

# Synthesis of 6-membered-ring fused thiazine-dicarboxylates and thiazole-pyrimidines *via* one-pot three-component reactions

Reagan L. Mohlala<sup>1,2</sup>; E. Mabel Coyanis<sup>1\*</sup>; Muhammad Q. Fish<sup>1</sup>, Manuel A. Fernandes<sup>2</sup>; Moira L. Bode<sup>2\*</sup>

<sup>1</sup> Advanced Materials Division, Mintek, Private Bag X3015, Randburg, 2125, South Africa

<sup>2</sup> Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, PO Wits, 2050, South Africa;

\*Correspondence: mabelc@mintek.co.za (E.M. Coyanis); Moira.Bode@wits.ac.za (M.L. Bode)

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## 1. Biological evaluation results

The synthesized pyrimidines and their analogues **8**, **13**, **19** and **20** were evaluated for toxicity in the MT-4 cell line at 100  $\mu$ M. MT-4 cells were originally isolated from an adult Japanese patient with acute lymphoblastic leukemia [1]. By determining the cytotoxicity of the compounds, it shows that they have the potential to kill cancer cells, as most of the compounds have values up to 7.3  $\mu$ M. Auranofin [2] was used as a cytotoxic control compound, starting at a concentration of 100  $\mu$ M. DMSO was used as a background control. Most of the compounds were considered as relatively toxic, with CC50 values below 100  $\mu$ M. Further biological studies using a range of cancer cell lines and primary cells would need to be performed to determine if the observed toxicity is directed to particular cancer types or if it is stochastic. On the other hand, six of the thiazole-pyrimidine derivatives and analogues, namely **13a**, **13j**, **13h**, **13o**, **19a/b** and **20**, were found to have CC50 values above 200  $\mu$ M, as shown in Table S1, whilst at least compounds 10c, 10m and 13e were found to have CC50 values of above 100  $\mu$ M. These compounds, namely **3a**, **13j**, **13h**, **13o**, **19a/b** and **20**, can be considered to be relatively nontoxic and were subjected to antiviral activity evaluation. The antiviral activities were performed in infected MT4 cells, using dolutegravir and raltegravir as control compounds. The tests were carried out in a cell-based assay, and each compound was analyzed at a single dose of 100  $\mu$ M. Based on the outcome, none of these compounds showed any significant activity.

**Table S1:** CC<sub>50</sub> values and antiviral percentage inhibition of synthesised thiazine derivatives 10a-u, 13a-j, 19, 20 and 8

Compound	CC <sub>50</sub> (μM)	% HIV Antiviral inhibition (in infected MT4 cells)
10a	7.3	
10b	12	
10c	102	
10d	51	
10e	16	
10f	33	
10g	33	
10h	51	
10i	49	
10j	80	
10k	25	
10l	20	
10m	120	
10n	17	
10o	21	
10p	16	
10q	39	
10r	26	
10s	57	
10t	33	
10u	27	
13a	> 200	-7.18
13b	59	

<b>13c</b>	17	
<b>13d</b>	101	
<b>13e</b>	> 200	0.98
<b>13f</b>	36	
<b>13g</b>	> 200	-2.63
<b>13h</b>	14	
<b>13i</b>	17	
<b>13j</b>	> 200	-9.18
<b>19a</b>	> 200	-4.83
<b>19b</b>	> 200	1.19
<b>20</b>	> 200	0.55
<b>8</b>	27	-7.18

CC<sub>50</sub> - concentration of compounds that causes 50% reduction of cell growth

Most of the synthesised thiazines **10** and pyrimidines **13** were shown to have toxicity towards a leukaemia cell line used in this study, with the most toxic being **10a**, indicating potential anticancer effects. Future work will be directed towards determining if this toxicity is specific or stochastic.

## 2. Biological evaluation methods

### 2.1 Cytotoxicity

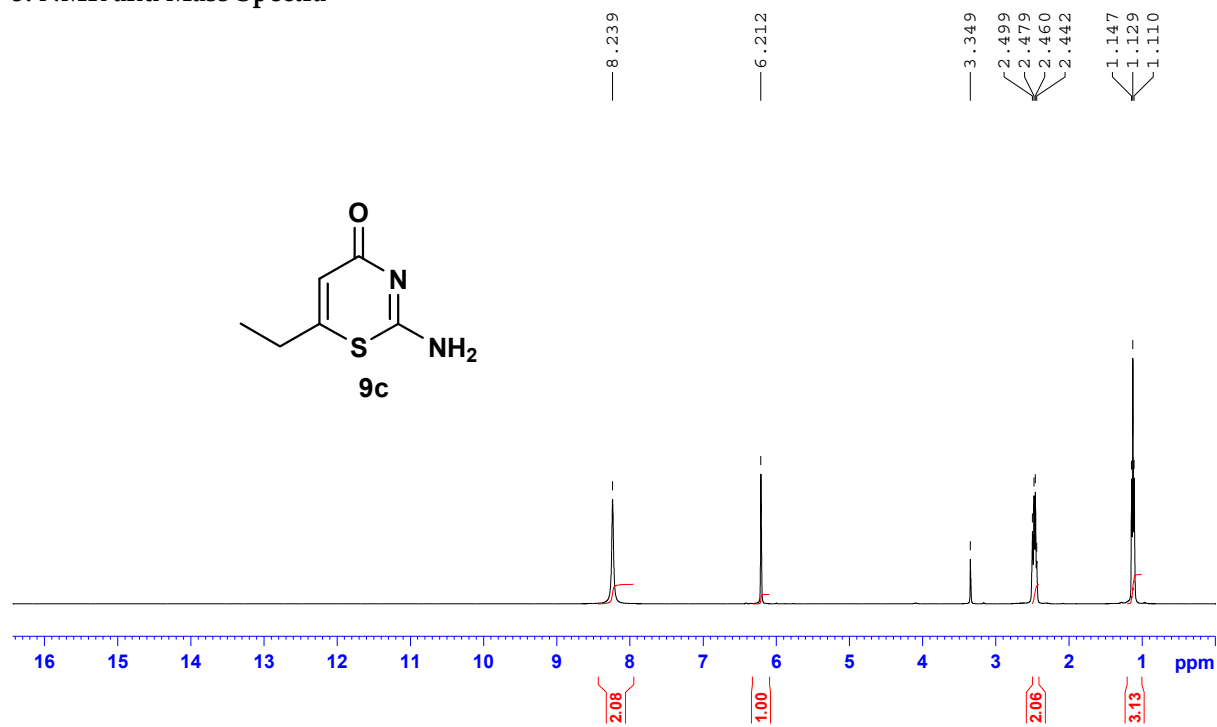
The day of cytotoxicity testing, MT-4 cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 catalogue number 120) were counted and seeded at  $5 \times 10^4$  cells per well. A total of 100  $\mu\text{L}$  of cells was added to each well of 96-well cell culture plates (TTP) for testing. The volume in the last row of wells of each plate was further increased to 197  $\mu\text{L}$  of media containing MT-4 cells. The plate was placed into the incubator to equilibrate to 37 °C and 5%  $\text{CO}_2$ . Test compounds were prepared in plate format, and each compound was at 25 mM stock concentration in DMSO. A total of 3.2  $\mu\text{L}$  compound was added to the final row of wells containing 197  $\mu\text{L}$  cells and mixed to ensure the solution was homogeneous with the cells. A two-fold serial dilution of an eight-times series was performed, and the final 100  $\mu\text{L}$  was discarded. The plate was placed at 37 °C and 5%  $\text{CO}_2$  for 5 days. On the fifth day, the cell viability was assessed by using the Cell Titre 96® Aqueous One Solution Cell Proliferation assay (Promega) as per the manufacturer's instructions. Briefly, 20  $\mu\text{L}$  of MTS solution was added to each well and incubated at 37 °C for 4 hours. The absorbance of the plates was then read at 490 nm on a Spectra Max Spectrophotometer (Molecular Devices). The data analysis was completed on Origin 8.1 with the log value of the compounds molar concentration plotted against the percentage cell viability determined by the absorbance level by sigmoidal fitting. From the curve, a half maximal cytotoxicity CC50 for compounds was determined. Auranofin was used as a cytotoxic control compound, starting at 100  $\mu\text{M}$ , across a two-fold serial dilution of eight dilution series. DMSO was used as a background control and two-fold serially diluted from 1.5%.

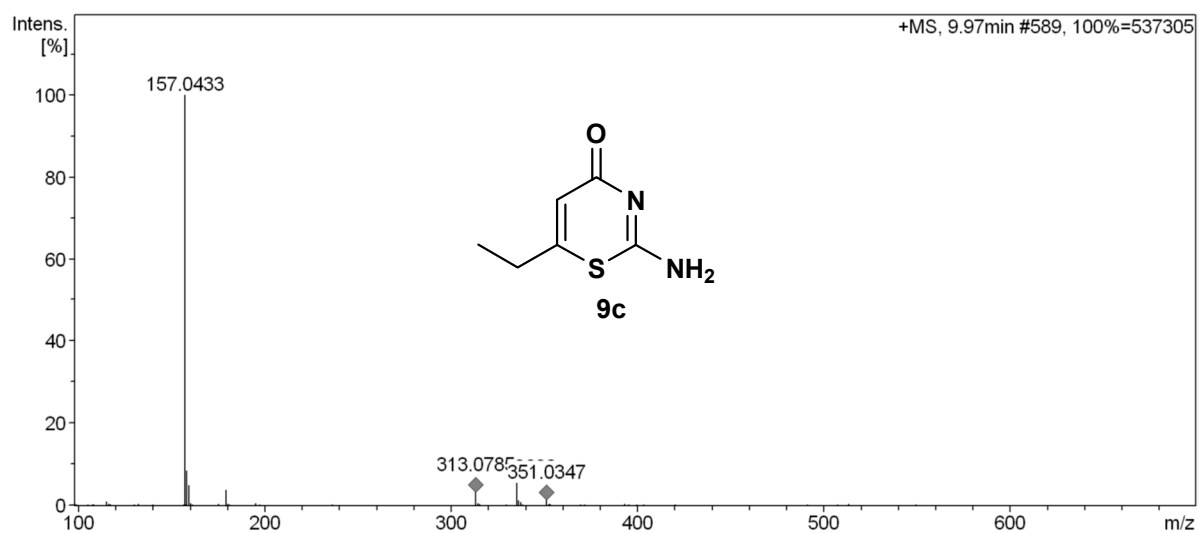
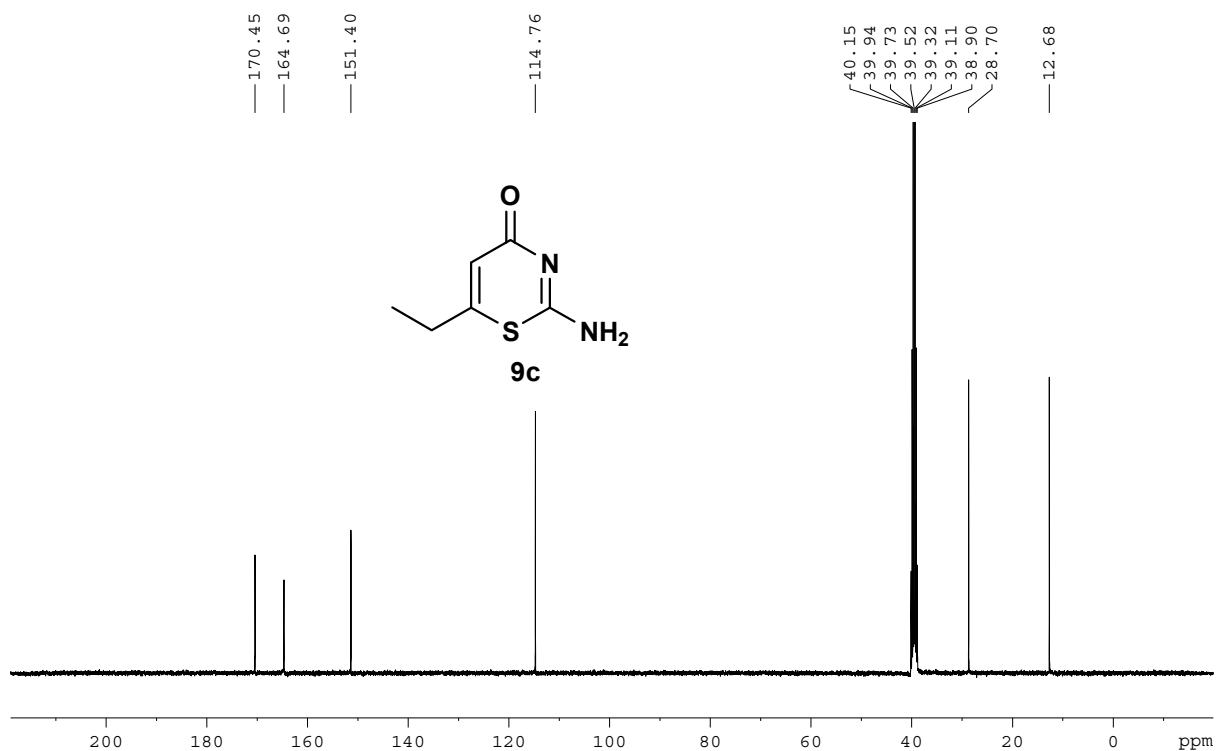
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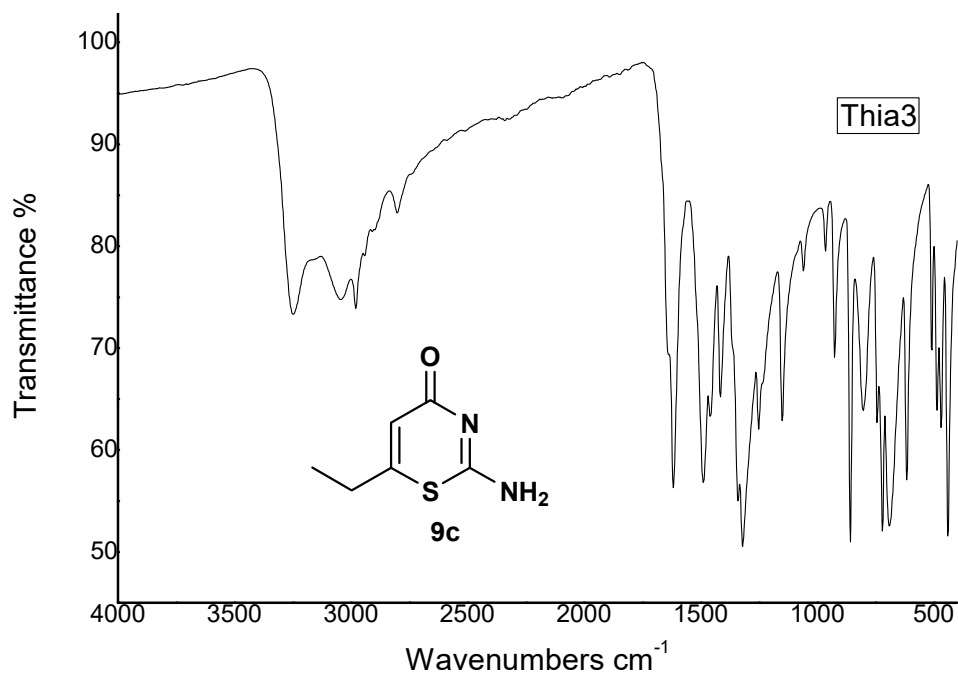
An HIV-1 chronically infected ACH-2 clone was obtained through the NIH HIV Reagent Program, Division of AIDS, NIAID, NIH: ACH-2 Cells, ARP-349, contributed by Dr. Thomas Folks. The cells were grown and maintained in RPMI 1640 (Sigma) supplemented with 10 mM HEPES, 2 mM L-glutamine, non-essential amino acids and 10% heat inactivated fetal bovine serum at 37 °C and 5%  $\text{CO}_2$ . The ACH-2 cells were activated by 1  $\mu\text{M}$  phorbol myristate acetate (PMA) [3]. Supernatants were harvested at 48 h by centrifugation at 4000 x g and stored at -80 °C until use. MT4 cells were grown and maintained in RPMI (Gibco) supplemented with 2 mM L-glutamine, non-essential amino acids and 10% heat-inactivated fetal bovine serum at 37 °C and 5%  $\text{CO}_2$ . Harvested supernatant containing HIV-1 was added to MT4 cells, and viral absorption was allowed for 24 h, similar to the method by Rosenwirth and co-workers [4]. After viral absorption, the cells were washed once with fresh media, a sample was taken and HIV1 infection was allowed to develop. Seven days after infection, MT4 supernatant samples were tested for the presence of p24 antigen, using the bioelisa HIV-1+2 Ag/Ab kit (biokit) as per the manufacturer's instructions. After HIV-1 infection was confirmed, supernatant samples were isolated by centrifugation at 4000 x g and immediately used to infect  $1 \times 10^5$  cells/ml of MT4 cells. The virus was allowed to adsorb, and 24 h later, the cells were washed with fresh media and, 100  $\mu\text{L}$  of cells,  $1 \times 10^4$  cells, was

aliquoted into a round-bottom 96-well plate (TPP). Uninfected cells were also prepared. A day 1, a sample was taken and stored at -80 °C until final testing. Compounds were diluted in fresh media to 200  $\mu$ M concentrations, and 100  $\mu$ L was added to each test well; the final concentration of compounds was 100  $\mu$ M. Control compounds include dolutegravir and raltegravir and were used at 10  $\mu$ M each; blank wells contained equal volumes of DMSO. Upper and lower asymptotes were determined by untreated MT4 cells either infected with HIV (100%) or not infected with HIV (0%). Four days after infection, p24 presence was determined by using the bioelisa HIV-1+2 Ag/Ab kit as per the manufacturer's instructions. The data were used to determine if the selected concentration of compounds would reduce the amount of p24 and thereby indicate a reduction in HIV infectivity.

### 3. NMR and Mass Spectra



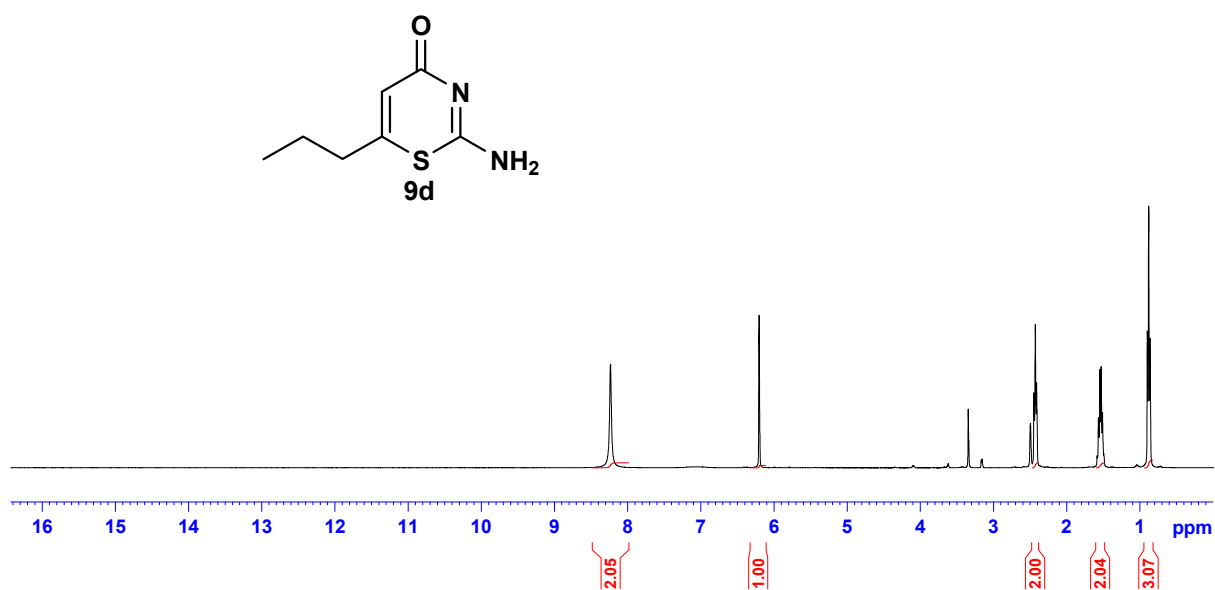




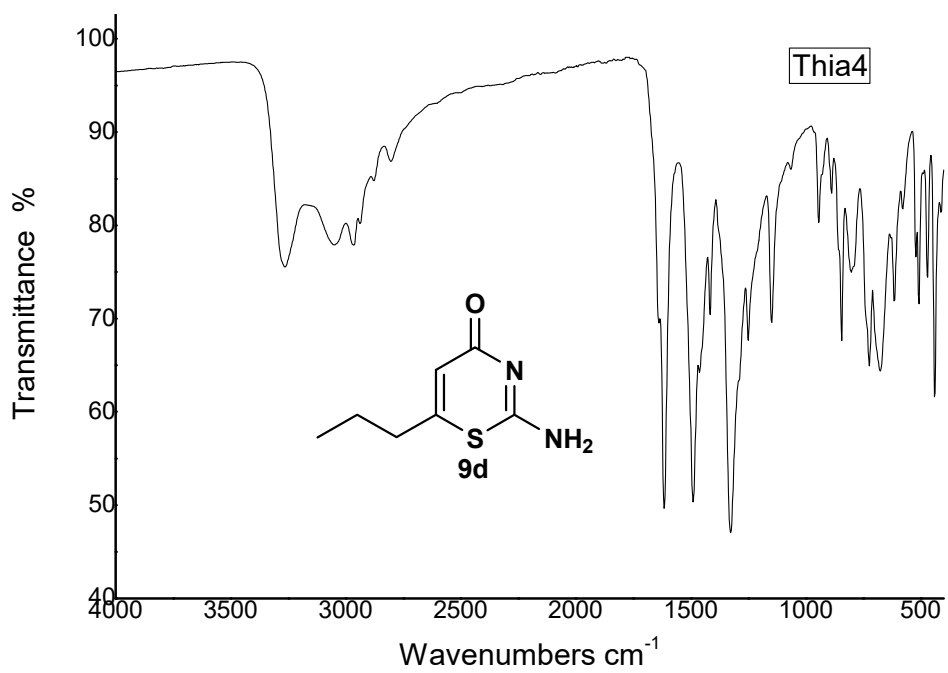
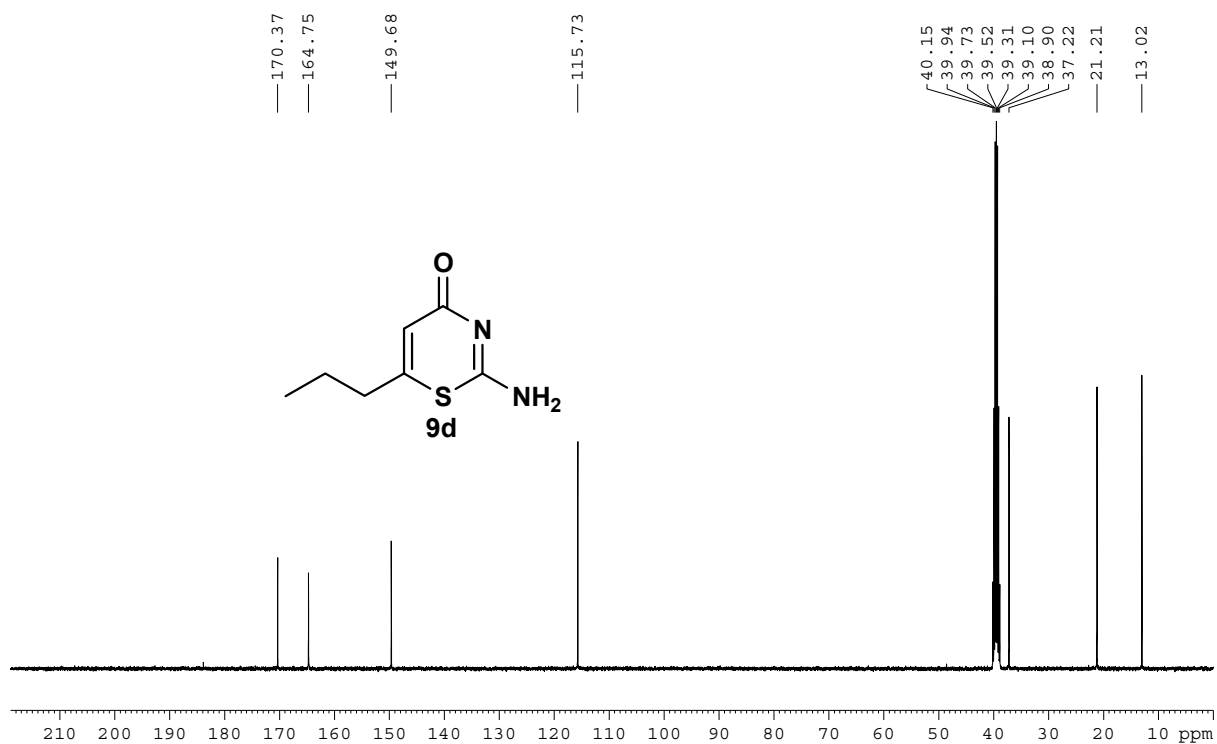
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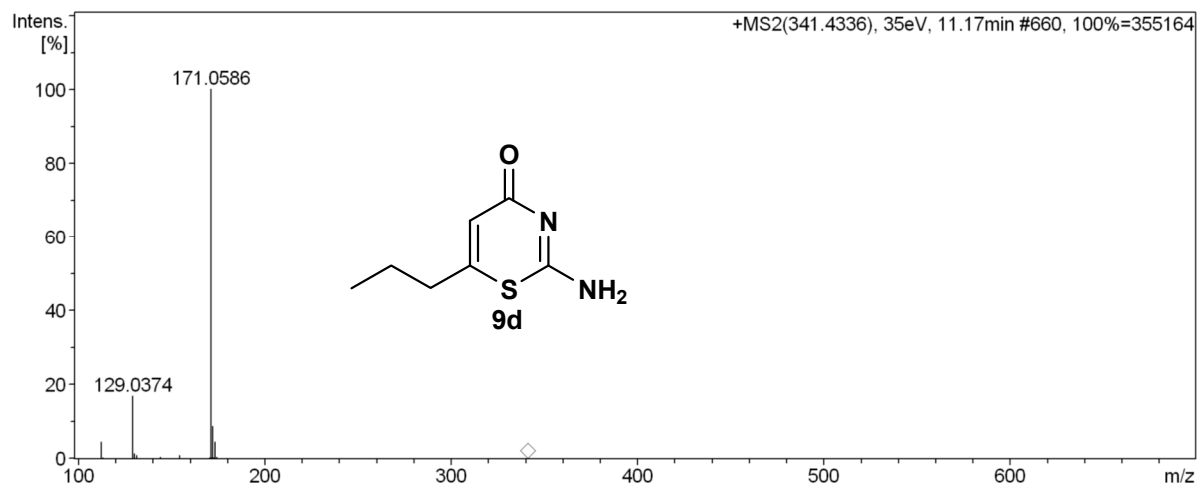
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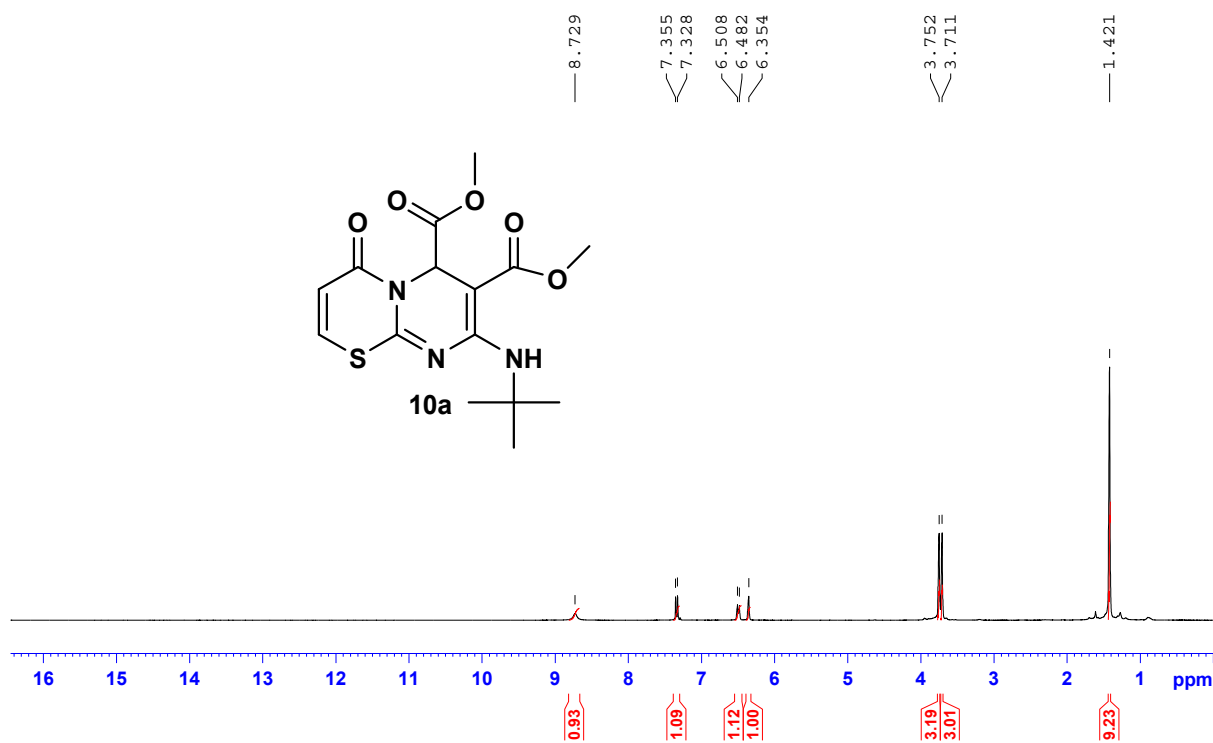


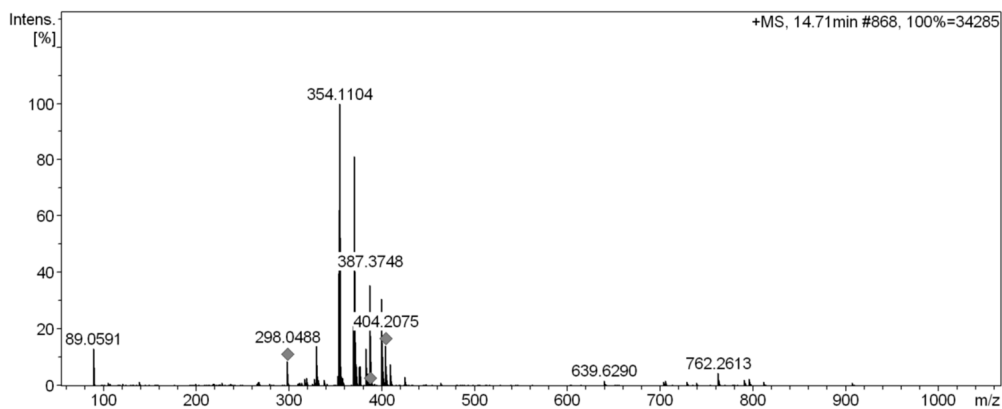
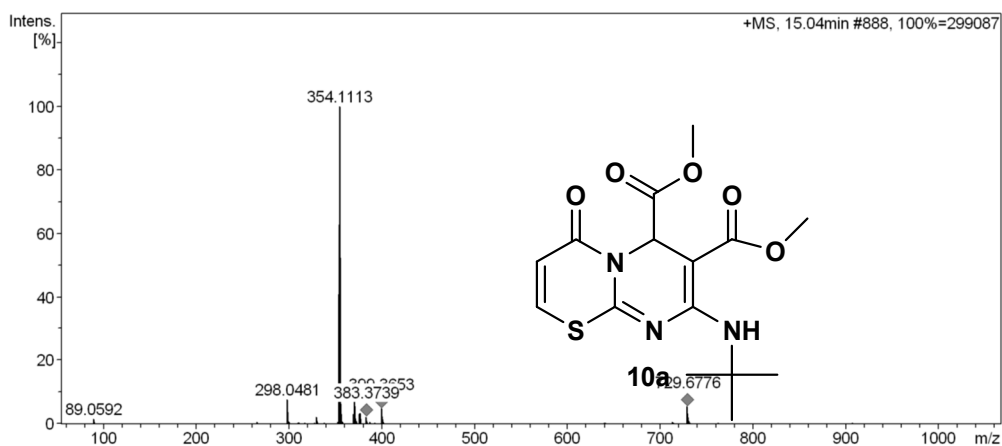
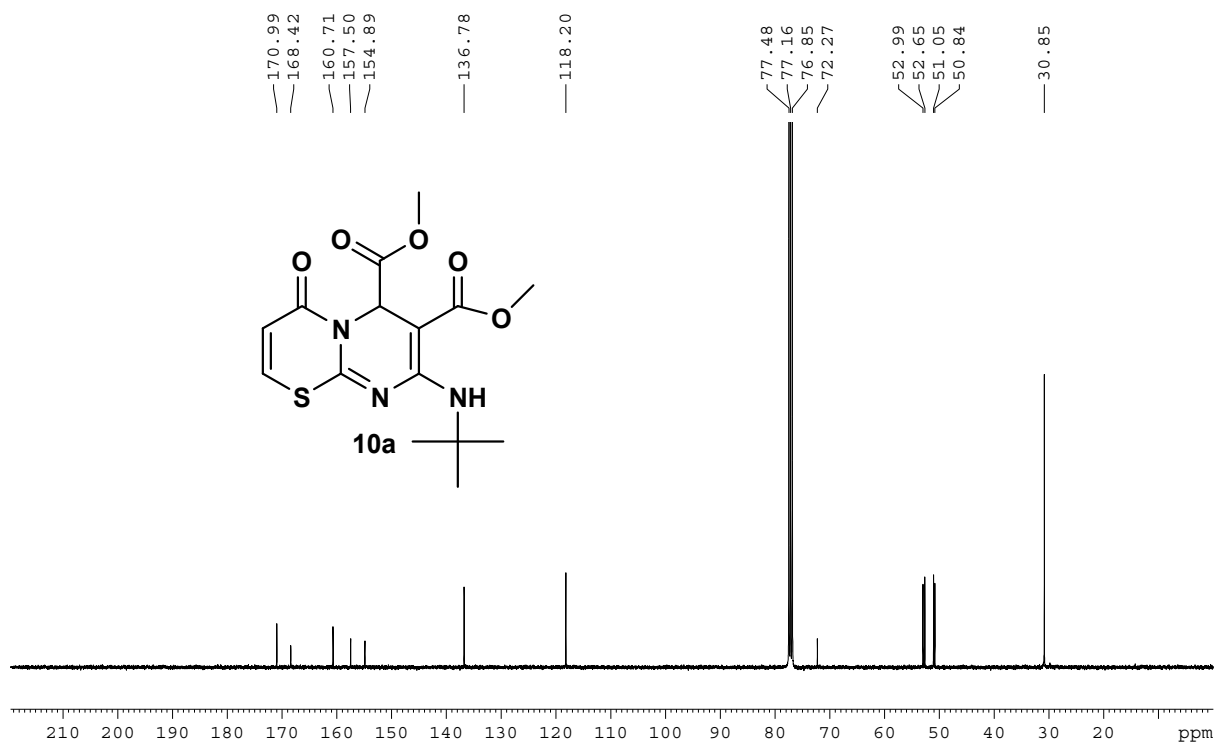


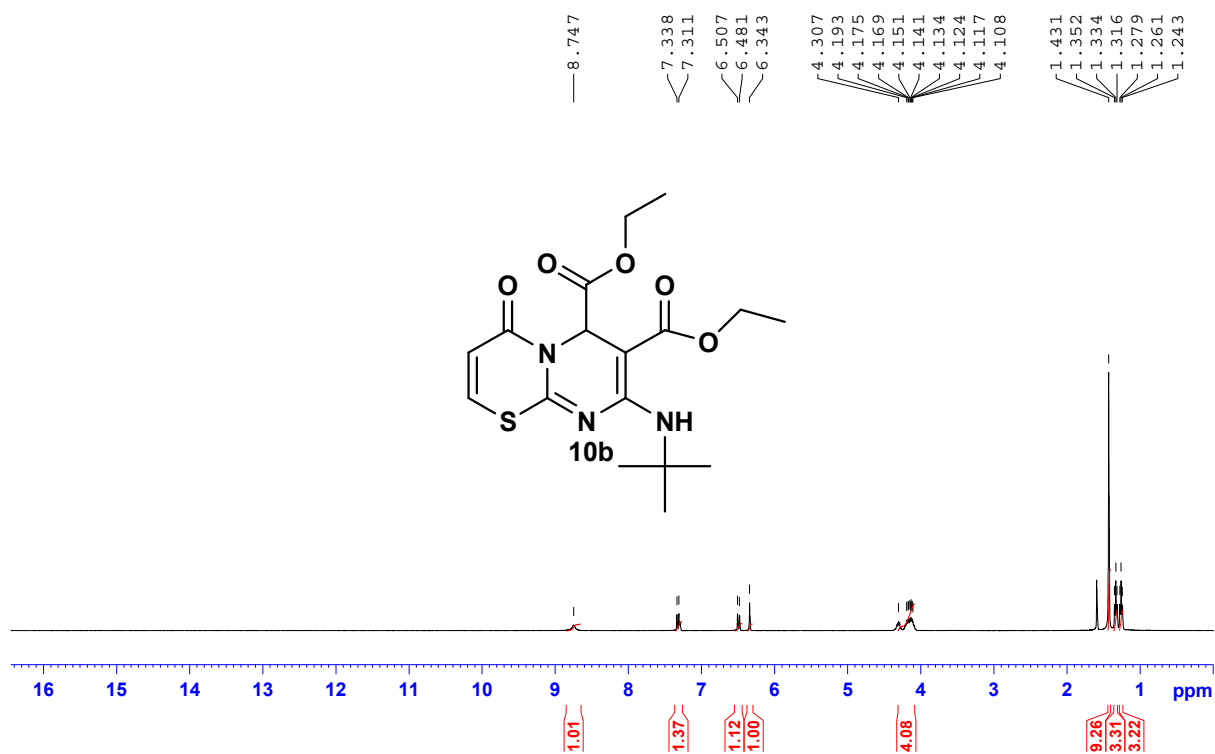
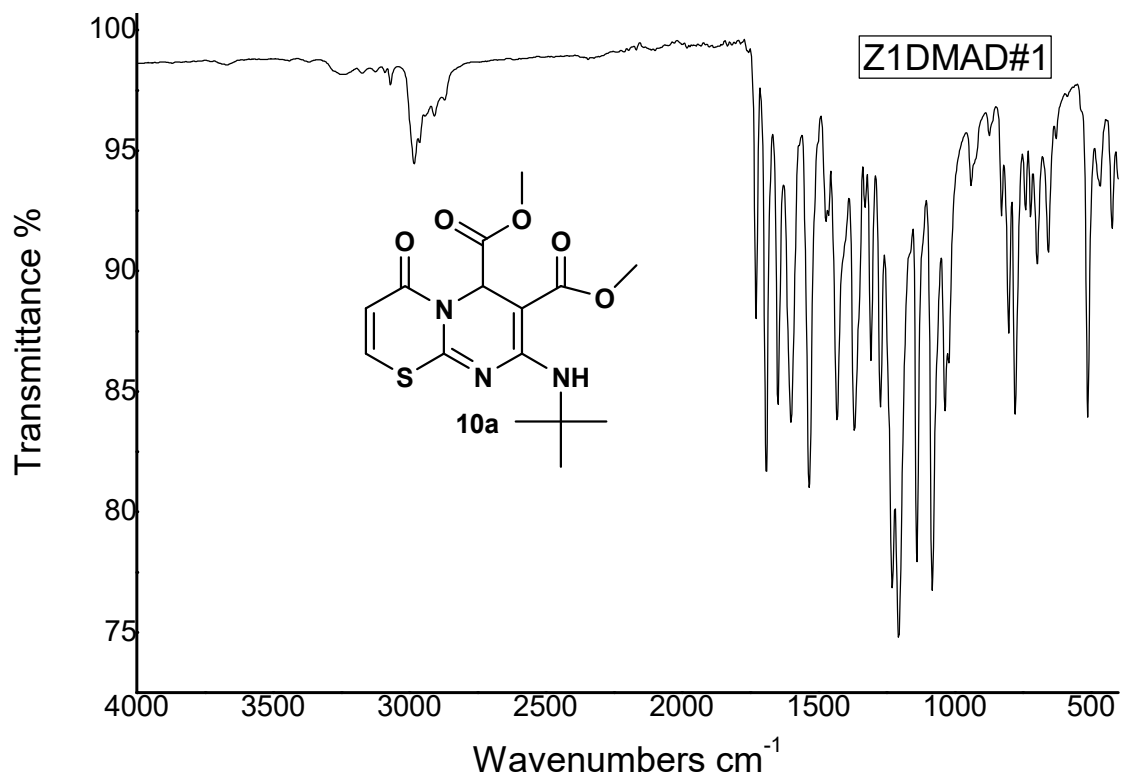


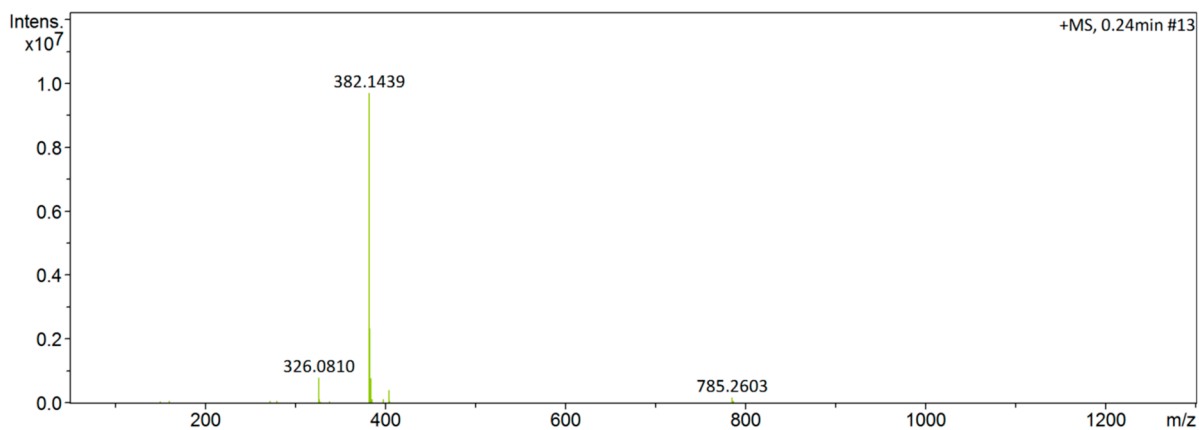
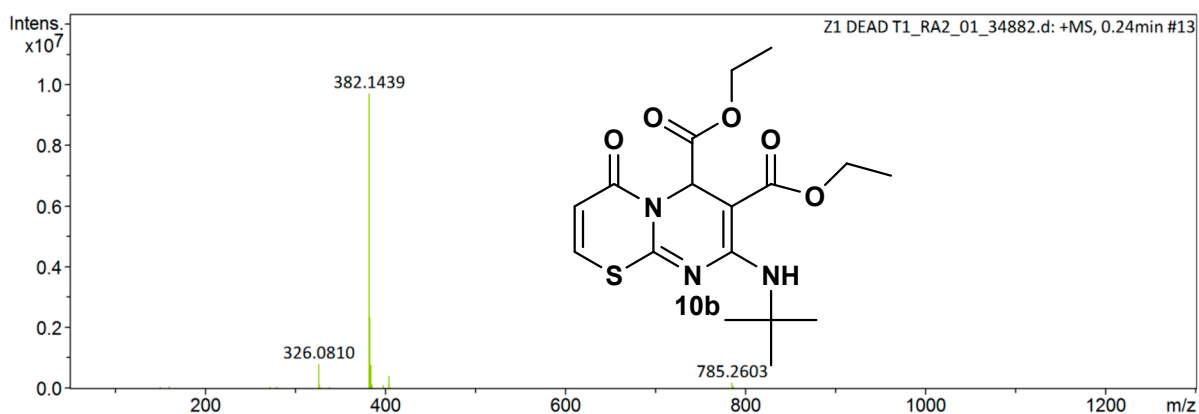
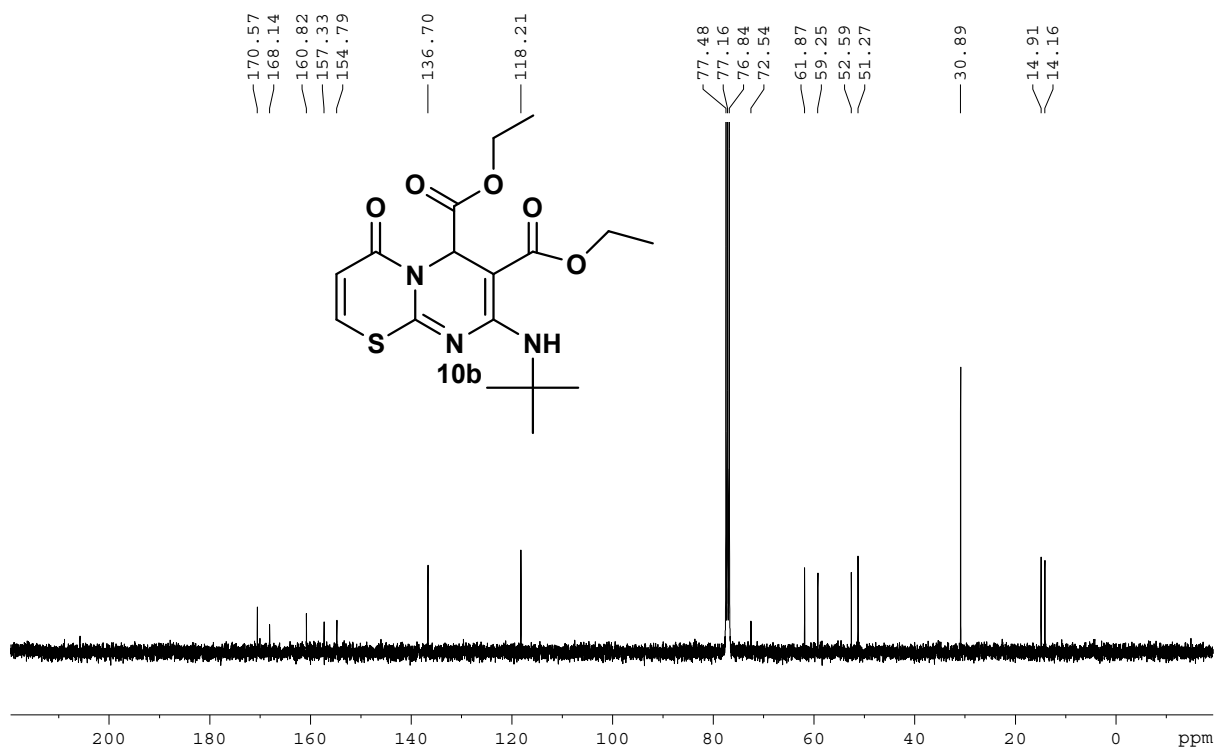


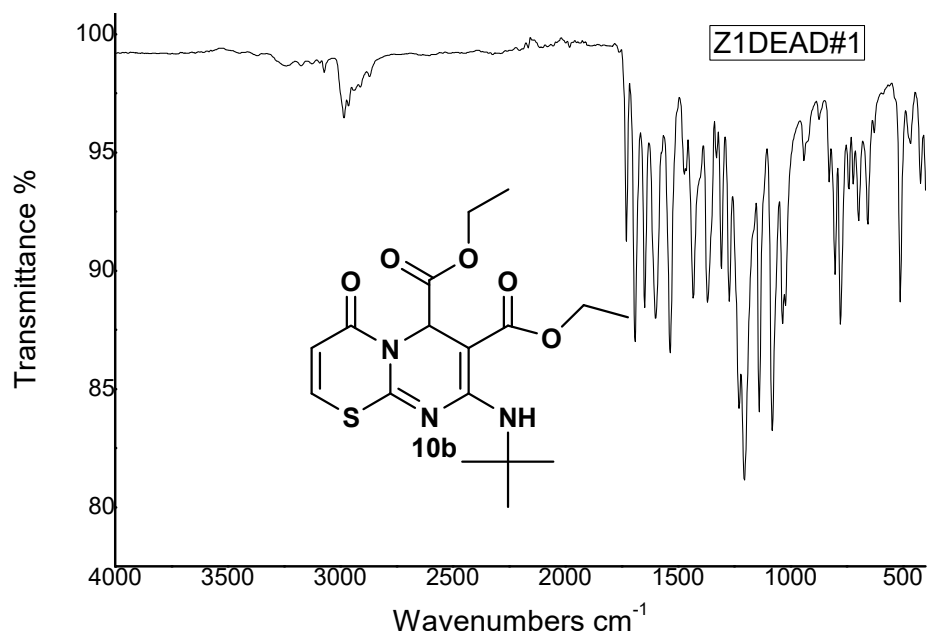
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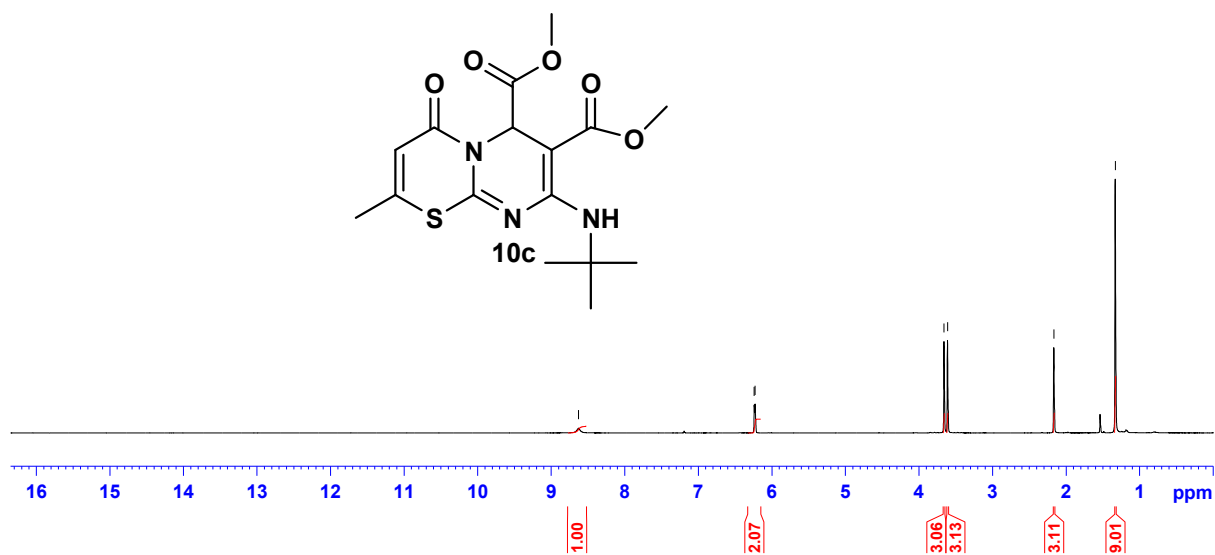
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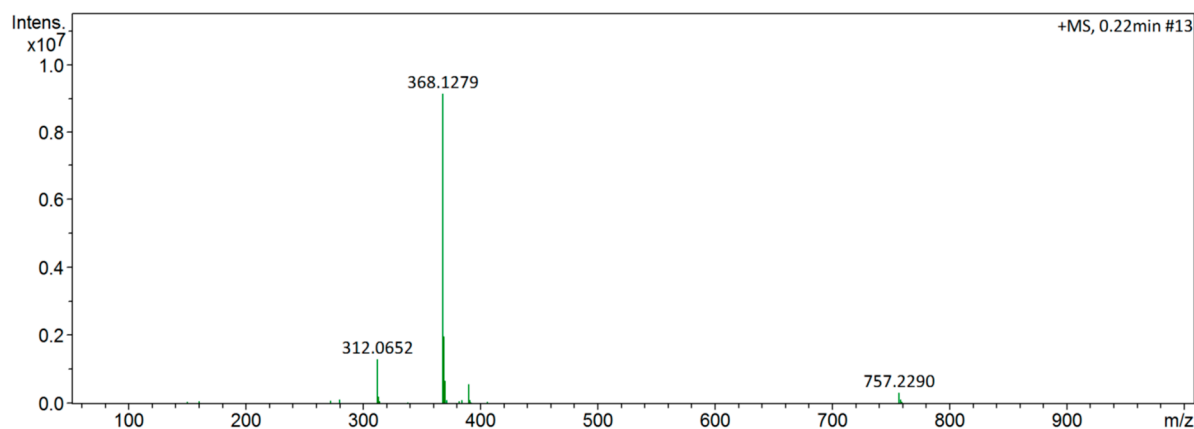
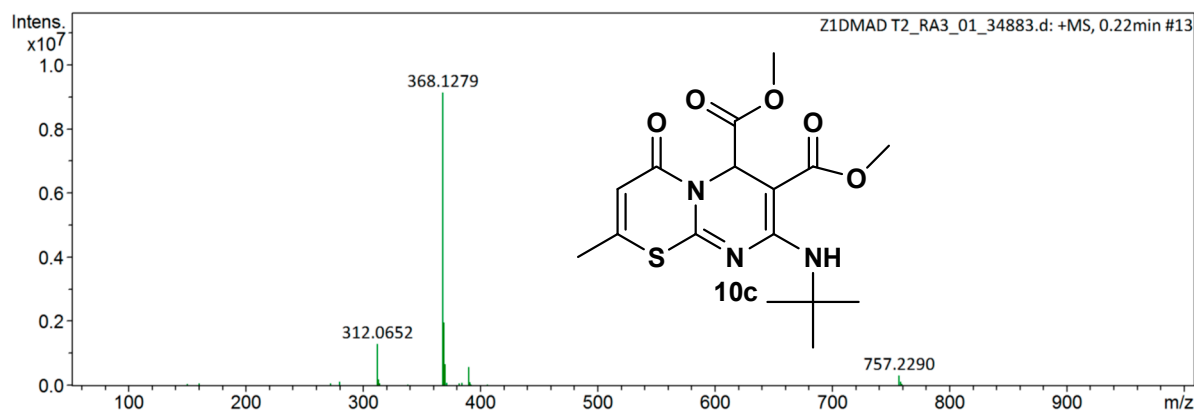
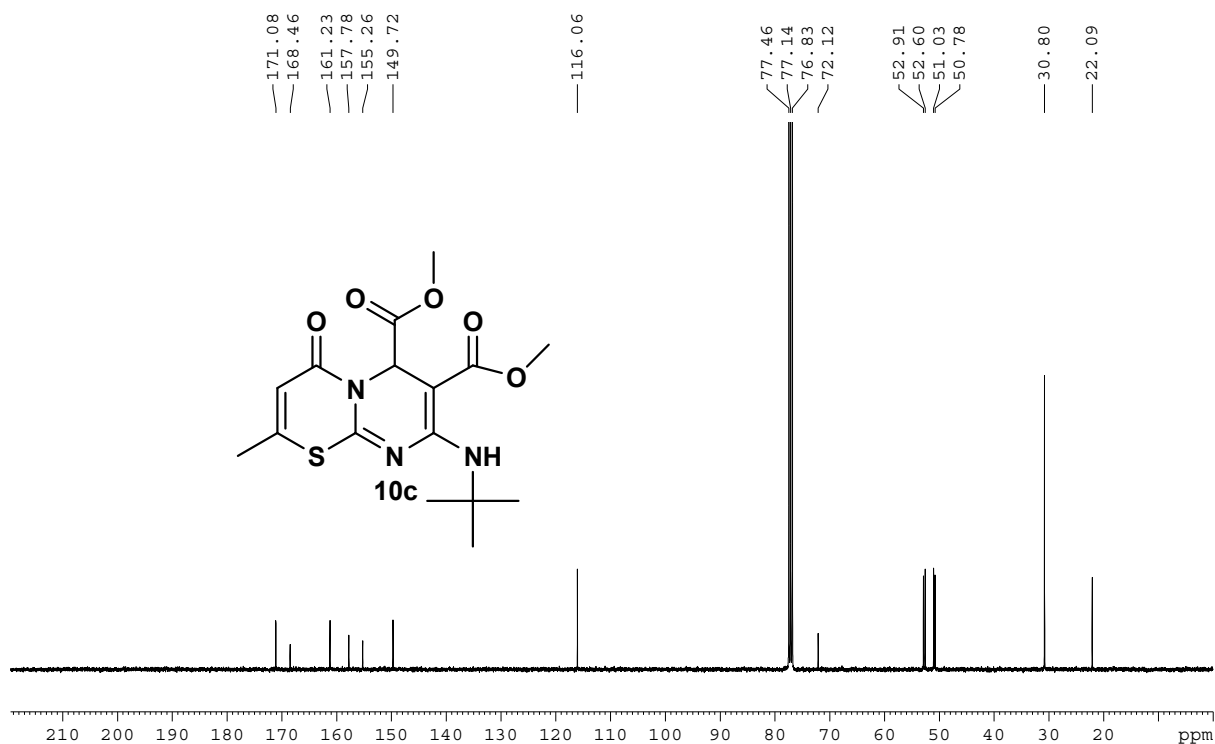
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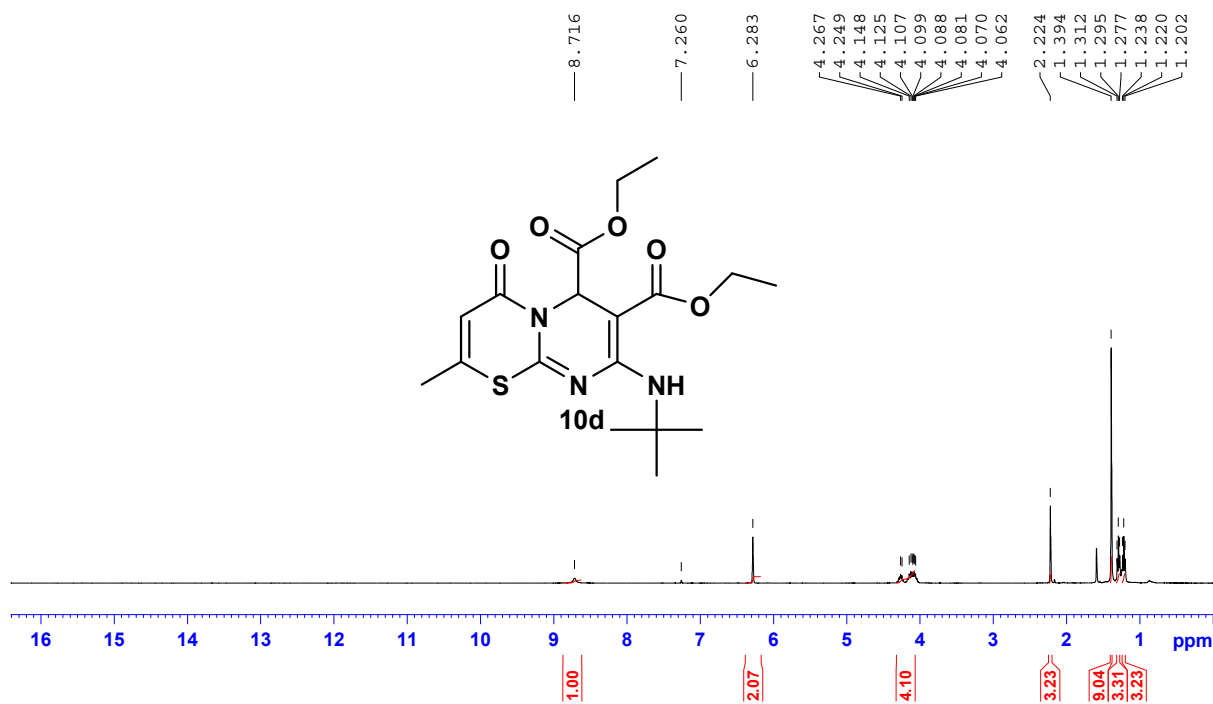
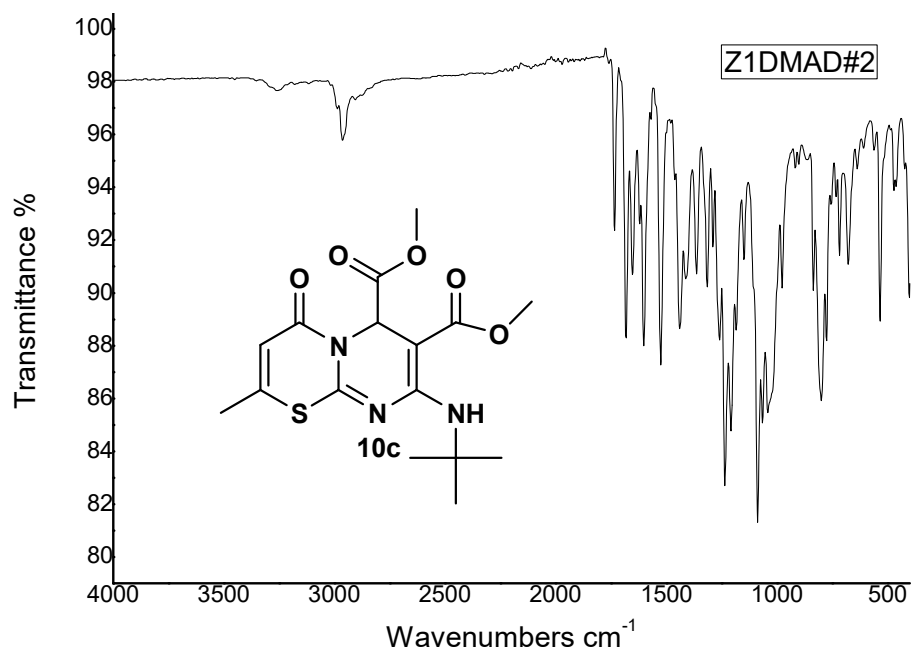
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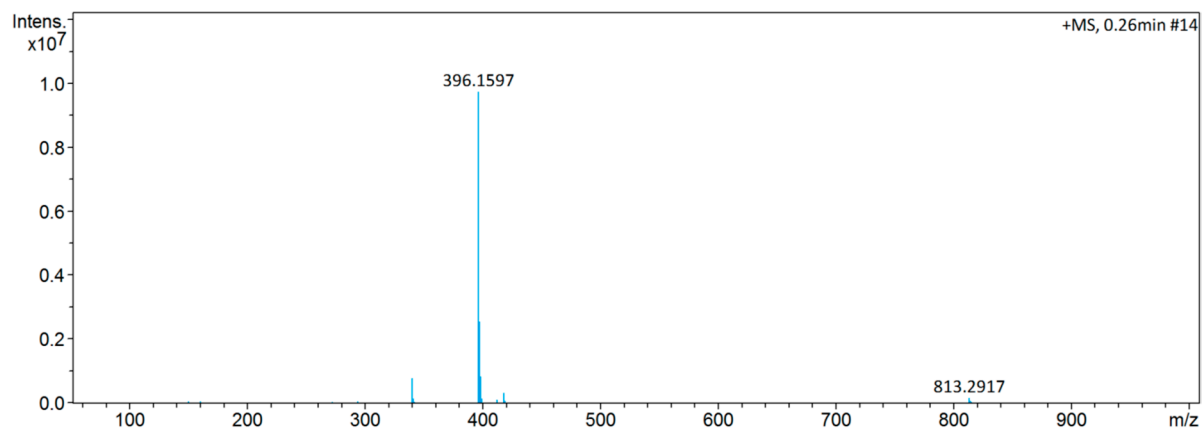
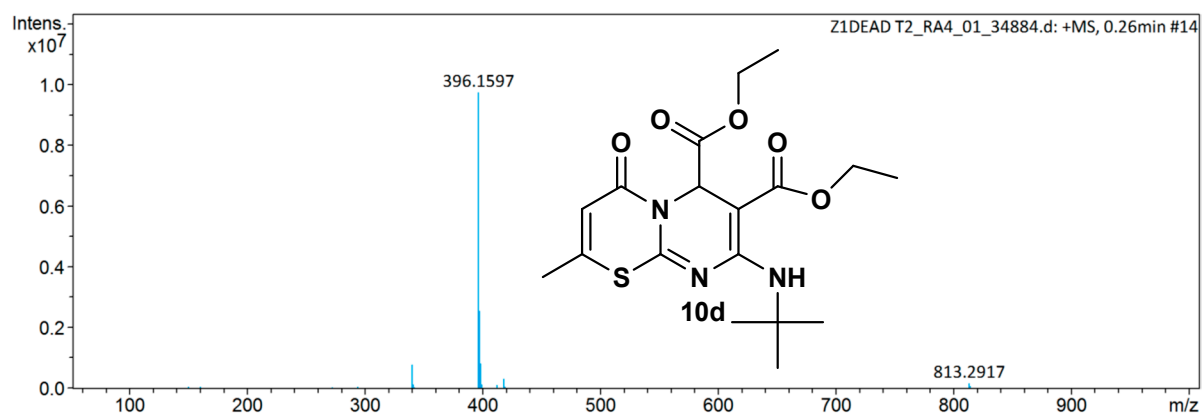
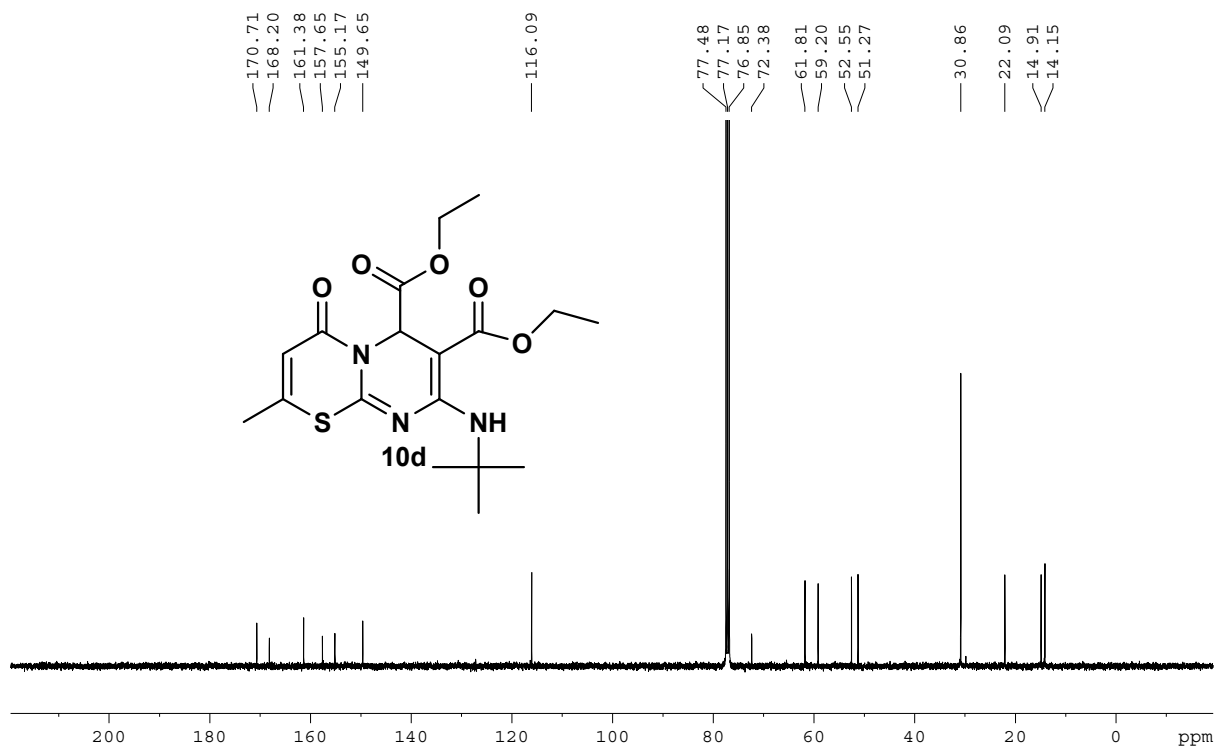
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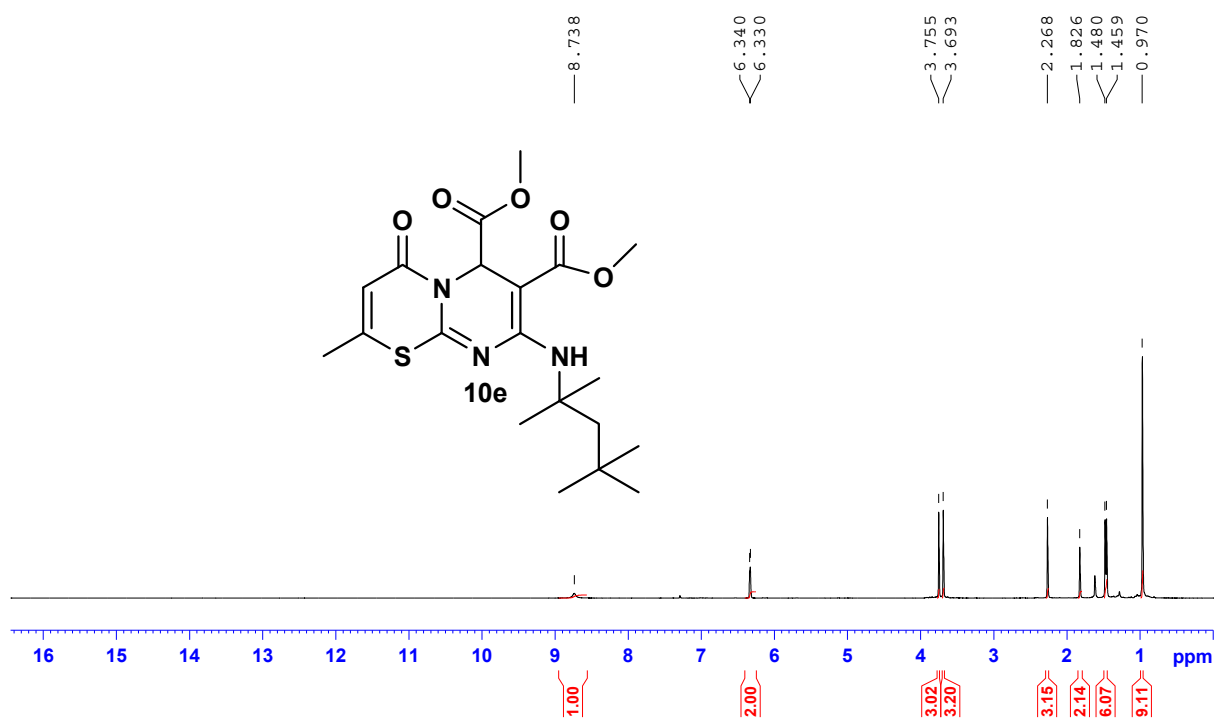
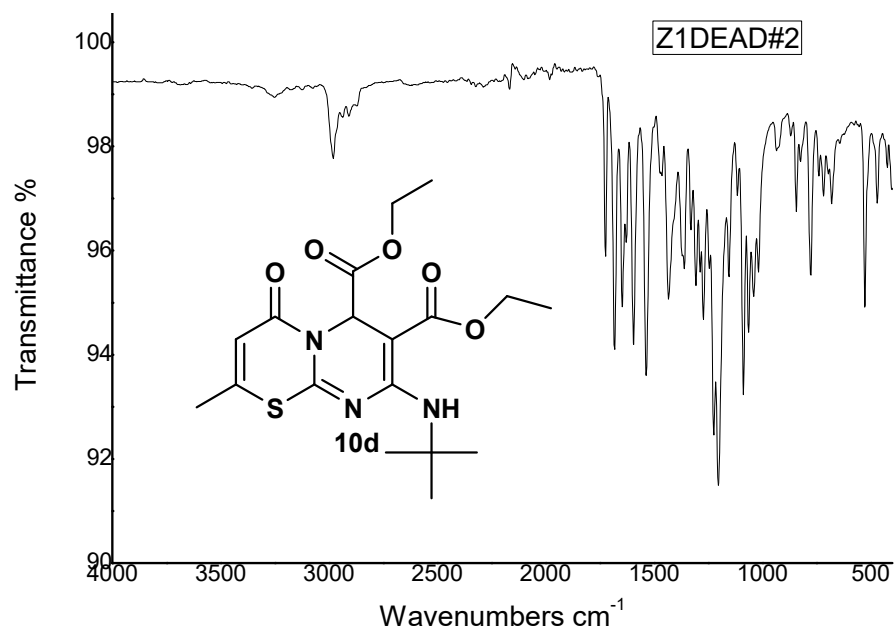


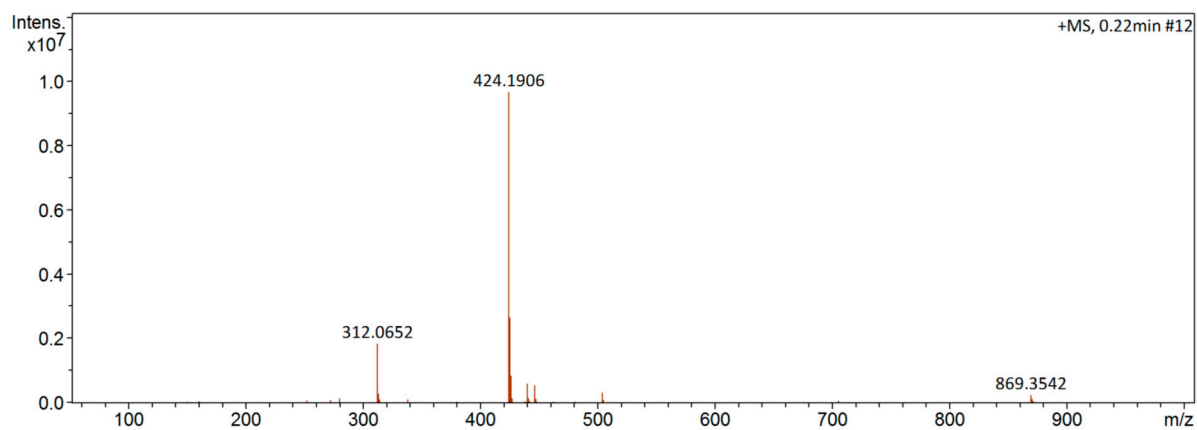
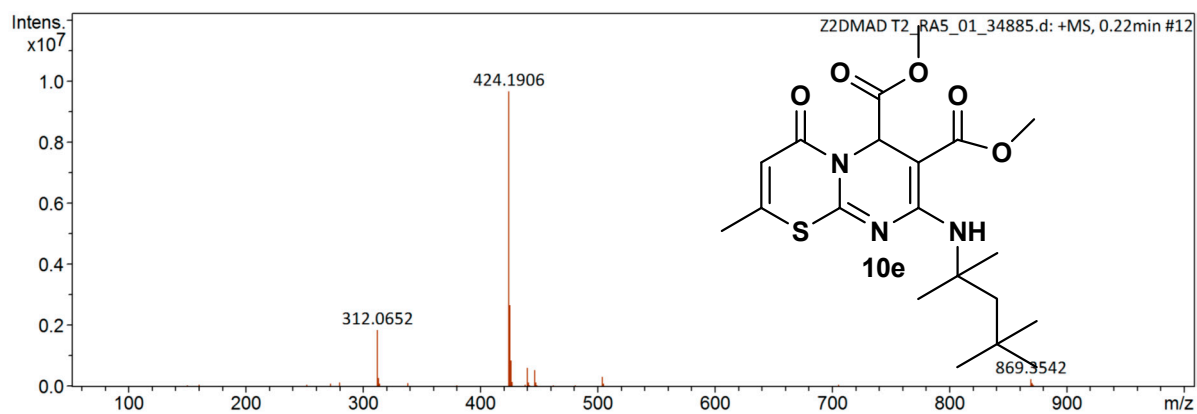


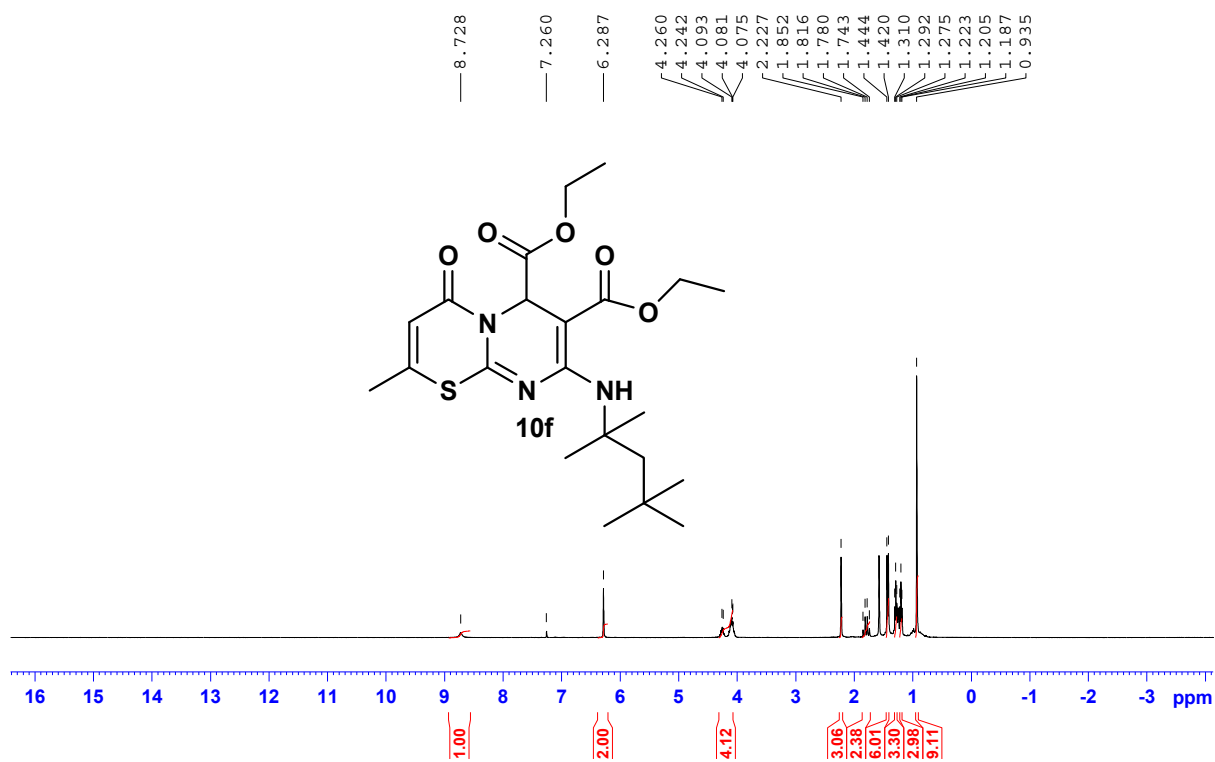
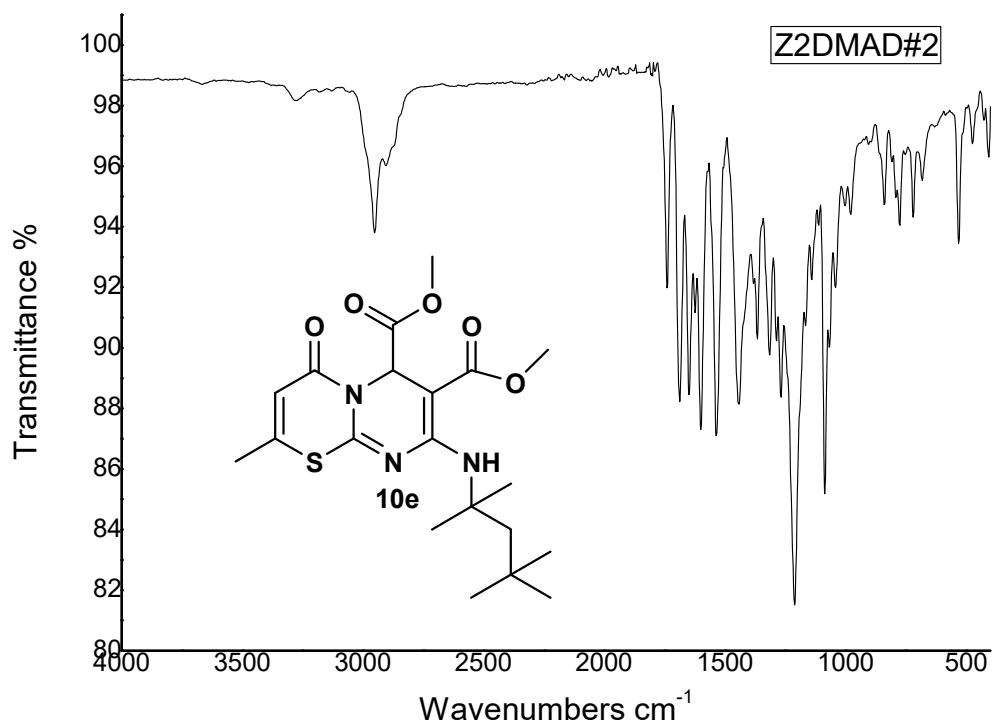


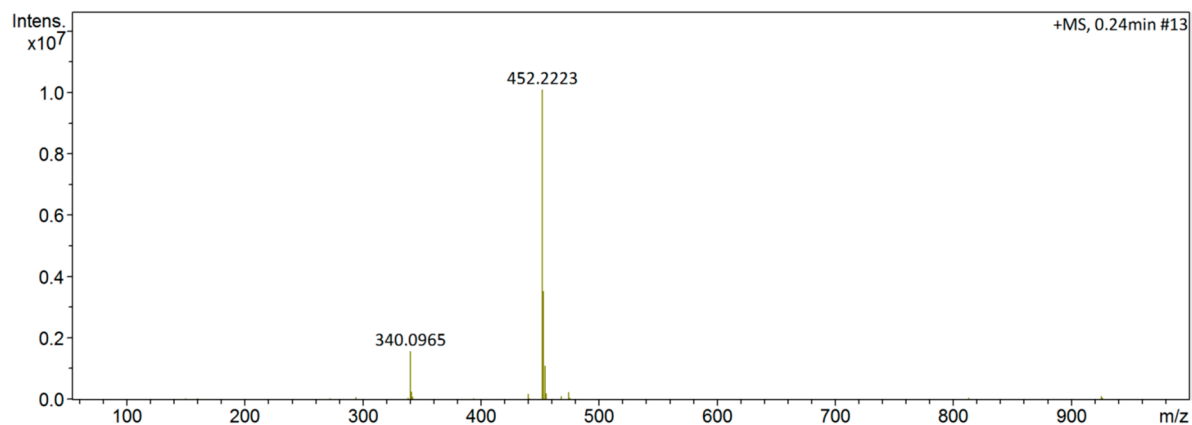
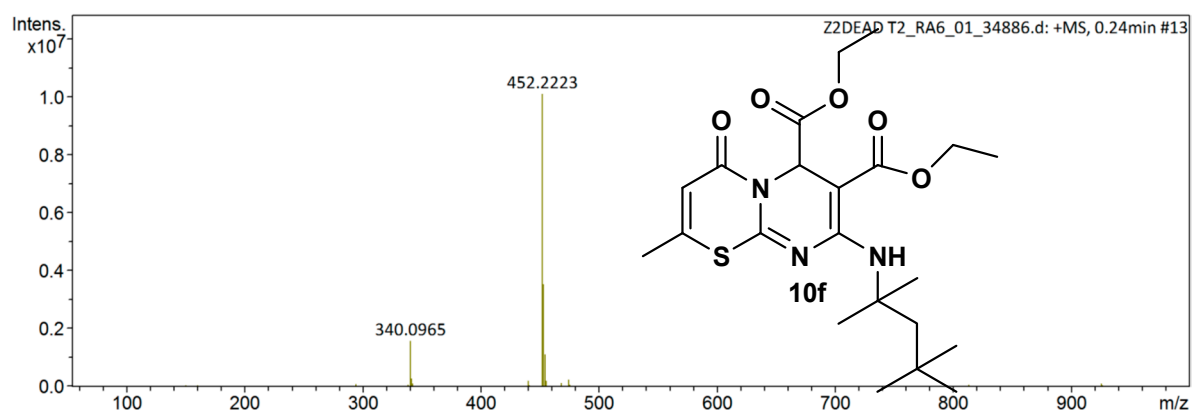
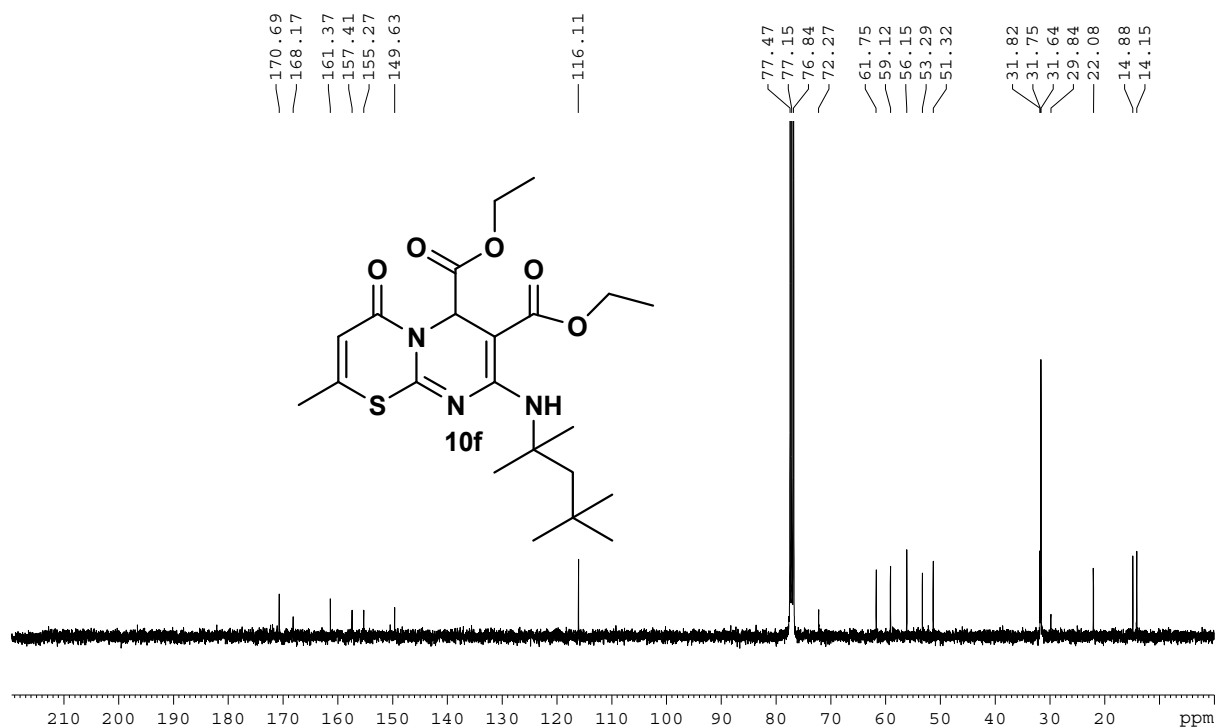


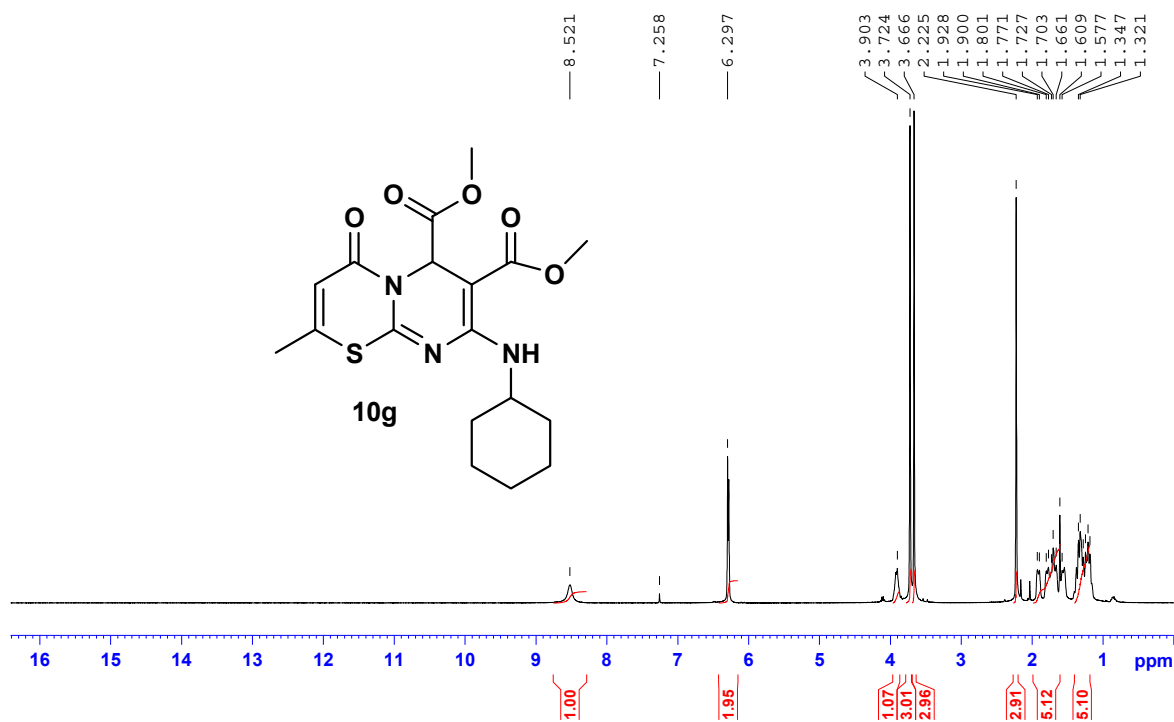
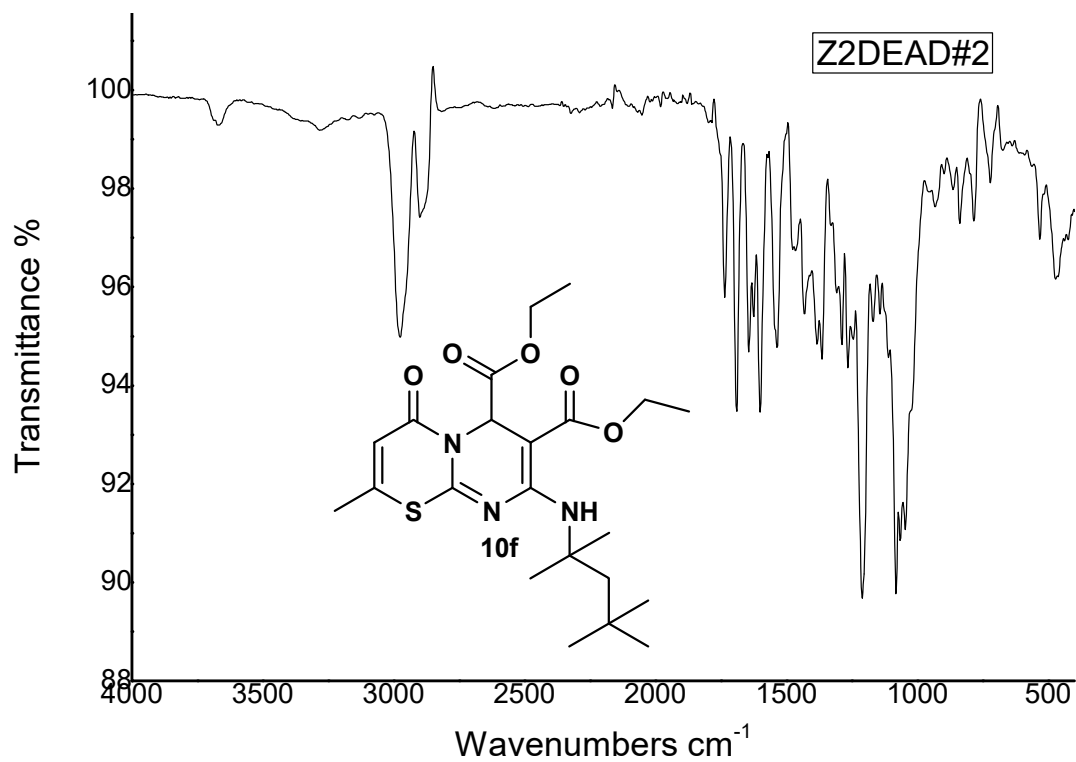


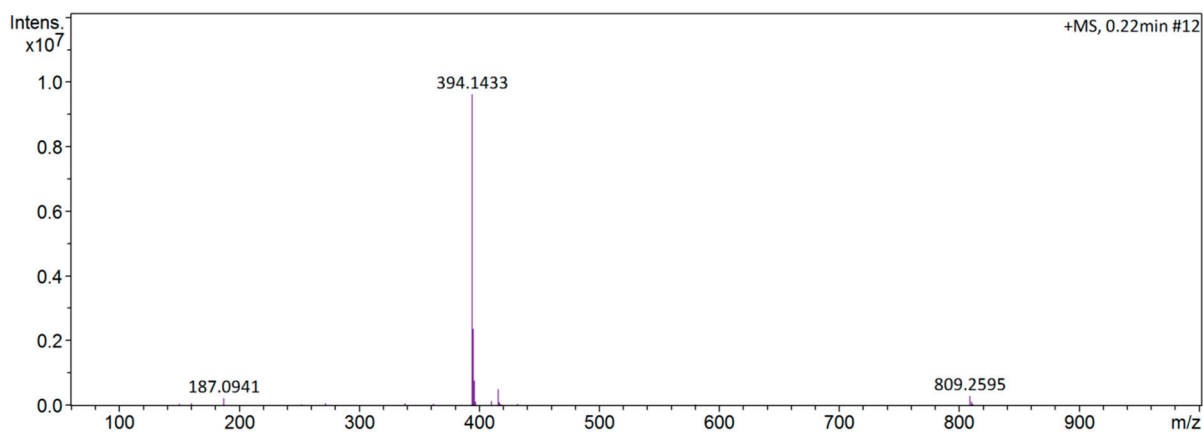
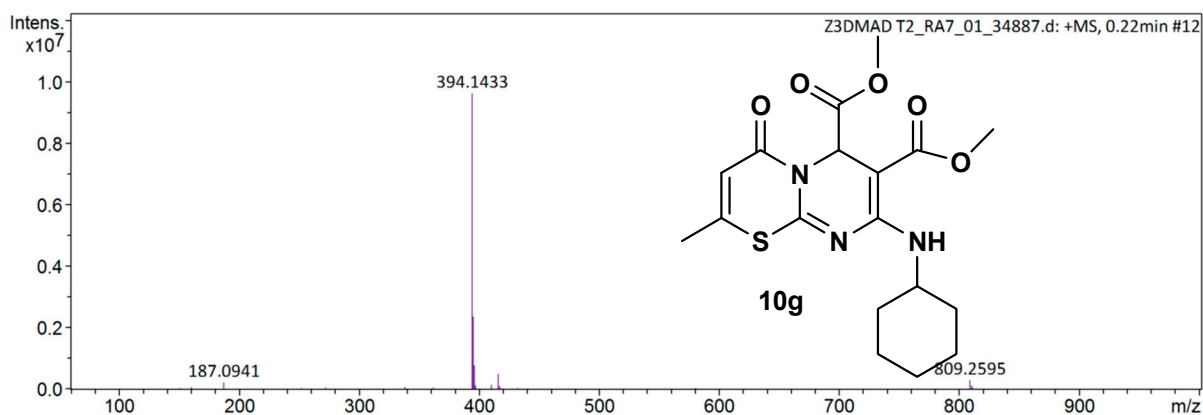
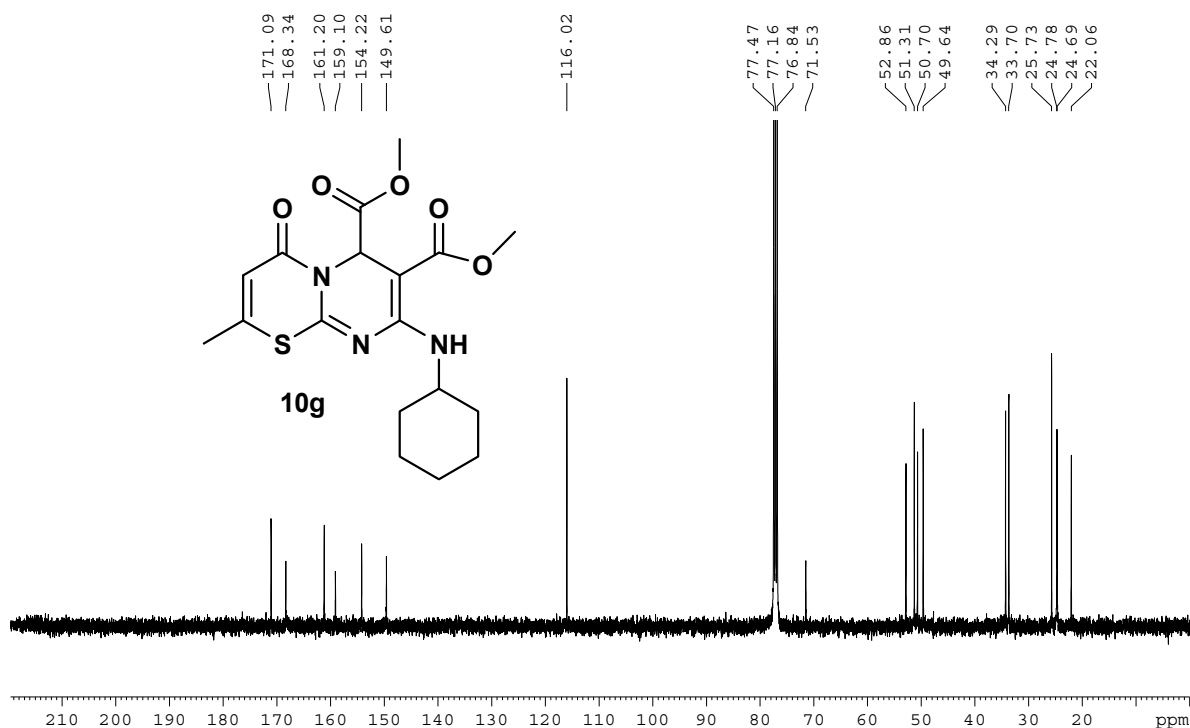


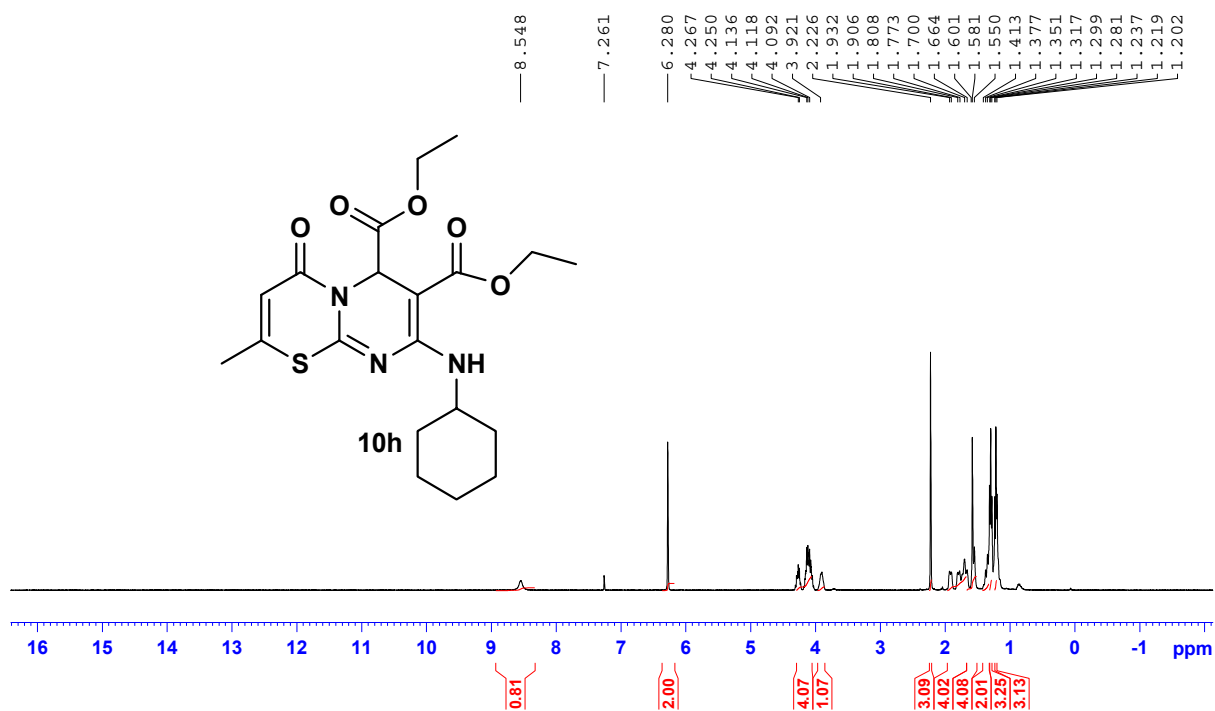
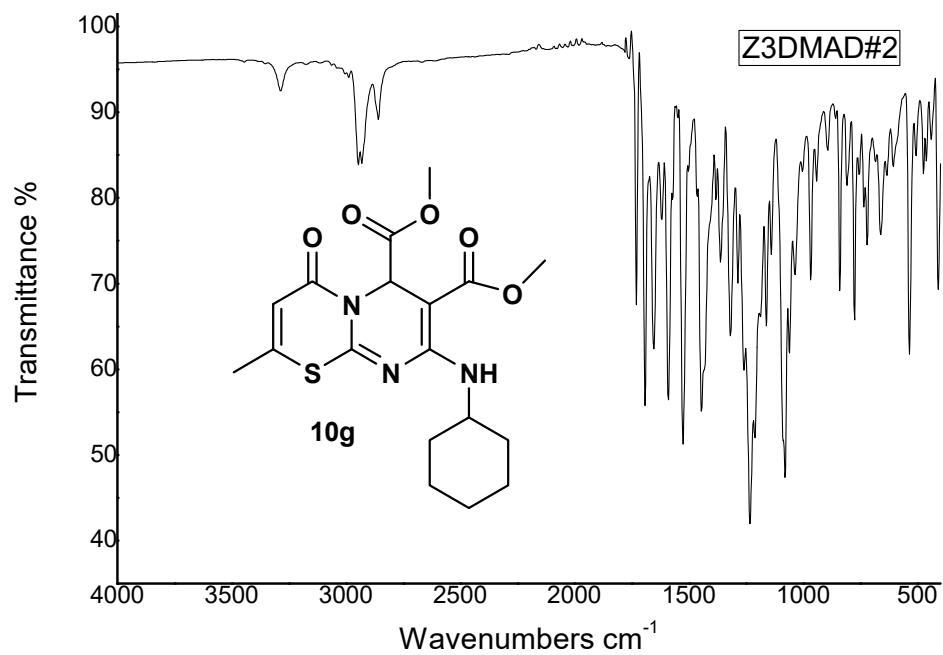




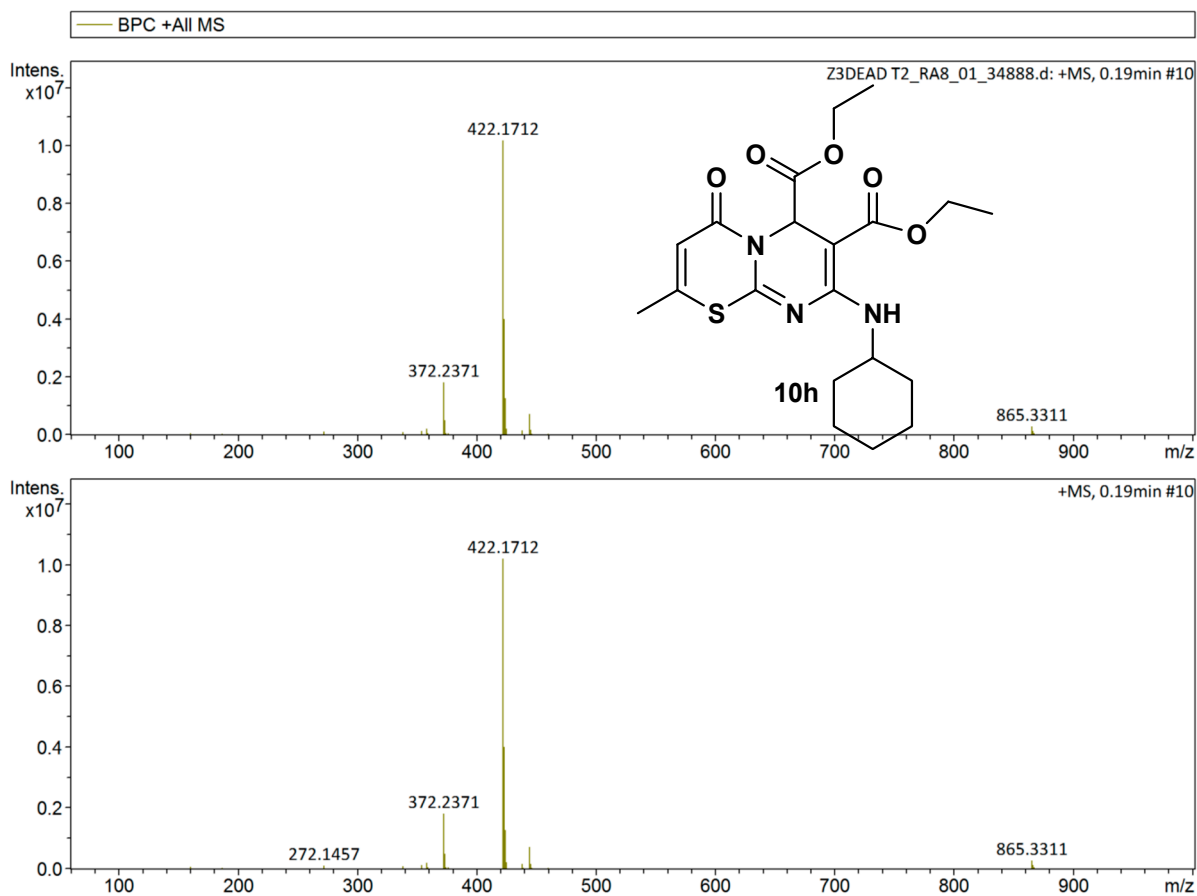


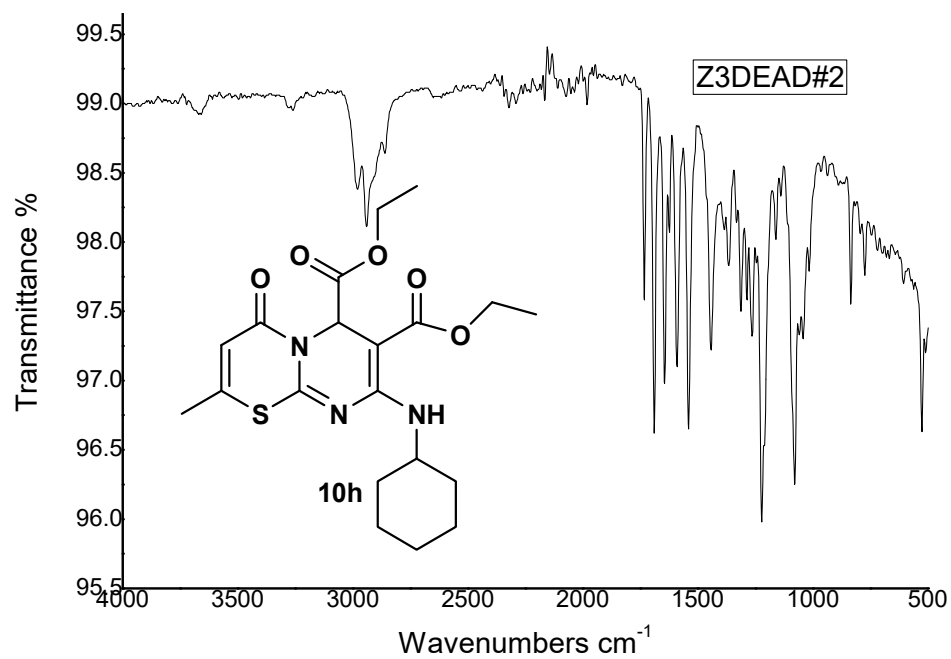






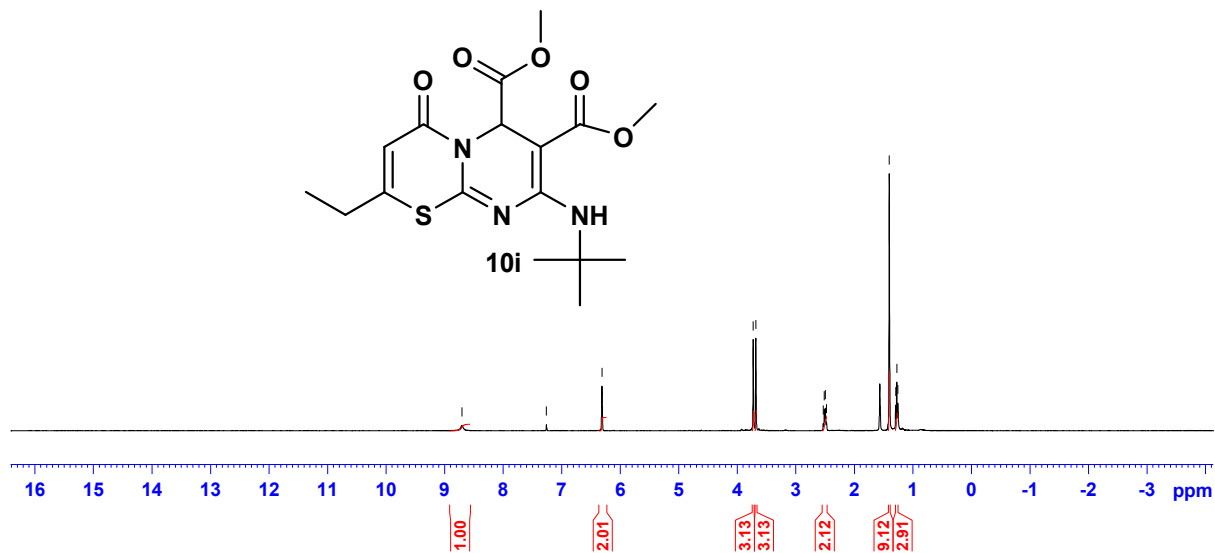


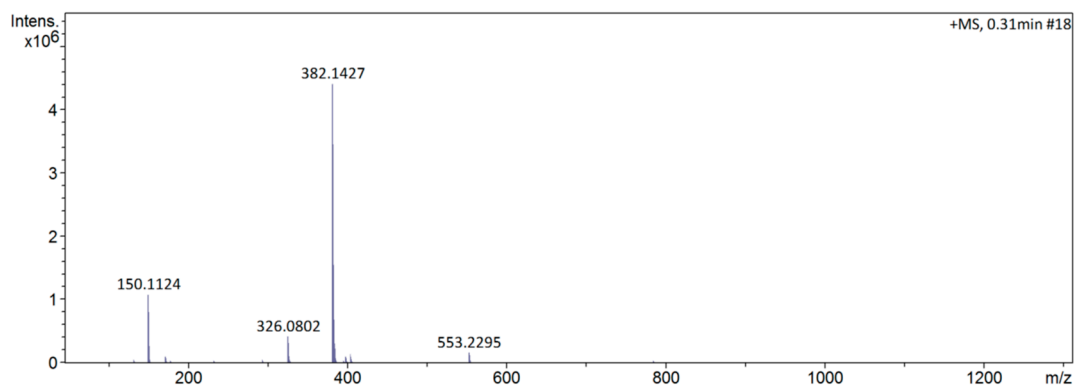
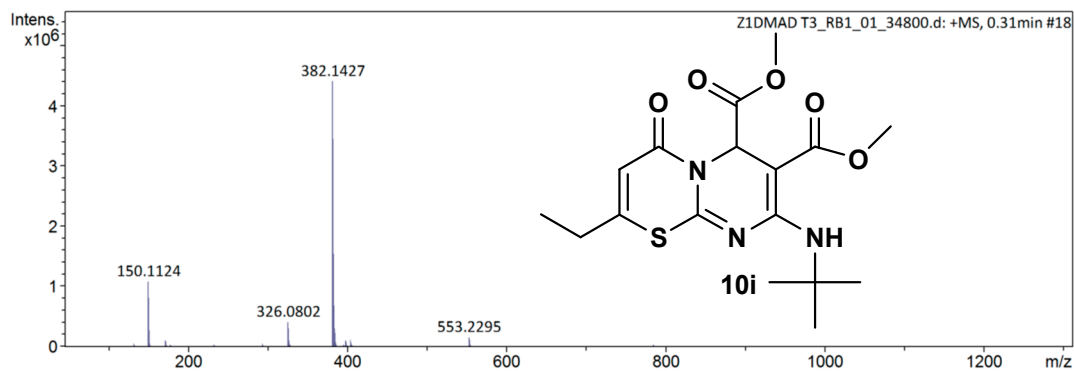
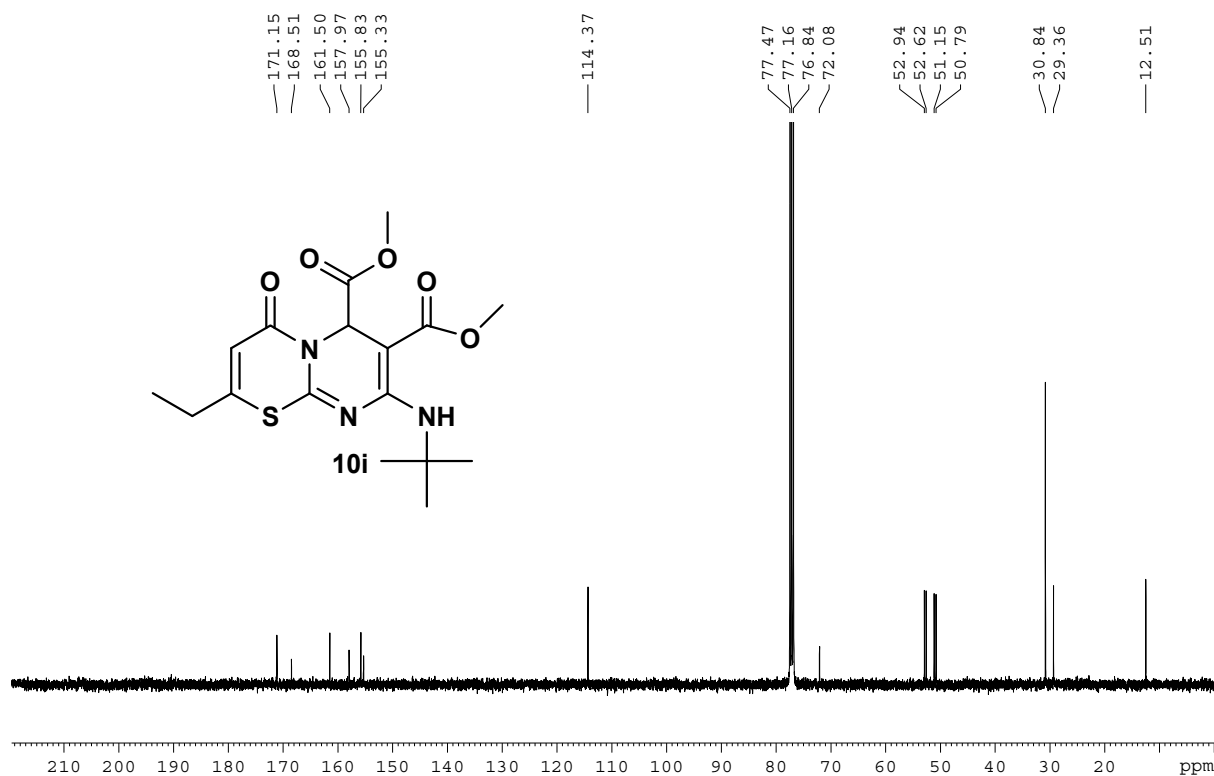


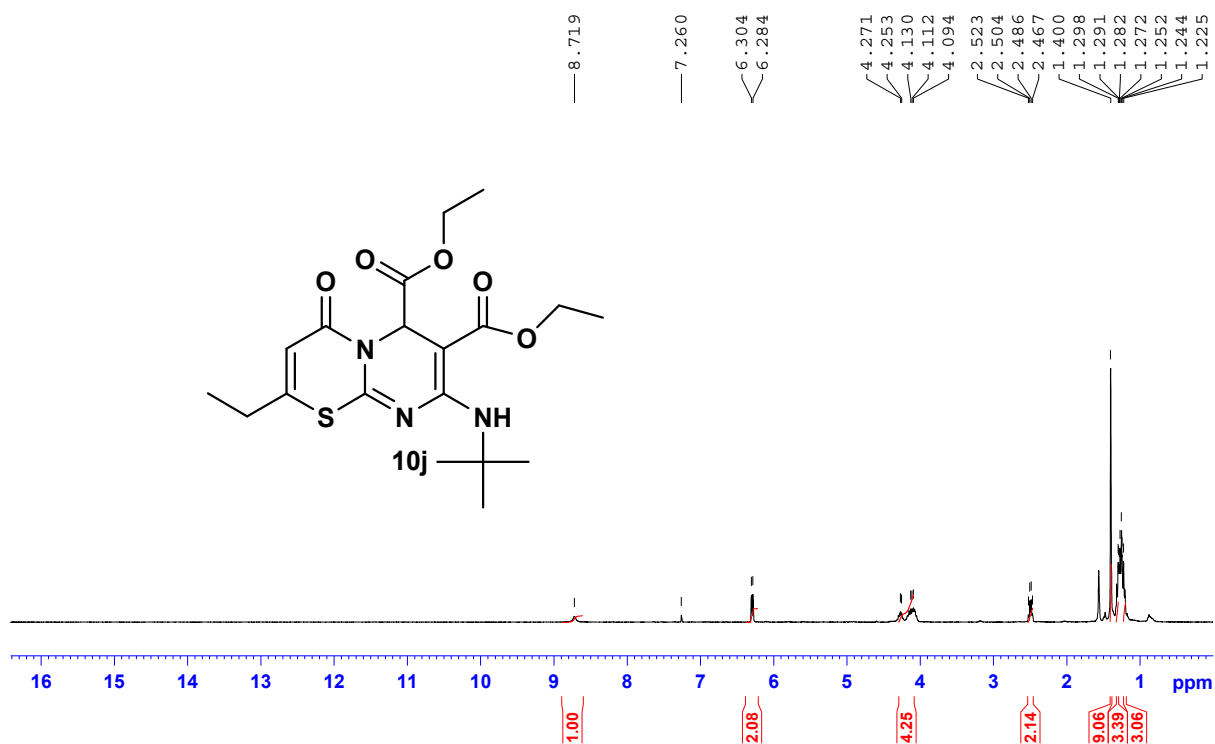
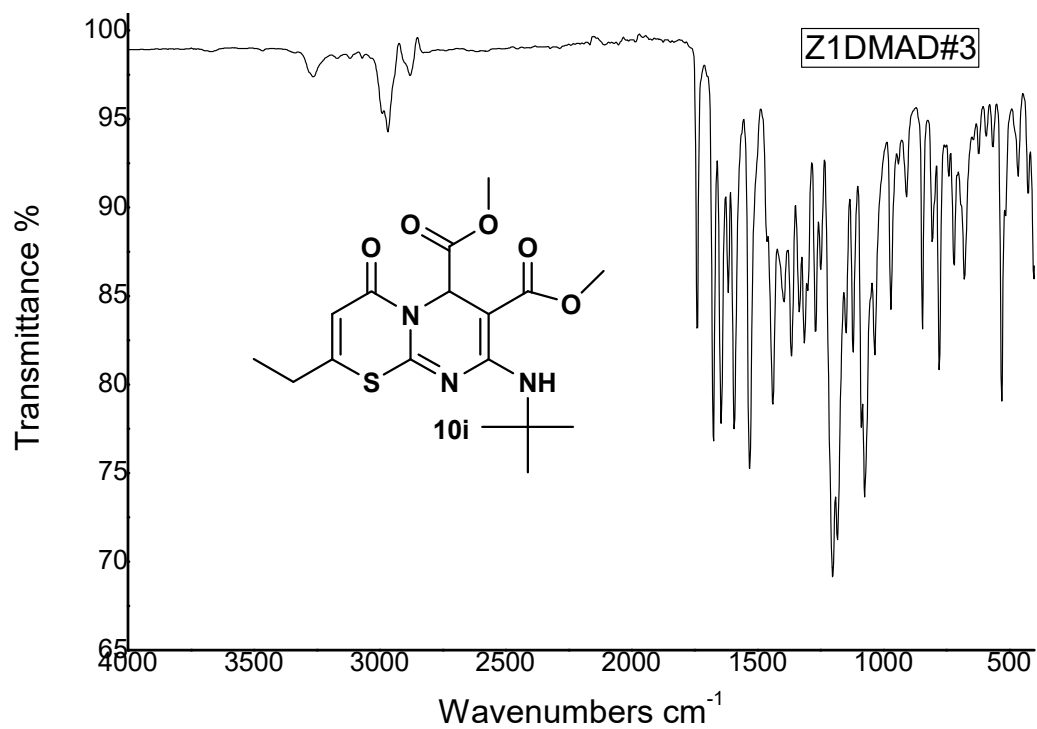


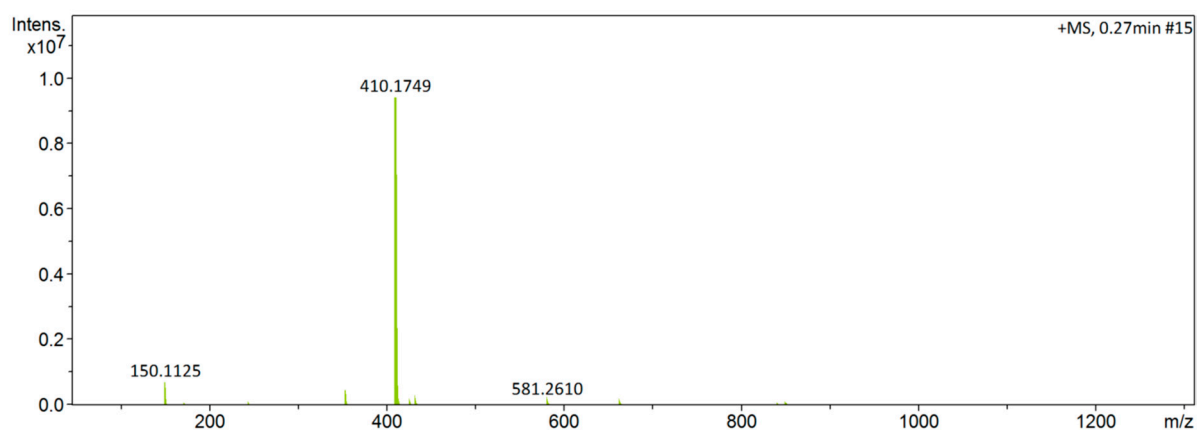
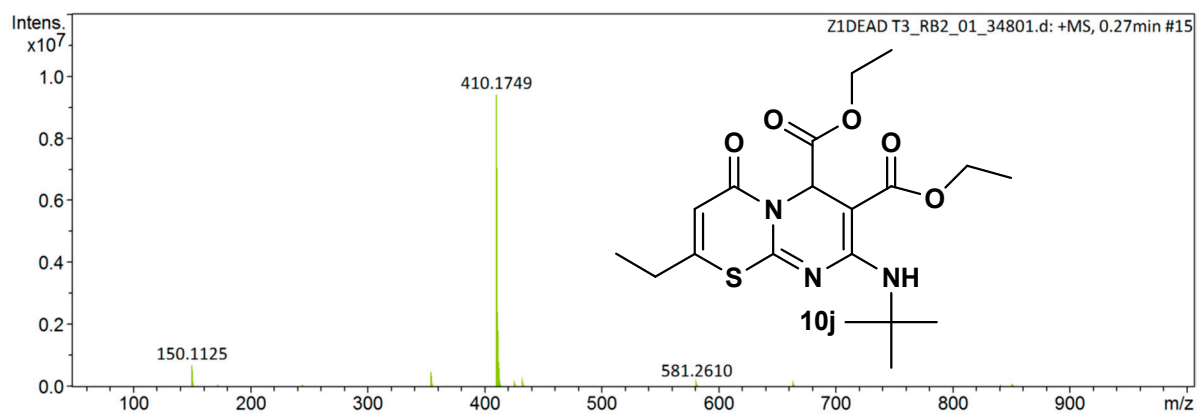
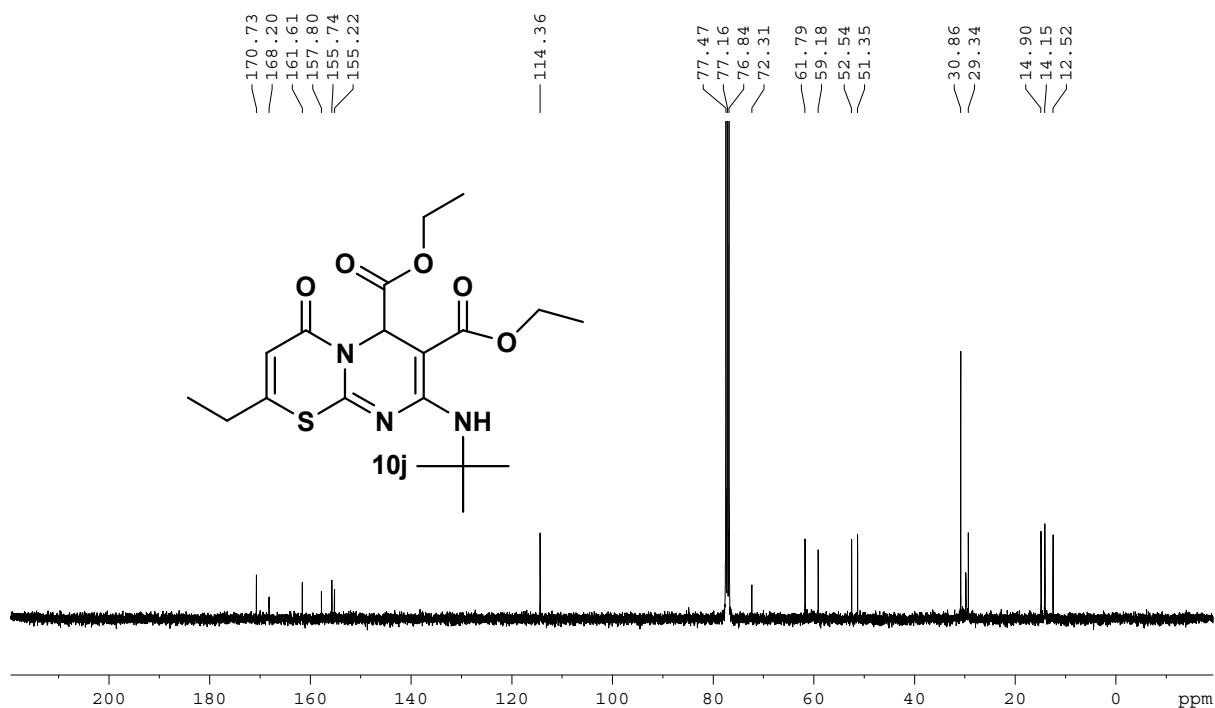
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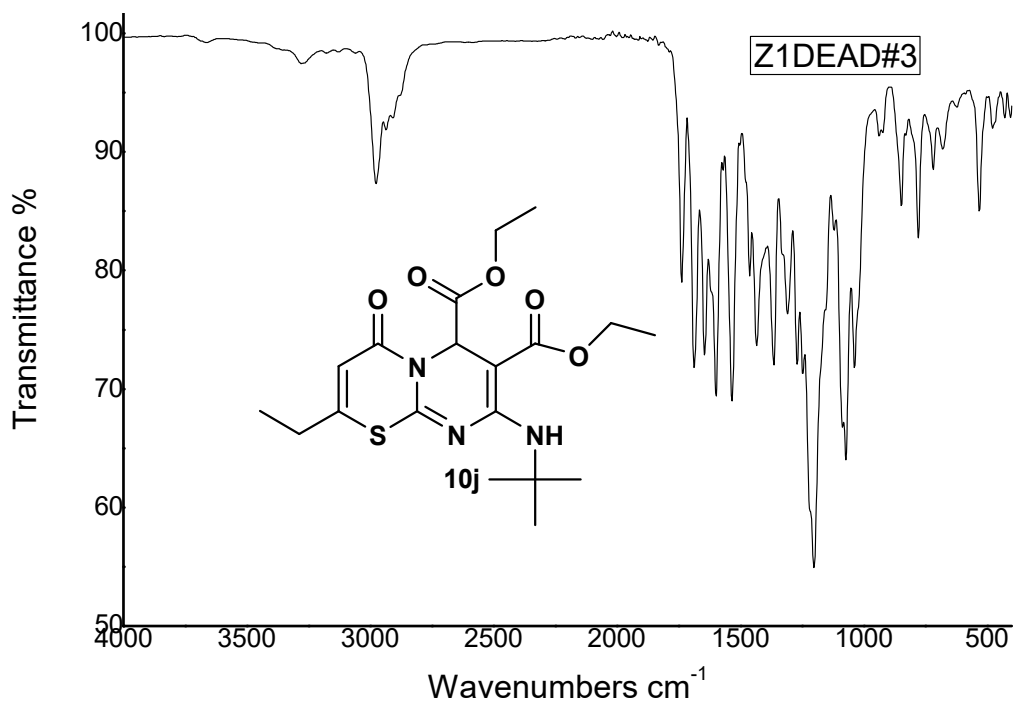
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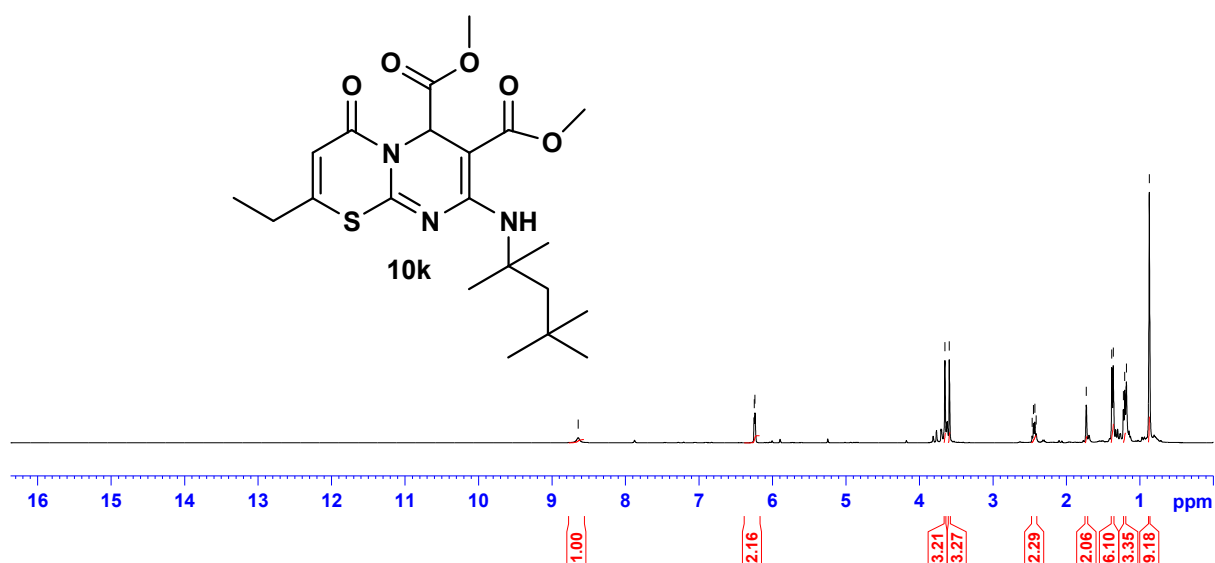
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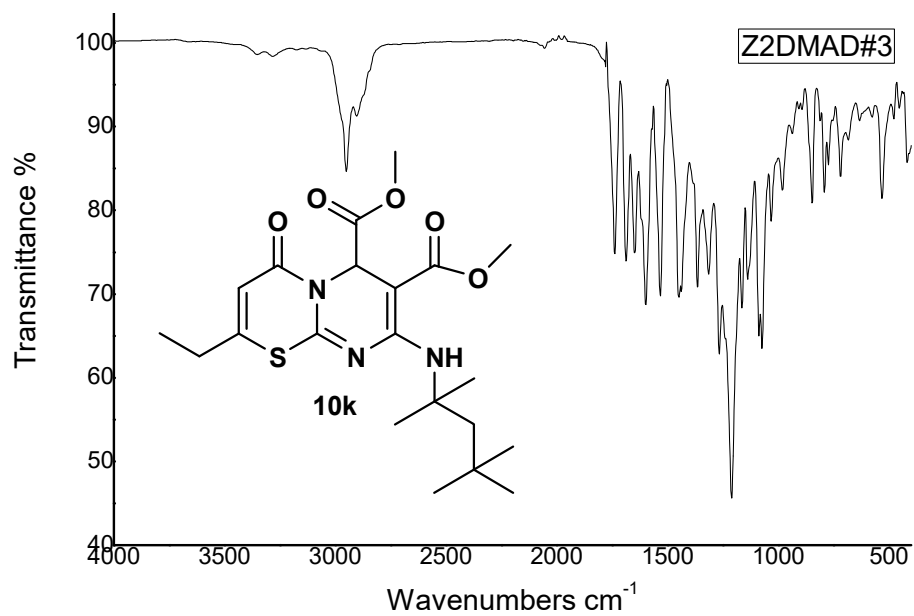
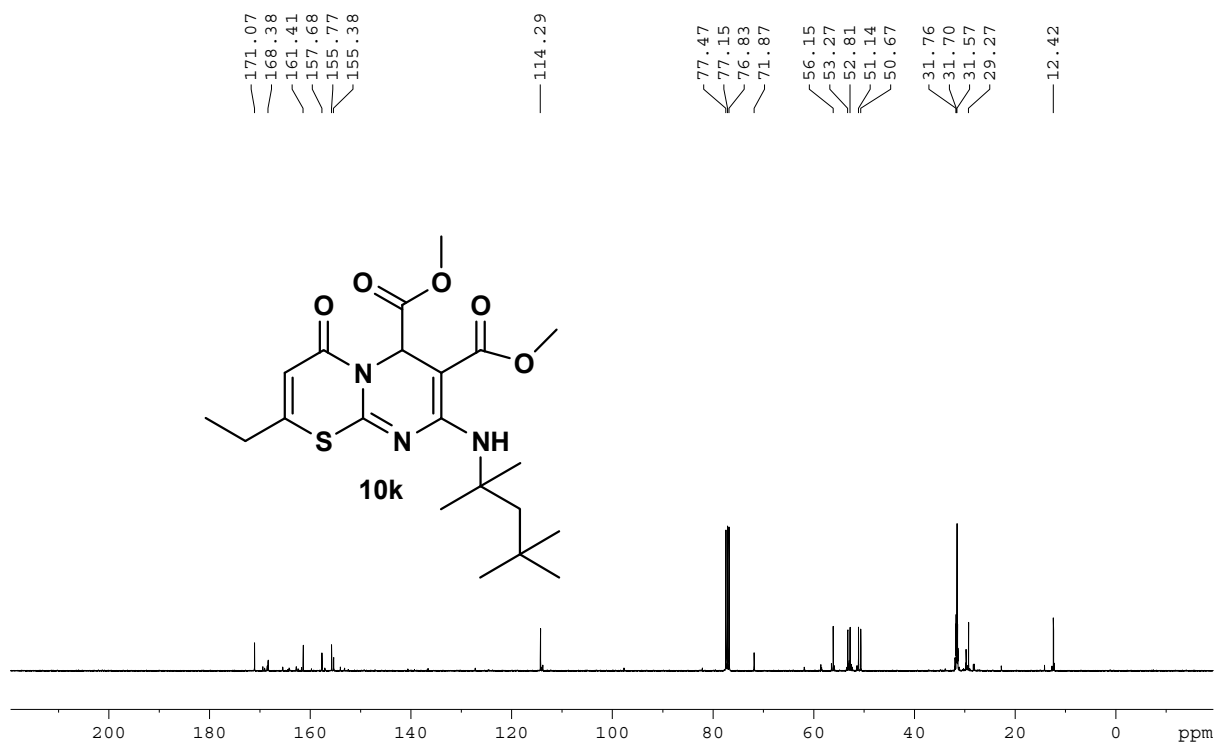
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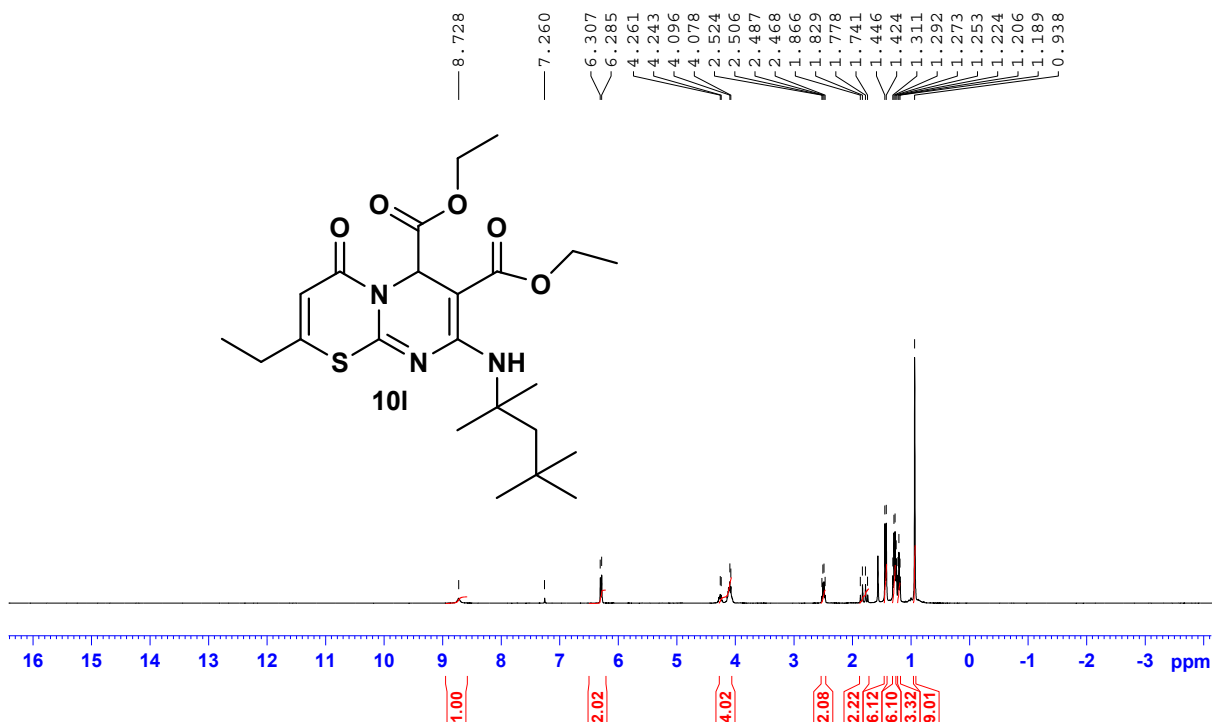
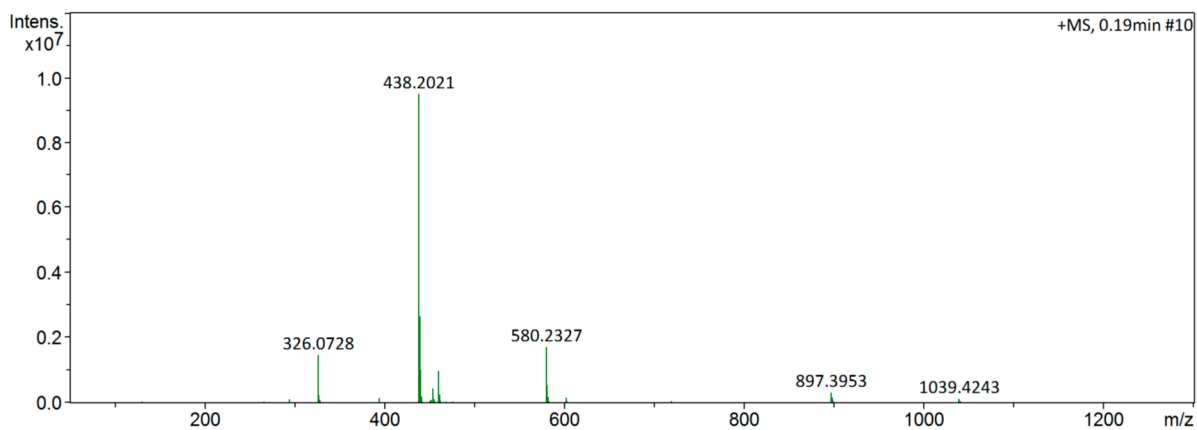
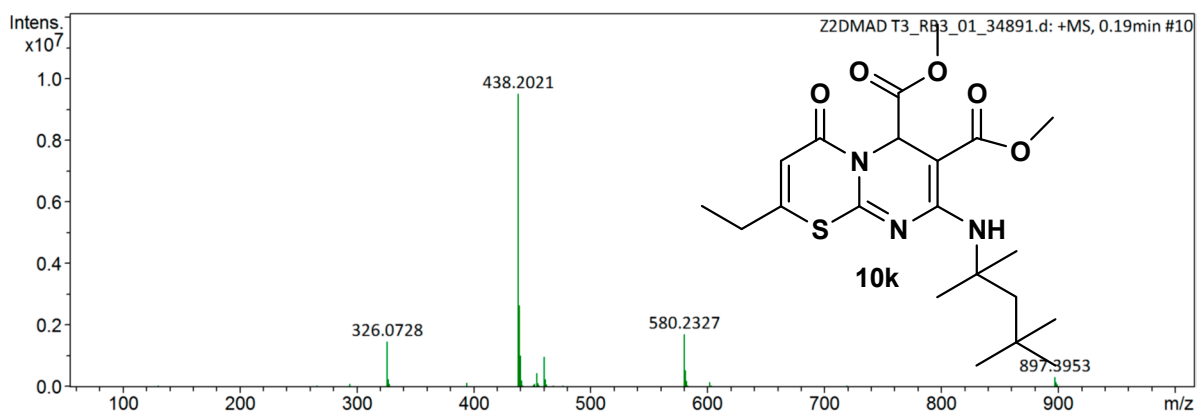
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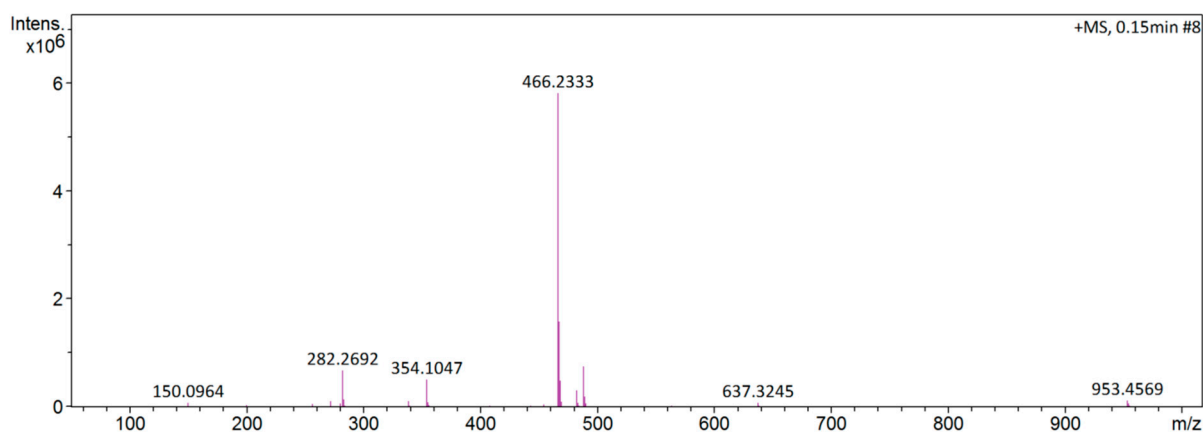
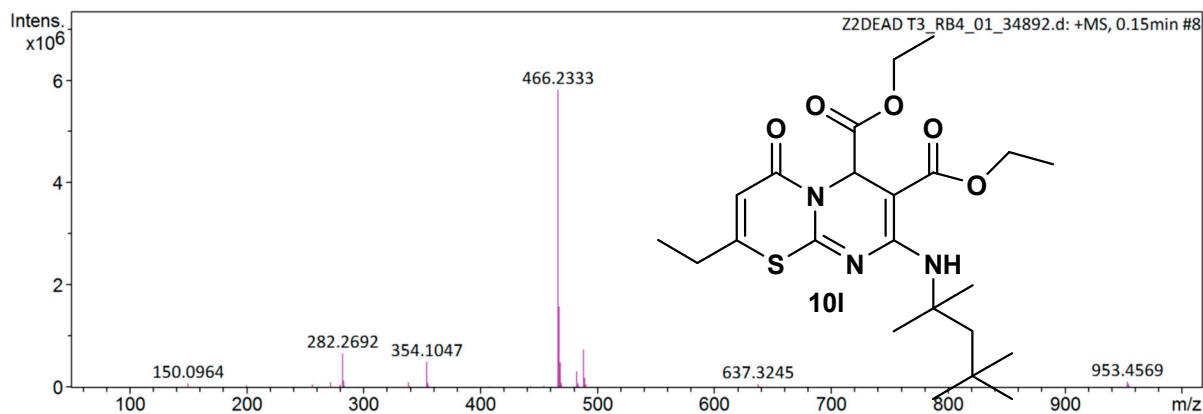
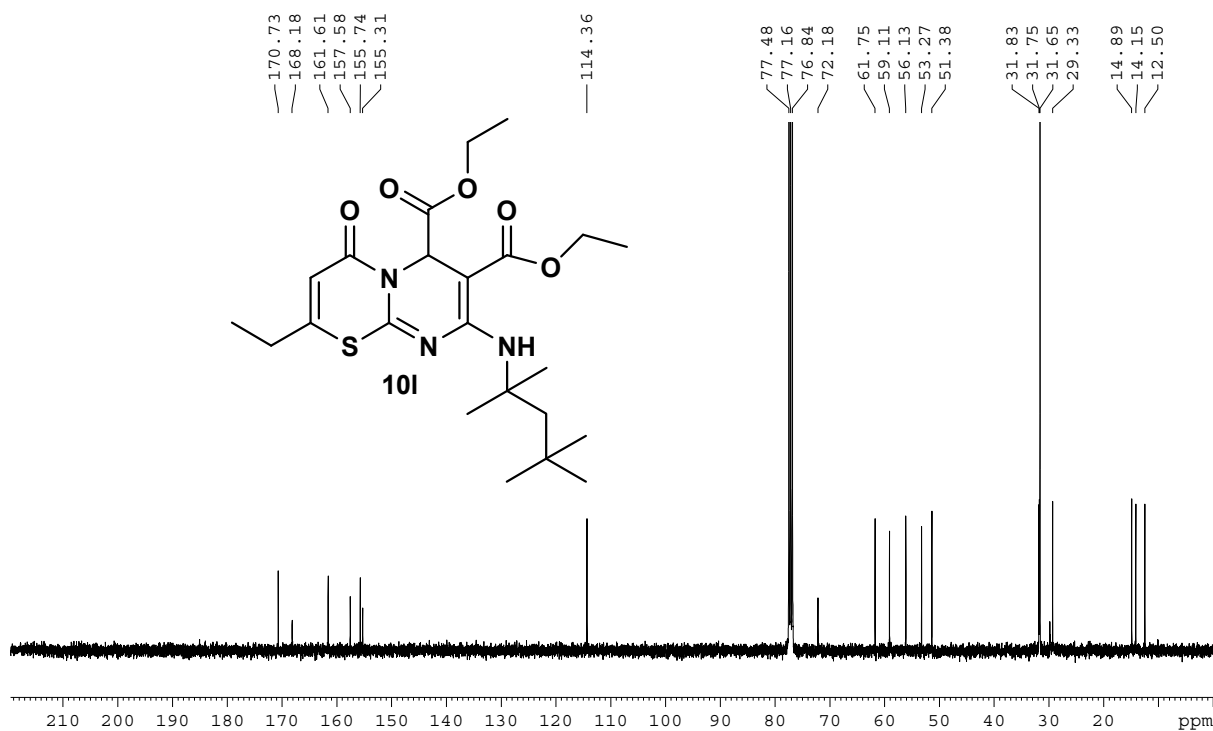
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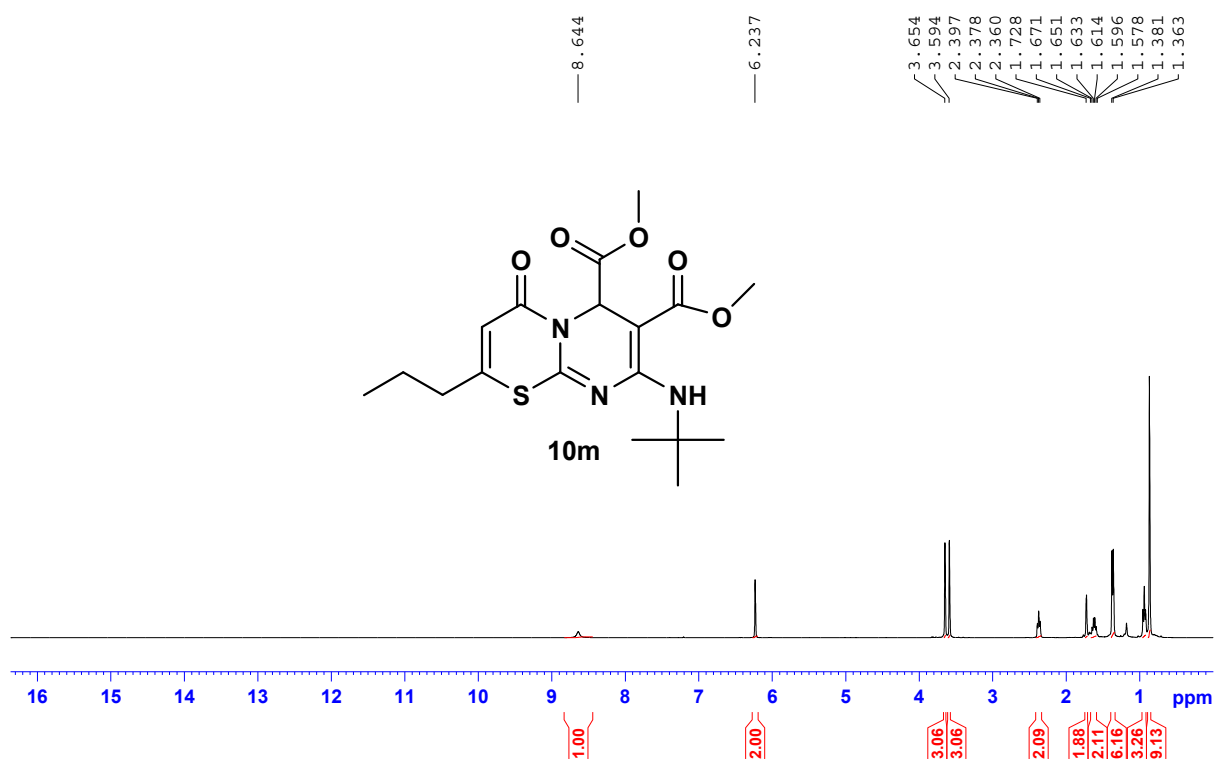
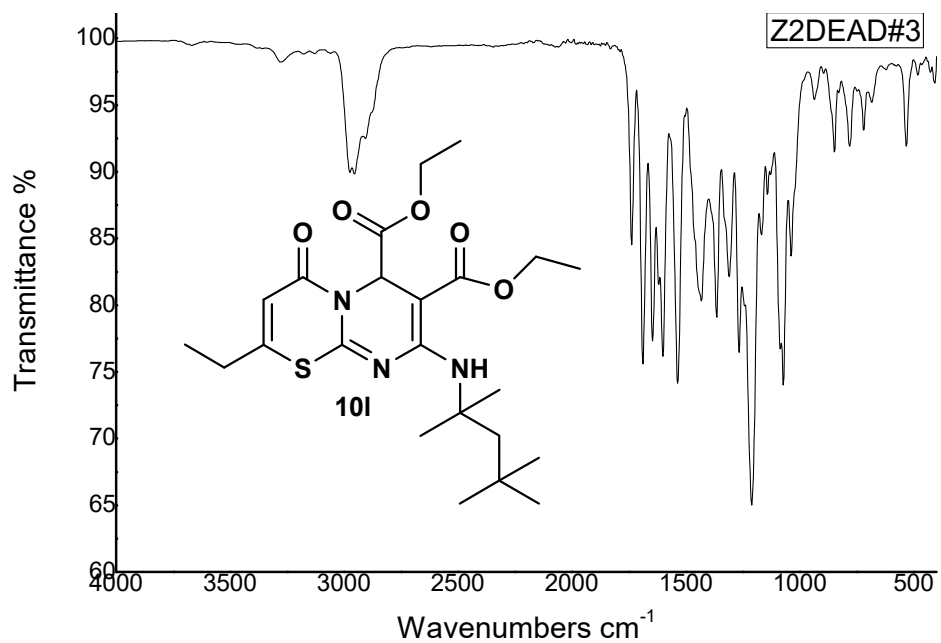












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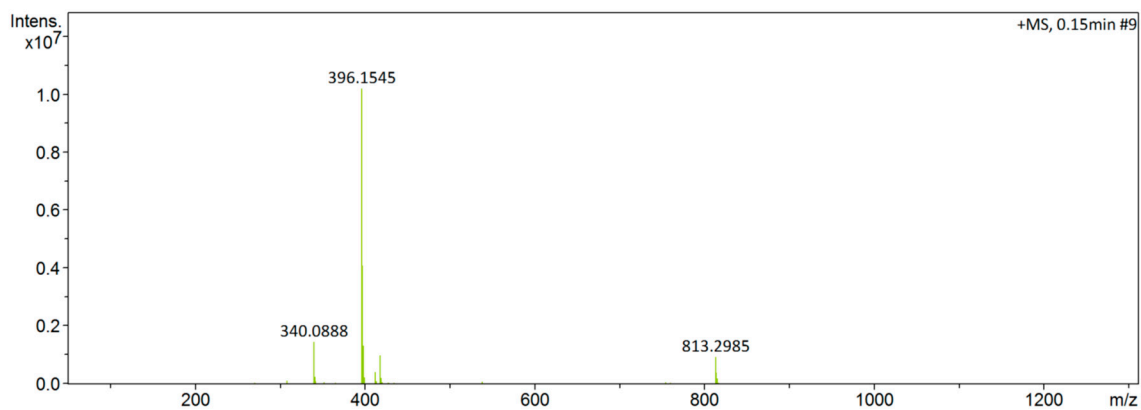
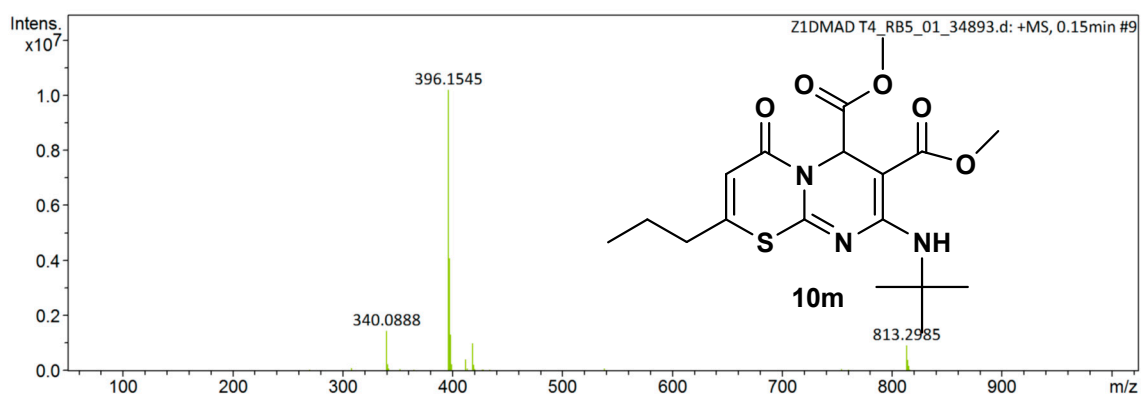
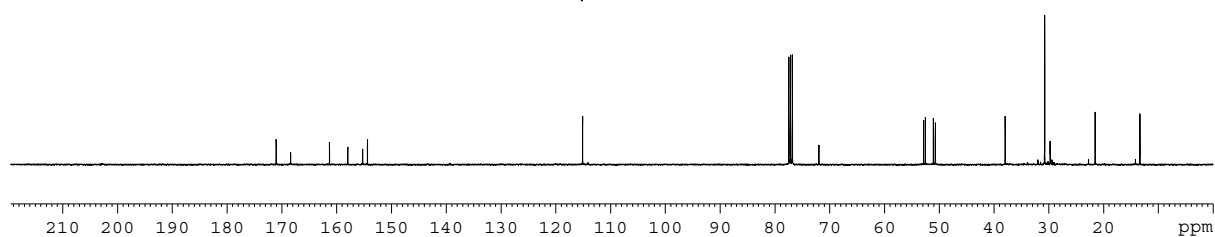
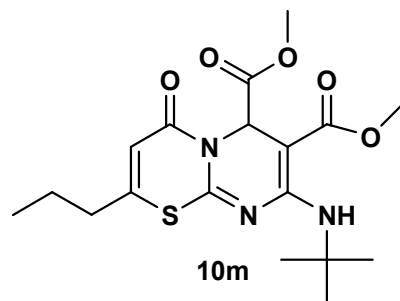
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50.73

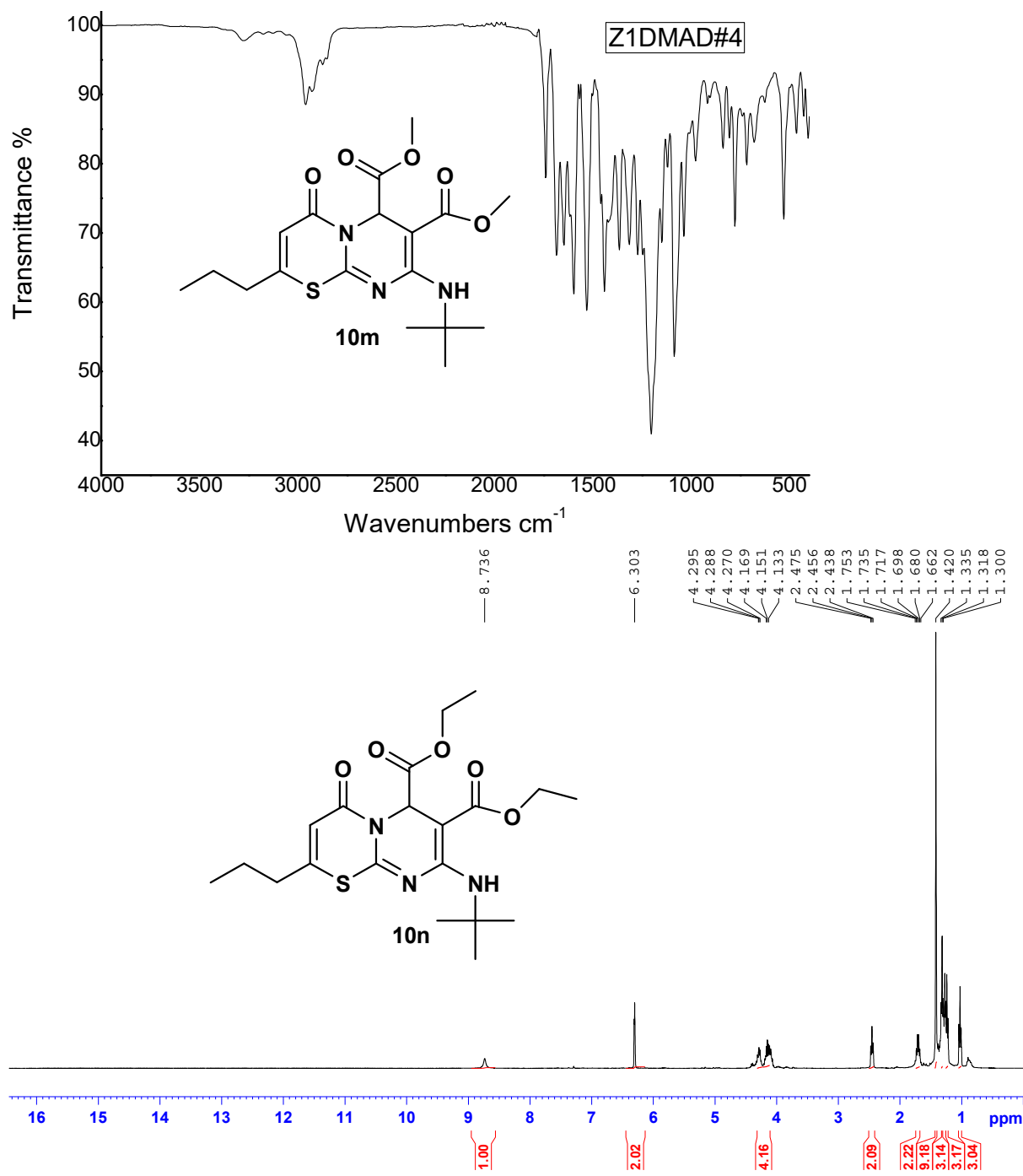
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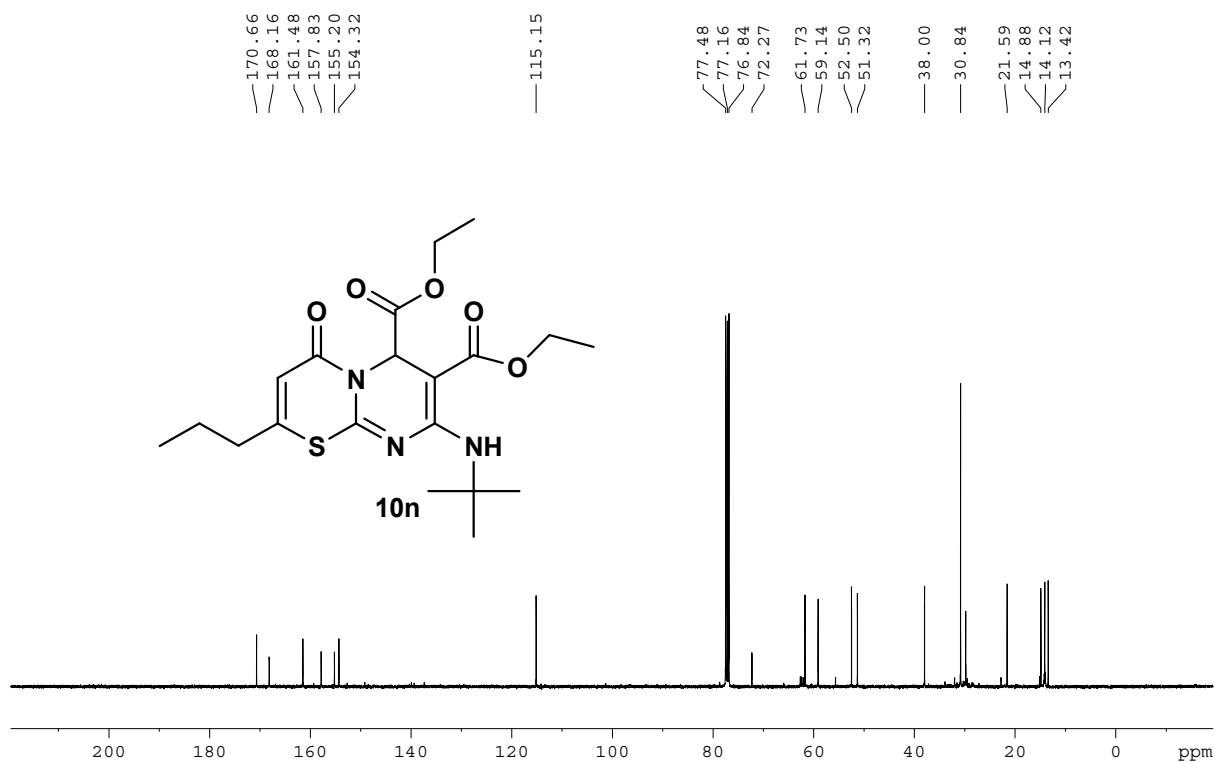
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21.58

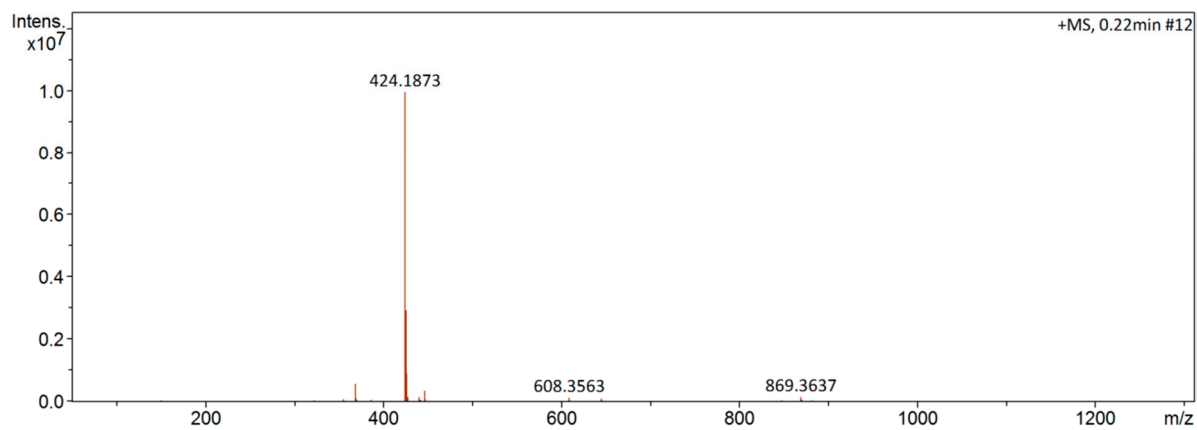
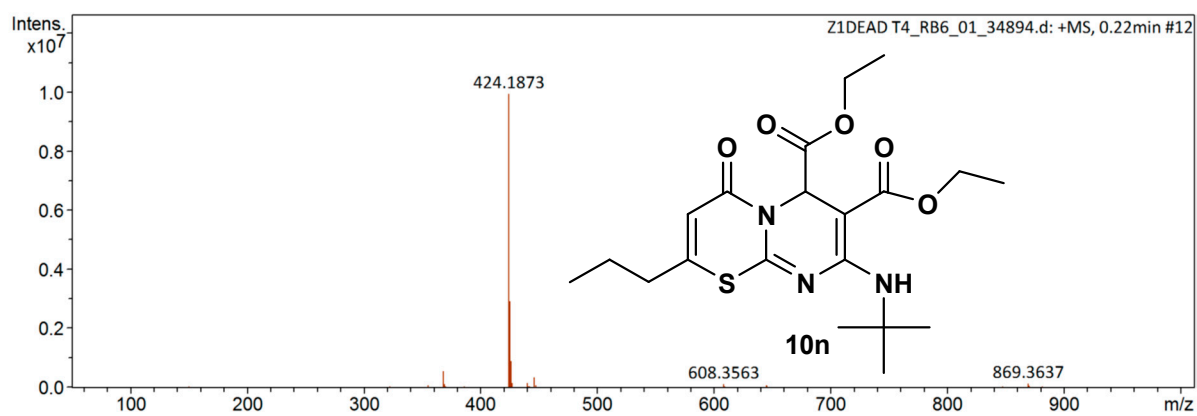
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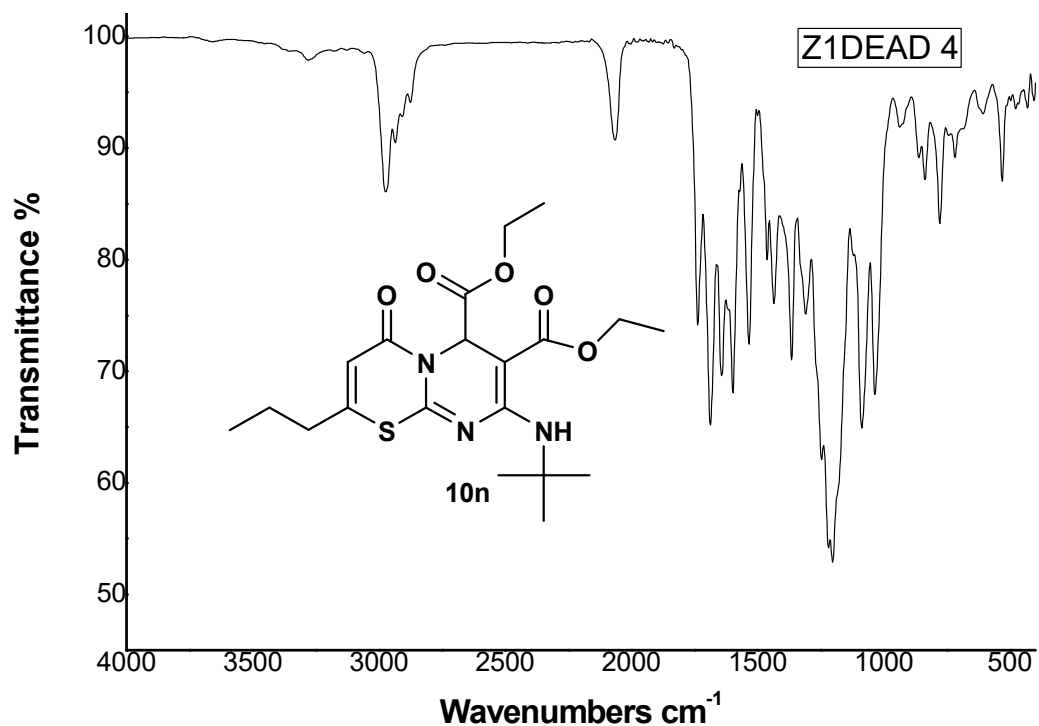






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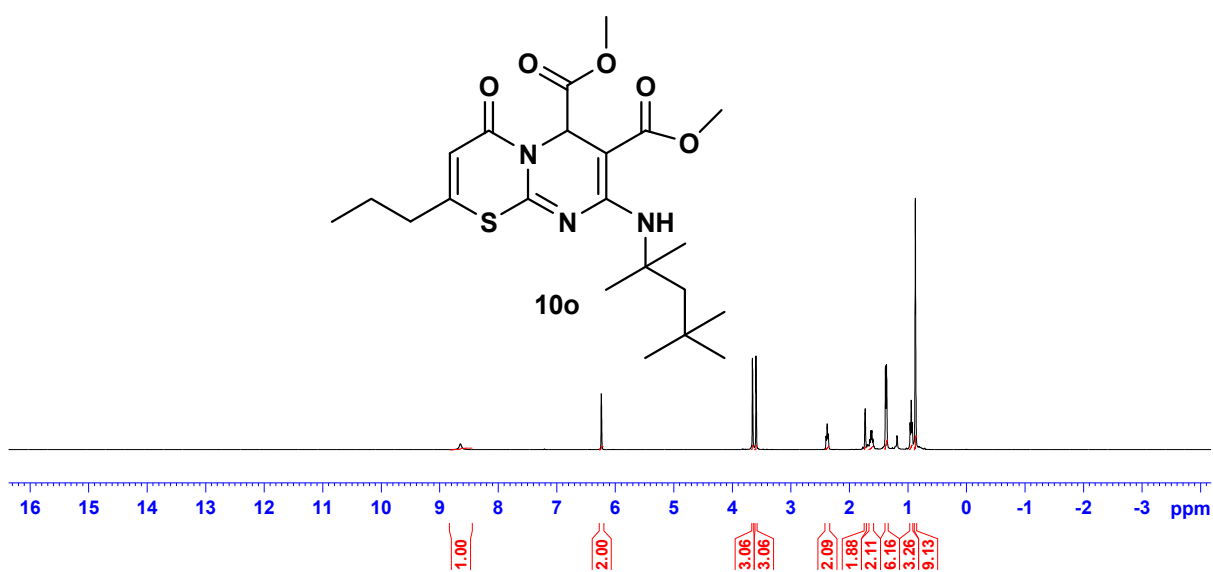


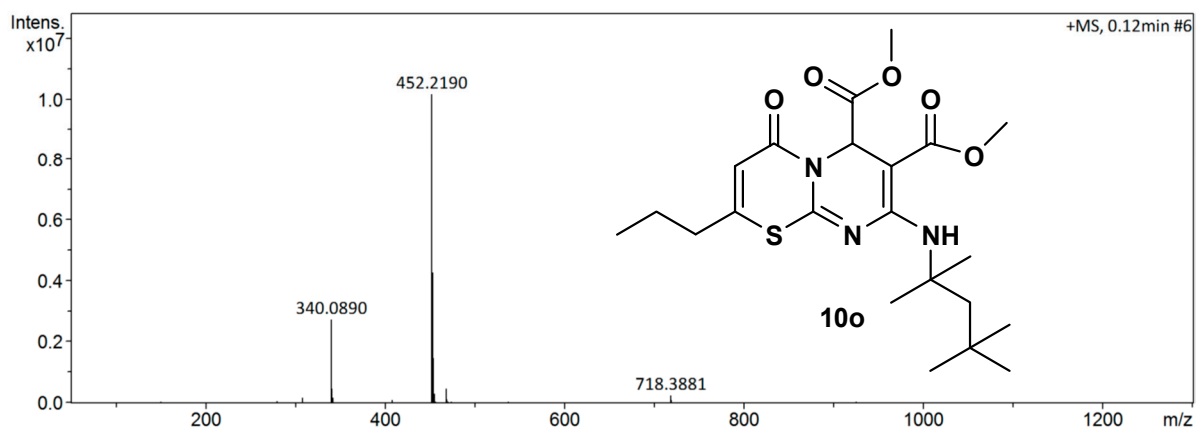
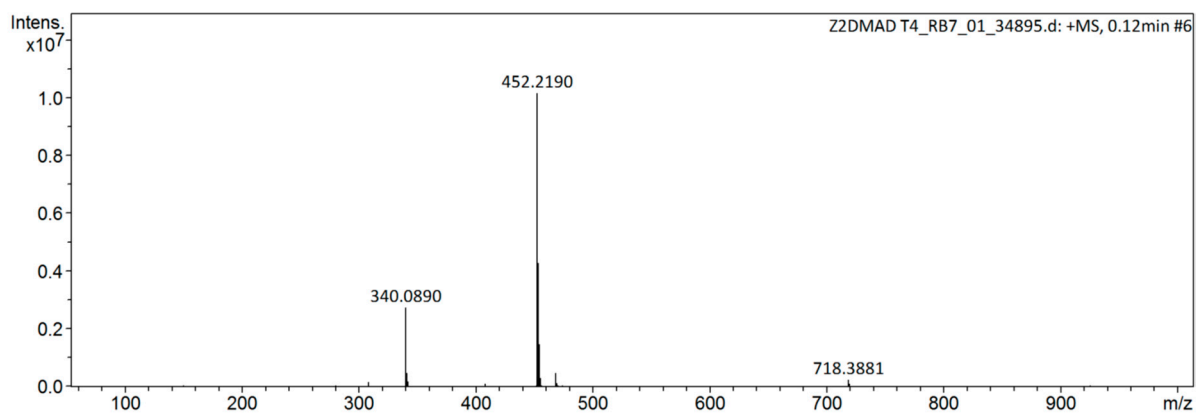
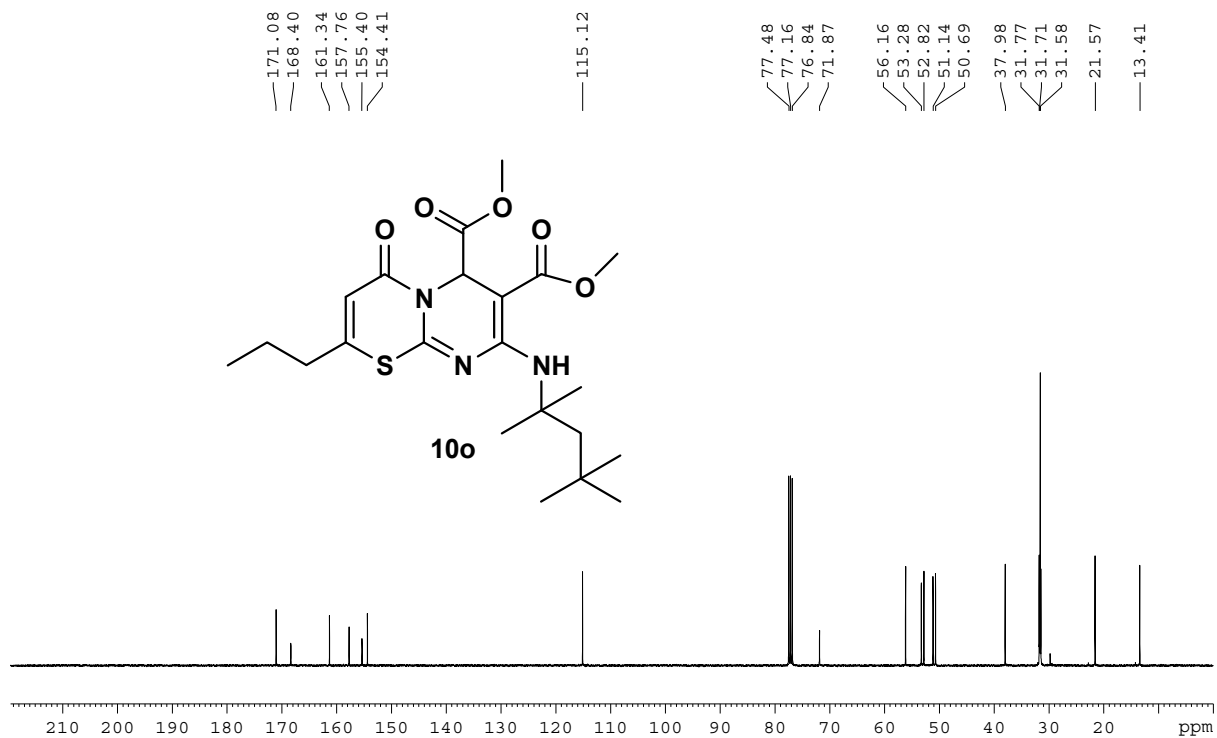


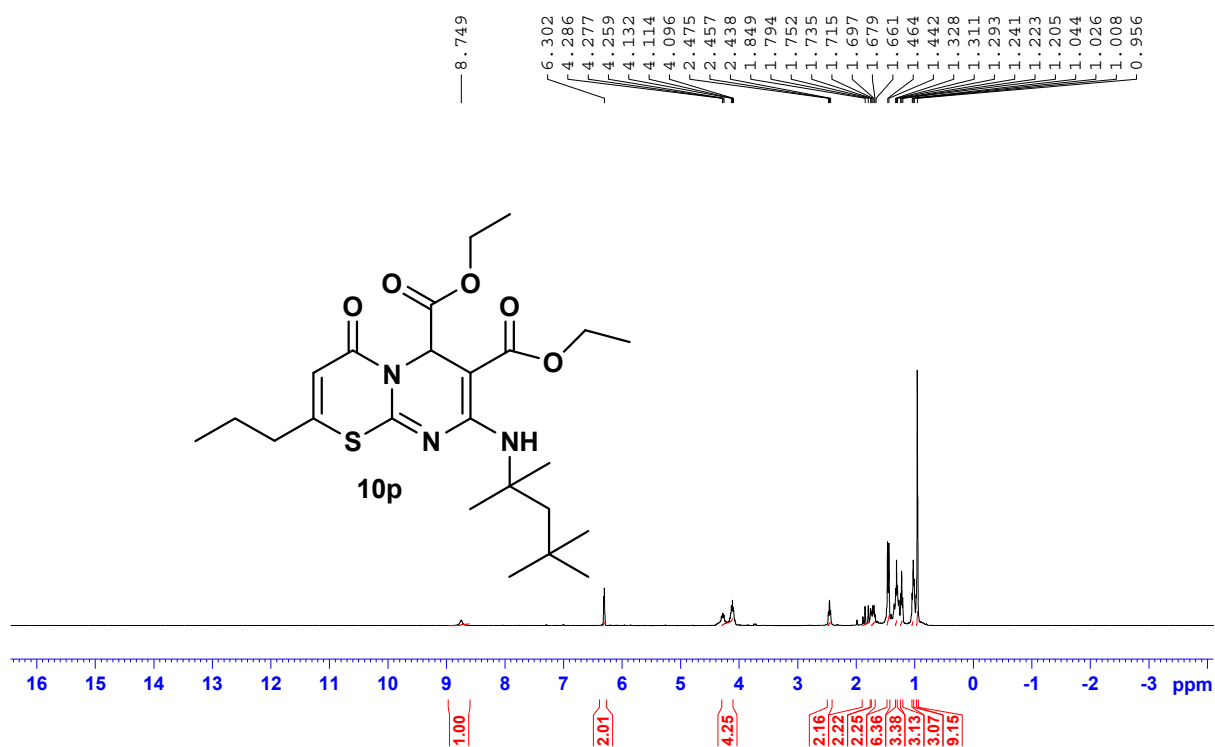
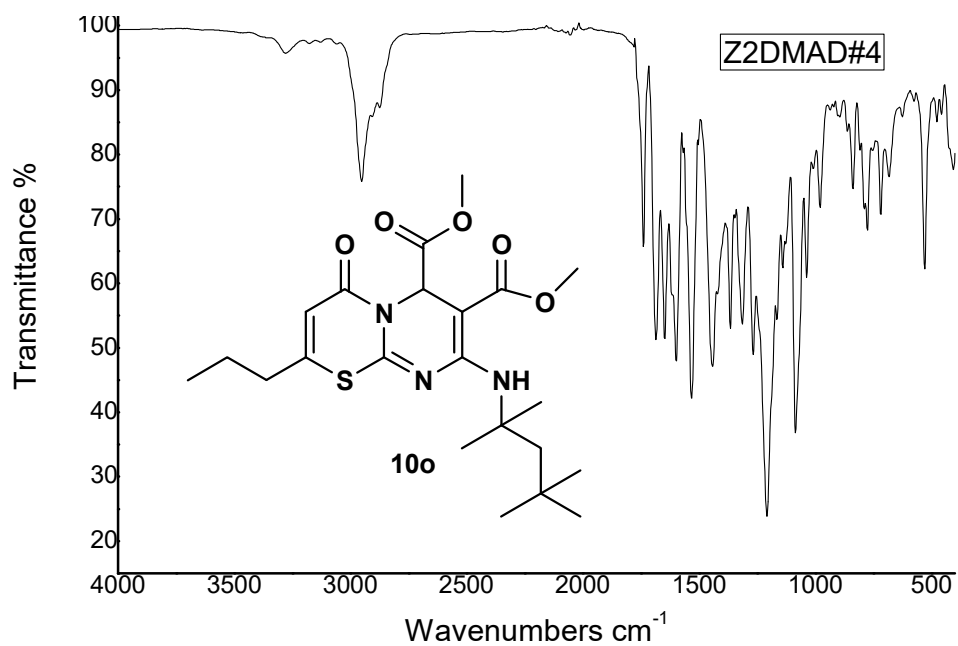
— 8.644

— 6.237

3.654  
3.594  
2.397  
2.378  
2.360  
1.728  
1.671  
1.651  
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0.872

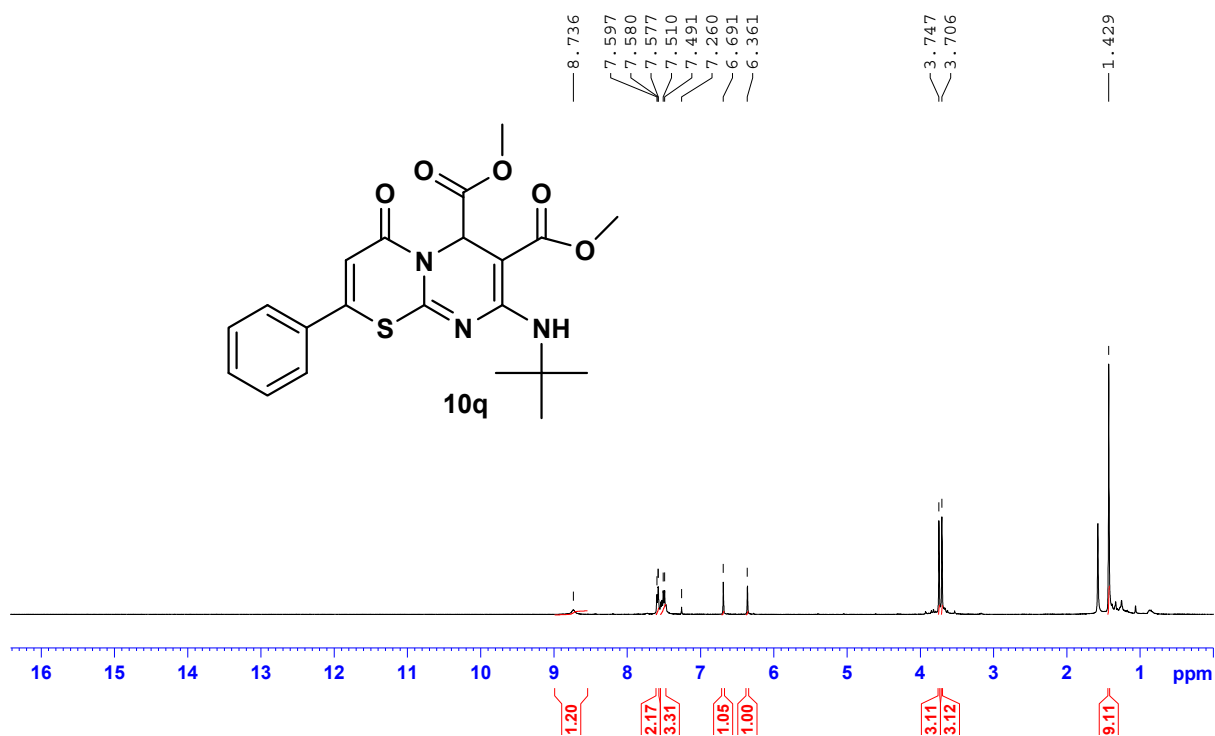
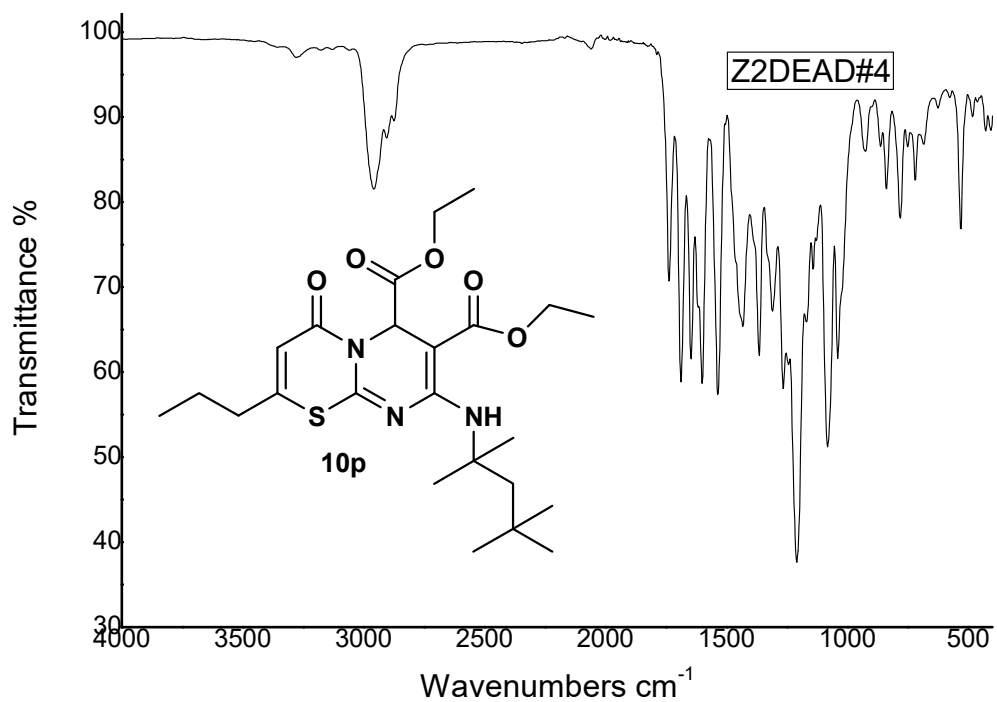


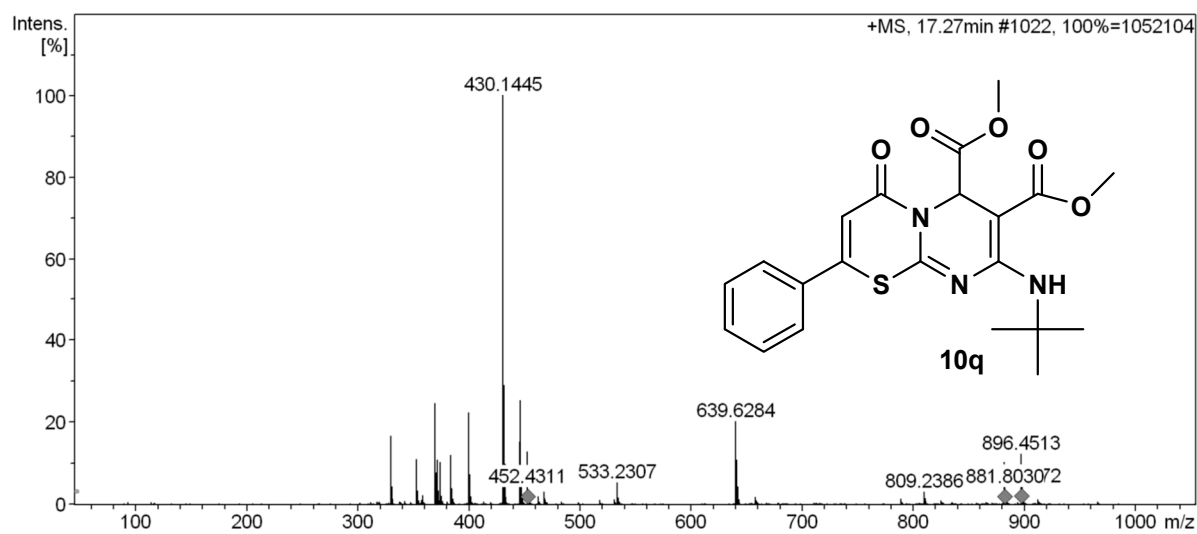
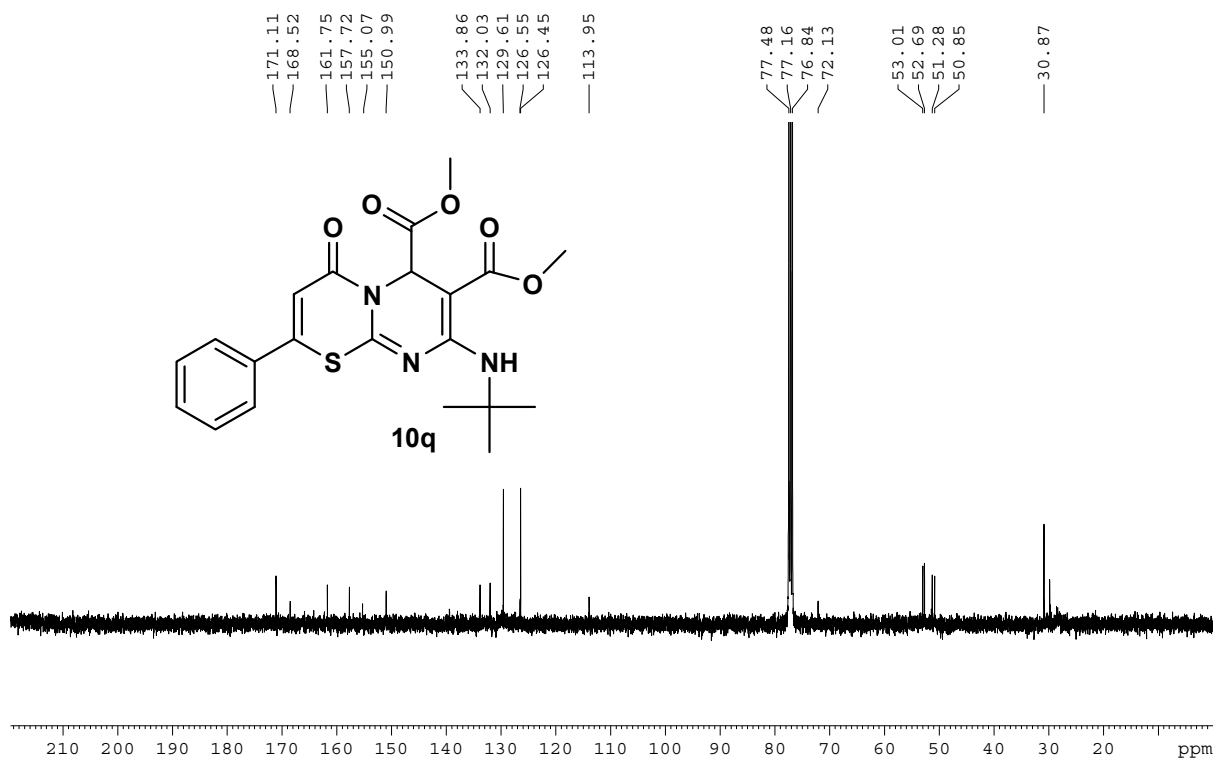


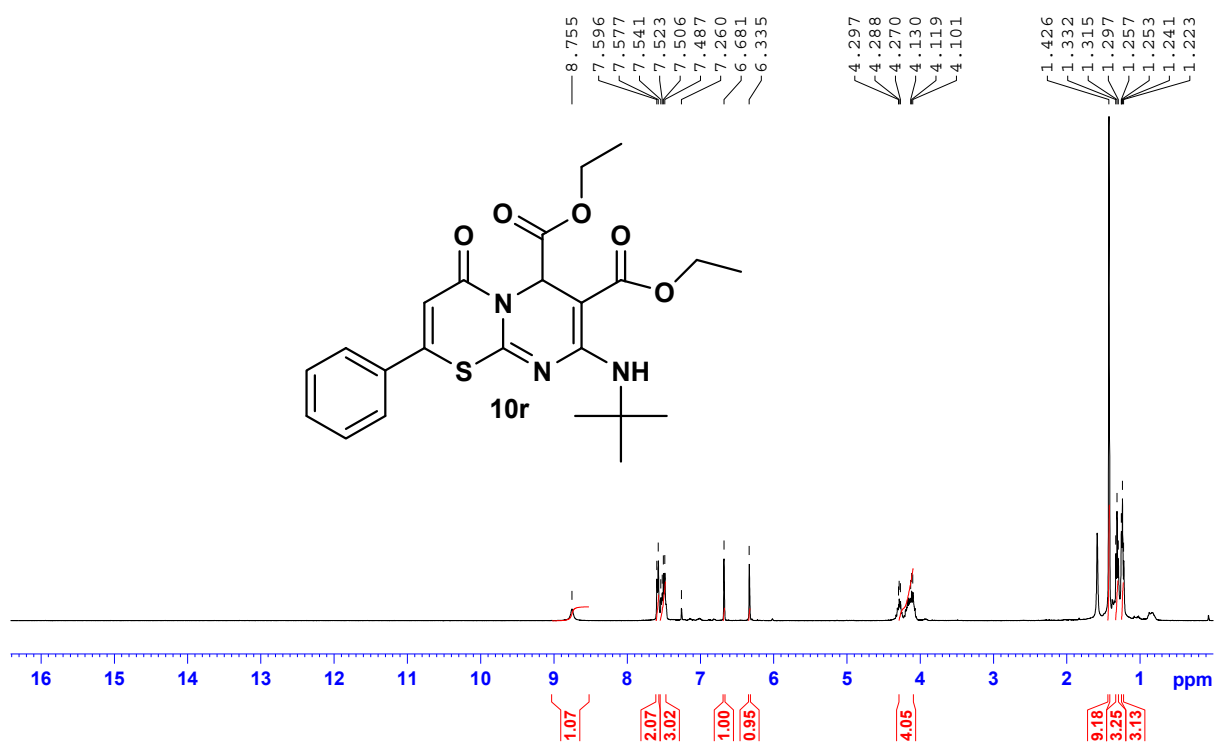
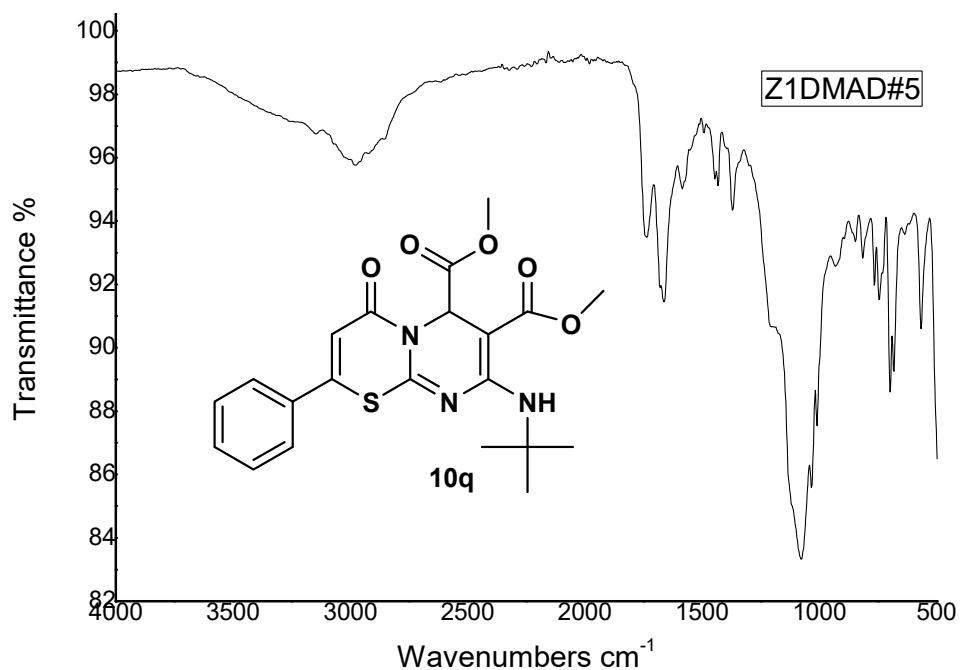


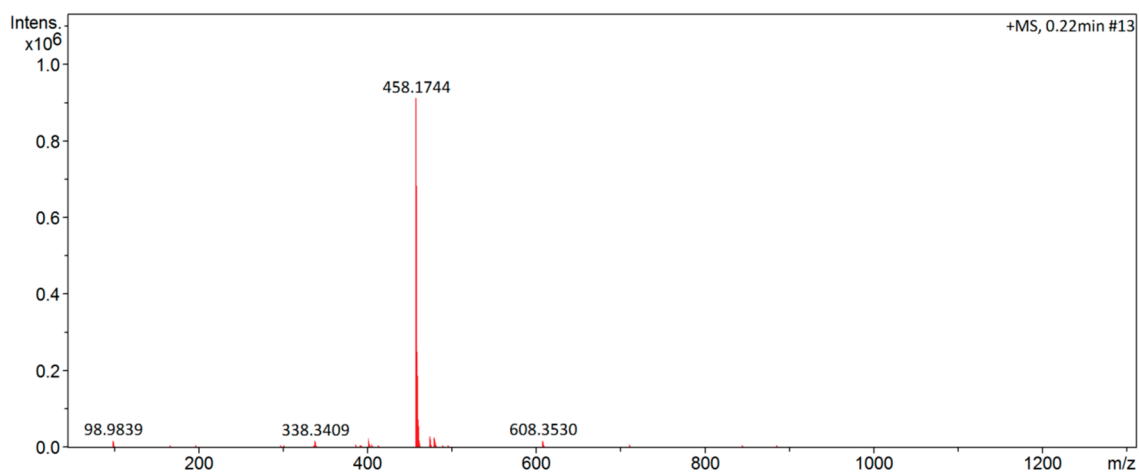
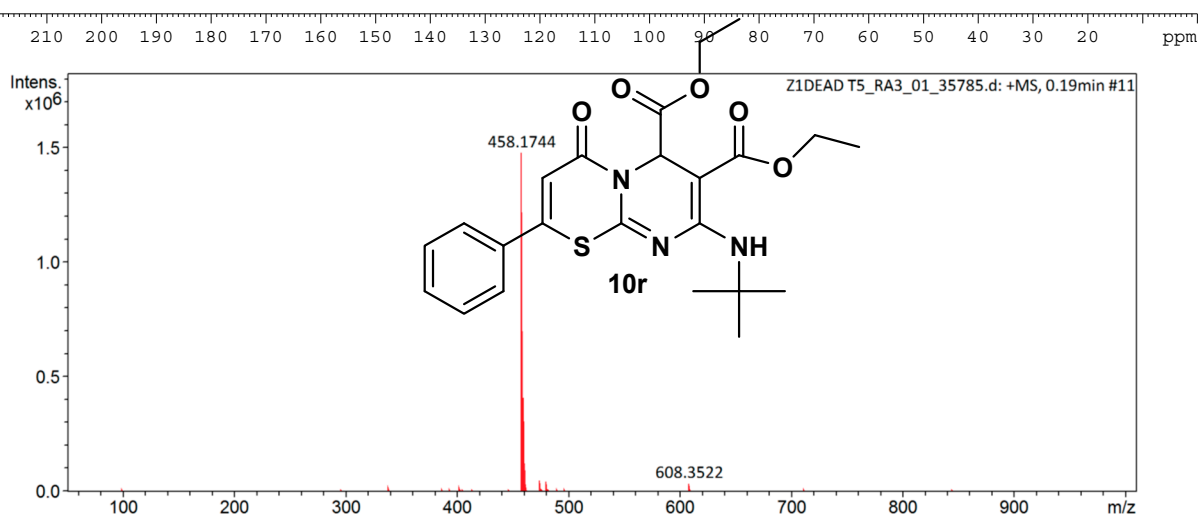
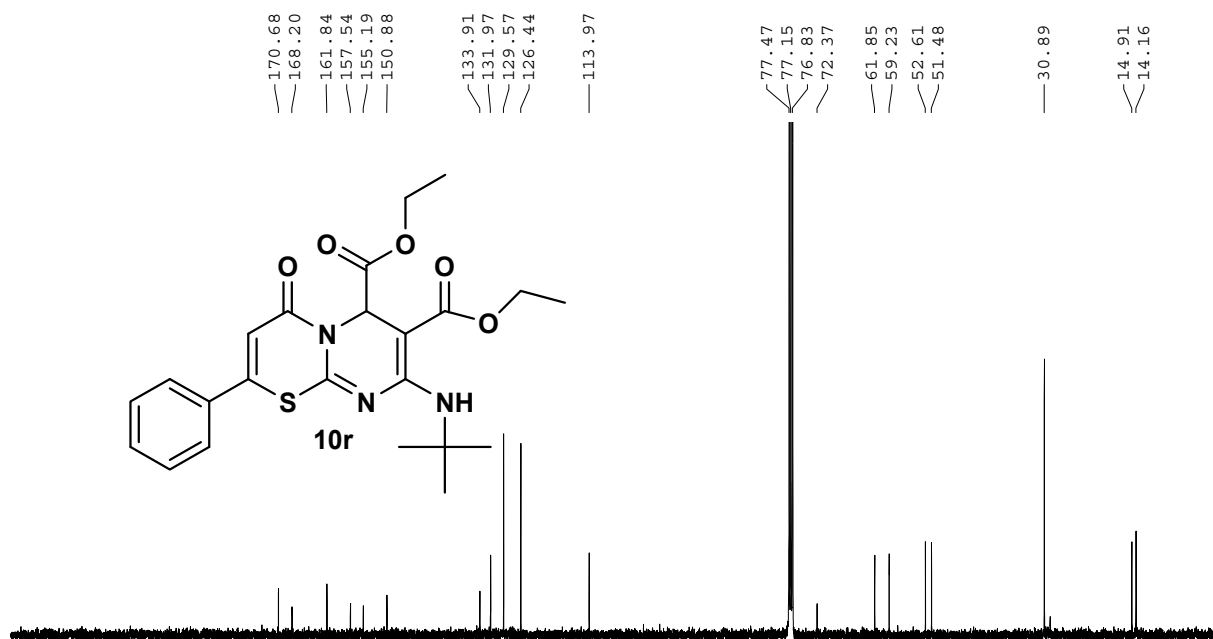


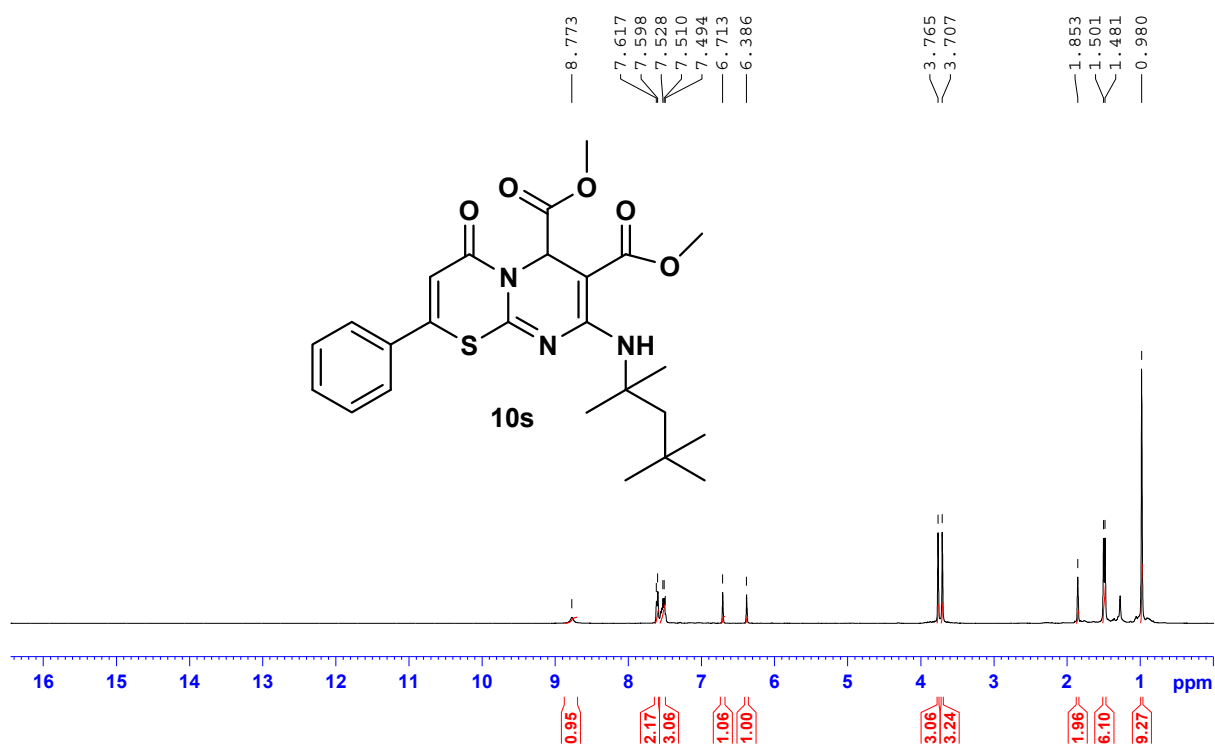
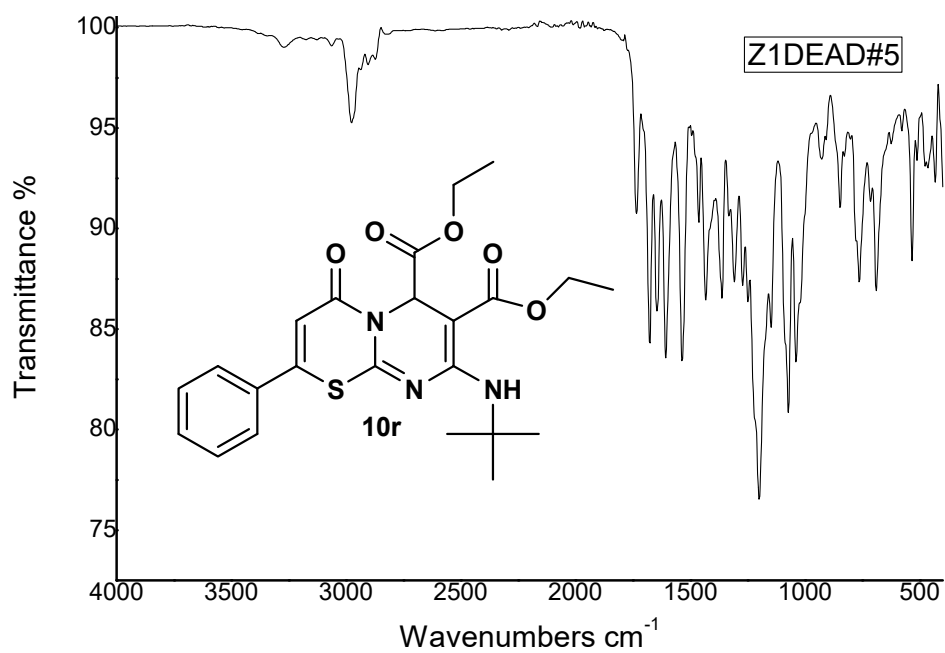


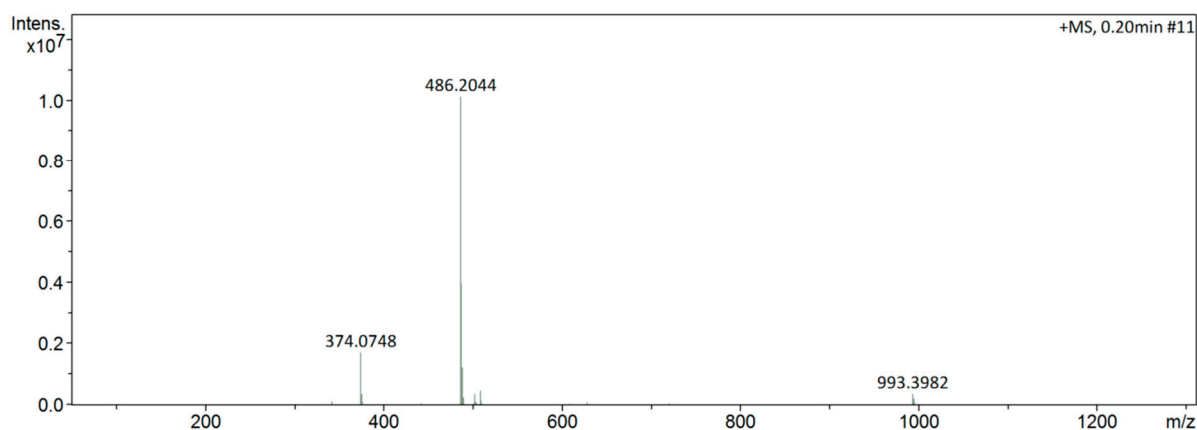
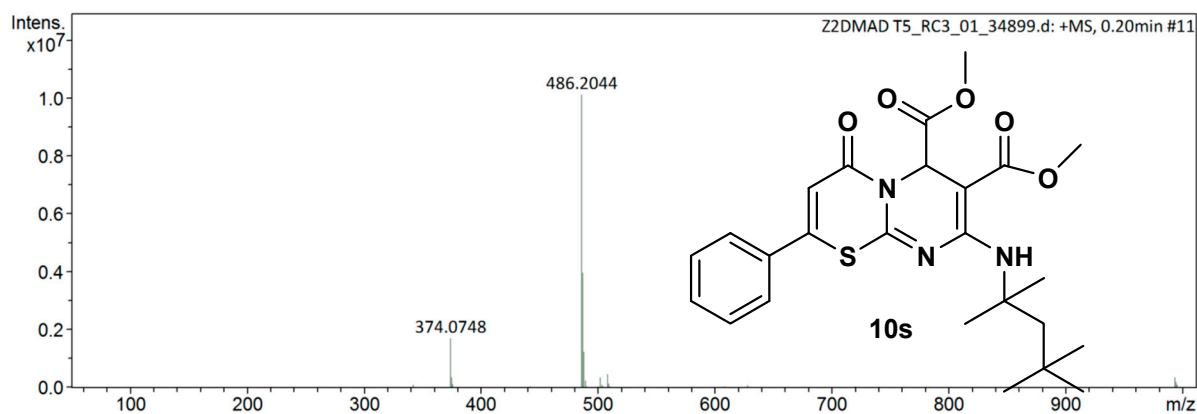
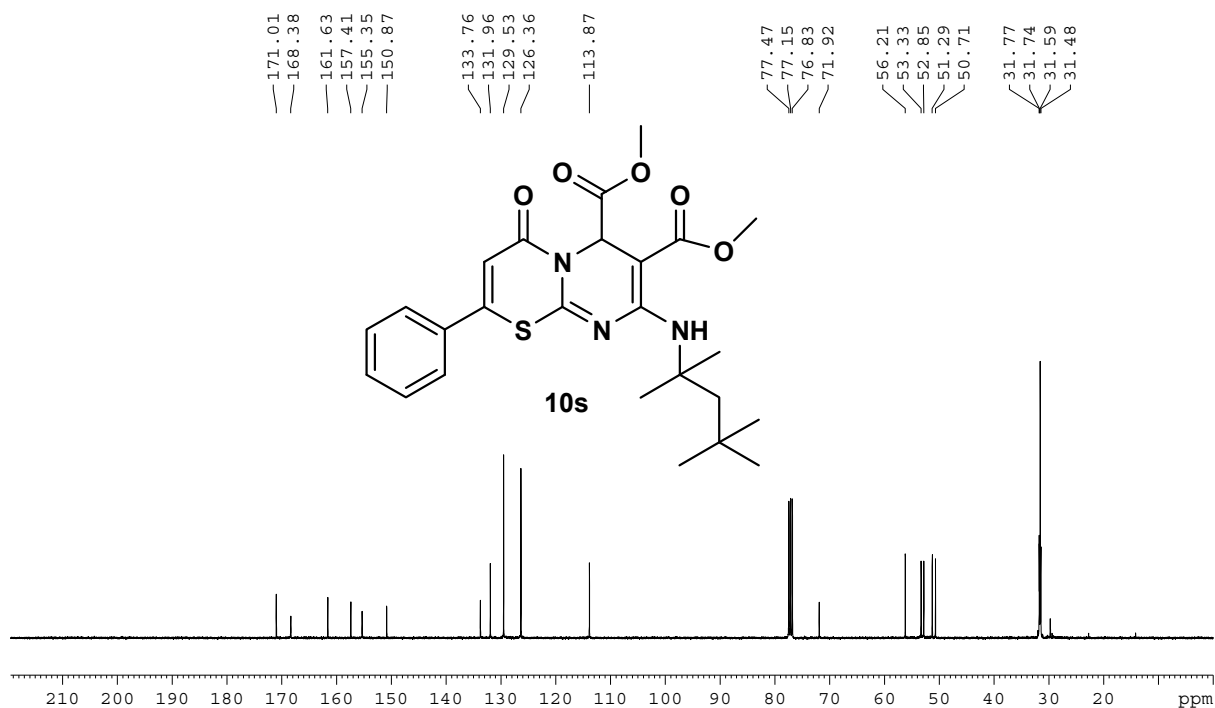


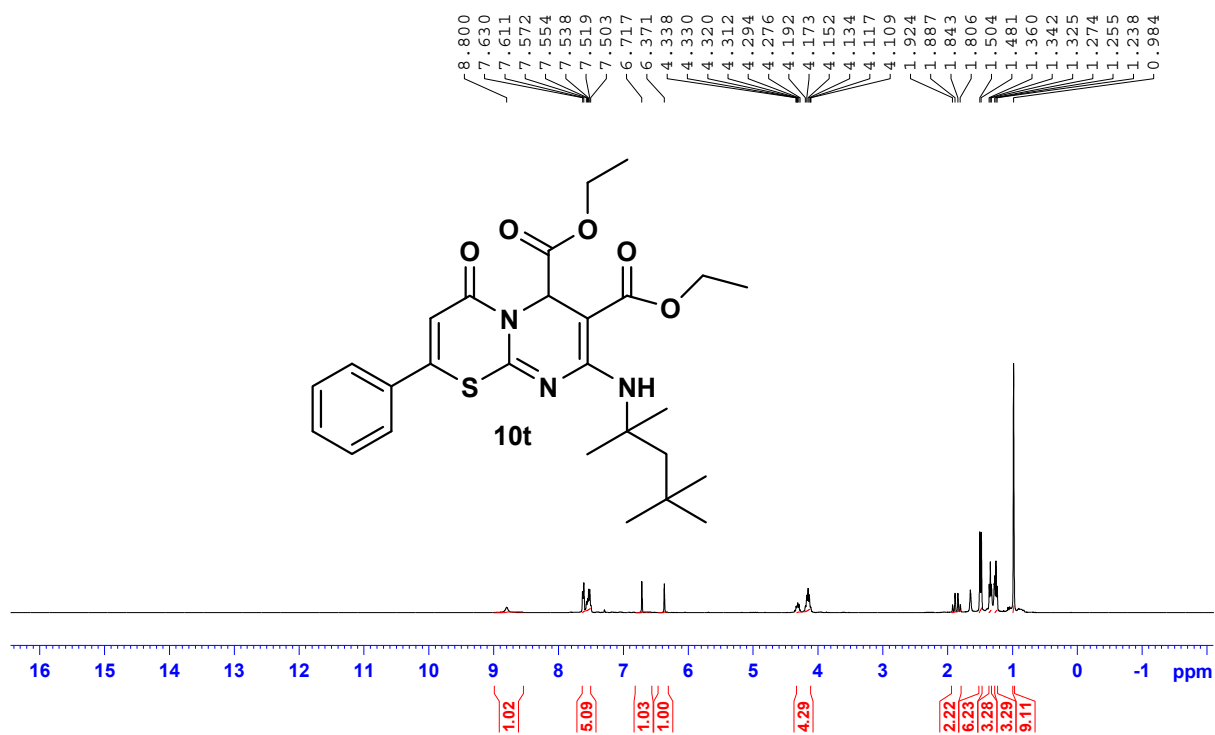
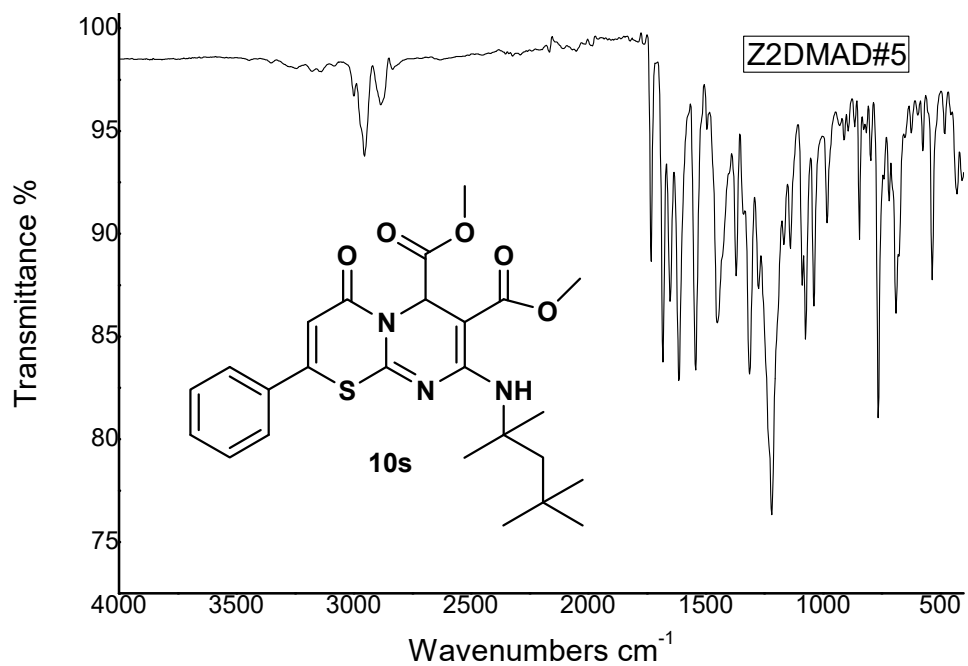




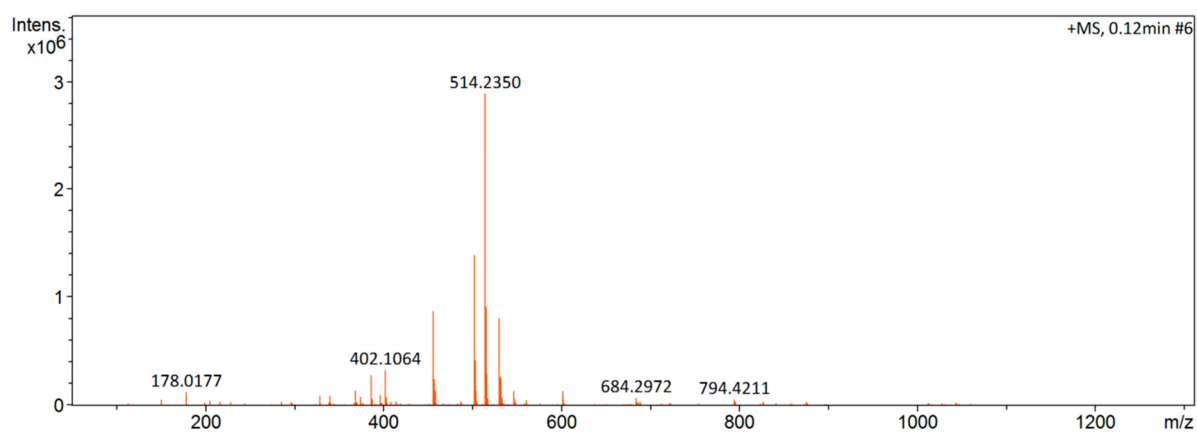
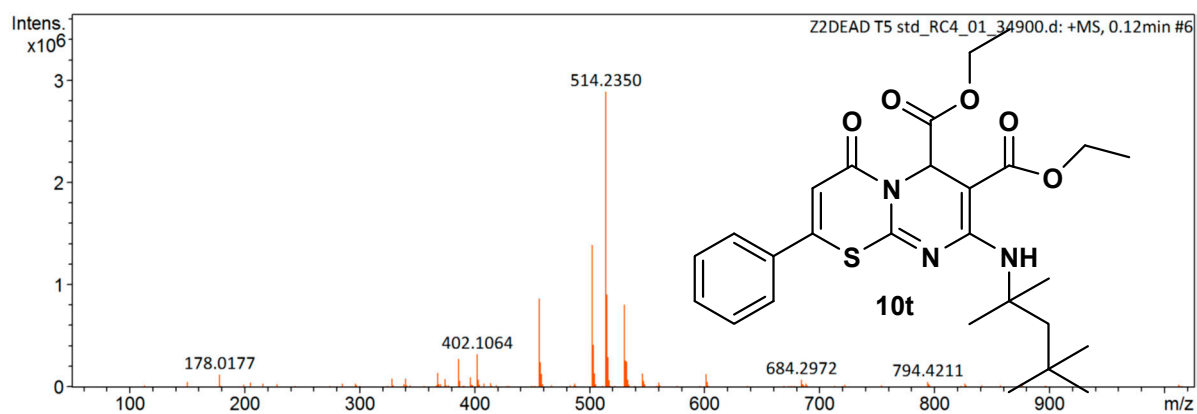
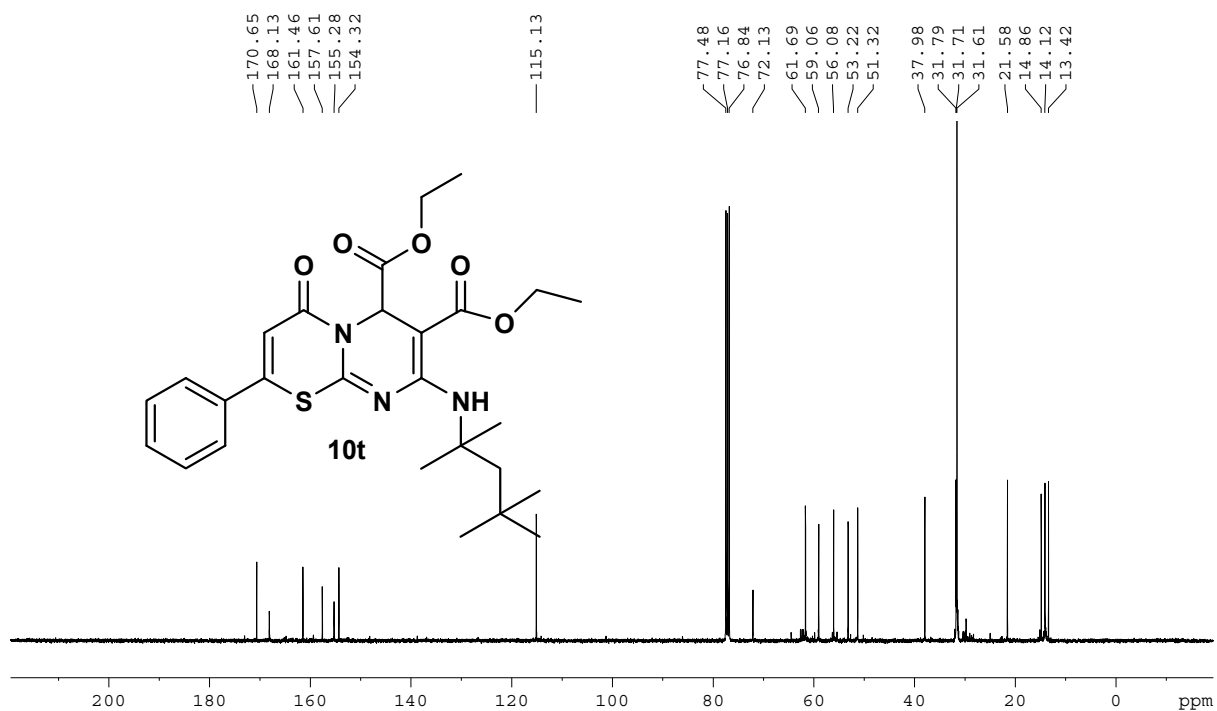


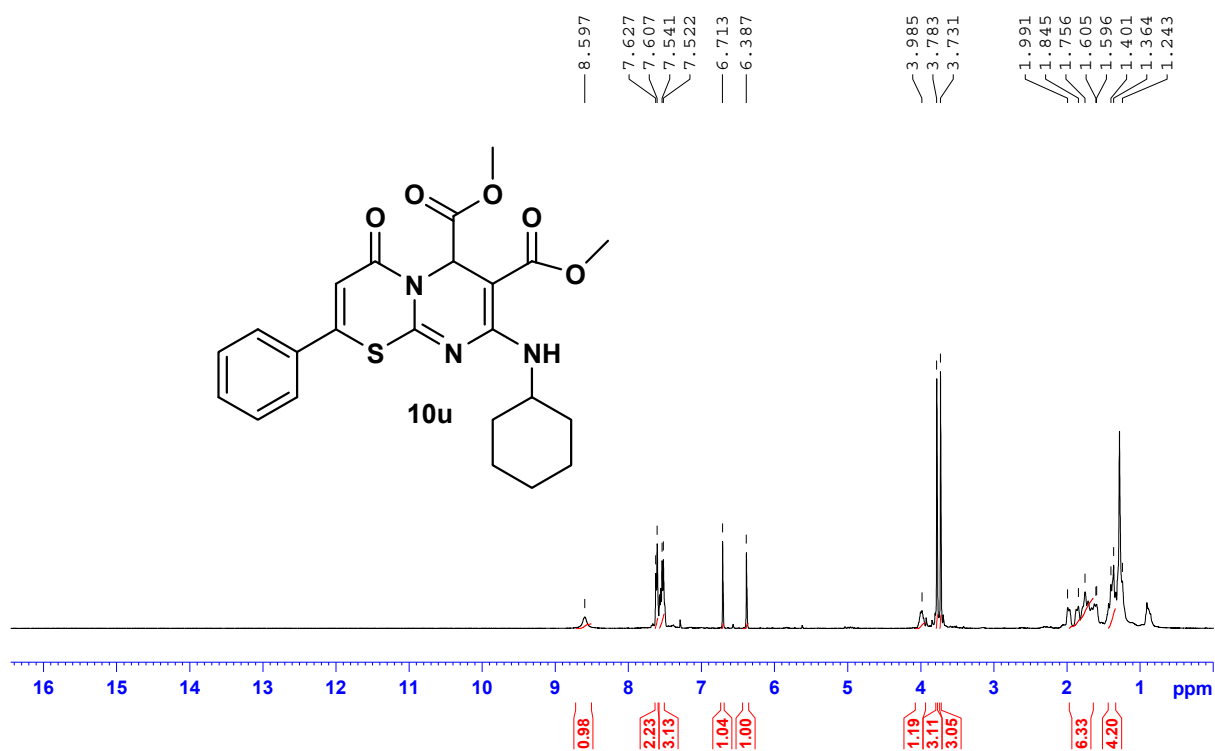
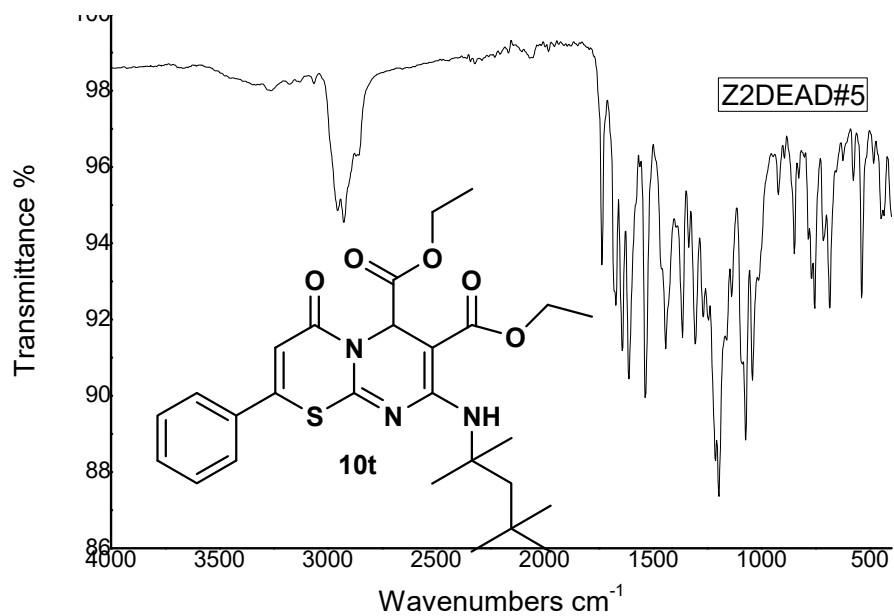


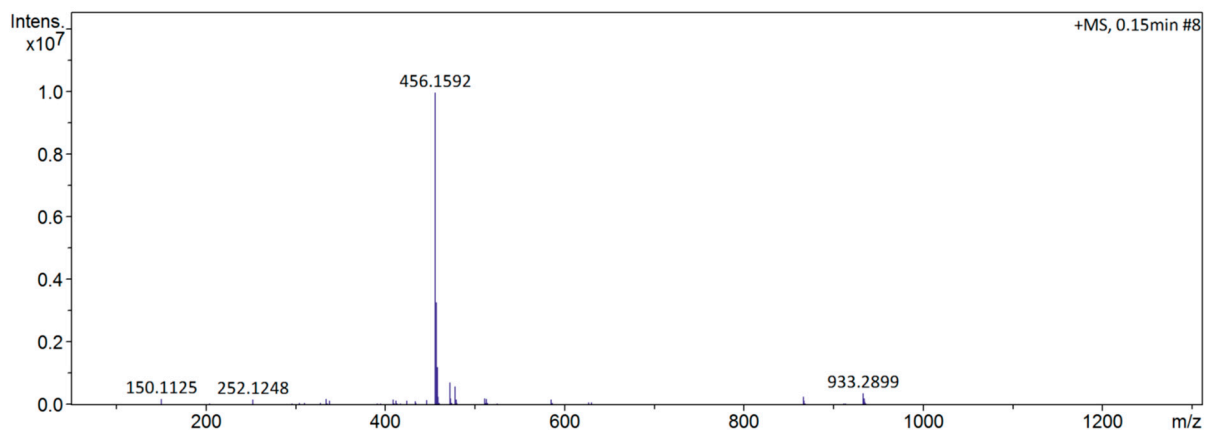
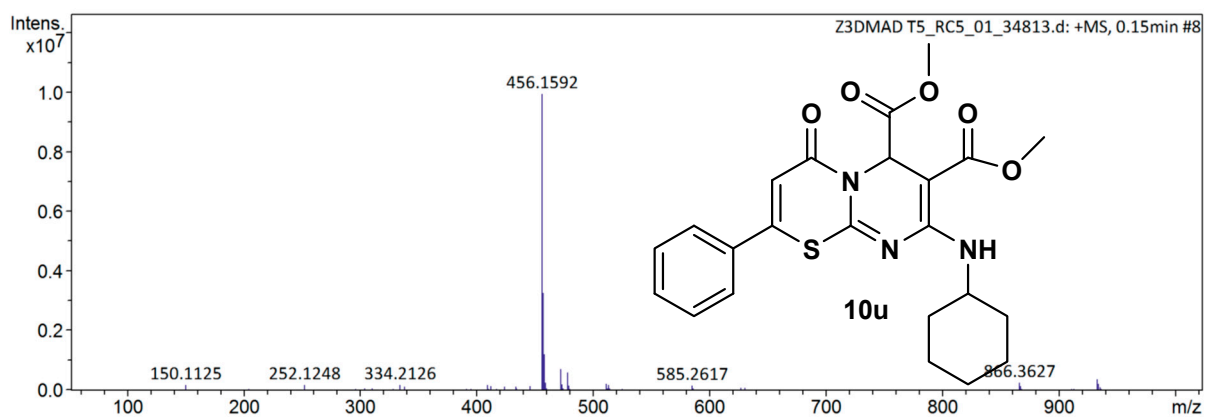
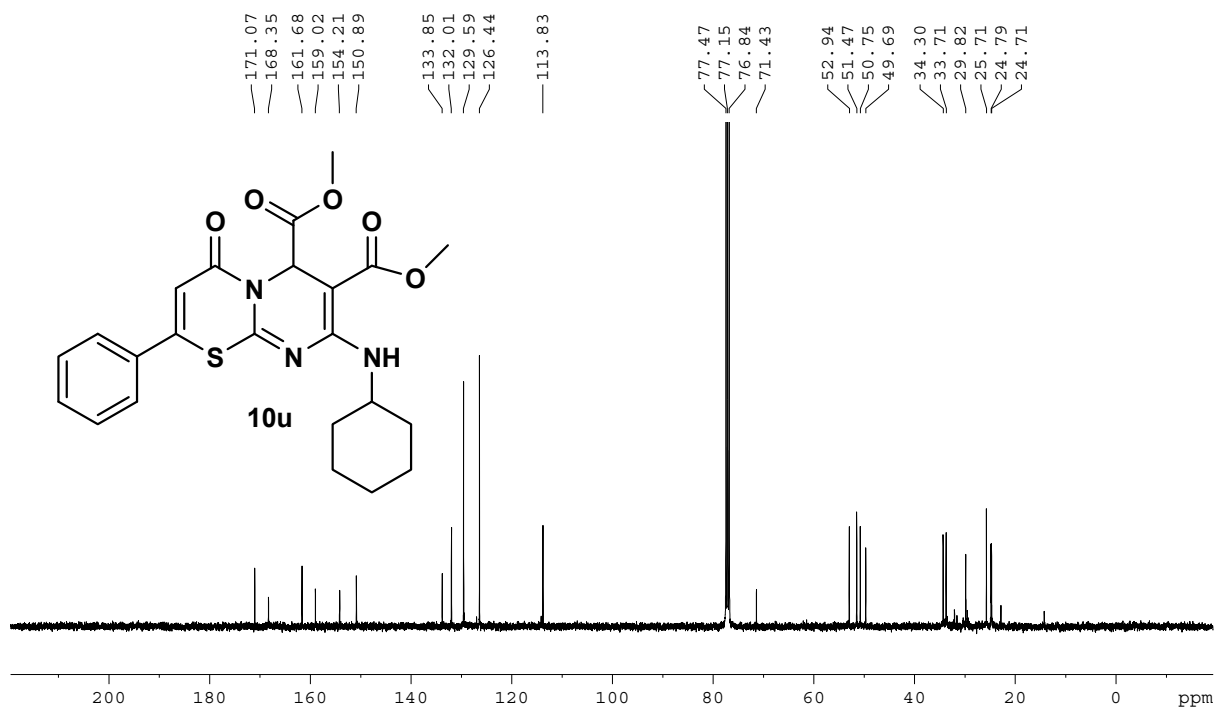


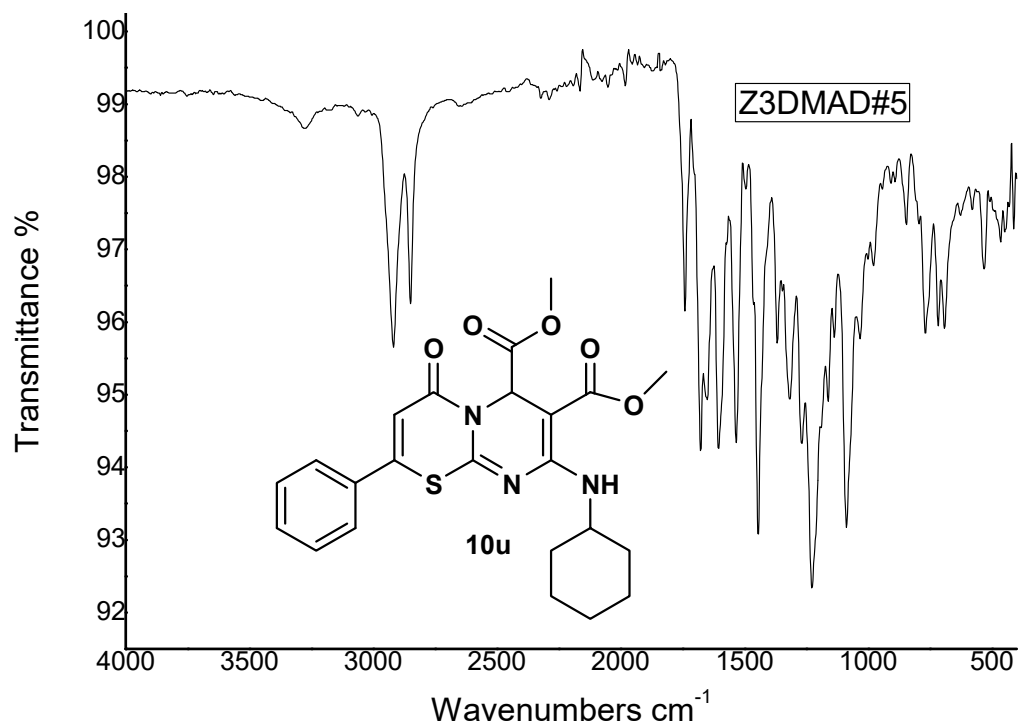




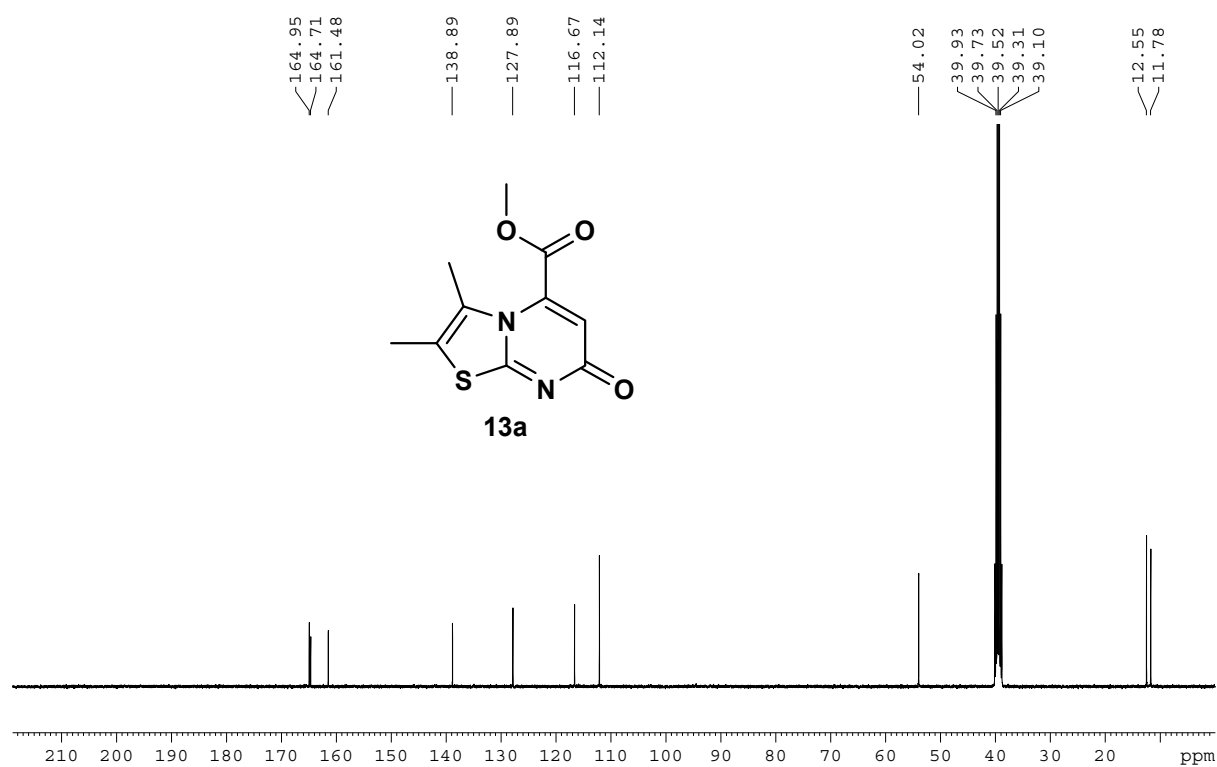
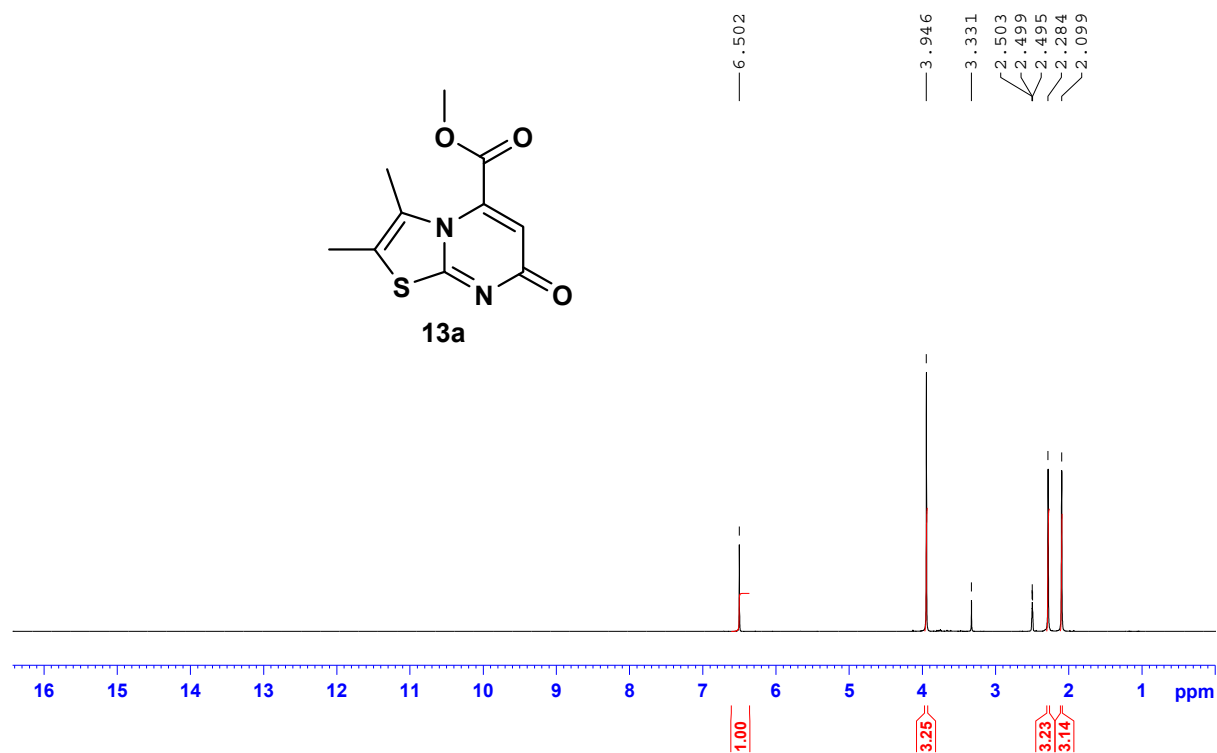


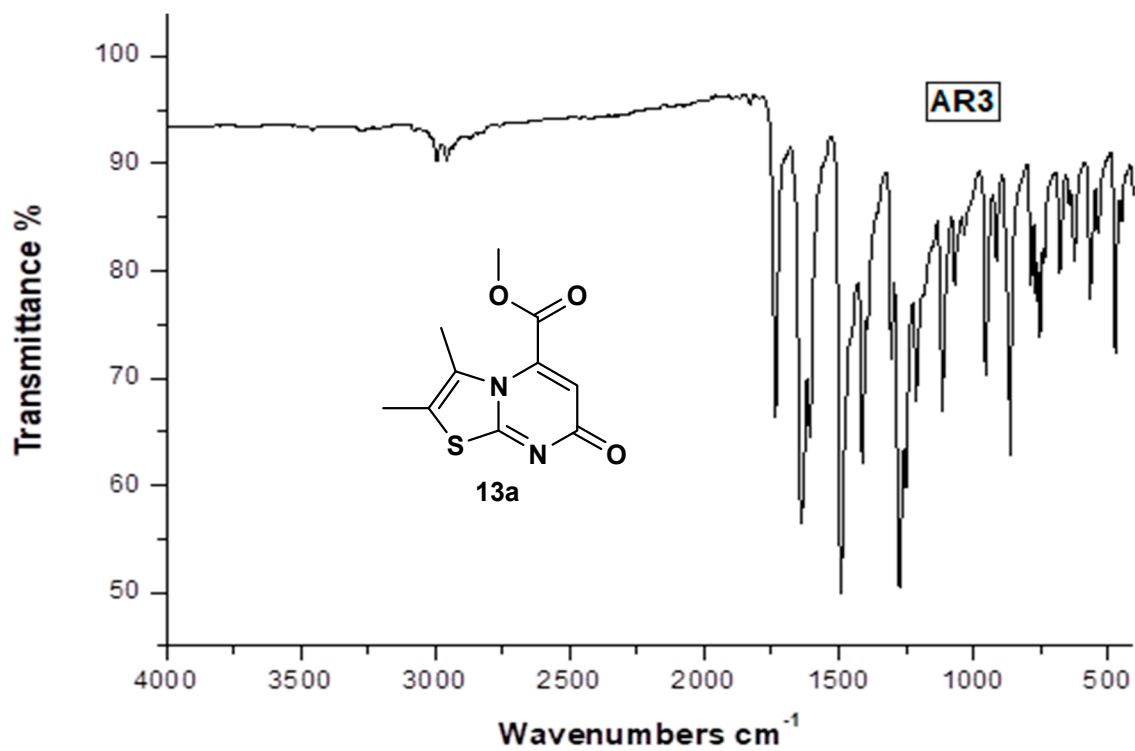
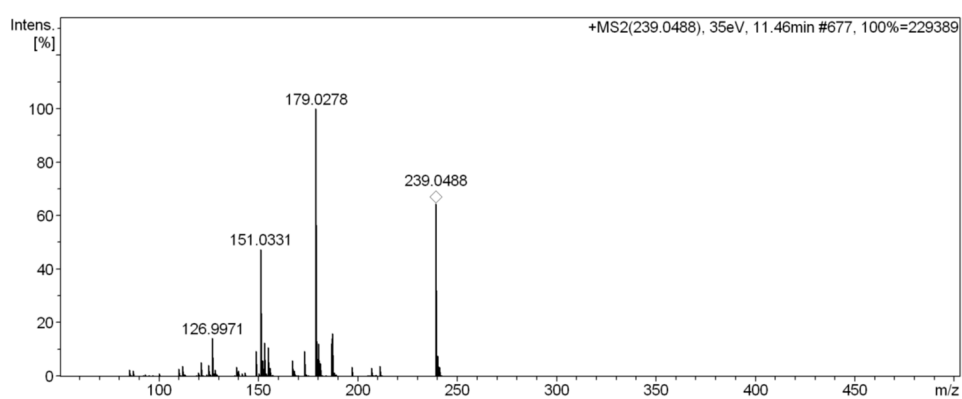
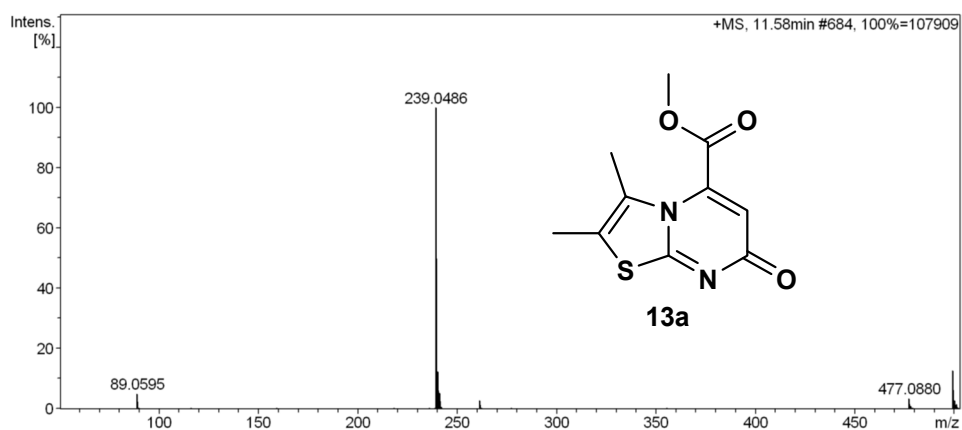


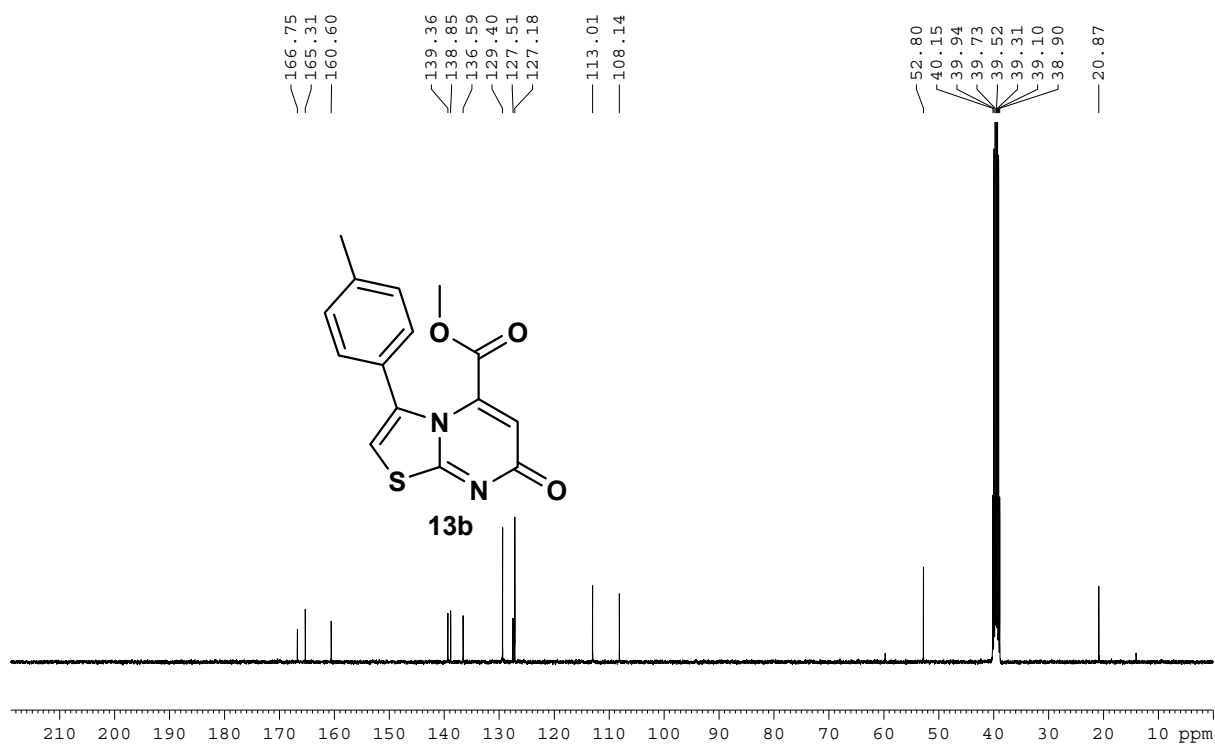
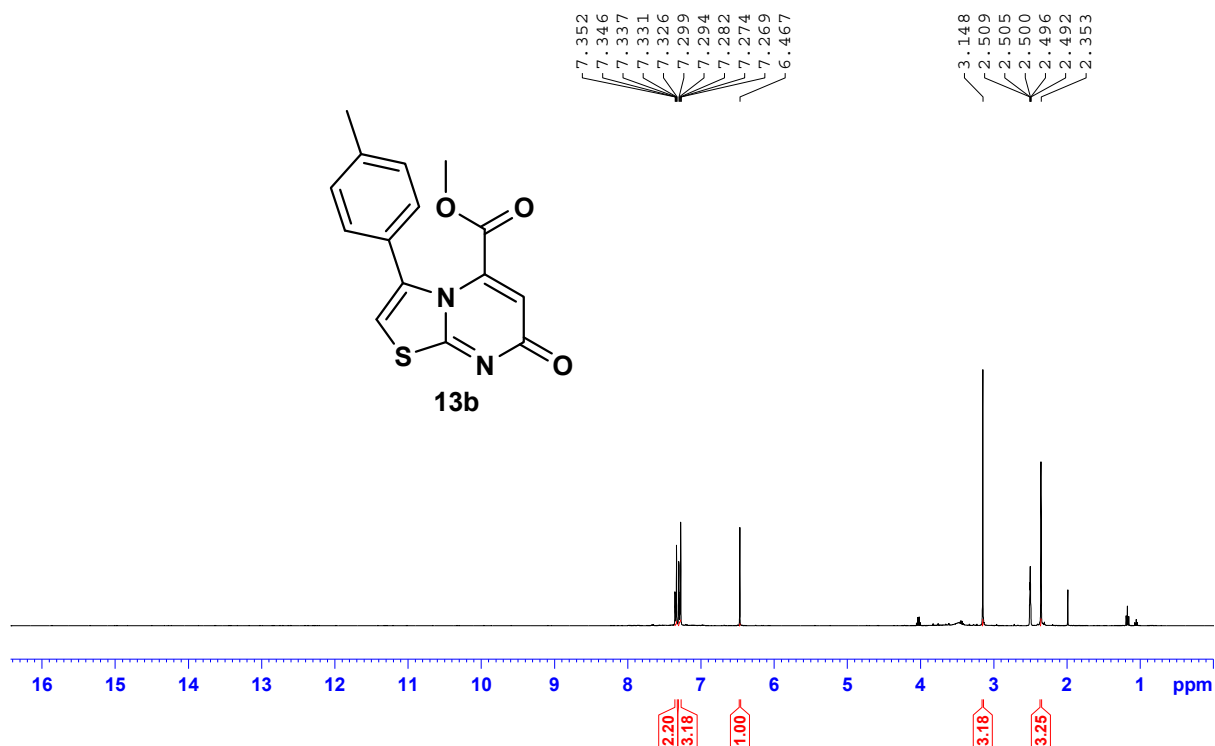


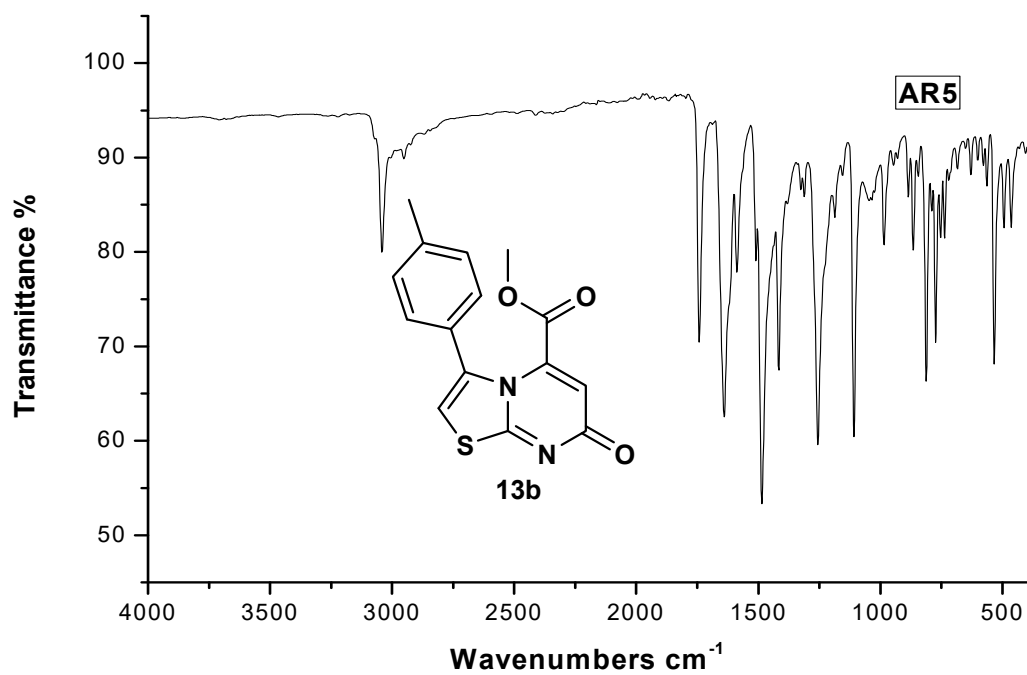
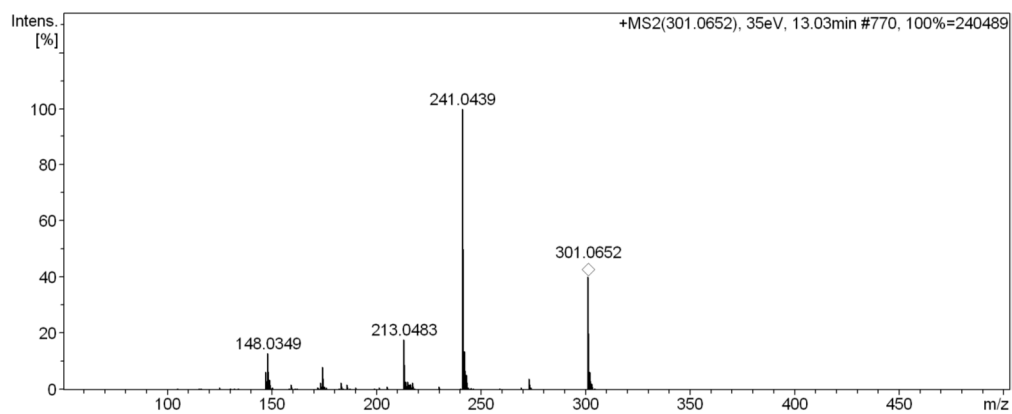
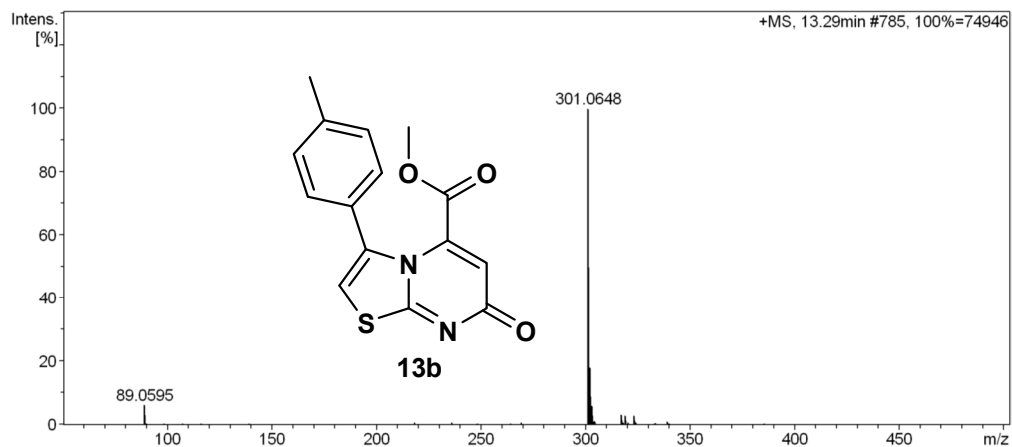


Methyl 7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate derivatives and their analogs.

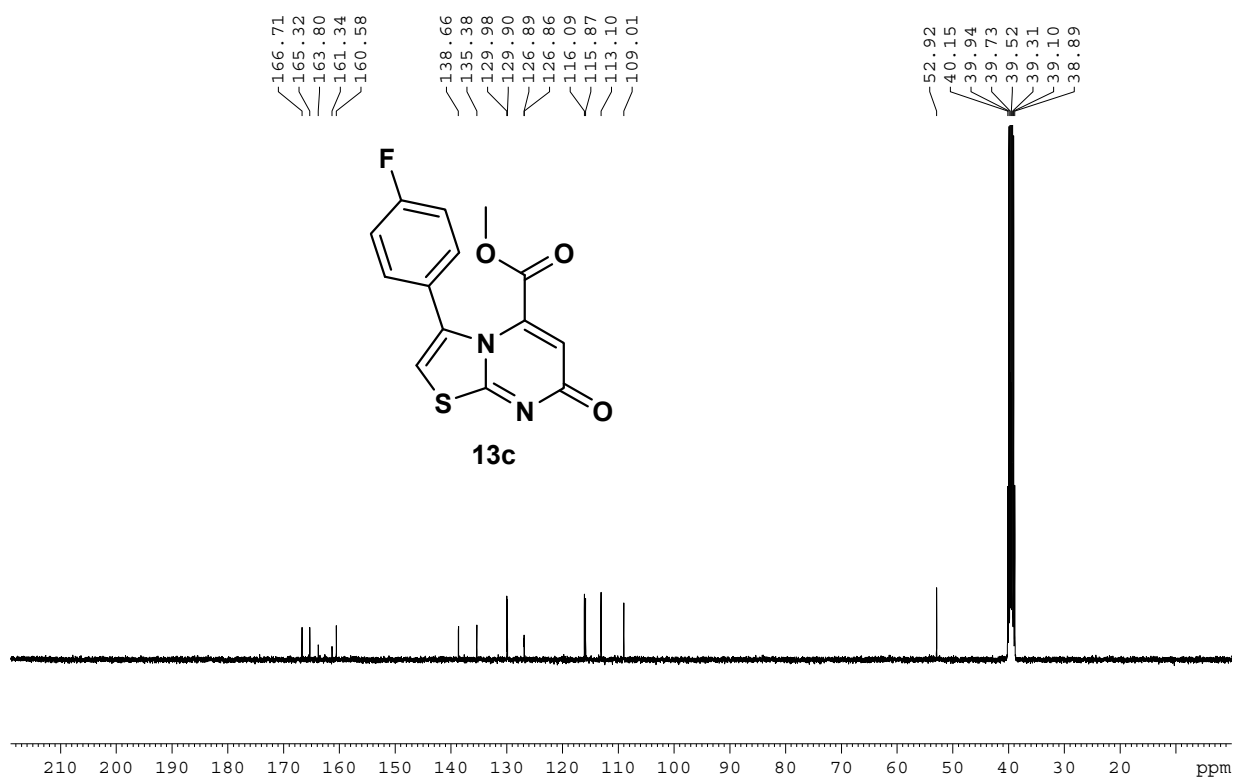
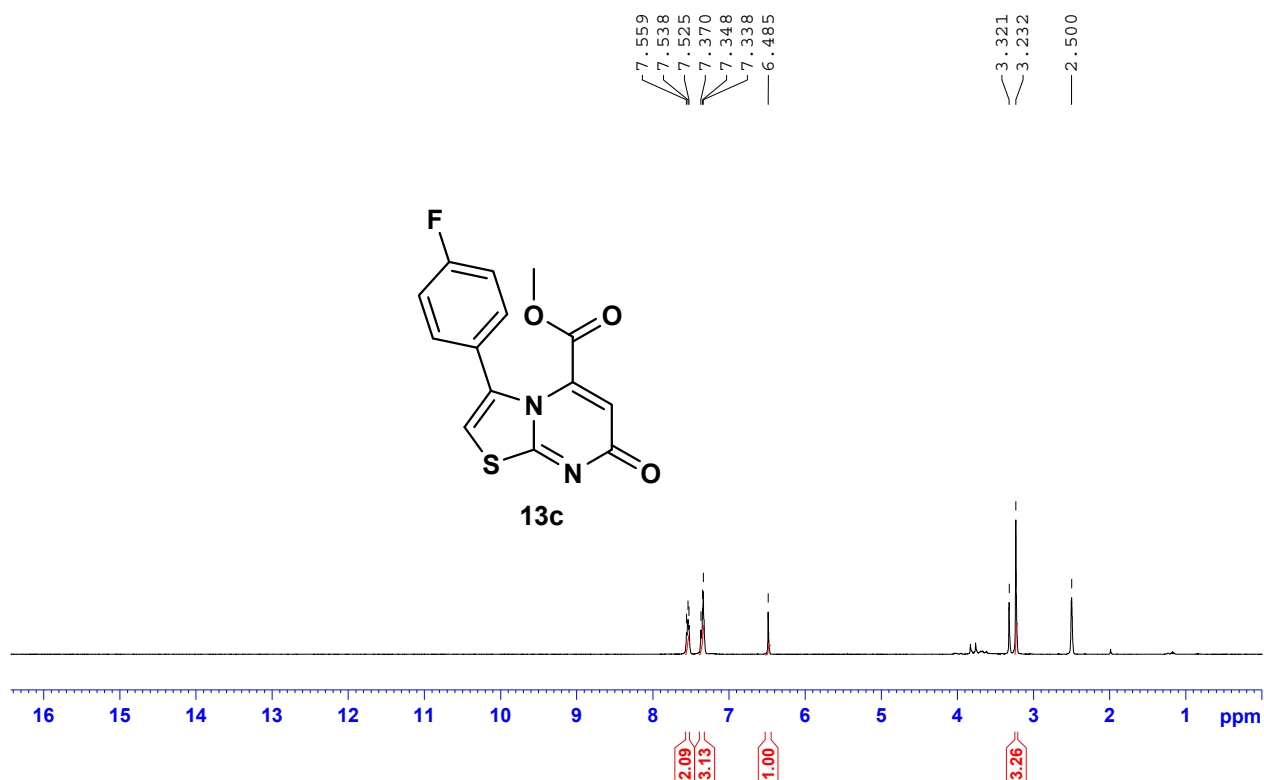


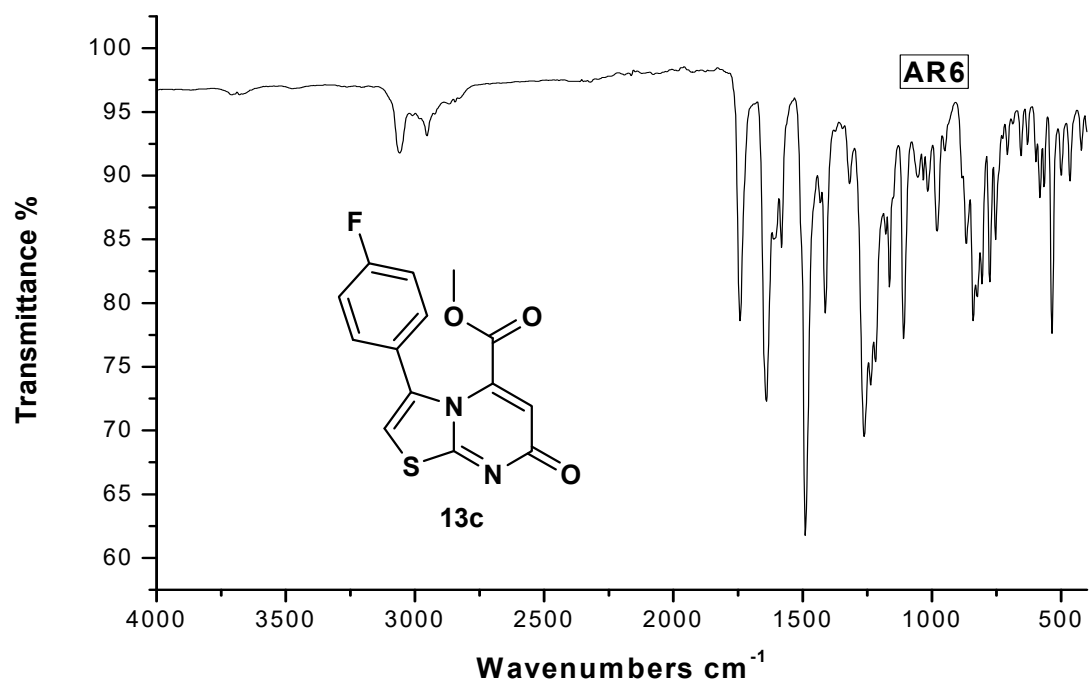
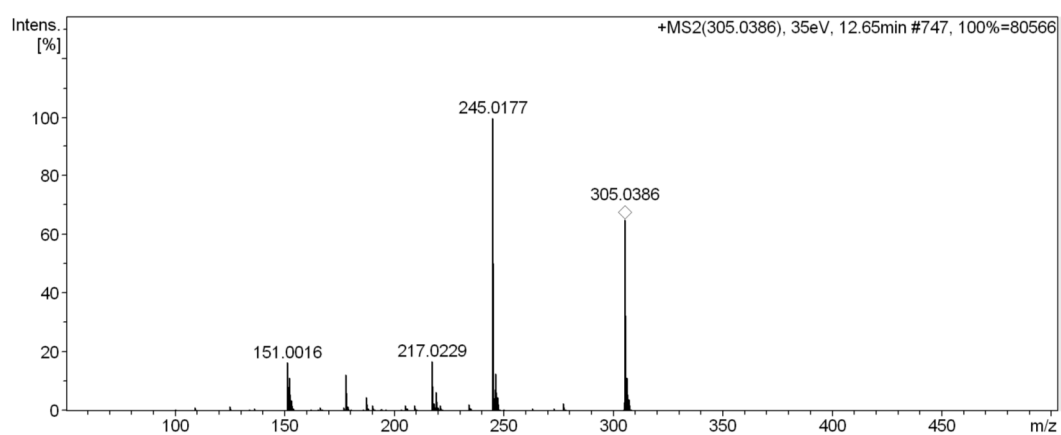
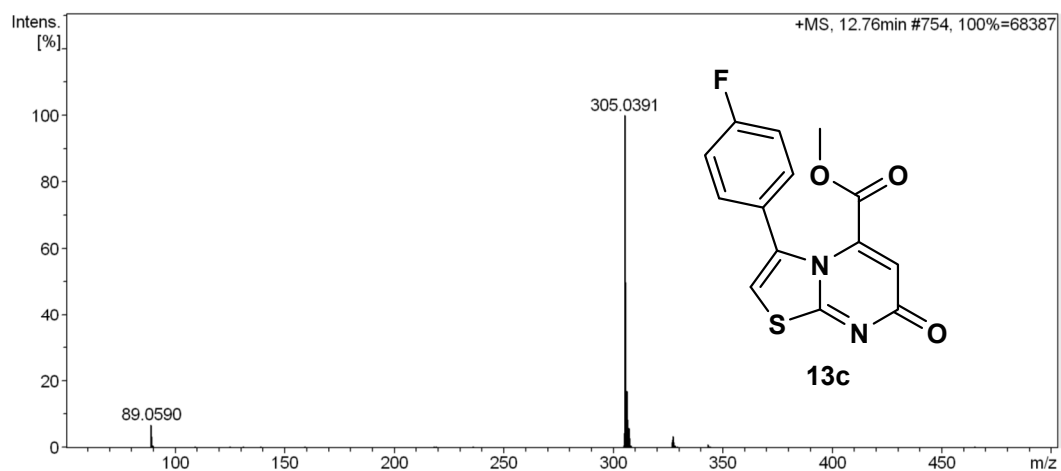


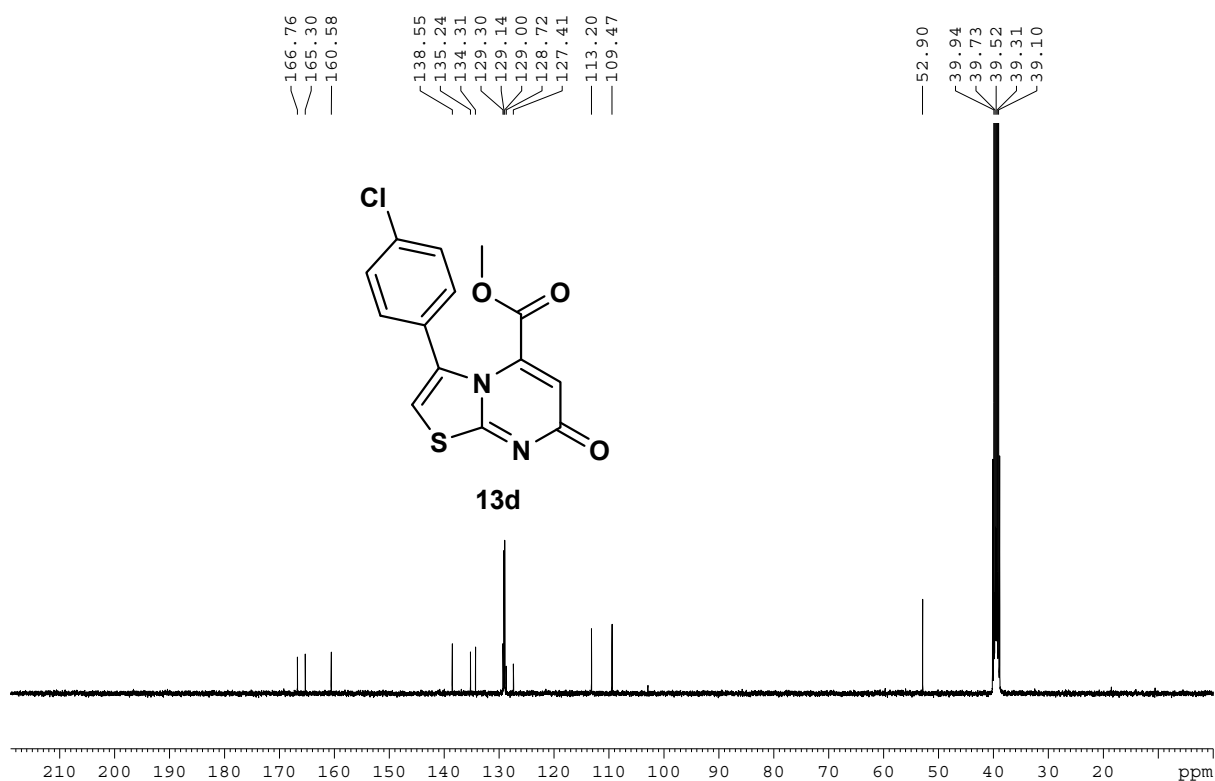
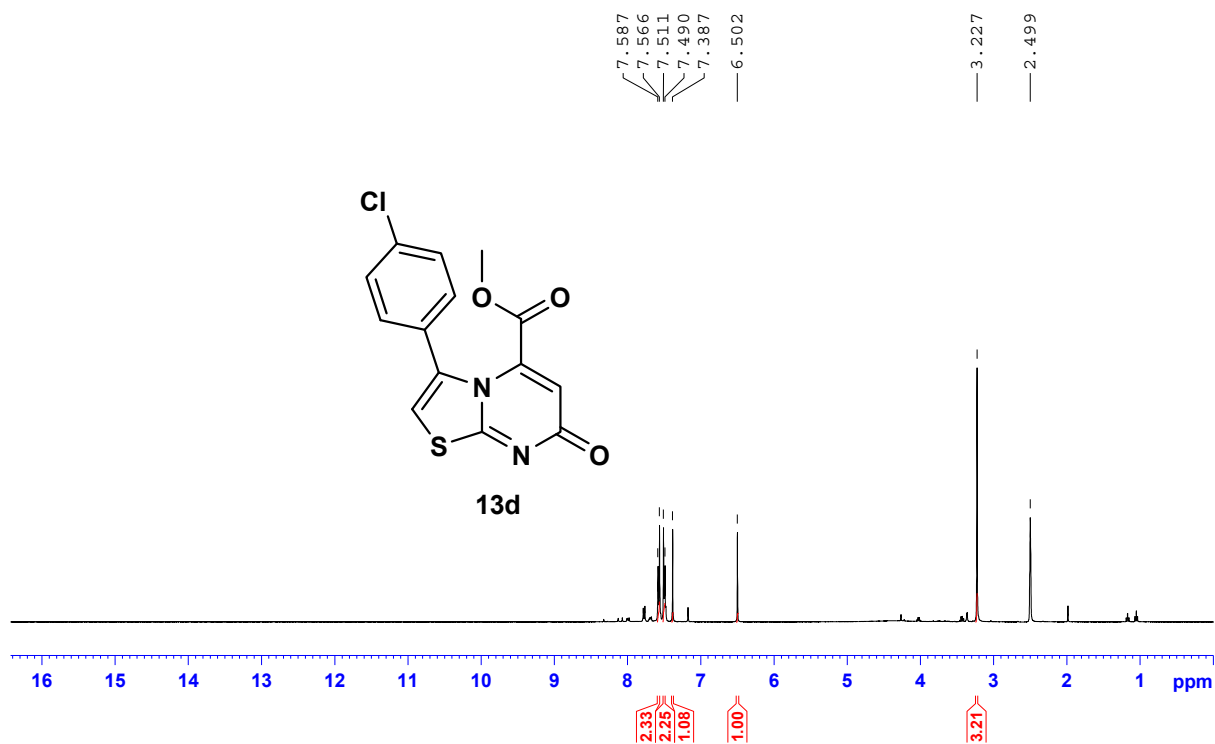


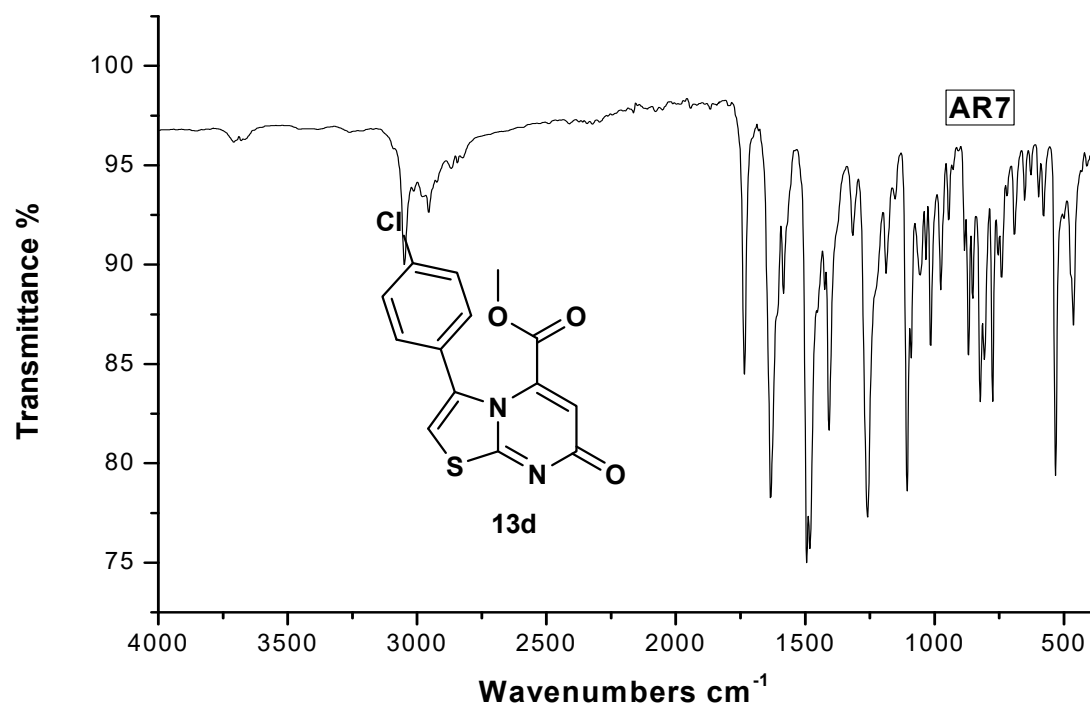
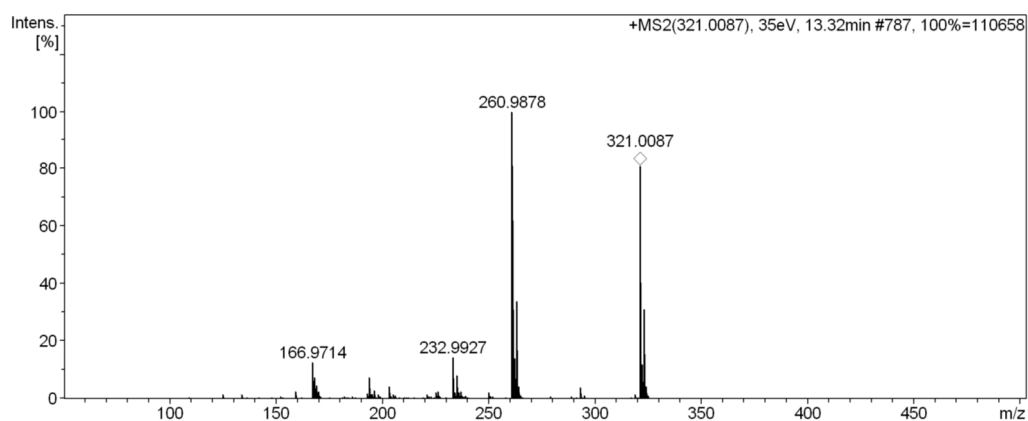
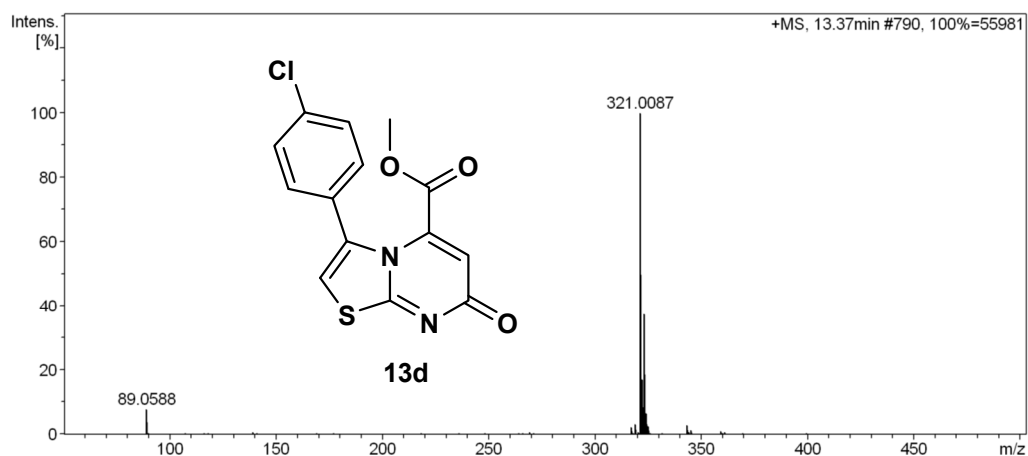


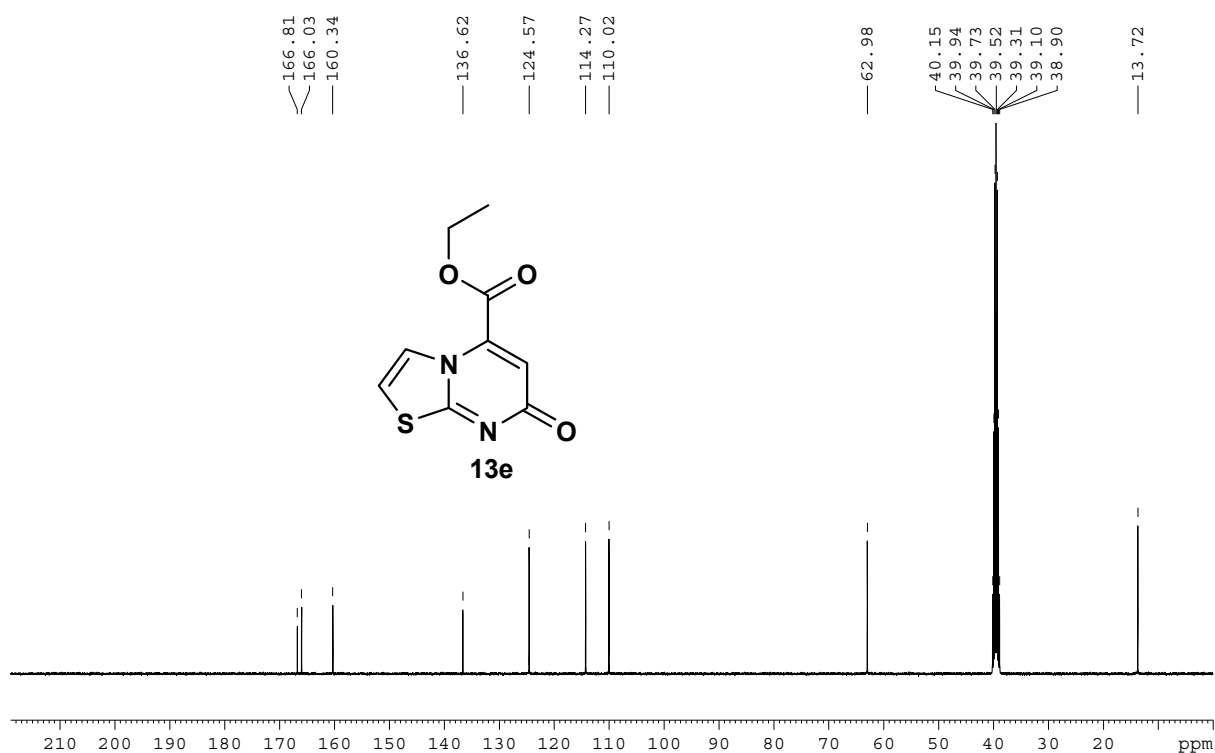
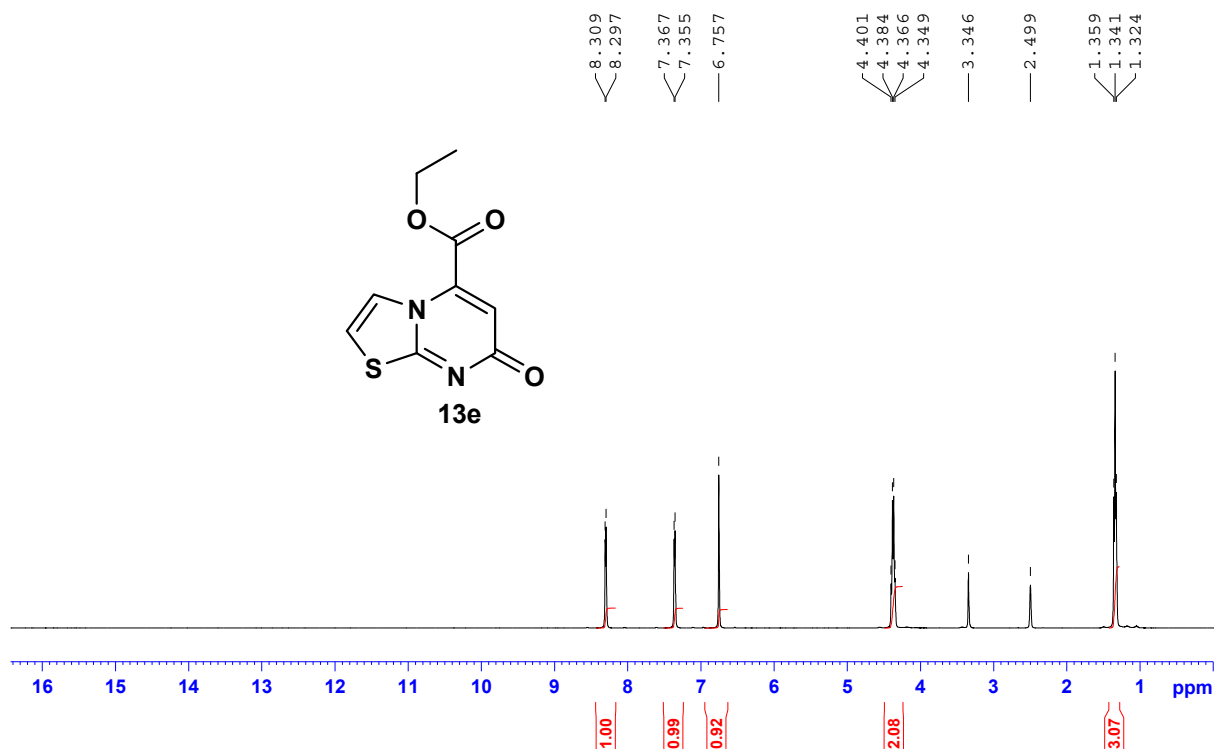


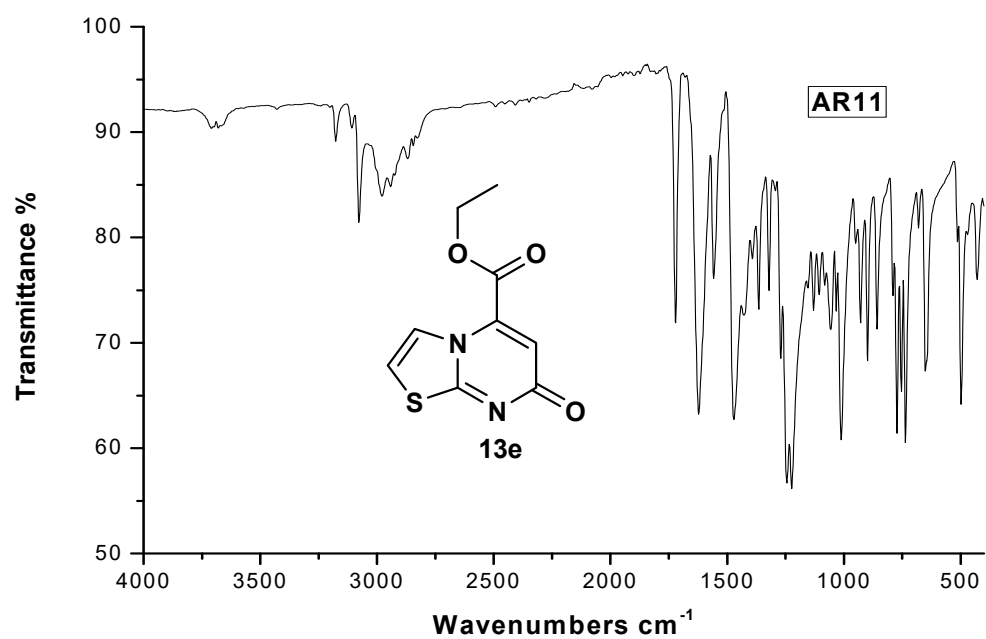
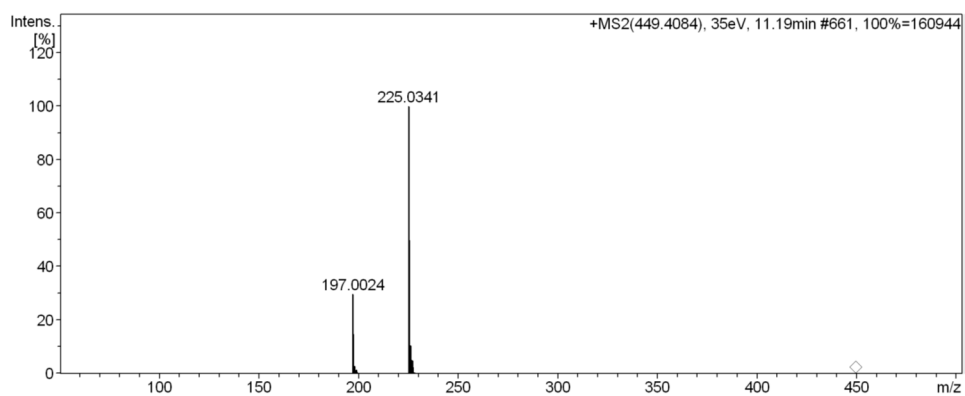
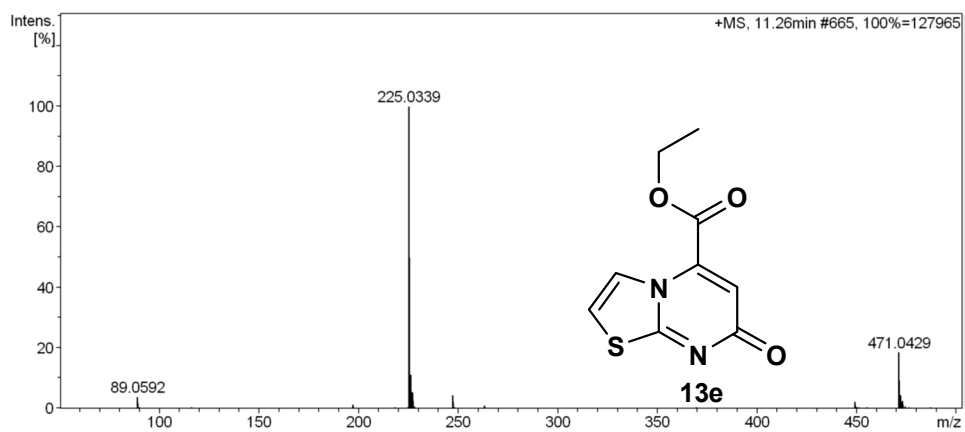


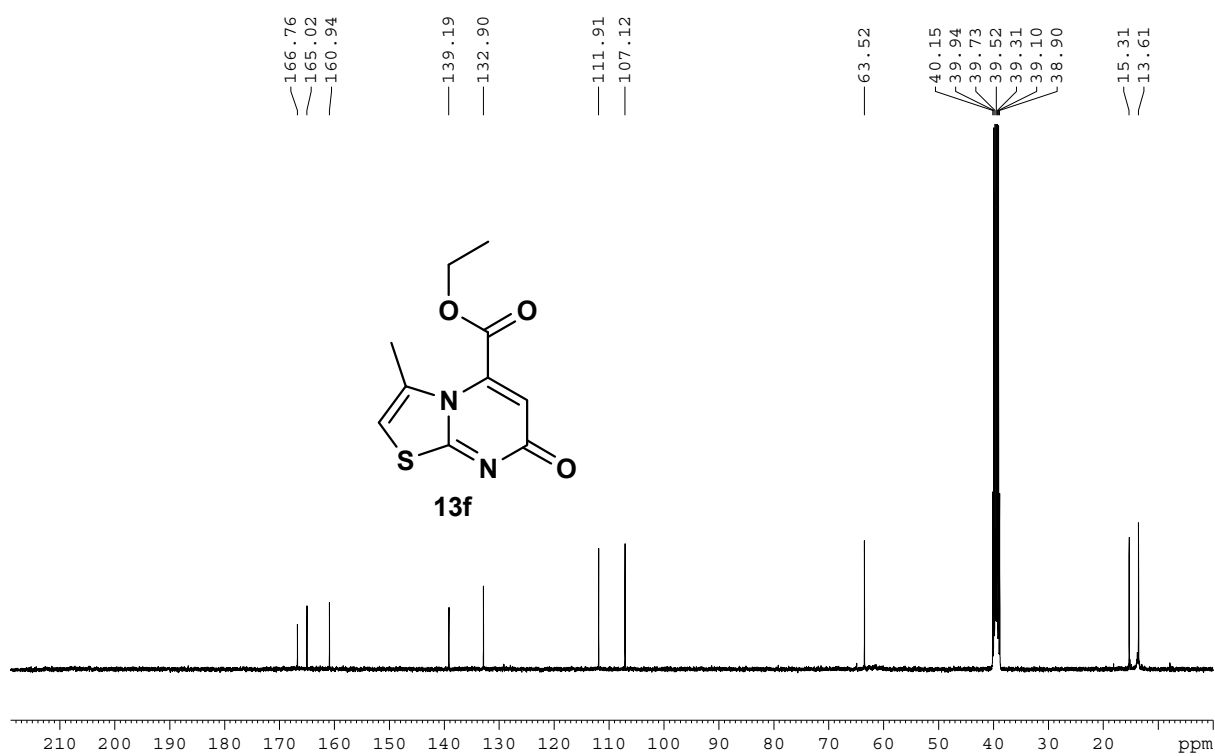
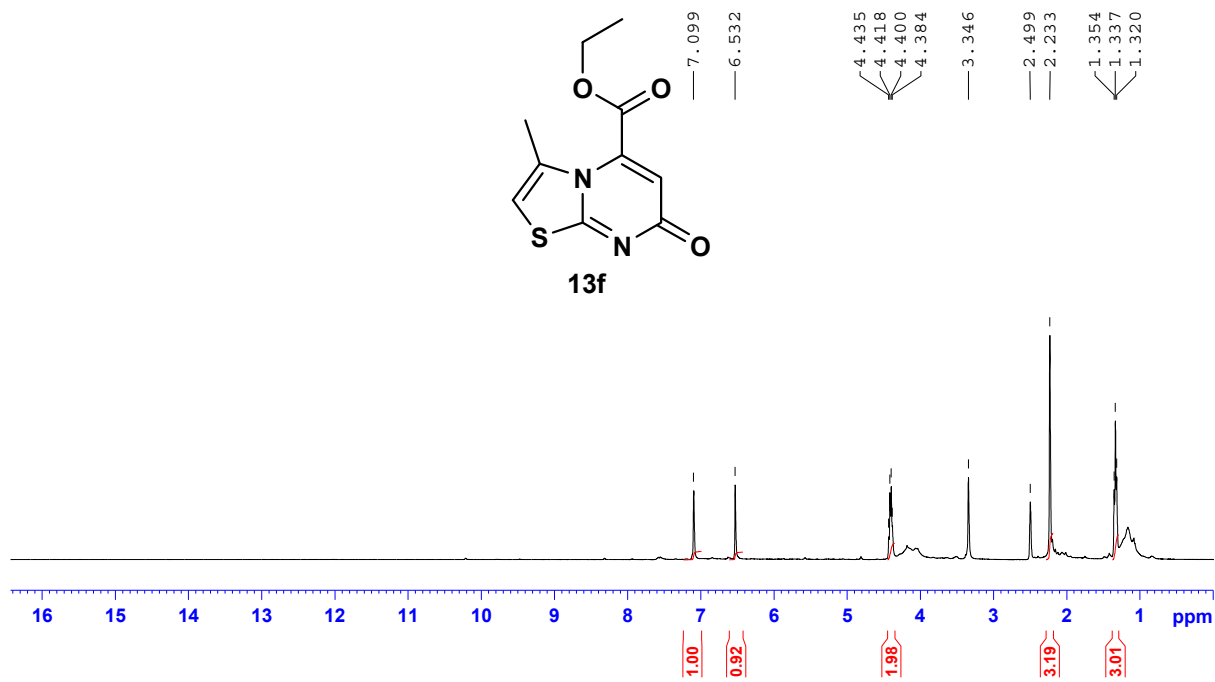


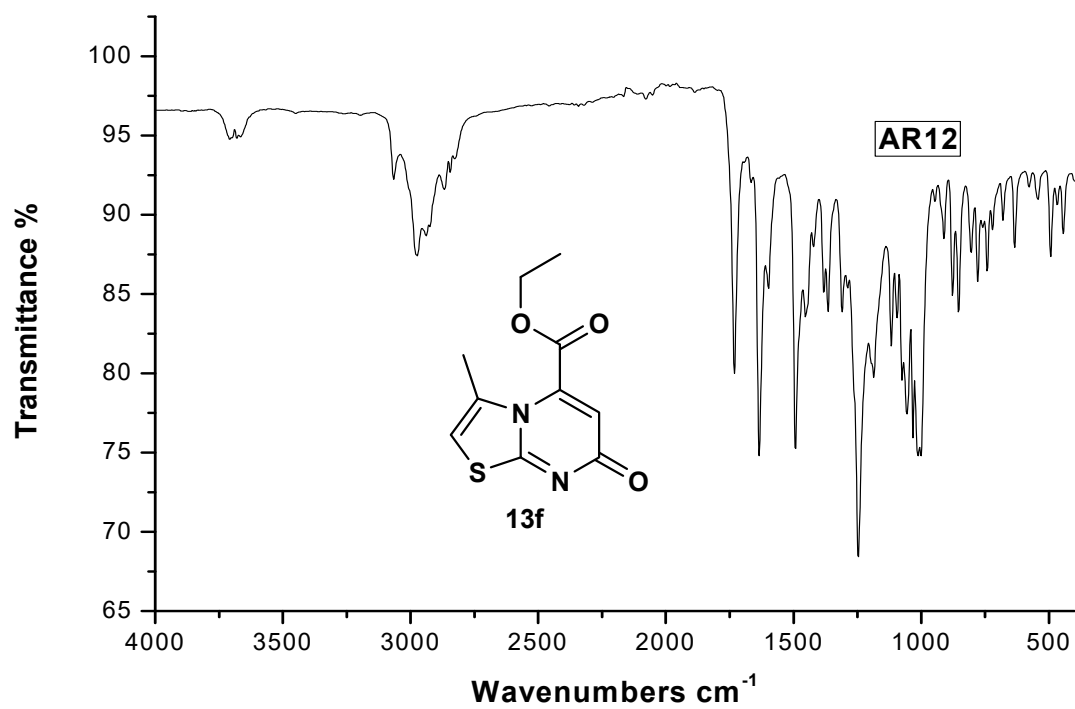
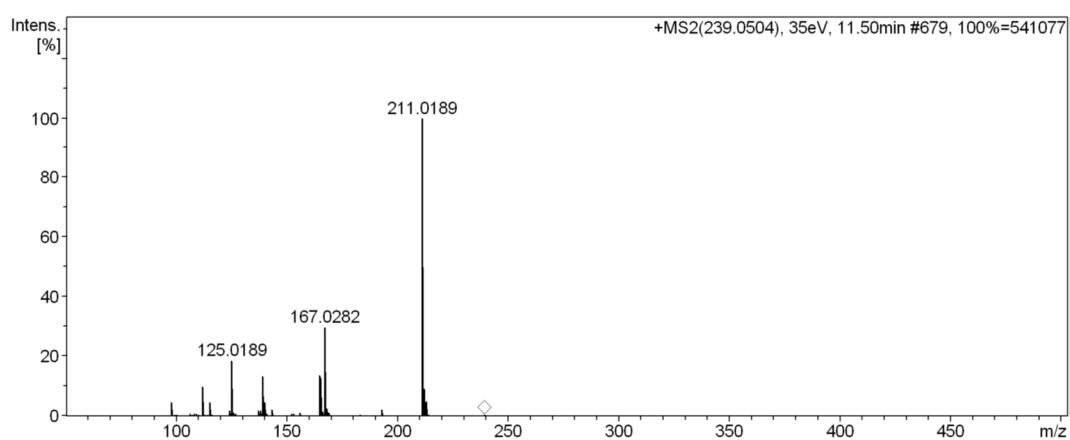
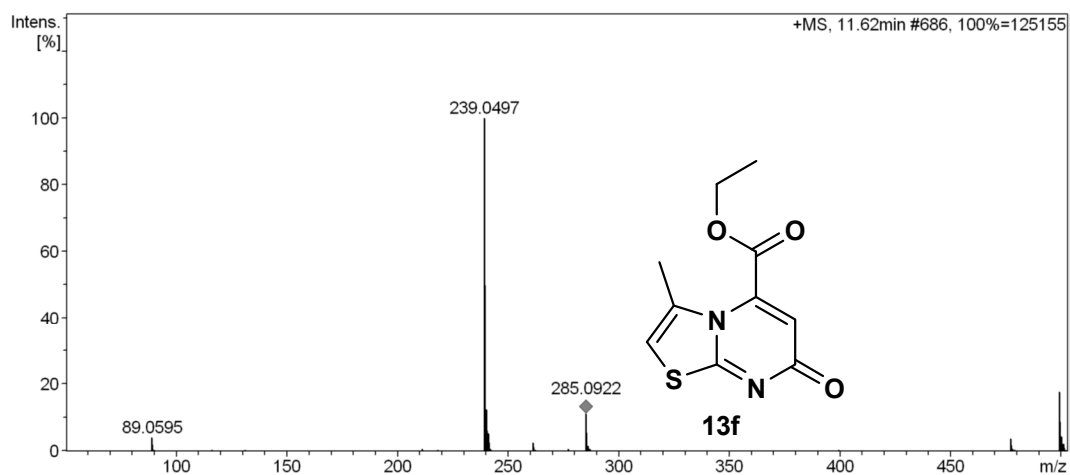




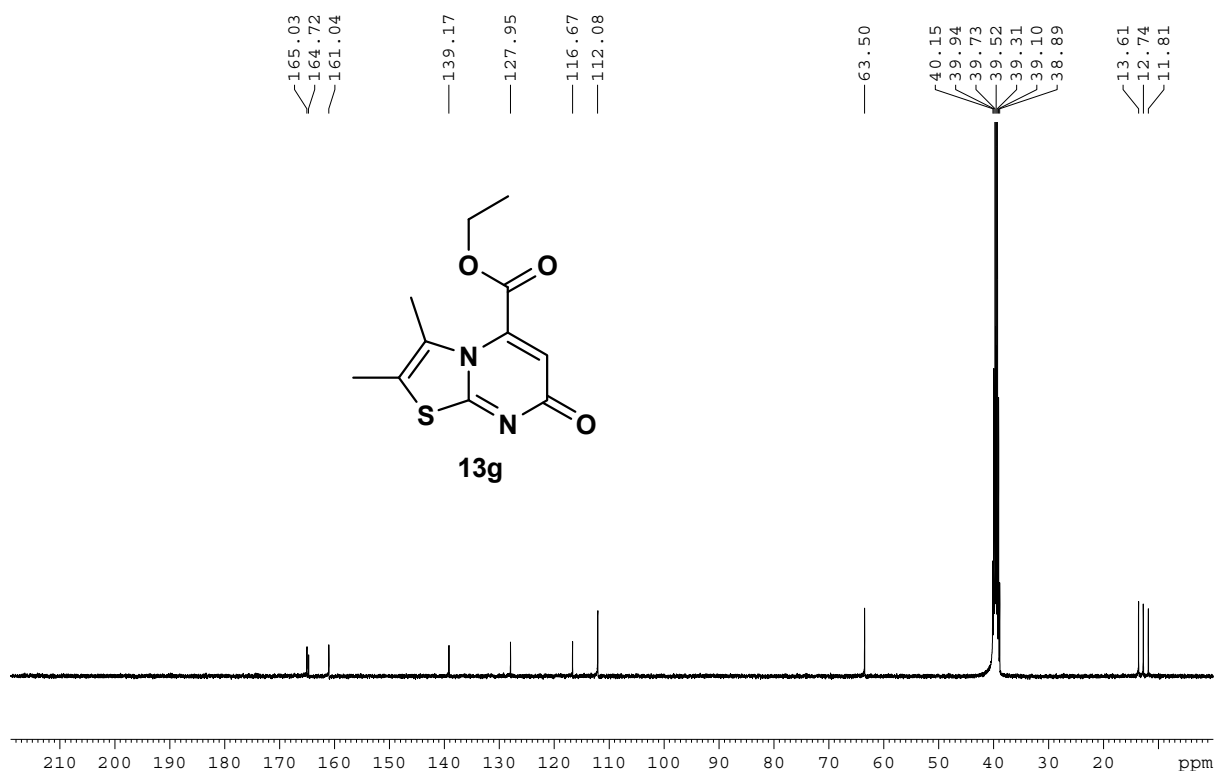
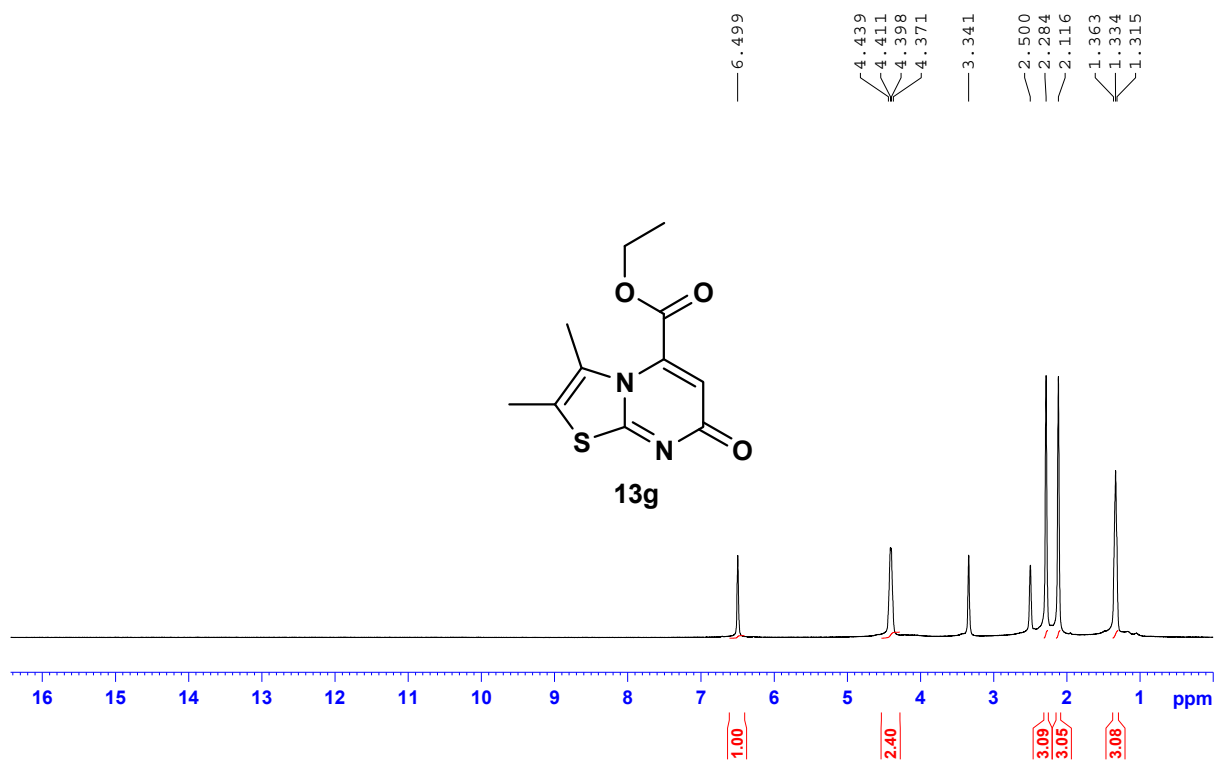


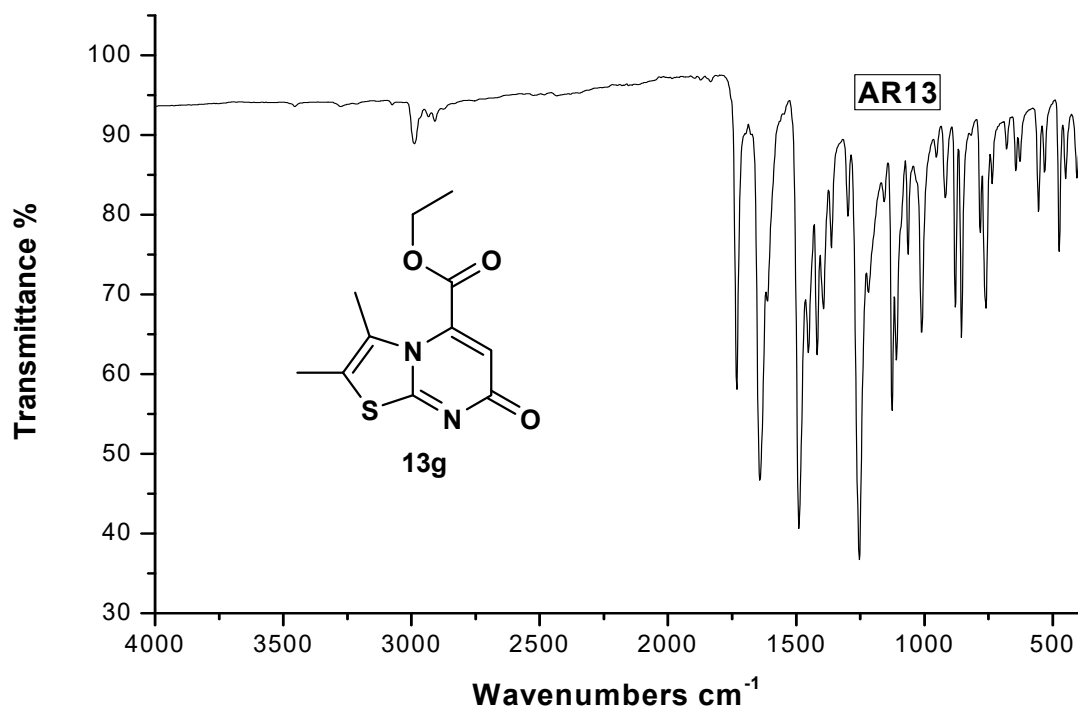
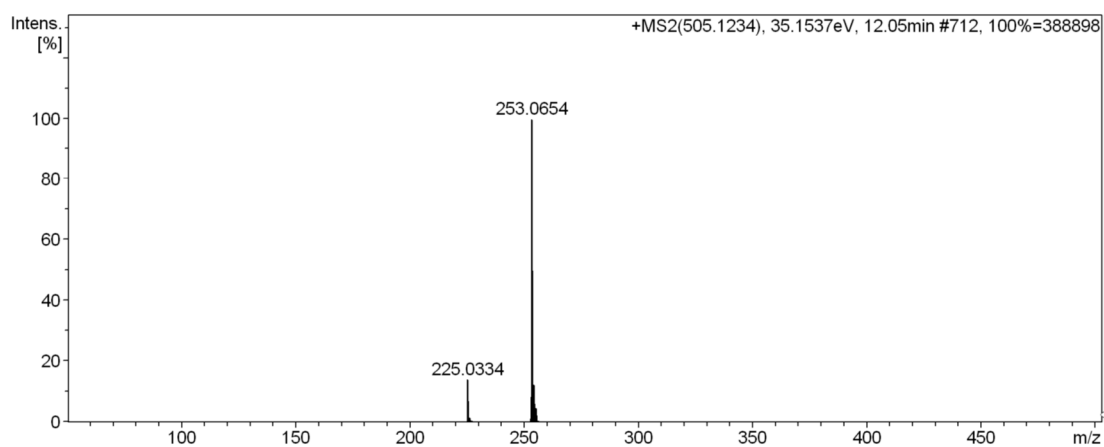
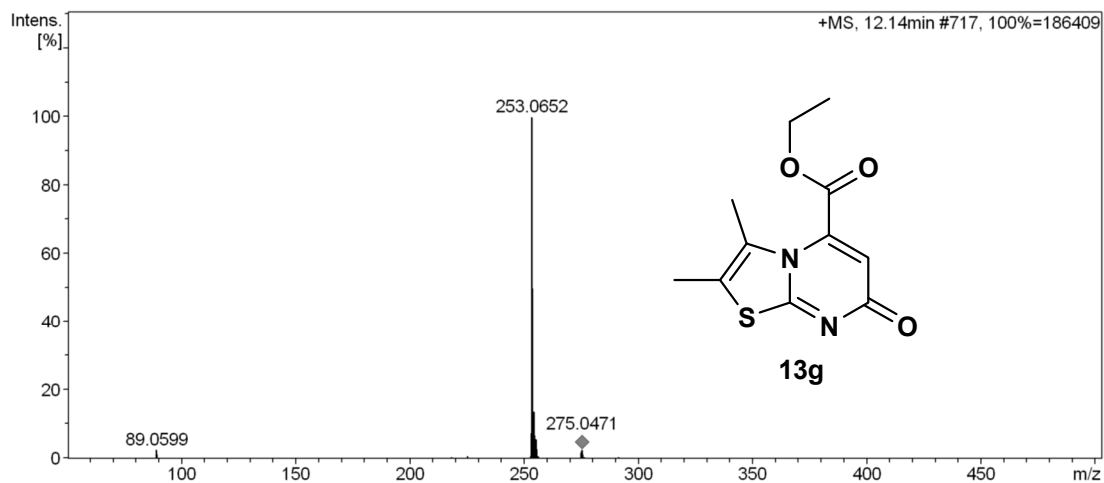


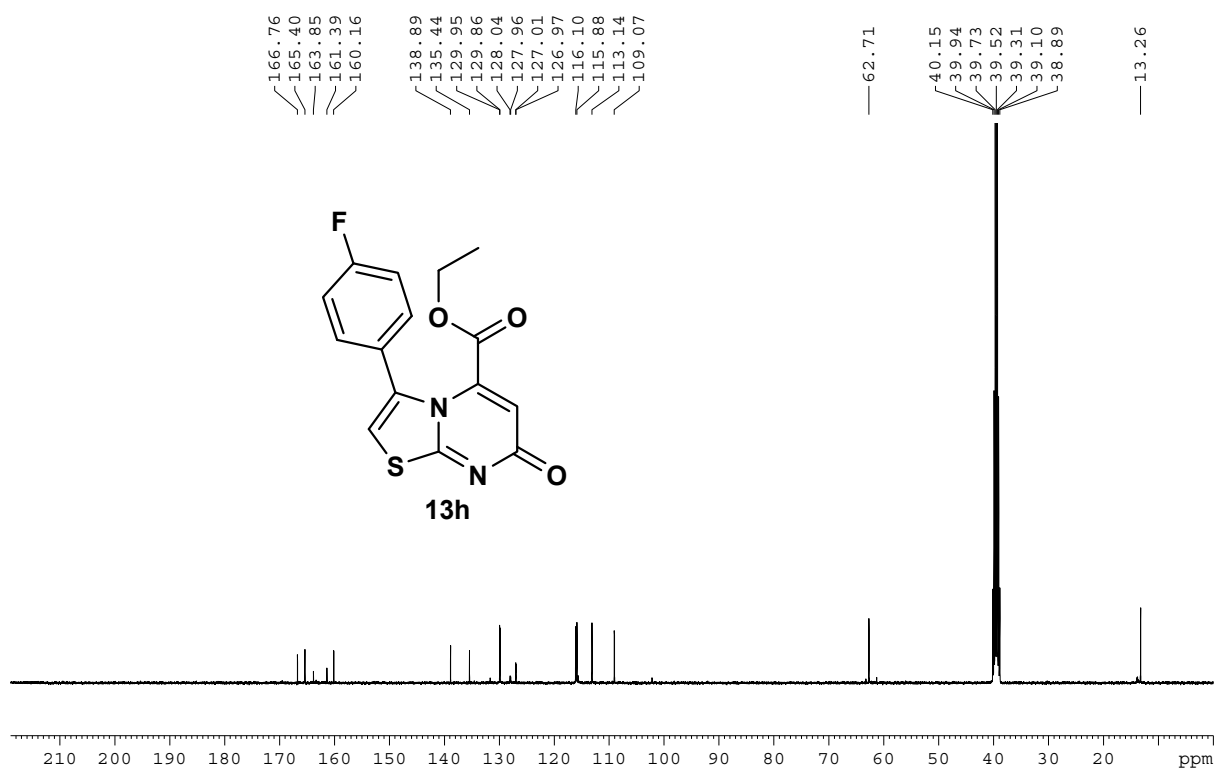
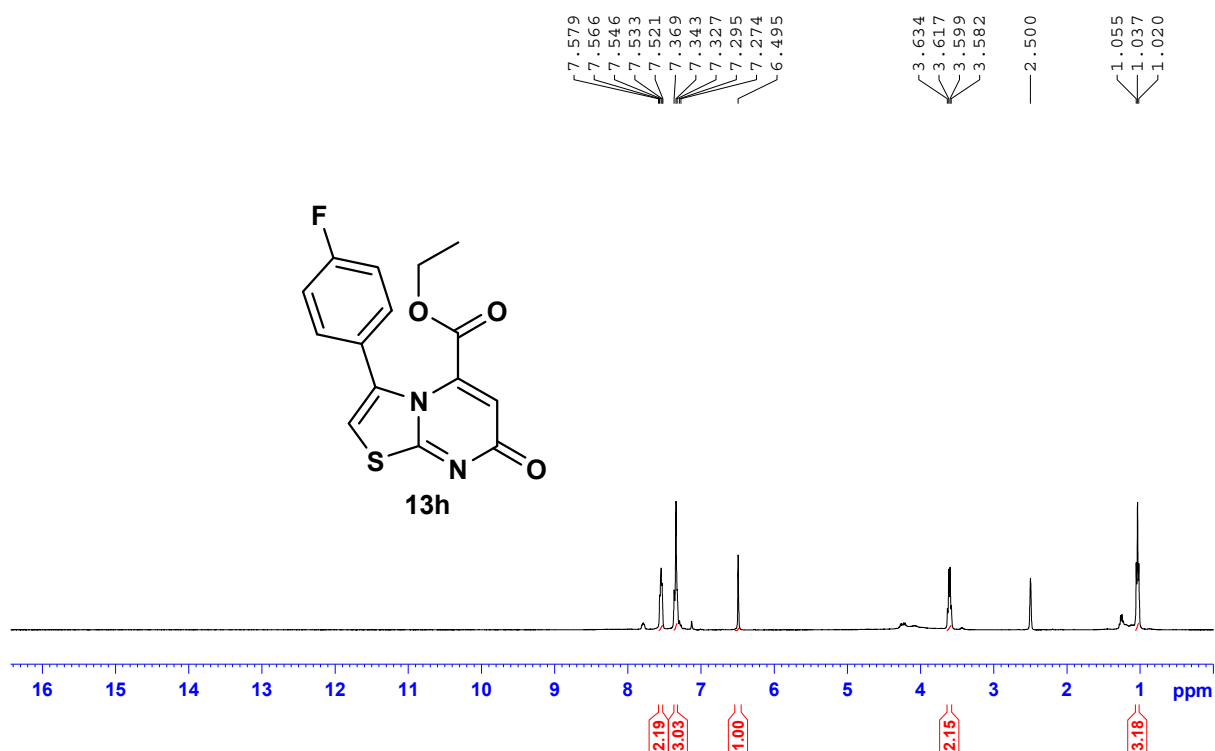


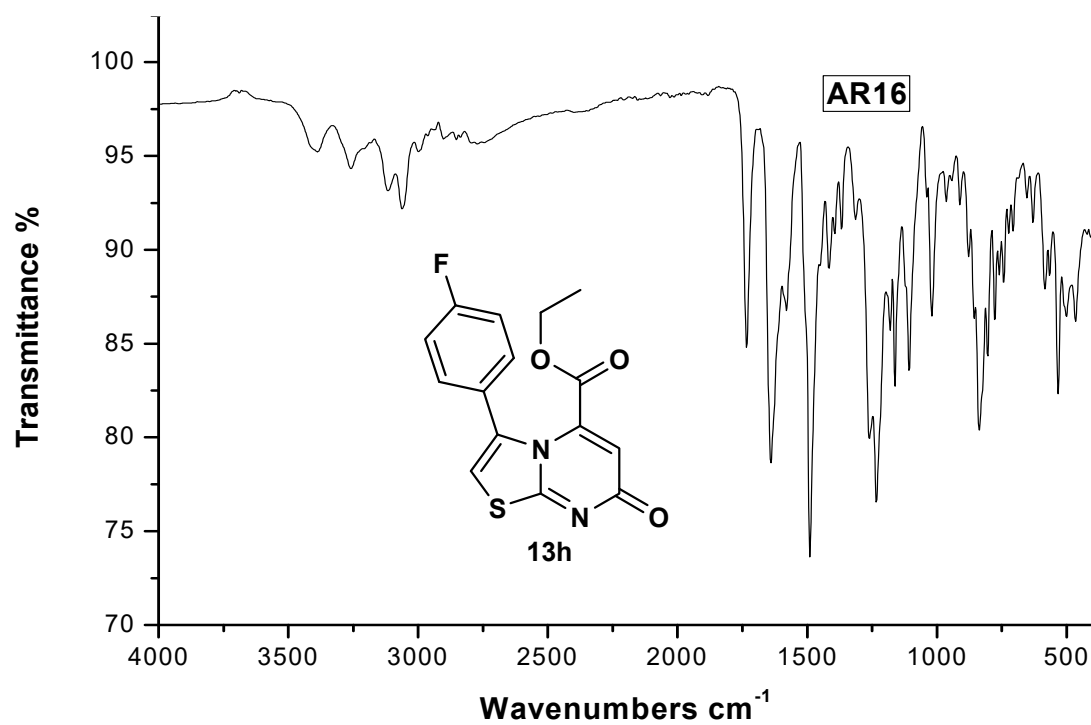
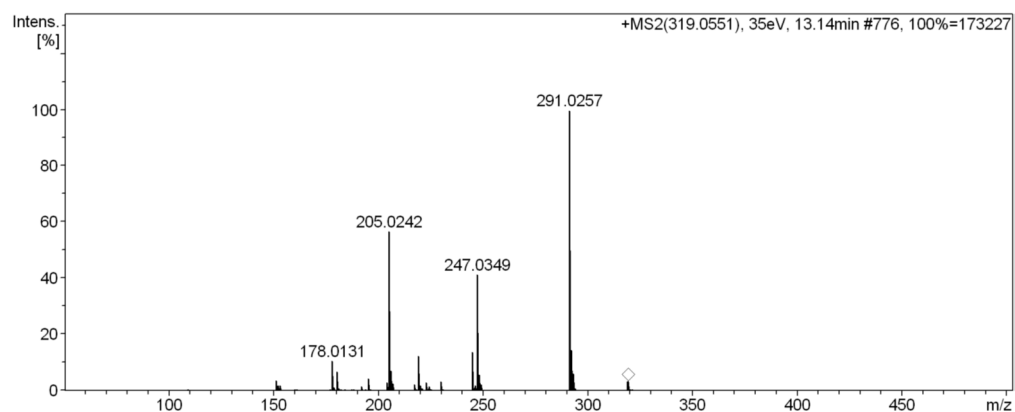
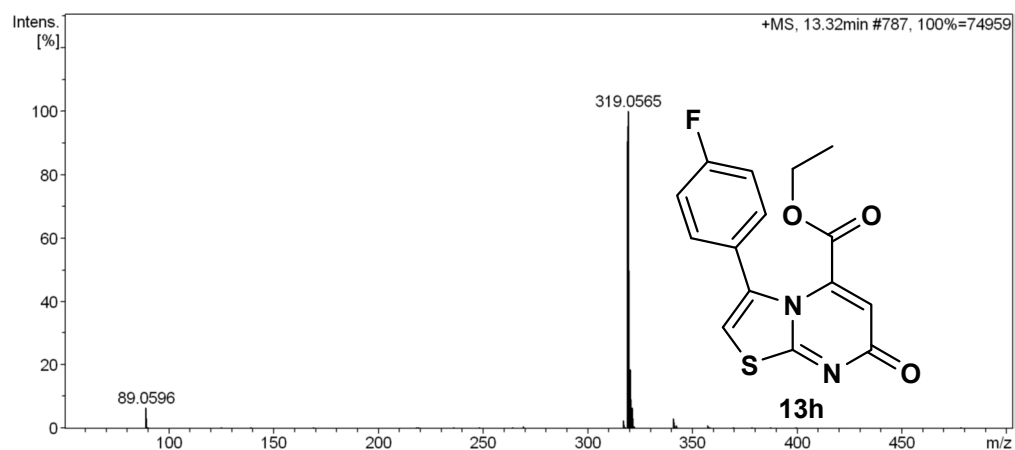


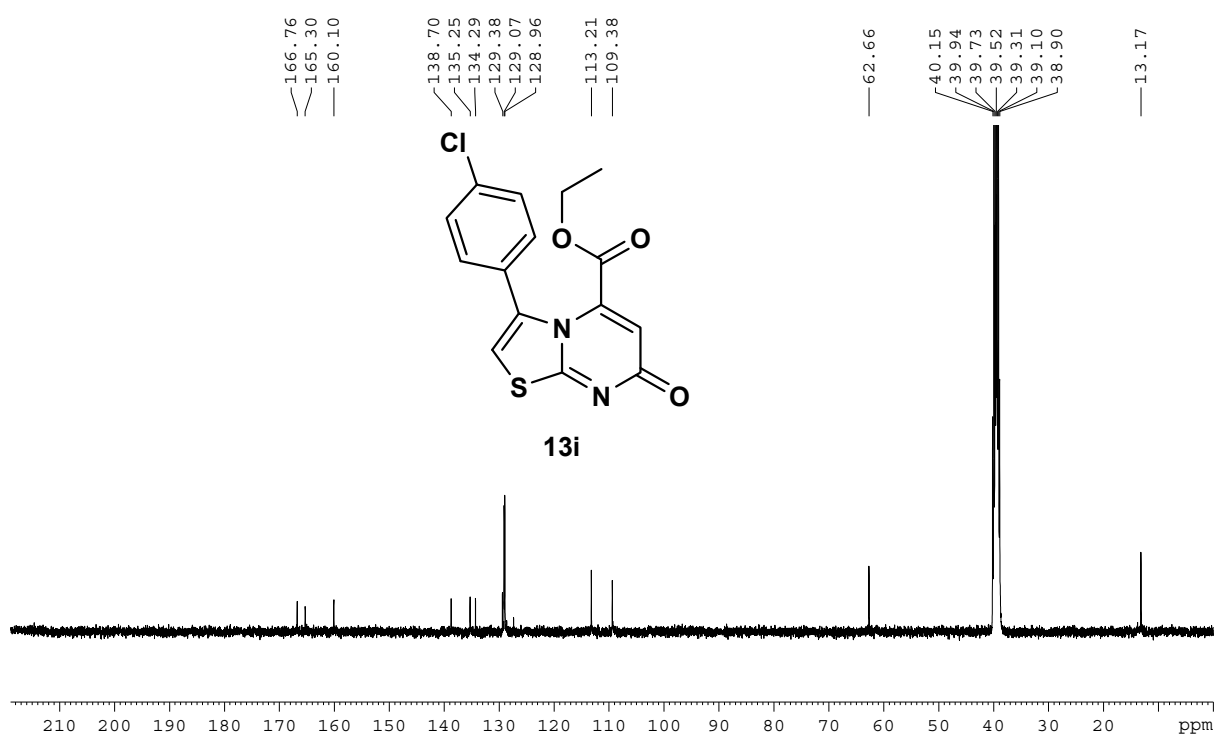
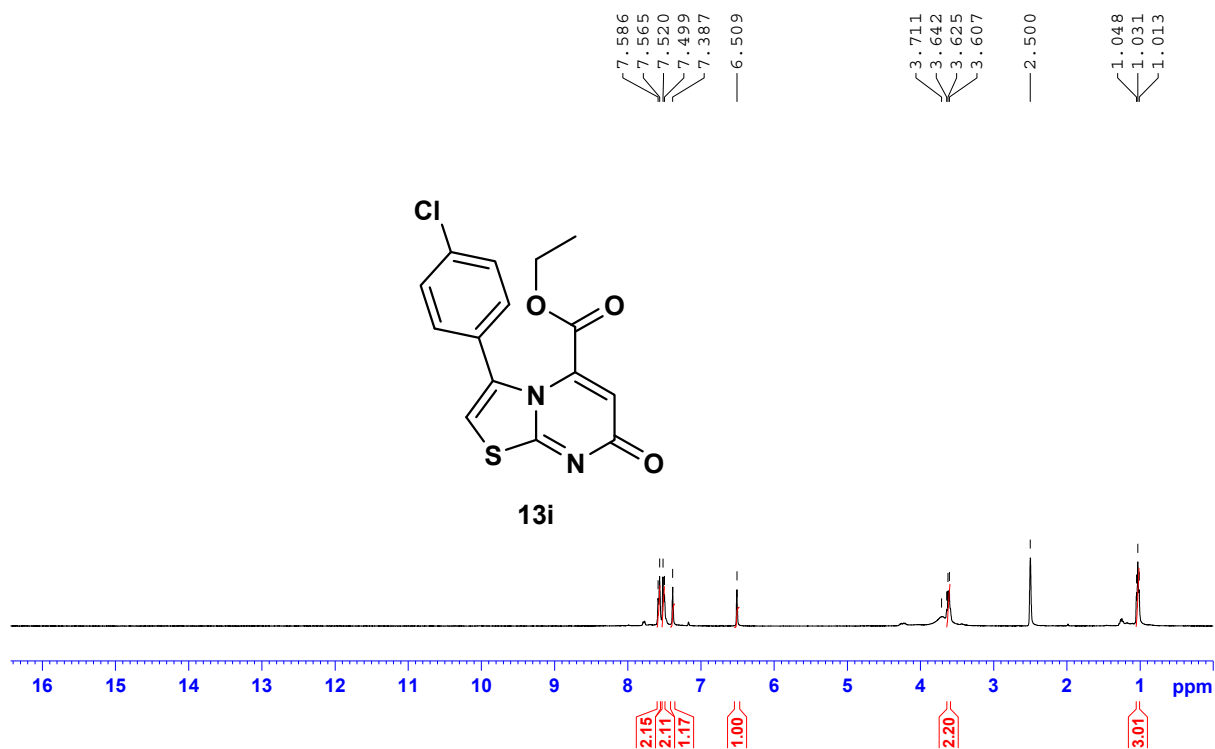


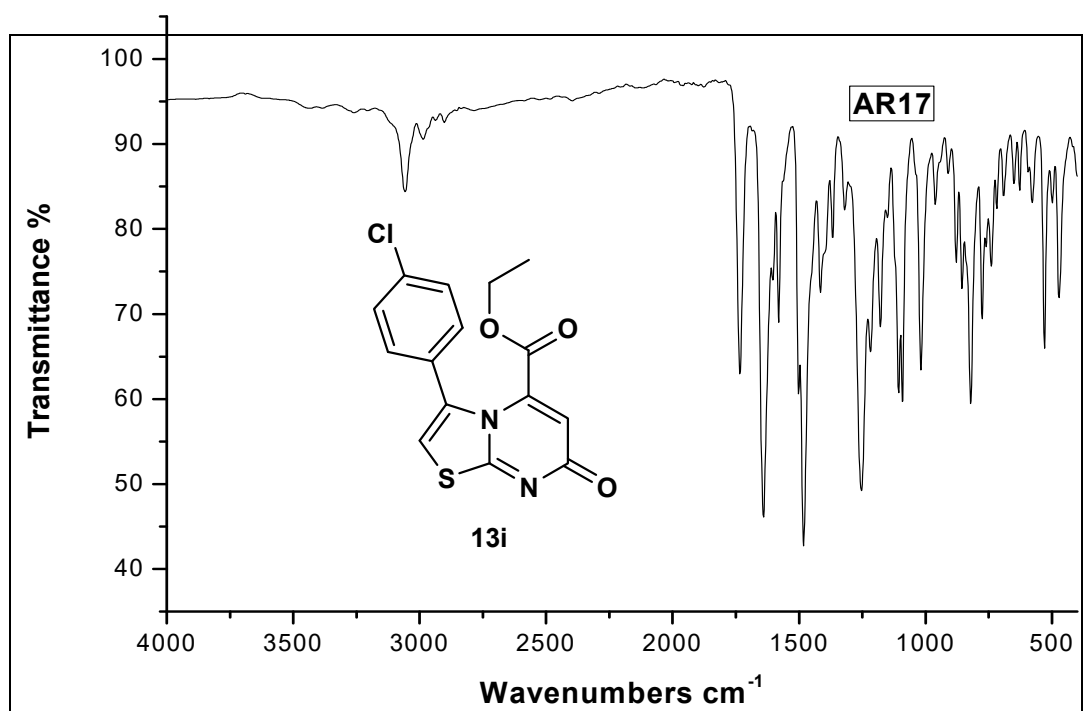
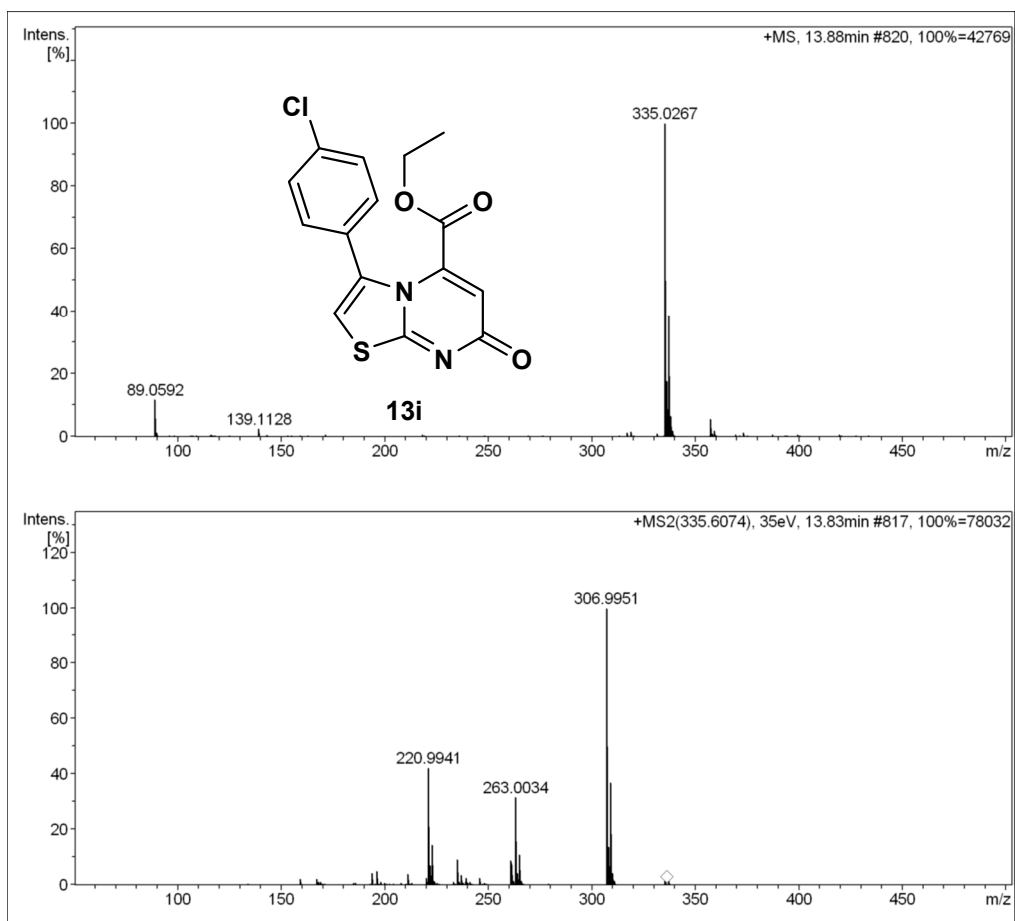


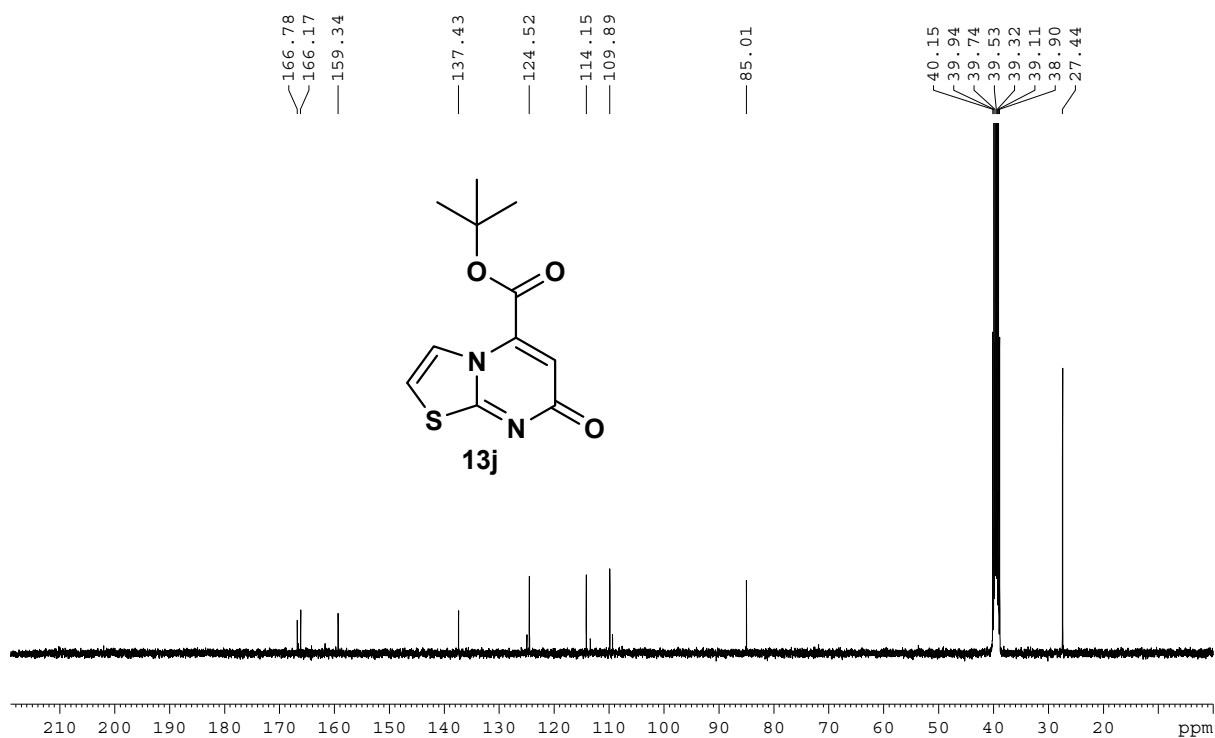
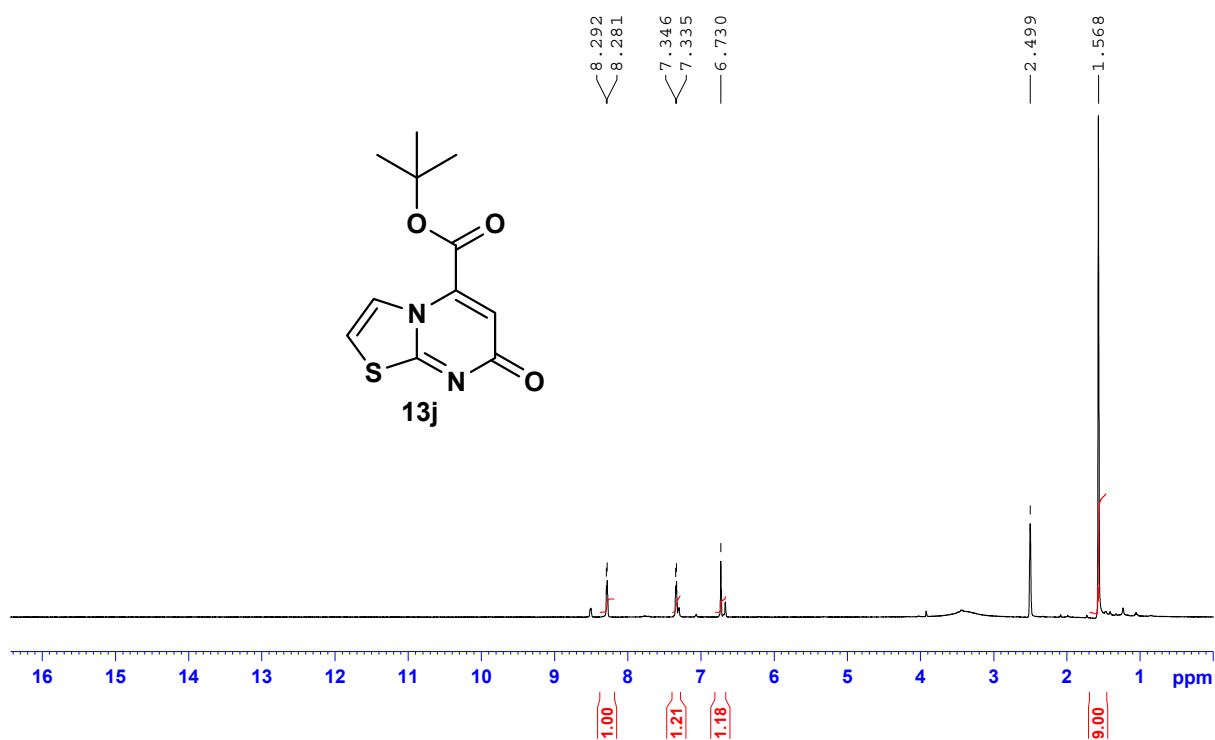


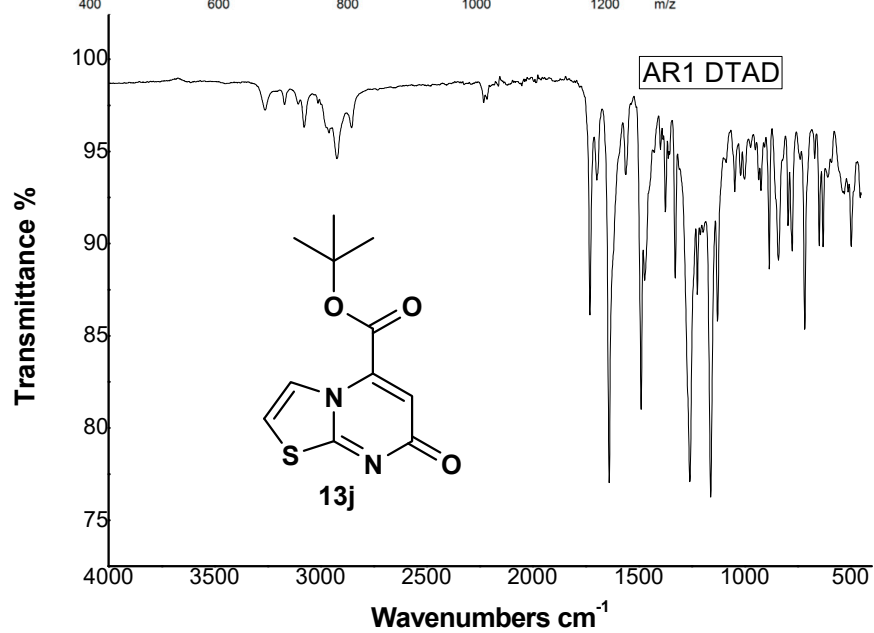
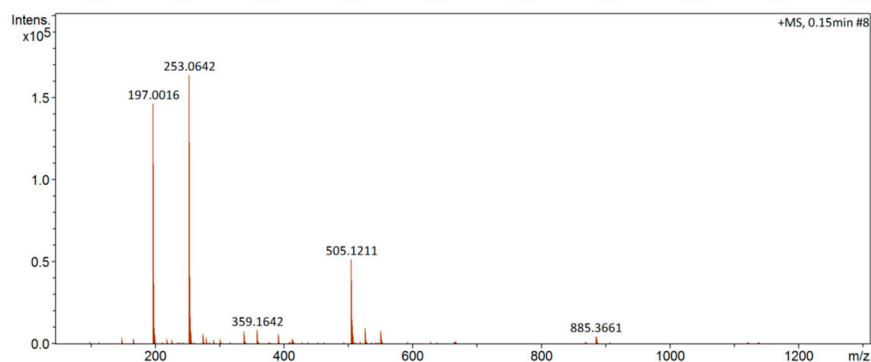
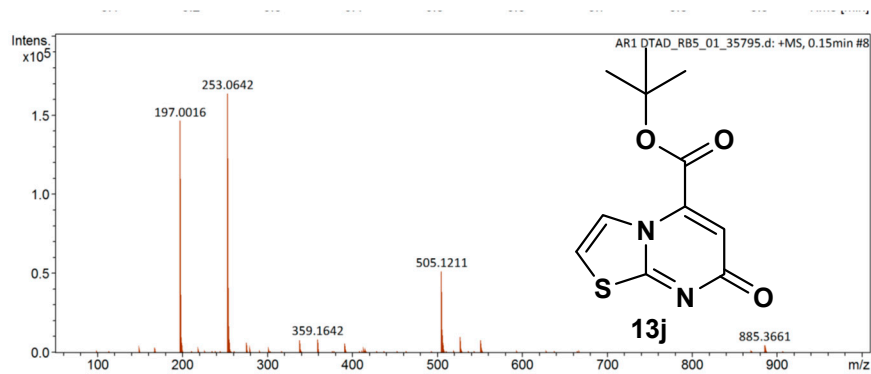




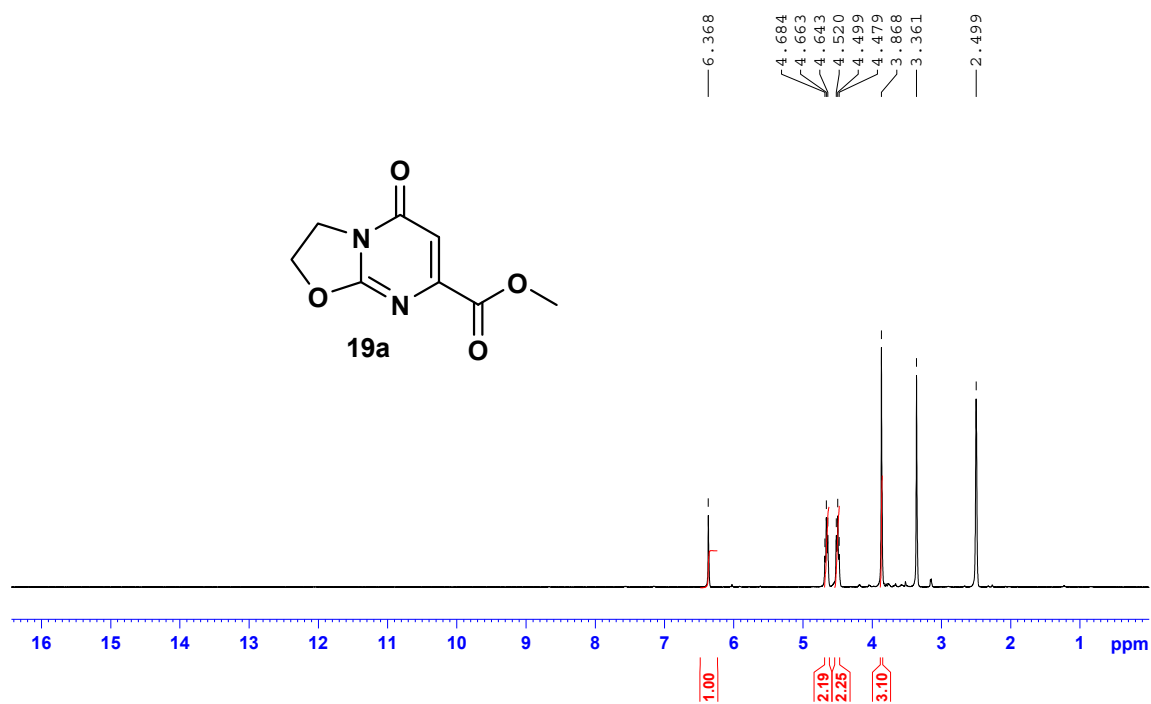
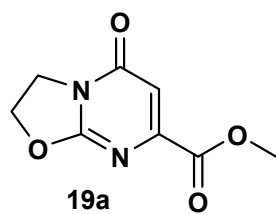


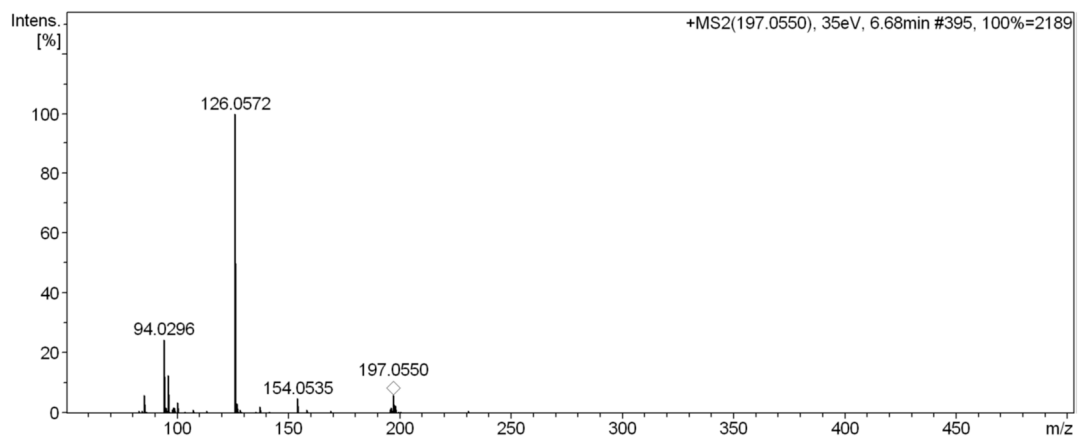
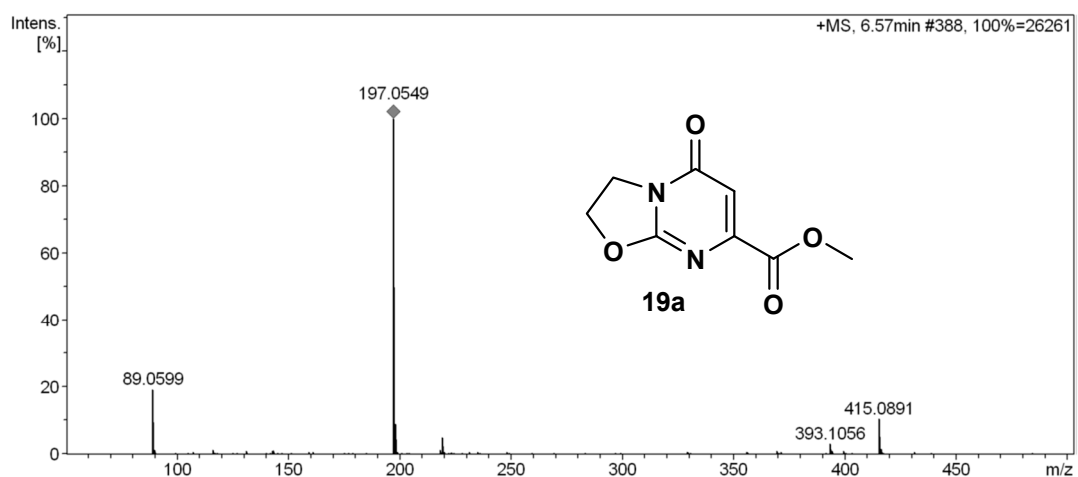
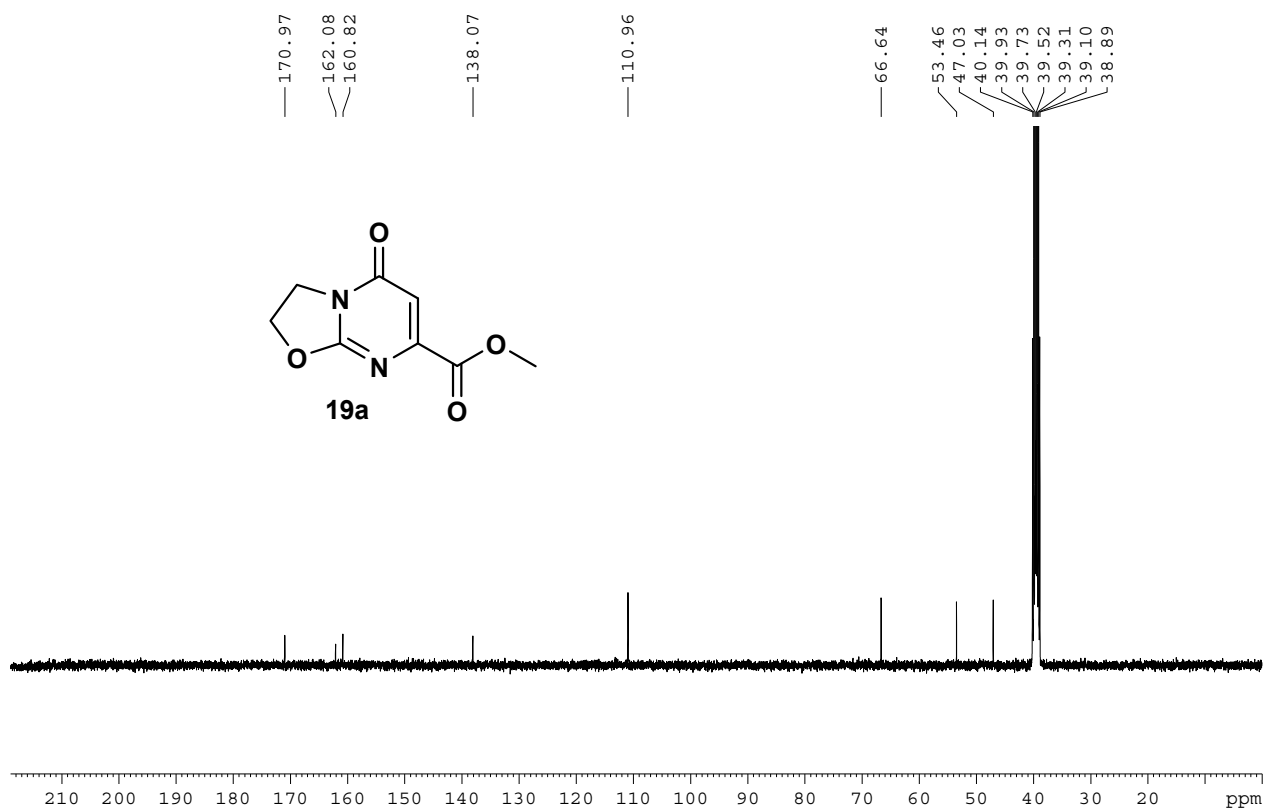


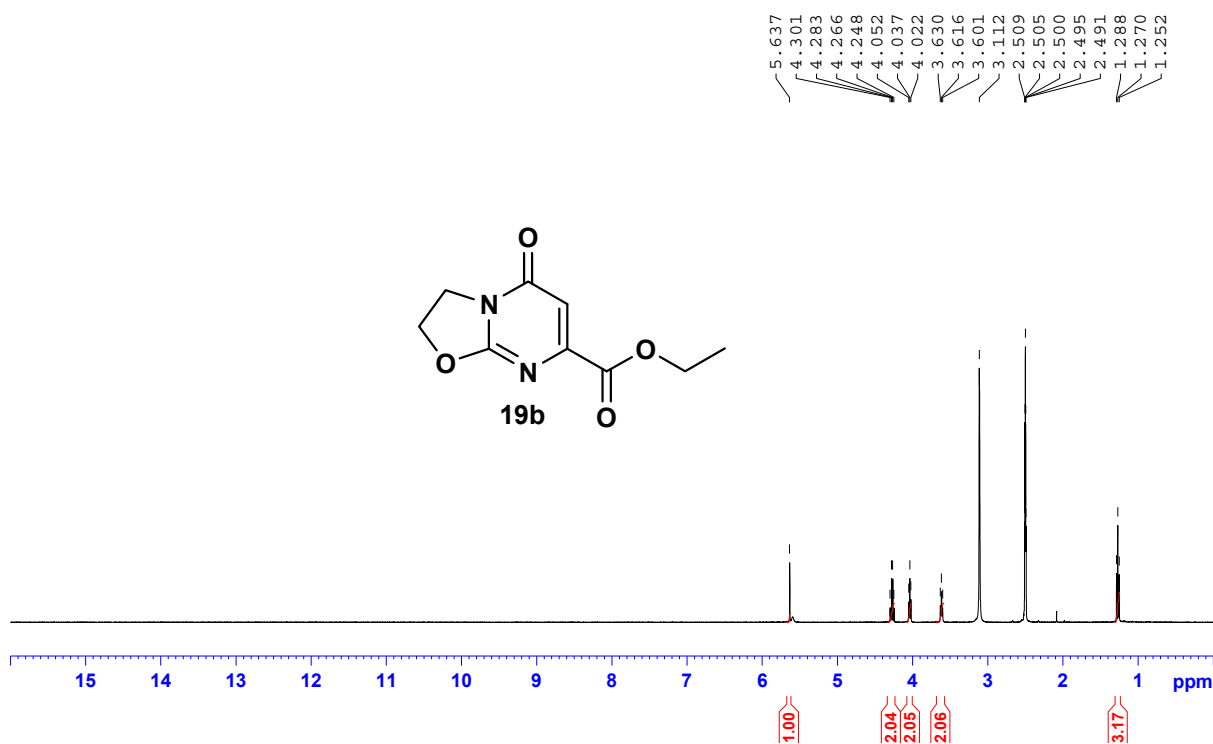
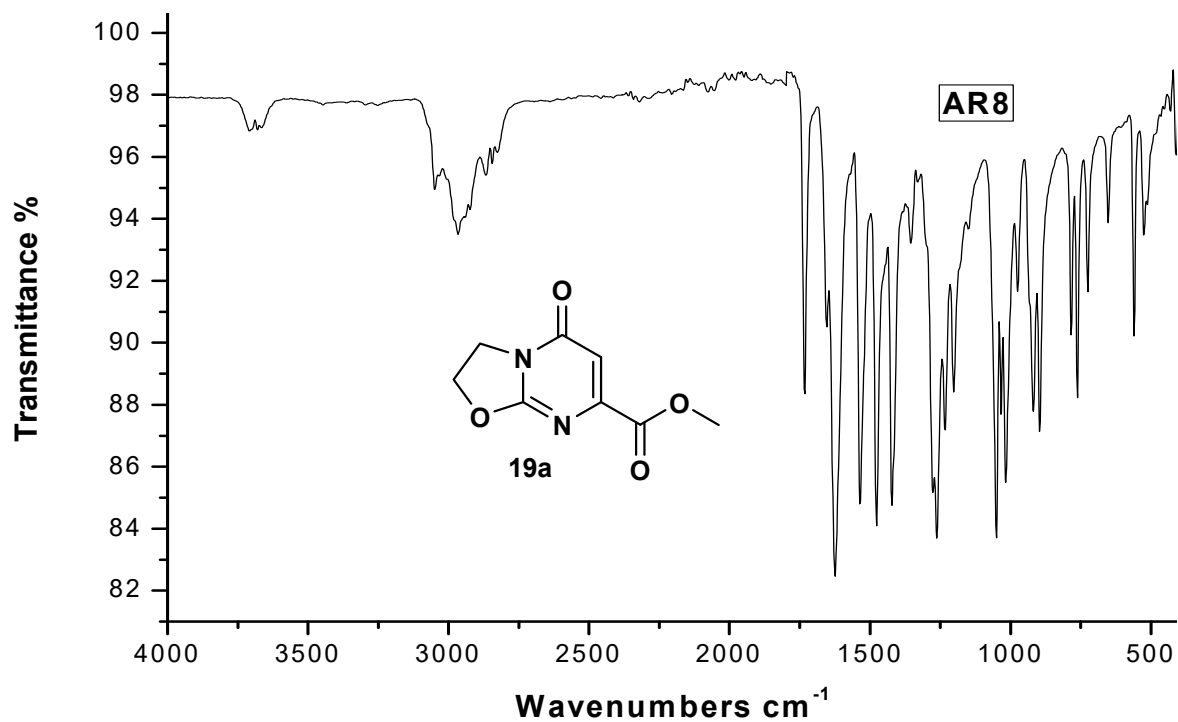


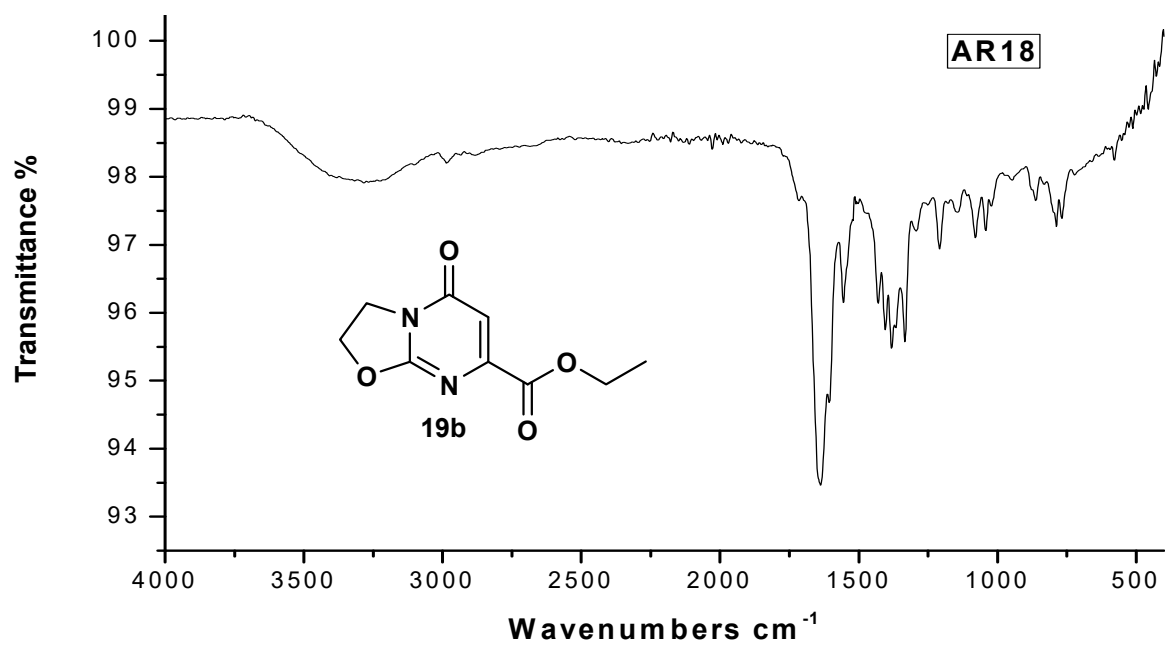
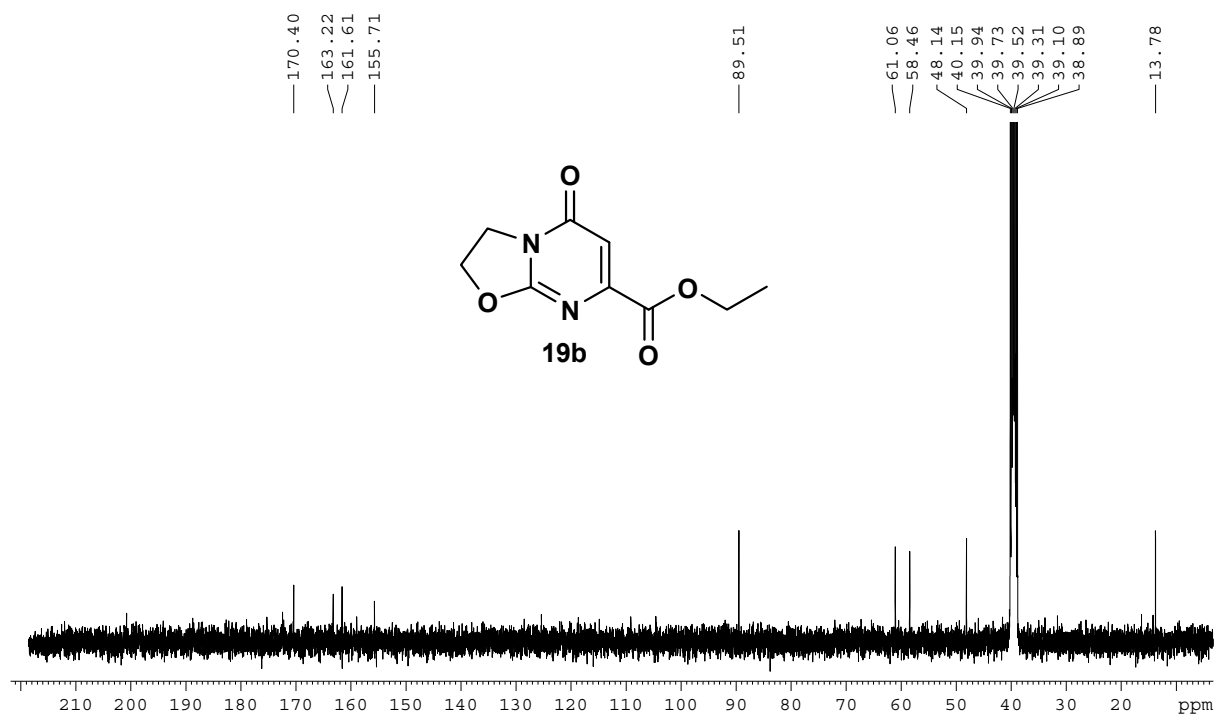


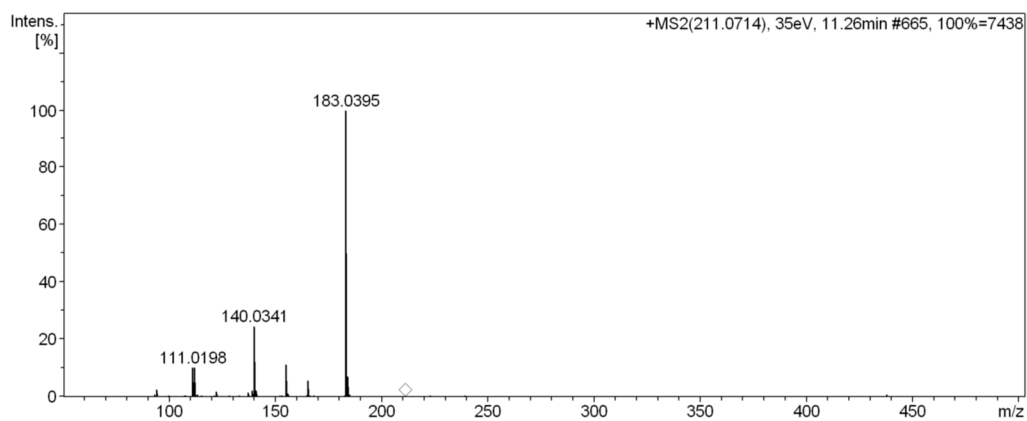
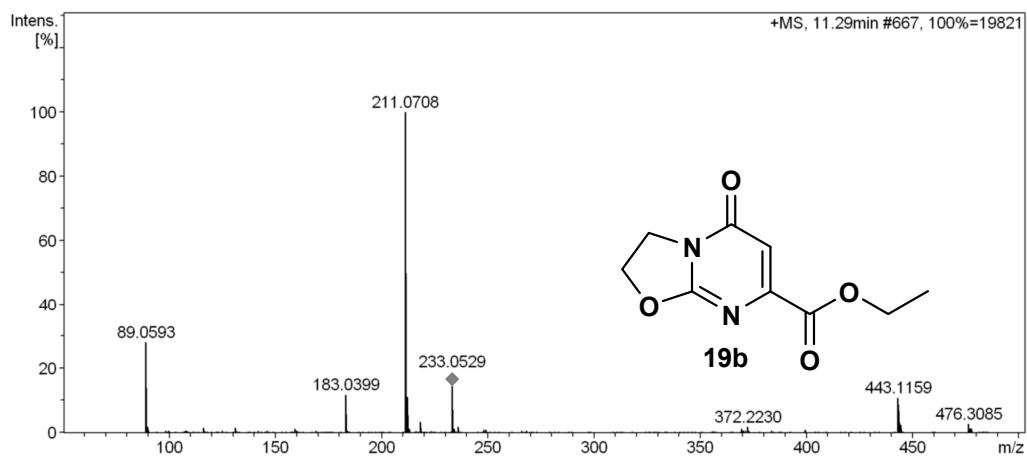












8.907

8.079

6.662

6.561

4.504

4.487

4.468

4.450

3.519

3.501

3.484

3.466

2.504

2.500

2.495

1.422

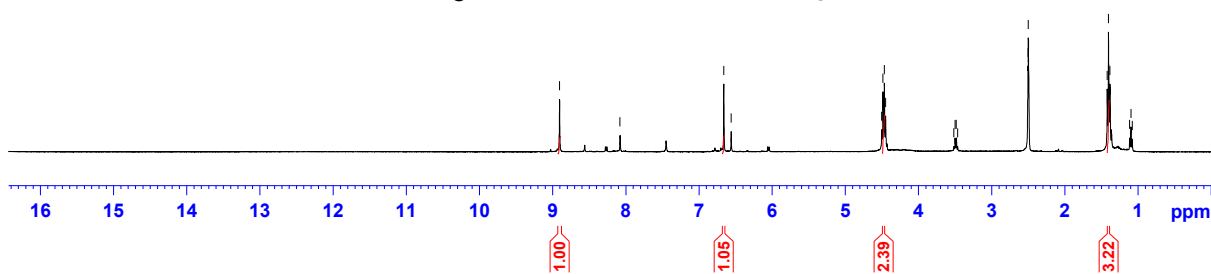
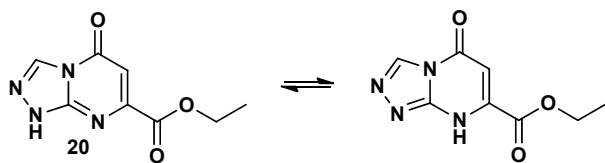
1.404

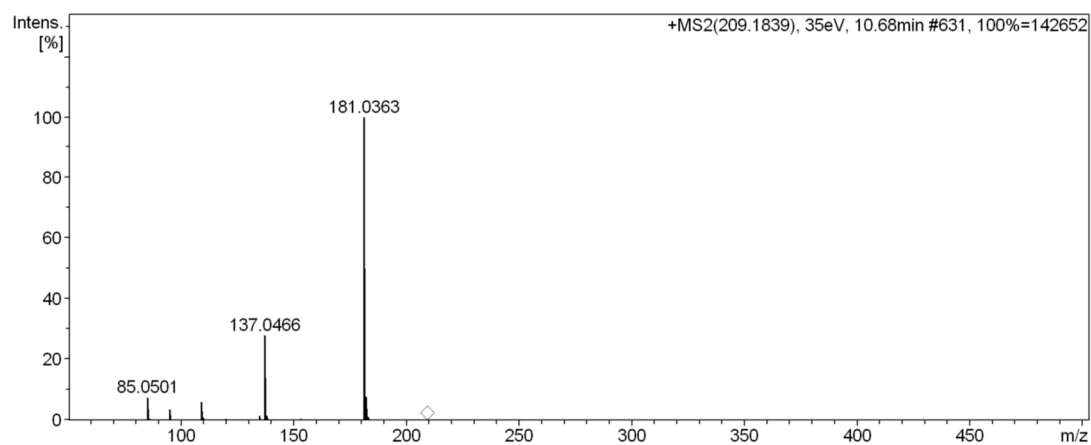
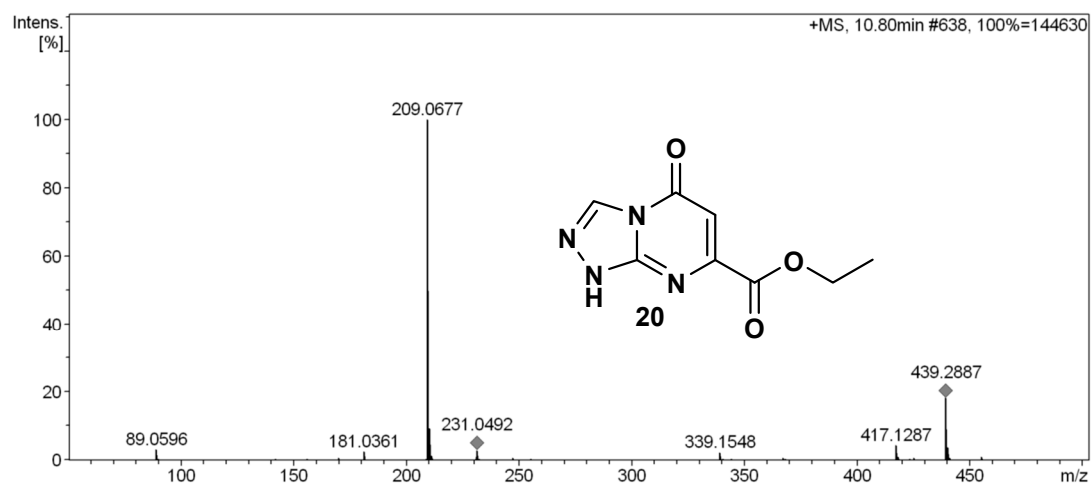
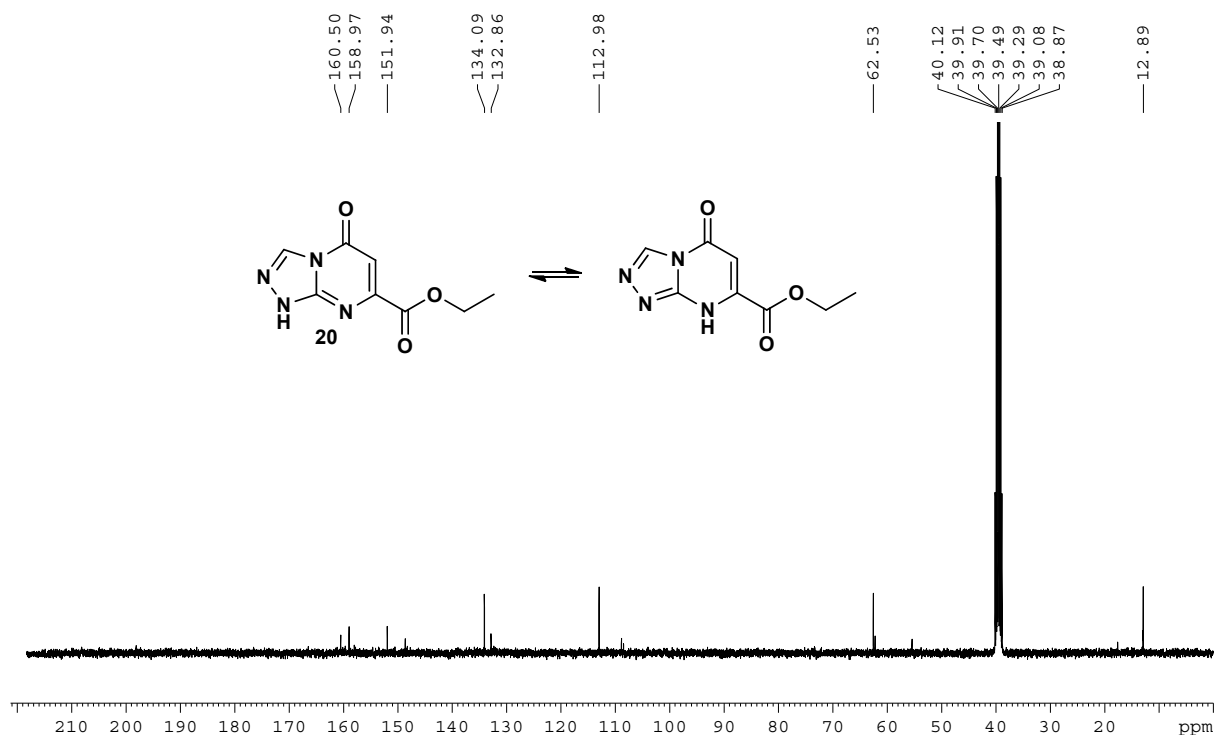
1.386

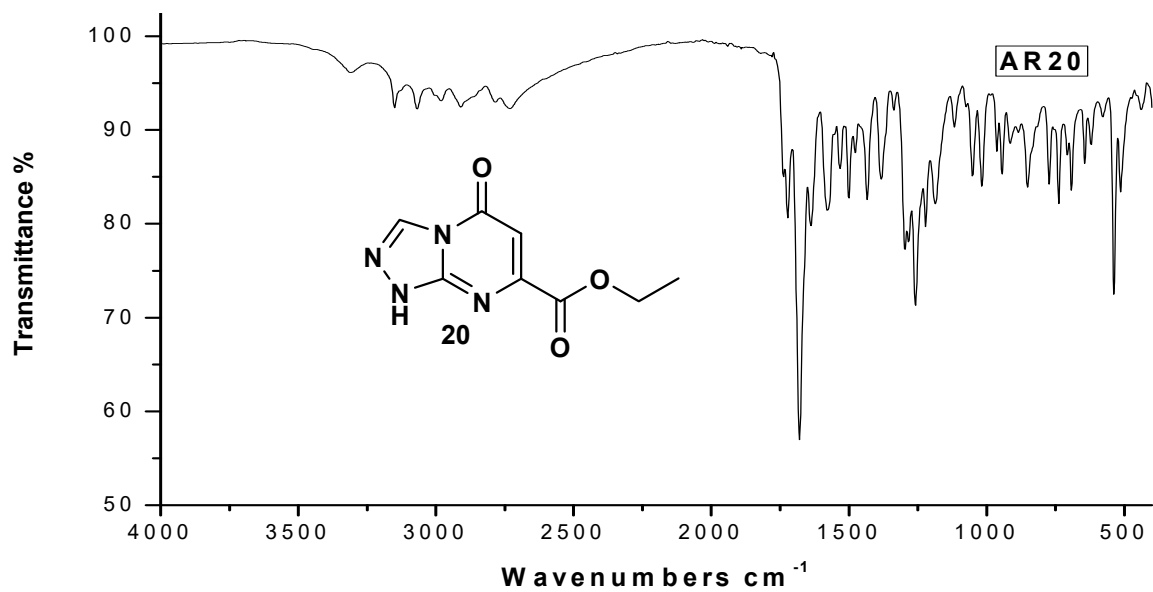
1.115

1.098

1.080







8.680

7.535

4.470

4.452

4.435

4.417

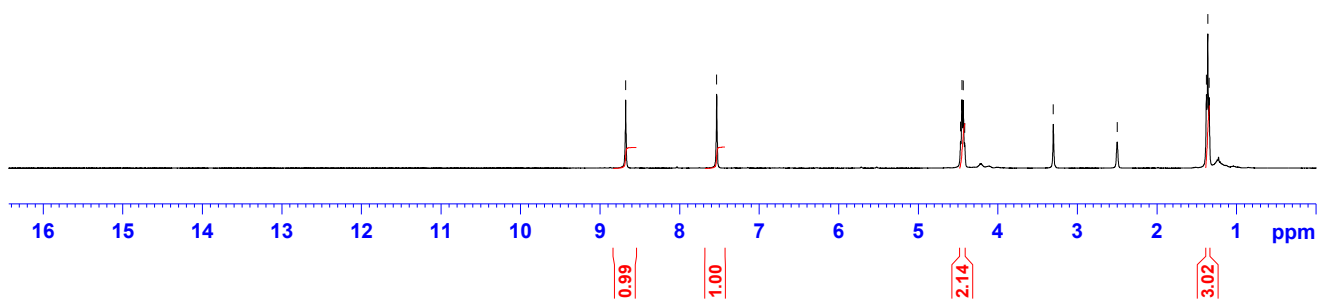
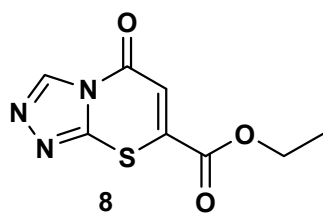
3.303

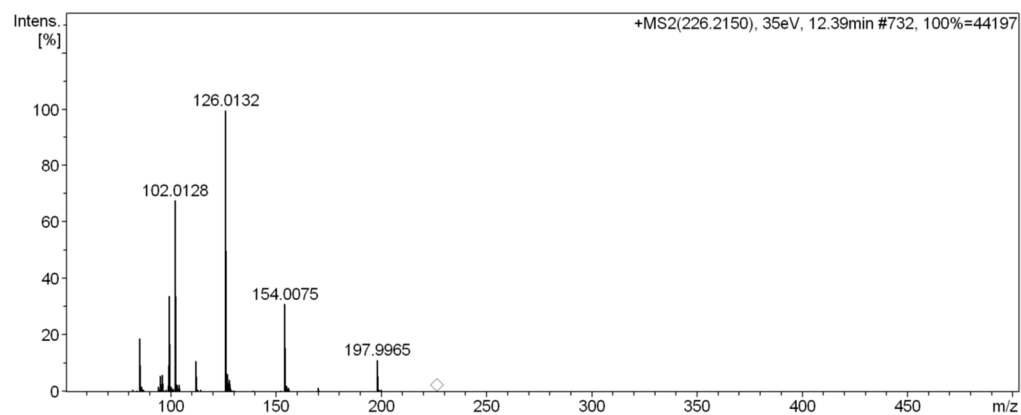
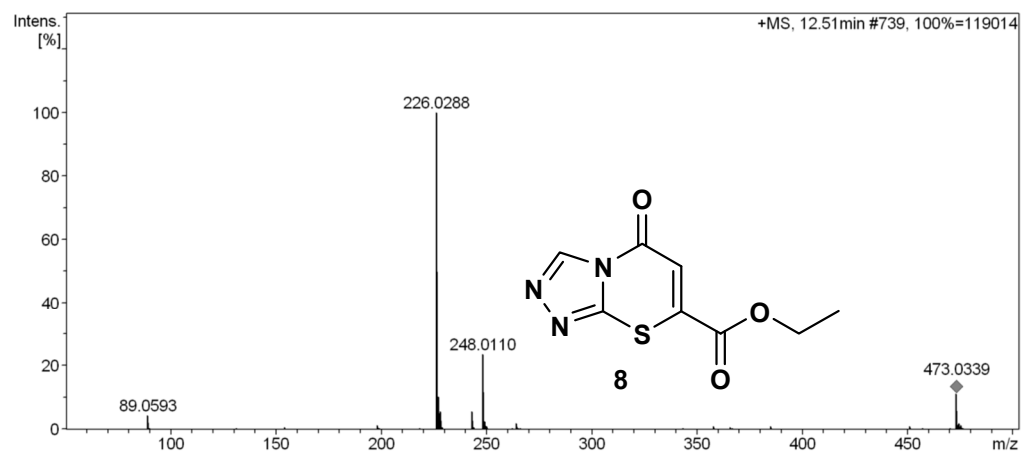
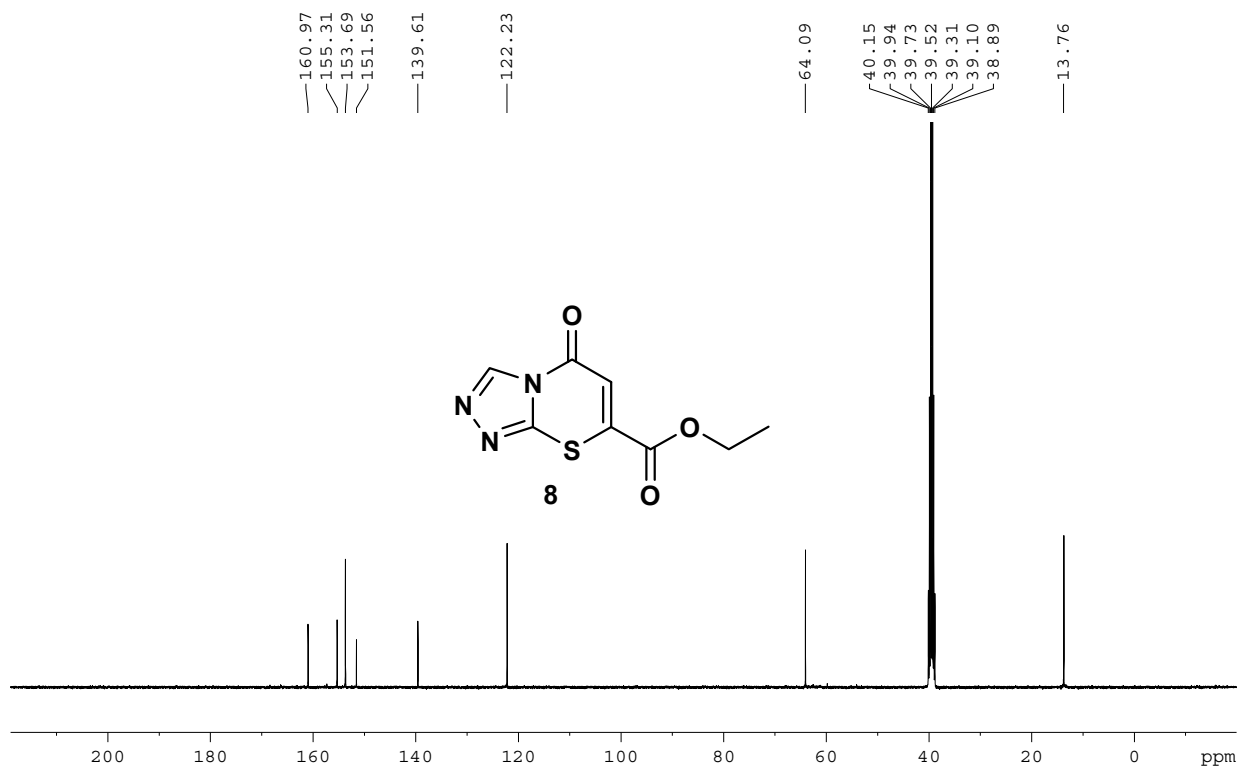
2.501

1.379

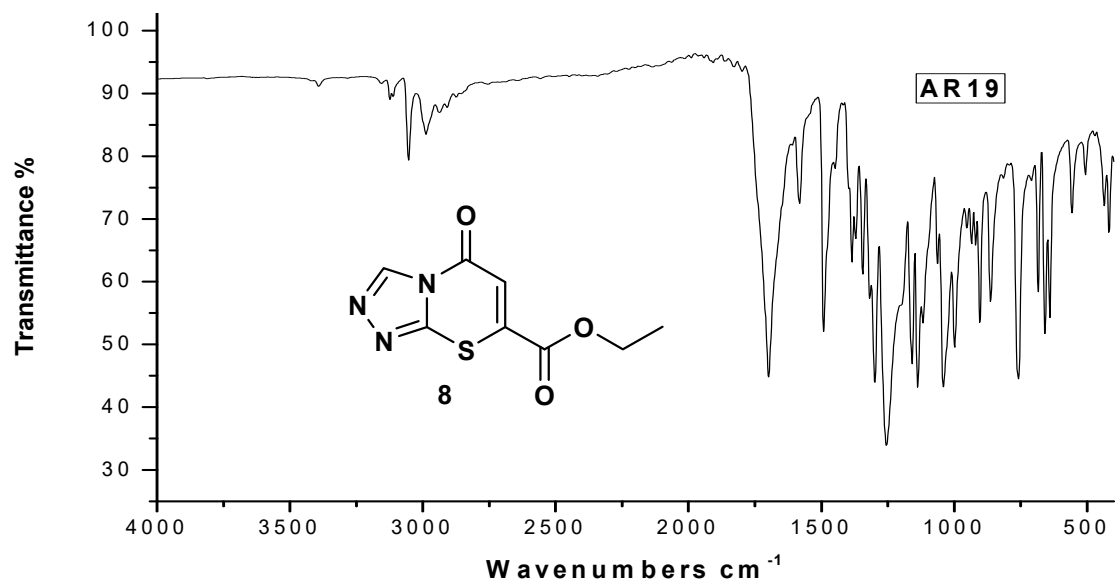
1.362

1.344









**Table S2.** Detailed mass spectrometry data of the synthesised compounds

Compound	Chemical Formula [M+1]	Calculated m/z	Measured m/z	Mass error Abs	Mass error (ppm)
10a	C <sub>15</sub> H <sub>20</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	354.1118	354.1113	-0.0005	-1.411
10b	C <sub>17</sub> H <sub>24</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	382.1431	382.1439	0.0008	2.093
10c	C <sub>16</sub> H <sub>22</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	368.1275	368.1279	0.0004	1.086
10d	C <sub>18</sub> H <sub>26</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	396.1588	396.1597	0.0009	2.272
10e	C <sub>20</sub> H <sub>30</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	424.1901	424.1906	0.0005	1.179
10f	C <sub>22</sub> H <sub>34</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	452.2214	452.2223	0.0009	1.990
10g	C <sub>18</sub> H <sub>24</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	394.1431	394.1433	0.0002	0.507
10h	C <sub>20</sub> H <sub>28</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	422.1744	422.1712	-0.0032	-7.579
10i	C <sub>17</sub> H <sub>24</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	382.1430	382.1427	-0.0003	-0.785
10j	C <sub>19</sub> H <sub>28</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	410.1744	410.1749	0.0005	1.218
10k	C <sub>21</sub> H <sub>32</sub> N <sub>3</sub> O <sub>5</sub> S	438.2057	438.2021	-0.0036	-8.215
10l	C <sub>23</sub> H <sub>36</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	466.2370	466.2333	-0.0037	-7.935
10m	C <sub>18</sub> H <sub>26</sub> N <sub>3</sub> O <sub>5</sub> S	396.1588	396.1545	-0.0043	-10.854
10n	C <sub>20</sub> H <sub>30</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	424.1901	424.1873	-0.0028	-6.600
10o	C <sub>22</sub> H <sub>34</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	452.2214	452.2190	0.0024	1.105
10p	C <sub>24</sub> H <sub>38</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	480.2527	480.2526	-0.0001	-0.208
10q	C <sub>21</sub> H <sub>24</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	430.1431	430.1445	0.0015	3.254
10r	C <sub>23</sub> H <sub>28</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	458.1744	458.1744	0.0000	0.000
10s	C <sub>25</sub> H <sub>32</sub> N <sub>3</sub> O <sub>5</sub> S <sup>+</sup>	486.2057	486.2044	-0.0013	-2.673

<b>10t</b>	$C_{27}H_{36}N_3O_5S^+$	514.2370	514.2350	-0.0020	-3.889
<b>10u</b>	$C_{23}H_{26}N_3O_5S^+$	456.1588	456.1592	0.0004	0.876
<b>13a</b>	$C_{10}H_{11}N_2O_3S^+$	239.0485	239.0486	0.0001	0.418
<b>13b</b>	$C_{15}H_{13}N_2O_3S^+$	301.0641	301.0648.	0.0007	2.325
<b>13c</b>	$C_{14}H_{10}FN_2O_3S^+$	305.0391	305.0391	0.0000	0.000
<b>13d</b>	$C_{14}H_{10}ClN_2O_3S^+$	321.0095	321.0087	-0.0008	-2.492
<b>13e</b>	$C_9H_9N_2O_3S^+ +$	225.0328	225.0339	0.0011	4.888
<b>13f</b>	$C_{10}H_{11}N_2O_3S^+$	239.0485	239.0497	0.0012	5.019
<b>13g</b>	$C_{11}H_{13}N_2O_3S^+$	253.0641	253.0652	0.0011	4.346
<b>13h</b>	$C_{15}H_{12}FN_2O_3S^+$	319.0547	319.0565.	0.0018	5.641
<b>13i</b>	$C_{15}H_{12}ClN_2O_3S^+$	335.0252	335.0267	0.0015	4.477
<b>13j</b>	$C_{11}H_{13}N_2O_3S^+$	253.0641	253.0642	0.0001	0.395
<b>19a</b>	$C_8H_9N_2O_4^+$	197.0557	197.0549	-0.0008	-4.059
<b>19b</b>	$C_9H_{11}N_2O_4^+$	211.0713	211.0708	-0.0005	-2.368
<b>20</b>	$C_8H_9N_4O_3^+$	209.0669	209.0677	0.0008	3.826
<b>5</b>	$C_8H_8N_3O_3S^+$	226.0281	226.0288.	0.0007	3.096

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