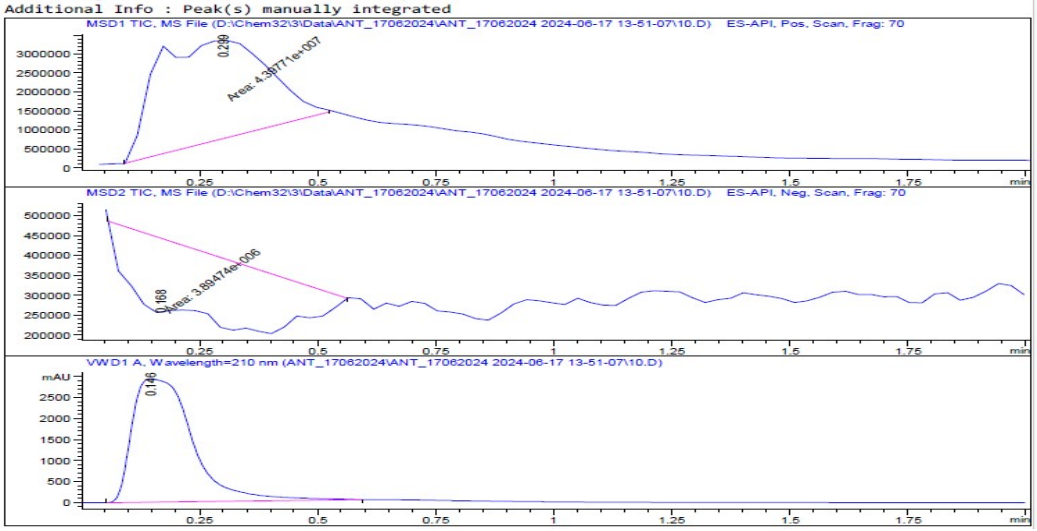
(E)-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3,4-diphenylthiazol-2(3*H*)-imine (4a):





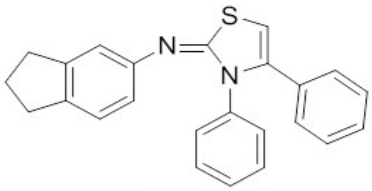
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\10.D
Sample Name: BBP-AI-01

Acq. Operator	: SYSTEM	Seq. Line	: 3
Acq. Instrument	: LCMS	Location	: 10
Injection Date	: 17-Jun-24 1:58:30 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Method	: D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS .M (Sequence Method)		
Last changed	: 17-Jun-24 1:51:07 PM by SYSTEM		
Additional Info	: Peak(s) manually integrated		



Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\10.D
Sample Name: BBP-AI-01

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.



MW= 368.4

Retention Time (MS)	MS Area	Mol. Weight or Ion
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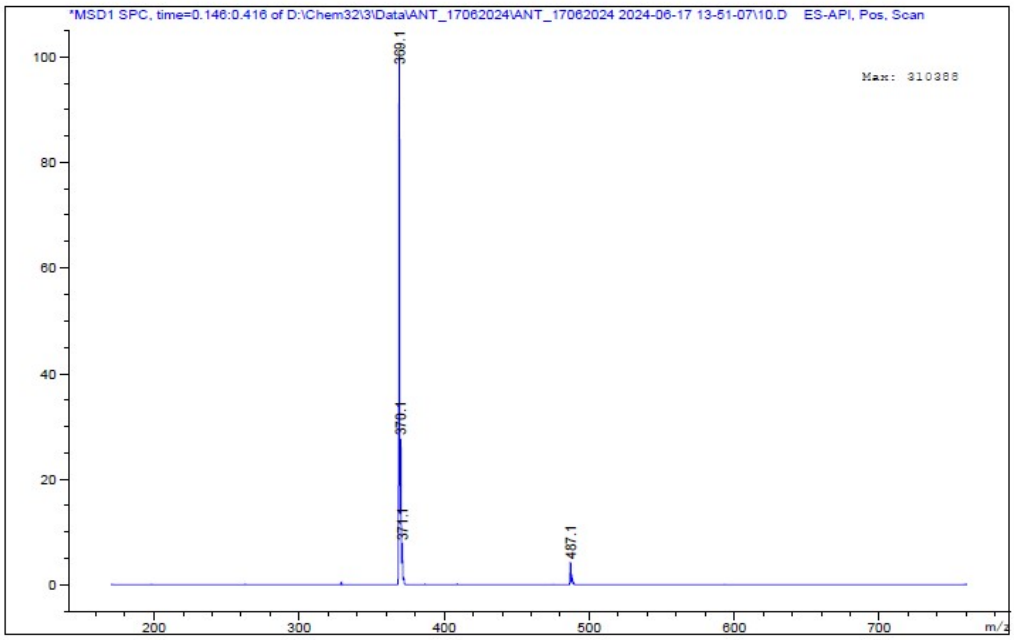
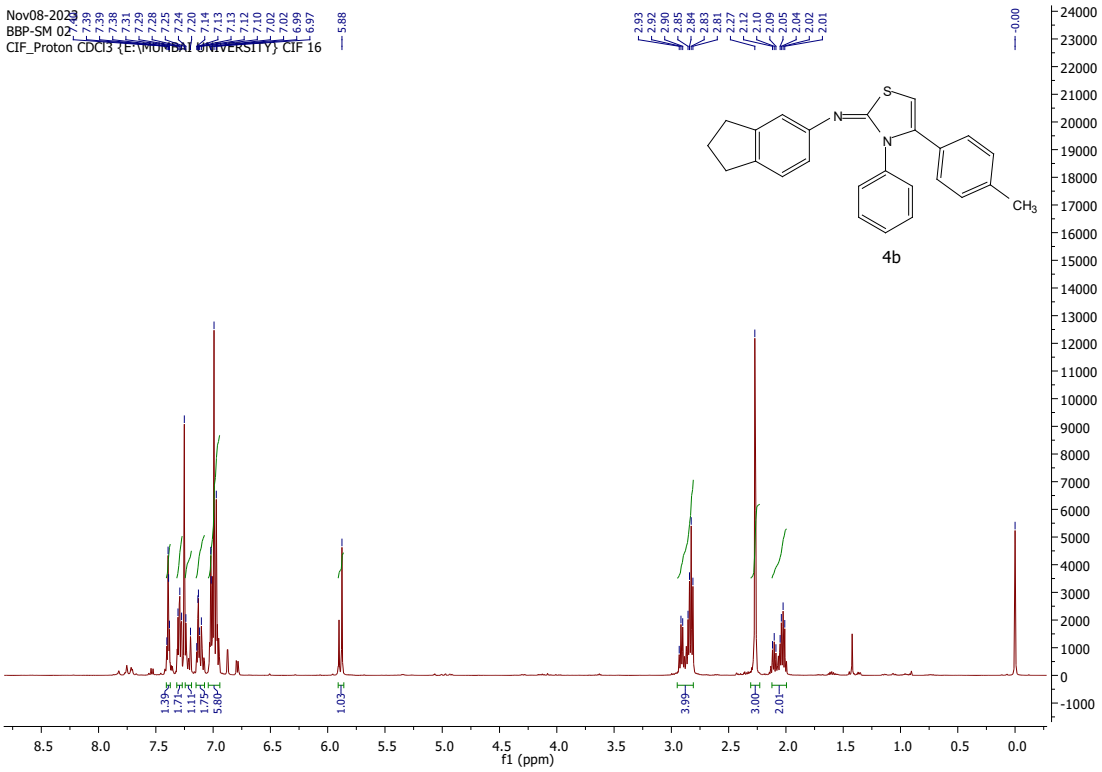
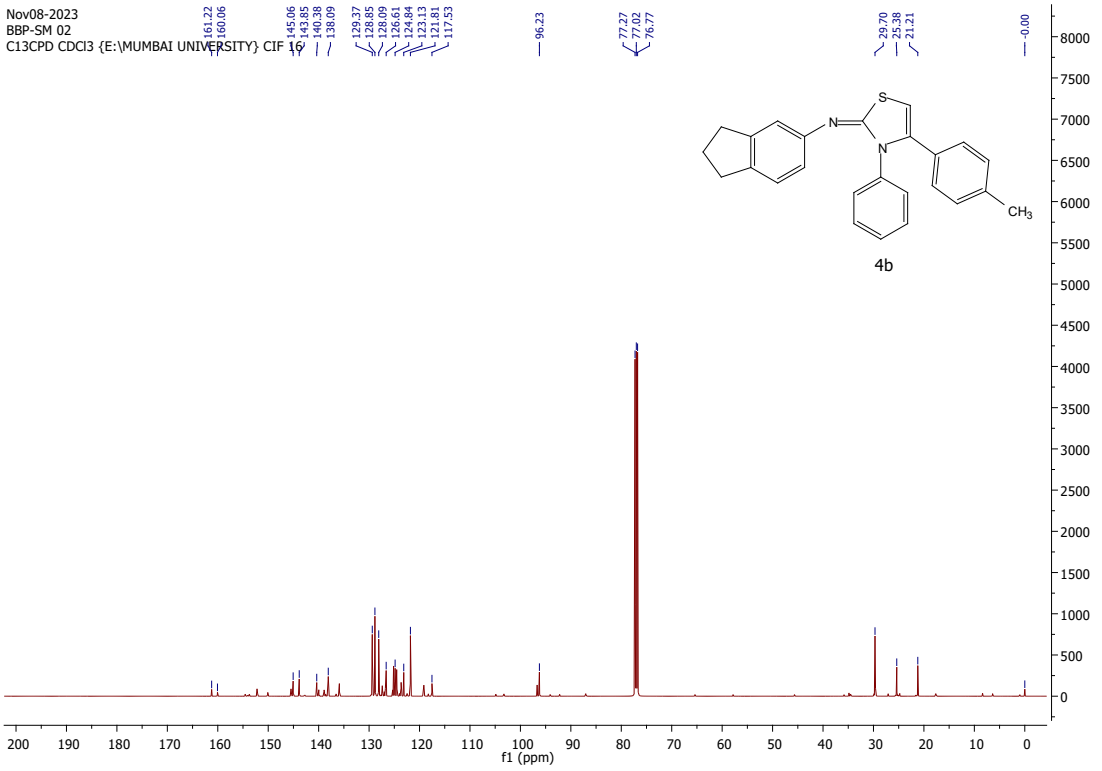
		369.10 I



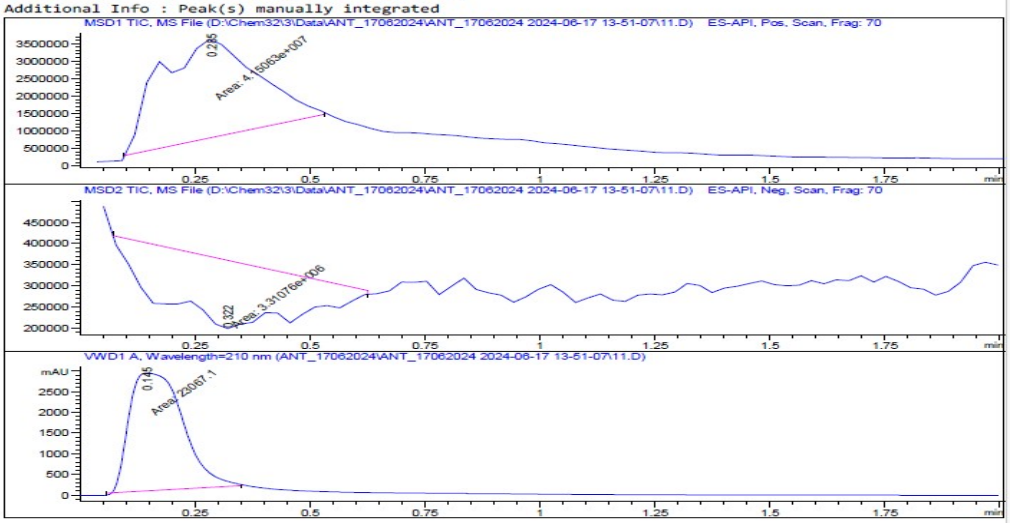
***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-phenyl-4-(*p*-tolyl)thiazol-2(3*H*)-imine (4b):**





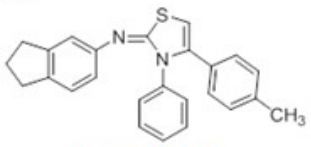
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\11.D
Sample Name: BBP-AI-02

Acq. Operator	: SYSTEM	Seq. Line	: 4
Acq. Instrument	: LCMS	Location	: 11
Injection Date	: 17-Jun-24 2:01:37 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Method	: D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS .M (Sequence Method)		
Last changed	: 17-Jun-24 1:51:07 PM by SYSTEM		
Additional Info	: Peak(s) manually integrated		



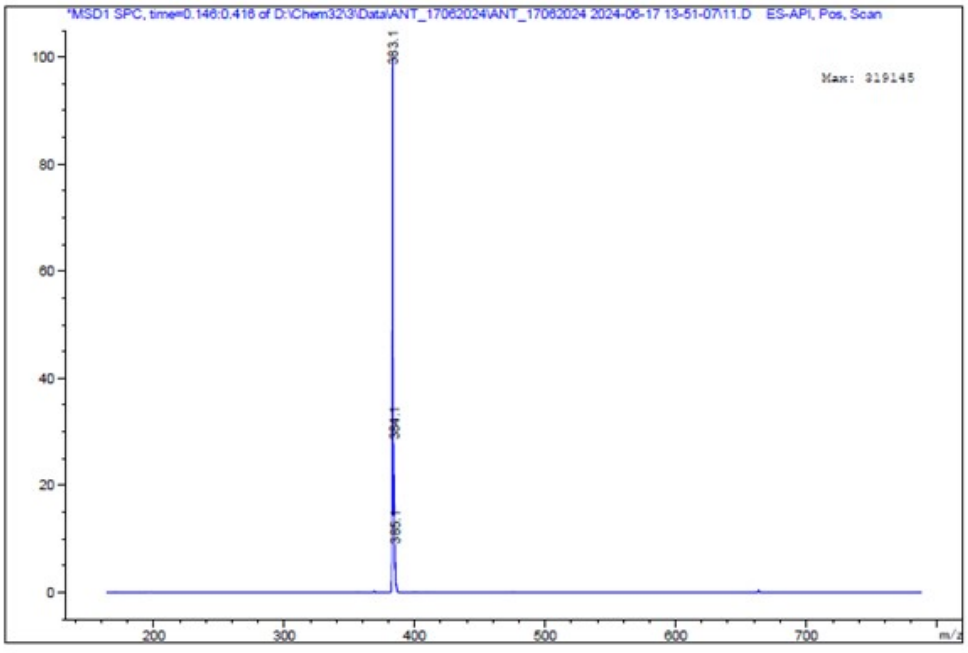
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\11.D
Sample Name: BBP-AI-02

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

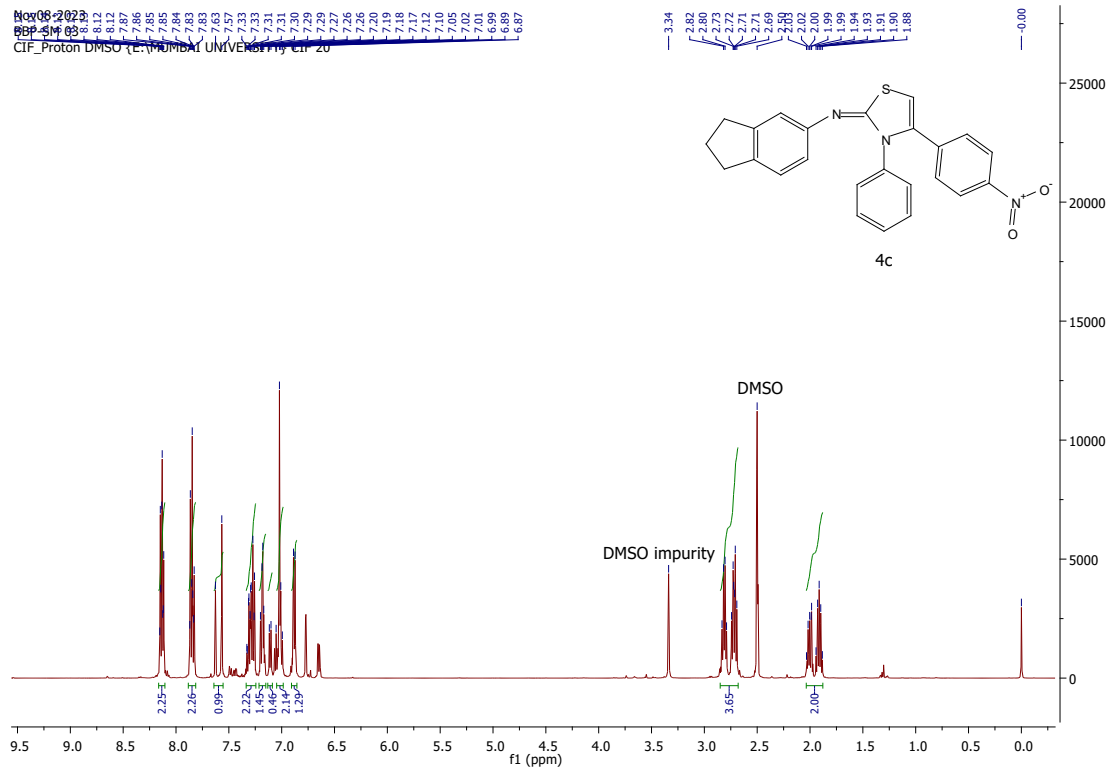


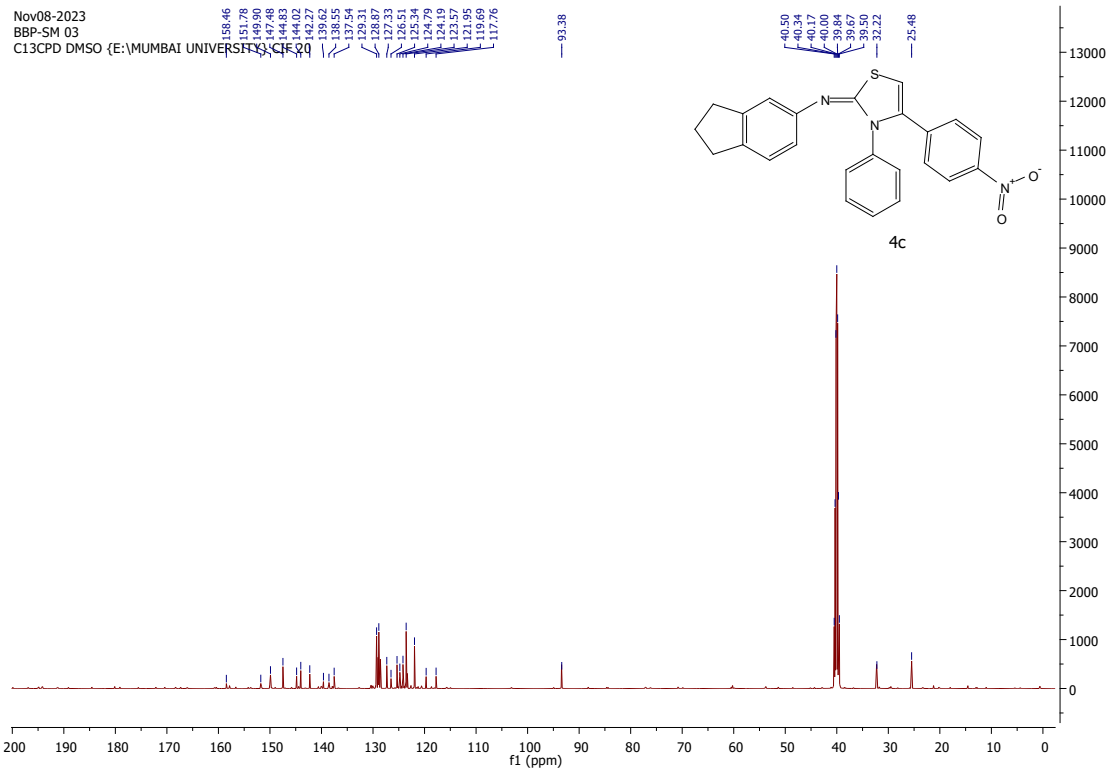
MW = 382.5

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.285	41506296	384.10 I
		383.10 I



***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-4-(4-nitrophenyl)-3-phenylthiazol-2(3*H*)-imine (4c):**





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Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\12.D
Sample Name: BBP-AI-03

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Acq. Operator    : SYSTEM                          Seq. Line :   5
Acq. Instrument  : LCMS                             Location :   12
Injection Date   : 17-Jun-24 2:04:36 PM                Inj :    1
                                                 Inj Volume : 20.000 µl
Method           : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS
                   .M (Sequence Method)
Last changed     : 17-Jun-24 1:51:07 PM by SYSTEM
Additional Info  : Peak(s) manually integrated
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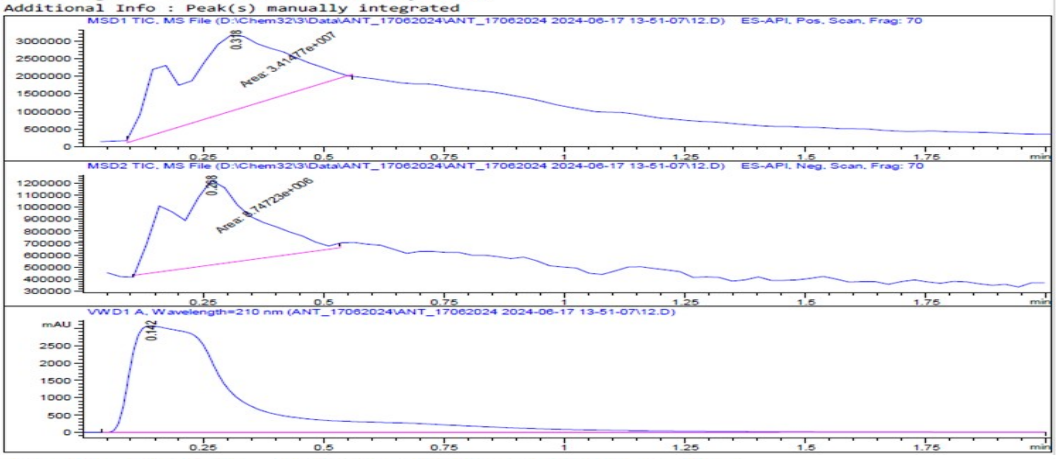
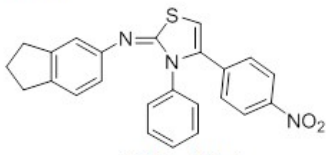


Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\12.D
Sample Name: BBP-AI-03

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.



MW= 413.4

Retention Time (MS)	MS Area	Mol. Weight or Ion
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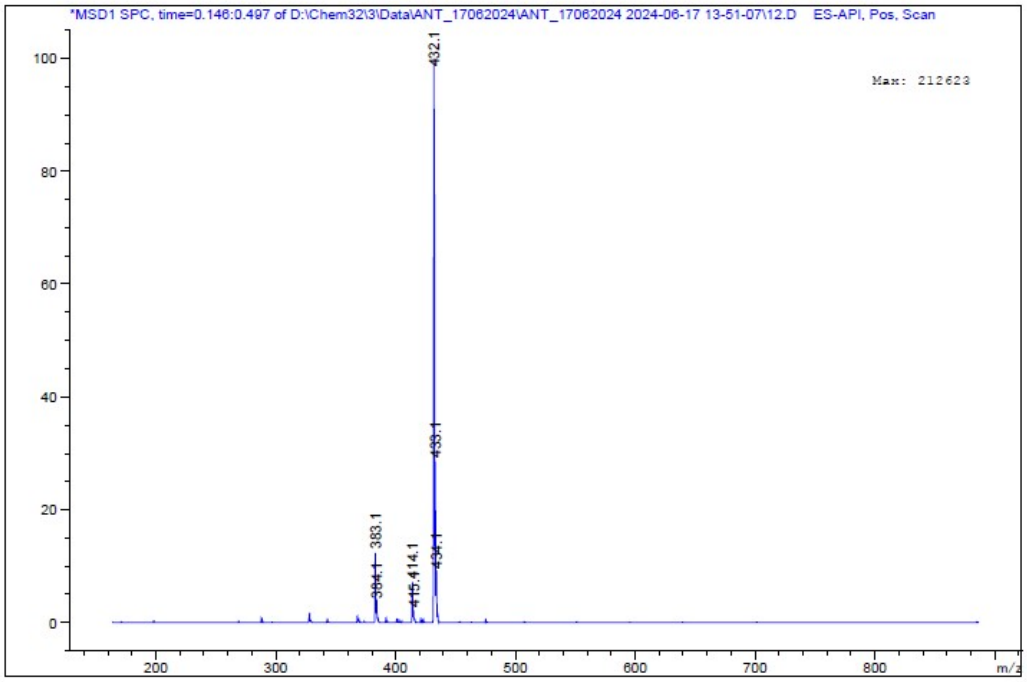
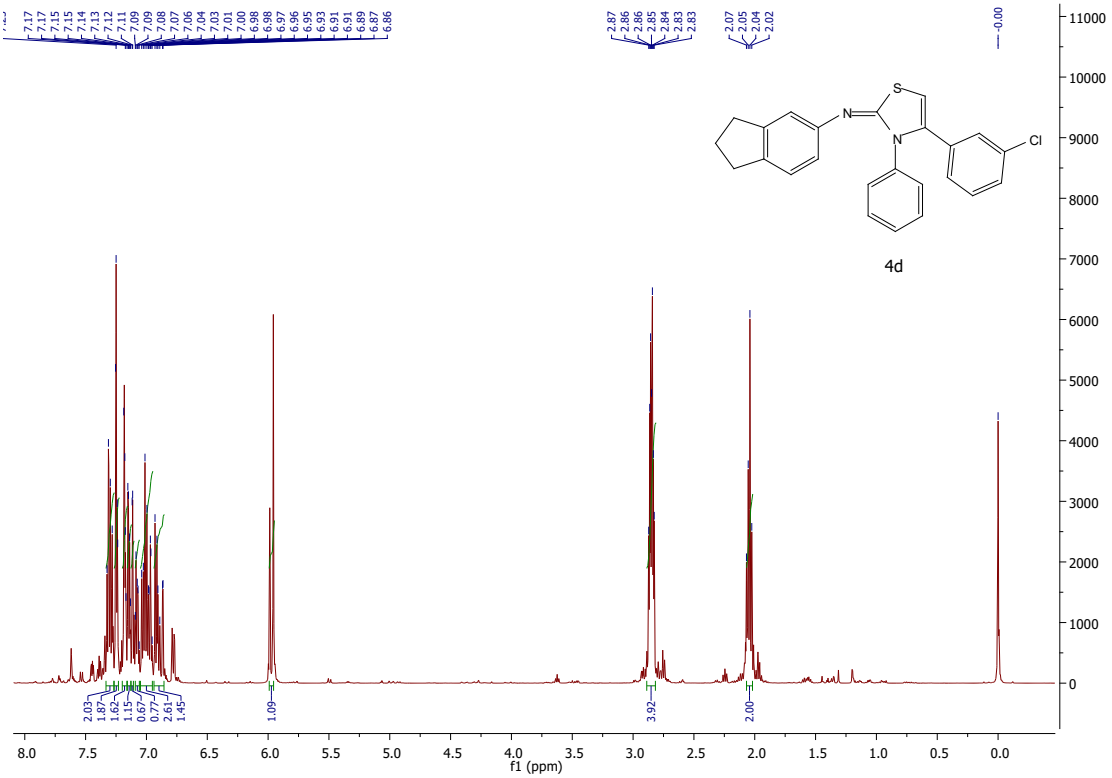
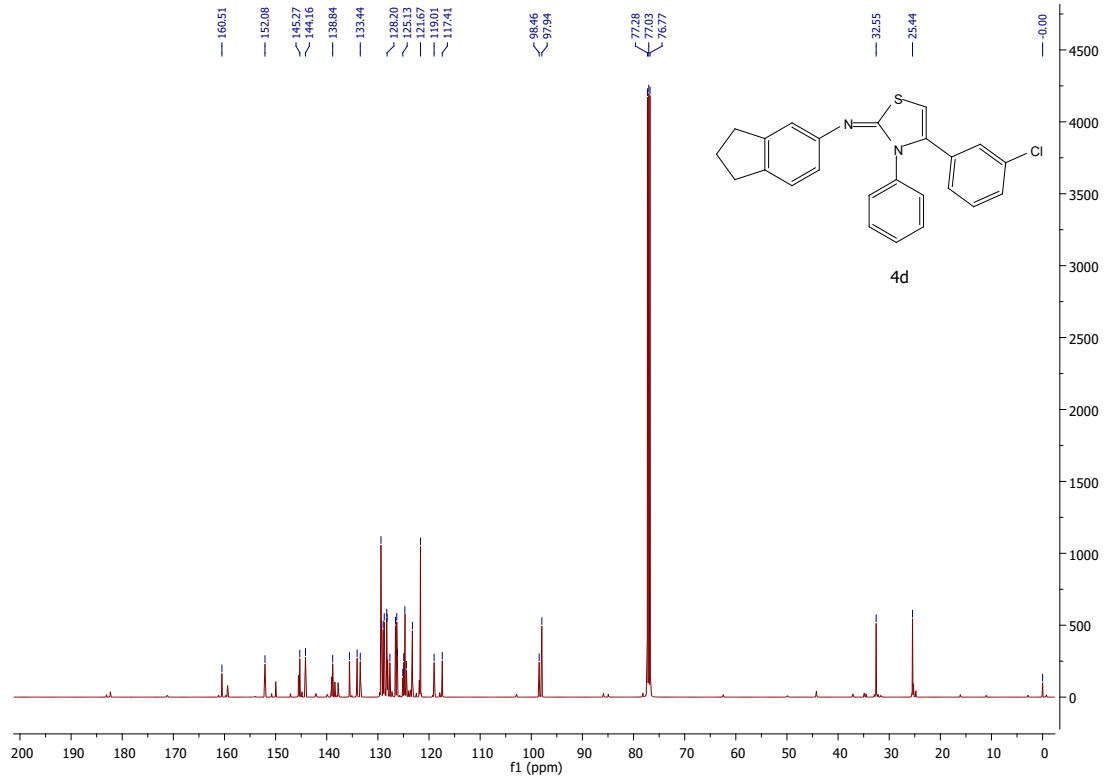
		432.10 I
		383.10 I



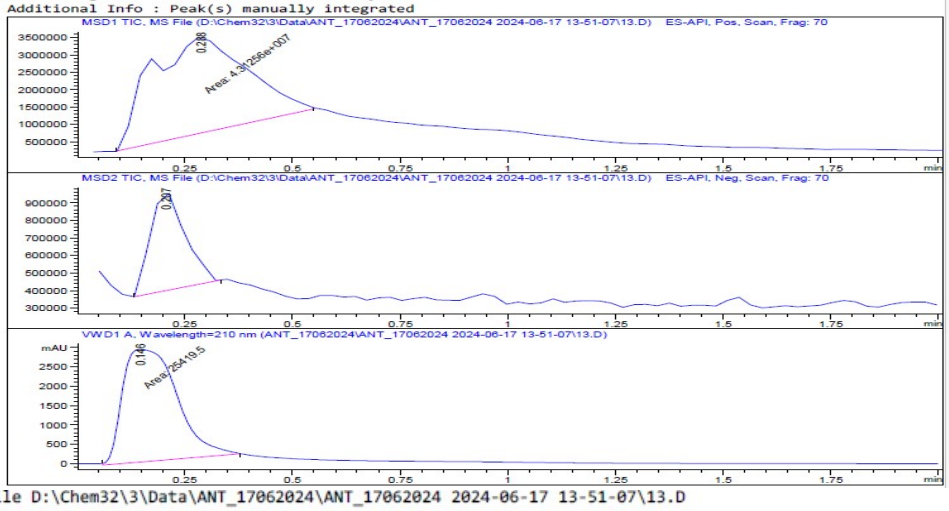
***(E)*-4-(4-chlorophenyl)-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-phenylthiazol-2(3*H*)-imine (4d):**





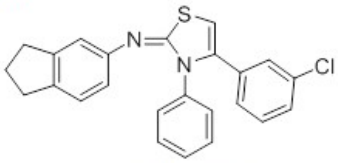
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\13.D
Sample Name: BBP-AI-04

Acq. Operator	: SYSTEM	Seq. Line	: 6
Acq. Instrument	: LCMS	Location	: 13
Injection Date	: 17-Jun-24 2:07:39 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Method	: D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS .M (Sequence Method)		
Last changed	: 17-Jun-24 1:51:07 PM by SYSTEM		
Additional Info	: Peak(s) manually integrated		



Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\13.D
Sample Name: BBP-AI-04

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.



MW = 402.0

Retention Time (MS)	MS Area	Mol. Weight or Ion
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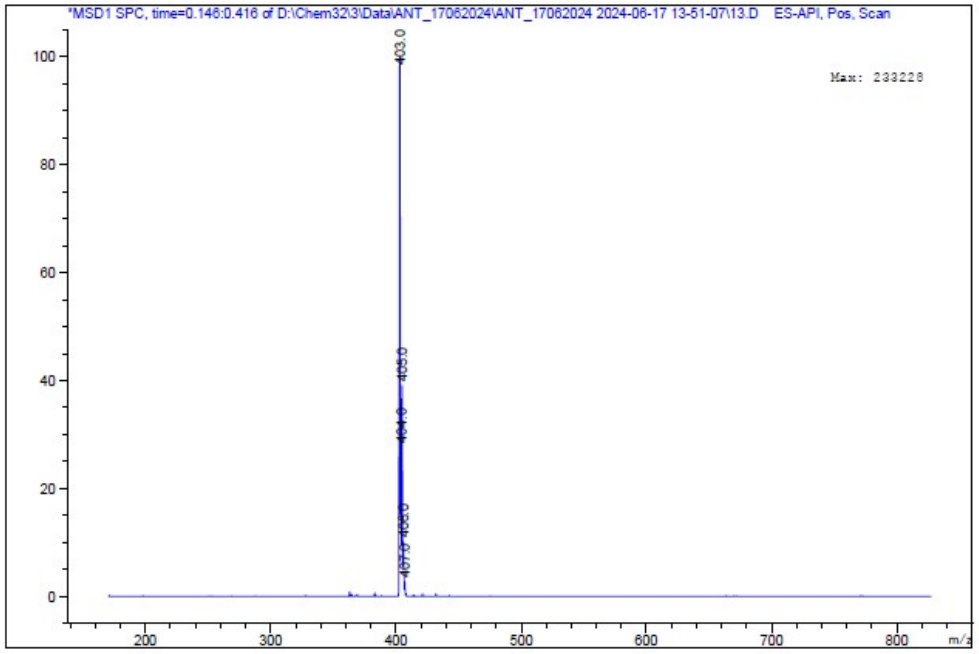
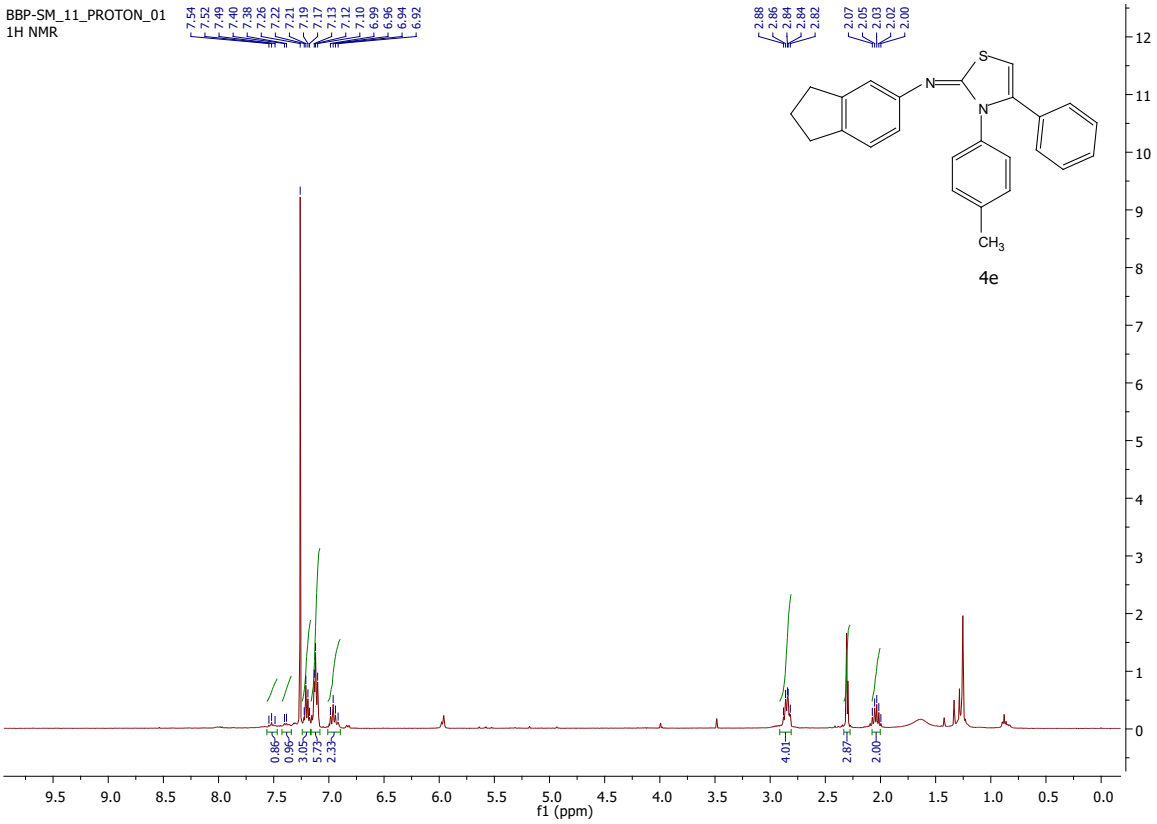
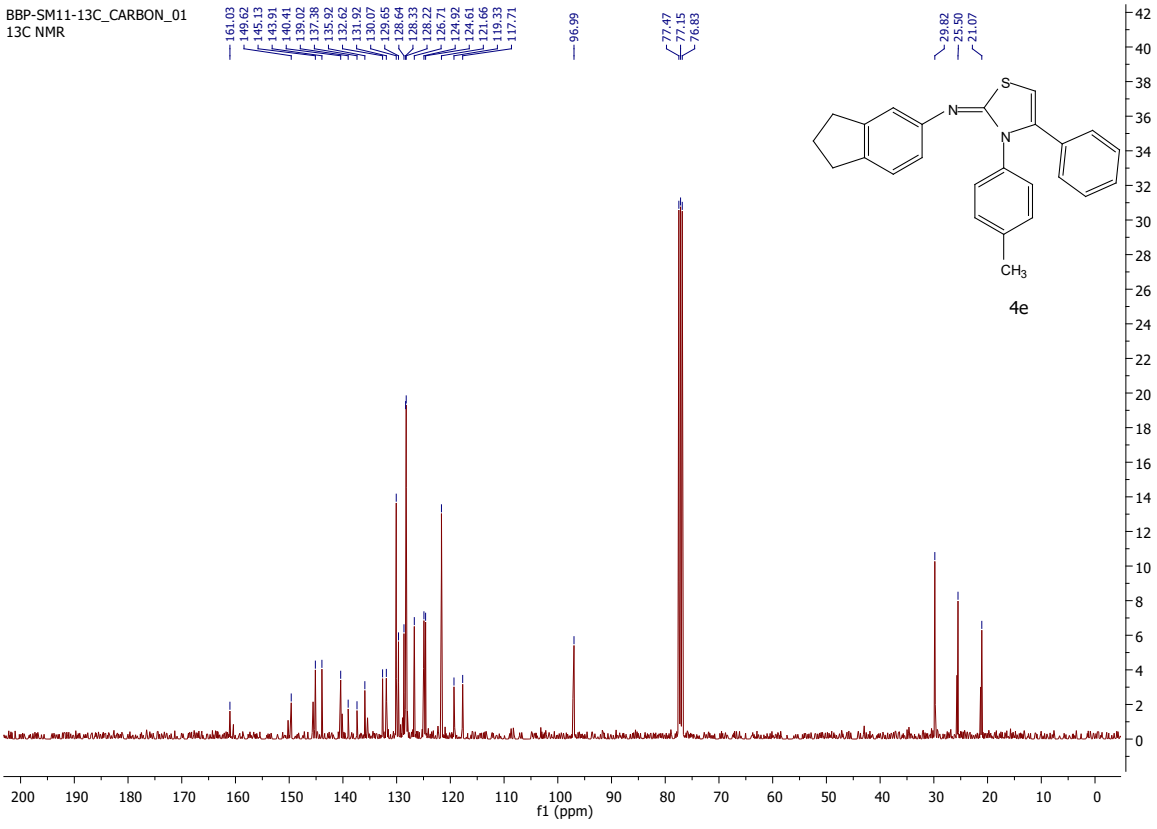
		405.00 I
		404.00 I
		403.00 I



***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-4-phenyl-3-(*p*-tolyl)thiazol-2(3*H*)-imine (4e):**

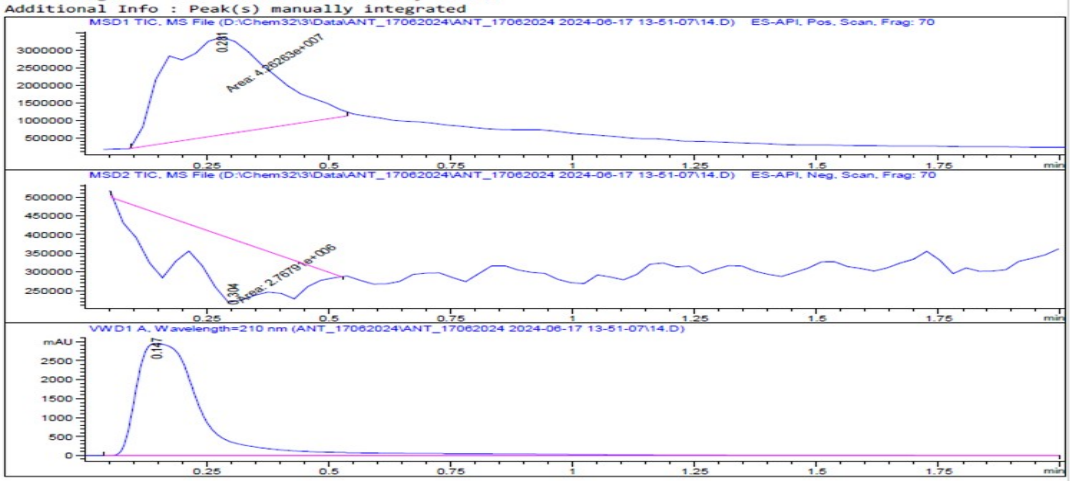




Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\14.D
Sample Name: BBP-AI-05

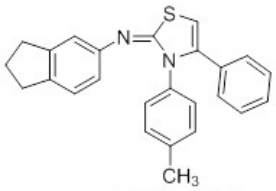
Acq. Operator	: SYSTEM	Seq. Line	: 7
Acq. Instrument	: LCMS	Location	: 14
Injection Date	: 17-Jun-24 2:10:43 PM	Inj	: 1
		Inj Volume	: 20.000 µl

Method : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS .M (Sequence Method)
Last changed : 17-Jun-24 1:51:07 PM by SYSTEM
Additional Info : Peak(s) manually integrated



Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\14.D
Sample Name: BBP-AI-05

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.



MW = 382.2

Retention Time (MS)	MS Area	Mol. Weight or Ion
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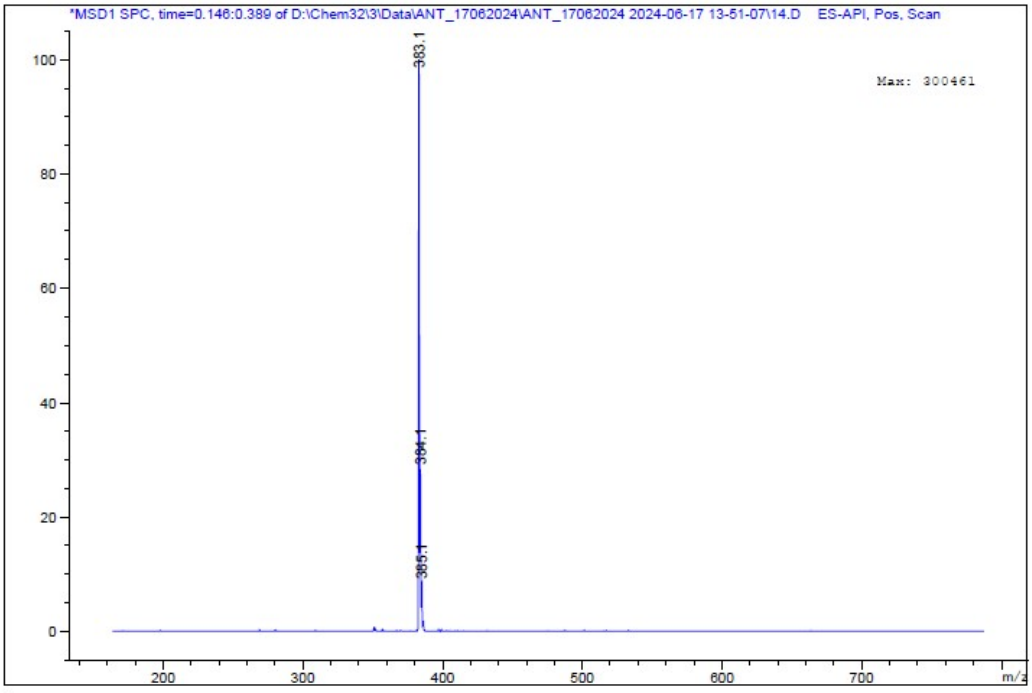
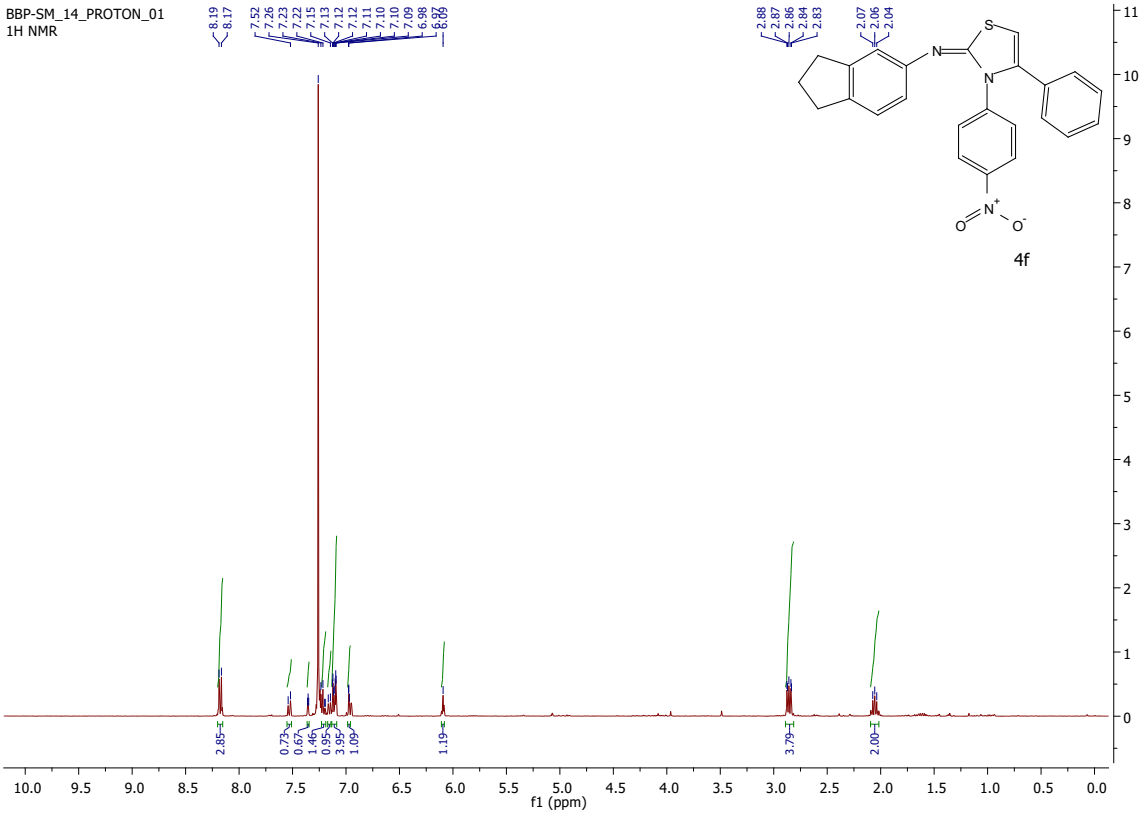
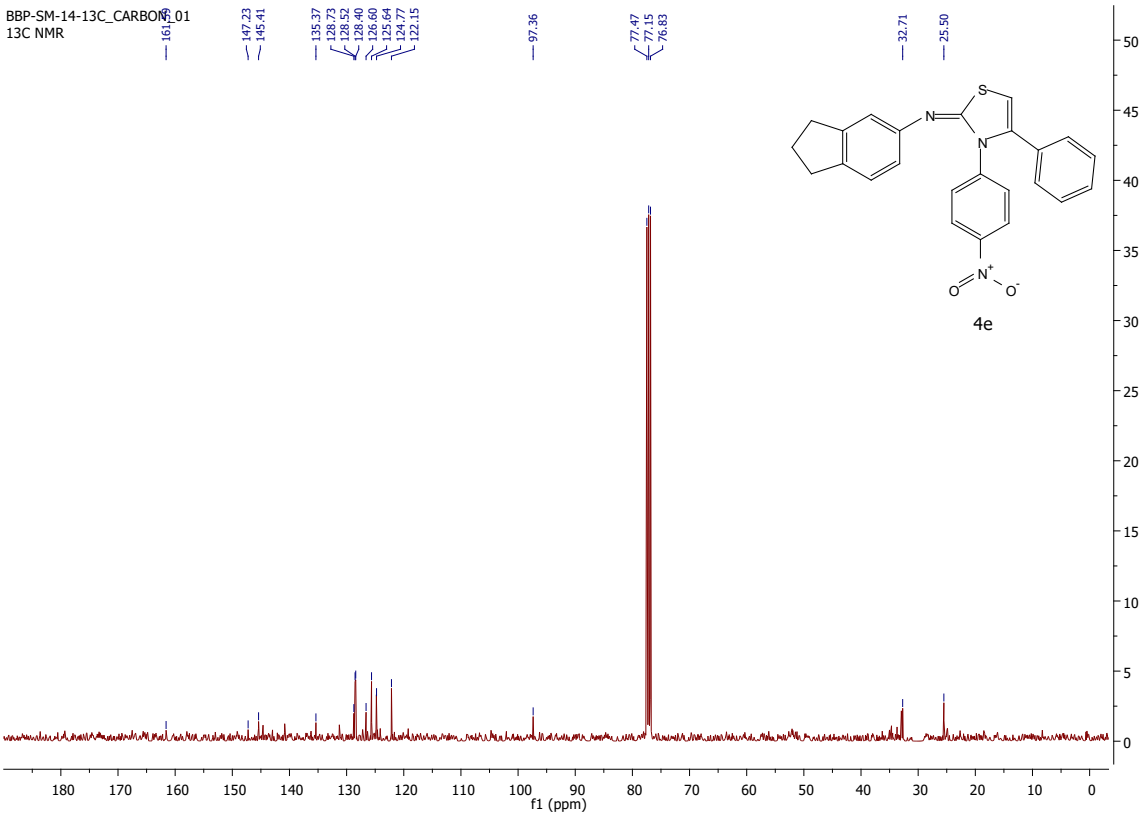
		383.10 I



***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-(4-nitrophenyl)-4-phenylthiazol-2(3*H*)-imine (4f):**

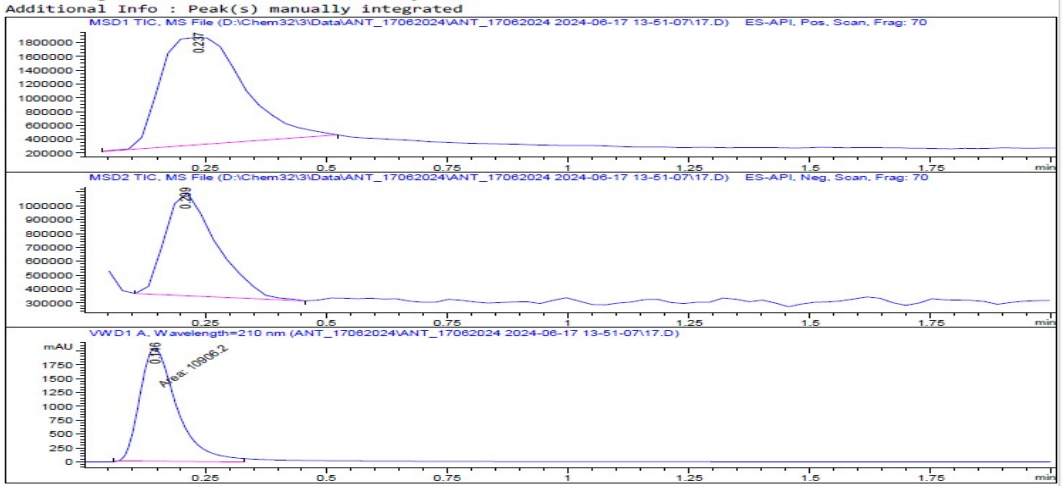




Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\17.D
Sample Name: BBP-AI-08

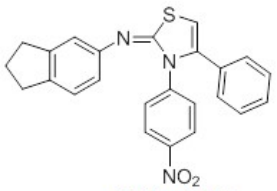
Acq. Operator	: SYSTEM	Seq. Line	: 10
Acq. Instrument	: LCMS	Location	: 17
Injection Date	: 17-Jun-24 2:19:55 PM	Inj	: 1
		Inj Volume	: 20.000 µl

Method : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS .M (Sequence Method)
Last changed : 17-Jun-24 1:51:07 PM by SYSTEM
Additional Info : Peak(s) manually integrated



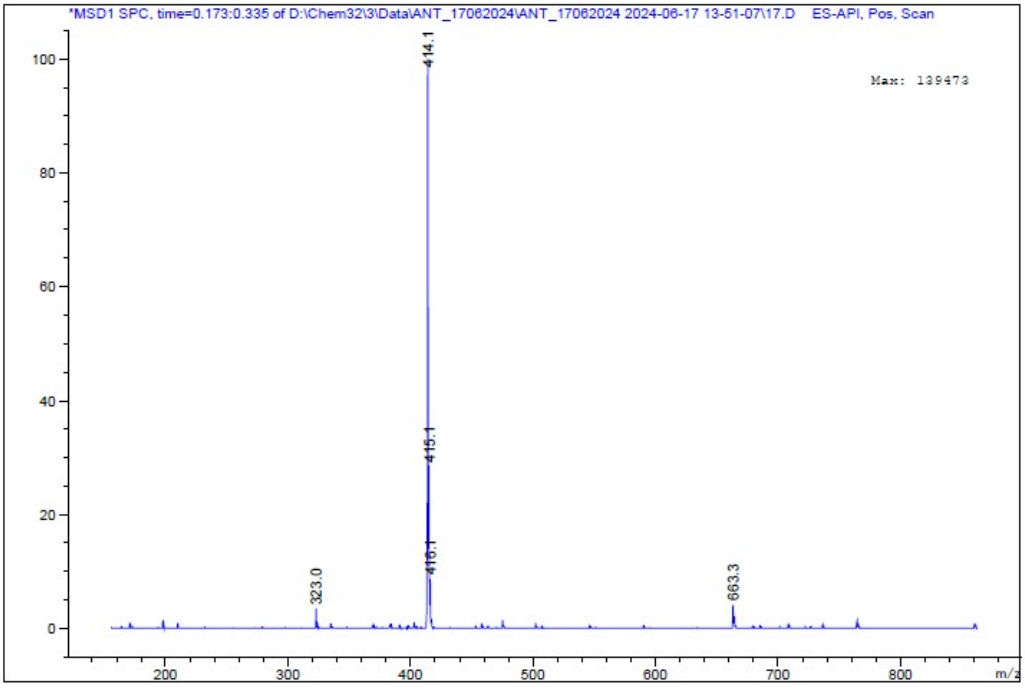
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\17.D
Sample Name: BBP-AI-08

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

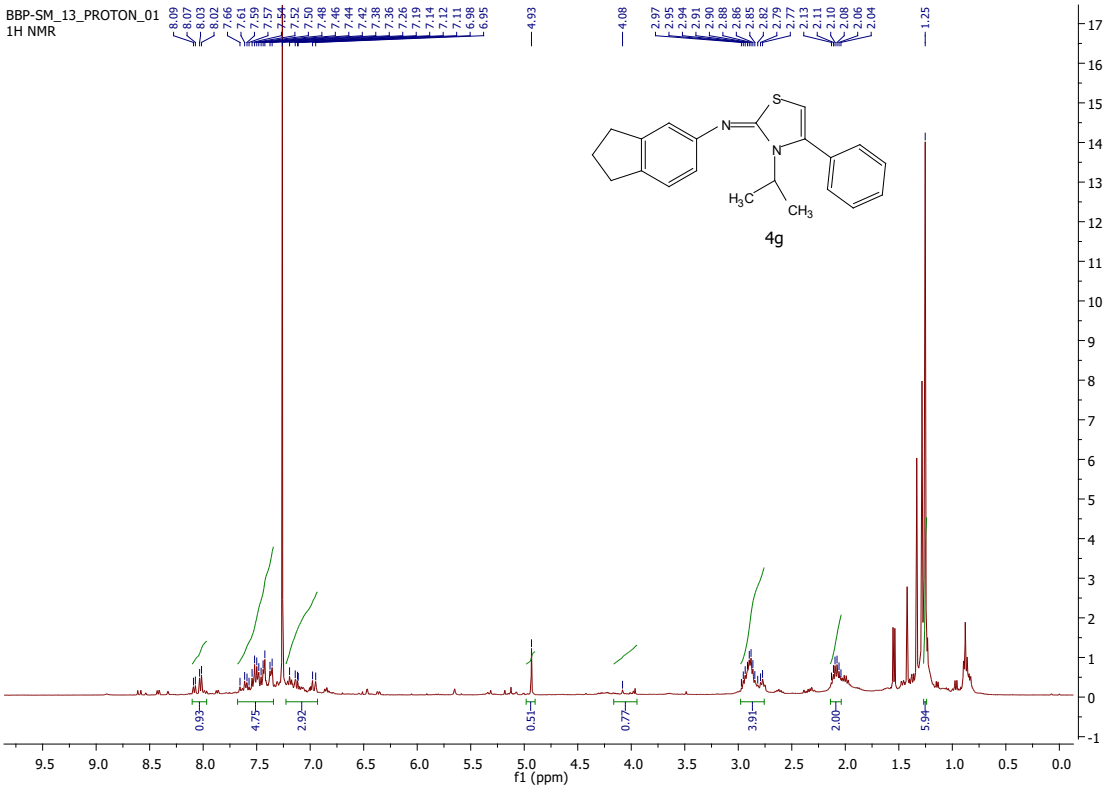


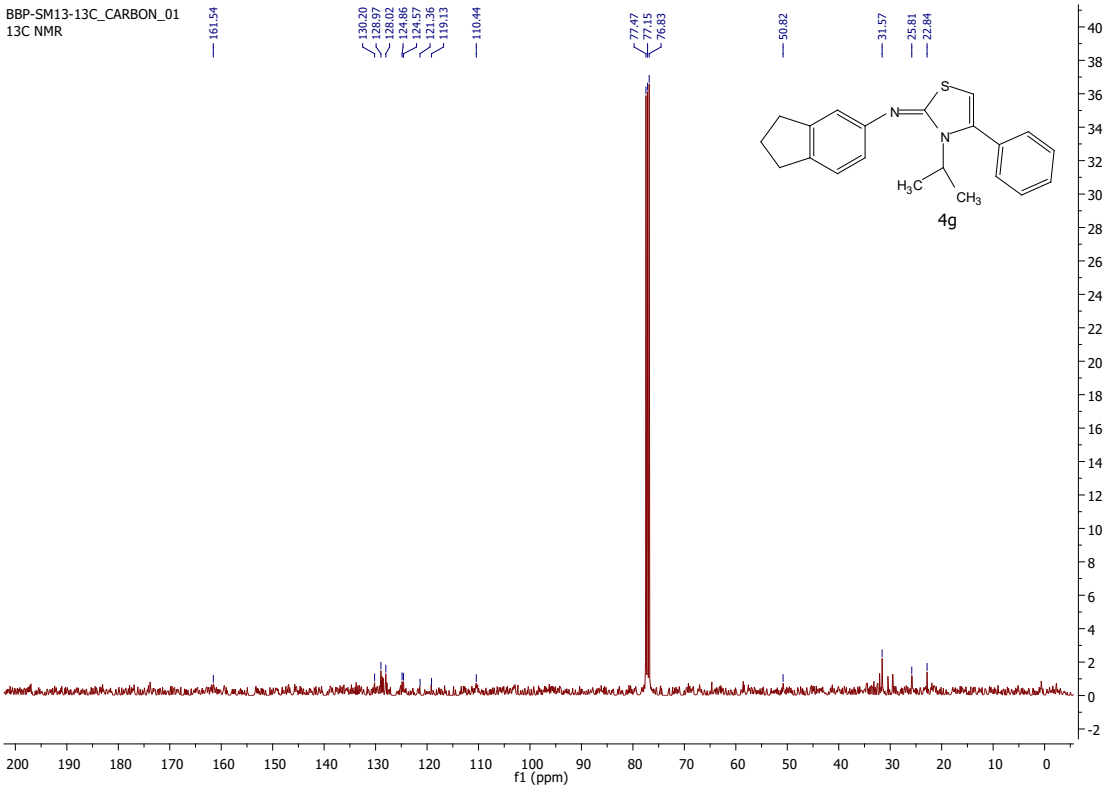
MW = 413.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.237	18583826	415.10 I
		414.10 I



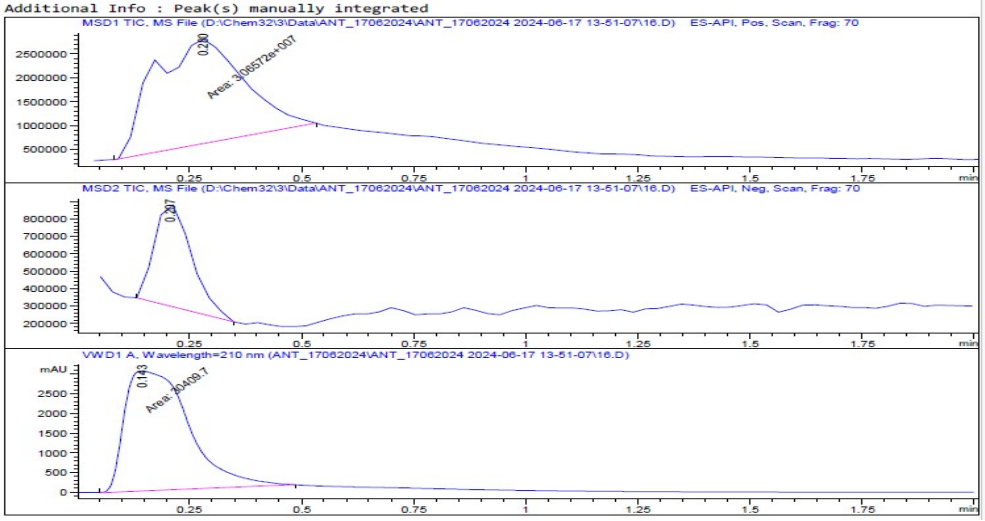
***(E)*-*N*-(2,3-dihydro-1*H*-inden-5-yl)-3-isopropyl-4-phenylthiazol-2(3*H*)-imine (4g):**





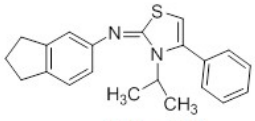
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\16.D
Sample Name: BBP-AI-07

```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   9
Acq. Instrument : LCMS                           Location :   16
Injection Date  : 17-Jun-24 2:16:49 PM               Inj :   1
                                                 Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 1:51:07 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```



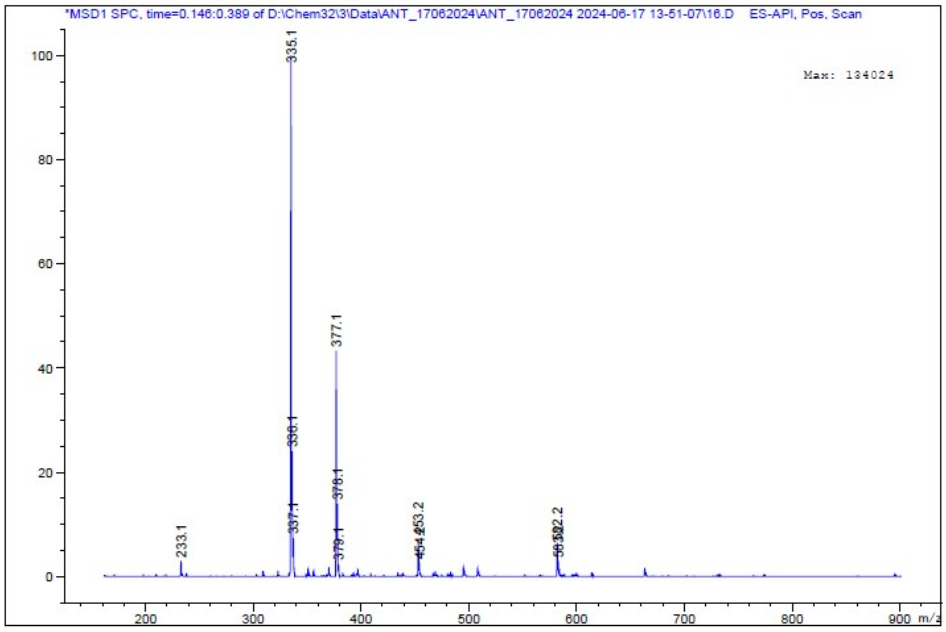
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\16.D
Sample Name: BBP-AI-07

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

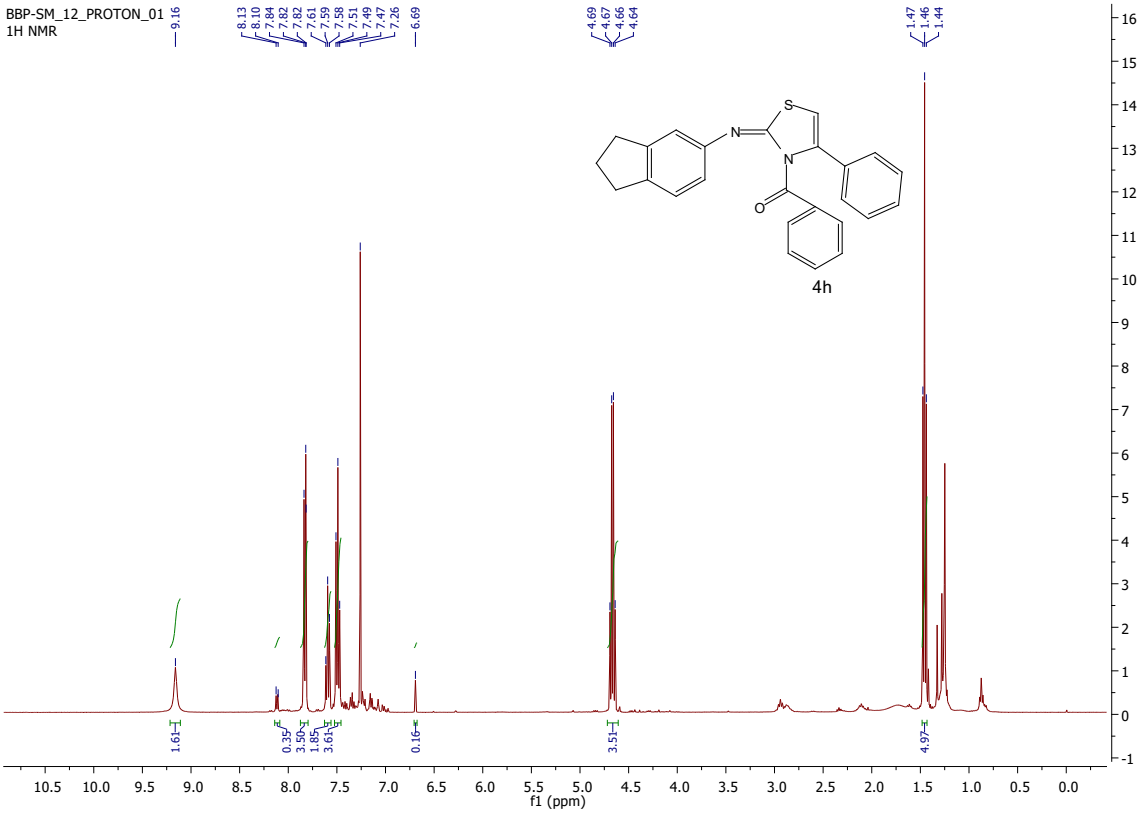


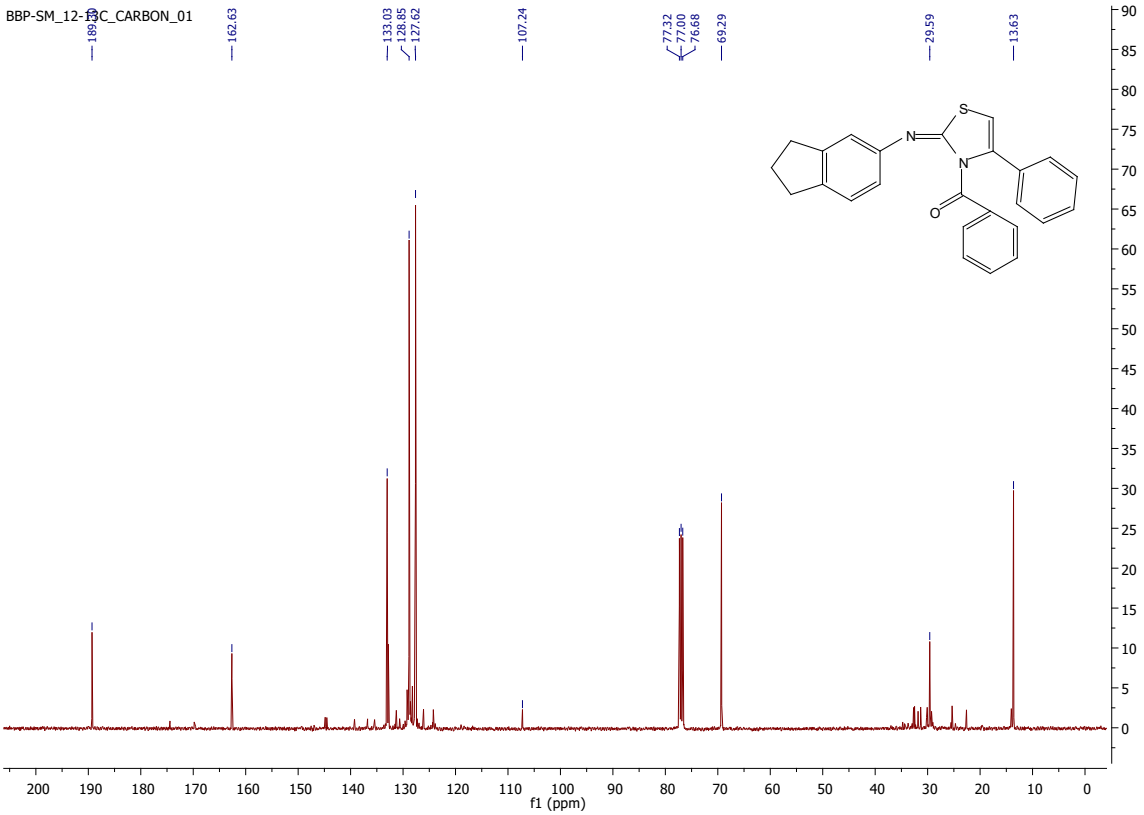
MW = 334.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.280	30657174	378.10 I
		377.10 I
		336.10 I
		335.10 I



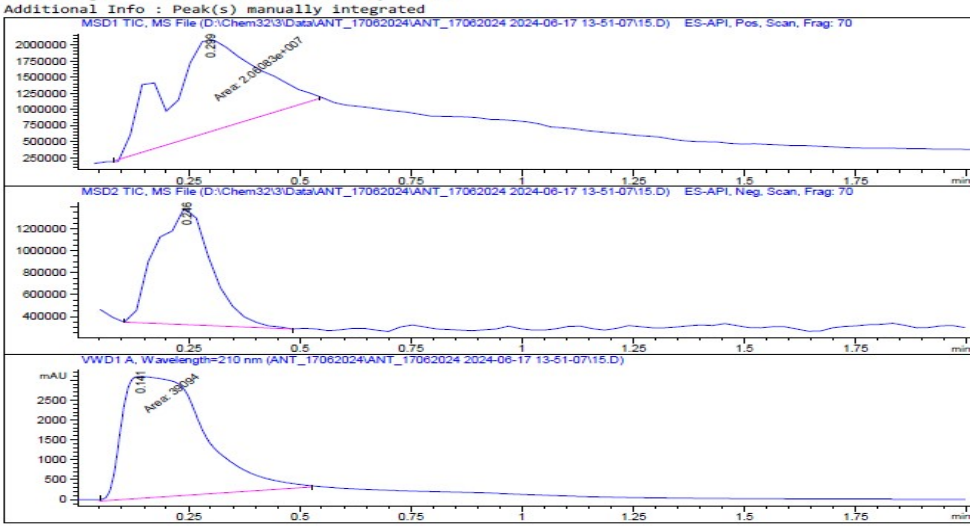
***(E)*-(2-((2,3-dihydro-1*H*-inden-5-yl)imino)-4-phenylthiazol-3(2*H*)-yl)(phenyl)methanone (4h):**





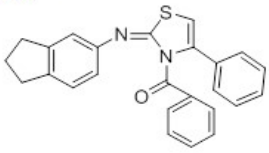
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\15.D
Sample Name: BBP-AI-06

```
==================================================================
Acq. Operator    : SYSTEM                          Seq. Line :   8
Acq. Instrument  : LCMS                             Location :   15
Injection Date   : 17-Jun-24 2:13:47 PM                Inj :    1
                                                 Inj Volume : 20.000 µl
Method           : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\DIRECT MASS
                   .M (Sequence Method)
Last changed     : 17-Jun-24 1:51:07 PM by SYSTEM
Additional Info  : Peak(s) manually integrated
```



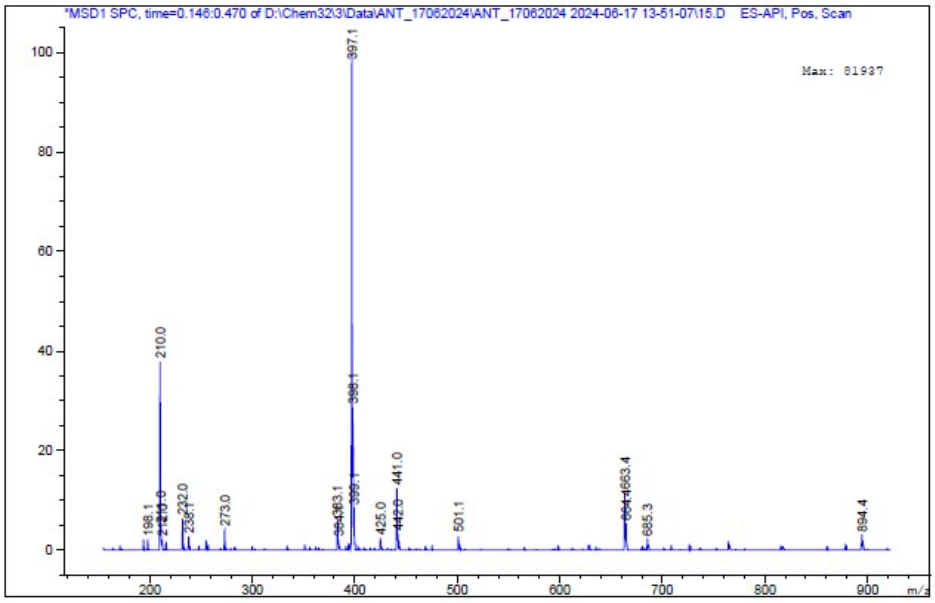
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 13-51-07\15.D
Sample Name: BBP-AI-06

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

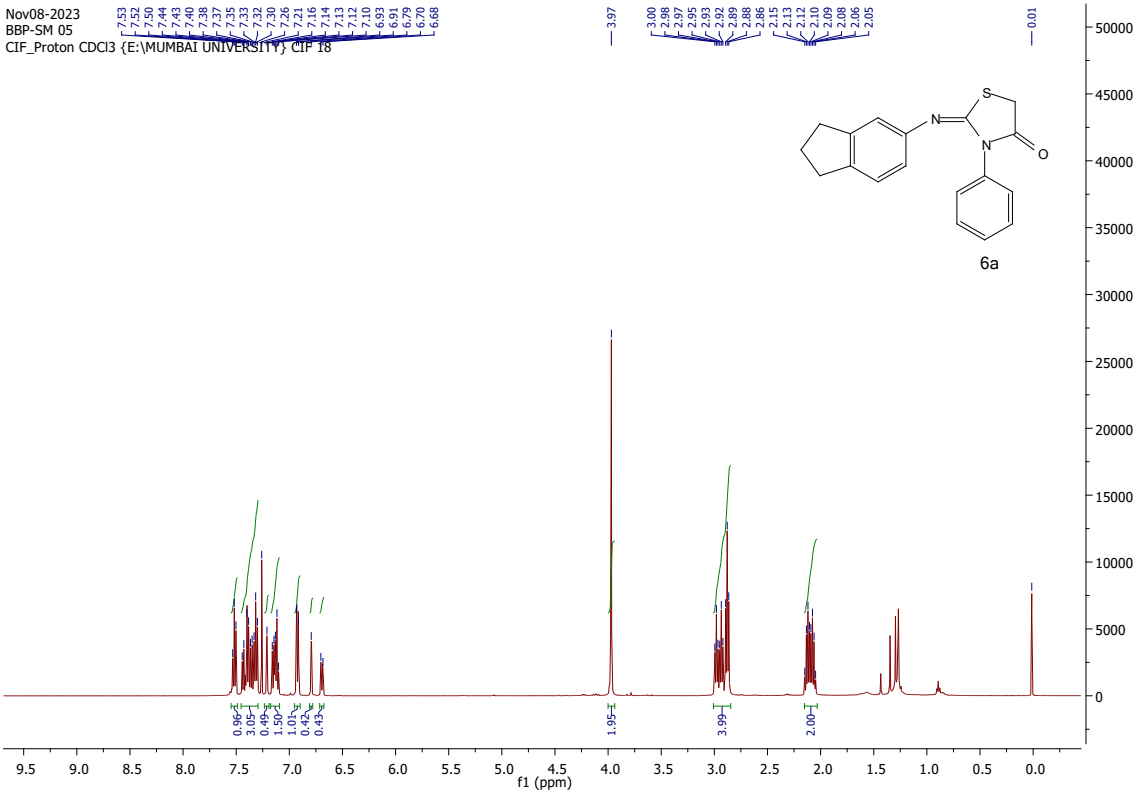


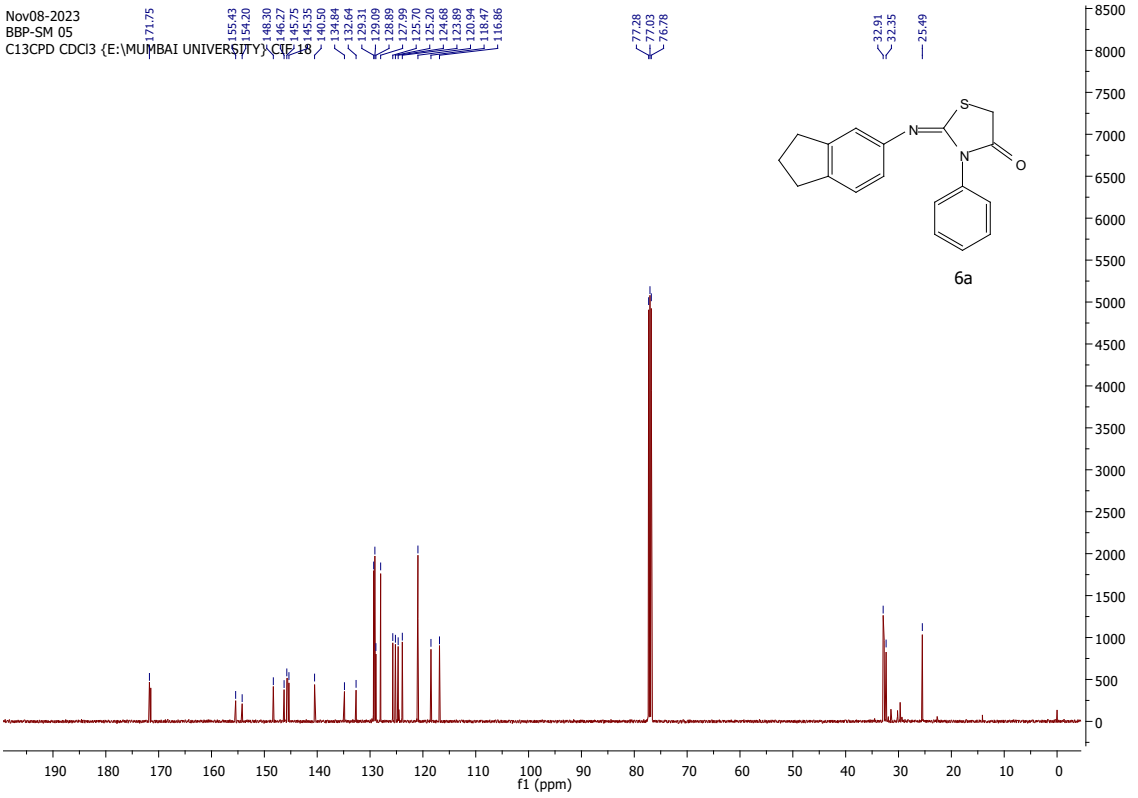
MW = 396.5

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.299	20608332	663.40 I
		441.00 I
		398.10 I
		397.10 I
		210.00 I



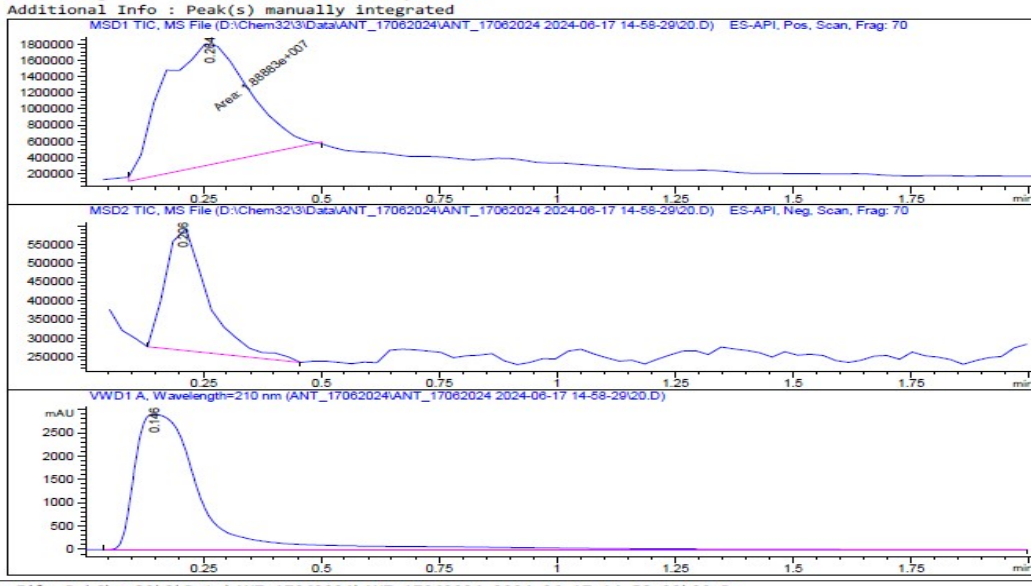
***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-phenylthiazolidin-4-one (6a):**





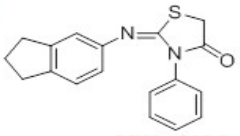
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\20.D
Sample Name: BBP-AI-09

Acq. Operator	: SYSTEM	Seq. Line	: 3
Acq. Instrument	: LCMS	Location	: 20
Injection Date	: 17-Jun-24 3:05:49 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Method	: D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS .M (Sequence Method)		
Last changed	: 17-Jun-24 2:58:29 PM by SYSTEM		
Additional Info	: Peak(s) manually integrated		



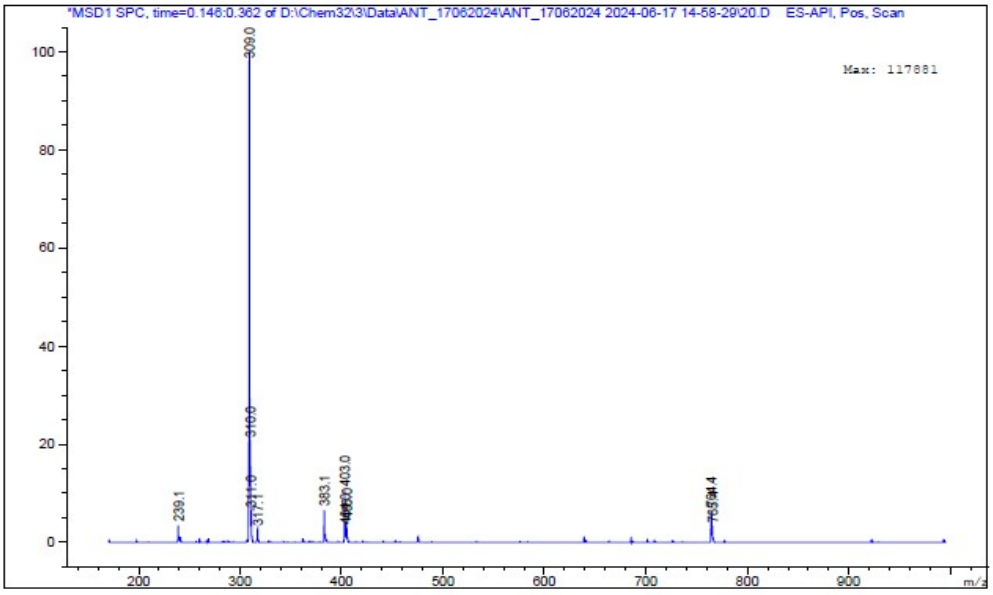
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\20.D
Sample Name: BBP-AI-09

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

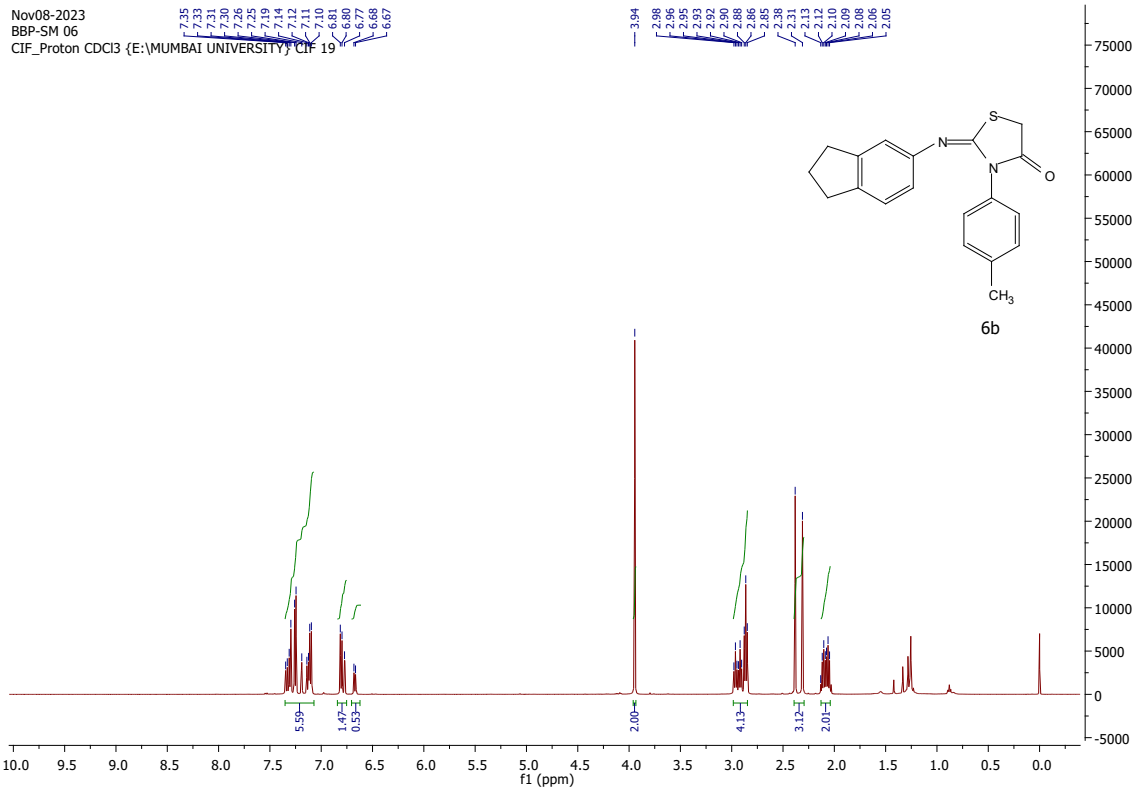


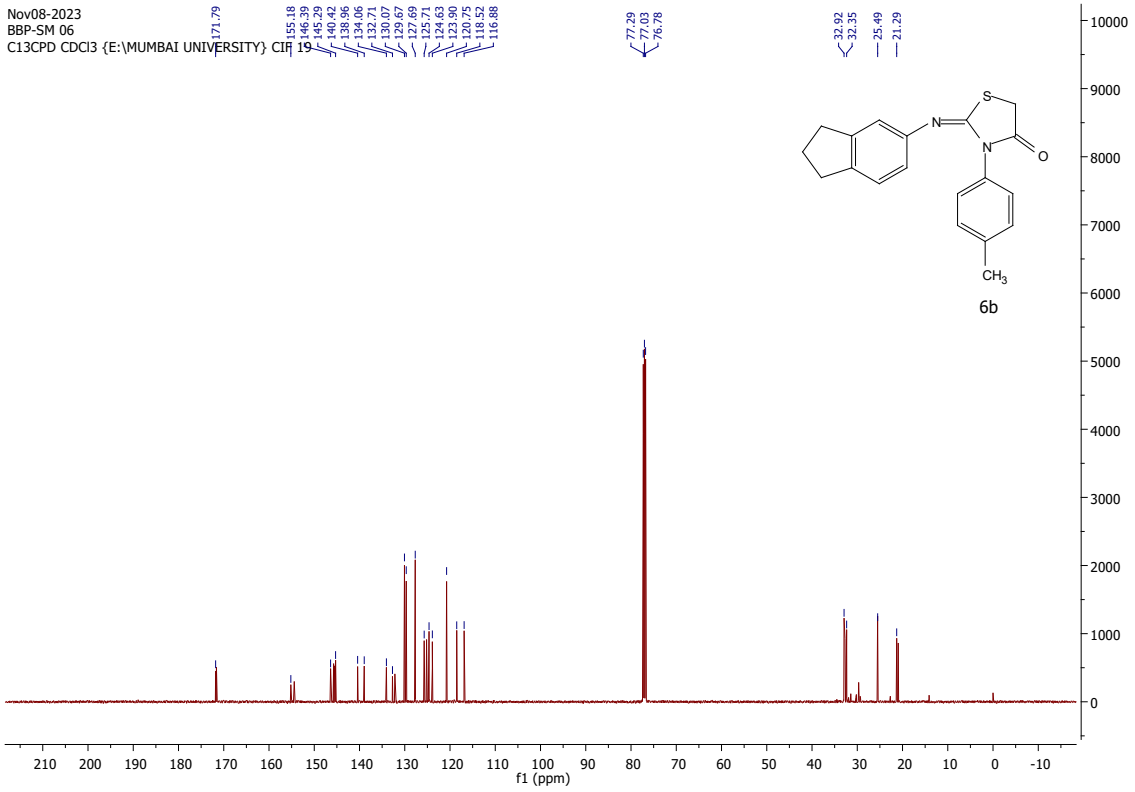
MW = 308.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.264	18888288	403.00 I
		310.00 I
		309.00 I



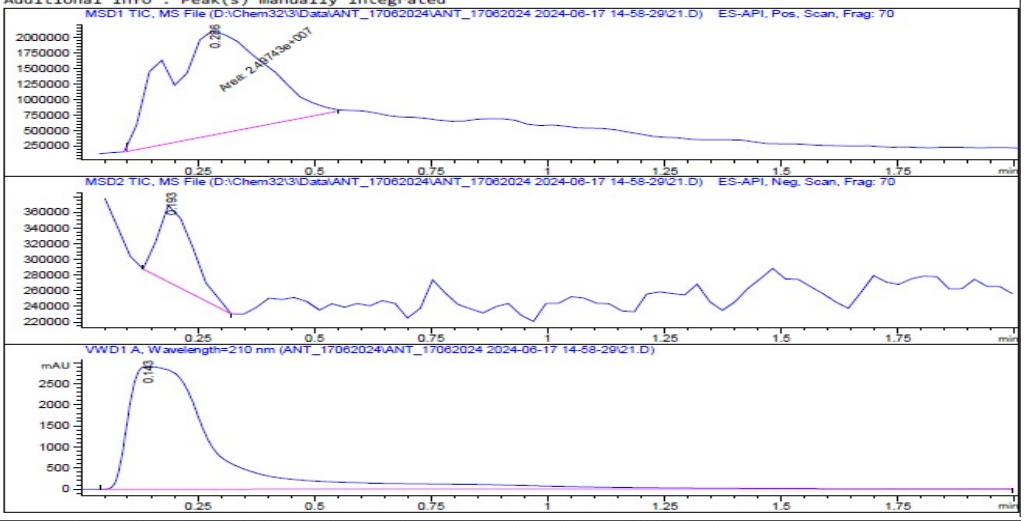
***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-(p-tolyl)thiazolidin-4-one (6b):**





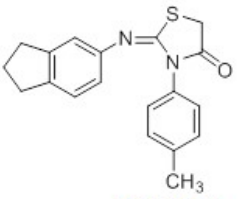
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\21.D
Sample Name: BBP-AI-10

```
Acq. Operator   : SYSTEM                          Seq. Line :   4
Acq. Instrument : LCMS                             Location :   21
Injection Date  : 17-Jun-24 3:08:53 PM                Inj :    1
                                                  Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```



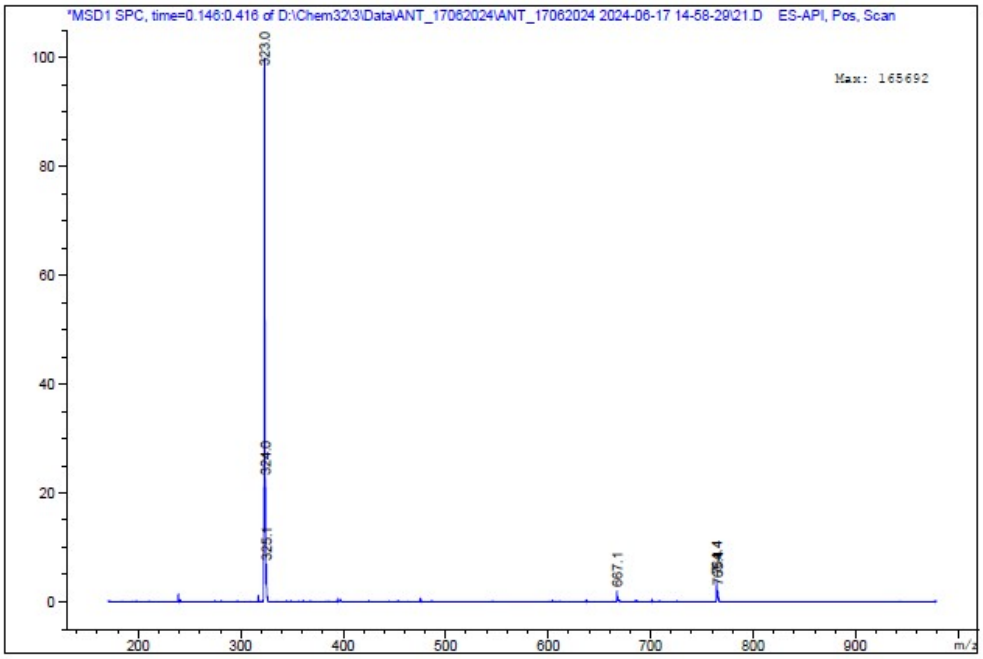
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\21.D
Sample Name: BBP-AI-10

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

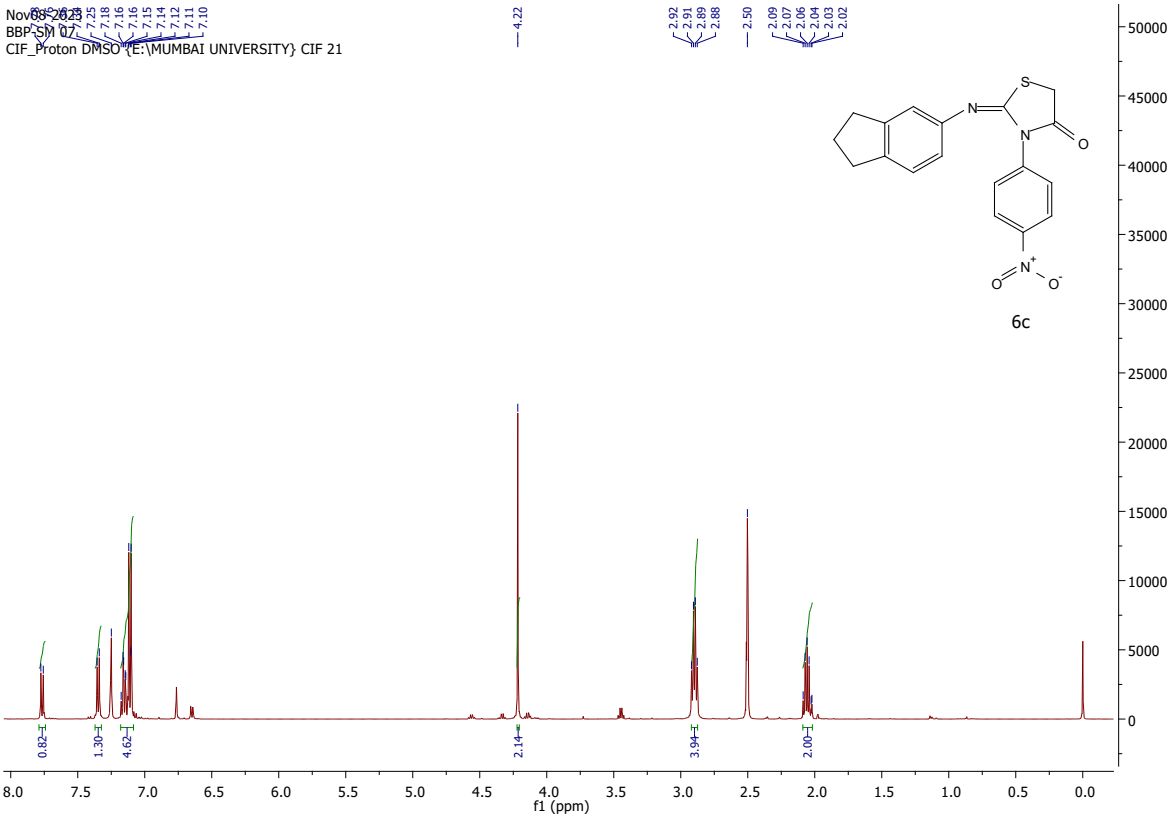


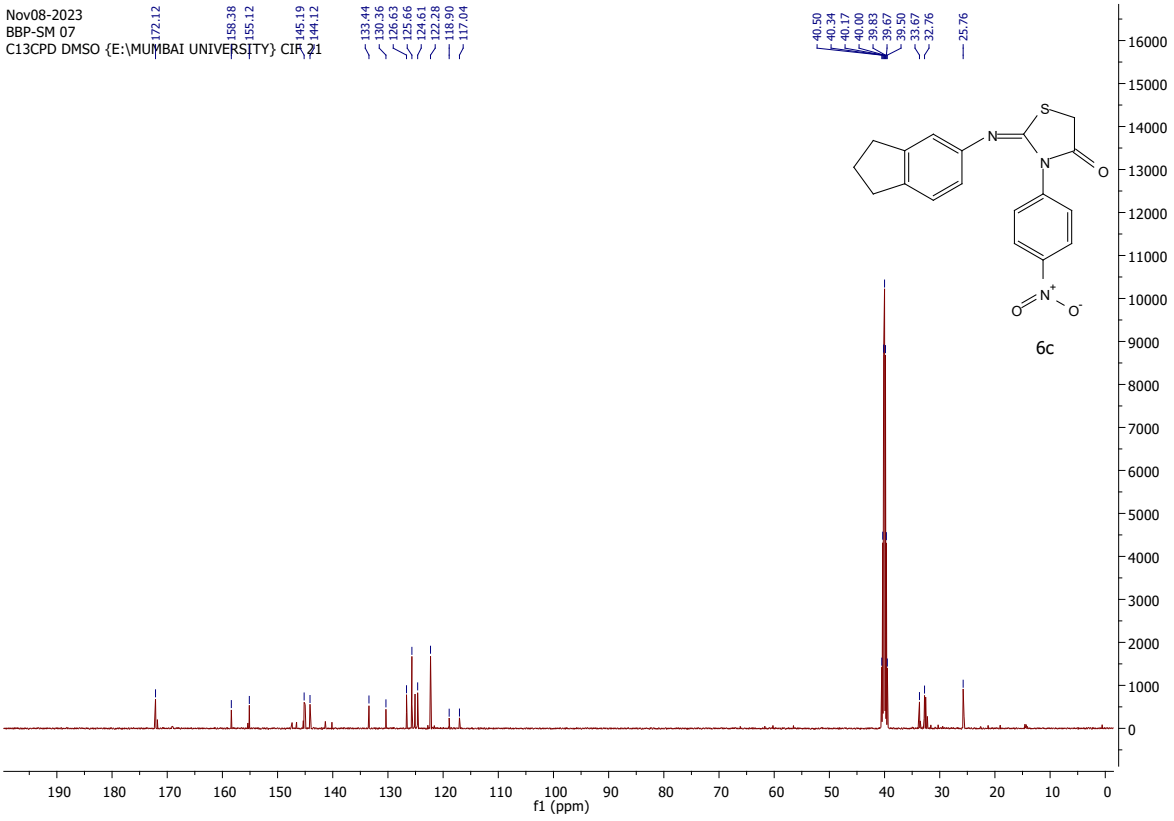
MW = 322.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.286	24974340	324.00 I
		323.00 I



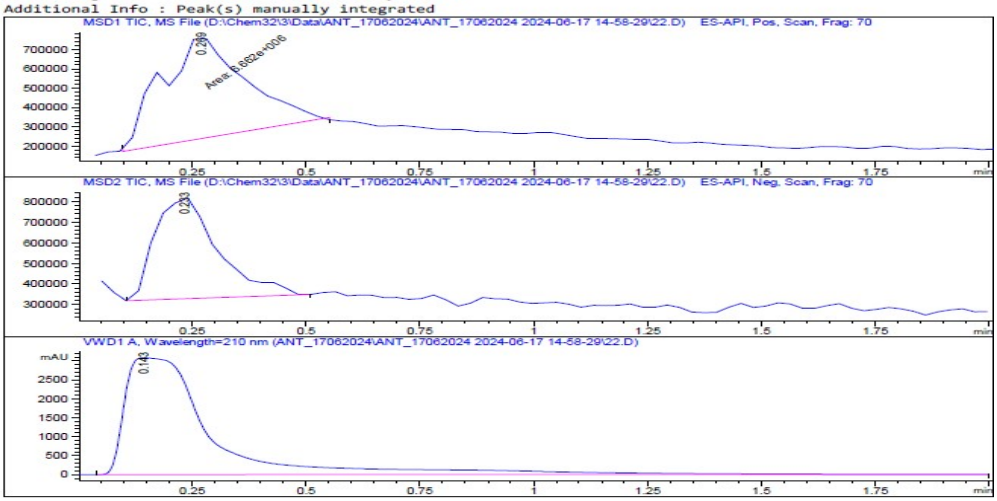
***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-(4-nitrophenyl)thiazolidin-4-one (6c):**





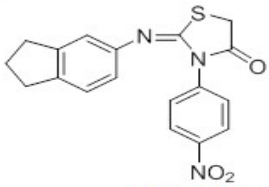
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\22.D
Sample Name: BBP-AI-11

```
Acq. Operator   : SYSTEM                          Seq. Line :   5
Acq. Instrument : LCMS                             Location :   22
Injection Date  : 17-Jun-24 3:12:01 PM                Inj :    1
                                                  Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```



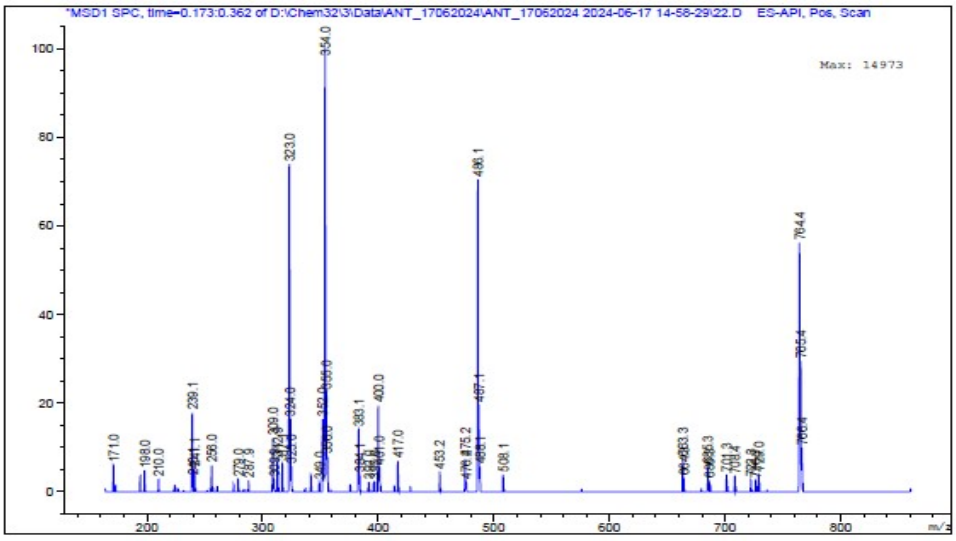
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\22.D
Sample Name: BBP-AI-11

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

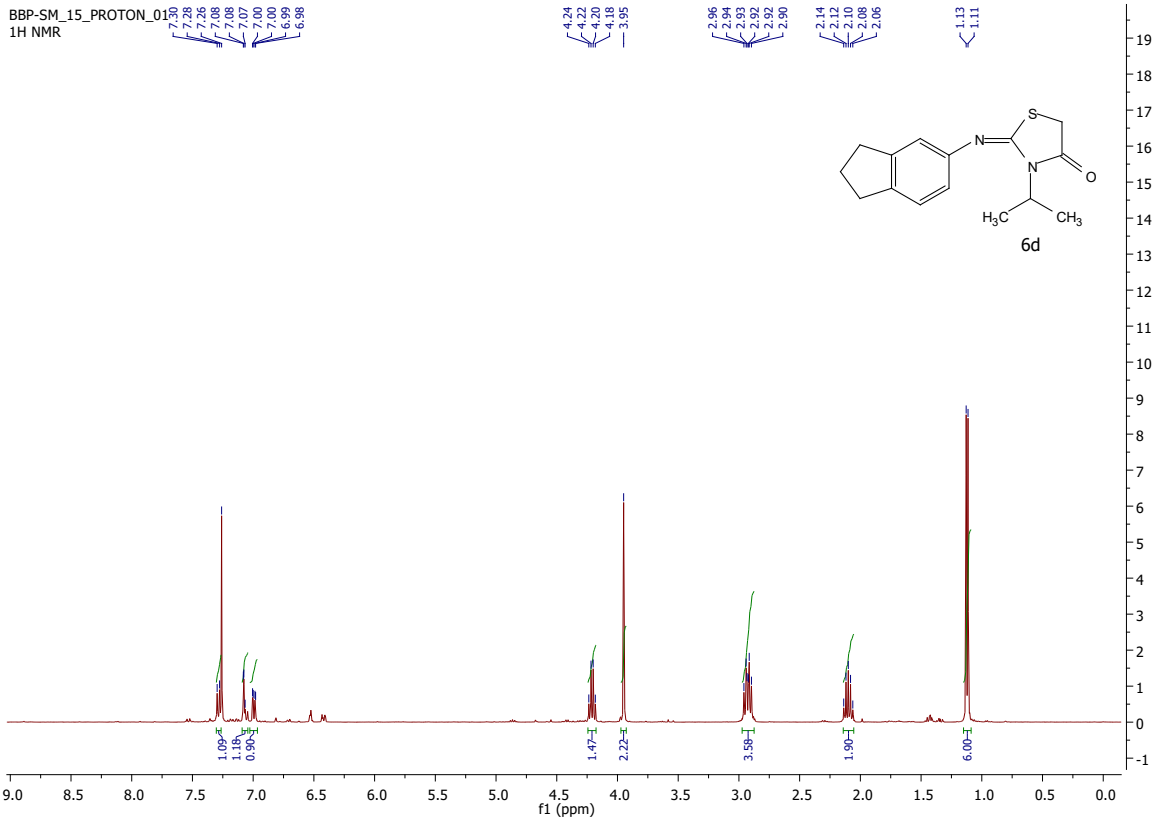


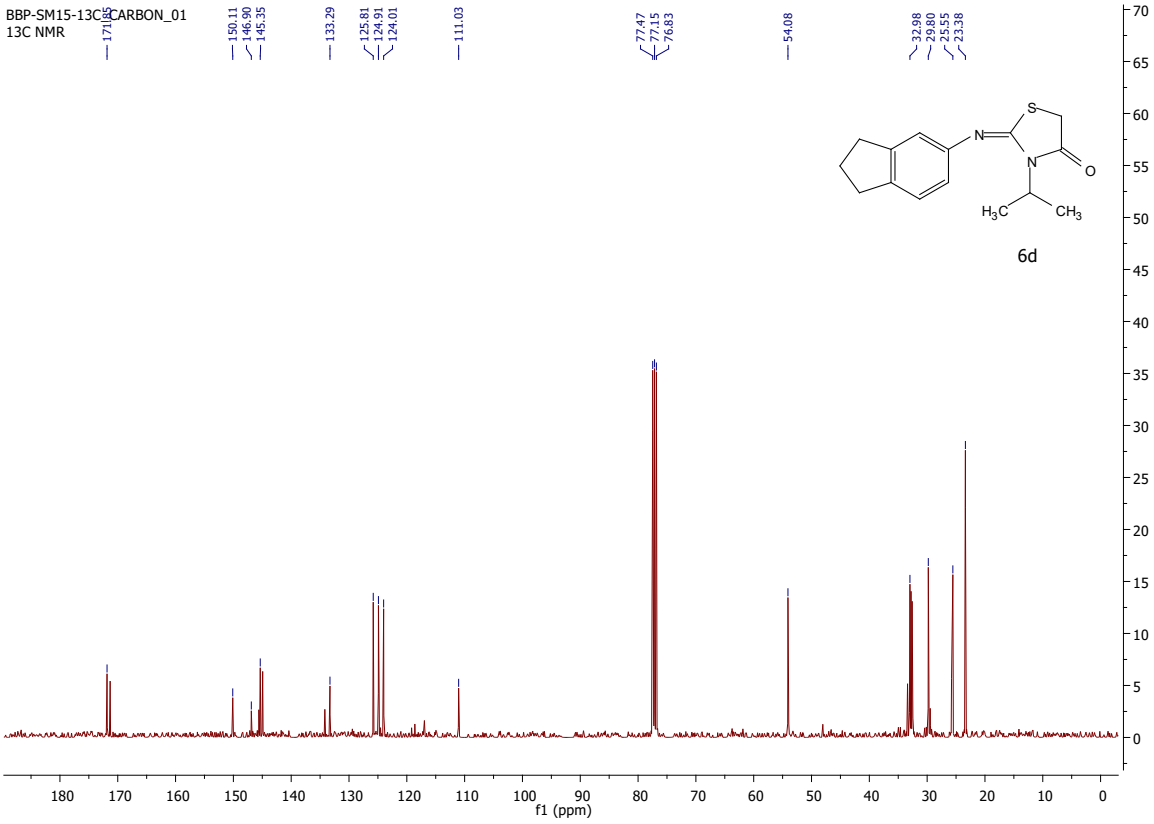
MW = 353.0

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.269	6661996	765.40 I
		764.40 I
		487.10 I
		486.10 I
		400.00 I
		383.10 I
		355.00 I
		354.00 I
		352.00 I
		324.00 I
		323.00 I
		309.00 I
		239.10 I



***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-isopropylthiazolidin-4-one (6d):**

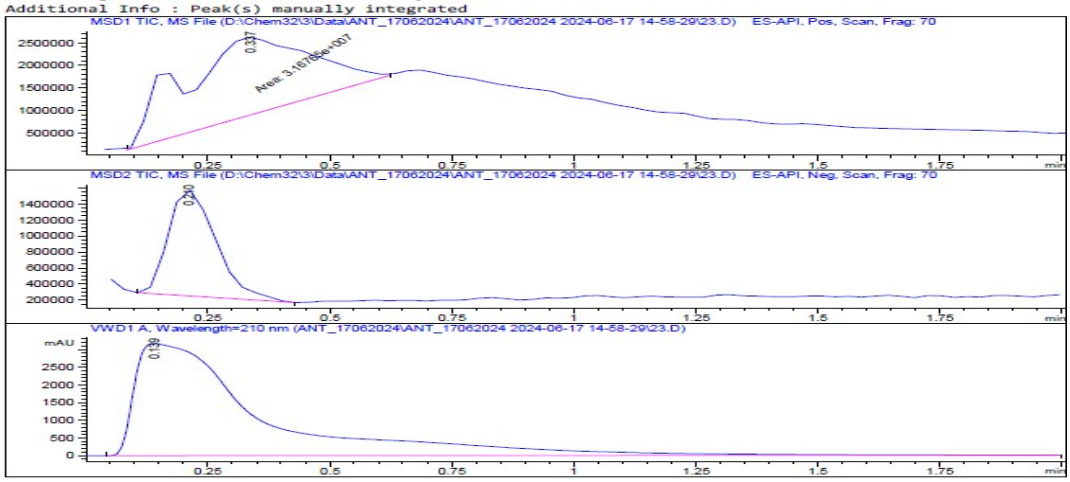




Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\23.D
Sample Name: BBP-AI-12

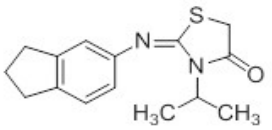
Acq. Operator	: SYSTEM	Seq. Line	: 6
Acq. Instrument	: LCMS	Location	: 23
Injection Date	: 17-Jun-24 3:15:02 PM	Inj	: 1
		Inj Volume	: 20.000 µl

Method : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS .M (Sequence Method)
Last changed : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated



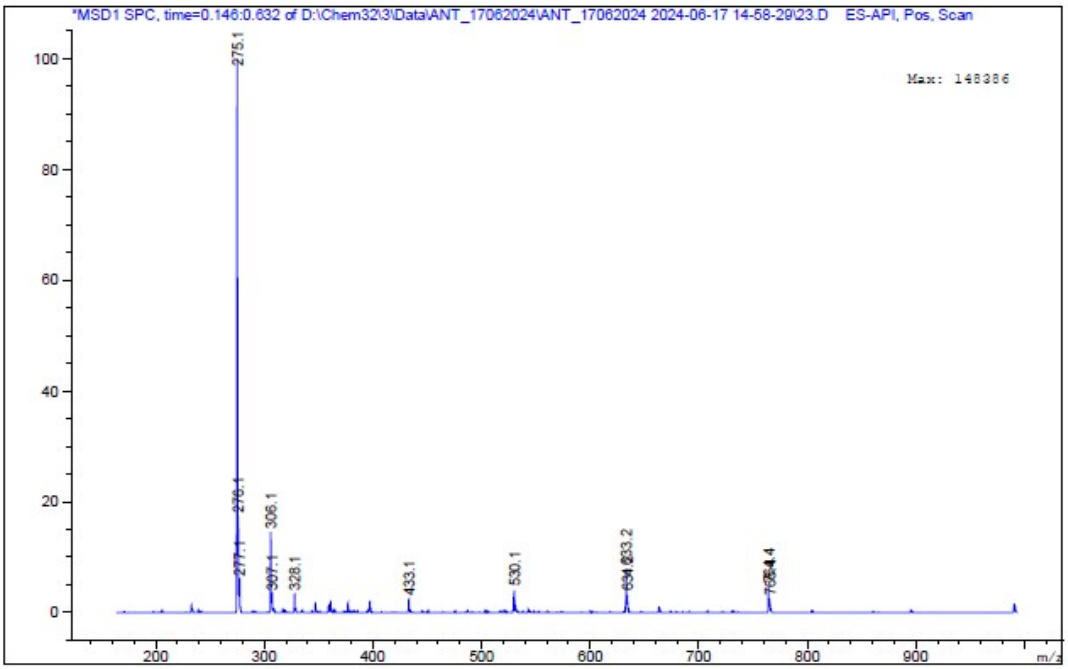
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\23.D
Sample Name: BBP-AI-12

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

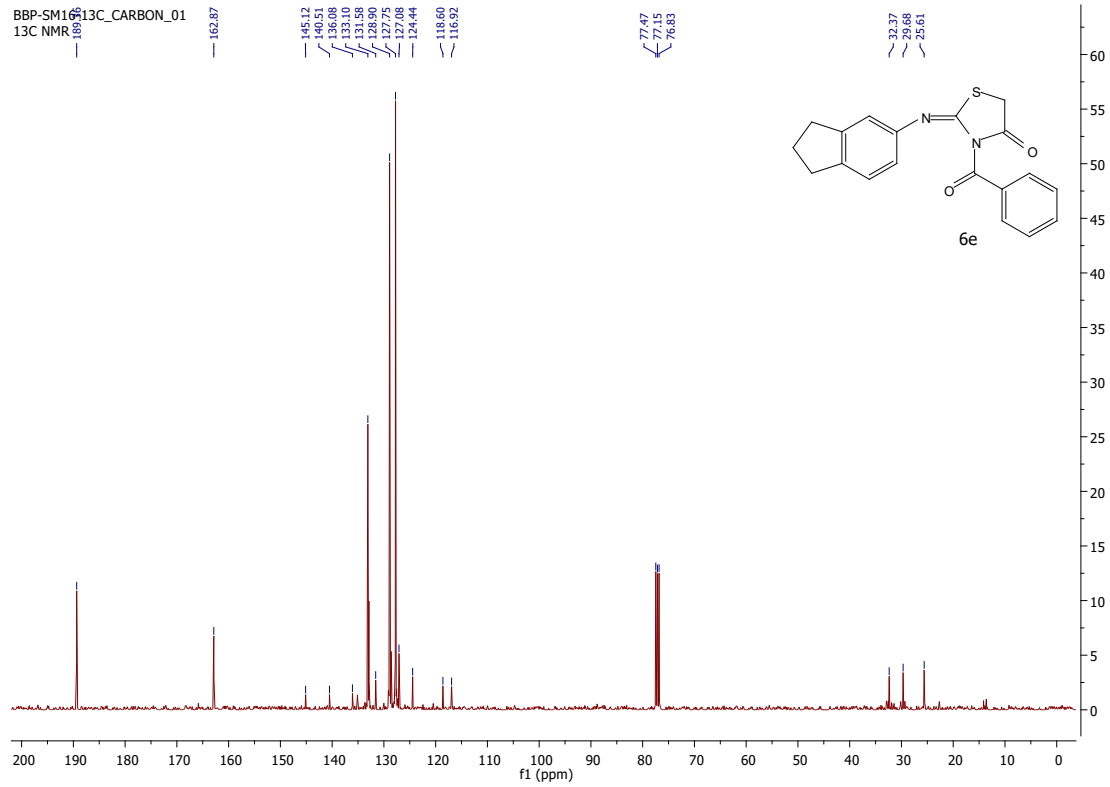


MW = 274.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.337	31676514	306.10 I
		276.10 I
		275.10 I

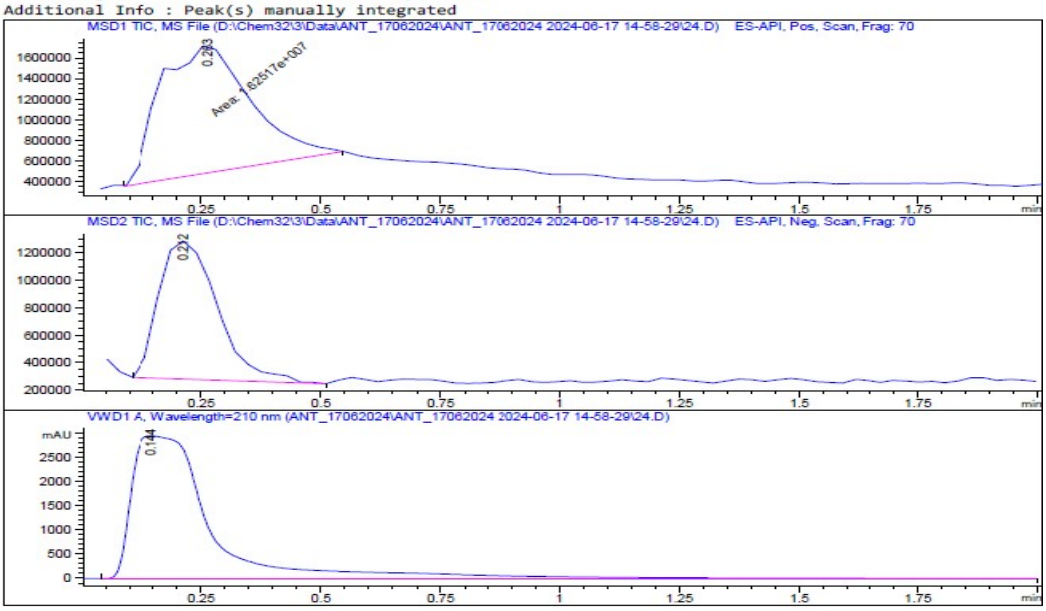


***(E)*-3-benzoyl-2-((2,3-dihydro-1*H*-inden-5-yl)imino)thiazolidin-4-one (6e):**



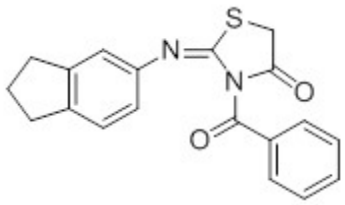

```
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\24.D
Sample Name: BBP-AI-13

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : LCMS                          Location :   24
Injection Date  : 17-Jun-24 3:18:03 PM             Inj :   1
                                               Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```



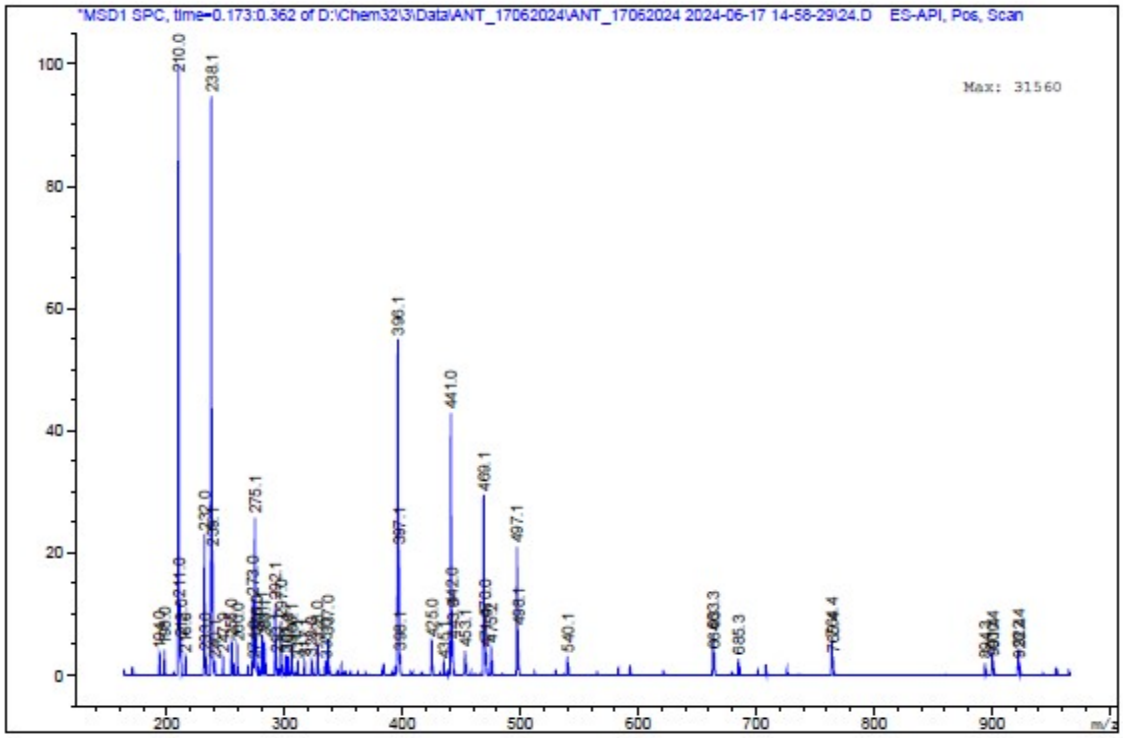
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\24.D
Sample Name: BBP-AI-13

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

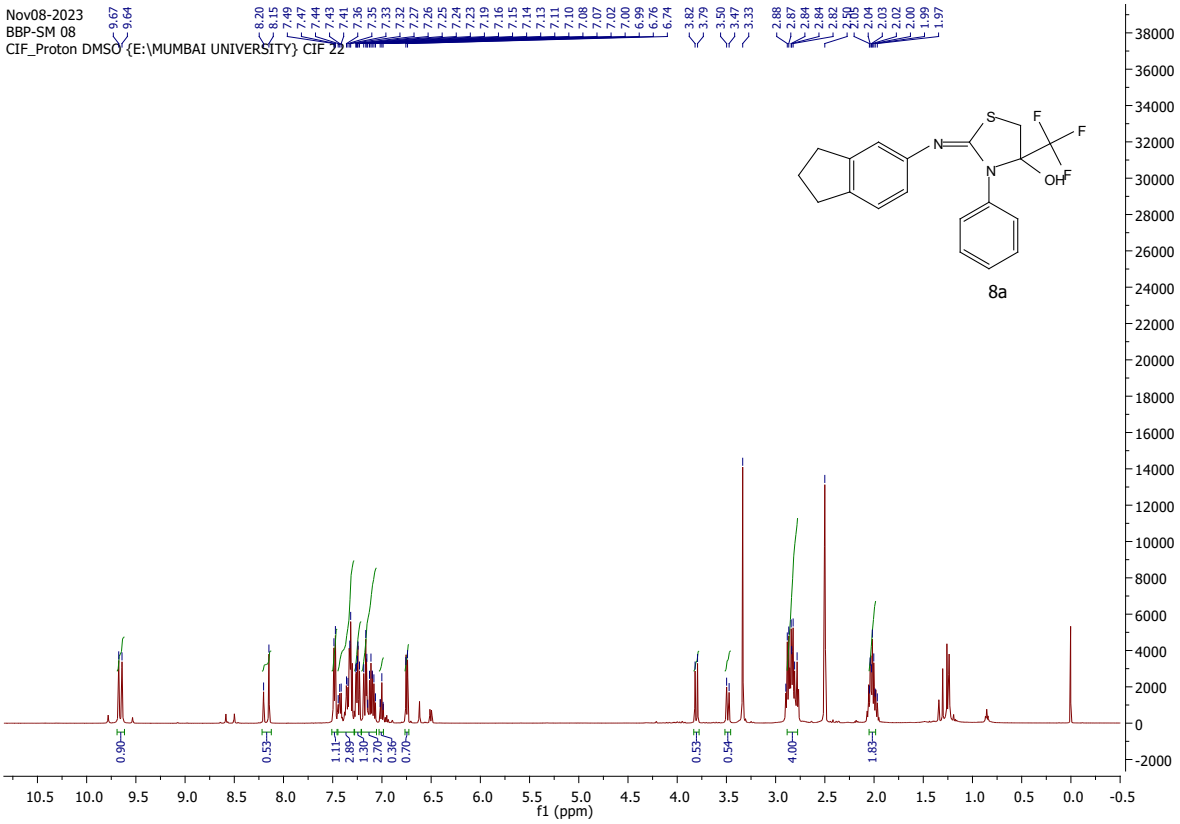


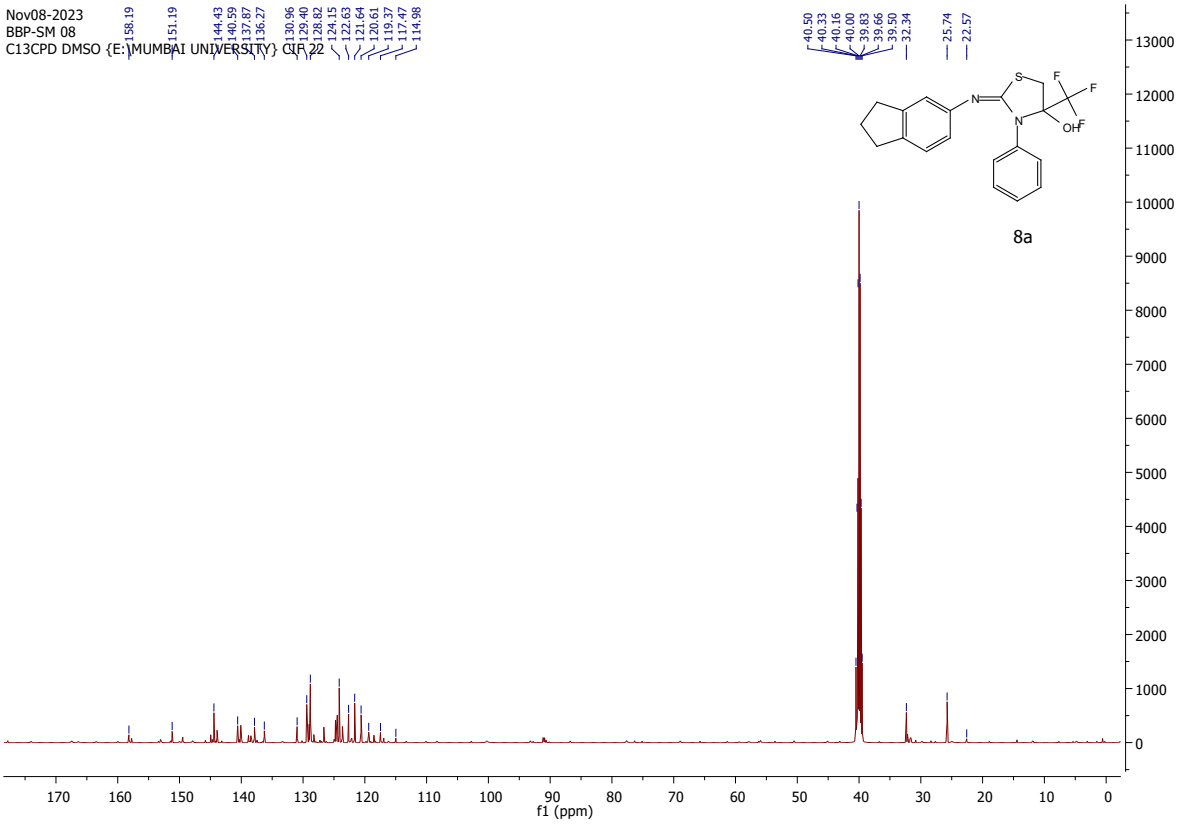
MW = 337.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.263	16251726	497.10 I
		469.10 I
		442.00 I
		441.00 I
		397.10 I
		396.10 I
		292.10 I
		275.10 I
		273.00 I
		239.10 I
		238.10 I
		232.00 I
		211.00 I
		210.00 I



***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-phenyl-4-(trifluoromethyl)thiazolidin-4-ol (8a):**





Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\25.D
Sample Name: BBP-AI-14

```
Acq. Operator   : SYSTEM                          Seq. Line :   8
Acq. Instrument : LCMS                             Location :   25
Injection Date  : 17-Jun-24 3:21:07 PM                Inj :   1
                                                Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated
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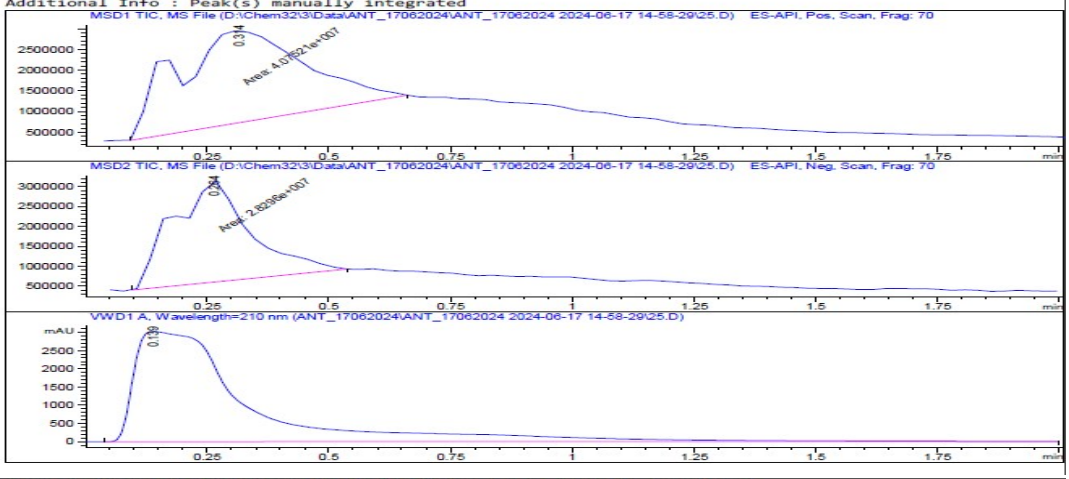
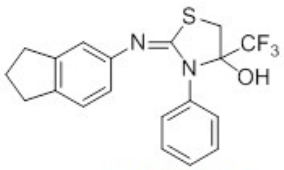


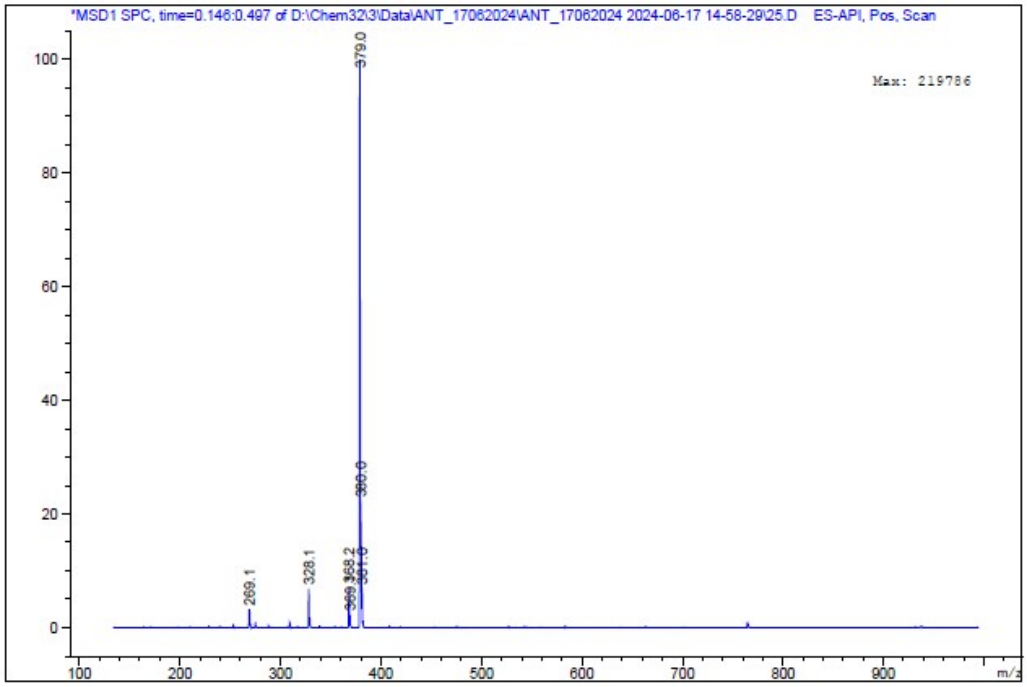
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\25.D
Sample Name: BBP-AI-14

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

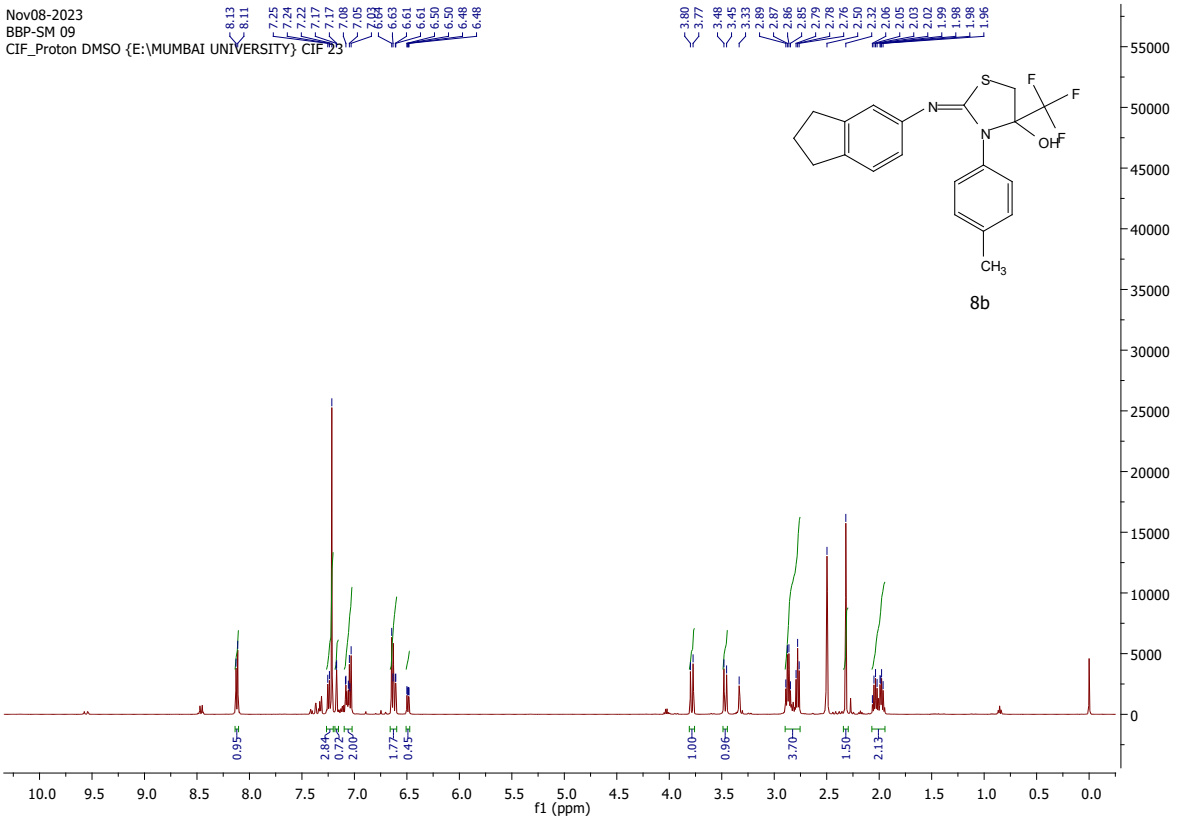


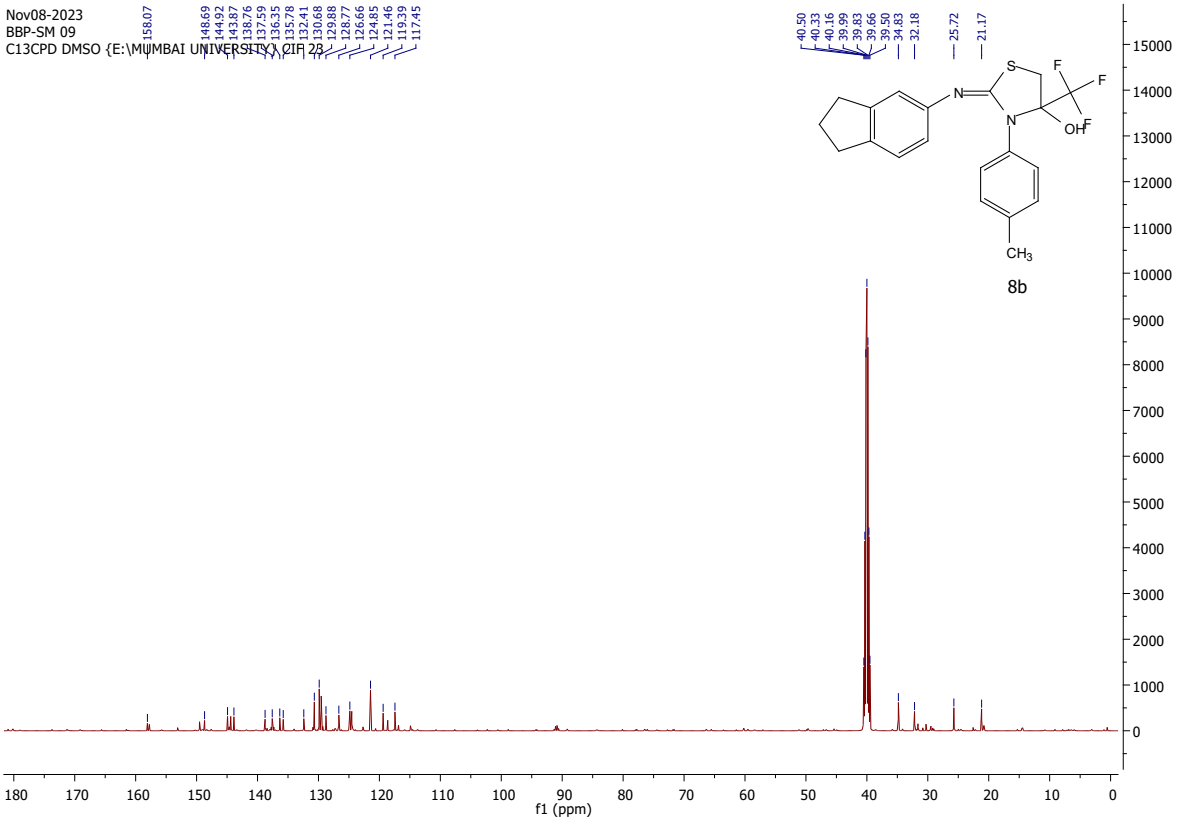
MW = 378.1

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.314	40752080	380.00 I
		379.00 I



***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-(*p*-tolyl)-4-(trifluoromethyl)thiazolidin-4-ol (8b):**





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Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\26.D
Sample Name: BBP-AI-15

=====================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   9
Acq. Instrument : LCMS                             Location :   26
Injection Date  : 17-Jun-24 3:24:10 PM                Inj :   1
                                                  Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
Additional Info : Peak(s) manually integrated
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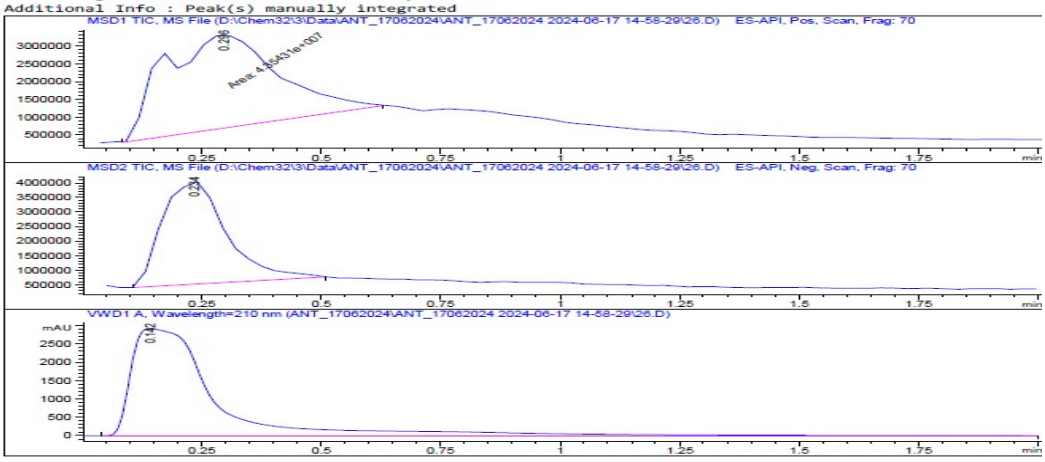
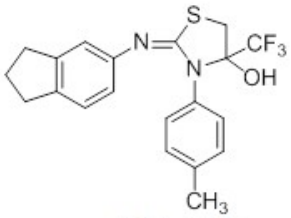


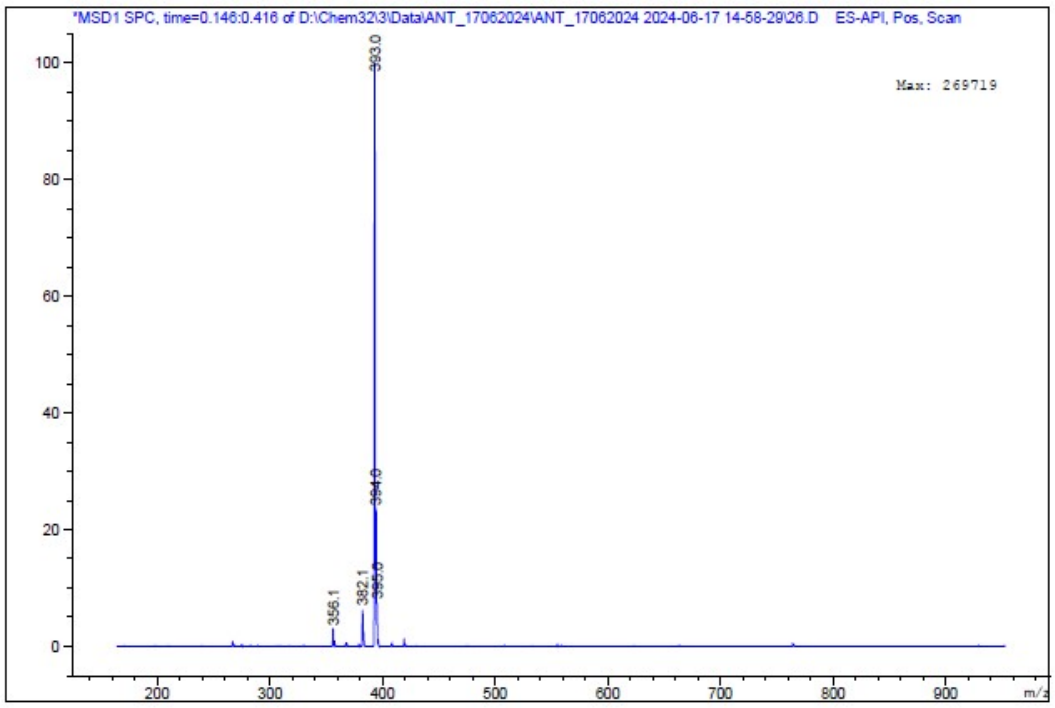
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\26.D
Sample Name: BBP-AI-15

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

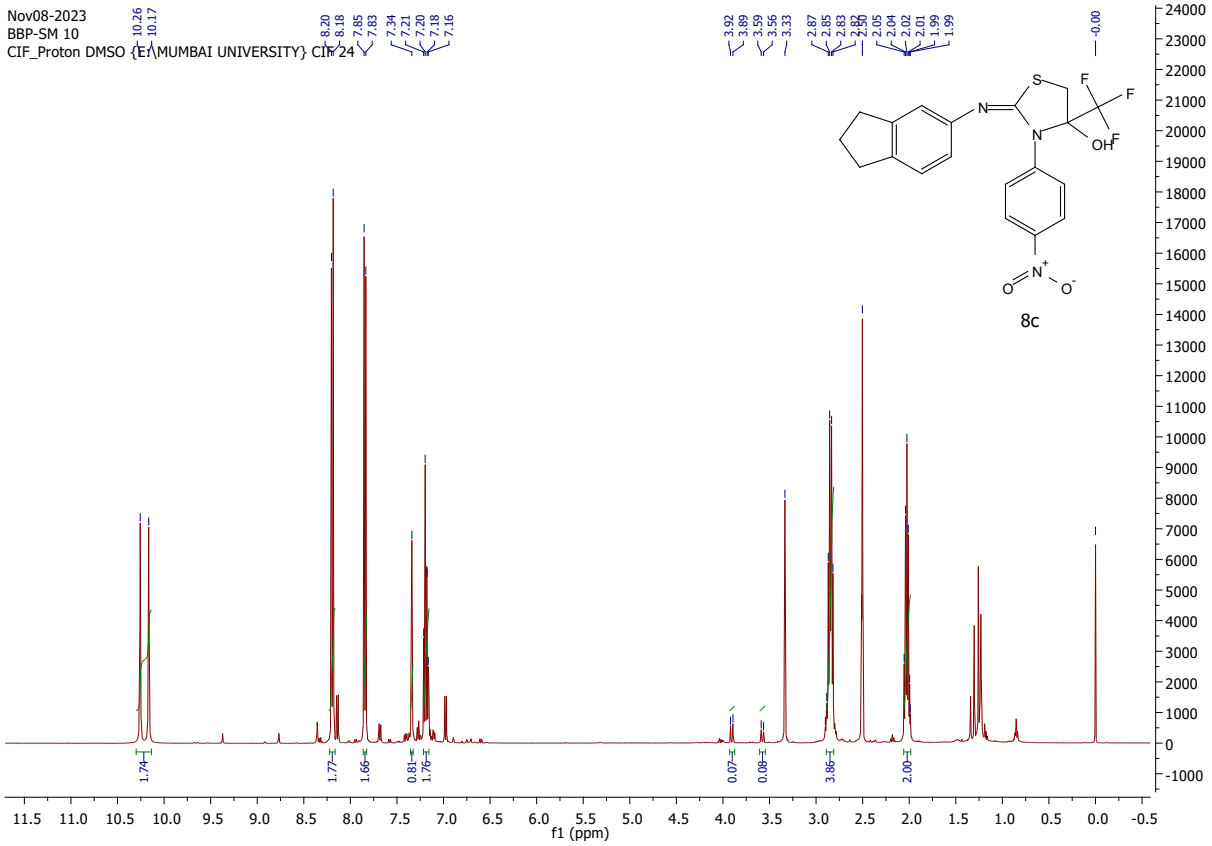


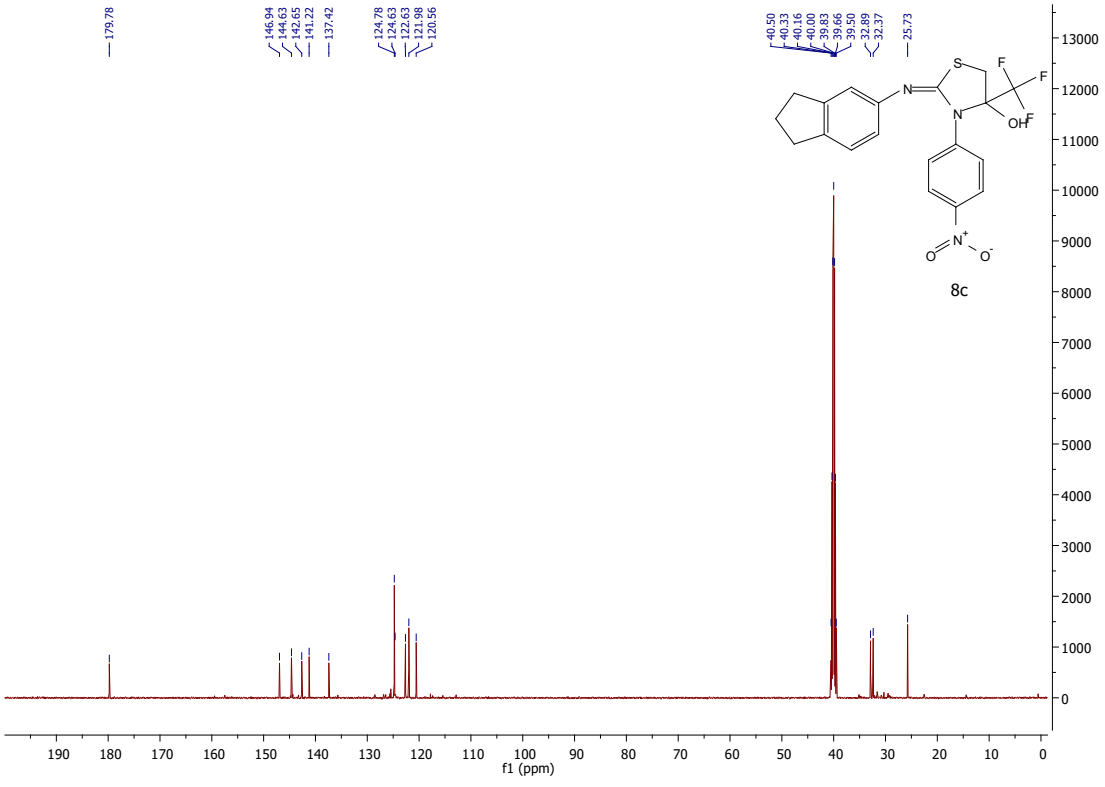
MW = 392.1

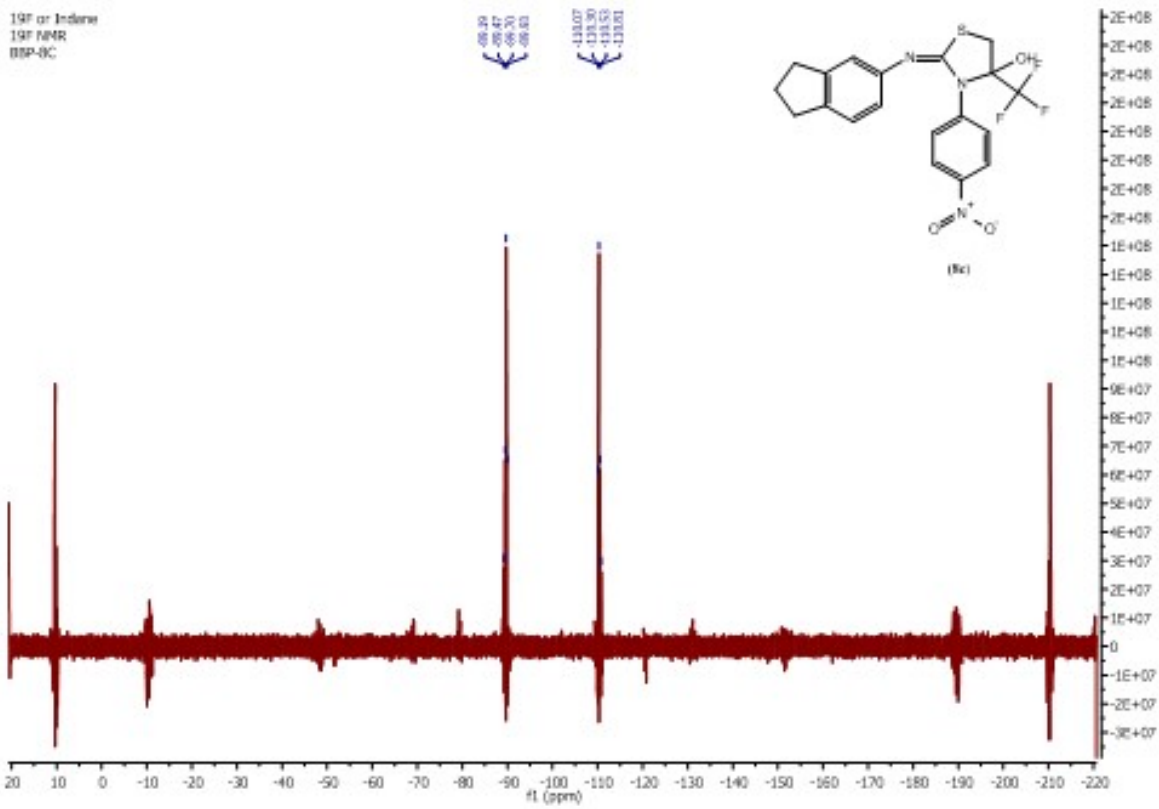
Retention Time (MS)	MS Area	Mol. Weight or Ion
0.296	43543104	394.00 I
		393.00 I



***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-(4-nitrophenyl)-4-(trifluoromethyl)thiazolidin-4-ol (8c):**








```
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\27.D
Sample Name: BBP-AI-16

    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :  10
    Acq. Instrument : LCMS                          Location :  27
    Injection Date  : 17-Jun-24 3:27:13 PM          Inj :  1
                                                    Inj Volume : 20.000 µl
    Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                      .M (Sequence Method)
    Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
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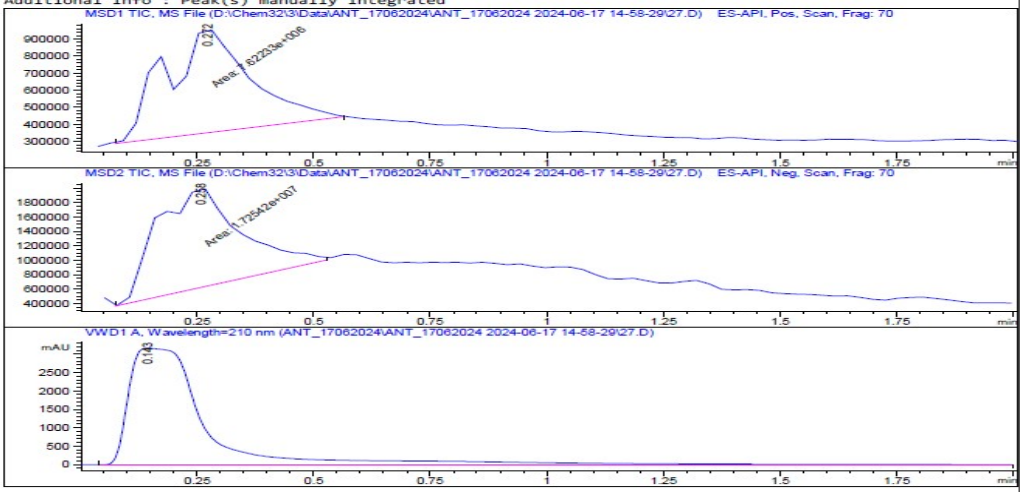
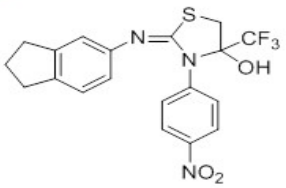


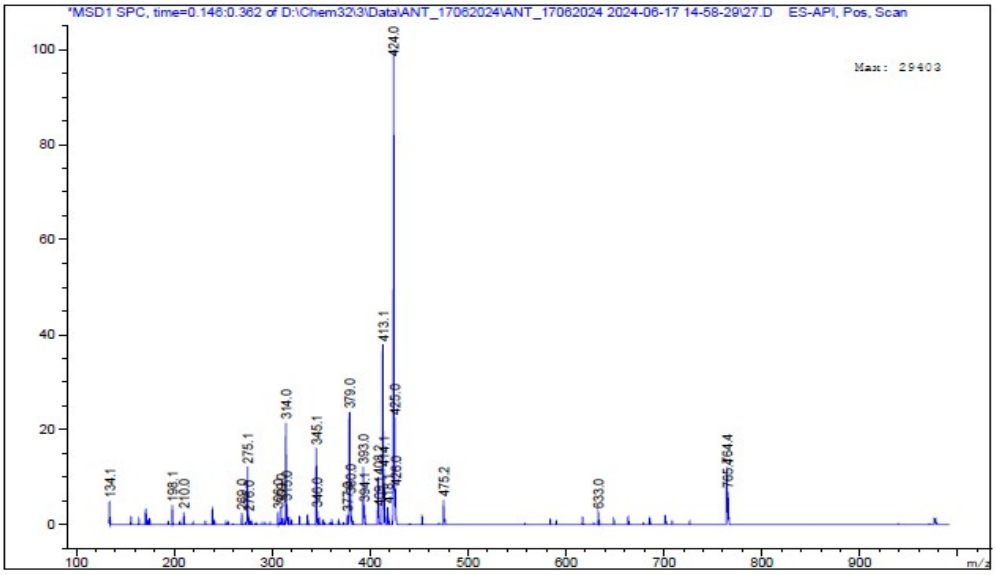
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\27.D
Sample Name: BBP-AI-16

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

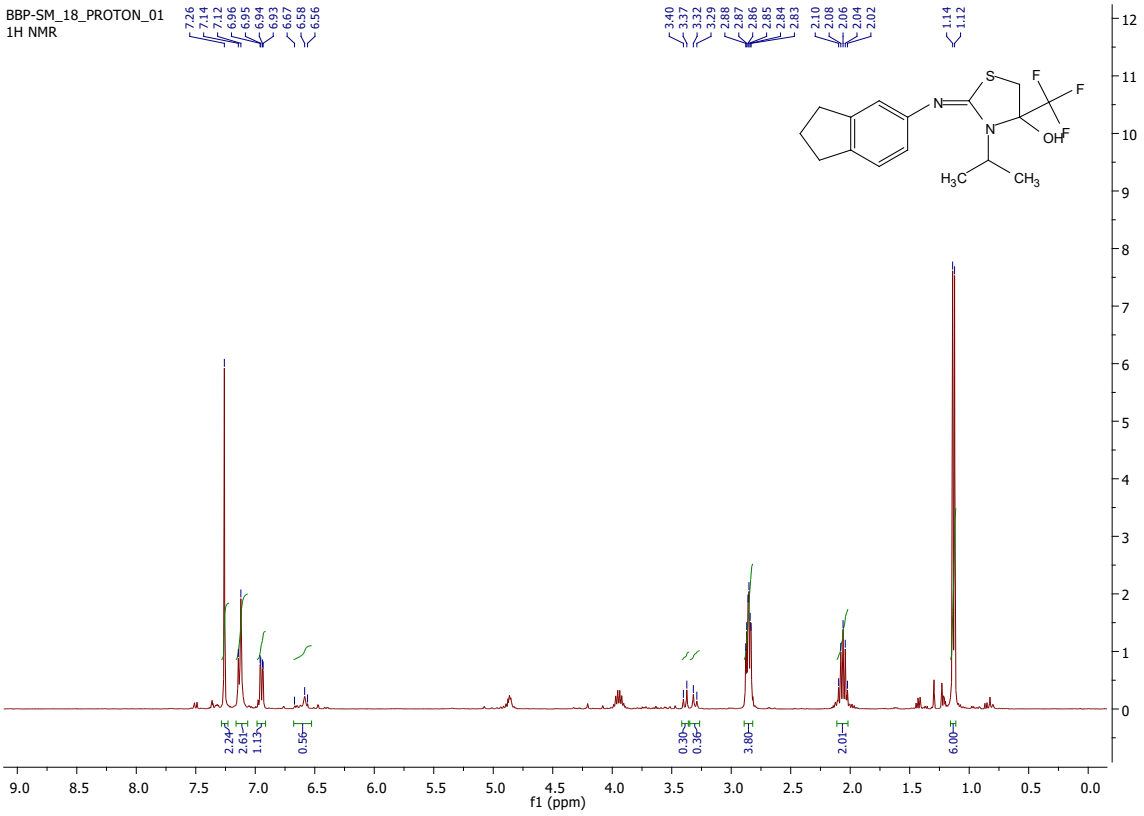


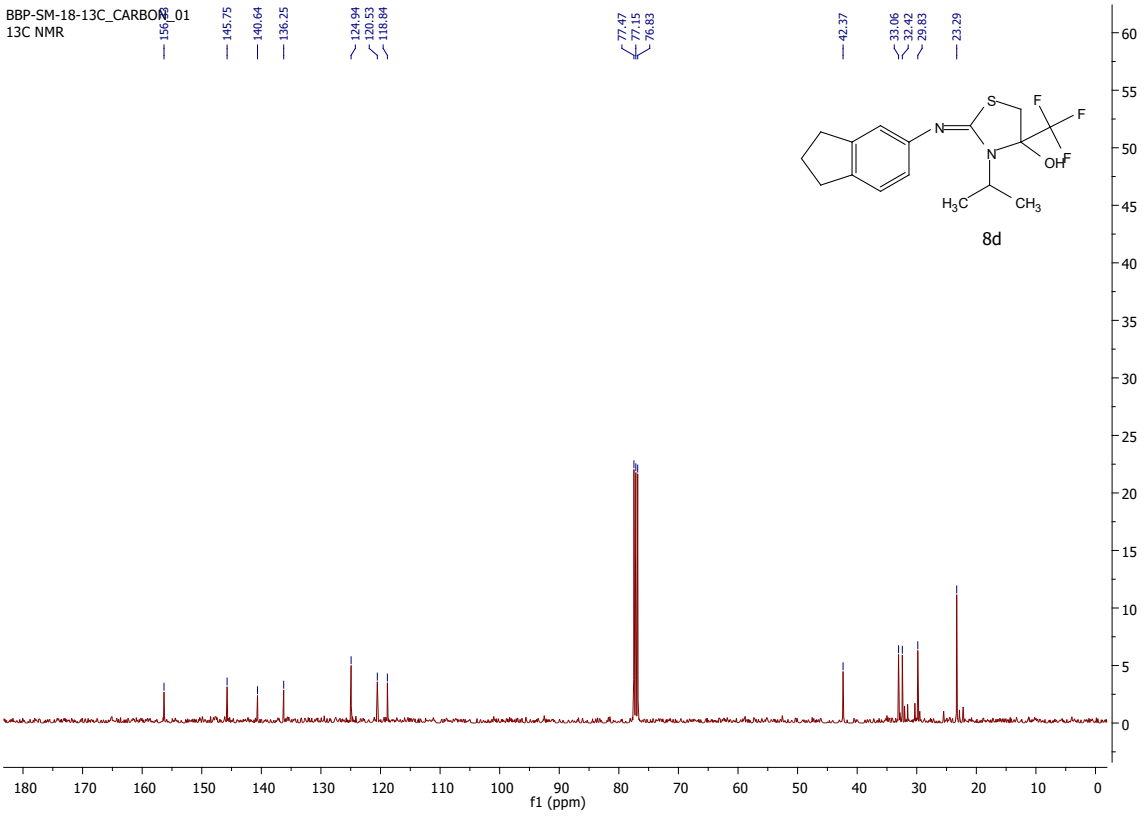
MW = 423.0

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.272	7622332	764.40 I
		425.00 I
		424.00 I
		414.10 I
		413.10 I
		393.00 I
		379.00 I
		345.10 I
		314.00 I
		275.10 I



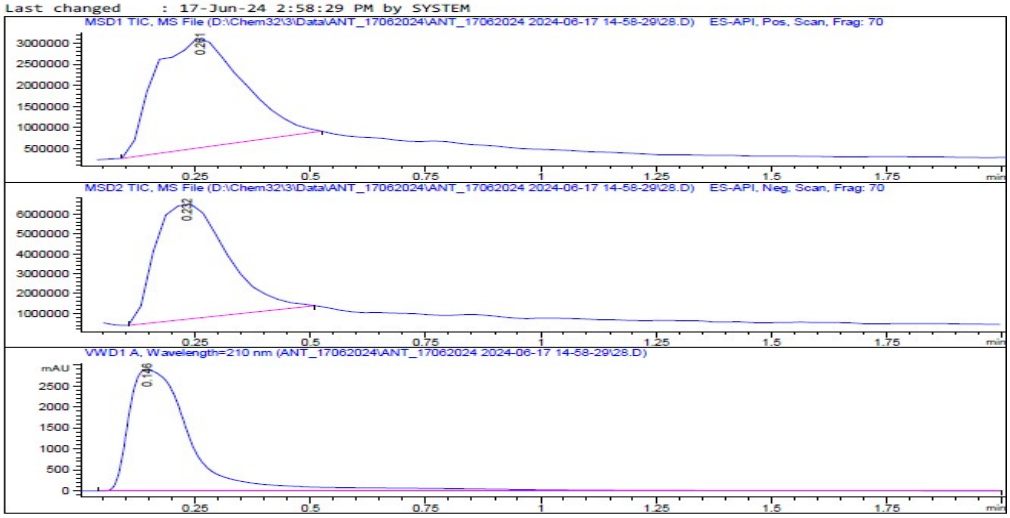
***(E)*-2-((2,3-dihydro-1*H*-inden-5-yl)imino)-3-isopropyl-4-(trifluoromethyl)thiazolidin-4-ol (8d):**


BBP-SM_18_PROTON_01
1H NMR
7.26
7.14
7.12
6.96
6.95
6.94
6.93
6.67
6.58
6.56
3.40
3.37
3.32
3.29
2.88
2.87
2.86
2.85
2.84
2.83
2.10
2.08
2.06
2.04
2.02
1.14
1.12
S
F
F
N
N
OH
F
H3C
CH3
2.24
2.61
1.13
0.56
0.30
0.36
3.80
2.01
6.00
f1 (ppm)


BBP-SM-18-13C_CARBON_01
13C NMR
156.98
145.75
140.64
136.25
124.94
120.53
118.84
77.47
77.15
76.83
42.37
33.06
32.42
29.83
23.29
S
F
F
N
N
OH
F
H3C
CH3
8d
f1 (ppm)

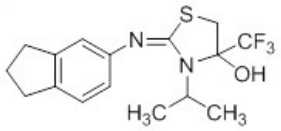
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\28.D
Sample Name: BBP-AI-17

Acq. Operator	: SYSTEM	Seq. Line	: 11
Acq. Instrument	: LCMS	Location	: 28
Injection Date	: 17-Jun-24 3:30:14 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Method	: D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS .M (Sequence Method)		
Last changed	: 17-Jun-24 2:58:29 PM by SYSTEM		



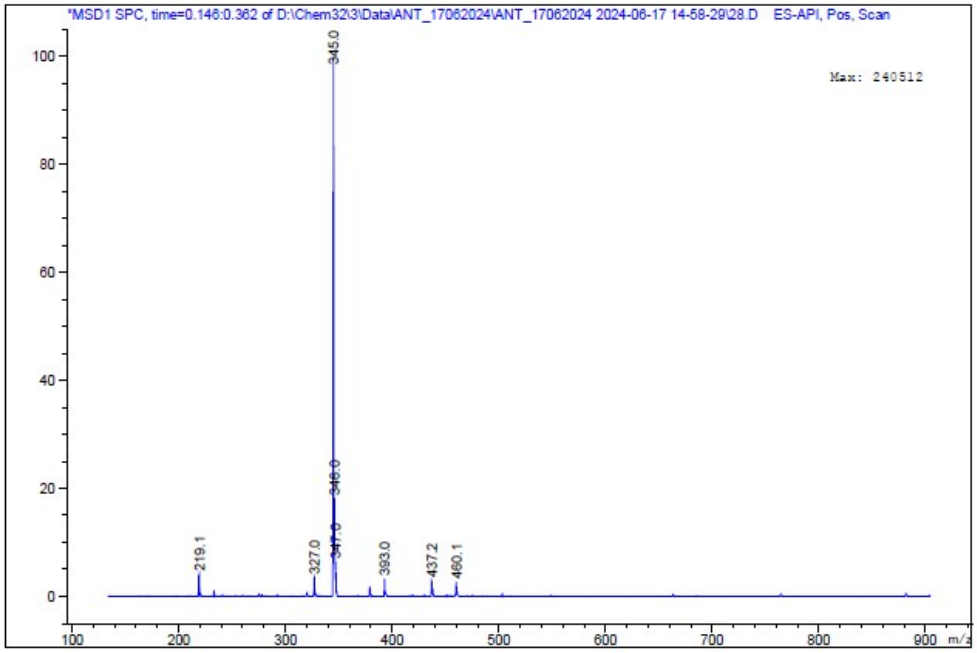
Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\28.D
Sample Name: BBP-AI-17

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.

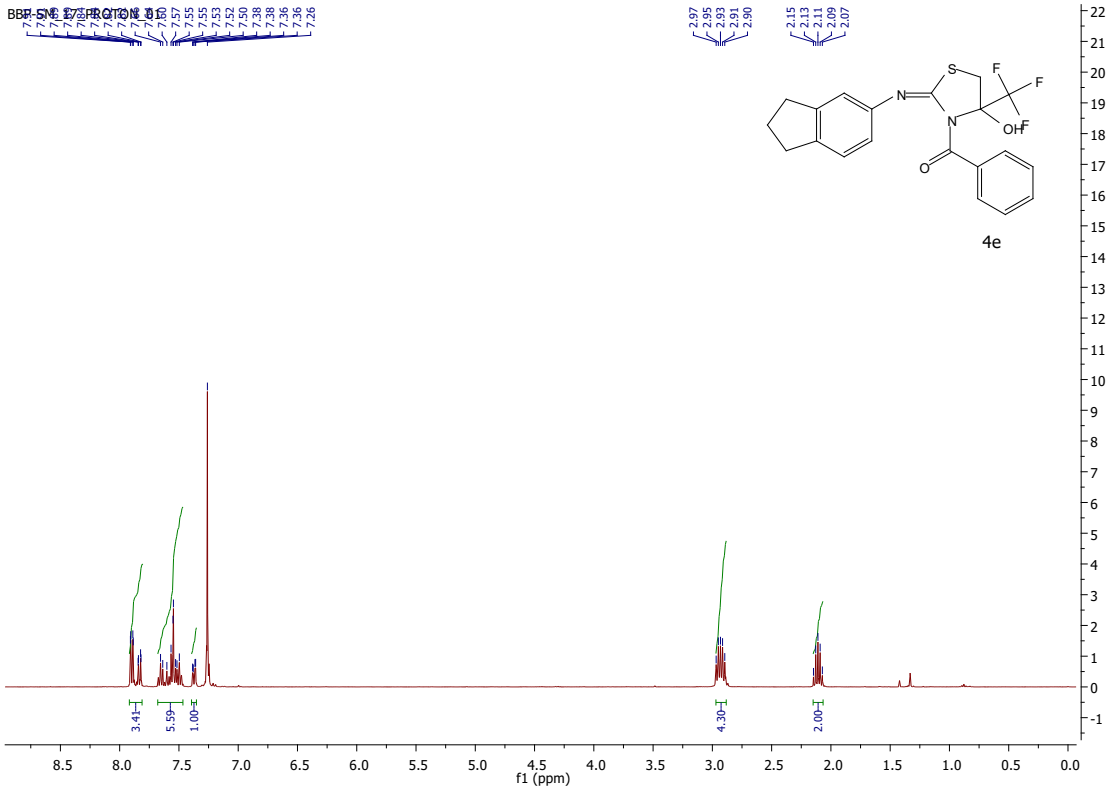


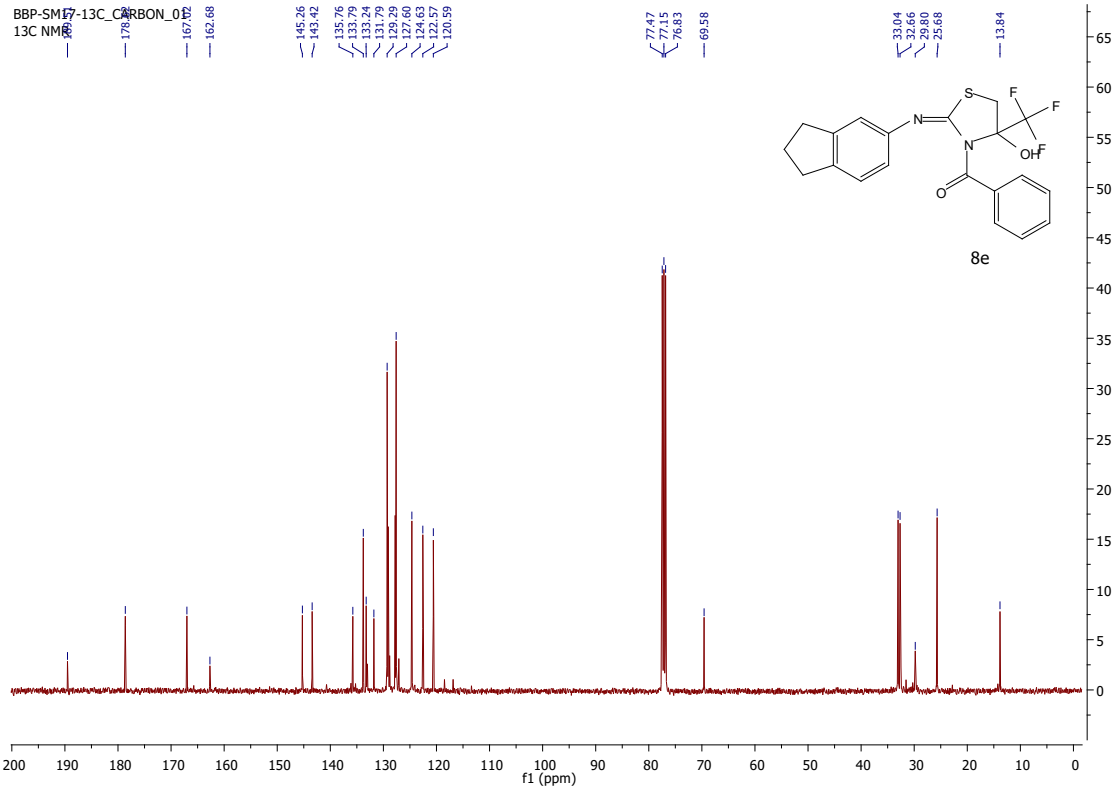
MW = 344.0

Retention Time (MS)	MS Area	Mol. Weight or Ion
0.261	34539392	346.00 I
		345.00 I



***(E)*-(2-((2,3-dihydro-1*H*-inden-5-yl)imino)-4-hydroxy-4-(trifluoromethyl)thiazolidin-3-yl)(phenyl)-methanone (8e):**





Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\29.D
Sample Name: BBP-AI-18

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Acq. Operator   : SYSTEM                          Seq. Line :  12
Acq. Instrument : LCMS                             Location :  29
Injection Date  : 17-Jun-24 3:33:21 PM                Inj :   1
                                                Inj Volume : 20.000 µl
Method          : D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\DIRECT MASS
                  .M (Sequence Method)
Last changed    : 17-Jun-24 2:58:29 PM by SYSTEM
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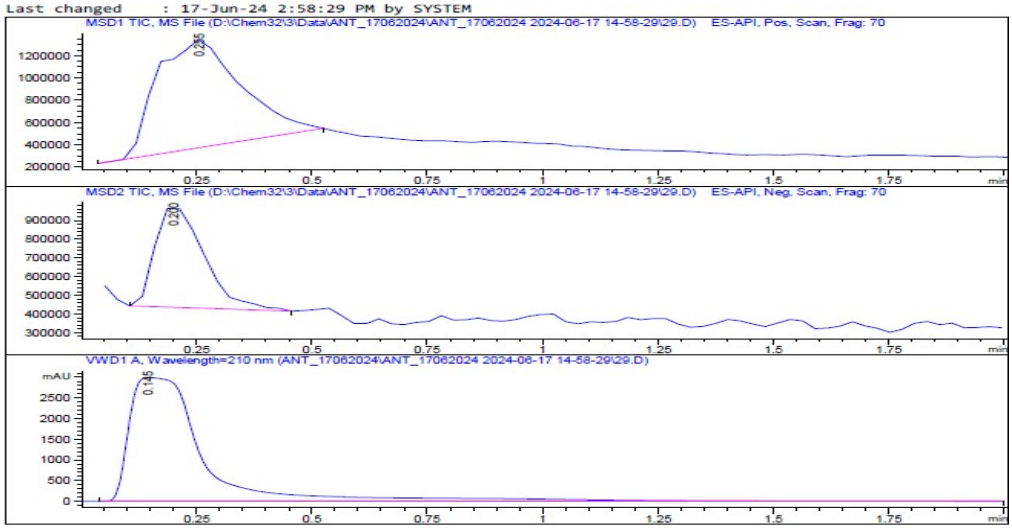
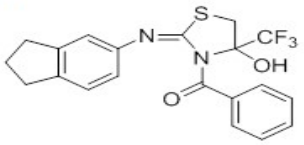
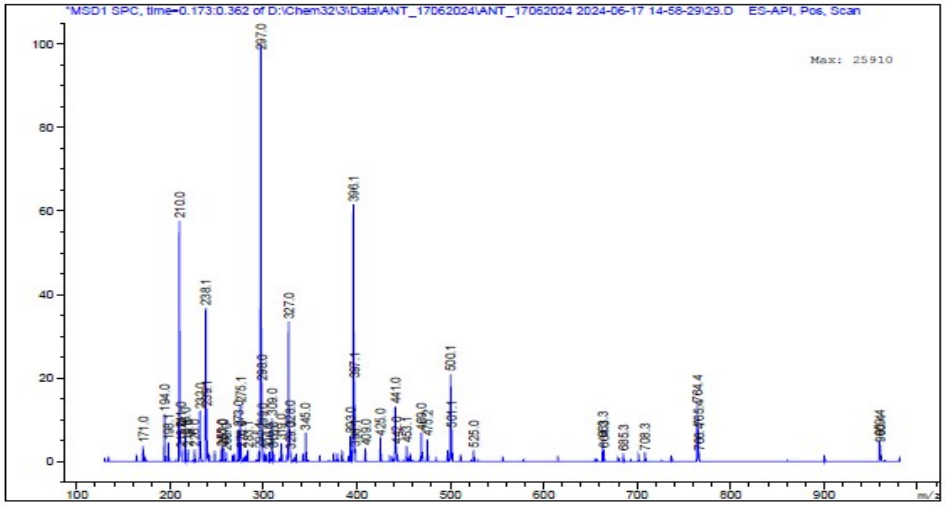


Data File D:\Chem32\3\Data\ANT_17062024\ANT_17062024 2024-06-17 14-58-29\29.D
Sample Name: BBP-AI-18

MS Signal: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"
Spectra averaged over upper half of peaks.
Noise Cutoff: 1000 counts.
Reportable Ion Abundance: > 10%.



Retention Time (MS)	MS Area	Mol. Weight or Ion
0.255	12272275	764.40 I
		500.10 I
		441.00 I
		397.10 I
		396.10 I
		327.00 I
		309.00 I
		298.00 I
		297.00 I
		275.10 I
		239.10 I
		238.10 I
		232.00 I
		210.00 I
		194.00 I



Cell culture

Adriamycin sensitive-resistant (MCF-7/ADR) breast cancer cell and (A-549/ADR) human lung cancer cell lines were grown in appropriate medium containing 10% fetal bovine serum and 2 mM L-glutamine. In order to cultivate resistant and sensitive cells, serum concentrations were optimized. 100 µL of 5000 cells/well were injected onto 96-well microtiter plates for the current screening experiment. Prior to adding experimental drugs, the microtiter plates were incubated for 24 hours at 37°C, 5% CO2, 95% air and 100% relative humidity following cell inoculation.

Sulforhodamine B (SRB) Assay for Cytotoxicity

The synthesized compounds were solubilized in a suitable solvent to prepare a stock of 10-2 concentration. During the experiment, four 10-fold serial dilutions were performed using the entire medium. To achieve the desired final compound concentrations, 10 µl of each compound dilution was introduced to the corresponding microtiter wells, which already included 90 µl medium.

Plates were incubated for 48 hours under standard conditions after the addition of drug and the assay was terminated by adding cold TCA. 50 µl of cold 30% (w/v) TCA (final concentration: 10% TCA) was carefully added to the cells to set them in place, and they were then incubated for 60 minutes at 4°C. The supernatant was disposed of; the plates were washed five times with tap water and allowed to air dry. After gentle addition of 50 µl of sulforhodamine B (SRB) solution at 0.4% (w/v) in 1% acetic acid to each well, the plates were left incubated at room temperature for 20 minutes. Unbound dye was recovered during staining and any remaining dye was removed by washing five times with 1% acetic acid. Plates were allowed to air dry. After eluting the bound dye with 10 mM trizma base, the absorbance was measured at 540 nm using an Elisa plate reader with a reference wavelength of 690 nm.

Percent growth was determined on a plate-by-plate basis for test wells compared to control wells. The ratio of the test well's average absorbance to that of the control wells X 100 was used to calculate the percentage growth. The percentage growth at each compound concentration level was computed using the six absorbance measurements: time zero (Tz), control growth (C) and test growth in the presence of drug at the four concentration levels (Ti). For each test article, the dosage response parameters were calculated. Growth inhibition of 50% (GI_{50}) drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

Experimental protocol performed for *in-vitro* cytotoxicity:

	Human Brast Cancer Cell Line MCF-7															
	% Control Growth															
	µMolar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
Samples	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM
4c	8.35	80.2	435.8	648.5	8.54	90.9	463.5	761.6	7.44	75.9	415.2	536.6	8.11	82.3	438.1	648.0
6c	8.29	89.3	683.9	2803.6	8.68	100.1	652.8	2446.2	7.40	91.7	687.1	2281.5	8.12	93.7	674.6	2510.0
8a	7.74	81.8	714.2	983.2	8.58	98.8	642.6	102.4	7.61	86.1	657.1	657.9	7.98	88.9	671.3	581.0
8b	8.42	90.3	777.1	2937.4	9.18	99.8	723.4	2381.9	8.55	83.2	736.9	2285.7	8.71	91.1	745.8	2535.0
8c	6.07	76.4	170.8	244.7	5.62	83.8	167.4	-2043.1	3.59	75.9	156.9	-1852.5	5.10	78.7	165.0	-1216.0
ADR	1.46	49.0	-199.1	-3657.5	0.26	49.7	-144.6	-3711.8	-3.87	33.5	-256.2	-4257.5	-0.72	44.1	-200.0	-3875.0

	Human Brast Cancer Cell Line A-549															
	% Control Growth															
	µMolar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
Samples	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM
4c	7.94	70.0	537.3	-529.6	8.60	88.8	563.8	-669.0	7.64	68.1	581.1	323.3	8.06	75.6	560.7	-291.0
6c	8.20	67.8	641.9	1991.4	7.54	77.0	654.1	1564.9	6.45	71.5	562.5	1969.8	7.40	72.1	619.5	1842.0
8a	7.79	54.5	841.8	-5562.4	8.40	79.7	745.7	-5292.2	7.83	71.9	700.0	-3869.5	8.01	68.7	762.5	-4908.0
8b	7.71	68.7	756.0	2001.9	8.50	87.1	697.7	1860.4	6.74	68.2	658.4	2013.9	7.65	74.7	704.0	1958.7
8c	7.57	74.0	28.0	-5180.3	8.87	66.4	10.4	-5355.6	7.44	60.1	-52.6	-4716.9	7.96	66.9	-4.73	-5084.2
ADR	6.64	33.5	7.7	-1352.5	1.97	27.7	-85.9	-2149.0	-1.32	25.0	-102.3	-1770.9	2.43	28.7	-60.17	-1757.4

	Monkey Normal Kidney Cell Line VERO															
	% Control Growth															
	µMolar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
Samples	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM	0.1 µM	1 µM	10 µM	100 µM
4c	7.58	56.76	548.5	-7089	8.24	81.05	614.6	-1469	7.865	81.25	631.2	-951	7.89	73.02	598.1	-3170

6c	7.86	86.26	994.8	2762	7.65	79.58	827.6	2940	7.76	81.56	898.5	3001	7.76	82.47	906.9	2901
8a	8.20	87.23	715.5	-7449	7.04	76.94	739.7	-3755	7.26	79.26	710.2	-3328	7.50	81.14	721.8	-4844
8b	8.04	97.39	812.0	-7079	7.30	79.51	777.0	-2203	7.62	82.58	786.3	-1107	7.65	86.49	791.8	-3463
8c	7.46	92.19	6.5	-9435	7.35	81.28	156.5	-8773	6.87	82.36	146.7	-7688	7.23	85.28	103.2	-8632
ADR	6.89	32.69	-837.3	-8969	2.11	37.07	-309.9	-7797	0.69	36.53	-239.6	-6904	3.23	35.43	-462.3	-7890

Standard protocol performed for *in-silico* Molecular Docking.

System Preparation

The EGFR kinase domain (PDB ID:1M17) was prepared in PyMOL/PyRx by removing crystallo graphic waters and non-essential heteroatoms and by adding missing hydrogens. Erlotinib and the test ligand 8c were built and energy-minimized, then converted to PDBQT format using the dockit AutoDock Vina pipeline, which assigned Gasteiger charges and defined rotatable bonds. Docking

Docking Setup

Blind docking was carried out in AutoDock Vina using a search space encompassing the entire protein surface. The grid box was centered at (8.96,7.213,58.875) with dimensions 95.016 ˚A × 67.26 ˚A × 52.842 ˚A, ensuring unrestricted sampling of potential binding sites. Interaction profiles of the best-ranked poses were analyzed with LigPlot+ to identify hydrogen bonds and hydrophobic contacts. Erlotinib was used as a control to validate the docking setup and scoring behavior.

Standard protocol performed for *in-silico* MD Simulation Finding.

Simulation Protocol

DockedEGFR–ligand complexes were parameterized using CHARMM-GUI with the CHARMM36m force field for the protein and CGenFF parameters for ligands. Each system was solvated in a TIP3P water box with a 10 ˚A buffer and neutralized with 0.15 M NaCl. Following energy minimization, 5 ns NVT and 5 ns NPT equilibration were performed at 300 K and 1 bar using a V-rescale thermostat and Parrinello–Rahman barostat, respectively. Production MD simulations were carried out for 100 ns using GROMACS 2023.1 with a 2 fs timestep, PME electrostatics, LINCS constraints, and periodic boundary conditions. Trajectories were saved every 10 ps for post-simulation analysis.

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