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Coumarin-based derivatives targeting *Trypanosoma cruzi* cruzain and *Trypanosoma brucei* cathepsin L-like proteases

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**** SUPPLEMENTARY INFORMATION ****

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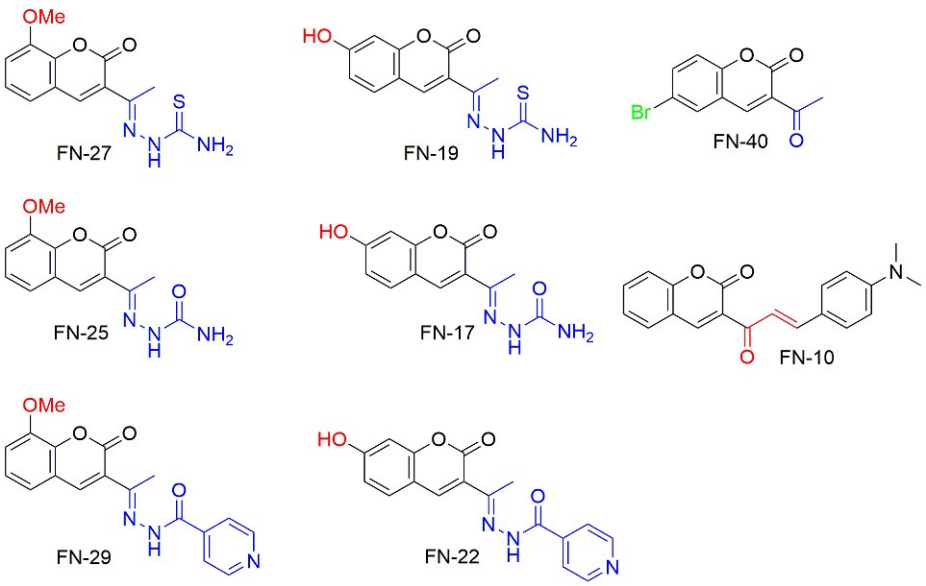

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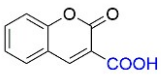

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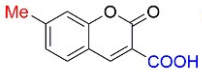

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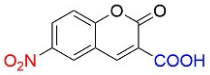

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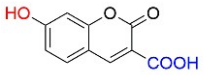

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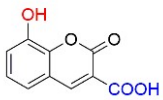

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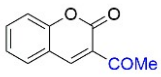

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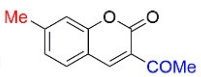

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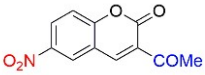

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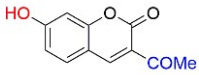

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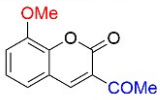

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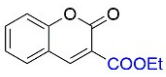

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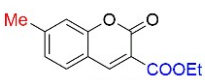

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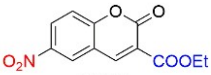

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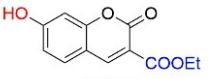

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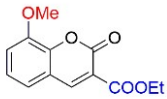

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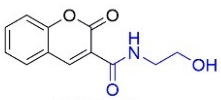

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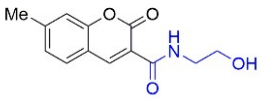

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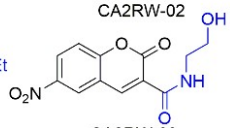

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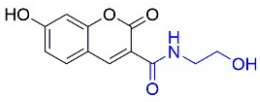

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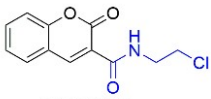

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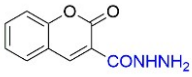

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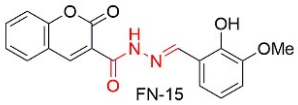

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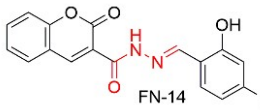

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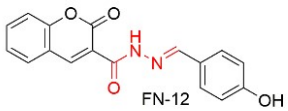
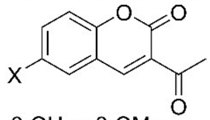


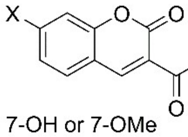
FN-12

FN-09 or FN-11

Coumarins-3-cetone derivatives



X - 6-OH or 6-OMe



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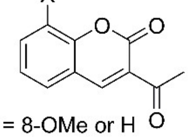
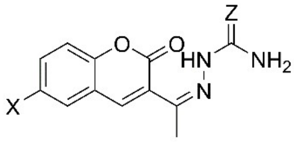


X = 8-OMe or H



Z = O, S or NH
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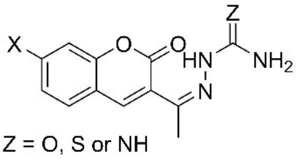

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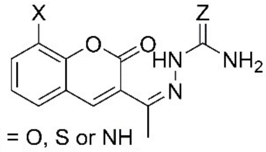

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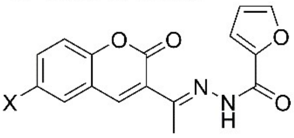

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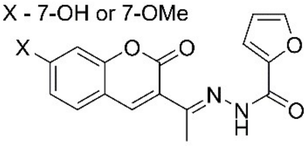

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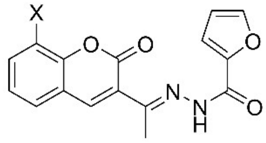

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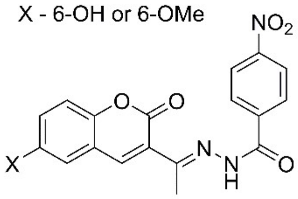

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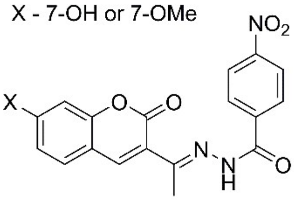

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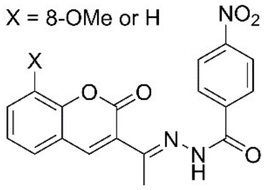

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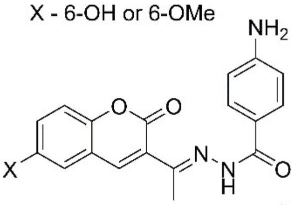

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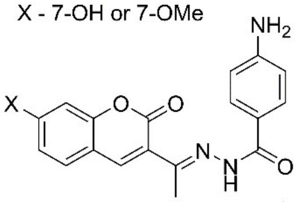

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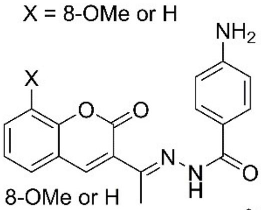

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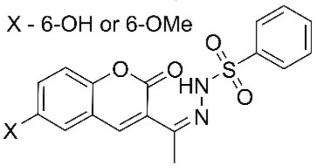

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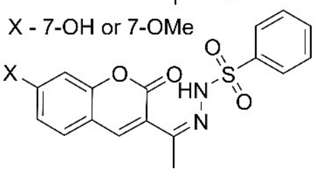

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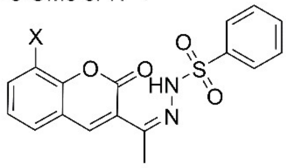

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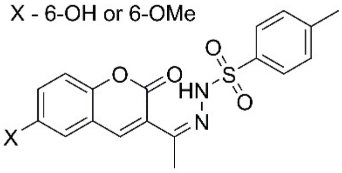

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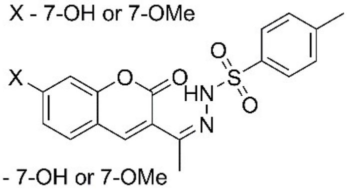

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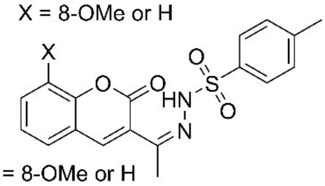

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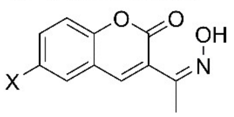

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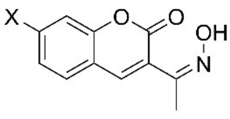

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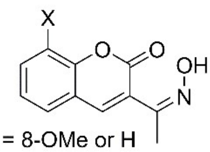

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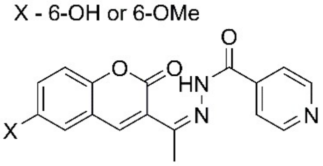

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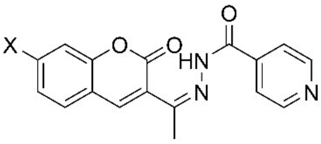

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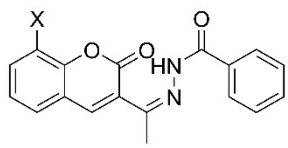
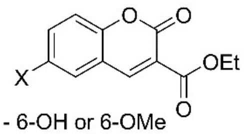


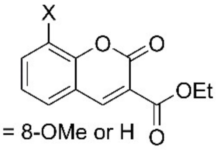
X = 8-OMe or H

3-coumarins-esthers derivatives



X - 6-OH or 6-OMe

X - 7-OH or 7-OMe



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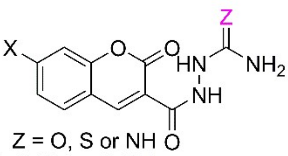
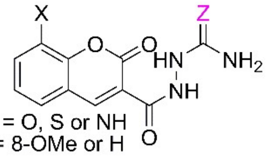
Z = O, S or NH
X - 6-OH or 6-OMe



Z = O, S or NH
X - 7-OH or 7-OMe



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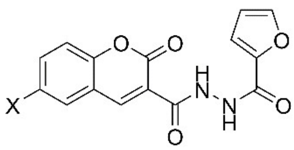
X = 8-OMe or H



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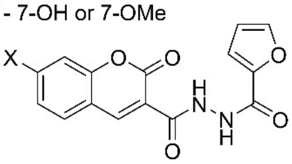

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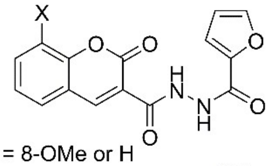

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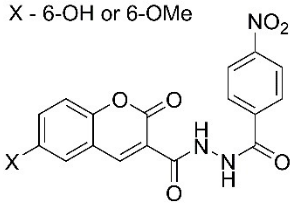
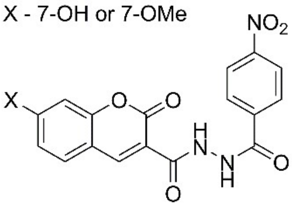


X - 6-OH or 6-OMe



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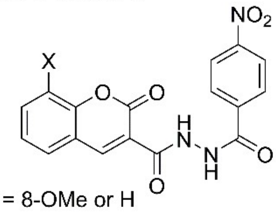

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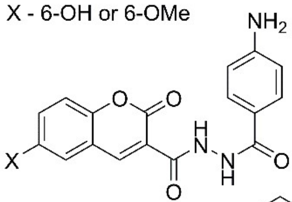

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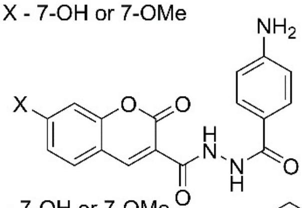

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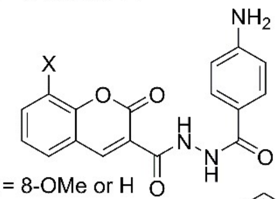

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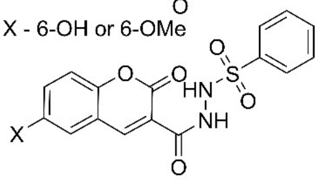

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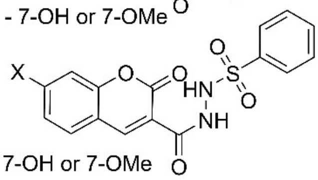

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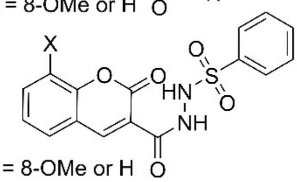

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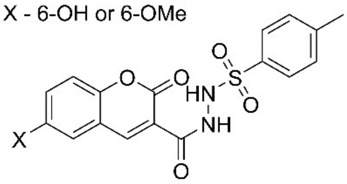

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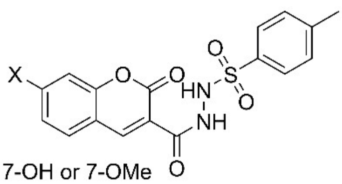

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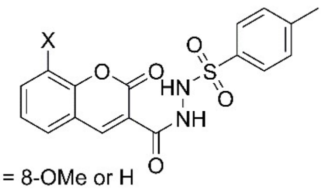

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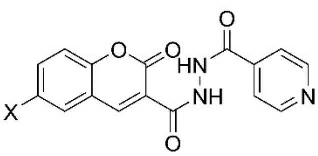
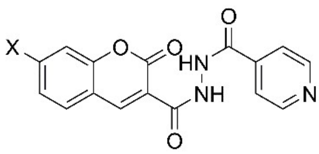
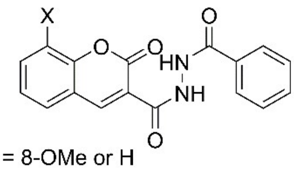


X - 6-OH or 6-OMe



X - 7-OH or 7-OMe



X = 8-OMe or H

Table 1S. Lipinski rule of five parameters for the most promising coumarin analogs.

Compound	Molecular weight (g/mol)	Nº H-bond acceptors	Nº H-bond donors	Log $P_{o/w}$	Violations
FN-06	204.18	4	1	1.55	0
FN-07	218.21	4	0	1.83	0
FN-10	319.35	3	0	3.53	0
FN-17	261.23	5	3	0.64	0
FN-19	277.30	4	3	1.40	0
FN-25	275.26	5	2	1.09	0
FN-27	291.33	4	2	1.57	0
FN-29	337.33	6	1	2.25	0
LS-04	309.27	7	5	-0.16	0
MP-01	218.21	4	0	2.17	0
MP-03	233.18	5	0	1.03	0
MP-04	202.21	1	3	2.30	0
MP-05	188.18	3	0	1.86	0
RW-01	233.22	4	2	1.21	0
RW-02	247.25	4	2	1.53	0
RW-03	278.22	6	2	0.41	0
RW-04	249.22	5	3	0.79	0
RW-05	251.67	3	1	2.21	0

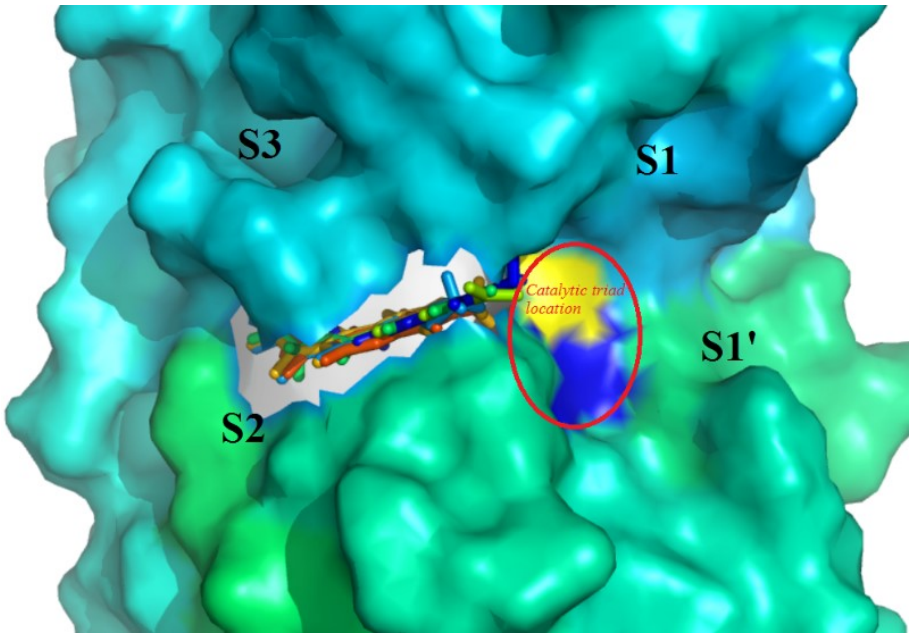


Fig. 1S – Coumarin moieties into S2 subsite from the cysteine protease.

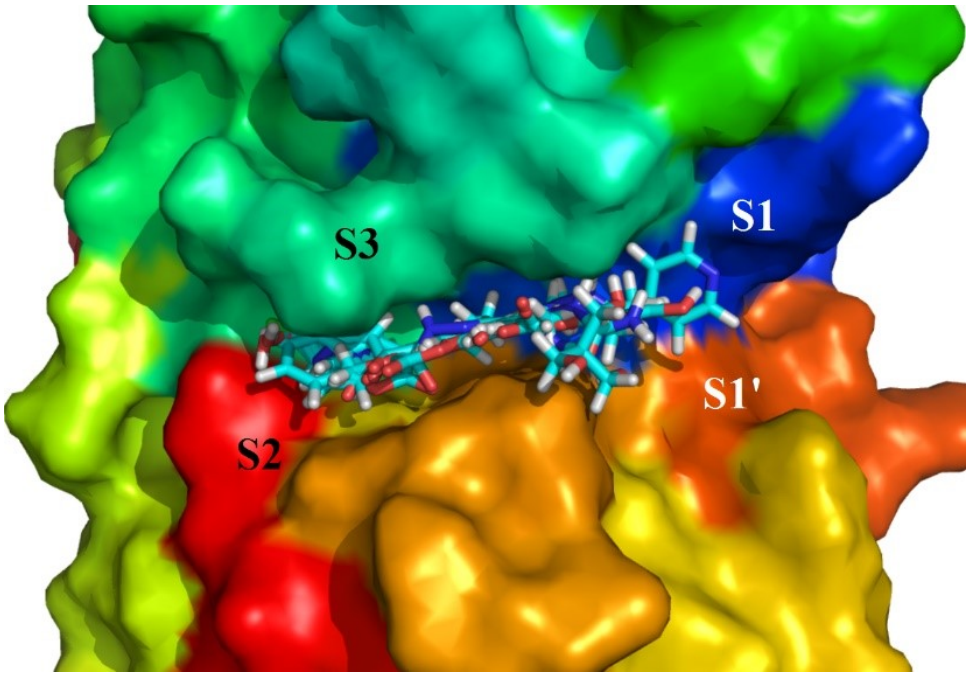


Fig. 2S – Coumarin-based compounds in complex with cysteine protease, except FN-27.

Table 2S. All *Trypanosoma brucei* proteins/enzymes investigated as potential targets for FN-10.

PDB entry	Protein/Target Name	FitScore
3NVL	2,3-Bisphosphoglycerate-Independent Phosphoglycerate Mutase	41.73
4BI9	3-Ketoacyl-CoA Thiolase, Putative	58.49
16PK	3-Phosphoglycerate Kinase	38.37
3EB9	6-Phosphogluconolactonase	50.84
5C5V	Acidocalcisomal Pyrophosphatase	35.53
5VN4	Adenine Phosphoribosyltransferase, Putative	71.81
3H9U	Adenosylhomocysteinase	72.44
4EFC	Adenylosuccinate Lyase	61.54
7DL8	ALBA1-Domain Protein	56.90
4EFD	Aminopeptidase	21.00
4LWO	Arginine *N*-Methyltransferase, Putative	50.74
4LNS	Asparagine Synthetase A	42.78
4W5K	Aspartate Aminotransferase, Mitochondrial	52.99
4I15	Class 1 Phosphodiesterase PDEB1	53.09
4HWY	Cysteine Peptidase C (CPC)	45.32
4DK2	Deoxyuridine Triphosphatase	54.07
5XFW	Dihydroorotate Dehydrogenase (Fumarate)	58.19
2HKE	Diphosphomevalonate Decarboxylase, Putative	52.37
6GIM	DNA Duplex	76.32
2PTW	Enolase	55.65
6R36	Farnesyl Pyrophosphate Synthase	28.67
1F2J	Fructose-Bisphosphate Aldolase, Glycosomal	49.33
3DWV	Glutathione Peroxidase-Like Protein	83.31
4P8R	Glyceraldehyde 3-Phosphate Dehydrogenase, Cytosolic	66.22
2VEI	Glycosomal Triosephosphate Isomerase	30.98
3O6O	Heat Shock Protein 83	77.99
6IF4	Histone Acetyltransferase	46.19
6MXC	Hypoxanthine-Guanine Phosphoribosyltransferase	57.42
4I70	Inosine-Adenosine-Guanosine-Nucleoside Hydrolase	57.08
6IA7	Intraflagellar Transport Protein 22	55.59
3ESF	Iron-Containing Superoxide Dismutase B2	47.47
7E3N	Isocitrate Dehydrogenase [NADP]	58.77
5NTD	Leucyl Aminopeptidase	75.89
5L9A	L-Threonine 3-Dehydrogenase	78.75
4AFP	Metacaspase Mca2	53.94
4ZT6	Methionyl-Trna Synthetase	68.23
4EU1	Mitochondrial Aspartate Aminotransferase	52.35
2GIA	Mitochondrial Rna-Binding Protein 1	35.93
3I3G	*N*-Acetyltransferase	56.83
4FKY	Nucleoside Diphosphate Kinase	74.35
4BP8	Oligopeptidase B	47.45
1SZR	Ornithine Decarboxylase	47.79
3JV1	P22 Protein	44.26
6GMP	Parvulin 42	47.02
6SPT	Peroxin 14	64.39
3CVN	Peroxisome Targeting Signal 1 Receptor	27.49

5H2R	Phosphodiesterase	69.17
1VBJ	**Prostaglandin $F_{2\alpha}$ Synthase**	**85.80**
1YAR	Proteasome Alpha Subunit	66.87
6GEY	Pteridine Reductase	65.26
3ZS7	Pyridoxal Kinase	41.16
4KCU	Pyruvate Kinase 1	50.32
1FX2	Receptor-Type Adenylate Cyclase Gresag 4.1	62.14
3BNW	Riboflavin Kinase, Putative	46.27
6FXS	Ribose 5-Phosphate Isomerase, Putative	57.86
4NLB	Ribosomal RNA Processing Protein 6	59.46
7C45	RNAse D Complex with RNA U12	42.13
5TVM	S-Adenosylmethionine Decarboxylase Alpha Chain	60.84
3LSS	Seryl-Trna Synthetase	58.31
3G1Q	Sterol 14-Alpha-Demethylase	76.90
6LP1	Succinyl-CoA:3-Ketoacid-Coenzyme A Transferase	53.01
5KLH	Surface Glycoprotein	48.77
1R26	Thioredoxin	60.95
5FUW	Thymdine Kinase	68.69
6TIM	Triosephosphate Isomerase	59.97
2WOW	Trypanothione Reductase	67.44
6GXY	Tryparedoxin	64.91
4B6M	Tubulin-Specific Chaperone, Putative	54.86
3M4U	Tyrosine Specific Protein Phosphatase, Putative	36.76
1GY8	UDP-Galactose 4-Epimerase	64.69
4BQH	UDP-N-Acetylglucosamine Pyrophosphorylase	50.79
3GUE	UTP-Glucose-1-Phosphate Uridylyltransferase 2	62.07

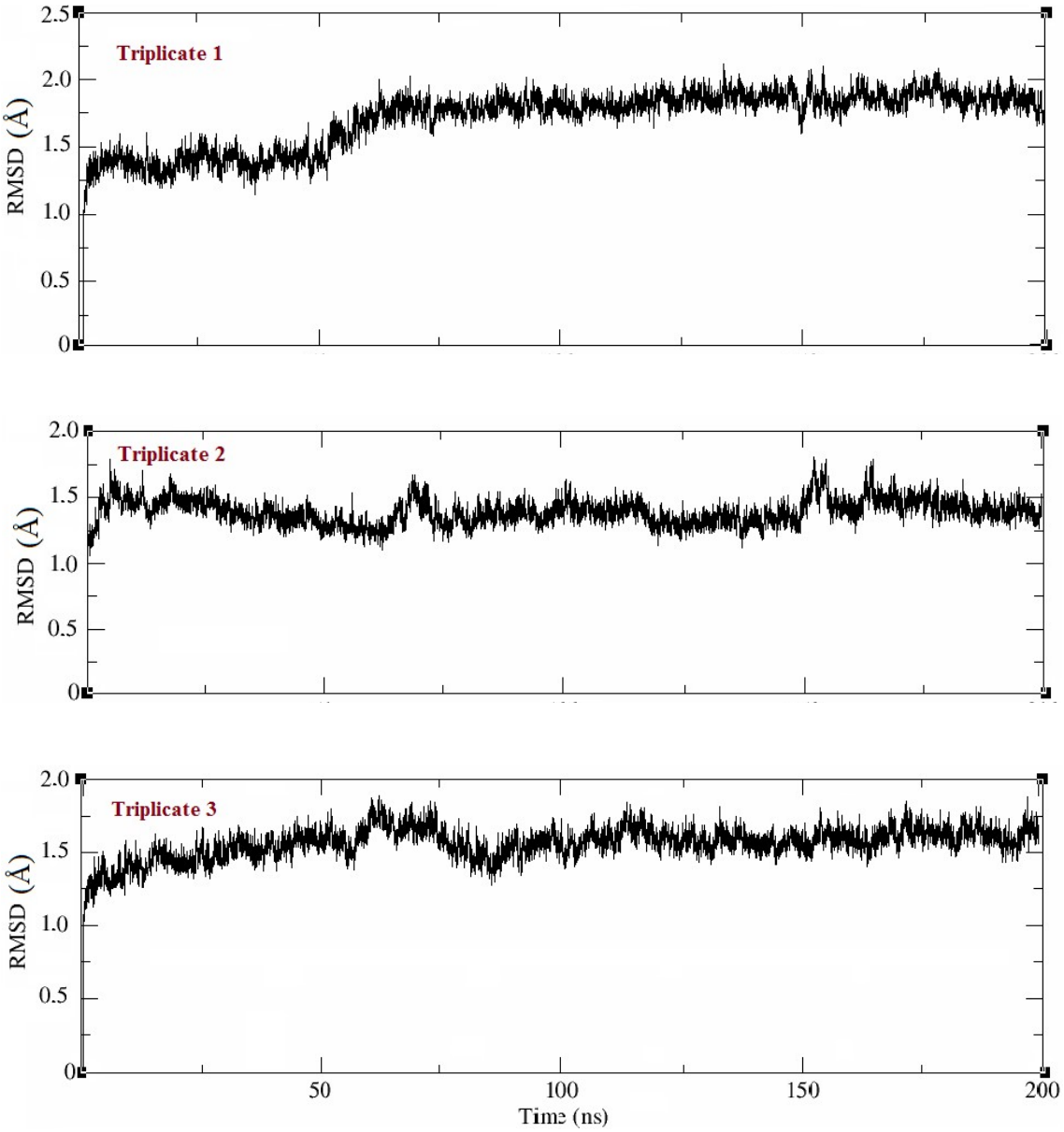
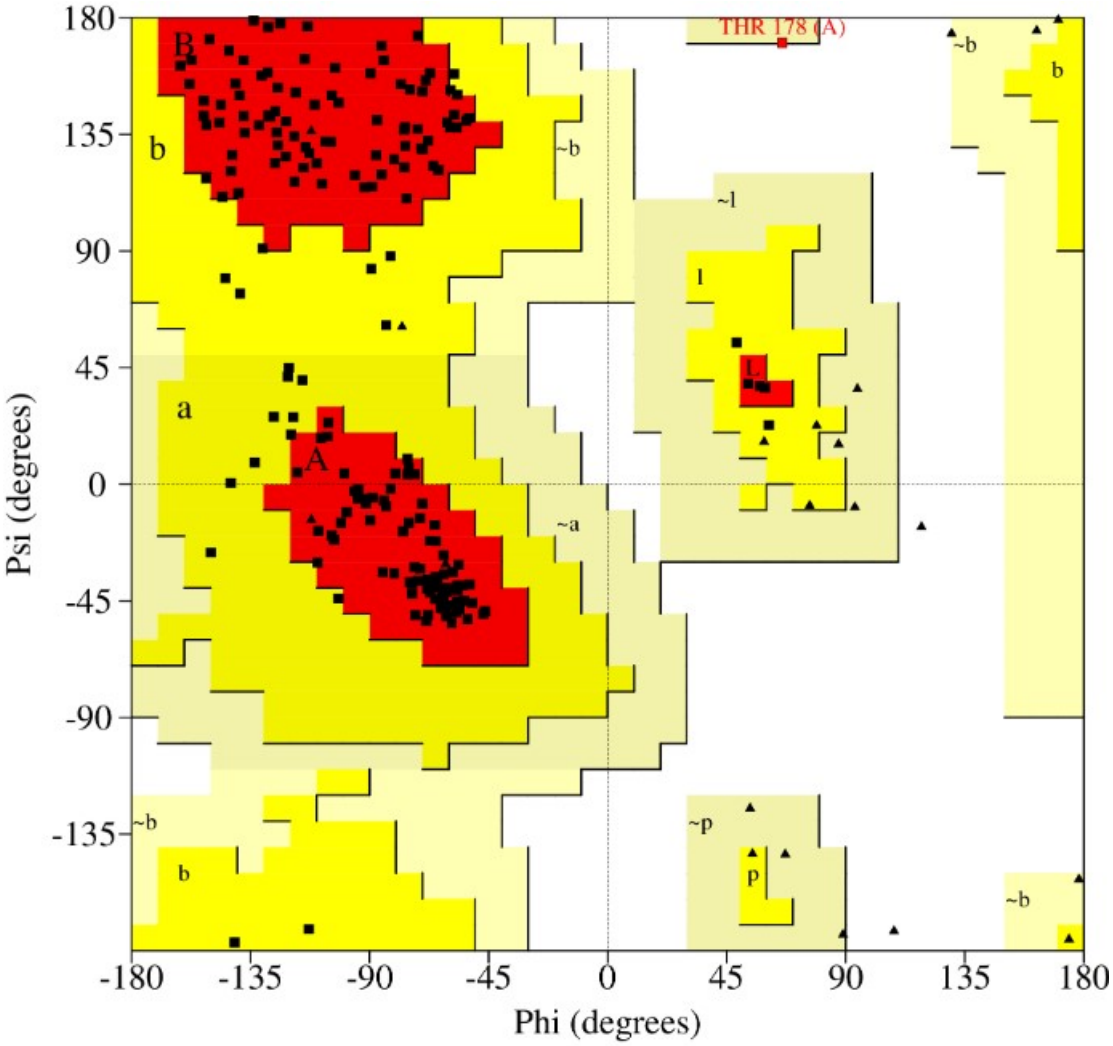


Fig. 3S – RSMD charts of dynamics simulations performed in triplicates using *Tbr*CATL as model.

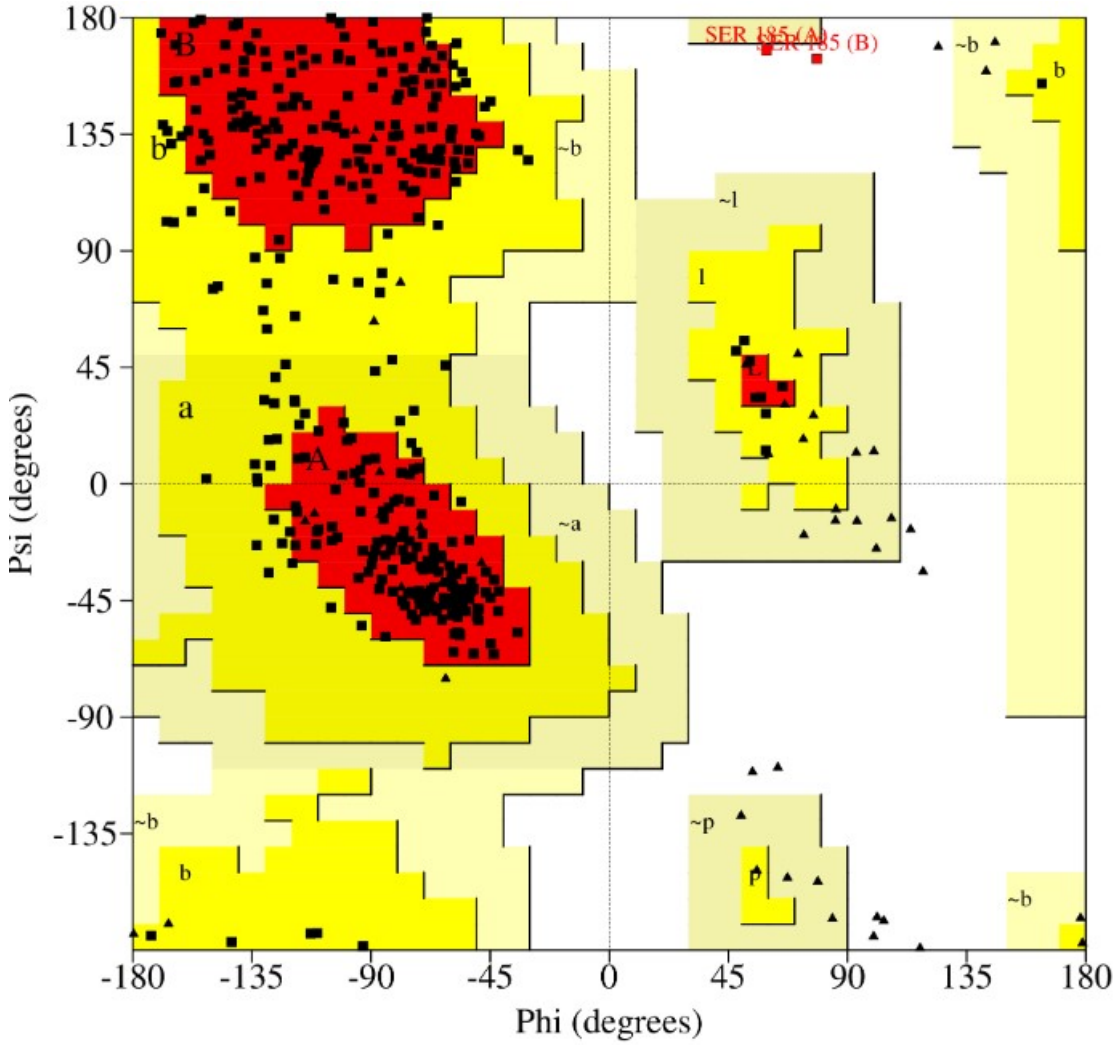
Fig. 4S – Ramachandran plot for CRZ-FN27 complex.



Plot statistics

Residues in most favoured regions [A,B,L]	161	88.0%
Residues in additional allowed regions [a,b,l,p]	21	11.5%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.5%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	183	100.0%

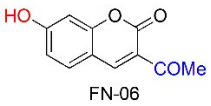
Fig. 5S – Ramachandran plot for *Tbr*CATL-FN27 complex.



Plot statistics

Residues in most favoured regions [A,B,L]	285	79.2%
Residues in additional allowed regions [a,b,l,p]	73	20.3%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	2	0.6%
Number of non-glycine and non-proline residues	360	100.0%

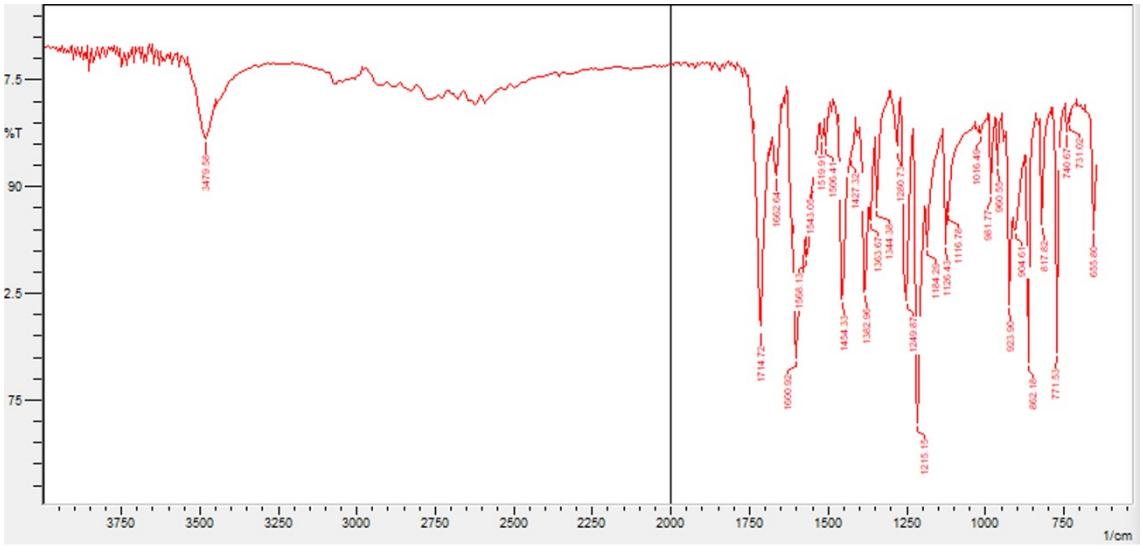
Chemical Characterization



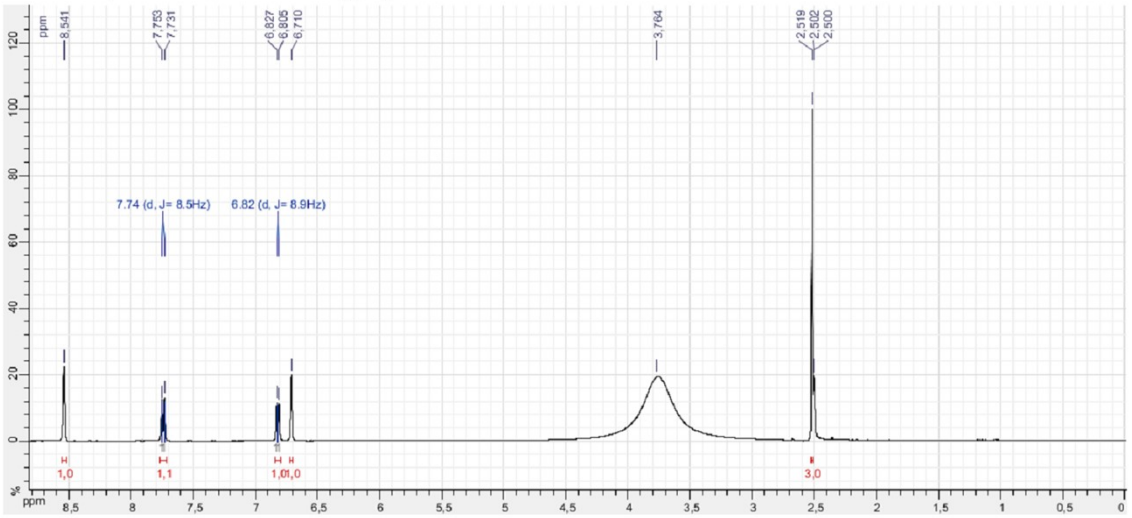
FN-06

3-Acetyl-7-hydroxy-2H-chromen-2-one ***(FN-06)***

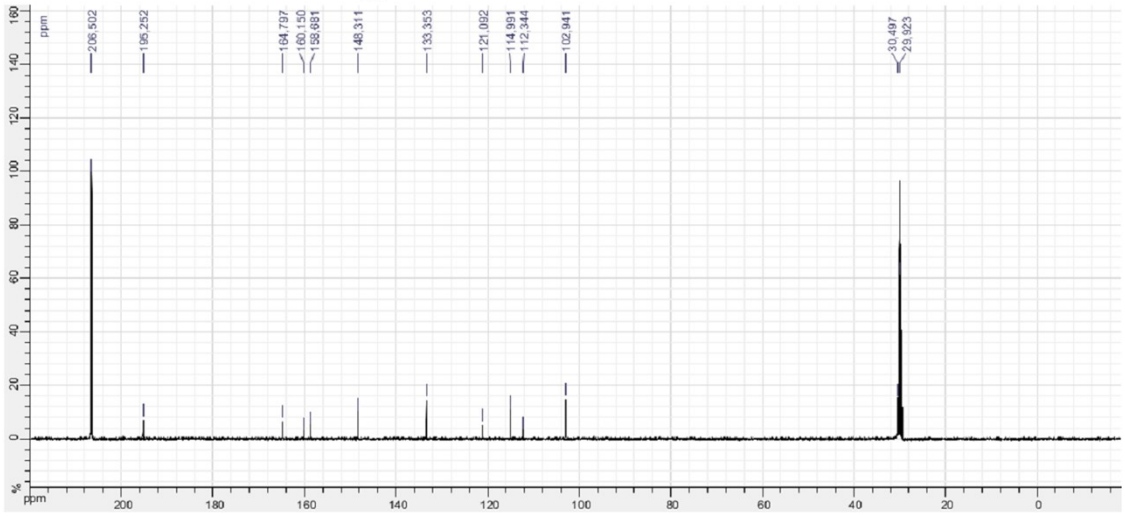
ATR-FTIR



^{1}H NMR (400 MHz, DMSO-d_6, ppm)



^{13}C NMR (100 MHz, Acethone-d_6, ppm)



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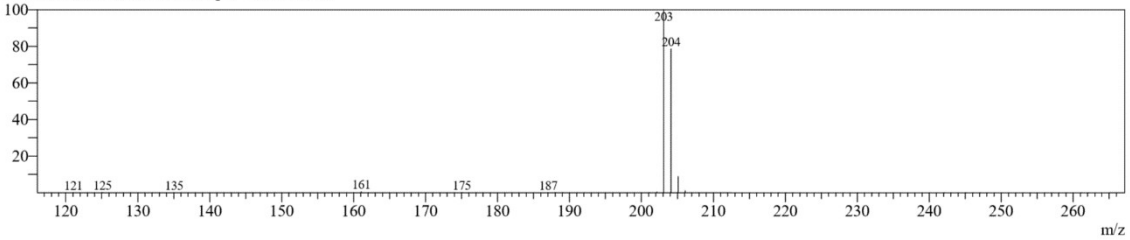
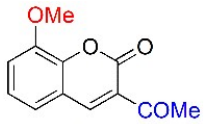
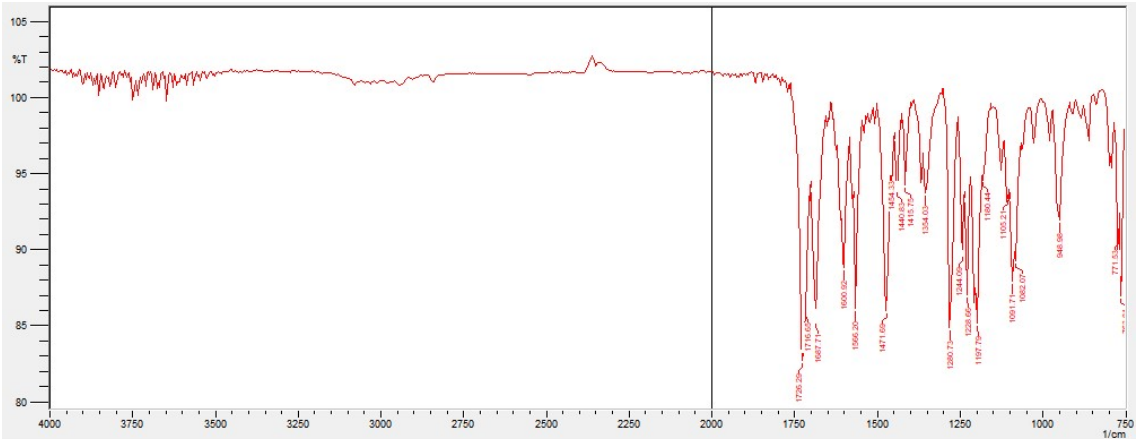




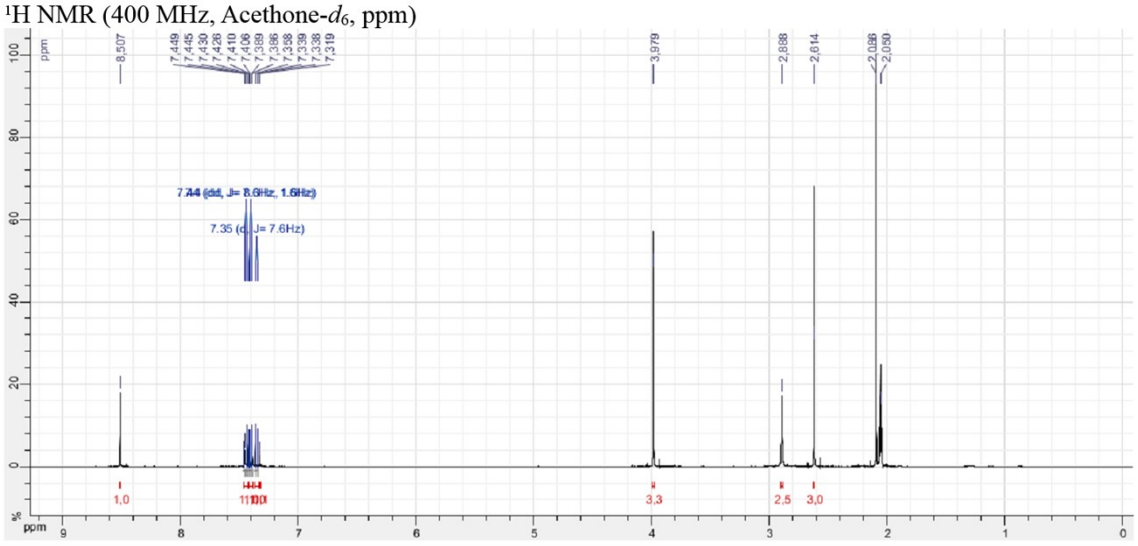
FN-07

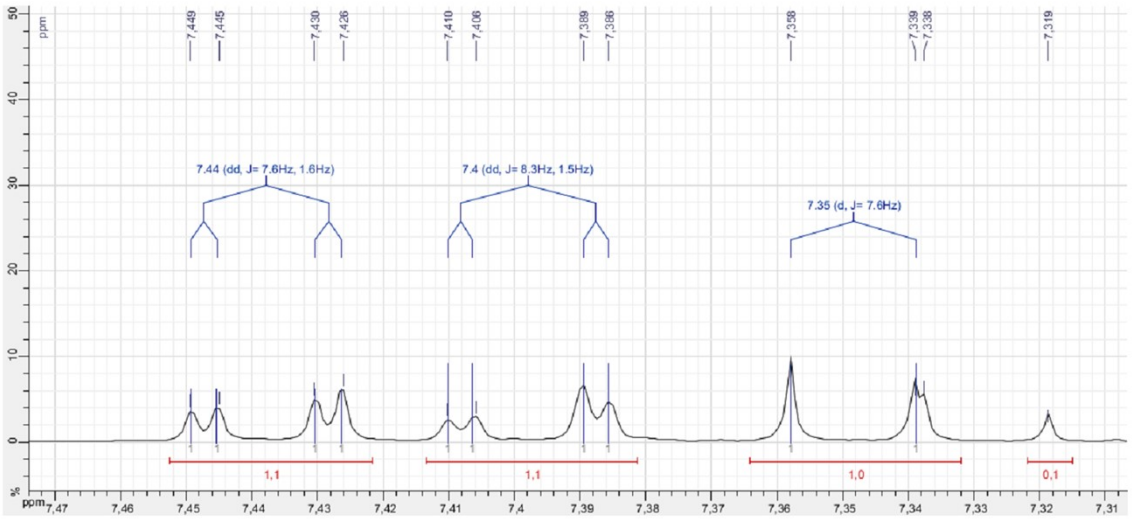
3-Acetyl-8-methoxy-2H-chromen-2-one ***(FN-07)***

ATR-FTIR

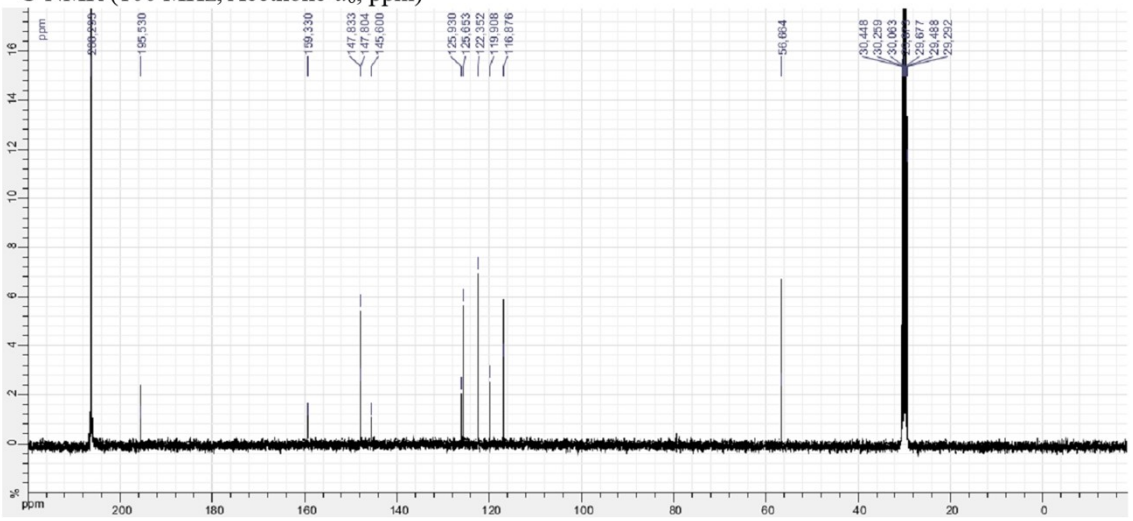


^{1}H NMR (400 MHz, Acethone-d_6, ppm)





^{13}C NMR (100 MHz, Acethone-d_6, ppm)



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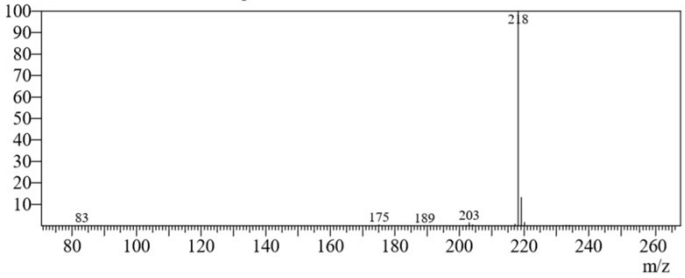
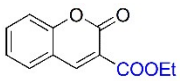
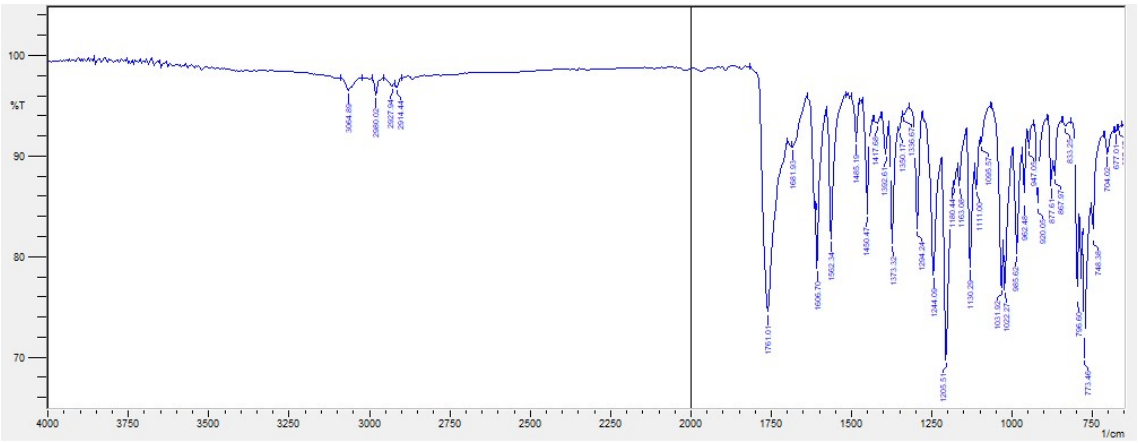




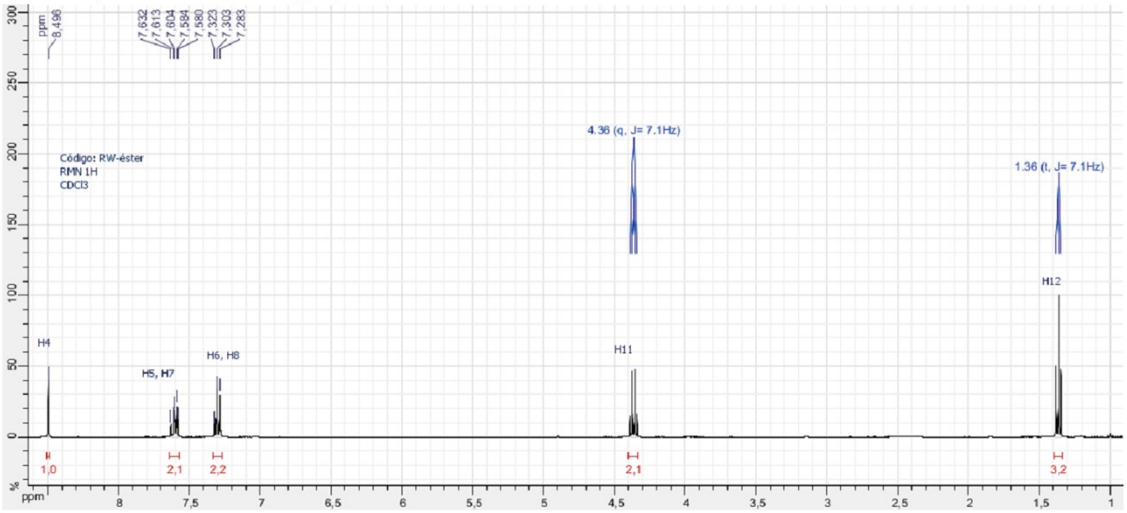
MP-01

Ethyl 2-oxo-2H-chromene-3-carboxylate ***(MP-01)***

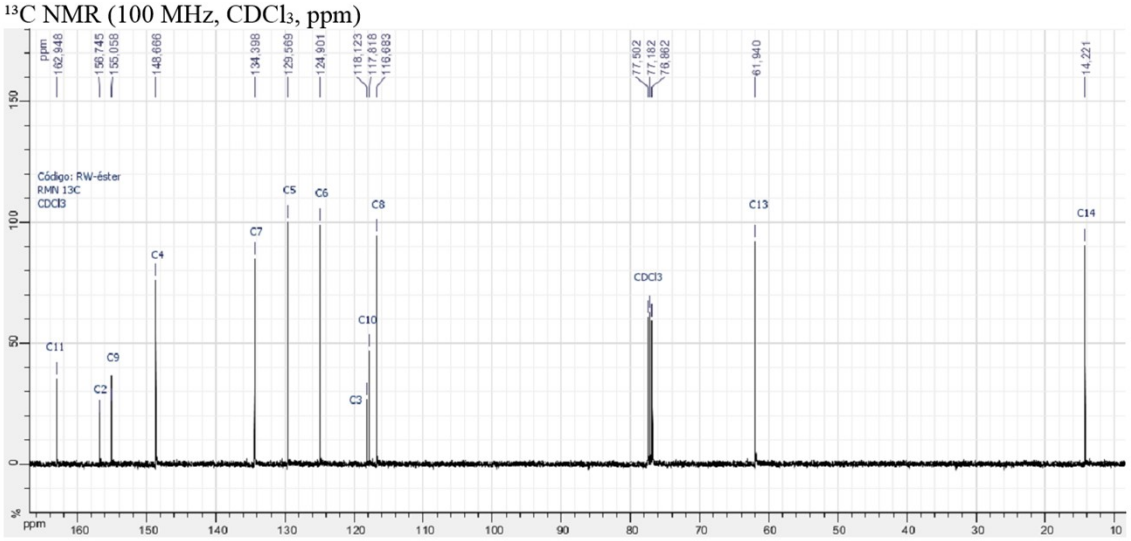
ART-FTIR



^{1}H NMR (400 MHz, $CDCl_3$, ppm)



^{13}C NMR (100 MHz, $CDCl_3$, ppm)



MS

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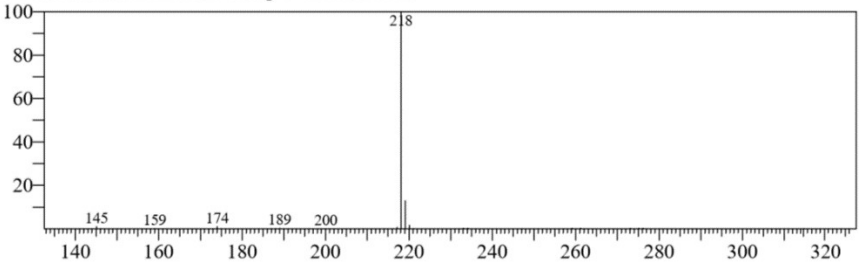
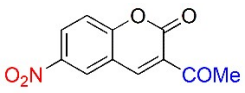
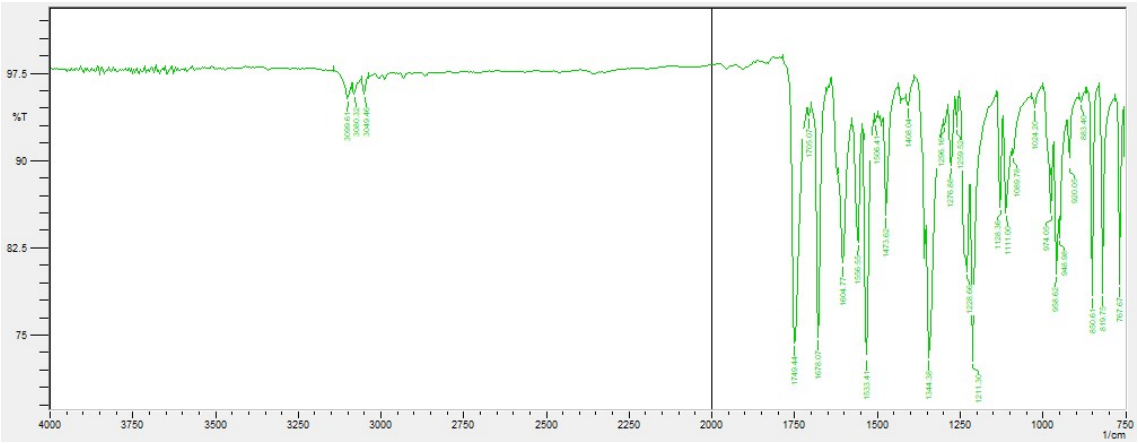




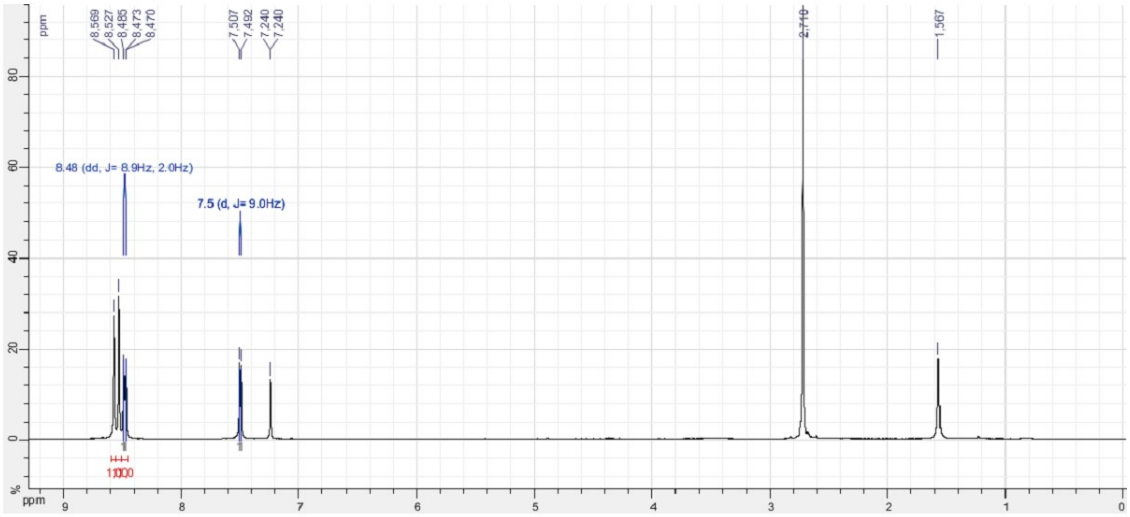
MP-03

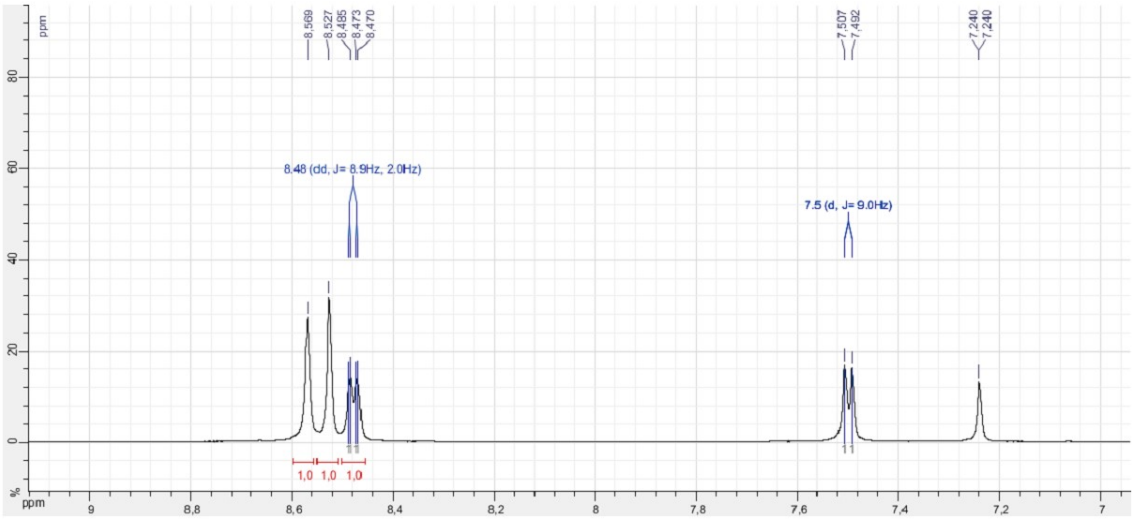
3-Acetyl-6-nitro-2H-chromen-2-one ***(MP-03)***

ATR-FTIR

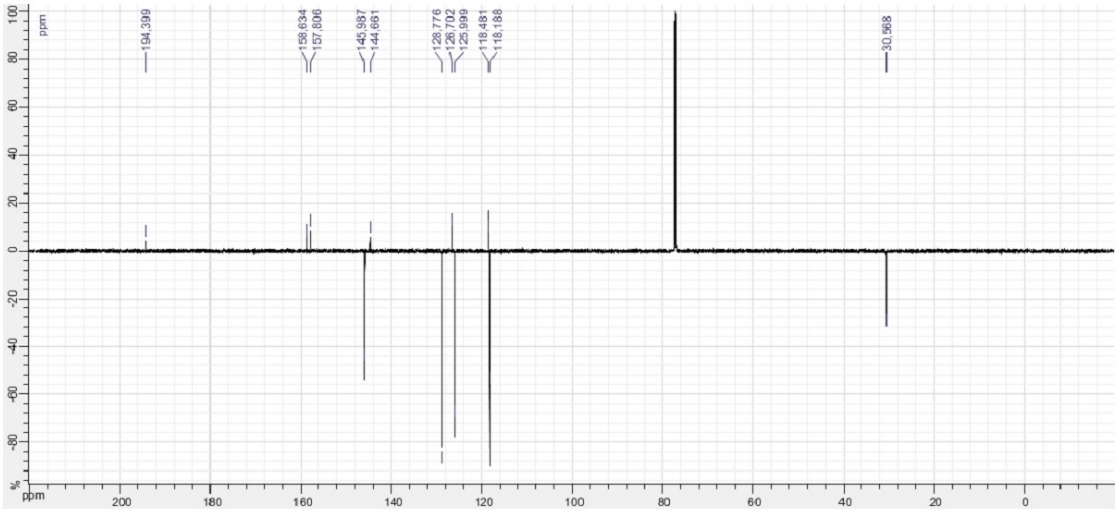


^{1}H NMR (400 MHz, $CDCl_3$, ppm)

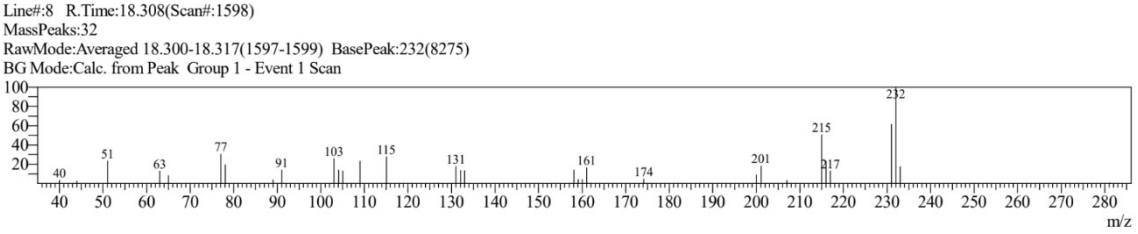


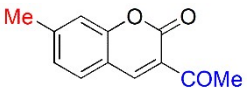


DEPT ^{13}C (100 MHz, $CDCl_3$, ppm)



MS

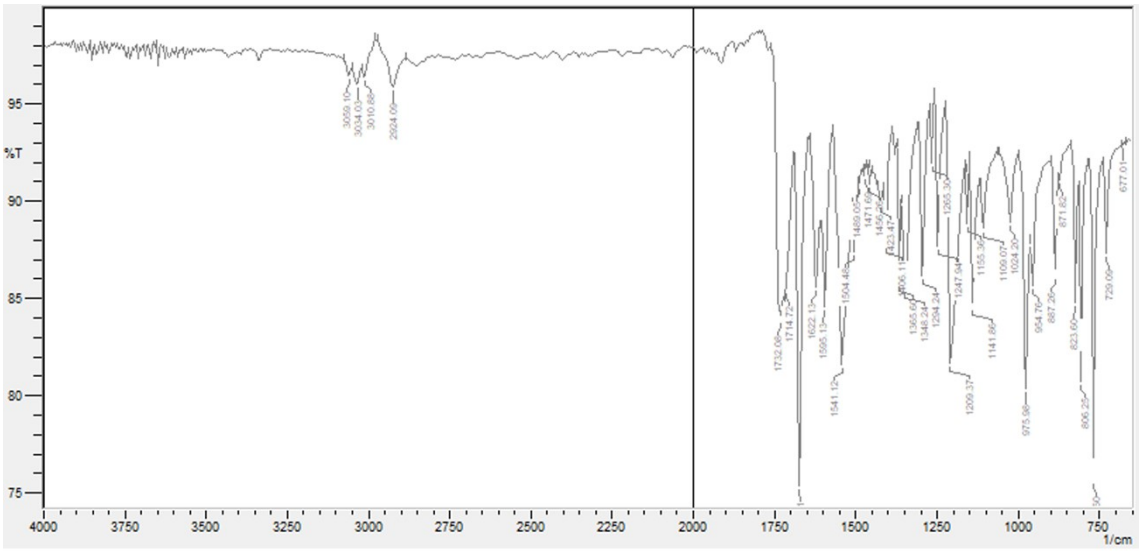




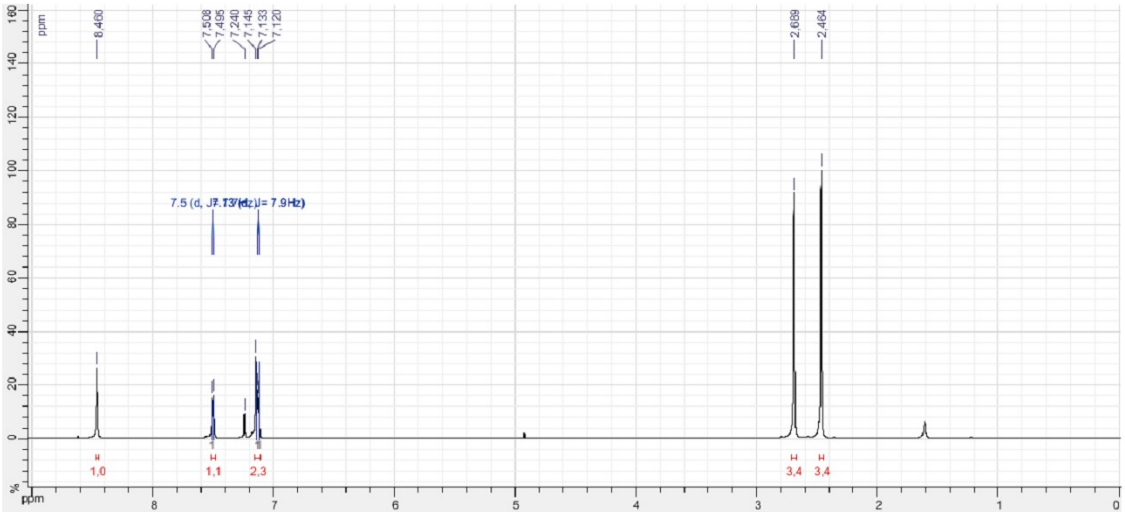
MP-04

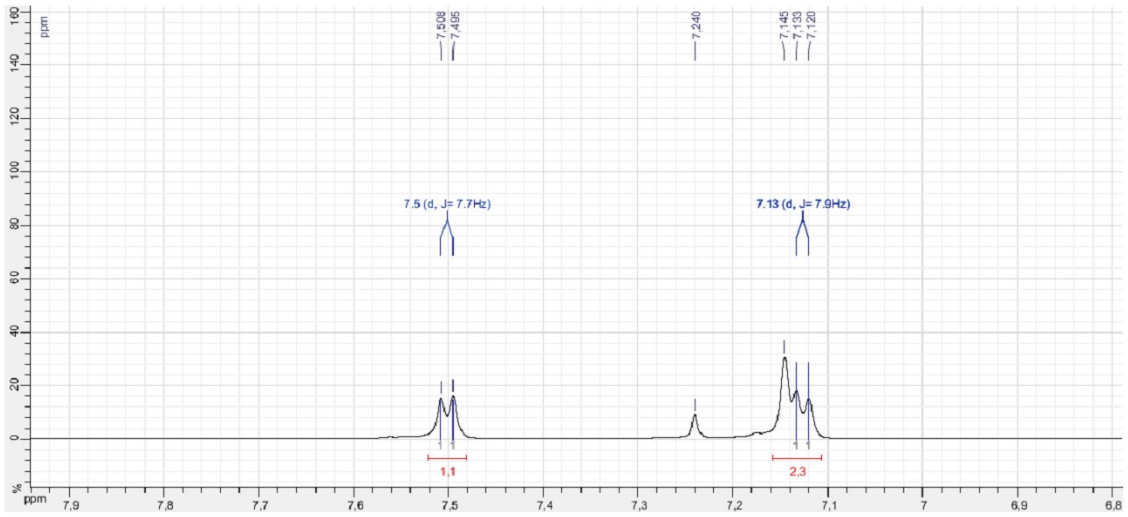
3-Acetyl-7-methyl-2H-chromen-2-one ***(MP-04)***

ATR-FTIR

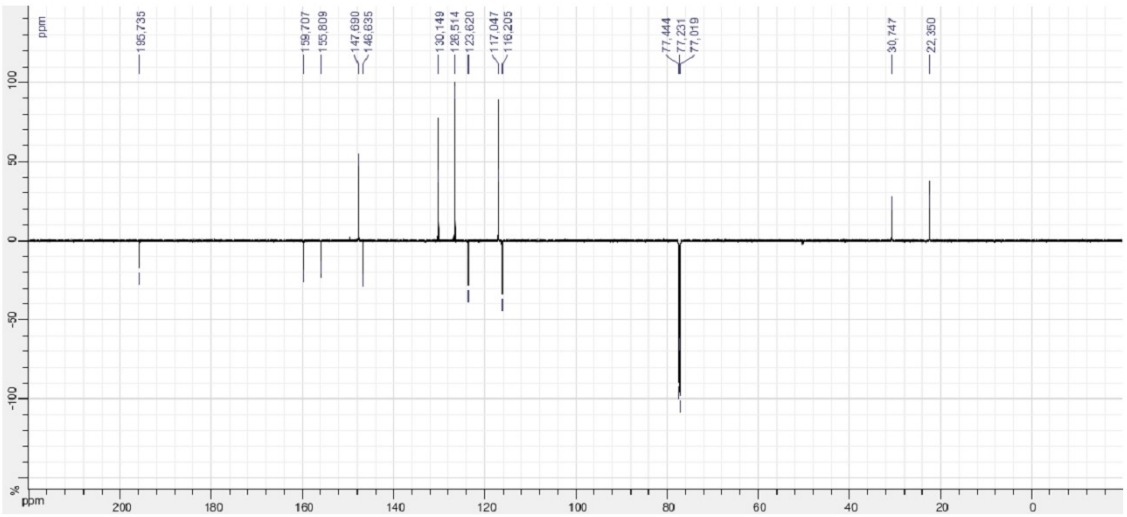


^{1}H NMR (400 MHz, $CDCl_3$, ppm)



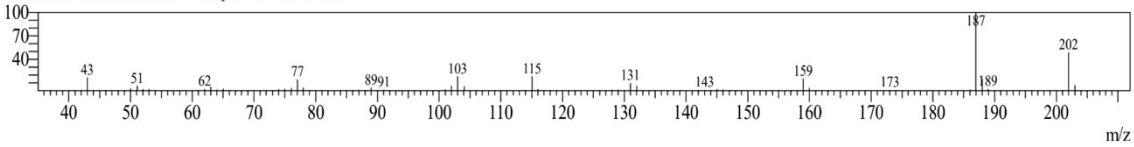


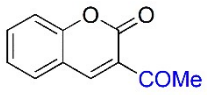
DEPT ^{13}C ($CDCl_3$, ppm)



MS

Line#:3 R.Time:13.483(Scan#:1019)
MassPeaks:115
RawMode:Averaged 13.475-13.492(1018-1020) BasePeak:187(7050568)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

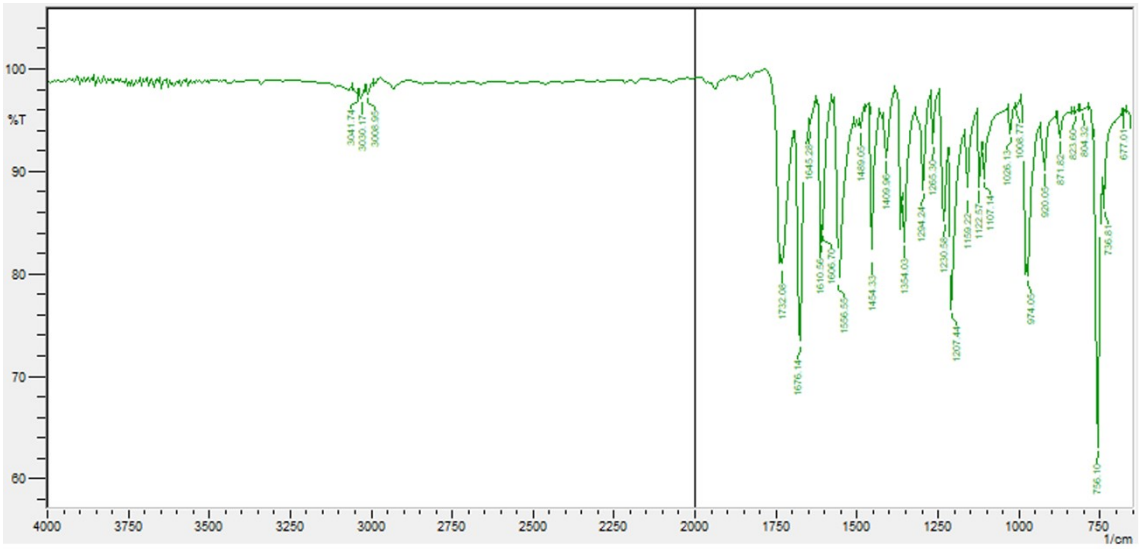




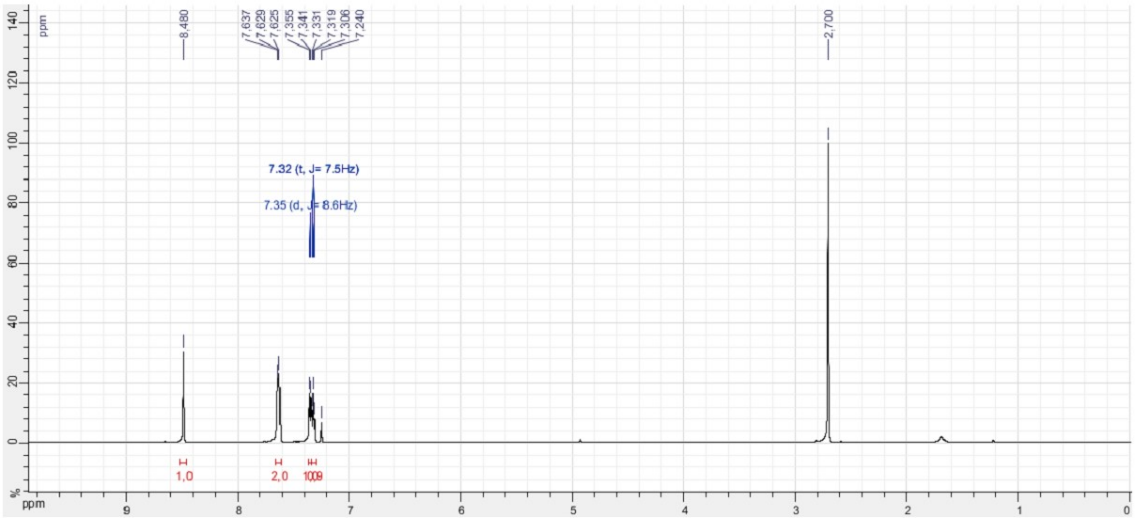
MP-05

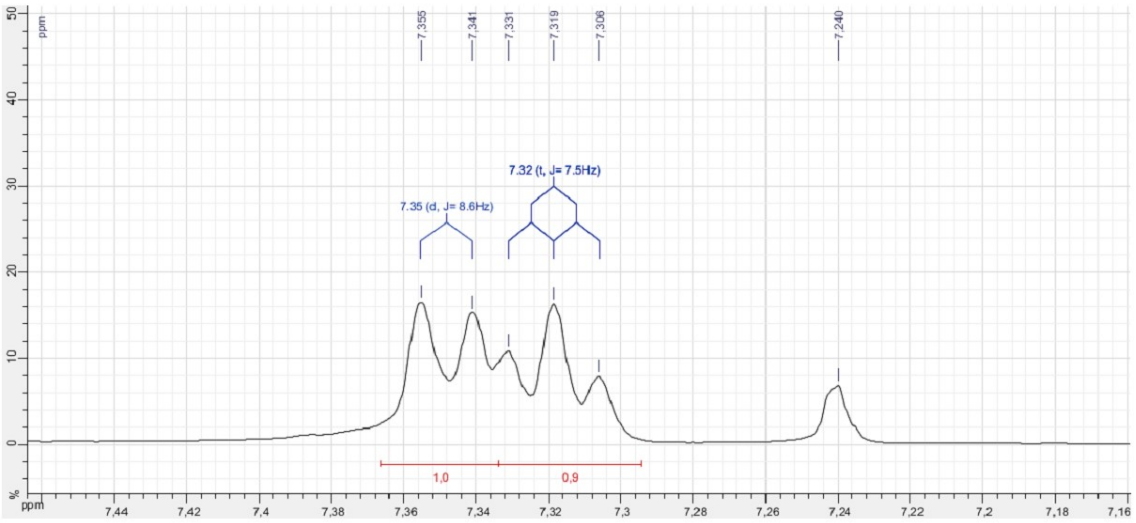
3-Acetyl-2H-chromen-2-one ***(MP-05)***

ATR-FTIR

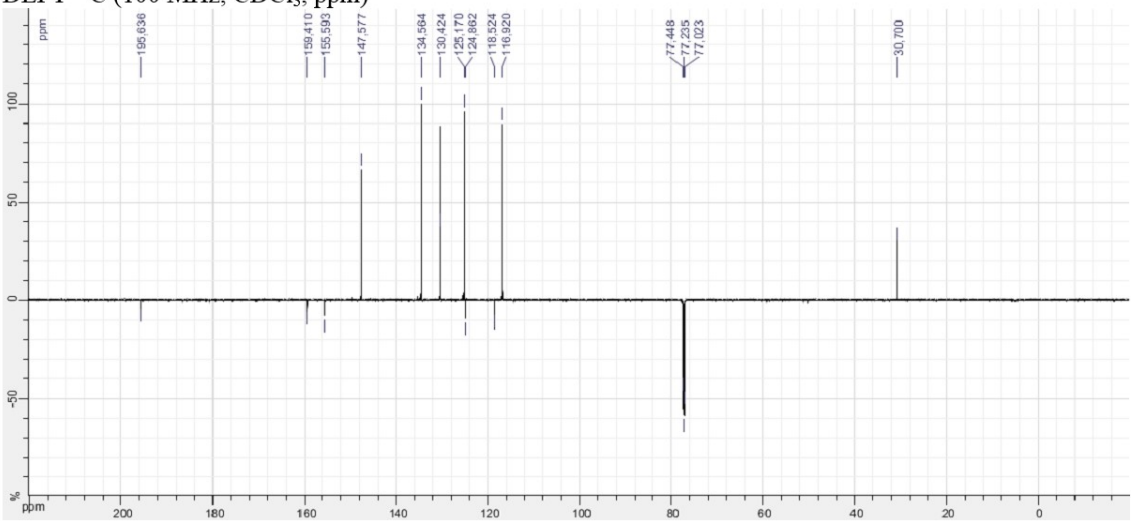


^{1}H NMR (400 MHz, $CDCl_3$, ppm)

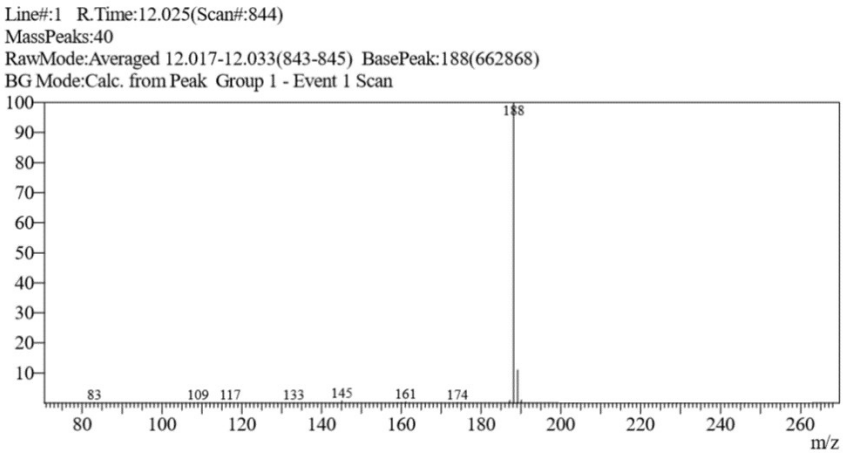


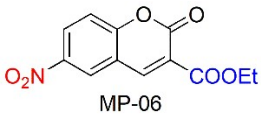


DEPT ^{13}C (100 MHz, $CDCl_3$, ppm)



MS

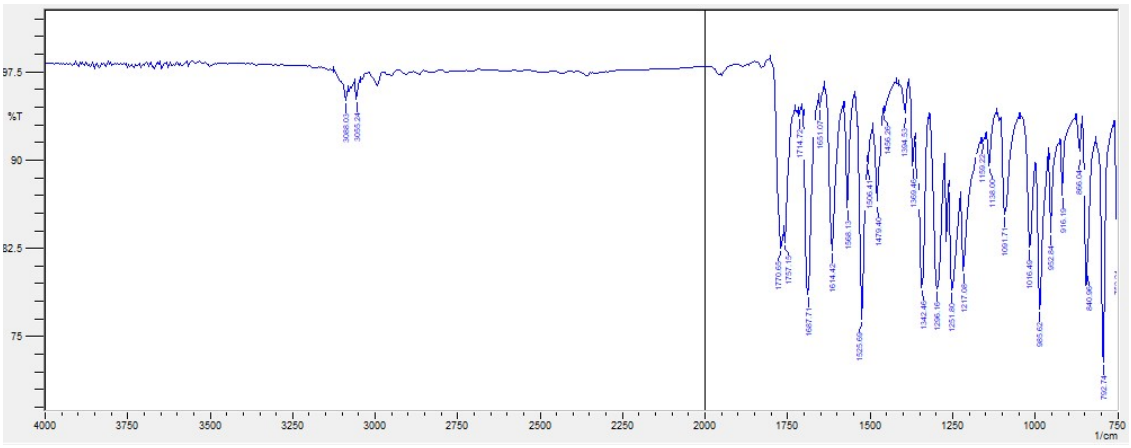




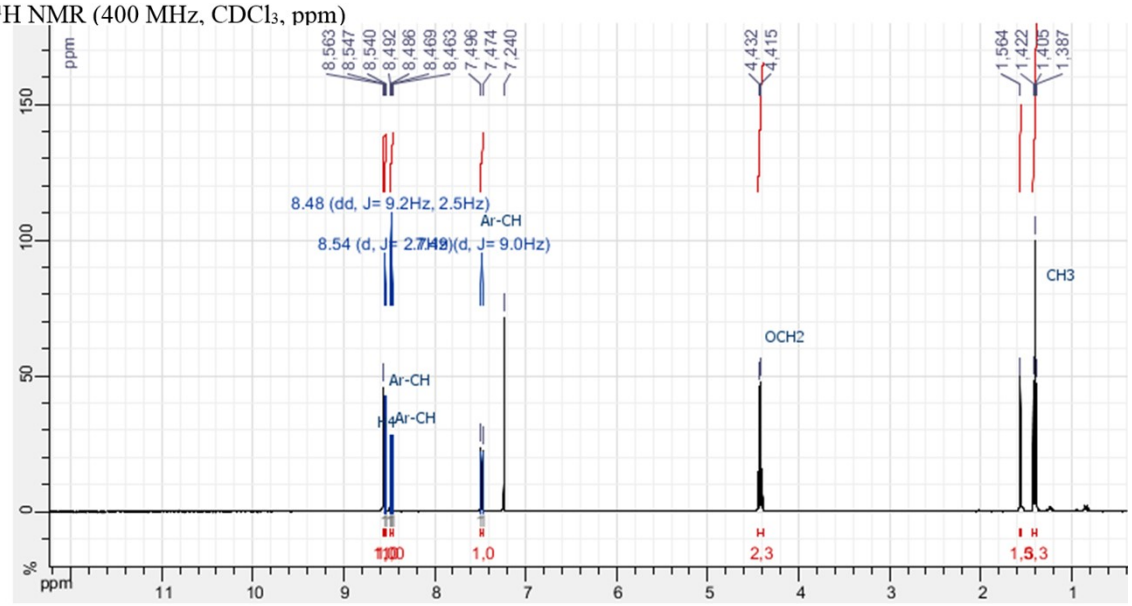
MP-06

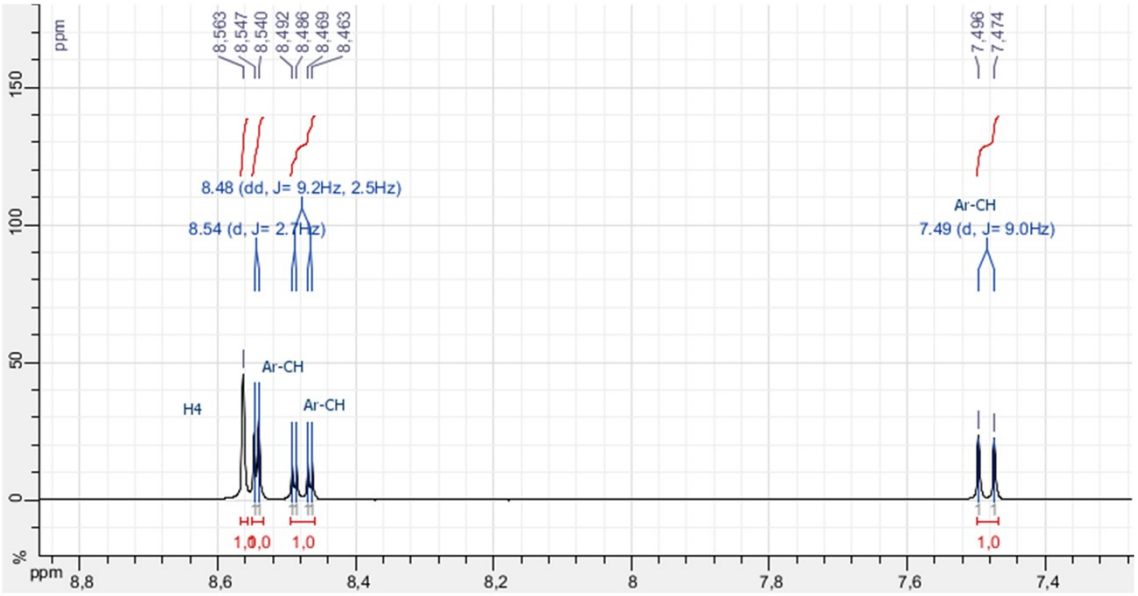
Ethyl 6-nitro-2-oxo-2H-chromene-3-carboxylate ***(MP-06)***

ATR-FTIR

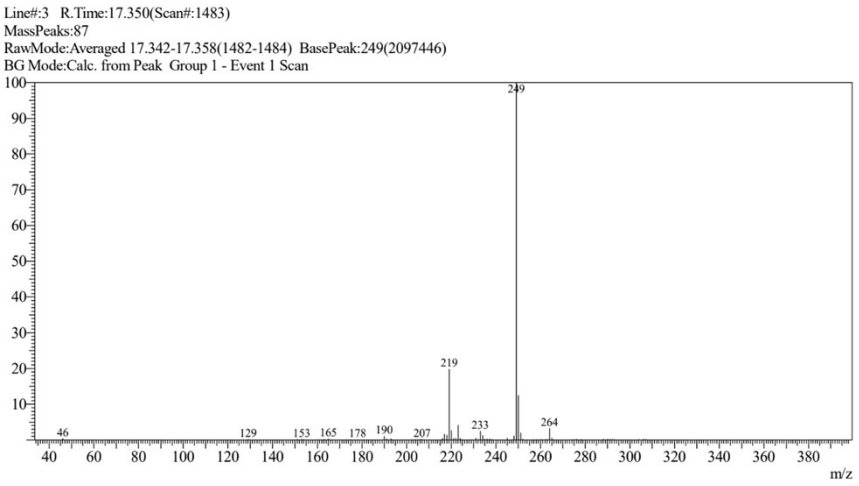


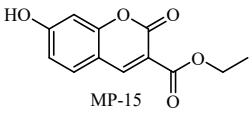
^{1}H NMR (400 MHz, $CDCl_3$, ppm)




ppm
8,563
8,547
8,540
8,492
8,486
8,469
8,463
7,496
7,474
8.48 (dd, J= 9.2Hz, 2.5Hz)
8.54 (d, J= 2.7Hz)
Ar-CH
7.49 (d, J= 9.0Hz)
H4
Ar-CH
Ar-CH
1,0
1,0
1,0
150
100
50
0
%
ppm
8,8
8,6
8,4
8,2
8
7,8
7,6
7,4

MS

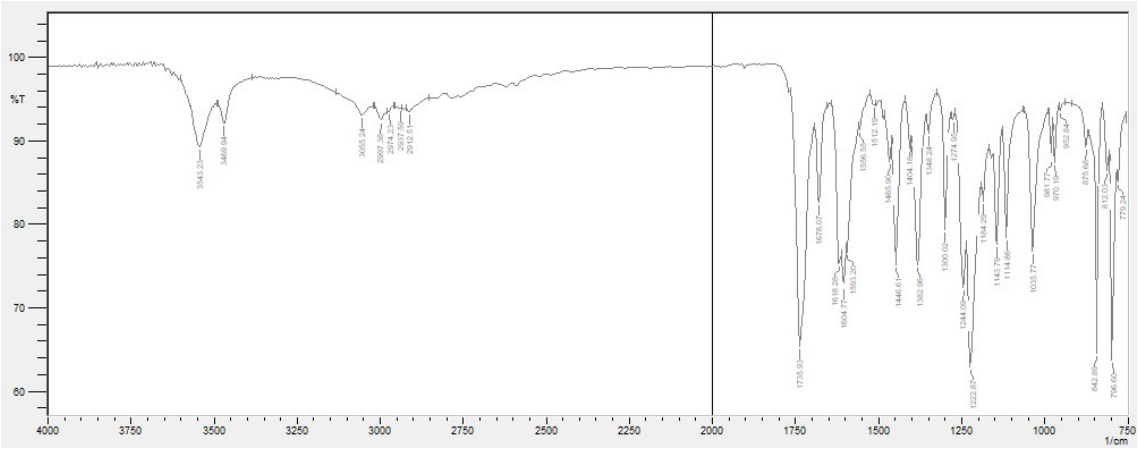

Line#:3 R.Time:17.350(Scan#:1483)
MassPeaks:87
RawMode:Averaged 17.342-17.358(1482-1484) BasePeak:249(2097446)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
249
219
46
129
153
165
178
190
207
233
264
m/z



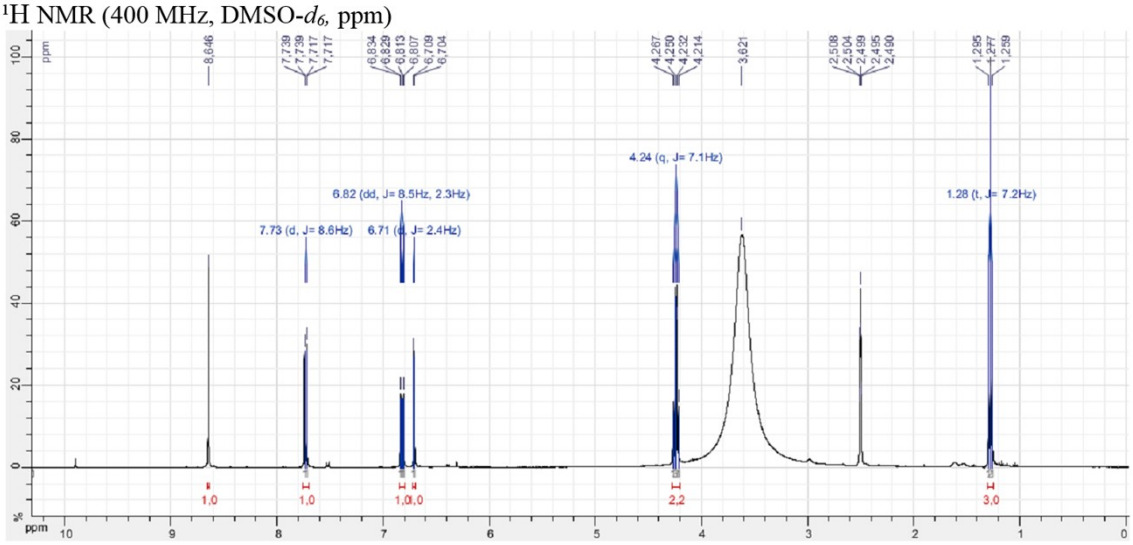
MP-15

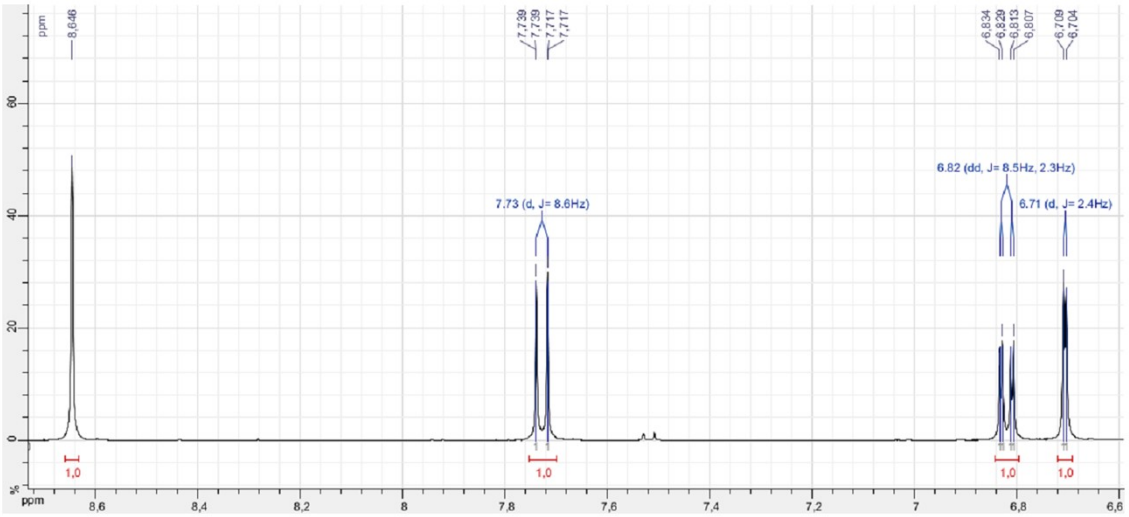
Ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate ***(MP-15)***

ATR-FTIR



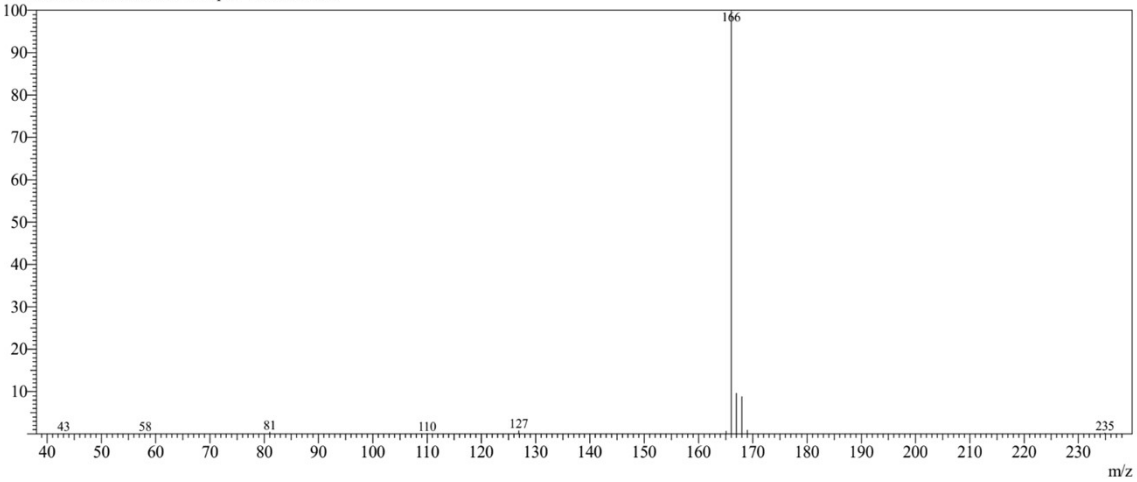
^{1}H NMR (400 MHz, DMSO-d_6, ppm)

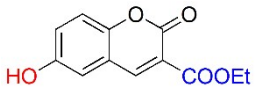




MS

Line#:1 R.Time:10.992(Scan#:720)
MassPeaks:13
RawMode:Averaged 10.983-11.000(719-721) BasePeak:166(179094)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

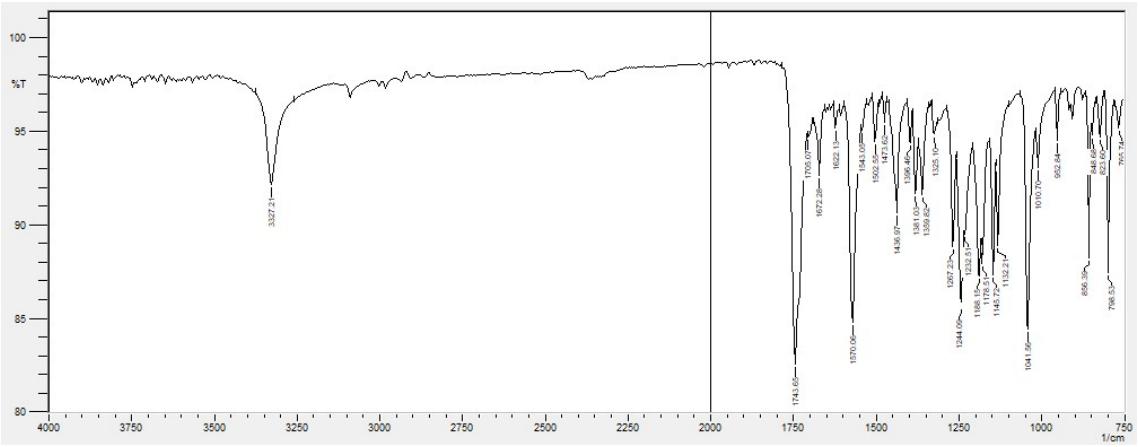




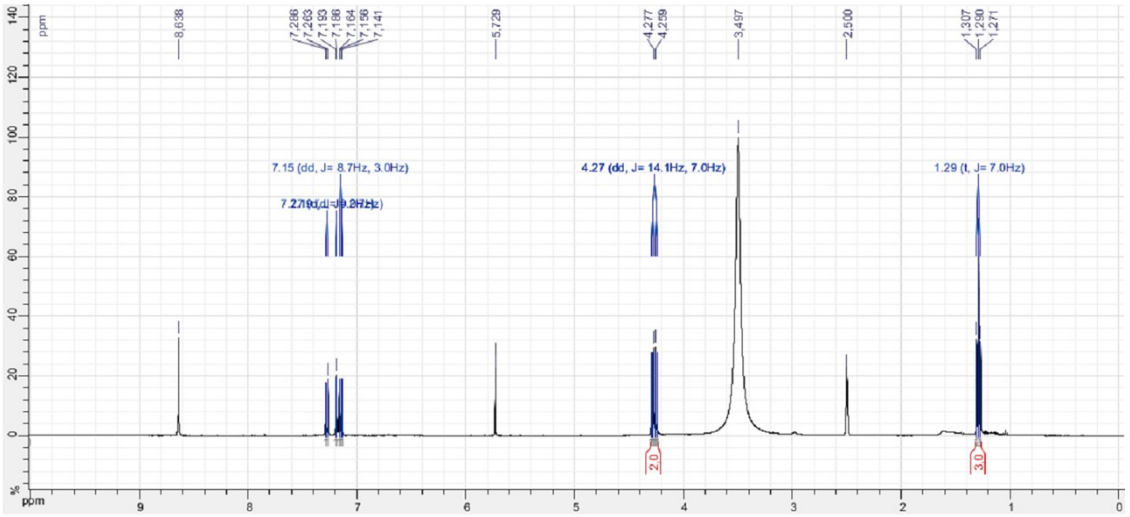
MP-17

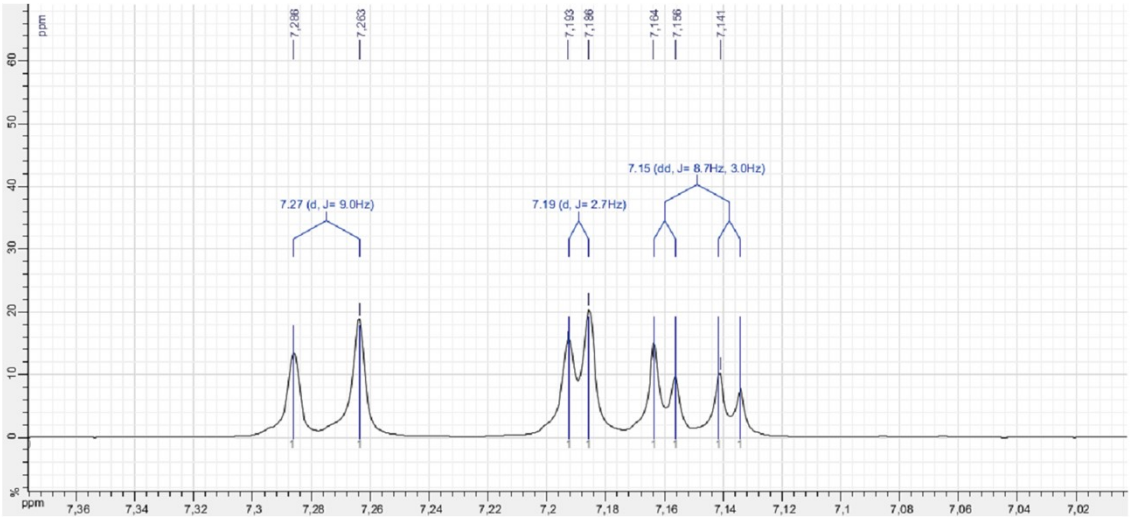
Ethyl 6-hydrixy-2-oxo-2H-chromene-3-carboxylate ***(MP-17)***

ATR-FTIR



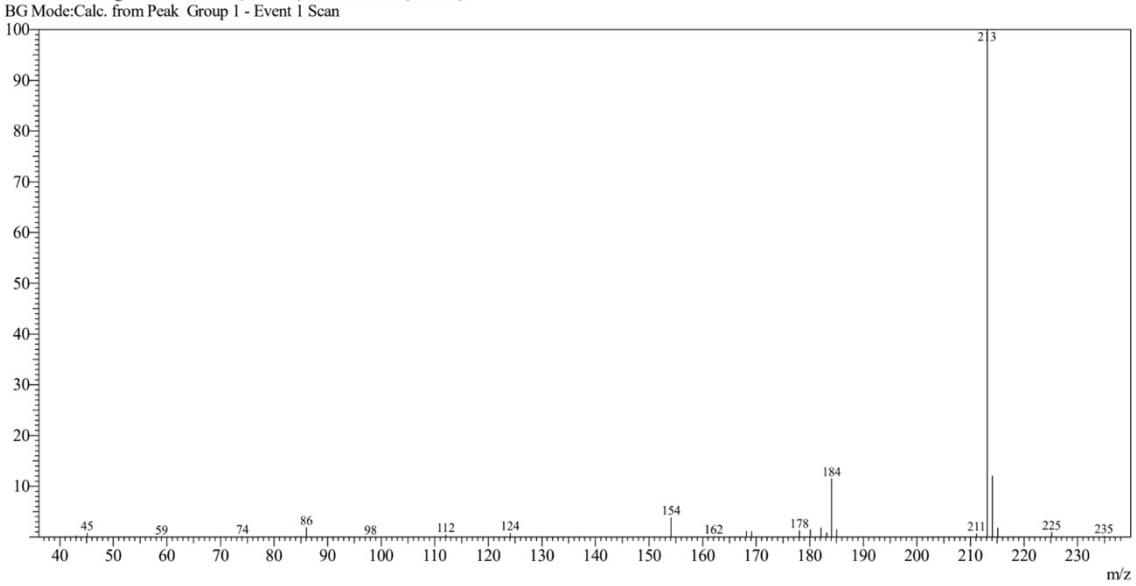
^{1}H NMR ^{1}H (400 MHz, DMSO-d_6, ppm)

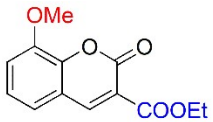




MS

Line#:3 R.Time:11.742(Scan#:810)
MassPeaks:30
RawMode:Averaged 11.733-11.750(809-811) BasePeak:213(131453)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

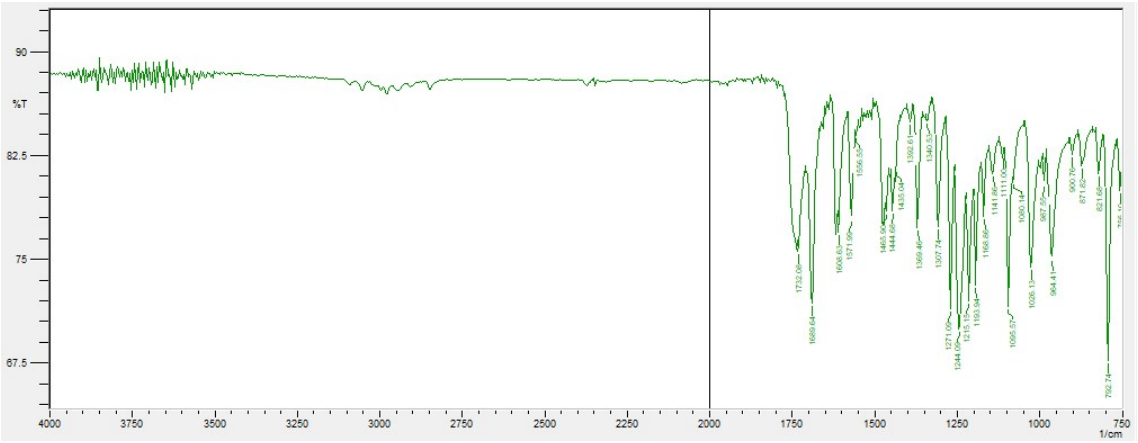




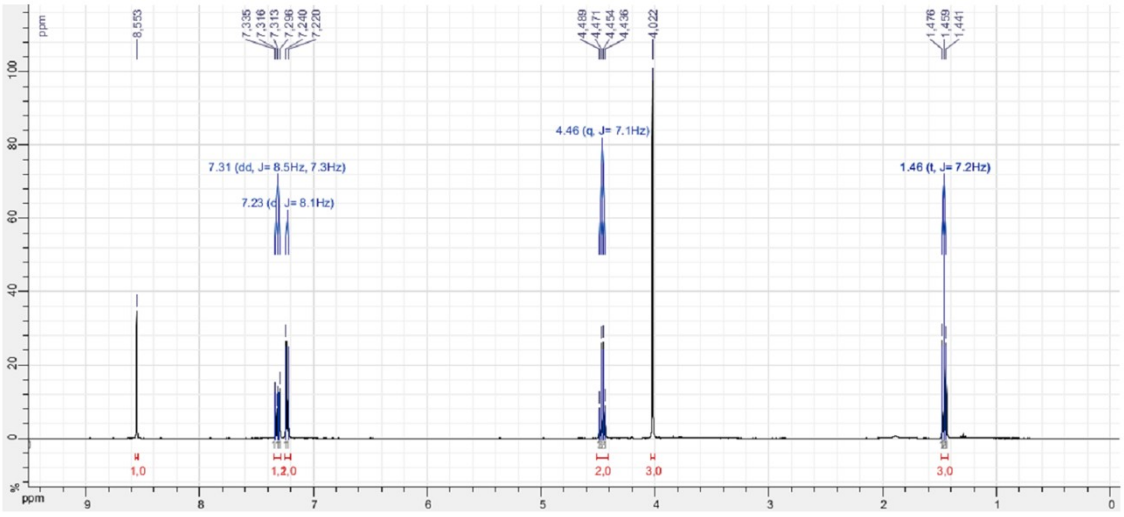
MP-21

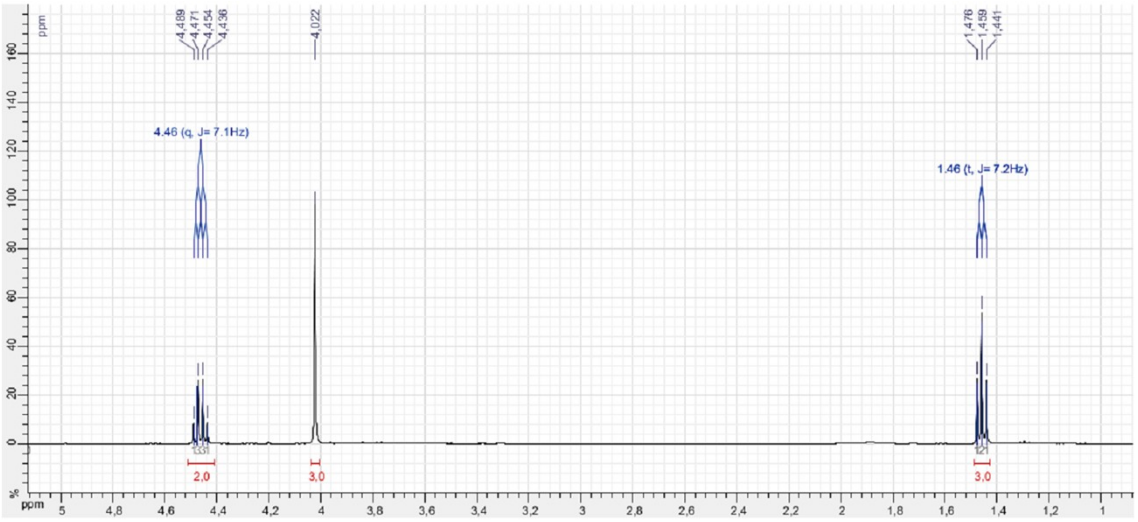
Ethyl 8-methoxy-2-oxo-2H-chromene-3-carboxylate ***(MP-21)***

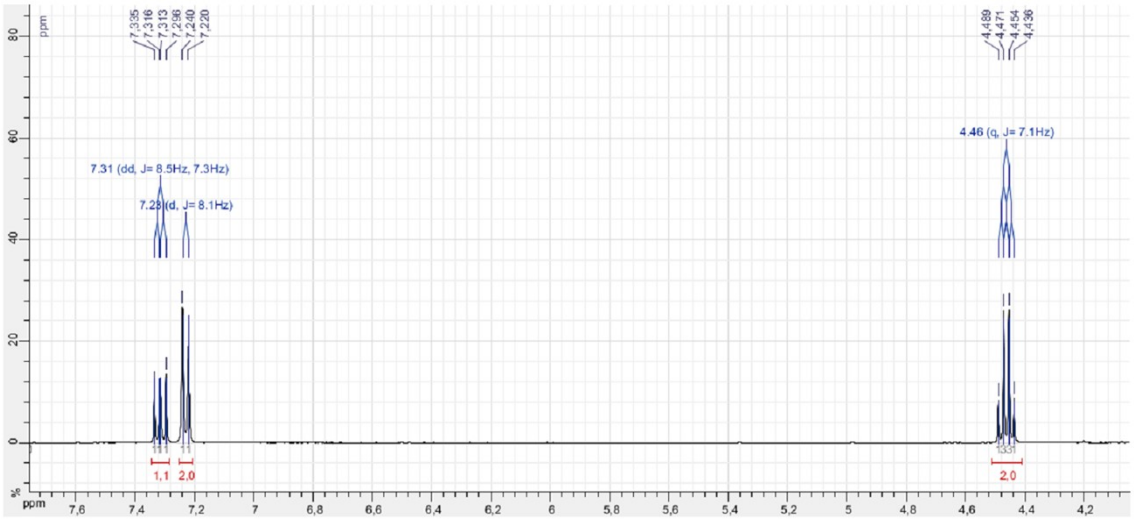
ATR-FTIR



^{1}H NMR (400 MHz, DMSO-d_6, ppm)

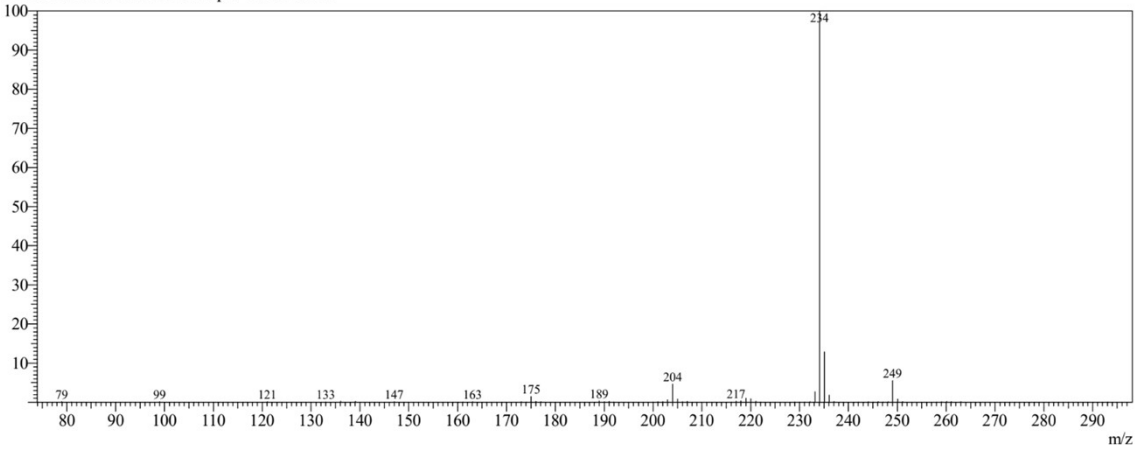


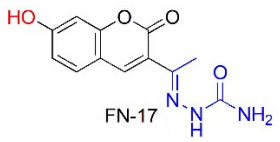




MS

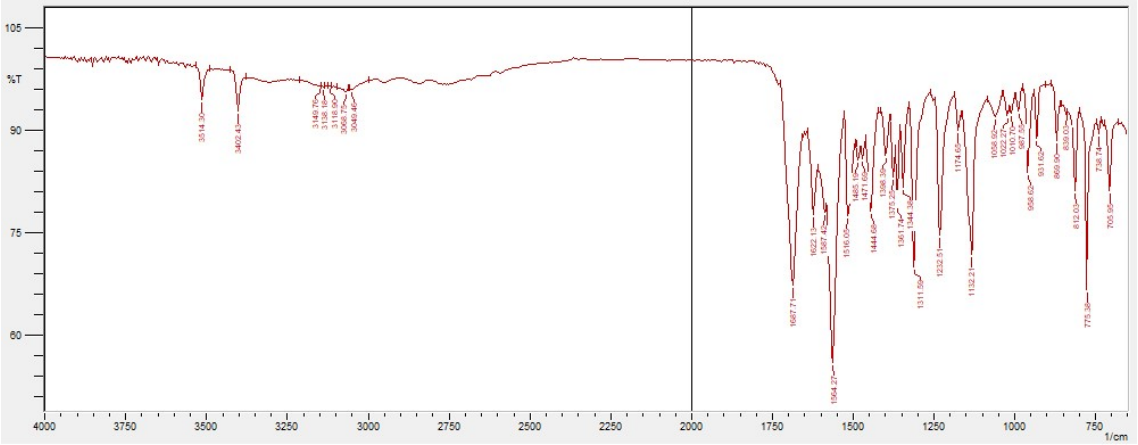
Line#:1 R.Time:16.108(Scan#:1334)
MassPeaks:75
RawMode:Averaged 16.100-16.117(1333-1335) BasePeak:234(1791747)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



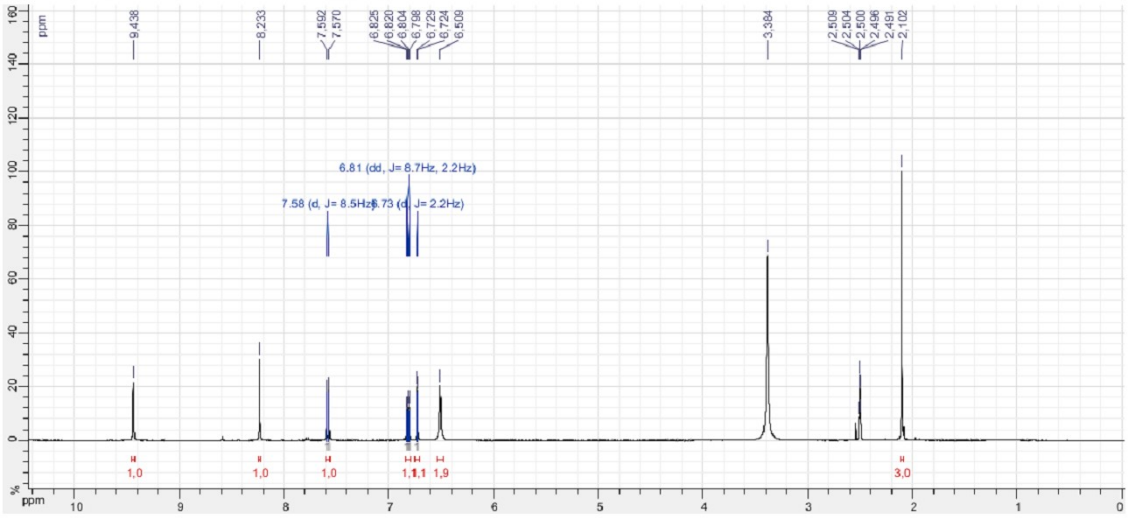


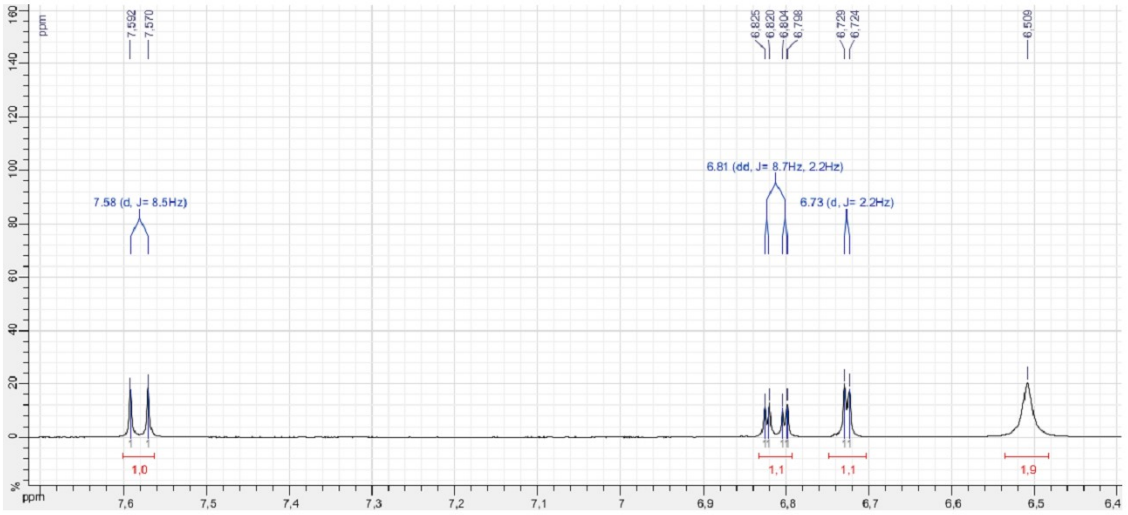
(1E)-1-(1-(7-Hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)semicarbazide ***(FN-17)***

ATR-FTIR

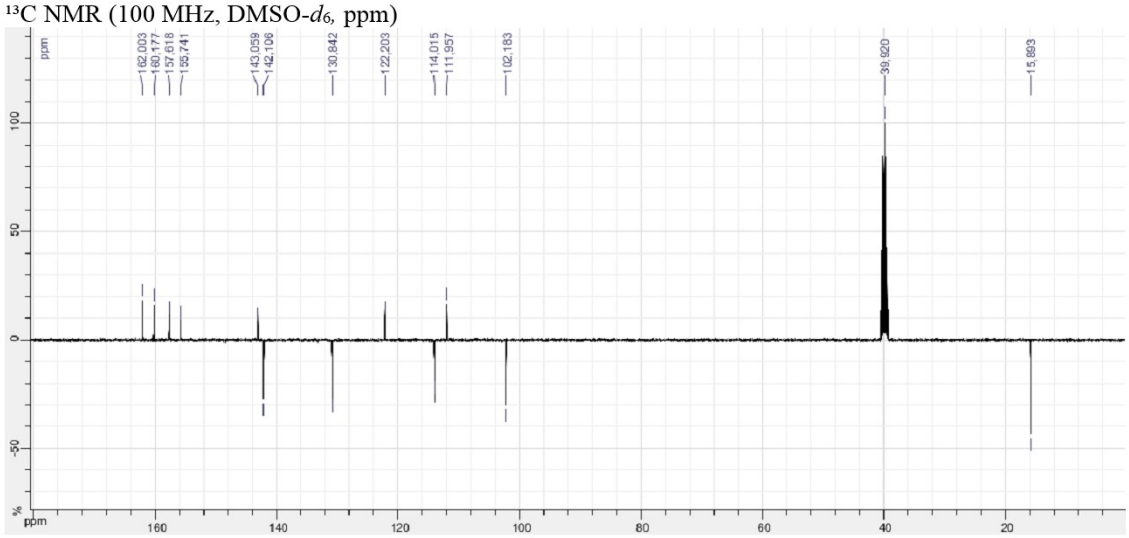


^{1}H NMR (400 MHz, DMSO-d_6, ppm)

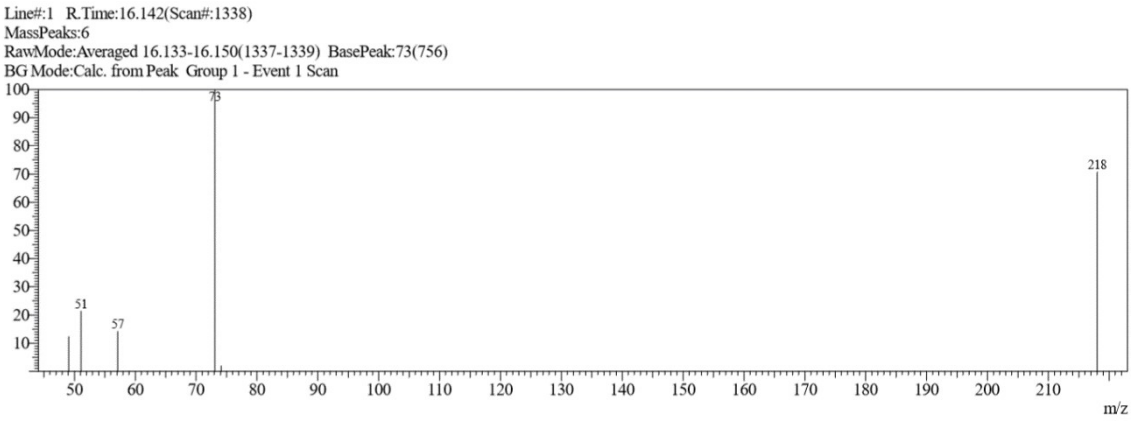


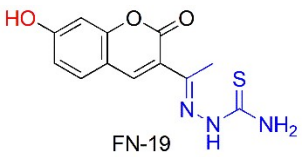


^{13}C NMR (100 MHz, DMSO-d_6, ppm)



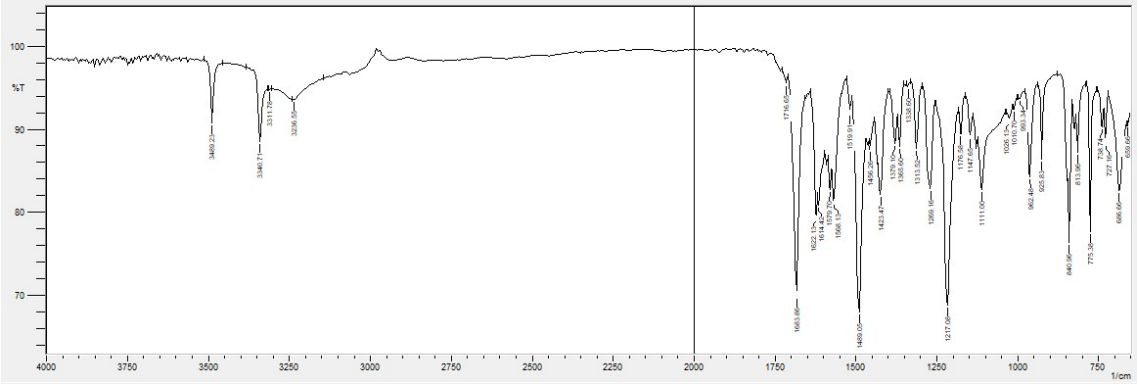
MS



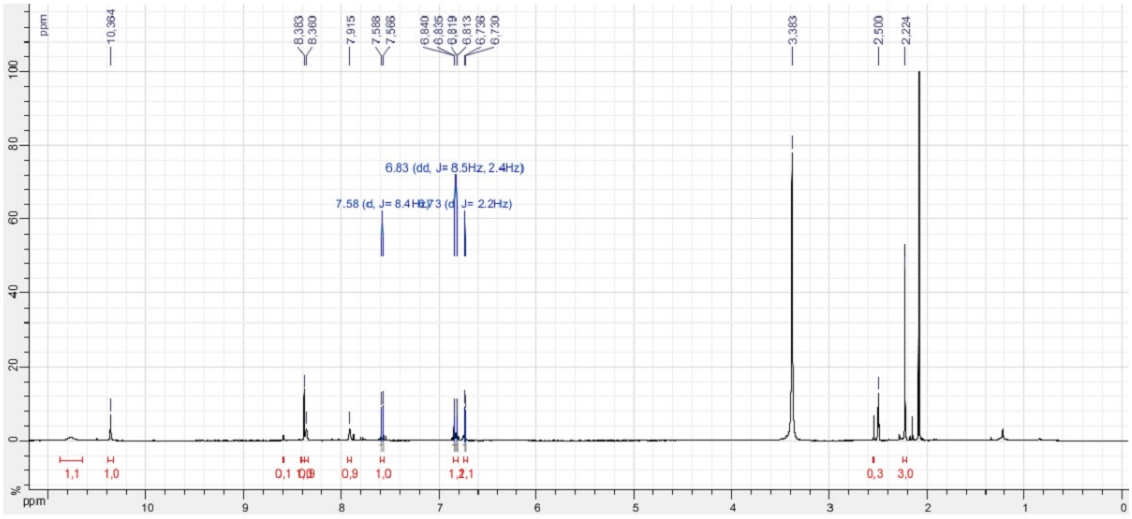


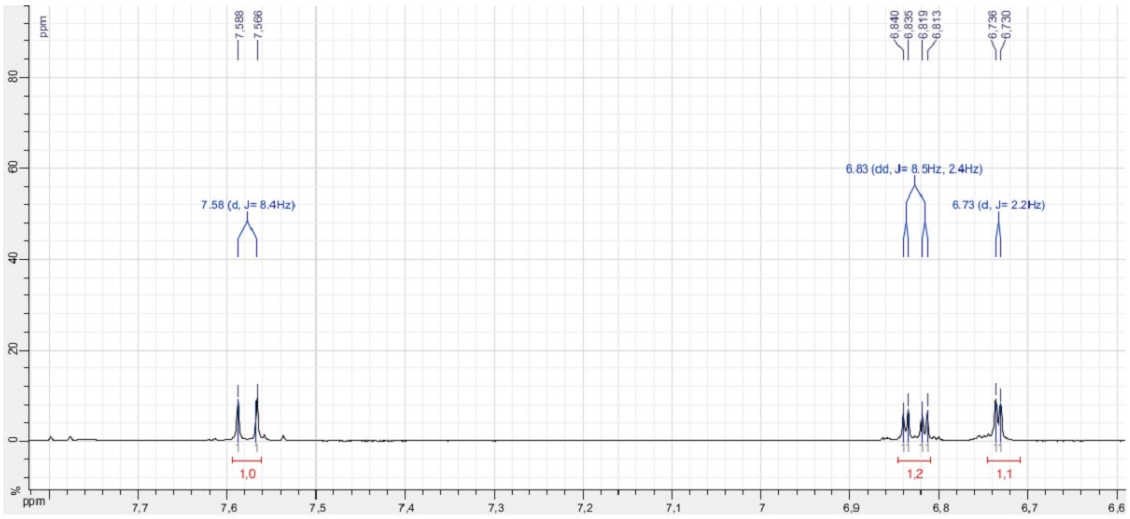
(1E)-1-(1-(7-Hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)thiosemicarbazide ***(FN-19)***

ATR-FTIR

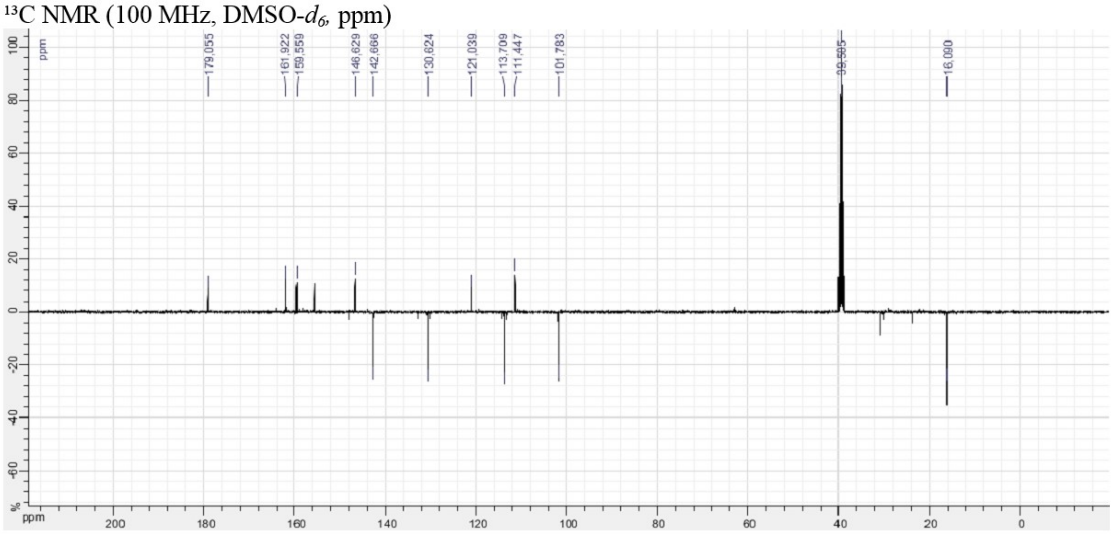


^{1}H NMR (400 MHz, DMSO-d_6, ppm)

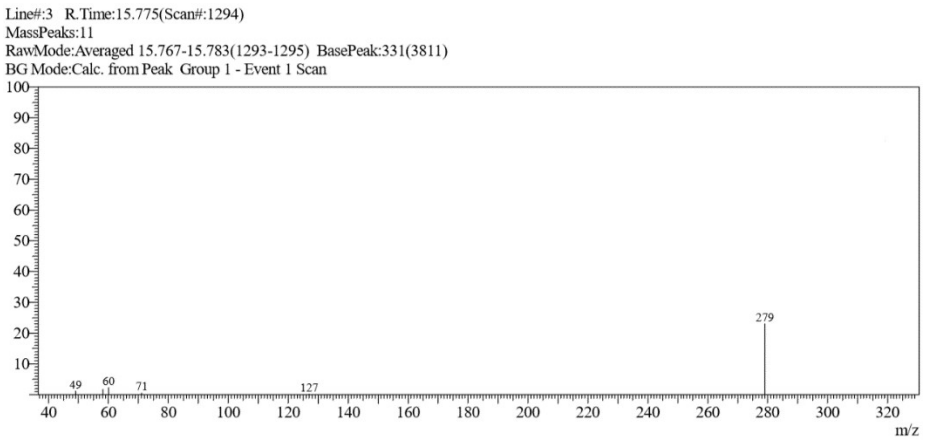


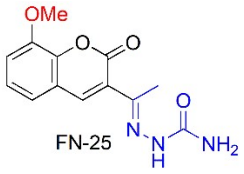


^{13}C NMR (100 MHz, DMSO-d_6, ppm)



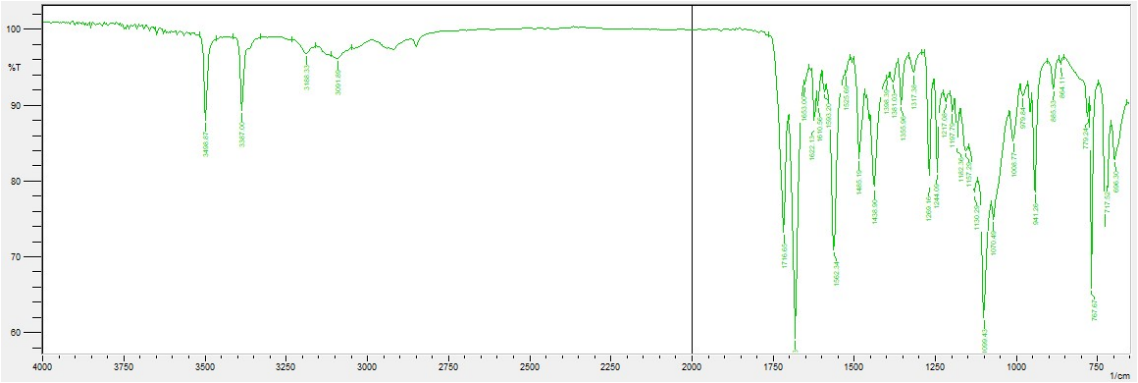
MS



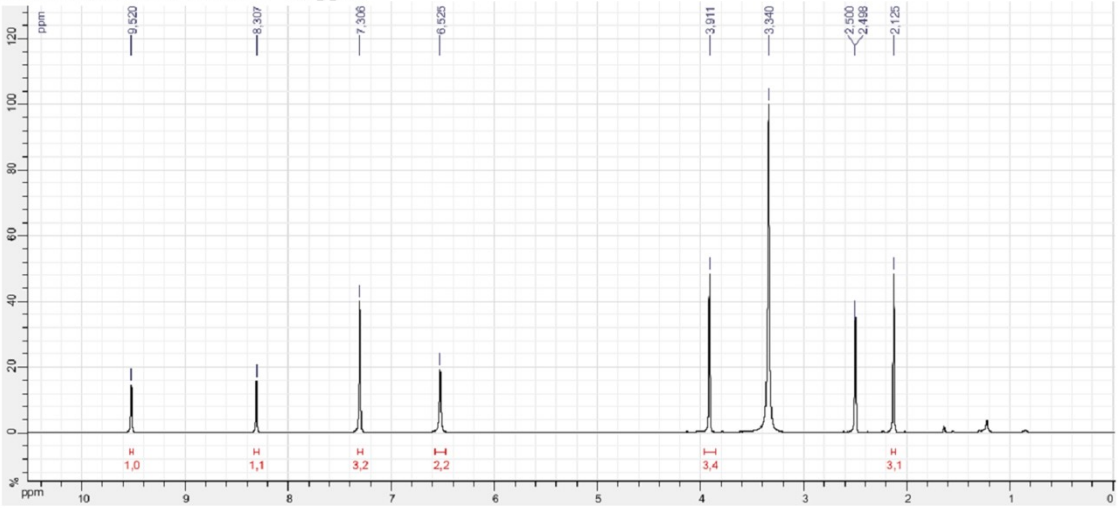


(1E)-1-(1-(8-Methoxy-2-oxo-2H-chromen-3-yl)ethylidene)semicarbazide ***(FN-25)***

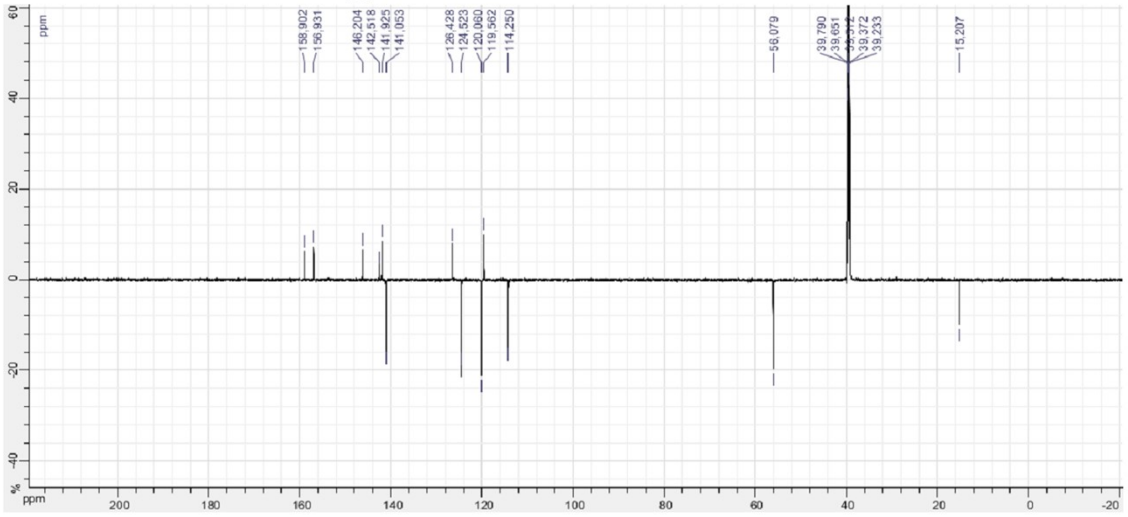
ART-FTIR



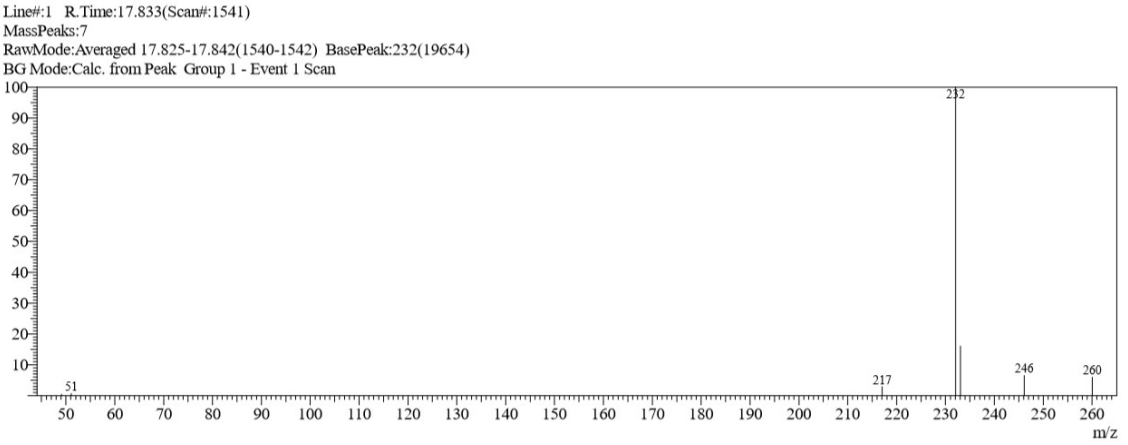
^{1}H NMR (400 MHz, DMSO-d_6, ppm)

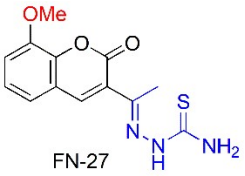


DEPT ^{13}C (100 MHz, DMSO-d_6, ppm)



MS

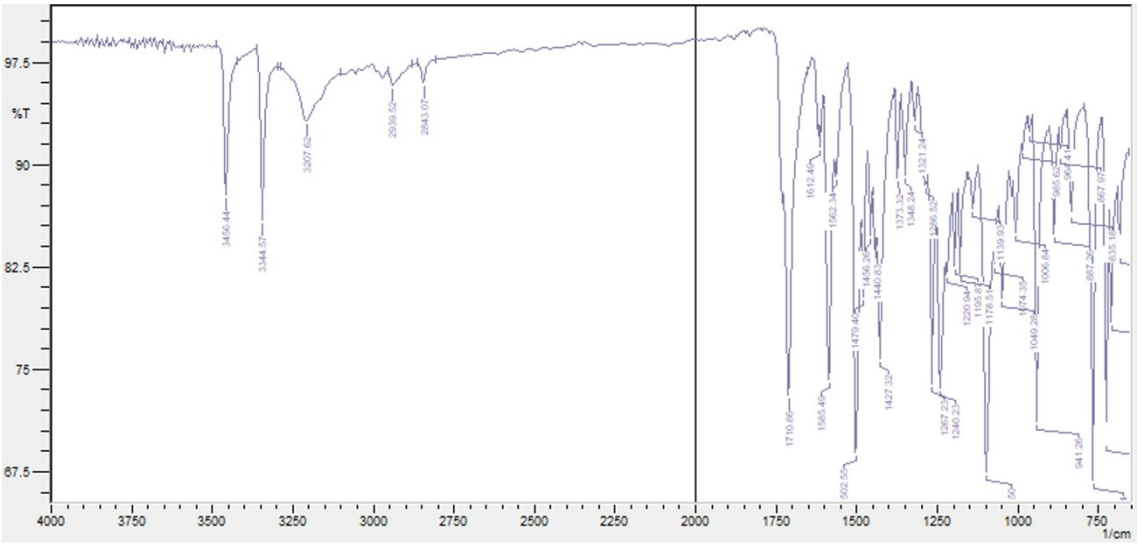




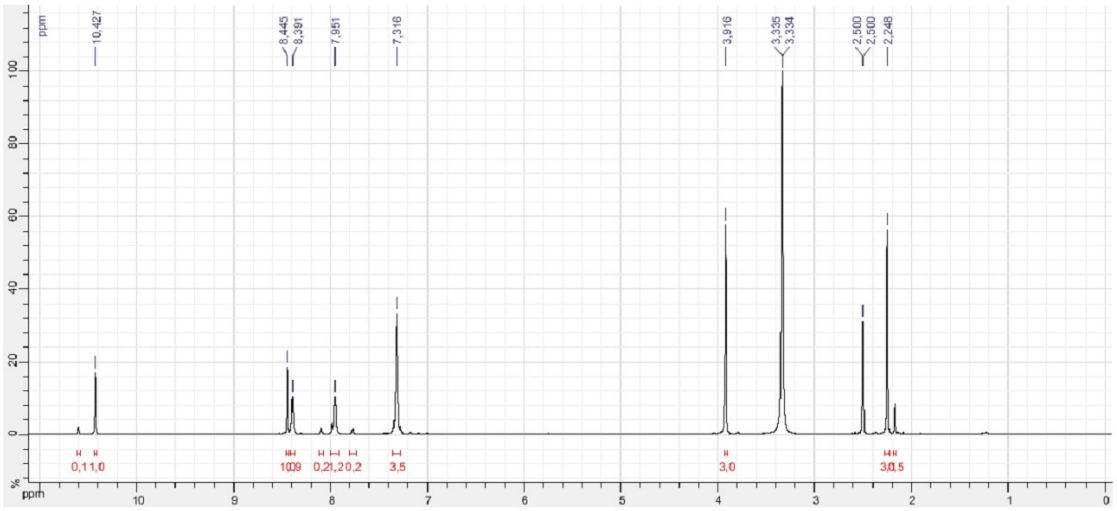
FN-27

(1E)-1-(1-(8-Methoxy-2-oxo-2H-chromen-3-yl)ethylidene)thiosemicarbazide ***(FN-27)***

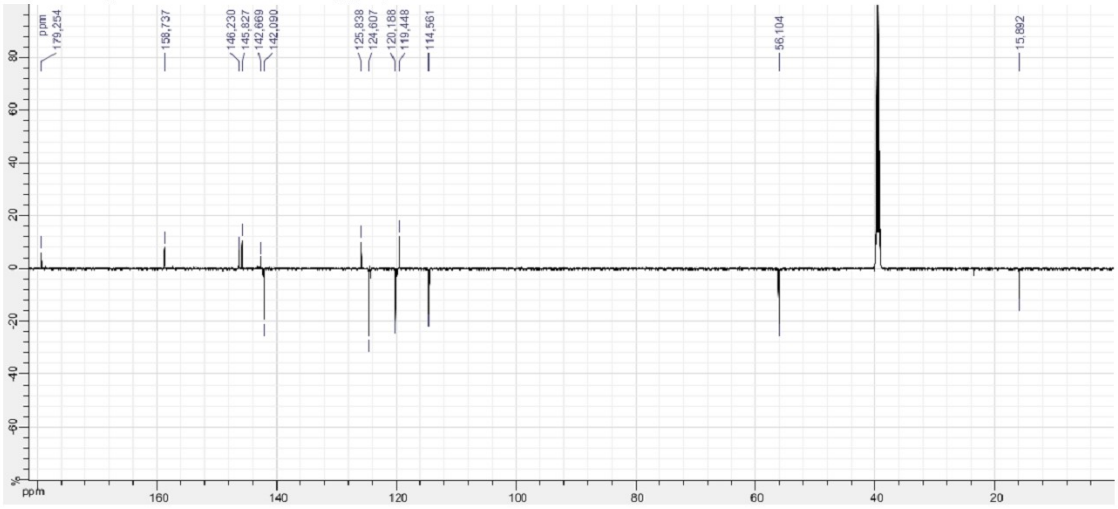
ATR-FTIR



^{1}H NMR (400 MHz, DMSO-d_6, ppm)

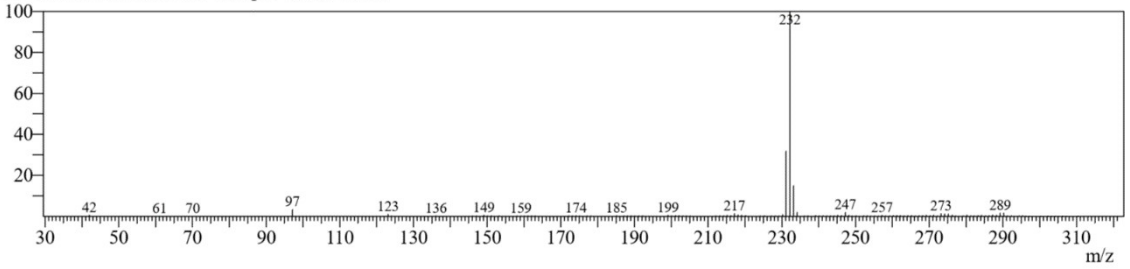


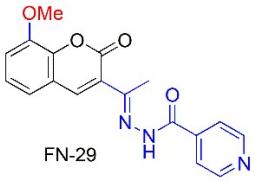
^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm)



MS

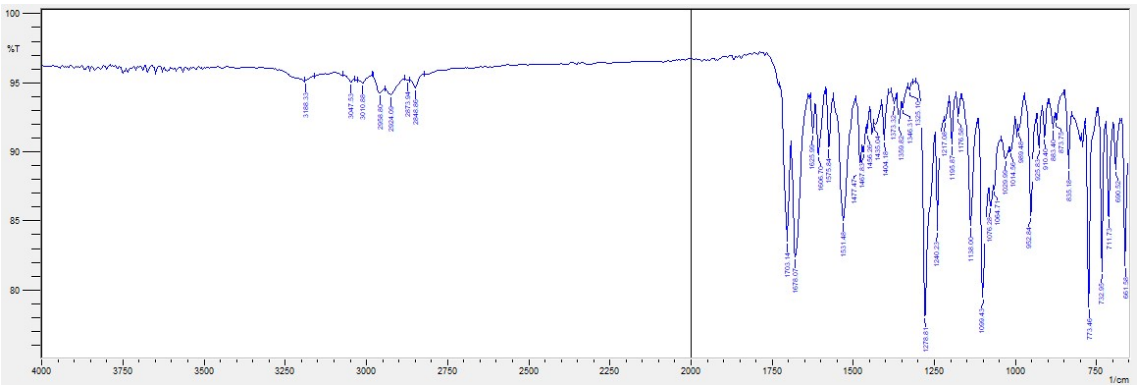
Line#:1 R.Time:18.308(Scan#:1598)
MassPeaks:177
RawMode:Averaged 18.300-18.317(1597-1599) BasePeak:232(312707)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan





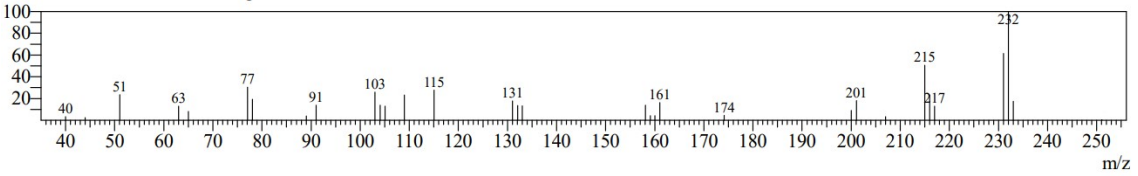
(13E)-N'-(1-(8-Methoxy-2-oxo-2H-chromen-3-yl)ethylidene)isonicotinohydrazide ***(FN-29)***

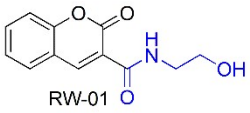
ATR-FTIR



MS

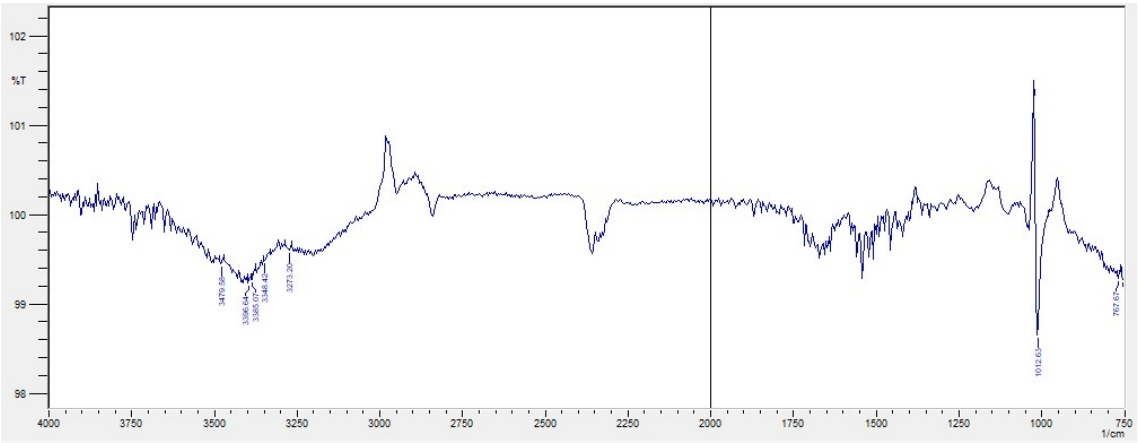
Line#:8 R.Time:18.308(Scan#:1598)
MassPeaks:32
RawMode:Averaged 18.300-18.317(1597-1599) BasePeak:232(8275)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



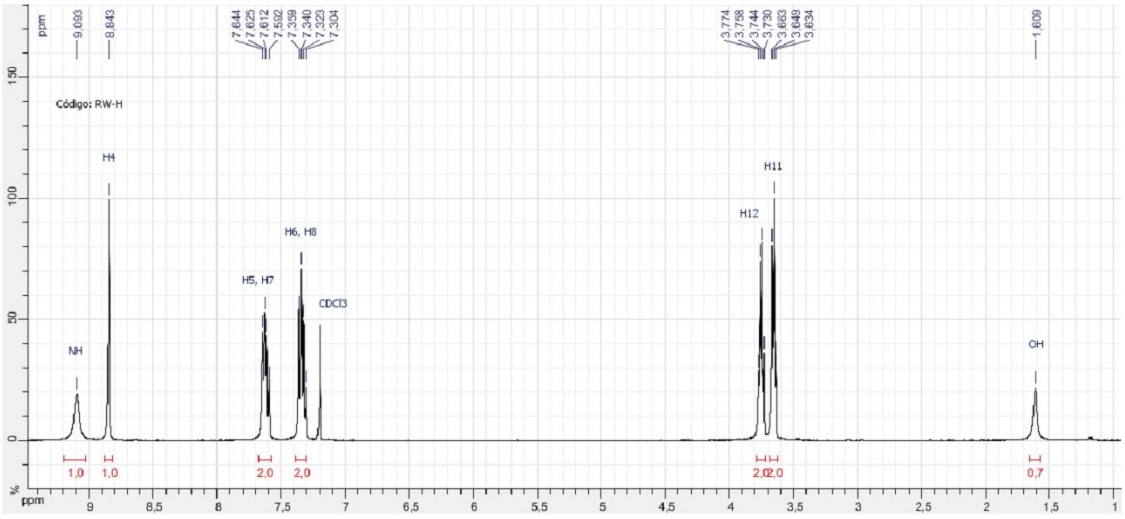


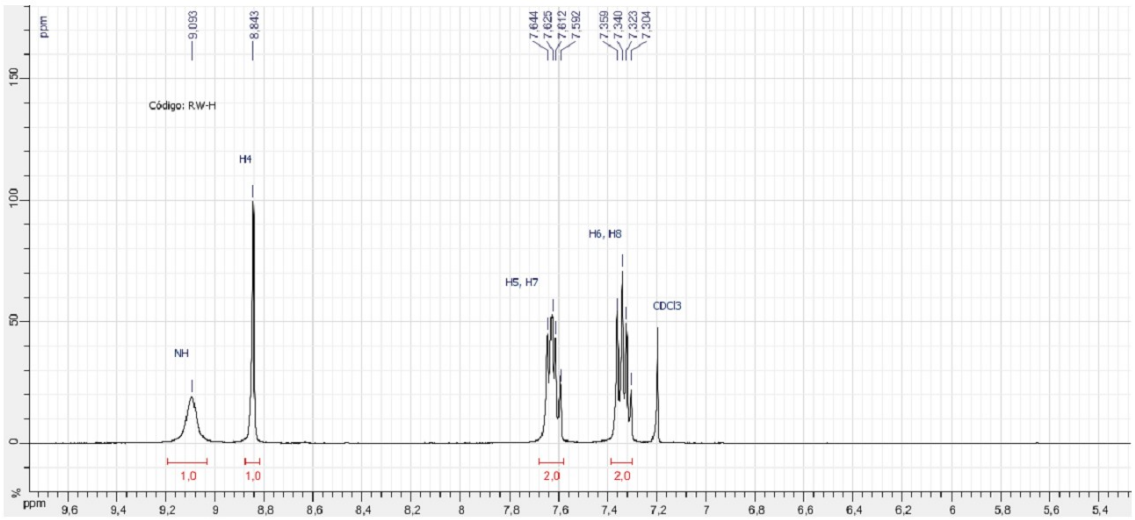
N-(2-Hydroxyethyl)-2-oxo-2H-chromene-3-carboxamide ***(RW-01)***

ATR-FTIR



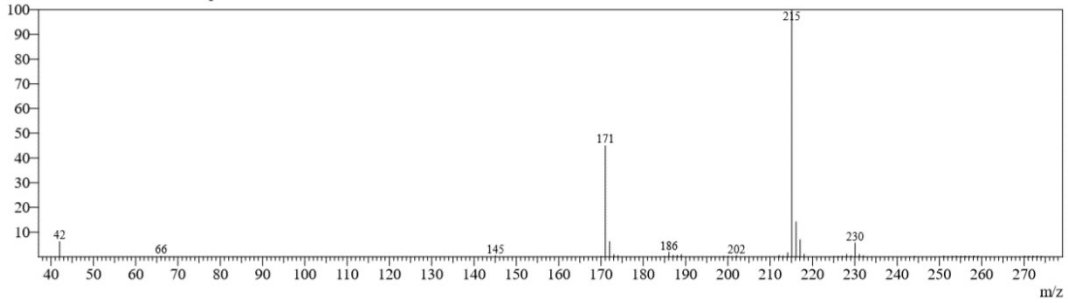
^{1}H NMR (400 MHz, $CDCl_3$, ppm)

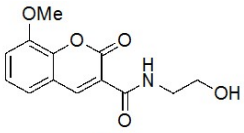




MS

Line#:5 R.Time:17.750(Scan#:1531)
MassPeaks:46
RawMode:Averaged 17.742-17.758(1530-1532) BasePeak:215(558122)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

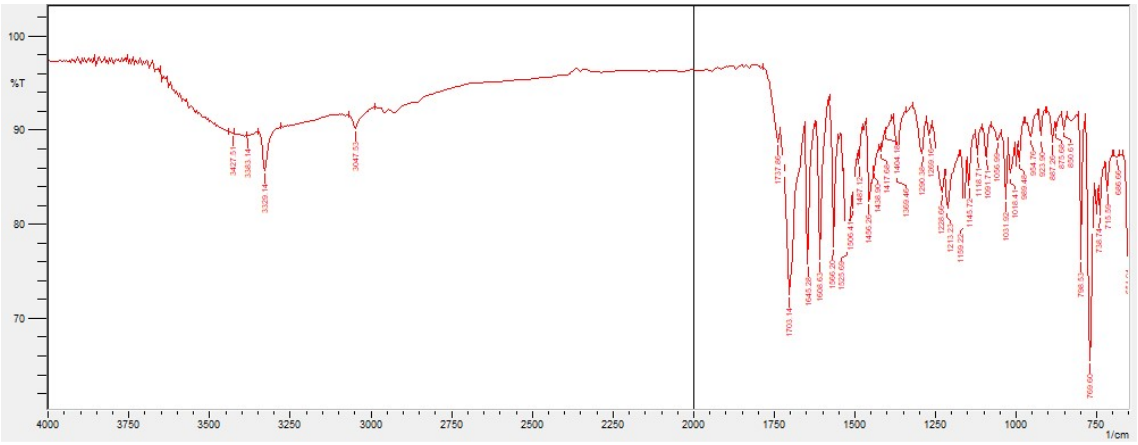




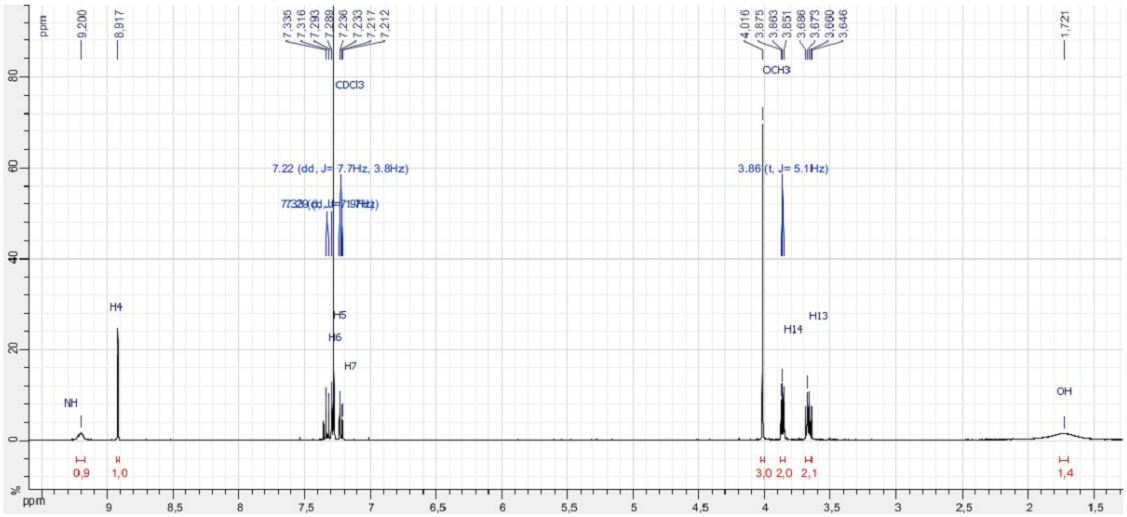
RW-02

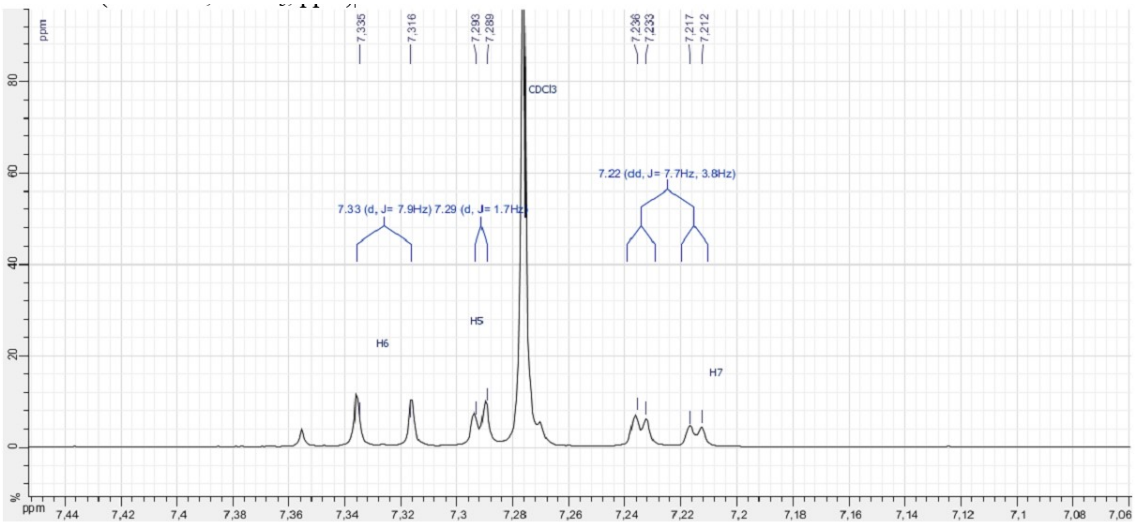
N-(2-Hydroxyethyl)-8-methoxy-2-oxo-2H-cromeno-3-carboxamida ***(RW-02)***

ATR-FTIR



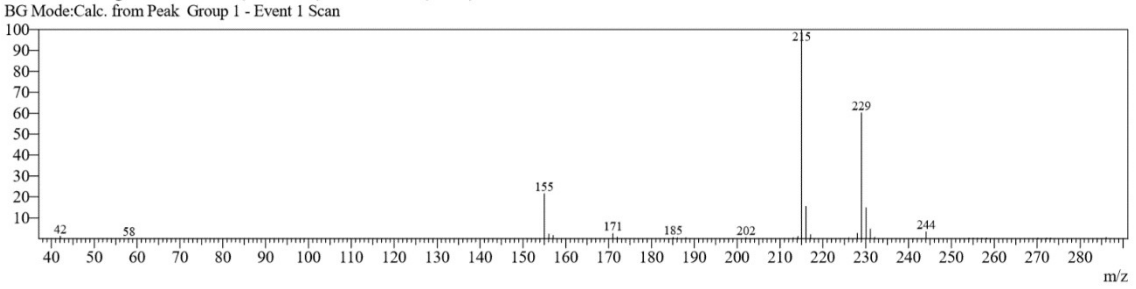
^{1}H NMR (400 MHz, $CDCl_3$, ppm)

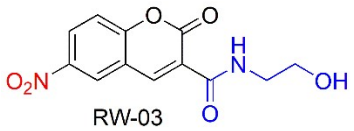




MS

Line#:3 R.Time:17.067(Scan#:1449)
MassPeaks:32
RawMode:Averaged 17.058-17.075(1448-1450) BasePeak:215(96113)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

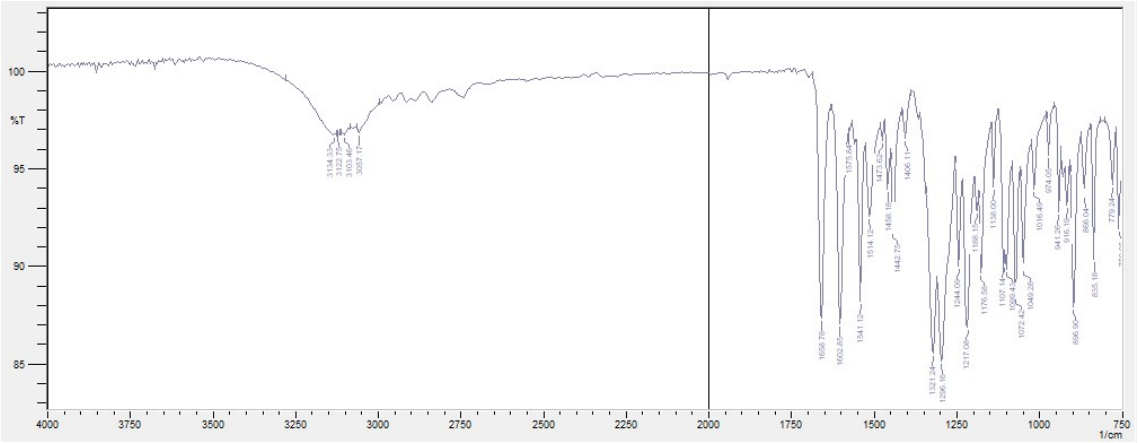




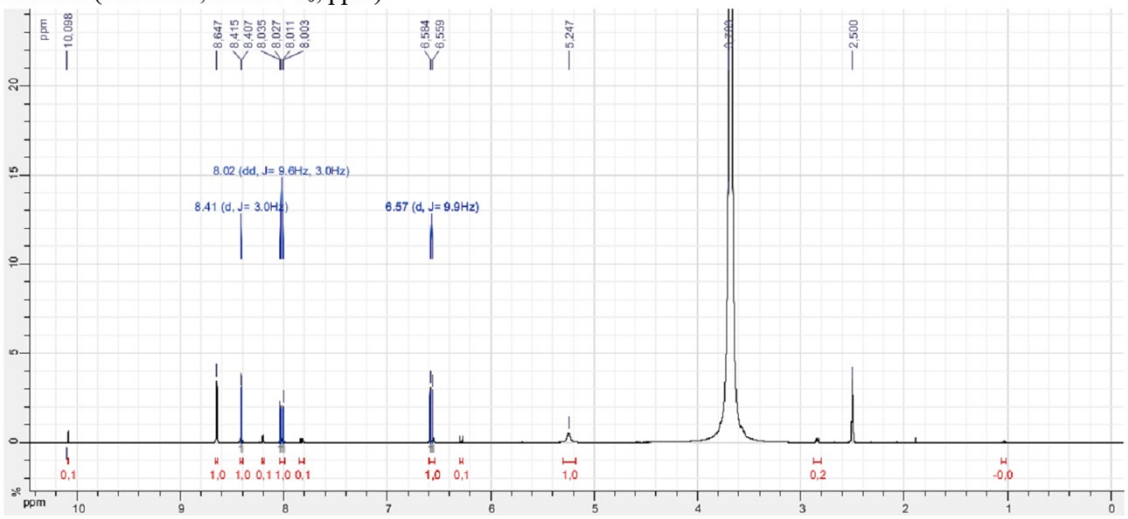
RW-03

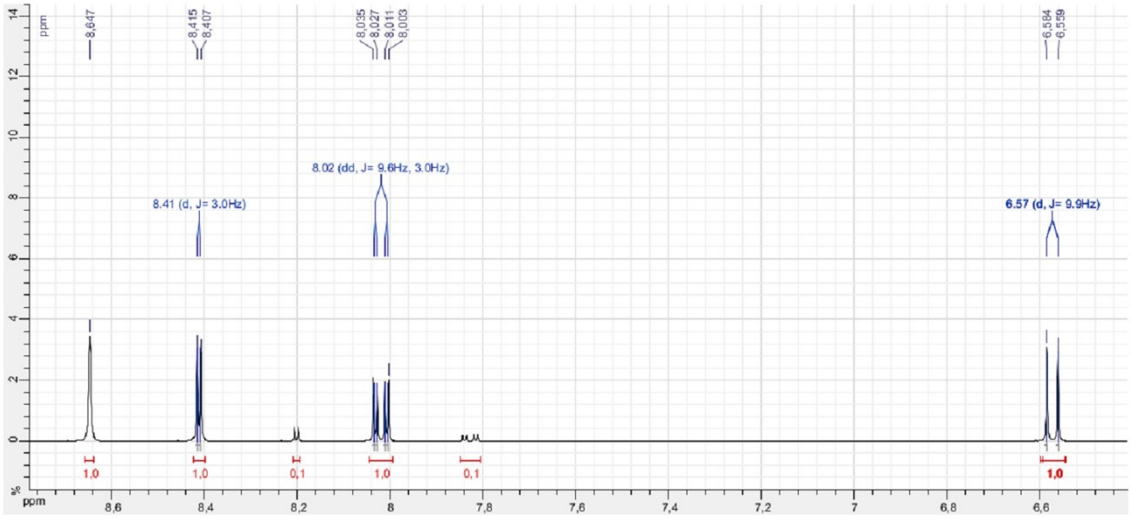
N-(2-Hydroxyethyl)-6-nitro-2-oxo-2H-chromene-3-carboxamide ***(RW-03)***

ATR-FTIR



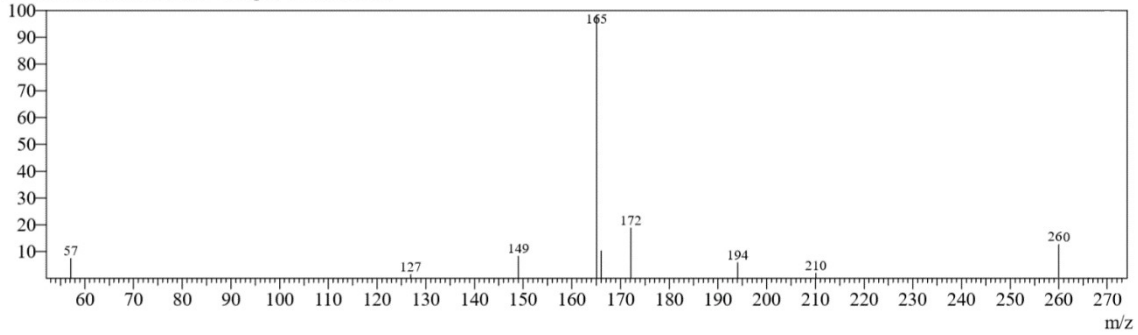
^{1}H NMR (400 MHz, DMSO-d_6, ppm)

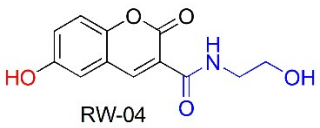




MS

Line#:2 R.Time:16.725(Scan#:1408)
MassPeaks:12
RawMode:Averaged 16.717-16.733(1407-1409) BasePeak:298(5840)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

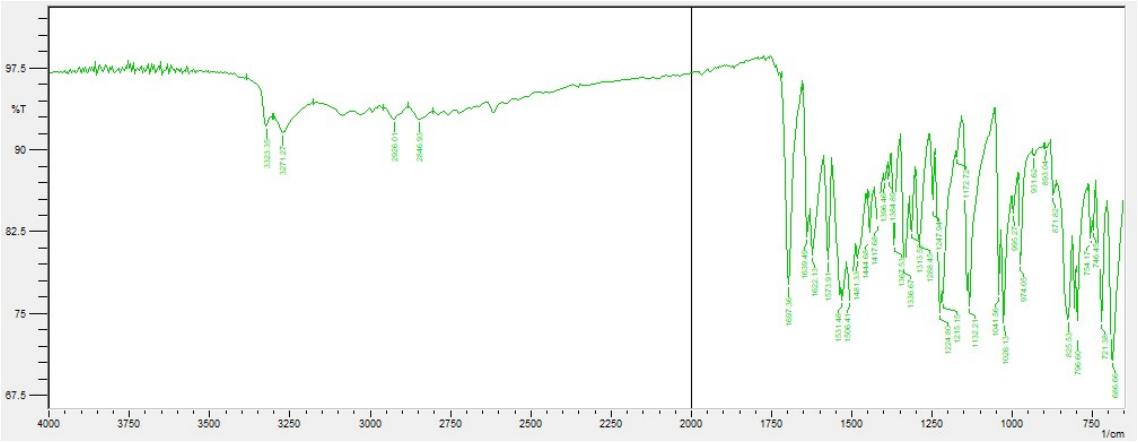




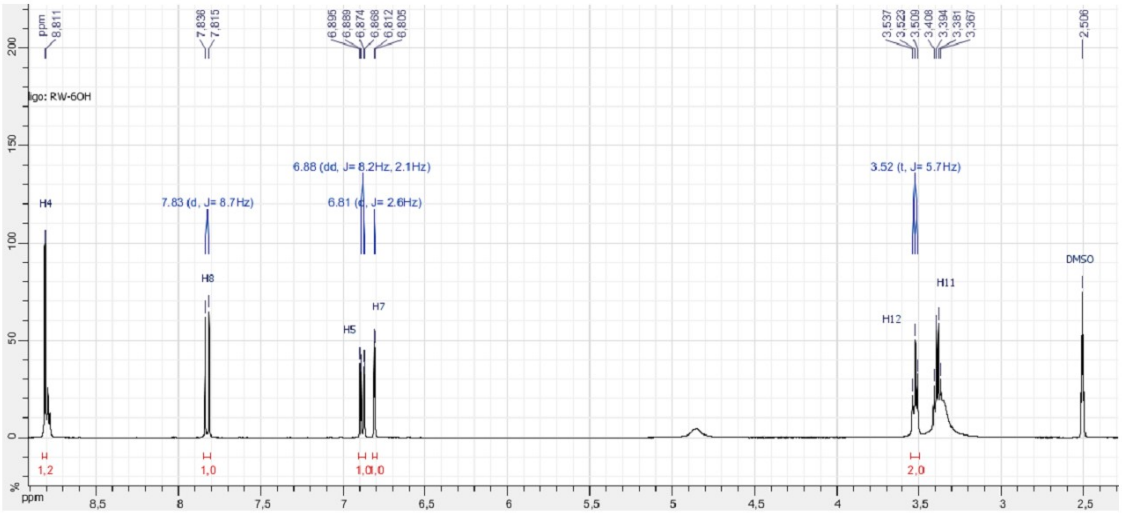
RW-04

N-(2-hydroxyethyl)-6-hydroxy-2-oxo-2H-chromene-3-carboxamide ***(RW-04)***

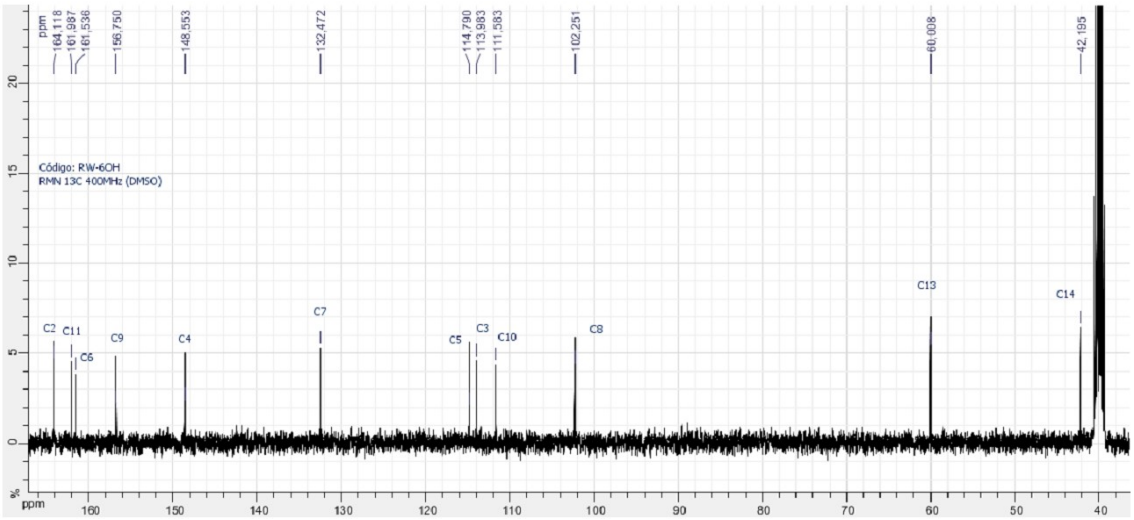
ART-FTIR



^{1}H NMR (400 MHz, DMSO-*d*$_6$, ppm)

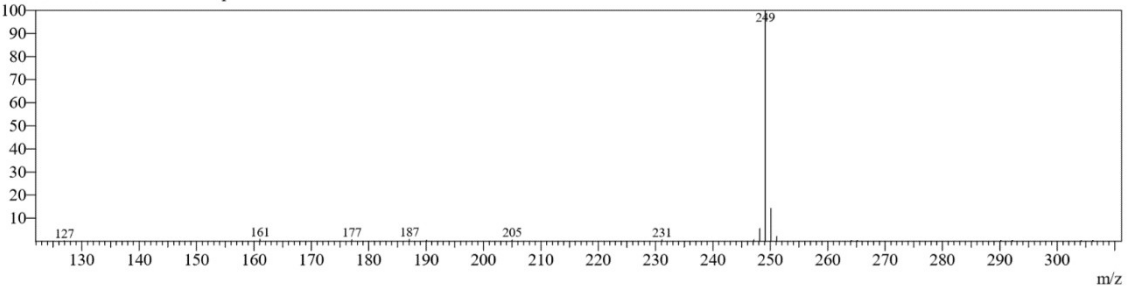


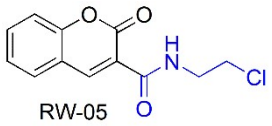
^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm)



MS

Line#:3 R.Time:21.808(Scan#:2018)
MassPeaks:18
RawMode:Averaged 21.800-21.817(2017-2019) BasePeak:249(165774)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

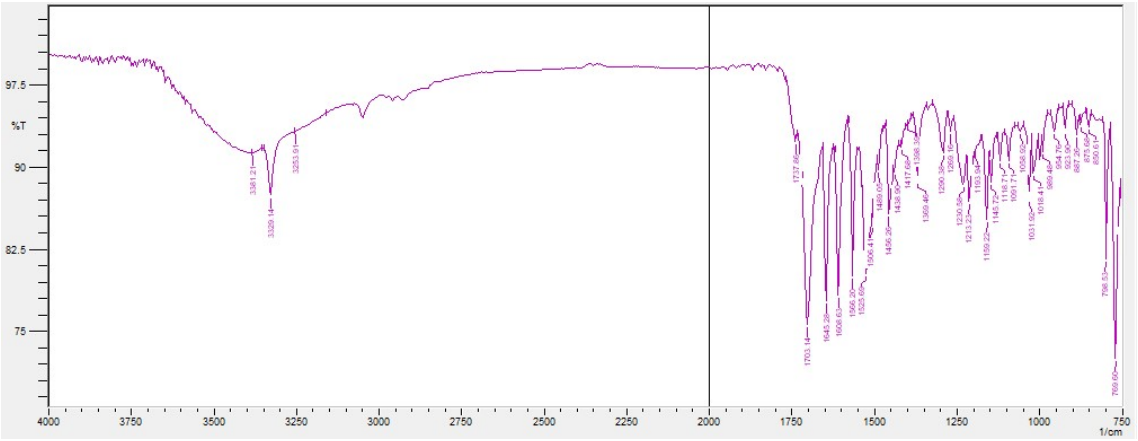




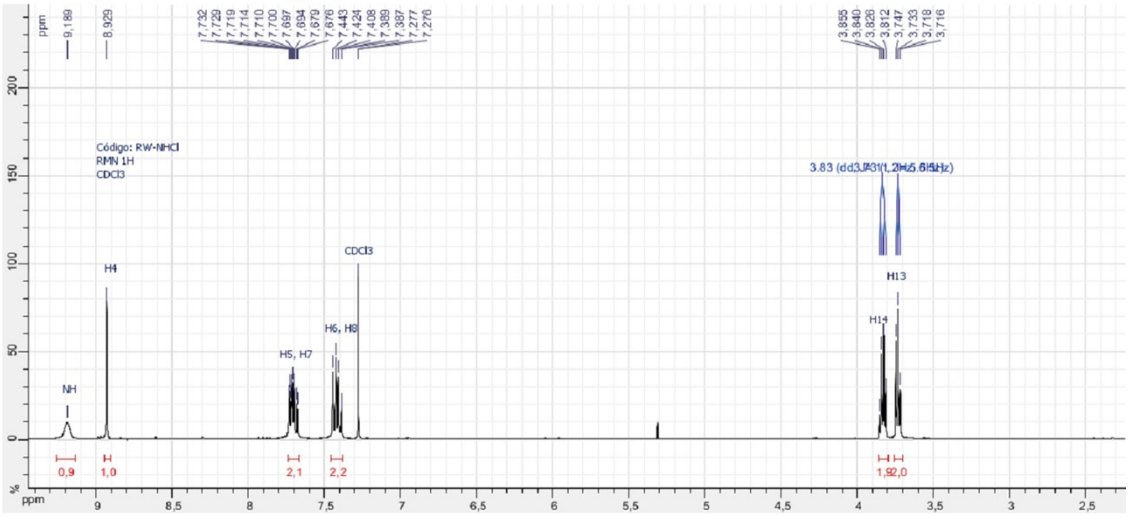
RW-05

N-(2-Chloroethyl)-2-oxo-2H-chromene-3-carboxamide ***(RW-05)***

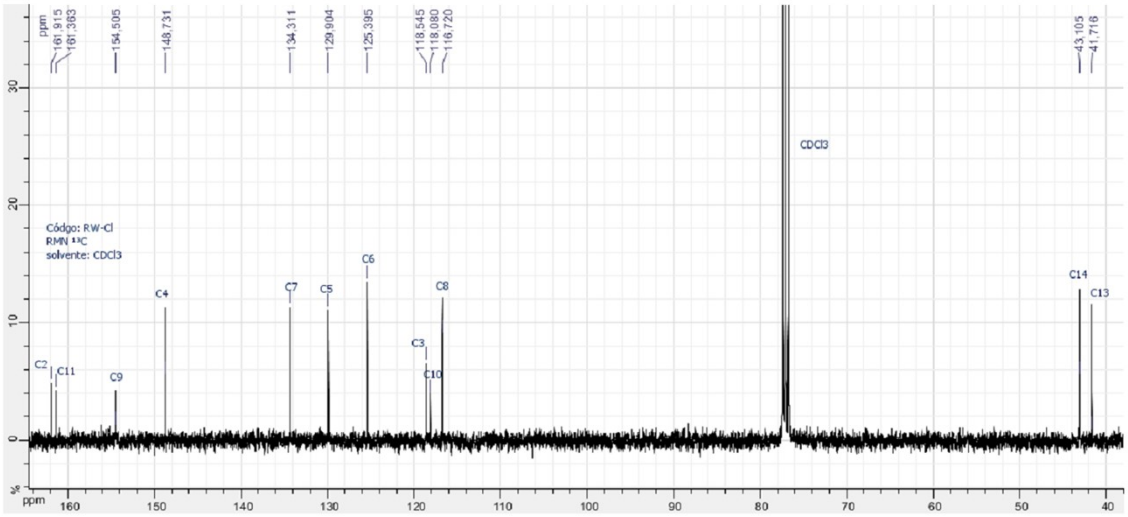
ATR-FTIR



^{1}H NMR (400 MHz, $CDCl_3$, ppm)

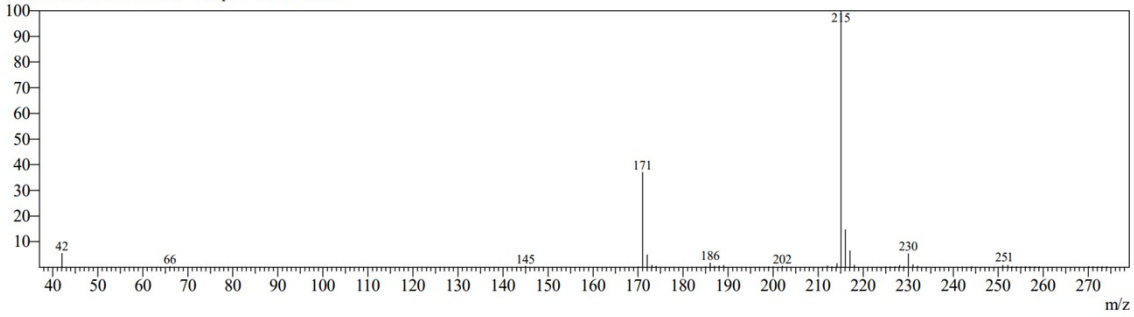


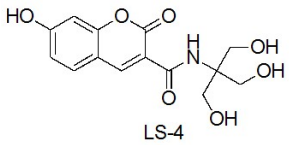
^{13}C NMR (100 MHz, $CDCl_3$, ppm)



MS

Line#:3 R.Time:17.750(Scan#:1531)
MassPeaks:48
RawMode:Averaged 17.742-17.758(1530-1532) BasePeak:215(608088)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

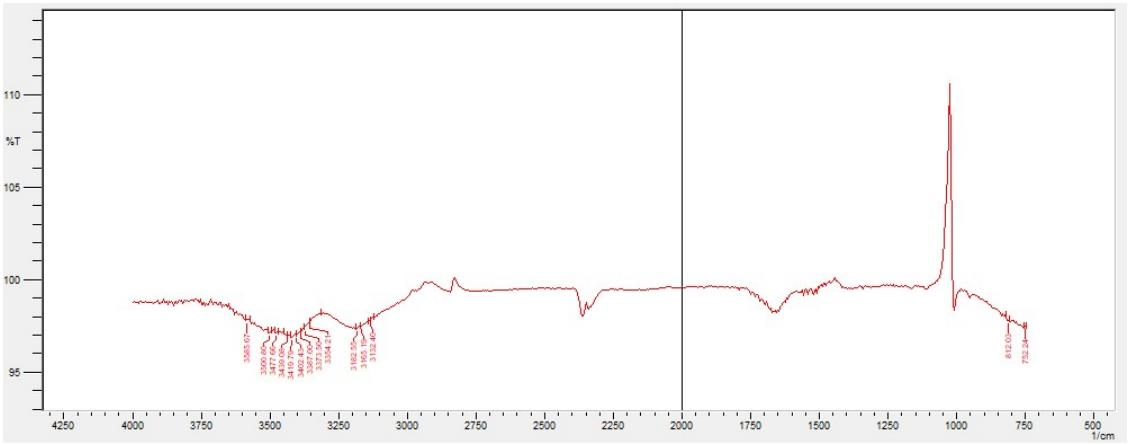




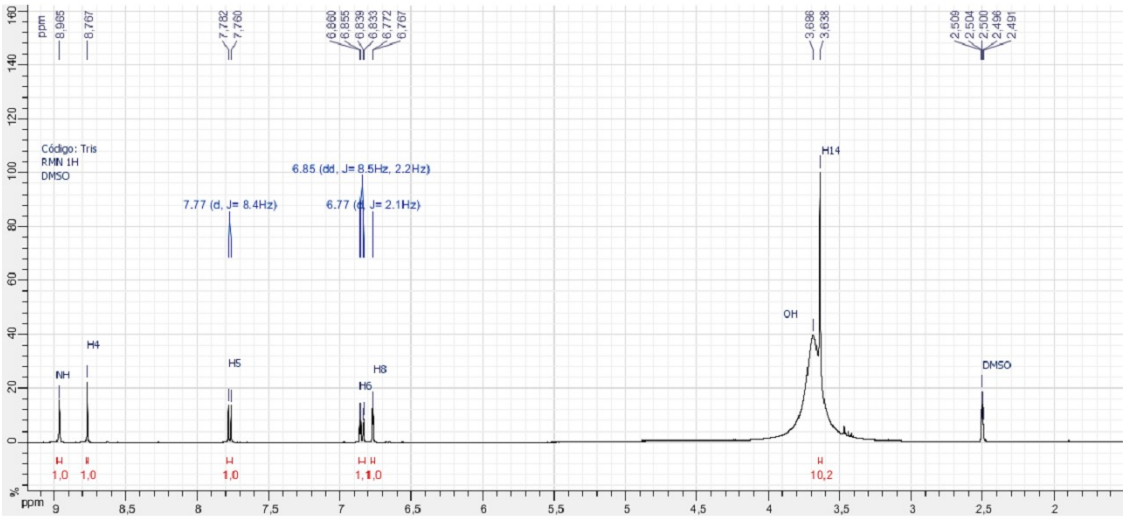
LS-4

7-Hydroxy-N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-2-oxo-2H-chromene-3-carboxamide ***(LS-04)***

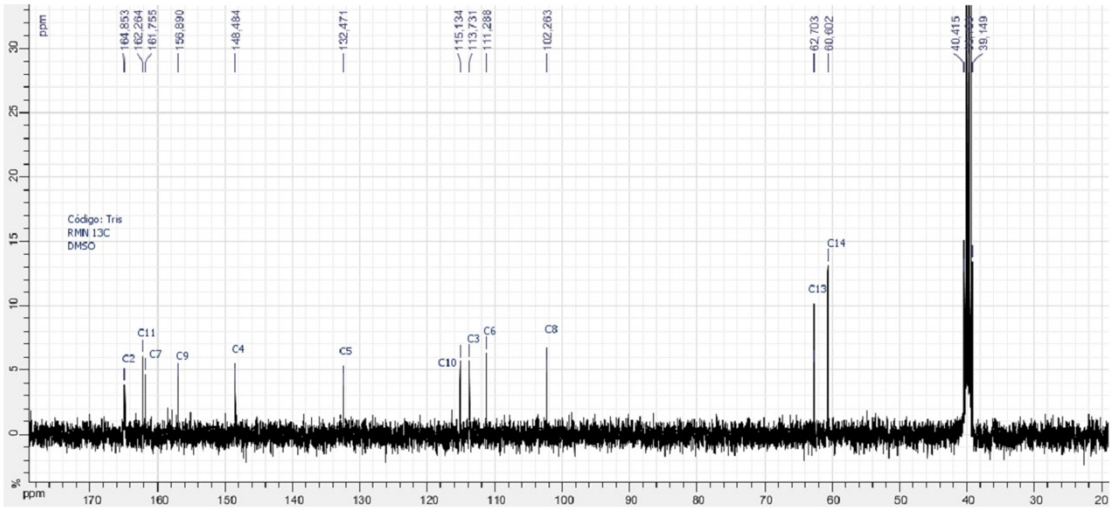
ATR-FTIR



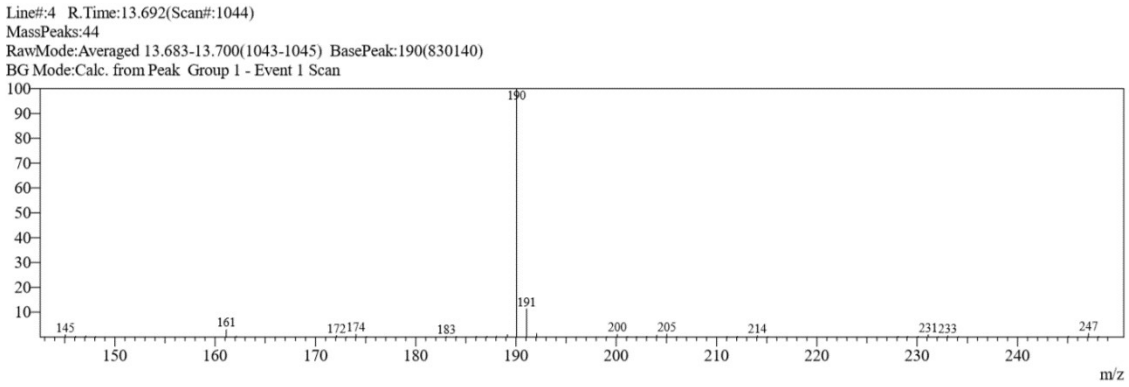
^{1}H NMR (400 MHz, DMSO-d_6, ppm)

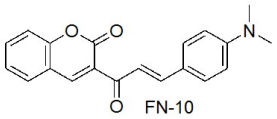


^{13}C NMR (100 MHz, DMSO-d_6, ppm)



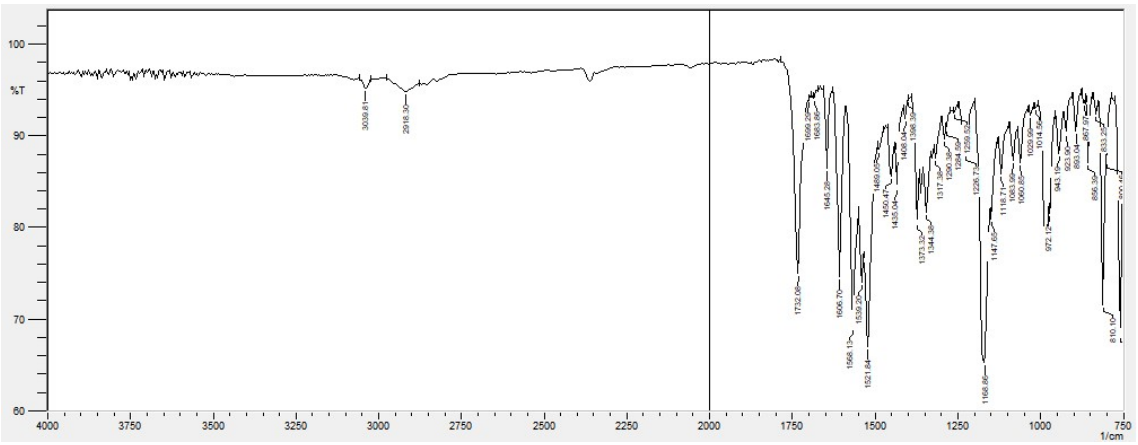
MS





3-((E)-3-(4-(Dimethylamino)phenyl)acryloyl)-2H-chromen-2-one ***(FN-10)***

ATR-FTIR



MS