A facile one pot multi component synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetates and their antiviral activity

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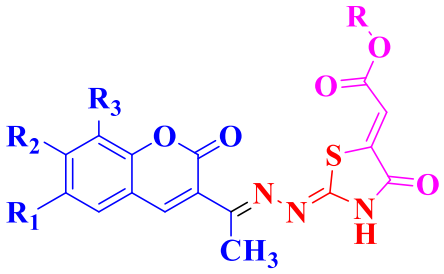


IV (1-31)

Abstract: An efficient one-pot synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives has successfully been achieved via a three component cyclization reaction of various substituted 3-acetyl coumarins, thiosemicarbazide and dialkyl acetylenedicarboxylates, in presence of acetic acid. The isolated products were obtained in pure form with high yields through simple workup. The newly synthesised compounds structure was established on the basis of spectral (IR, ^{1}H NMR, ^{13}C NMR, ESI-mass) elemental analysis and single crystal X-ray data. All synthesised compounds were screened for their antiviral activity against a broad spectrum of human viruses in different cell cultures. Of the novel synthesised compounds, thirteen compounds exerted activity against

Punta Toro virus, including compound **IV-19**, for which an antiviral potency was noted against a broad panel of DNA and RNA viruses as well.

Keywords: One- pot three - component synthesis, 3-acetylcoumarin, thiosemicarbazide, thiazolidinone and Antiviral activity.

1. Introduction

According to the world health organization, infectious diseases caused by the different members of DNA- and RNA-containing viruses are related with significant proportions of human deaths across the globe. Antiviral agents are considered to be very useful weapons for controlling these infectious diseases. However, the treatment of viral infections still remains an important challenge because of the rapid mutability of the virus resulting in escape mutants and drug-resistant virus strains [1-3]. Due to the emergence of anti-viral resistance, there is a continuous interest in developing novel antiviral agents that have less side effects, high therapeutic indices and are active against different circulating resistant viruses.

Multi component reaction (MCR) chemistry is becoming a promising field of chemistry, due to the many positive implications of economic and ecological issues [4,5]. This MCR approach provides an elegant and rapid synthesis platform with various diversified complex molecules in one-pot, high synthetic efficiency, and minimal waste generation by reducing the reaction stages and the reaction work-up like solvent extraction, purification, selectivity and atom economy.

Numerous biologically active molecules contain various hetero atoms like nitrogen, oxygen, sulphur and selenium. Due to their biological importance, enormous interest was given to the synthesis of these heterocyclic scaffolds resulting in one of the most fertile research domains for medicinal chemists all over the world. Besides all the other heterocyclic systems, functionalized thiazolidinone is an N- and S-containing privileged heterocyclic scaffold, with versatile pharmacological properties such as anticancer [6, 7], antiviral [8,9,10], herbicidal [11], antioxidant [12,], antiinflammatory [13, 14], antimicrobial [15-19], antimalarial [20] and ubiquitously present in a large number of clinically used drugs.

In addition, coumarins and their derivatives are an important class of natural and synthetic heterocyclic compounds with vital pharmacological and material application properties including anti-inflammatory [21], antileishmanial [22], steroid hydroxylase [23], MALDI-FT ICR MS detection of hydrophobic compounds [24], stokes shift calculation [25], anti HIV [26, 27], solid state dye lasers [28], fluorescent chemosensor [29], antitumor agents [30, 31], antituberculosis[32, 33]. From this synthetic, medicinal and molecular diversity point of view and in continuation of our research towards one pot multicomponent synthesis of potential heterocyclic systems [34 - 37] constructing a chemical entity having both coumarin and thiazolidinone heterocyclic motifs along with a potential functional group into a single molecular framework, would be of great interest as these two combined nuclei might result in interesting biological properties.

Here we developed an efficient one pot multi component approach for the synthesis of alkyl - 4-oxo-(coumarinyl ethylidene hydrazono)-thiazolidin-5-ylidene acetates and studied their antiviral properties against a broad spectrum of human viruses in different cell culture medium.

2. Experimental:

2.1 Materials and Methods:

The reagents like thiosemicarbazide, dimethyl acetylene dicarboxylate, diethyl acetylene dicarboxylate, di *tert*. butyl acetylene dicarboxylate and the solvents used in this method are procured from the commercial sources and used without any further purification unless until mentioned. The progress of the reaction was monitored through thin layer chromatography technique by using silica gel plates (60 F_{254}, Merck) and the spot visualization was done with ultraviolet radiation and Iodine vapour. The melting points were recorded on digital Stuart melting point apparatus (SMP-30, Stuart, Staffordshire, UK). The FT-IR spectra were recorded on Perkin- Elmer spectrophotometer (Spectrum-100S). All the ^{1}H NMR and ^{13}C NMR were recorded on Bruker at 400 MHz and 100 MHz frequencies and the chemical shift values are given with respect to tetramethylsilane (TMS) as an internal standard. The elemental analysis was performed on Carlo- Erba 1108 instrument. The crystallographic data

was collected on Bruker Kappa Apex II difractometer with graphite monochromated MoKα radiation. The compounds structure was solved by using X-shell software. The single crystal XRD data was deposited in Cambridge Crystallographic Data Centre and the CCDC number was assigned (CCDC: **1418103**).

2.2. General procedure for the synthesis of alkyl - 4-oxo-(coumarinyl ethylidene hydrazono)-thiazolidin-5-ylidene acetates (IV 1-31):

A mixture substituted 3-acetyl coumarin (1 mmol) and thiosemicarbazide (1mmol) were taken in the round bottom flask having 5 mL of acetic acid solvent. The reaction mixture was stirred at room temperature for about 15 minutes. To this reaction mixture dialkylacetylene dicarboxylate (1 mmol) was added. The reaction mixture was stirred and then the temperature of the reaction mixture was raised to 60-65 ^{o}C. After completion of the reaction (monitored by the TLC) the reaction mixture was cooled, the solid separated was filtered and recrystallized from a methanol solvent.

2.2.1. (Z)-methyl 2-((Z)-4-oxo-2-((E)-(1-(2-oxo-2H-chromen-3-yl)ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (IV-1) :

Yield : (82%); m.p.: 247 – 249 ^{o}C; IR (KBr, υ cm^{-1}): 3431(-NH), 1723(lactone -C=O), 1643 (ester –C=O); 1517 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, -CH_3); 3.76 (s, 3H, -OCH_3); 6.68 (s, 1H, -C=H); 7.40 (t, *J* = 7.6 Hz, 1H, -Ar-H); 7.43 – 7.47 (m, 1H, -Ar-H); 7.66 – 7.71 (m, 1H, -Ar-H); 7.91 – 7.93 (m, 1H, -Ar-H); 8.27(s, 1H, -C_4-H of coumarin); 12.98 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 22.5; 57.7; 119.7; 121.2; 123.9; 130.0; 131.3; 134.7; 138.0; 147.2; 147.3; 148.0; 158.8; 164.1; 165.7; 167.4; 170.9; *Anal*. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31; S, 8.63; Found: C, 54.92; H, 3.58; N, 11.26; S, 8.56; HRMS (ESI) m/z calculated for $C_{17}H_{13}N_3NaO_5S$ [M + Na]$^{+}$: 394.0474, found. 394.0472.

2.2.2. (Z)-methyl 2-((Z)-2-((E)-(1-(6-chloro-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-2) :

Yield: (85%); m.p.: 267 – 269 ^{o}C; IR (KBr, υ cm^{-1}): 3152 (-NH), 1722 (lactone – C=O), 1623 (ester – C=O), 1505 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.36 (s, 1H, -CH_3); 3.79 (s, 1H, -OCH_3); 6.67 (s, 1H, -C=H); 7.50 (d, *J* = 8.8 Hz, 1H, -Ar-H) 7.70 – 7.72 (m, 1H, -Ar-H); 8.08 (d, *J* = 2.4 Hz, 1H, -Ar-H); 8.23 (s, 1H, -C_4-H of coumarin); 12.98 (s, 1H, -NH); *Anal*. Calcd for $C_{17}H_{12}ClN_3O_5S$: C, 50.31; H, 2.98; N, 10.35; s, 7.90; Found: C, 50.37; H,

2.93; N, 10.31; S, 7.84; HRMS (ESI) m/z calculated for $C_{17}H_{12}ClN_3NaO_5S$ $[M + Na]^+$: 428.0084, found: 428.0083.

2.2.3. (Z)-methyl 2-((Z)-2-((E)-(1-(6,8-dichloro-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-3):

Yield: (86%); m.p.: 265 – 267 ^oC; IR (KBr, υ cm^{-1}): 3152 (-NH), 1725(lactone –C=O), 1614 (ester –C=O); ^1H NMR (400MHz, DMSO-d_6, δ ppm): 2.36 (s, 3H, $-CH_3$); 3.76 (s, 3H, $-OCH_3$), 6.66 (s, 1H, -C=H); 7.70 (t, *J* = 6.4 Hz, 1H, -Ar-H); 8.08 (s, 1H, -Ar-H); 8.23 (s, 1H, $-C_4$-H of coumarin); 12.99 (s, 1H, -NH); *Anal.* Calcd for $C_{17}H_{11}Cl_2N_3O_5S$: C, 46.38; H, 2.52; N, 9.54; S, 7.28; Found: C, 46.32; H, 2.58; N, 9.59; S, 7.22; MS (ES m/z): 441 $[M+ H]^+$.

2.2.4. (Z)-methyl 2-((Z)-2-((E)-(1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-4):

Yield: (92%); m.p.: 276 – 278 ^oC; IR (KBr, υ cm^{-1}): 3132 (-NH), 1708 (lactone-C=O),1708 (ester –C=O), 1602 (amide-C=O); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H, $-CH_3$); 3.77 (s, 3H, $-OCH_3$); 6.93 (s, 1H, -C=H); 7.42 – 7.45 (m, 1H, -Ar-H); 7.81 – 7.84 (m, 1H, -Ar-H); 8.16 (d, *J* = 2.4Hz, 1H, -Ar-H); 8.23 (s, 1H, $-C_4$-H of coumarin); 12.97 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 17.1; 52.4; 116.3; 118.3; 120.6; 122.5; 131.3; 135.0; 140.7; 152.6; 171.9; *Anal.* Calcd for $C_{17}H_{12}BrN_3O_5S$: C, 45.35; H, 2.69; N, 9.33; S, 7.12; Found:, C, 45.39; H, 2.74; N, 9.38; S, 7.16; HRMS (ESI) m/z calculated for $C_{17}H_{12}BrN_3NaO_5S$ $[M + Na]^+$: 471.9579, found: 471.9575.

2.2.5. (Z)-methyl 2-((Z)-2-((E)-(1-(6,8-dibromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-5):

Yield: (90%); m.p.: 248 – 250 ^oC; IR (KBr, υ cm^{-1}): 3150 (-NH), 1728 (lactone -C=O), 1614 (amide –C=O); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.36 (s, 3H, $-CH_3$); 3.77 (s, 3H,-OCH_3); 6.68 (s, 1H, -C=H); 7.45 (s, 1H, -Ar-H); 7.83 (s, 1H, -Ar-H); 8.23 (s, 1H, $-C_4$-H of coumarin); 12.99 (s, 1H, -NH); *Anal.* Calcd for $C_{17}H_{11}Br_2N_3O_5S$: C, 38.59; H, 2.10; N, 7.94; S, 6.06; Found: C, 38.63; H, 2.15; N, 7.90; S, 6.17; MS (ESI m/z): 530 $[M+H]^+$.

2.2.6. (Z)-methyl 2-((Z)-4-oxo-2-((E)-(1-(3-oxo-3H-benzo[f]chromen-2-yl) ethylidene) hydrazono)thiazolidin-5-ylidene)acetate (IV-6):

Yield: (91%); m.p.: 312 - 314 ^oC; IR (KBr, υ cm^{-1}): 3138 (-NH), 1717 (lactone –C=O), 1617 (amide –C=O), 1570 (-C=N); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.31(s, 3H,-CH_3);

3.71 (s, 3H, -OCH_3); 7.65 – 7.67 (m, 2H, -Ar-H); 7.77 (t, *J* = 7.6 Hz, 2H, -Ar-H); 8.08 (d, *J* = 8.8 Hz, 1H, -Ar-H); 8.23 (d, *J* = 9.2 Hz, 1H, -Ar-H); 8.43 (s, 1H, -Ar-H); 9.11 (s, 1H-C_4-H of coumarin); 10.43 (s, 1H, -NH); *Anal*. Calcd. for $C_{21}H_{15}N_3O_5S$: C, 59.85; H, 3.59; N, 9.97; S, 7.61; Found: C, 59.80; H, 3.64; N, 9.91; S, 7.65; MS (ES m/z): 422 $[M+H]^+$.

2.2.7. (Z)-methyl 2-((Z)-2-((E)-(1-(8-methoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-7):

Yield: (79%); m.p.: 260 – 262 ºC; IR (KBr, υ cm^{-1}): 3155 (-NH), 1705 (lactone –C=O), 1611 (amide –C=O), 1523 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.37 (s, 3H, -CH_3); 3.76 (s, 3H, ester-OCH_3); 3.93 (s, 3H, -OCH_3); 6.68 (s, 1H, =CH); 7.31 – 7.38 (m, 2H, -Ar-H); 7.44 – 7.46 (m, 1H, -Ar-H); 8.24 (s, 1H,C_4-H of coumarin); 12.97 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ17.1; 52.4; 56.1; 114.5; 114.9; 119.2; 120.4; 122.4; 124.7; 126.1; 142.2; 142.9; 146.3; 158.5; 165.8; *Anal*. Calcd for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; S, 7.99; Found: C, 53.81; H, 3.72; N, 10.52; S, 7.93; HRMS (ESI) m/z calculated for $C_{18}H_{15}N_3O_6S$ $[M + H]^+$: 402.0760, found: 402.0763.

2.2.8. (Z)-methyl 2-((Z)-2-((E)-(1-(6-bromo-8-methoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-8):

Yield: (90%); m.p.: 267 -269 ºC; IR (KBr, υ cm^{-1}): 3420 (-NH), 1717 (lactone –C=O), 1634 (ester –C=O), 1587 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.36 (s, 3H, -CH_3); 3.77 (s, 3H, - ester -OCH_3); 3.96 (s, 3H, -OCH_3); 6.68 (s, 1H, -C=H); 7.52 (d, *J* = 2Hz, 1H, -Ar-H); 7.74 (d, *J* = 2 Hz, 1H, -Ar-H); 8.20 (s, 1H, -C_4-H of coumarin); 12.98 (s, 1H, -NH); *Anal*. Calcd for $C_{18}H_{14}BrN_3O_6S$: C, 45.01; H, 2.94; N, 8.75; S, 6.68; Found: C, 45.10; H, 3.00; N, 8.79; S, 6.63; HRMS (ESI) m/z calculated for $C_{18}H_{15}BrN_3O_6S$ $[M+H]^+$: 479.9865, found: 479.9870.

2.2.9. (Z)-methyl 2-((Z)-2-((E)-(1-(8-ethoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-9):

Yield: (82%); m.p.: 255 – 257 ºC; IR (KBr, υ cm^{-1}): 3150 (-NH), 1711 (lactone -C=O), 1605 (amide –C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.42 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.37 (s, 3H, CH_3); 3.76 (s, 3H, -OCH_3); 4.17 – 4.23 (m, 2H, CH_2); 6.68 (s, 1H, =CH); 7.29 – 7.37 (m, 2H, -Ar-H); 7.43 – 7.45 (m, 1H, -Ar-H); 8.23 (s, 1H, -C_4-H of coumarin); 12.97 (s, 1H, -NH); *Anal*. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72; Found: C, 54.98; H, 4.18; N, 10.16; S, 7.78; MS (ESI m/z): 416 $[M+H]^+$.

2.2.10. (Z)-methyl 2-((Z)-2-((E)-(1-(6-bromo-8-ethoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-10):

Yield: (88%); m.p.: 257 – 259 ºC; IR (KBr, υ cm^{-1}): 3141 (-NH), 1740 (lactone -C=O), 1702 (ester -C=O), 1605 (amide –C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.40 (t, *J* = 6.8 Hz, 3H, -CH_3); 2.36 (s, 3H, -CH_3); 3.77 (s, 3H, -OCH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.68 (s, 1H, =CH); 7.50 (d, *J* = 2 Hz, 1H, -Ar-H); 7.73 (d, *J* = 2 Hz, 1H, -Ar-H); 8.19 (s, 1H, -C_4-H of coumarin); 12.98 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 14.4; 17.2; 52.4; 65.1; 114.6; 116.2; 118.2; 120.5; 122.4; 127.2; 140.9; 142.4; 142.6; 146.4; 161.9; 165.8; Anal.Calcd for $C_{19}H_{16}BrN_3O_6S$: C, 46.17; H, 3.26; N, 8.50; S, 6.49; Found: C, 46.11; H, 3.21; N, 8.40; S, 6.54; MS (ES m/z): 496 $[M+2H]^+$.

2.2.11. Ethyl (Z)-2-((E)-4-oxo-2-(((Z)-1-(2-oxo-2H-chromen-3-yl)ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (IV-11):

Yield: (82%); m.p.: 236 - 238 ºC; IR (KBr, υ cm^{-1}): 3247 (-NH); 1738 (lactone –C=O); 1713 (ester –C=O); 1688 (amide –C=O); 1614 (-C=N); 1233 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.25 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.38(s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, - CH_2); 6.65 (s,1H, =CH); 7.39 – 7.48 (m, 2H, -Ar-H); 7.69 (t, *J* = 7.2 Hz, 1H, -Ar-H)7.92 (d, *J* = 6.8 Hz, 1H, -Ar-H); 8.27 (s, 1H, -C_4-H of coumarin); 12.96 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 13.9, 17.1, 61.2, 114.6, 115.8, 116.0, 118.5, 123.9, 124.7, 126.1, 129.4, 131.7, 132.7, 141.1, 141.9, 153.5, 158.8, 171.9; *Anal.* Calcd for $C_{18}H_{15}N_3O_5S$: C, 56.10; H, 3.92; N, 10.90; S, 8.32; Found: C, 56.17; H, 3.99; N, 10.95; S, 8.38; HRMS (ESI) m/z calculated for $C_{18}H_{15}N_3NaO_5S$ $[M + Na]^+$: 408.0630, found: 408.0637.

2.2.12. Ethyl (Z)-2-((E)-2-(((Z)-1-(6-chloro-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-12):

Yield: (86%); m.p.: 256 - 258 ºC; IR (KBr, υ cm^{-1}): 3245 (-NH); 1715 (lactone-C=O); 1630 (ester –C=O); 1603 (amide –C=O); 1572 (-C=N); 1286 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.26 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.33 (s, 3H, -CH_3); 4.22 – 4.27 (m, 2H, -CH_2); 6.62 (s, 1H, =CH); 7.51 (d, *J*= 8.8 Hz, 1H, Ar-H); 7.70 – 7.73 (m, 1H, Ar-H); 7.86 (d, *J* = 2.8 Hz, 1H, Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.64 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 14.0; 23.0; 61.3; 114.7; 118.3; 119.6; 127.0; 127.7; 128.6; 132.1; 140.4; 142.5; 151.7; 156.7; 159.9; 165.4; *Anal.* Calcd. for $C_{18}H_{14}ClN_3O_5S$: C,

51.49; H, 3.36; N, 10.01; S, 7.64; Found: C, 51.54; H, 3.44; N, 9.95; S, 7.69; MS (ESI m/z): 420 $[M + H]^+$ (100%).

2.2.13. Ethyl (Z)-2-((E)-2-(((Z)-1-(6,8-dichloro-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-13):

Yield: (85%); m.p.: 254 - 256 ^oC; IR (KBr, υ cm^{-1}): 3251 (-NH); 1741 (lactone -C=O); 1697 (ester –C=O); 1607 (amide -C=O); 1567 (-C=N); 1240 (-C-O-C); ^1H NMR (400 MHz, DMSO-d_6,δ ppm): 1.25 (t, J = 6.4 Hz, 3H, $-CH_2CH_3$); 2.36 (s, 3H, $-CH_3$); 4.20 - 4.24 (m, 2H, $-CH_2$); 6.65 (s, 1H, =CH); 7.86 (d, J = 2.4Hz, 1H, Ar-H); 8.06 (t, J = 2.8 Hz, 1H, Ar-H); 8.23 (s, 1H, $-C_4$-H of coumarin); 12.69 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 13.9; 23.0; 61.3; 114.7; 118.2; 119.6; 126.9; 127.7; 128.3; 128.6; 132.0; 140.3; 142.5; 151.7; 156.7; 165.3; *Anal.* Calcd for $C_{18}H_{13}Cl_2N_3O_5S$: C, 47.59; H, 2.88; N, 9.25; S, 7.06; Found: C, 47.51; H, 2.92; N, 9.19; S, 7.13; MS (ESI m/z): 454 $[M]^+$ (100%); 456$[M+2H]^+$.

2.2.14. Ethyl (Z)-2-((E)-2-(((Z)-1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-14):

Yield: (88%); m.p.: 258 - 260 ^oC; IR (KBr, υ cm^{-1}): 3165 (-NH); 1728 (lactone-C=O); 1697 (ester –C=O); 1635 (amide –C=O); 1614 (-C=N); 1246.46 (C-O-C); ^1H NMR (400 MHz, DMSO-d_6,δ ppm): 1.26 (t, J = 6.8 Hz, 3H, $-CH_2CH_3$); 2.36 (s, 3H, CH_3); 4.18 – 4.27 (m, 2H,-CH_2); 6.63 (s, 1H, =CH); 7.44 (d, J= 8.8 Hz, 1H, Ar-H); 7.82 (d, J = 9.2 Hz, 1H, Ar-H); 8.00 (t, J = 2 Hz,1H, Ar-H); 8.21 (s, 1H, $-C_4$-H of coumarin); 12.96 (s, 1H,-NH); *Anal.* Calcd for $C_{18}H_{14}BrN_3O_5S$: C, 46.56; H, 3.04; N, 9.05; S, 6.91; Found: C, 46.50; H, 3.10; N, 9.12; S, 6.85; MS (ESI m/z): 464 $[M]^+$ (100%).

2.2.15. Ethyl (Z)-2-((E)-2-(((Z)-1-(6,8-dibromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-15):

Yield: (91%); m.p.: 246 - 248 ^oC; IR (KBr, υ cm^{-1}): 3249 (-NH); 1741 (lactone-C=O); 1697 (ester –C=O); 1634 (amide –C=O); 1614 (-C=N); 1241 (-C-O-C); ^1H NMR (400 MHz, DMSO-d_6,δ ppm): 1.24 (t, J = 4.4 Hz, 3H, $-CH_3$); 2.36 (s, 3H, $-CH_3$); 4.18 – 4.23 (m, 2H, -CH_2); 6.92 (s, 1H,=CH); 7.44 (d, J = 8.8 Hz, 1H, -Ar-H); 7.81- 7.84 (m, 1H, -Ar-H); 8.58 (s, 1H, $-C_4$-H of coumarin); 12.99 (s, 1H, -NH); *Anal.* Calcd for $C_{18}H_{13}Br_2N_3O_5S$: C, 39.80; H, 2.41; N, 7.74; S, 5.90; Found: C, 39.74; H, 2.46; N, 7.70; S, 5.95; MS (ESI m/z): 544 $[M + H]^+$.

2.2.16. Ethyl (Z)-2-((Z)-4-oxo-2-(((E)-1-(3-oxo-3H-benzo[f]chromen-2-yl)ethylidene) hydrazono)thiazolidin-5-ylidene)acetate (IV-16):

Yield: (88%); m.p.: 278 - 280 ^{o}C; IR (KBr, υ cm^{-1}): 3154 (-NH); 1711 (lactone-C=O); 1632 (ester –C=O); 1607 (-amide –C=O); 1565 (-C=N); 1238 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.24 (t, J = 6.8 Hz, 3H, -CH_2CH_3); 2.45 (s, 3H, -CH_3); 4.18 – 4.23 (m, 2H, -CH_2); 6.61(s, 1H, =CH); 7.63 – 7.68 (m, 2H, -Ar-H); 7.79 (t, J = 8 Hz, 1H, -Ar-H); 8.10 (d, J = 8 Hz, 1H, -Ar-H); 8.27 (d, J = 8.8 Hz, 1H, -Ar-H); 8.55 (d, J = 8 Hz, 1H, -Ar-H); 9.00 (s, 1H, -C_4-H of coumarin); 12.97 (s, 1H, -NH); *Anal.* Calcd for $C_{22}H_{17}N_3O_5S$: C, 60.68; H, 3.93; N, 9.65; S, 7.36; Found: C, 60.62; H, 3.97; N, 9.61; S, 7.40; MS (ESI m/z): 436 [M + H]$^{+}$.

2.2.17. Ethyl (Z)-2-((E)-2-(((Z)-1-(8-methoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-17):

Yield: (80%); m.p.: 252 -254 ^{o}C; IR (KBr, υ cm^{-1}): 3436 (-NH); 1708 (lactone-C=O); 1643 (ester –C=O); 1614 (amide –C=O); 1578 (-C=N); 1235 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6,δ ppm):** 1.24 (t, J = 6.8 Hz, 3H, -CH_2CH_3); 2.37(s, 3H, -CH_3); 3.93 (s, 3H, -OCH_3); 4.20 – 4.26 (m, 2H, -CH_2); 6.65 (s, 1H, =CH); 7.26 – 7.28 (m, 1H, Ar-H); 7.33 – 7.36 (m, 1H, Ar-H); 7.44 – 7.46 (m, 1H, Ar-H); 8.24 (s, 1H, -C_4-H of coumarin); 12.95 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 13.9; 17.2; 56.2; 61.3; 114.7; 115.0; 118.8; 119.2; 119.9; 120.5; 124.7; 124.8; 126.0; 126.2; 142.2; 142.4; 146.3; 158.5; 165.3; *Anal.* Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72; Found: C, 54.91; H, 4.16; N, 10.18; S, 7.75; MS (ESI m/z): 416 [M + H]$^{+}$ (100%).

2.2.18. Ethyl (Z)-2-((E)-2-(((Z)-1-(6-bromo-8-methoxy-2-oxo-2H-chromen-3-yl)ethylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-18):

Yield: (83%); m.p.: 264 - 266 ^{o}C; IR (KBr, υ cm^{-1}): 3081 (-NH); 1724 (lactone-C=O); 1632 (ester –C=O); 1618 (amide –C=O); 1600 (-C=N); 1235 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.25 (t, J = 6.8 Hz, 3H, -CH_3); 2.36 (s, 3H, -CH_3); 3.96 (s, 3H, -OCH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.65 (s, 1H, =CH); 7.52 (d, J = 2.4 Hz, 1H, -Ar-H); 7.74 (d, J =2 Hz, 1H,-Ar-H); 8.20 (s, 1H,-C_4-H of coumarin); 12.97 (s,1H, -NH); *Anal.* Calcd for $C_{19}H_{16}BrN_3O_6S$: C, 46.17; H, 3.26; N, 8.50; S, 6.49; Found: C, 46.13; H, 3.21; N, 8.59, S, 6.42; MS (ESI m/z): 496 [M + 2H]$^{+}$.

2.2.19. Ethyl (Z)-2-((E)-2-(((Z)-1-(8-ethoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-19):

Yield: (82%); m.p.: 244 - 246 ºC; IR (KBr, υ cm^{-1}): 3426 (-NH); 1735 (lactone-C=O); 1708 (ester –C=O); 1696 (amide –C=O); 1607 (-C=N); 1240 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2-CH_3); 1.42 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3); 2.37 (s, 3H, -CH_3); 4.17 – 4.25 (m, 4H, -CH_2), 6.65 (s, 1H, =CH); 7.29 – 7.35 (m, 2H, -Ar-H); 7.37 – 7.45 (m, 1H, Ar-H); 8.23 (s, 1H, -C4-H of coumarin); 12.96 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 13.9; 14.6; 17.2; 61.3; 64.5; 114.7; 115.9; 119.3; 120.5; 124.8; 126.2; 142.2; 142.7; 143.0; 145.5; 158.6; 165.4; *Anal*. Calcd for $C_{20}H_{19}N_3O_6S$: C, 55.94; H, 4.46; N, 9.78; S, 7.47; Found: C, 55.90; H, 4.49; N, 9.71; S, 7.41; MS (ESI m/z): 452 $[M + Na]^+$(100%).

2.2.20. Ethyl (Z)-2-((E)-2-(((Z)-1-(6-bromo-8-ethoxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-20):

Yield: (86%); m.p.: 236 - 238 ºC; IR (KBr, υ cm^{-1}): 3251 (-NH); 1726 (lactone –C=O); 1709 (ester-C=O); 1613 (amide –C=O); 1567 (-C=N); 1237 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2-CH_3); 1.40 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.36 (s,3H, -CH_3); 4.00 – 4.03 (m, 2H, -O-CH_2); 4.21 – 4.25 (m, 2H, -O-CH_2); 6.69 (s, 1H, -C=H); 7.50 (d, *J* = 2Hz, 1H, -Ar-H); 7.73 (d, *J* = 2 Hz, 1H, -Ar-H); 8.19 (s, 1H, -C_4-H of coumarin); 12.97 (s, 1H, -NH); *Anal*. Calcd for $C_{20}H_{18}BrN_3O_6S$: C, 47.25; H, 3.57; N, 8.27; S, 6.31; Found: C, 47.21; H, 3.51; N, 8.31; S, 6.38; MS (ESI m/z): 510 $[M + 2H]^+$.

2.2.21. Ethyl (Z)-2-((E)-2-(((Z)-1-(7-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-21):

Yield: (82%); m.p.: 260 – 262 ºC; IR (KBr, υ cm^{-1}): 3212 (-OH); 3162 (-NH); 1728 (lactone-C=O); 1693 (ester -C=O); 1609 (amide –C=O); 1216 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3); 2.36 (s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.64 (s, 1H, =CH); 6.76 (d, *J* = 2.4 Hz, 1H, -Ar-H); 6.82 – 6.85 (m, 1H, -Ar-H); 7.73 (d, *J* = 8.8Hz, 1H, -Ar-H); 8.18 (s, 1H, -C_4-H of coumarin); 10.83 (s, 1H, -OH); 12.91 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 14.4; 17.7; 61.8; 110.9; 111.6; 113.1; 115.0; 120.9; 128.2; 143.2; 143.5; 154.2; 159.8; 160.2; 160.7; 162.8; 165.8; 166.1; *Anal*. Calcd for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; S, 7.99; Found: C, 53.81; H, 3.72; N, 10.51; S, 7.93; MS (ESI m/z): 402 $[M + H]^+$.

2.2.22. Ethyl (Z)-2-((E)-2-(((Z)-1-(7-hydroxy-8-methyl-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-22):

Yield: (77%); m.p.: 273 – 275 ºC; IR (KBr, υ cm^{-1}): 3315 (-OH); 3175 (-NH); 1730 (lactone –C=O); 1678 (ester –C=O); 1633 (amide – C=O)1605 (-C=N); 1230 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6,δ ppm): 1.25 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.18 (s, 3H, -Ar-CH_3); 2.37 (s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.64 (s, 1H, =CH); 6.89 (d, *J* = 8.4 Hz, 1H, -Ar-H); 7.57 (d, *J* = 8.4Hz, 1H, -Ar-H); 8.16 (s, 1H, -C_4-H of coumarin); 10.75 (s, 1H, -OH); 12.92 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 7.8; 13.9; 17.2; 61.3; 110.5; 111.1; 112.6; 114.5; 120.5; 127.8; 142.8; 142.9; 153.7; 159.3; 159.7; 160.2; 162.3; 165.3; 165.7; *Anal*. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72; Found: C, 54.98; H, 4.16; N, 10.08; S, 7.71; MS (ESI m/z): 416 $[M+ H]^+$.

2.2.23. tert-butyl (Z)-2-((E)-4-oxo-2-(((Z)-1-(2-oxo-2H-chromen-3-yl) ethylidene) hydrazono) thiazolidin -5-ylidene)acetate (IV-23):

Yield: (78%); m.p.: 240 - 242 ºC; IR (KBr, υ cm^{-1}): 3122 (-NH); 1721 (lactone –C=O); 1685 (ester –C=O); 1637 (amide –C=O); 1602 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*. butyl); 2.38 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.39 – 7.43 (m, 1H, -Ar-H); 7.47 (d, *J* = 8.4 Hz, 1H, -Ar-H); 7.66 – 7.71 (m, 1H, -Ar-H); 7.90 – 7.93 (m, 1H, -Ar-H); 8.28 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 17.1; 27.6; 82.1; 116.0; 116.5; 118.6; 124.8; 126.0; 129.5; 132.8; 141.7; 142.0; 153.6; 158.8; 160.7; 161.8; 164.6; 165.7; *Anal*. Calcd for $C_{20}H_{19}N_3O_5S$: C, 58.10; H, 4.63; N, 10.16; S, 7.76; Found: C, 58.16; H, 4.60; N, 10.11; S, 7.71; HRMS (ESI) m/z calculated for $C_{20}H_{19}N_3NaO_5S[M + Na]^+$: 436.0943, found: 436.0942.

2.2.24. tert-butyl (Z)-2-((E)-2-(((Z)-1-(6-chloro-2-oxo-2H-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-24):

Yield: (85%); m.p.: 272 - 274 ºC; IR (KBr, υ cm^{-1}): 3232 (-NH), 1728 (lactone -C=O), 1605 (-amide –C=O); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 1.47 (s, 9H, *tert*. butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.50 (d, *J* = 8.8 Hz, 1H, -Ar- H); 7.70 – 7.72 (m,1H, -Ar-H); 8.07 (d, *J* = 2.4 Hz, 1H, -Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 17.1; 27.6; 82.1; 116.4; 118.0; 120.0; 127.2; 128.4; 128.4; 132.2; 140.7; 152.2; 158.4; 161.6; 164.6; *Anal*. Calcd for $C_{20}H_{18}ClN_3O_5S$: C, 53.63; H, 4.05; N, 9.38; S, 7.16; found: C, 53.70; H, 4.14; N, 9.30; S, 7.20; MS (ESI m/z): 470 $[M + Na]^+$ (100%).

2.2.25. tert-butyl (Z)-2-((E)-2-(((Z)-1-(6,8-dichloro-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-25):

Yield: (85%); m.p.: 269 - 270 ºC; IR (KBr, υ cm^{-1}): 3141 (-NH); 1732 (lactone –C=O); 1675 (ester –C=O); 1621 (amide –C=O); 1601 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*. butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.50 (d, *J* = Hz, 1H, - Ar-H); 7.70 – 7.73 (m, 1H, -Ar-H); 8.07 (d, *J* = 2.4Hz, 1H, -Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 8.29 (s, 1H, -Ar-H); 12.92 (s, 1H, -NH). *Anal*. Calcd for $C_{20}H_{17}Cl_2N_3O_5S$: C, 49.80; H, 3.55; N, 8.71; S, 6.65; Found: C, 49.86; H, 3.59; N, 8.77;S, 6.54; MS (ES m/z): 483 $[M+H]^+$.

2.2.26. tert-butyl (Z)-2-((E)-2-(((Z)-1-(6-bromo-2-oxo-2H-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-26):

Yield: (84%); m.p.: 274 – 276 ºC; IR (KBr, υ cm^{-1}): 3202 (-NH); 1722 (lactone –C=O); 1605 (-C=N); 1241 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*. butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.44 (d, *J* = 8.8 Hz, 1H, -Ar-H); 7.81 – 7.84 (m, 1H, -Ar-H); 8.20 (d, *J* = 2.4 Hz, 1H); 8.24 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 17.1; 27.6; 82.1; 115.9; 116.4; 124.7; 125.9; 129.4; 132.7; 141.6; 141.9; 153.5; 158.7; 160.7; 161.8; 164.5; 165.6; *Anal*. Calcd for $C_{20}H_{18}BrN_3O_5S$: C, 48.79; H, 3.69; N, 8.53; S, 6.51; Found: C, 48.84; H, 3.61; N, 8.59; S, 6.54; MS (ESI m/z): 494 $[M + 2H]^+$.

2.2.27. tert-butyl (Z)-2-((E)-2-(((Z)-1-(6,8-dibromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-27):

Yield : (88%); m.p.: 242 - 244 ºC; IR (KBr, υ cm^{-1}): 3064 (-NH); 1725 (lactone –C=O); 1617 (amide –C=O); 1235 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 1.47 (s, 9H, - *tert*. butyl); 2.36 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.44 (d, *J* = 8.8 Hz, 1H, -Ar-H); 7.81 – 7.83 (m, 1H, -Ar-H); 8.20 (s, 1H, -C_4-H of coumarin); 12.92 (s, 1H, -NH); *Anal*. Calcd for $C_{20}H_{17}Br_2N_3O_5S$: C, 42.05; H, 3.00; N, 7.36; S, 5.61; Found: C, 42.10; H, 2.96; N, 7.32; S, 5.66; MS (ES m/z): 572 $[M+H]^+$.

2.2.28. tert-butyl (Z)-2-((E)-4-oxo-2-(((Z)-1-(3-oxo-3H-benzo[f]chromen-2-yl)ethylidene) hydrazono)thiazolidin-5-ylidene)acetate (IV-28):

Yield: (80%); m.p.: 318 – 320 ºC; IR (KBr, υ cm^{-1}): 3085 (-NH); 1720 (lactone -C=O); 1626 (ester –C=O); 1612 (amide –C=O); 1238 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.46 (s, 9H, *tert*. butyl- H); 2.45 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.64 – 7.69 (m, 2H, -Ar-

H); 7.78 (t, *J* = 8 Hz, 1H, -Ar-H); 8.11 (d, *J* = 8.4 Hz, 1H,-Ar-H); 8.28 (d, *J* = 8.8 Hz, 1H, -Ar-H); 8.56 (d, *J* = 8.4 Hz, 1H, -Ar-H); 9.01 (s, 1H, C_4-H of coumarin); 12.95 (s, 1H, =NH); *Anal*. Calcd for $C_{24}H_{21}N_3O_5S$: C, 62.19; H, 4.57; N, 9.07; S, 6.92; Found: C, 62.14; H, 4.53; N, 8.97; S, 6.96; MS (ES m/z): 464 $[M+H]^+$.

2.2.29. tert-butyl(Z)-2-((E)-2-(((Z)-1-(8-methoxy-2-oxo-2H-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-29):

Yield: (84%); m.p.: 138 - 140 °C; IR (KBr, υ cm^{-1}): 3215(-NH); 1722 (-lactone – C=O); 1614 (amide – C=O); 1247 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*. butyl - H); 2.37 (s, 3H, -CH_3); 3.93 (s, -3H, -OCH_3); 6.54 (s, 1H, =CH); 7.31 – 7.38 (m, 2H, -Ar-H); 7.43 – 7.45 (m, 1H, -Ar-H); 8.29 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 17.7; 28.1; 56.7; 82.7; 115.5; 117.0; 119.7; 121.1; 125.3; 126.7; 142.2; 142.3; 143.5; 146.8; 159.1; 162.3; 165.1; 166.3; *Anal*. Calcd for $C_{21}H_{21}N_3O_6S$: C, 56.87; H, 4.77; N, 9.48, S, 7.23; Found: C, 56.82; H, 4.79; N, 9.42; S, 7.26; MS (ESI m/z): 444 $[M + H]^+$.

2.2.30. tert-butyl(Z)-2-((E)-2-(((Z)-1-(6-bromo-8-methoxy-2-oxo-2H-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-30):

Yield: (85%); m.p.: 249 – 250 °C; IR (KBr, υ cm^{-1}): 3196 (-NH); 1734 (lactone-C=O); 1685 (ester –C=O); 1627 (amide –C=O); 1603 (-C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*.butyl-H), 2.35 (s, 3H, -CH_3); 3.95 (s, 3H, -OCH_3); 7.52 (d, *J* = 2 Hz, 1H, -Ar-H); 7.74 (d, *J* = 2 Hz, 1H, -Ar-H); 8.21 (s, 1H, -C_4-H of coumarin); 12.91 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 17.1; 27.6; 56.7; 82.1; 116.1; 116.5; 117.5; 120.4; 122.4; 127.2; 140.9; 141.7; 142.3; 147.1; 158.0; 161.0; 161.5; 164.6; 165.7; *Anal*. Calcd for $C_{21}H_{20}BrN_3O_6S$: C, 48.28; H, 3.86; N, 8.04; S, 6.14; Found: C, 48.32; H, 3.81; N, 8.12; S, 6.18; MS (ES m/z): 523 $[M + H]^+$.

2.2.31. tert-butyl (Z)-2-((E)-2-(((Z)-1-(8-ethoxy-2-oxo-2H-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (IV-31):

Yield: (81%); m.p.: 225 - 227 °C; IR (KBr, υ cm^{-1}): 3147 (-NH); 1717 (lactone –C=O); 1608 (-C=N); 1250 (-C-O-C); ^{1}H NMR (400 MHz, DMSO- d_6, δ ppm): 1.42 (t, *J* = 6.8 Hz, 3H, -CH_3); 1.47 (s, 9H, *tert*. butyl –H); 2.37 (s, 3H, -CH_3); 4.17 – 4.22 (m, 2H, -O-CH_2); 6.54 (s, 1H, =CH); 7.29 – 7.37 (m, 2H, -Ar-H); 7.43 (d, *J* = 7.2Hz, 1H, -Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H,=NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 14.6; 17.2; 27.6;

64.5; 82.2; 115.9; 116.5; 119.3; 120.5; 124.8; 126.1; 141.7; 142.3; 143.0; 145.5; 158.6; 160.8; 161.9; 164.6; 165.7; *Anal*. Calcd for $C_{22}H_{23}N_3O_6S$: C, 57.76; H, 5.07; N, 9.18; S, 7.01; Found: C, 57.79; H, 4.98; N, 9.24; S, 6.97; HRMS (ESI m/z calculated for $C_{22}H_{23}N_3NaO_6S$ $[M + Na]^+$: 480.1205, found: 480.1204.

2.3. Antiviral activity evaluation:

The antiviral activity of the new compounds was determined in different cell types. (i) HEL cells were seeded in 96-well plates and incubated for 6 days at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV^r, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), diluted in medium to obtain a virus input of 100 $CCID_{50}$ (1 $CCID_{50}$ being the virus dose that is able to infect 50% of the cell cultures), was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 37°C, microscopical analysis was performed to score the virus-induced cytopathicity. (ii) Vero cells were seeded in 96-well plates at 30,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 3 days (for Coxsackie virus B4 and Sindbis virus) or 6 days (for Parainfluenza virus 3, Punta Toro virus and Reovirus) of incubation at 37°C microscopical analysis was performed to score the virus-induced cytopathicity. (iii) HeLa cells were seeded in 96-well plates at 15,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 3 days (for Coxsackie virus B4 and Vesicular stomatitis virus) or 6 days (for Respiratory syncytial virus) of incubation at 37°C microscopical analysis was performed to score the virus-induced cytopathicity. (iv) MDCK cells were seeded in 96-well plates at 7,500 cells per well and incubated for 1 day at 35°C. Medium was aspirated and replaced by

serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 35°C the virus-induced cytopathicity was determined by visual scoring of the CPE, as well as by measuring the cell viability with the colorimetric formazan-based MTS assay. (v) CRFK cells were seeded in 96-well plates at 30,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Feline herpes virus* or *Feline corona virus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

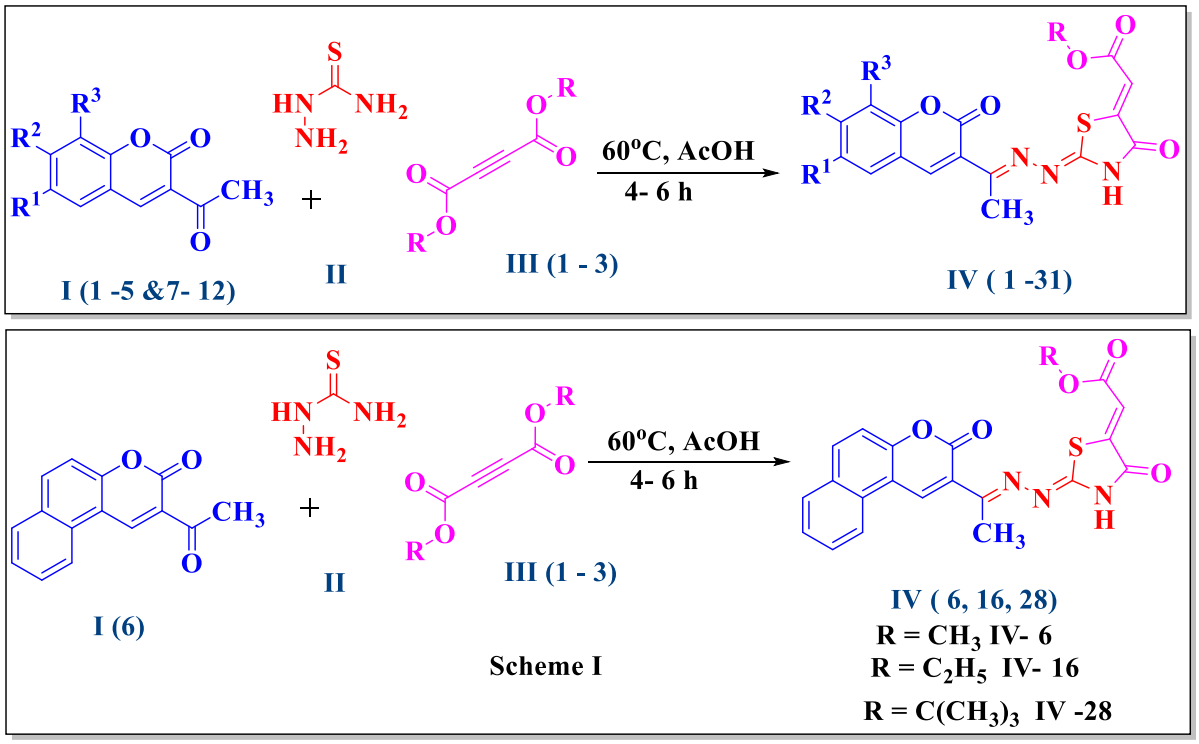
3. Results and discussion

3.1. Chemistry

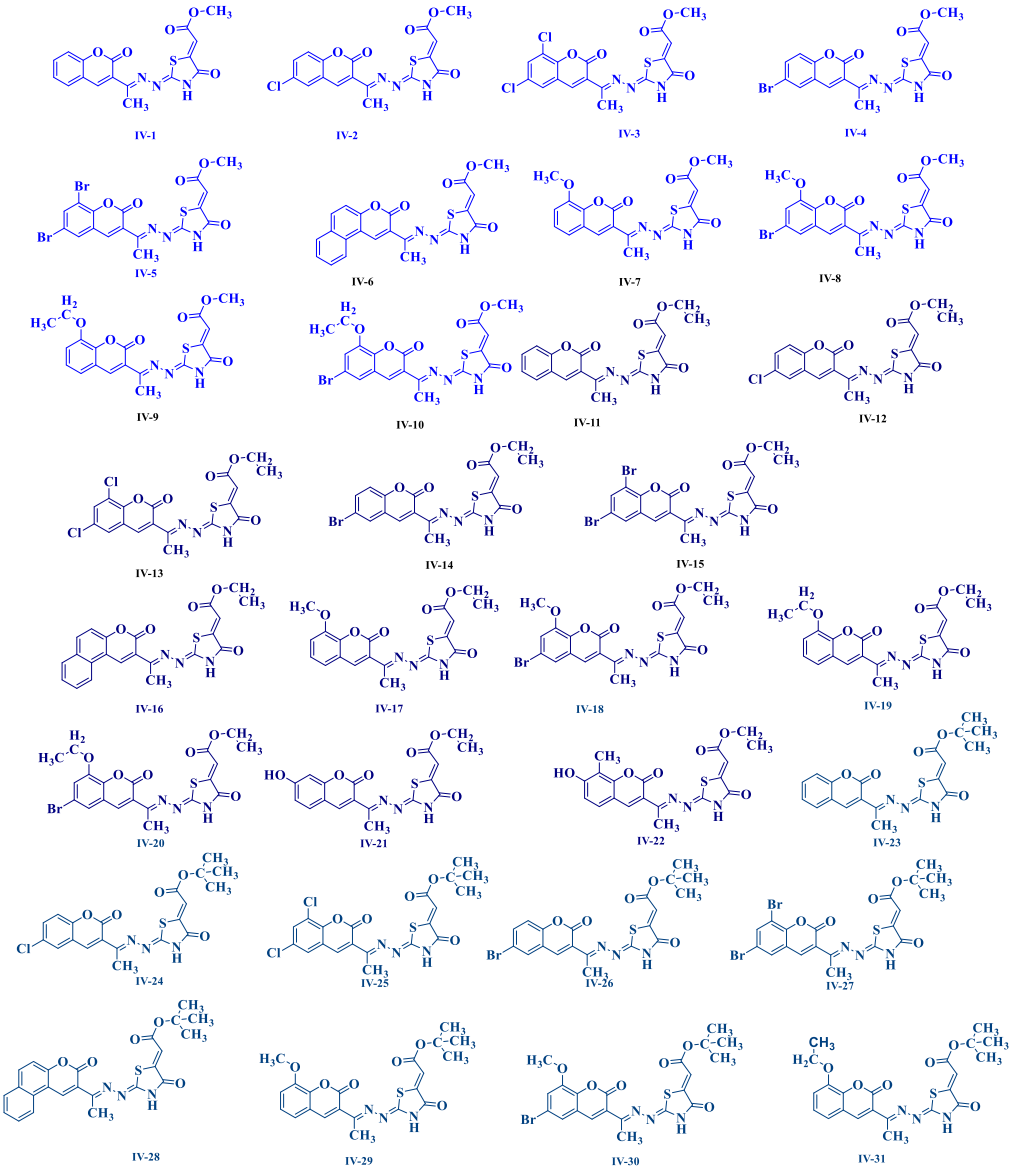
A proficient and novel synthesis of alky 4-oxo-coumarinyl ethylidene hydrazono- thiazolidin-5-ylidene acetate derivatives were obtained via a one pot multicomponent approach. The schematic representation of the target molecules is depicted in **Scheme 1**. The starting materials of substituted 3-acetyl coumarins were synthesised using various substituted salicylaldehydes and ethylacetoacetate by Knoevenegel condensation method [38, 39]. Initially we performed the reaction in presence of ethanol with substituted 3-acetylcoumarin, thiosemicarbazide and dialkyl acetylene dicarboxylate. The reaction took place at longer time intervals to obtain the desired product, whereas in the presence of acetic acid the reaction was completed within 3-4 h with good yields.

All the synthesised compounds were well characterised by physical and analytical spectral data (IR, ^{1}H NMR, ^{13}C NMR, ESI - Mass). The analytical data of the novel substituted 4-oxo-coumarinyl ethylidene hydrazono- thiazolidin-5-ylidene acetate were presented in the experimental section. The IR spectrum of the compound IV-14 exhibited characteristic absorption bands at 3165, 1728, 1697, 1635 and 1614 cm^{-1} with respect to the –NH, coumarin lactone, ester carbonyl, amide carbonyl and imine (-C=N) functional groups.

Further support was obtained by using ^{1}H NMR, ^{13}C NMR, mass spectral data and single crystal X-ray data. The ^{1}H NMR of compound IV-8 exhibited characteristic singlet peaks at δ 2.36, 3.77, 3.96, 6.68, 8.20 and 12.98 ppm corresponding to $–CH_3$, $-OCH_3$ of ester, OCH_3 of coumarin ring, vinylic proton (=C-H), C_4-H of coumarin, and -NH proton respectively. The ^{13}C NMR exhibited characteristic peaks at δ 13.98, 120.52, 158.72, 162.32, 165.66 ppm for methyl, vinylic, lactone carbonyl, imino and ester carbons respectively. The LC-MS of the compound IV-12 indicated the molecular ion peaks as [M+1] and [M+3] at m/z 420.16 and 422.18 a.m.u.



Scheme I



Scheme 1. The synthetic strategy to make the alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin -5-ylidene acetate derivatives.

Single crystal X-ray data of the compound IV-31 further confirms the structure (**Figure 2a**). In the title compound, the 2*H*-chromene ring system is essentially planar [r.m.s deviation = 0.003 Å] and are twisted with a little angle of 2.70 (10) to the 1,3-thiazolidine ring. The C8---C3---O1---C2, C3---O1---C2---C1, C9---C10---C12---N1, C10---C12---N1---N2, S1—C14---N2---N1, C14---N2---N1---C12, S1---C16---C17---C18 and C16---C17---C18---O2 torsion angles are 8.5(4), - 174.6(3), 5.1(4), -179.9(2), -0.4(4), -172.8(2), 0.9(4) and 166.7(2) respectively. Selected geometric parameters are given in **Table 1**.

In the crystal, there exist two intramolecular C—H···O hydrogen bonds. N—H···O hydrogen bonds link the molecules to each other, forming centro symmetric R^2_2 (8) dimers (**Table 2, Figure. 2b**). Furthermore, C---H…π and π-π interactions are not observed.

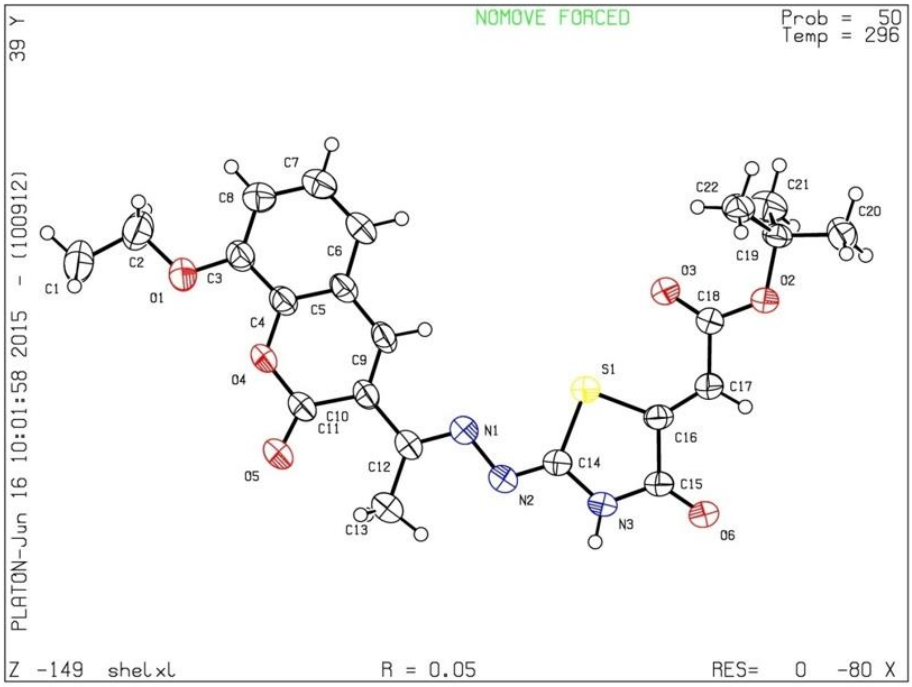
Table 1: Single crystal X-ray diffraction data of compound IV- 31

Chemical formula	$C_{22}H_{23}N_3O_6S$
M_r	457.49
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	296
a, *b*, *c* (Å)	5.6858 (3), 28.274 (2), 13.7996 (10)
β (°)	97.095 (3)
V (Å^3)	2201.5 (3)
Z	4
F(000)	960
D_x (Mg m^{-3})	1.380
Radiation type	Mo $K\alpha$
No. of reflections for cell measurement	3354
θ range (°) for cell measurement	5.2–45.5
μ (mm^{-1})	0.19
Crystal shape	Block
Colour	Yellow
Crystal size (mm)	0.60 × 0.30 × 0.20
Diffractometer	Bruker axs kappa apex2 CCD Diffractometer
Radiation source	fine-focus sealed tube
Monochromator	Graphite
Scan method	ω and ϕ scan
Absorption correction	Multi-scan *SADABS* (Bruker, 1999)
T_{min}, T_{max}	0.894, 0.963
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	17534, 5392, 3321
R_{int}	0.039
θ values (°)	θ_{max} = 28.4, θ_{min} = 1.4
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.670
Range of *h*, *k*, *l*	h = -7→7, k = -35→37, l = -18→18
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.054, 0.177, 1.07
No. of reflections	5392
No. of parameters	294
No. of restraints	0
H-atom treatment	H-atom parameters constrained
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.091P)^2 + 0.0395P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	< 0.001
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.40, -0.34

Table 2: Hydrogen Bonds (Å)

D—H···A	D—H	H···A	D···A	D—H···A
N3---H3N…O6	0.86	1.98	2.828(3)	170
C21-H21C…O3	0.96	2.55	3.104 (3)	117
C22---H22B...O3	0.96	2.36	2.908 (3)	115

a



b

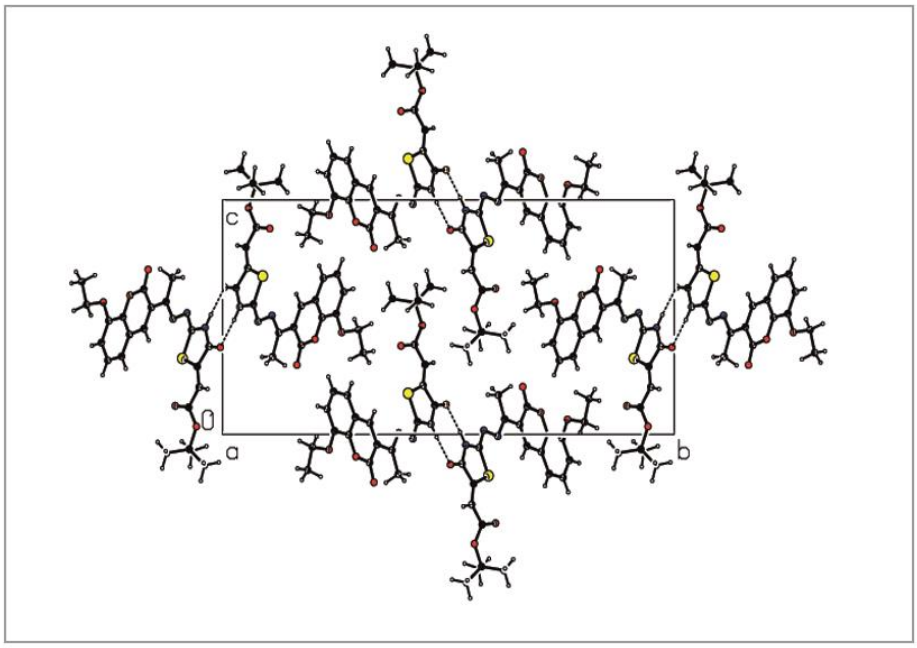


Figure 2. a) The molecular structure of compound IV-31 with the atom labelling (CCDC 418103). b) View of the dimers formed by the pairs of N-H---O hydrogen bonds with R^2_2 (8) motifs, down the axis.

3.2.Antiviral activity

The compounds alkyl 4-oxo-(coumarinyl ethylidene hydrazono)-thiazolidin-5-ylidene acetates (IV-1 – IV-31) were evaluated against a wide range of human RNA and DNA viruses in different types of cell cultures. The HEL cell culture was used to evaluate the compounds against herpes simplex virus type I (KOS) [HSV-1 KOS], herpes simplex virus type 2 (G) [HSV-2 G], vaccinia virus [VV], vesicular stomatitis virus (VSV), thymidine kinase-deficient herpes simplex virus type 1 (HSV-1 TK^- KOS ACV^r) and adeno virus type 2 (AV-2). Based on the microscopical analysis of the virus-induced cytopathicity (CPE) the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine and alovudine). As summarized in **Table 3**, only one compound (IV-19) showed moderate activity against the tested herpes simplex viruses, with EC_{50} values of about 10 µM.

Vesicular stomatitis virus (VSV) was also tested in the HeLa cell culture, together with the RNA viruses coxsackie virus B4 (CV B4) and respiratory syncytial virus (RSV). Here, dextran sulphate (DS-10000) and ribavirin were included as reference compounds. **Table 4** shows that compound IV-19, in contrast to HEL cells, inhibited VSV cytopathogenicity of HeLa cells with an EC_{50} of **9.5** µM. Both for compounds IV-24 and IV-25 antiviral potency was recorded against VSV and RSV in the lower micromolar range (**Table 4**).

Next, Vero cells were utilized to evaluate the compounds against parainfluenza type 3 virus (PI-3V), reovirus type 1 (RV-1), sindbis virus (SV), coxsackie virus B4 (CV B4) and punto toro virus (PTV), together the reference compounds DS-10000 and ribavirin. As depicted in **Table 5**, compound IV-2 had the broadest antiviral spectrum in Vero cell cultures. It was active against 4 of the 5 tested viruses (PI-3V, SV, CV B4 and PTV). Comparable activity was noted for compound IV-3, except for CV B4. Again, compound IV-19 showed antiviral activity against several of the tested viruses in these Vero cells: PI-3V, CV B4 and PTV. A remarkable observation was the activity of the compounds against punto toro virus. For 13 out of the 31 synthesised compounds, a clear inhibition of PTV cytopathogenicity was measured, with EC_{50} values ranging between **1.9 and 9.5 µM**.

Table 5: Cytotoxicity and antiviral activity of compounds in Vero cell cultures.

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
IV 1	100	>20	>20	>20	>20	>20
IV 2	≥100	**33**	>100	**39**	**39**	**9.5**
IV 3	≥100	**45**	>100	**46**	>100	**8.9**
IV 4	100	>20	>20	>20	>20	**9.5**
IV 5	100	**10**	>20	>20	>20	>20
IV 6	100	>20	>20	>20	>20	>20
IV 7	≥100	>100	>100	>100	>100	>100
IV 8	100	>20	>20	>20	>20	**3.2**
IV 9	≥20	>20	>20	>20	>20	>20
IV 10	100	>20	>20	>20	>20	**3.0**
IV 11	100	>20	>20	>20	>20	>20
IV 12	100	>20	>20	>20	>20	**8.9**
IV 13	100	>20	>20	>20	>20	**8.4**
IV 14	100	>20	>20	>20	>20	**7.0**
IV 15	100	>20	>20	>20	>20	>20
IV 16	100	>20	>20	>20	>20	>20
IV 17	100	>20	>20	>20	**7.0**	**1.9**
IV 18	≥20	>20	>20	>20	>20	**1.9**
IV 19	100	**10**	>20	>20	**7.0**	**8.3**
IV 20	≥20	>20	>20	>20	>20	**6.2**
IV 21	≥20	>20	>20	>20	>20	>20
IV 22	≥20	>20	>20	>20	>20	>20
IV 23	≥20	>20	>20	>20	**8.9**	>20
IV 24	≥20	>20	>20	>20	>20	>20
IV 25	≥20	>20	>20	>20	>20	>20
IV 26	>100	>100	>100	>100	>100	>100
IV 27	20	>4	>4	>4	>4	>4
IV 28	≥20	>20	>20	>20	>20	>20
IV 29	≥20	>20	>20	>20	>20	**8.9**
IV 30	20	>4	>4	>4	>4	>4
IV 31	≥20	>20	**9.5**	>20	>20	>20
DS-10000 (μg/ml)	>100	>100	>100	50	8.9	20
Ribavirin	>250	126	>250	250	>250	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Finally, the same compounds were tested for their potency against influenza virus (IV). The MDCK cell line was used to infect with influenza A virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and influenza B virus (strain B/Ned/537/05). As reference compounds, zanamivir, ribavirin, amantadine and rimantadine were selected. As

summarized in **Table 6**, only the three compounds IV-7, IV-9 and IV-21 exerted activity against influenza virus with EC_{50} values in the lower micromolar range, both by determination of the CPE as by measuring the cell viability with the colorimetric formazan-based MTS assay[40,41].

Table 6: Cytotoxicity and antiviral activity of compounds in MDCK cell cultures.

Compound	Cytotoxicity (µM)		Antiviral EC_{50}[c] (µM)					
	Minimum cytotoxic concentration[a]	CC_{50}[b]	Influenza A/H1N1 A/Ned/378/05		Influenza A/H3N2 A/HK/7/87		Influenza B B/Ned/537/05	
			CPE	MTS	CPE	MTS	CPE	MTS
IV 1	100	>100	>20	>20	>20	>20	>20	>20
IV 2	20	8.6	>4	>4	>4	>4	>4	>4
IV 3	20	9.5	>4	>4	>4	>4	>4	>4
IV 4	≥100	>100	>100	>100	>100	>100	>100	>100
IV 5	20	37.7	>4	>4	>4	>4	>4	>4
IV 6	100	>100	>20	>20	>20	>20	>20	>20
IV 7	100	>100	**11.0**	**7.4**	**6.5**	**4.1**	**27.0**	**13.8**
IV 8	20	72.6	>4	>4	>4	>4	>4	>4
IV 9	≥20	39.0	**10.5**	**6.3**	**7.9**	**4.2**	>20	>20
IV 10	≥20	38.0	>20	>20	>20	>20	>20	>20
IV 11	≥20	40.8	>20	>20	>20	>20	>20	>20
IV 12	100	>100	>20	>20	>20	>20	>20	>20
IV 13	≥20	>100	>20	>20	>20	>20	>20	>20
IV 14	100	57.2	>20	>20	>20	>20	>20	>20
IV 15	20	12.4	>4	>4	>4	>4	>4	>4
IV 16	20	38.8	>4	>4	>4	>4	>4	>4
IV 17	20	43.7	>4	>4	>4	>4	>4	>4
IV 18	20	48.4	>4	>4	>4	>4	>4	>4
IV 19	20	10.1	>4	>4	>4	>4	>4	>4
IV 20	≥4	27.2	>4	>4	>4	>4	>4	>4
IV 21	≥100	>100	**20.9**	**9.1**	**34**	**8.7**	**29.5**	**24.9**
IV 22	100	>100	>20	>20	>20	>20	>20	>20
IV 23	≥4	44.7	>4	>4	>4	>4	>4	>4
IV 24	4	2.1	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
IV 25	4	8.5	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
IV 26	>100	>100	>100	>100	>100	>100	>100	>100
IV 27	0.8	0.6	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
IV 28	0.8	1.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
IV 29	≥4	9.0	>4	>4	>4	>4	>4	>4
IV 30	0.8	1.9	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
IV 31	20	9.4	>4	>4	>4	>4	>4	>4
Zanamivir	>100	>100	0.2	0.3	0.5	0.6	0.4	0.4
Ribavirin	≥100	>100	8.9	8.2	8.9	5.3	6.8	8.4
Amantadine	>200	>200	8.0	19.1	0.8	1.6	>200	>200
Rimantadine	>200	>200	200	>200	0.3	0.1	>200	>200

[a] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[b] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Evaluation of these alkyl 4-oxo-coumarinyl ethylidene hydrazono- thiazolidin -5-ylidene acetate derivatives against feline corona virus and feline herpes virus was less successful as none of the compounds showed any detectable activity against these feline viruses (**Table 7**).

The structure activity relationship studies of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin -5-ylidene acetate derivatives clearly exhibiting that the presence of substituents on coumarin at 6^{th} and 8^{th} positions and ethoxy ester on thiazolidinone ring are crucial for antiviral activity. Out of twelve ethoxy ester having thiazolidinones, seven coumpounds exhibited excellent activity against *Punta Toro* virus. The compounds IV-17, IV-18, IV-19 and IV-20 possessed 8-methoxy, 6-bromo 8-methoxy, 8-ethoxy and 6-bromo 8-ethoxy exhibited excellent EC_{50} values of 1.9, 1.9, 8.3 and 6.2µM concentrations. It is noteworthy to mention that coumarin having bromo at 6^{th} position and methoxy / ethoxy functional group at 8^{th} position is exhibiting broad spectrum of antiviral activity. Similarly the compounds having halogenated electron withdrawing groups (Cl and Br) were found to be active against Para influenza, Sindbis, Coxsackie, and Punta Toro Viruses.

4. Conclusion

In the search for novel potentially antiviral agents, a one pot synthesis was used to generate alky -4-oxo- (coumarinyl ethylidene hydrazono)-thiazolidin-5-ylidene acetate (IV- 1-31) derivatives. Three groups of analogs have been synthesised and evaluated against a broad panel of viruses in different cell lines. It was found that among the tested compounds: (i) IV-19 was the compound that showed antiviral activity against most viruses, both DNA as RNA viruses, (ii) about 1/3 of the analogs showed potency against punta toro virus, and (iii) three compounds were highly selective for influenza virus.

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Author contributions: KV and RRV designed the project, RRV managed and supervised the project, KV performed a majority of experiments, Kurt V supported with the antiviral assays, VV and CVK contributed towards optimization of experiments and important scientific discussions, all authors wrote the article together.

Conflicts of interest: We declare no conflict of competing or financial intertest regarding this manuscript.

Supplementary material associated with this article having analytical spectral data like 1H NMR, 13CNMR and Mass spectral data can be found online....

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Highlights

- One Pot multicomponent synthesis of 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetates.
- Single crystal X-ray data and NMR for structural analysis.
- Antiviral activity against awide range of human RNA and DNA viruses in different types of cell cultures.
- Compound (IV-19) showed antiviral activity against most viruses, both DNA as RNA viruses, about 1/3 of the analogs showed potency against punta toro virus and three compounds were highly selective for influenza virus.

Table 3: Cytotoxicity and antiviral activity of compounds in HEL cell cultures.

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r	Adeno virus-2
IV 1	100	>20	>20	>20	>20	>20	>20
IV 2	≥100	>100	>100	>100	>100	>100	>100
IV 3	100	>20	>20	>20	>20	>20	>20
IV 4	100	>20	>20	>20	>20	>20	>20
IV 5	100	>20	>20	>20	>20	>20	>20
IV 6	100	>20	>20	>20	>20	>20	>20
IV 7	100	>20	>20	>20	>20	>20	>20
IV 8	100	>20	>20	>20	>20	>20	>20
IV 9	≥20	>20	>20	>20	>20	>20	>20
IV 10	20	>4	>4	>4	>4	>4	>4
IV 11	100	>20	>20	>20	>20	>20	>20
IV 12	100	>20	>20	>20	>20	>20	>20
IV 13	100	>20	>20	>20	>20	>20	>20
IV 14	100	>20	>20	>20	>20	>20	>20
IV 15	20	>4	>4	>4	>4	>4	>4
IV 16	100	>20	>20	>20	>20	>20	>20
IV 17	100	>20	>20	>20	>20	>20	>20
IV 18	100	>20	>20	>20	>20	>20	>20
IV 19	100	**11**	**10**	>20	>20	**10**	>20
IV 20	100	>20	>20	>20	>20	>20	>20
IV 21	100	>20	>20	>20	>20	>20	>20
IV 22	≥20	>20	>20	>20	>20	>20	>20
IV 23	>100	>100	>100	>100	>100	>100	>100
IV 24	20	>4	>4	>4	>4	>4	>4
IV 25	20	>4	>4	>4	>4	>4	>4
IV 26	>100	>100	>100	>100	>100	>100	>100
IV 27	20	>4	>4	>4	>4	>4	>4
IV 28	≥4	>4	>4	>4	>4	>4	>4
IV 29	20	>4	>4	>4	>4	>4	>4
IV 30	≥4	>4	>4	>4	>4	>4	>4
IV 31	20	>4	>4	>4	>4	>4	>4
Brivudin	>250	0.05	181	20	>250	150	-
Cidofovir	>250	1.6	1.0	22	>250	1.6	7.9
Acyclovir	>250	0.4	0.2	>250	>250	98	-
Ganciclovir	>100	0.06	0.06	>100	>100	2.9	-
Zalcitabine	>250	-	-	-	-	-	7.5
Alovudine	>250	-	-	-	-	-	16

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 4: Cytotoxicity and antiviral activity of compounds in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
IV 1	100	>20	>20	>20
IV 2	100	>20	>20	>20
IV 3	100	>20	>20	>20
IV 4	100	>20	>20	>20
IV 5	100	>20	>20	>20
IV 6	100	>20	>20	>20
IV 7	100	>20	>20	>20
IV 8	100	>20	>20	>20
IV 9	100	>20	>20	>20
IV 10	100	>20	>20	>20
IV 11	100	>20	>20	>20
IV 12	>100	>100	>100	>100
IV 13	100	>20	>20	>20
IV 14	≥100	>100	>100	>100
IV 15	>100	>100	>100	>100
IV 16	≥100	>100	>100	>100
IV 17	100	>20	>20	>20
IV 18	100	>20	>20	>20
IV 19	100	**9.5**	>20	>20
IV 20	100	>20	>20	>20
IV 21	>100	>100	>100	>100
IV 22	>100	>100	>100	>100
IV 23	100	>20	>20	>20
IV 24	100	**20**	>20	**11**
IV 25	100	**9.5**	>20	**10**
IV 26	>100	>100	>100	>100
IV 27	20	>4	>4	>4
IV 28	100	>20	>20	>20
IV 29	20	>4	>4	>4
IV 30	20	>4	>4	>4
IV 31	20	>4	>4	>4
DS-10000 (µg/ml)	>100	1.8	27	3.0
Ribavirin	>250	22	81	7.5

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 7: Anti-Feline Corona Virus (FIPV) and anti-Feline Herpes Virus activity and cytotoxicity in CRFK cell cultures.

Compound	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
(IV 1)	62.5	>20	>20
(IV 2)	>100	>100	>100
(IV 3)	>100	>100	>100
(IV 4)	47.0	>20	>20
(IV 5)	73.4	>20	>20
(IV 6)	>100	>100	>100
(IV 7)	>100	>100	>100
(IV 8)	55.4	>20	>20
(IV 9)	>100	>100	>100
(IV 10)	46.7	>20	>20
(IV 11)	50.0	>20	>20
(IV 12)	>100	>100	>100
(IV 13)	>100	>100	>100
(IV 14)	>100	>100	>100
(IV 15)	>100	>100	>100
(IV 16)	>100	>100	>100
(IV 17)	85.0	>20	>20
(IV 18)	>100	>100	>100
(IV 19)	52.2	>20	>20
(IV 20)	48.8	>20	>20
(IV 21)	>100	>100	>100
(IV 22)	>100	>100	>100
(IV 23)	50.0	>20	>20
(IV 24)	20.3	>20	>20
(IV 25)	37.6	>20	>20
(IV 26)	>100	>100	>100
(IV 27)	9.5	>4	>4
(IV 28)	23.1	>20	>20
(IV 29)	13.7	>4	>4
(IV 30)	9.7	>4	>4
(IV 31)	39.1	>20	>20
HHA (μg/ml)	>100	24.3	15.1
UDA (μg/ml)	>100	7.3	4.3
Ganciclovir	>100	>100	8.6

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay

A facile one pot multi component synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetates and their antiviral activity

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Keywords: One- pot three - component reaction, 3-acetylcoumarin, thiosemicarbazide, thiazolidinone and antiviral activity.

Content

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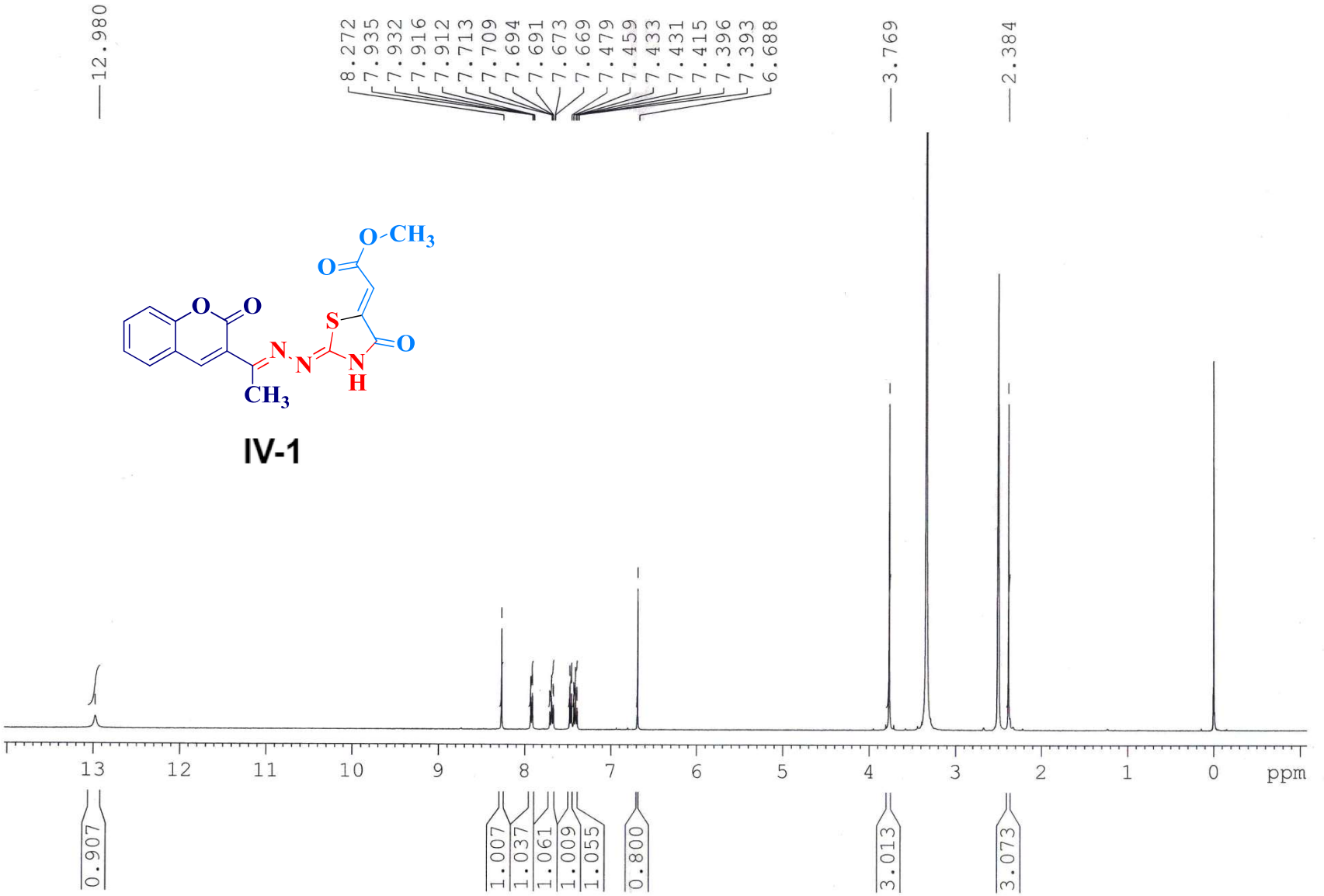

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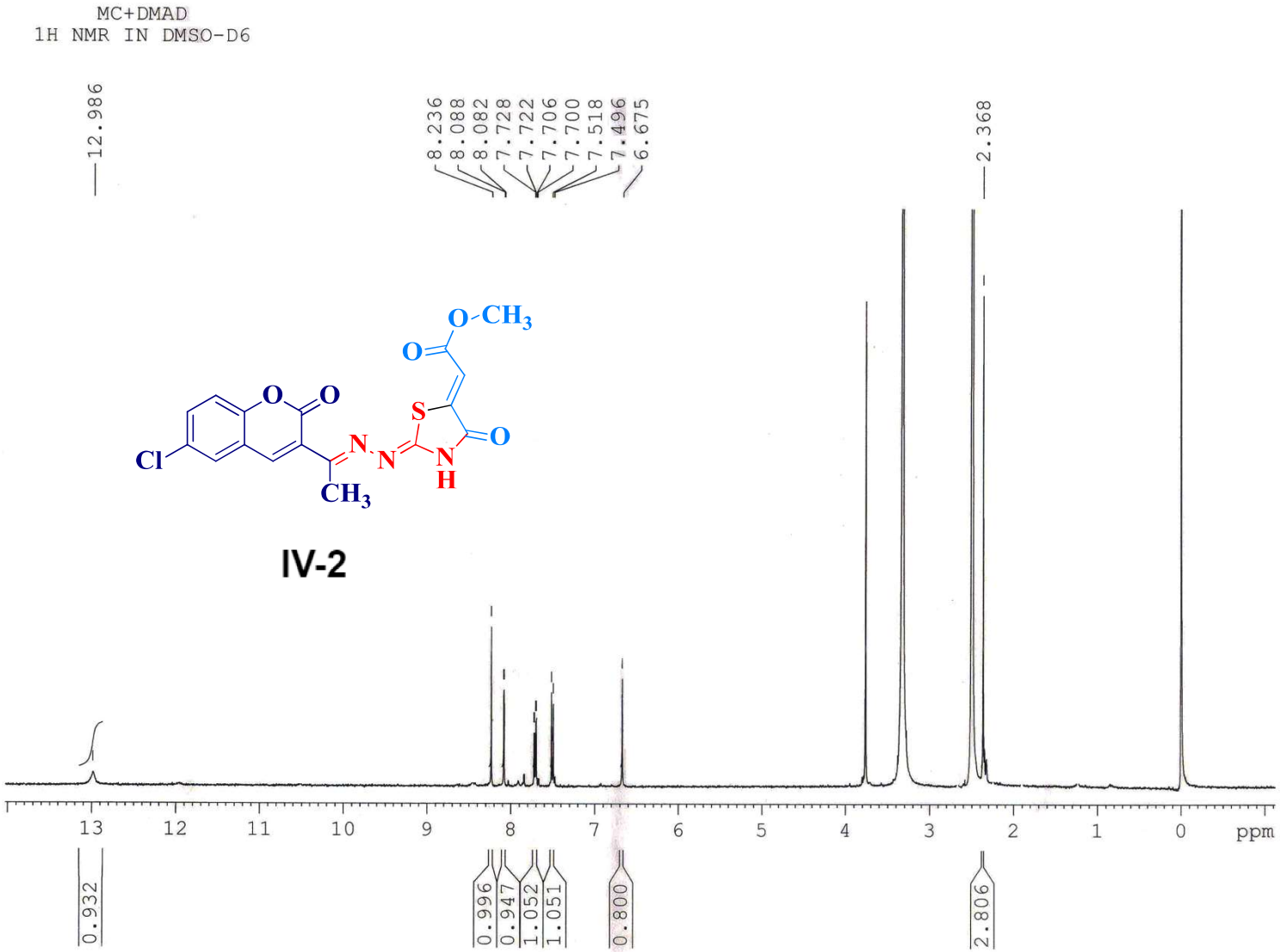
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H
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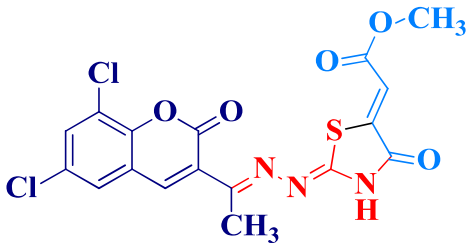

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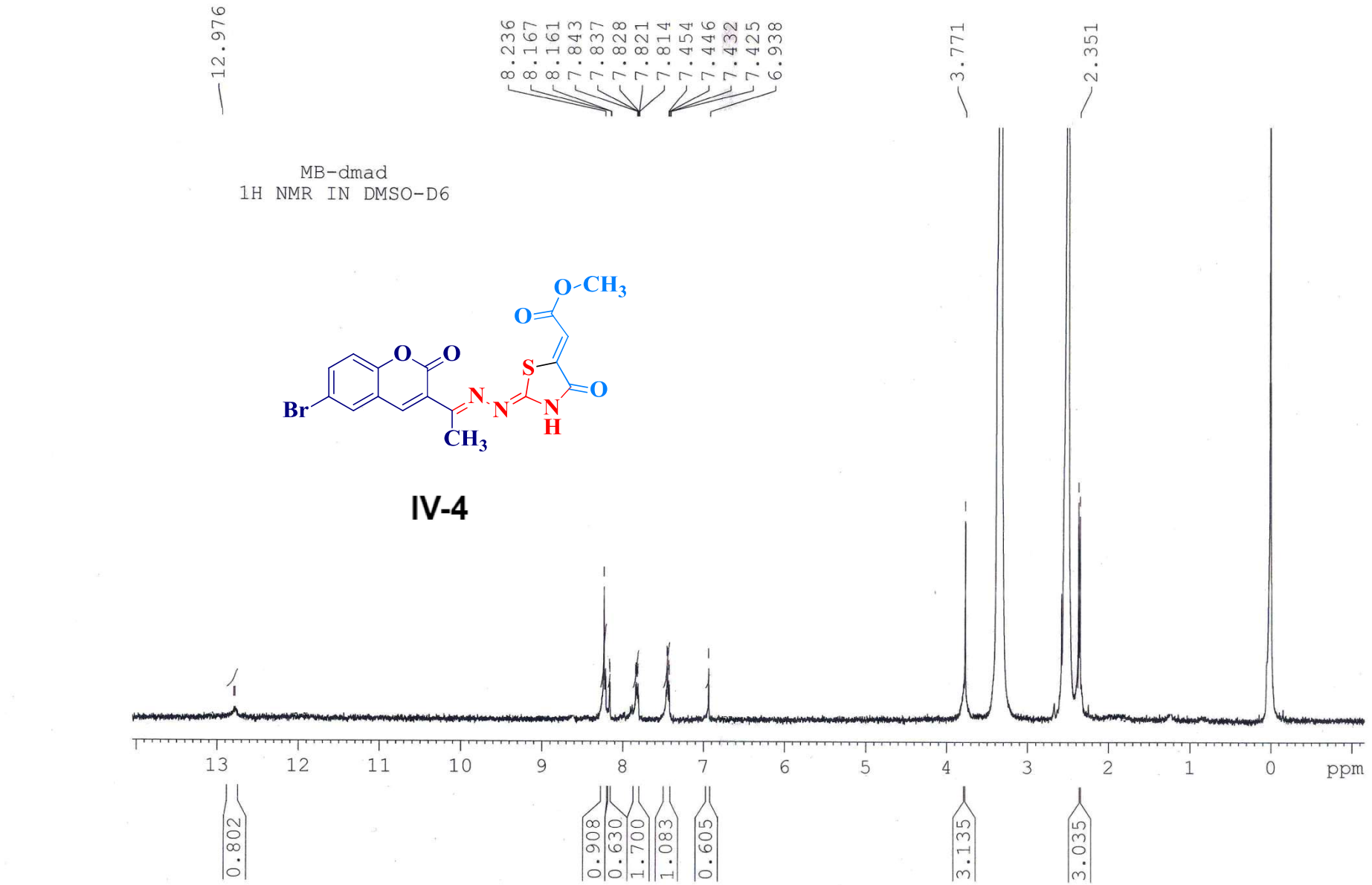
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7.425
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3.771
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MB-dmad
1H NMR IN DMSO-D6
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N
H
Br
CH3
IV-4
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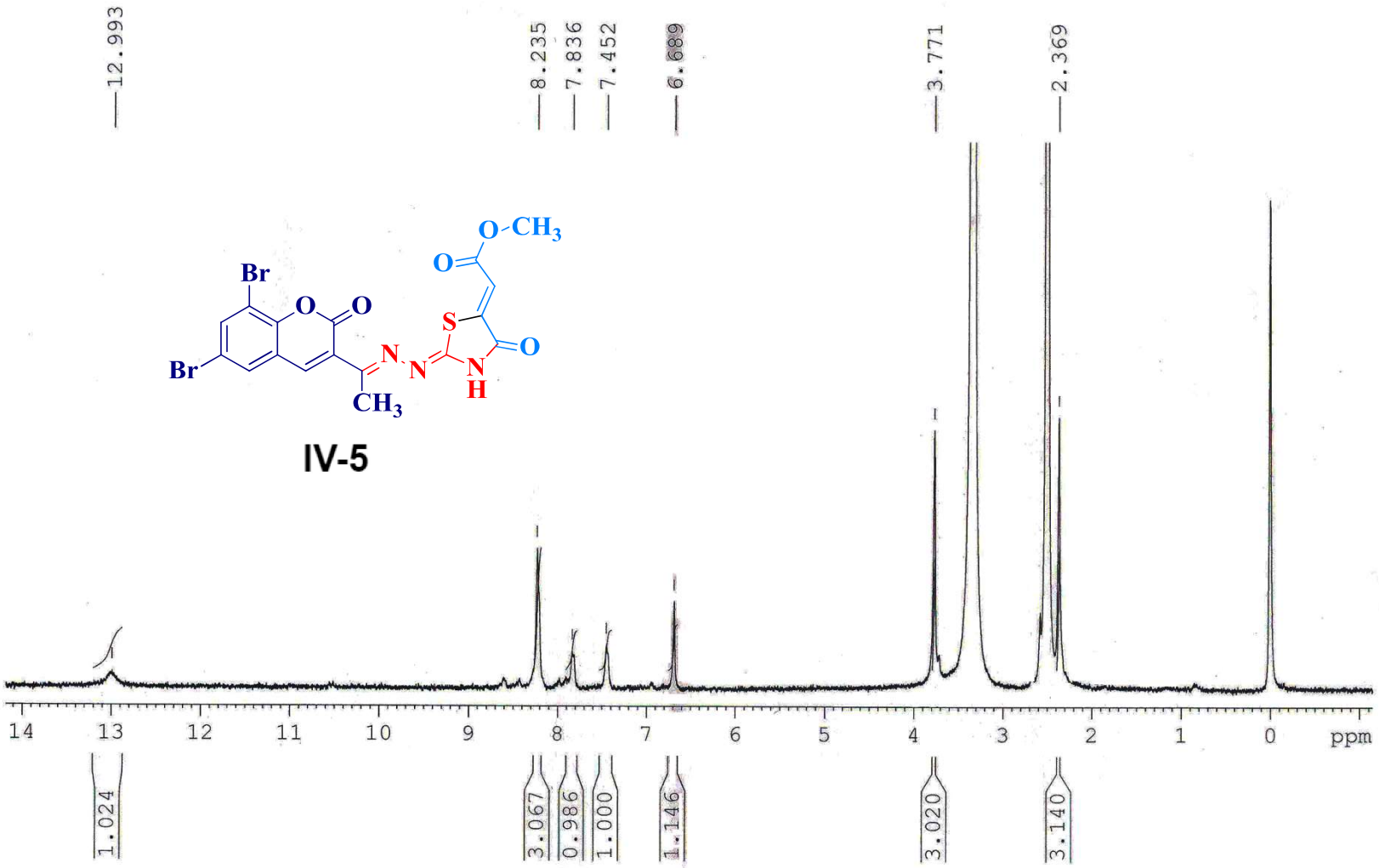
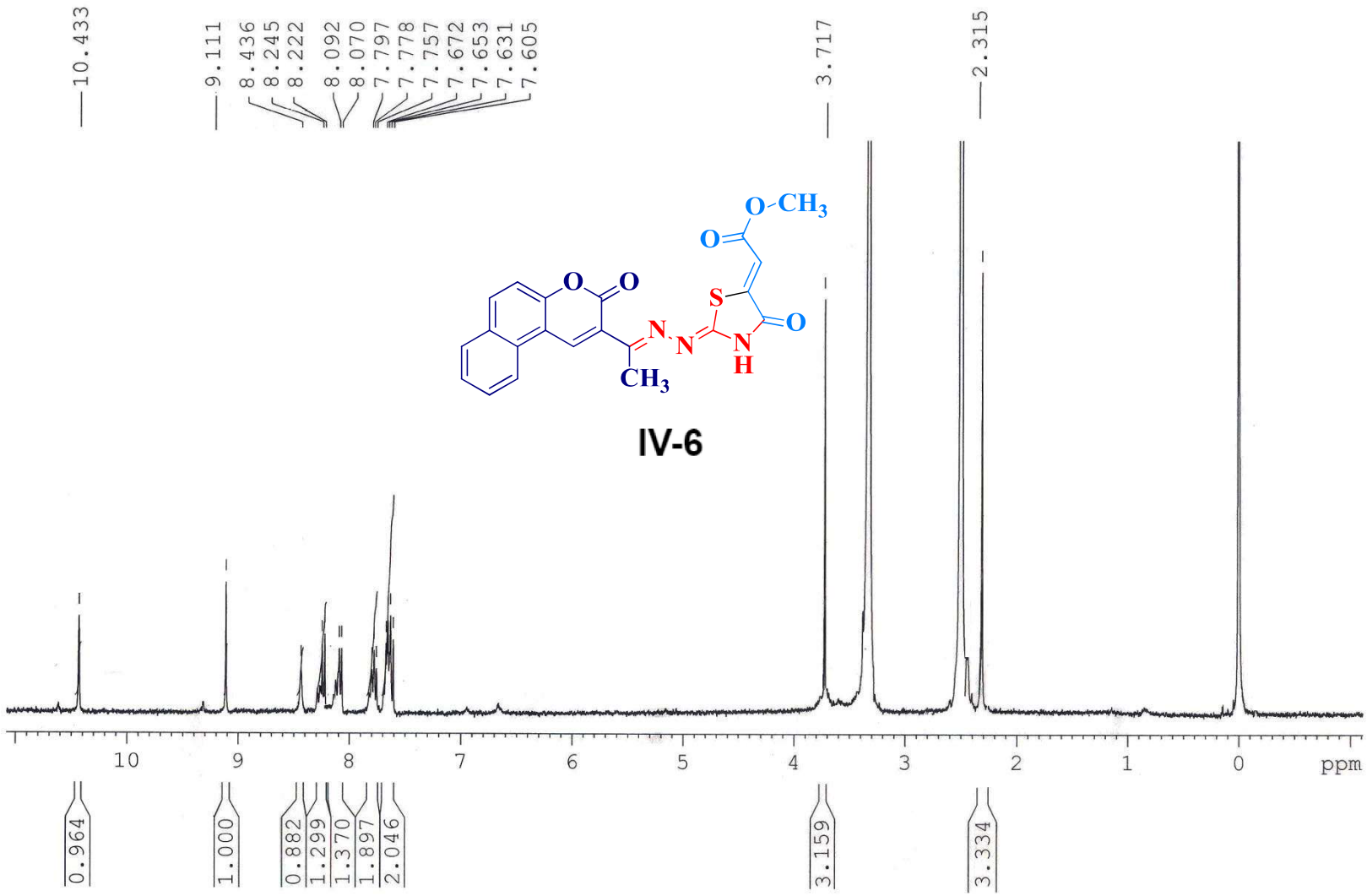


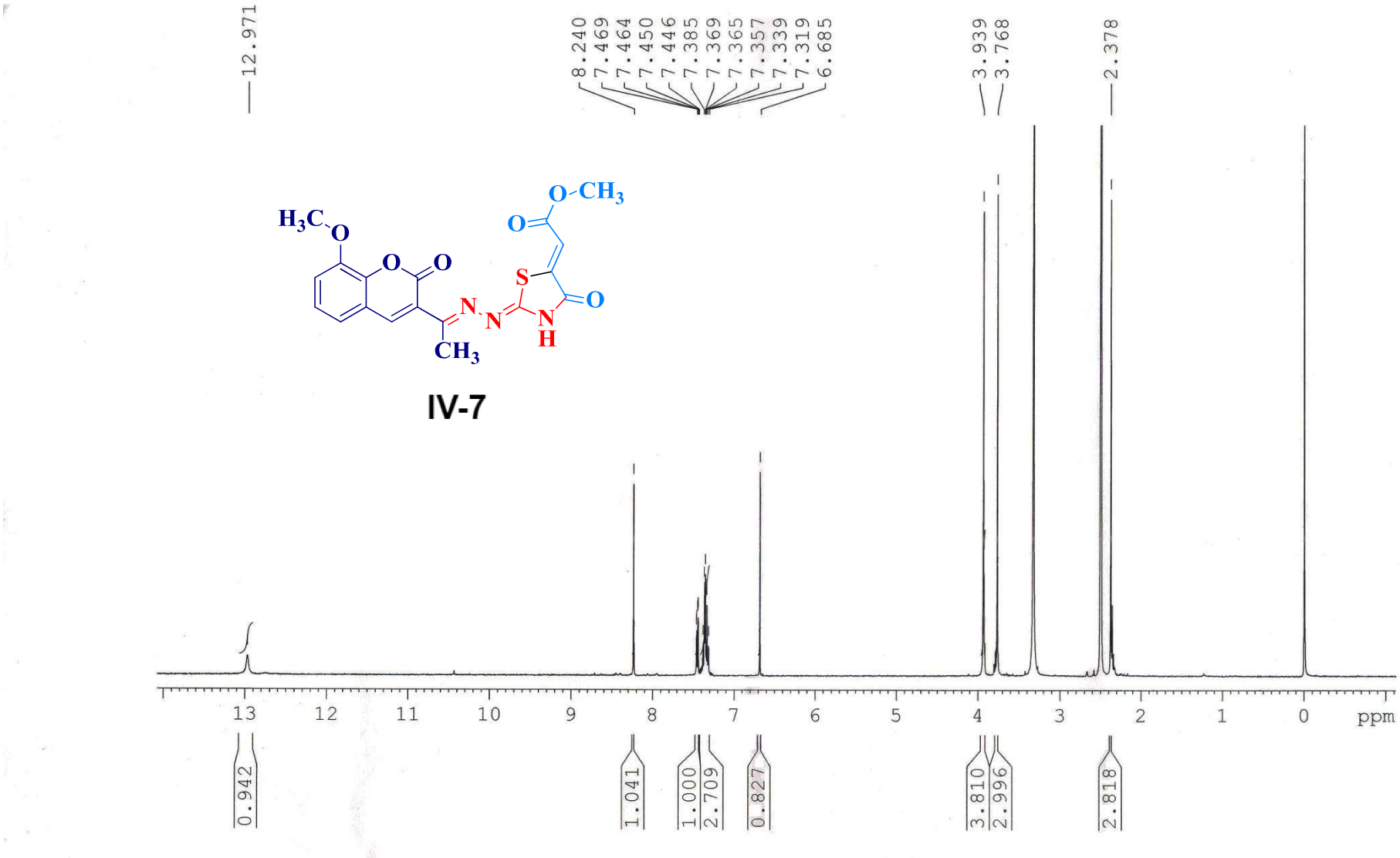
IV-5

NAP+DMAD
1H NMR IN DMSO-D6



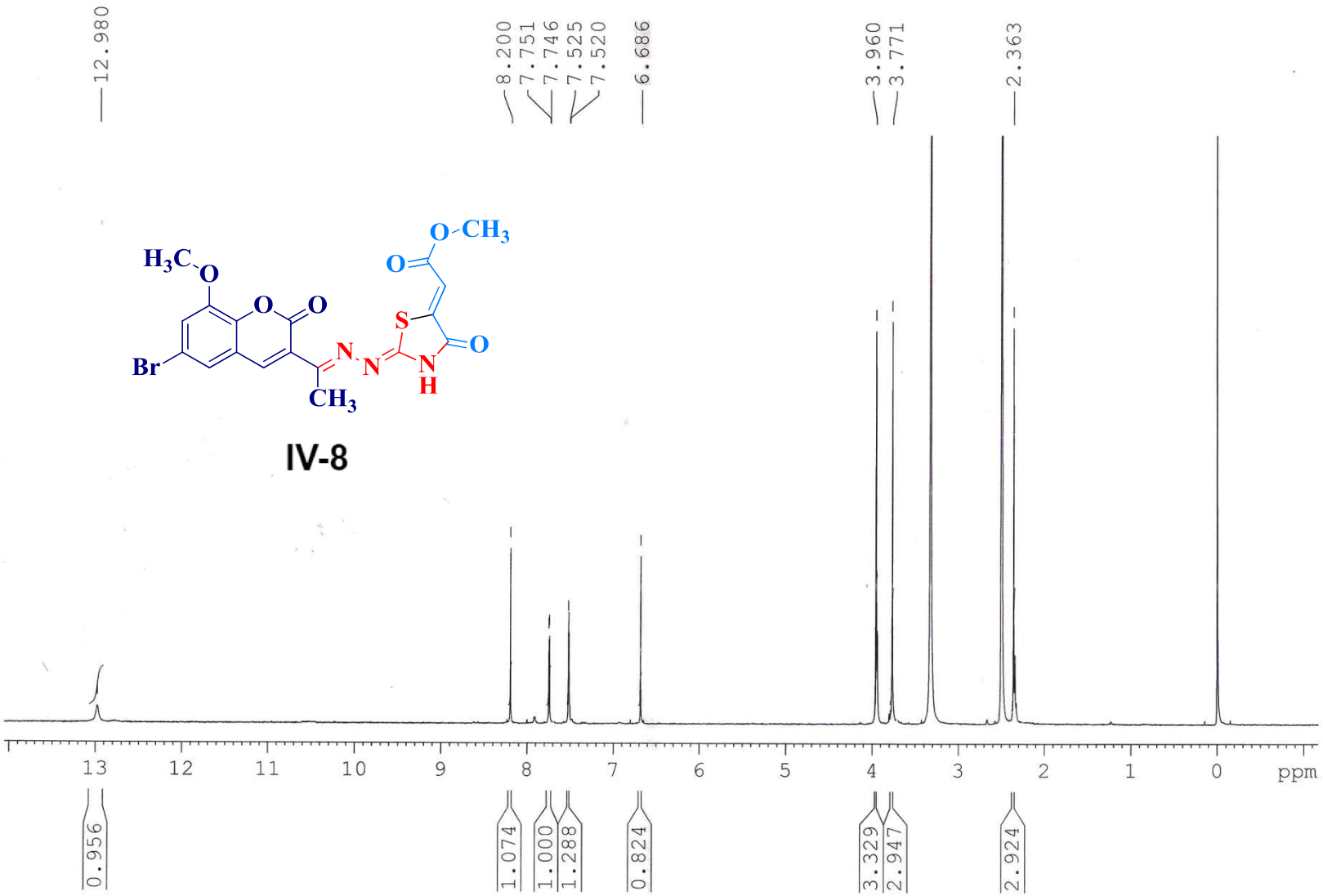
IV-6

OV+DMAD
1H NMR IN DMSO-D6



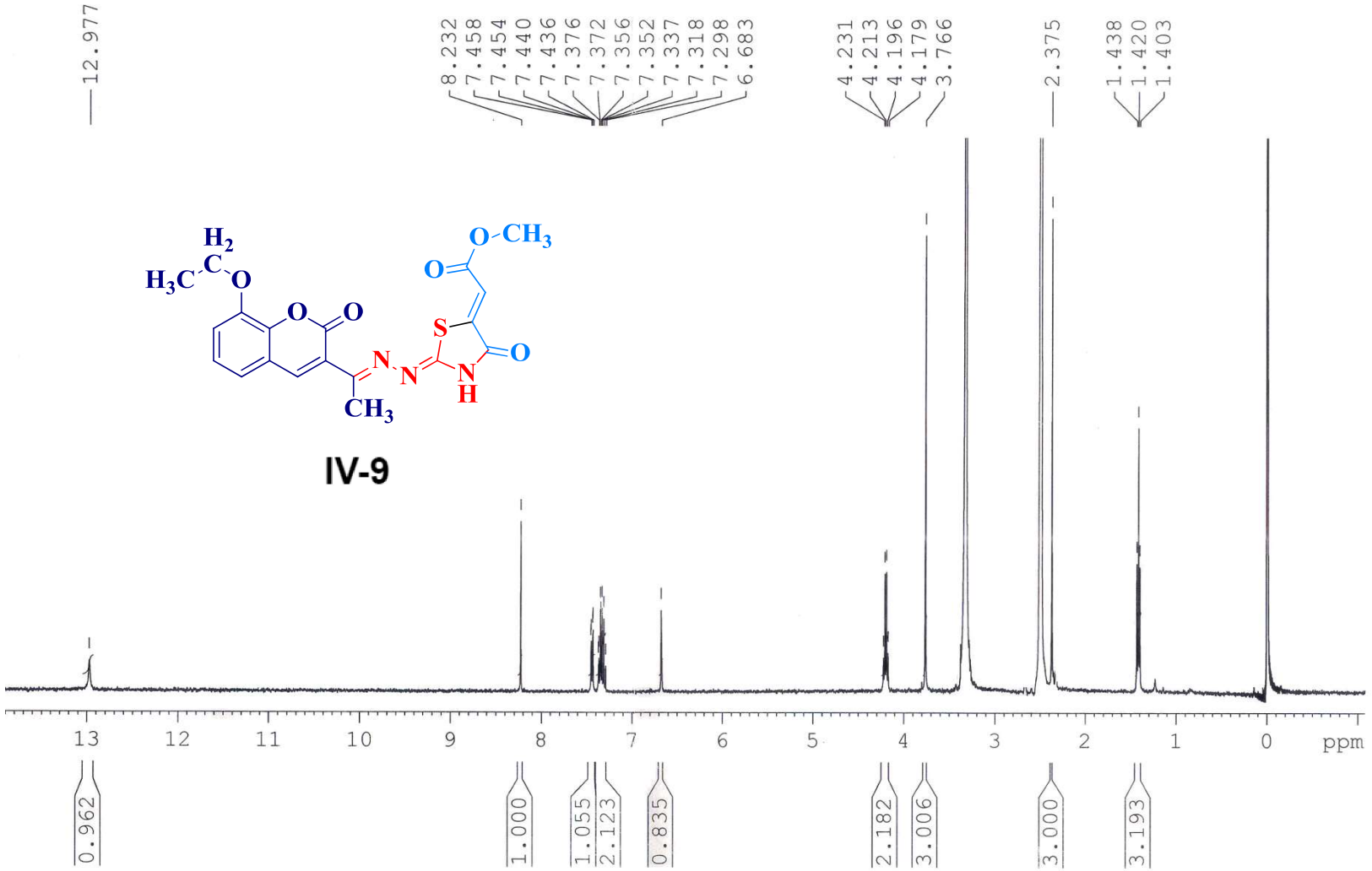
IV-7

BrOV-dmad
1H NMR IN DMSO-D6



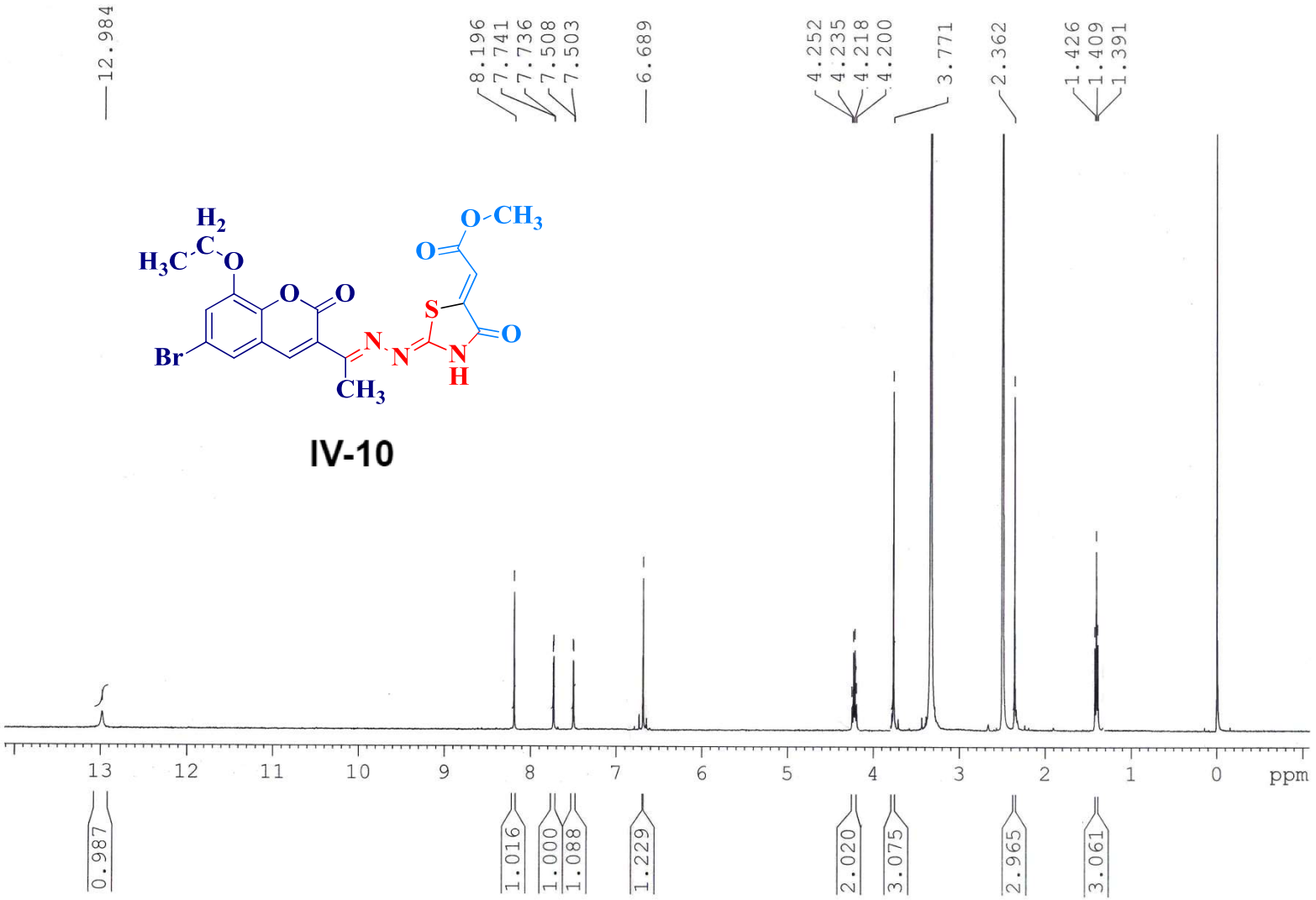
IV-8

OEE+DMAD
1H NMR IN DMSO-D6



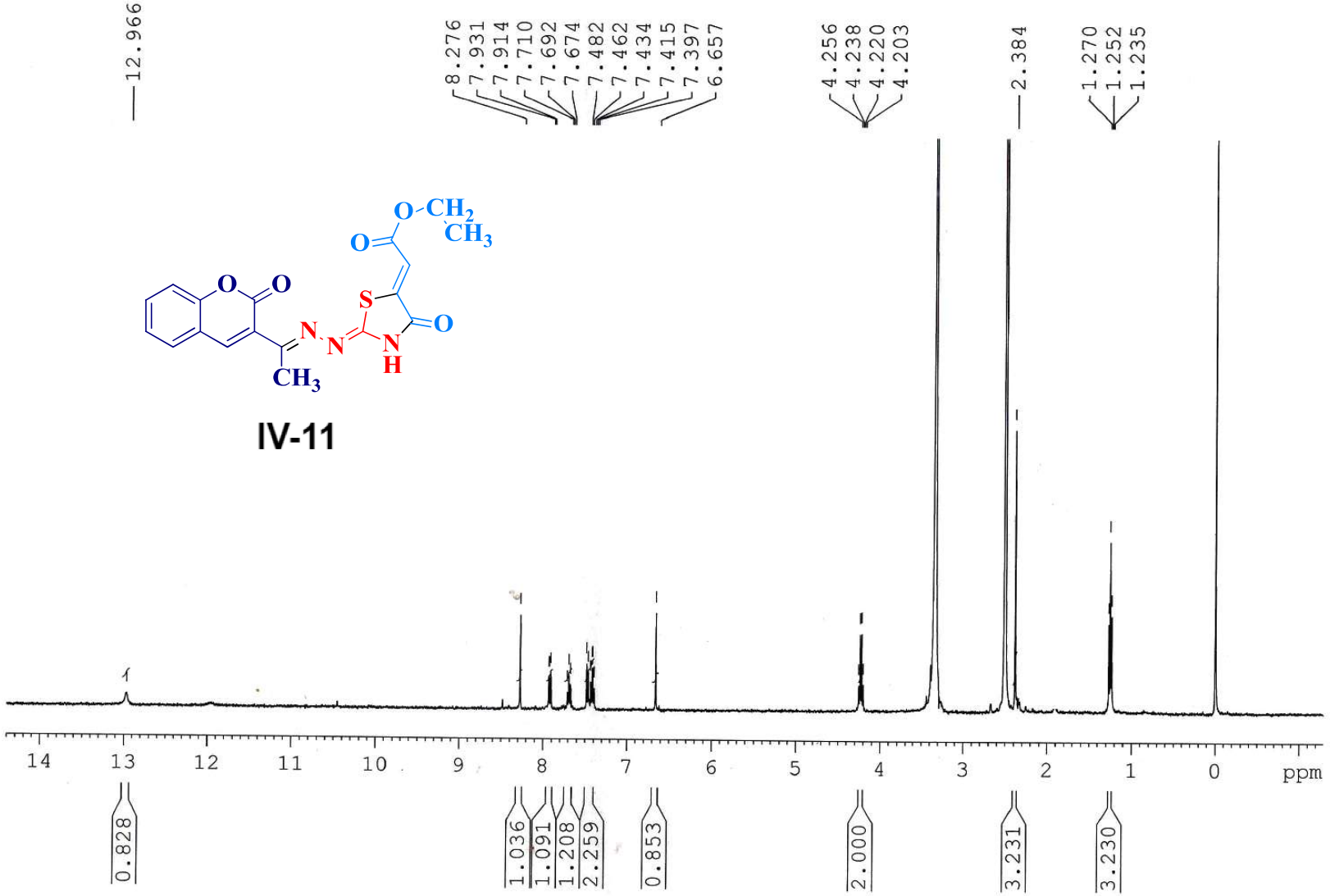
IV-9

BroEt-dmad
1H NMR IN DMSO-D6



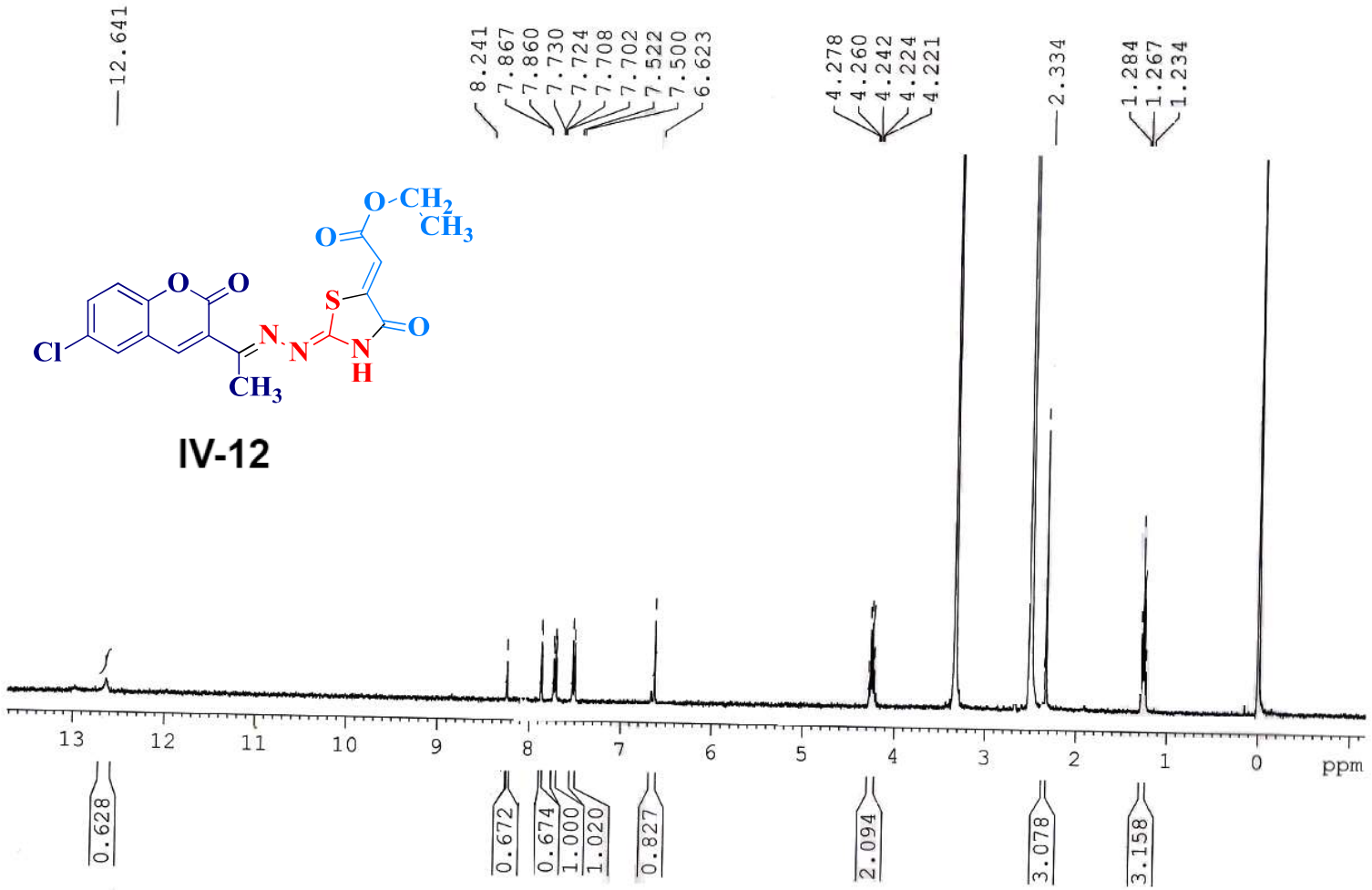
IV-10

DEAD
1H NMR IN DMSO-D6



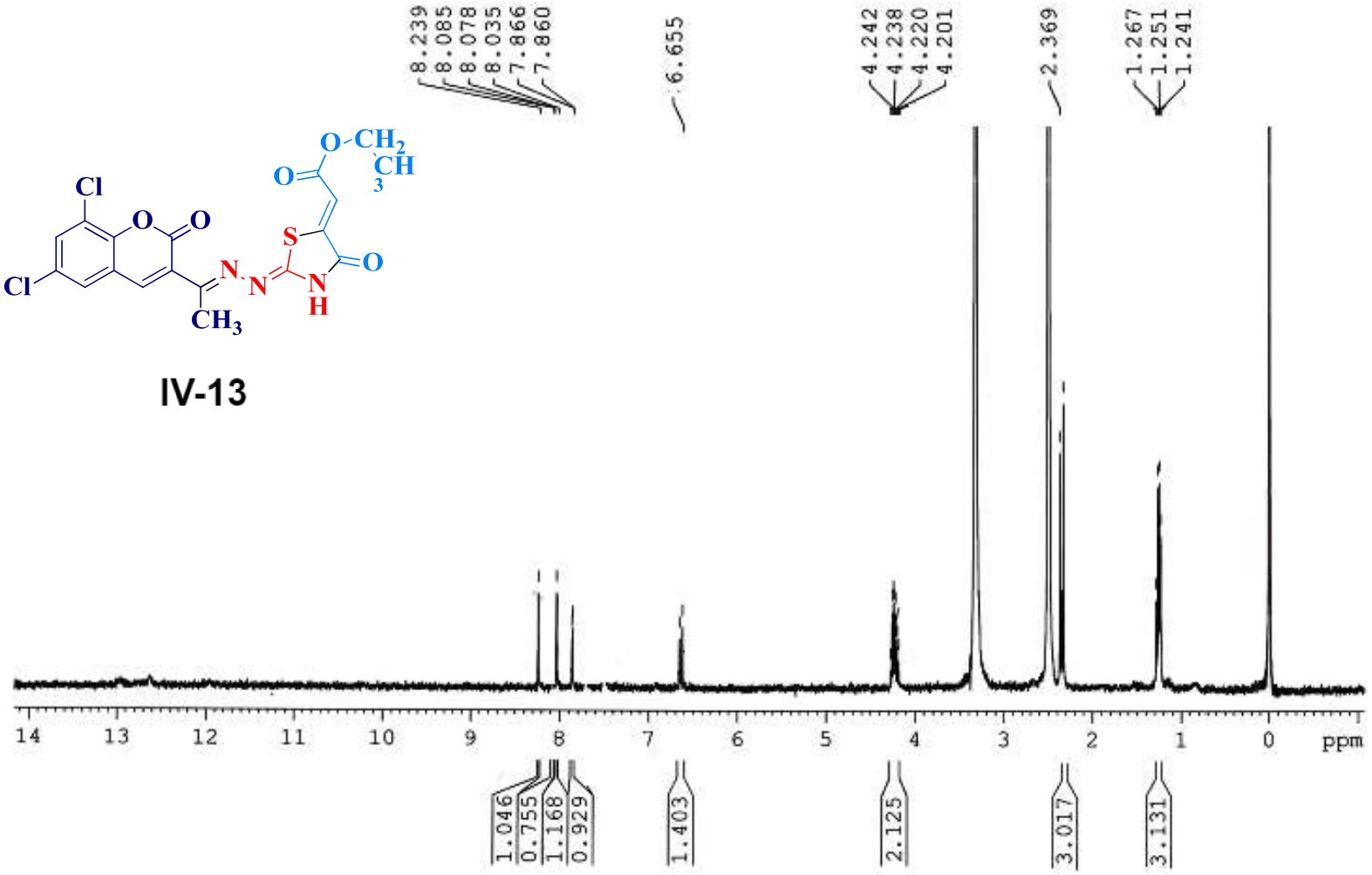
IV-11

MC
1H NMR IN DMSO-D6



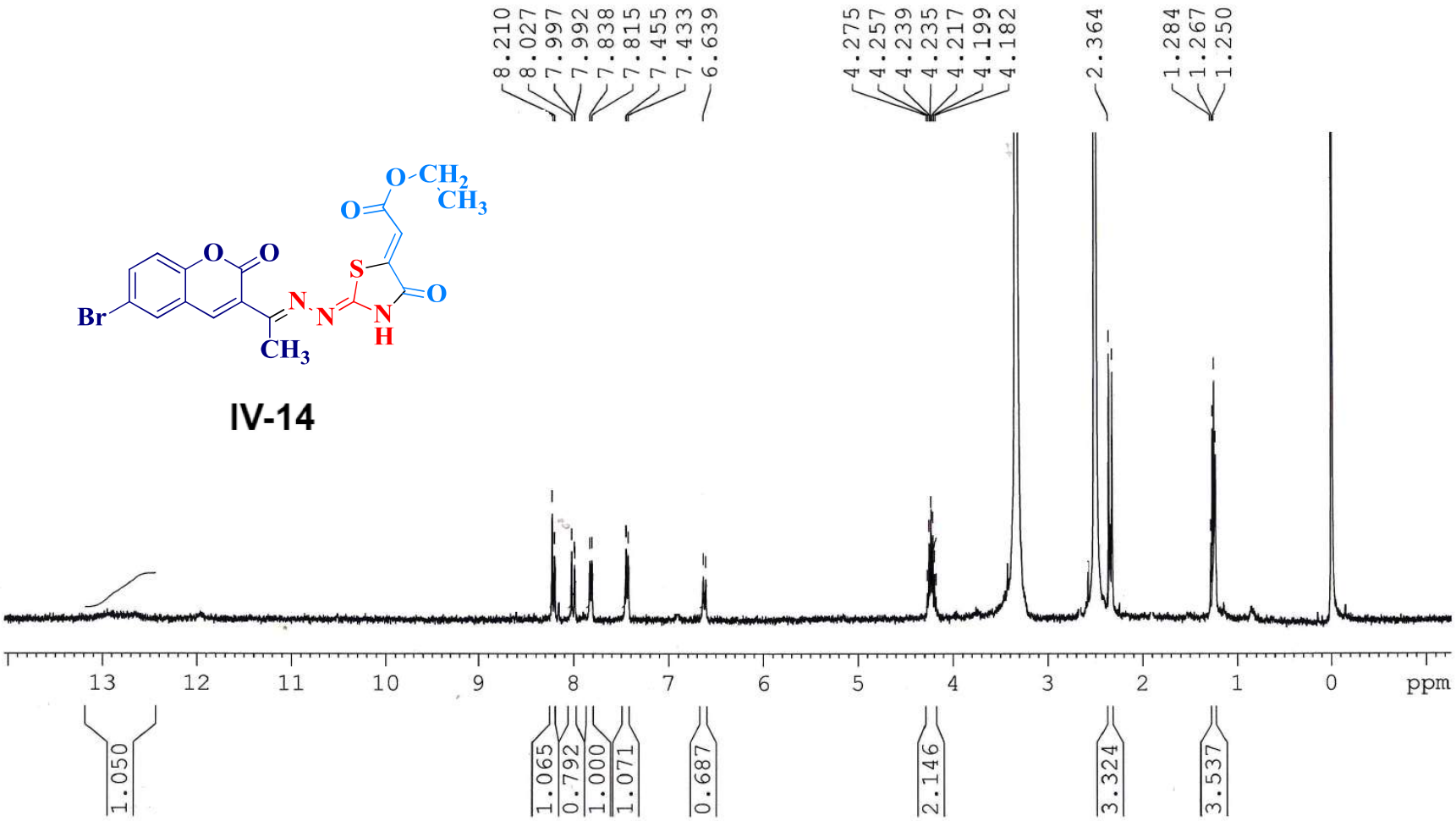
IV-12

DCD
1H NMR IN DMSO-D6

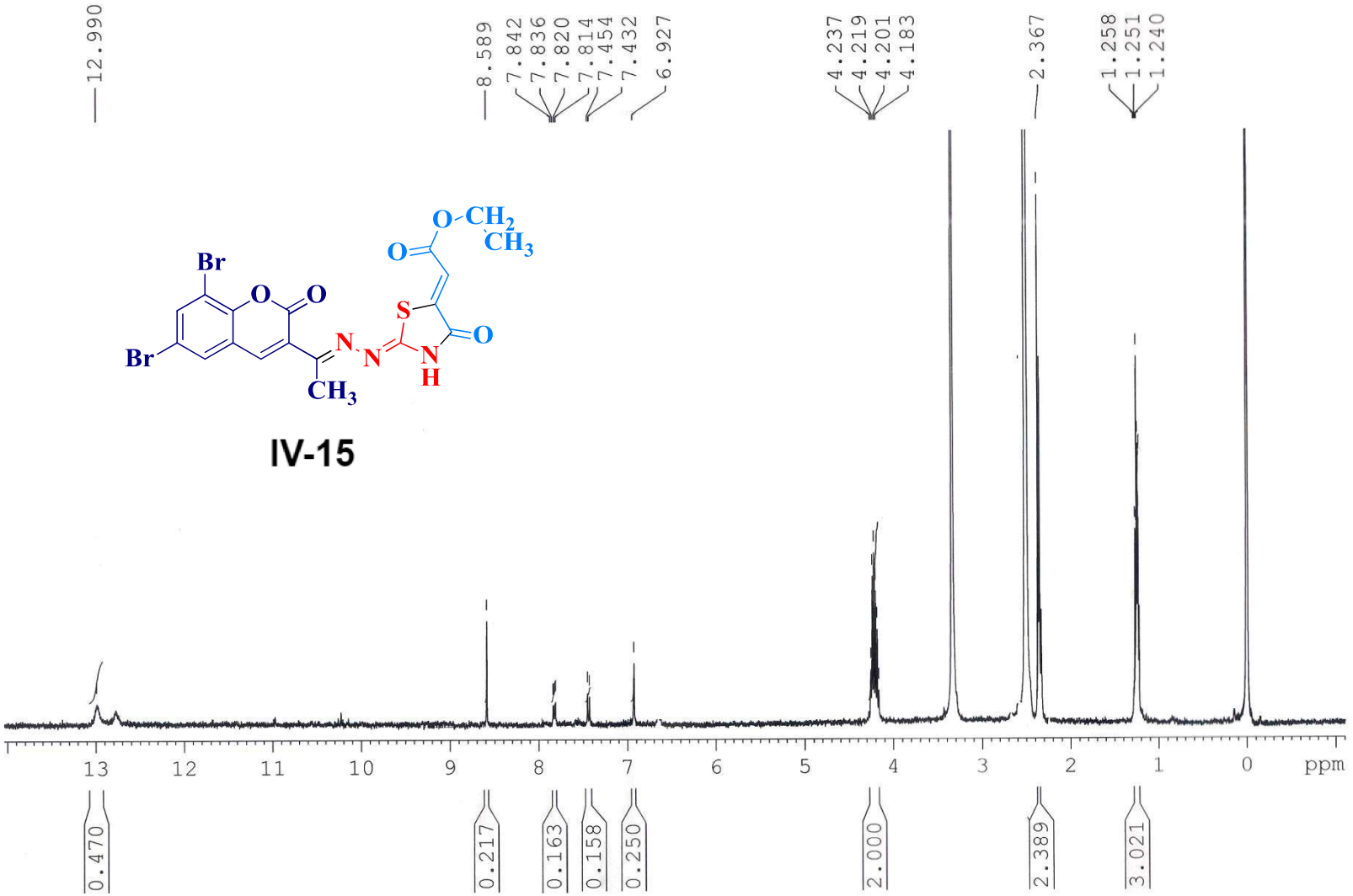


IV-13

MBD
1H NMR IN DMSO-D6

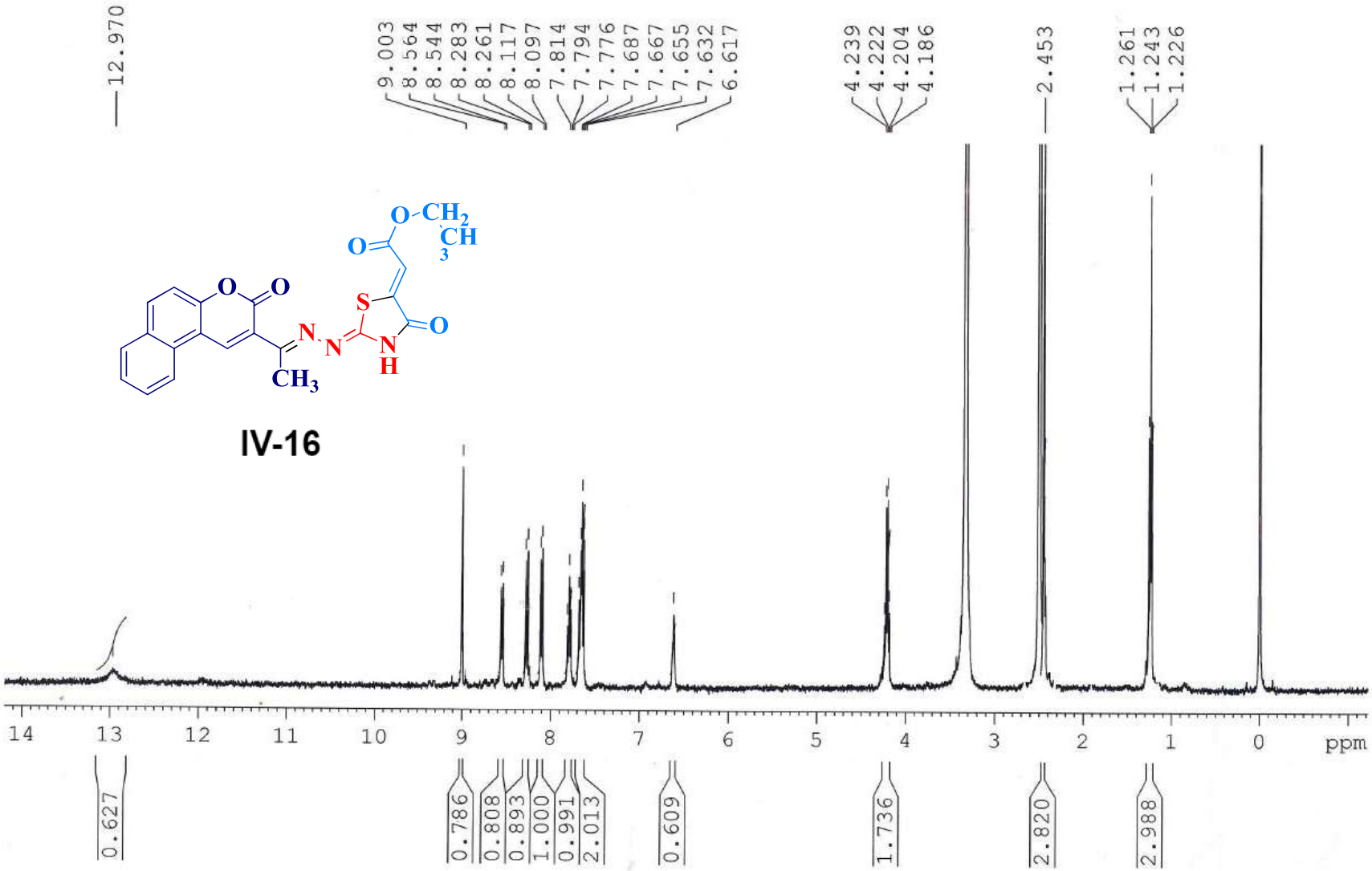


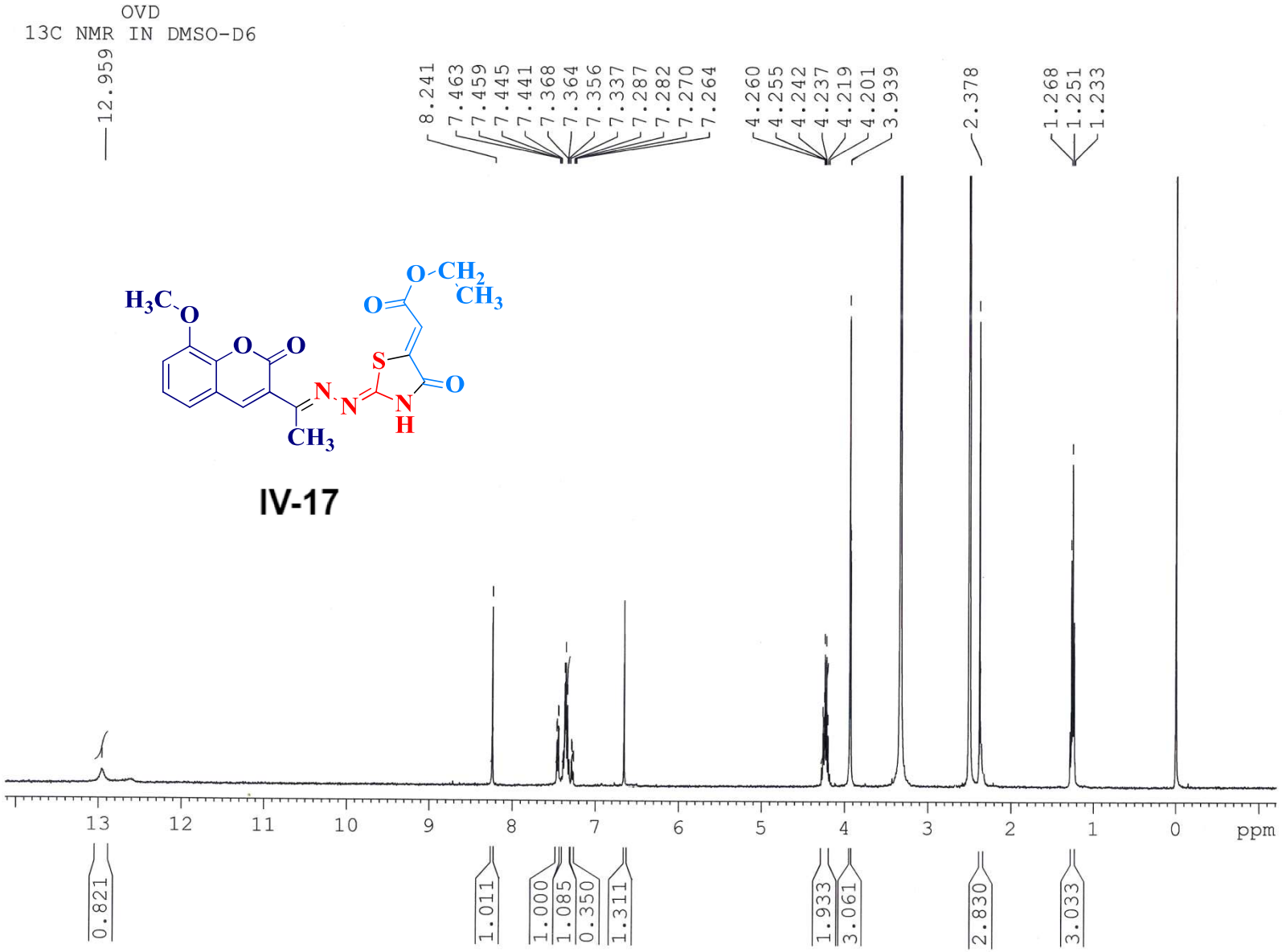
DBD
1H NMR IN DMSO-D6



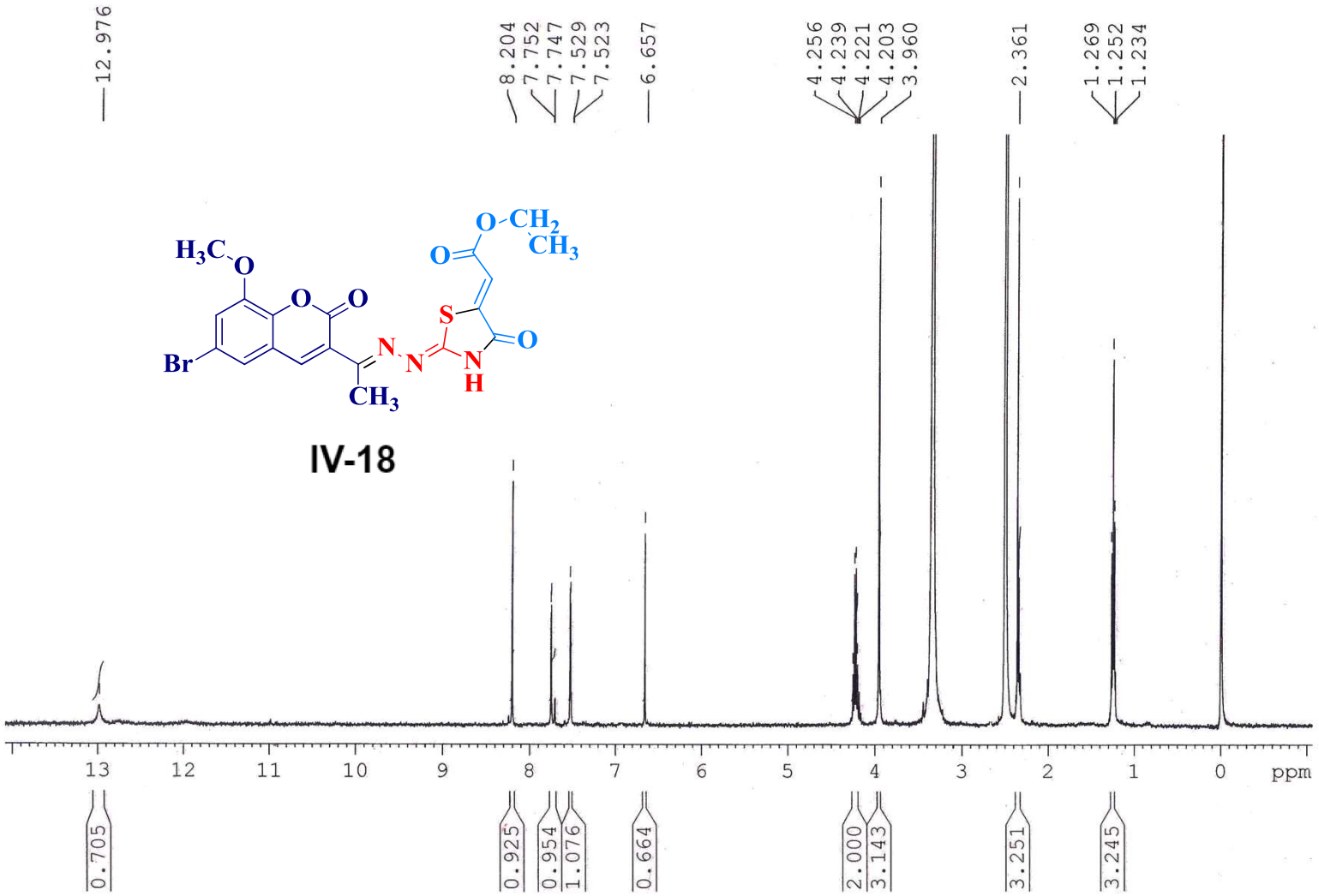
IV-15

NaPD
1H NMR IN DMSO-D6



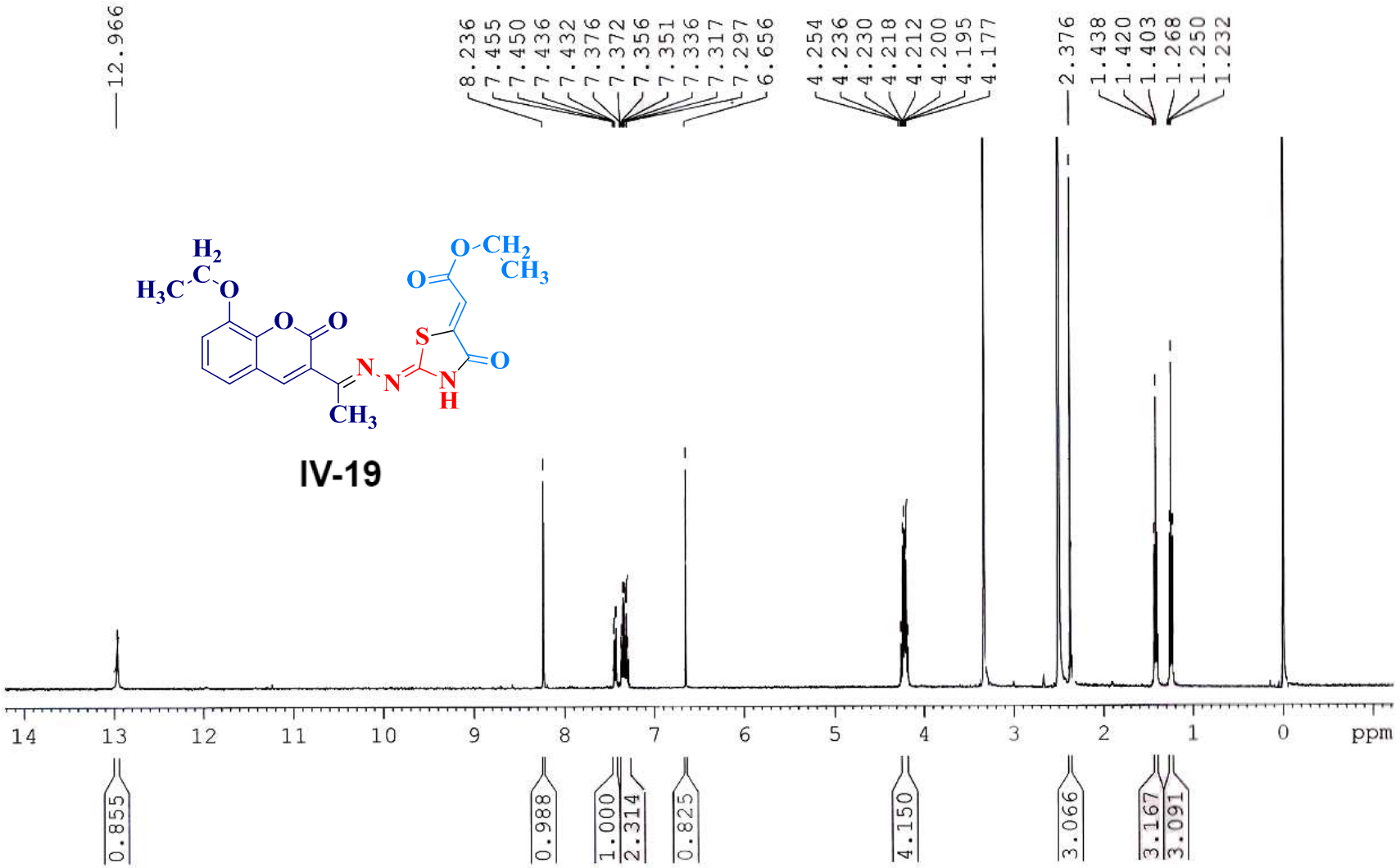

OVD
13C NMR IN DMSO-D6
12.959
8.241
7.463
7.459
7.445
7.441
7.368
7.364
7.356
7.337
7.287
7.282
7.270
7.264
4.260
4.255
4.242
4.237
4.219
4.201
3.939
2.378
1.268
1.251
1.233
IV-17
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.821
1.011
1.000
1.085
0.350
1.311
1.933
3.061
2.830
3.033

BrOV
1H NMR IN DMSO-D6

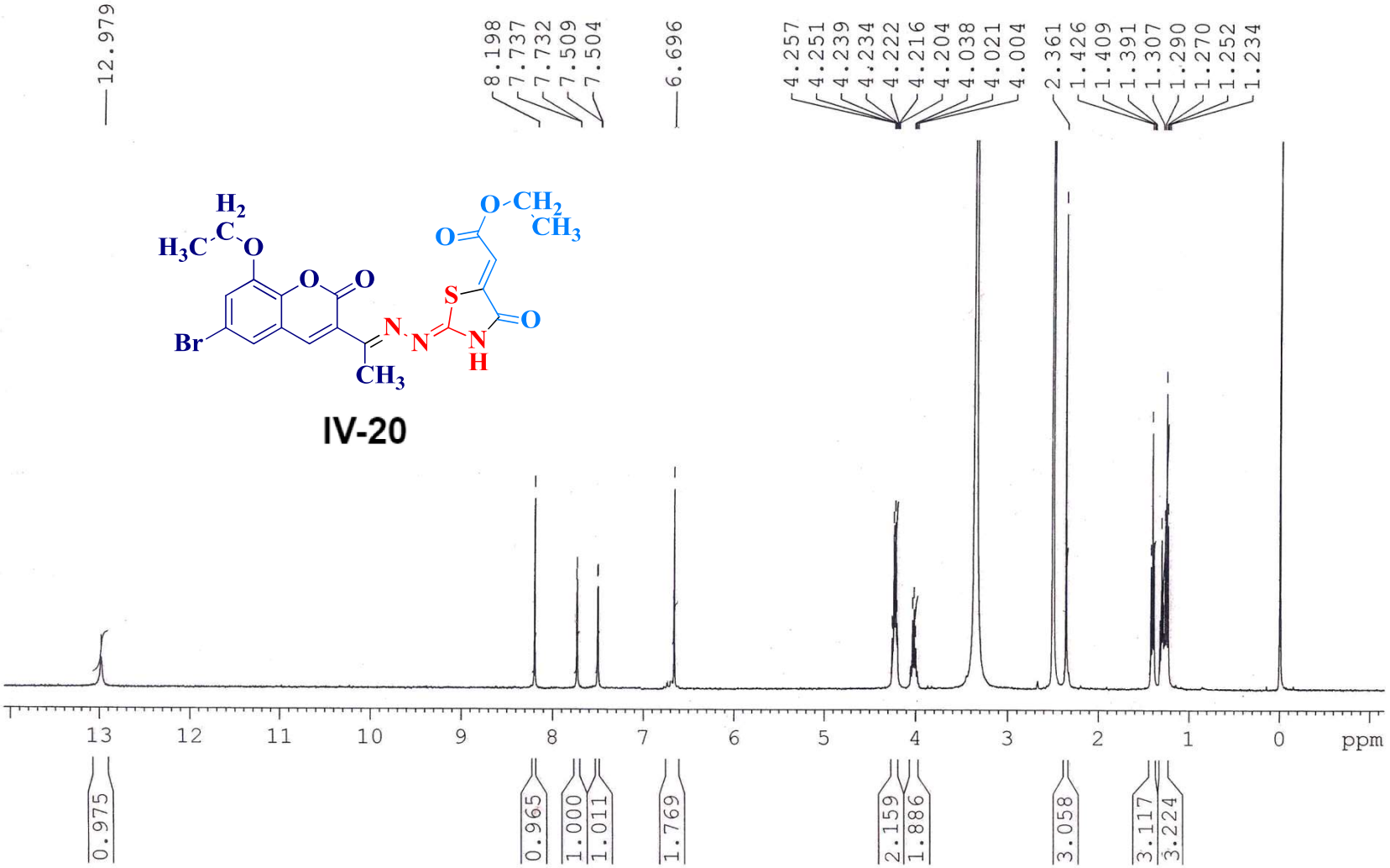


IV-18

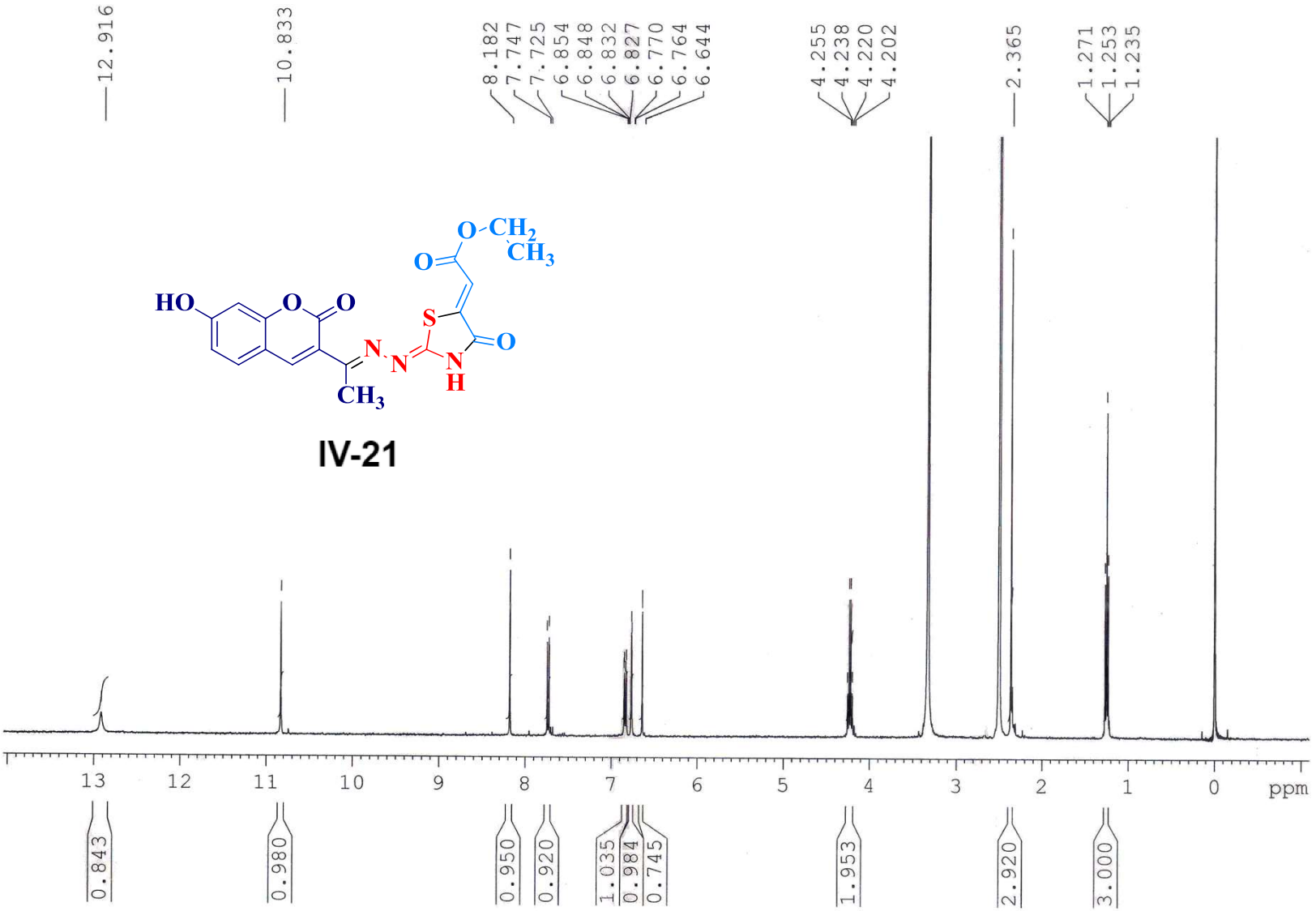
O-Et
1H NMR IN DMSO-D6



Br-OET
1H NMR IN DMSO-D6

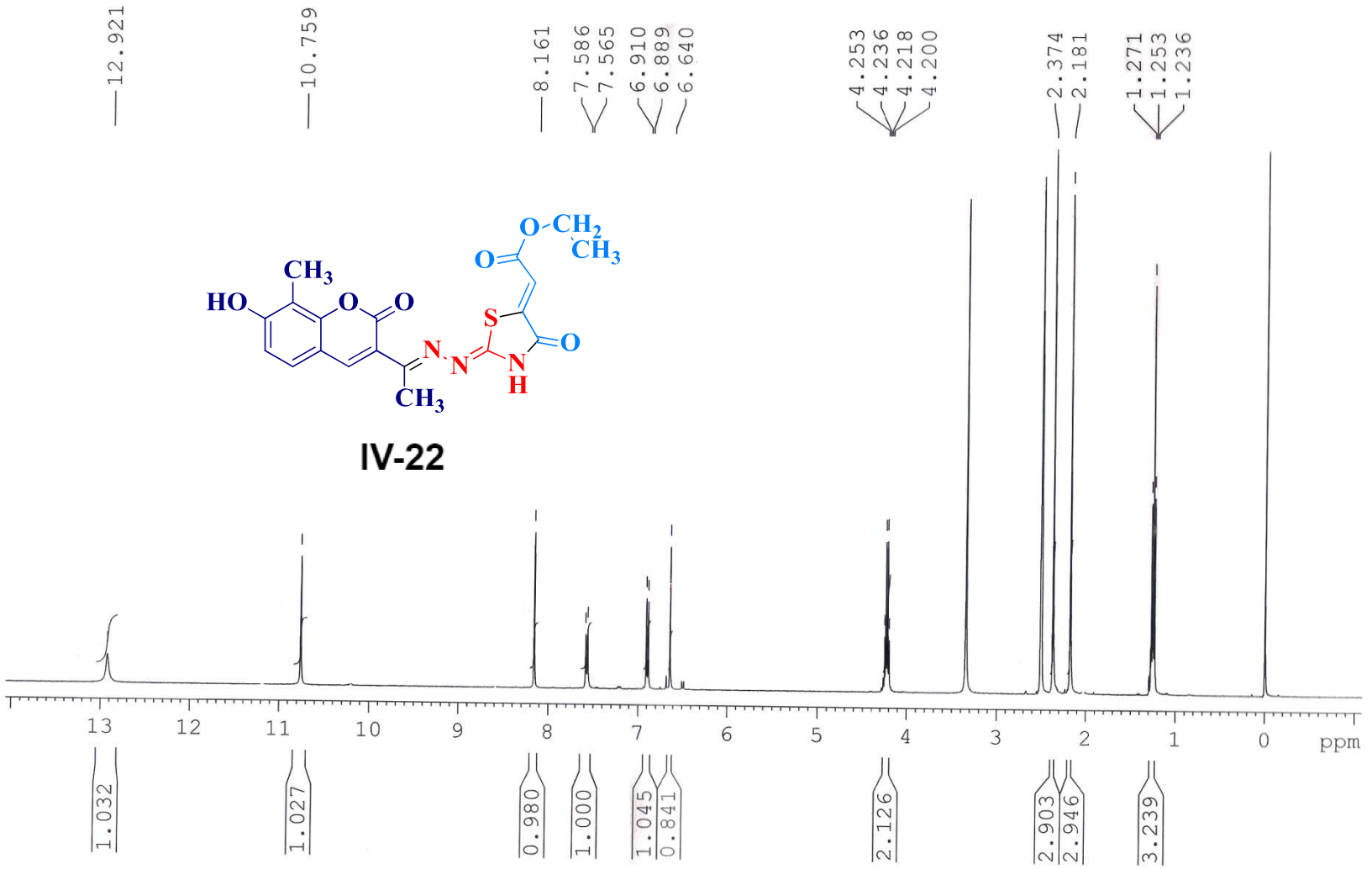


OH+DEAD
NMR IN DMSO-D6



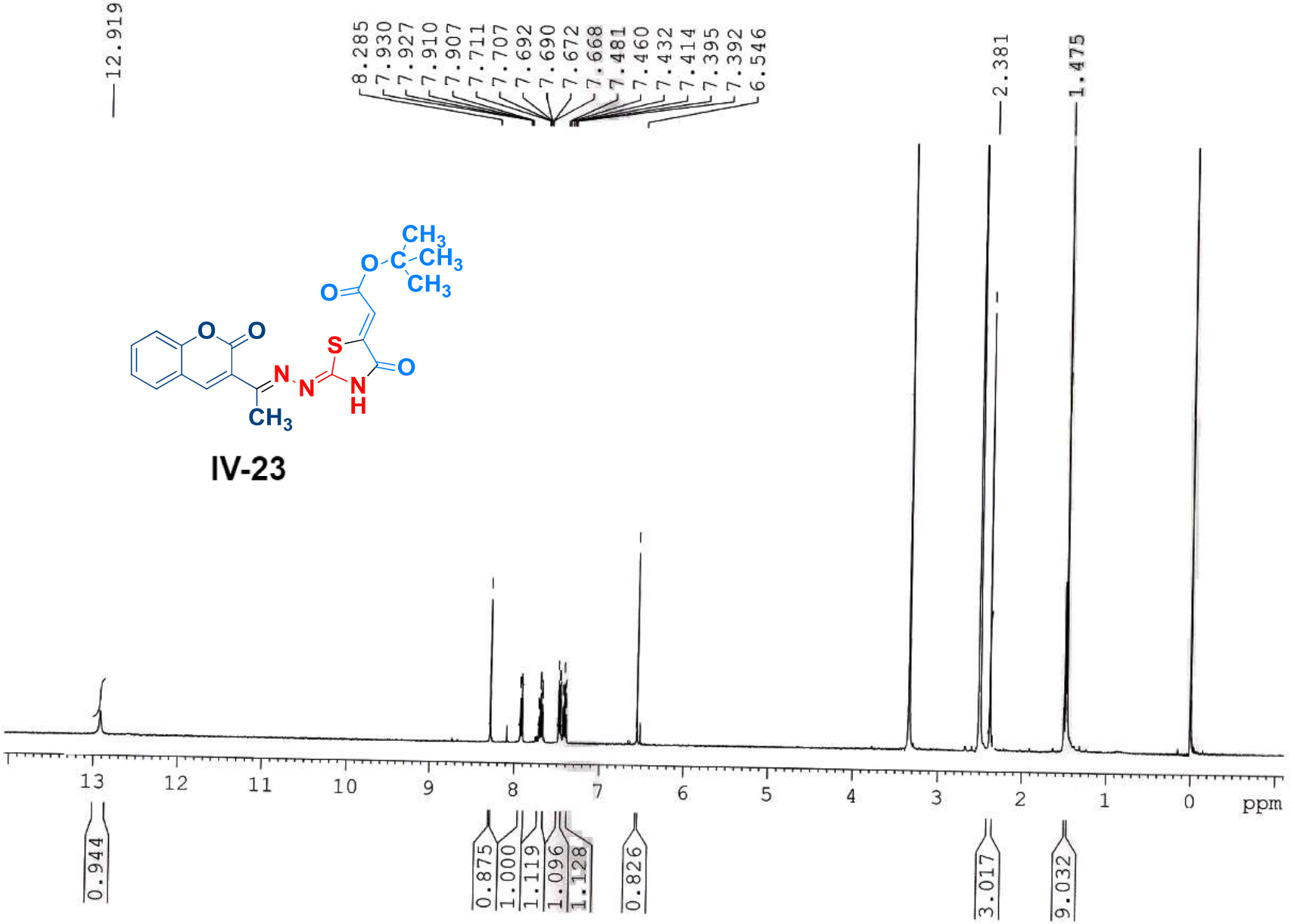
IV-21

MER
1H NMR IN DMSO-D6



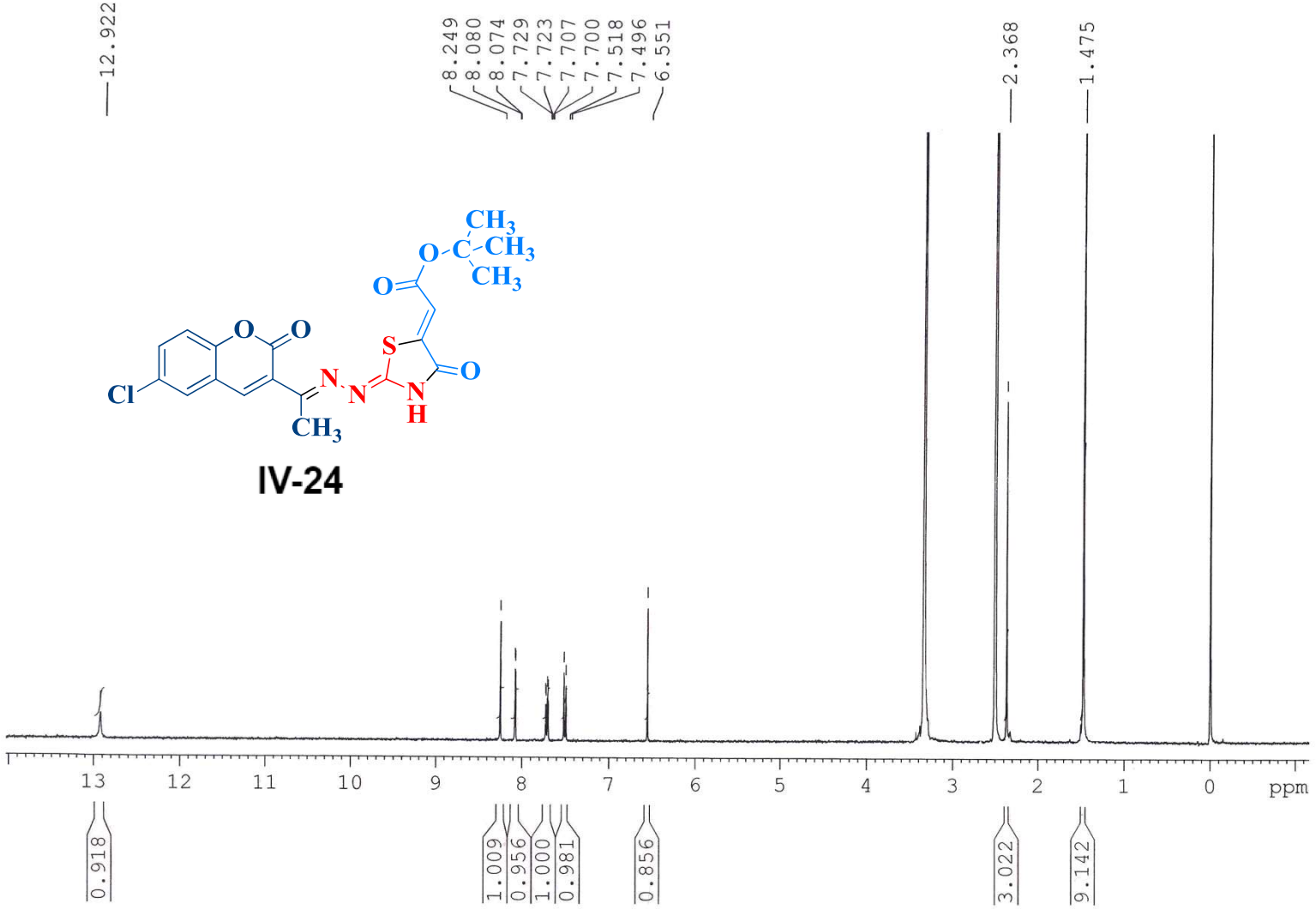
IV-22

DTB
1H NMR IN DMSO-D6



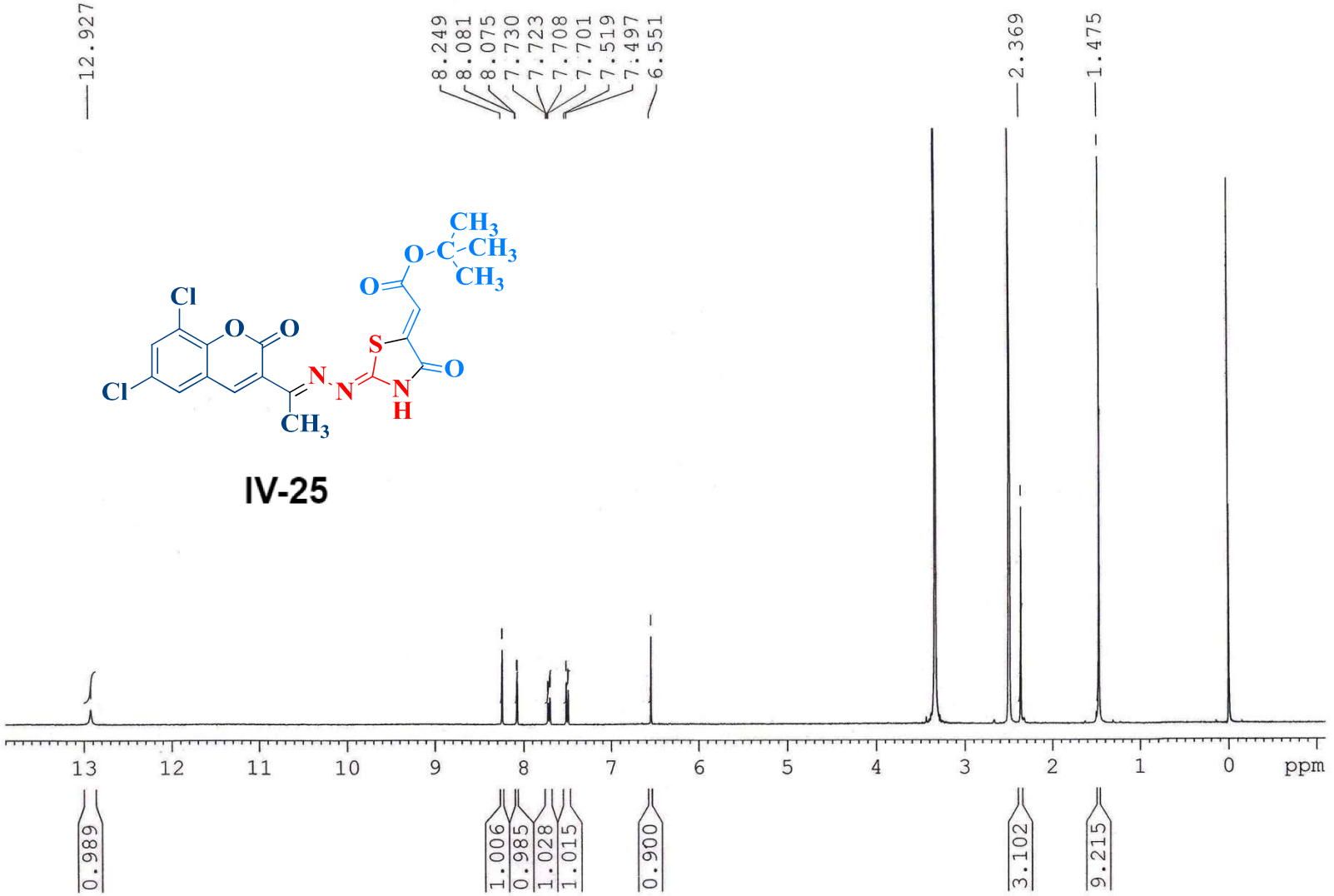
IV-23

MC
1H NMR IN DMSO-D6



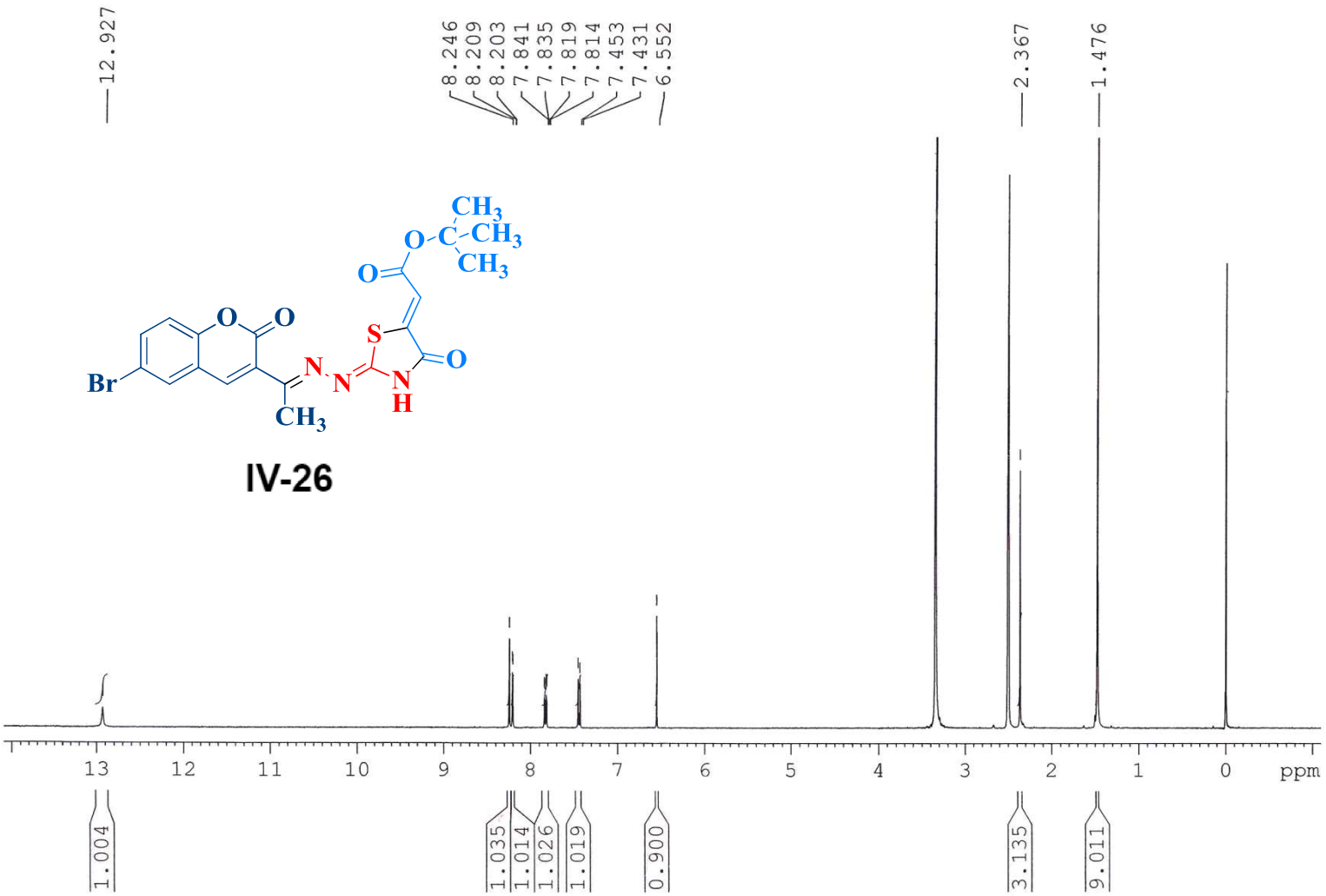
IV-24

DC
1H NMR IN DMSO-D6



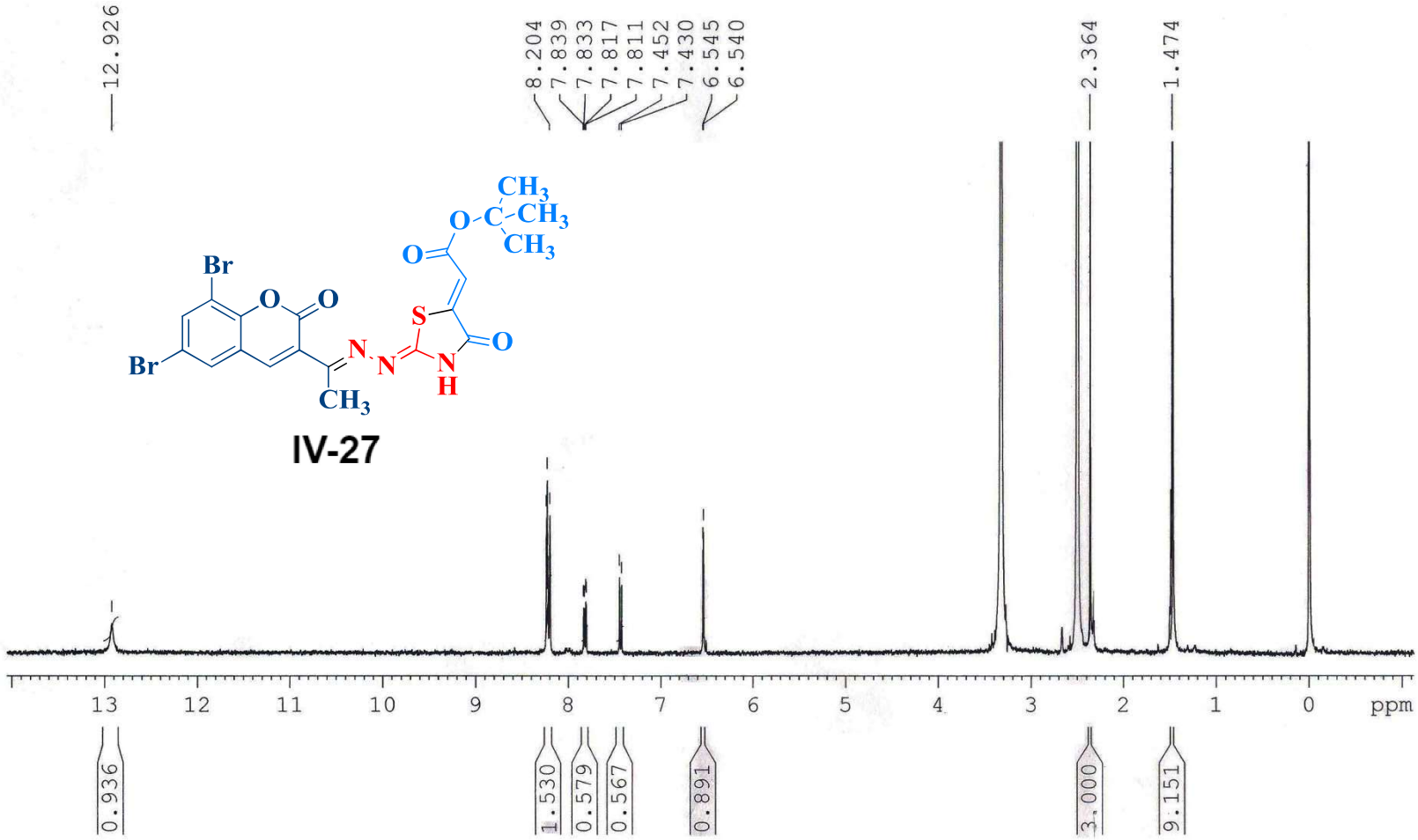
IV-25

MB
1H NMR IN DMSO-D6

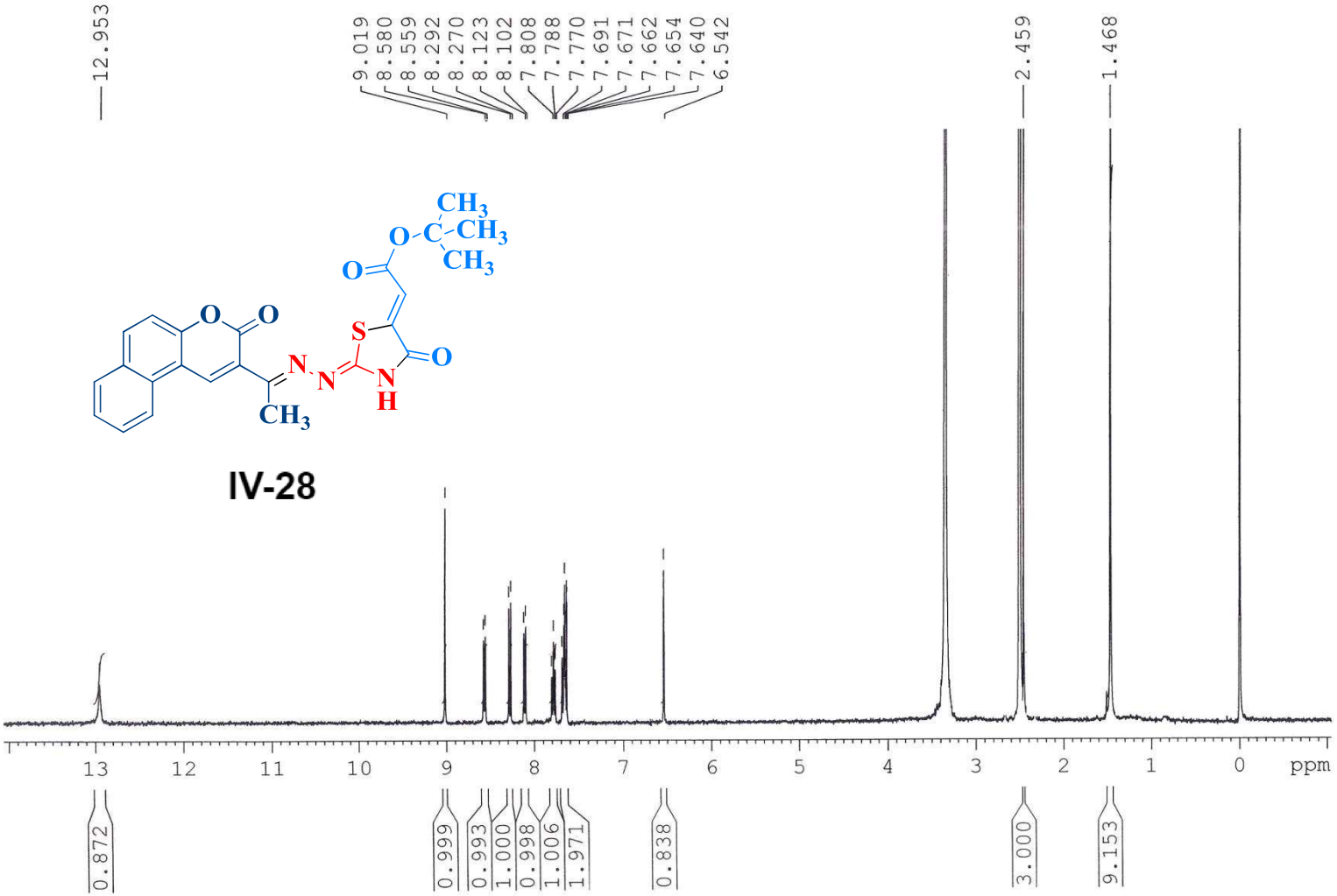


IV-26

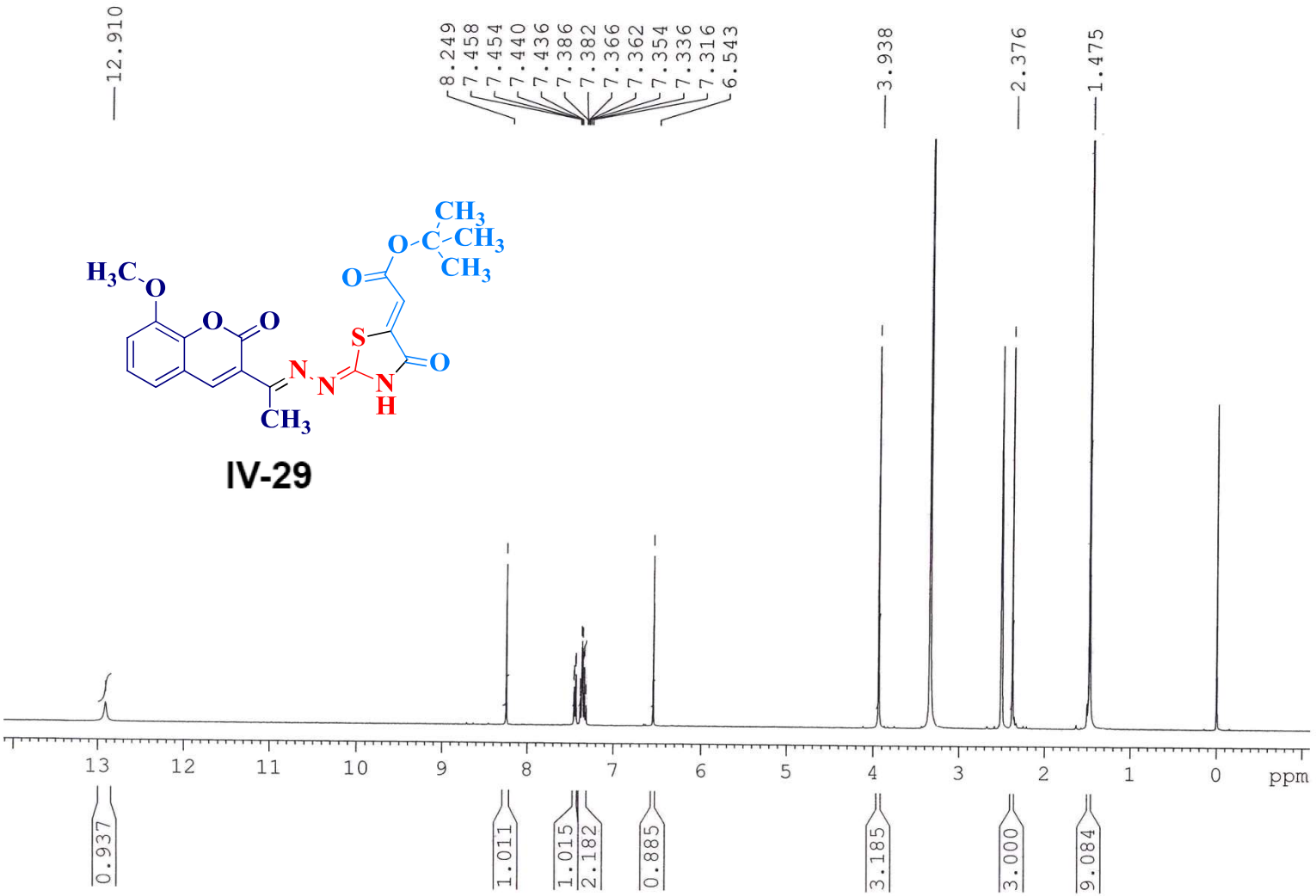
DB+DTBAD
1H NMR IN DMSO-D6



NAP
1H NMR IN DMSO-D6

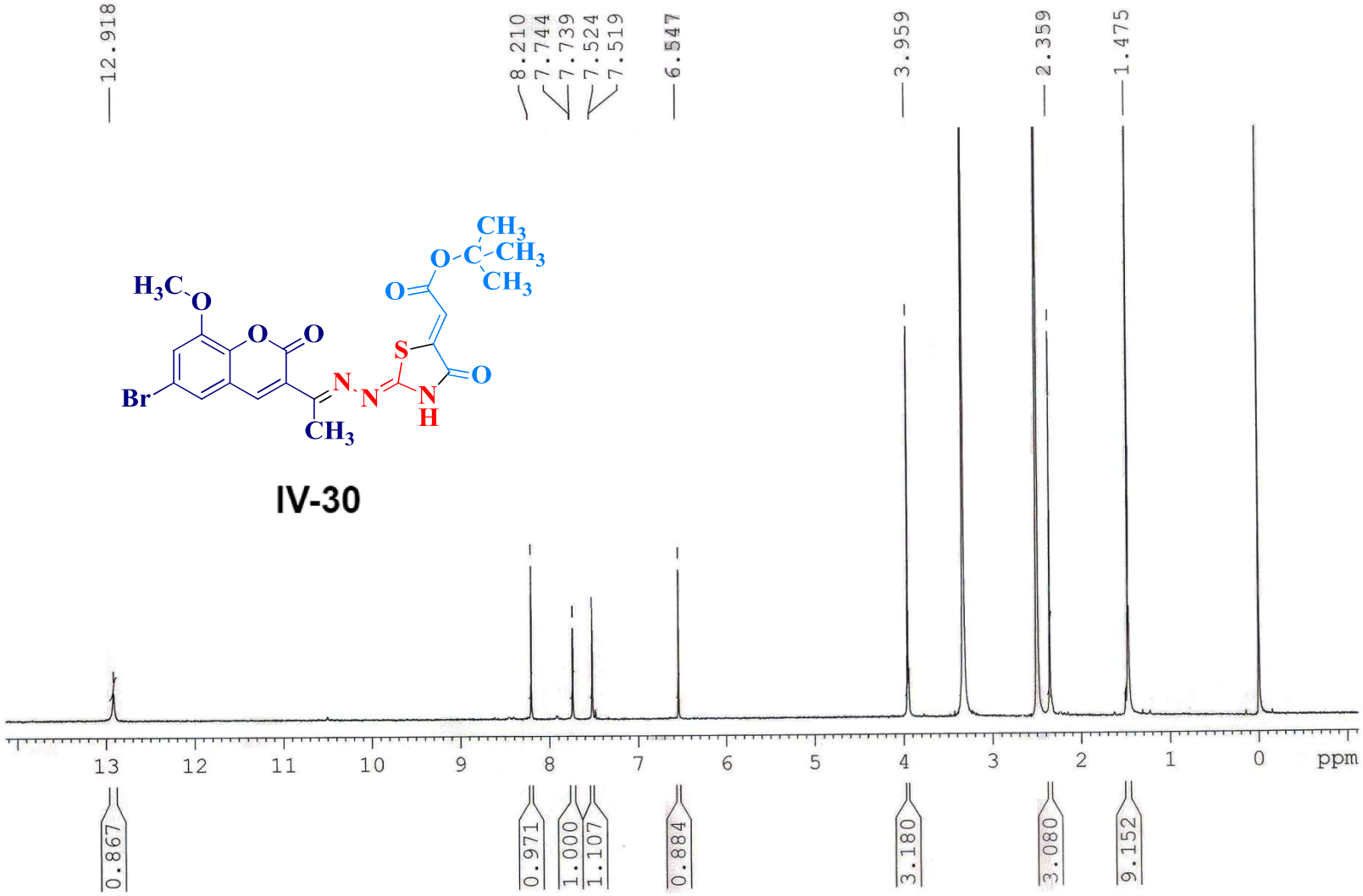


OV
1H NMR IN DMSO-D6



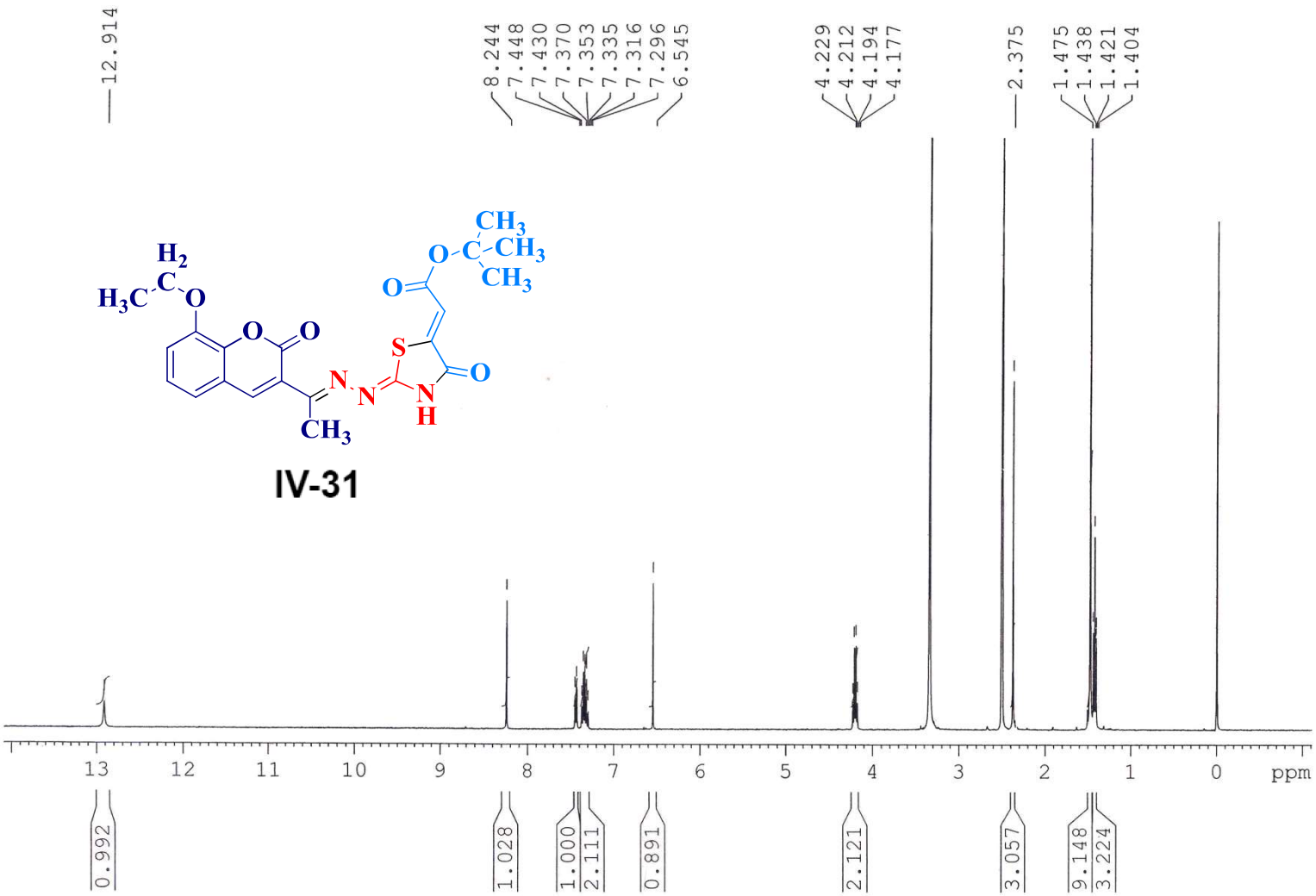
IV-29

Br+OV+DTB
1H NMR IN DMSO-D6



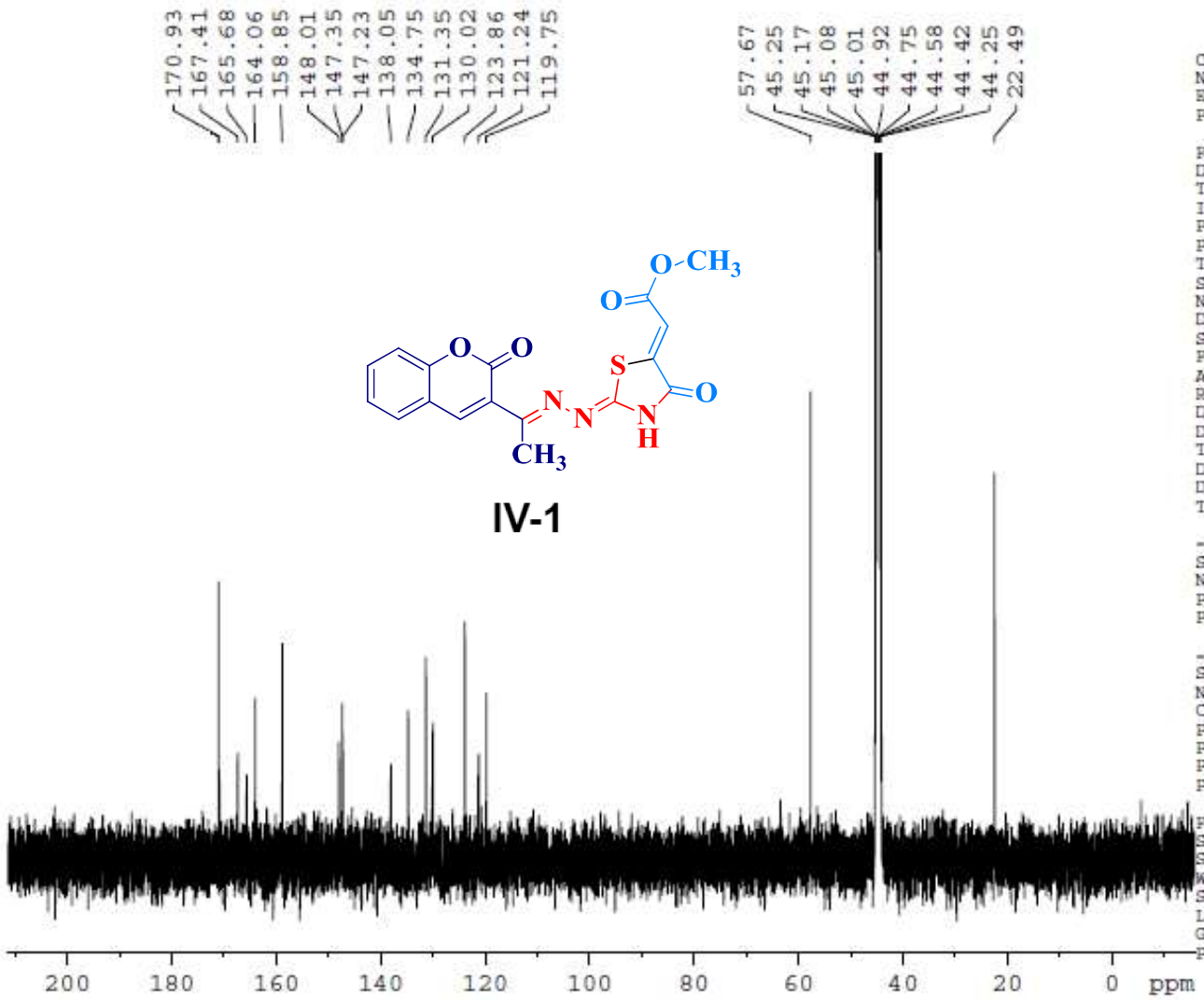
IV-30

DET
1H NMR IN DMSO-D6



IV-31

KMS-D-SBM
C13CPD CDCl3 {C:\Bruker\TopSpin3.2} nmr 40



IV-1

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Current Data Parameters
NAME                  Sagar
EXPNO                   172
PROCNO                    1

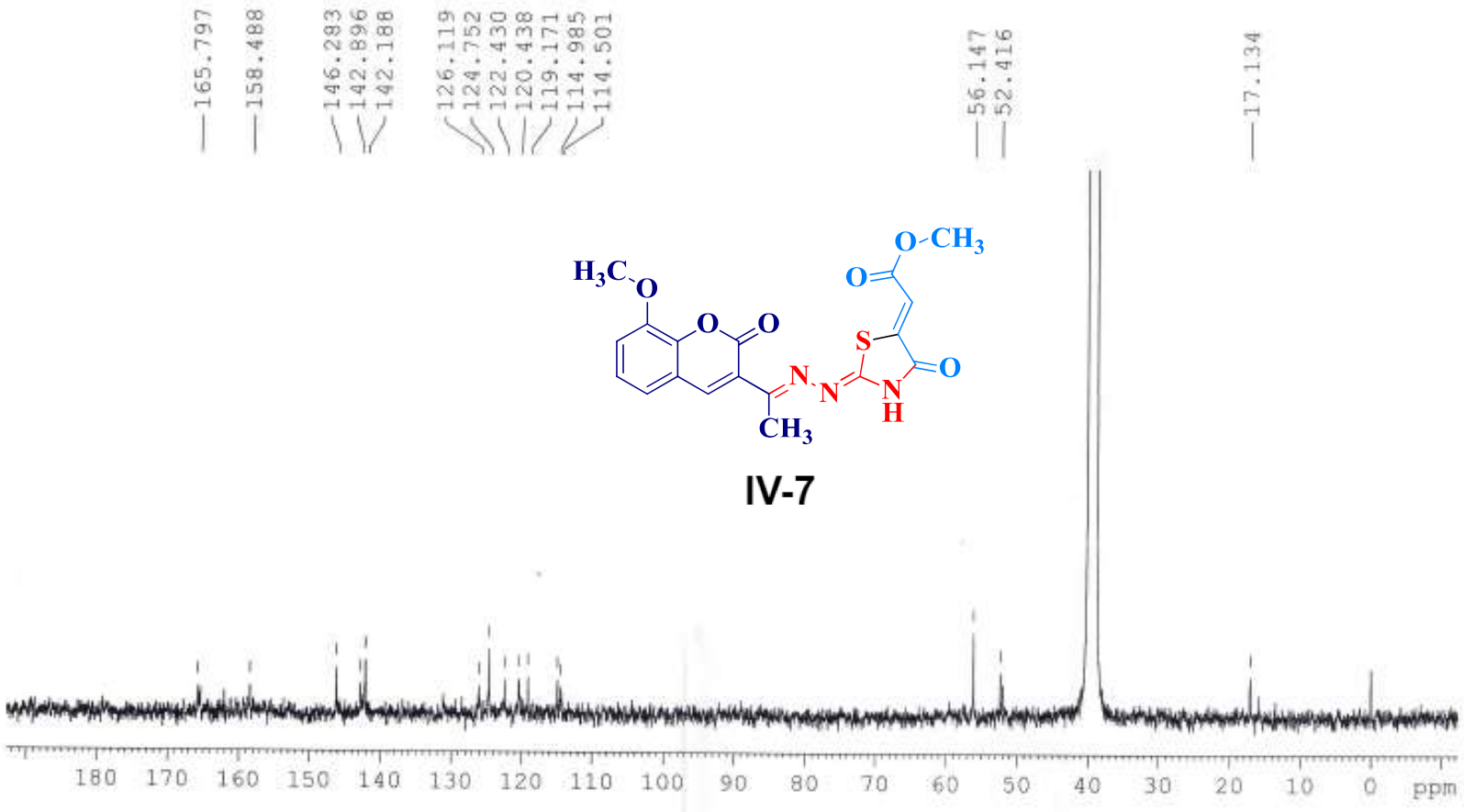
F2 - Acquisition Parameters
Date_              20150824
Time                  23.42
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                    1024
DS                       4
SWH              29761.904 Hz
FIDRES           0.454131 Hz
AQ               1.1010048 sec
RG                     203
DW                16.800 usec
DE                 6.50 usec
TE                 0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          125.7955112 MHz
NUC1                    13C
P1                   8.65 usec
PLW1         120.50000000 W

======== CHANNEL f2 ========
SFO2          500.2320009 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2               80.00 usec
PLW2          27.16399956 W
PLW12          0.34685999 W
PLW13          0.22199000 W

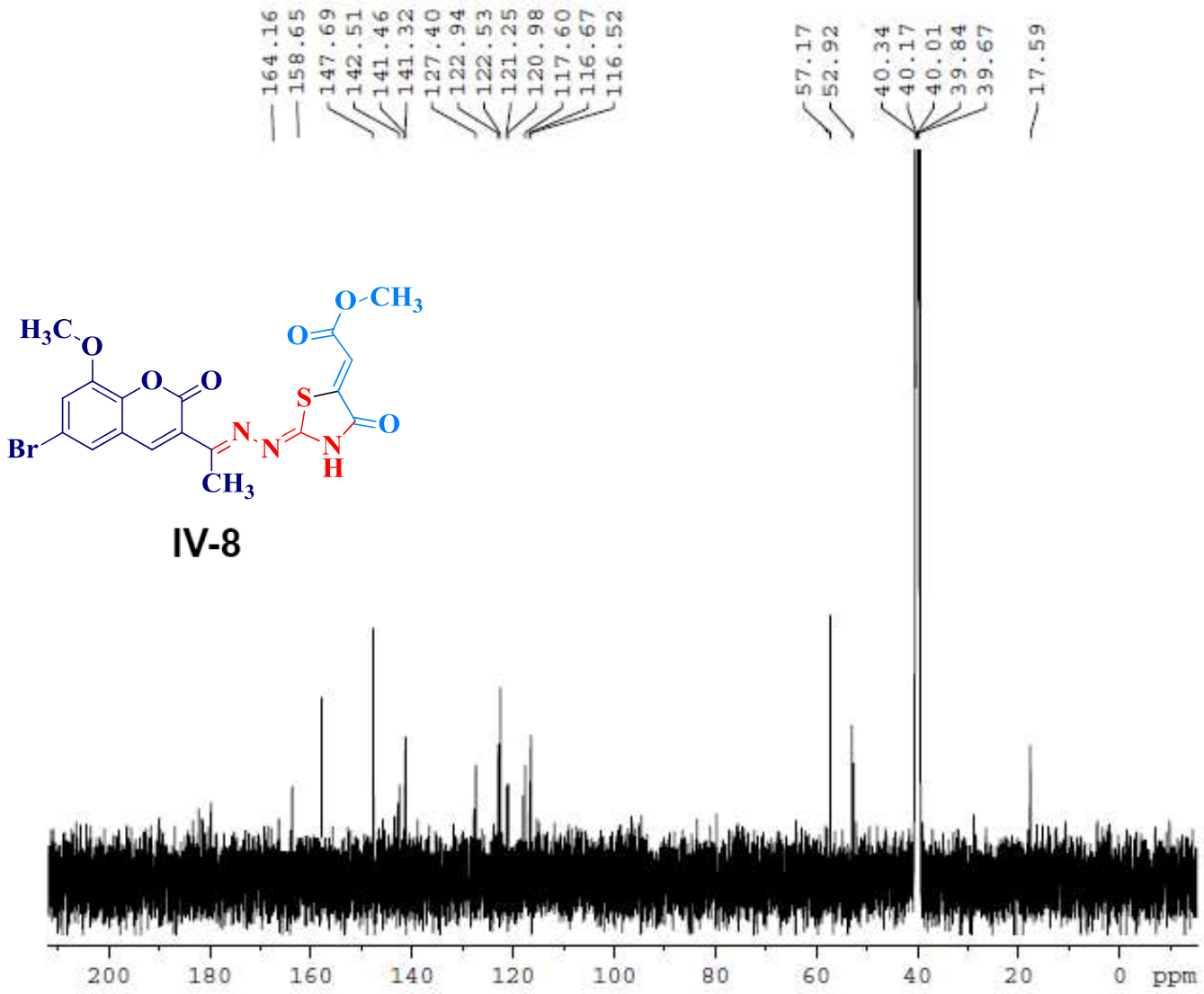
F2 - Processing parameters
SI                   32768
SF            125.7829335 MHz
WDW                     EM
SSB                  0
LB                    1.00 Hz
GB                   0
PC                    1.40
```

OV-DMAD
13C NMR IN DMSO-D6



IV-7

BroVDM
C13CPD DMSO {C:\Bruker\TopSpin3.2} nmr 42



IV-8

```
Current Data Parameters
NAME                  Sagar
EXPNO                   176
PROCNO                    1

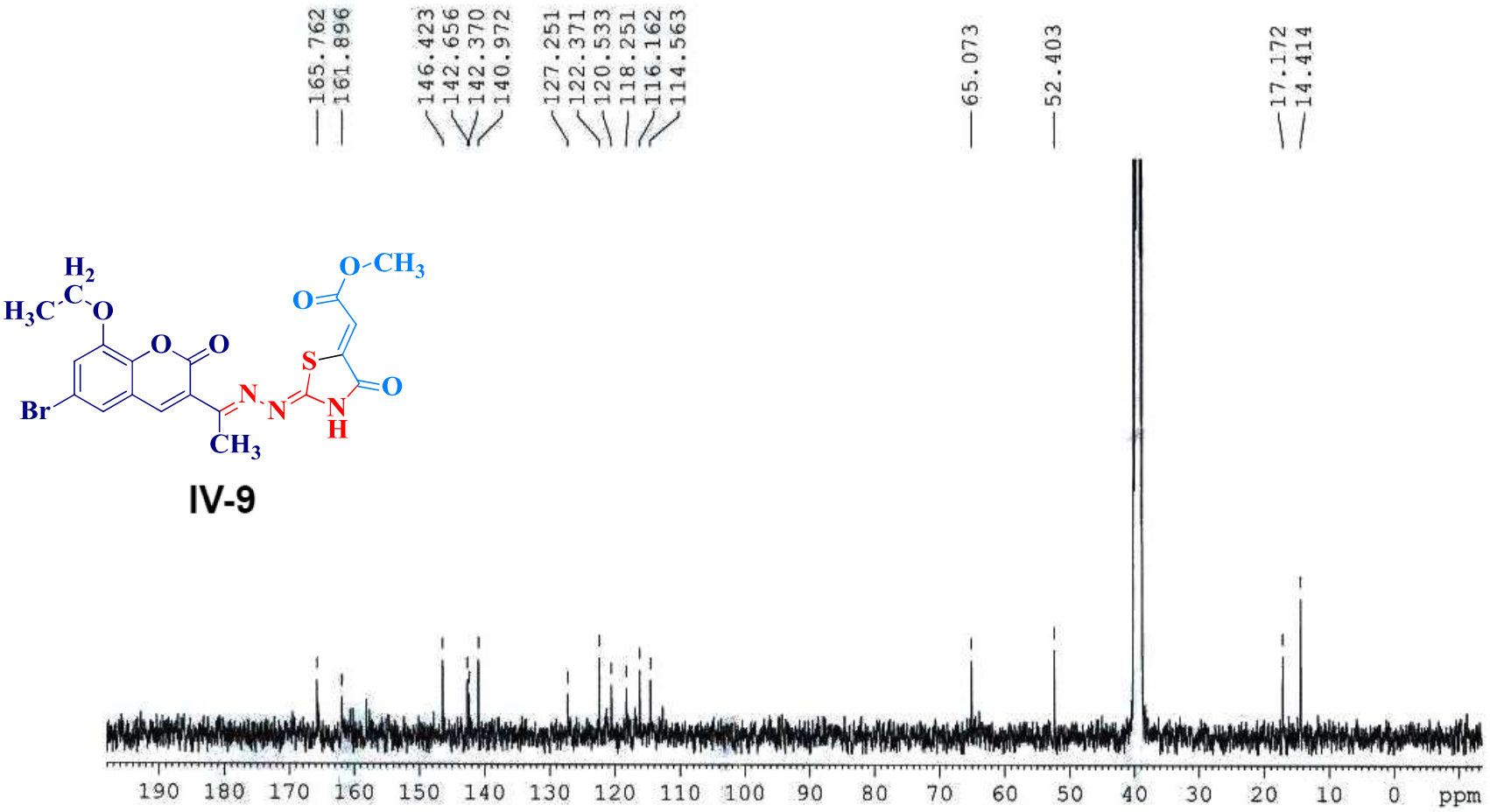
F2 - Acquisition Parameters
Date_              20150825
Time                   2.33
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                     1024
DS                        4
SWH               29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010048 sec
RG                      203
DW                 16.800 usec
DE                  6.50 usec
TE                0 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            125.7955112 MHz
NUC1                    13C
P1                   8.65 usec
PLW1           120.50000000 W

======== CHANNEL f2 ========
SFO2            500.2320009 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                80.00 usec
PLW2            27.16399956 W
PLW12            0.34685999 W
PLW13            0.22199000 W

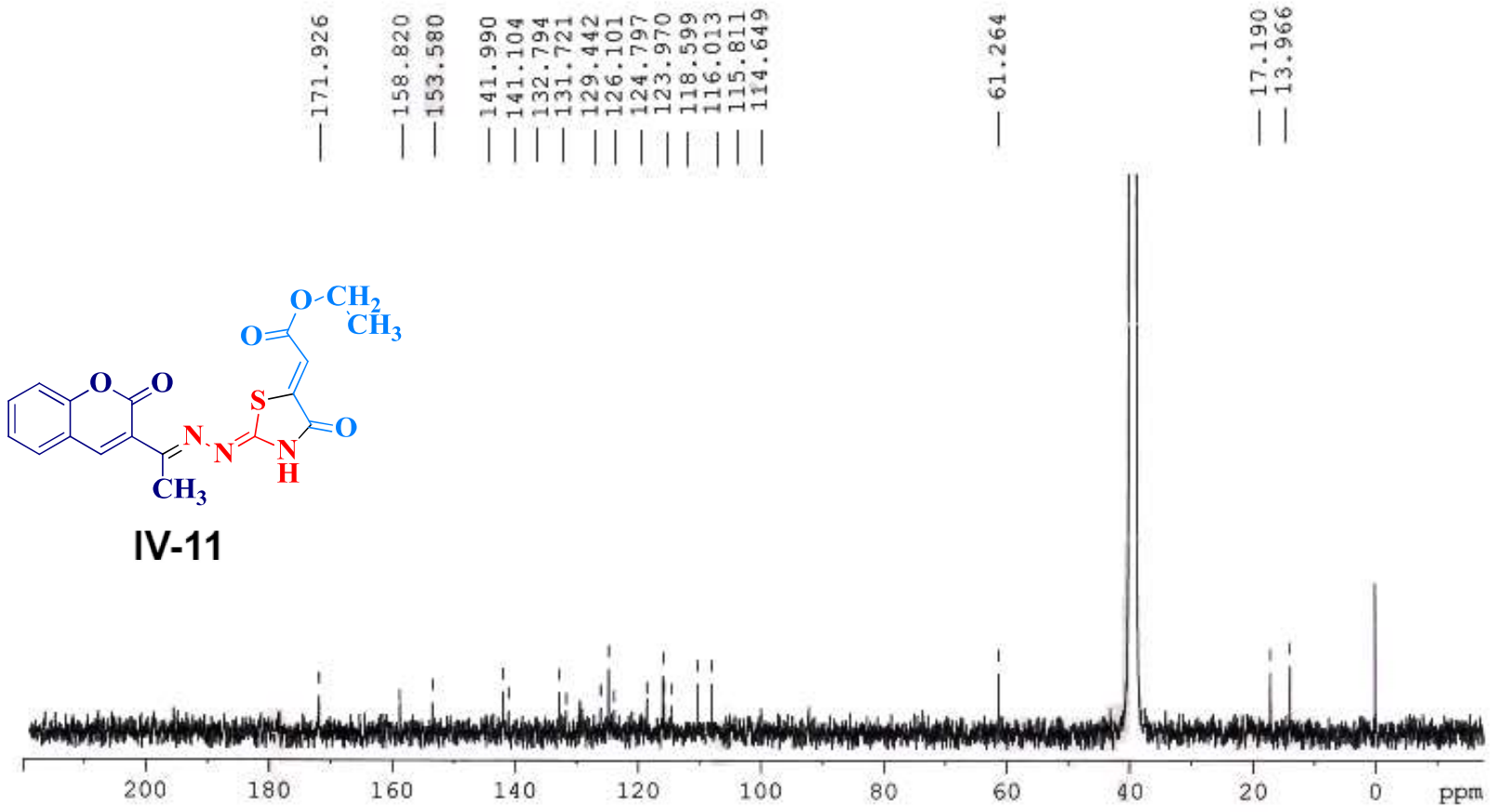
F2 - Processing parameters
SI                      32768
SF              125.7829335 MHz
WDW                        EM
SSB               0
LB                    1.00 Hz
GB                0
PC                    1.40
```

BROET-DM
13C NMR IN DMSO-D6



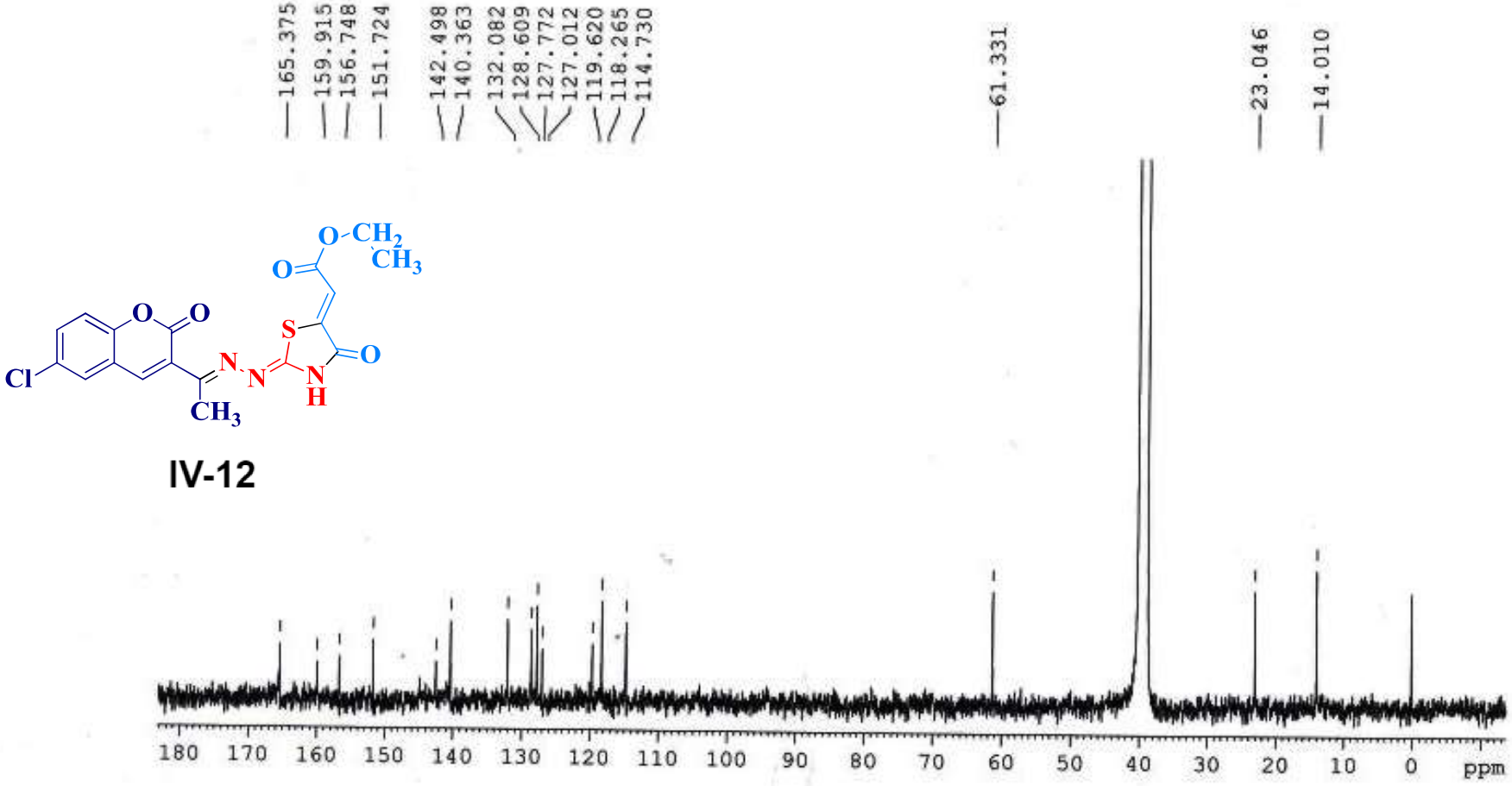
IV-9

DEAD
13C NMR IN DMSO-D6



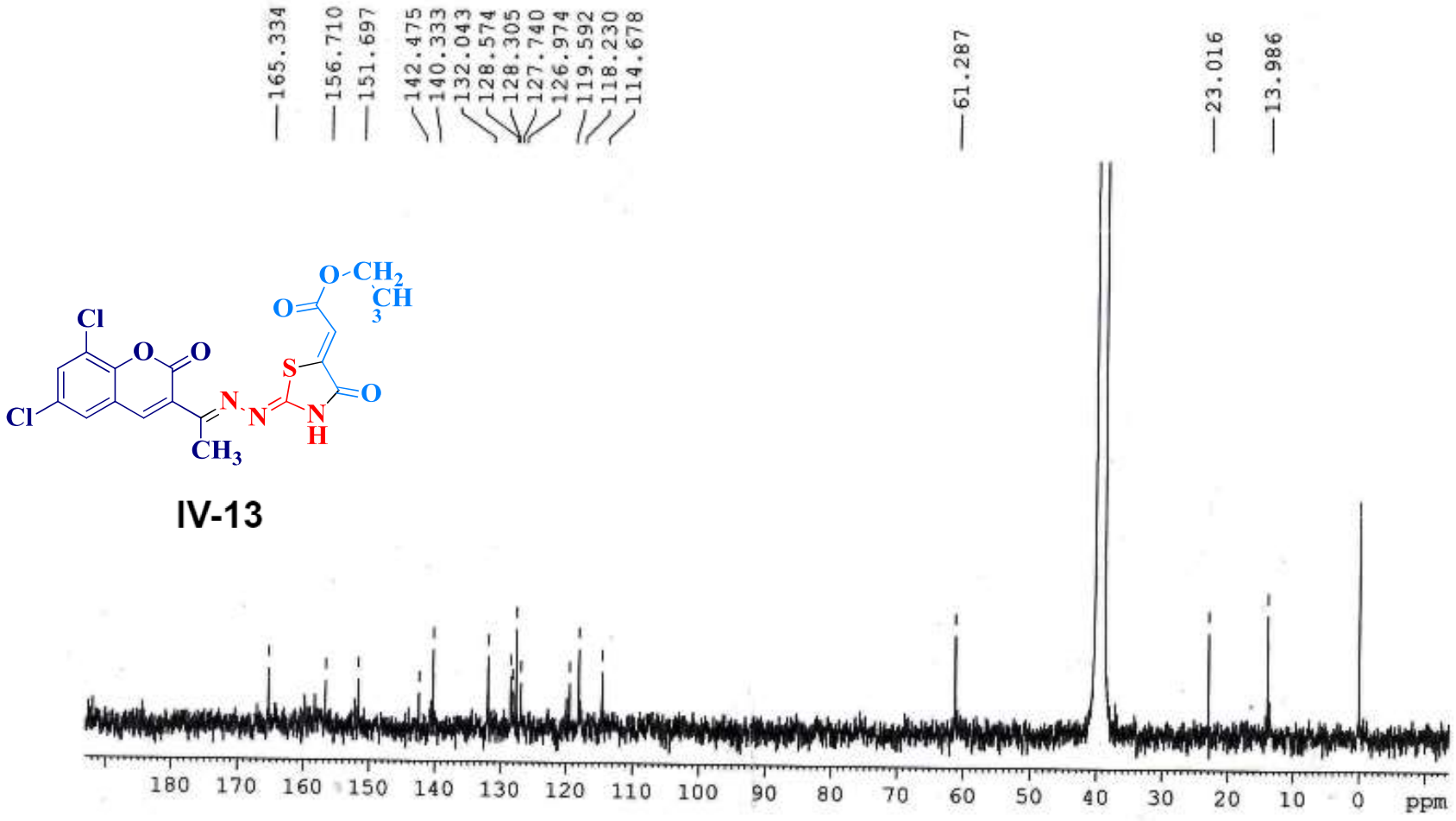
IV-11

DE-MC
13C NMR IN DMSO-D6

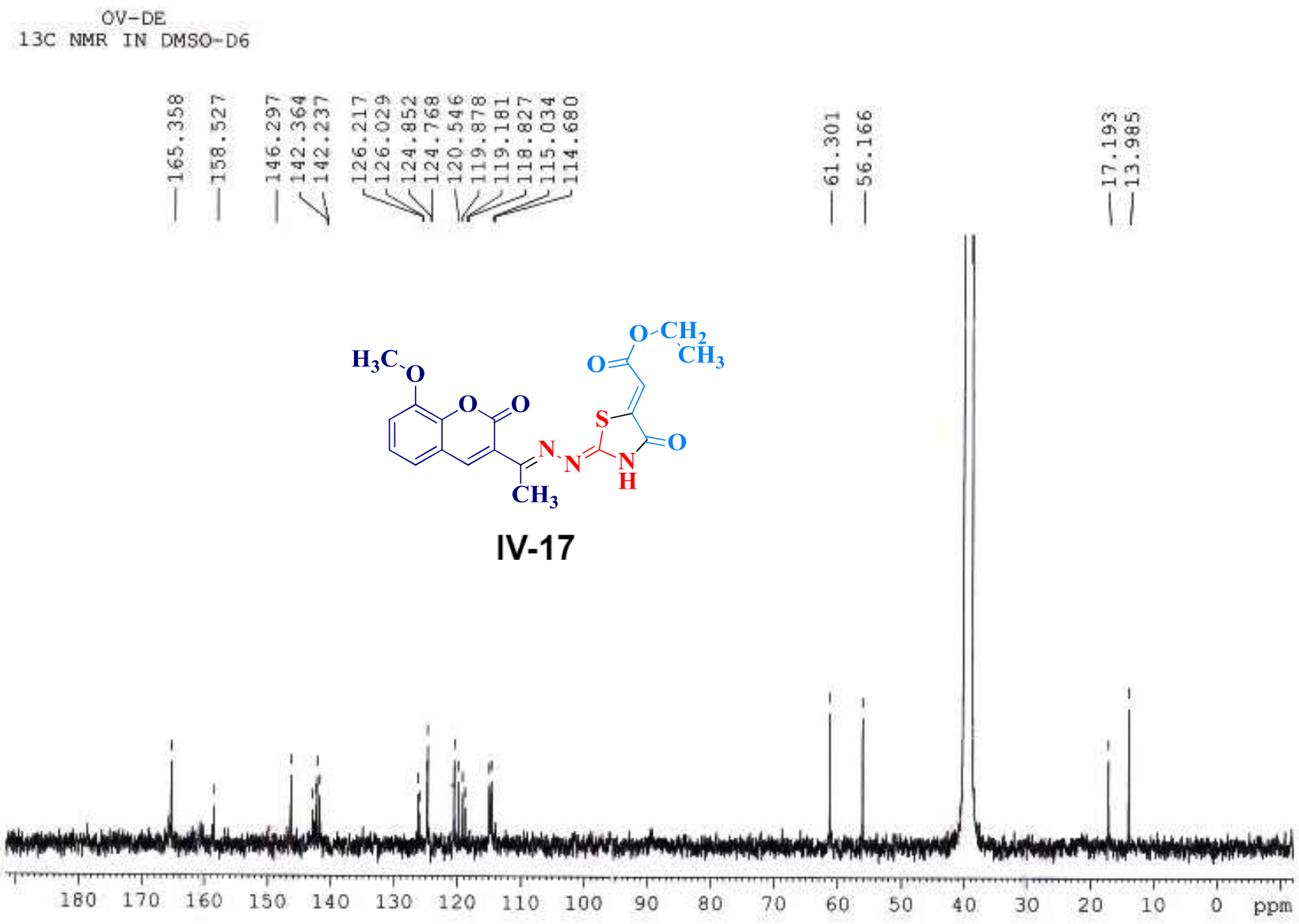


IV-12

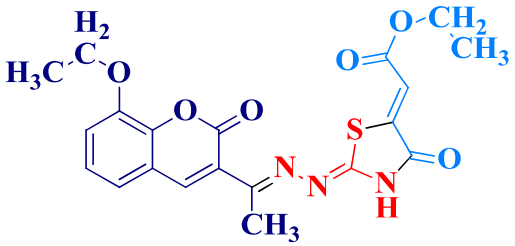
DE-DC
13C NMR IN DMSO-D6



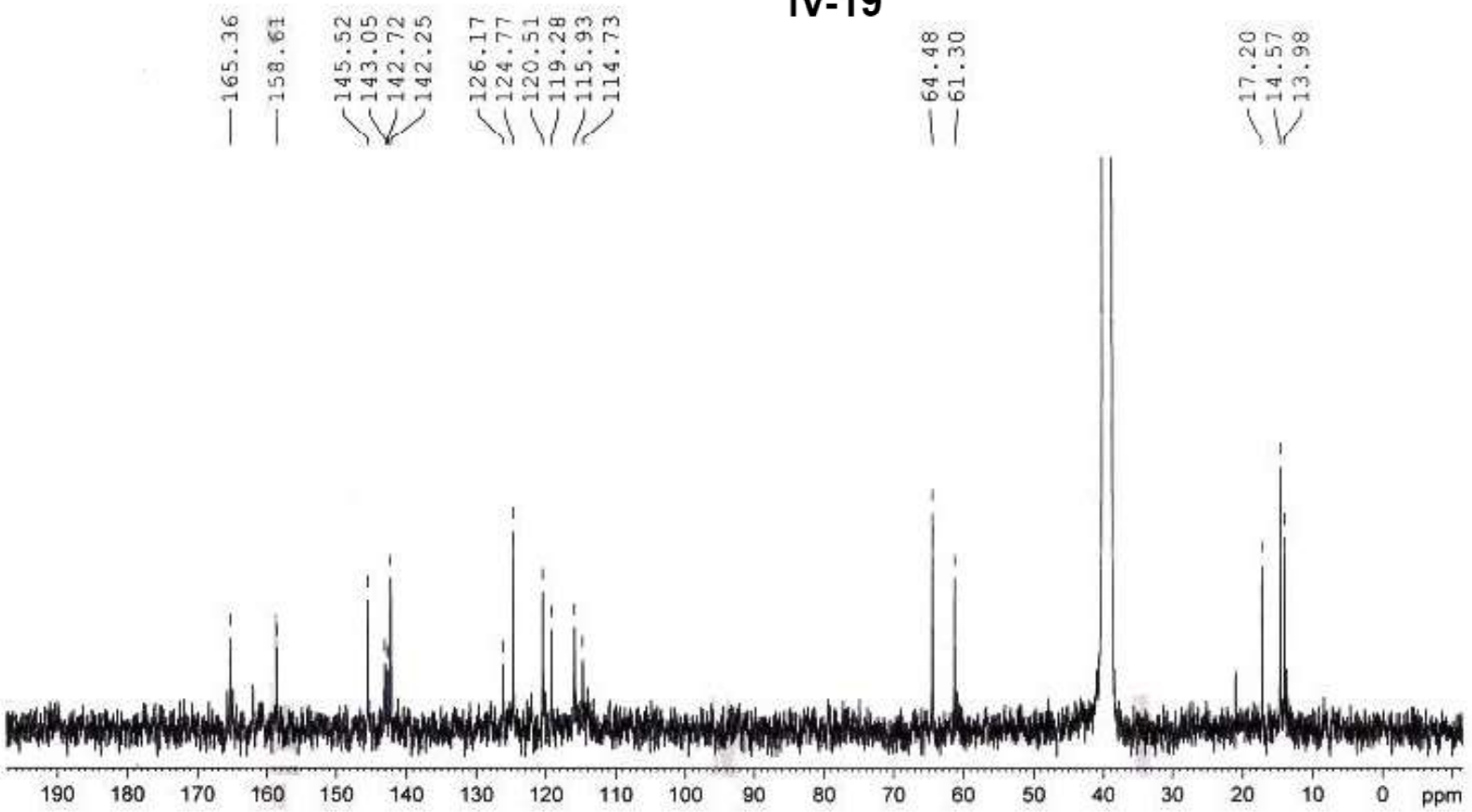
IV-13


OV-DE
13C NMR IN DMSO-D6
165.358
158.527
146.297
142.364
142.237
126.217
126.029
124.852
124.768
120.546
119.878
119.181
118.827
115.034
114.680
61.301
56.166
17.193
13.985
IV-17
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

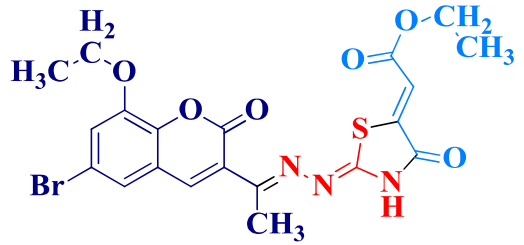
OET-DE
13C NMR IN DMSO-D6



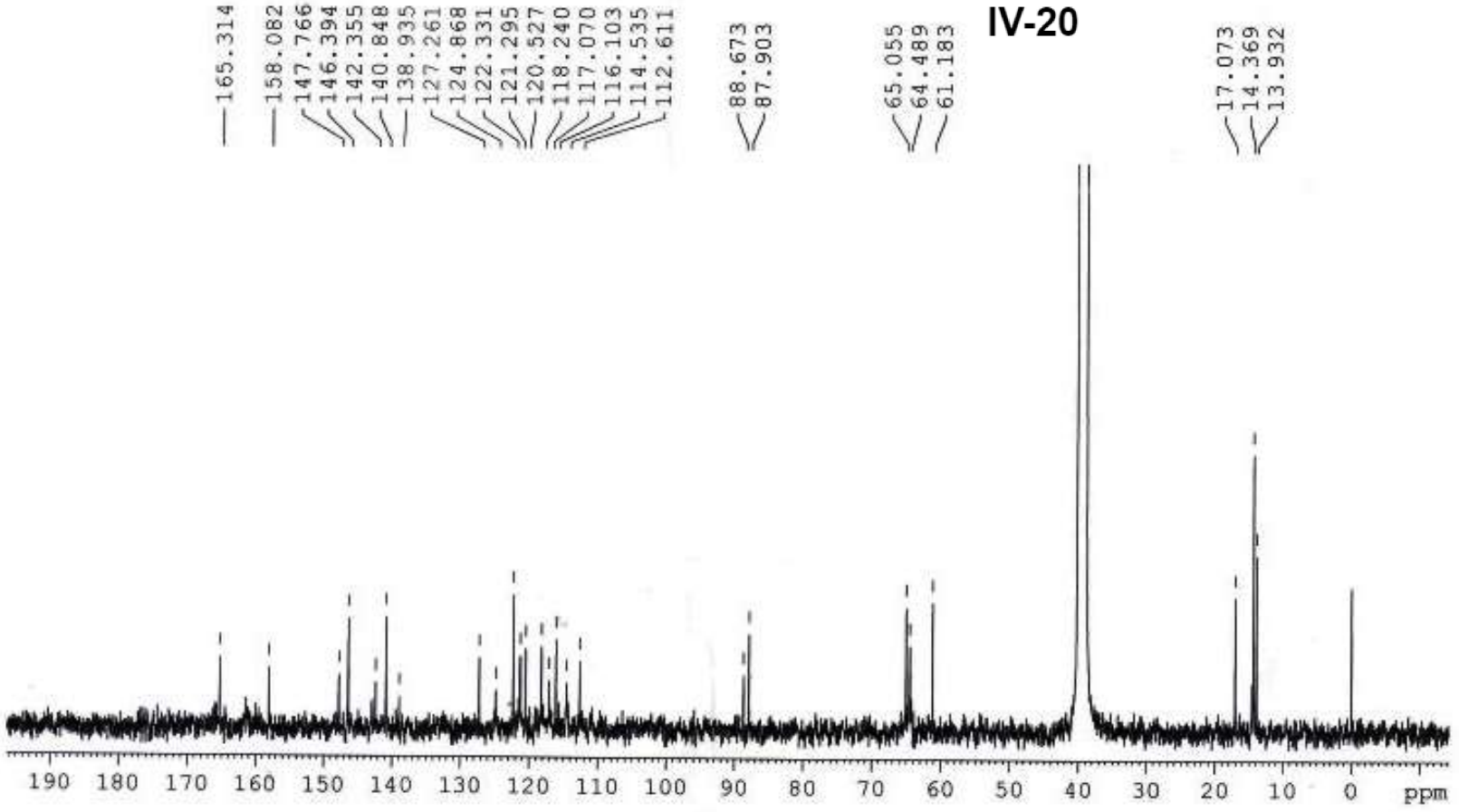
IV-19



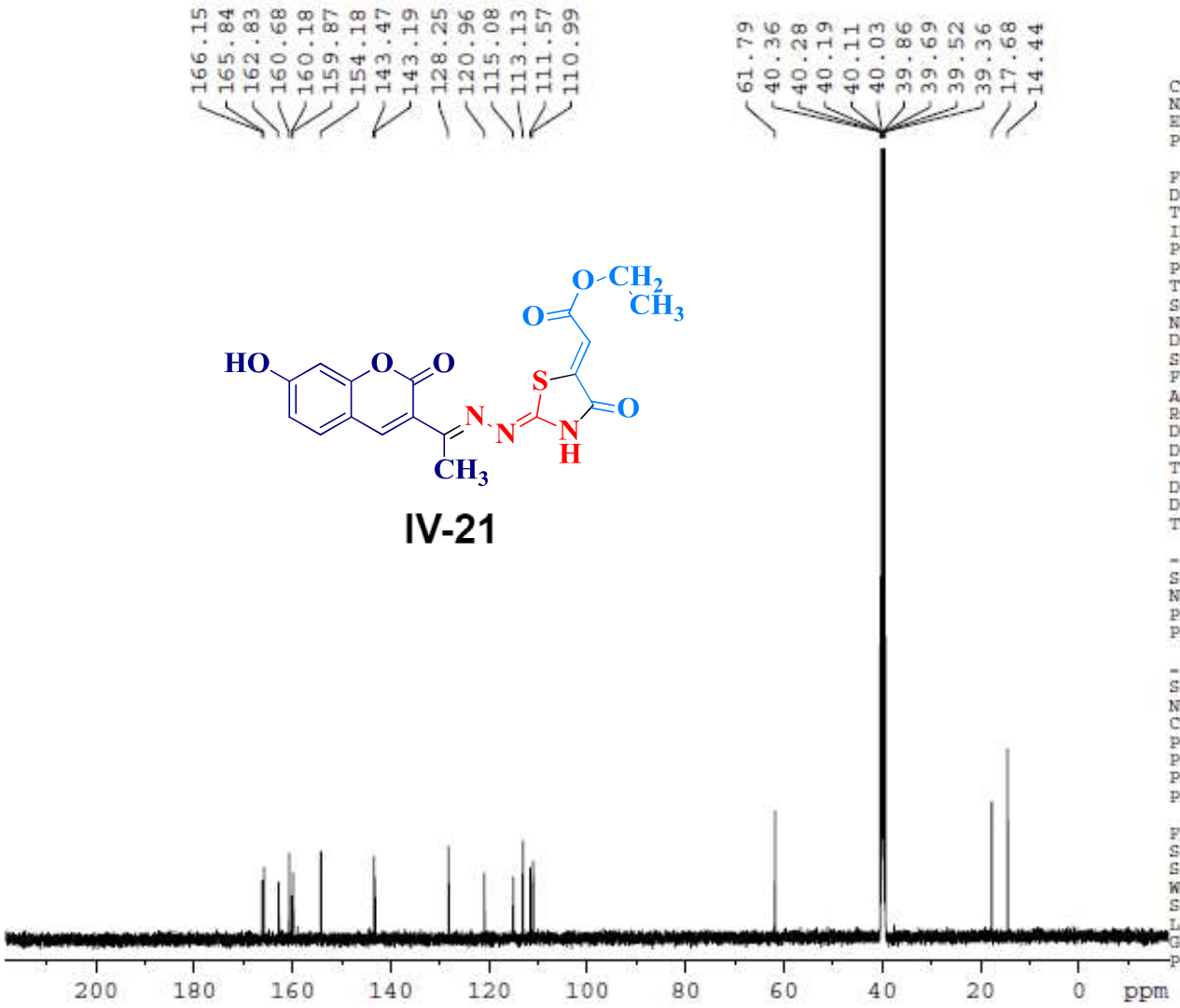
DE-BrOEt
13C NMR IN DMSO-D6



IV-20



C13CPD DMSO {C:\Bruker\TopSpin3.2} nmr 26



IV-21

```
Current Data Parameters
NAME                  Asha
EXPNO                    6
PROCNO                   1

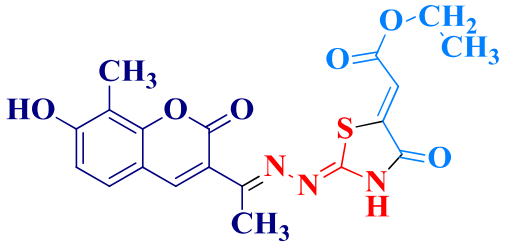
P2 - Acquisition Parameters
Date_              20151104
Time_                 9.59
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG              zgpg30
TD                    65536
SOLVENT                DMSO
NS                       411
DS                         4
SWH               29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010048 sec
RG                       203
DW                  16.800 usec
DE                   6.50 usec
TE                   301.5 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           125.7955112 MHz
NUC1                    13C
P1                   8.65 usec
PLW1          120.50000000 W

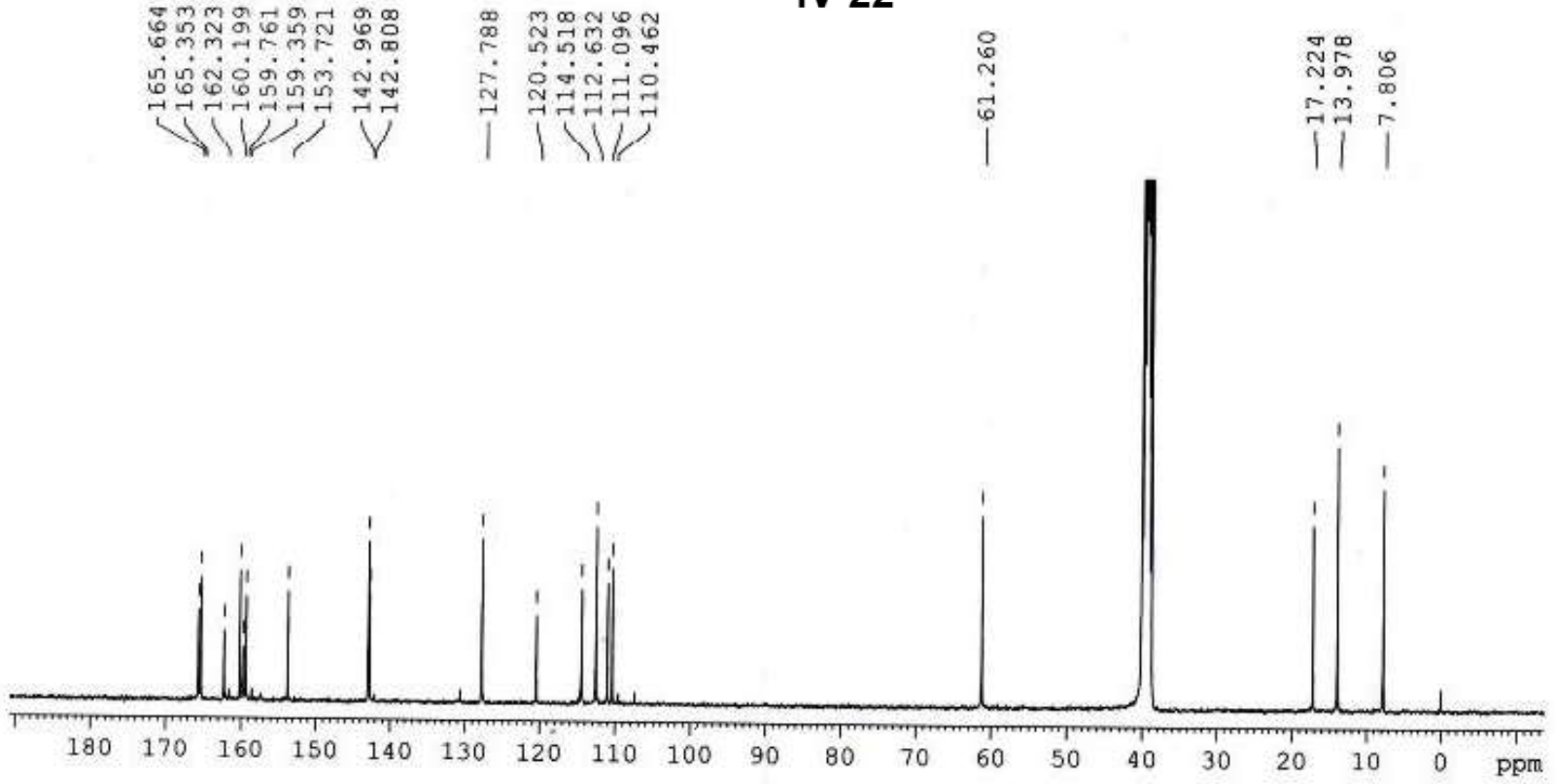
======== CHANNEL f2 ========
SFO2           500.2320009 MHz
NUC2                    1H
CPDPRG[2            waltz16
PCPD2                80.00 usec
PLW2           27.16399956 W
PLW12           0.34685999 W
PLW13           0.22199000 W

P2 - Processing parameters
SI                      32768
SF             125.7829335 MHz
WDW                        EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
```

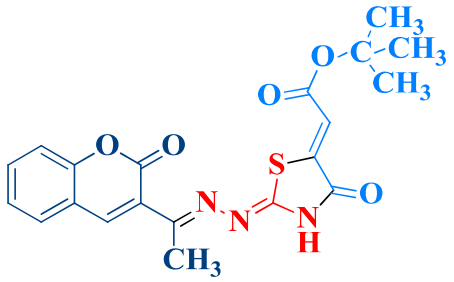
-DE-MERE
13C NMR IN DMSO-D6



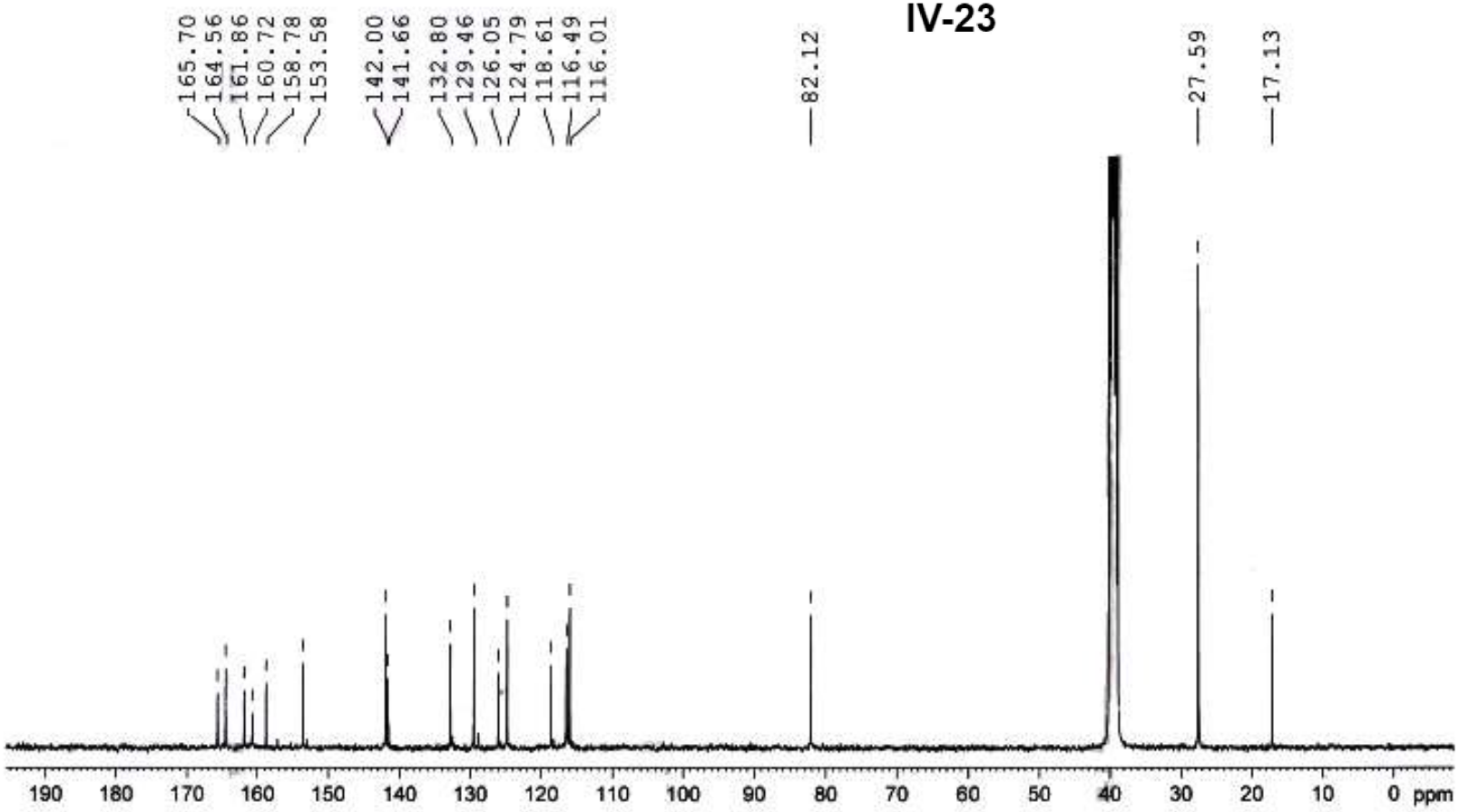
IV-22

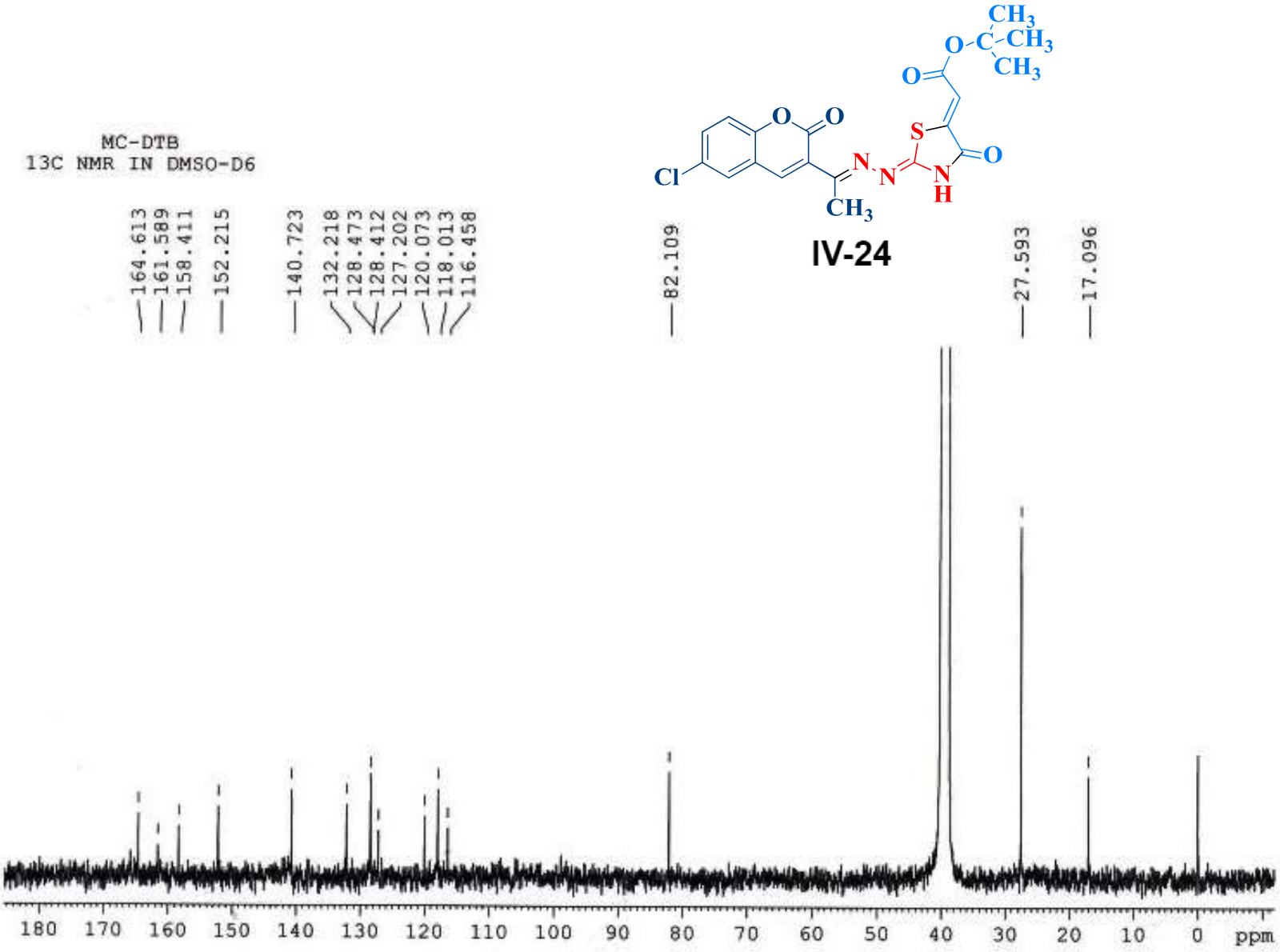


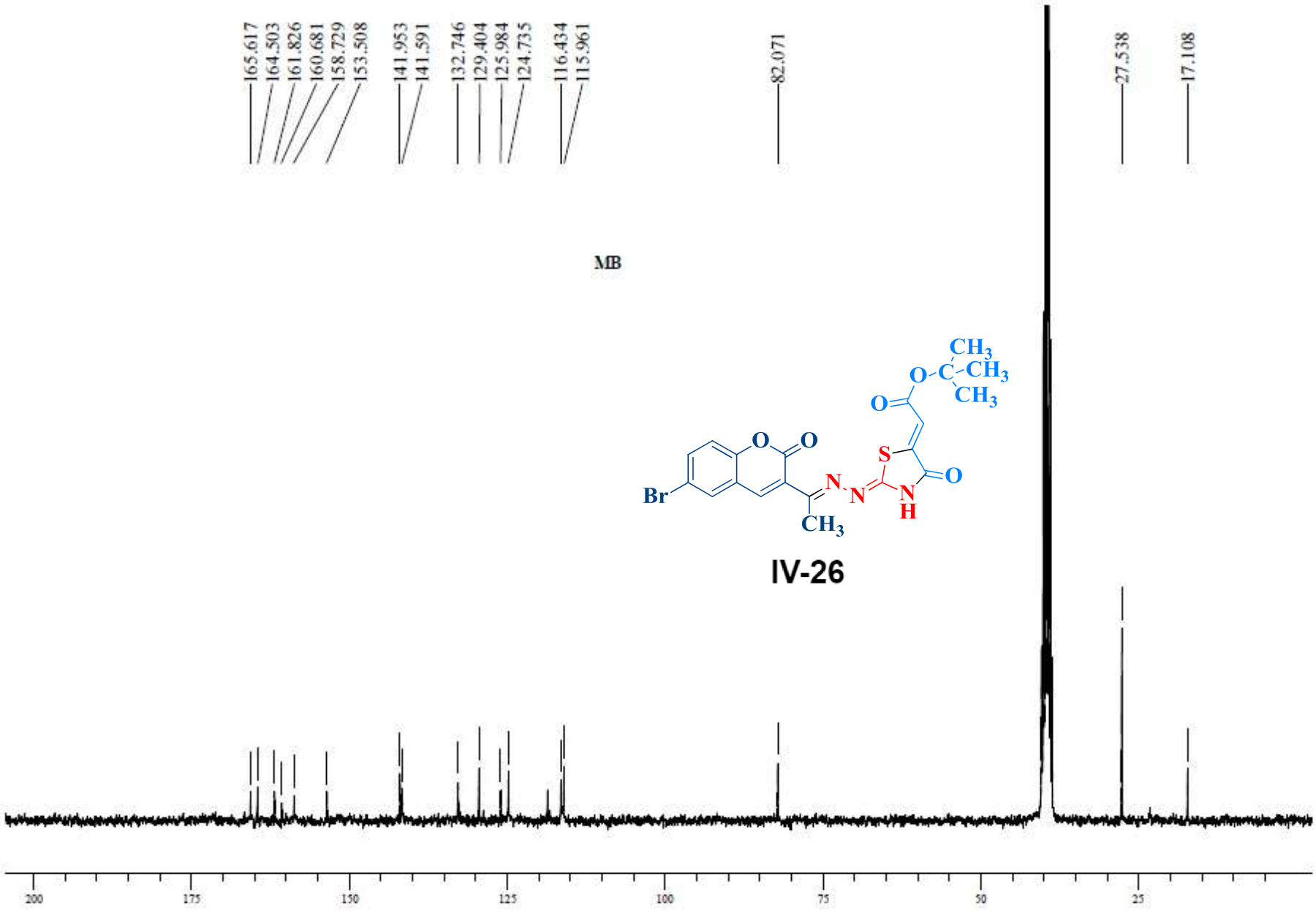
SDTB
13C NMR IN DMSO-D6

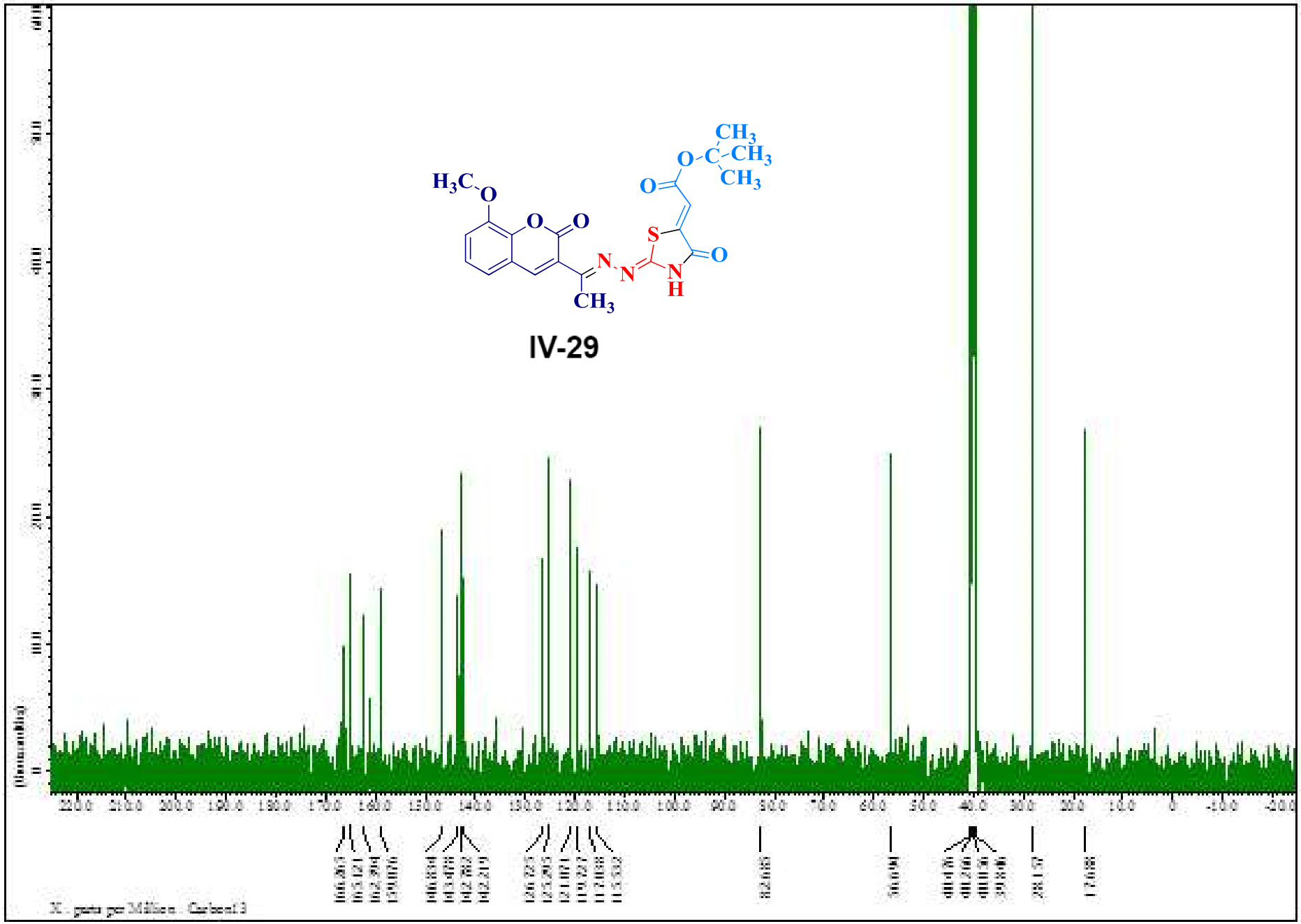


IV-23

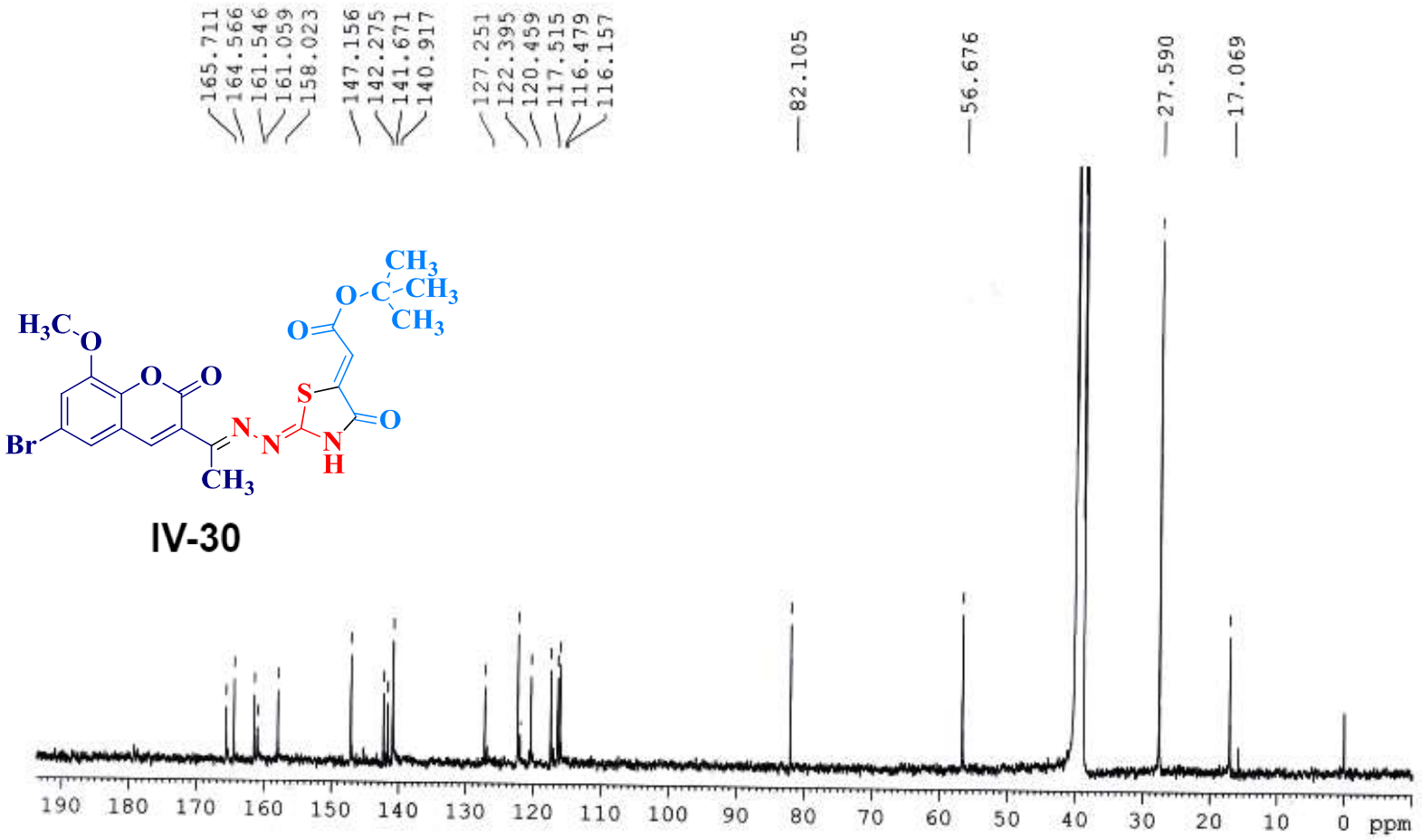



MC-DTB
13C NMR IN DMSO-D6
164.613
161.589
158.411
152.215
140.723
132.218
128.473
128.412
127.202
120.073
118.013
116.458
82.109
27.593
17.096
IV-24
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


165.617
164.503
161.826
160.681
158.729
153.508
141.953
141.591
132.746
129.404
125.984
124.735
116.434
115.961
82.071
27.538
17.108
MB
Br
O
O
O
O
S
N
N
N
H
O
CH3
O
C
CH3
CH3
CH3
IV-26
200
175
150
125
100
75
50
25

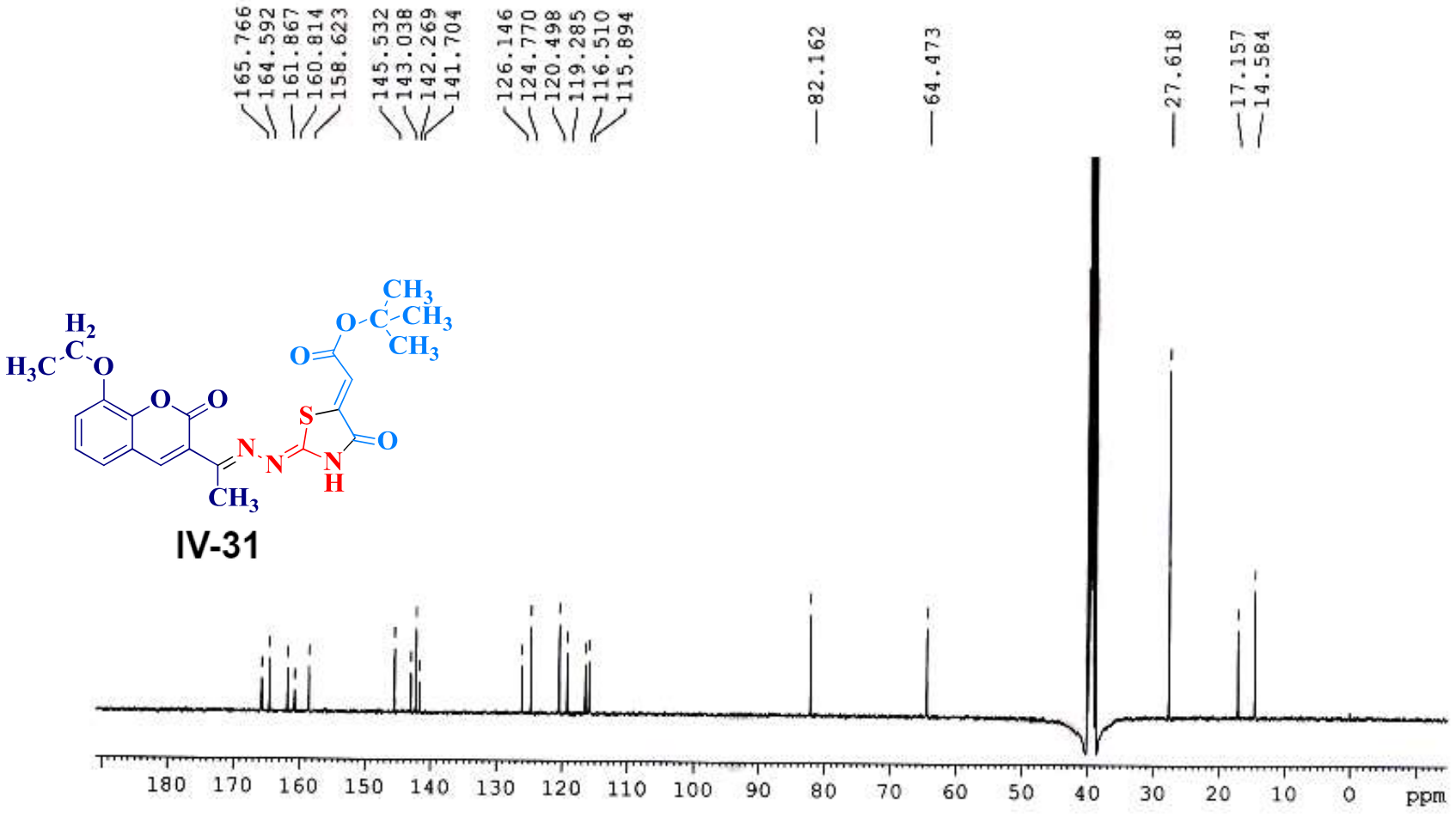

IV-29

BrOV-DTB
13C NMR IN DMSO-D6



IV-30

OV-OET+DTB
13C NMR IN DMSO-D6



IV-31

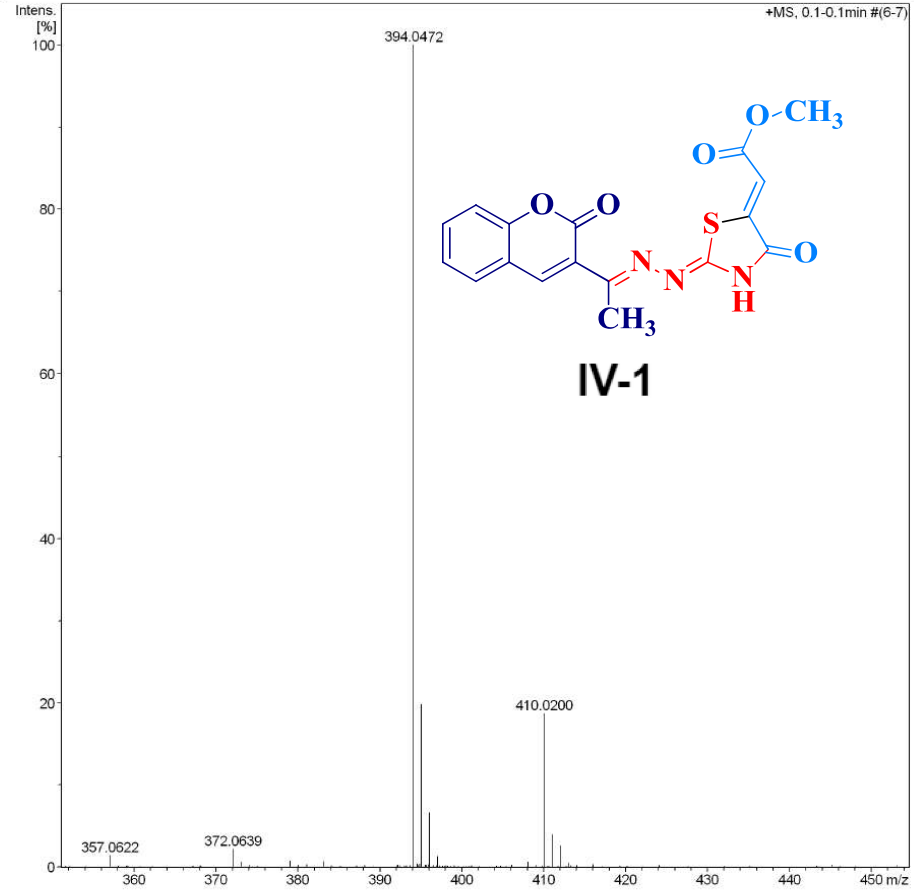
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-242+Zn.d	Acquisition Date	3/19/2015 10:03:04 AM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-242+Zn	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	250 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1100 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source



IV-1

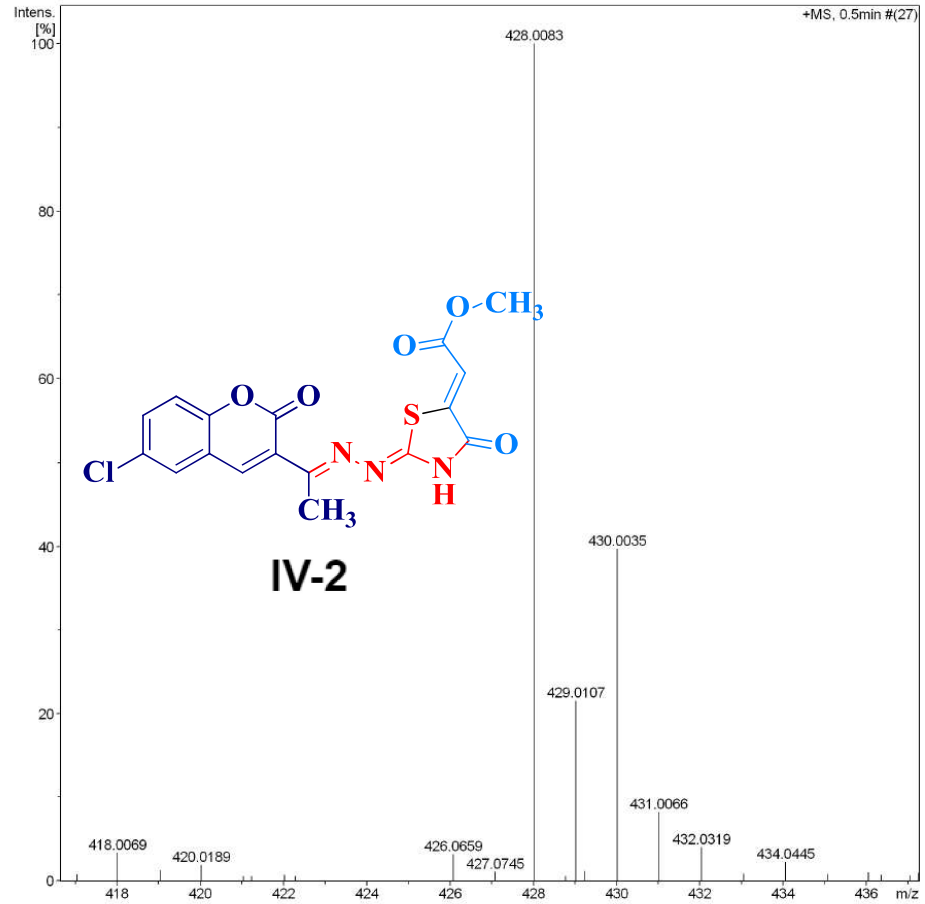
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-242+Zn5.d	Acquisition Date	3/19/2015 10:06:45 AM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-242+Zn5	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	250 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1100 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source



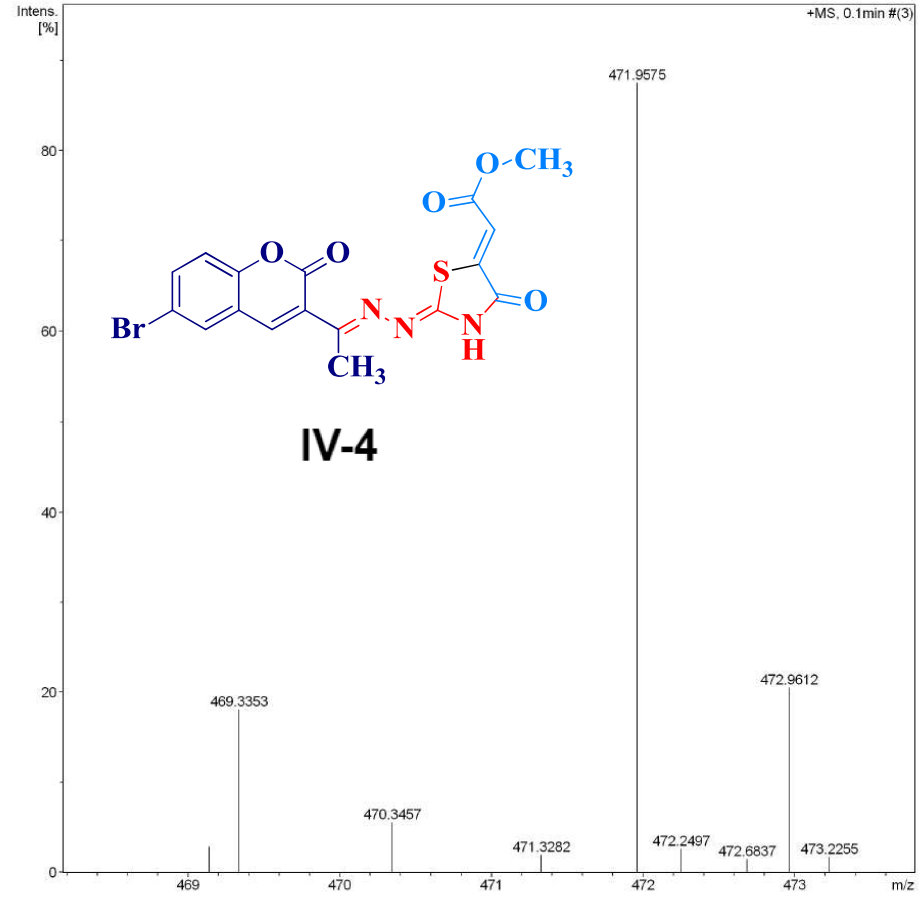
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-242+Cu6.1.d	Acquisition Date	3/19/2015 11:00:41 AM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-242+Cu6.1	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	250 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1100 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source

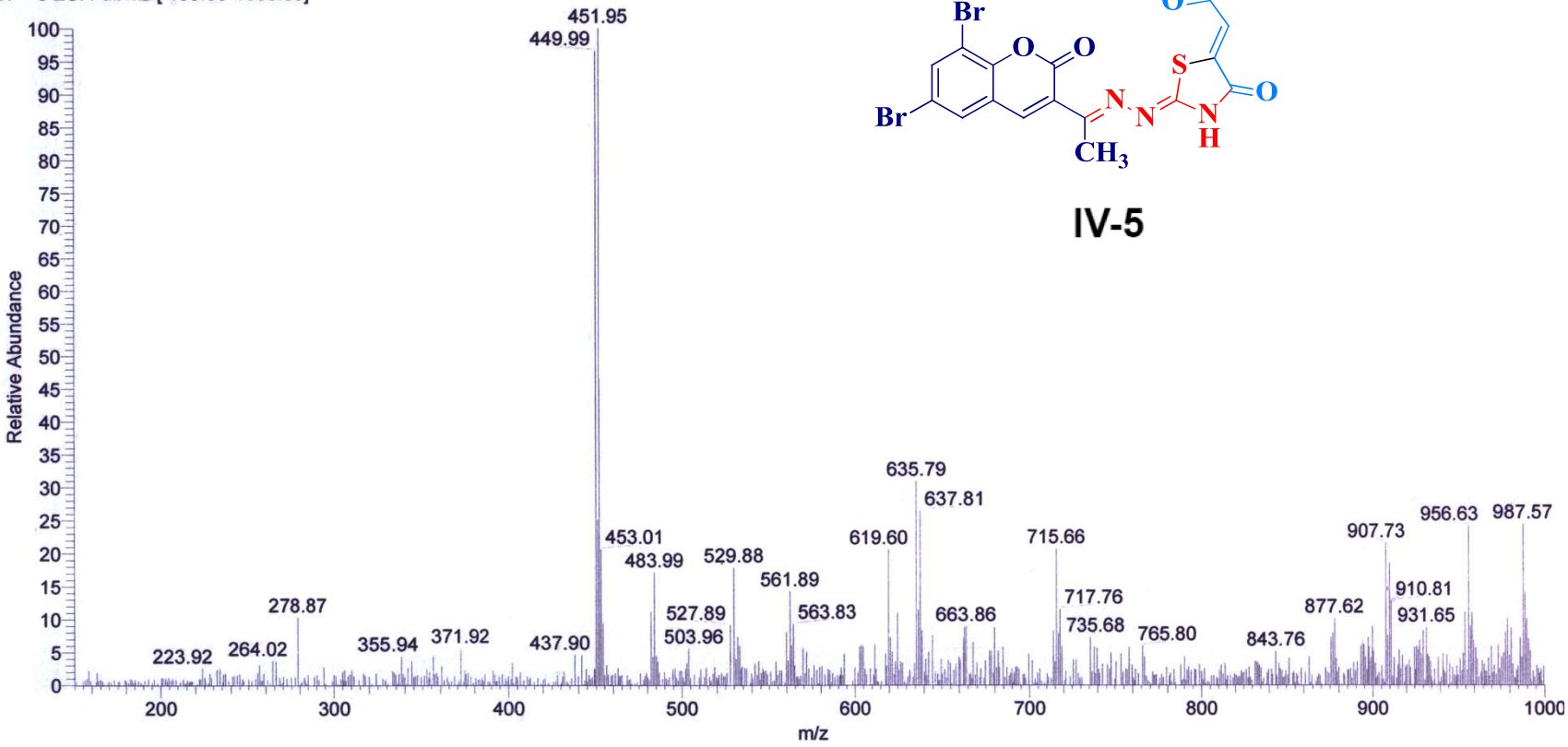


IV-4

SAIF, CSIR-CDRI, Lucknow

Data File: 15I09APR46
Original Data Path: 15I09APR46.RAW
Current Data Path: C:\XCALIBUR\DATA\APR2015\08APR2015\\
Sample ID: RM-DBDM
Acquisition Date: 04/09/15 13:43:46

15I09APR46 #19-46 RT: 0.29-0.71 AV: 28 SB: 2 0.00 , 0.00 NL: 1.87E6
T: + c ESI Full ms [150.00-1000.00]



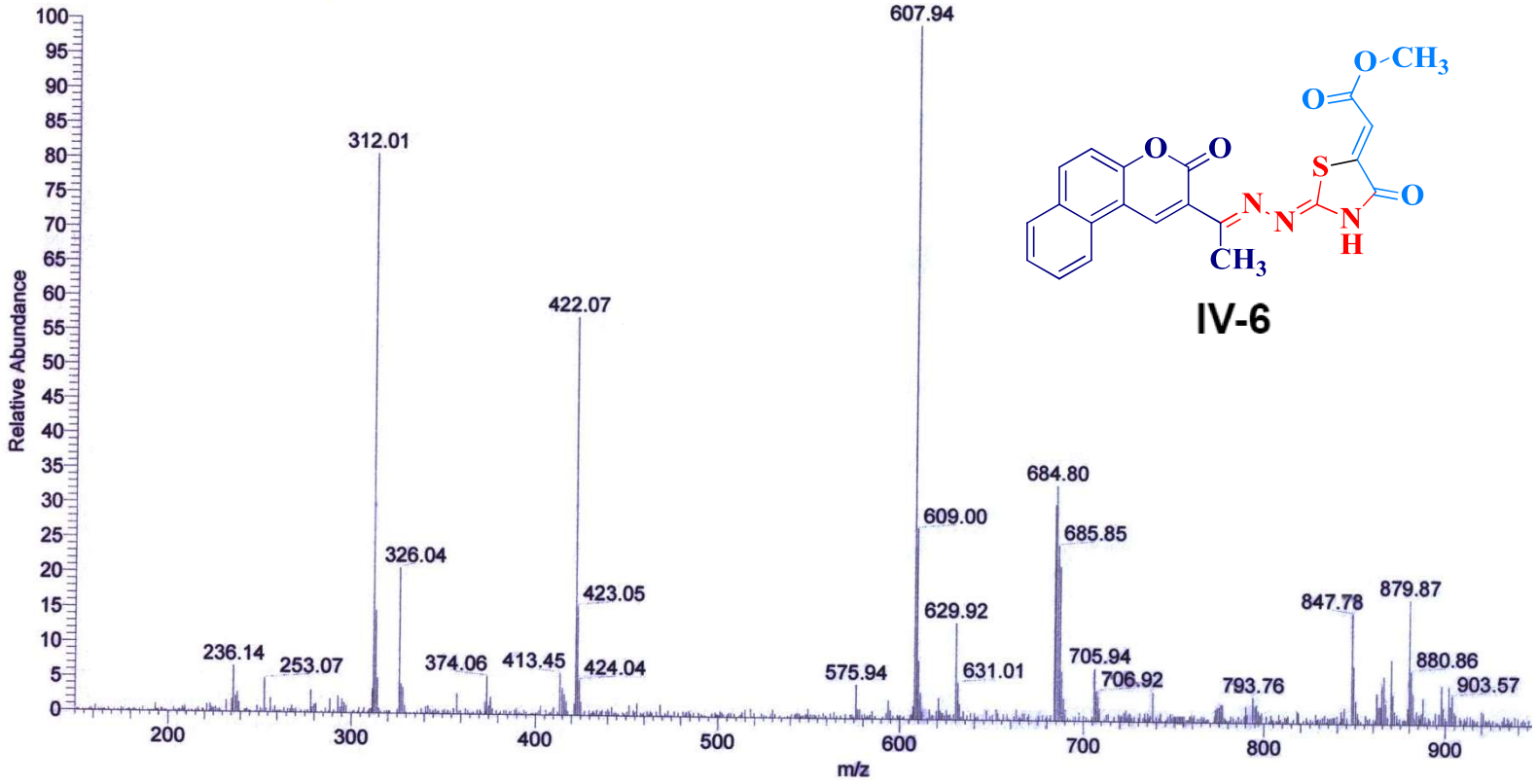
IV-5

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

SAIF, CSIR-CDRI, Lucknow

Data File: 15I09APR47
Original Data Path: 15I09APR47.RAW
Current Data Path: C:\XCALIBUR\DATA\APR2015\08APR2015\\
Sample ID: RM-NAPDM
Acquisition Date: 04/09/15 13:45:46

15I09APR47 #19-46 RT: 0.29-0.69 AV: 28 SB: 2 0.00 , 0.00 NL: 5.68E6
T: + c ESI Full ms [150.00-1000.00]



IV-6

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

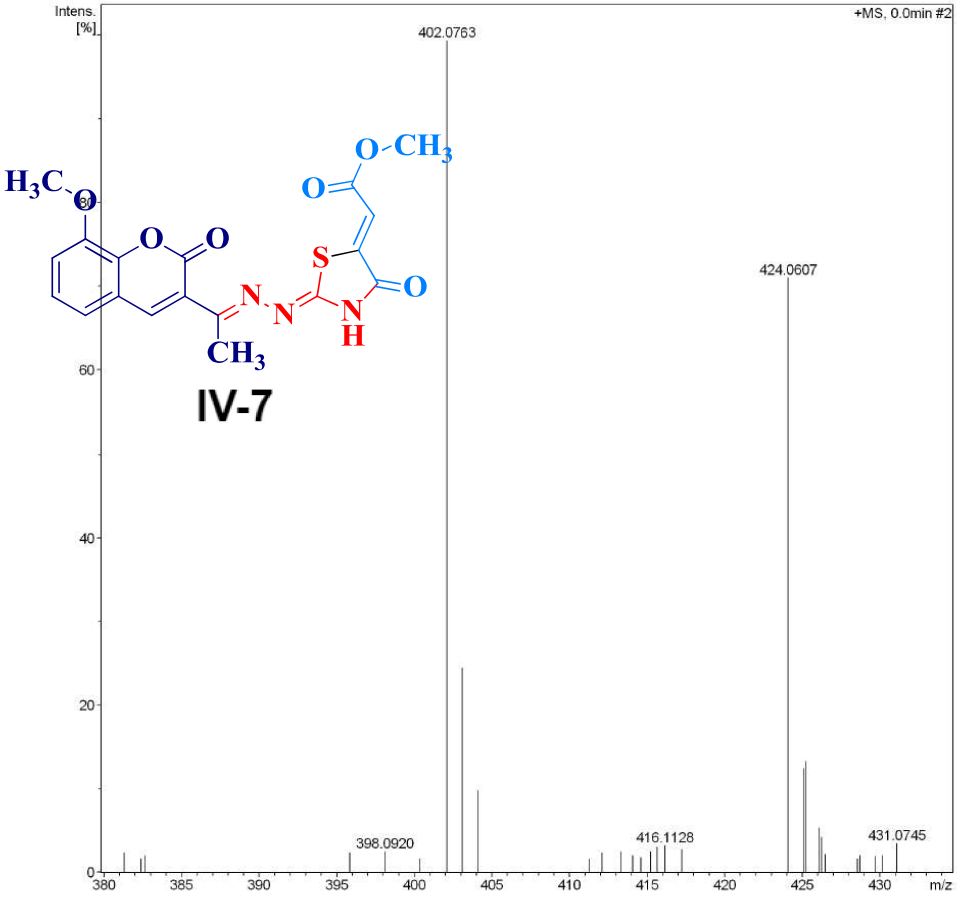
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-242+Fe5.d	Acquisition Date	3/19/2015 11:39:13 AM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-242+Fe5	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	250 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1100 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source



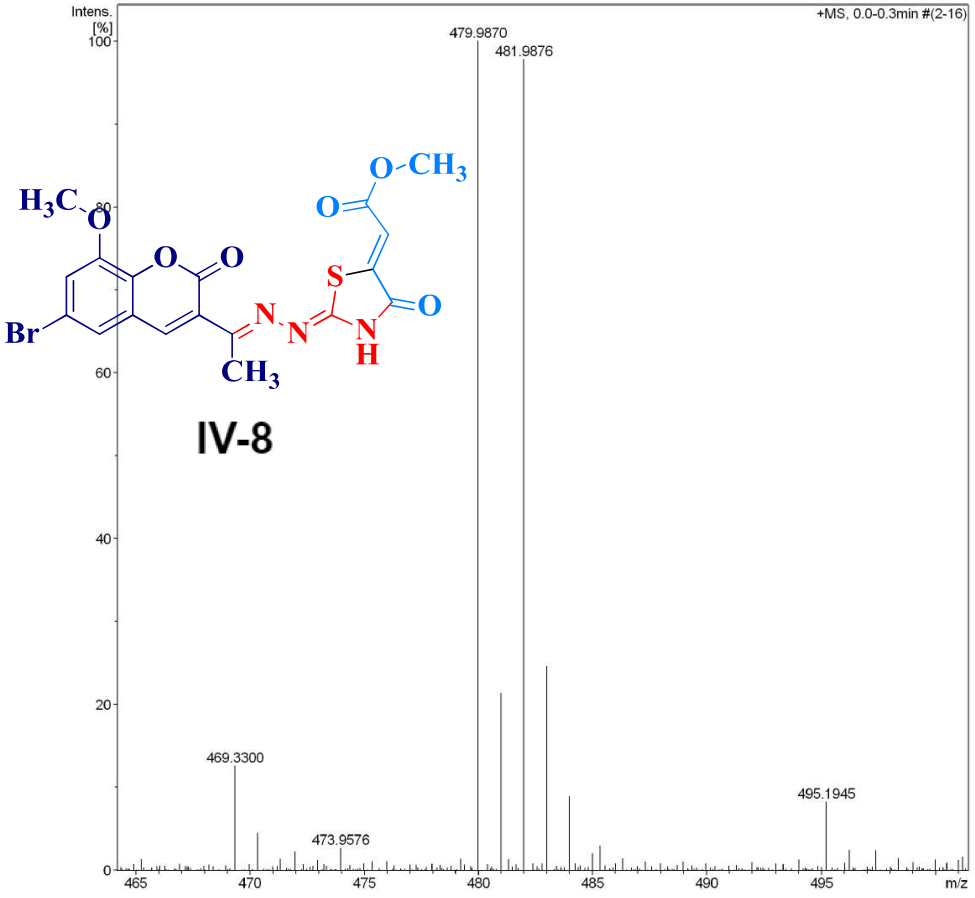
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-242+Fe6.2.d	Acquisition Date	3/19/2015 11:47:52 AM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-242+Fe6.2	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	250 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1100 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source

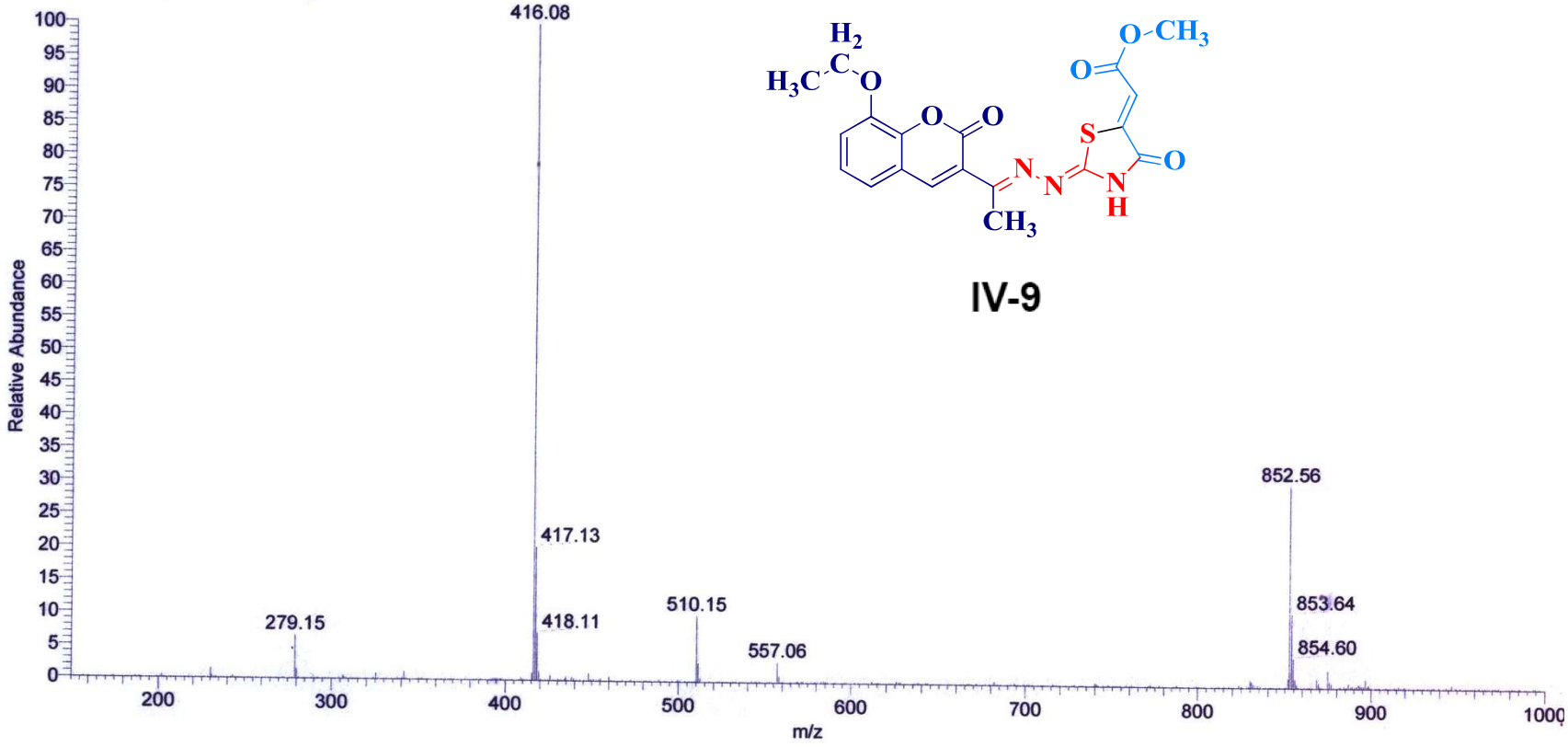


IV-8

SAIF, CSIR-CDRI, Lucknow

Data File: 15I15APR41
Original Data Path: 15I15APR41.RAW
Current Data Path: C:\Xcalibur\data\APR2015\15APR2015\\
Sample ID: RM-OETDM
Acquisition Date: 04/15/15 13:33:52

15I15APR41 #19-47 RT: 0.30-0.70 AV: 29 SB: 2 0.00 , 0.00 NL: 3.42E7
T: + c ESI Full ms [150.00-1000.00]



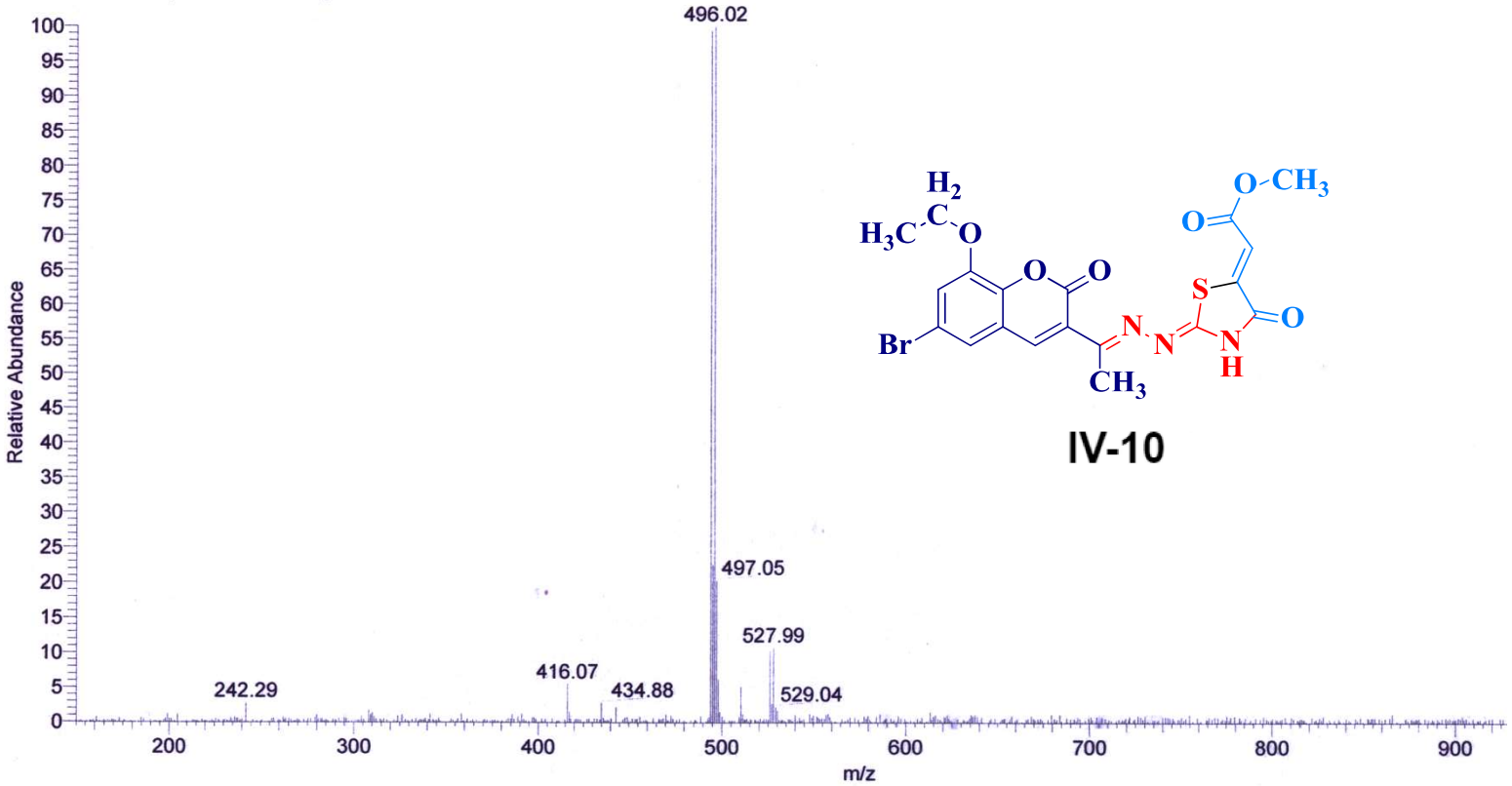
IV-9

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

SAIF, CSIR-CDRI, Lucknow

Data File: 15I15APR40
Original Data Path: 15I15APR40.RAW
Current Data Path: C:\Xcalibur\data\APR2015\15APR2015\\
Sample ID: RM-BROETDM
Acquisition Date: 04/15/15 13:31:49

15I15APR40 #19-46 RT: 0.30-0.71 AV: 28 SB: 2 0.00 , 0.00 NL: 6.12E6
T: + c ESI Full ms [150.00-1000.00]



IV-10

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

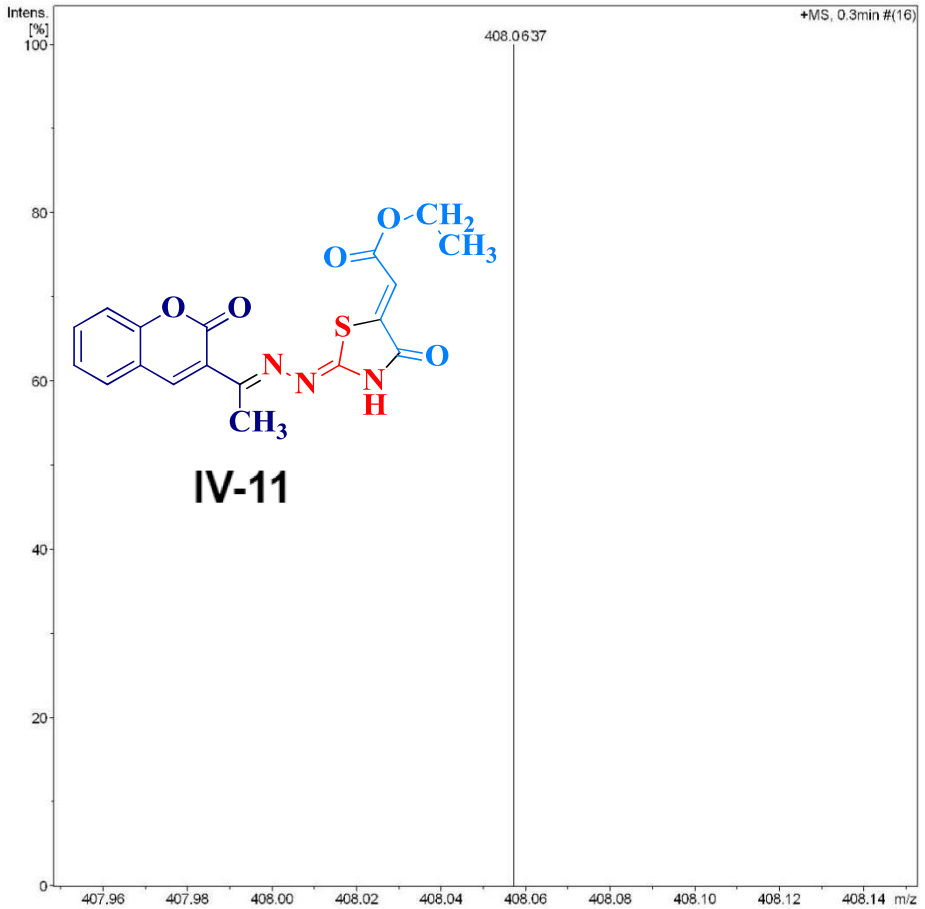
Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-DEAD.d	Acquisition Date	6/22/2014 3:58:03 PM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-DEAD	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source



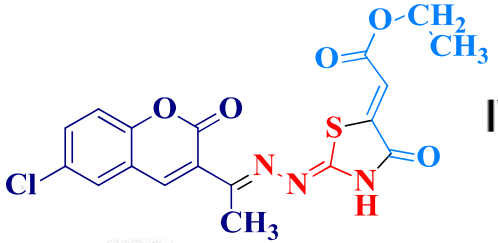
IV-11

021405A7434

Medicinal Chemistry Laboratory-Analytical Research

Sample ID:GVK-DU-1928-E11884-138-2
Acq.Method:RND-FA-4.5 MIN

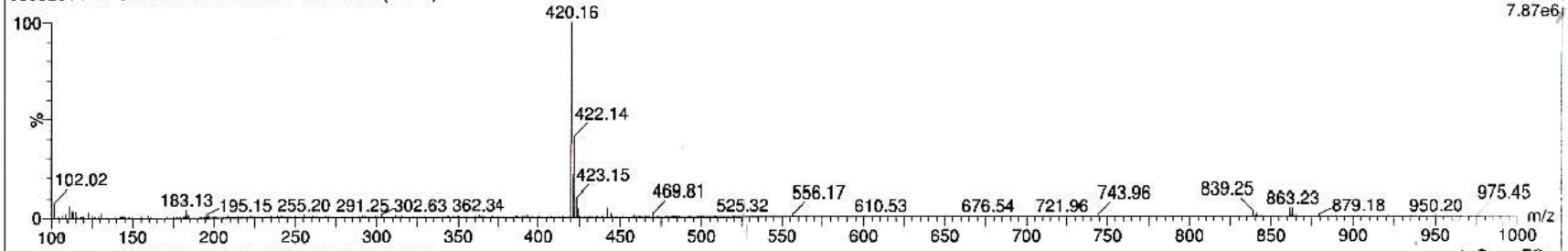
Date of Analysis: 08-May-201416:15:45
Instrument ID: ANL-MCL3-LCMS-007



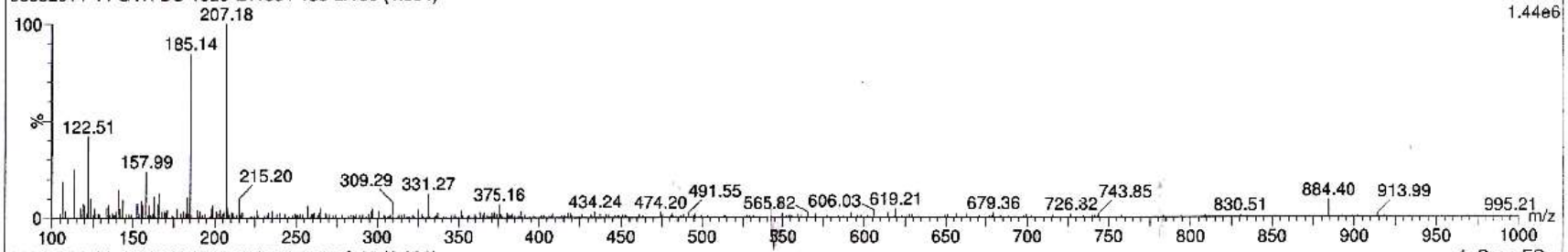
IV-12

08052014-44-GVK-DU-1928-E11884-138-2A 82 (2.103)

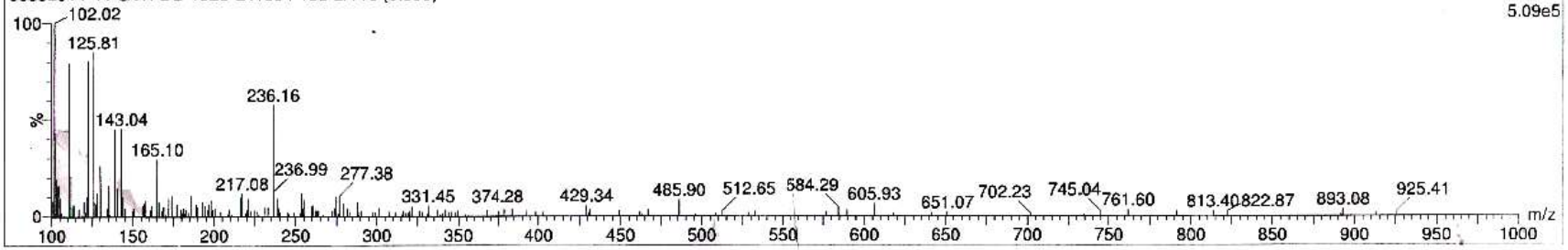
08052014-44-GVK-DU-1928-E11884-138-2A 79 (2.025)

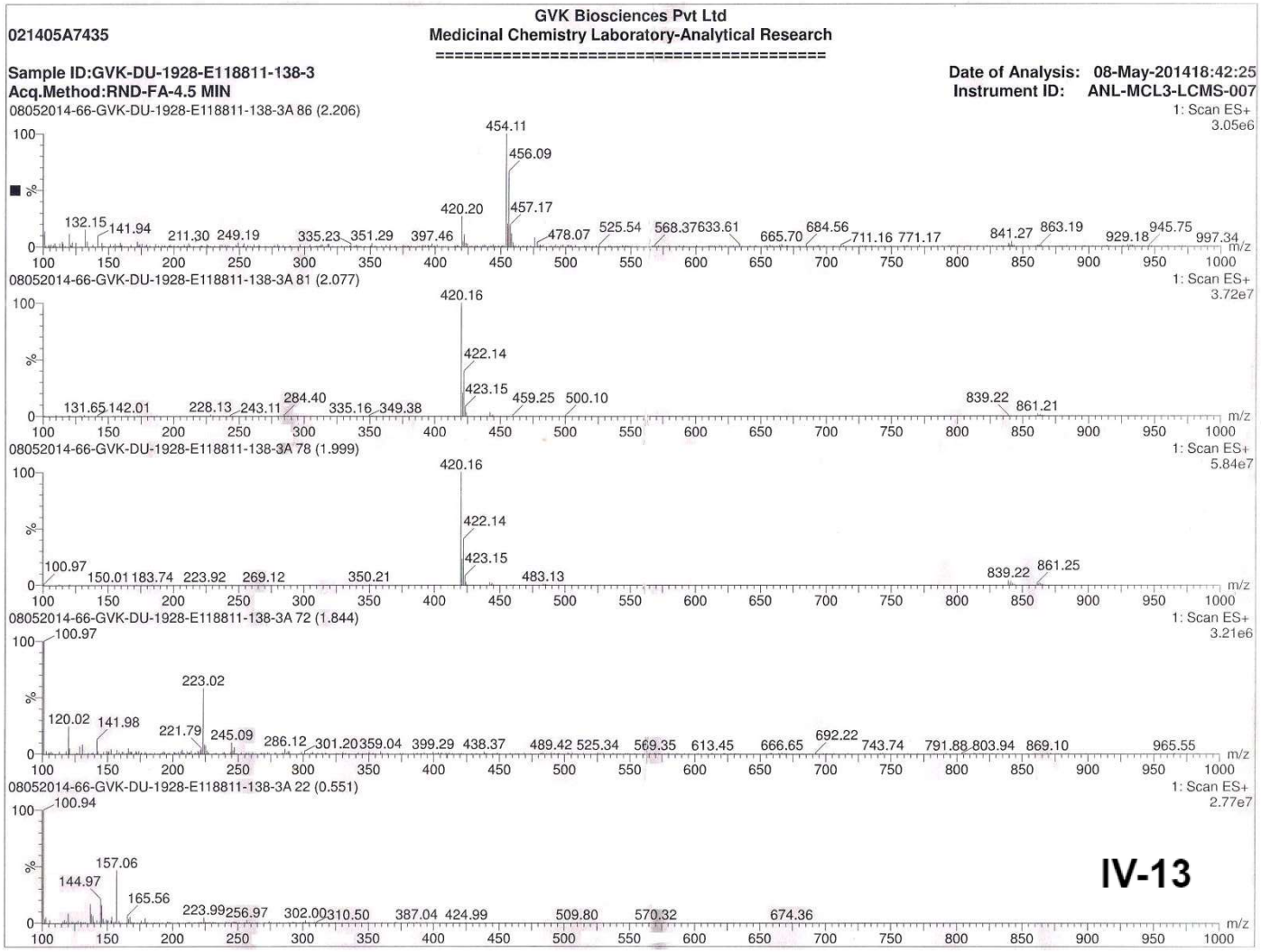


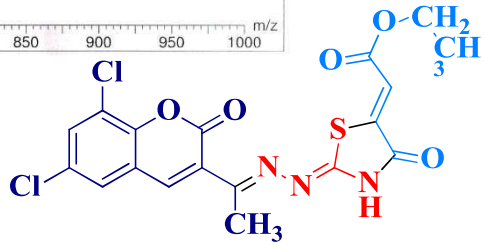
08052014-44-GVK-DU-1928-E11884-138-2A 60 (1.534)



08052014-44-GVK-DU-1928-E11884-138-2A 16 (0.396)




GVK Biosciences Pvt Ltd
Medicinal Chemistry Laboratory-Analytical Research
021405A7435
Sample ID:GVK-DU-1928-E118811-138-3
Acq.Method:RND-FA-4.5 MIN
Date of Analysis: 08-May-201418:42:25
Instrument ID: ANL-MCL3-LCMS-007
08052014-66-GVK-DU-1928-E118811-138-3A 86 (2.206)
1: Scan ES+ 3.05e6
08052014-66-GVK-DU-1928-E118811-138-3A 81 (2.077)
1: Scan ES+ 3.72e7
08052014-66-GVK-DU-1928-E118811-138-3A 78 (1.999)
1: Scan ES+ 5.84e7
08052014-66-GVK-DU-1928-E118811-138-3A 72 (1.844)
1: Scan ES+ 3.21e6
08052014-66-GVK-DU-1928-E118811-138-3A 22 (0.551)
1: Scan ES+ 2.77e7
IV-13



GVK Biosciences Pvt Ltd

Medicinal Chemistry Laboratory-Analytical Research

021405A7433

Sample ID:GVK-DU-1928-E118811-138

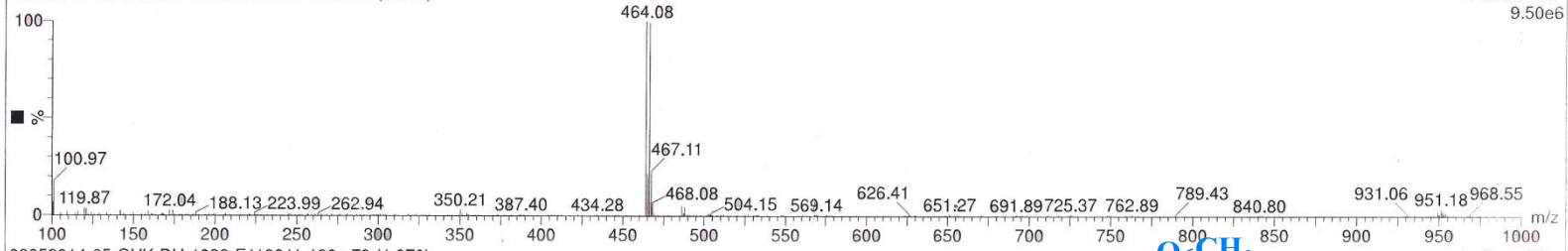
Acq.Method:RND-FA-4.5 MIN

Date of Analysis: 08-May-201418:36:30

Instrument ID: ANL-MCL3-LCMS-007

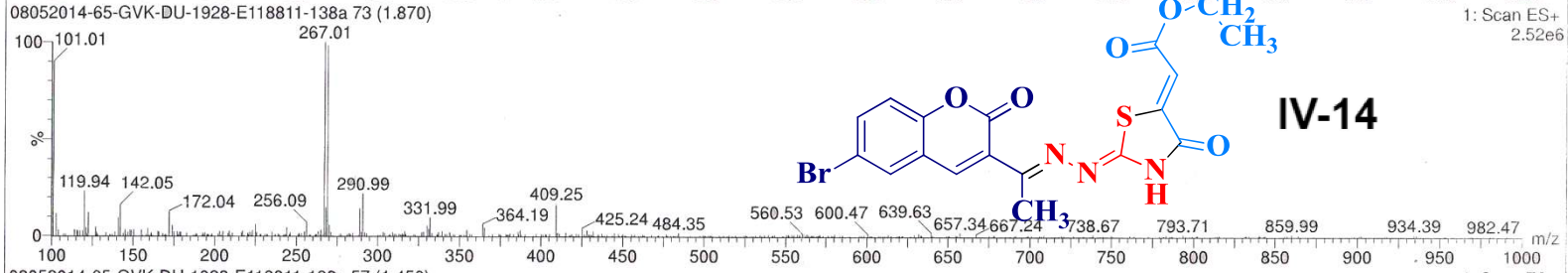
08052014-65-GVK-DU-1928-E118811-138a 82 (2.103)

1: Scan ES+ 9.50e6



08052014-65-GVK-DU-1928-E118811-138a 73 (1.870)

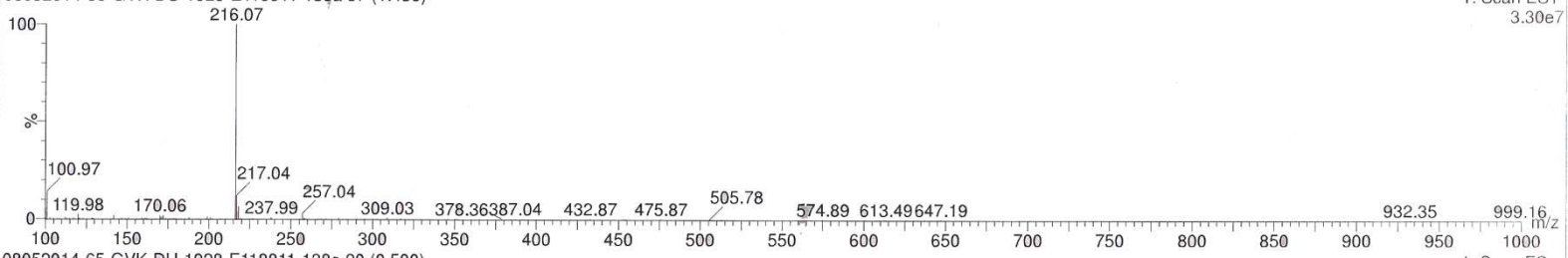
1: Scan ES+ 2.52e6



IV-14

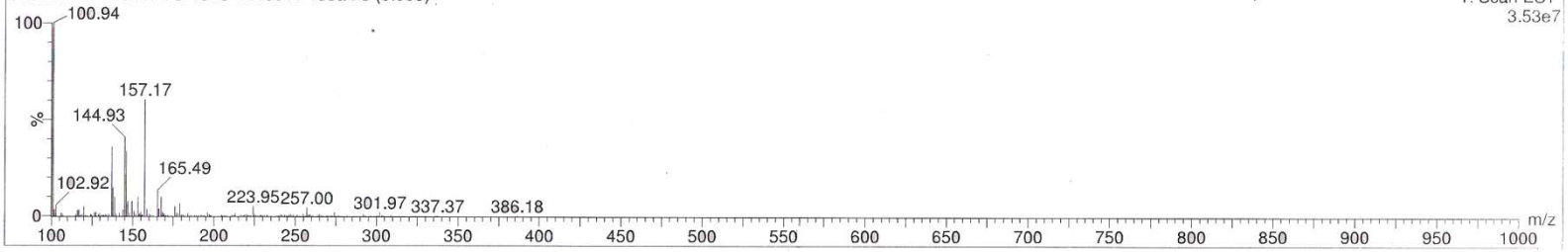
08052014-65-GVK-DU-1928-E118811-138a 57 (1.456)

1: Scan ES+ 3.30e7



08052014-65-GVK-DU-1928-E118811-138a 20 (0.500)

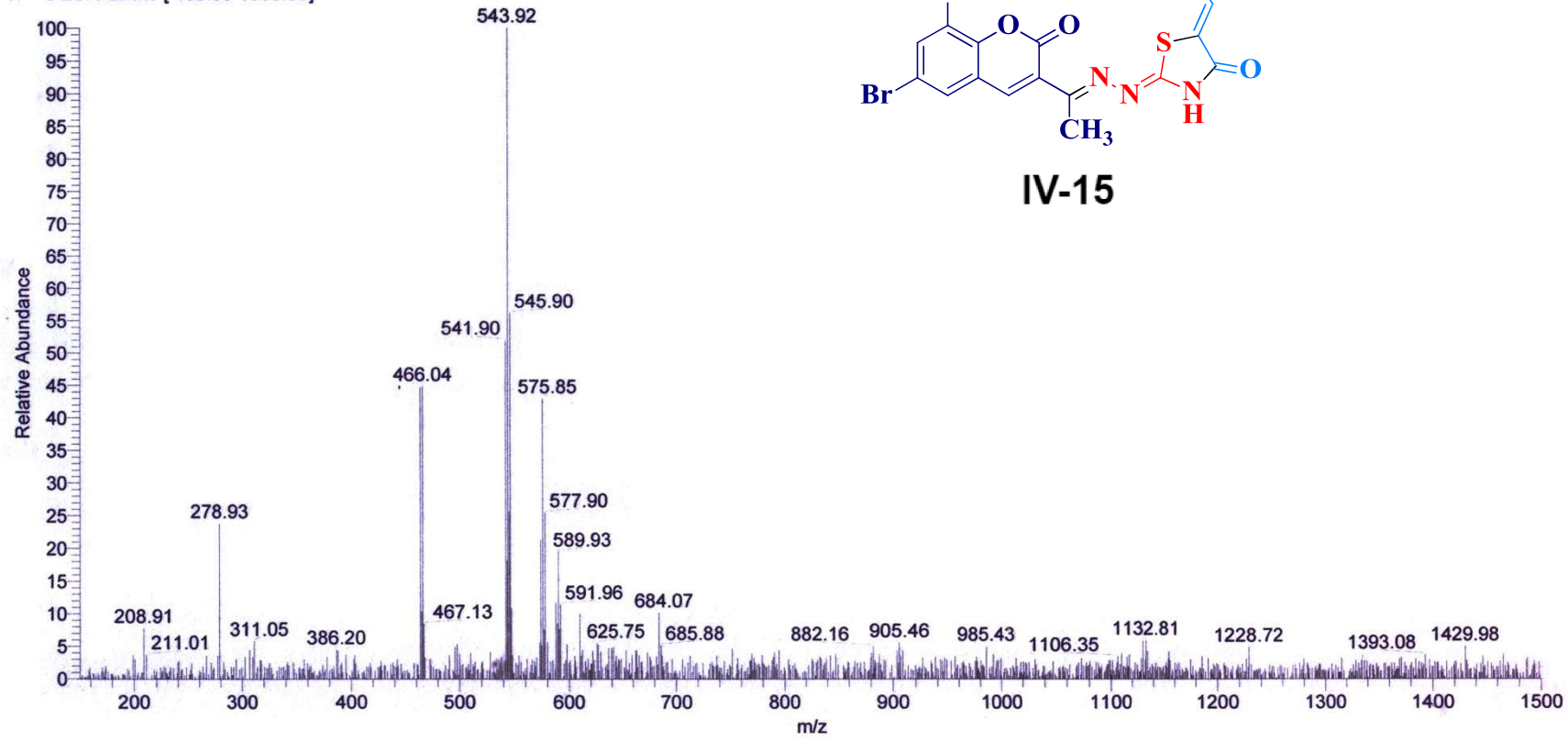
1: Scan ES+ 3.53e7



SAIF, CSIR-CDRI, Lucknow

Data File: 15I16APR49
Original Data Path: 15I16APR49.RAW
Current Data Path: C:\Xcalibur\data\APR2015\16APR2015\
Sample ID: RM-DBDE
Acquisition Date: 04/16/15 13:36:13

15I16APR49 #15-35 RT: 0.30-0.71 AV: 21 SB: 2 0.00 , 0.00 NL: 1.39E6
T: + c ESI Full ms [150.00-1500.00]



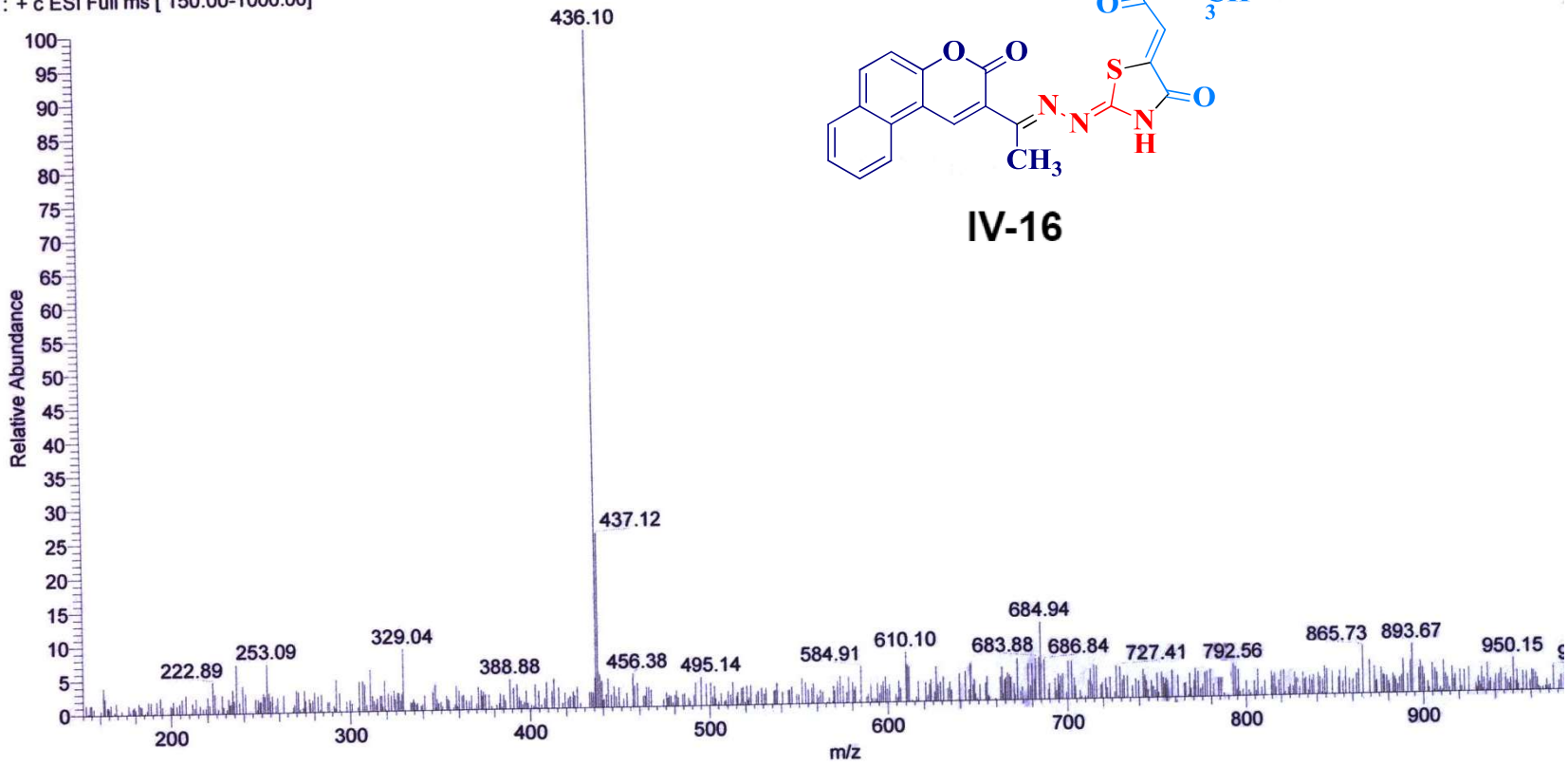
IV-15

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

SAIF, CSIR-CDRI, Lucknow

Data File: 15I16APR48
Original Data Path: 15I16APR48.RAW
Current Data Path: C:\Xcalibur\data\APR2015\16APR2015\
Sample ID: RM-NADE
Acquisition Date: 04/16/15 13:34:17

15I16APR48 #19-45 RT: 0.30-0.70 AV: 27 SB: 2 0.01 , 0.01 NL: 1.07E6
T: + c ESI Full ms [150.00-1000.00]



IV-16

ION TRAP LCQ ADVANTAGE MA
THERMO ELECTRON CORPORATION

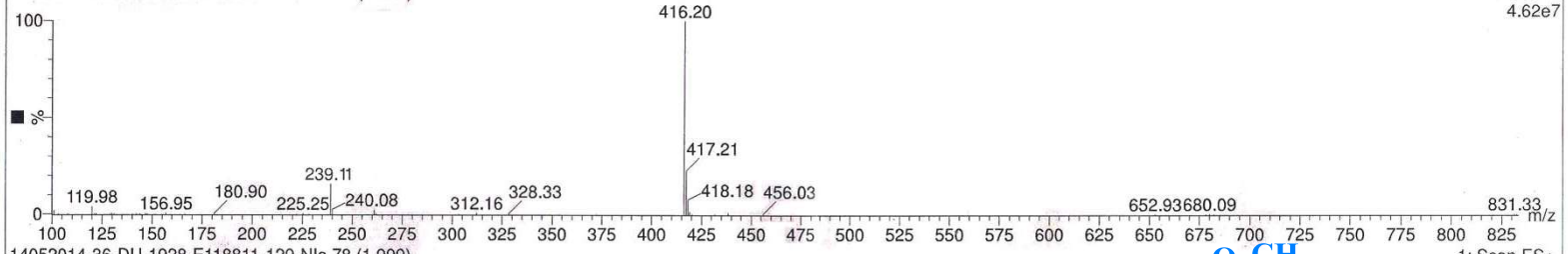
GVK Biosciences Pvt Ltd
Medicinal Chemistry Laboratory-Analytical Research

021405B4575

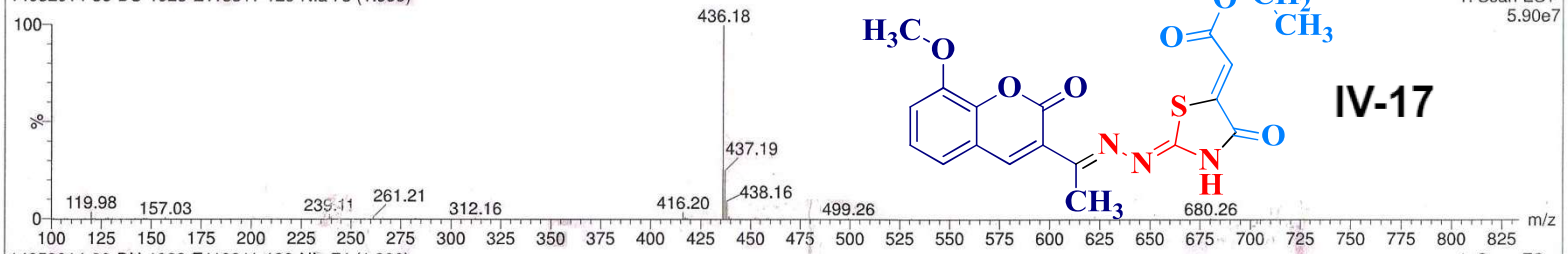
Sample ID:DU-1928-E118811-129-NI
Acq.Method:RND-FA-4.5 MIN

Date of Analysis: 14-May-201414:45:11
Instrument ID: ANL-MCL3-LCMS-007

14052014-36-DU-1928-E118811-129-NIa 75 (1.922)

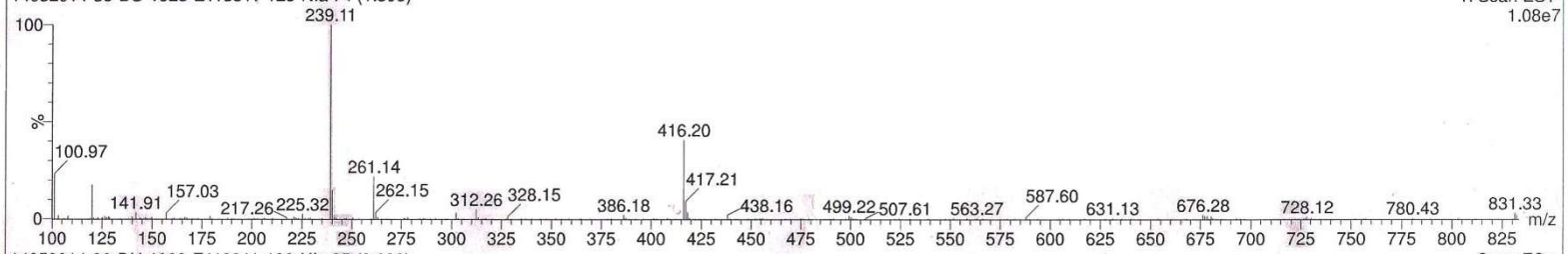


14052014-36-DU-1928-E118811-129-NIa 78 (1.999)

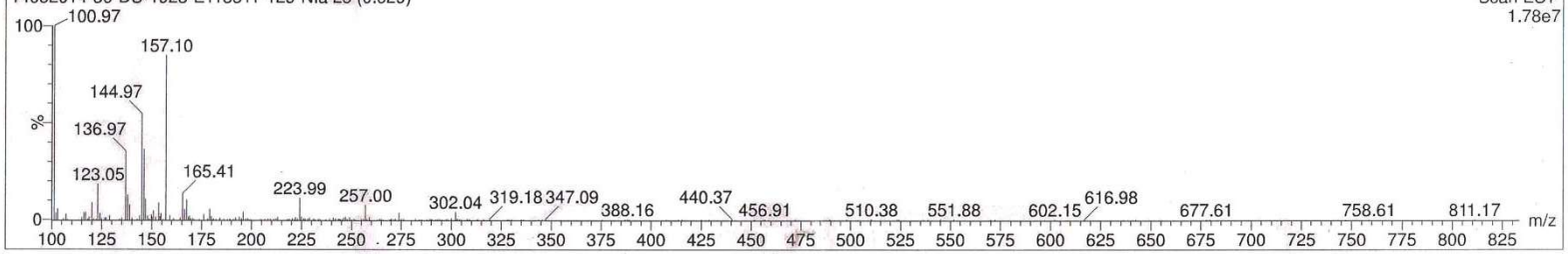


IV-17

14052014-36-DU-1928-E118811-129-NIa 74 (1.896)



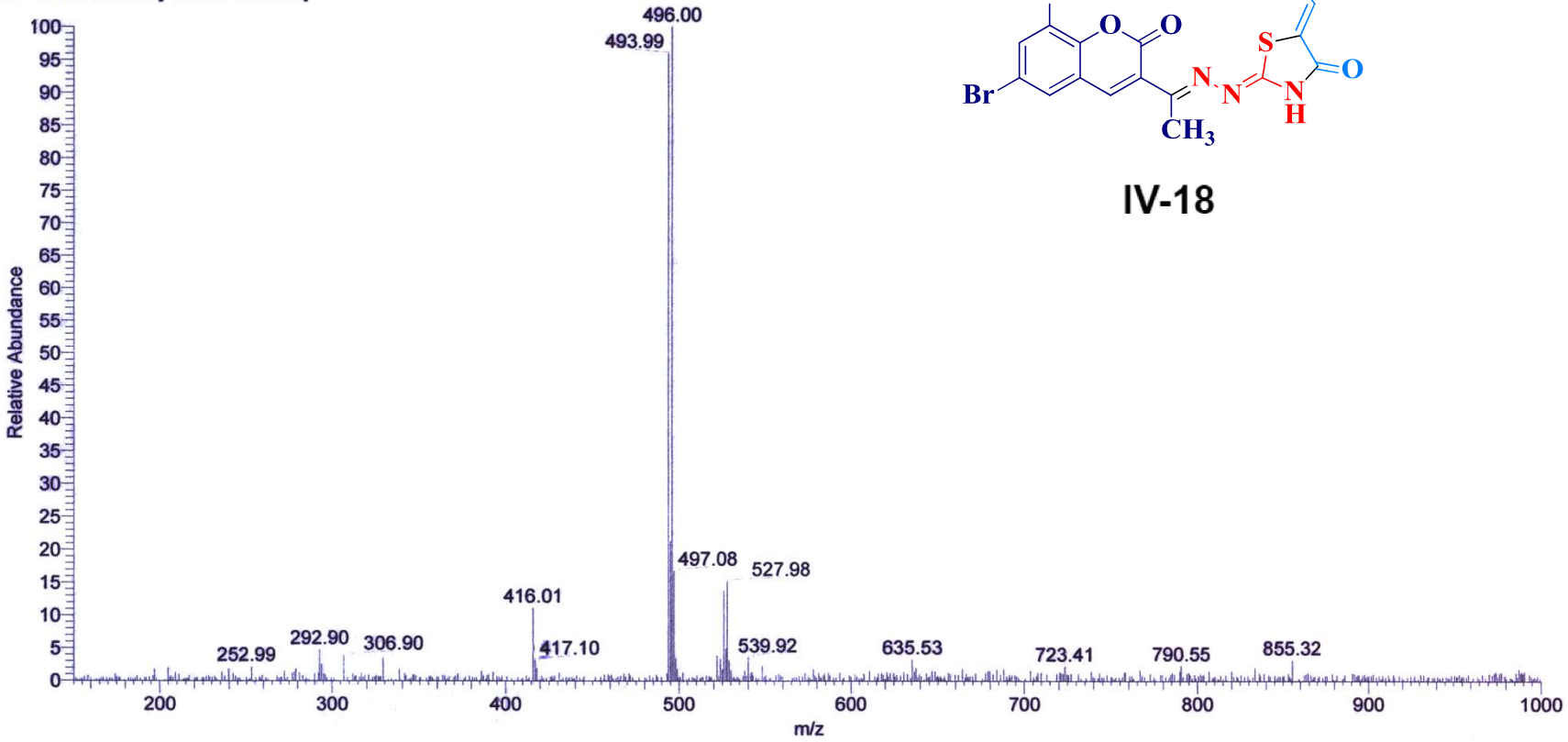
14052014-36-DU-1928-E118811-129-NIa 25 (0.629)



SAIF, CSIR-CDRI, Lucknow

Data File: 15I16APR47
Original Data Path: 15I16APR47.RAW
Current Data Path: C:\Xcalibur\data\APR2015\16APR2015\
Sample ID: RM-BROVDE
Acquisition Date: 04/16/15 13:32:17

15I16APR47 #20-46 RT: 0.30-0.70 AV: 27 SB: 2 0.00 , 0.00 NL: 4.03E6
T: + c ESI Full ms [150.00-1000.00]



IV-18

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

GVK Biosciences Pvt Ltd

021405B3313

Medicinal Chemistry Laboratory-Analytical Research

==========

Sample ID:GVK-DU-1928-E118811-128-ET

Acq.Method:RND-FA-4.5 MIN

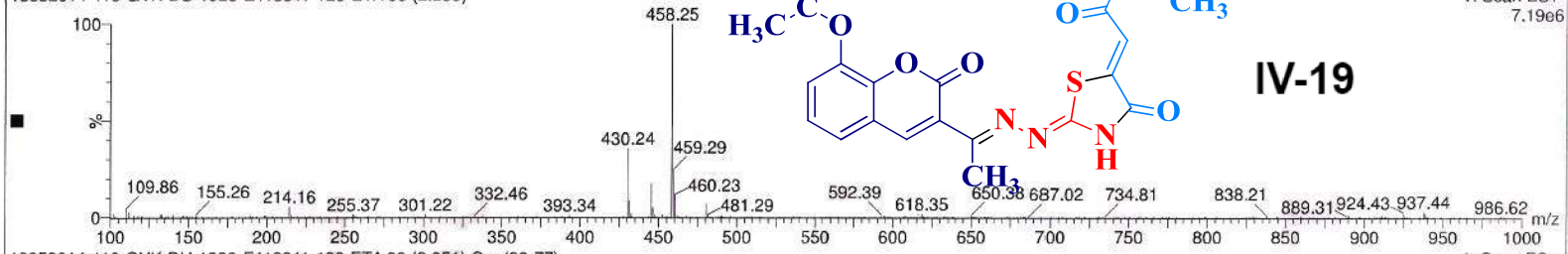
Date of Analysis: 13-May-201422:48:53

Instrument ID: ANL-MCL3-LCMS-007

IV-19

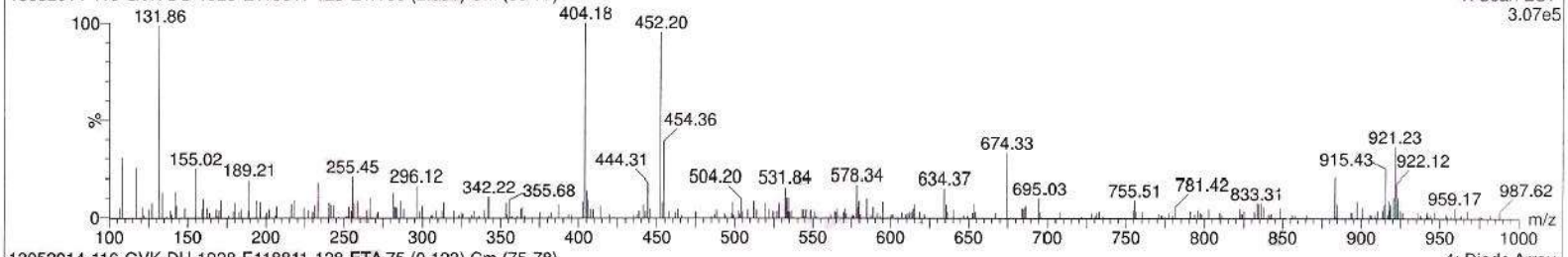
13052014-116-GVK-DU-1928-E118811-128-ETA 86 (2.206)

1: Scan ES+
7.19e6



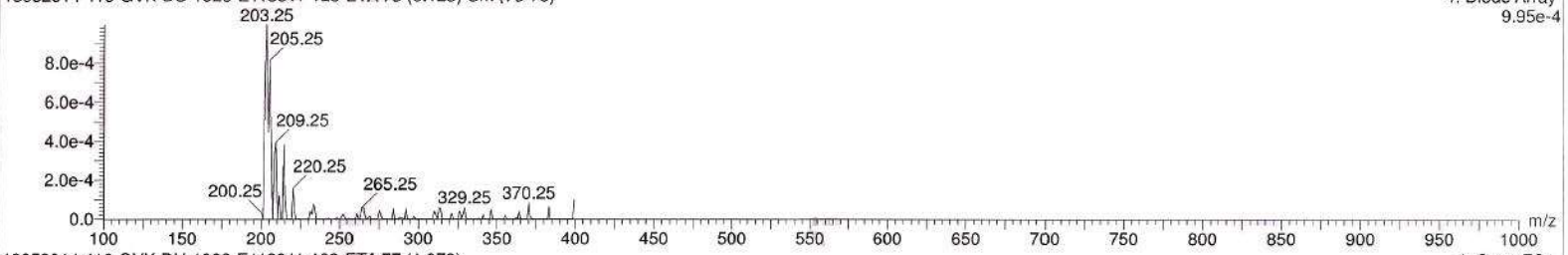
13052014-116-GVK-DU-1928-E118811-128-ETA 80 (2.051) Cm (80-77)

1: Scan ES+
3.07e5



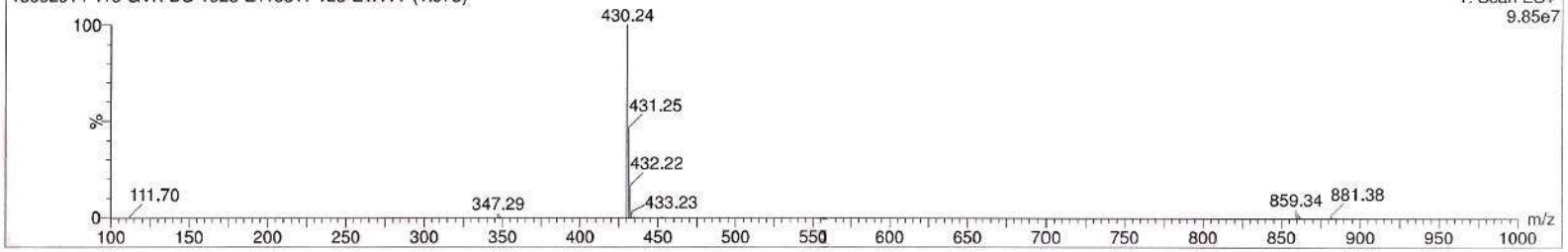
13052014-116-GVK-DU-1928-E118811-128-ETA 75 (0.123) Cm (75-78)

4: Diode Array
9.95e-4



13052014-116-GVK-DU-1928-E118811-128-ETA 77 (1.973)

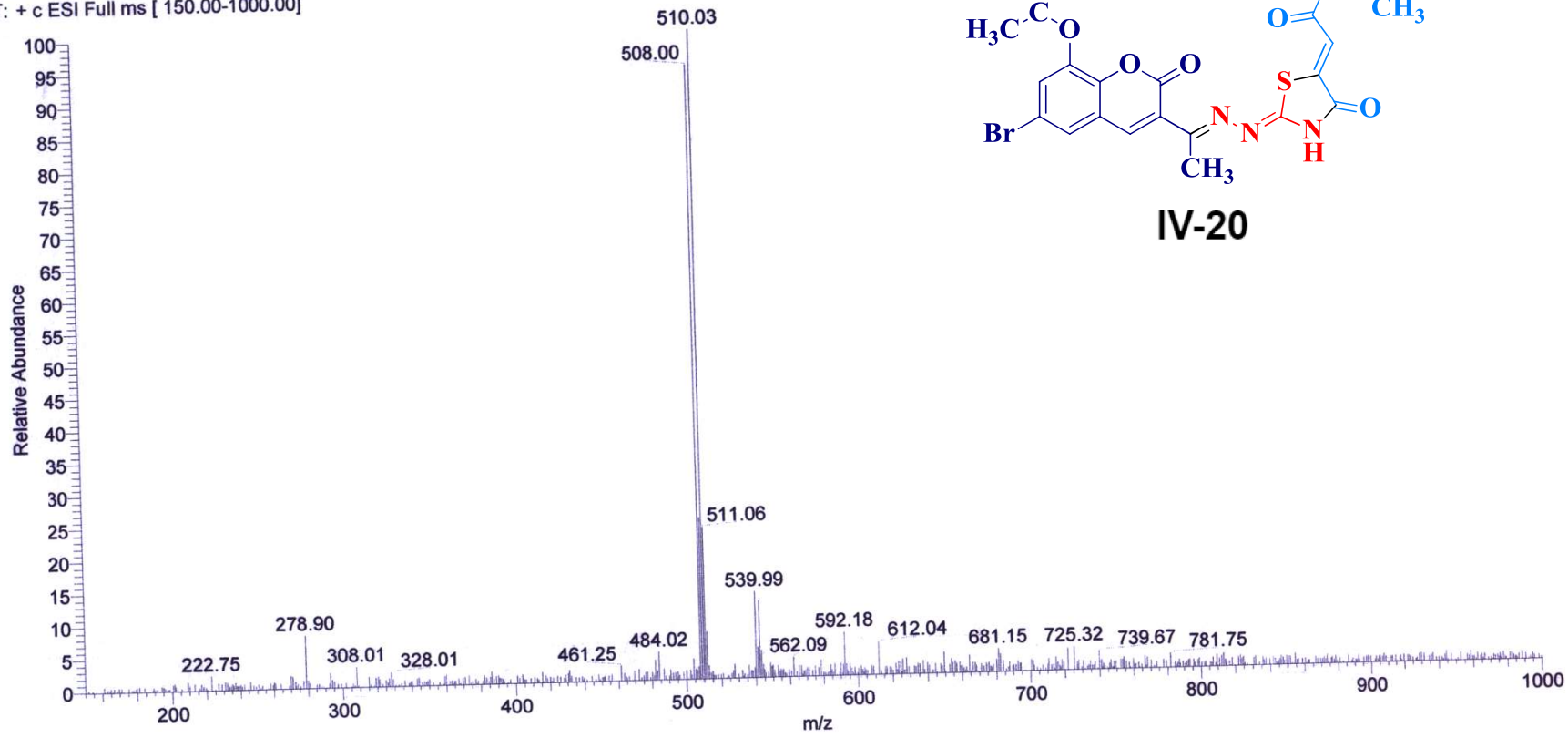
1: Scan ES+
9.85e7



SAIF, CSIR-CDRI, Lucknow

Data File: 15I10APR47
Original Data Path: 15I10APR47.RAW
Current Data Path: C:\XCALIBUR\DATA\APR2015\08APR2015\
Sample ID: RM-OETBR
Acquisition Date: 04/10/15 14:19:46

15I10APR47 #19-45 RT: 0.30-0.70 AV: 27 SB: 2 0.01 , 0.01 NL: 2.94E6
T: + c ESI Full ms [150.00-1000.00]

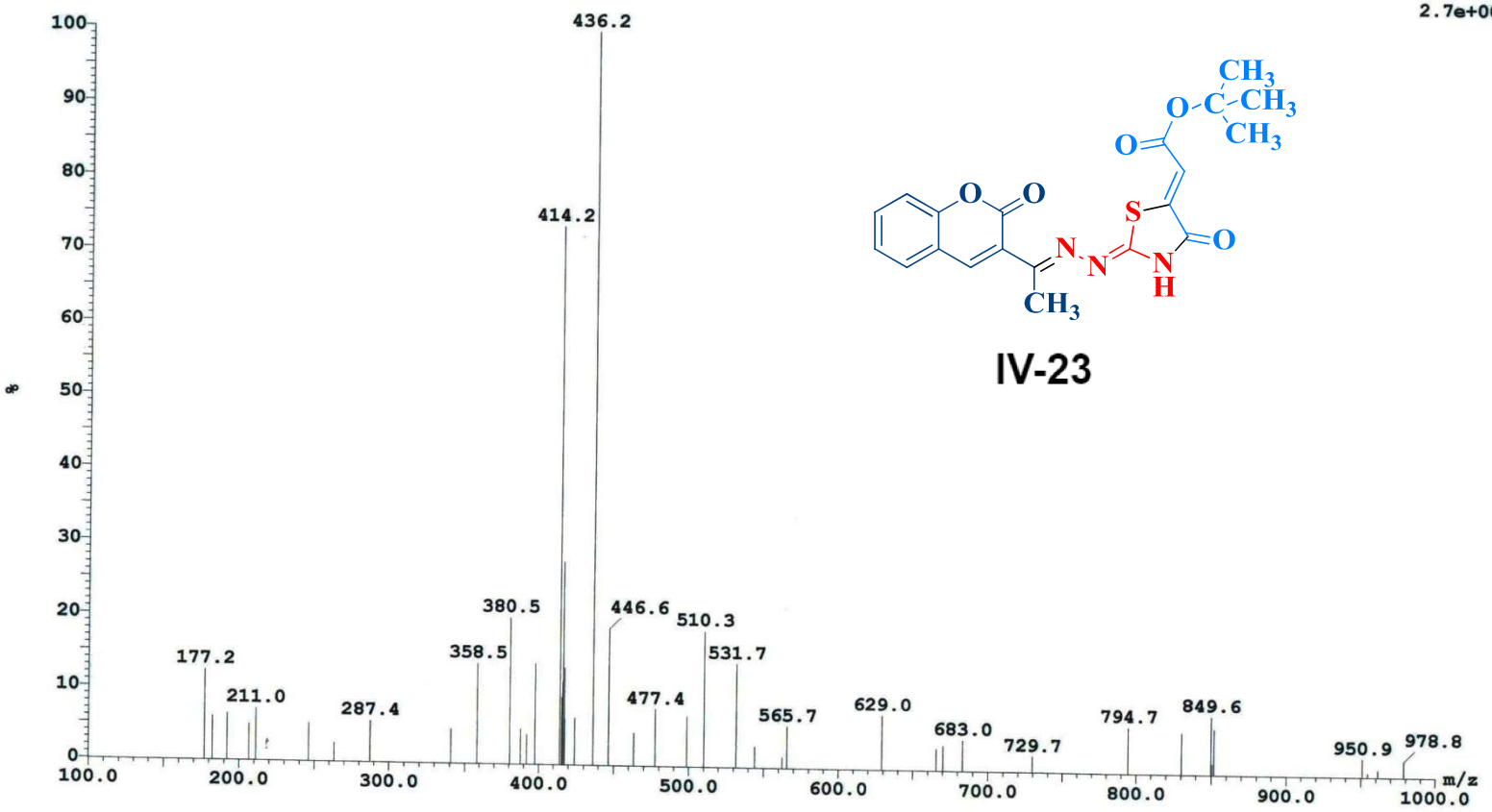


IV-20

ION TRAP LCQ ADVANTAGE MAX
THERMO ELECTRON CORPORATION

1:(Time: 0.32) Center (Top,4, Ar); Smooth (Mn, 2x0.75); Subtract (1,40.00 ,0.010); Combine (13:17-(6:7+23:24))

1:MS ES+
2.7e+004



IV-23

Openlynx Report SAIF, CDRI-LUCKNOW
File:14I08DEC386
Vial:1:C,3

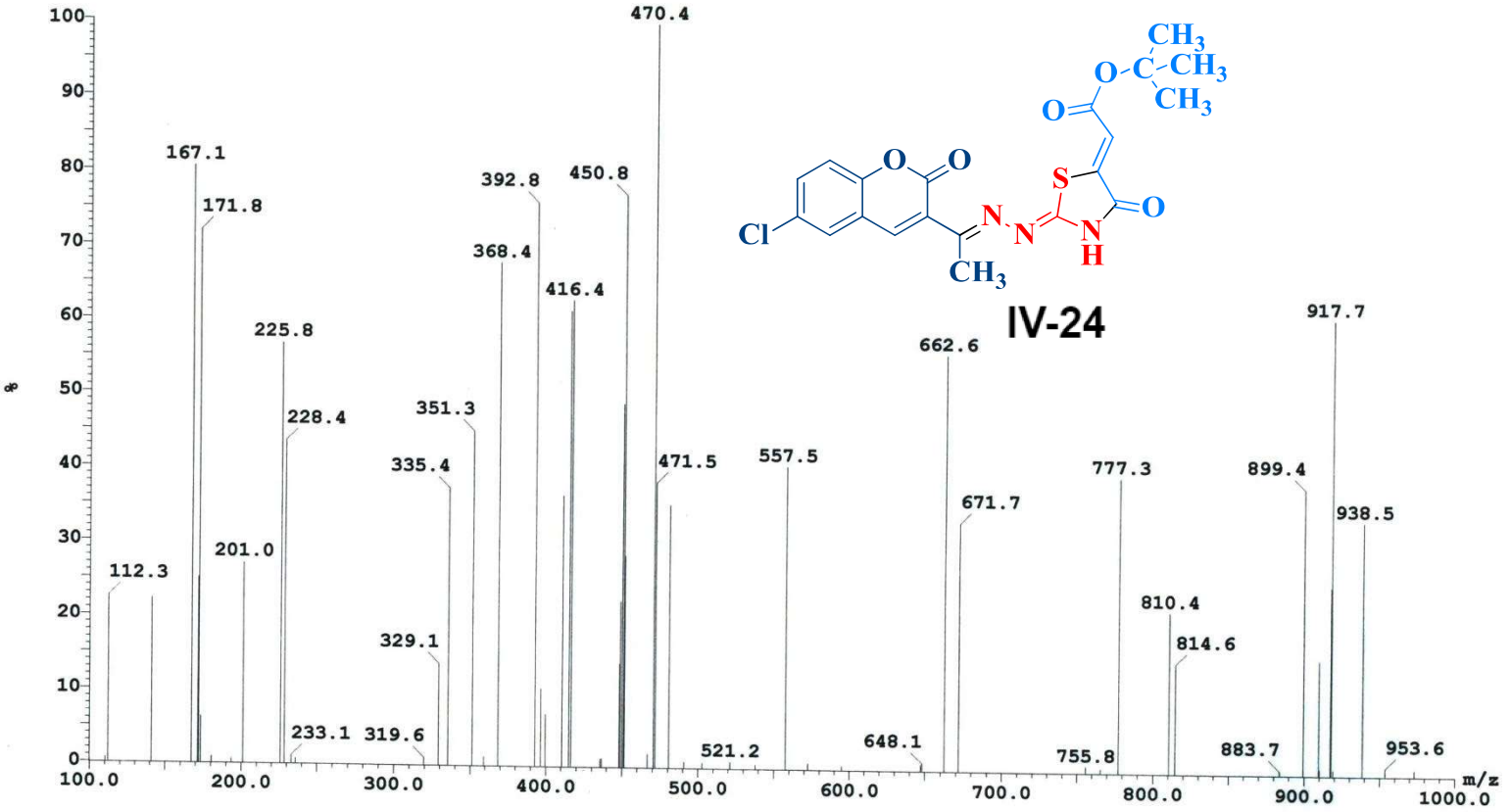
Date:08-Dec-2014
ID:MSR-NTP-10

Time:12:58:46

Printed: Mon Dec 08 13:02:03 2014

1:(Time: 0.29) Center (Top,4, Ar); Smooth (Mn, 2x0.75); Subtract (1,40.00 ,0.010); Combine (12:16-(5:6+22:23))

1:MS ES+



Openlynx Report SAIF, CDRI-LUCKNOW
File:14I08DEC389
Vial:1:C,6

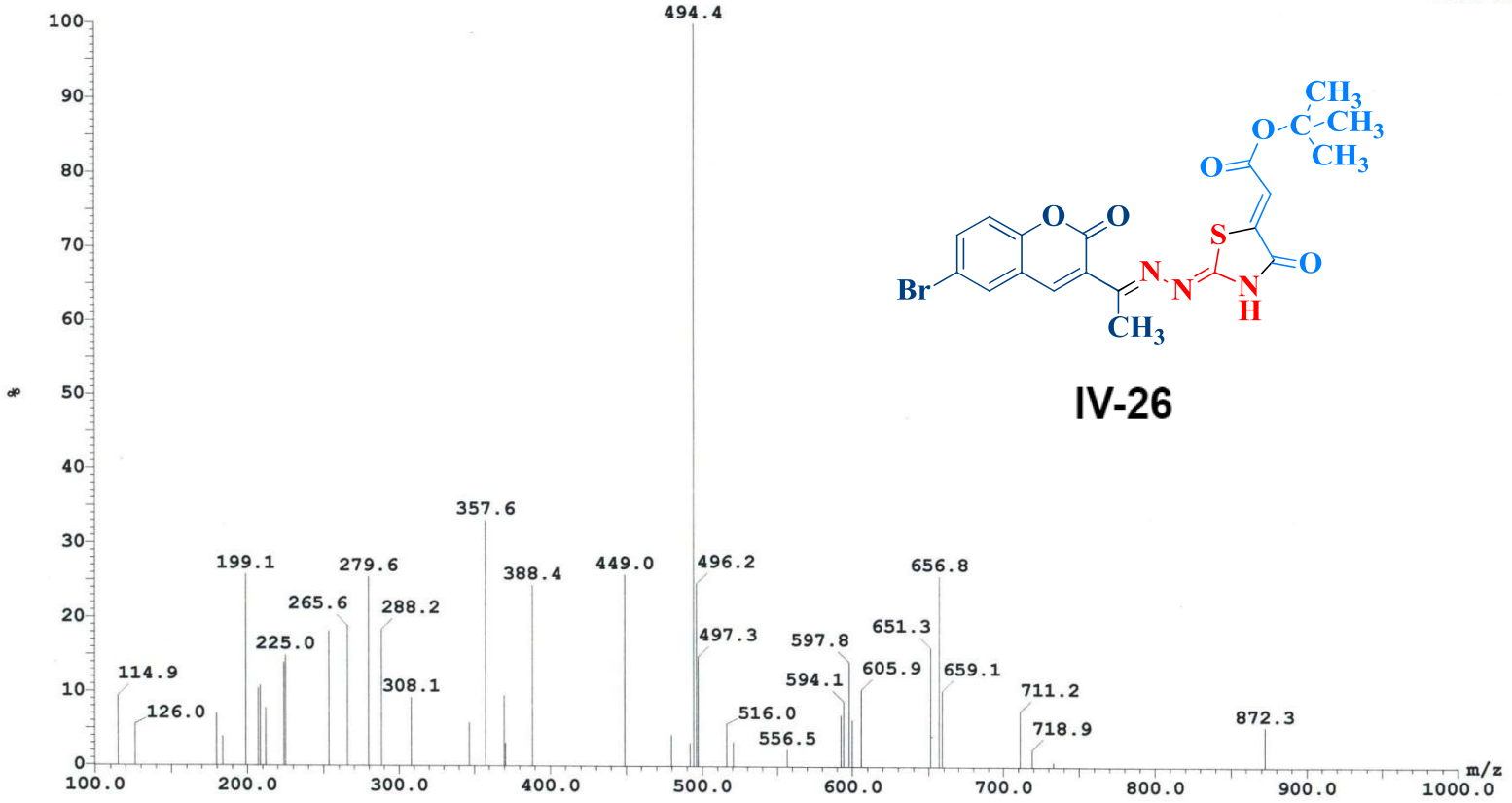
Date:08-Dec-2014
ID:MSR-NTP-13

Time:13:08:38

Printed: Mon Dec 08 13:11:55 2014

1:(Time: 0.38) Center (Top,4, Ar); Smooth (Mn, 2x0.75); Subtract (1,40.00 ,0.010); Combine (16:20-(9:10+26:27))

1:MS ES+
1.5e+004



IV-26

Openlynx Report SAIF, CDRI-LUCKNOW
File:14I08DEC393
Vial:1:D,2

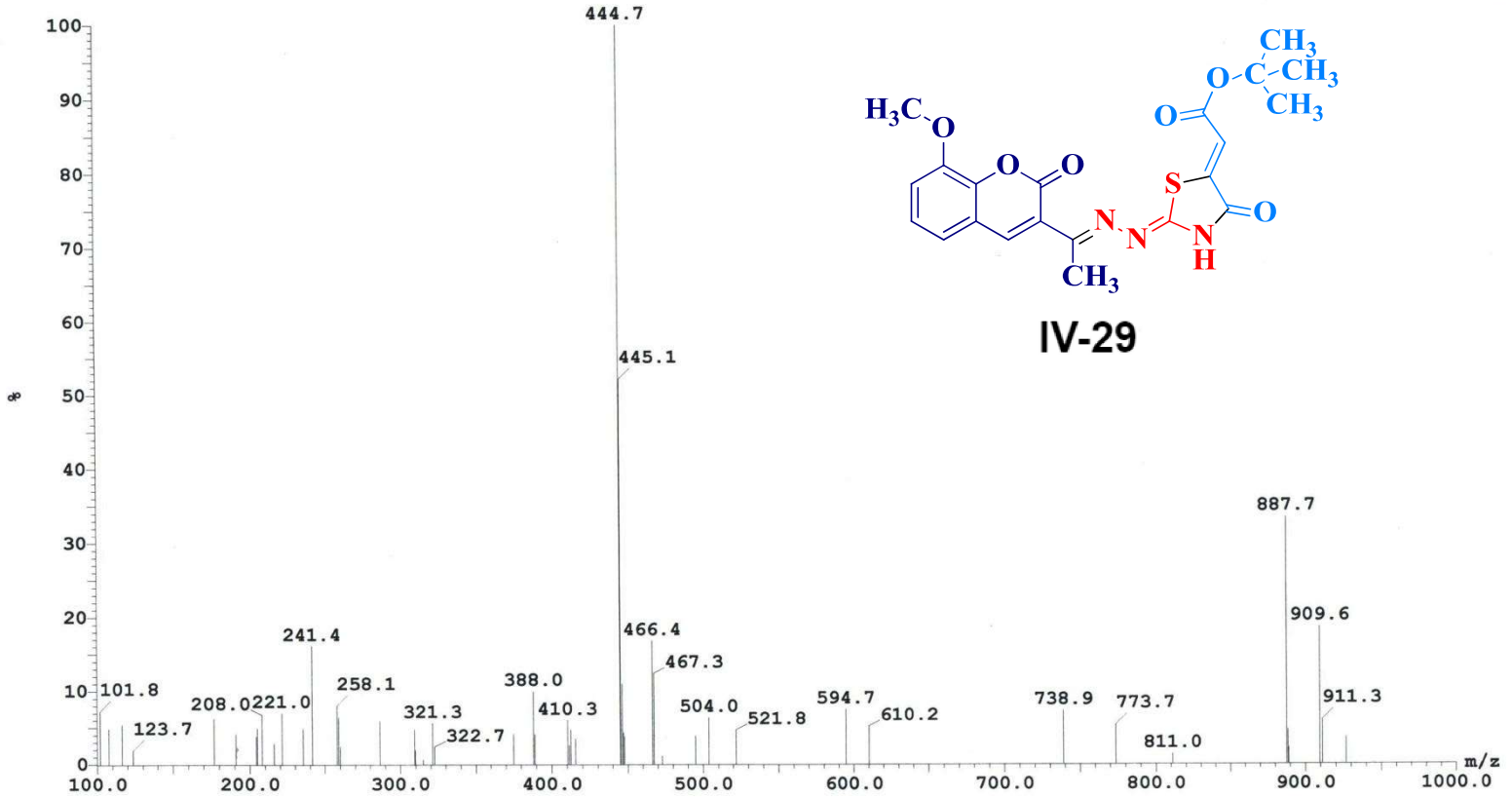
Date:08-Dec-2014
ID:MSR-NTP-15

Time:13:21:48

Printed: Mon Dec 08 13:25:06 2014

1:(Time: 0.34) Center (Top,4, Ar); Smooth (Mn, 2x0.75); Subtract (1,40.00 ,0.010); Combine (14:18-(7:8+24:25))

1:MS ES+
4.9e+004



IV-29

Display Report

Analysis Info

Analysis Name	D:\Data\prof.v.k gupta\NA-257-I .d	Acquisition Date	12/29/2014 6:38:53 PM
Method	tune_low.m	Operator	IIT ROORKEE
Sample Name	NA-257-I	Instrument	micrOTOF-Q II 10328
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Source