

Morpholinated curcuminoids against urinary bladder cancer cells: synthesis and anticancer evaluation

Pawel Bakun^{1, 2*}, Malgorzata Kucinska³, Paulina Kobylka^{2, 3}, Joanna Kuźmińska^{2, 4}, Tomasz Koczorowski¹, Dariusz T. Mlynarczyk¹, Lukasz Popenda⁵, Katarzyna Górka³, Malgorzata Kasperkowiak⁶, Marek Murias³, Anna Jelińska⁴, Tomasz Goslinski^{1*}

¹ Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

² Doctoral School, Poznan University of Medical Sciences, Bukowska 70, 60-812 Poznan, Poland

³ Chair and Department of Toxicology, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

⁴ Chair and Department of Pharmaceutical Technology, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland

⁵ NanoBioMedical Centre, Adam Mickiewicz University, Wszechnicy Piastowskiej 3, 61-614 Poznan, Poland

⁶ Centre for Advanced Technologies, Adam Mickiewicz University, Uniwersytetu Poznańskiego 10, 61-614 Poznan, Poland

*Correspondence: pbakun@ump.edu.pl (P.B.), tomasz.goslinski@ump.edu.pl (T.G.)

Supplementary Information

Table of contents:

1. <i>In silico</i> analysis	3
2. Experimental	7
Compound 2a-B, Morpholinethoxycurcumin-BF ₂	7
Compound 2a, Morpholinethoxycurcumin	9
Compound 2b-B, Morpholinethoxyizocurcumin-BF ₂	11
Compound 2b, Morpholinethoxyizocurcumin	13
Compound 2c-B	15
Compound 2c	17
Compound 3-B	19
Compound 4a-B	21
Compound 4a	23
Compound 4b-B	25
Compound 4b	27
Compound 4c-B	29
Compound 4c	31
Compound 4d-B	33
Compound 4d	35
Compound 4e-B	37
Compound 4e	39
Compound 4f-B	41
Compound 4f	43
Compound 4g-B	45
Compound 4g	47
Compound 4h-B	49
Compound 4h	51
Compound 4i-B	53
Compound 4i	55
Compound 4j-B	57
Compound 4k-B	59
Compound 4l-B	61
Compound 4m-B	63
3. Microtox data	65
4. HPLC gradients	66
5. Solubility study	67
6. References	68

1. *In silico* analysis

In silico analysis was performed using the SwissTarget Prediction, which nicely showed how the morpholine group and BF₂-moiety might change the biological activity. Using the SwissTargetPrediction, the most probable biological targets of selected active compounds were determined (**Figure S1**). Moreover, curcumin was used as a reference compound. By the introduction of the morpholine group to the structure, the resulting derivative was more likely to affect the kinases with the probability of 40%, 46.7%, 40%, and 46.7% for compounds **2a-B**, **2a**, **2b-B**, and **2b**, respectively, compared to curcumin and non-morpholinated compound **2c-B**. Interestingly, the position of the morpholine group in the phenyl ring did not affect the probability. On the one hand, the analysis showed that the presence of the morpholine group at the meta position in the non-BF₂ analog is the most favorable to target the enzymes. On the other hand, it clearly showed that the presence of BF₂ moiety in morpholine curcumin derivative increased the probability of targeting the family A G protein-coupled receptor since the compounds **2a-B**, **2b-B**, and **2c-B** show a higher probability of 26%, 20%, and 20%, respectively. The presence of morpholine groups can also decrease the number of potential targets, increasing the specificity. Curcumin has a pleiotropic activity that can simultaneously be an advantage and also a drawback. As discussed by Nelson *et al.*, curcumin can affect multiple biological targets, and this lack of selectivity can result in activation/inhibition of both “good” and “bad” targets (*vide supra*) [51] and has an impact on body homeostasis.

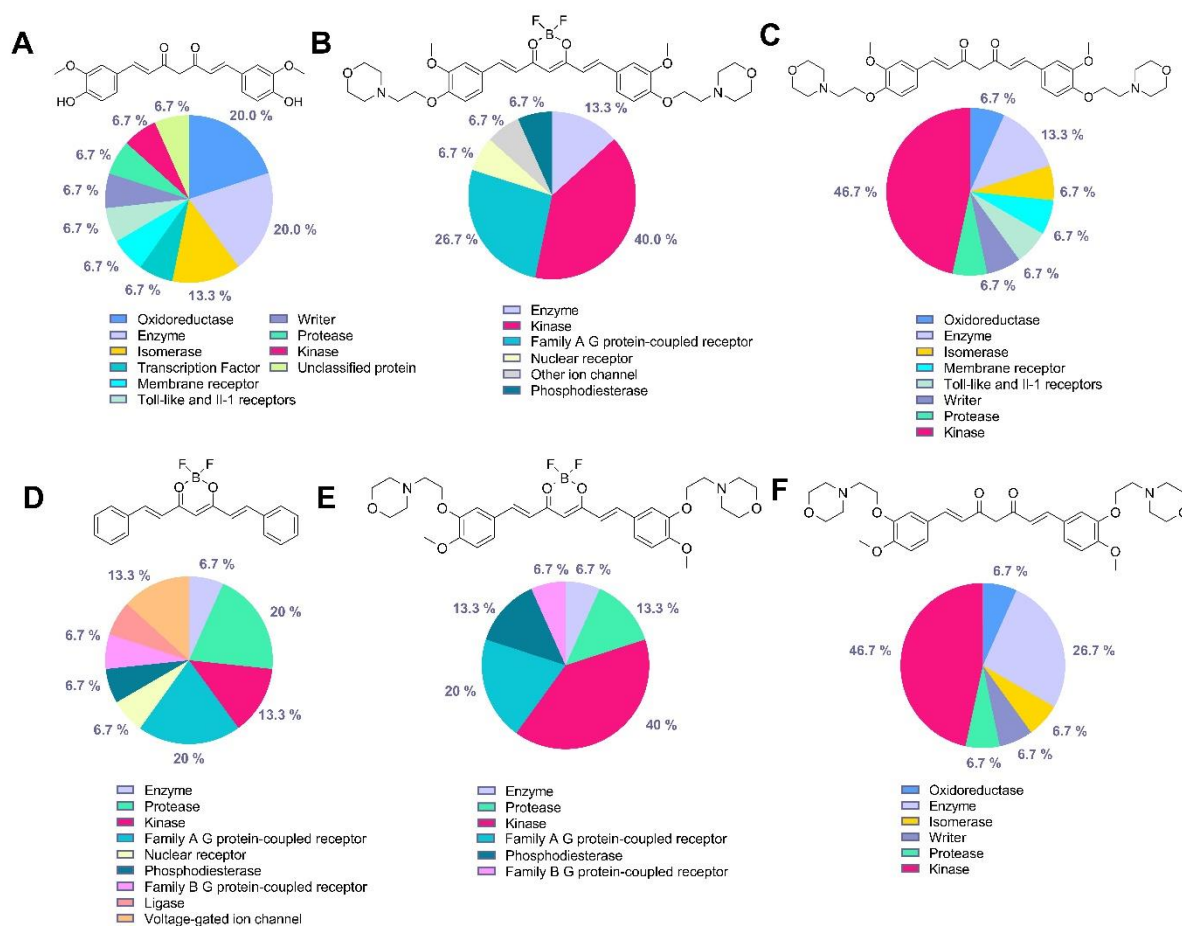


Figure S1. The potential cellular targets for the curcumin as reference compound (panel A), curcumin derivative **2a-B** (panel B), **2a** (panel C), **2c-B** (panel D), **2b-B** (panel E), and **2b** (panel F) predicted by SwissTarget-Prediction web tool.

Further studies were performed for two selected curcumin derivatives, **2a-B** and **2a**. The basic physicochemical properties such as molecular weight (MW), molecular refractivity (MR), atom counts, and total polar surface area (TPSA), were determined. In **Figure S2** the bioavailability radar for compounds **2a-B**, **2a**, and curcumin was presented. The colored area in the graph represents the optimal range for each property, such as (i) lipophilicity (expected values of XLOGP3 between -0.7 and +5.0), (ii) size (favorable MW between 150 and 500 g/mol), (iii) polarity (expected TPSA between 20 and 130 Å²), (iv) solubility (log S should be not higher than 6), (v) saturation (fraction of carbons in the sp³ hybridization not less than 0.25), and (vi) flexibility: no more than 9 rotatable bonds [52]. Our *in silico* analysis showed that both compounds fit most of the criteria; however, they possess more than 9 rotatable bonds, which may decrease the bioavailability. Several models generally predict the bioavailability and drug-like properties based on different criteria. Medicinal chemistry has used several models, such as Lipinski's rule of five, Egan [53], Ghose [54], Muegge [55], Veber [56], or Palm [57] rules. For example, the Veber rule was designed by analyzing the bioavailability data of over 1000 drug candidates processed at GlaxoSmithKline company. The filter is based on two descriptors, total polar surface area (TPSA) and rotatable bonds that should be ≤ 140 Å² and ≤ 10 Å², respectively [58]. Ghose *et al.* recommended the following descriptors: molecular weight between 160 and 480; logP between -0.4 and 5.6; molar refractivity between 40 and 130, and total number of atoms between 20 and 70 [58]. Muegge's filter includes $200 \leq \text{molecular weight} \leq 600$, $-2 \leq \text{XLOGP3 (lipophilicity)} \leq 5$, the total polar surface area ≤ 150 , the number of rings ≤ 7 , the number of carbon > 4 , the number of heteroatoms > 1 , the number of rotatable bonds ≤ 15 , the hydrogen bond acceptors ≤ 10 , and the hydrogen bond donors ≤ 5 [59]. On the other hand, the Egan rule considers the following parameters: logP and TPSA. In this filter, the logP should be ≤ 5.88 , while TPSA ≤ 131.6 Å² for good human absorption [58]. It should be noted that similar to Lipinski's rule of five, the Egan filter used statistical analysis fits only for passively absorbed compounds in the intestine [58]. Thus, the compounds transported via active transport or different carrier-based delivery system are excluded by this method [58]. The Palm filter was designed on the evaluation of dynamic surface properties of drug molecules and drug absorption in two cell models [58]. According to the results, the polar surface area is a better descriptor for intestinal drug absorption than logP. This statement was also verified using 20 model drugs with different absorption rates. The expected TPSA values for the standard orally bioavailable drugs should be < 140 Å² according to Veber and < 63 Å² according to Palm [58]. The Mozziconacci filter was designed based on the Lipinski rule of five and by analyzing several chemical libraries for drug-likeness (number of halogen atoms ≤ 7 , number of nitrogens ≥ 1 , number of oxygens ≥ 1 , number of rings ≤ 6 , rotatable bonds ≤ 15) [58], thus some models also allow the number of rotatable bonds less than 15.

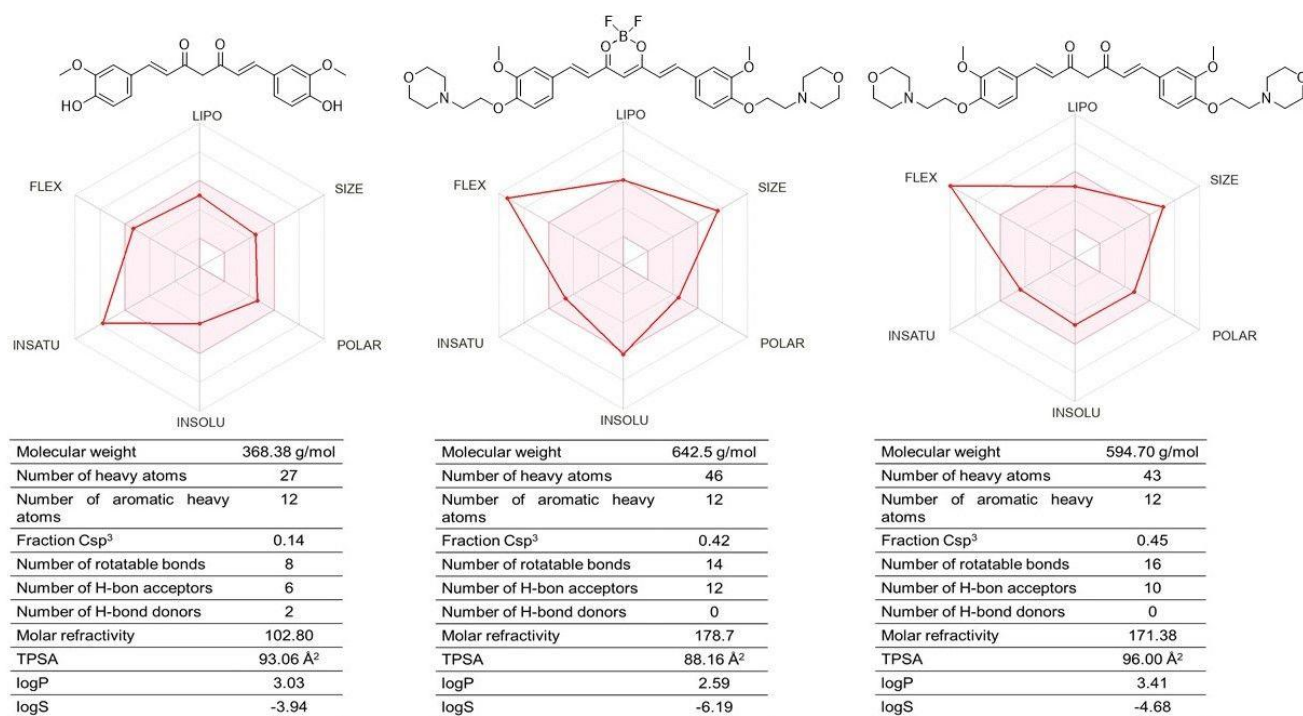


Figure S2. The selected physicochemical properties calculated for curcumin, **2a-B**, and **2a**.

The SwissADME uses different rule-based filters with diverse ranges of properties inside of which the molecule is defined as drug-like, the Lipinski, Ghose, Veber, Egan, and Muegge methods. **Table S1** presents the results for our compounds (**2a-B**, **2a**, and curcumin).

Table S1. The drug-likeness calculated in the SwissADME web tool.

Filter	Curcumin	A3	A4
Lipinski	Yes	Yes Violation (1): MW >500	Yes Violation (1): MW >500
Ghose	Yes	No Violations (3): MW >480, MR >130, number of atoms >70	No Violations (3): MW >480, MR >130, number of atoms >70
Veber	Yes	No Violation (1): number of rotatable bonds >10	No Violation (1): number of rotatable bonds >10
Egan	Yes	Yes	Yes
Muegge	Yes	No Violations (2): MW >600, number of H-bonds acceptors >10	No Violation (1): number of rotatable bonds >15
Bioavailability score	0.55	0.55	0.55

Interesting results were also found in the drug-likeness analysis. The curcumin fits all used methods and filters. However, these results did not reflect the real situation since the one crucial disadvantage of curcumin is its low bioavailability. It was found that the mode of curcumin transport in monolayers Caco-2 cells model involved both active and passive processes. The curcumin was transported via passive diffusion at concentrations <10 µg/mL and active transport at a concentration > 10 µg/mL [60].

As was mentioned above, some models did not consider active transport; thus, the calculation can be misleading and may not reflect the physiological situation. Therefore, since the curcumin derivatives **2a-B** and **2a** fit Lipinski and Egan rules (considering only passive transport), both compounds can face similar problems to curcumin regarding low bioavailability. However, these potential drawbacks can be overcome by using the nanocarrier and proper delivery systems.

The toxicity prediction using the ProTox-II (**Figure S3**) shows that the predicted organ toxicity of curcumin, **2a-B**, and **2a** is similar, and all compounds are predicted to be active for immunotoxicity. Moreover, all structures have similar predicted lethal doses and toxicity class 4.

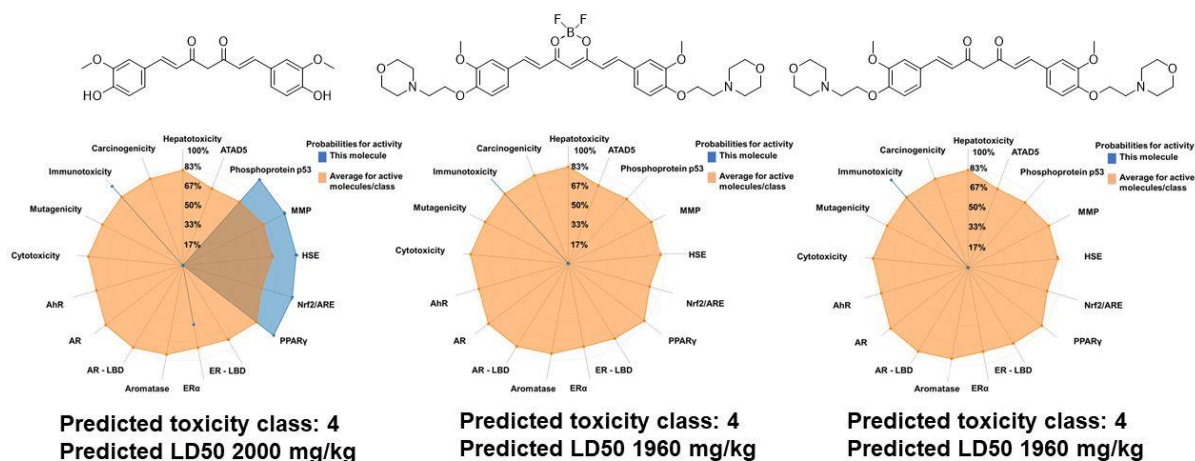
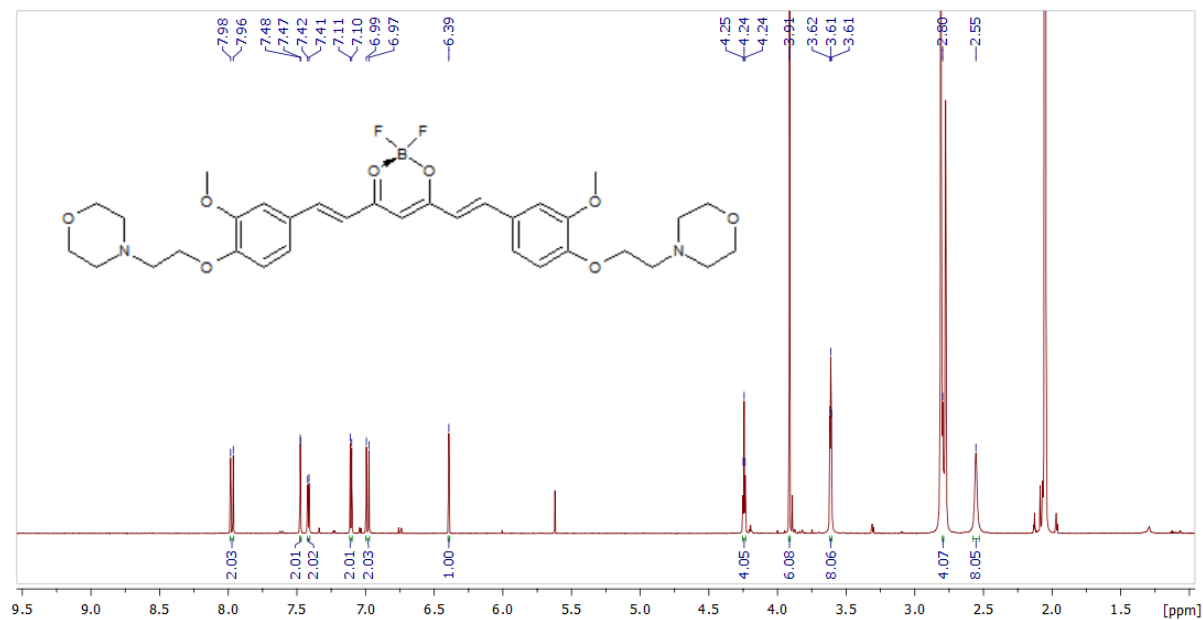


Figure S3. The toxicity radar predicted using the ProToxII software for curcumin, compound **2a-B**, compound **2a**. Abbreviations: AhR, aryl hydrocarbon receptor; AR, androgen receptor; AR-LBD, androgen receptor ligand-binding domain; ATAD5, ATPase Family AAA Domain Containing 5; ER, estrogen receptor; ER-LBD, estrogen receptor ligand binding domain; HSE, heat shock factor response element; LD, lethal dose; MMP, mitochondrial membrane potential; Nrf2/ARE, nuclear erythroid 2-related factor 2/antioxidant response element; PPAR γ , peroxisome proliferator-activated receptor.

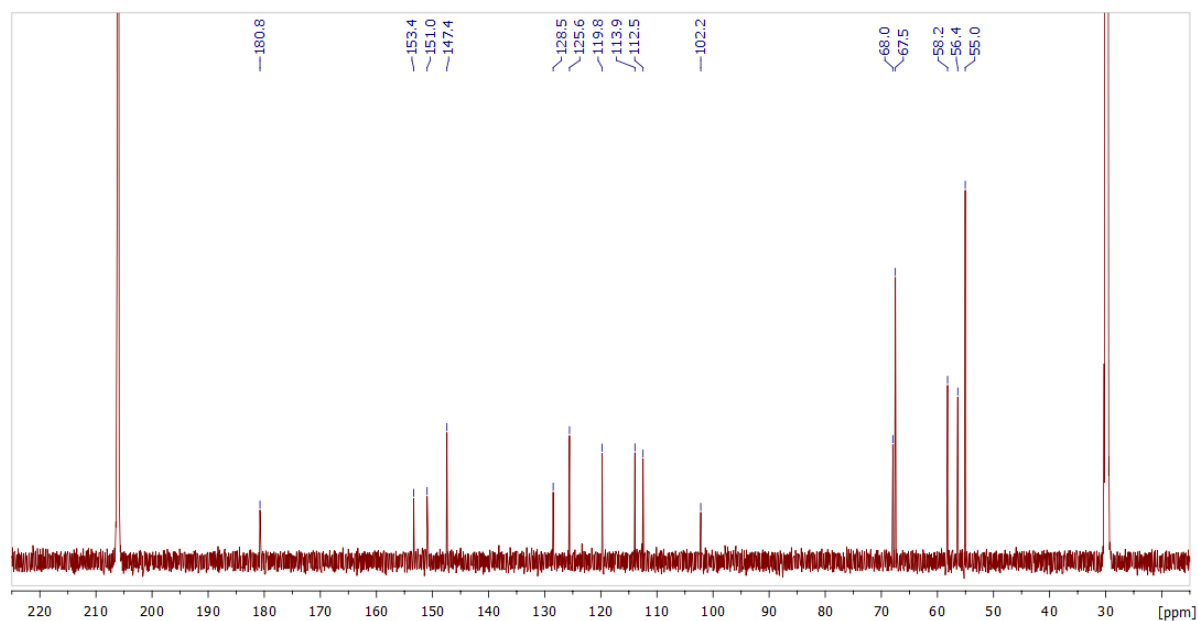
2. Experimental

Compound 2a-B, Morpholinethoxycurcumin-BF₂

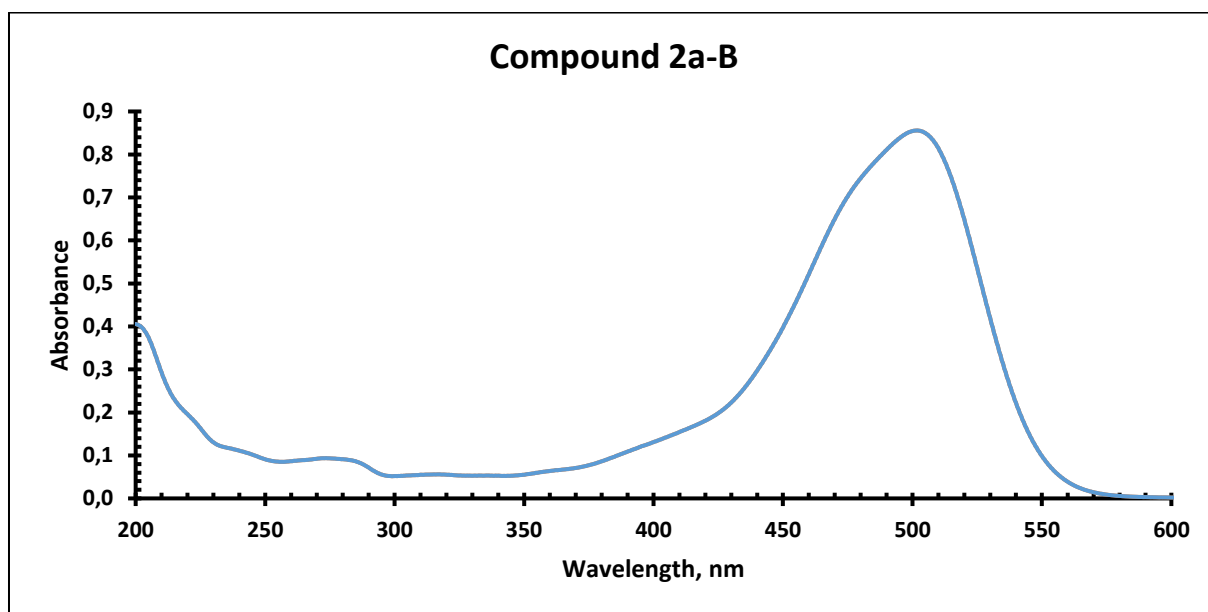
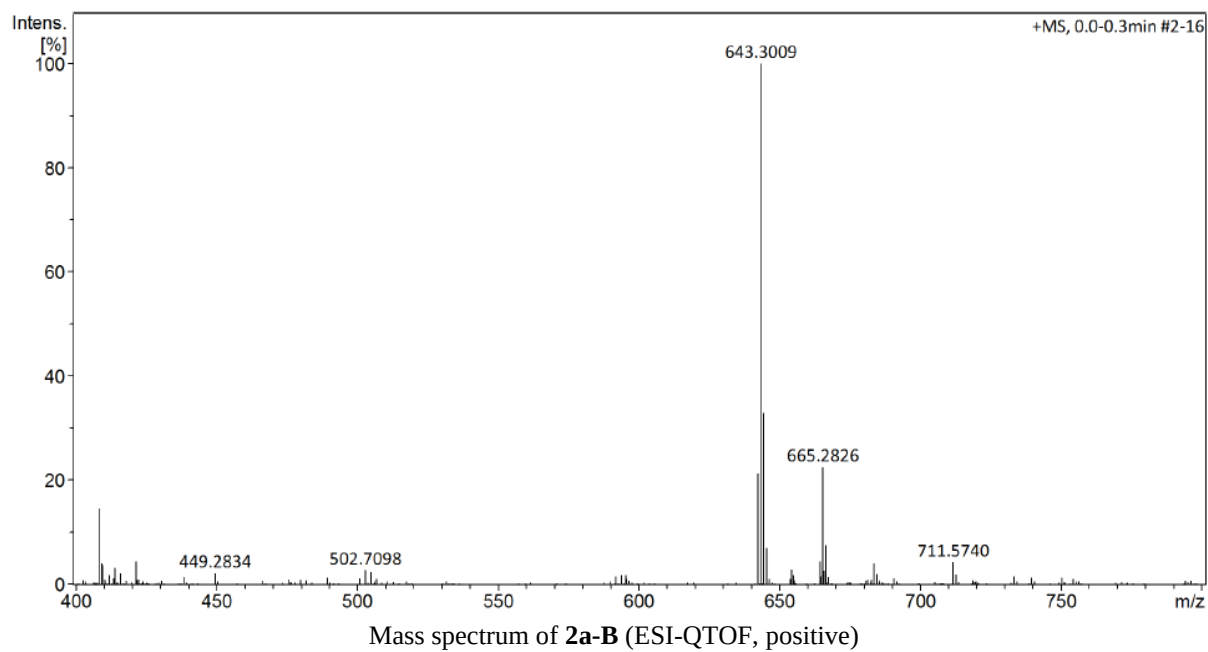
4-(2-{4-[(1*E*)-2-{2,2-difluoro-6-[(1*E*)-2-{3-methoxy-4-[2-(morpholin-4-yl)ethoxy]phenyl}ethenyl]-1,3,2-dioxaborinin-4-yl}ethenyl]-2-methoxyphenoxy}ethyl)morpholine



¹H NMR spectrum of **2a-B** (acetone-*d*₆, 25 °C)



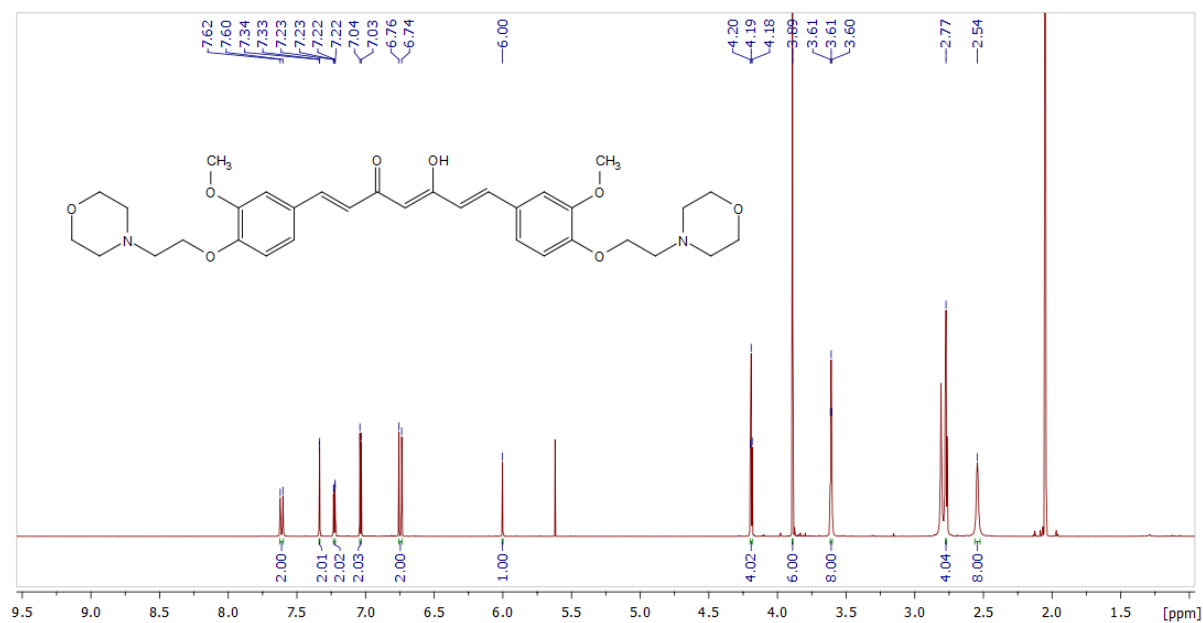
¹³C NMR spectrum of **2a-B** (acetone-*d*₆, 25 °C)



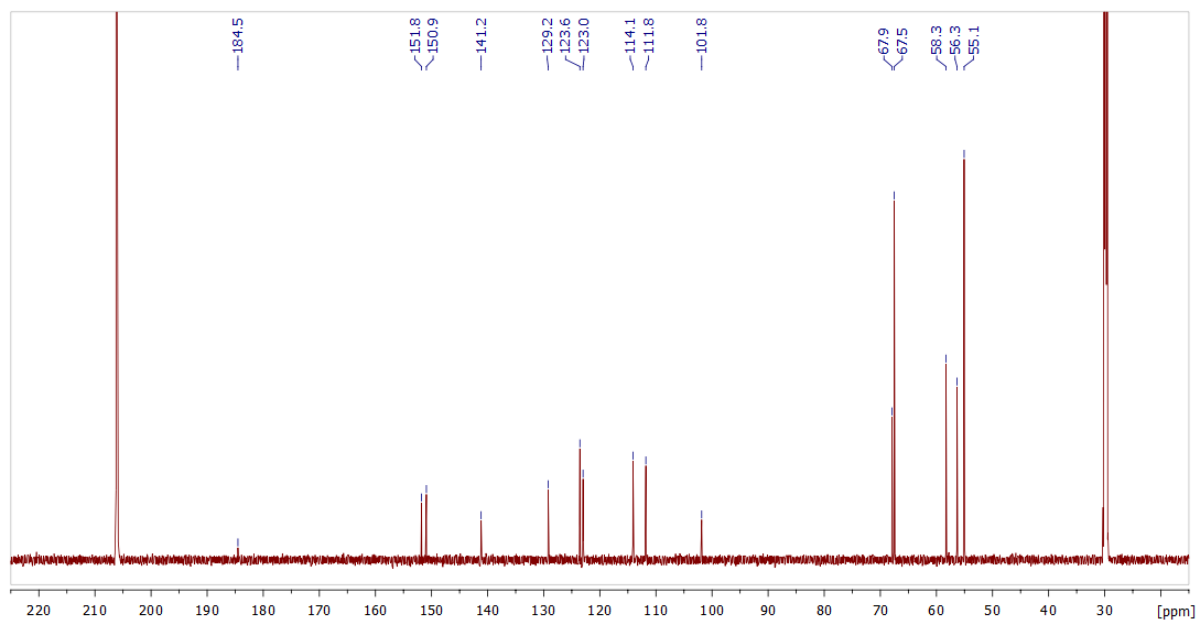
UV-vis absorbance spectrum of compound **2a-B** in acetonitrile.

Compound 2a, Morpholinethoxycurcumin

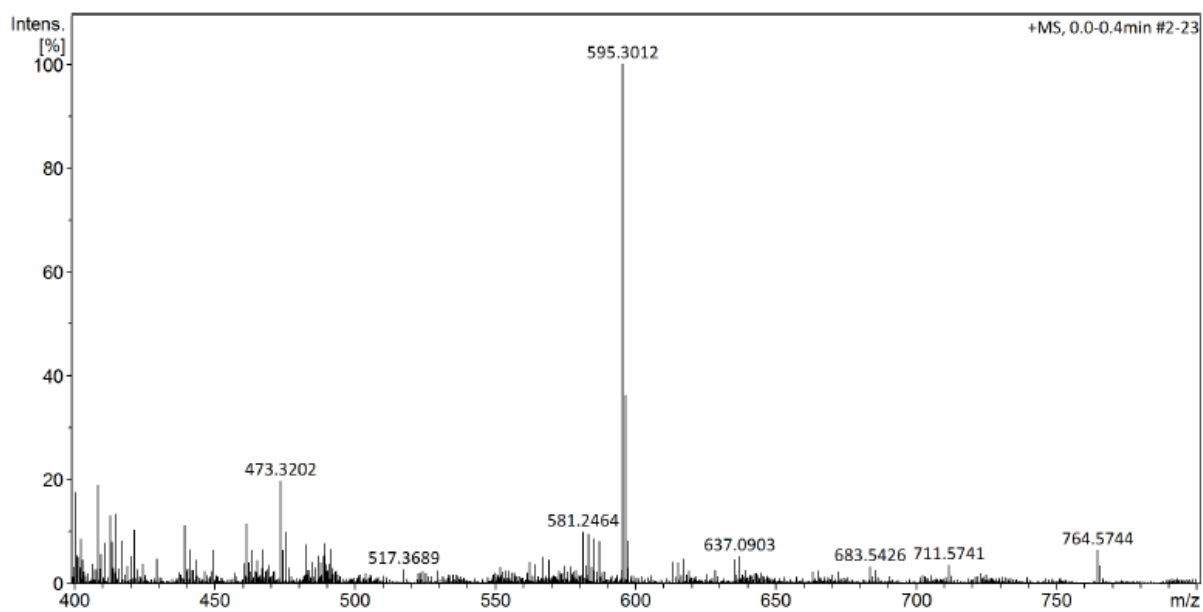
(1*E*,4*Z*,6*E*)-5-hydroxy-1,7-bis({3-methoxy-4-[2-(morpholin-4-yl)ethoxy]phenyl})hepta-1,4,6-trien-3-one



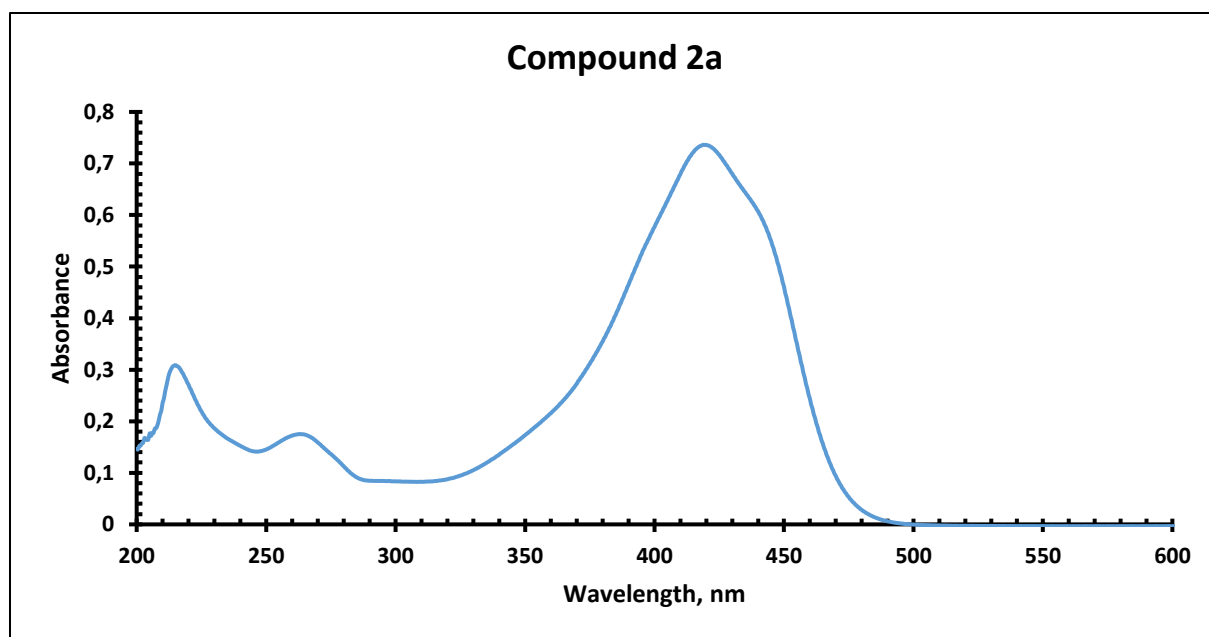
¹H NMR spectrum of **2a** (acetone-*d*₆, 25 °C)



¹³C NMR spectrum of **2a** (acetone-*d*₆, 25 °C)



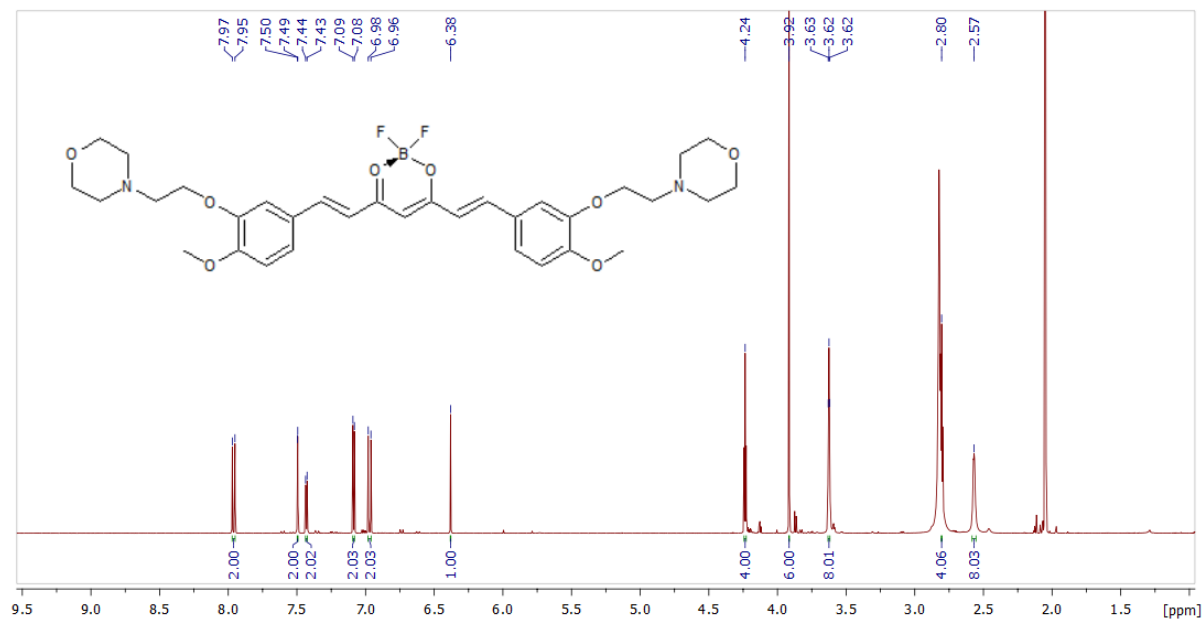
Mass spectrum of **2a** (ESI-QTOF, positive)



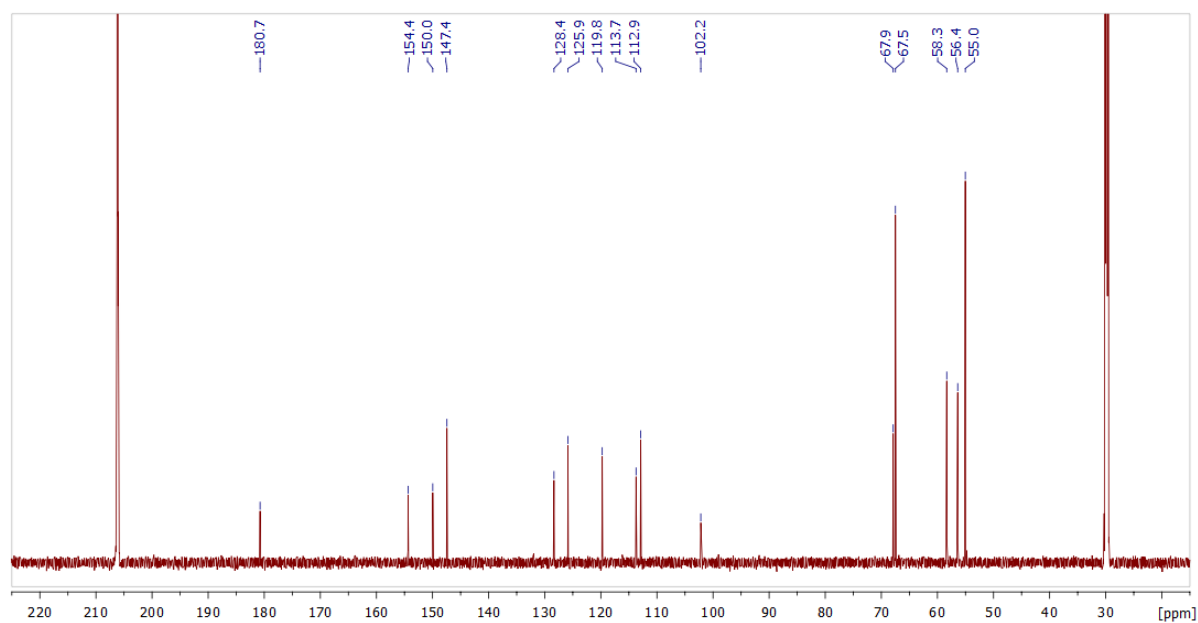
UV-vis absorbance spectrum of compound **2a** in acetonitrile.

Compound 2b-B, Morpholinethoxyizocurcumin-BF₂

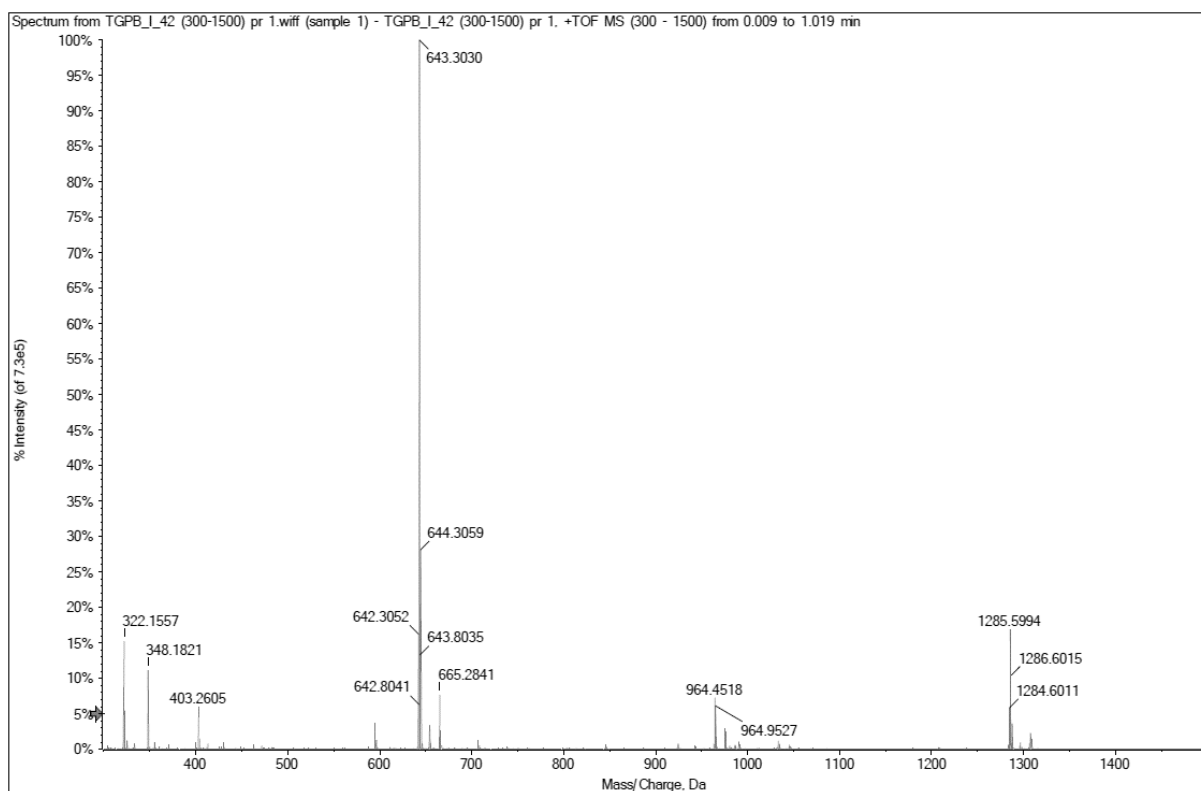
4-(2-{5-[(1*E*)-2-{2,2-difluoro-6-[(1*E*)-2-{4-methoxy-3-[2-(morpholin-4-yl)ethoxy]phenyl}ethenyl]-1,3,2-dioxaborinin-4-yl}ethenyl]-2-methoxyphenoxy}ethyl)morpholine



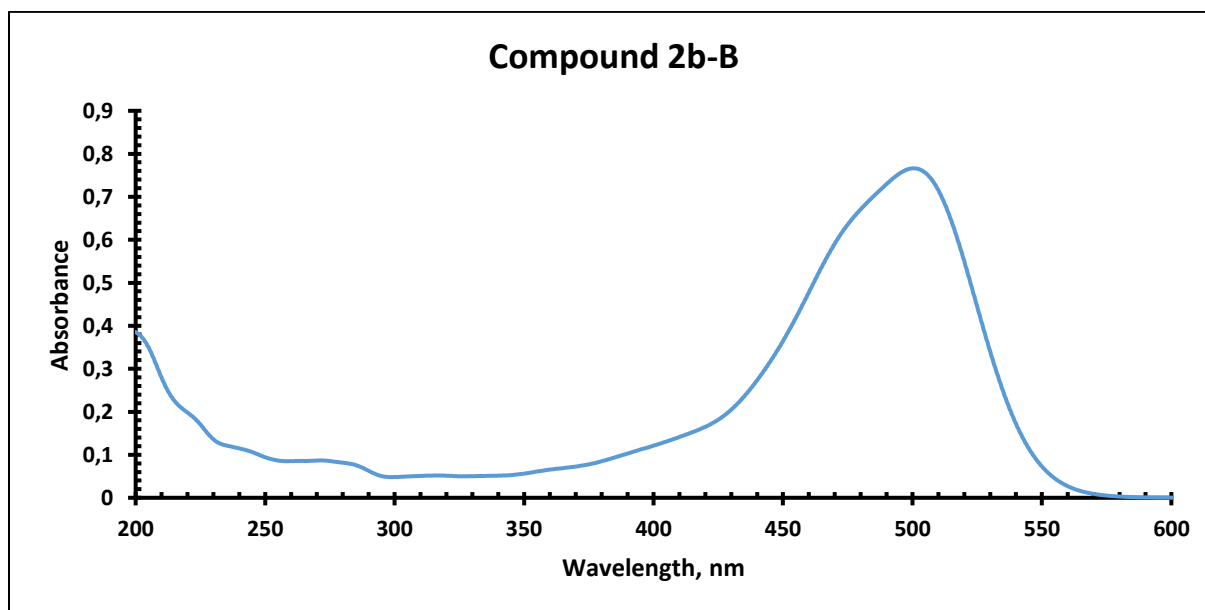
¹H NMR spectrum of **2b-B** (acetone-*d*₆, 25 °C)



¹³C NMR spectrum of **2b-B** (acetone-*d*₆, 25 °C)



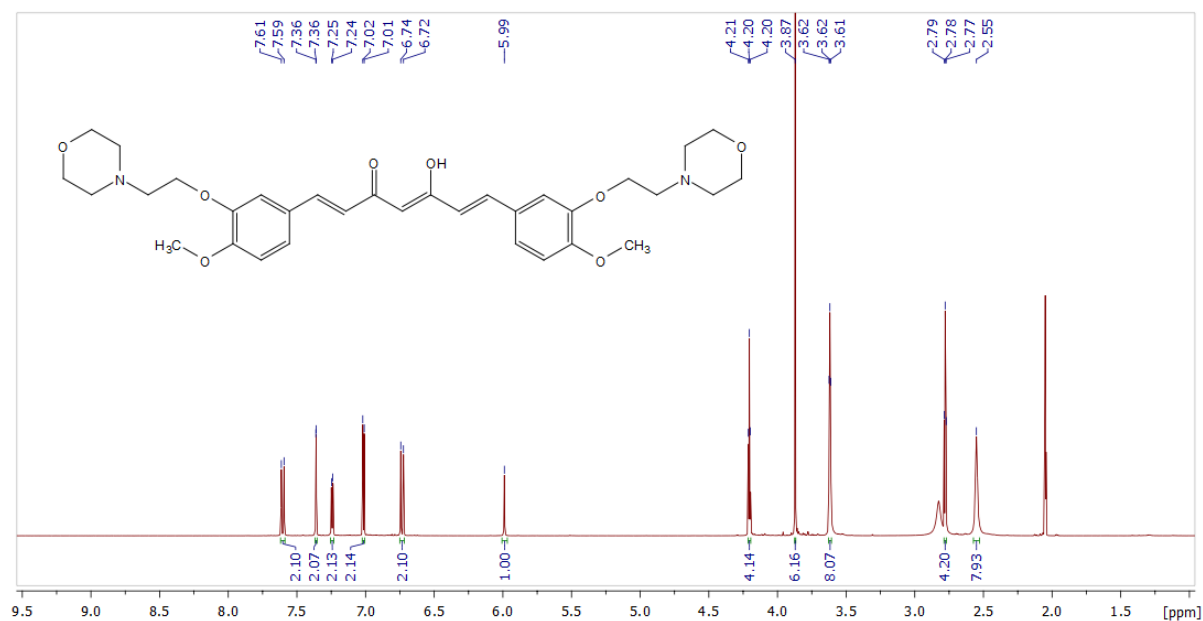
Mass spectrum of **2b-B** (ESI-QTOF, positive)



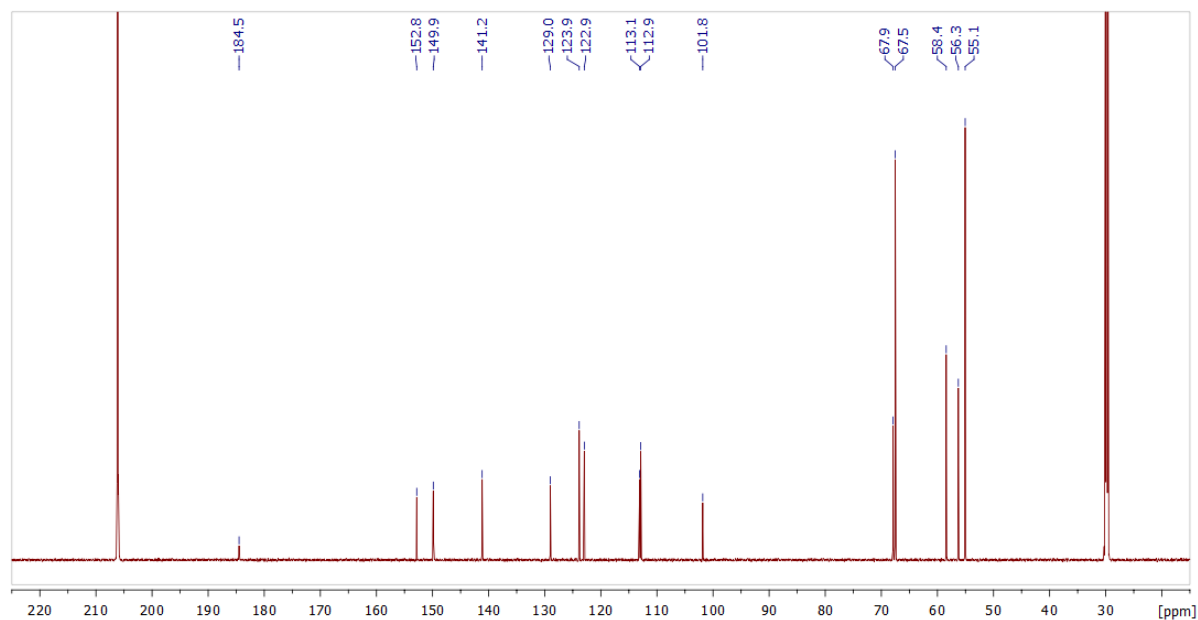
UV-vis absorbance spectrum of compound **2b-B** in acetonitrile.

Compound 2b, Morpholinethoxyizocurcumin

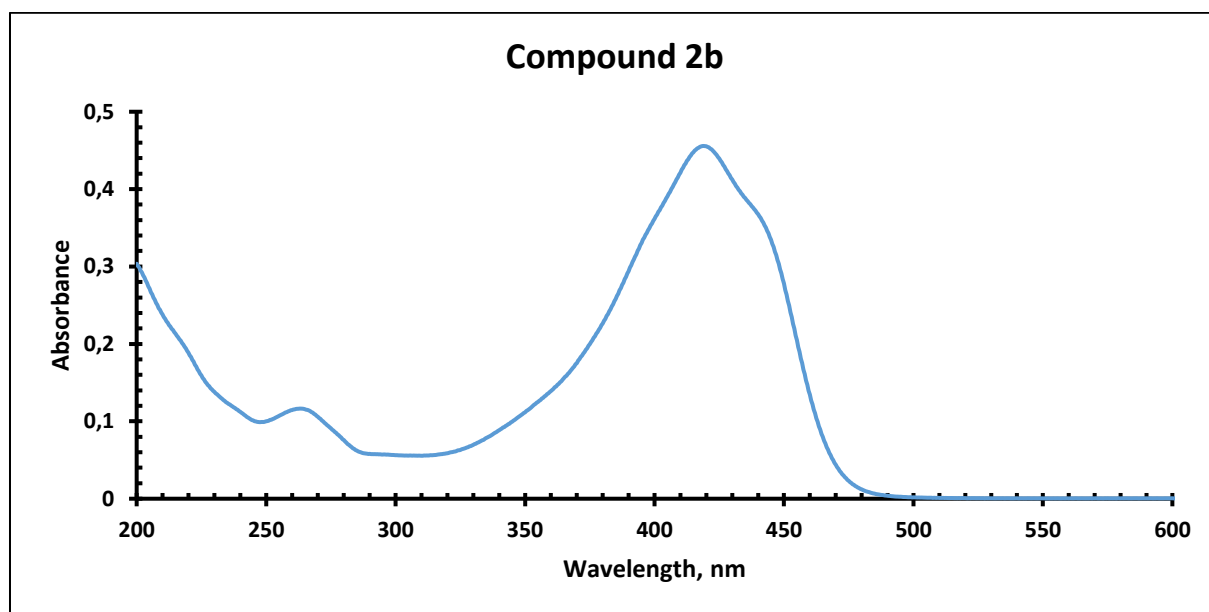
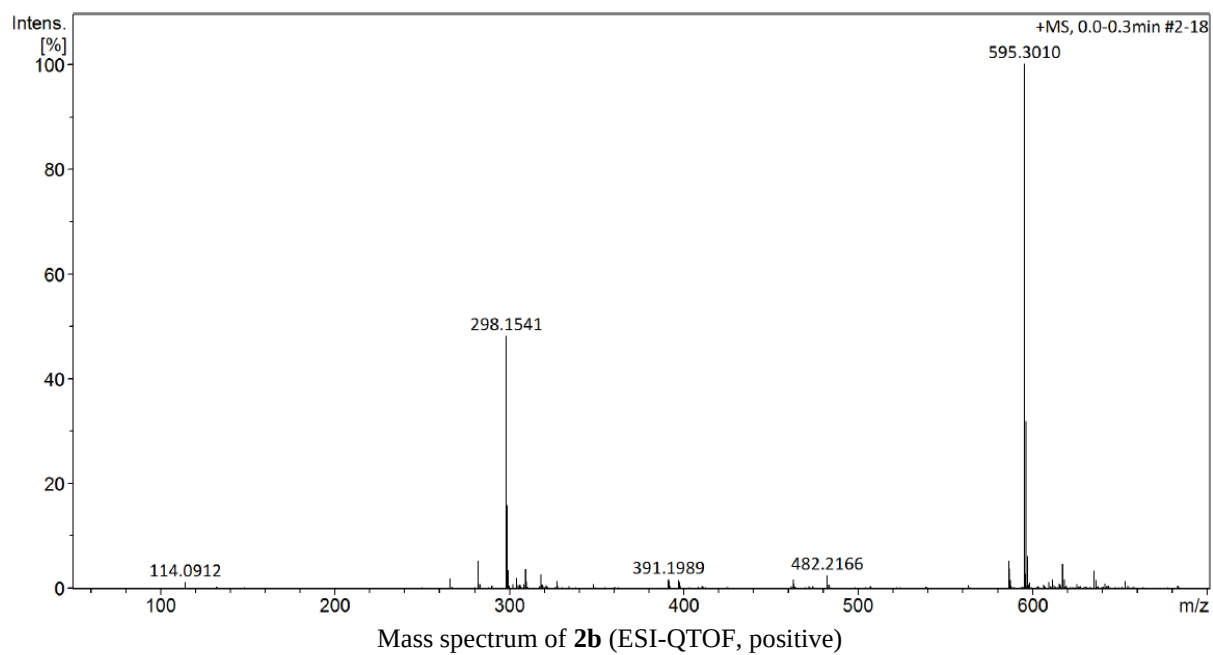
(1*E*,4*Z*,6*E*)-5-hydroxy-1,7-bis({4-methoxy-3-[2-(morpholin-4-yl)ethoxy]phenyl})hepta-1,4,6-trien-3-one



¹H NMR spectrum of **2b** (acetone-*d*₆, 25 °C)



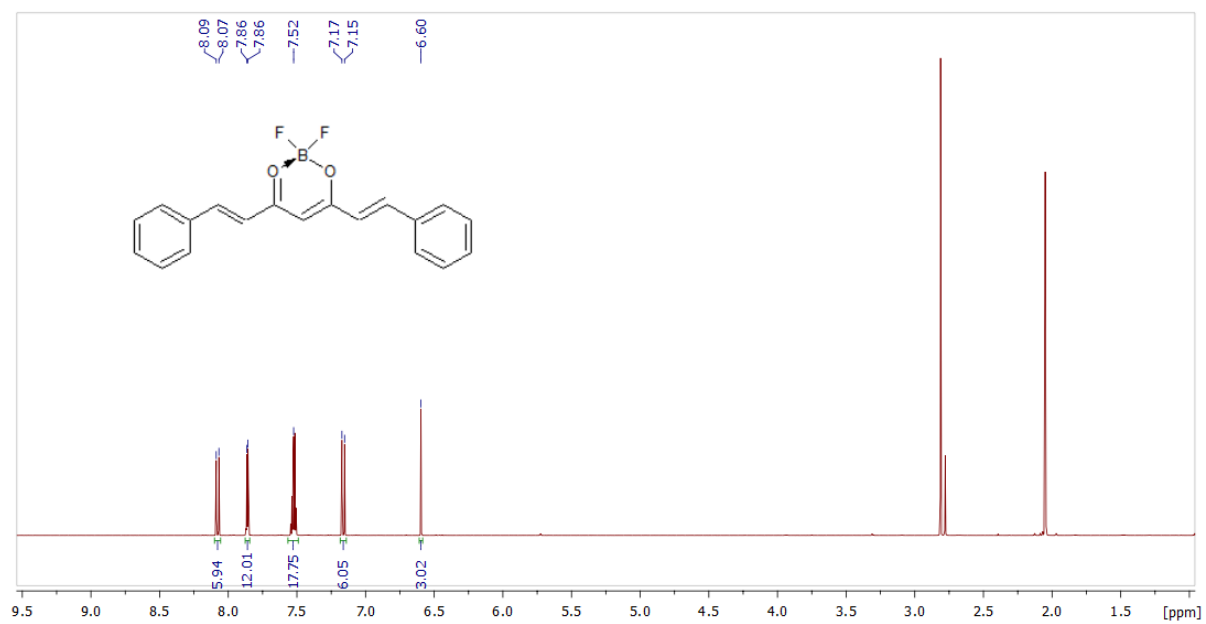
¹³C NMR spectrum of **2b** (acetone-*d*₆, 25 °C)



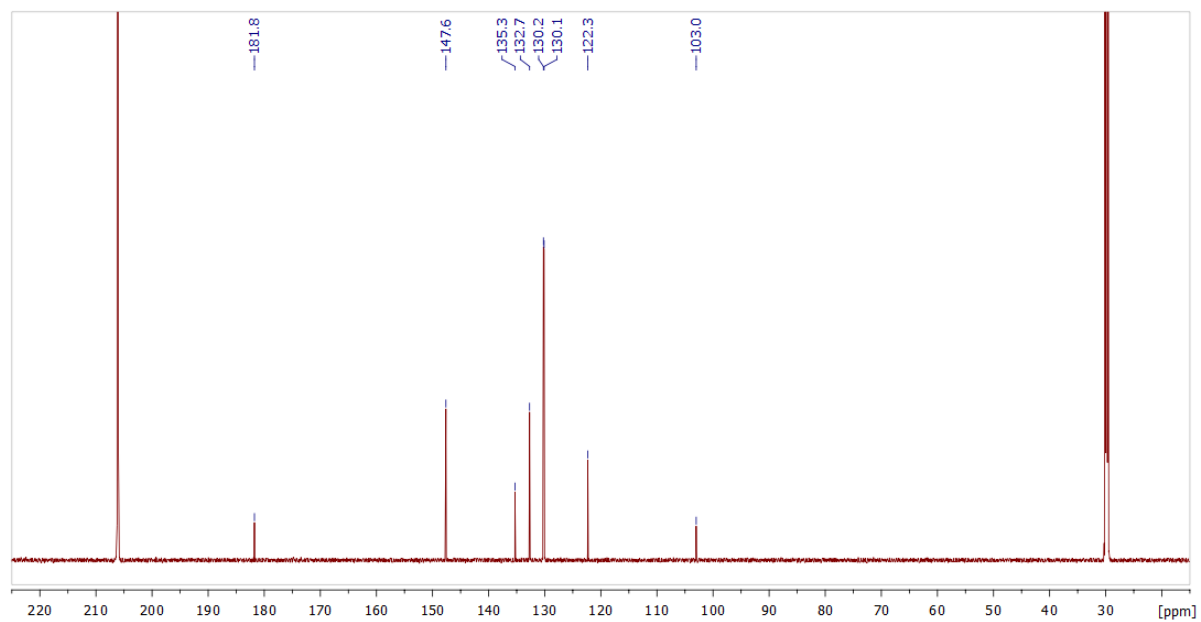
UV-vis absorbance spectrum of compound **2b** in acetonitrile.

Compound 2c-B

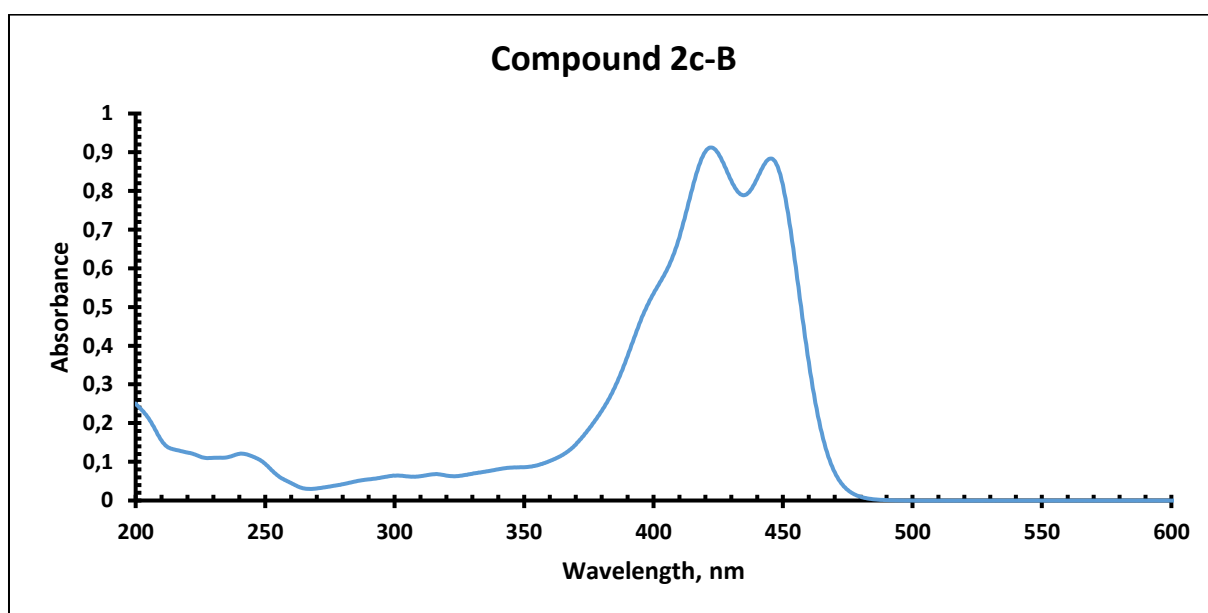
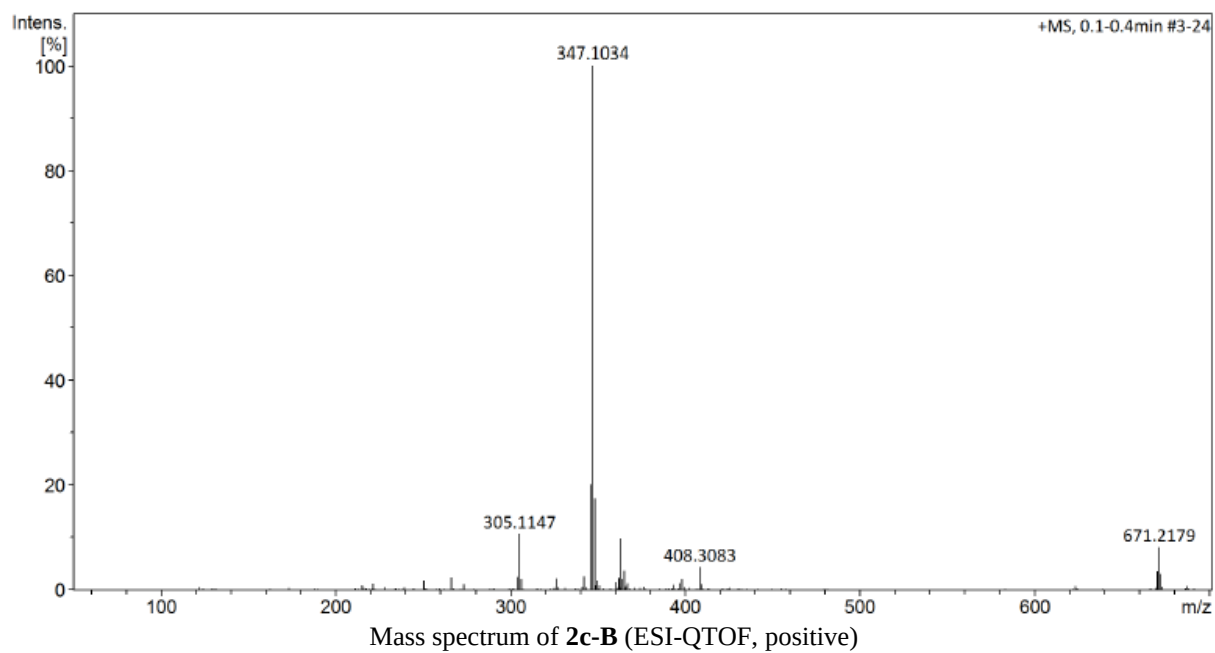
2,2-difluoro-4,6-bis[(1E)-2-phenylethenyl]-1,3,2-dioxaborinine



¹H NMR spectrum of **2c-B** (acetone-*d*₆, 25 °C)



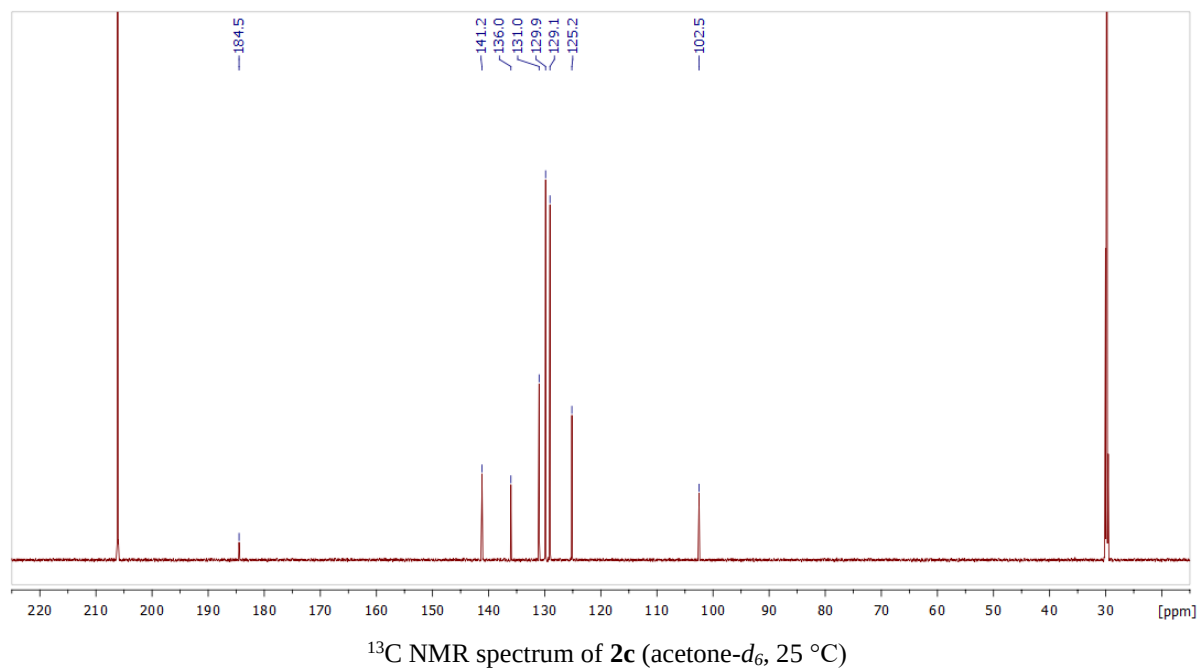
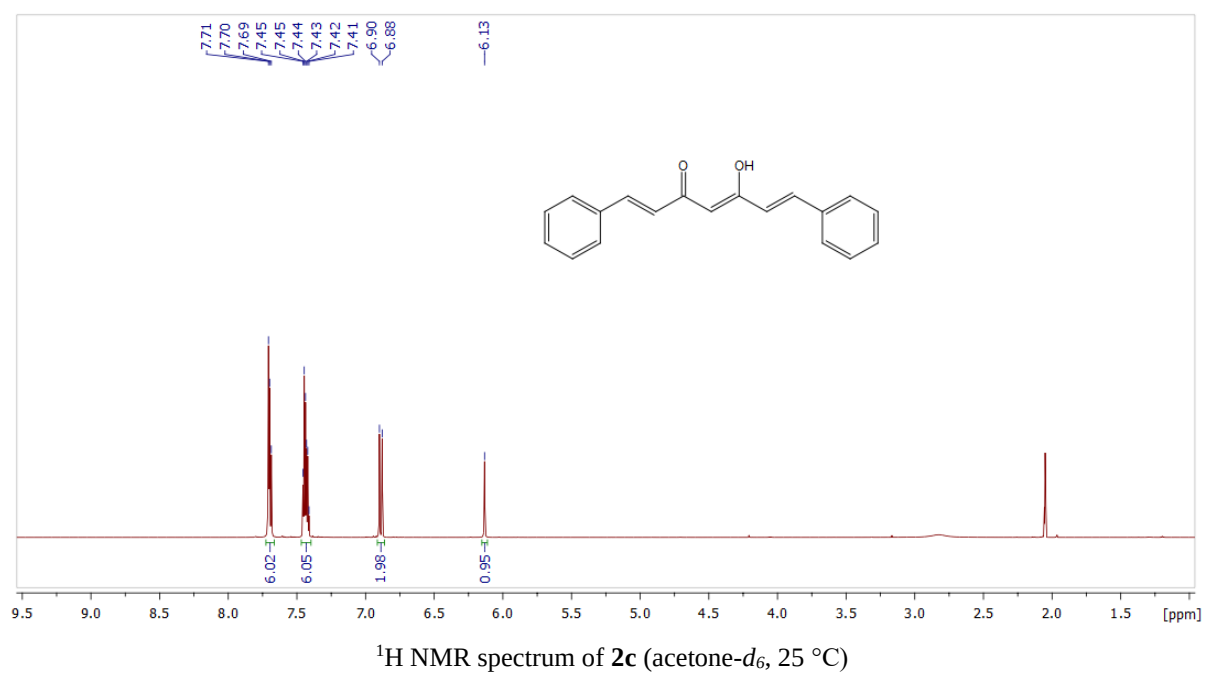
¹³C NMR spectrum of **2c-B** (acetone-*d*₆, 25 °C)

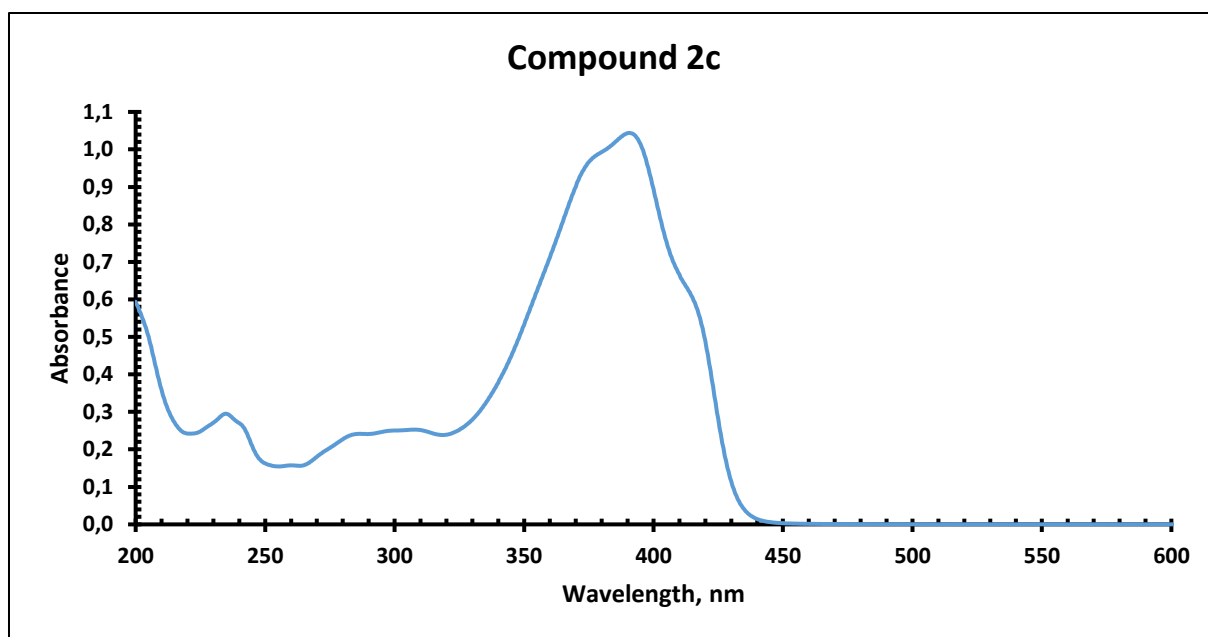
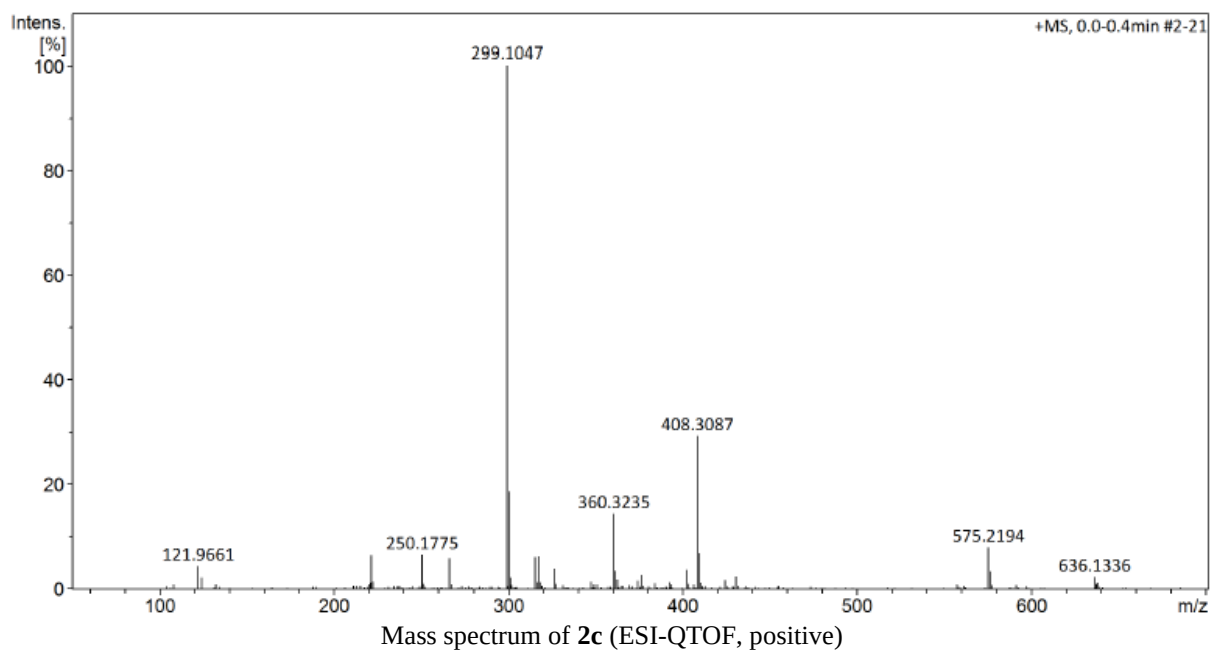


UV-vis absorbance spectrum of compound **2c-B** in acetonitrile.

Compound 2c

(1*E*,4*Z*,6*E*)-5-hydroxy-1,7-diphenylhepta-1,4,6-trien-3-one

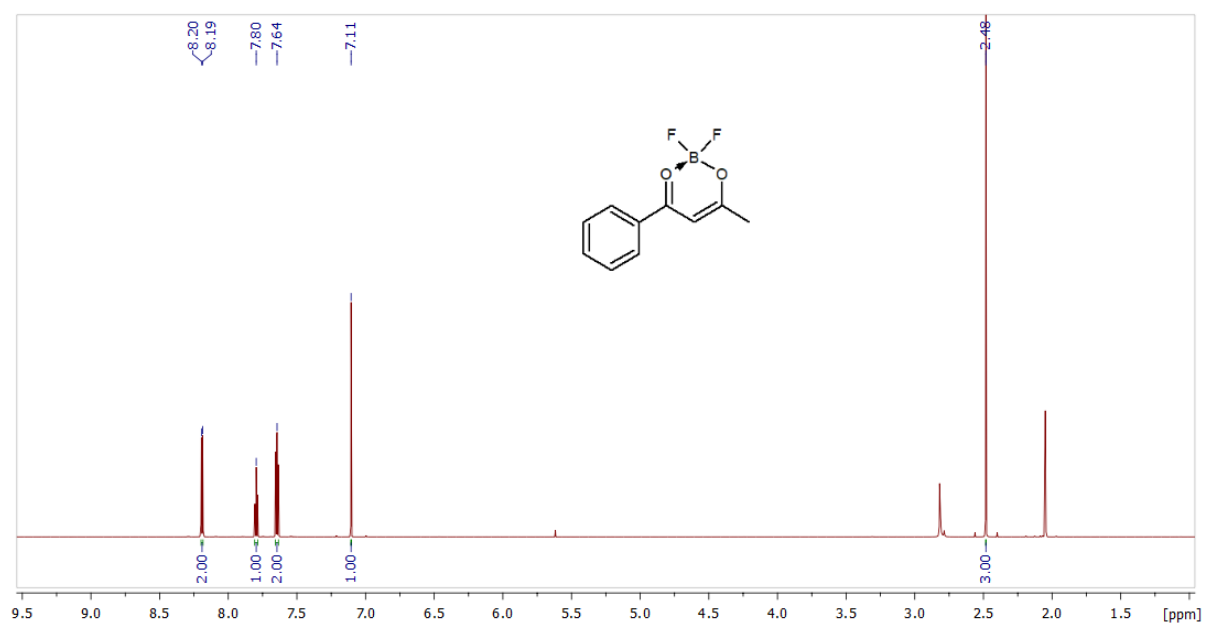




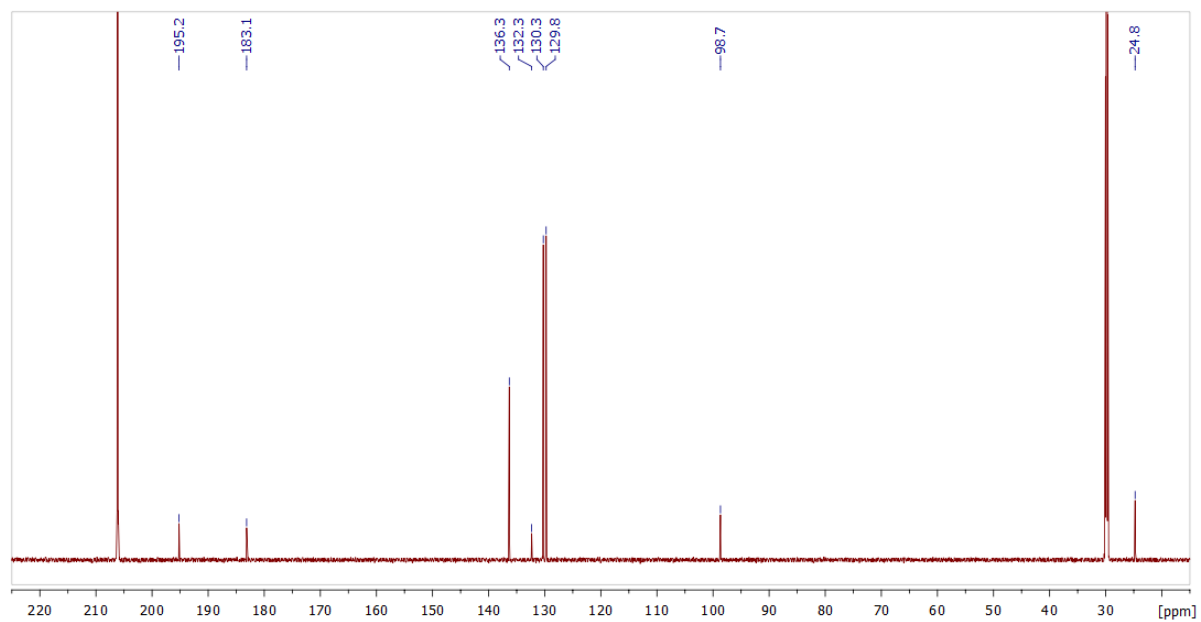
UV-vis absorbance spectrum of compound 2c in acetonitrile.

Compound 3-B

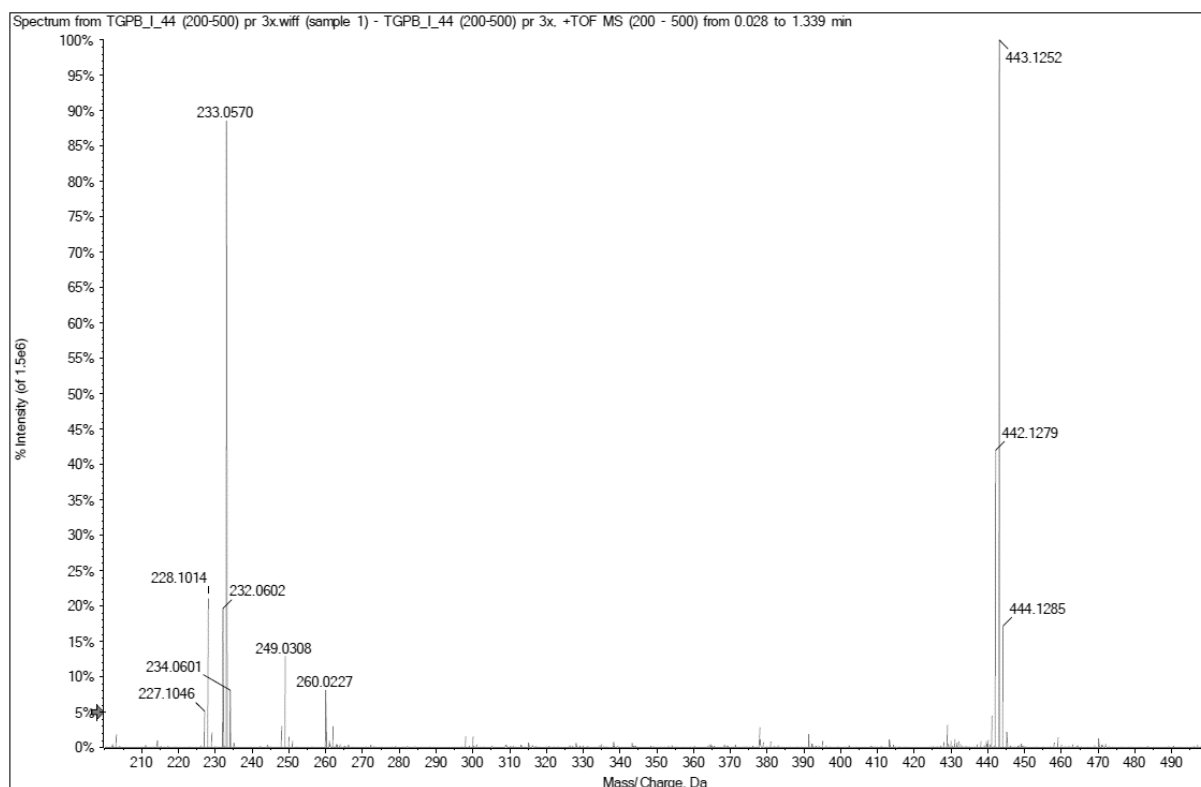
2,2-difluoro-4-methyl-6-phenyl-1,3,2-dioxaborinine



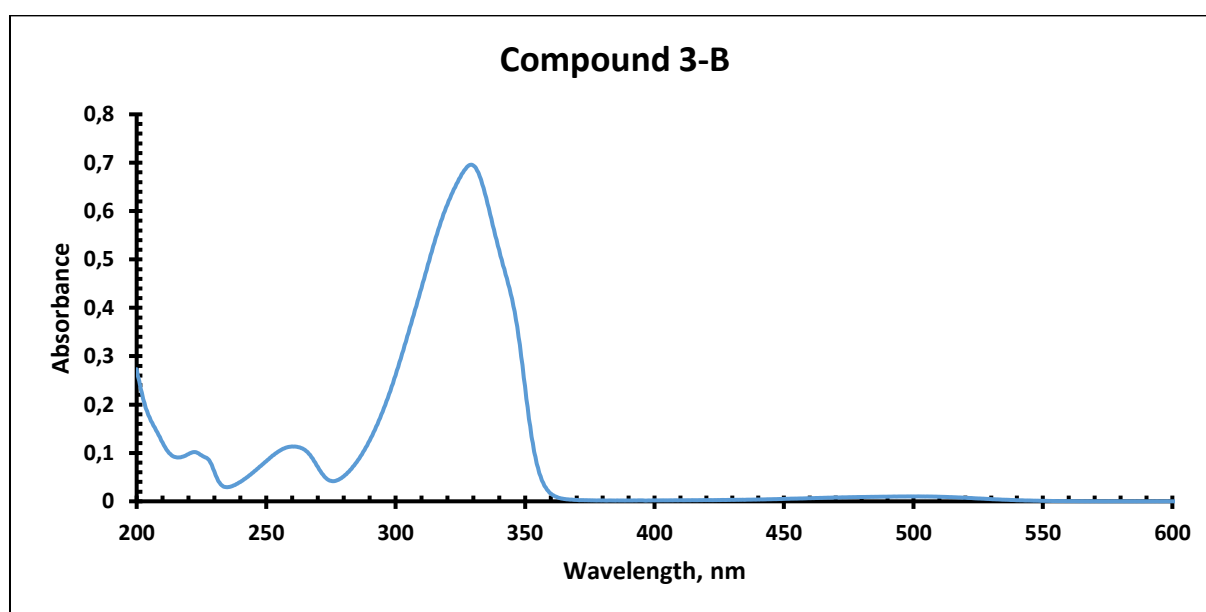
^1H NMR spectrum of **3-B** (acetone- d_6 , 25 °C)



^{13}C NMR spectrum of **3-B** (acetone- d_6 , 25 °C)



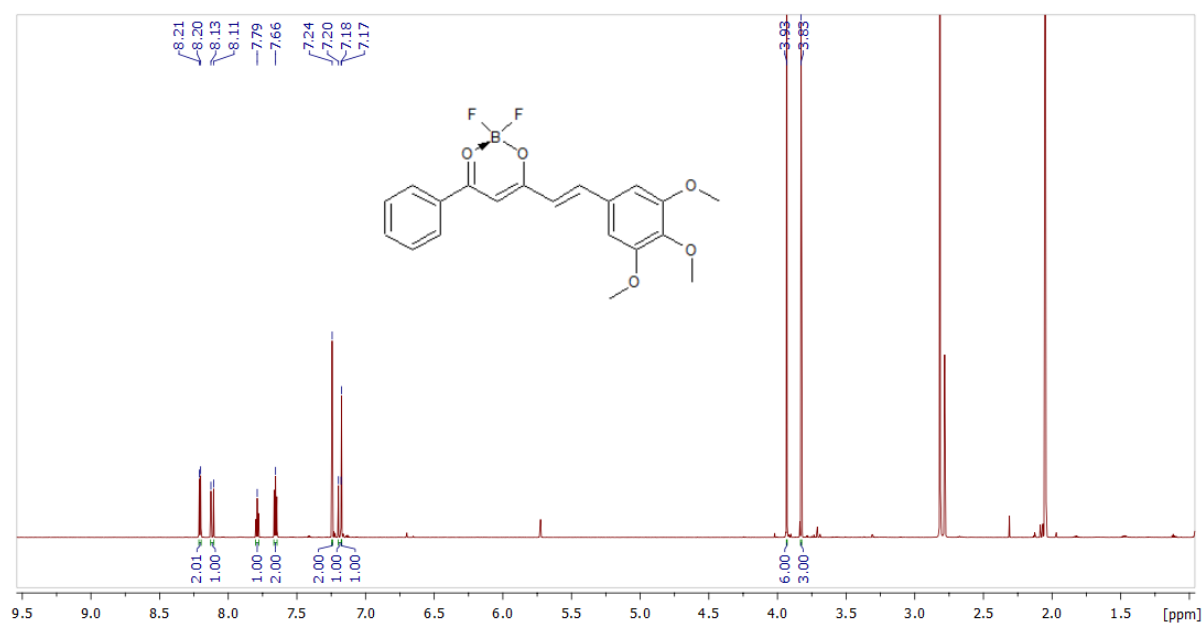
Mass spectrum of **3-B** (ESI-QTOF, positive)



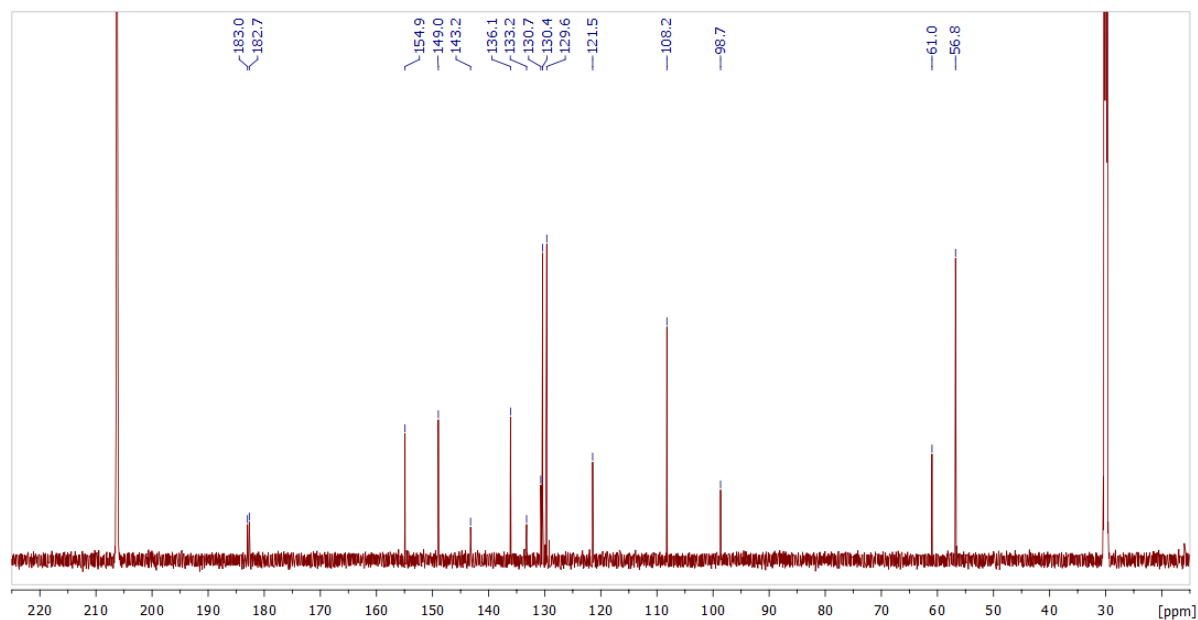
UV-vis absorbance spectrum of compound **3-B** in acetonitrile.

Compound 4a-B

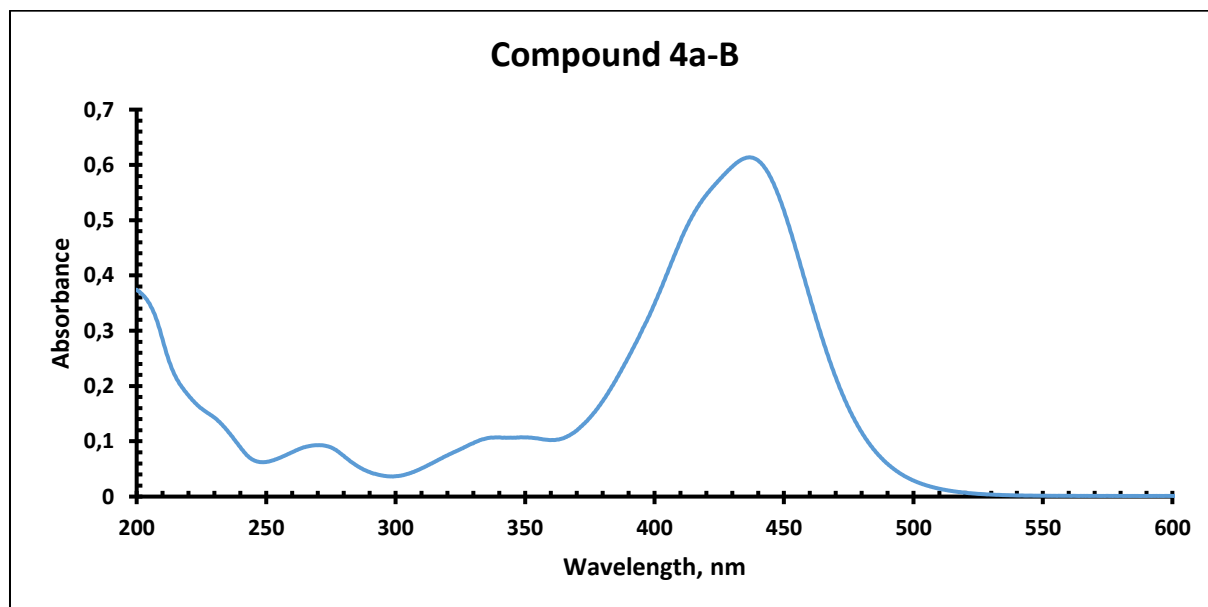
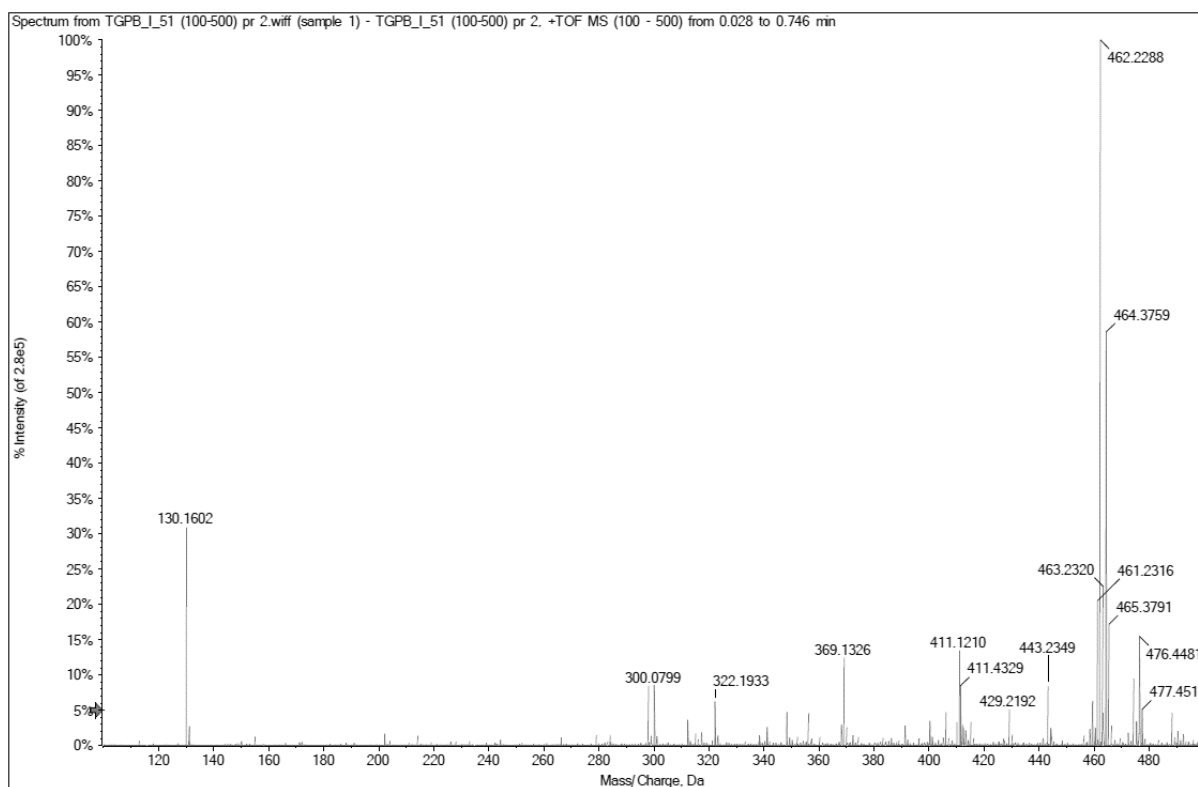
2,2-difluoro-4-phenyl-6-[(1*E*)-2-(3,4,5-trimethoxyphenyl)ethenyl]-1,3,2-dioxaborinine



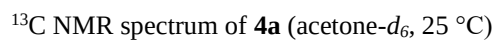
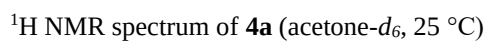
¹H NMR spectrum of **4a-B** (acetone-*d*₆, 25 °C)

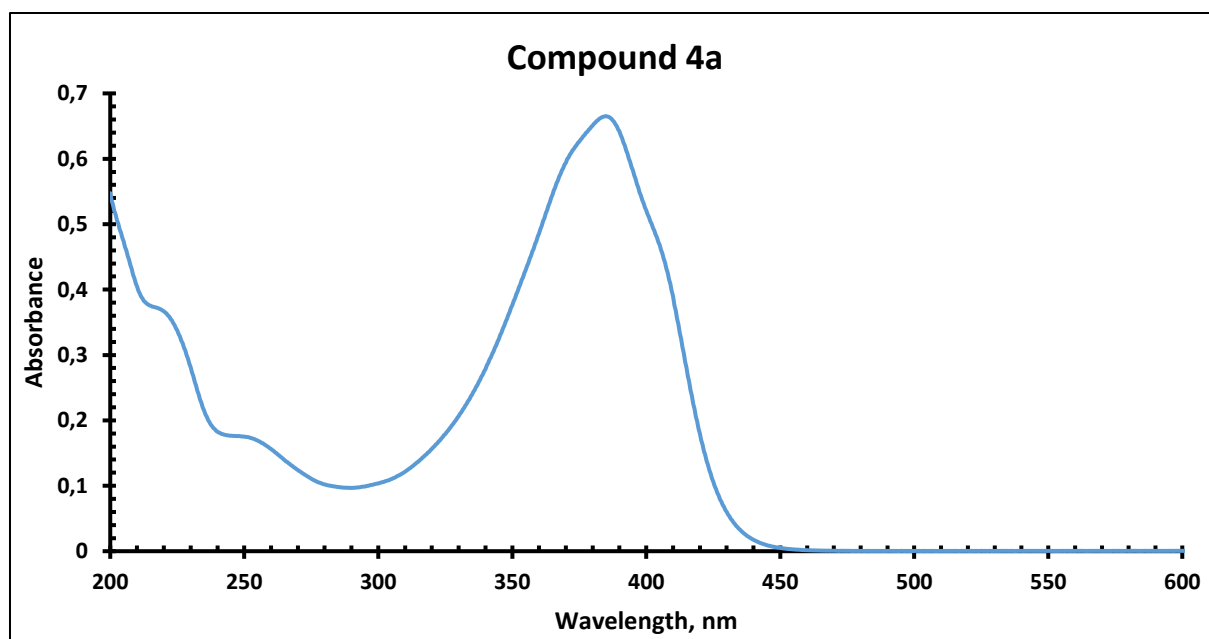
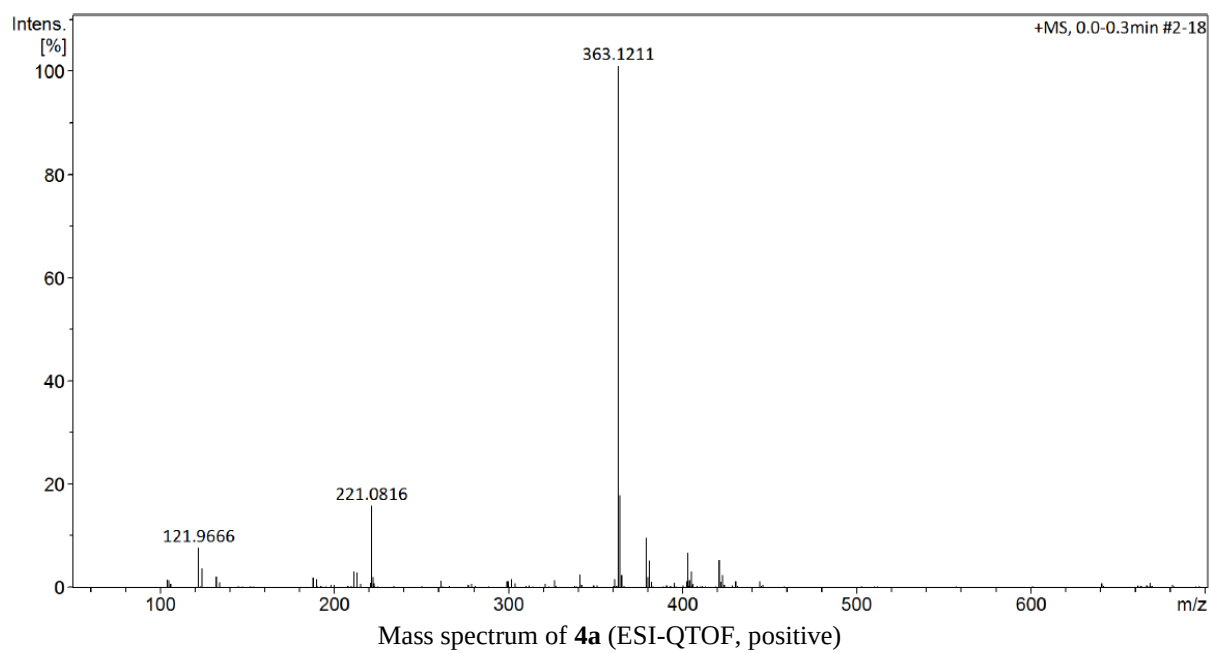


¹³C NMR spectrum of **4a-B** (acetone-*d*₆, 25 °C)



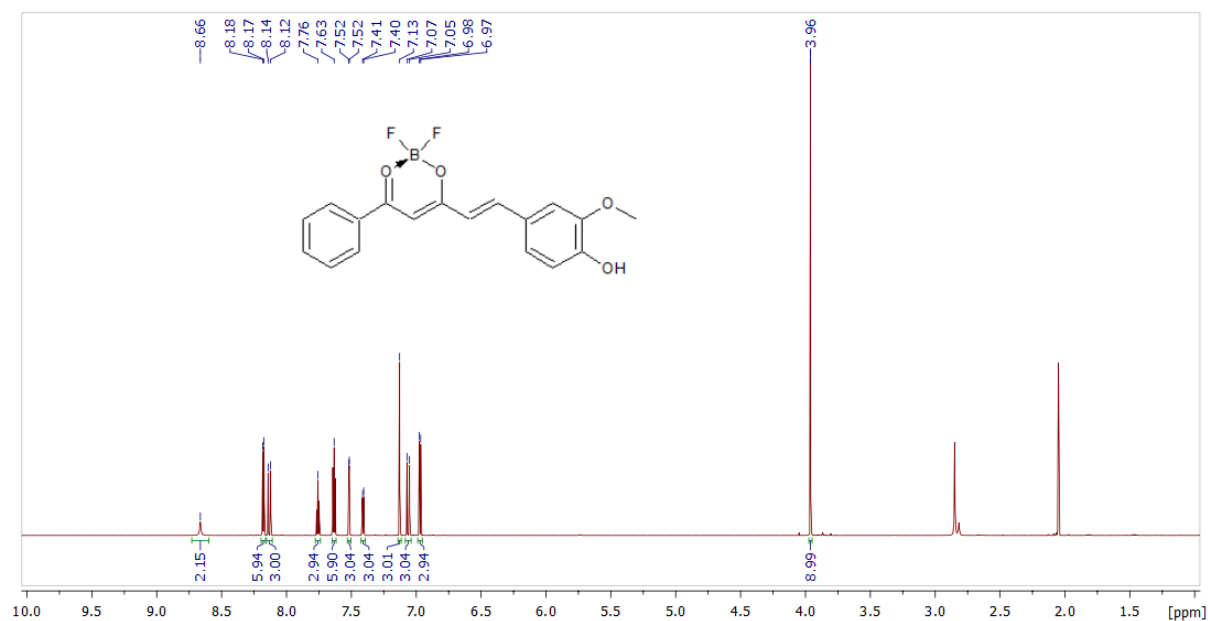
(2*Z*,4*E*)-3-hydroxy-1-phenyl-5-(3,4,5-trimethoxyphenyl)penta-2,4-dien-1-one



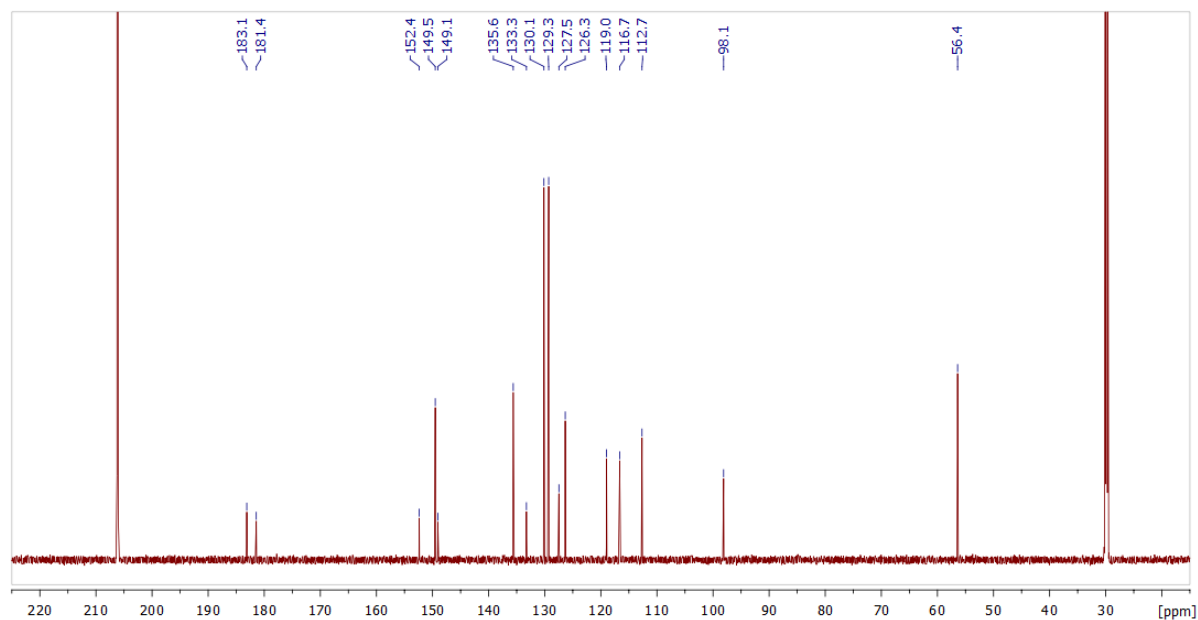


Compound 4b-B

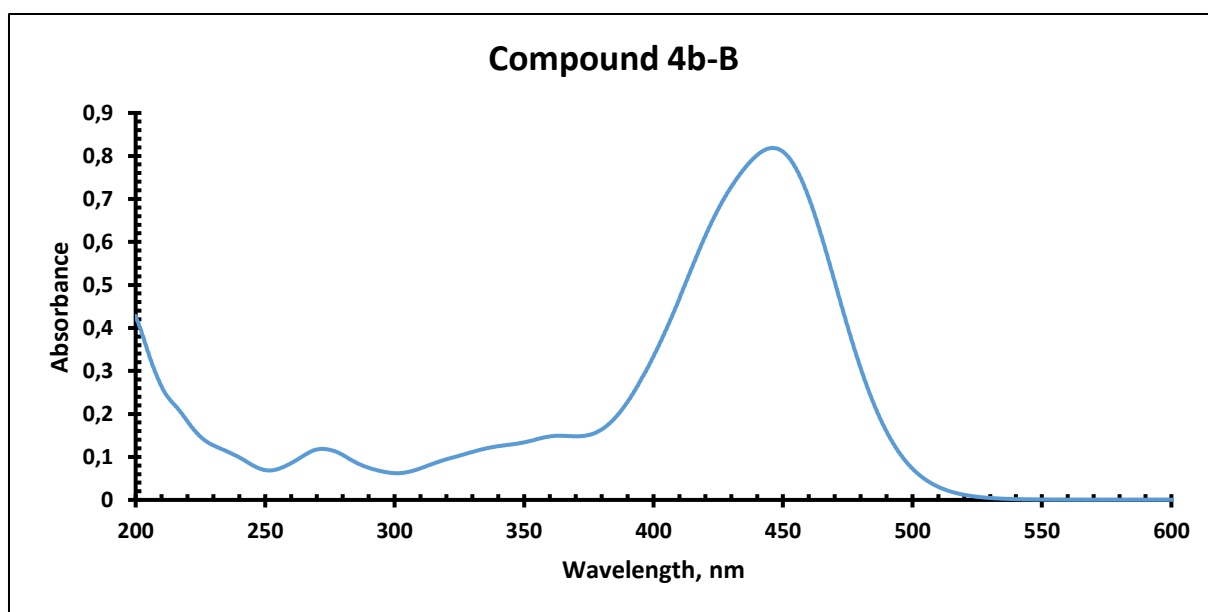
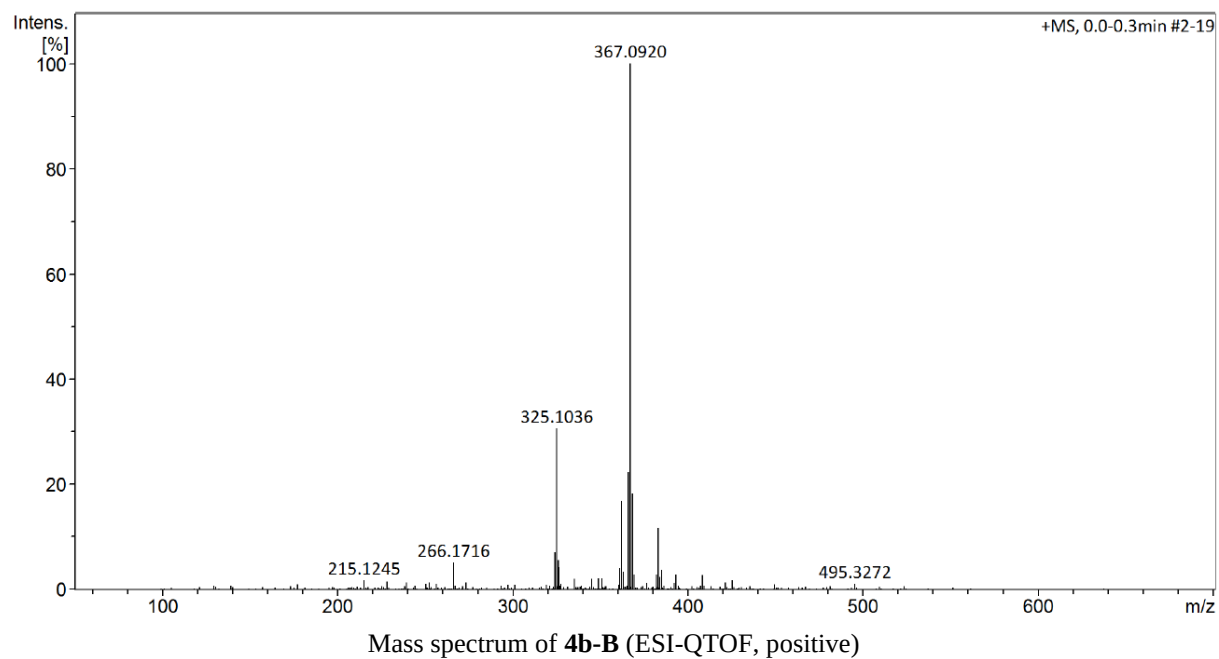
4-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2-methoxyphenol



¹H NMR spectrum of **4b-B** (acetone-*d*₆, 25 °C)



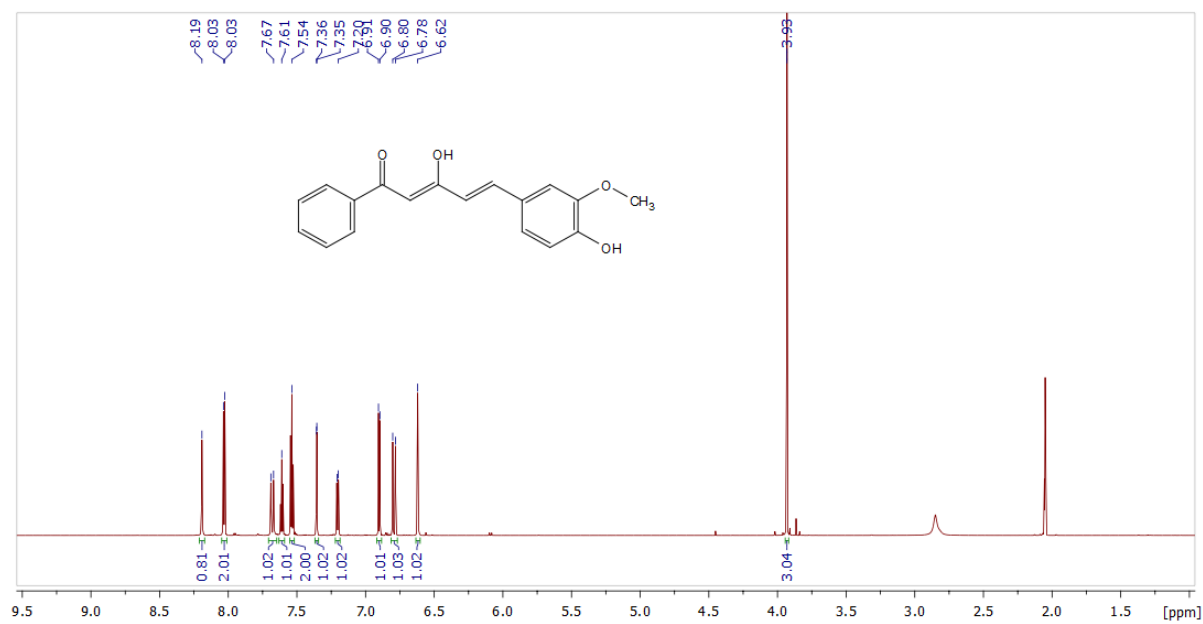
¹³C NMR spectrum of **4b-B** (acetone-*d*₆, 25 °C)



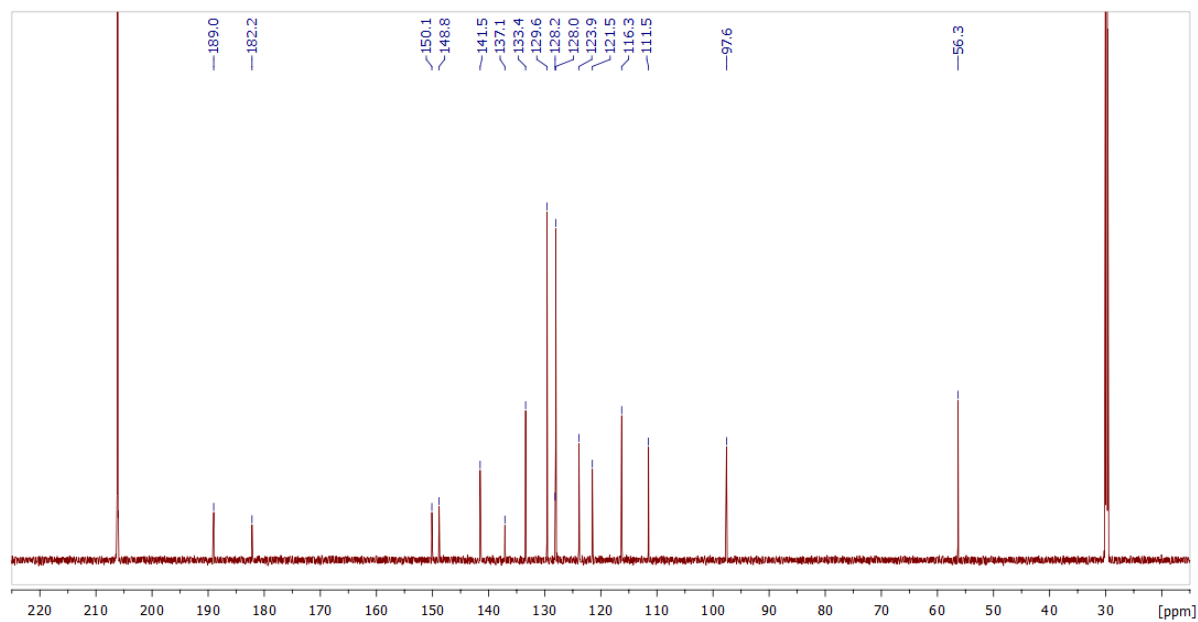
UV-vis absorbance spectrum of compound **4b-B** in acetonitrile.

Compound 4b

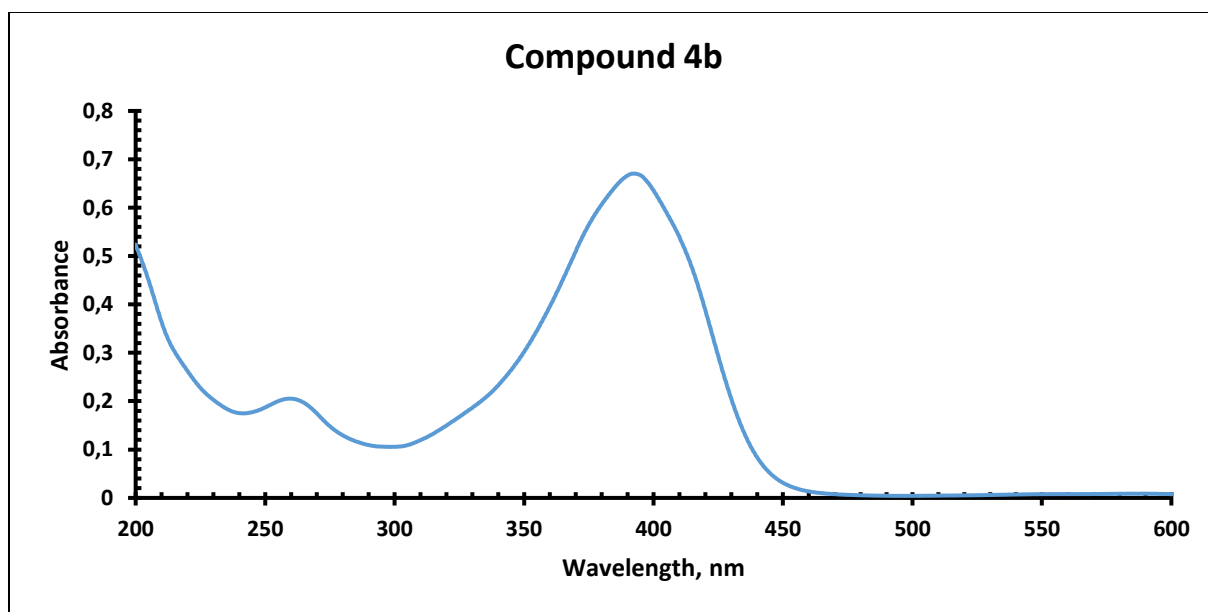
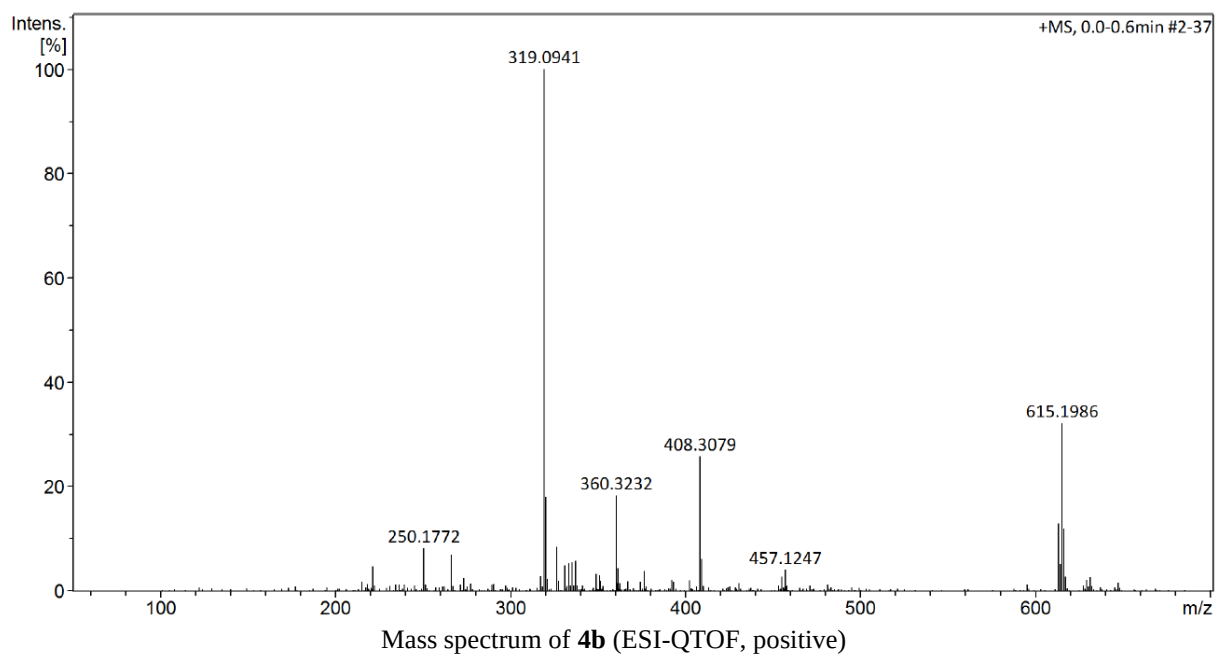
(2Z,4E)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-phenylpenta-2,4-dien-1-one



¹H NMR spectrum of **4b** (acetone-*d*₆, 25 °C)

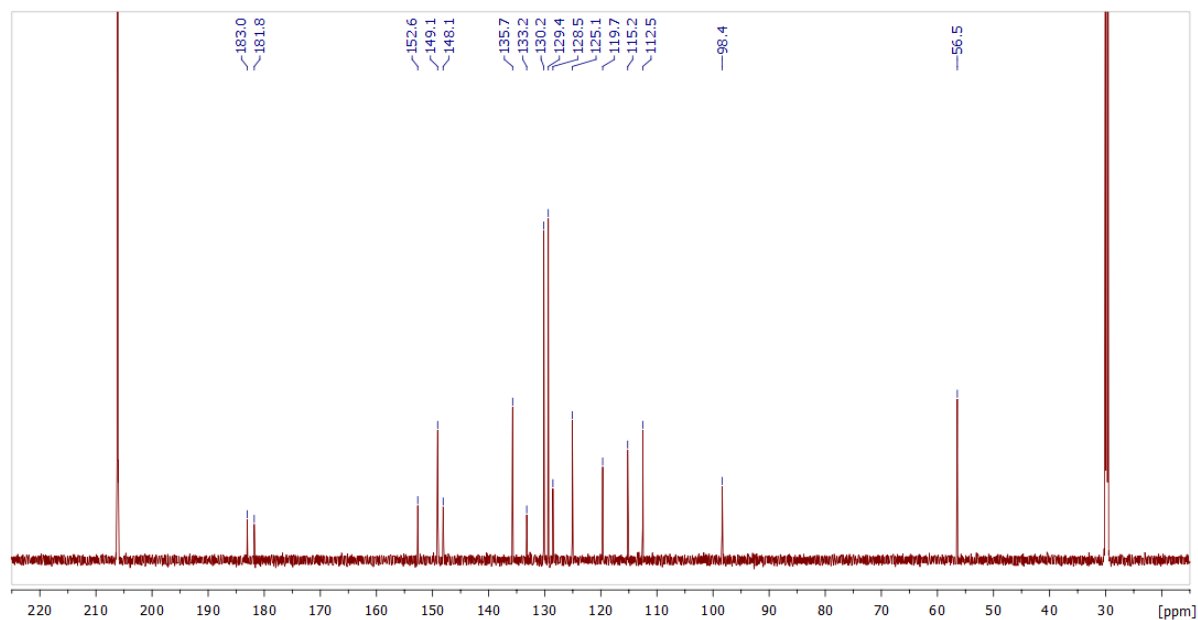
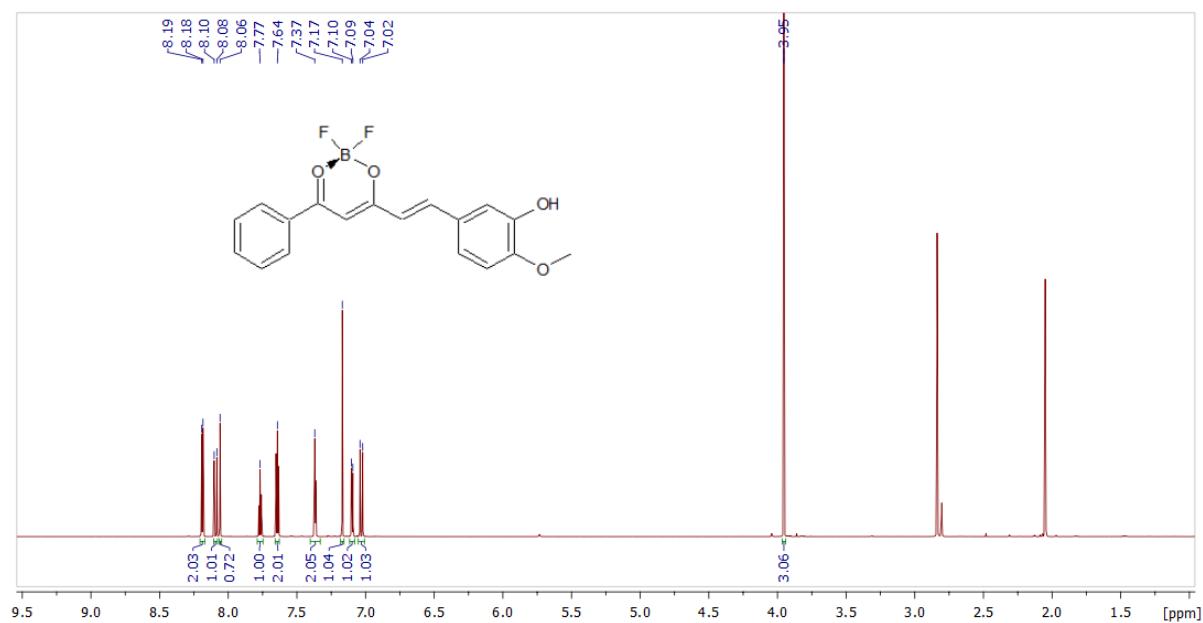


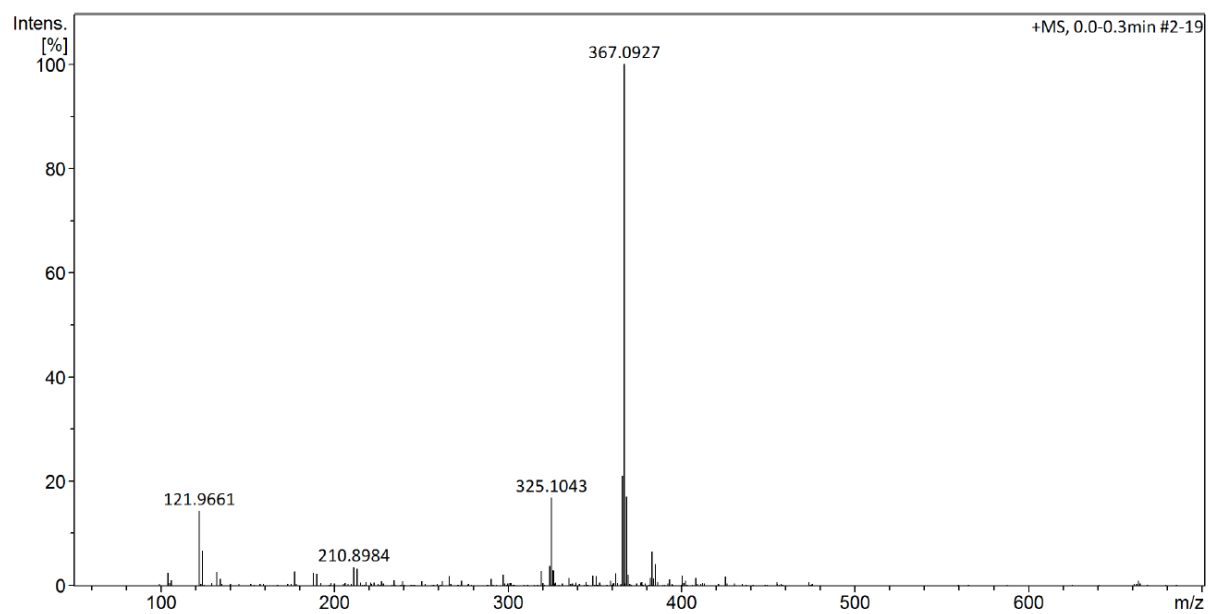
¹³C NMR spectrum of **4b** (acetone-*d*₆, 25 °C)



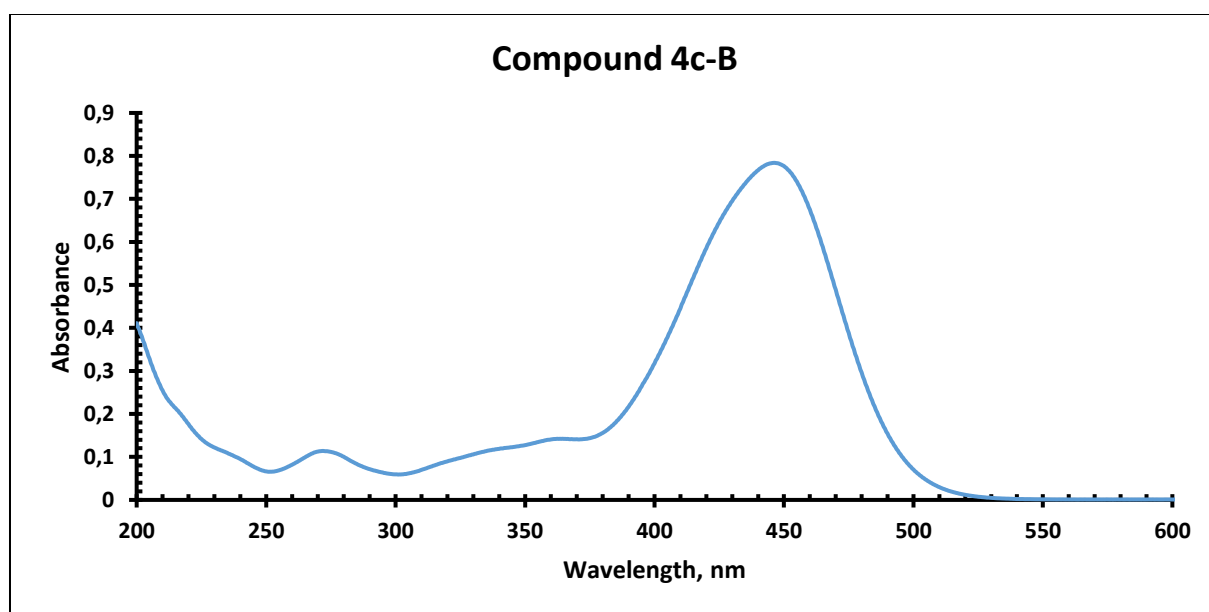
Compound 4c-B

5-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2-methoxyphenol





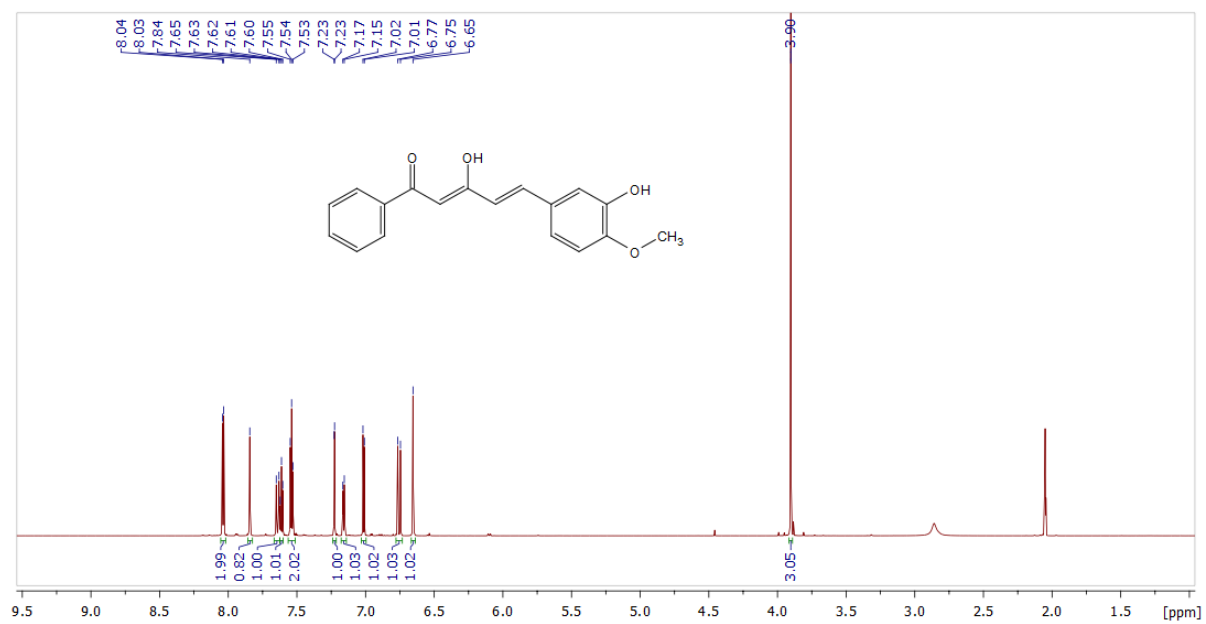
Mass spectrum of **4c-B** (ESI-QTOF, positive)



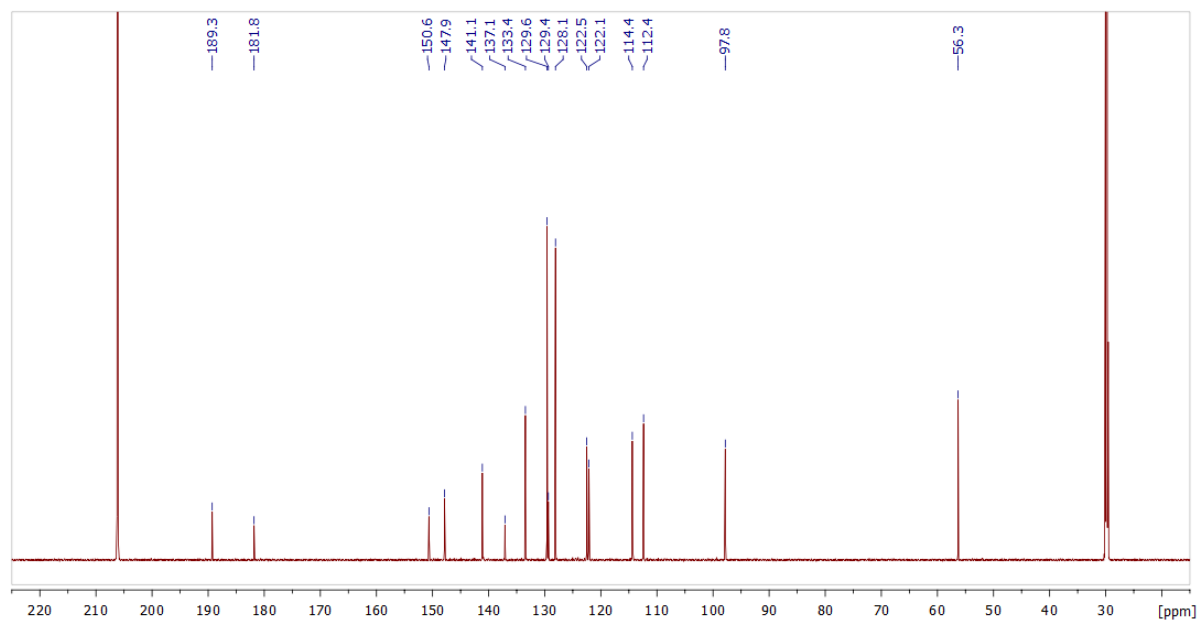
UV-vis absorbance spectrum of compound **4c-B** in acetonitrile.

Compound 4c

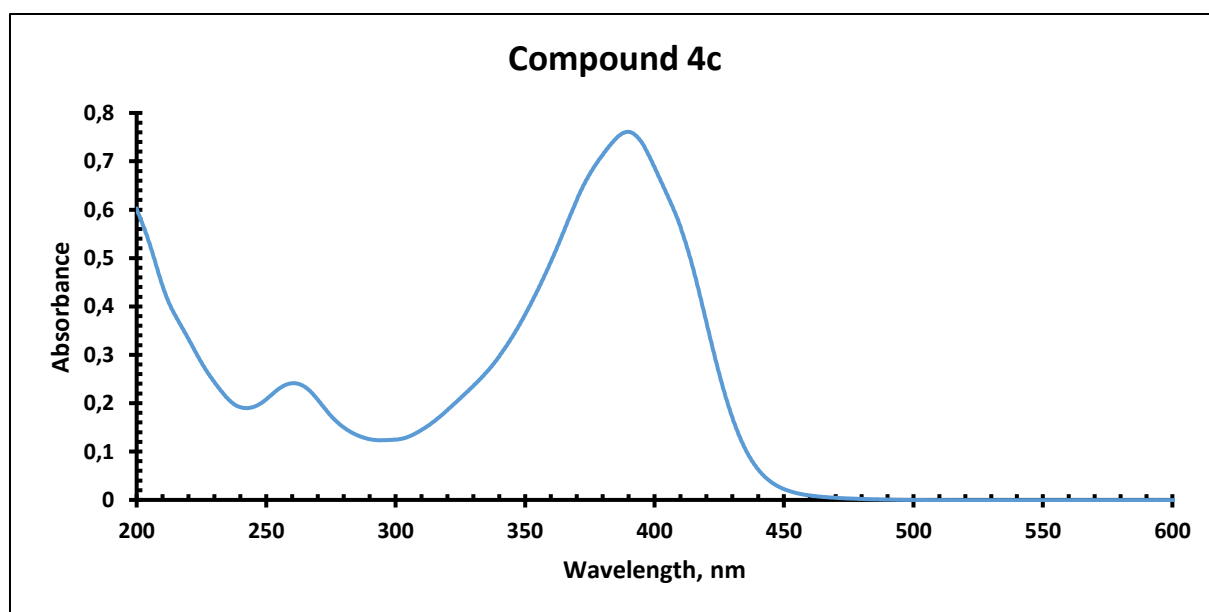
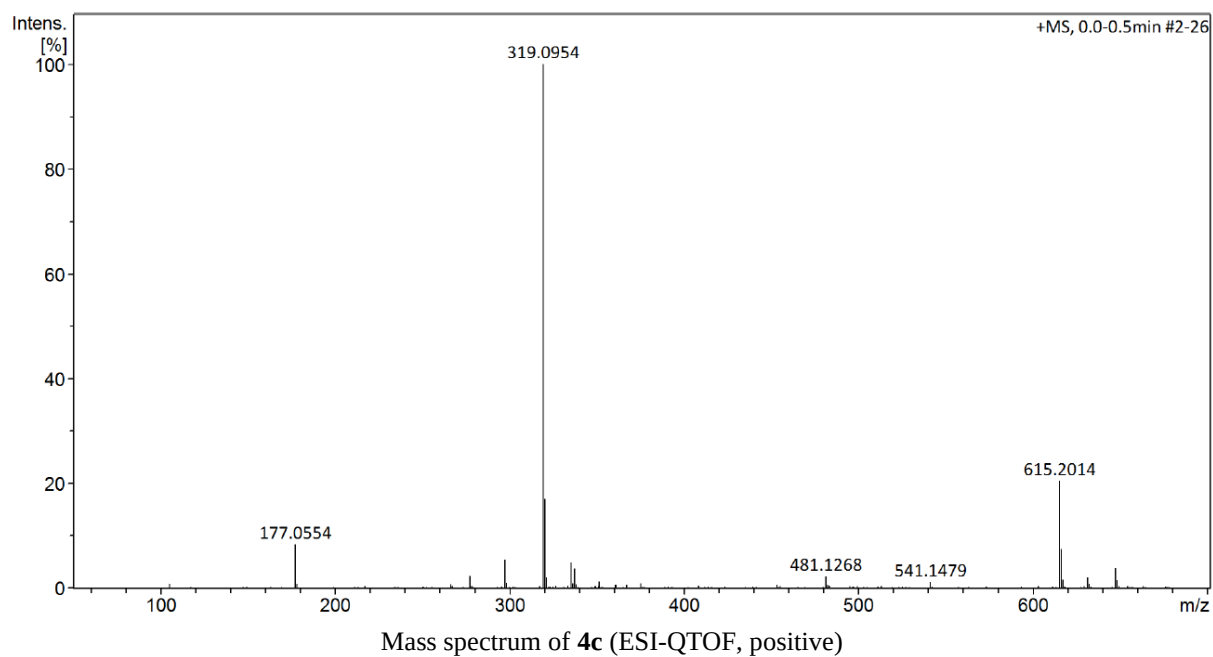
(2Z,4E)-3-hydroxy-5-(3-hydroxy-4-methoxyphenyl)-1-phenylpenta-2,4-dien-1-one



¹H NMR spectrum of **4c** (acetone-*d*₆, 25 °C)

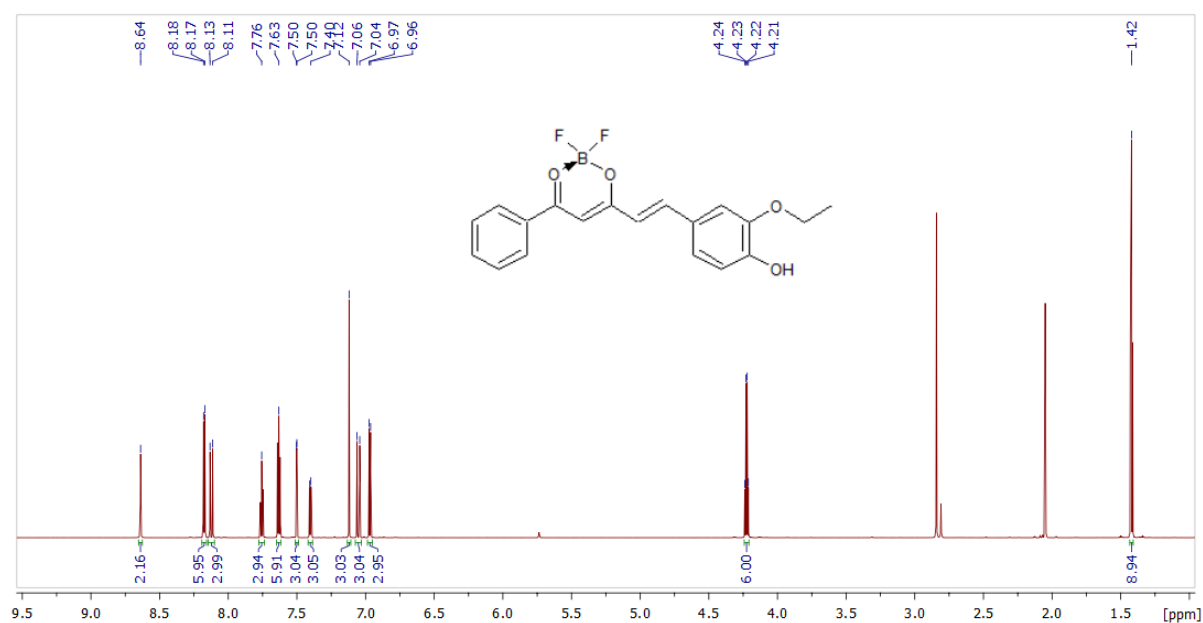


¹³C NMR spectrum of **4c** (acetone-*d*₆, 25 °C)

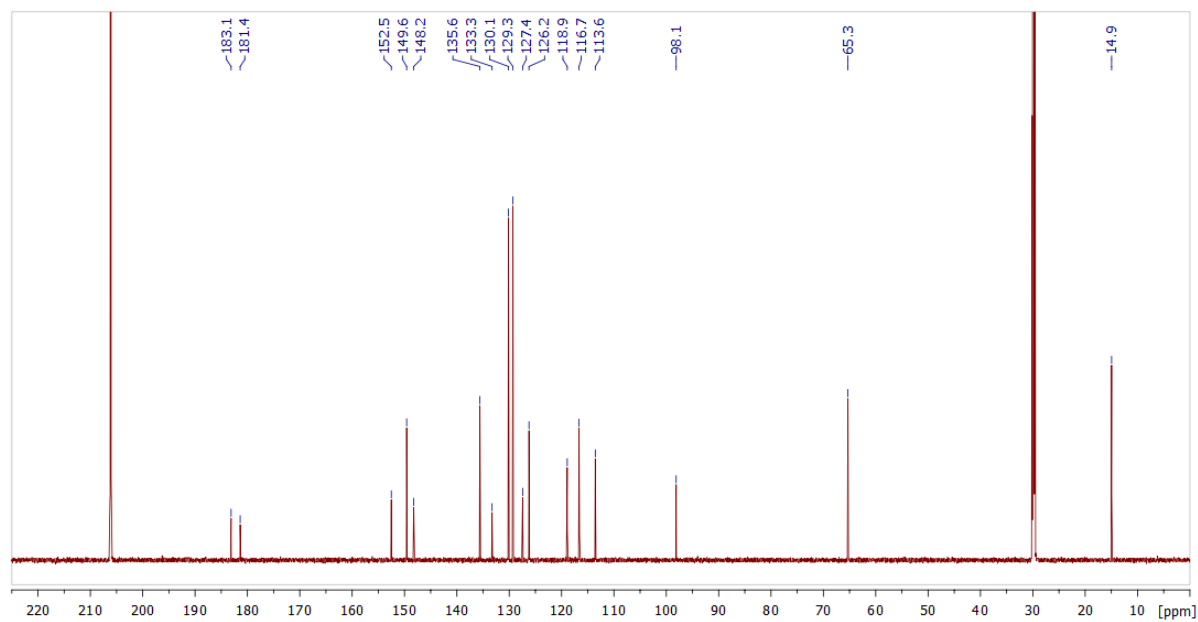


Compound 4d-B

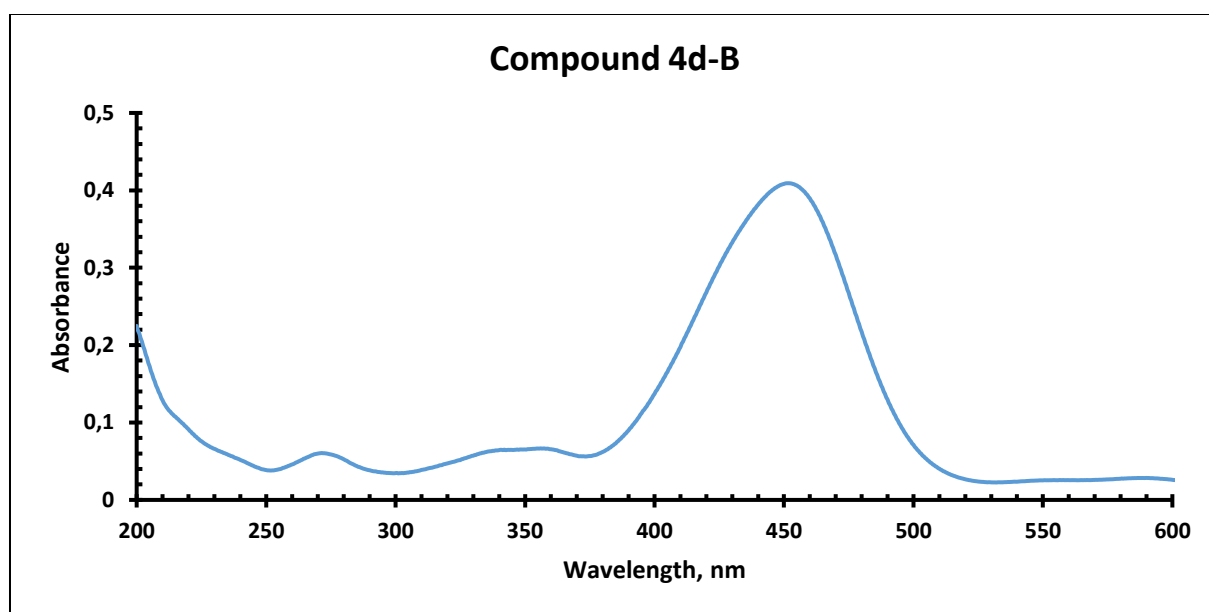
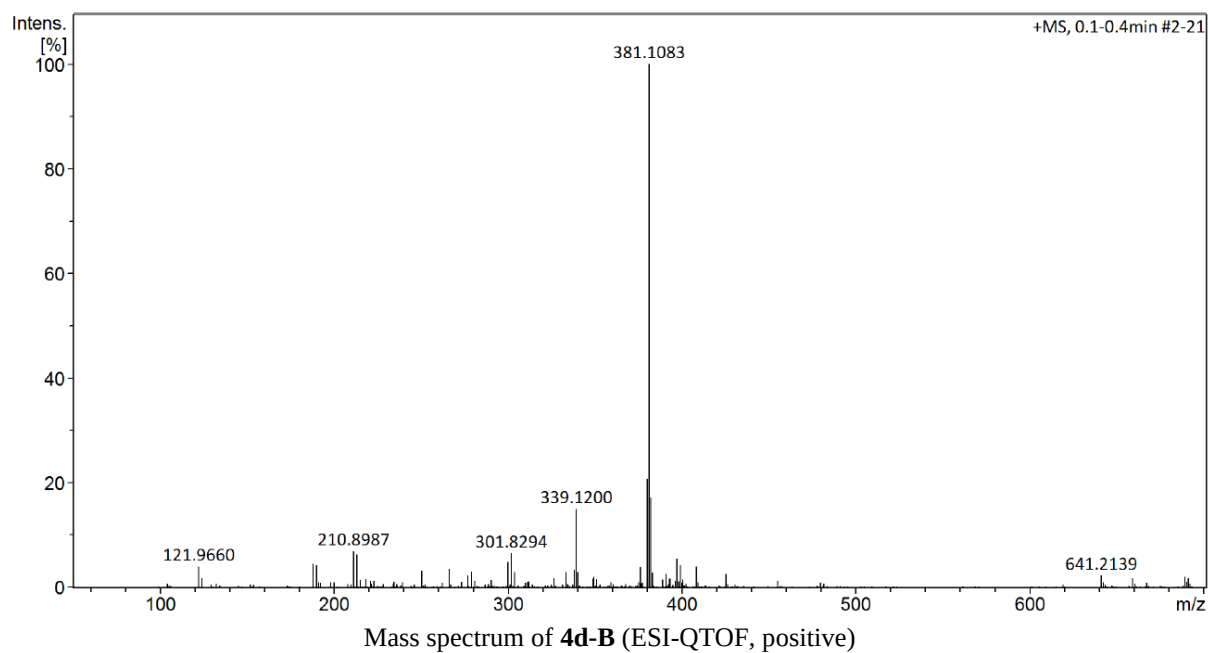
4-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2-ethoxyphenol



¹H NMR spectrum of **4d-B** (acetone-*d*₆, 25 °C)



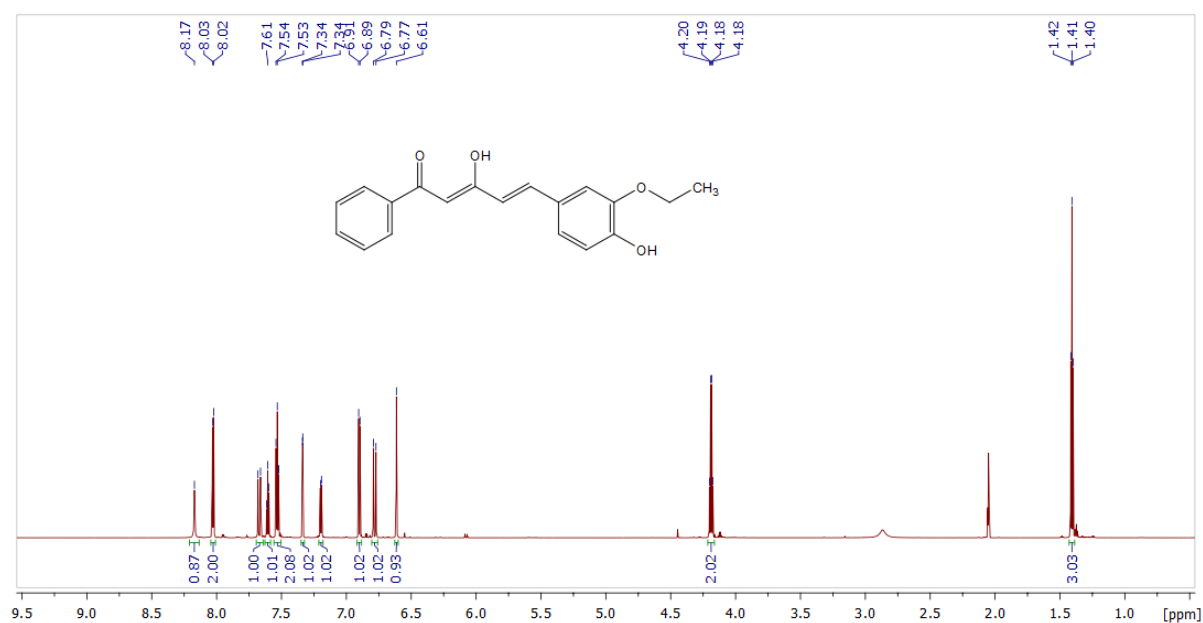
¹³C NMR spectrum of **4d-B** (acetone-*d*₆, 25 °C)



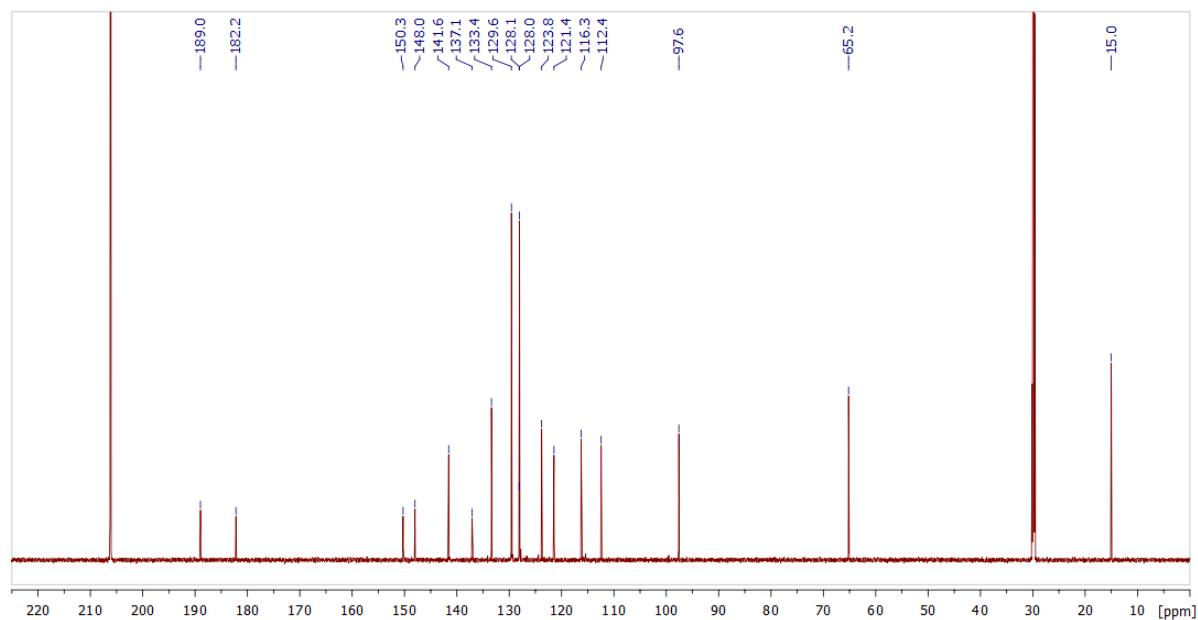
UV-vis absorbance spectrum of compound **4d-B** in acetonitrile.

Compound 4d

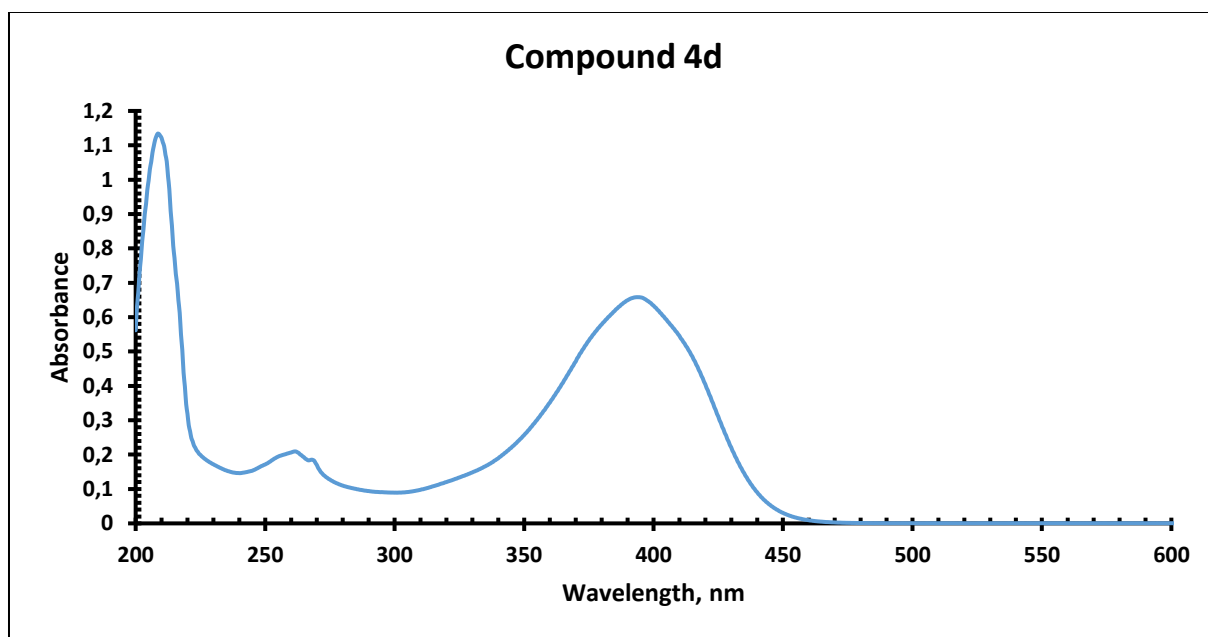
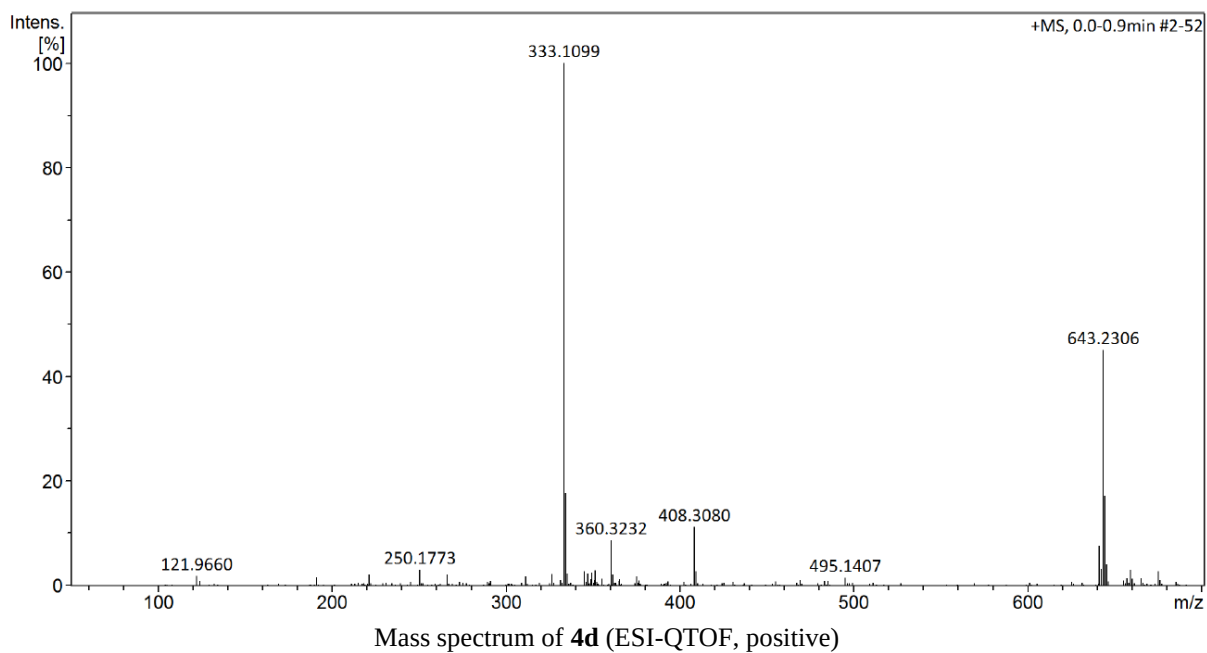
(2Z,4E)-5-(3-ethoxy-4-hydroxyphenyl)-3-hydroxy-1-phenylpenta-2,4-dien-1-one



¹H NMR spectrum of **4d** (acetone-*d*₆, 25 °C)

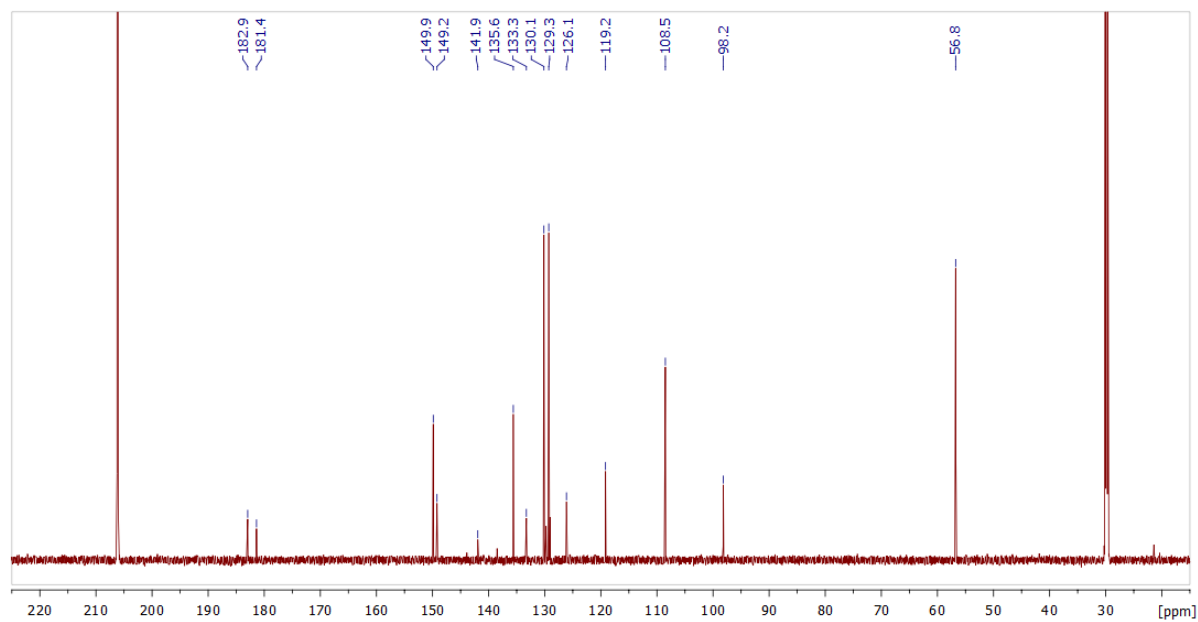
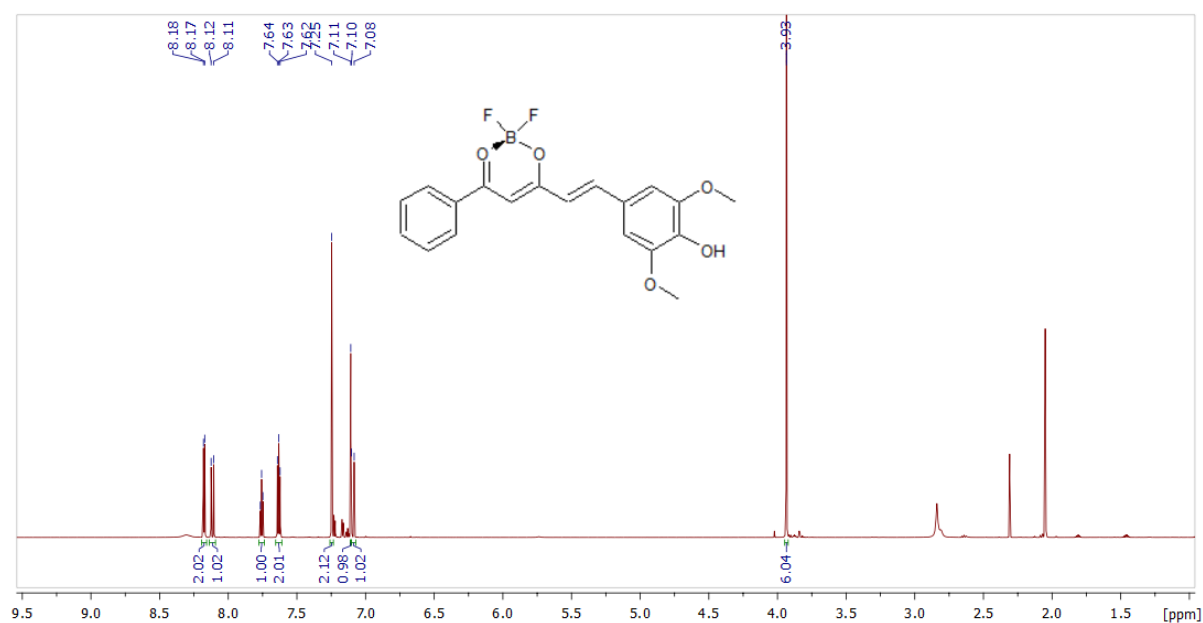


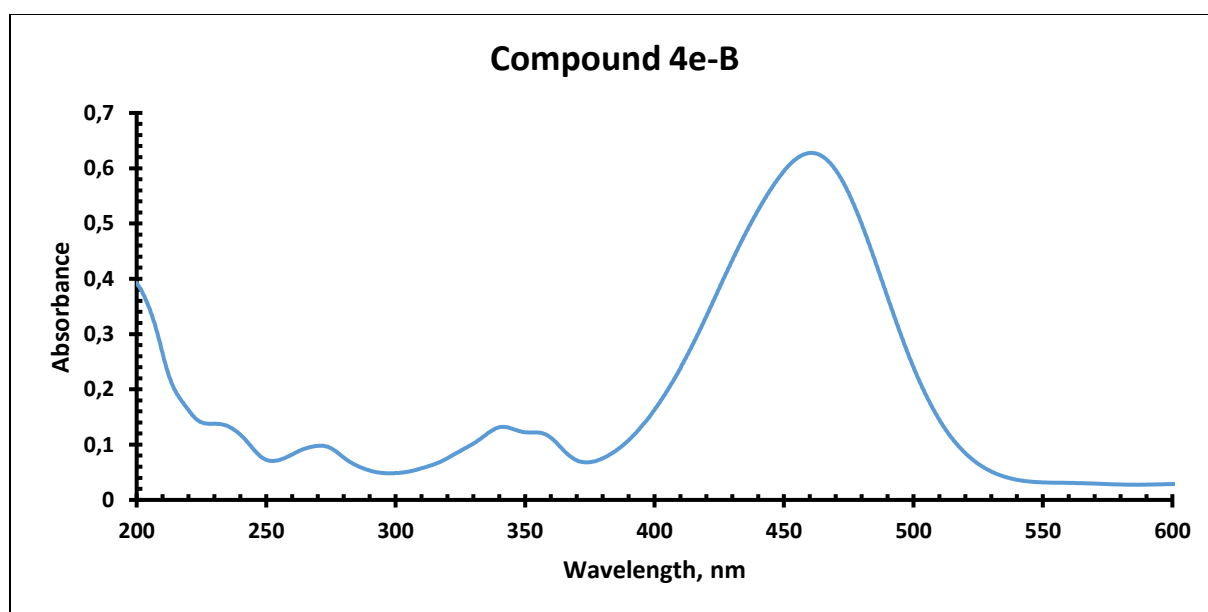
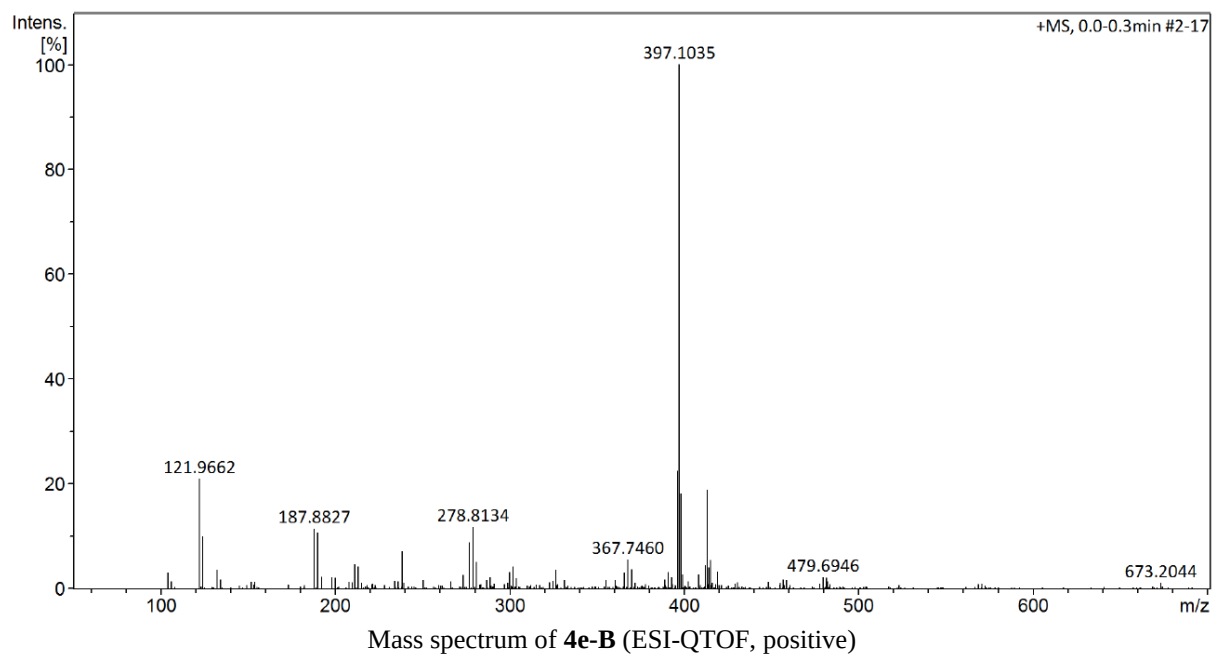
¹³C NMR spectrum of **4d** (acetone-*d*₆, 25 °C)



Compound 4e-B

4-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2,6-dimethoxyphenol

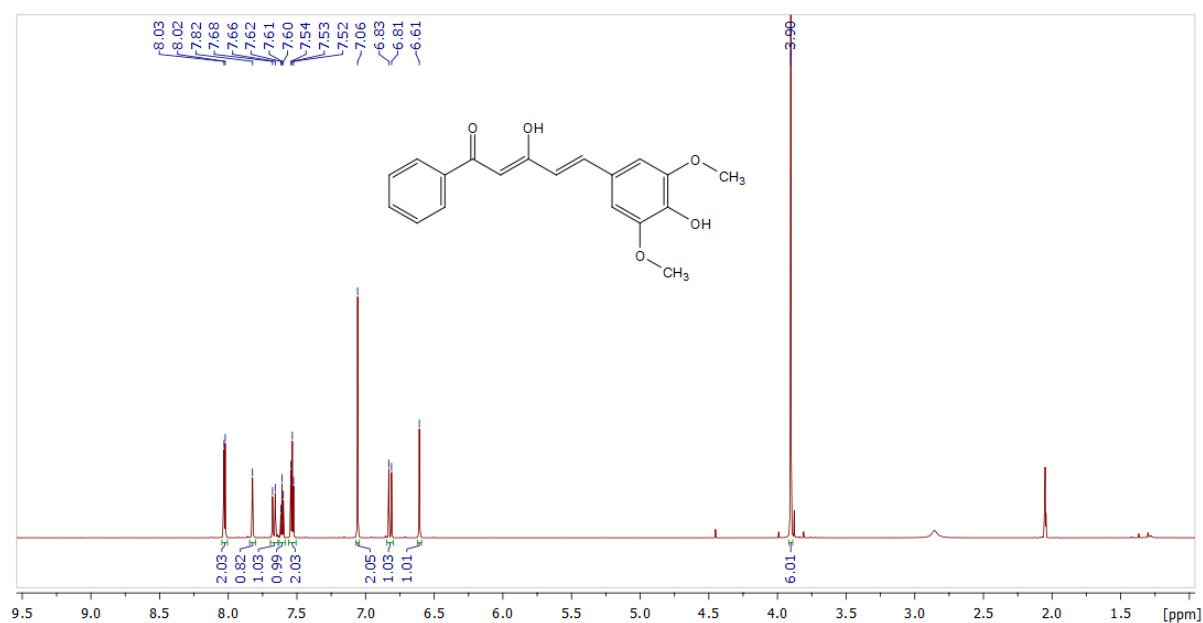




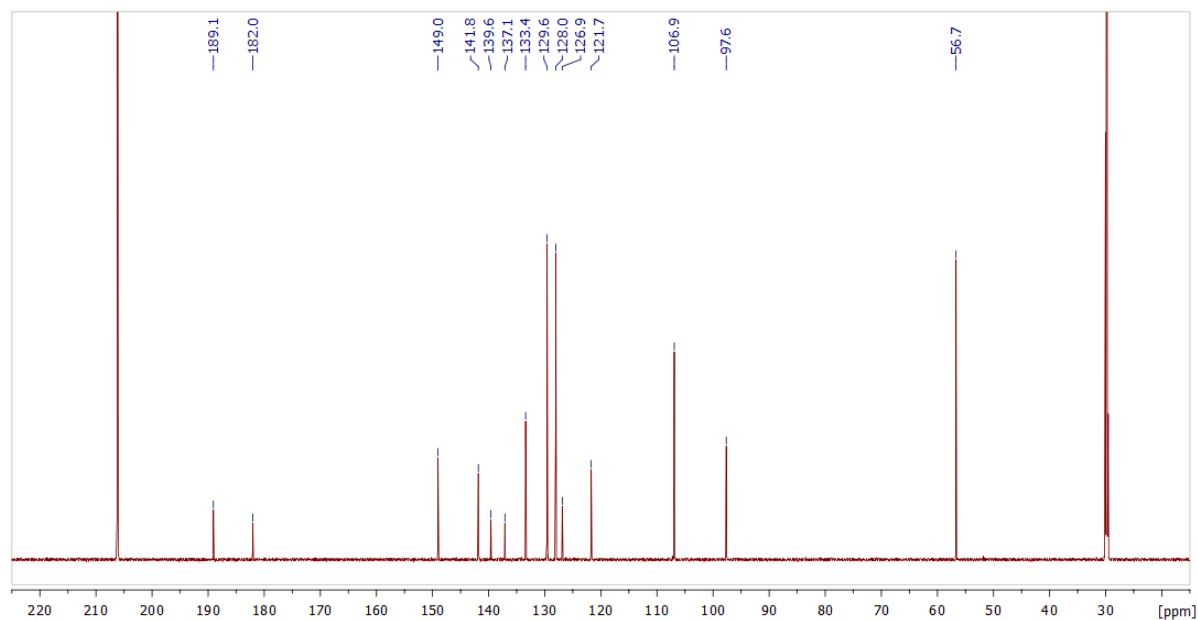
UV-vis absorbance spectrum of compound **4e-B** in acetonitrile.

Compound 4e

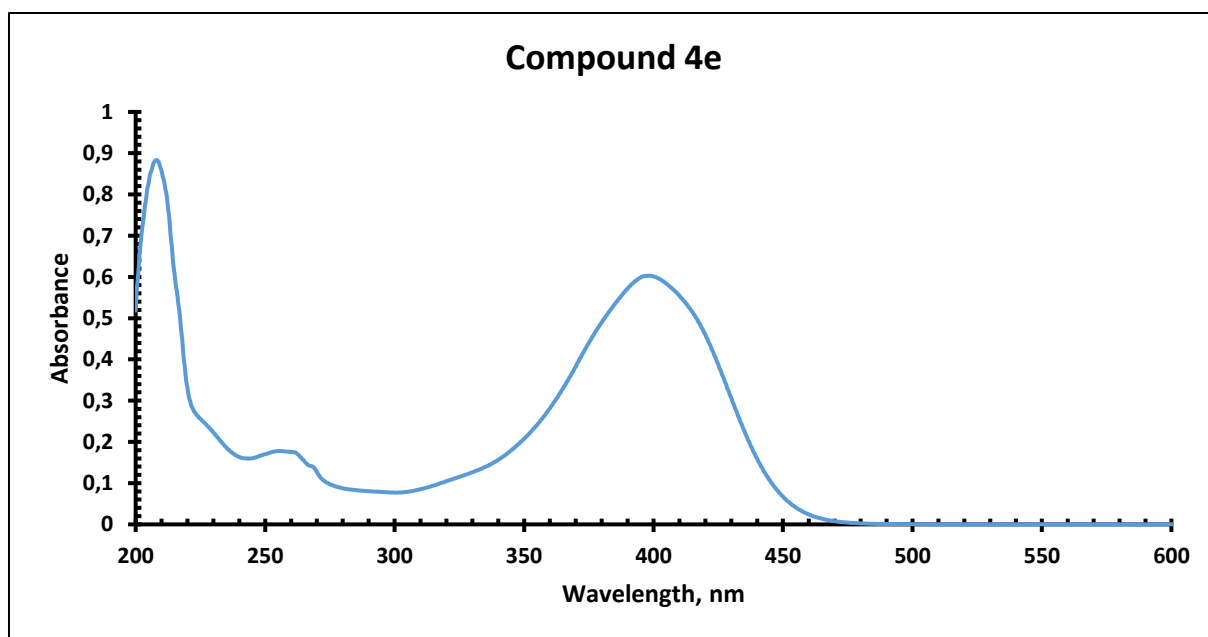
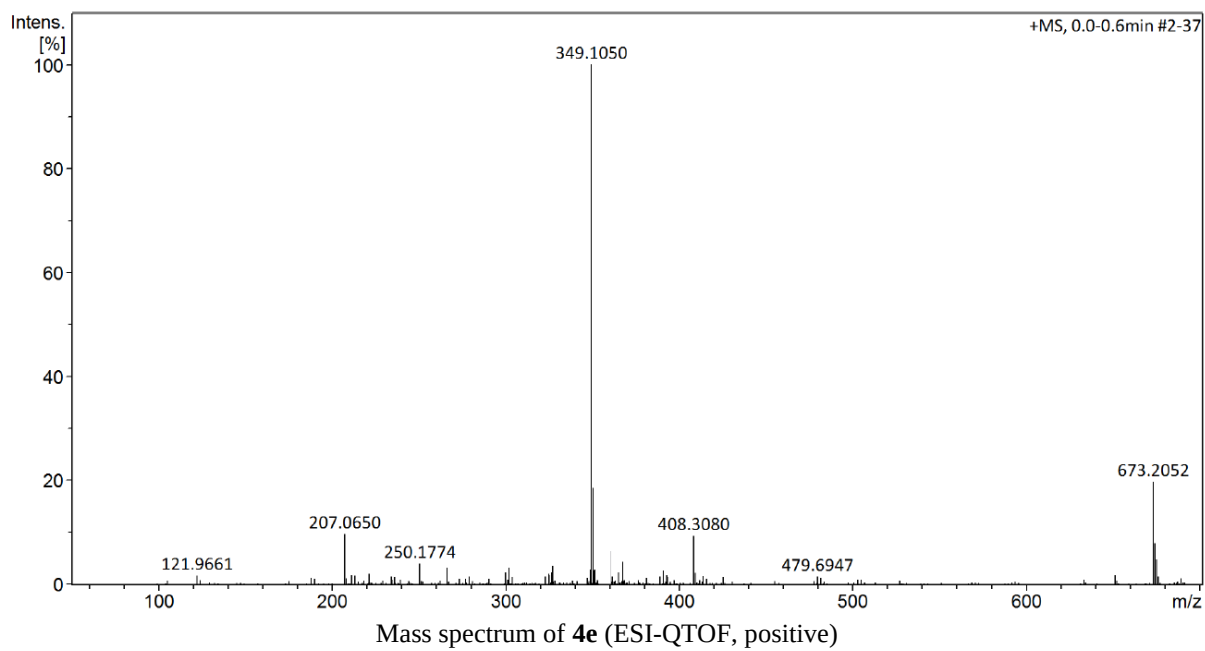
(2Z,4E)-3-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-1-phenylpenta-2,4-dien-1-one



¹H NMR spectrum of **4e** (acetone-*d*₆, 25 °C)

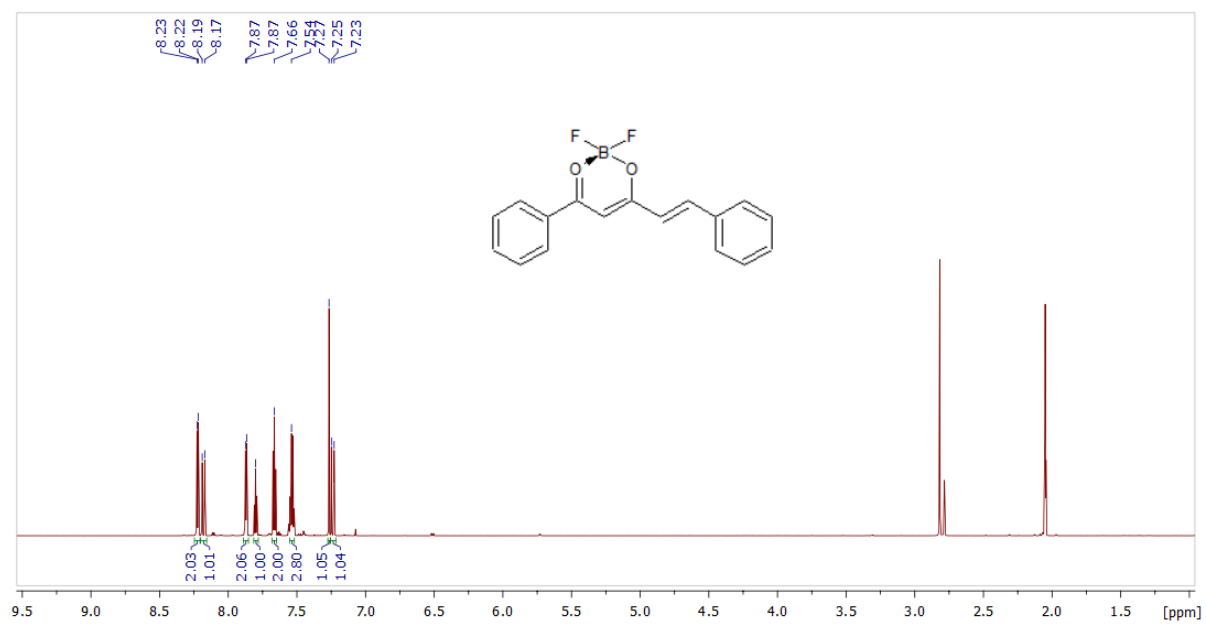


¹³C NMR spectrum of **4e** (acetone-*d*₆, 25 °C)

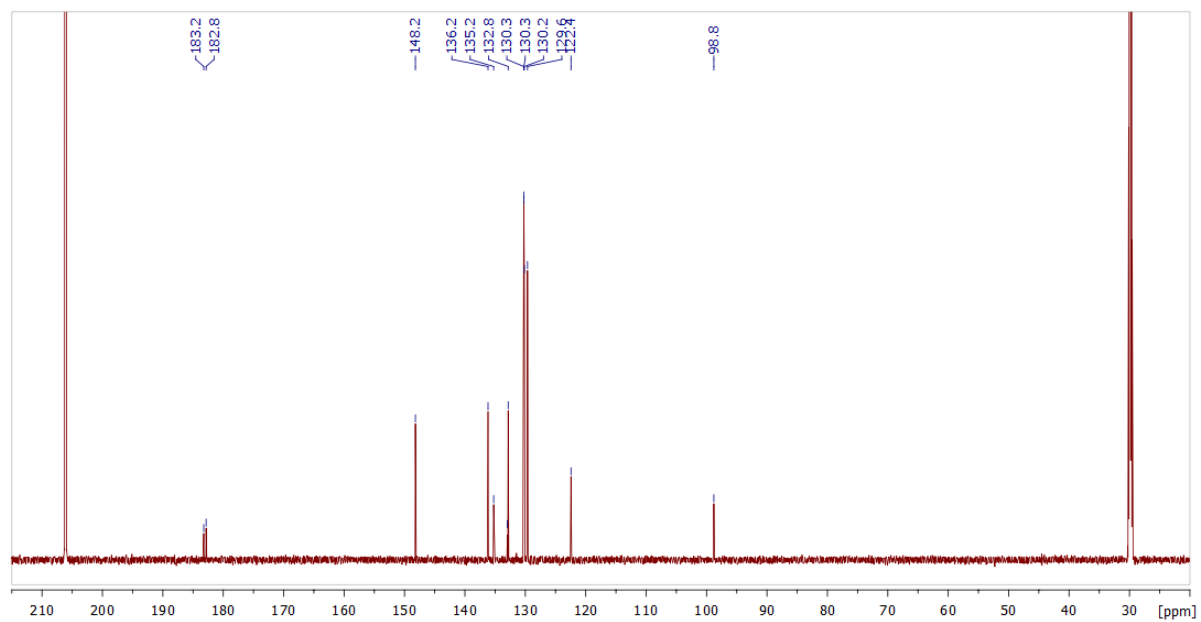


Compound 4f-B

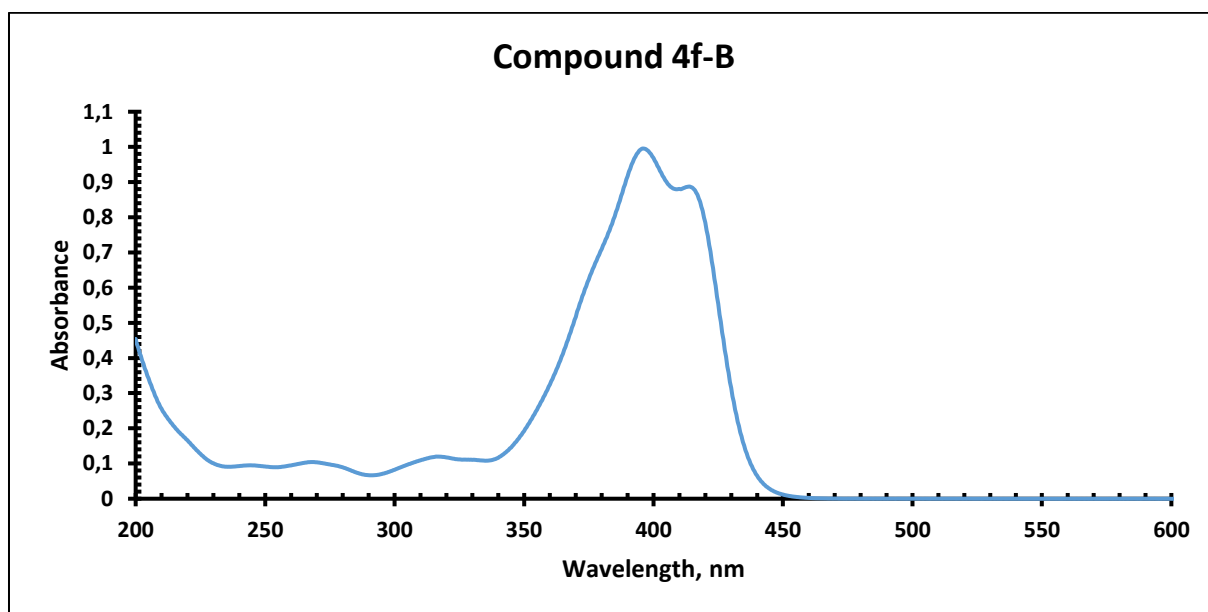
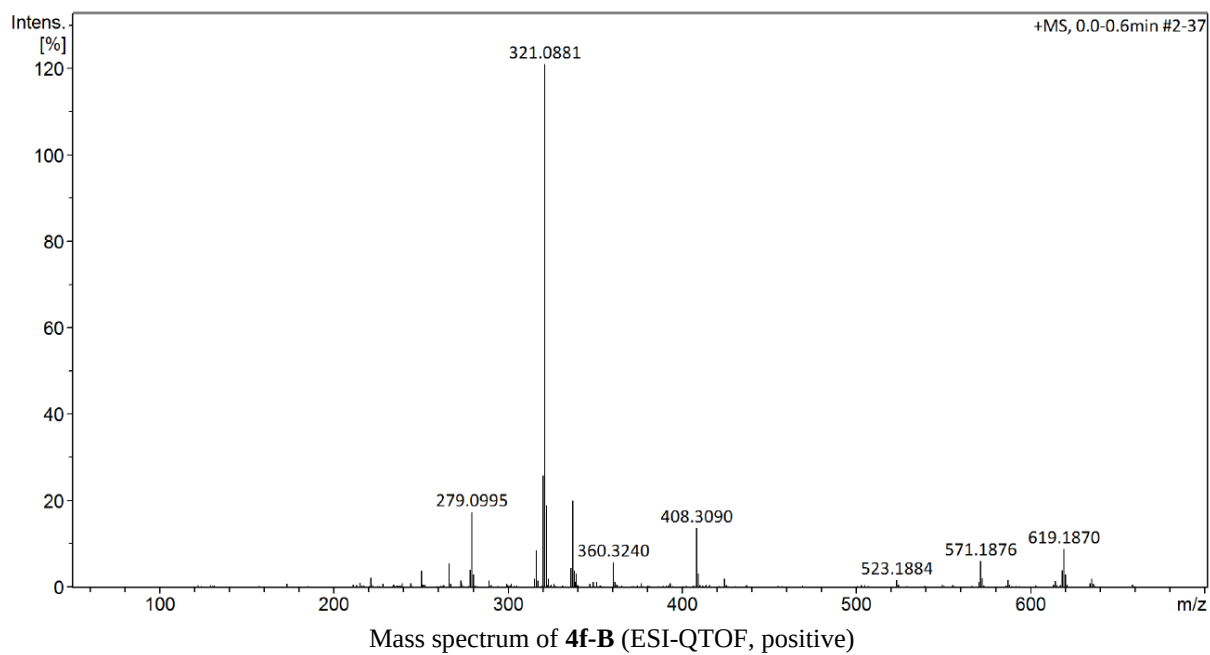
2,2-difluoro-4-phenyl-6-[(1E)-2-phenylethenyl]-1,3,2-dioxaborinine



^1H NMR spectrum of **4f-B** (acetone- d_6 , 25 °C)

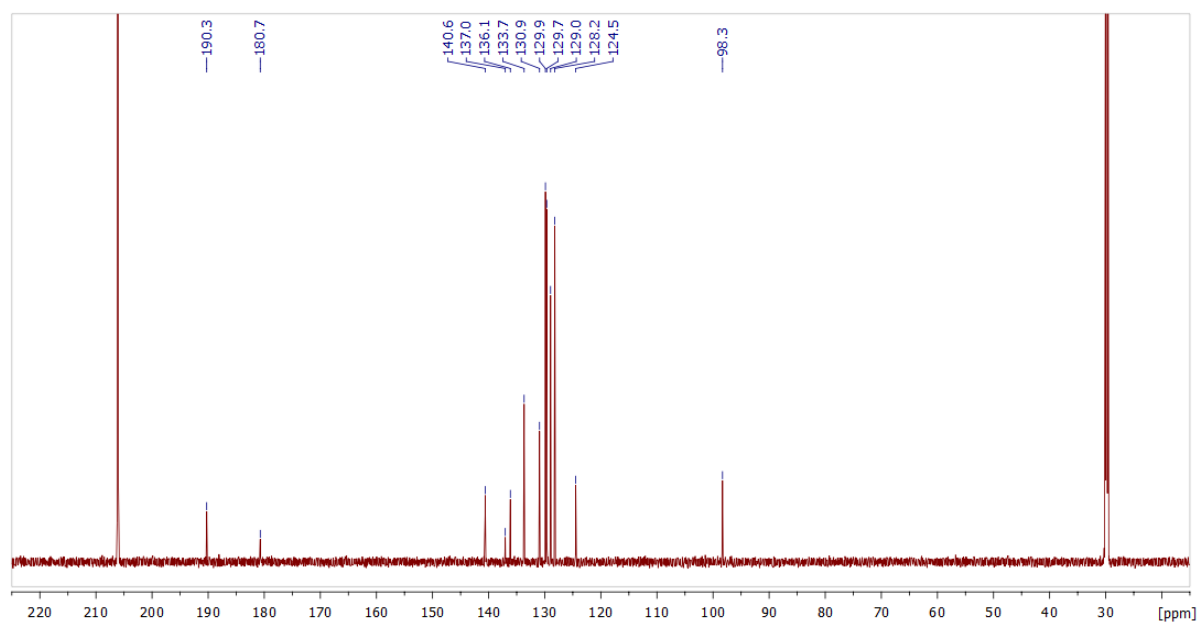
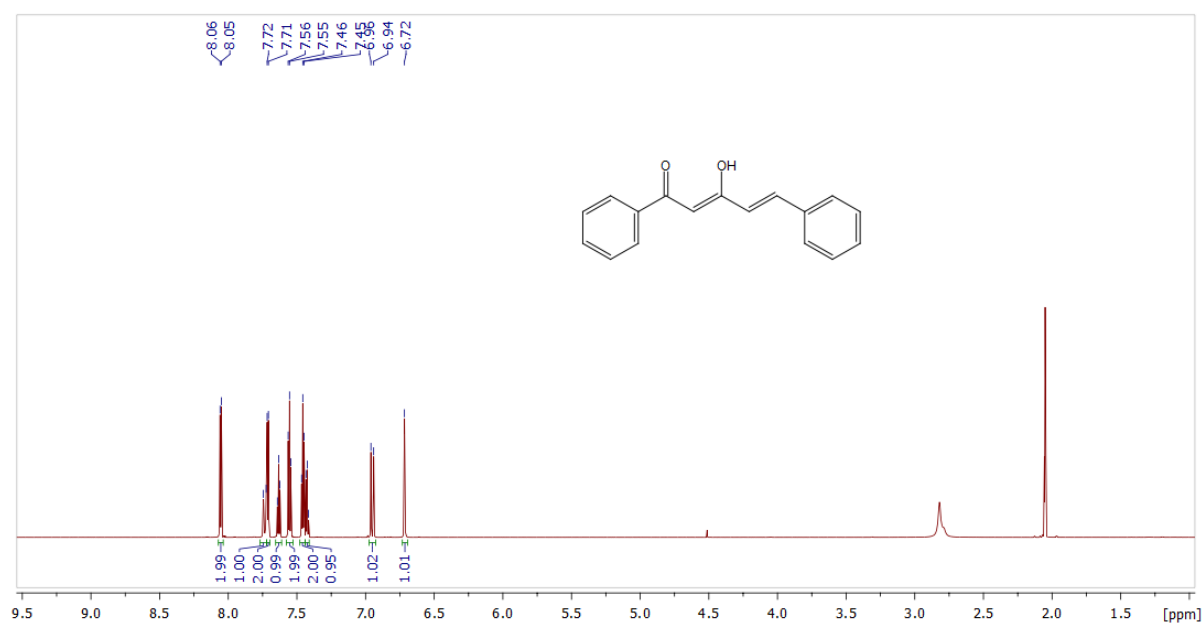


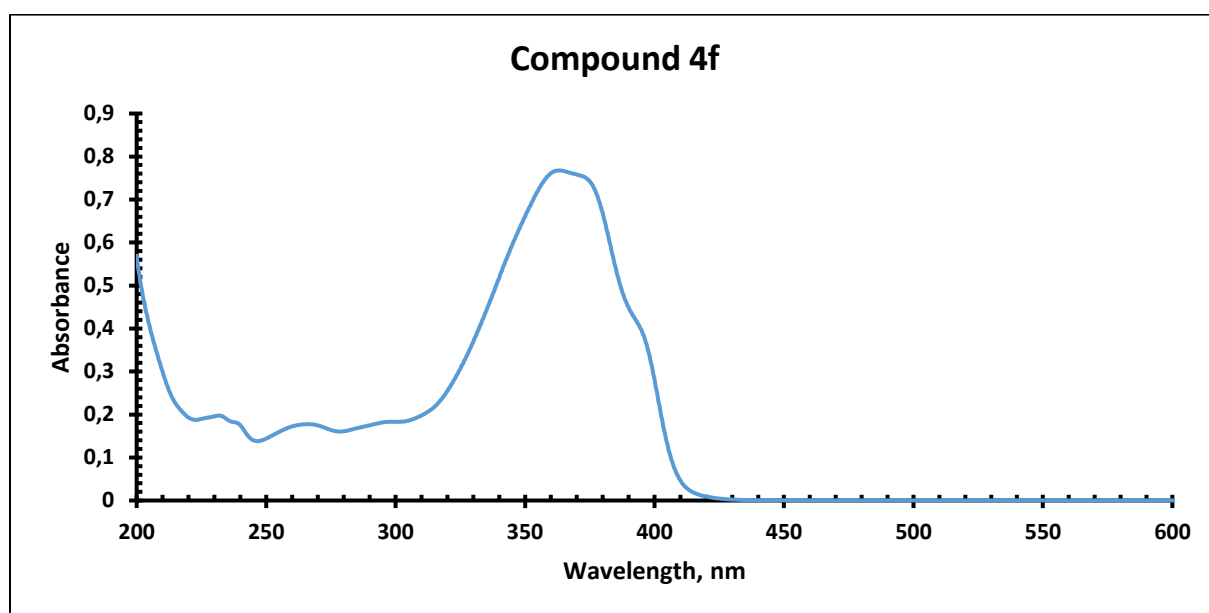
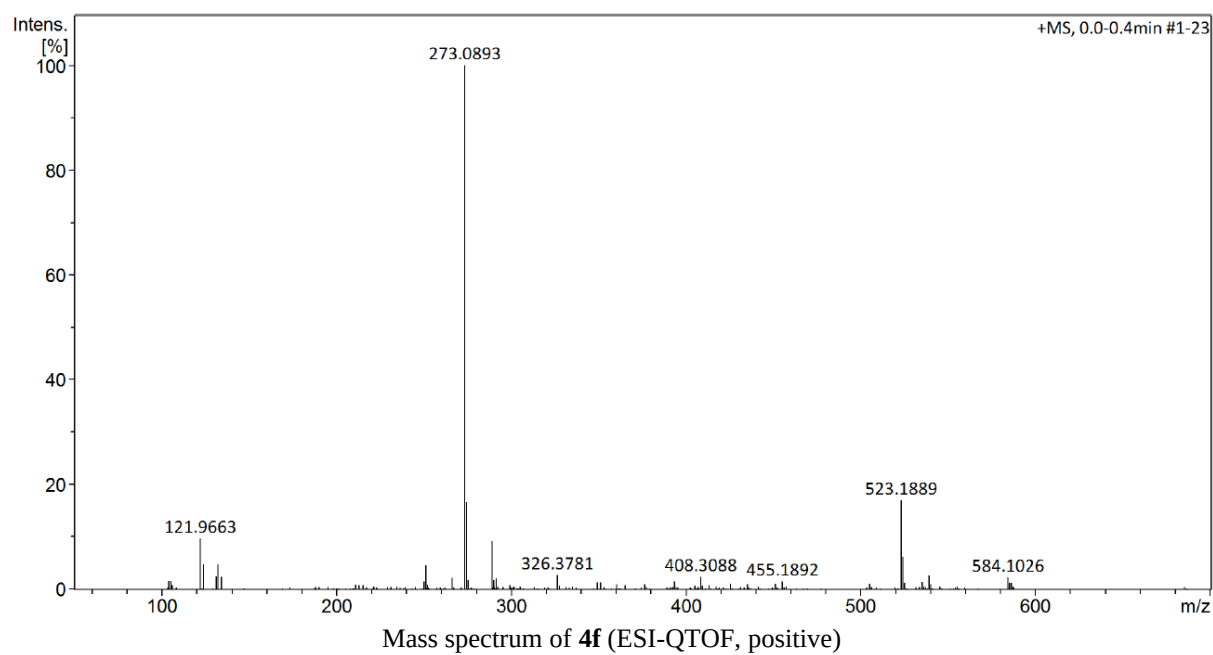
^{13}C NMR spectrum of **4f-B** (acetone- d_6 , 25 °C)



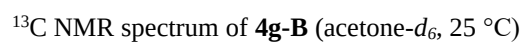
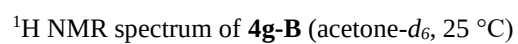
Compound 4f

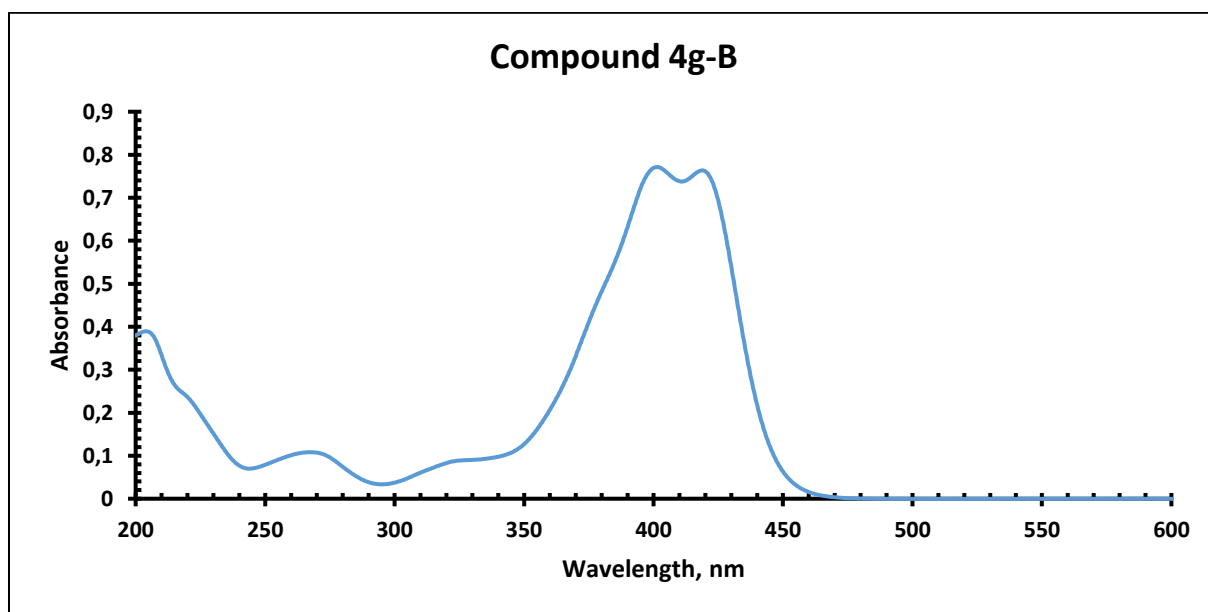
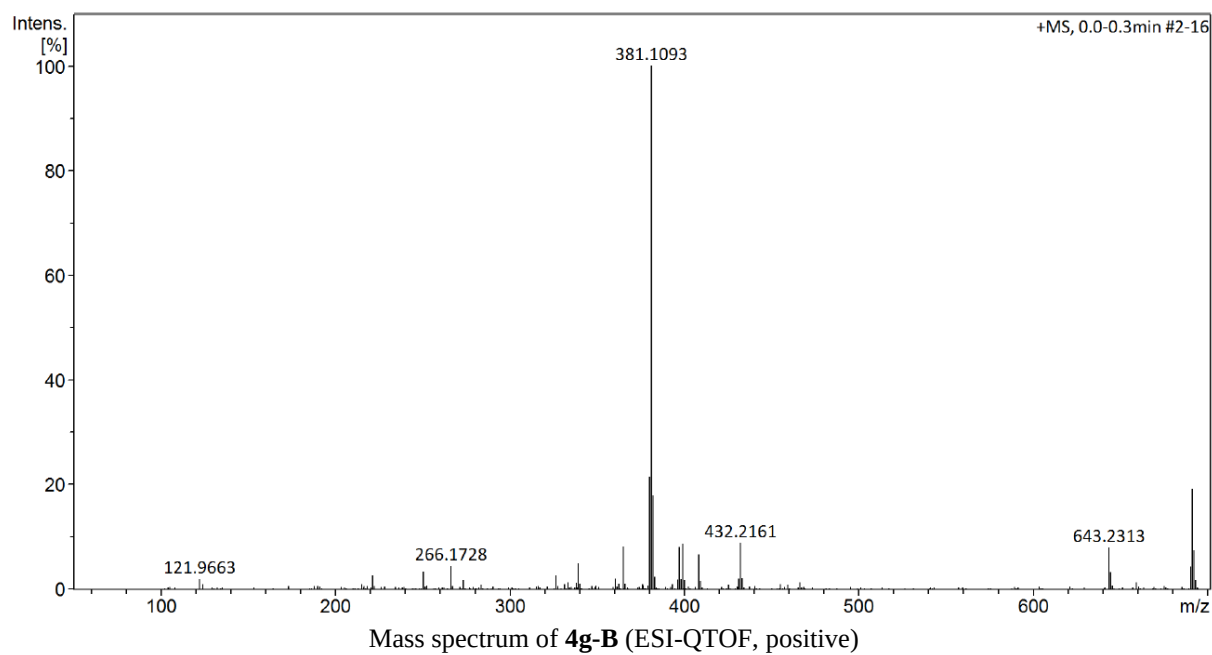
(2Z,4E)-3-hydroxy-1,5-diphenylpenta-2,4-dien-1-one





4-[(1E)-2-(3,5-dimethoxyphenyl)ethenyl]-2,2-difluoro-6-phenyl-1,3,2-dioxaborinine

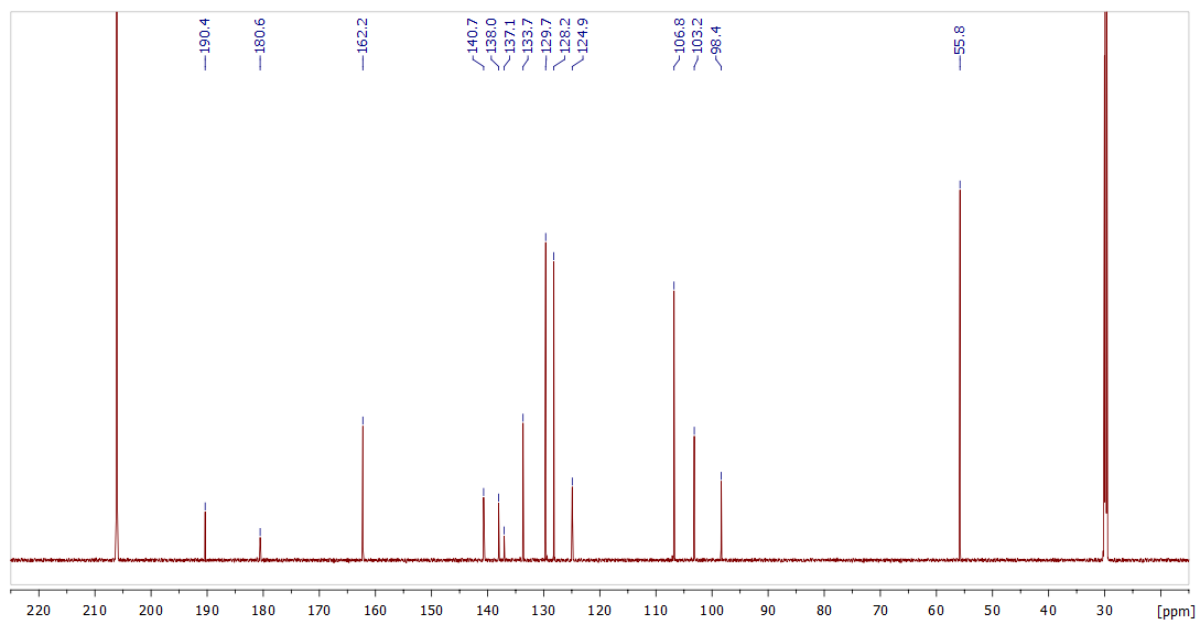
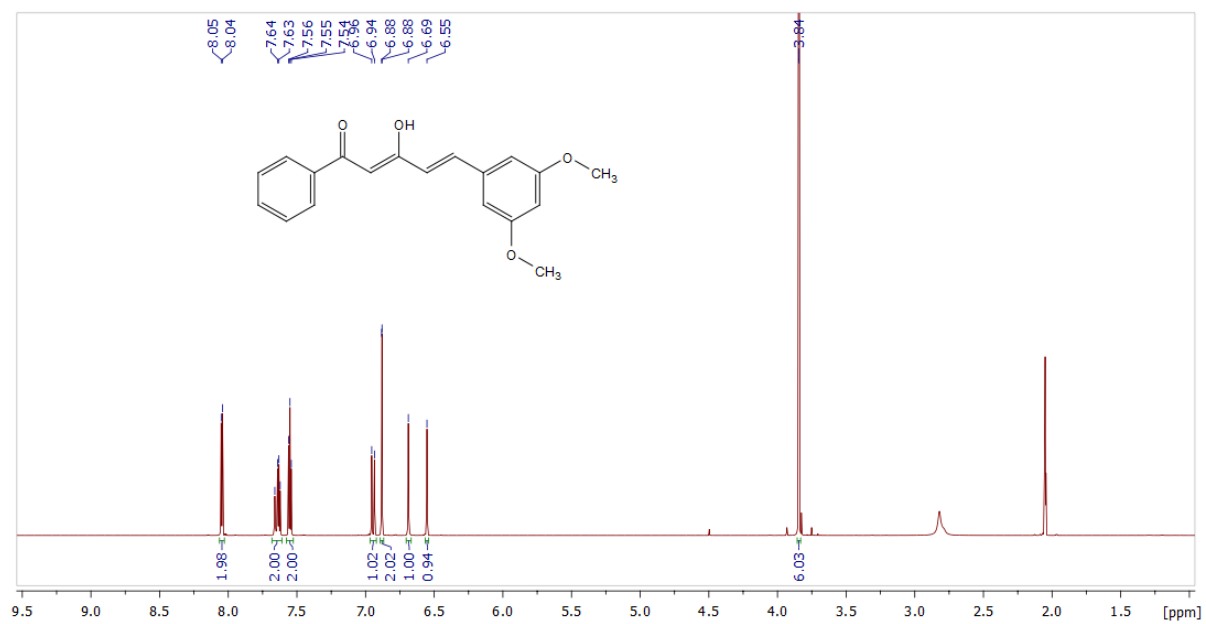


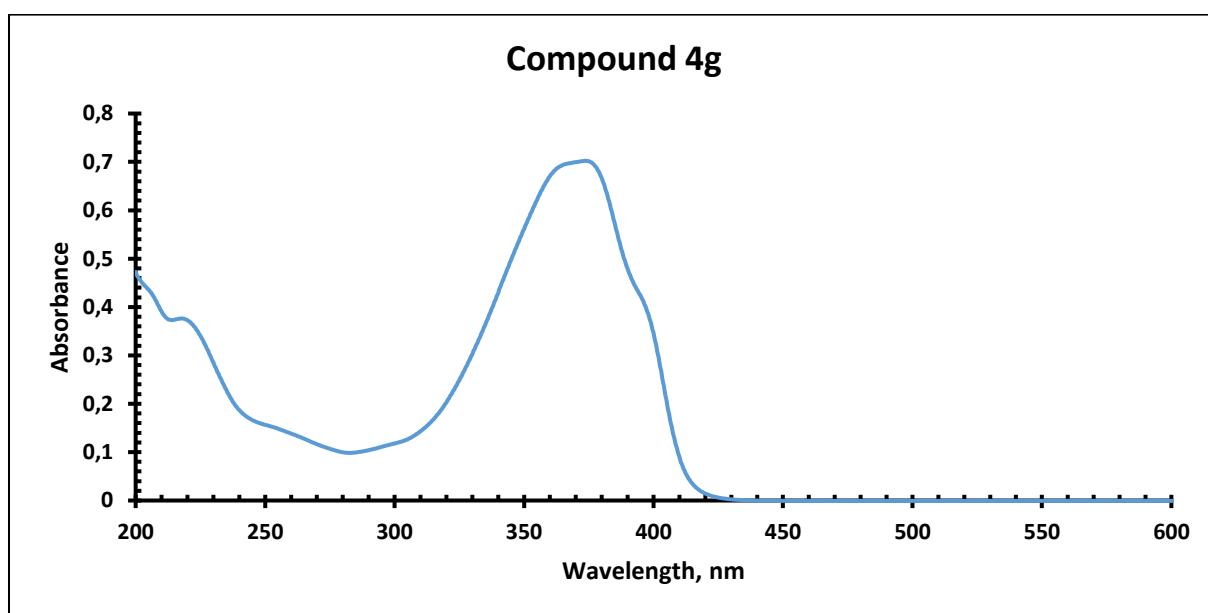
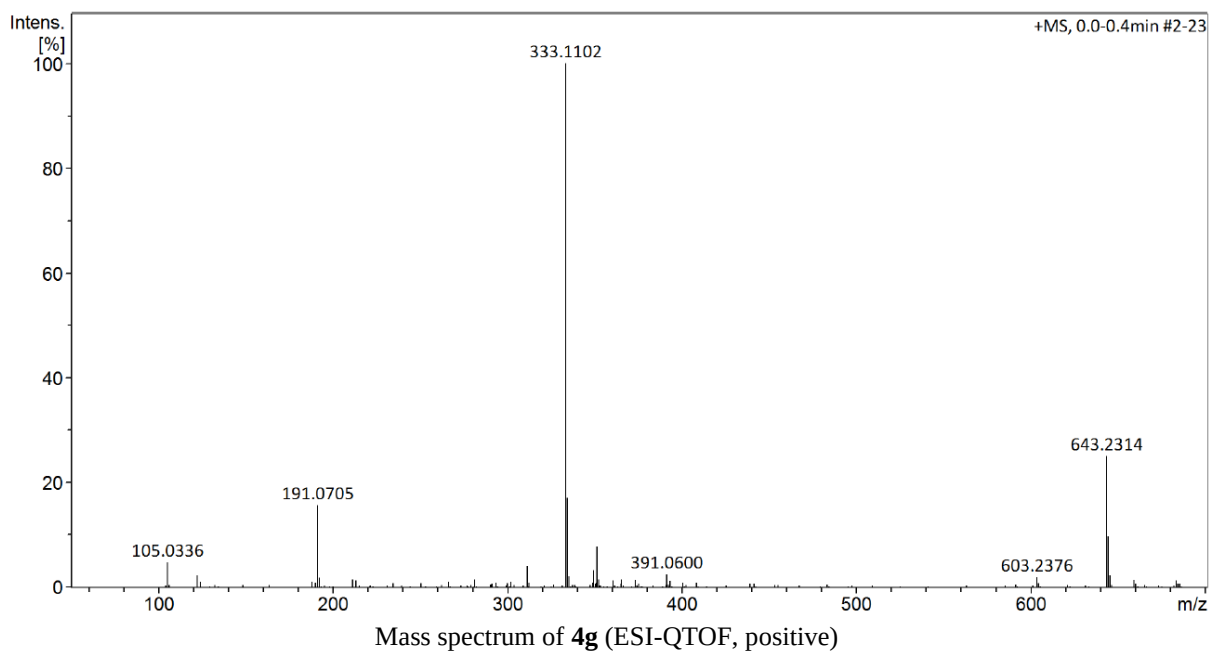


UV-vis absorbance spectrum of compound **4g-B** in acetonitrile.

Compound 4g

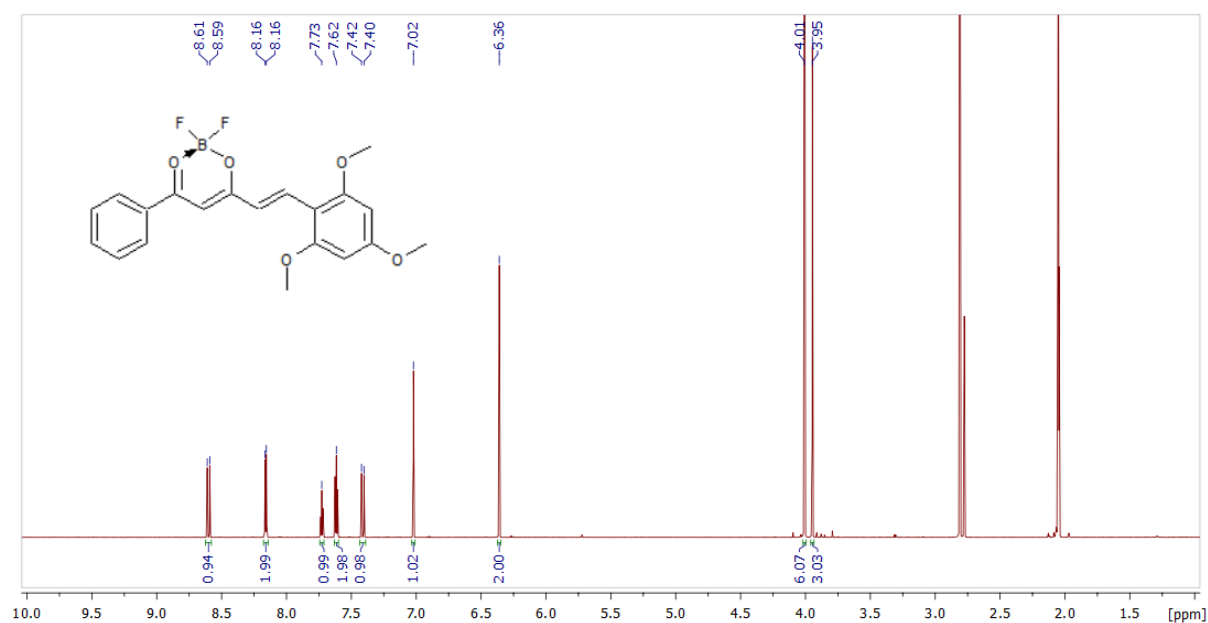
(2Z,4E)-5-(3,5-dimethoxyphenyl)-3-hydroxy-1-phenylpenta-2,4-dien-1-one



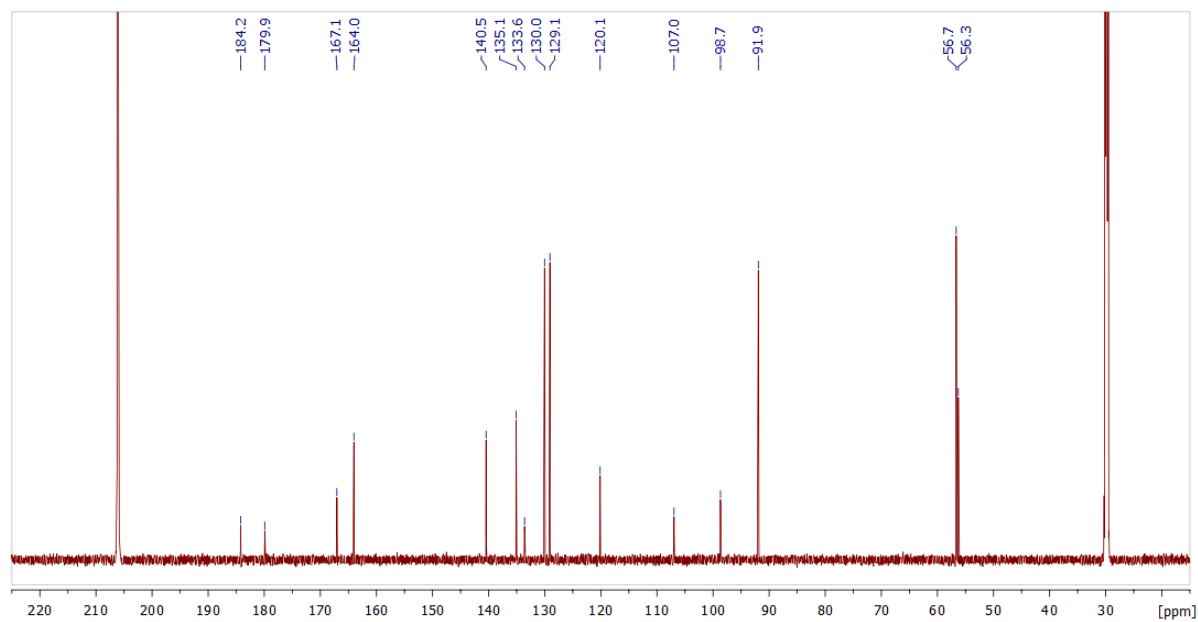


Compound 4h-B

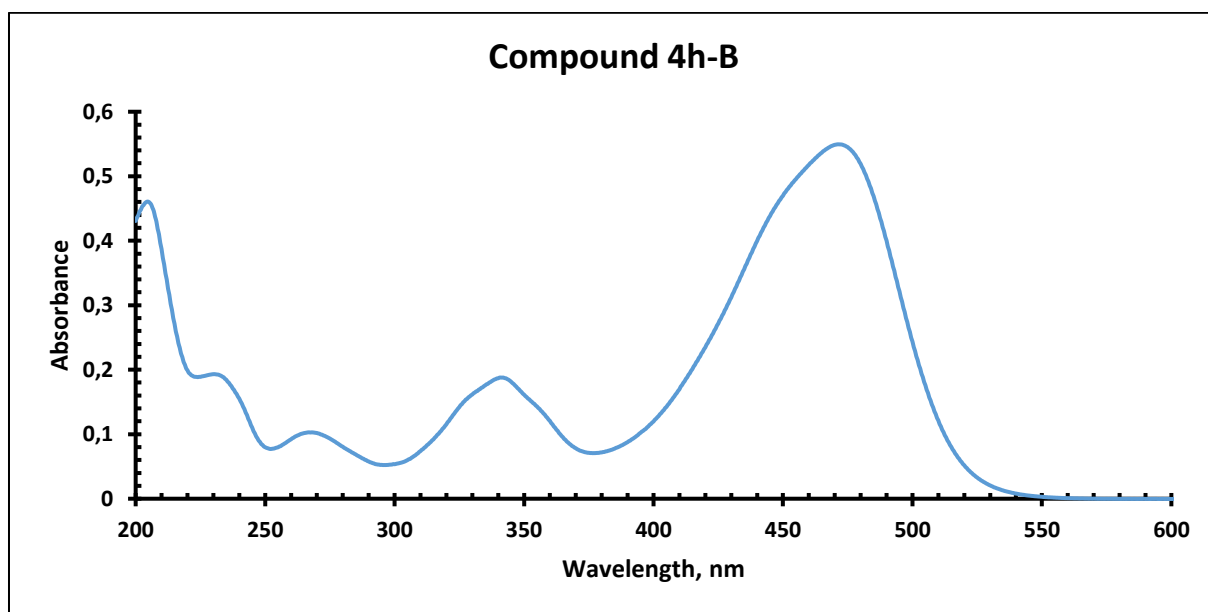
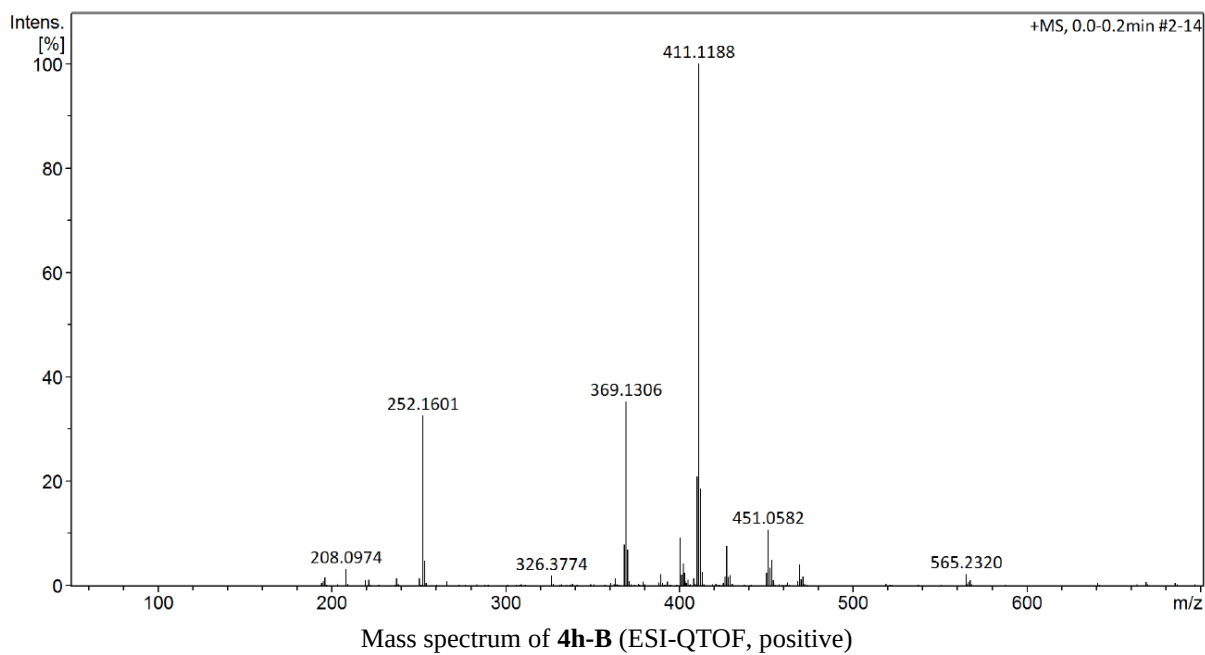
2,2-difluoro-4-phenyl-6-[(1*E*)-2-(2,4,6-trimethoxyphenyl)ethenyl]-1,3,2-dioxaborinine



¹H NMR spectrum of **4h-B** (acetone-*d*₆, 25 °C)



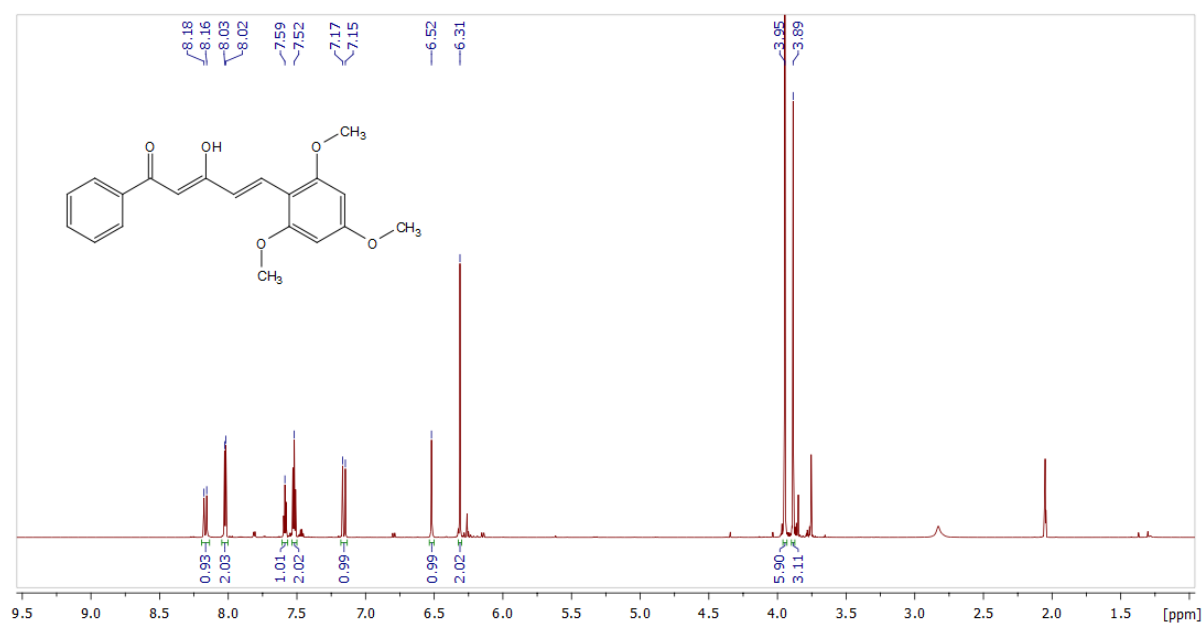
¹³C NMR spectrum of **4h-B** (acetone-*d*₆, 25 °C)



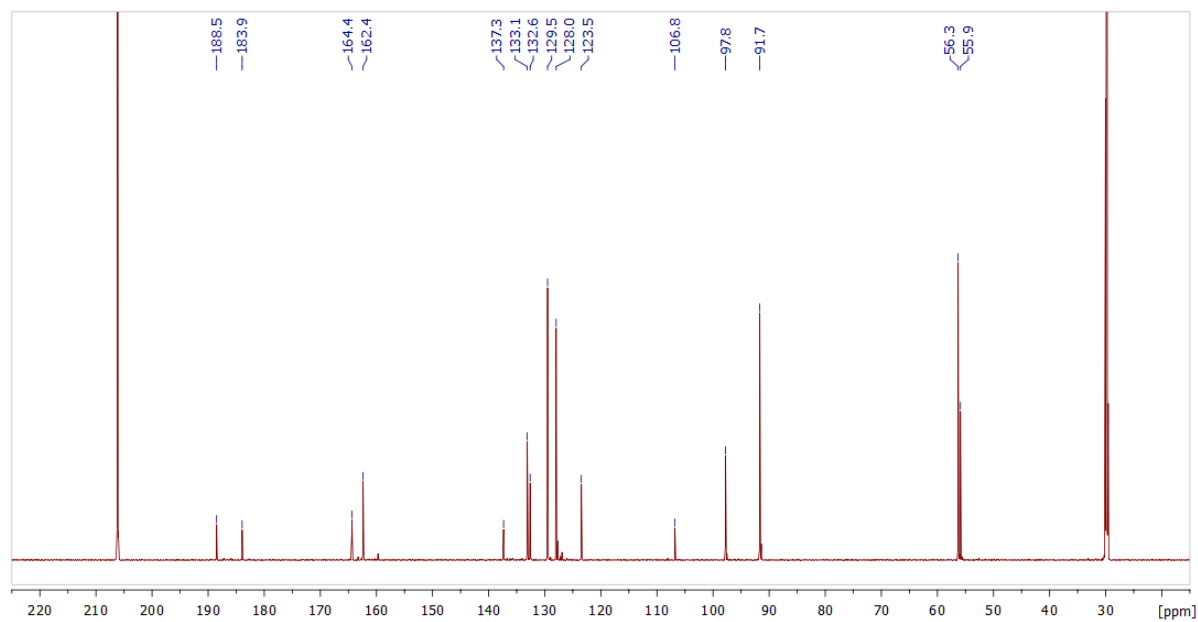
UV-vis absorbance spectrum of compound **4h-B** in acetonitrile.

Compound 4h

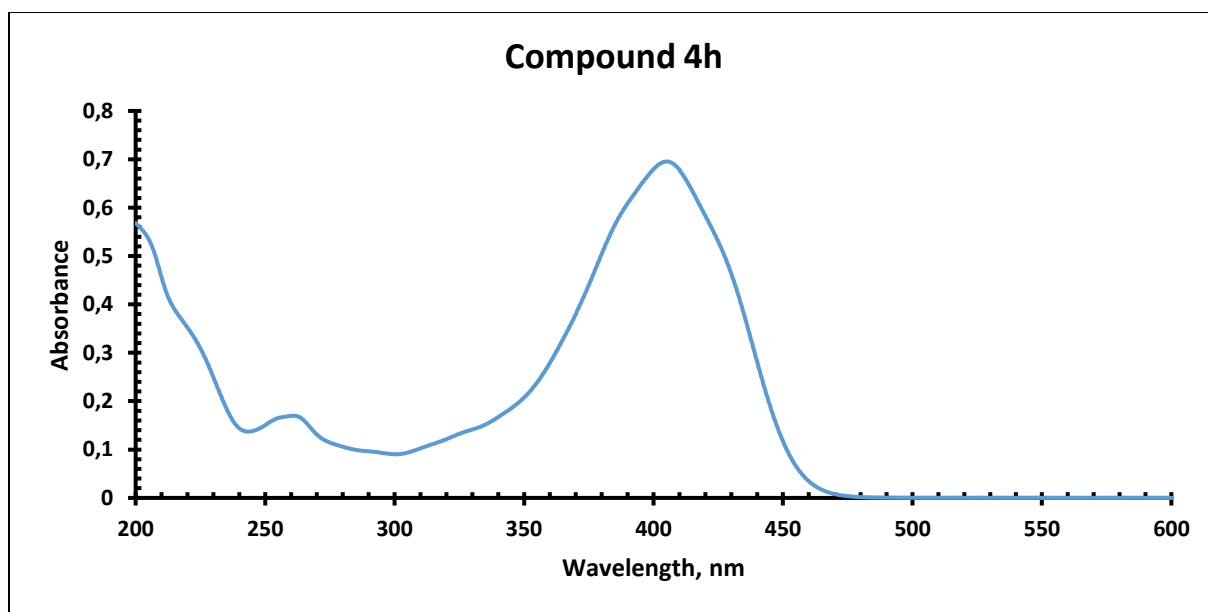
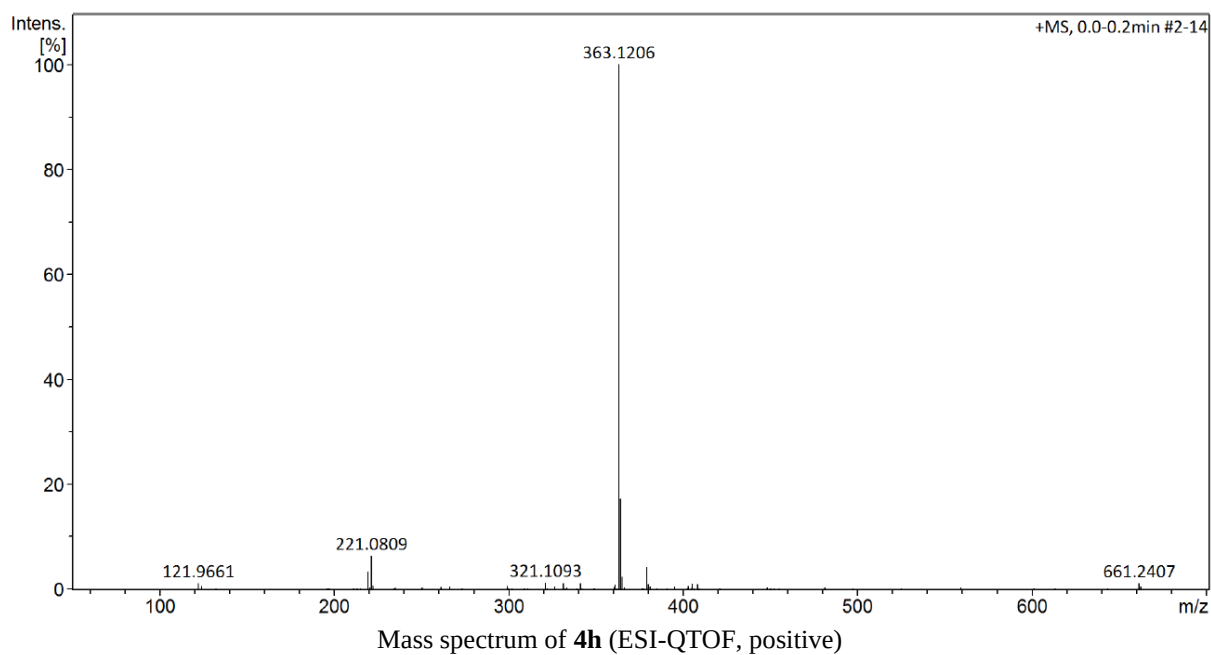
(2Z,4E)-3-hydroxy-1-phenyl-5-(2,4,6-trimethoxyphenyl)penta-2,4-dien-1-one



¹H NMR spectrum of **4h** (acetone-*d*₆, 25 °C)



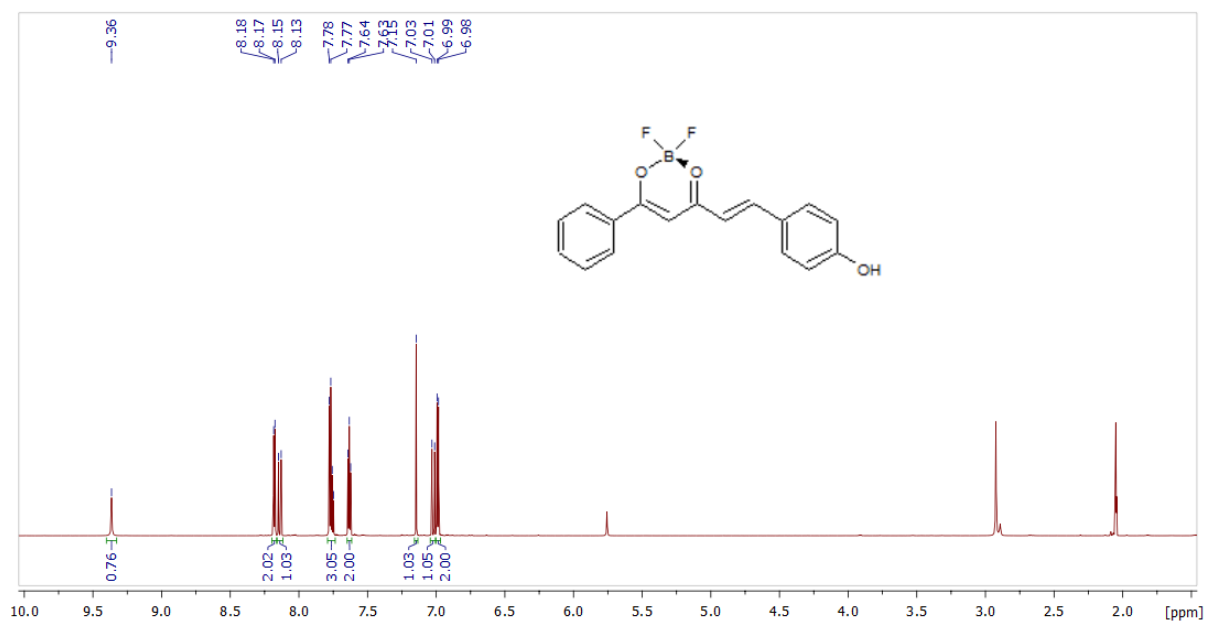
¹³C NMR spectrum of **4h** (acetone-*d*₆, 25 °C)



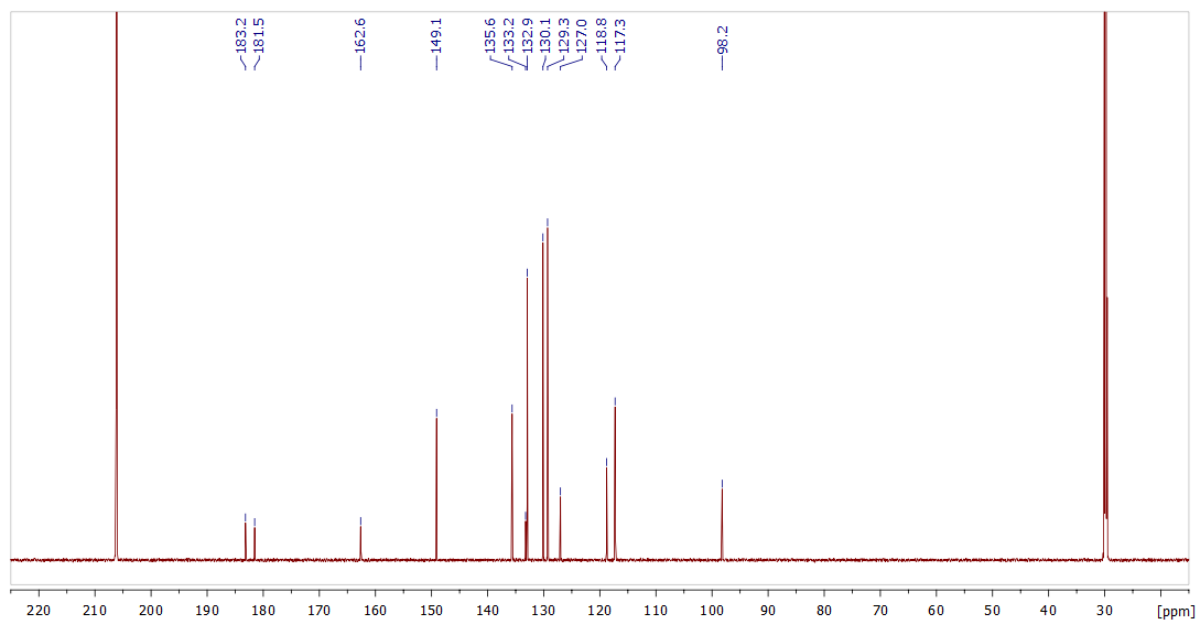
UV-vis absorbance spectrum of compound **4h** in acetonitrile.

Compound 4i-B

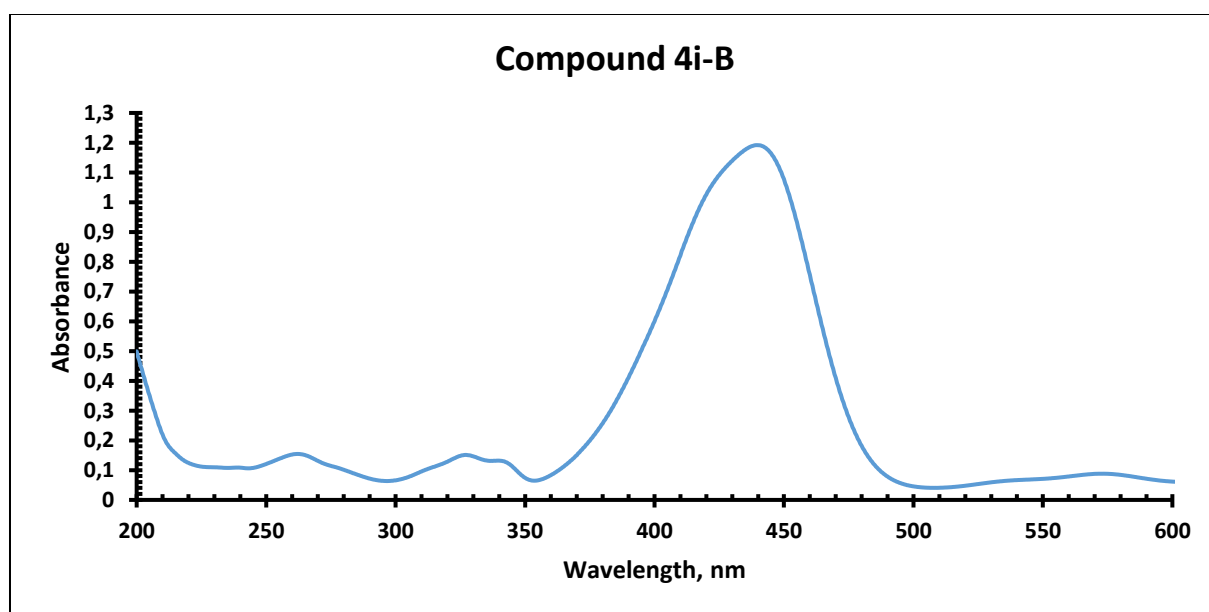
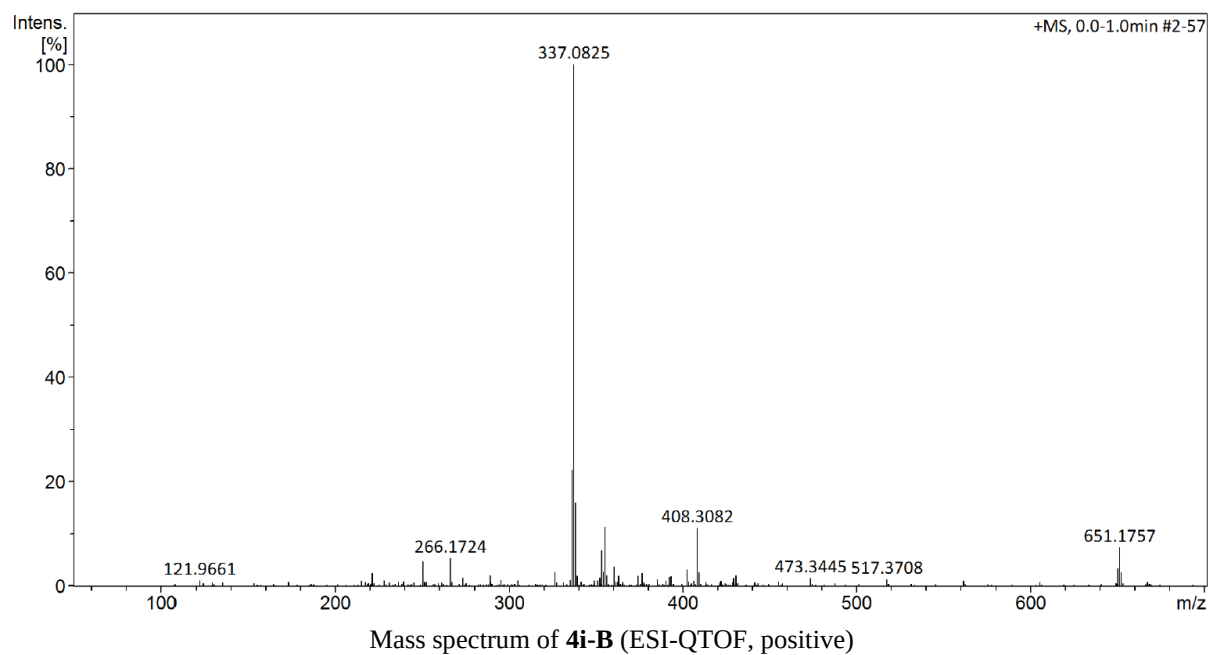
4-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]phenol



¹H NMR spectrum of **4i-B** (acetone-*d*₆, 25 °C)

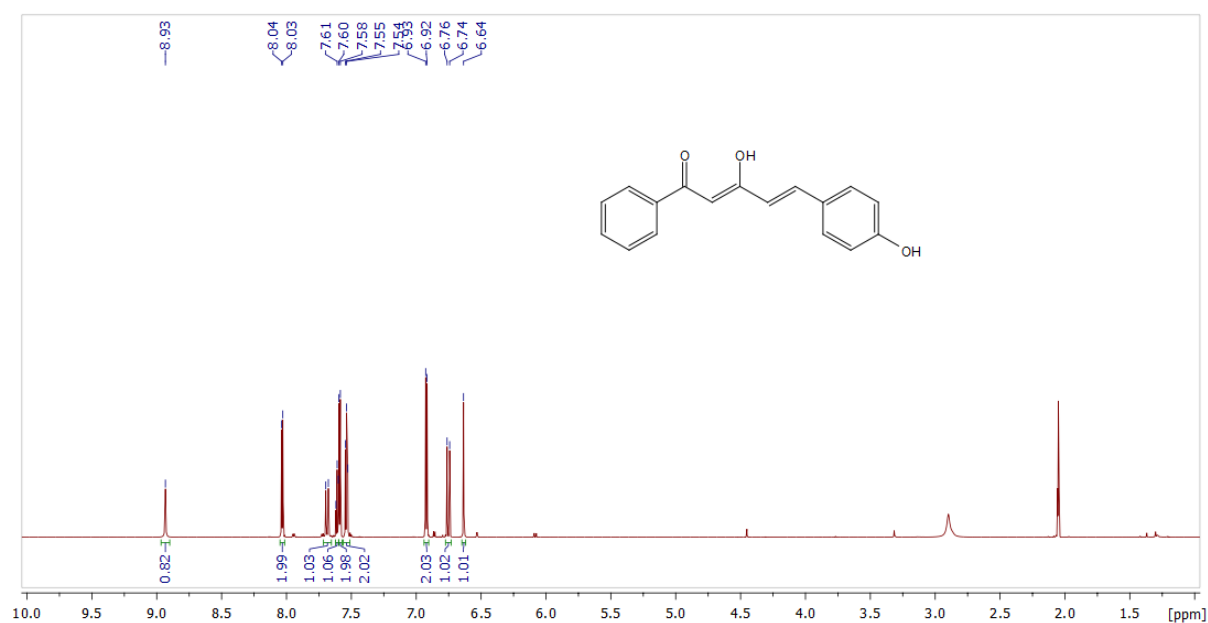


¹³C NMR spectrum of **4i-B** (acetone-*d*₆, 25 °C)

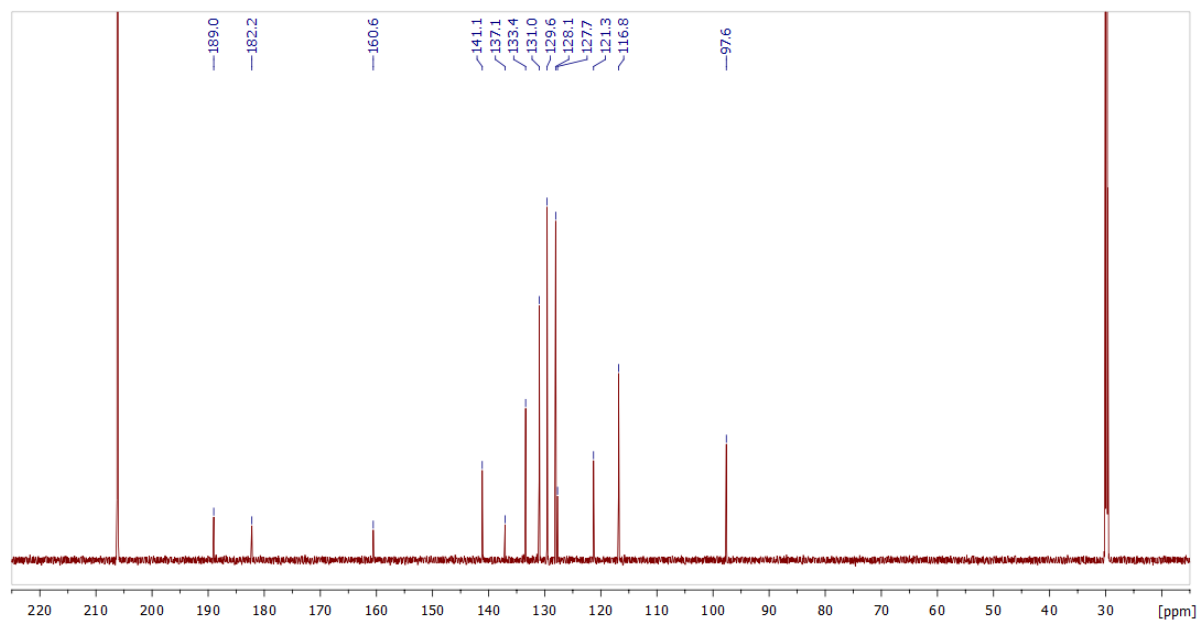


Compound 4i

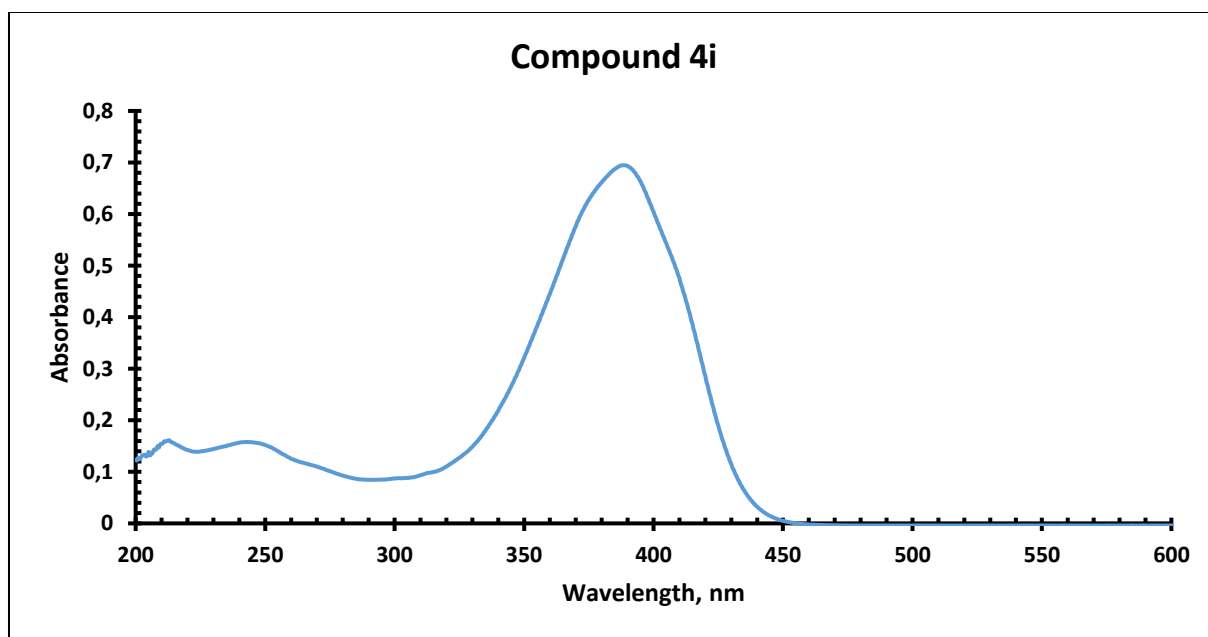
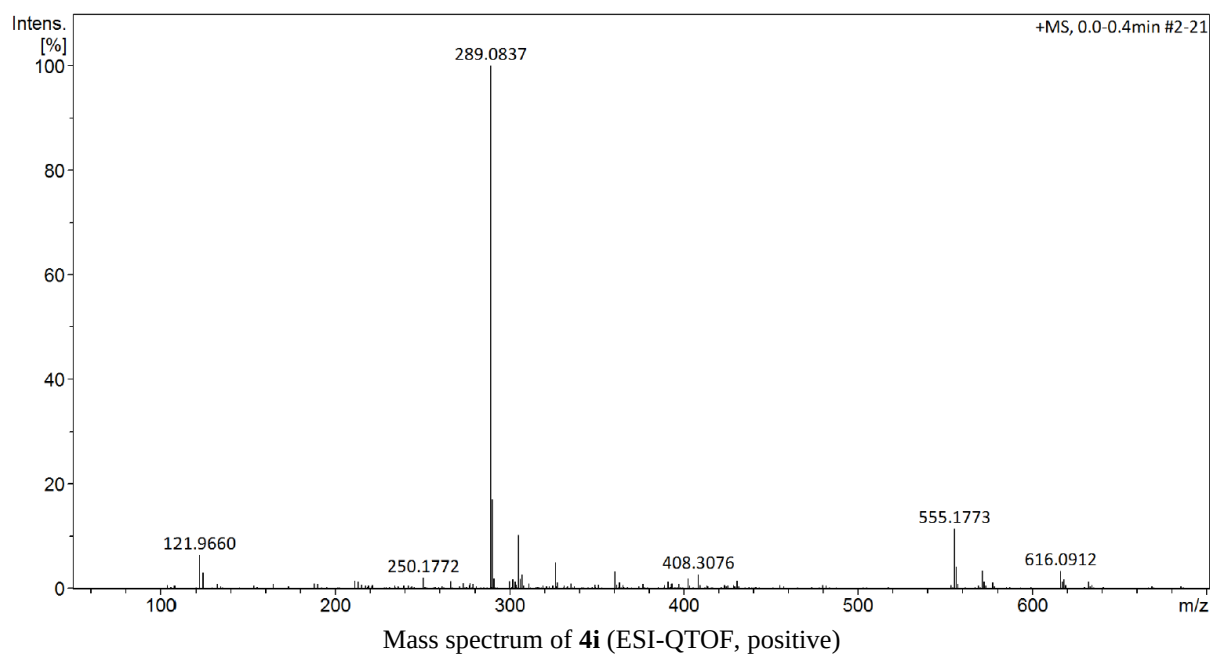
(2Z,4E)-3-hydroxy-5-(4-methoxyphenyl)-1-phenylpenta-2,4-dien-1-one



¹H NMR spectrum of **4i** (acetone-*d*₆, 25 °C)



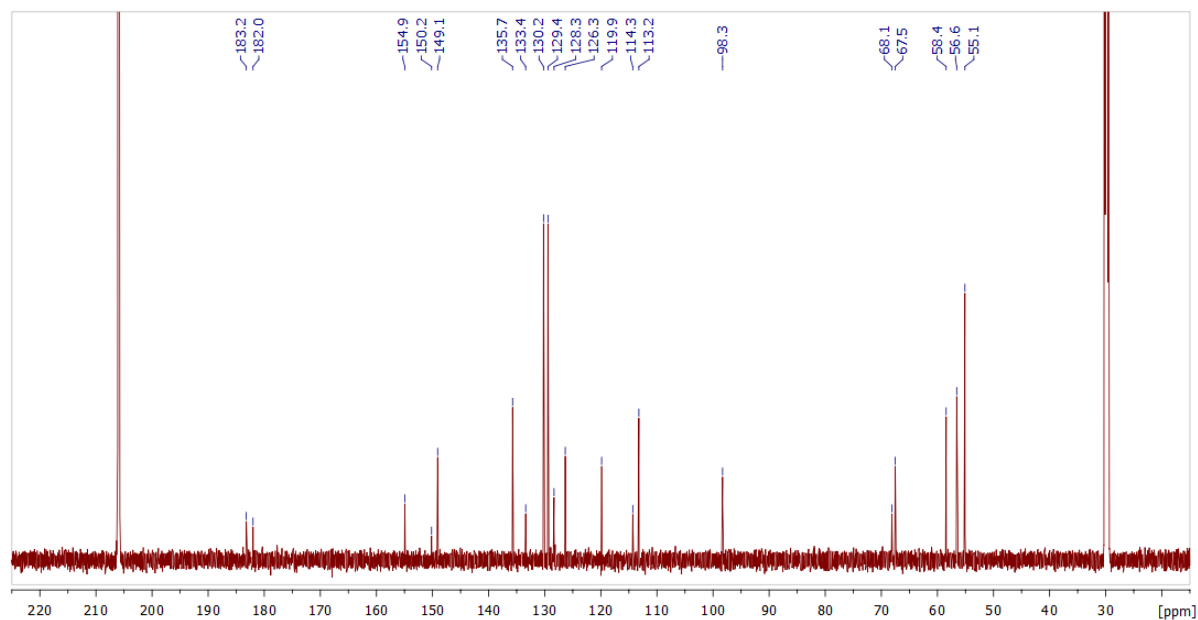
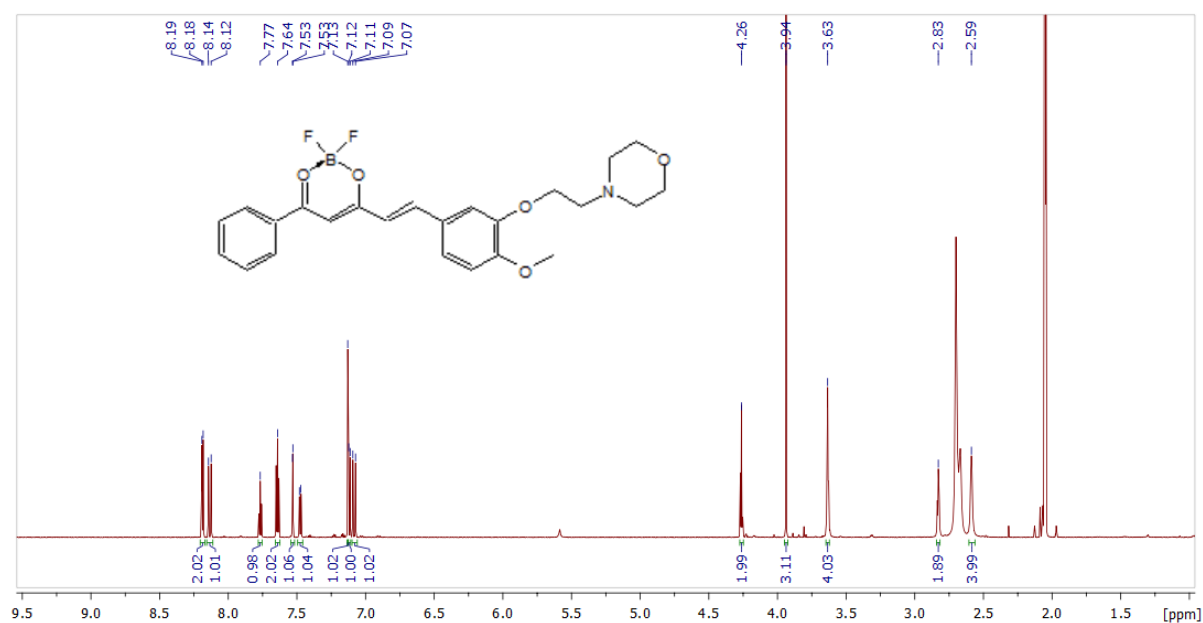
¹³C NMR spectrum of **4i** (acetone-*d*₆, 25 °C)

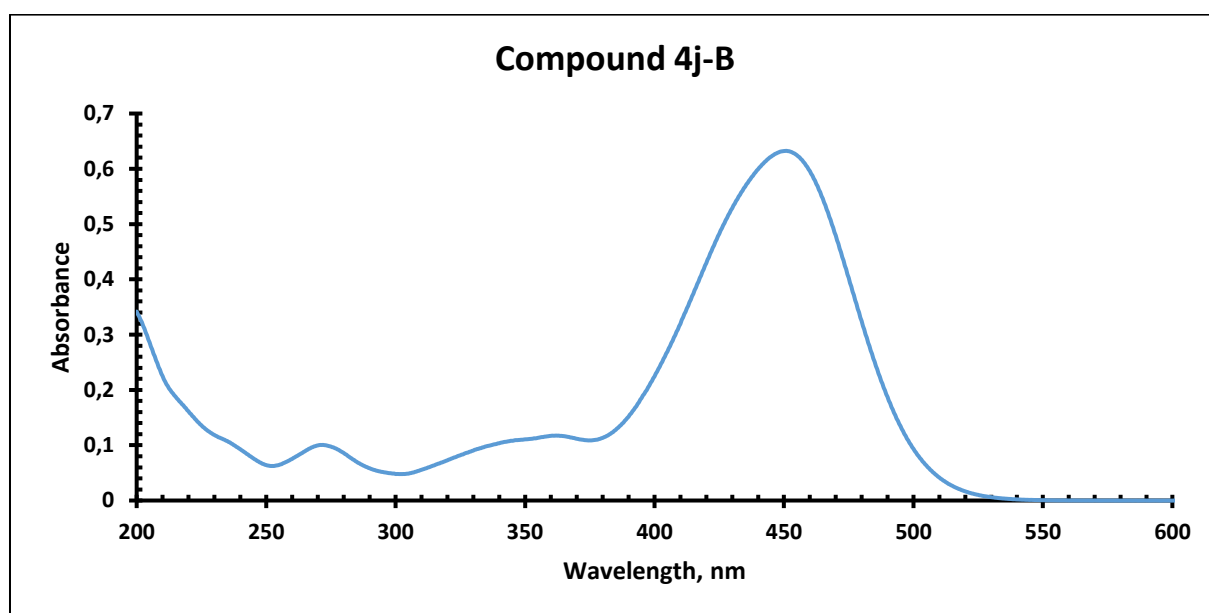
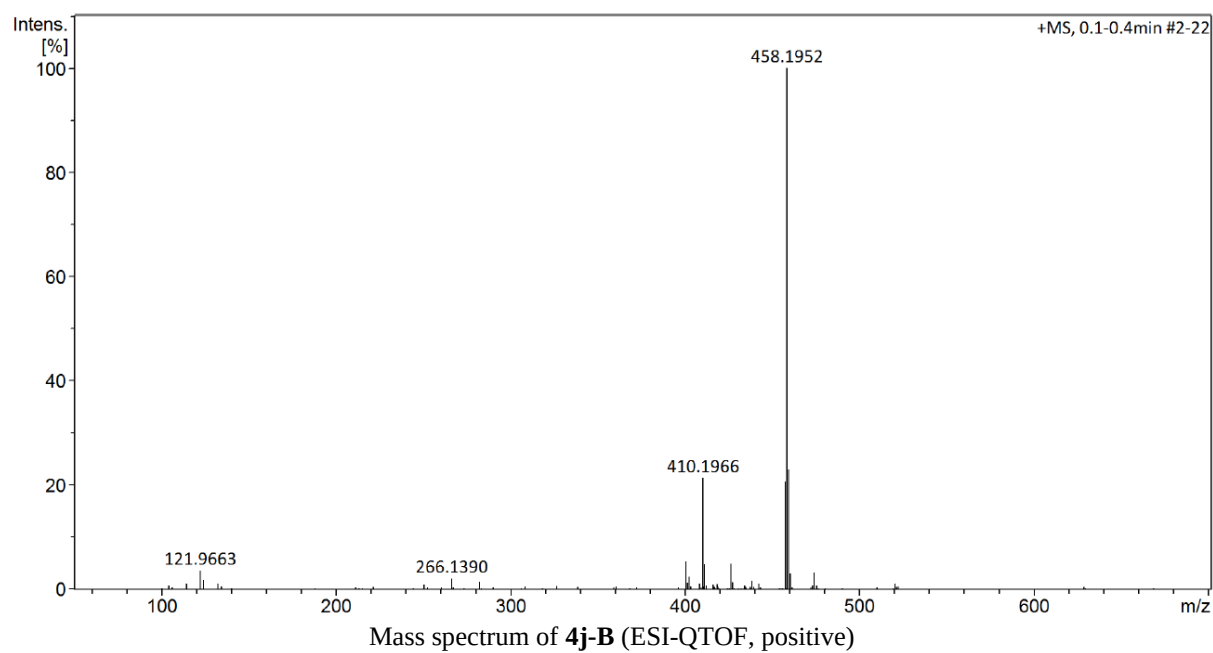


UV-vis absorbance spectrum of compound **4i** in acetonitrile.

Compound 4j-B

4-(2-{5-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2-methoxyphenoxy}ethyl)morpholine

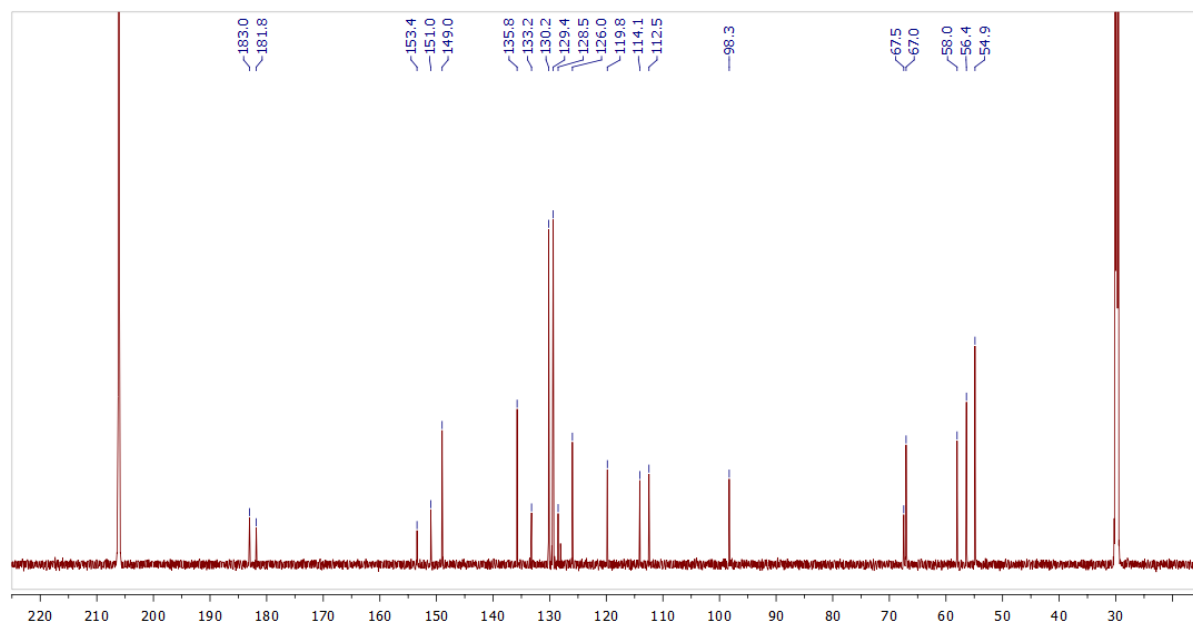
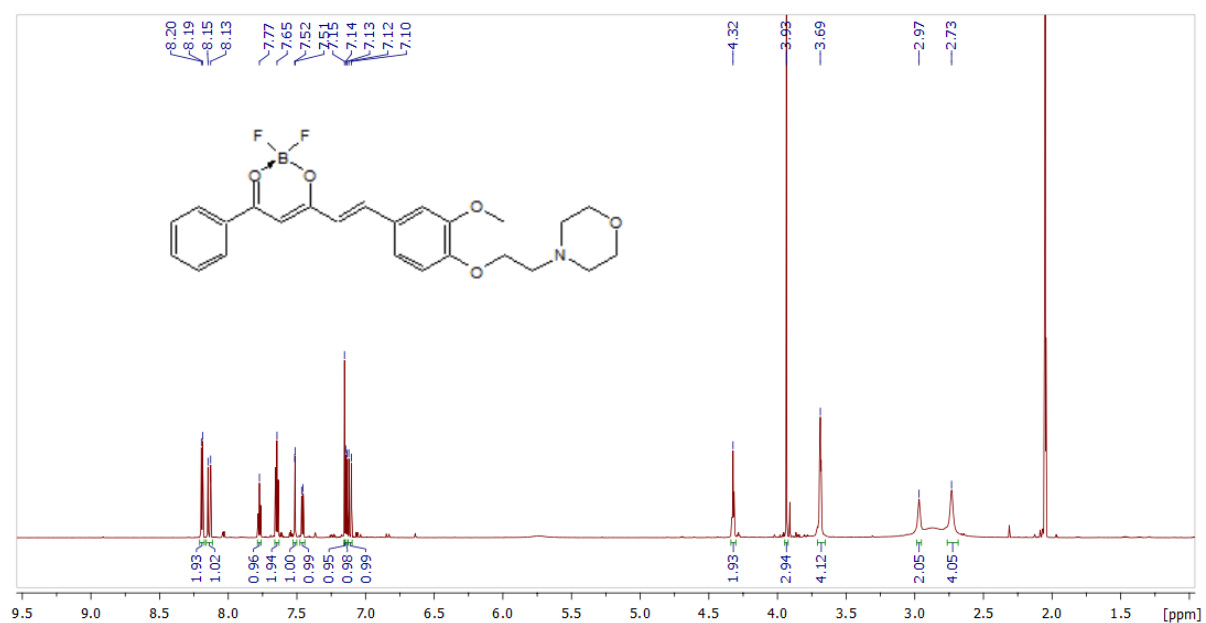


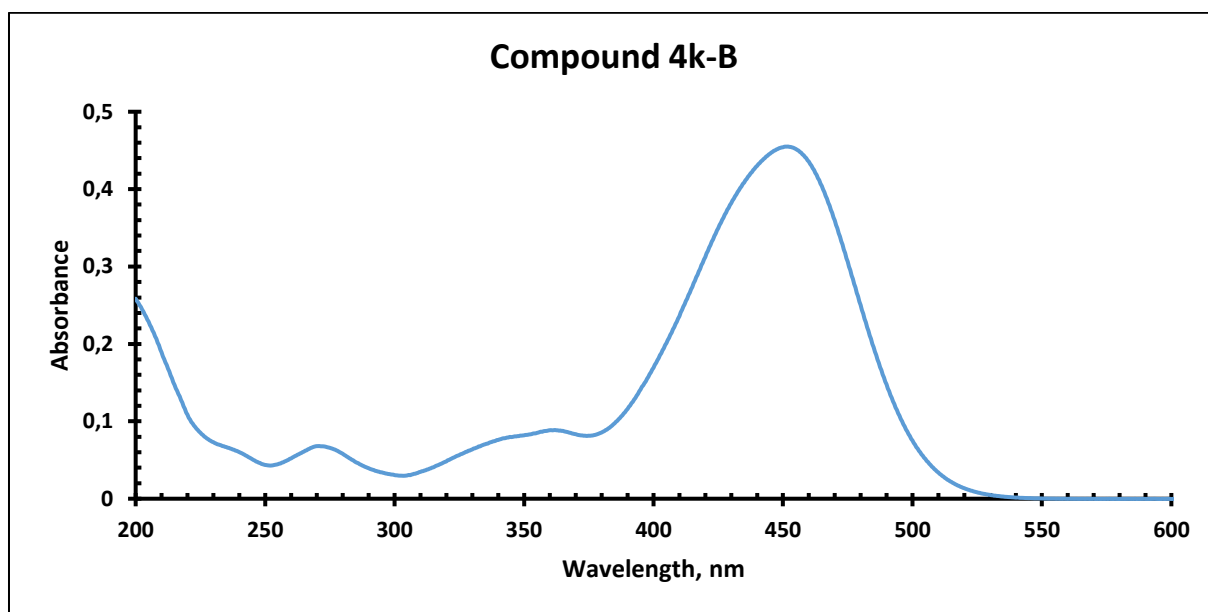
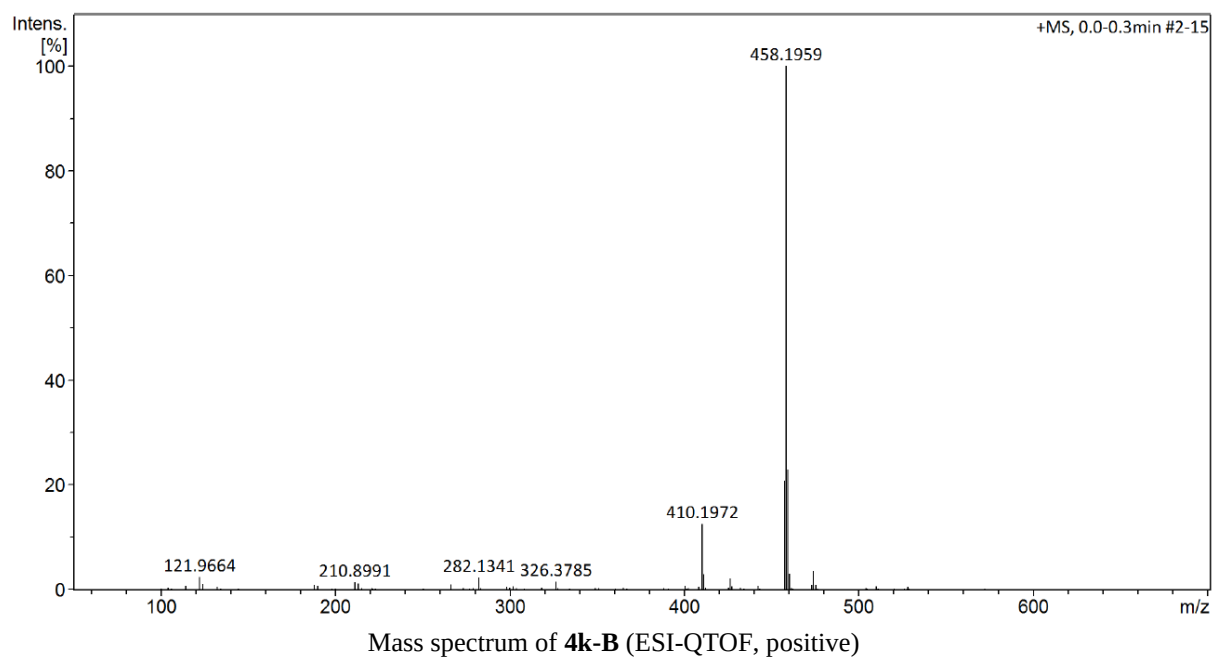


UV-vis absorbance spectrum of compound **4j-B** in acetonitrile.

Compound 4k-B

4-(2-{4-[(*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]-2-methoxyphenoxy}ethyl)morpholine

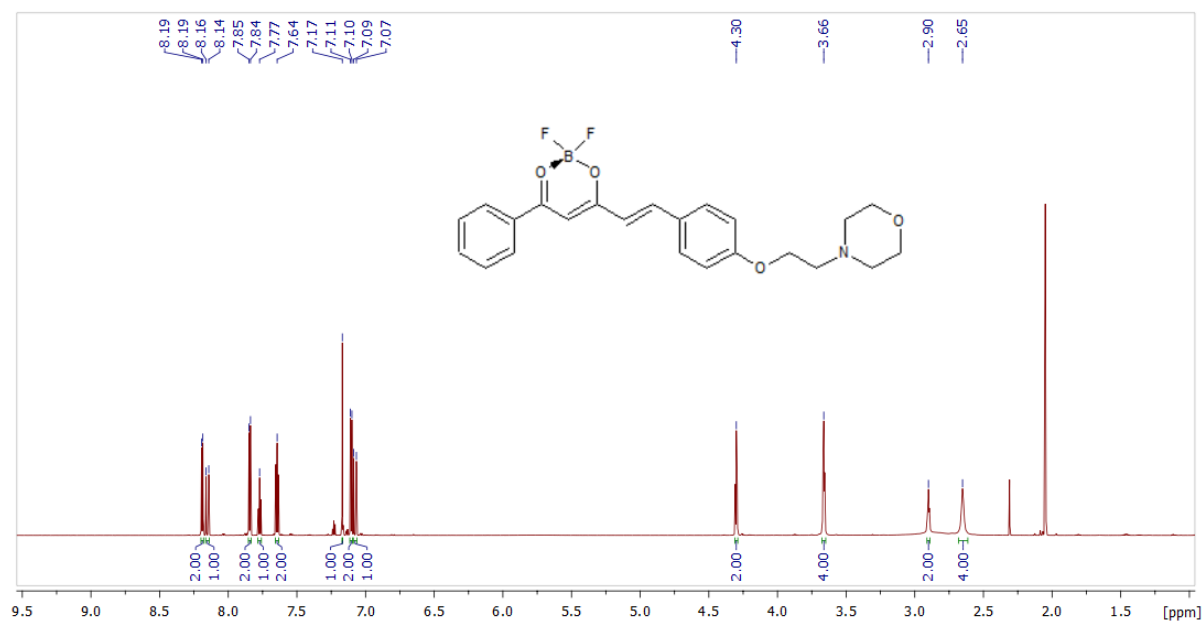




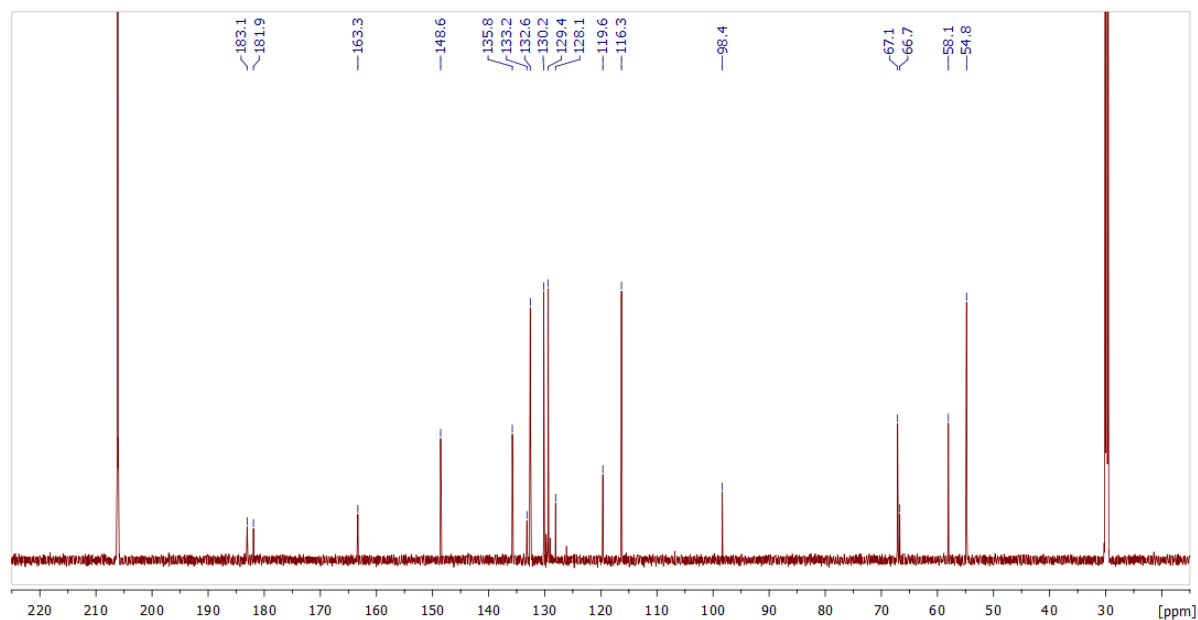
UV-vis absorbance spectrum of compound **4k-B** in acetonitrile.

Compound 4l-B

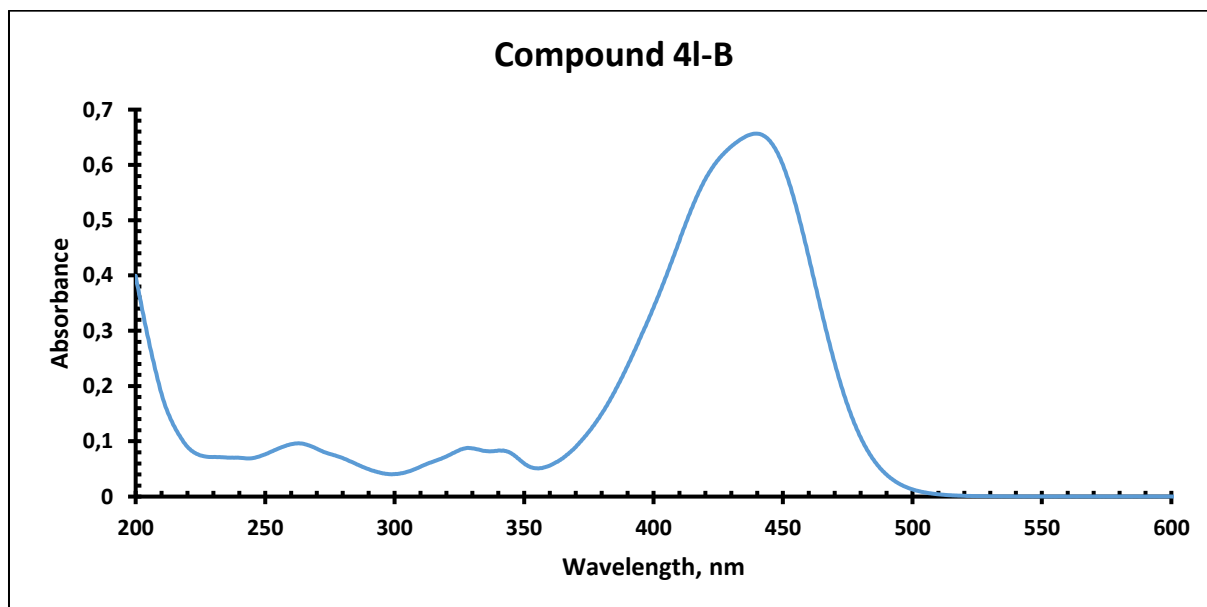
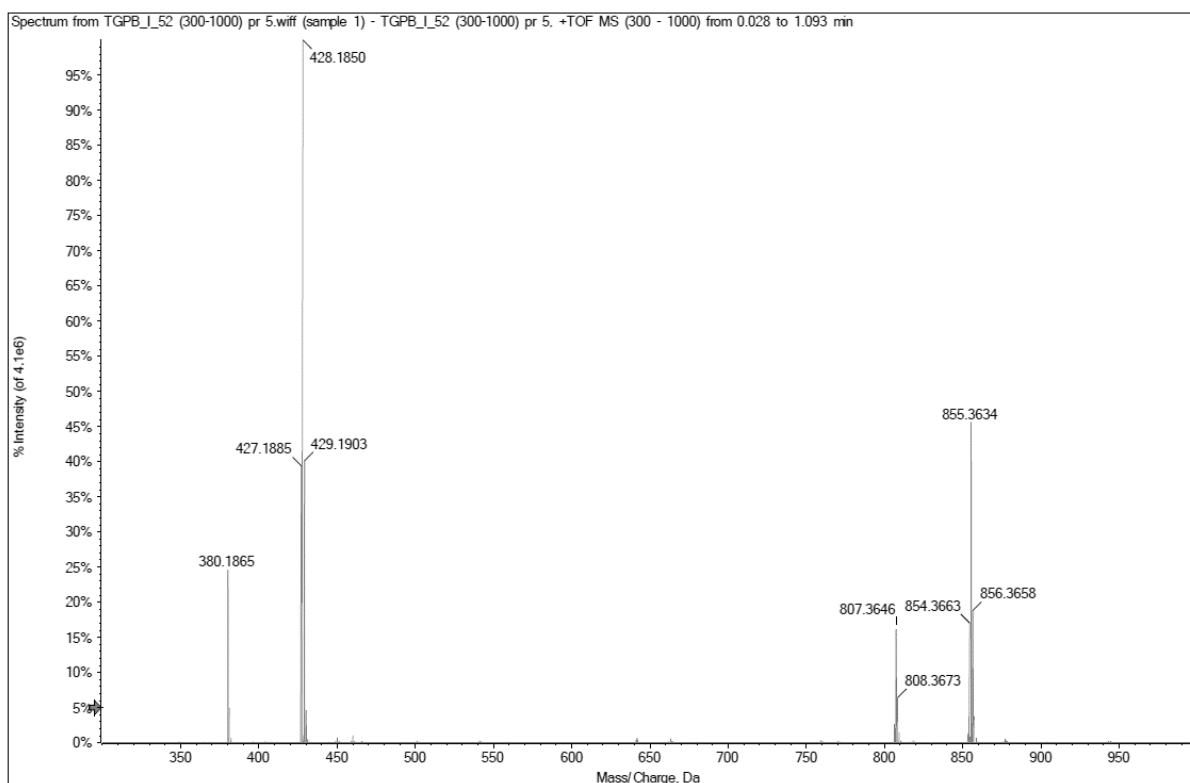
4-(2-{4-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]phenoxy}ethyl)morpholine



¹H NMR spectrum of **4l-B** (acetone-*d*₆, 25 °C)

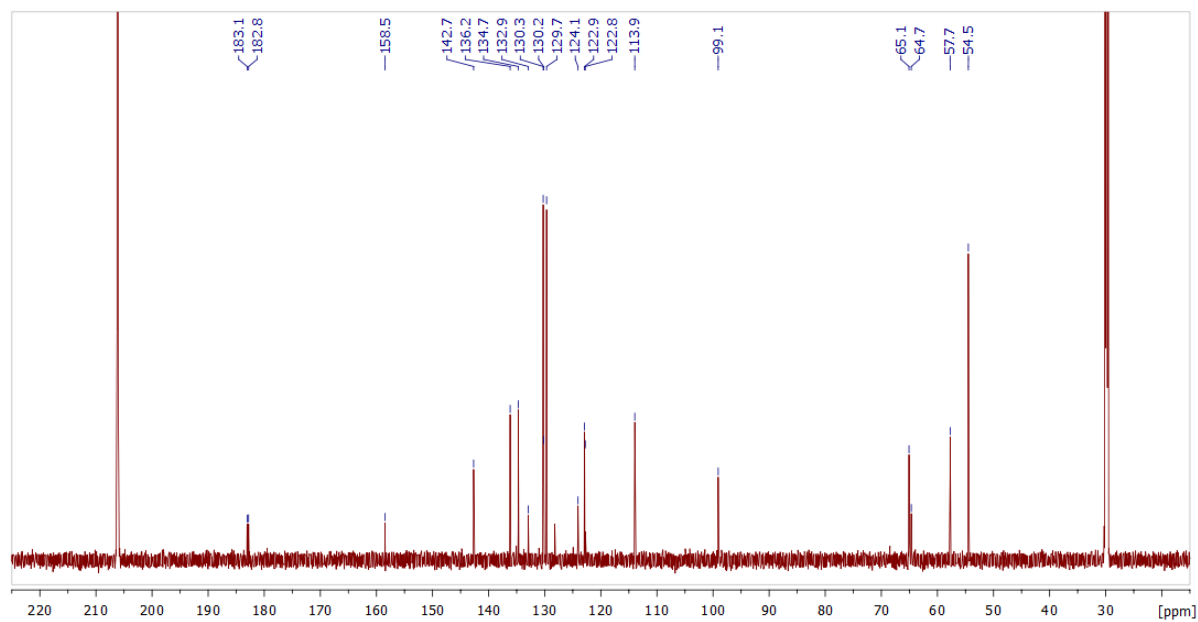
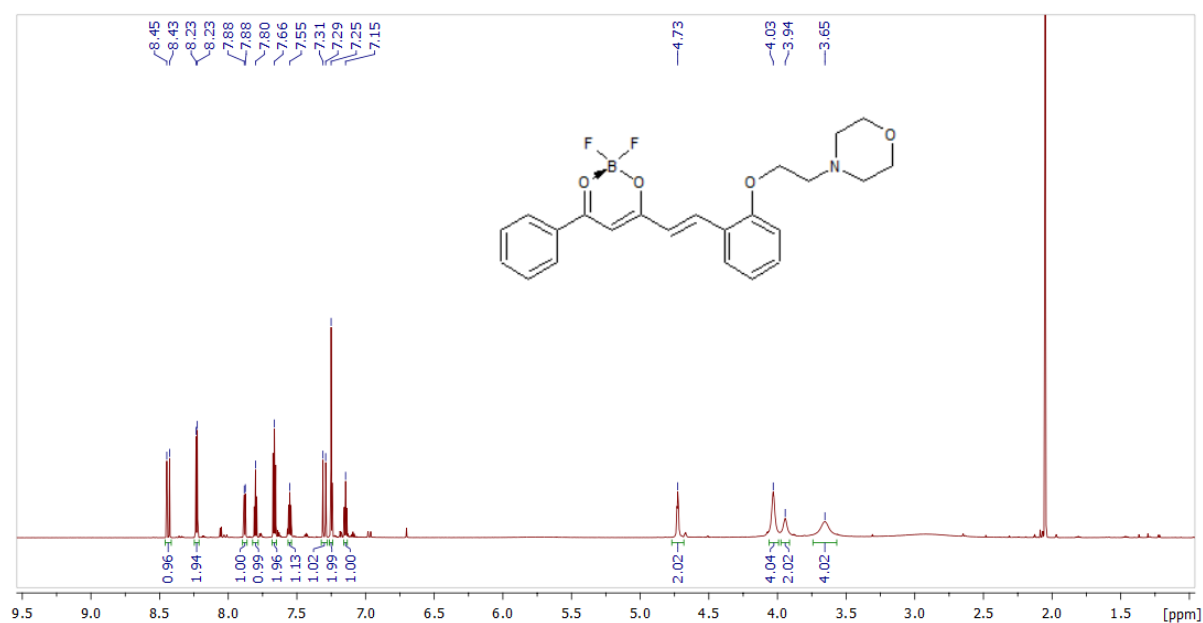


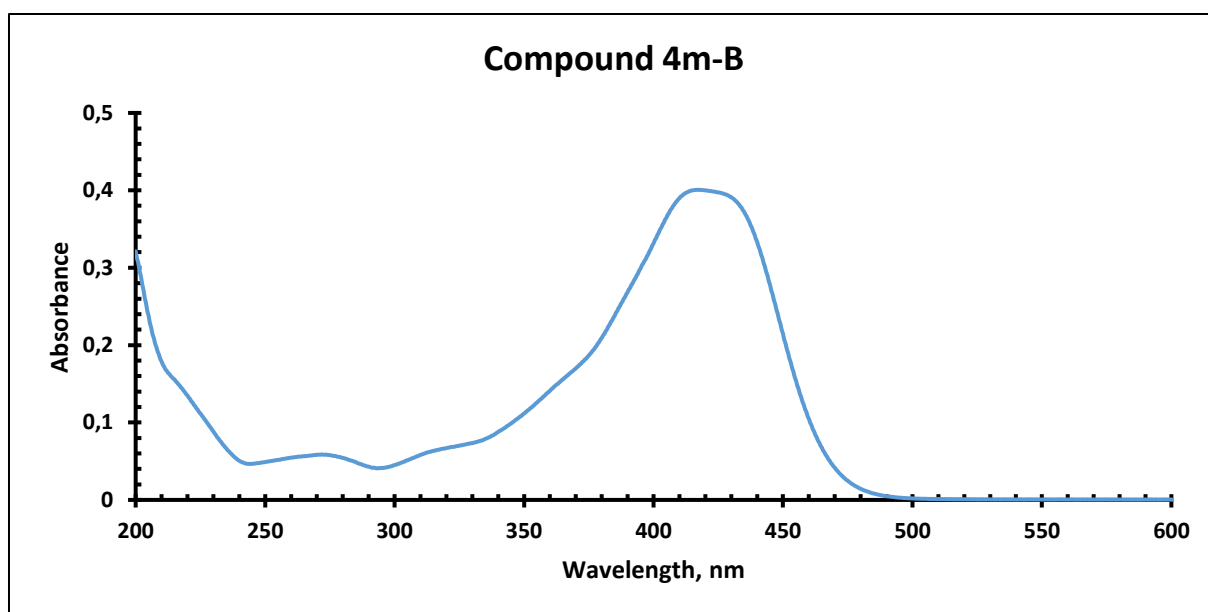
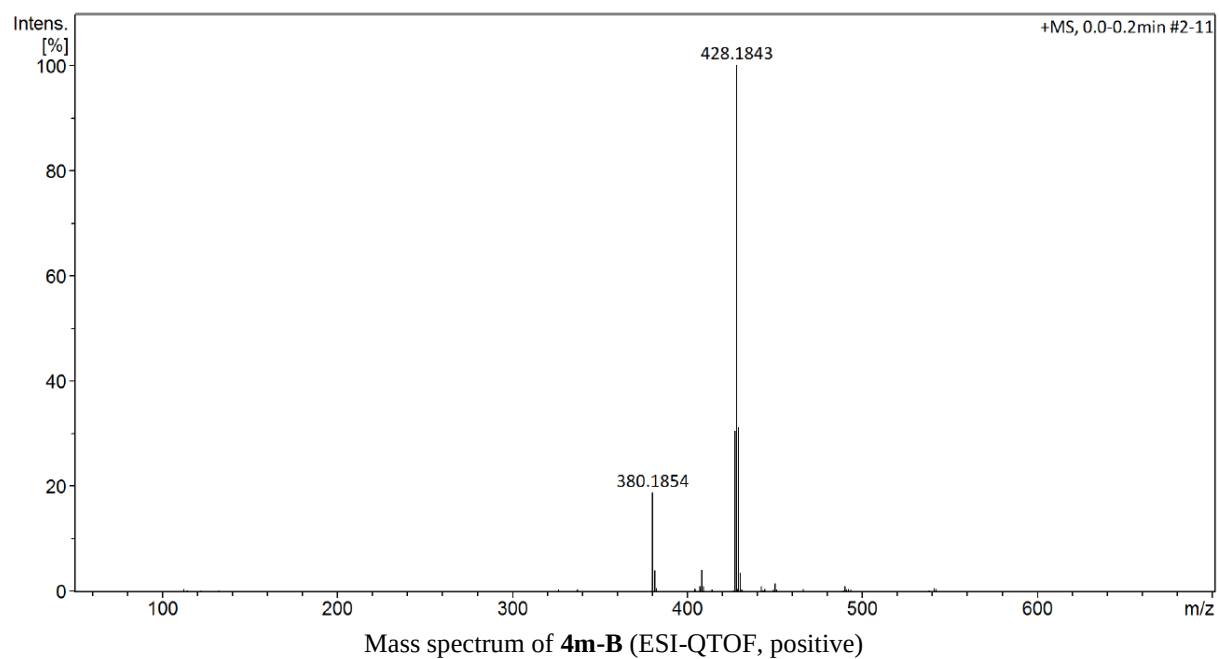
¹³C NMR spectrum of **4l-B** (acetone-*d*₆, 25 °C)



Compound 4m-B

4-(2-{2-[(1*E*)-2-(2,2-difluoro-6-phenyl-1,3,2-dioxaborinin-4-yl)ethenyl]phenoxy}ethyl)morpholine





UV-vis absorbance spectrum of compound **4m-B** in acetonitrile.

3. Microtox data

Table S2. Acute toxicity data obtained from Microtox[®] assay.

Compound	5 min				15 min			
	% Effect			EC ₂₀	% Effect			EC ₂₀
	1 μ M	10 μ M	100 μ M		1 μ M	10 μ M	100 μ M	
2a-B	12.51	23.56	32.46	7.10	10.56	24.58	32.07	7.06
2a	11.99	13.38	31.46	42.33	12.53	16.40	38.51	27.30
2b-B	1.76	21.38	63.00	9.37	1.31	18.25	61.09	13.68
2b	6.60	-2.98	52.16	47.51	1.99	-1.67	55.29	44.24
2c-B	-2.17	-8.67	18.52	>100	-2.91	-13.27	17.11	>100
2c	5.88	43.36	76.37	4.39	7.74	54.52	78.86	3.36
3-B	6.20	40.35	82.12	4.64	7.82	37.75	80.57	4.66
4a-B	-3.34	-2.23	71.16	34.61	-4.85	-4.93	78.36	33.73
4a	-0.99	6.80	31.98	60.77	1.64	2.60	32.26	61.67
4b-B	-0.78	11.29	6.63	NA	2.22	11.21	5.97	NA
4b	27.69	92.89	98.88	<1	39.34	93.25	99.19	<1
4c-B	28.10	92.07	34.01	<1	38.80	94.69	44.38	<1
4c	60.30	92.54	96.50	<1	77.53	94.86	97.49	<1
4d-B	3.61	28.18	56.65	7.00	3.00	28.25	56.20	7.06
4d	5.76	68.12	87.71	3.06	6.42	66.89	89.08	3.02
4e-B	-2.06	23.05	66.47	8.91	-4.85	23.56	67.17	8.87
4e	1.54	27.91	79.21	7.30	2.04	32.07	95.43	6.38
4f-B	21.25	23.28	29.46	<1	30.50	38.76	46.35	<1
4f	70.20	93.13	93.03	<1	81.46	96.28	95.28	<1
4g-B	2.23	6.97	19.71	>100	2.02	2.77	20.97	95.29
4g	5.49	32.06	37.72	5.91	-2.18	47.36	45.90	5.03
4h-B	-8.47	0.36	15.87	>100	-9.75	-9.19	12.80	>100
4h	1.96	-2.26	10.47	>100	-0.16	-6.05	3.72	>100
4i-B	8.84	21.15	32.44	9.16	0.41	30.47	33.42	1.09
4i	66.33	96.96	98.36	<1	69.33	97.28	98.63	<1
4j-B	-4.20	31.73	82.32	7.06	-6.42	30.98	87.03	7.36
4k-B	-9.27	9.39	48.06	44.60	-12.72	5.92	43.36	52.46
4l-B	16.37	21.57	90.00	6.82	15.78	19.66	94.57	10.41
4m-B	-1.00	60.10	85.69	4.09	-3.49	66.14	86.67	4.04

NA – unable to calculate value based on the obtained data.

4. HPLC gradients

Table S3. HPLC gradient A used for the purity analysis of morpholine derivatives.

Time [min]	0.4% acetic acid solution [%]	Acetonitrile [%]
0	92	8
5	70	30
8	50	50
10	30	70
12	50	50
15	70	30
20	92	8

Table S4. HPLC gradient B used for the purity analysis of non-morpholine derivatives.

Time [min]	0.4% acetic acid solution [%]	Acetonitrile [%]
0	50	50
5	30	70
8	25	75
10	20	80
12	30	70
15	50	50

5. Solubility study

The literature data indicating the solubility of curcumin are mainly based on spectroscopic measurements, which were performed on commercially available mixtures of curcumin with other curcuminoids. However, in the representative literature, the following information was found on the water solubility of curcumin: 0.6 $\mu\text{g/mL}$ [34], 1.34 $\mu\text{g/mL}$ [33], <8 $\mu\text{g/mL}$ [36], molar extinction coefficient remained undetermined – insoluble [35]. The self-association of curcuminoids and following aggregation in solutions is quite common especially in the acidic environment [17].

The assessment of the course of correlation between absorbance vs. concentration for compounds **2a-B**, **2b-B** and curcumin in water was performed. The linear course of correlation between absorbance vs. concentration was found for **2b-B**. The non-linear course of correlation between absorbance vs. concentration was found for **2a-B** and curcumin. It clearly demonstrates that **2b-B** with a solubility of at least 20 $\mu\text{g/mL}$ can be considered as a compound with better solubility than curcumin.

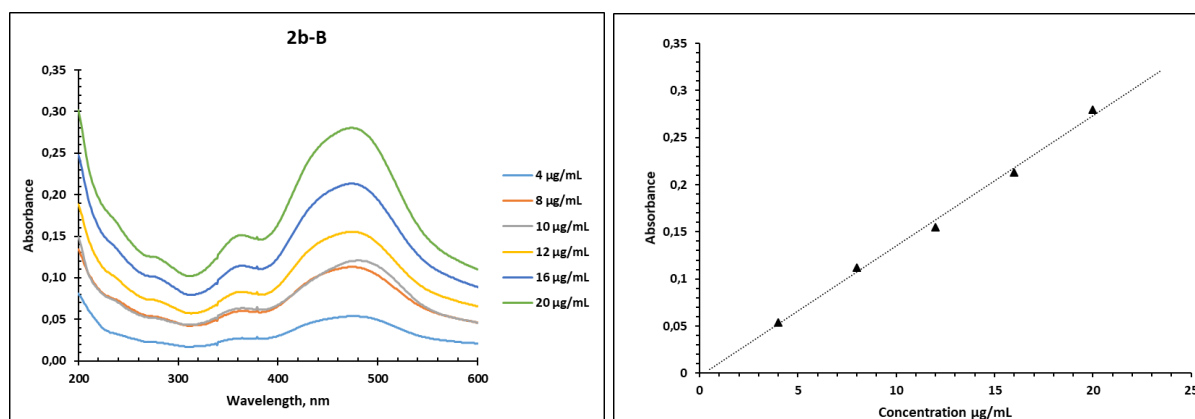


Figure S4. Spectra and correlation between absorbance at 479 nm and concentrations in the range of 4-20 $\mu\text{g/mL}$ ($6,22 \times 10^{-6}$ – $3,11 \times 10^{-5}$ mol/dm³) in H₂O for **2b-B**.

Table S5. Statistical data for aggregation study of curcumin, **2a-B** and **2b-B** in H₂O.

Compound	Solvent	a	b	t _a	t _b	t _{a,f}	r	n
curcumin	H ₂ O			aggregation				
2a-B	H ₂ O	6023.01	0.01739	38.32	5.360	3.182	1.00	5
2b-B	H ₂ O	8912.51	-0.00294	22.76	0.364	3.182	1.00	5

a – slope of the regression line $y = ax + b$;

b – the value of ordinate for $x = 0$;

r – correlation coefficient;

n – sample size

t_b value is presented in bold, when $t_b < t_{a,f}$; the value of b is not significant, e.i. $b=0$

6. References

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