

SUPPLEMENTARY MATERIAL

Design, Synthesis and *in vitro* antiproliferative activity of new thiazolidinedione-1, 3, 4-oxadiazole hybrids as thymidylate synthase inhibitors

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Procedure for synthesis of (E)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione (4)

A mixture of thiazolidinedione 3 (0.05 mole, 5.85 g) and anisaldehyde (0.05 mole, 6.0 ml) in ethanol (50 ml) was taken and cold NaOH solution (40%, 10 ml) was added drop wise into it. The reaction was kept overnight for 15 h. The resulting solid was acidified with hydrochloric acid, filtered, washed with excess water and dried. It was recrystallized with ethanol to give yellow crystals. Yield 90% mp 217-218 °C; ¹H NMR (300 MHz, DMSO-d₆, δ ppm): 3.82 (s, 3H), 7.09 (d, 2H, *J* = 8.7 Hz), 7.56 (d, 2H, *J* = 8.7 Hz), 7.75 (s, 1H), 11.92 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 55.95, 115.38, 120.75, 125.95, 132.30, 132.53, 161.45, 167.88, 168.40. ESI -ve MS (*m/z*): 234 (M-H)⁺.

Procedure for the synthesis of ethyl 2-((E)-5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetate (5).

A mixture of compound 4 (0.05 mole), ethyl chloroacetate (0.05 mole) and anhydrous potassium carbonate (0.075 mole) in dry acetone (100 ml) was stirred and refluxed for 15 h. After reaction completion, the content was filtered under hot condition. The filtrate so obtained was

concentrated to give compound **5** as yellow solid. Yield: 75% mp 132-133°C. ¹H NMR (300 MHz, DMSO-d₆, δ ppm): 1.21 (t, 3H, *J* = 6.9 Hz), 3.84 (s, 3H), 4.17 (q, 2H, *J* = 7.2, 14.1 Hz), 4.47 (s, 2H), 7.12 (d, 2H, *J* = 8.7 Hz), 7.60 (d, 2H, *J* = 8.7 Hz), 7.95 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 14.38, 42.60, 56.00, 62.15, 115.51, 117.61, 125.61, 132.94, 134.59, 161.88, 165.52, 167.24, 167.47. ESI +ve MS (*m/z*): 322 (*M*+H)⁺.

Procedure for synthesis of 2-((E)-5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetic acid (6).

A mixture of compound **5** (0.05 mole), glacial acetic acid (20 ml) and 11 N HCl (20 ml) was stirred at 90-100 °C for 8 h. After complete hydrolysis, the mixture was concentrated to afford yellow solid which was filtered, washed with cold water and dried. It was recrystallized with methanol to give pure acid derivative **6**. Yield 80% mp 221-222°C. ¹H NMR (300 MHz, DMSO-d₆, δ ppm): 3.86 (s, 3H), 4.37 (s, 2H), 7.13 (d, 2H, *J* = 8.4 Hz), 7.63 (d, 2H, *J* = 8.7 Hz), 7.95 (s, 1H), 13.44 (s, 1H). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 37.34, 50.66, 110.15, 112.47, 120.35, 127.54, 129.02, 156.49, 160.26, 162.13, 163.15. ESI -ve MS (*m/z*): 292 (*M*-H)⁺.

IR data of the target compounds 7-21:

(Z)-5-(4-methoxybenzylidene)-3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (7). IR (*v*_{max}, cm⁻¹): 3016, 2977, 2933, 2571, 1731, 1709, 1679, 1588, 1441, 1309, 1256, 1174, 1149, 1079, 860, 525;

(Z)-5-(4-methoxybenzylidene)-3-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (8). IR (*v*_{max}, cm⁻¹): 3014, 2932, 1732, 1682, 1587, 1568, 1509, 1456, 1421, 1373, 1308, 1256, 1175, 1143, 1094, 1024, 832;

(Z)-5-(4-methoxybenzylidene)-3-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (9). IR (ν_{max} , cm^{-1}): 3024, 1737, 1684, 1607, 1590, 1512, 1490, 1425, 1401, 1380, 1263, 1152, 1180, 1097, 824;

(Z)-5-(4-methoxybenzylidene)-3-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (10). IR (ν_{max} , cm^{-1}): 3025, 2977, 2933, 1739, 1684, 1604, 1588, 1566, 1511, 1406, 1382, 1323, 1260, 1177, 1151, 1097, 829;

(Z)-5-(4-methoxybenzylidene)-3-((5-p-tolyl-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (11). IR (ν_{max} , cm^{-1}): 3050, 2845, 1749, 1685, 1609, 1593, 1509, 1500, 1422, 1369, 1301, 1259, 1174, 1140, 1093, 1010, 830;

(Z)-5-(4-methoxybenzylidene)-3-((5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (12). IR (ν_{max} , cm^{-1}): 3263, 2950, 1749, 1686, 1585, 1549, 1511, 1489, 1411, 1261, 1174, 1160, 1086, 1070, 831;

(Z)-5-(4-methoxybenzylidene)-3-((5-o-tolyl-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (13). IR (ν_{max} , cm^{-1}): 3014, 2950, 1723, 1674, 1589, 1543, 1512, 1425, 1417, 1395, 1368, 1334, 1311, 1260, 1176, 1135, 1089, 1020, 822;

(Z)-5-(4-methoxybenzylidene)-3-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (14). IR (ν_{max} , cm^{-1}): 3087, 2838, 1733, 1679, 1587, 1527, 1509, 1374, 1348, 1305, 1258, 1178, 1141, 1091, 1067, 1022, 833;

(Z)-5-(4-methoxybenzylidene)-3-((5-m-tolyl-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (15). IR (ν_{max} , cm^{-1}): 2931, 1697, 1683, 1606, 1585, 1511, 1370, 1338, 1253, 1148, 1025, 829;

(Z)-5-(4-methoxybenzylidene)-3-((5-(phenoxymethyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (16). IR (ν_{max} , cm^{-1}): 3030, 2937, 1734, 1685, 1587, 1512, 1490, 1373, 1305, 1261, 1176, 1144, 1034, 829;

(Z)-3-((5-((3-chlorophenoxy)methyl)-1,3,4-oxadiazol-2-yl)methyl)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione (17). IR (ν_{max} , cm^{-1}): 3017, 2935, 1732, 1683, 1589, 1511, 1482, 1456, 1373, 1338, 1257, 1209, 1175, 1146, 1097, 1023, 825;

(Z)-3-((5-((2,3-dichlorophenoxy)methyl)-1,3,4-oxadiazol-2-yl)methyl)-5-(4-methoxybenzylidene)thiazolidine-2,4-dione (18). IR (ν_{max} , cm^{-1}): 3020, 2928, 1732, 1687, 1583, 1517, 1487, 1373, 1305, 1260, 1174, 1138, 1014, 825;

(Z)-5-(4-methoxybenzylidene)-3-((5-((naphthalen-1-yloxy)methyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (19). IR (ν_{max} , cm^{-1}): 3027, 2977, 2933, 1731, 1682, 1589, 1510, 1421, 1382, 1402, 1257, 1174, 1150, 1098, 826;

(Z)-5-(4-methoxybenzylidene)-3-((5-((naphthalen-3-yloxy)methyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (20). IR (ν_{max} , cm^{-1}): 3015, 2978, 2933, 1731, 1711, 1679, 1589, 1567, 1510, 1421, 1402, 1382, 1256, 1174, 1124, 1150, 1098, 1058, 825;

(Z)-5-(4-methoxybenzylidene)-3-((5-((quinolin-8-yloxy)methyl)-1,3,4-oxadiazol-2-yl)methyl)thiazolidine-2,4-dione (21). IR (ν_{max} , cm^{-1}): 3025, 2977, 1736, 1683, 1588, 1569, 1511, 1497, 1471, 1422, 1369, 1336, 1309, 1266, 1211, 1141, 1107, 1084, 1026, 1001, 817;

Antiproliferative activity

Cell Lines and Culture Medium

Two human cancer cell lines, MCF-7 and HCT-116 were cultured in Dulbecco's Modified Eagles Medium (DMEM) high glucose medium which was supplemented with 10% fetal bovine serum (FBS), 10,000 units/mL penicillin/streptomycin (Pen/Strep) and 1% glutamine). The above cell lines were cultured in 75 cm² flasks and maintained at 37 °C in an incubator humidified with 5% CO₂. The cell culture was performed using aseptic techniques in a Class II Safety Flow Hood.

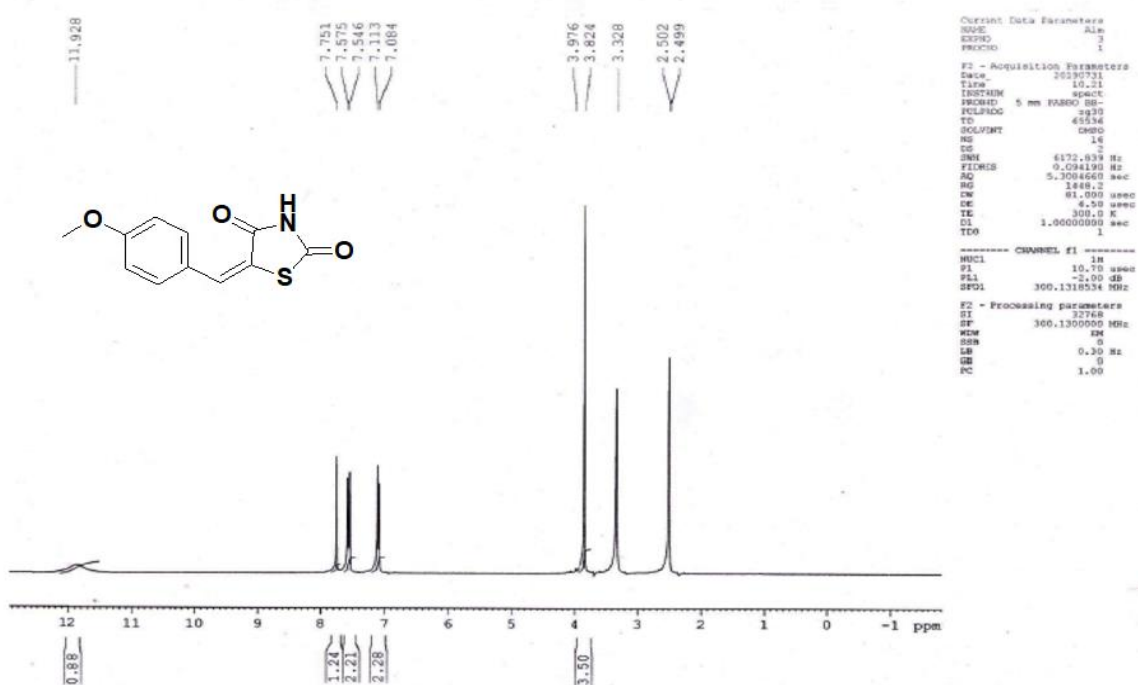
Cytotoxicity assay

Breast MCF-7 and colorectal HCT-116 cancer cells were added at (1×10^5 cells/mL) into a 96 well plate with three replicates and incubated overnight for attachment at 37°C, in 5% CO₂ humidified atmosphere. Drug concentrations at 6 serial dilutions (100, 50, 10, 1, 0.5 and 0.1 µM) were added in triplicates and incubated at 37°C, 5% CO₂ for 72 h. Drugs were dissolved in 0.1 % DMSO as a vehicle. Untreated cells were used as control. 5-fluorouracil (5-FU) was used as a positive control. Thereafter, each well for each time point was removed and replaced with 100 µM of full medium containing 10% of 3-(4,5- dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide (MTT) (10 mg/mL). Media was removed and 100µl of DMSO was added and cells were incubated for further 5 mins at 37 °C and 5% CO₂. Plates were quantified using the SpectraMax M3 plate reader at 570 nm. The percentage inhibition was calculated as $100 - [(Mean\ OD\ of\ treated\ cell \times 100) / Mean\ OD\ of\ vehicle\ treated\ cells\ (DMSO)]$. All the experiments were repeated at least three independent experiments. The results are presented in Table 2.

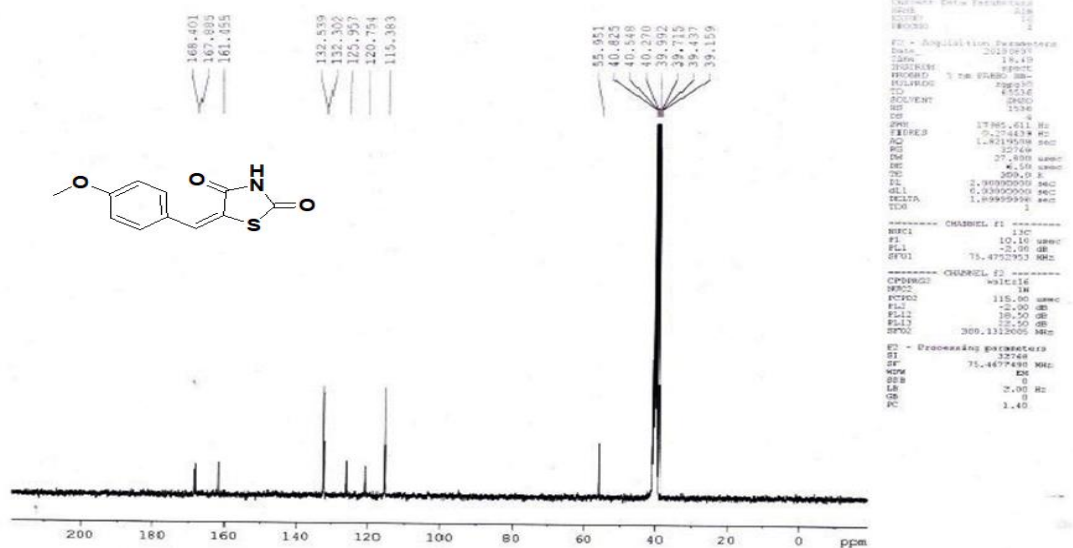
***In vitro* Thymidylate synthase assay**

It involves a mixture containing 2-mercaptoethanol (0.1 M), (6R,S)-tetrahydrofolate (0.0003 M), formaldehyde (0.012 M), MgCl₂ (0.02 M), dUMP (0.001 M), TrisHCl (0.04 M), and NaEDTA

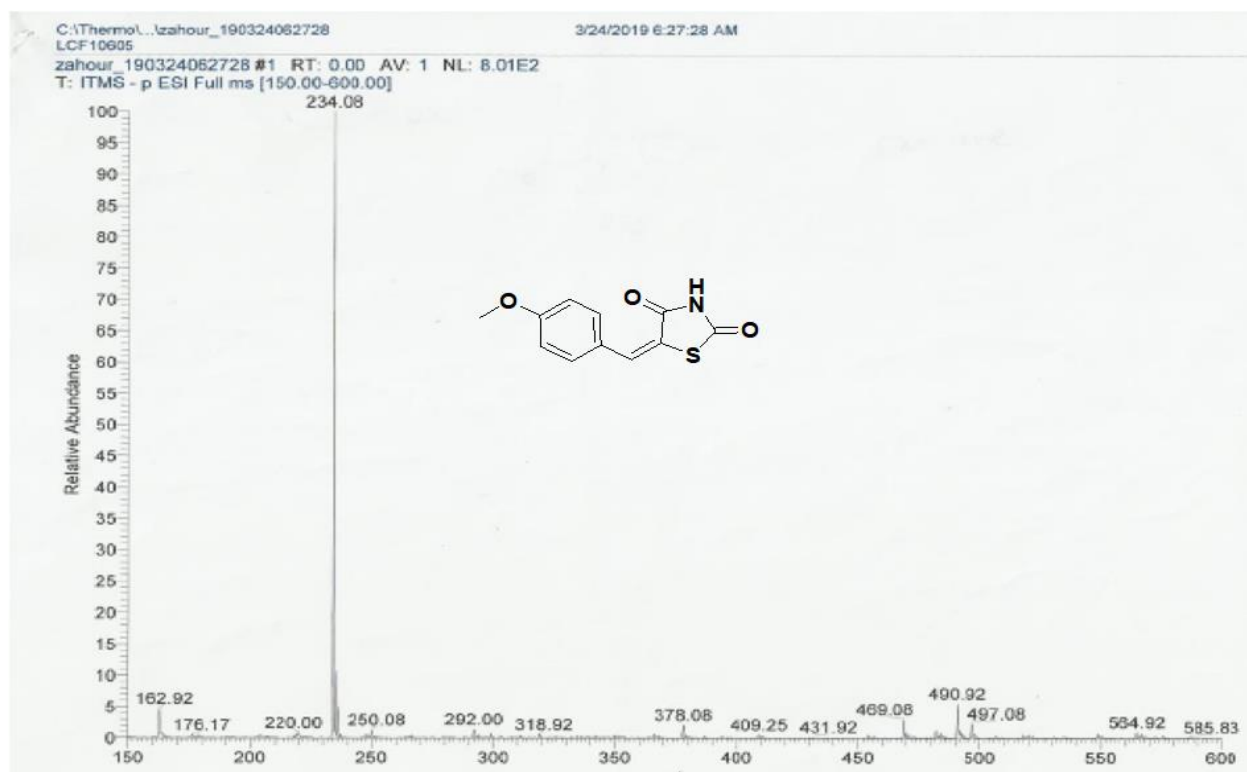
(0.00075 M). This assay was done spectrophotometrically at 30° C and pH 7.4. The reaction was initiated by the addition of an amount of enzyme giving a change in absorbance at 340 nm of 0.016/min in the absence of inhibitor. The percent inhibition was determined at a minimum of four inhibitor concentrations within 20% of the 50% point. The standard deviations for determination of the 50% points were within $\pm 10\%$ of the values given.



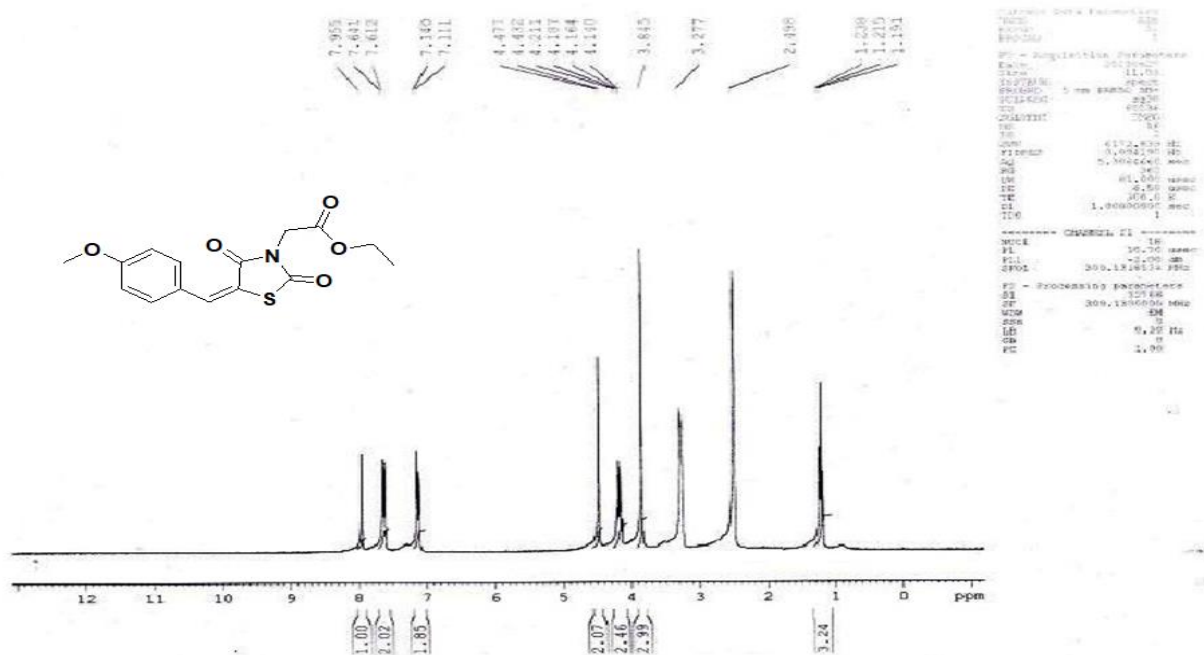
¹H NMR of compound 4



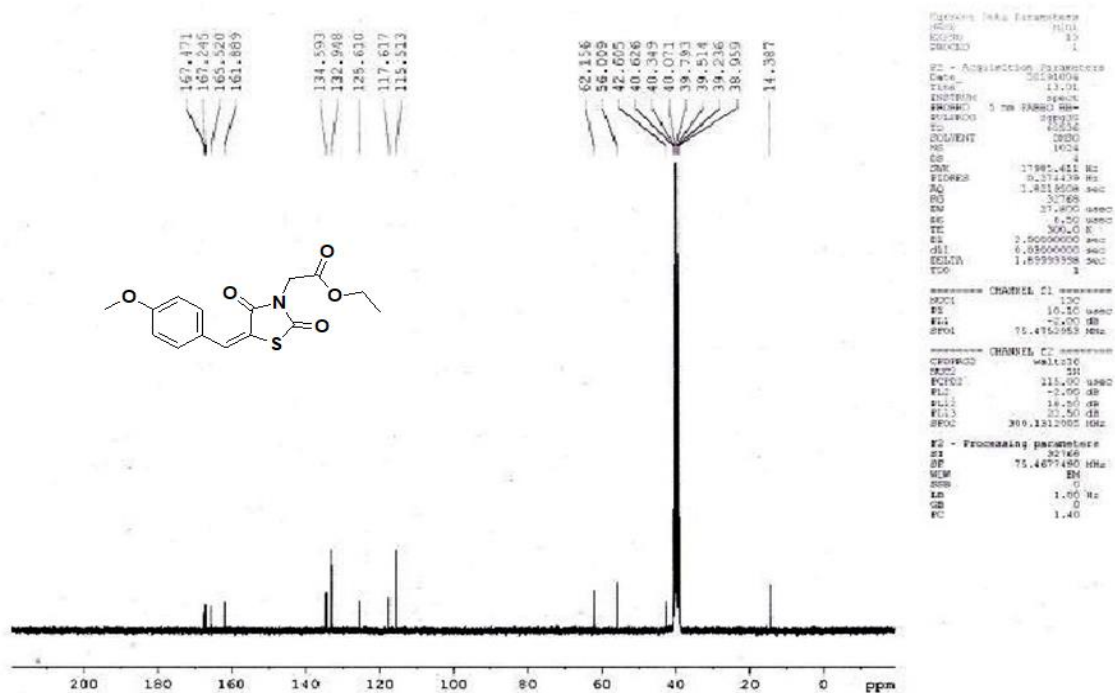
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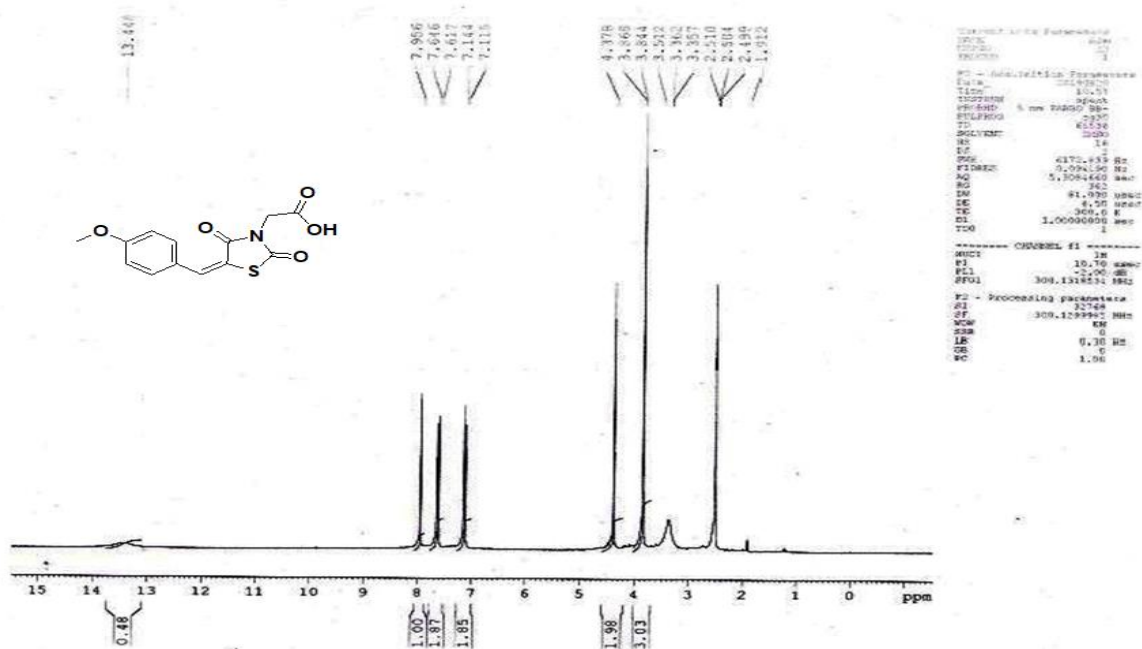
Mass of compound 4



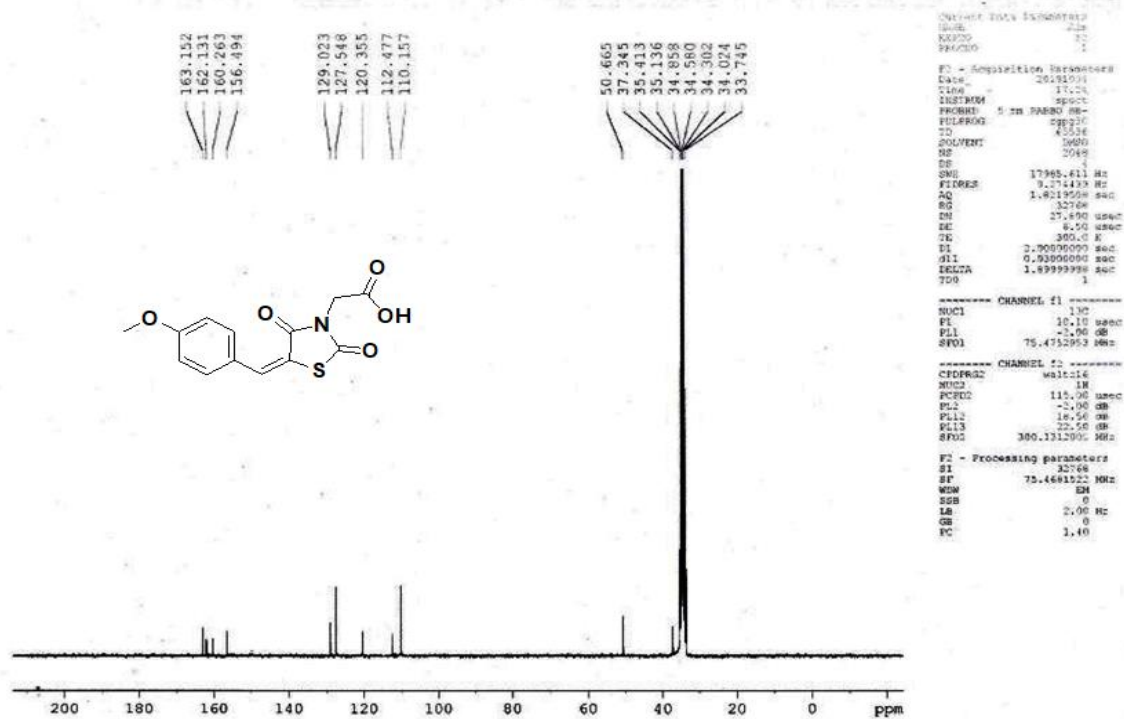
¹H NMR of compound 5



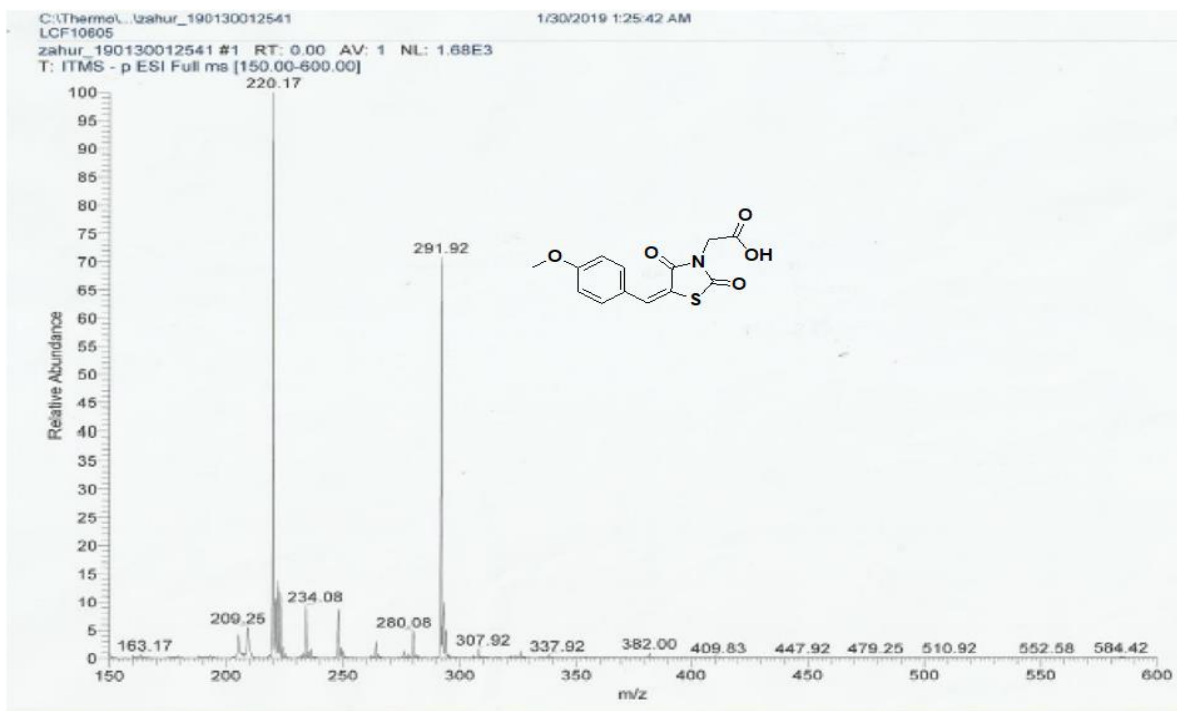
¹³C NMR of compound 5



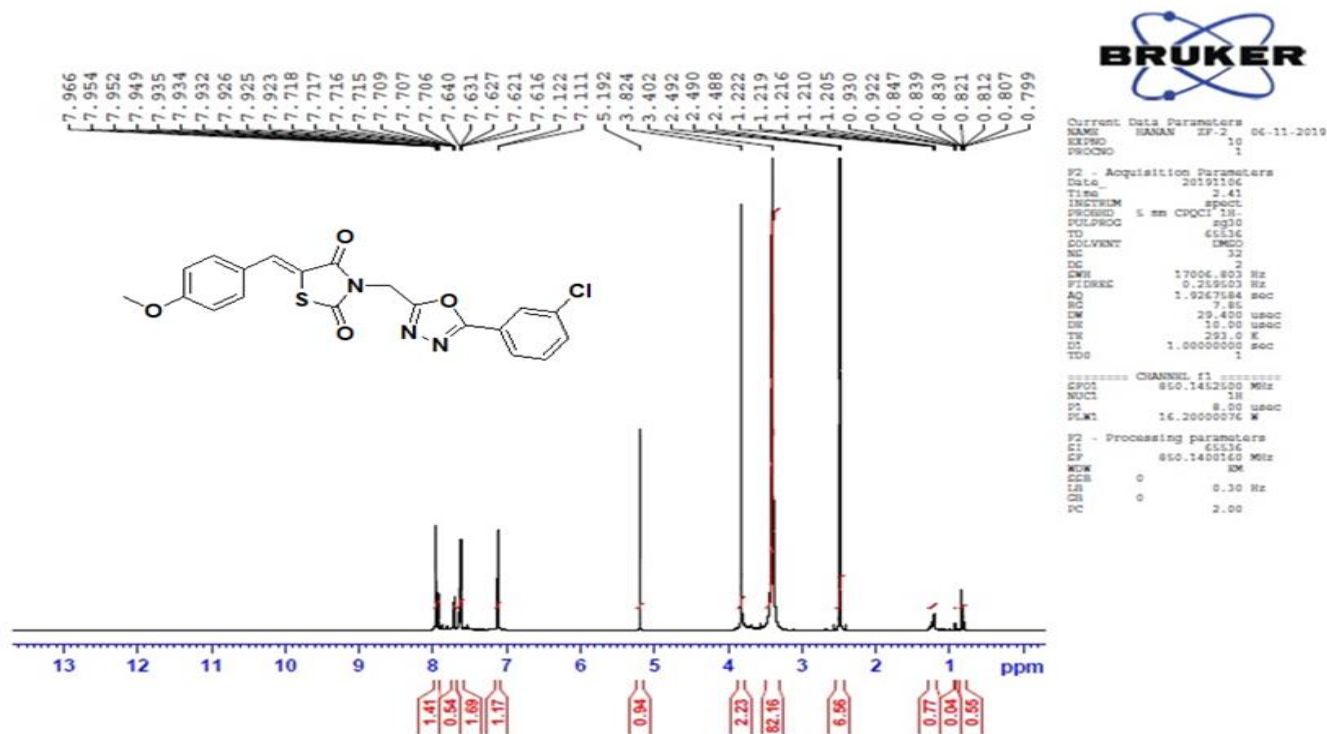
¹H NMR of compound 6



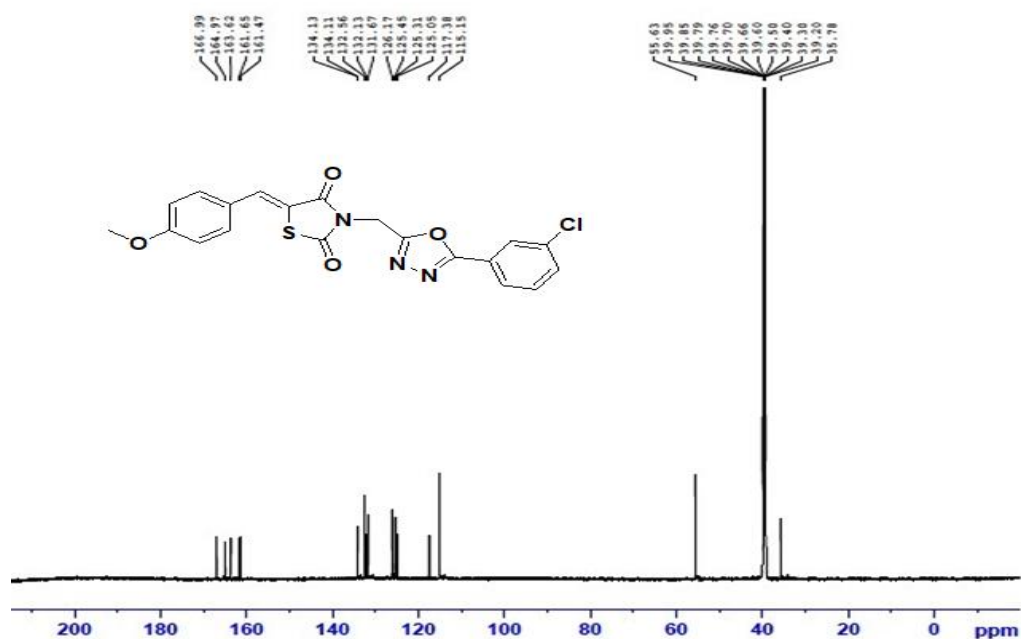
¹³C NMR of compound 6



Mass of compound 6



¹H NMR of compound 8



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PROCNO: 1

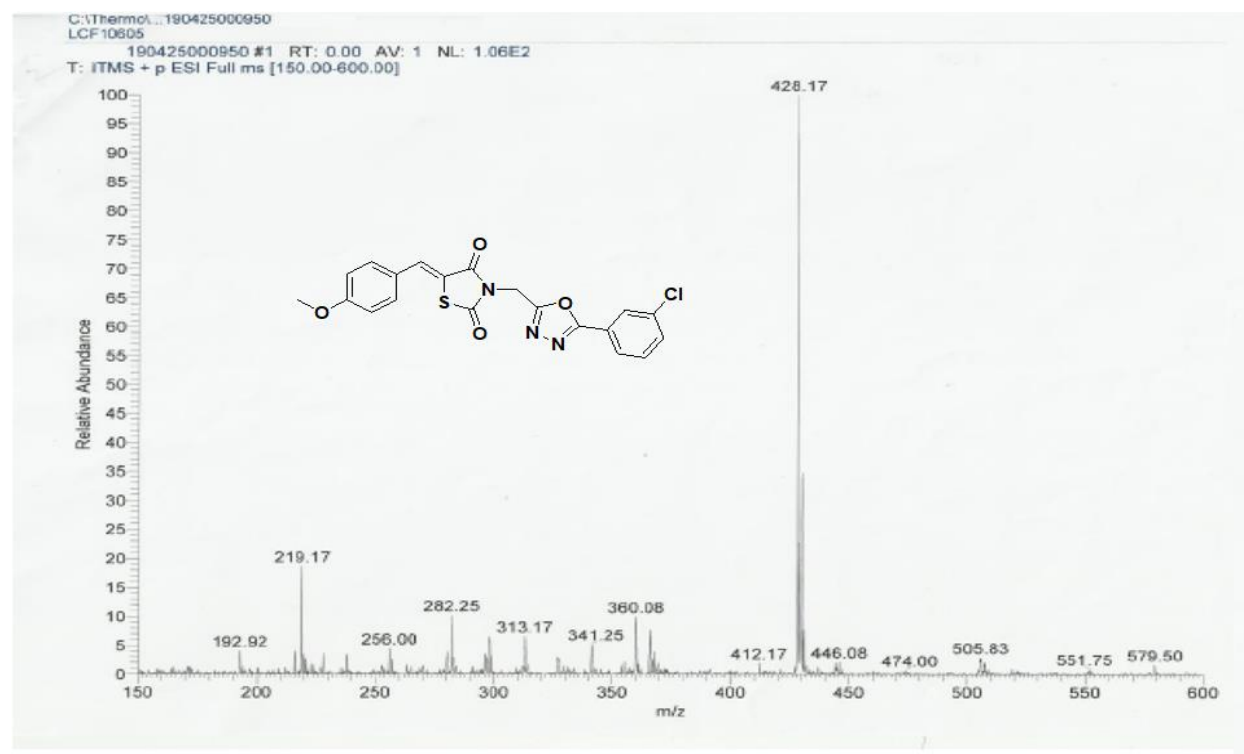
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AQ: 0.6422528 sec
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DE: 18.00 usec
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D11: 0.03000000 sec
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NUC1: 13C
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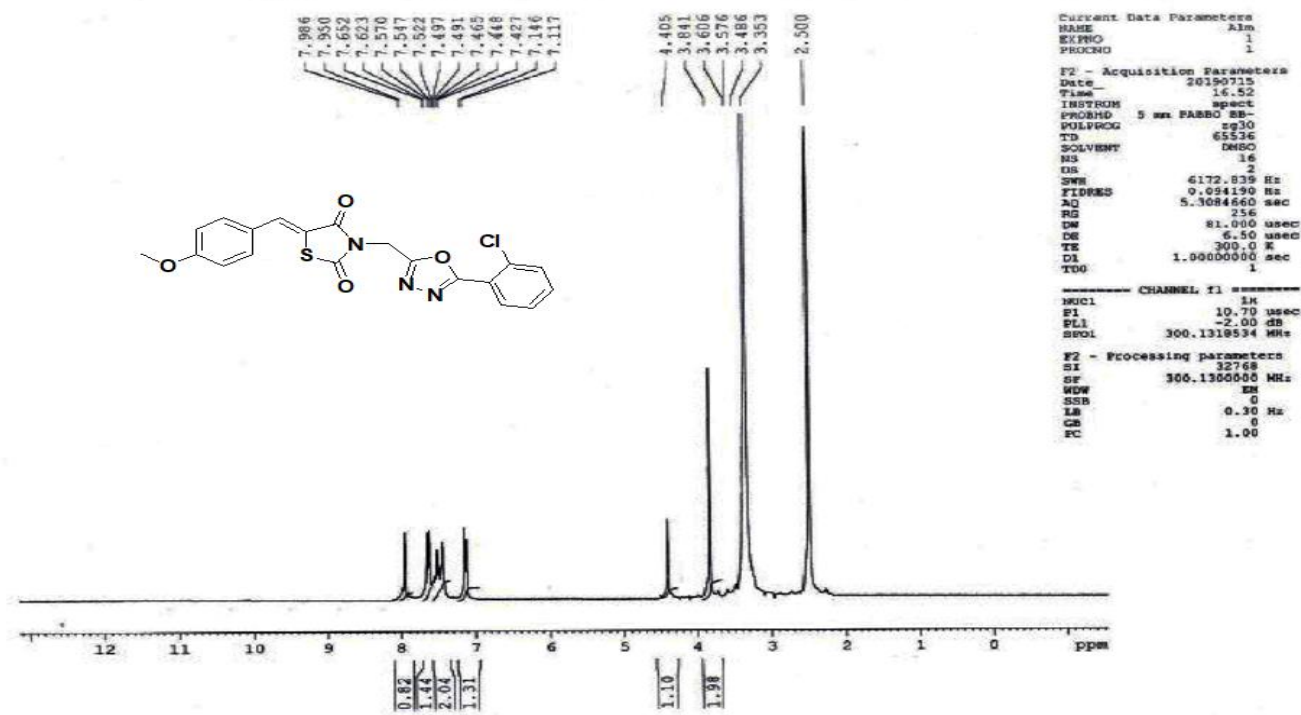
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NUC2: 1H
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PCPD2: 80.00 usec
PLM2: 16.20000076 W
PLM12: 0.16200000 W
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F2 - Processing parameters
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PC: 2.00

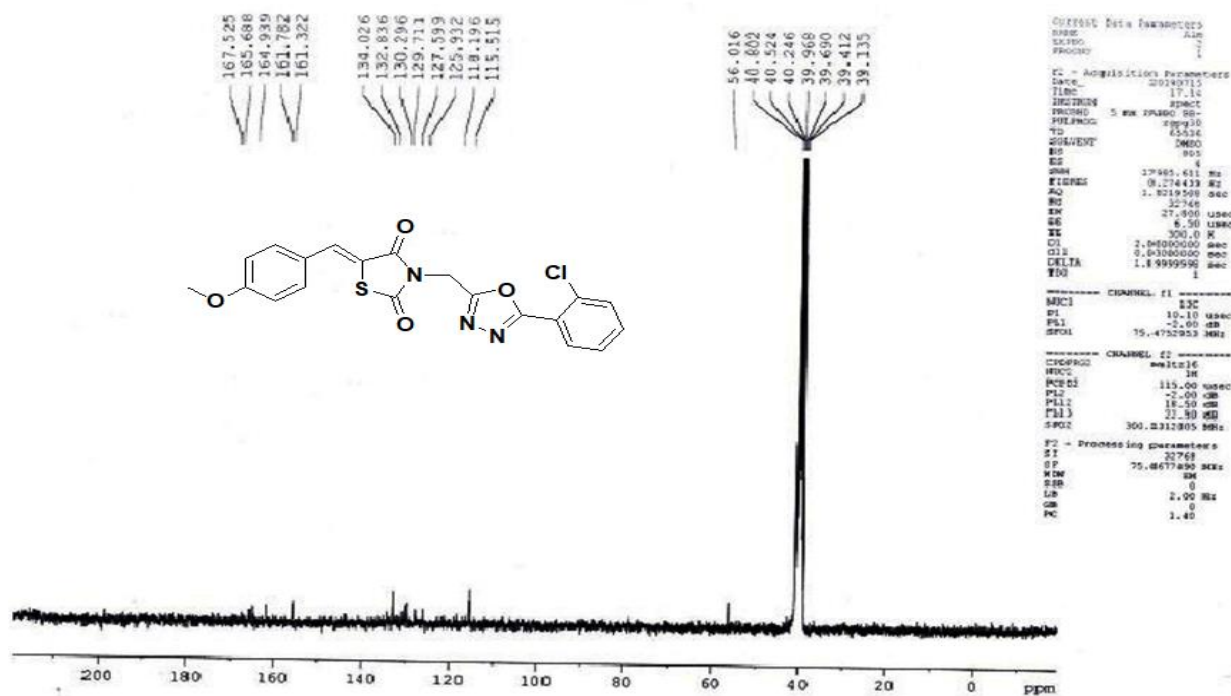
¹³C NMR of compound 8



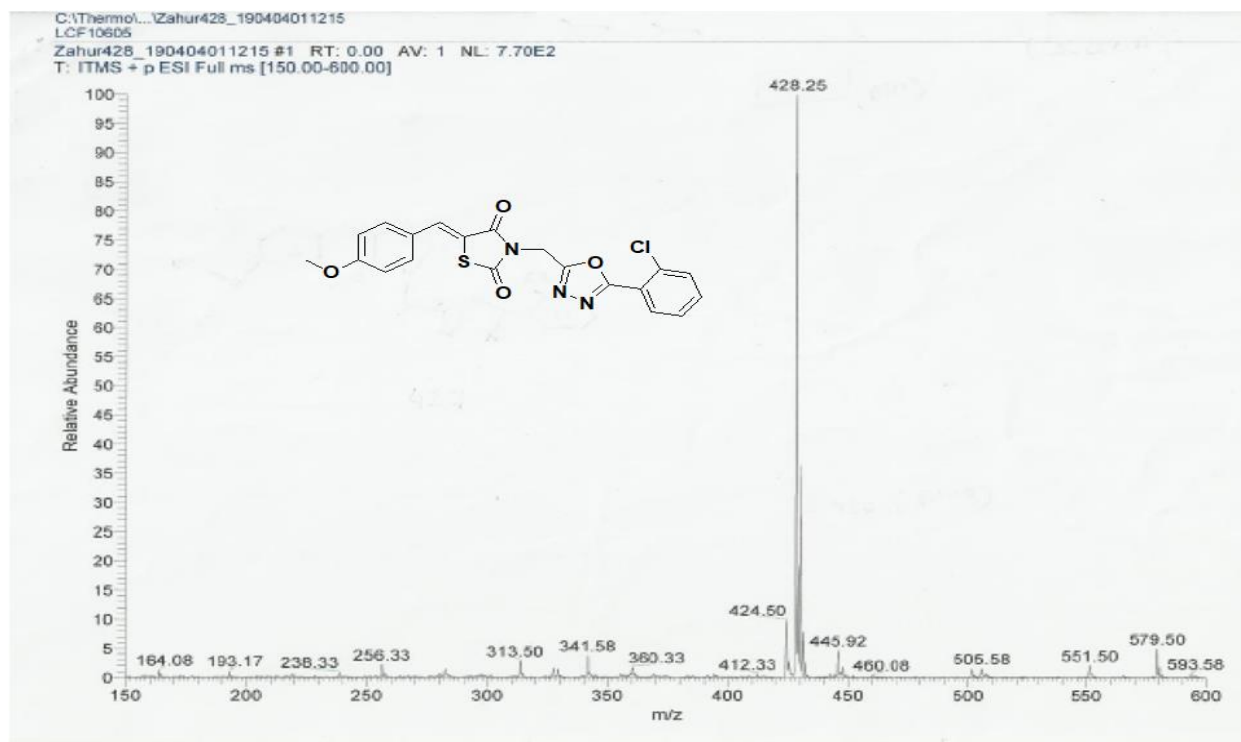
Mass of compound 8



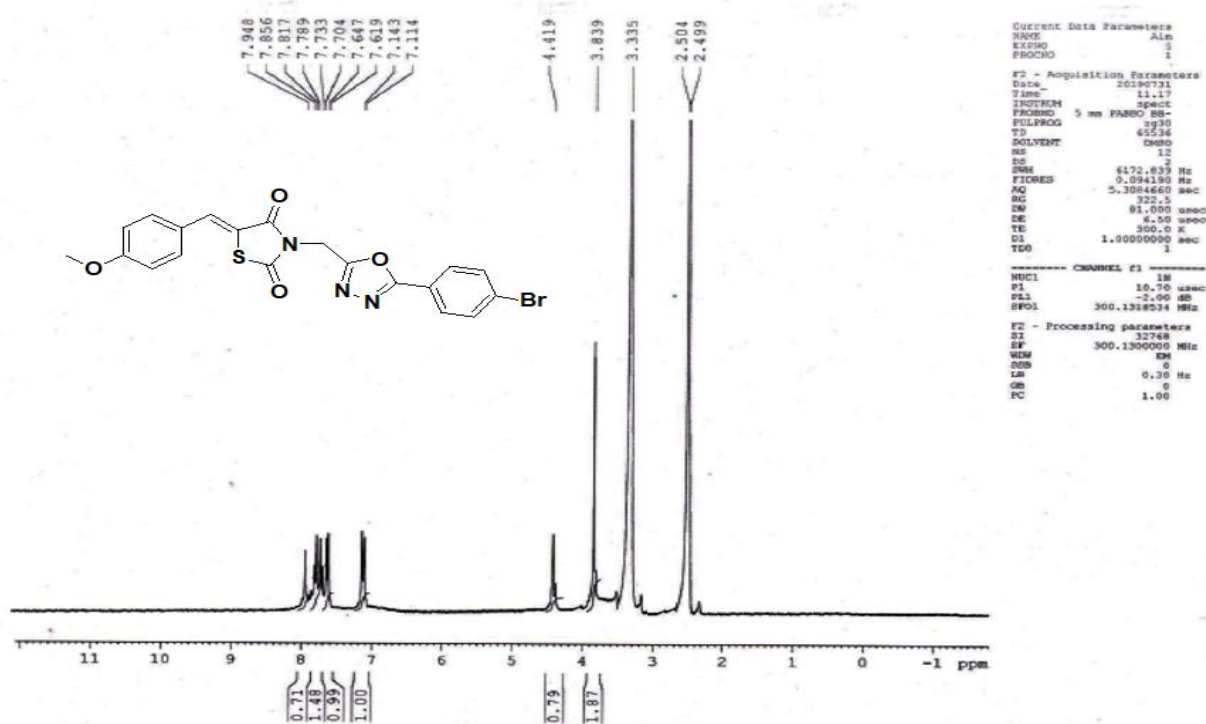
¹H NMR of compound 9



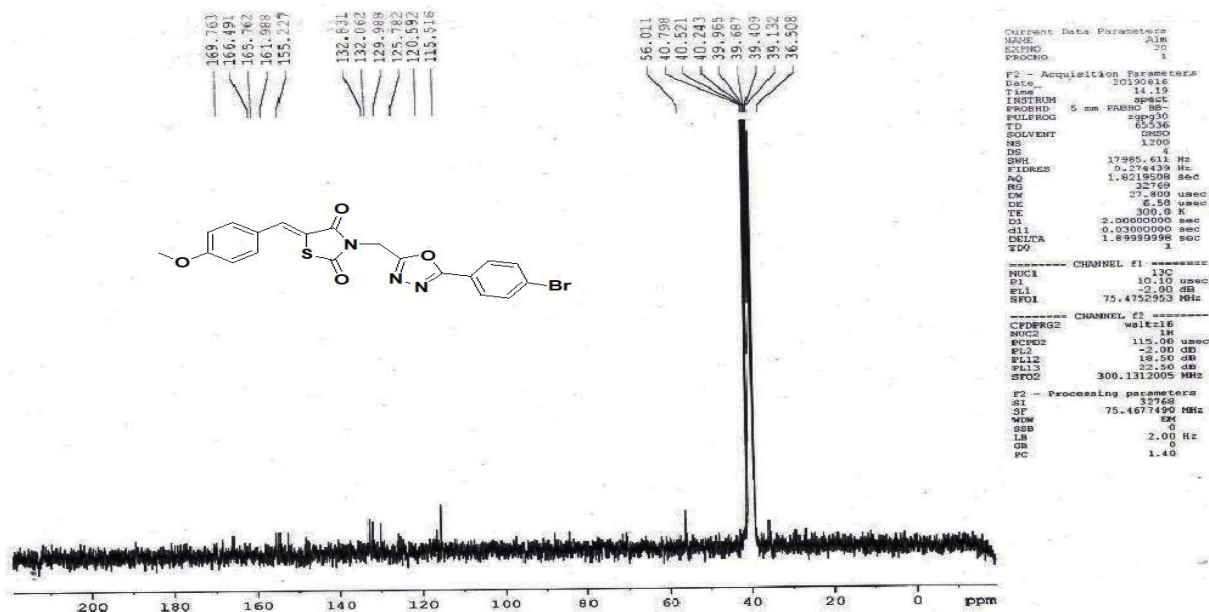
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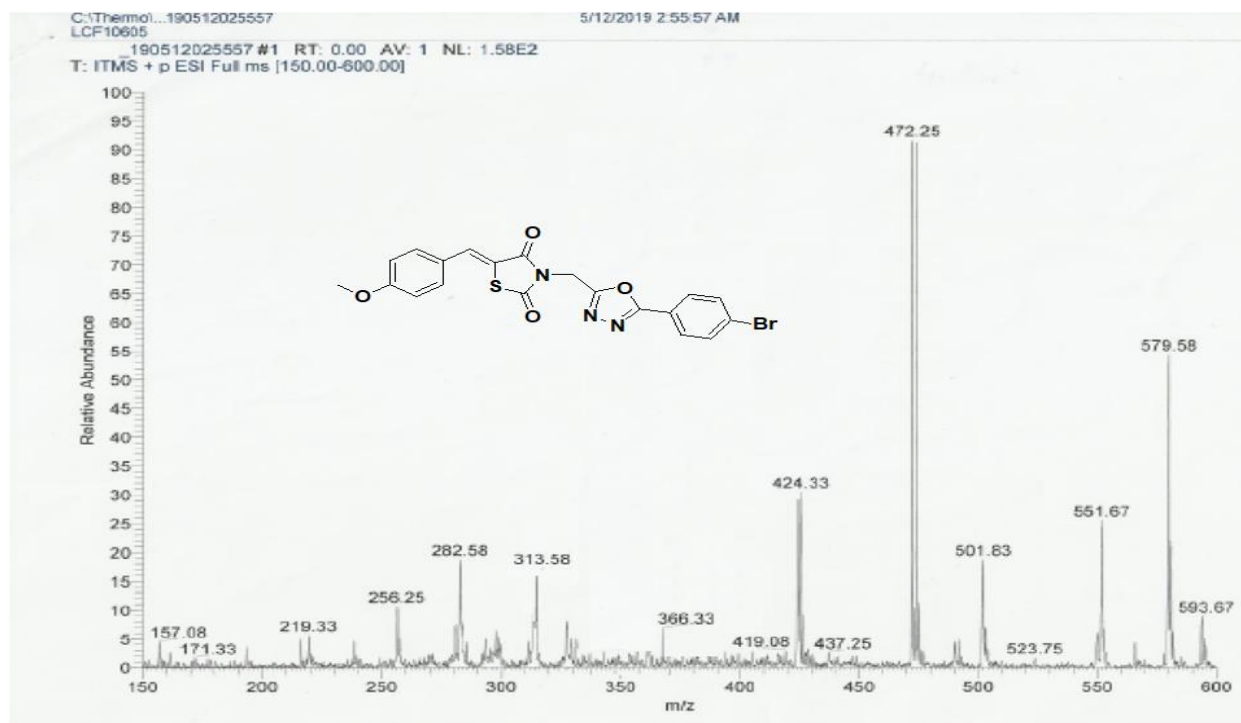
Mass of compound 9



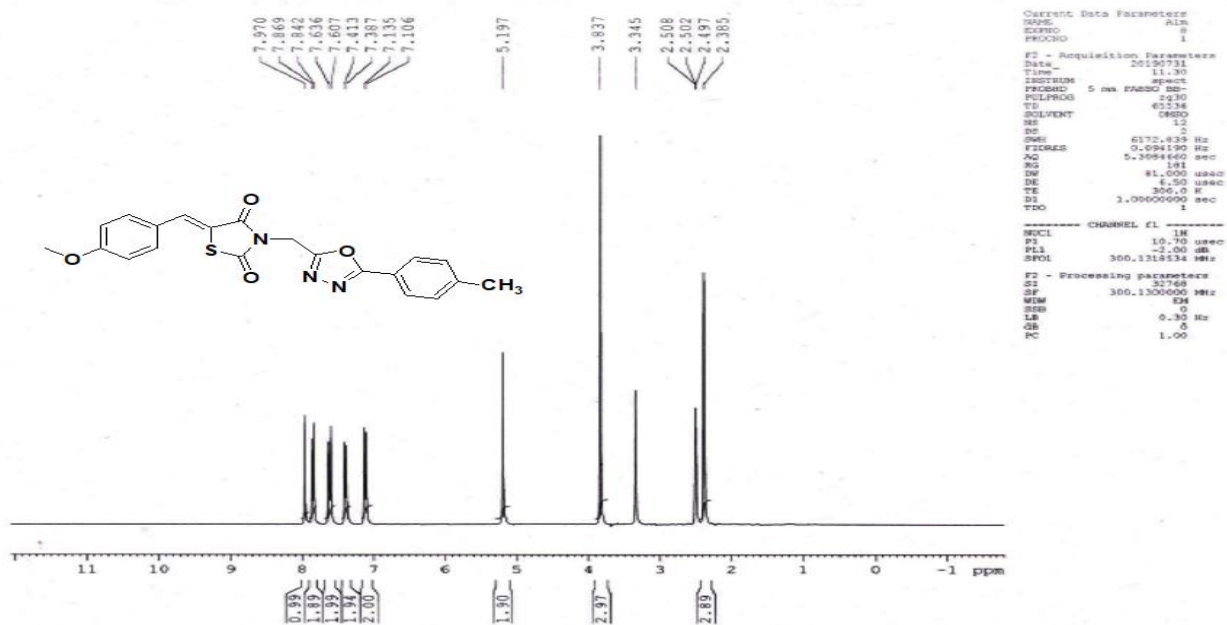
¹H NMR of compound 10



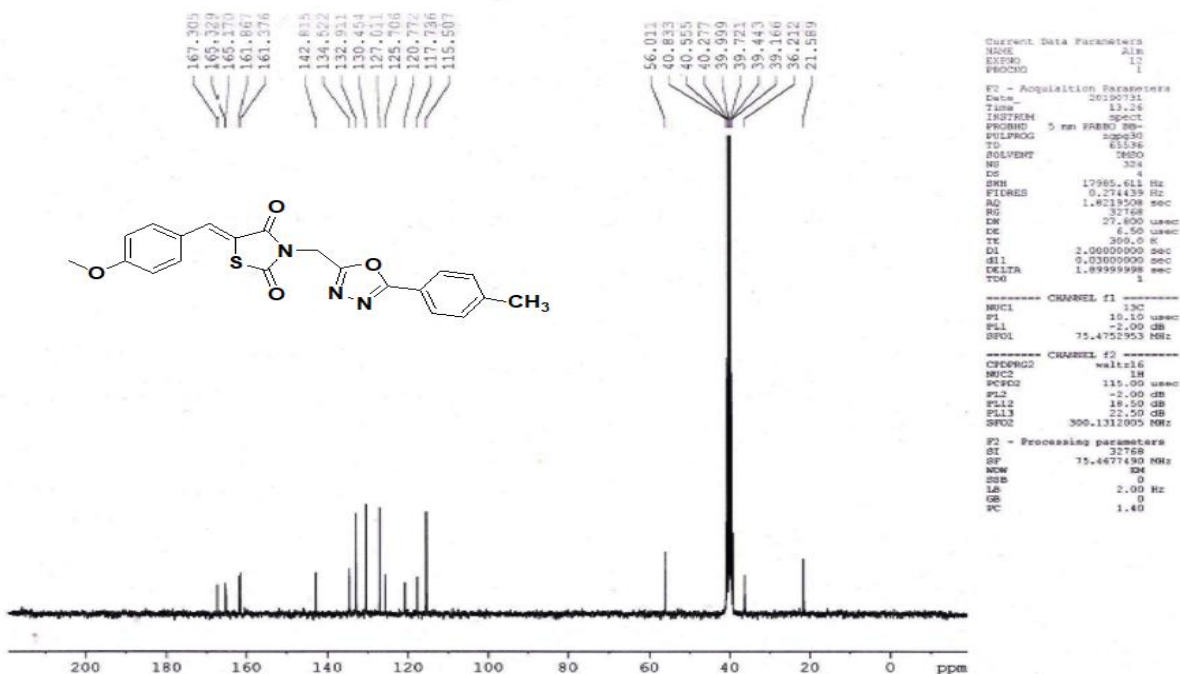
¹³C NMR of compound 10



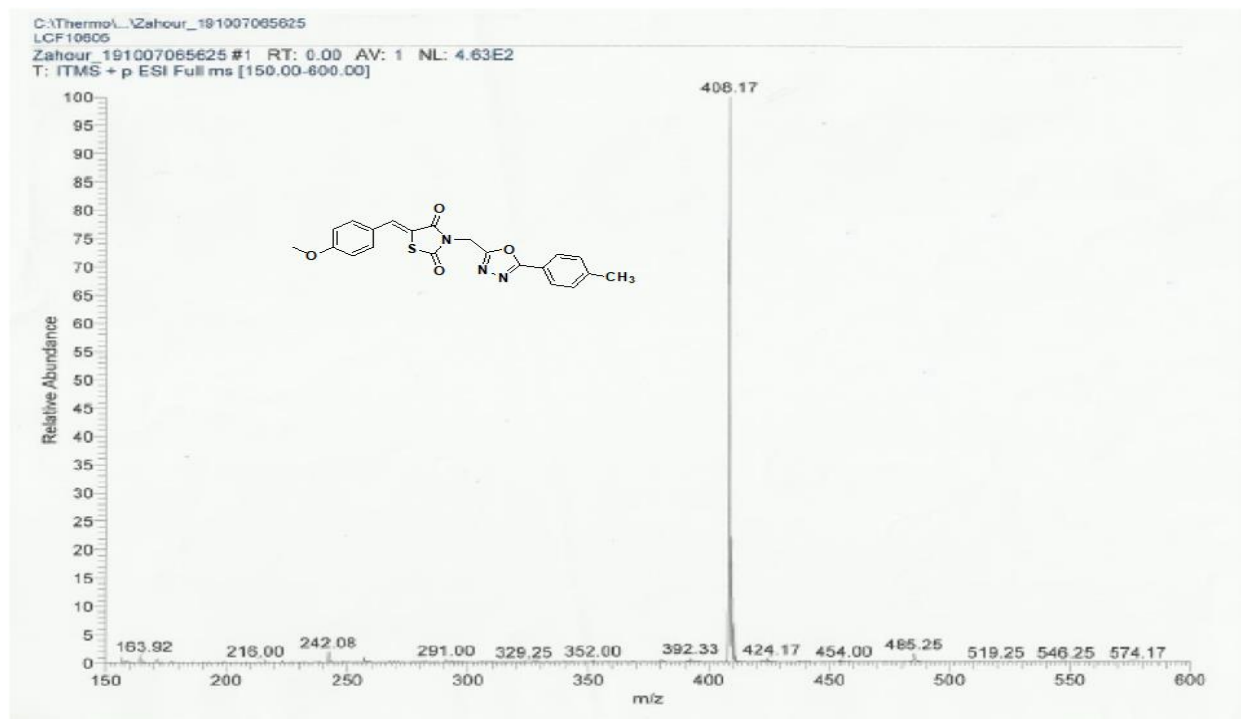
Mass of compound 10



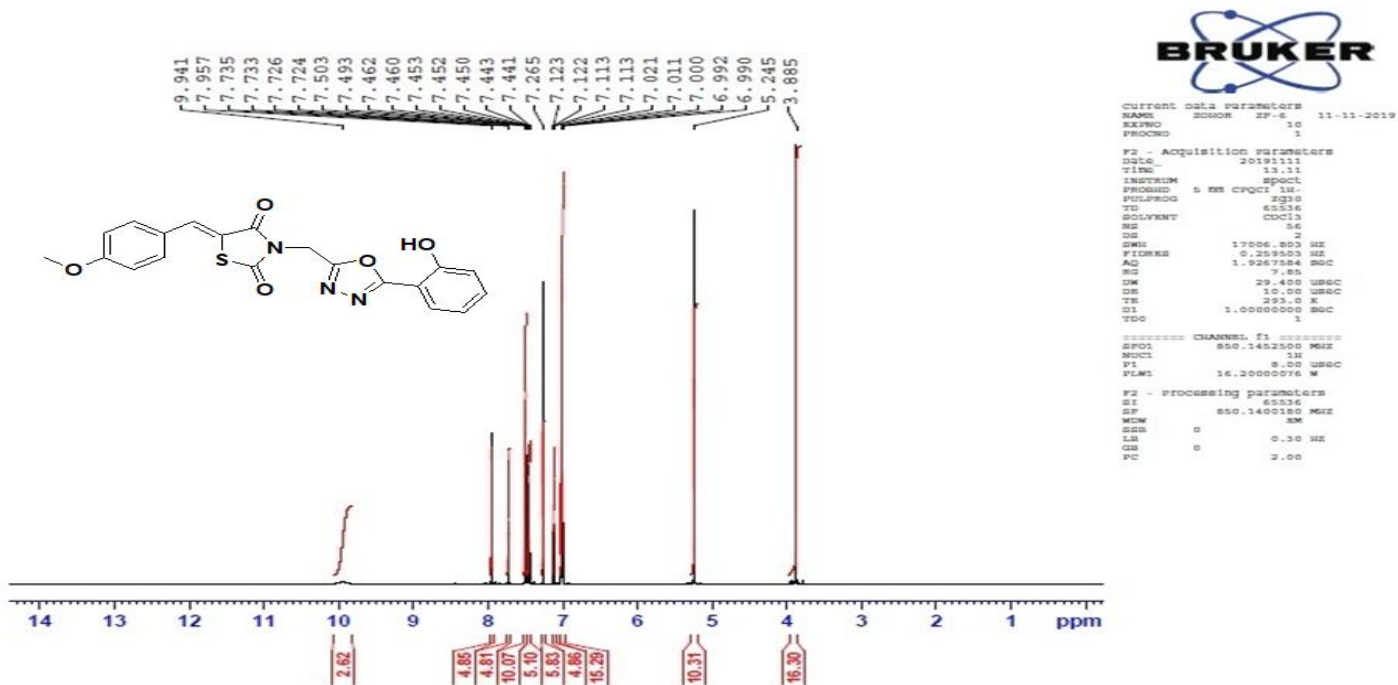
¹H NMR of compound 11



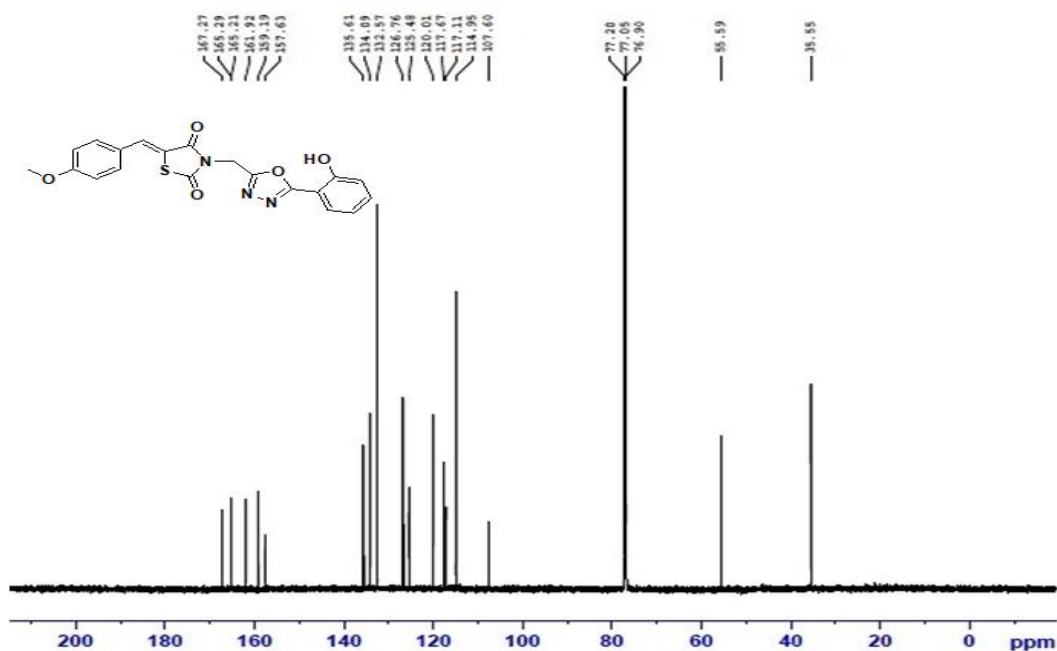
¹³C NMR of compound 11



Mass of compound 11



¹H NMR of compound 12



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PROCNO: 1

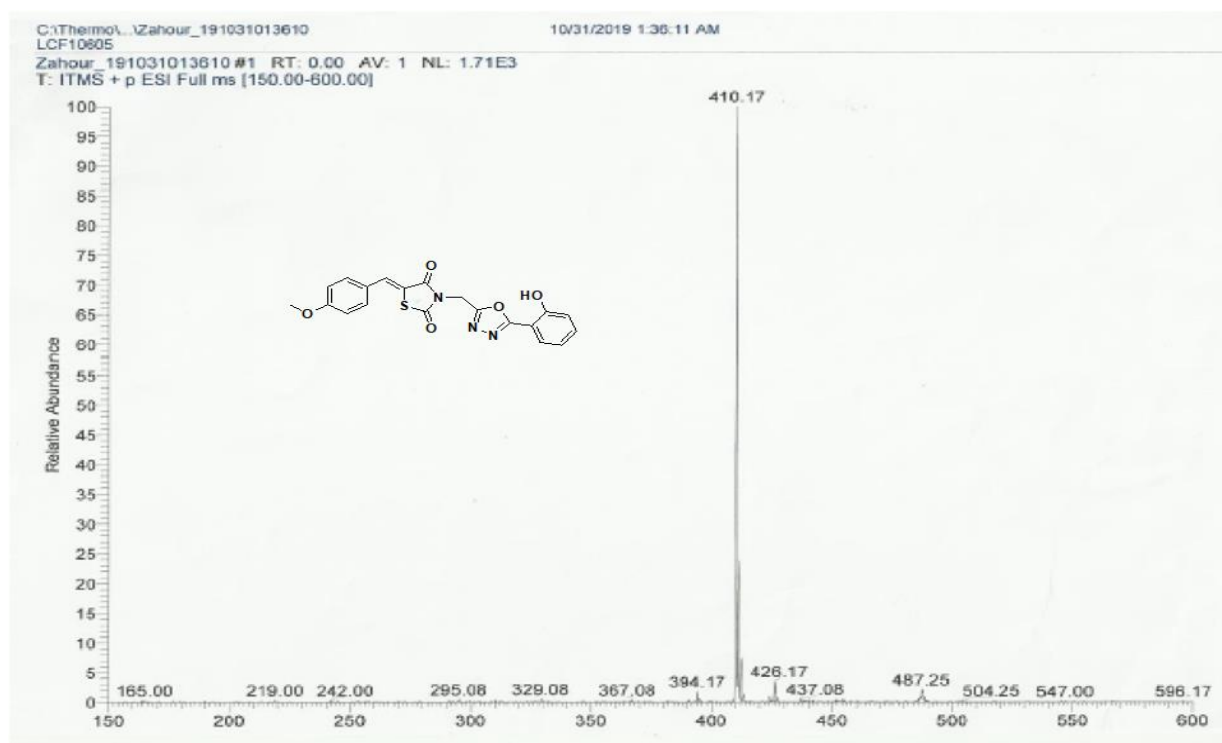
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SWH: 51020.406 MHz
FIDRES: 0.778510 Hz
AQ: 0.6422528 sec
RG: 186.93
DM: 9.800 USBC
DE: 18.00 USBC
TE: 293.0 K
D1: 2.00000000 sec
D11: 0.03000000 sec
TDO: 1

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NUC1: 13C
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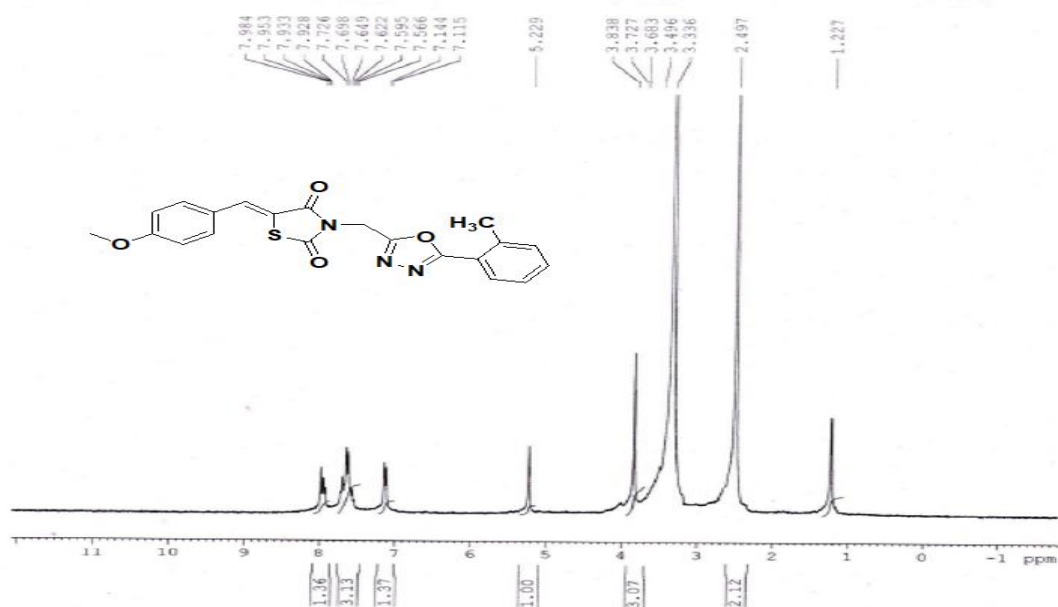
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PLW3: 0.10368000 W

F2 - Processing parameters
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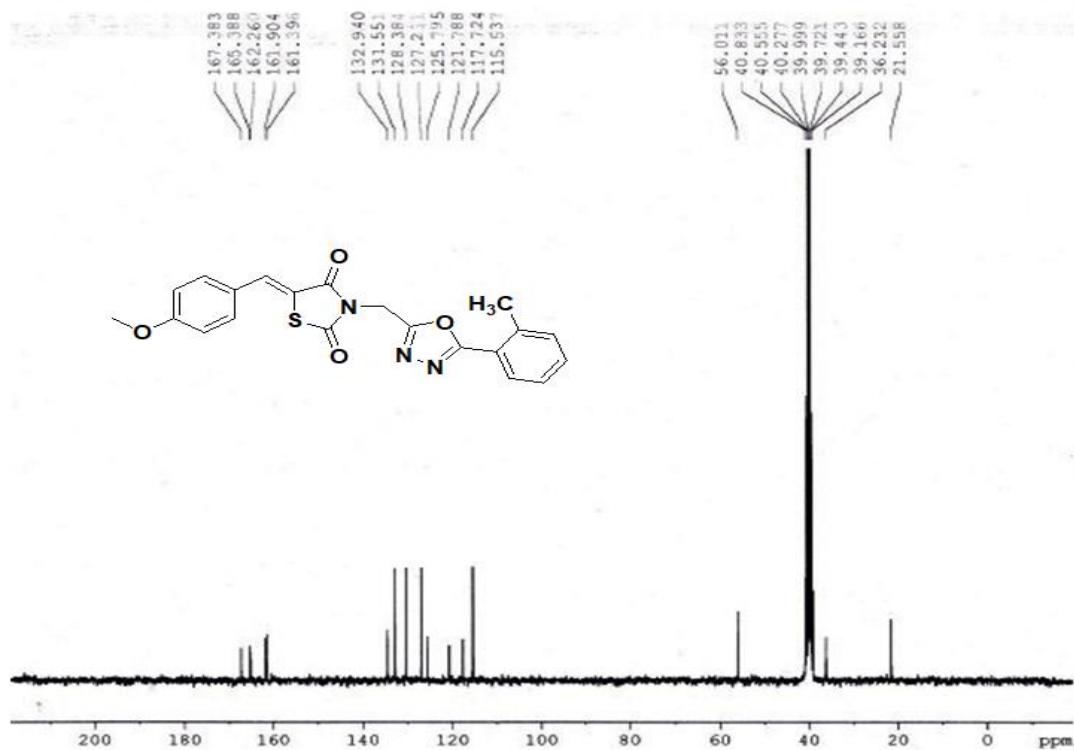
¹³C NMR of compound 12



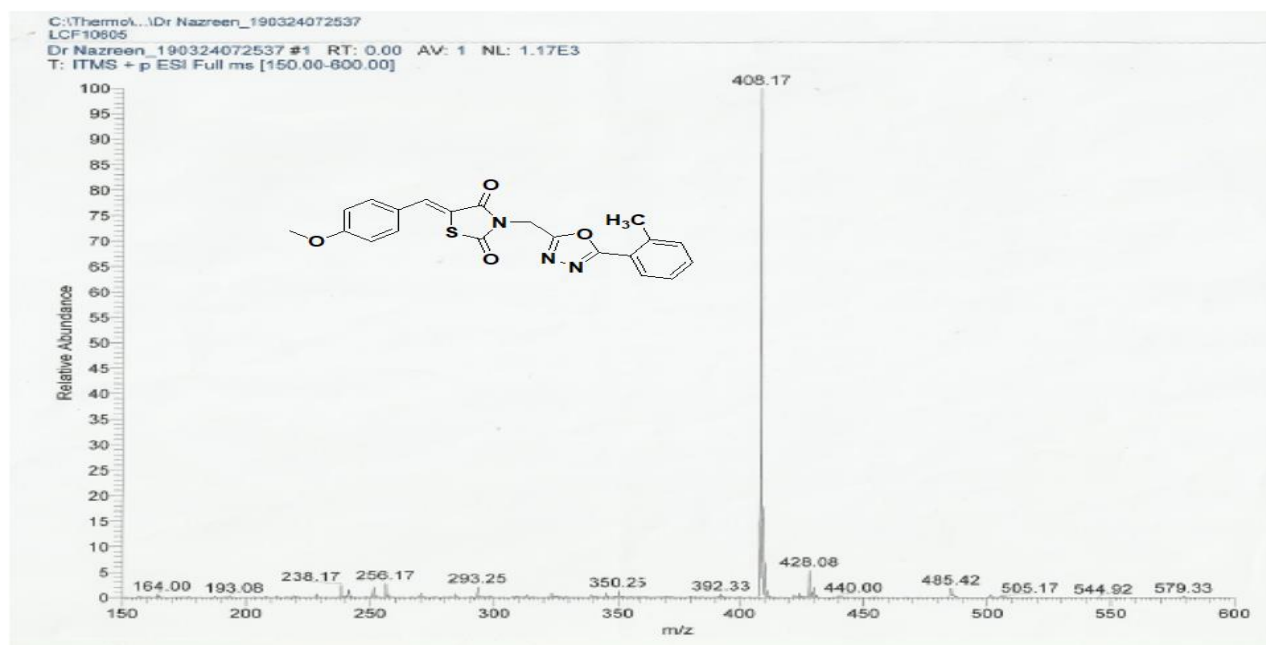
Mass of compound 12



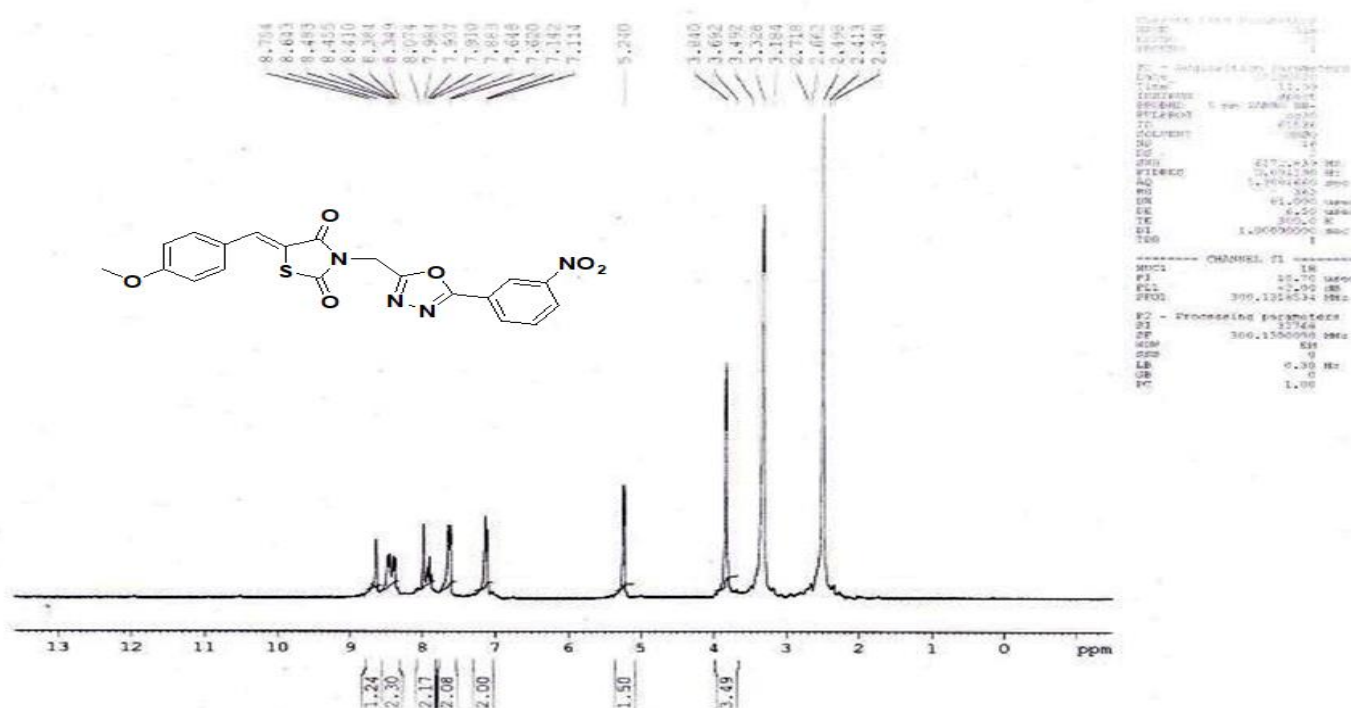
¹H NMR of compound 13



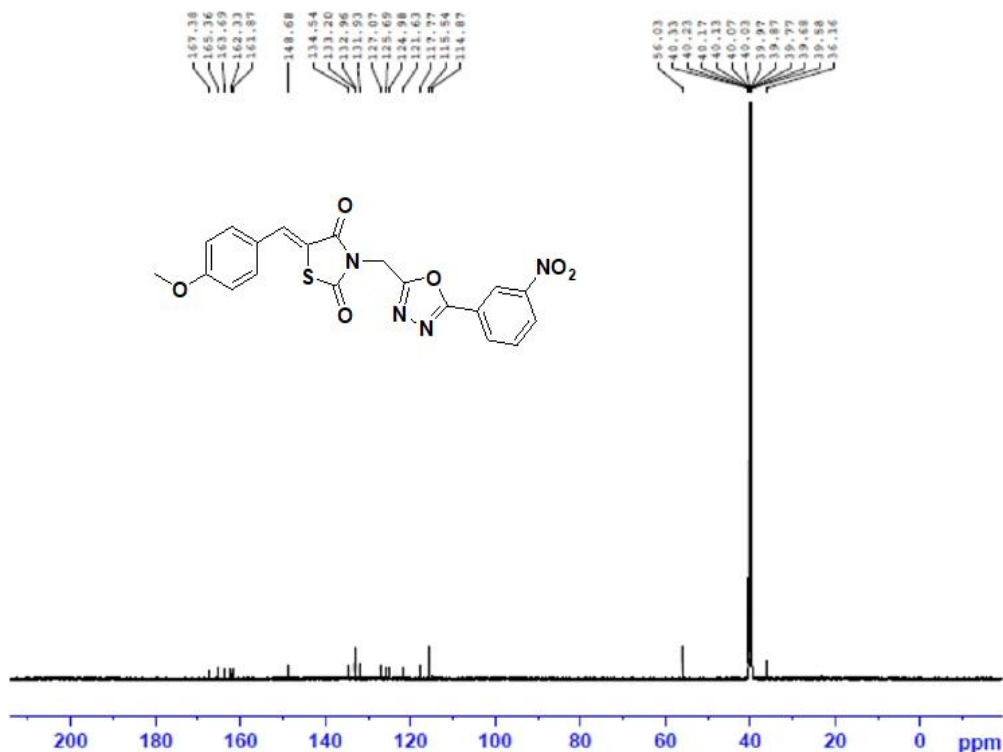
¹³C NMR of compound 13



Mass of compound 13



¹H NMR of compound 14



Current Data Parameters
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 REPO 40
 PROCNO 1

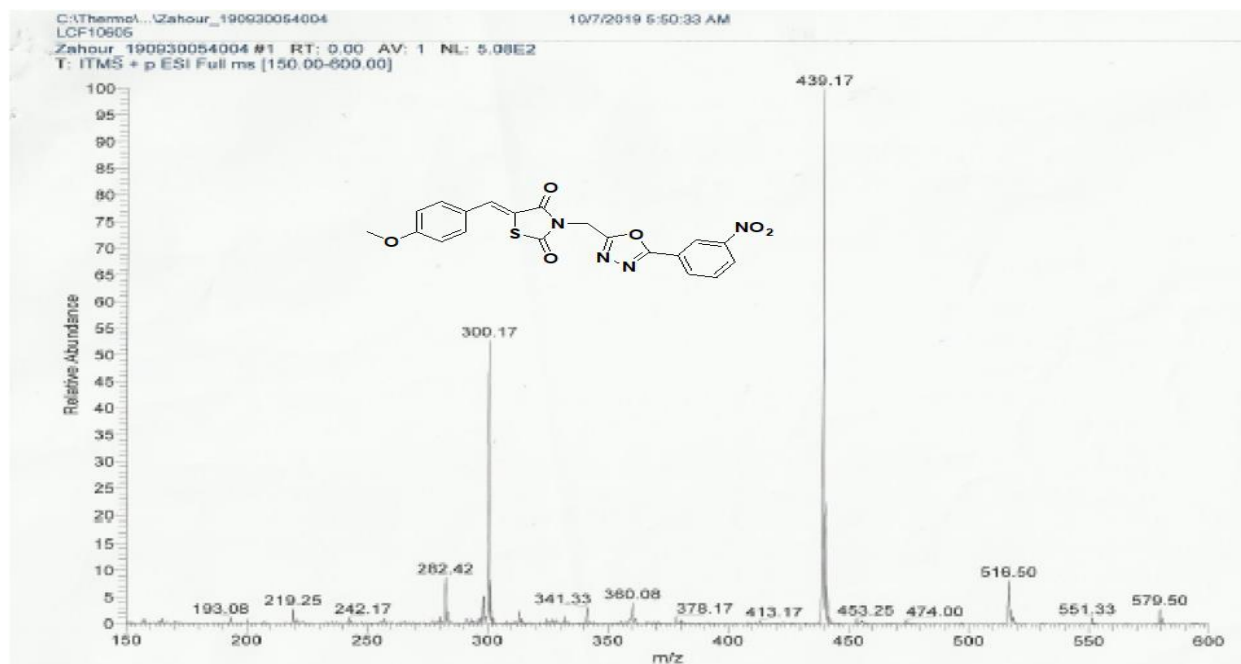
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 FIDRES 0.778010 HZ
 AQ 0.6422628 SEC
 RG 185.93
 DW 9.800 USEC
 DE 18.00 USEC
 TE 293.0 K
 D1 3.0000000 SEC
 D11 0.0300000 SEC
 TDO 1

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 PLW1 140.0000000 W

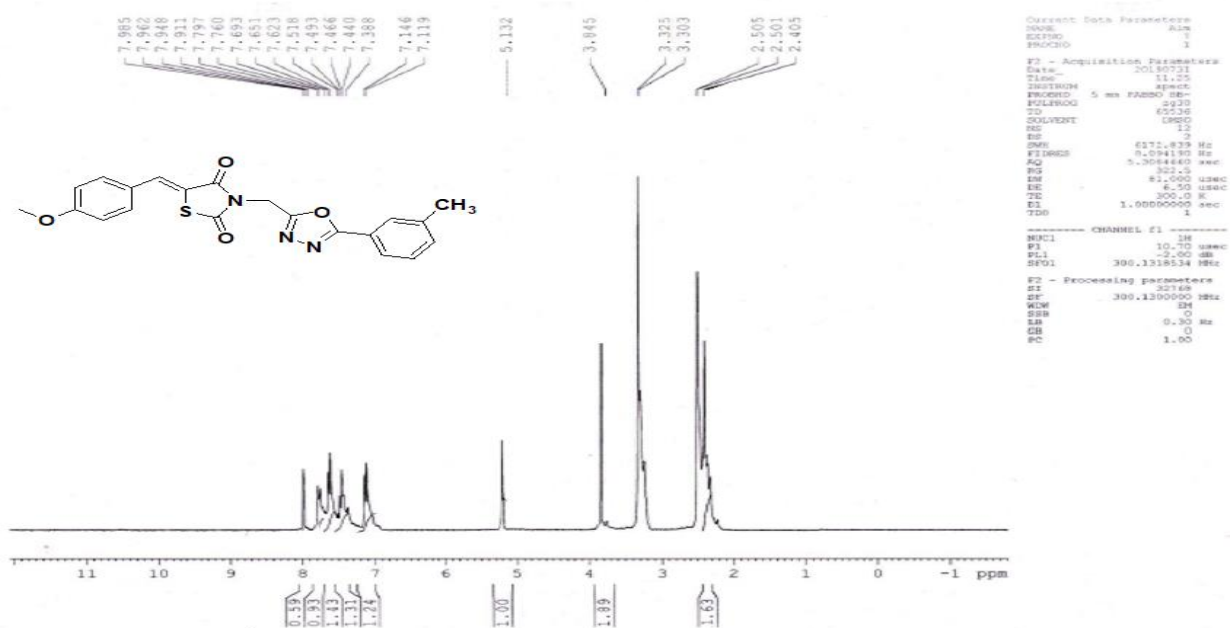
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 PLW12 0.16200000 W
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F2 - processing parameters
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 PC 2.00

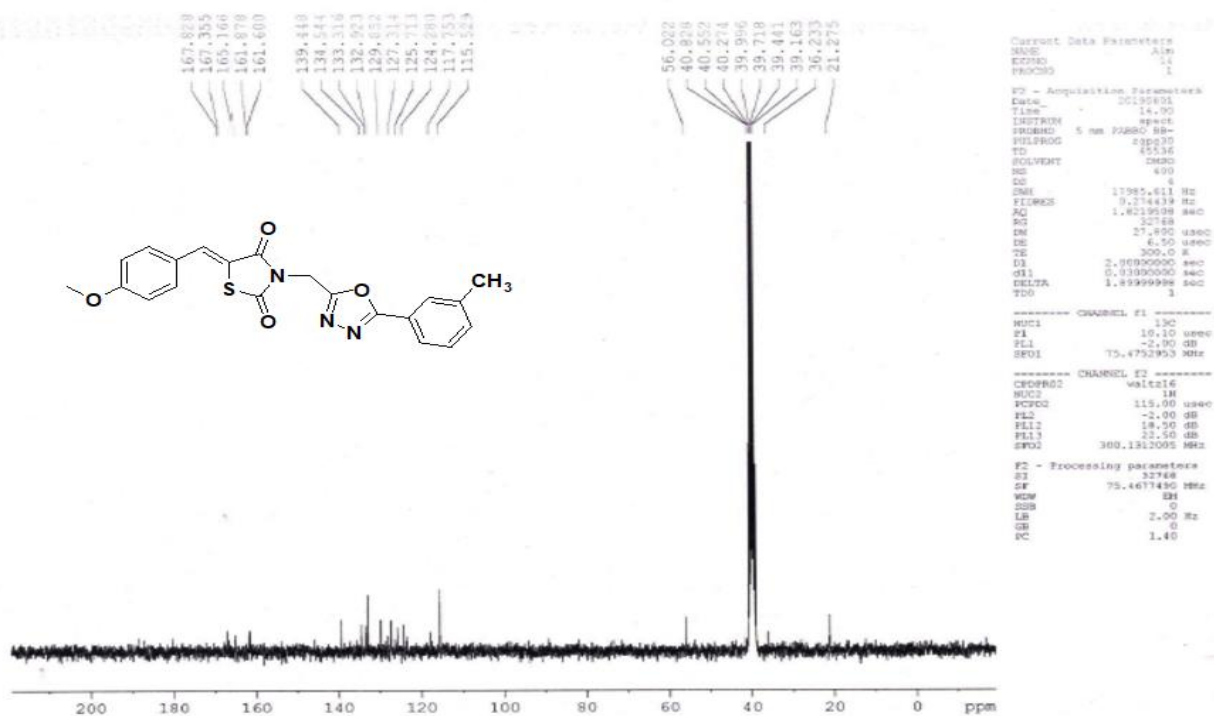
¹³C NMR of compound 14



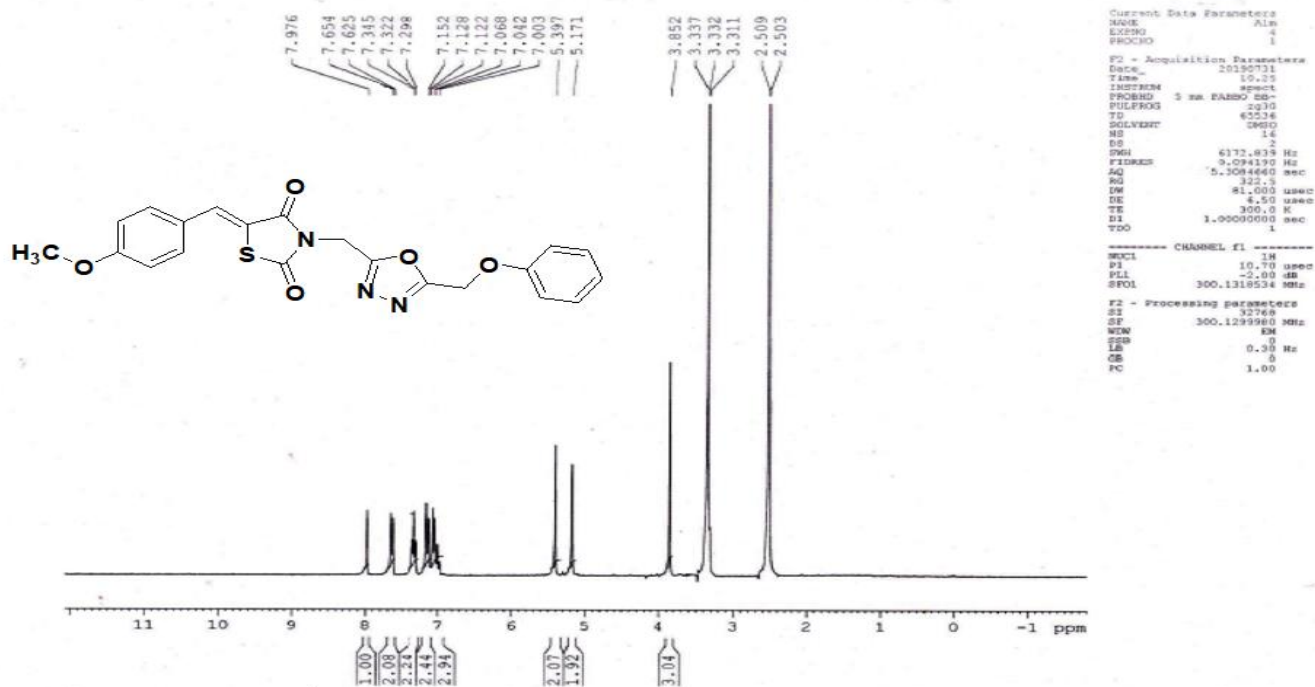
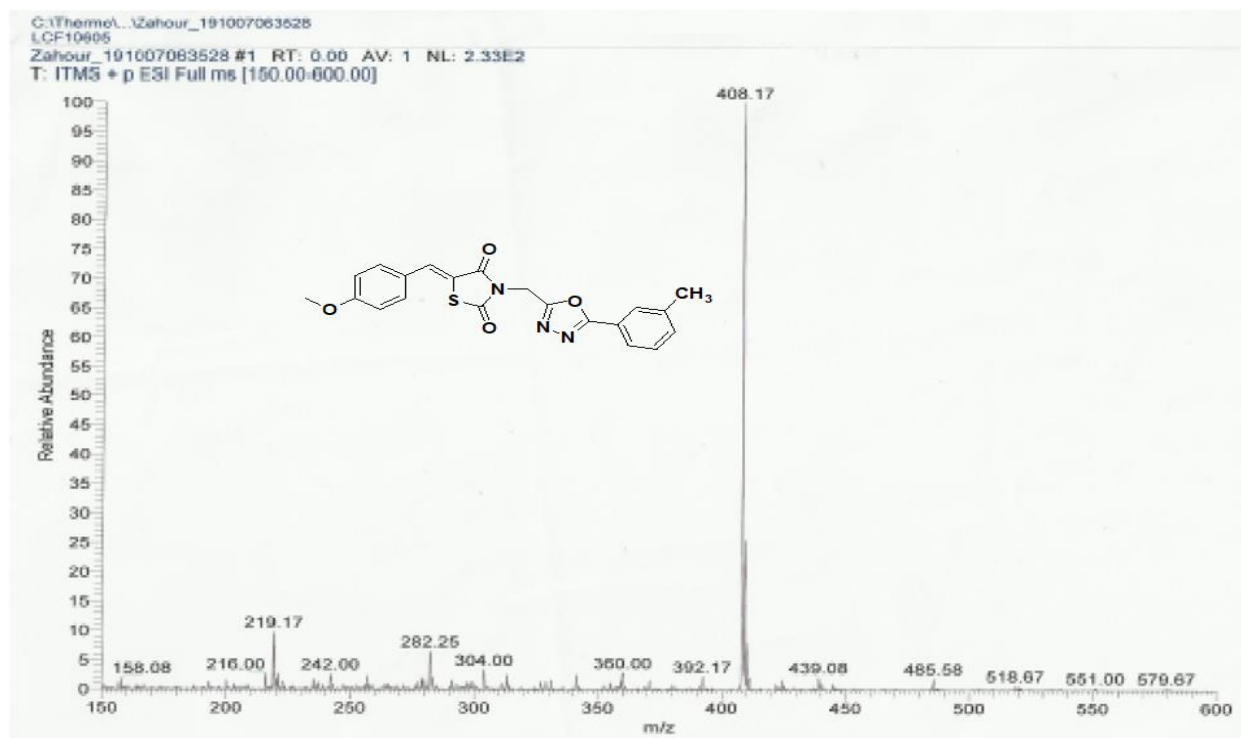
Mass of compound 14



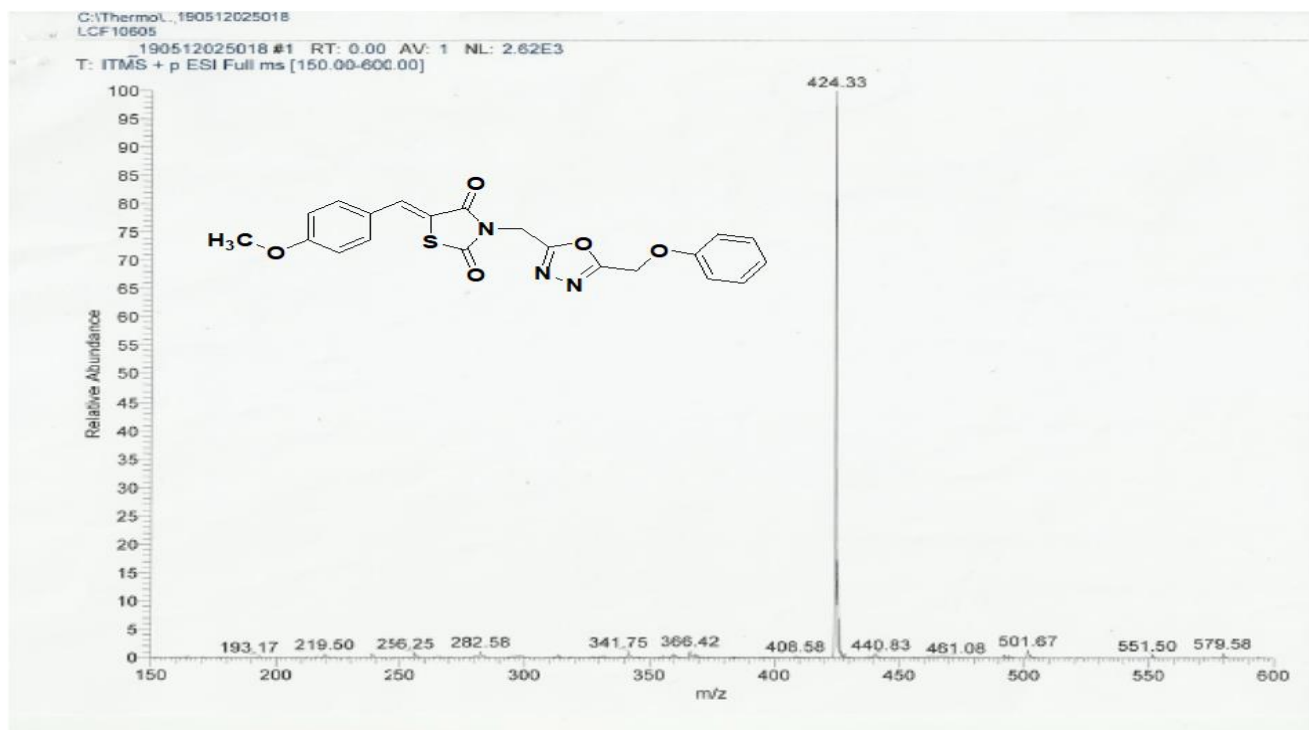
¹H NMR of compound 15



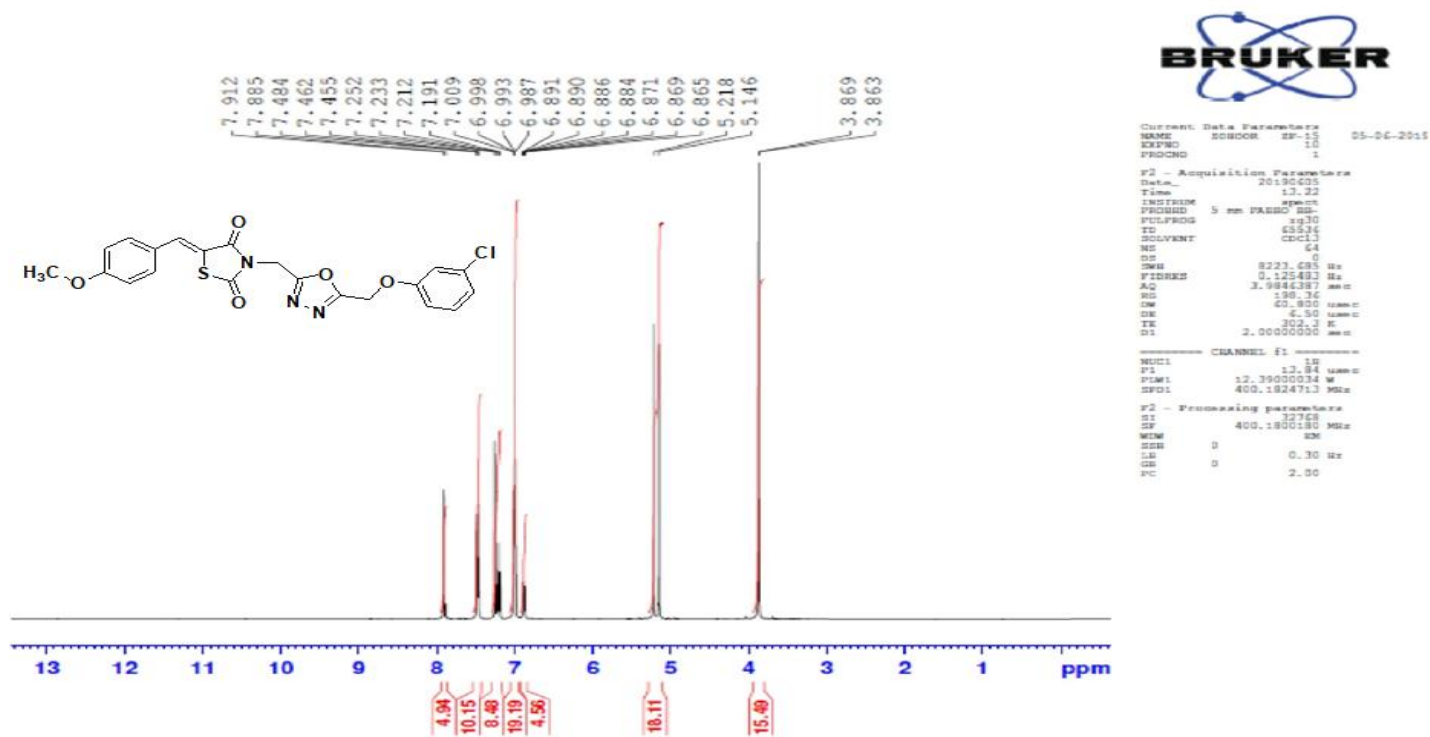
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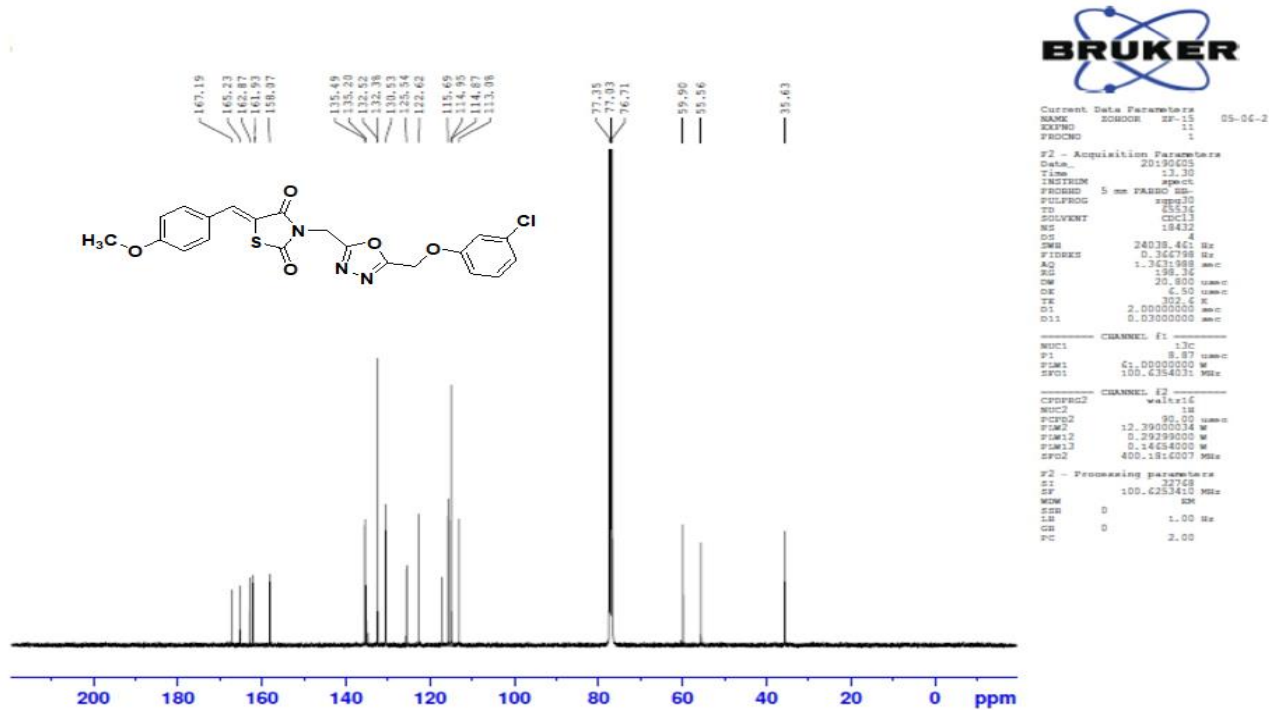
¹H NMR of compound 16



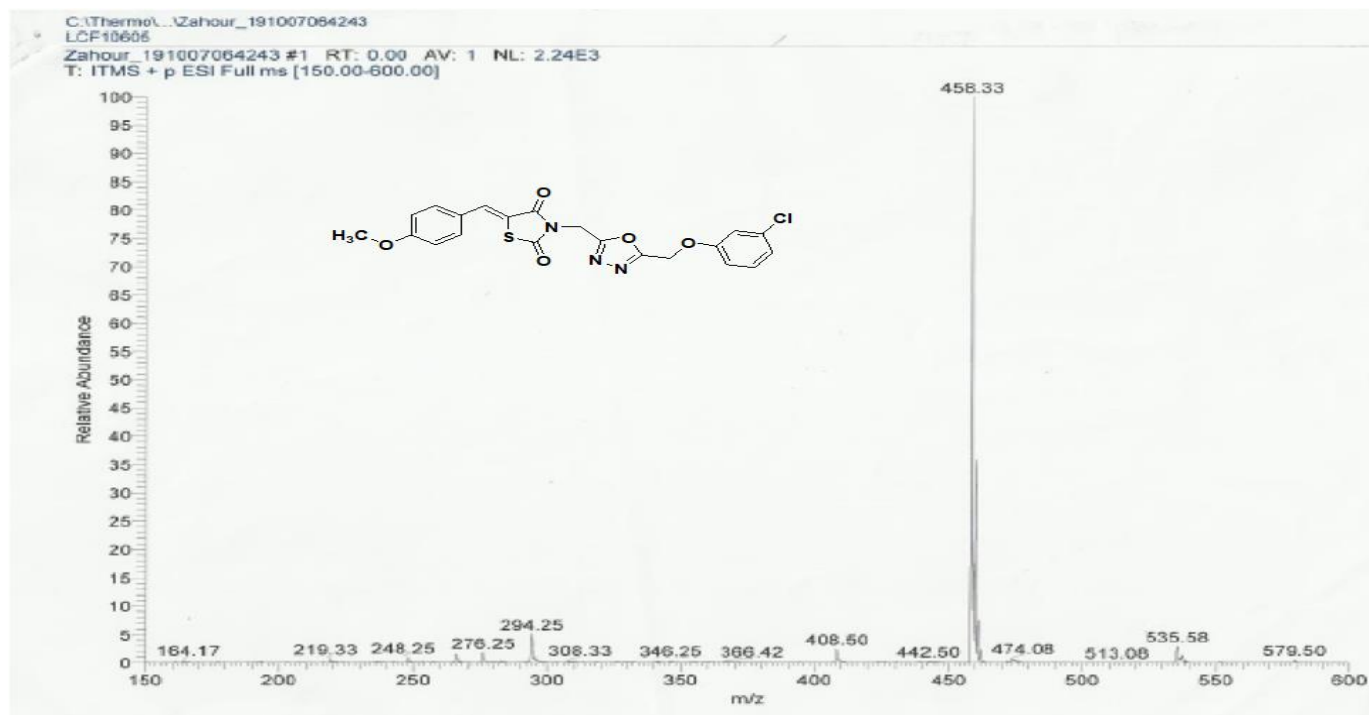
Mass of compound 16



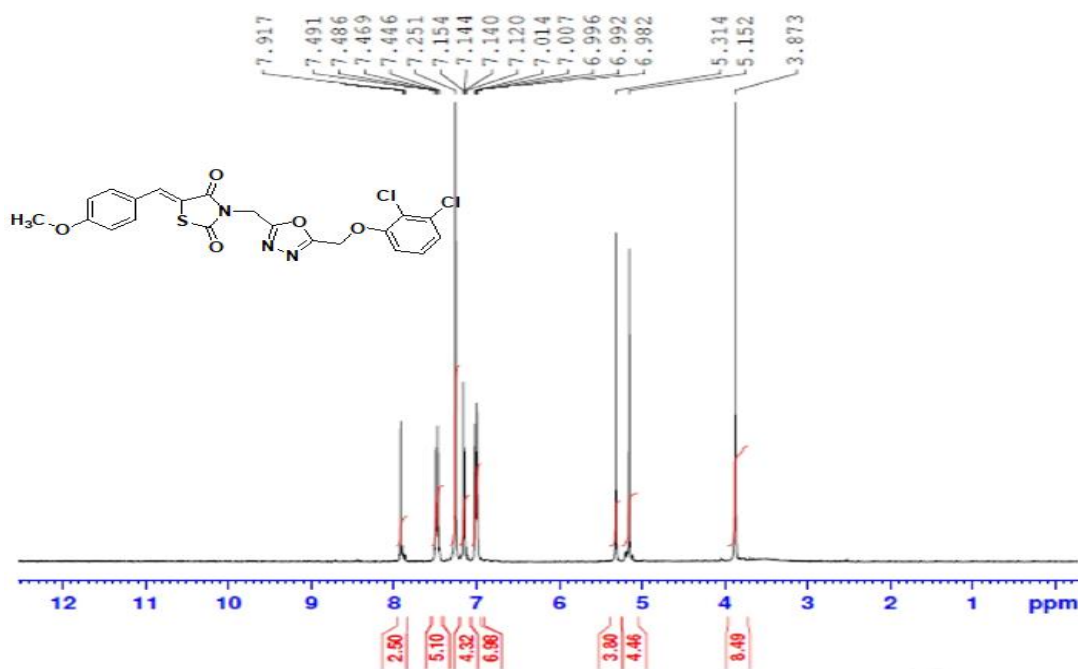
¹H NMR of compound 17



¹³C NMR of compound 17



Mass of compound 17



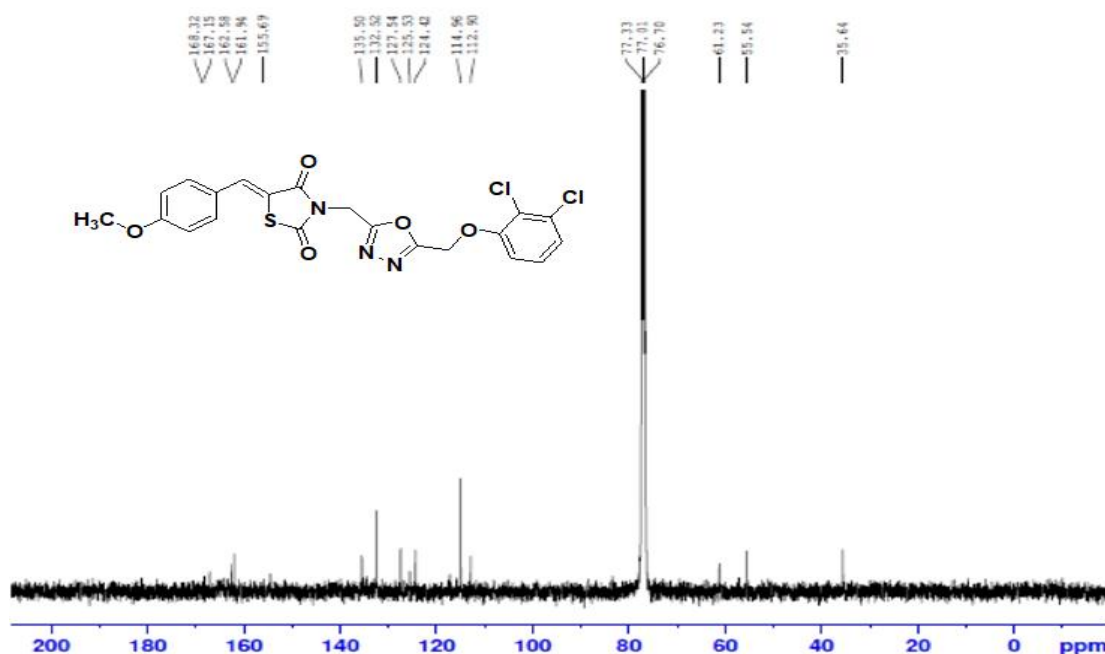
Current Data Parameters
NAME ZOSBOOR ZF-11 03-06-20
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190603
Time 11.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl₃
NS 128
DS 4
SWH 8223.481 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 384
DM 60.800 usec
DE 6.50 usec
TE 302.2 K
D1 2.00000000 sec

CHANNEL F1
NUC1 13C
P1 12.84 usec
PLM1 12.39000000 W
SFO1 400.1824713 MHz

F2 - Processing parameters
SI 32768
SF 400.1800150 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

¹H NMR of compound 18



Current Data Parameters
NAME ZOSBOOR ZF-11
EXPNO 11
PROCNO 1

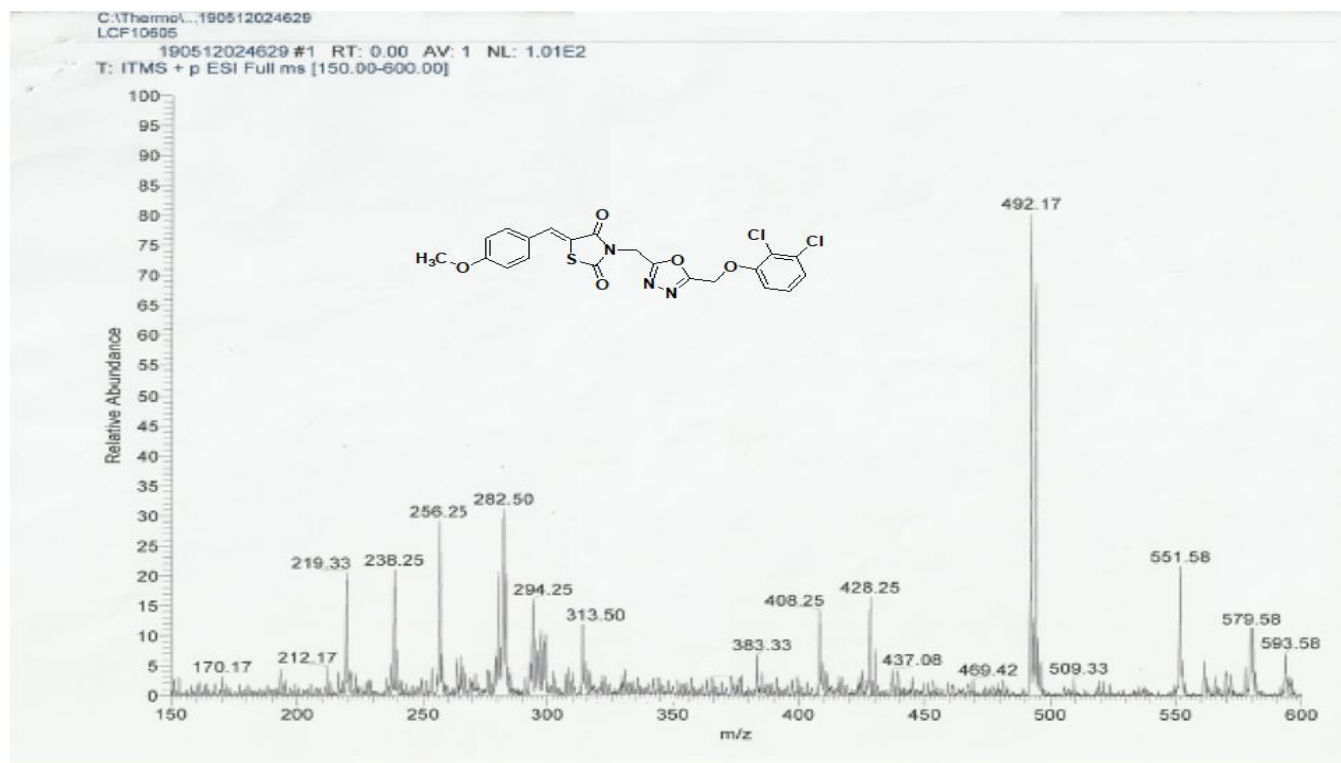
F2 - Acquisition Parameters
Date_ 20190603
Time 11.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl₃
NS 18432
DS 4
SWH 24038.461 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 384
DM 60.800 usec
DE 6.50 usec
TE 302.2 K
D1 2.00000000 sec
D11 0.53000000 sec

CHANNEL F1
NUC1 13C
P1 9.87 usec
PLM1 12.39000000 W
SFO1 100.6254031 MHz

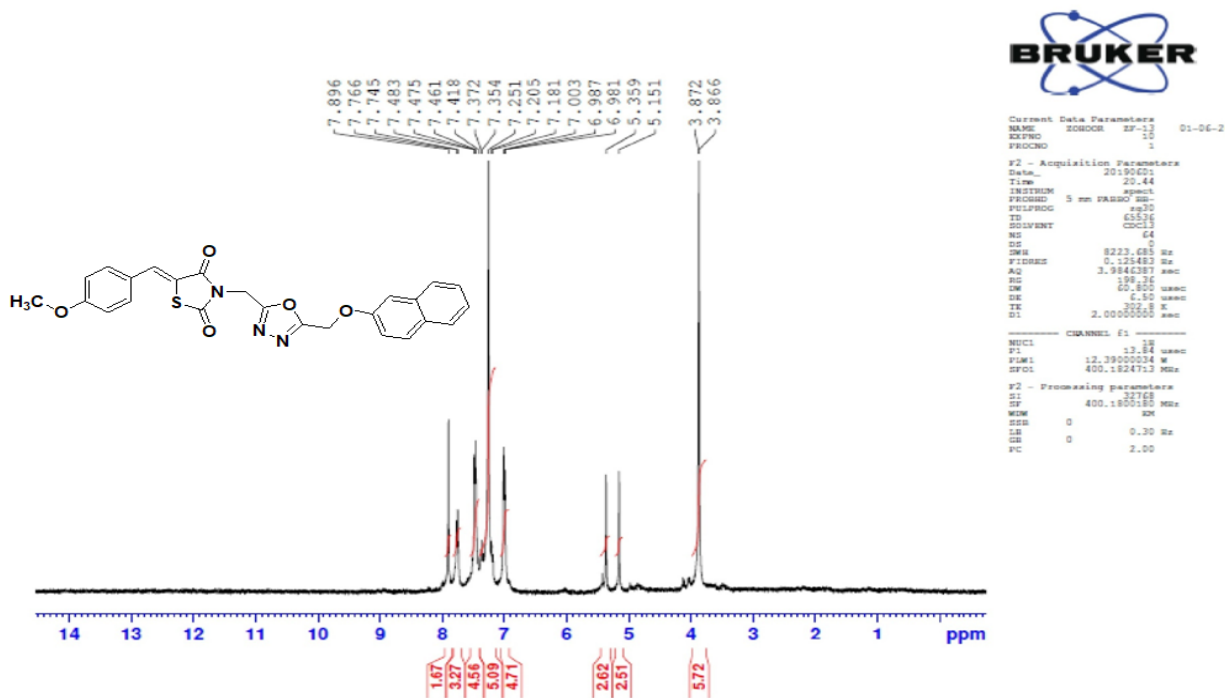
CHANNEL F2
CPDPRG2 waltz16
NUC2 13C
PCPD2 90.00 usec
PLM2 12.39000000 W
PLM12 0.232290000 W
PLM13 0.146340000 W
SFO2 400.1816007 MHz

F2 - Processing parameters
SI 32768
SF 100.6254031 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 2.00

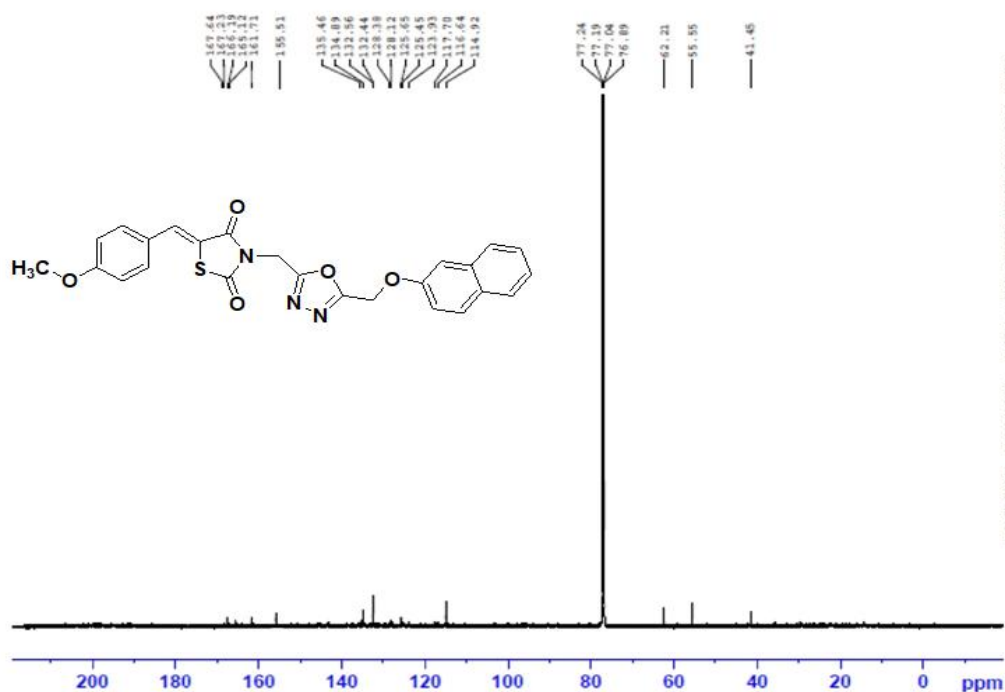
¹³C NMR of compound 18



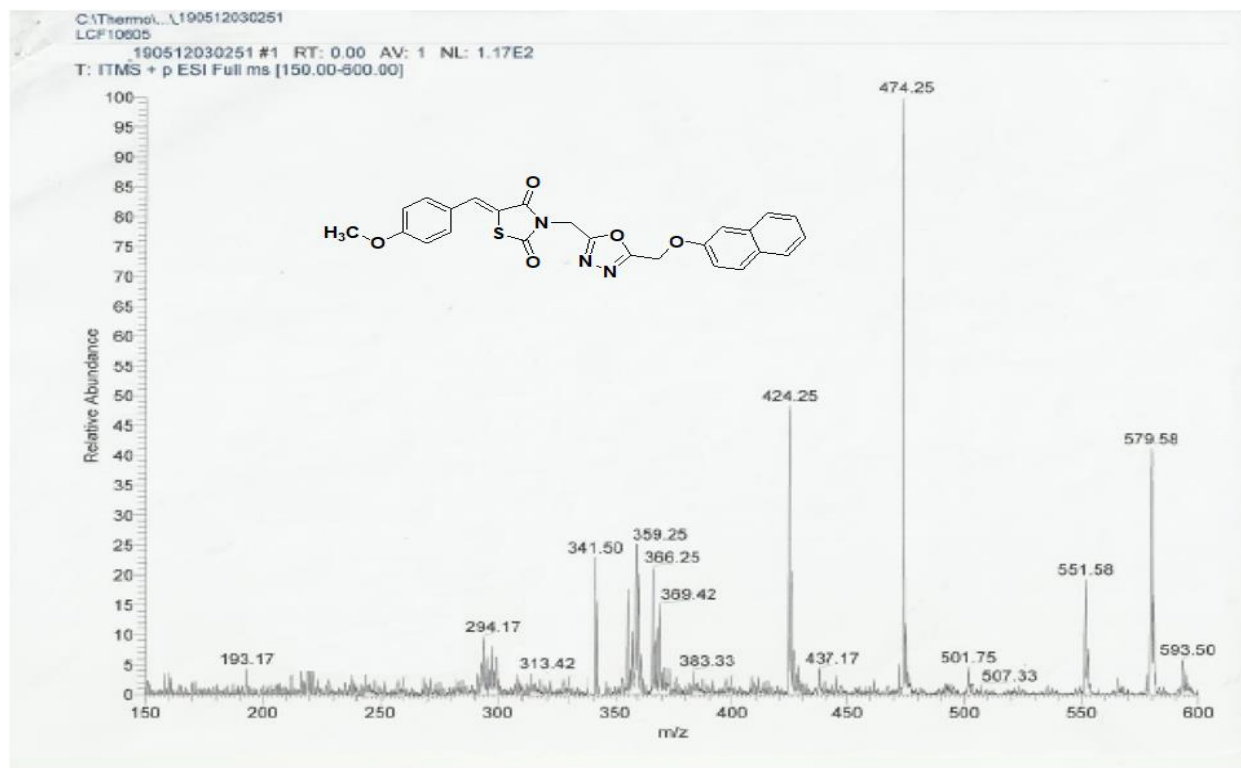
Mass of compound 18



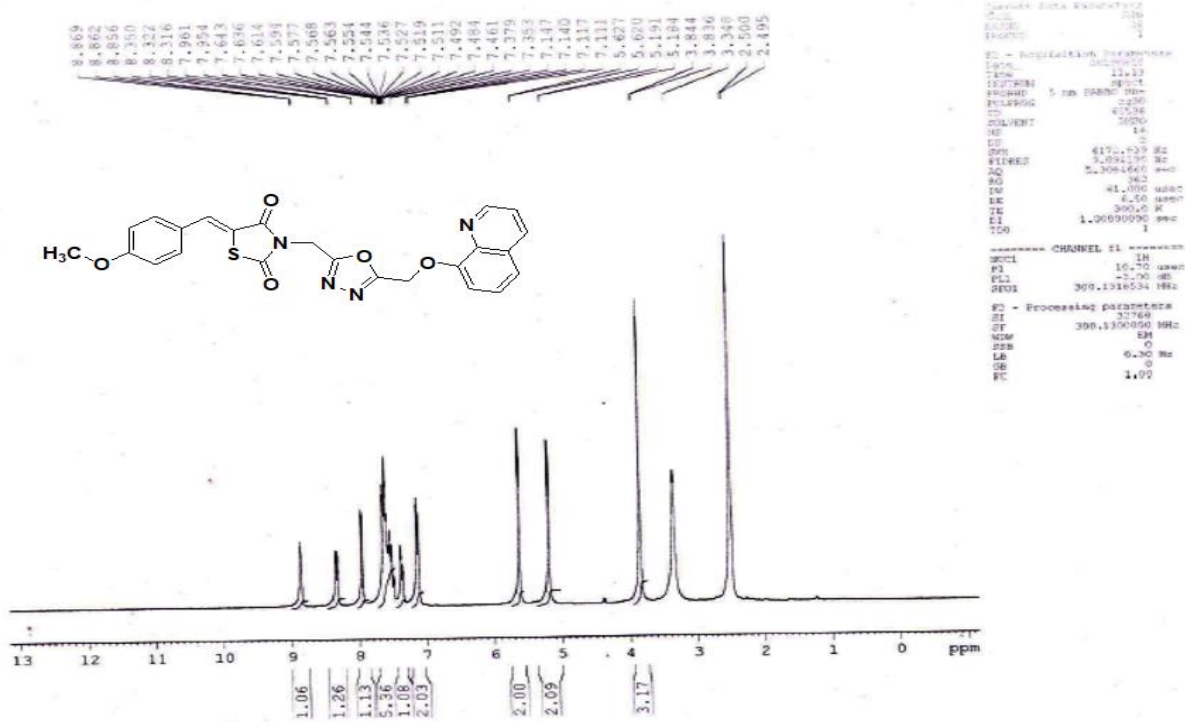
¹H NMR of compound 20



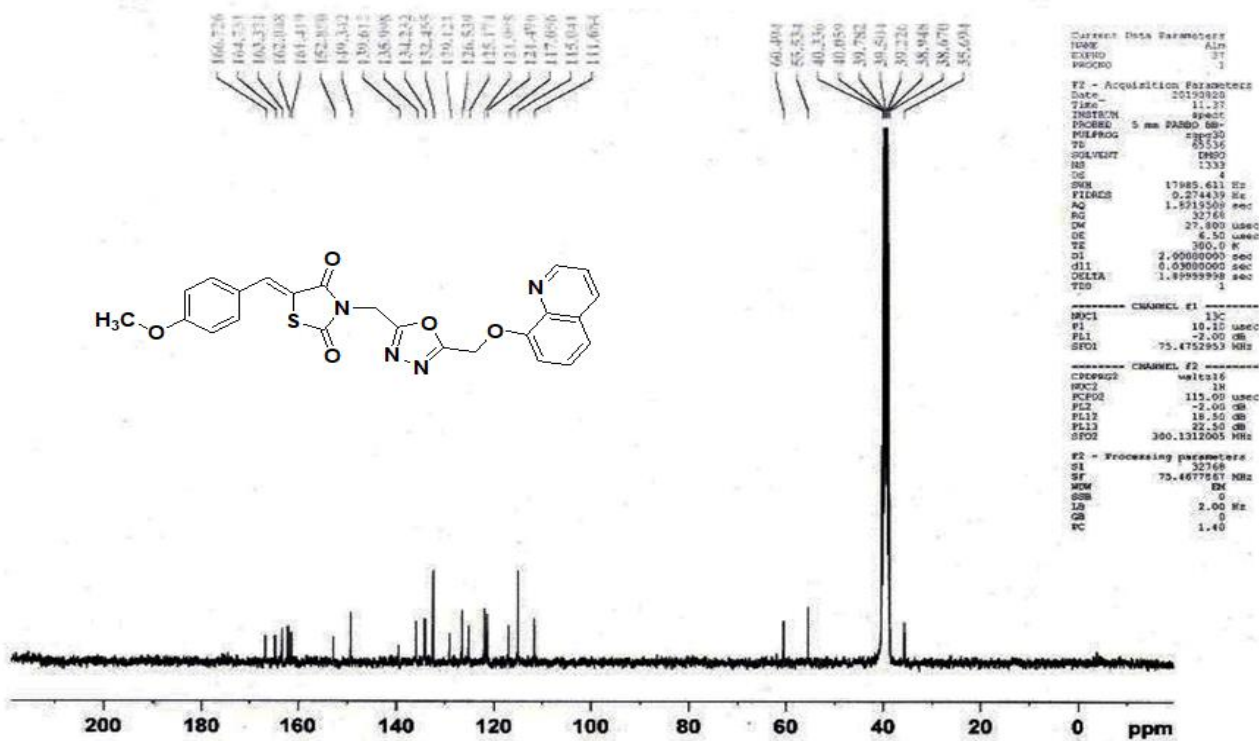
¹³C NMR of compound 20



Mass of compound 20



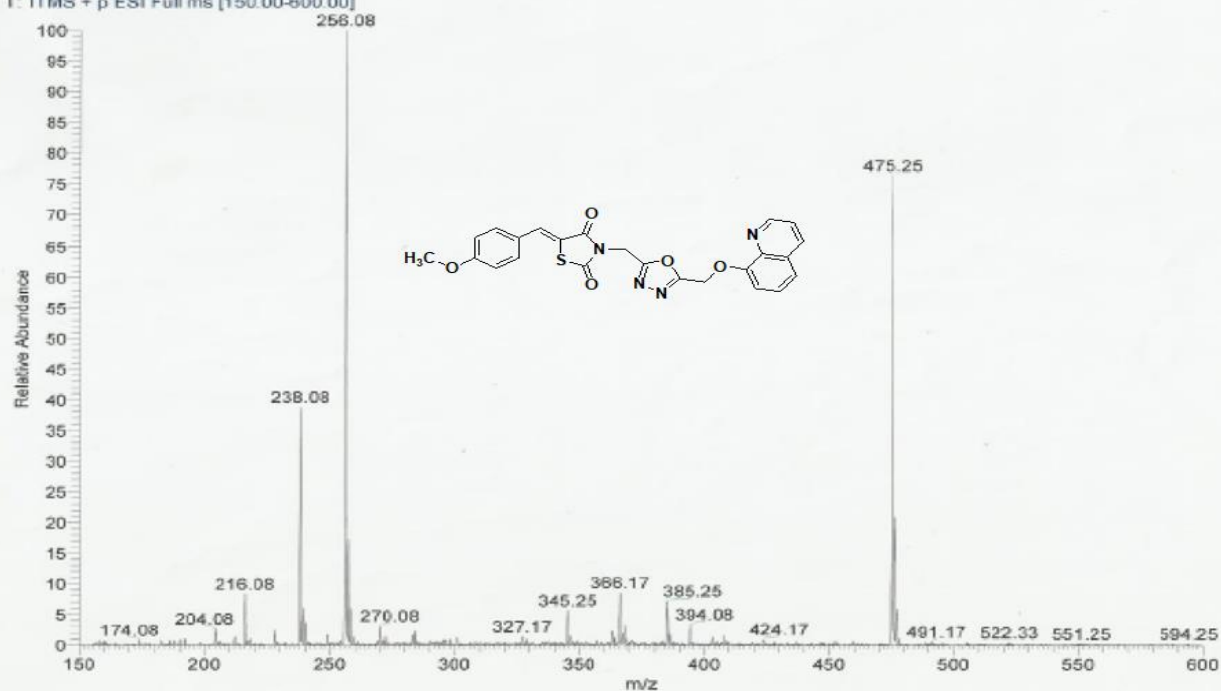
¹H NMR of compound 21



¹³C NMR of compound 21

C:\Thermol_190512030727
LCF10605

190512030727 #1 RT: 0.00 AV: 1 NL: 2.94E2
T: ITMS + p ESI Full ms [150.00-600.00]



Mass of compound 21