SUPPLEMENTARY INFORMATION

Design and Mechanistic Evaluation of Chalcone–Quinoline–Triazole Hybrids as Antifungal CYP51 Inhibitors

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Figure S1. UV–Vis spectrum of compound 4a

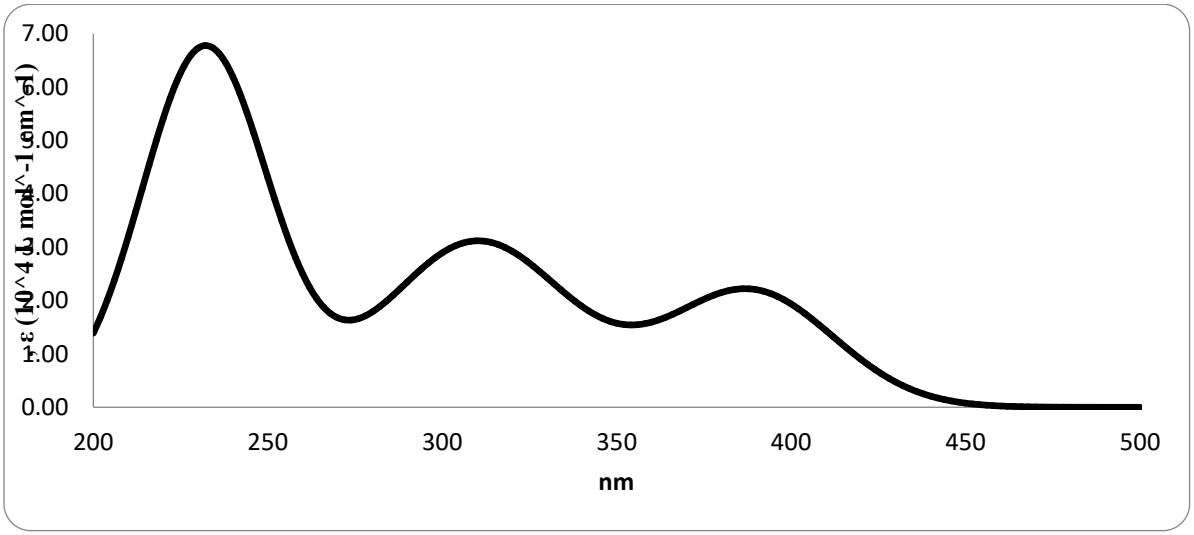


Figure S2. FTIR spectrum of compound 4a

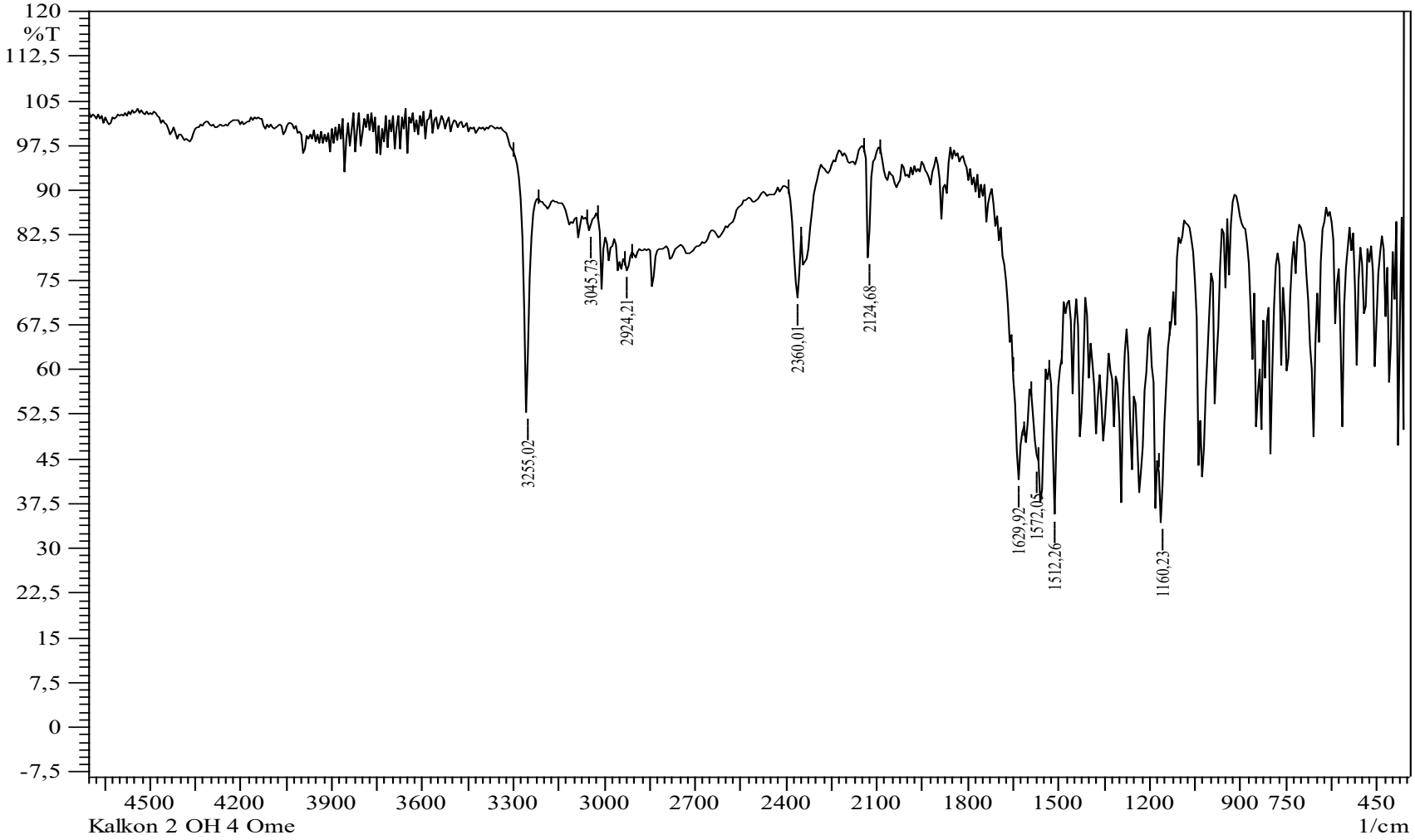


Figure S3. ^{1}H NMR spectrum of compound 4a

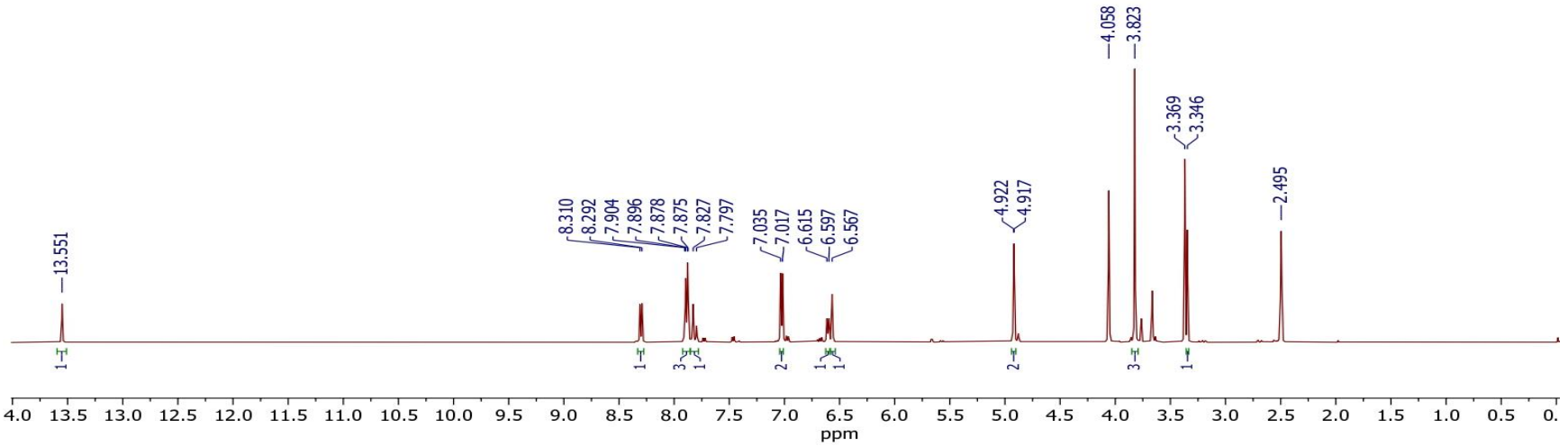


Figure S4. HRMS spectrum of compound 4a

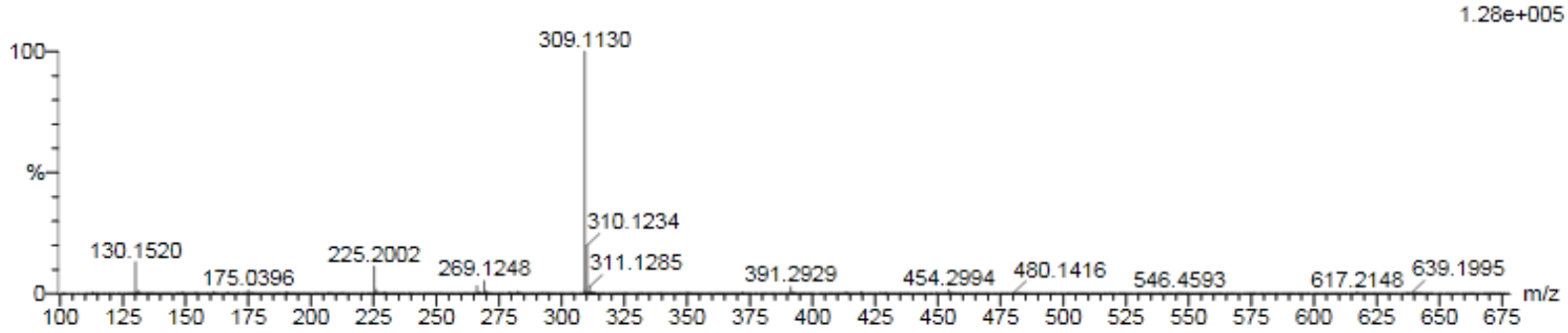
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200

KK 4PR OME 2OH 6 (0.119) Cm (5:7)
TOF MS ES+



	Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:					-1.5			
Maximum:			5.0	10.0	50.0			
	309.1130	309.1127	0.3	1.0	11.5	207.0	0.0	C19 H17 O4

Figure S5. UV–Vis spectrum of compound 4b

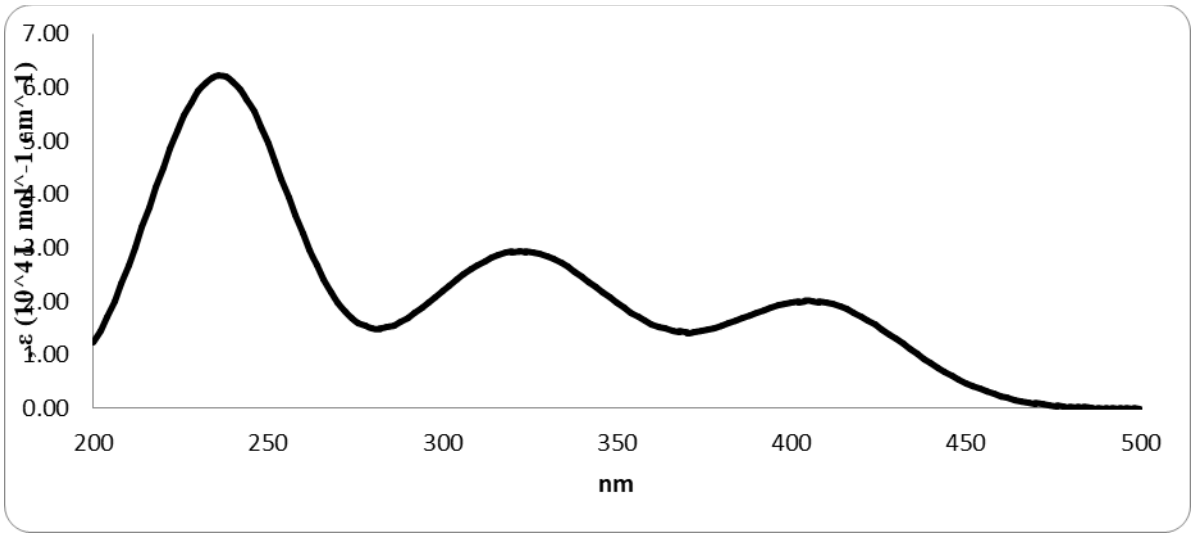


Figure S6. FTIR spectrum of compound 4b

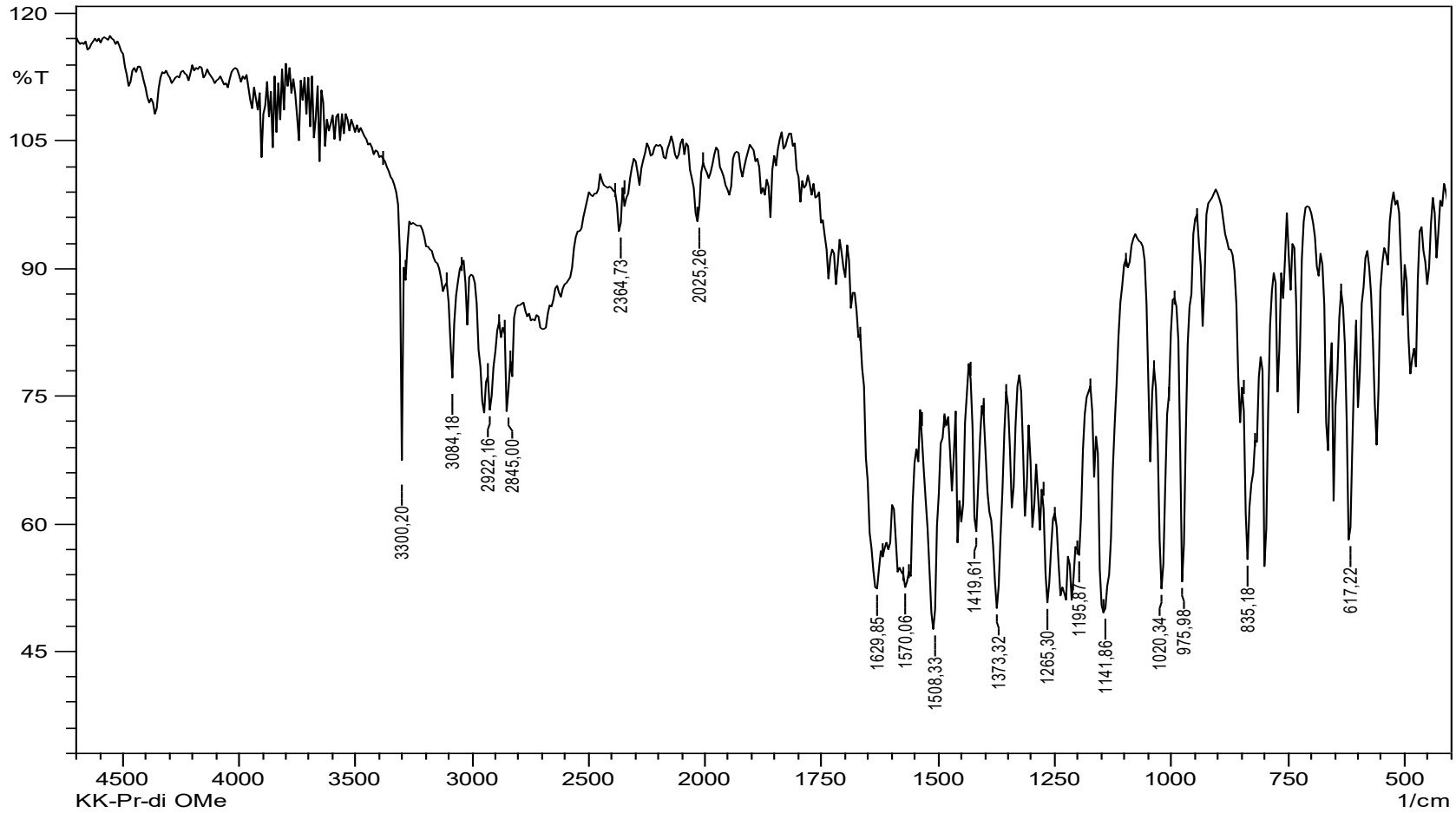


Figure S7. ^{1}H NMR spectrum of compound 4b

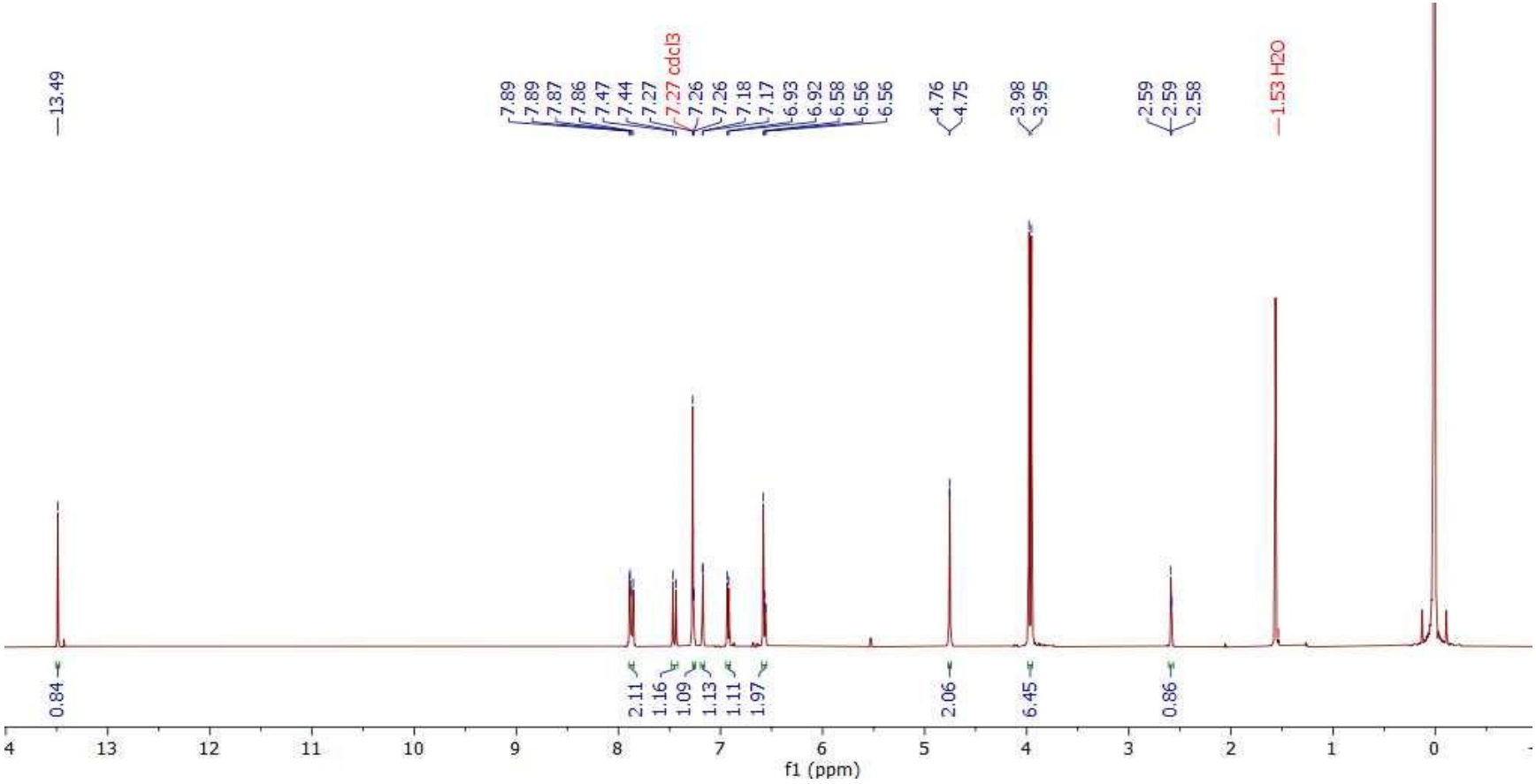
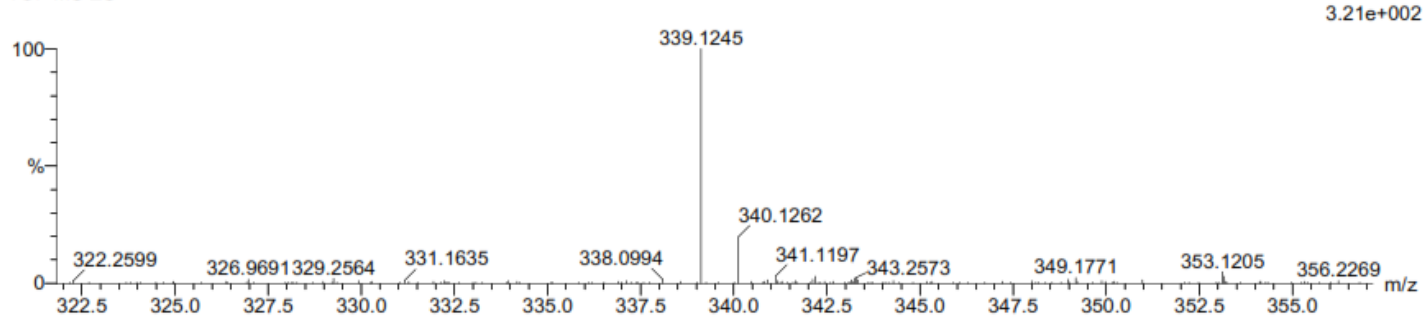


Figure S8. HRMS spectrum of compound 4b

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 mDa / DBE: min = -5.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
80 formula(e) evaluated with 15 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200
KK PR DIOME 83 (1.428) Cm (79:86)
TOF MS ES+



Minimum:				-5.0			
Maximum:		50.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.1245	339.1232	1.3	3.8	11.5	28.8	1.5	C20 H19 O5
	339.1291	-4.6	-13.6	2.5	29.1	1.9	C13 H23 O10
	339.1174	7.1	20.9	20.5	31.7	4.4	C27 H15
	339.1139	10.6	31.3	-1.5	31.2	4.0	C9 H23 O13
	339.1385	-14.0	-41.3	15.5	30.7	3.5	C24 H19 O2

Figure S9. UV–Vis spectrum of compound 4c

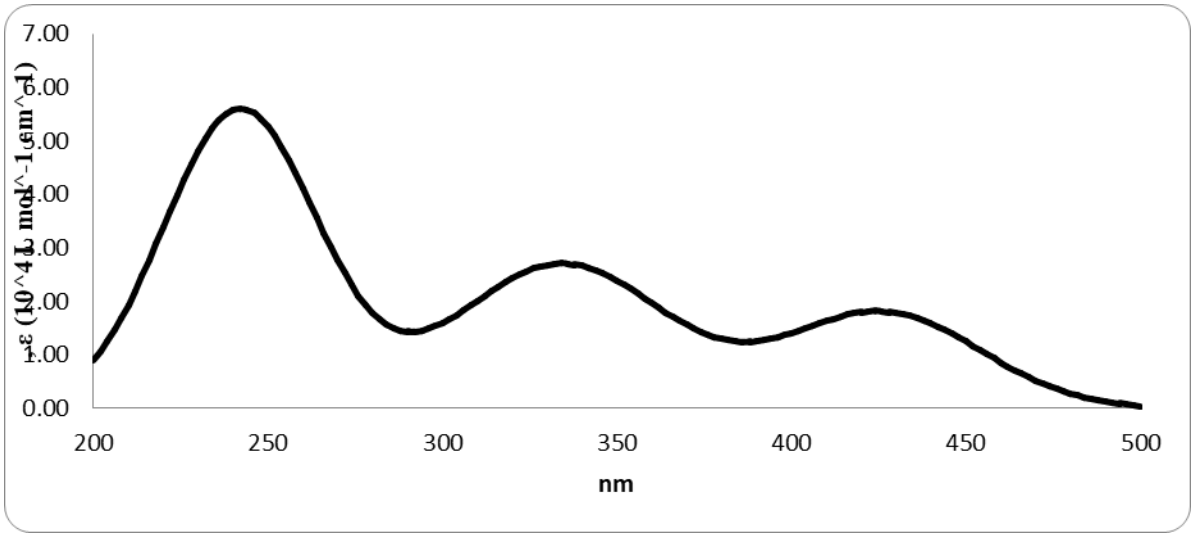


Figure S10. FTIR spectrum of compound 4c

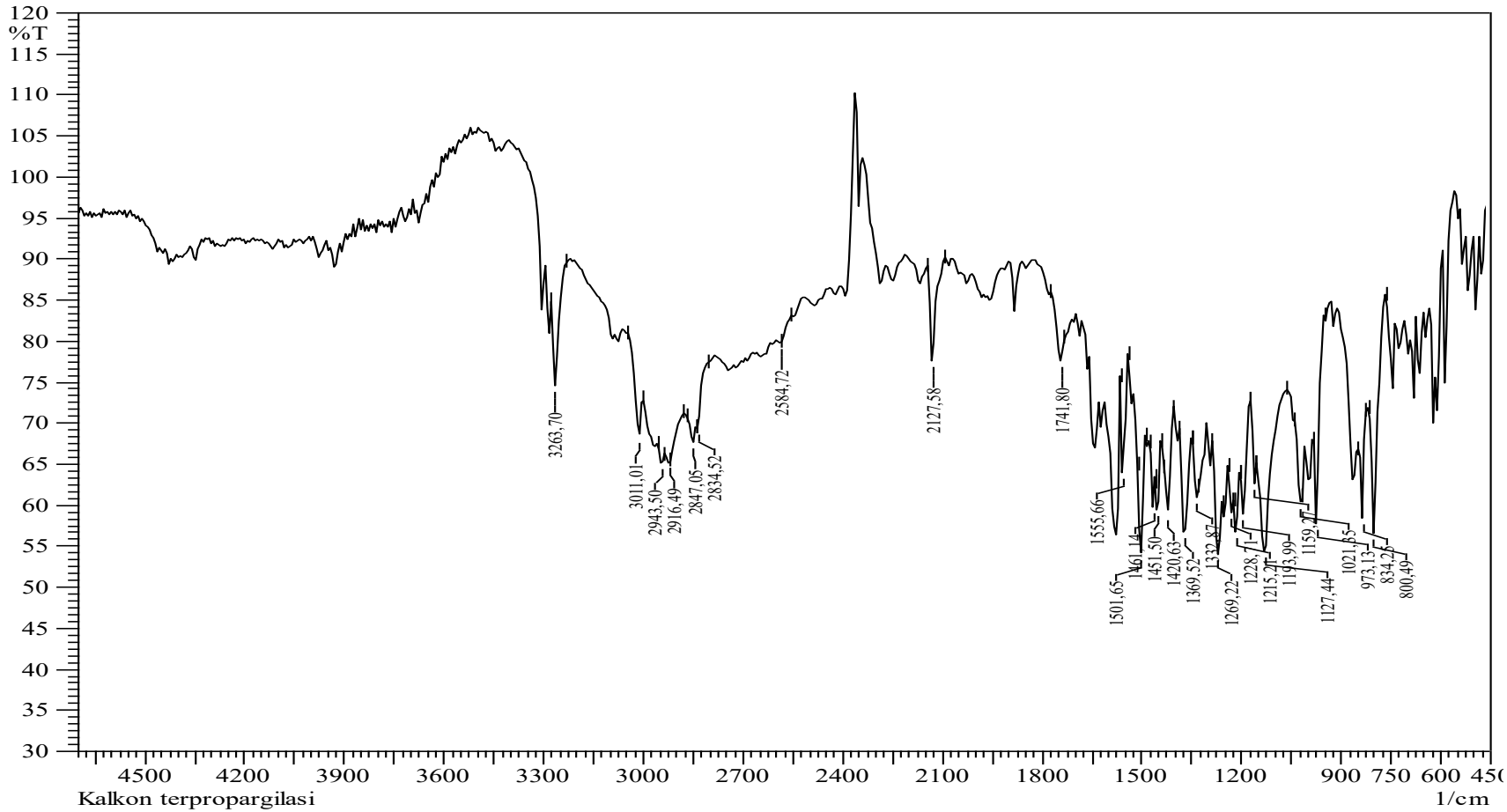


Figure S11. ^{1}H NMR spectrum of compound 4c

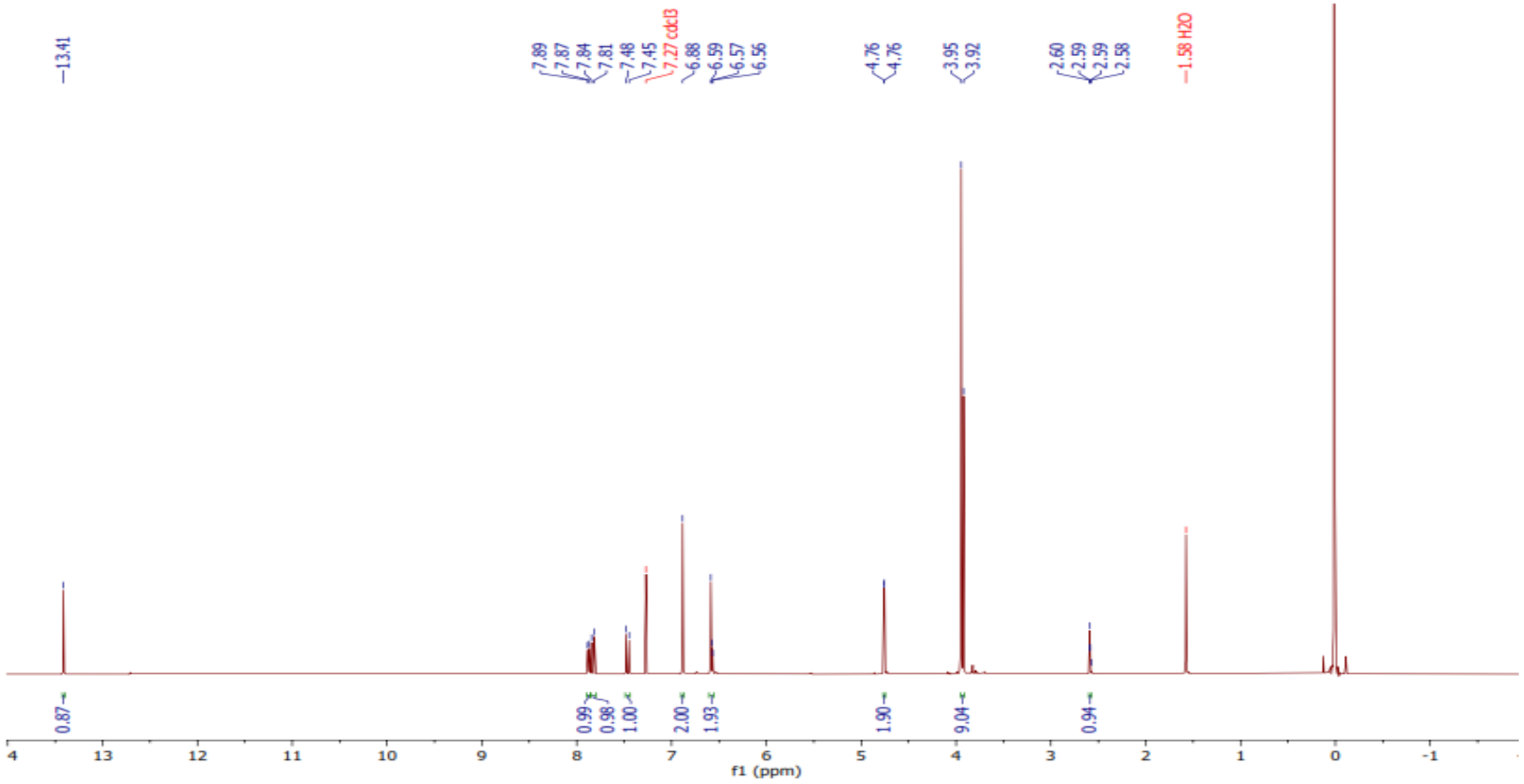


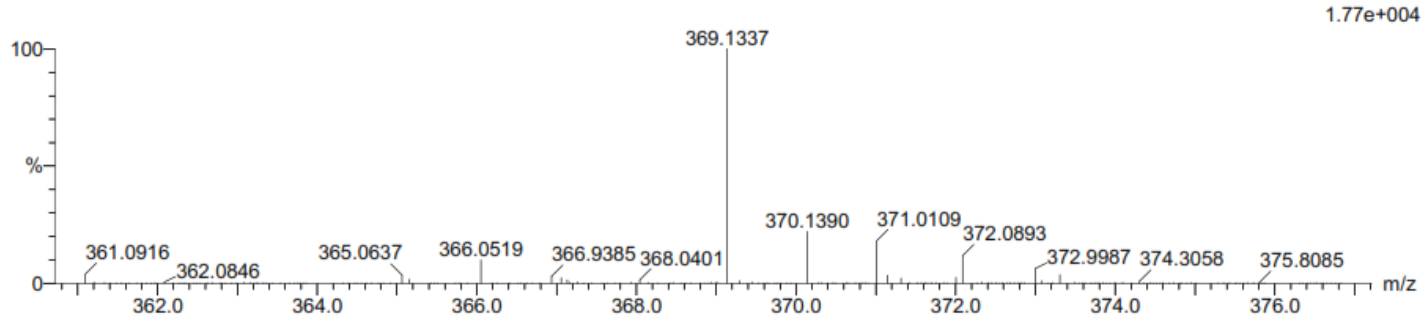
Figure S12. HRMS spectrum of compound 4c

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -5.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
88 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-200
TAF KK 4PR 24 (0.425) Cm (21:25)
TOF MS ES+



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-5.0			
Maximum:		10.0	10.0	50.0			
369.1337	369.1338	-0.1	-0.3	11.5	185.4	0.0	C21 H21 O6
	369.1279	5.8	15.7	20.5	190.3	4.9	C28 H17 O
	369.1397	-6.0	-16.3	2.5	189.1	3.8	C14 H25 O11
	369.1244	9.3	25.2	-1.5	191.9	6.5	C10 H25 O14

Figure S13. UV–Vis spectrum of compound 7a

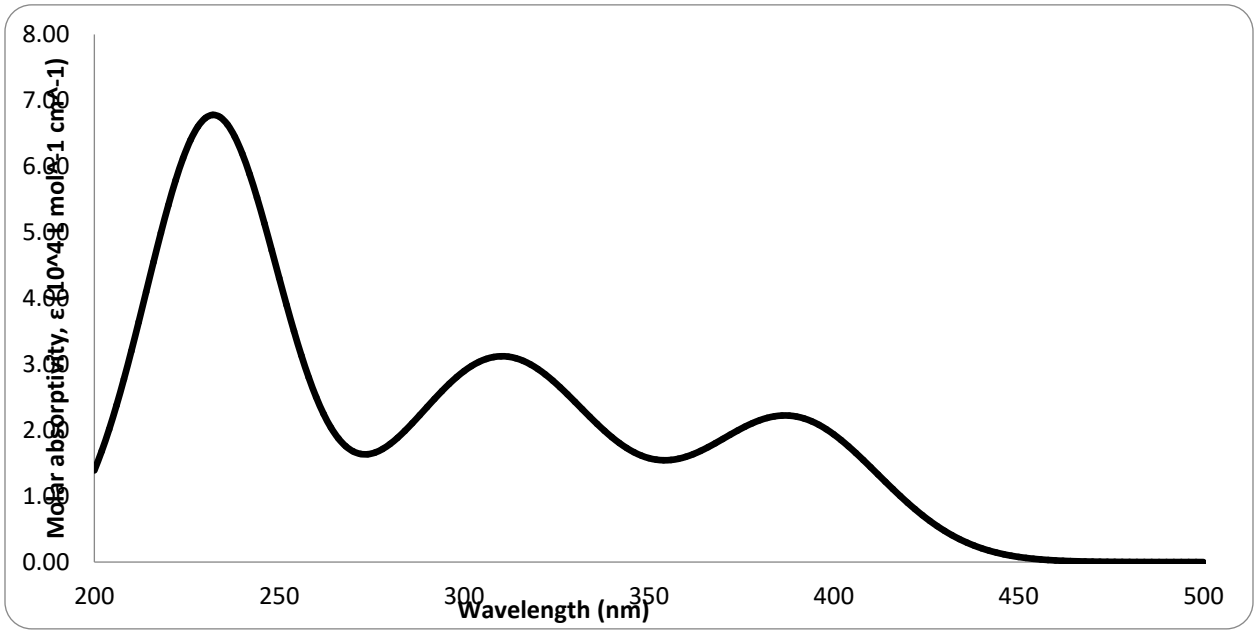


Figure S14. FTIR spectrum of compound 7a

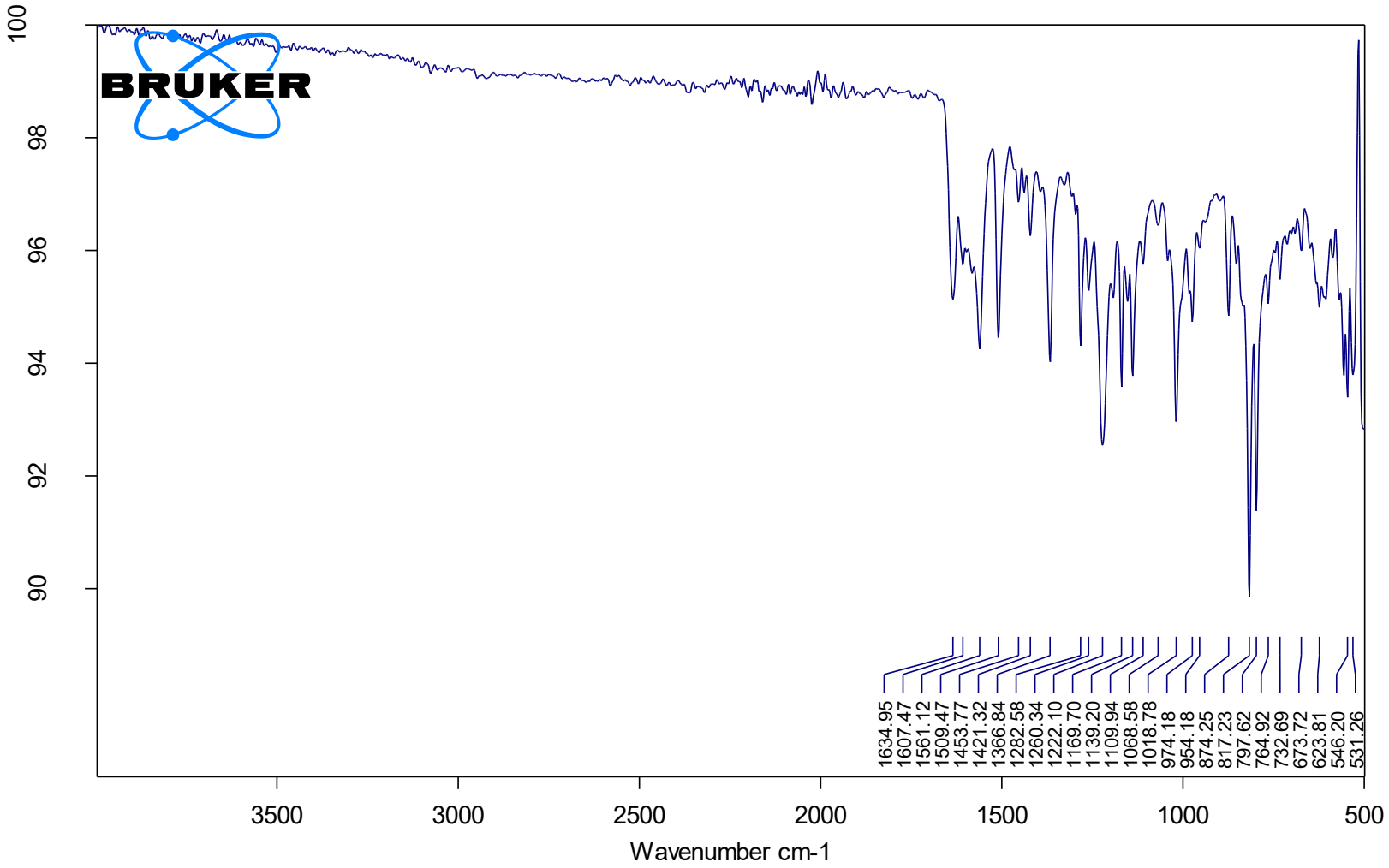


Figure S15. ^{1}H NMR spectrum of compound 7a

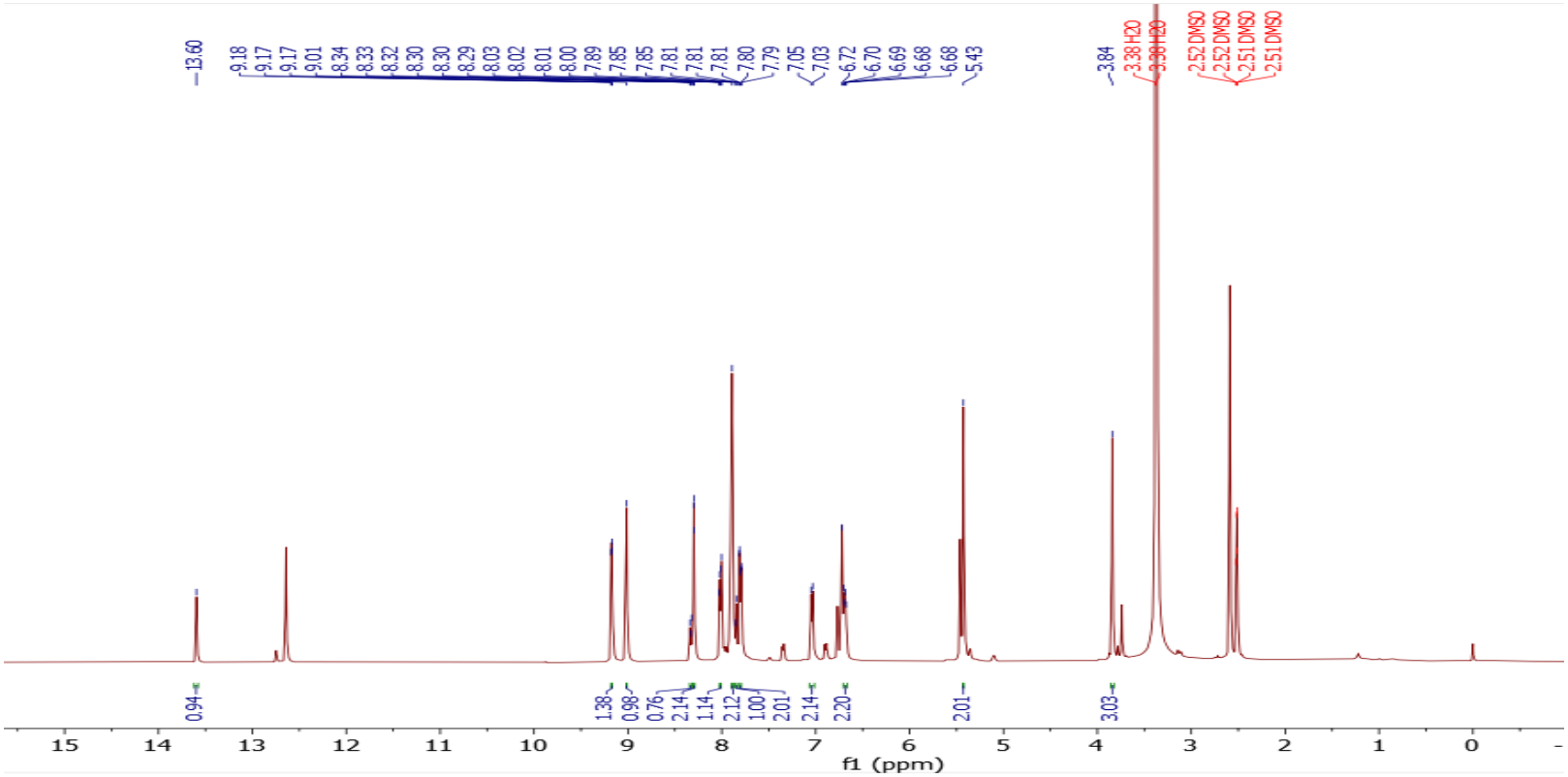


Figure S16. ^{13}C NMR spectrum of compound 7a

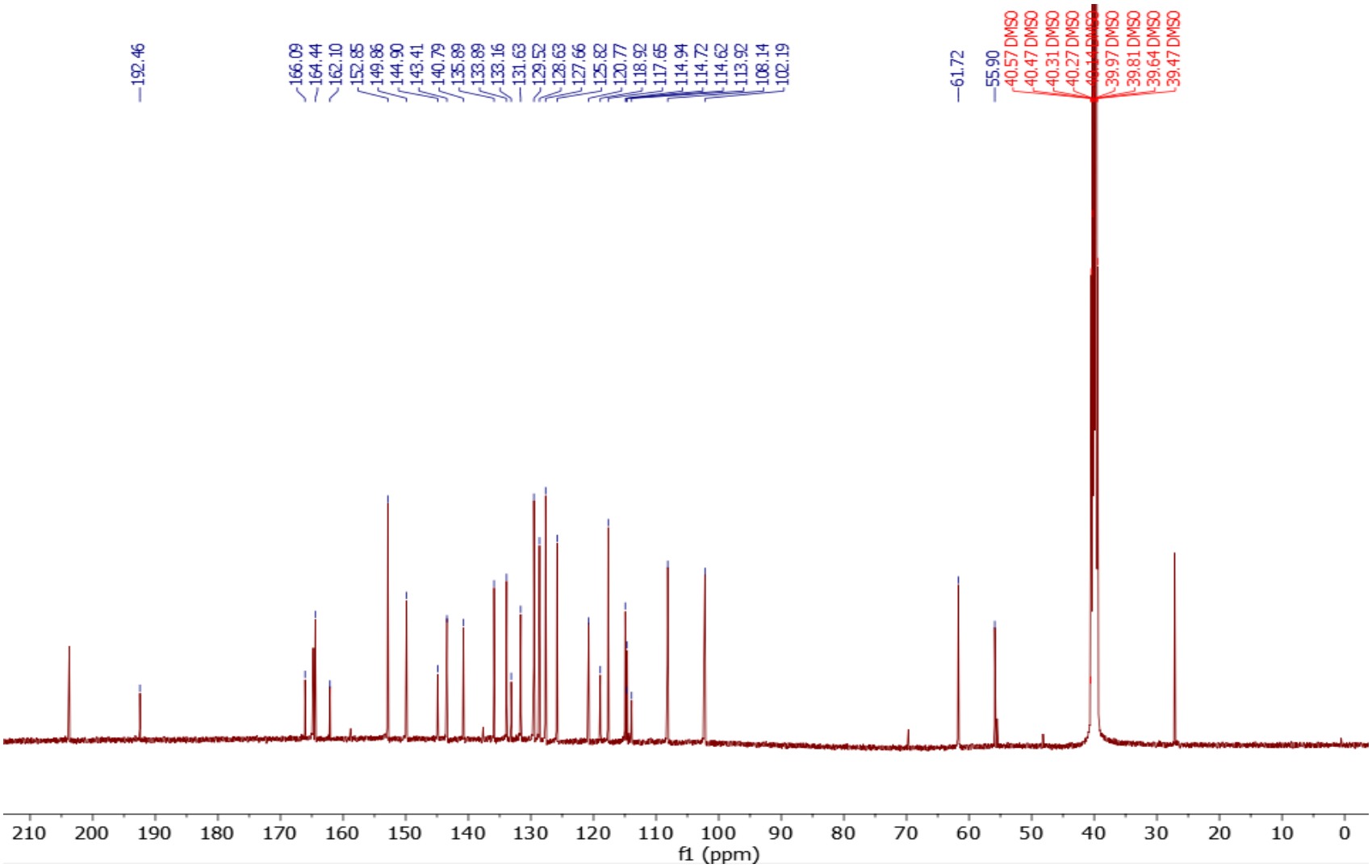


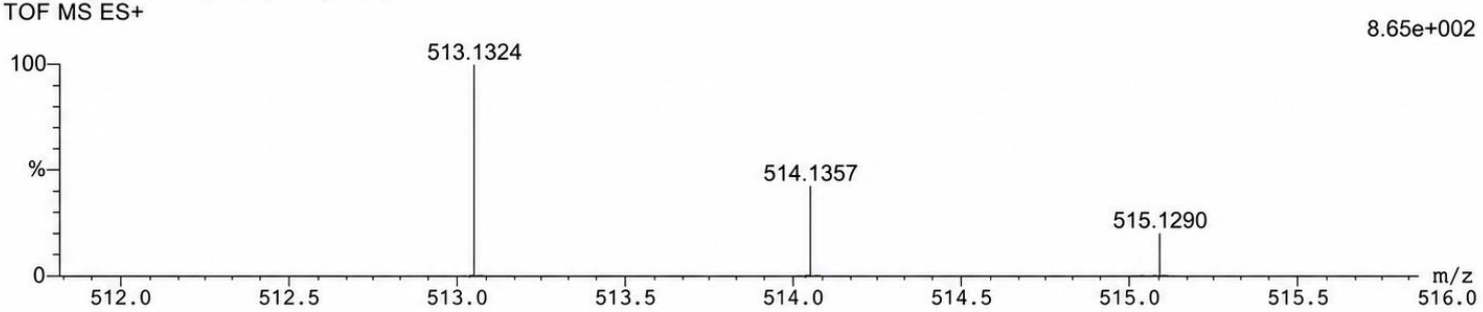
Figure S17. HRMS spectrum of compound 7a

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -5.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
28930 formula(e) evaluated with 499 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Cl: 0-8 Na: 0-1

KK-TZL-DIOME 7a (1.421) Cm (79:93)
TOF MS ES+



Minimum:				-5.0								
Maximum:		10.0	10.0	50.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
513.1324	513.1324	0.0	0.0	21.5	90.8	11.3	C28	H22	Cl	N4	O4	
513.1336	513.1336	-1.2	-2.3	23.5	90.1	12.1	C27	H20	Cl	N5	O3	Na
513.1311	513.1311	1.3	2.5	17.5	79.4	1.2	C26	H24	Cl	N3	O5	
513.1348	513.1348	-2.4	-4.7	18.5	80.5	3.2	C25	H26	Cl	N4	O4	
513.1299	513.1299	2.5	4.9	20.5	77.1	-0.1	C29	H18	Cl	N3	O3	

Figure S18. UV–Vis spectrum of compound 7b

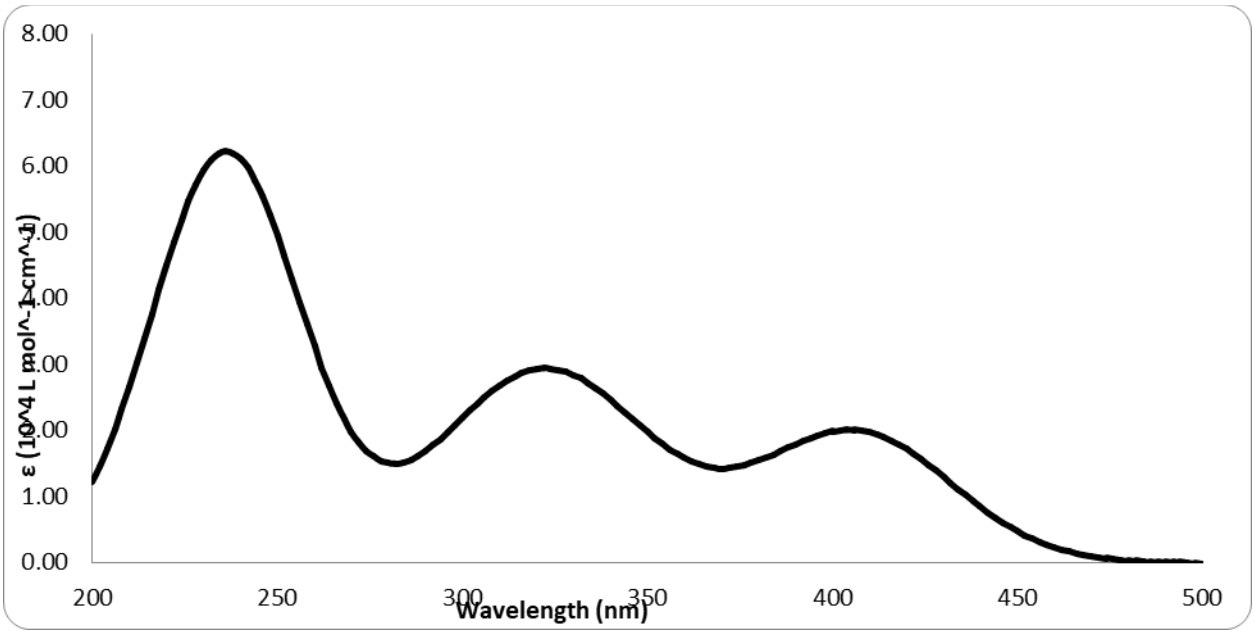


Figure S19. FTIR spectrum of compound 7b

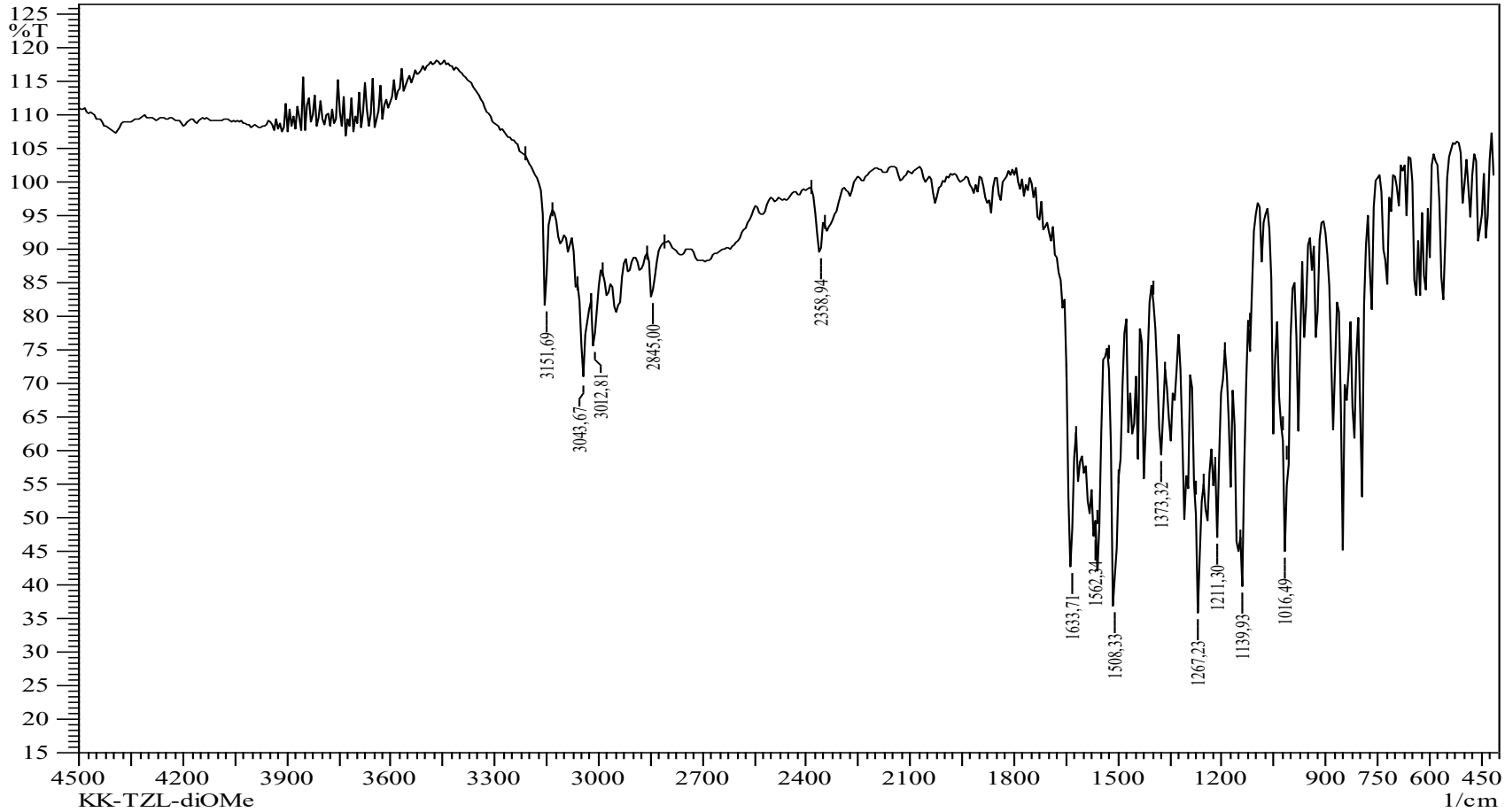


Figure S20. ^{1}H NMR spectrum of compound 7b

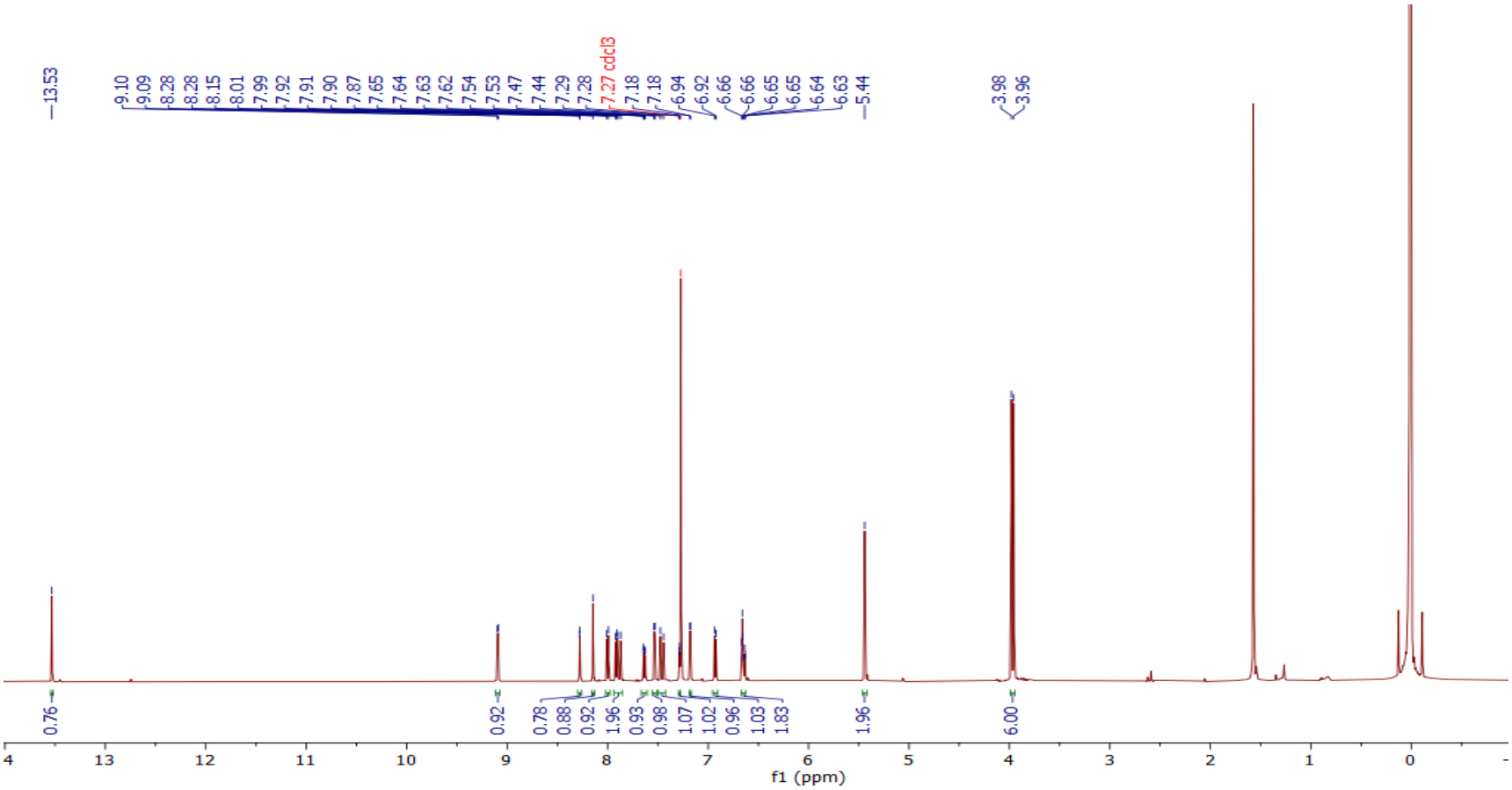


Figure S21. ^{13}C NMR spectrum of compound 7b

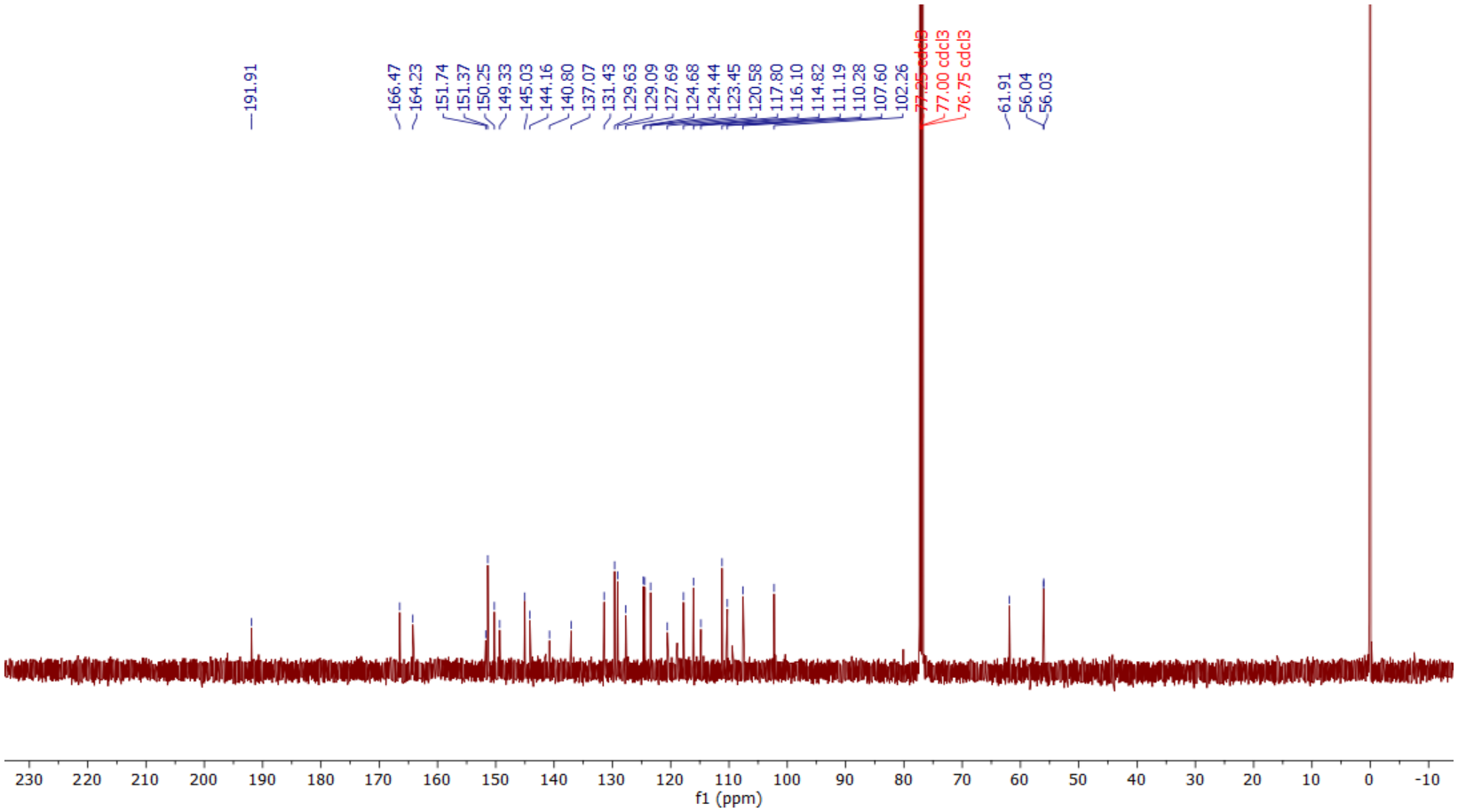


Figure S22. HRMS spectrum of compound 7b

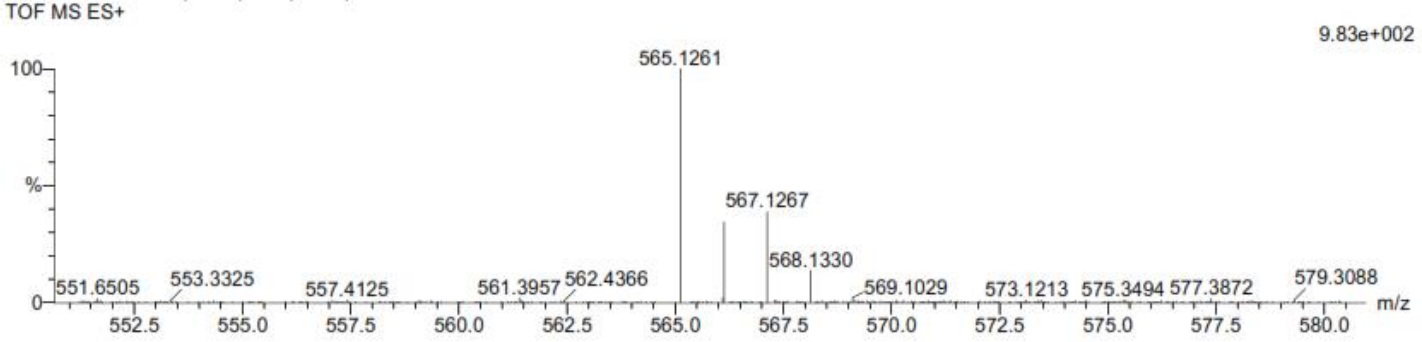
Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 mDa / DBE: min = -5.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
28930 formula(e) evaluated with 499 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Cl: 0-8 Na: 0-1

KK-TZL-DIOME 83 (1.428) Cm (79:93)
TOF MS ES+



Minimum:				-5.0							
Maximum:		10.0	10.0	50.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula				
565.1261	565.1257	0.4	0.7	21.5	84.3	11.3	C36	H28	Cl3		
	565.1263	-0.2	-0.4	23.5	85.2	12.3	C34	H22	O7	Na	
	565.1260	0.1	0.2	27.5	84.9	12.0	C32	H17	N6	O5	
	565.1265	-0.4	-0.7	17.5	74.2	1.3	C30	H26	O9	Cl	
	565.1268	-0.7	-1.2	24.5	74.6	1.6	C30	H19	N8	O	Cl Na
	565.1255	0.6	1.1	19.5	74.3	1.3	C29	H23	N4	O5	Cl Na
	565.1252	0.9	1.6	23.5	74.3	1.4	C27	H18	N10	O3	Cl
	565.1270	-0.9	-1.6	18.5	82.3	9.3	C26	H23	N8	O3	Cl2

Figure S23. UV–Vis spectrum of compound 7c

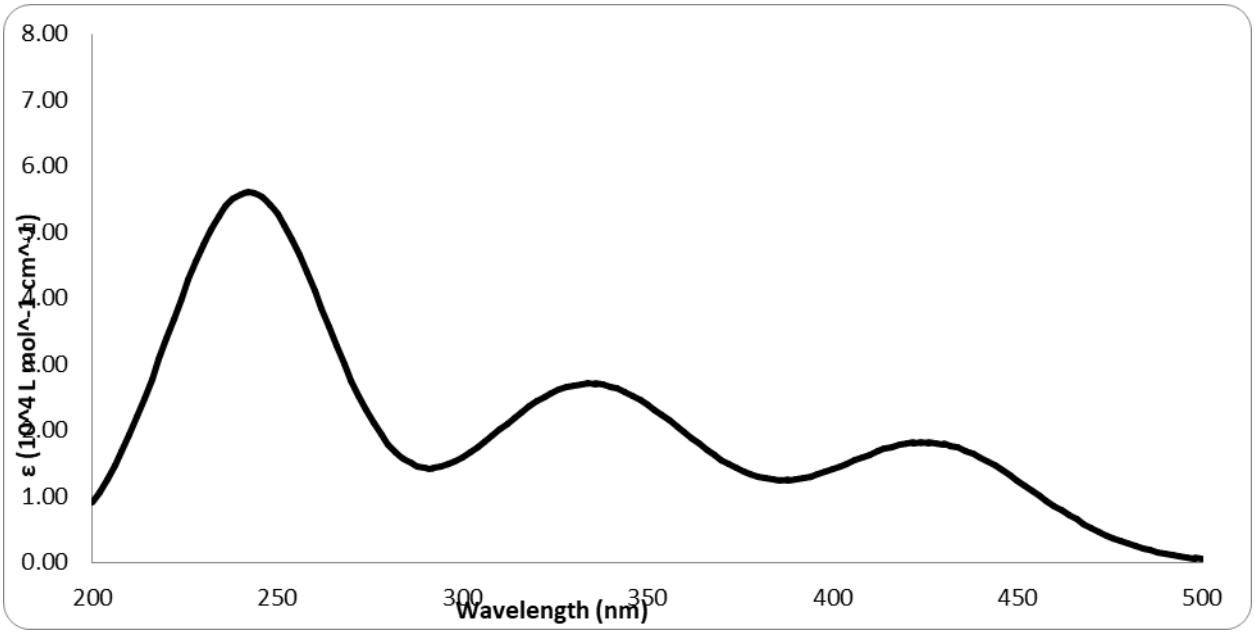


Figure S24. FTIR spectrum of compound 7c

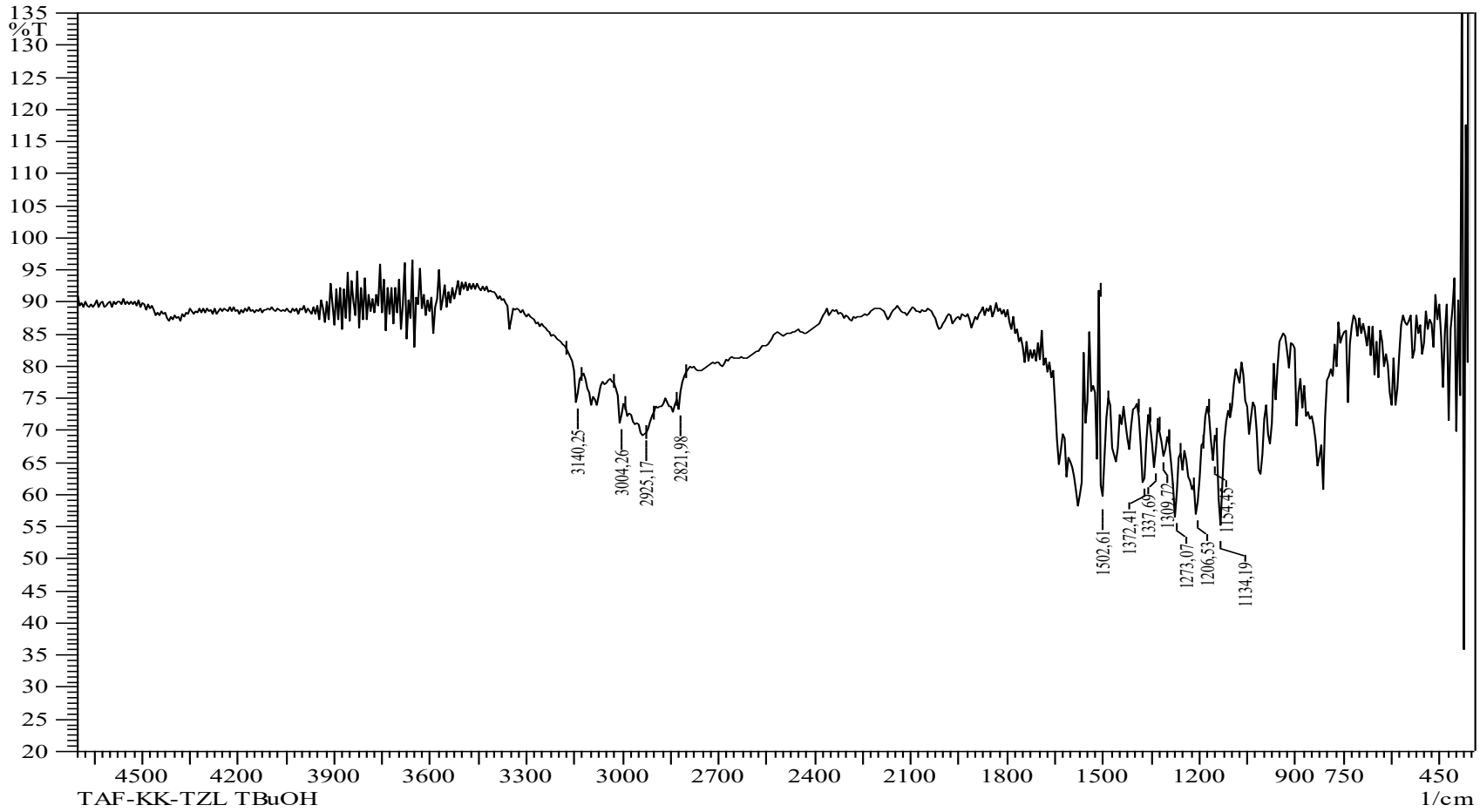


Figure S25. ^{1}H NMR spectrum of compound 7c

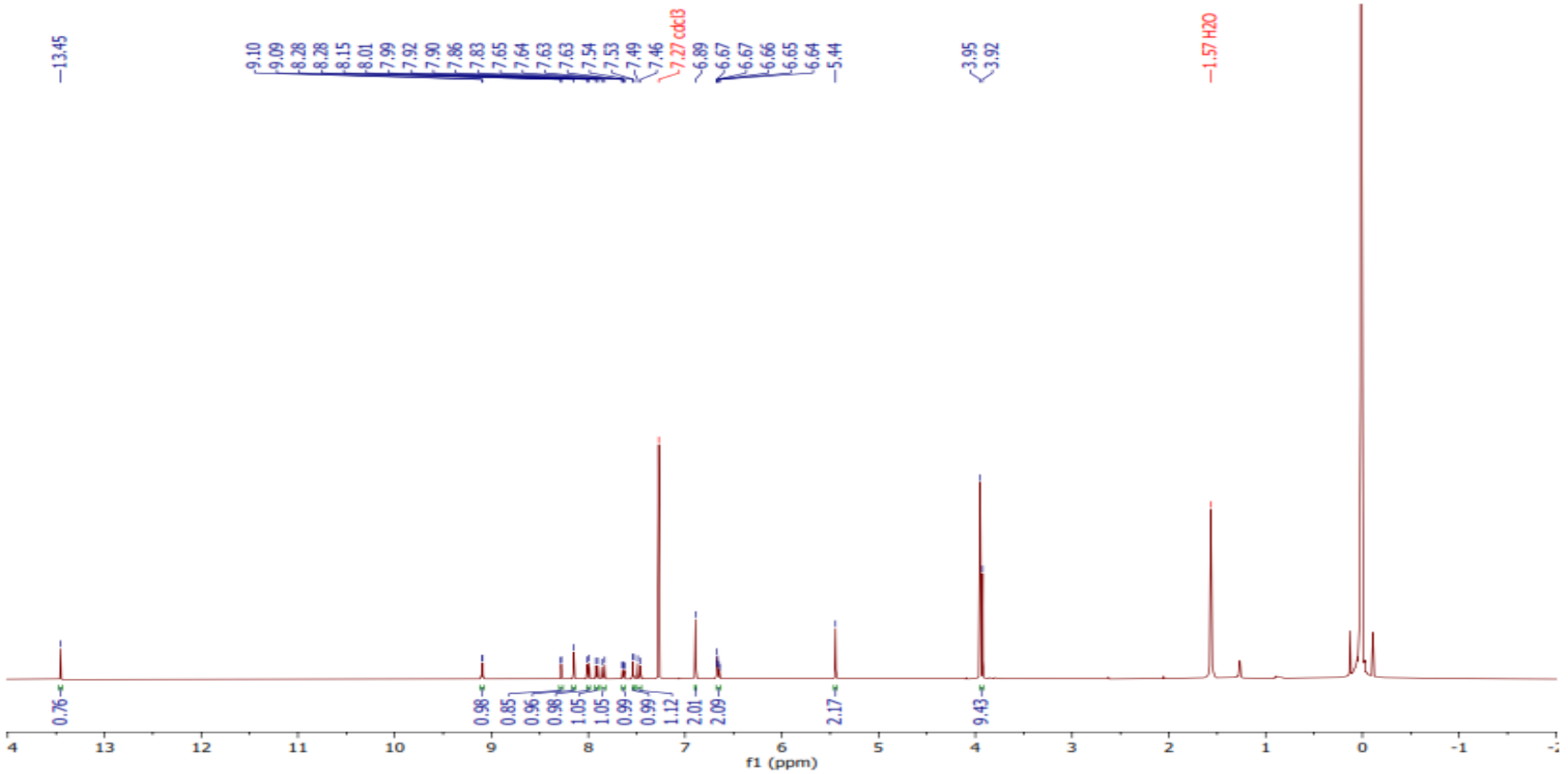


Figure S26. ^{13}C NMR spectrum of compound 7c

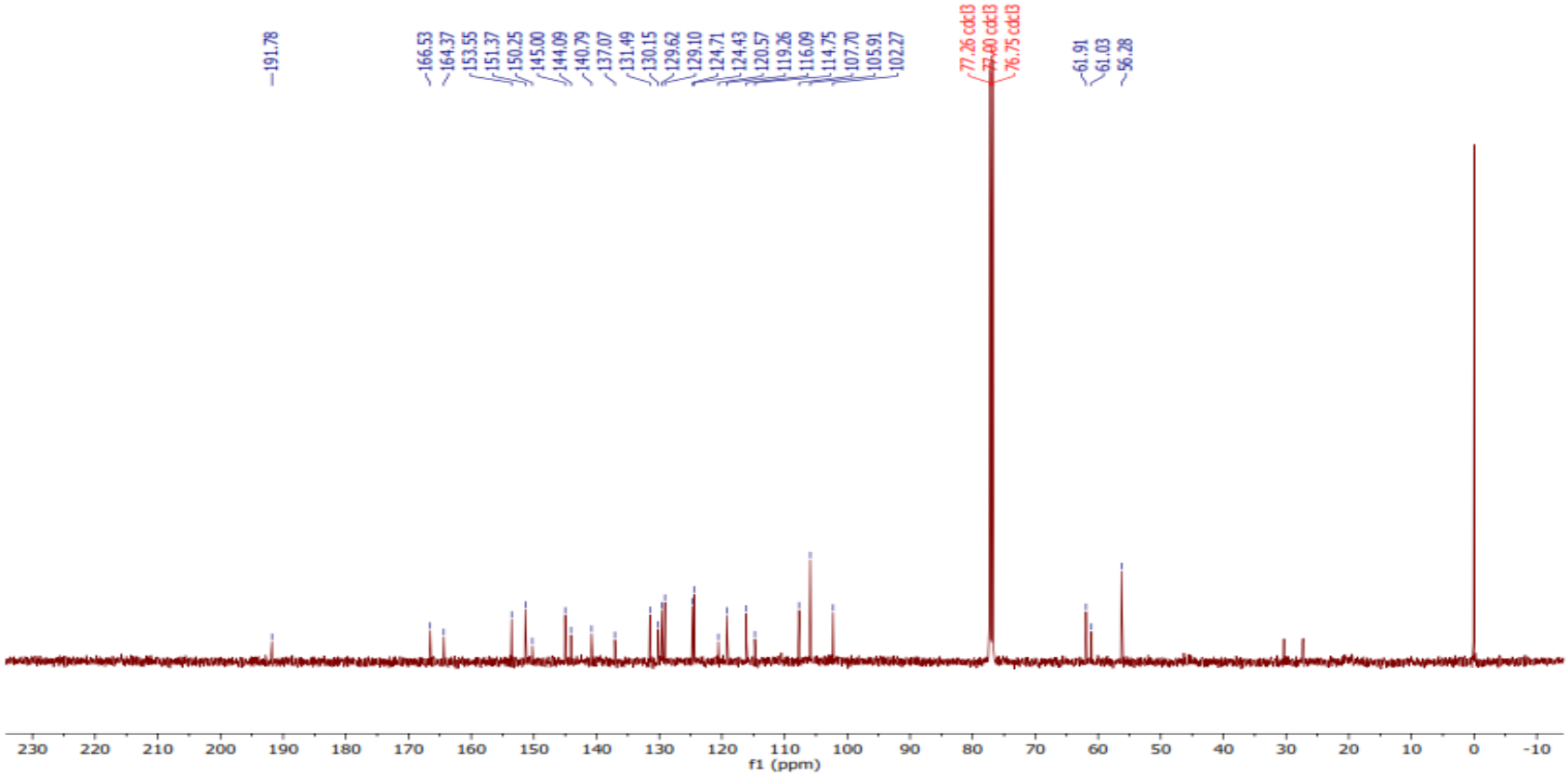
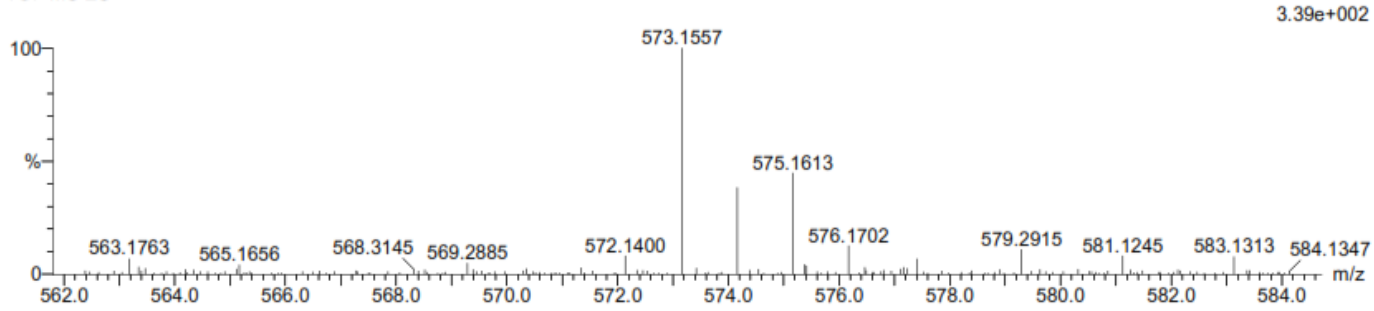


Figure S27. HRMS spectrum of compound 7c

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -5.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
16287 formula(e) evaluated with 251 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Cl: 0-8
TAF KK TZL QN 1 (0.034) Cm (1:2)
TOF MS ES+



Minimum:				-5.0							
Maximum:		10.0	10.0	50.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula				
573.1557	573.1576	-1.9	-3.3	33.5	52.3	6.0	C37	H17	N8		
	573.1563	-0.6	-1.0	28.5	52.2	6.0	C36	H21	N4	O4	
	573.1549	0.8	1.4	23.5	52.2	6.0	C35	H25	O8		
	573.1554	0.3	0.5	24.5	46.9	0.7	C31	H22	N8	O2	Cl
	573.1573	-1.6	-2.8	19.5	51.3	5.0	C30	H27	N6	O2	Cl2
	573.1541	1.6	2.8	19.5	47.2	0.9	C30	H26	N4	O6	Cl
	573.1559	-0.2	-0.3	14.5	51.6	5.3	C29	H31	N2	O6	Cl2
	573.1577	-2.0	-3.5	9.5	53.8	7.6	C28	H36	O6	Cl3	

Supplementary Pharmacophore modeling

To identify the key structural features essential for the antifungal activity of synthesised chalcone–quinoline hybrids, pharmacophore modelling was carried out. The generated pharmacophore model is shown in **Fig. S28** and highlights key interaction features including hydrogen bond donors/acceptors, hydrophobic and aromatic regions. The combined properties provide a spatial arrangement that is required for the best fit to the active site of lanosterol 14α-demethylase (CYP51), consistent with the molecular docking and biochemical data described in the previous sections.

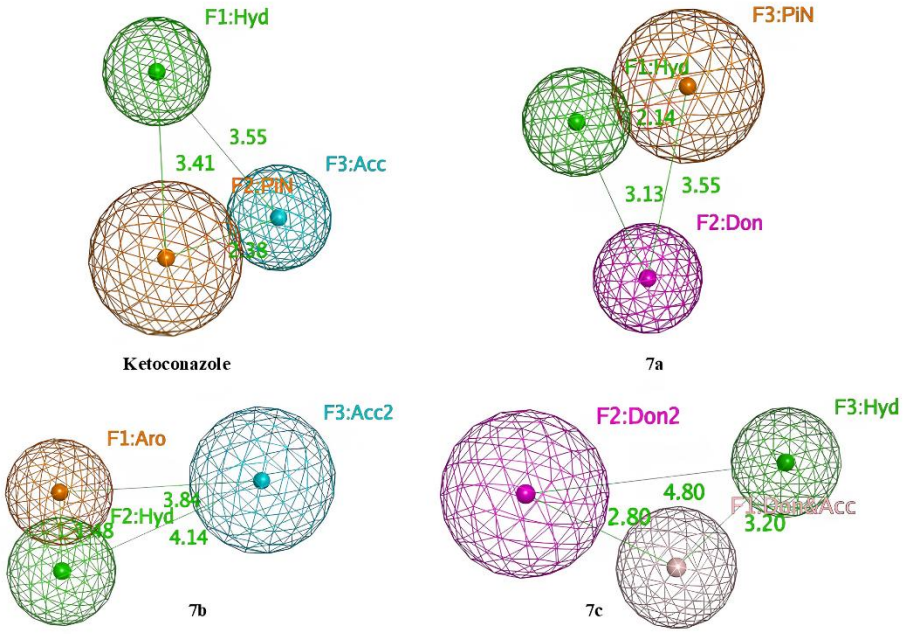


Fig. S28 Pharmacophore model illustrating key interaction features of the studied compounds. Color code: pink = donor and acceptor; green = hydrophobic; blue = acceptor; orange = aromatic.

According to the pharmacophore model, all the active molecules share a common structure with (i) an aromatic core, (ii) hydrogen bond acceptor/donor functions, and (iii) hydrophobic substituents. The chalcone moiety offers a large conjugated π-system which acts as an aromatic group while the carbonyl group acts as a hydrogen bond acceptor. The 1,2,3-triazole linker offers additional hydrogen bond acceptor sites and the quinoline ring enhances aromatic stacking interactions. This combination of properties is characteristic of many antifungal agents that bind to the CYP51 enzyme, and both hydrophobic and polar contacts are required for optimal binding in the enzyme cavity.

The most favourable alignment with the pharmacophore characteristics defined in Fig. 7 was found for compound **7b**. The 3,4-dimethoxyphenyl group has significant effects on both the hydrophobic interactions and the electron-donating effects, therefore increasing the electron density of the aromatic system and strengthening the π–π stacking interactions in the binding cavity of CYP51. Moreover, the methoxy groups may function as weak hydrogen bond acceptors, so enhancing the stability of ligand–protein interactions. The optimal disposition of the pharmacophoric elements corresponds to the highest docking score, the lowest MIC values and the most effective CYP51 inhibition observed for **7b**.

In contrast, compound **7a** had a suboptimal pharmacophore alignment with only one methoxy group. This limited substitution leads to a reduction in the hydrophobic surface area and also in the electron-donating capacity, which leads to a reduced interaction with the pharmacophore model. So, **7a** exhibited the lowest antifungal activity of the drugs tested. Although compound **7c** has an additional methoxy group, it also had increased steric bulk and possible conformational restrictions, which resulted in poor pharmacophore fitting. Three methoxy groups can hinder correct alignment within the binding site, reducing the extent of overlap with key pharmacophoric features.

The pharmacophore analysis also highlights the importance of the triazole linker in designing molecules. The triazole ring acts as a rigid link to maintain the relative orientation of the chalcone and quinoline parts, and also as a source of hydrogen bond acceptor sites. This property is particularly relevant to CYP51 inhibition, as triazole-containing antifungal drugs are known to interact with the heme iron or neighbouring residues within the active site of the enzyme. Recent studies have revealed that triazole based pharmacophores have a great impact on the antifungal activity by increasing the specificity of binding and stability of interaction.

Moreover, the existence of many aromatic and hydrophobic domains in the pharmacophore model aligns with the hydrophobic characteristics of the CYP51 binding pocket. It is known that residues such as Phe126, Tyr132, Phe228 and Leu376 found in the docking study (Table 4) are known to facilitate π–π and hydrophobic interactions. The ability of compound **7b** to fit well into these pharmacophoric features explains the high binding affinity and biological activity. Recent studies in antifungal drug design have revealed similar pharmacophore elements, where a balance of aromaticity, hydrogen bonding, and hydrophobicity play key roles in the effective suppression of CYP51.

A structure–activity relationship in the chalcone–quinoline hybrid series is provided by the pharmacophore model. The superior antifungal potency of compound **7b** is a result of its optimal fit with the key pharmacophoric features, including high hydrophobicity, hydrogen bonding potential and favorable aromatic interactions. These results are consistent with recent medicinal chemistry publications which emphasized the importance of multi-feature pharmacophore models for guiding the design of potent antifungal drugs.

Finally, the pharmacophore analysis depicted in **Fig. S28** complements the molecular docking and experimental antifungal results to provide a unified understanding of the molecular aspects of antifungal activity. The observed pharmacophoric properties can give a helpful framework for the rational design and optimisation of new generation chalcone-based antifungal drugs targeting CYP51.