Supplementary Information

Design and synthesis of novel triazole-isofroxadin molecules: Docking studies against inflammatory and tuberculosis targets

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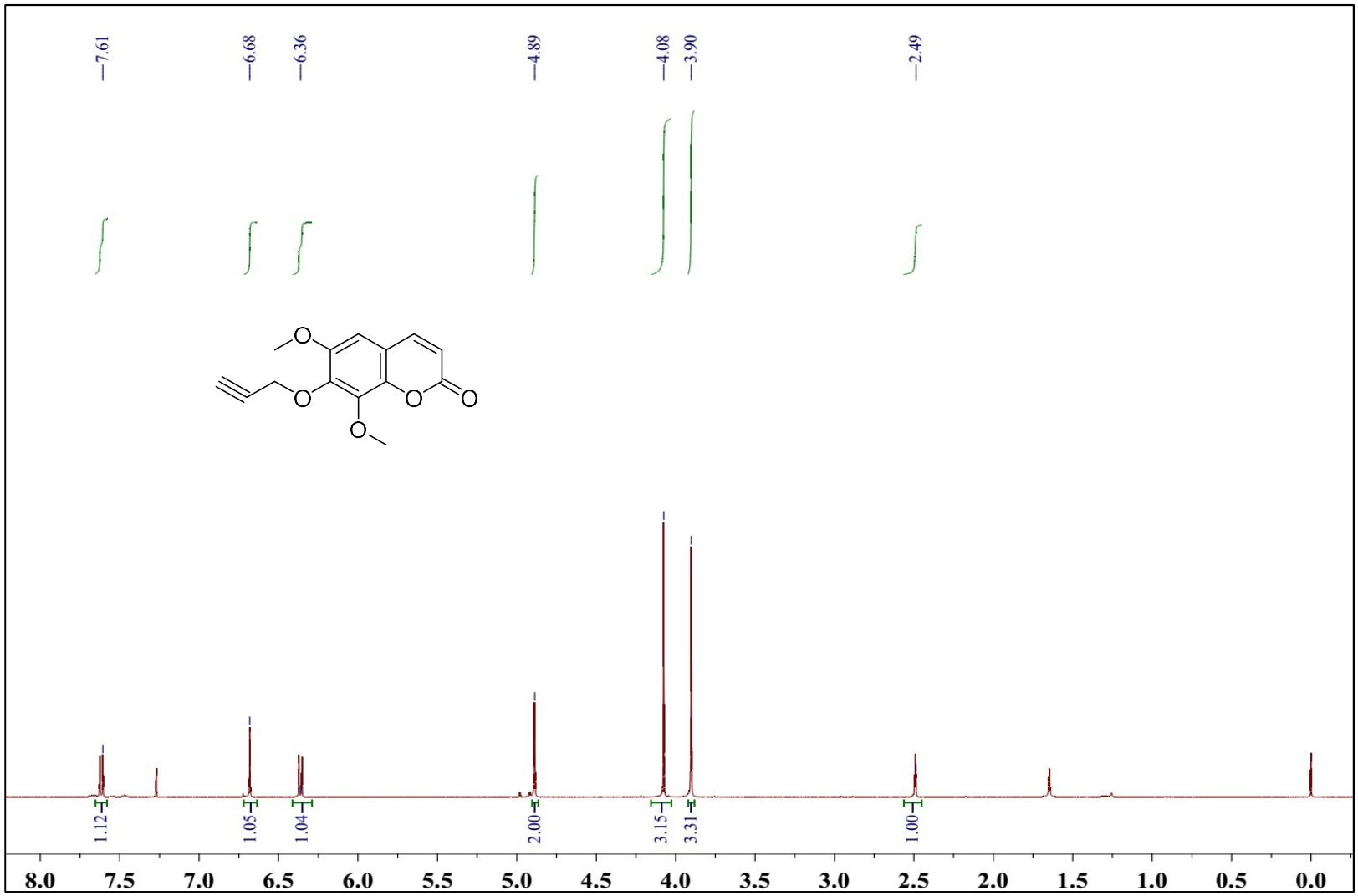
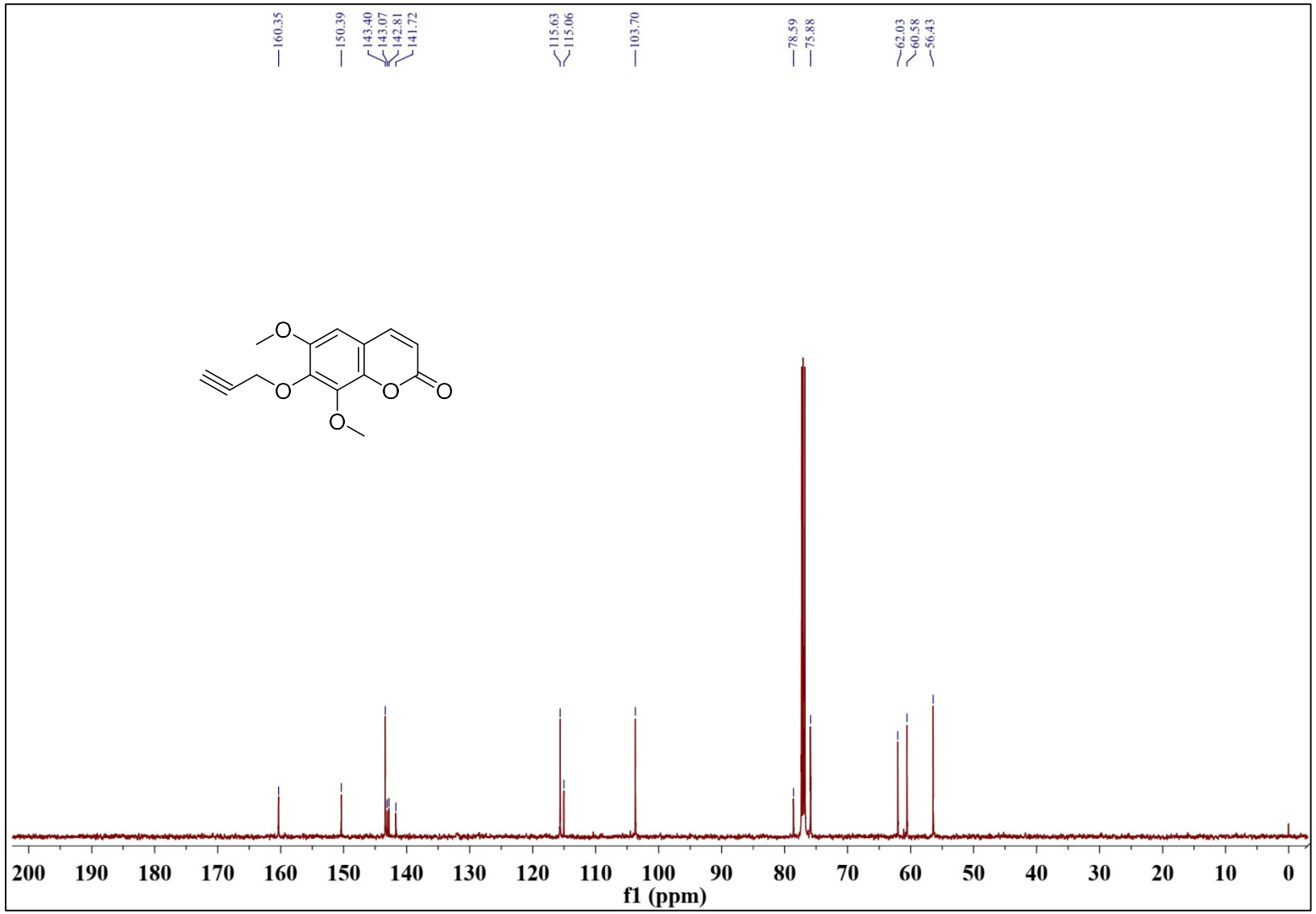
Received 10 January 2025; accepted (revised) 27 February 2025



^{1}H NMR of compound 5 in (400 MHz, $CDCl_3$)



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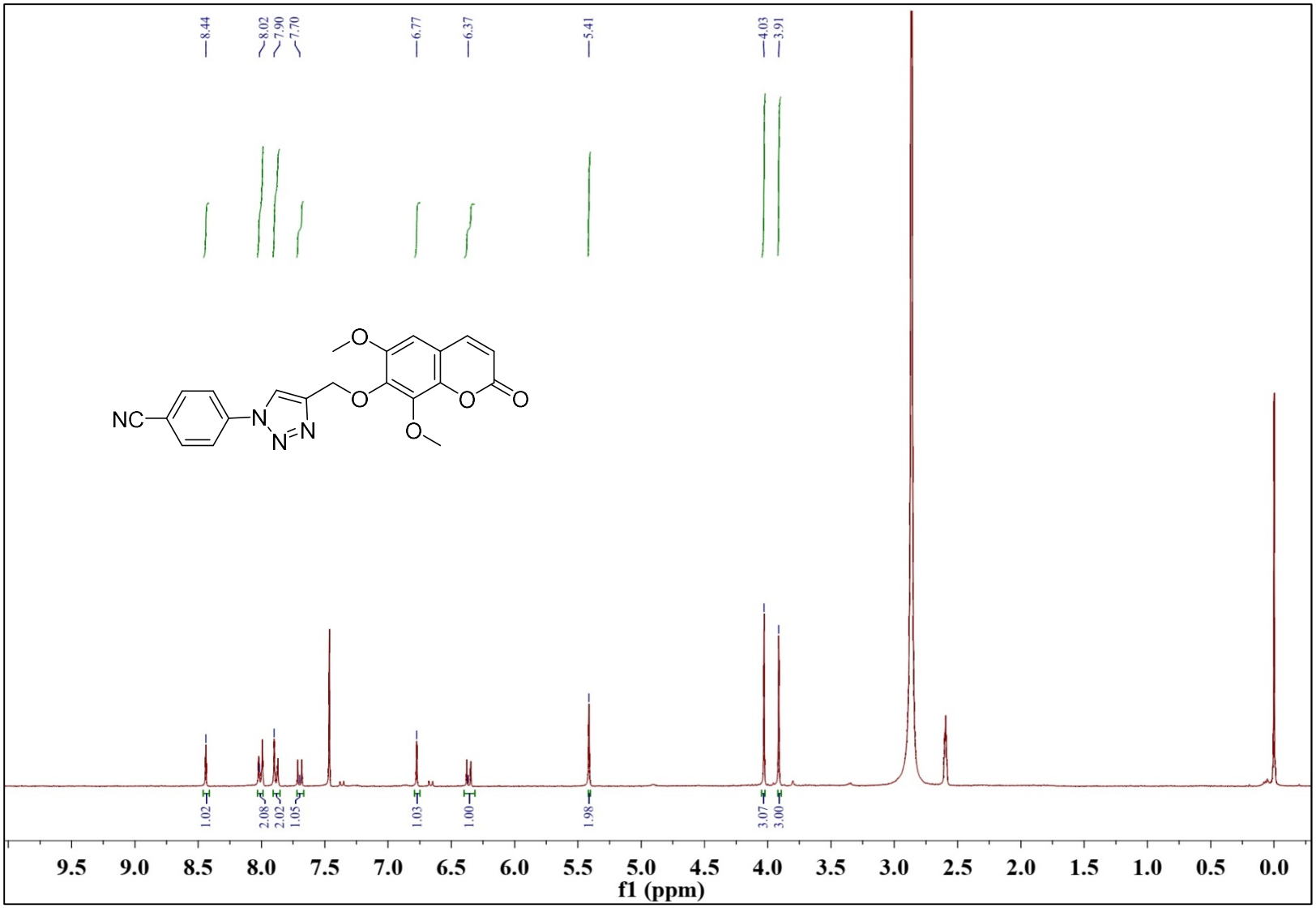

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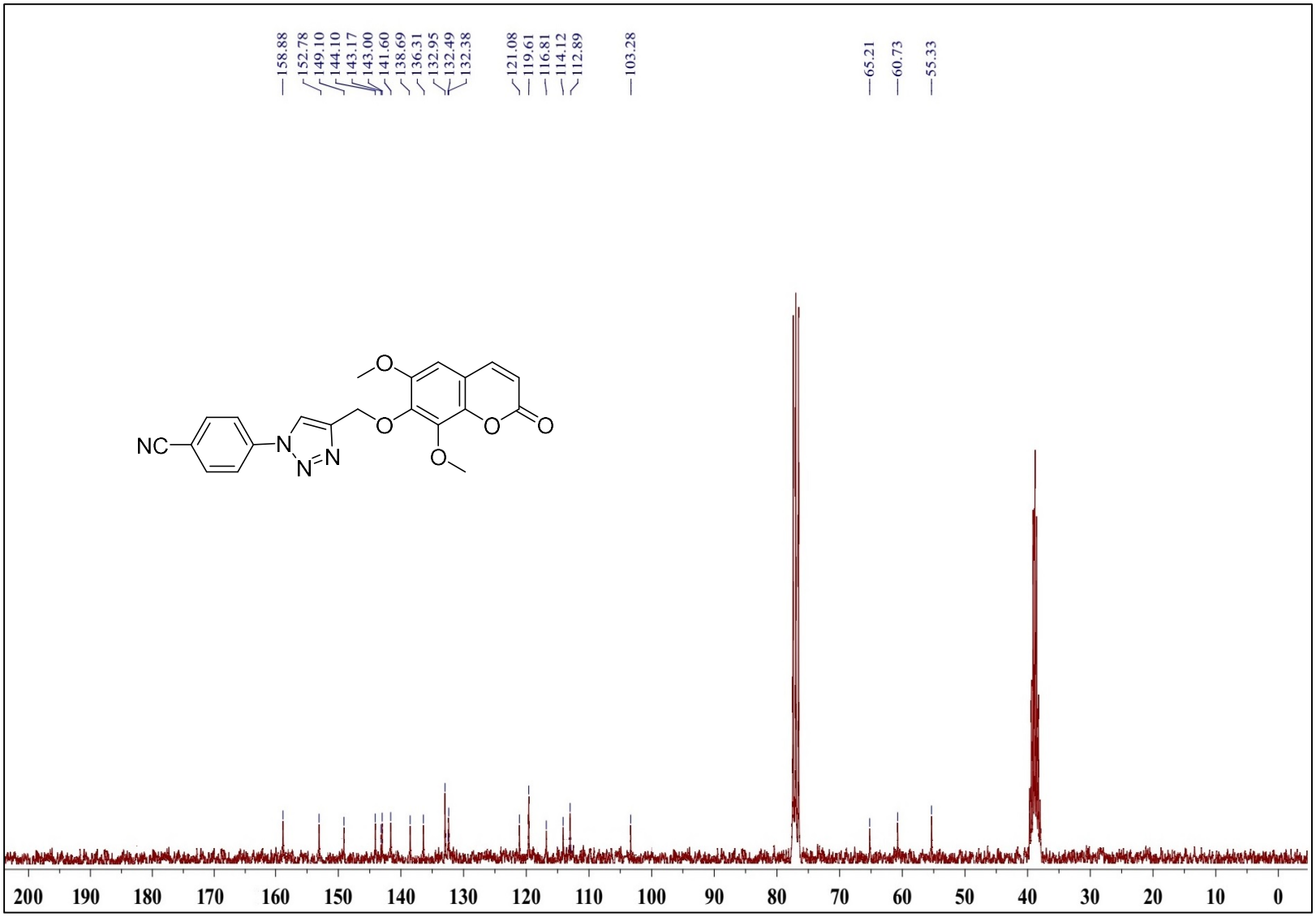

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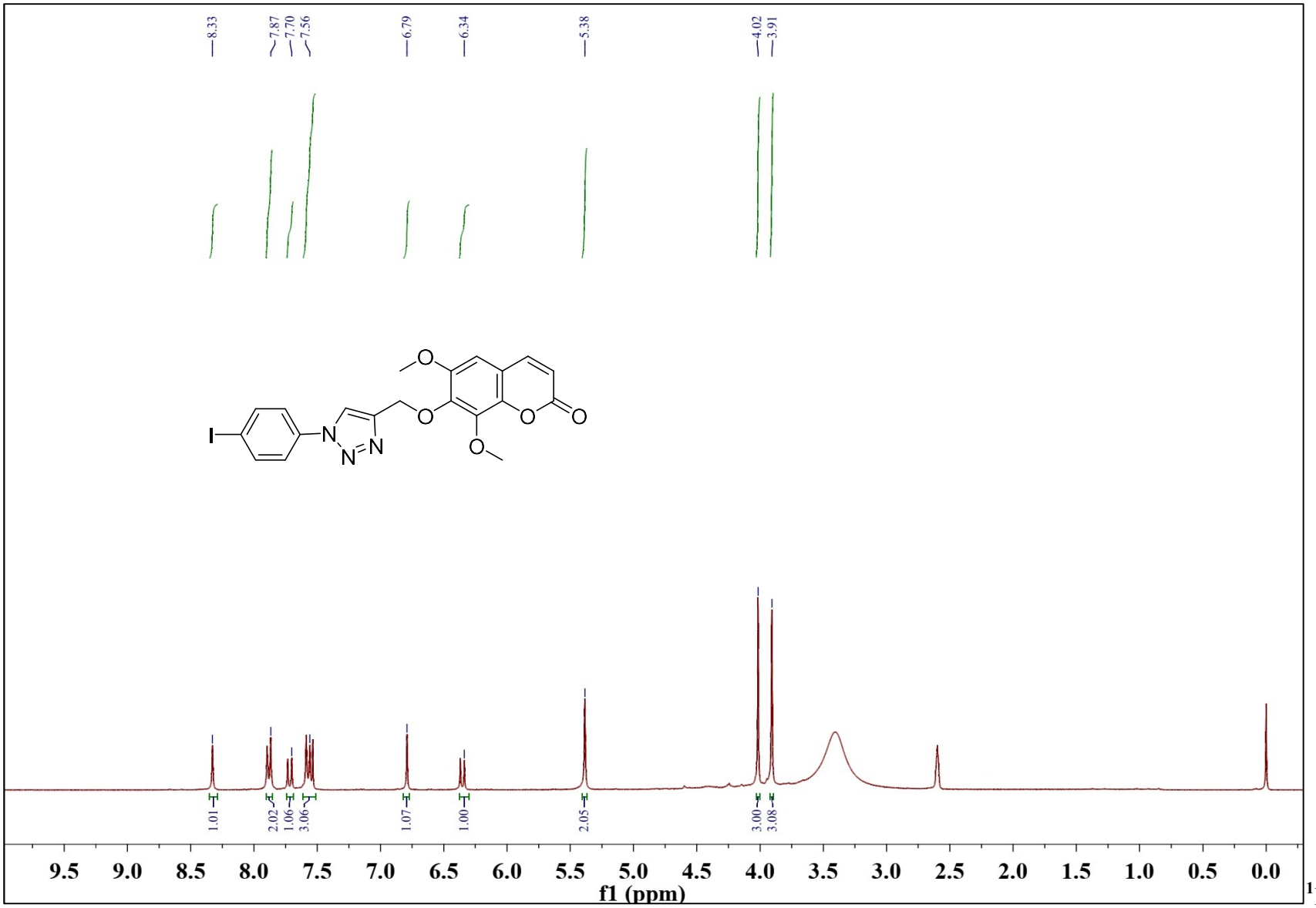

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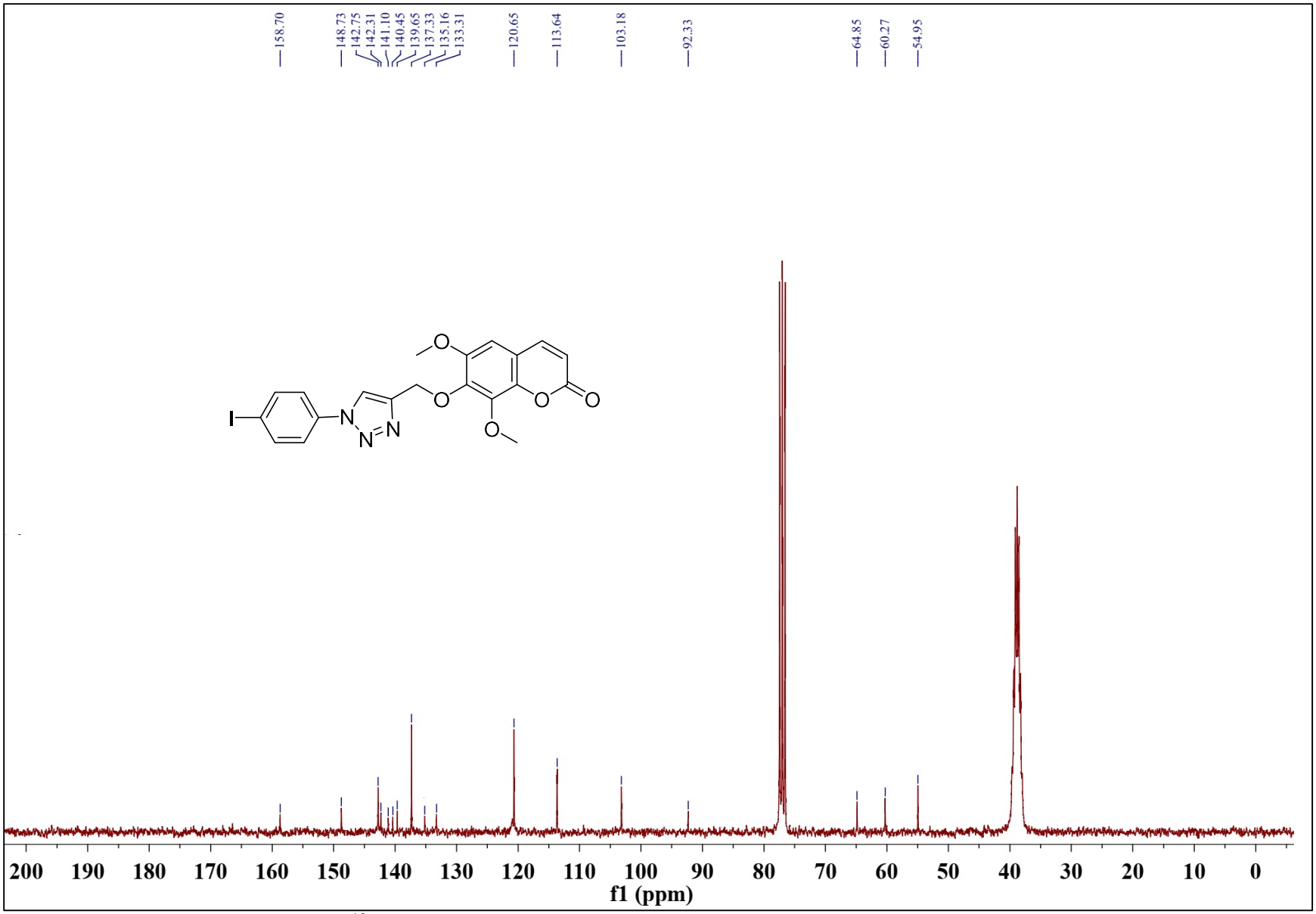

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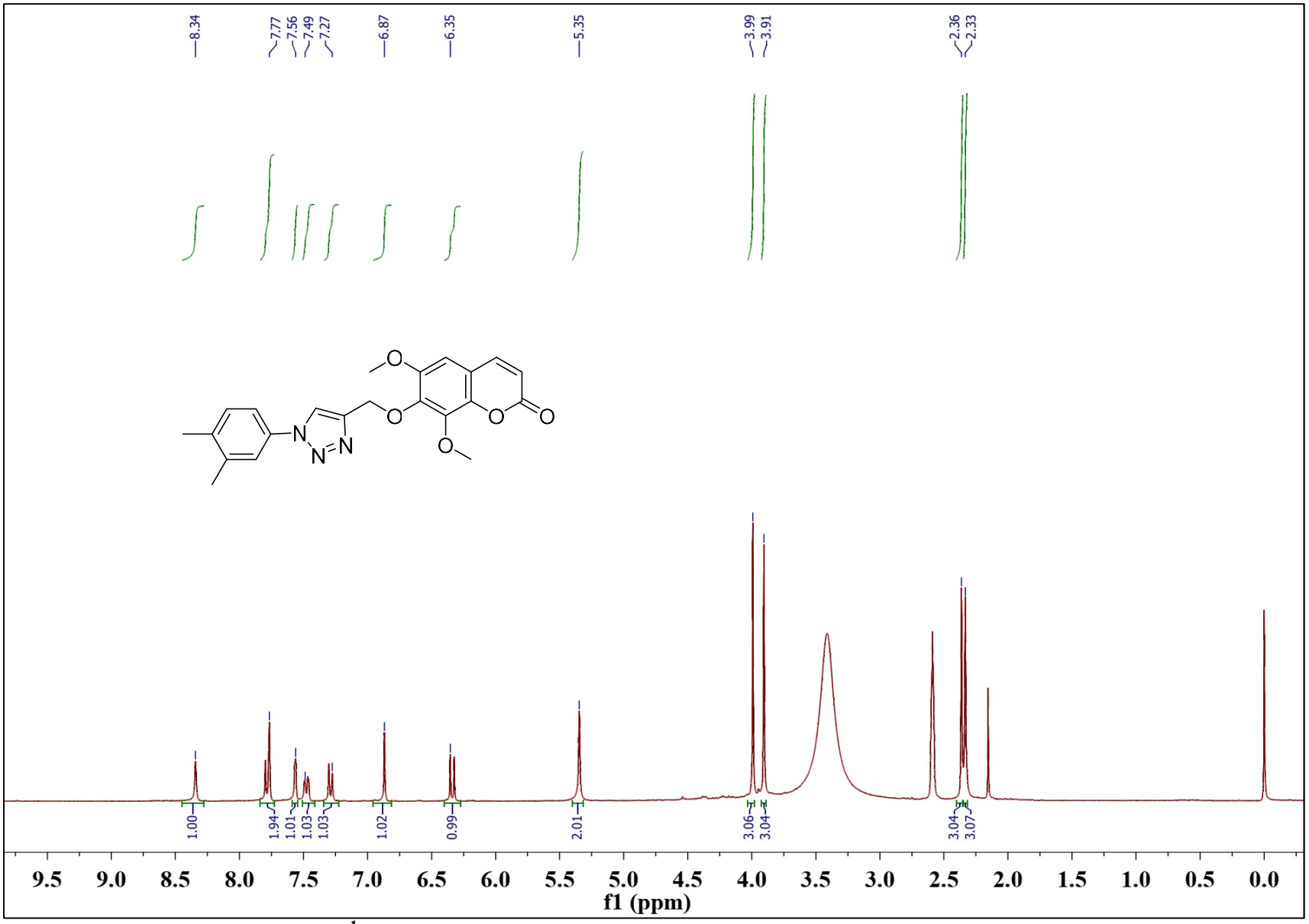

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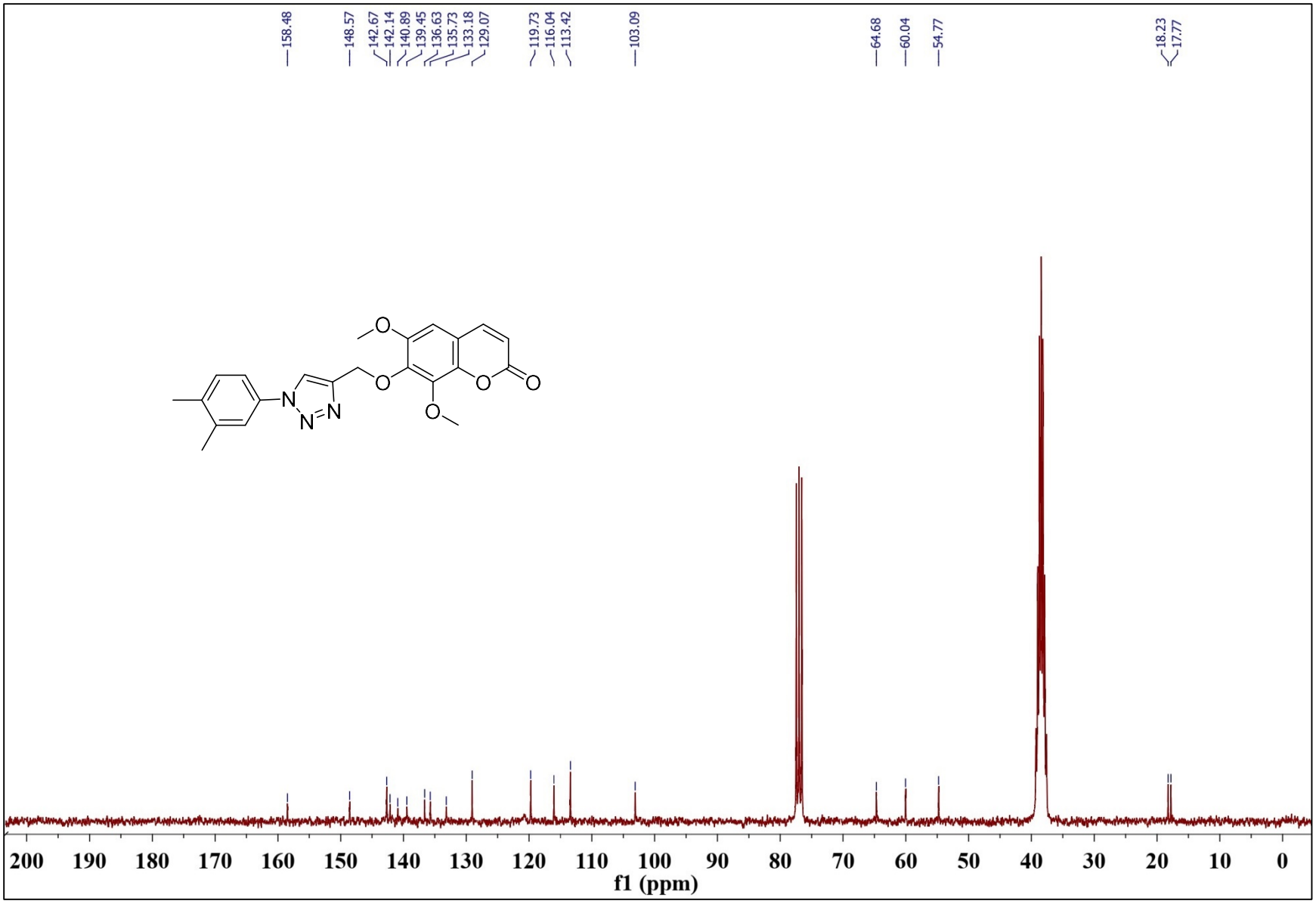

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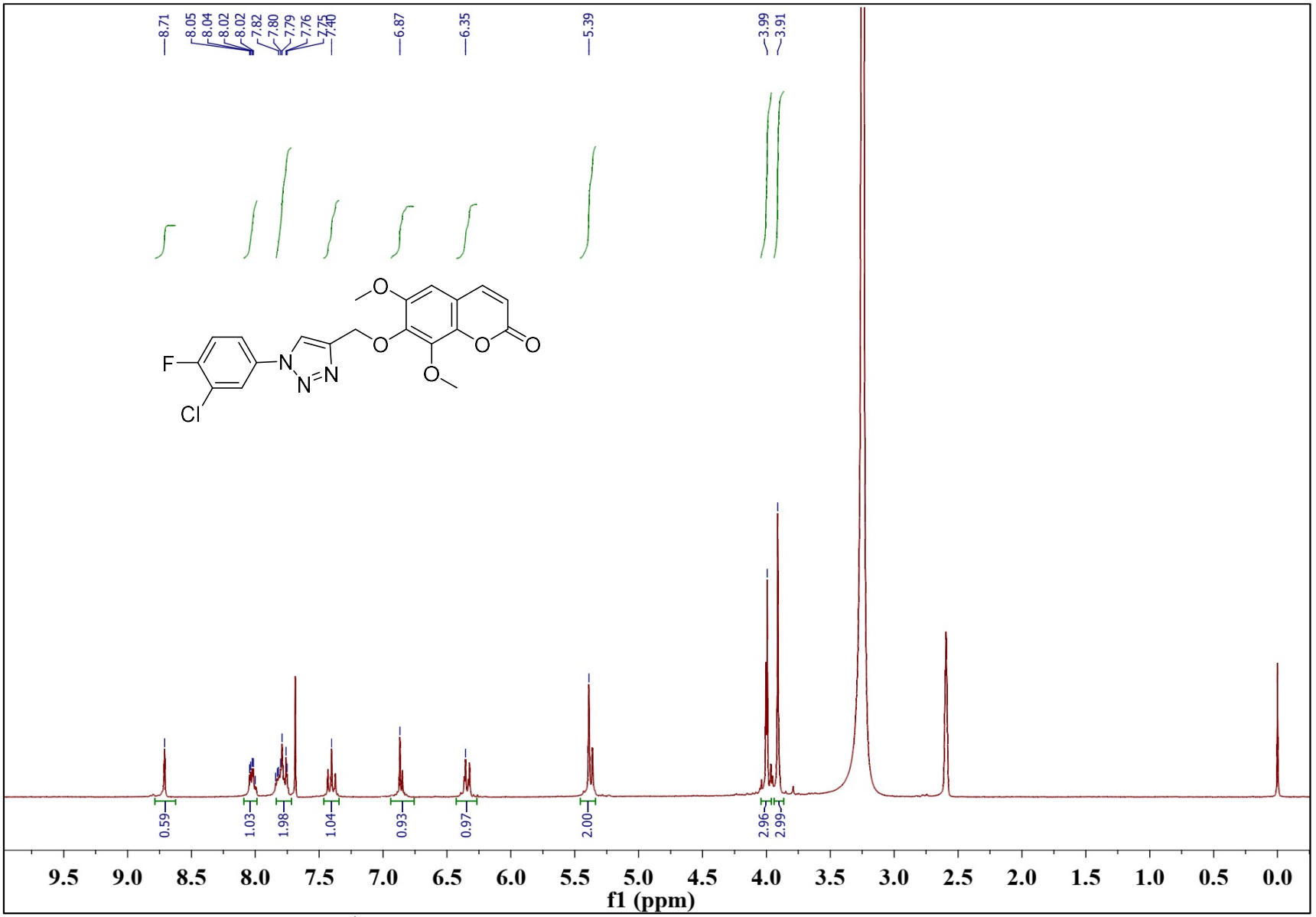

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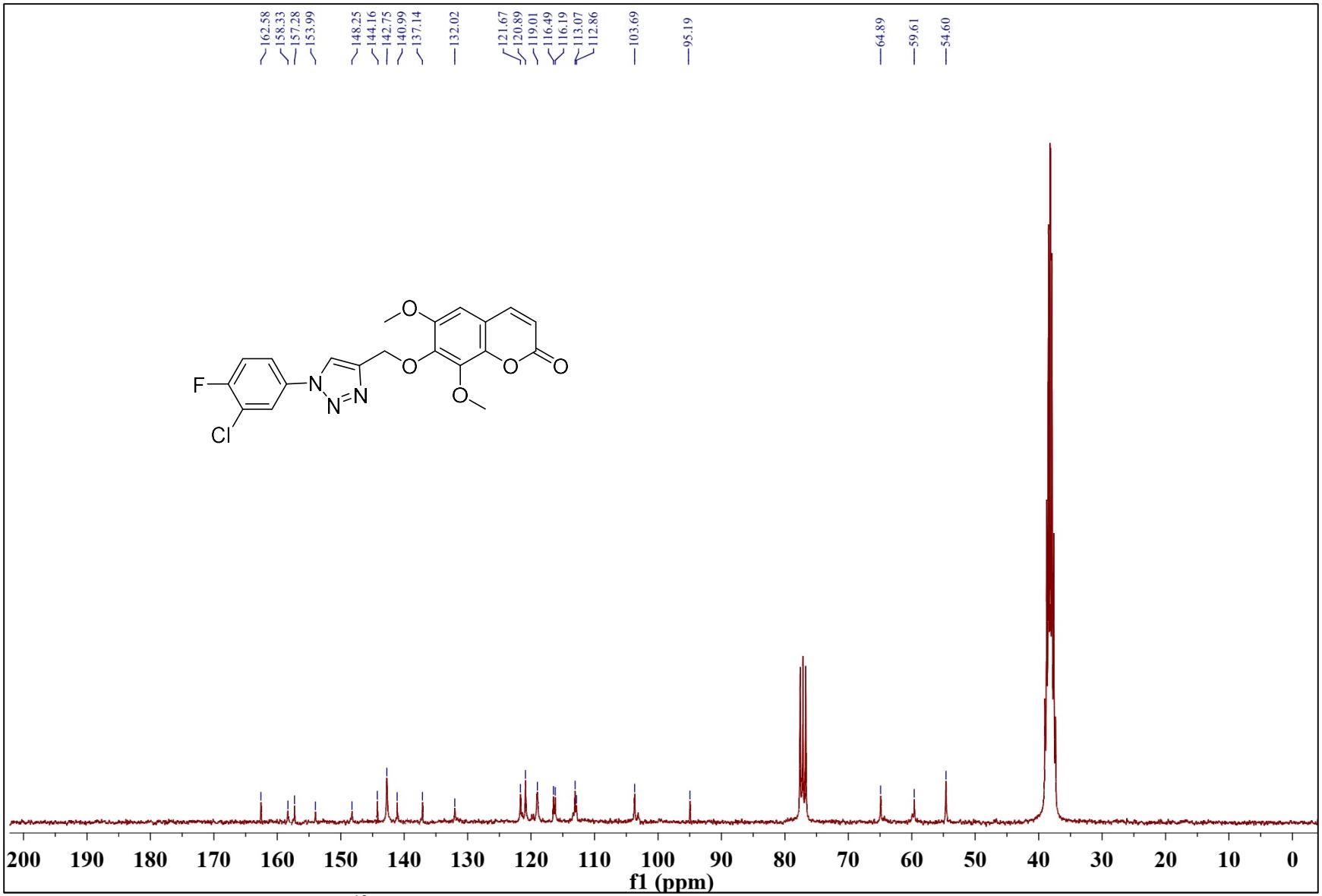

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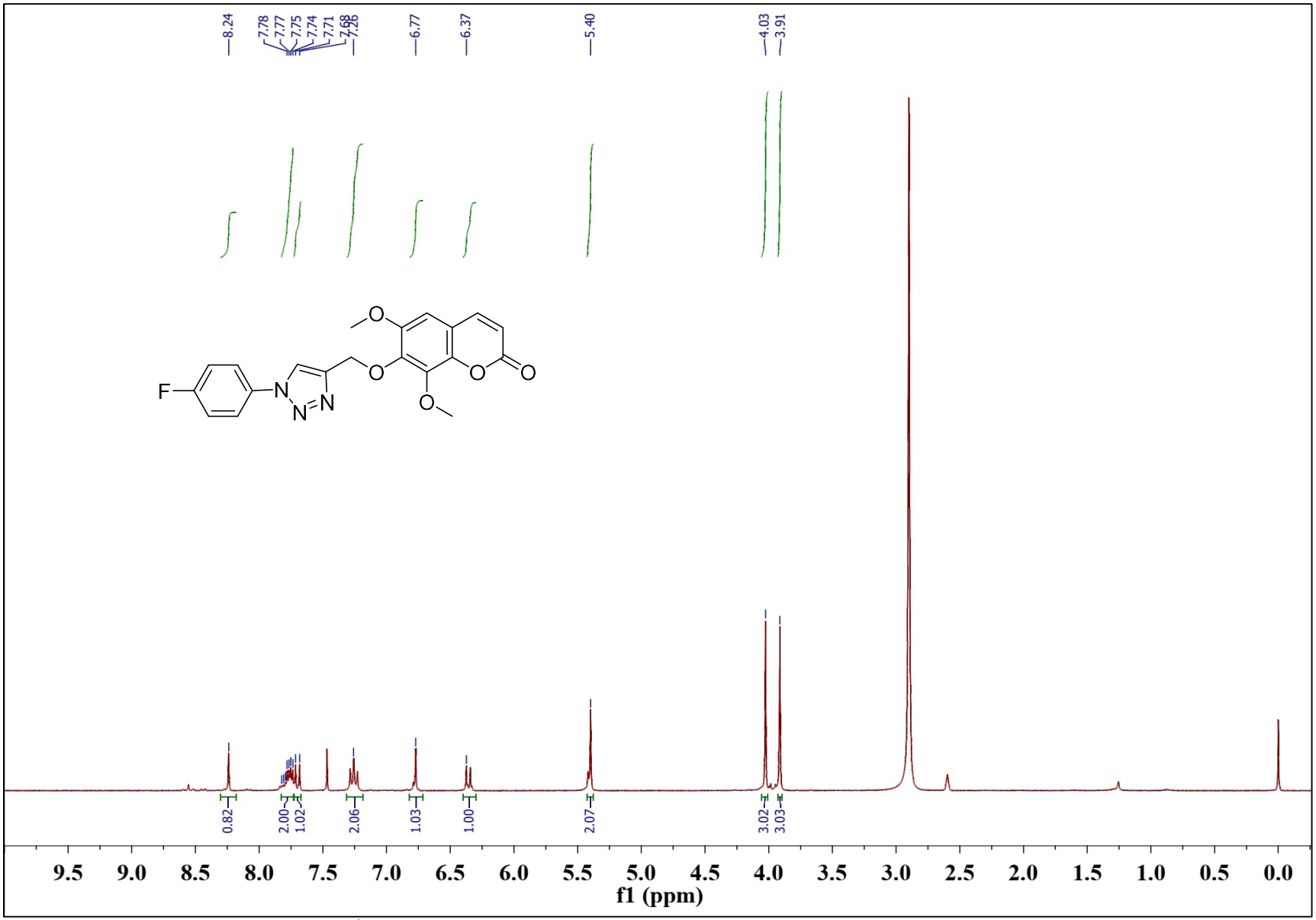

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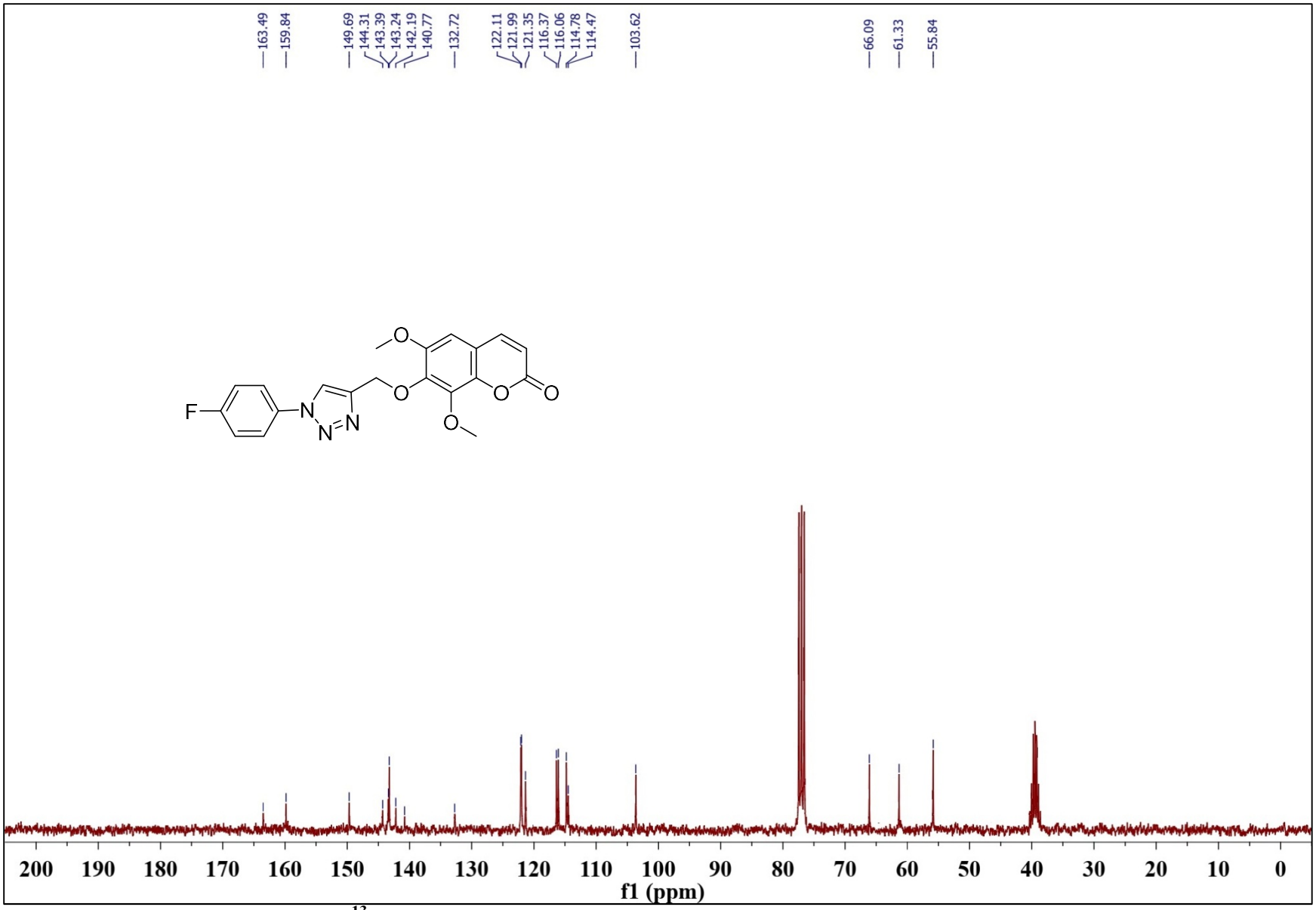

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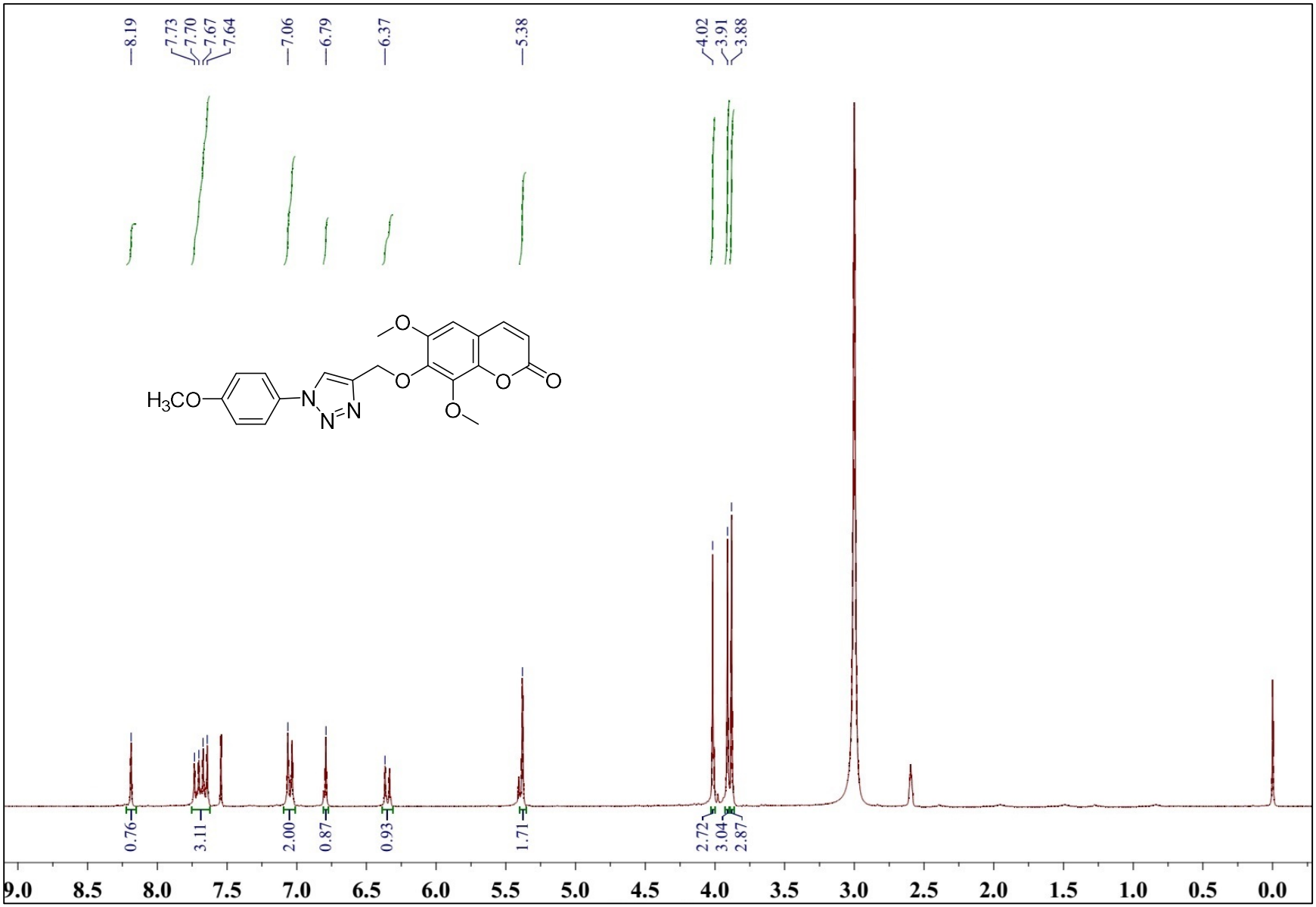

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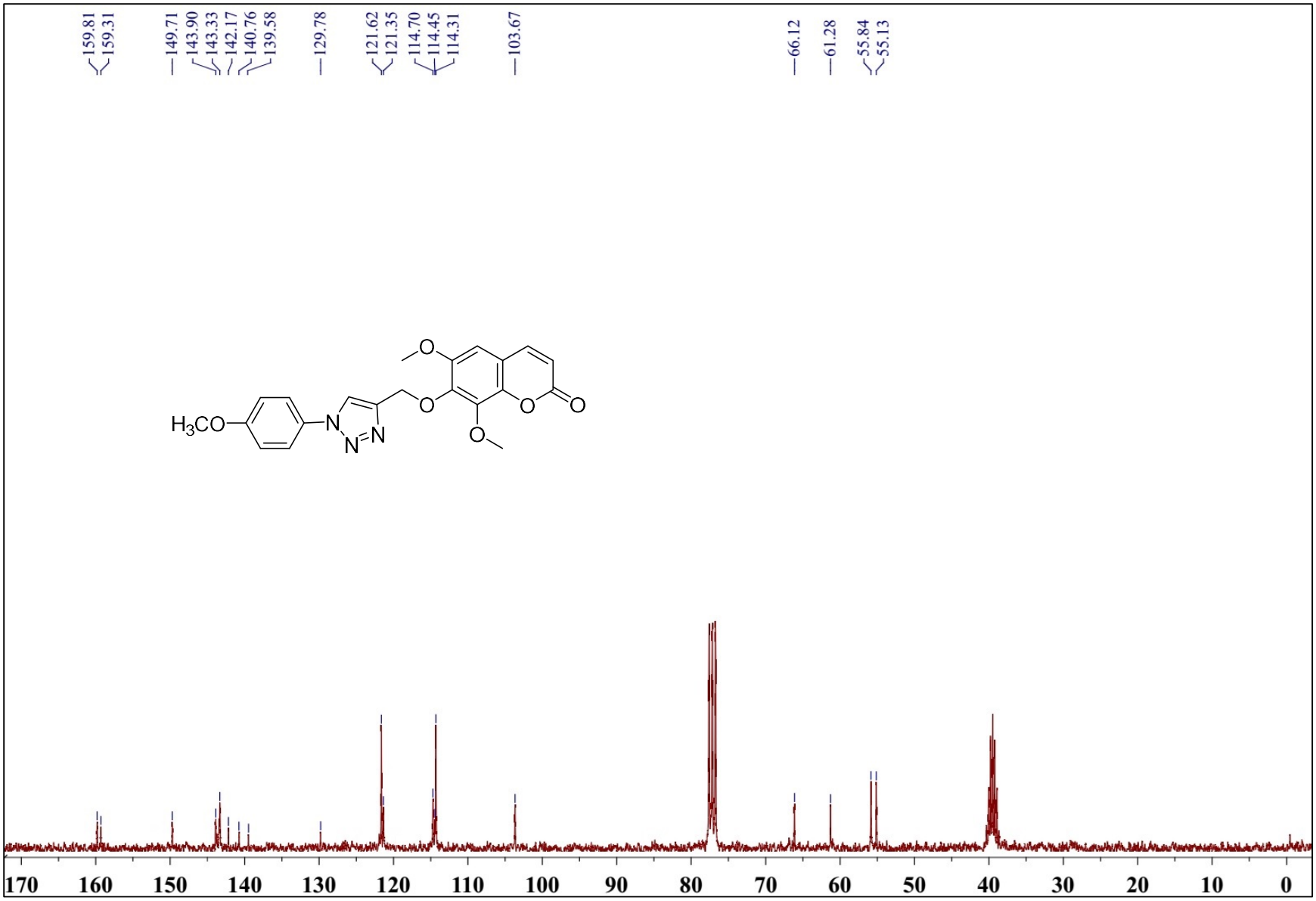

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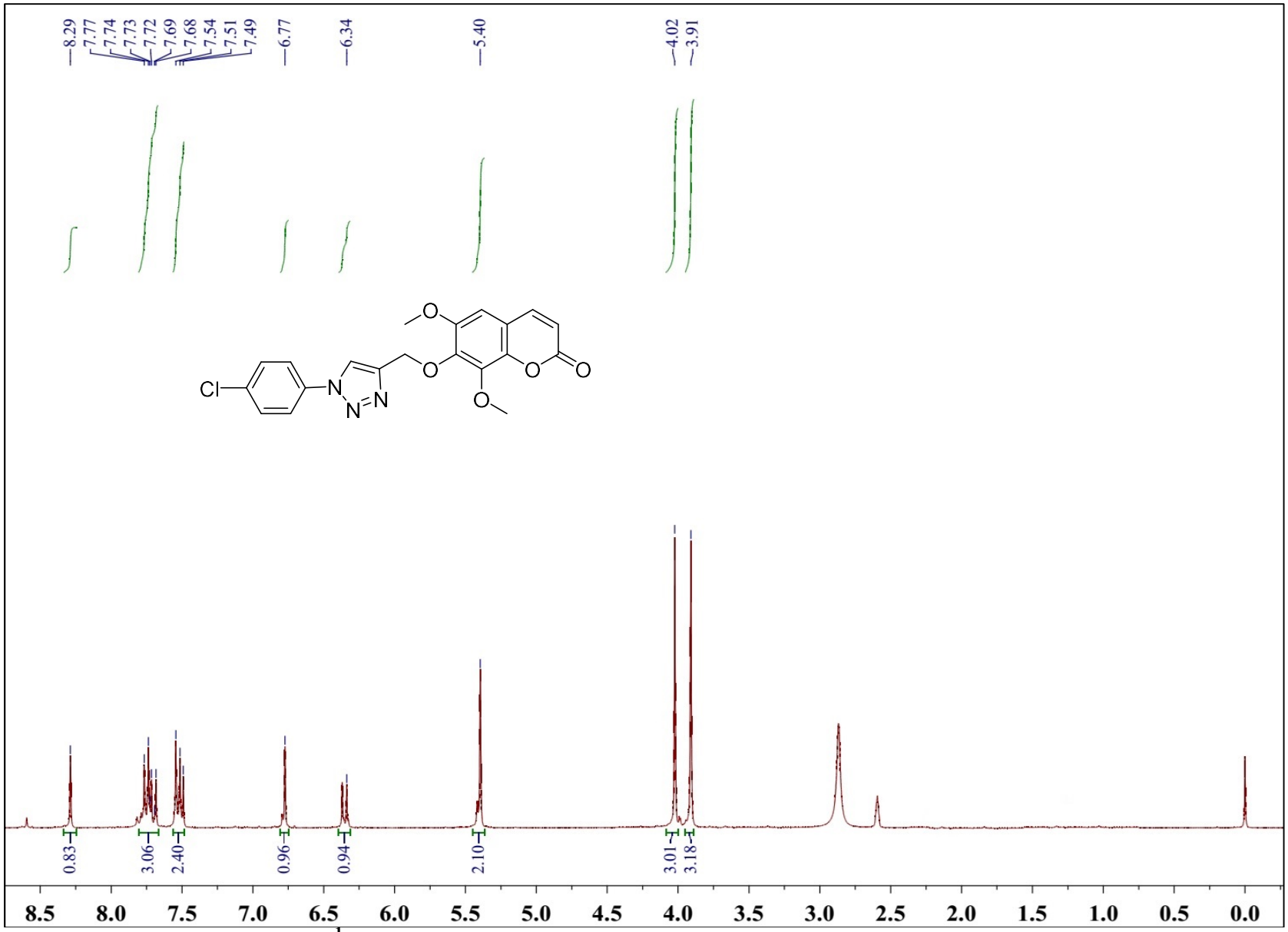

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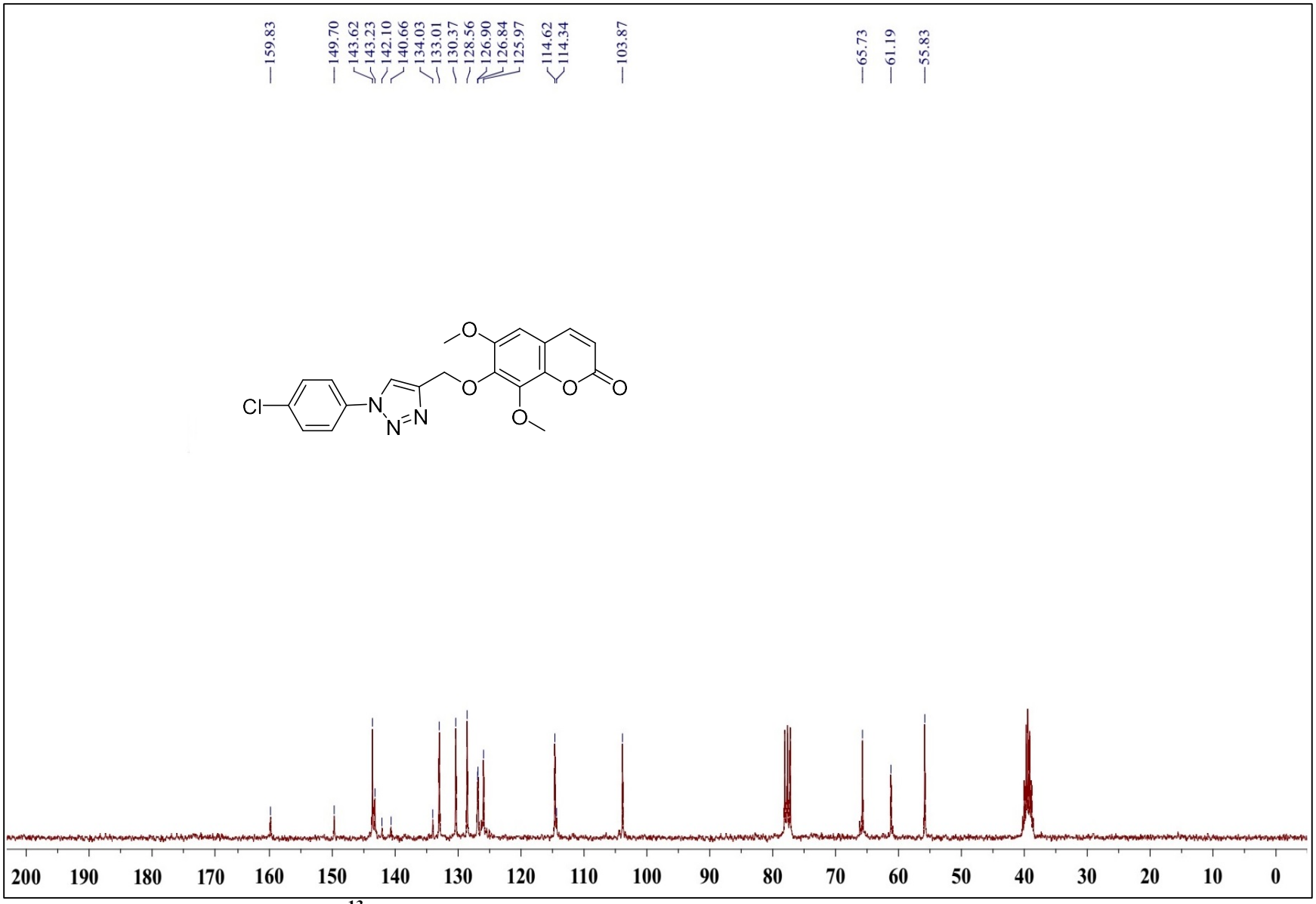

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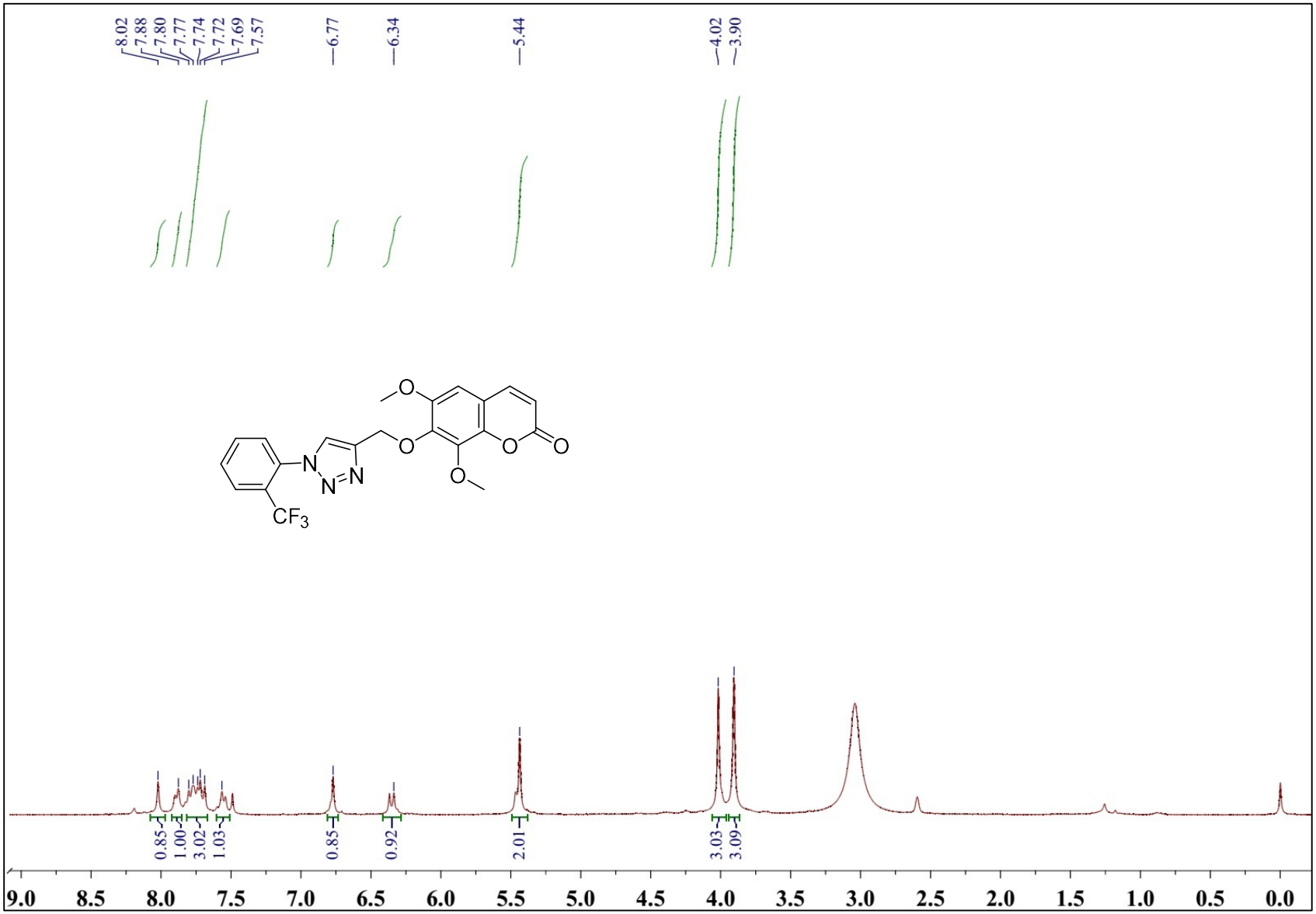

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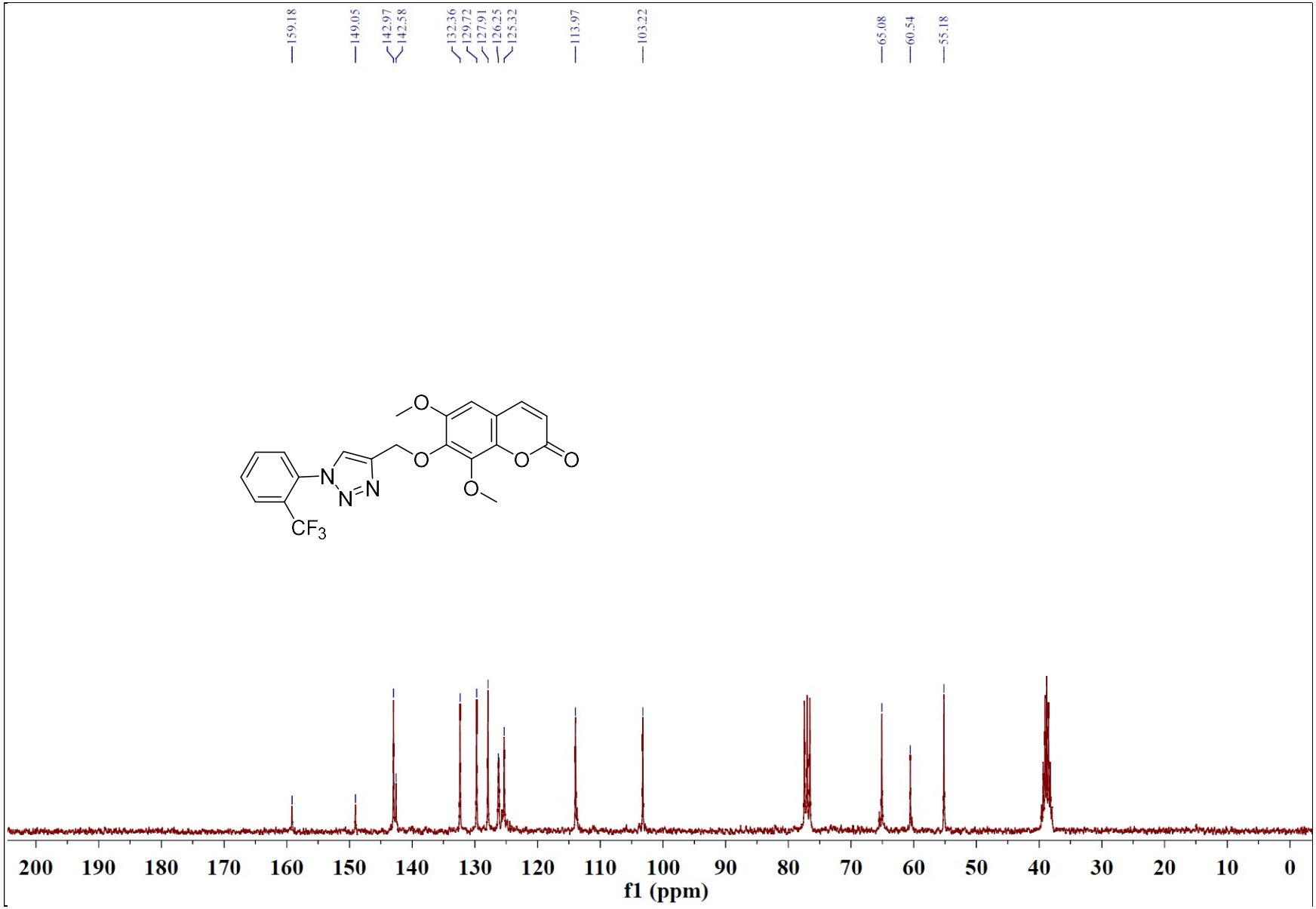

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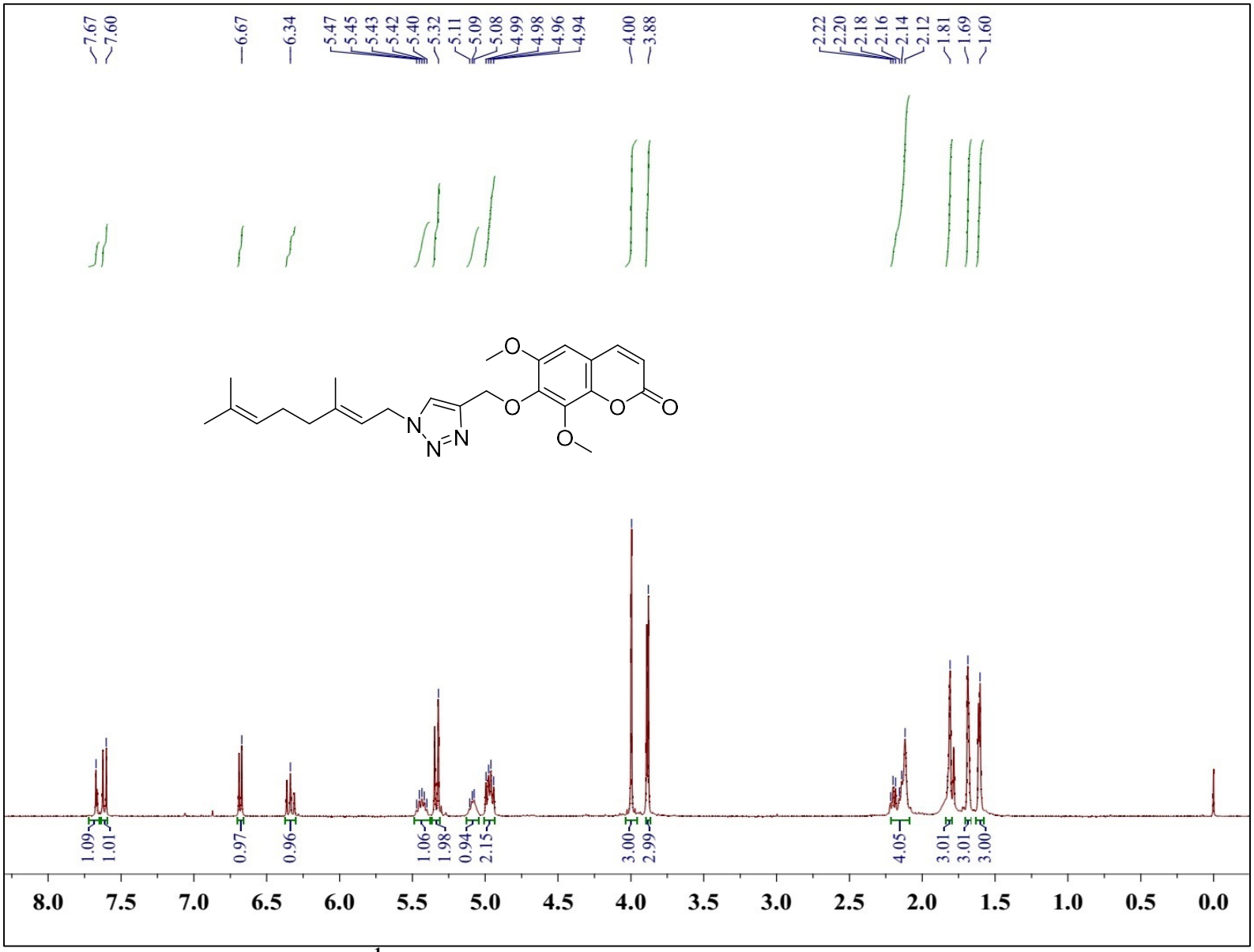

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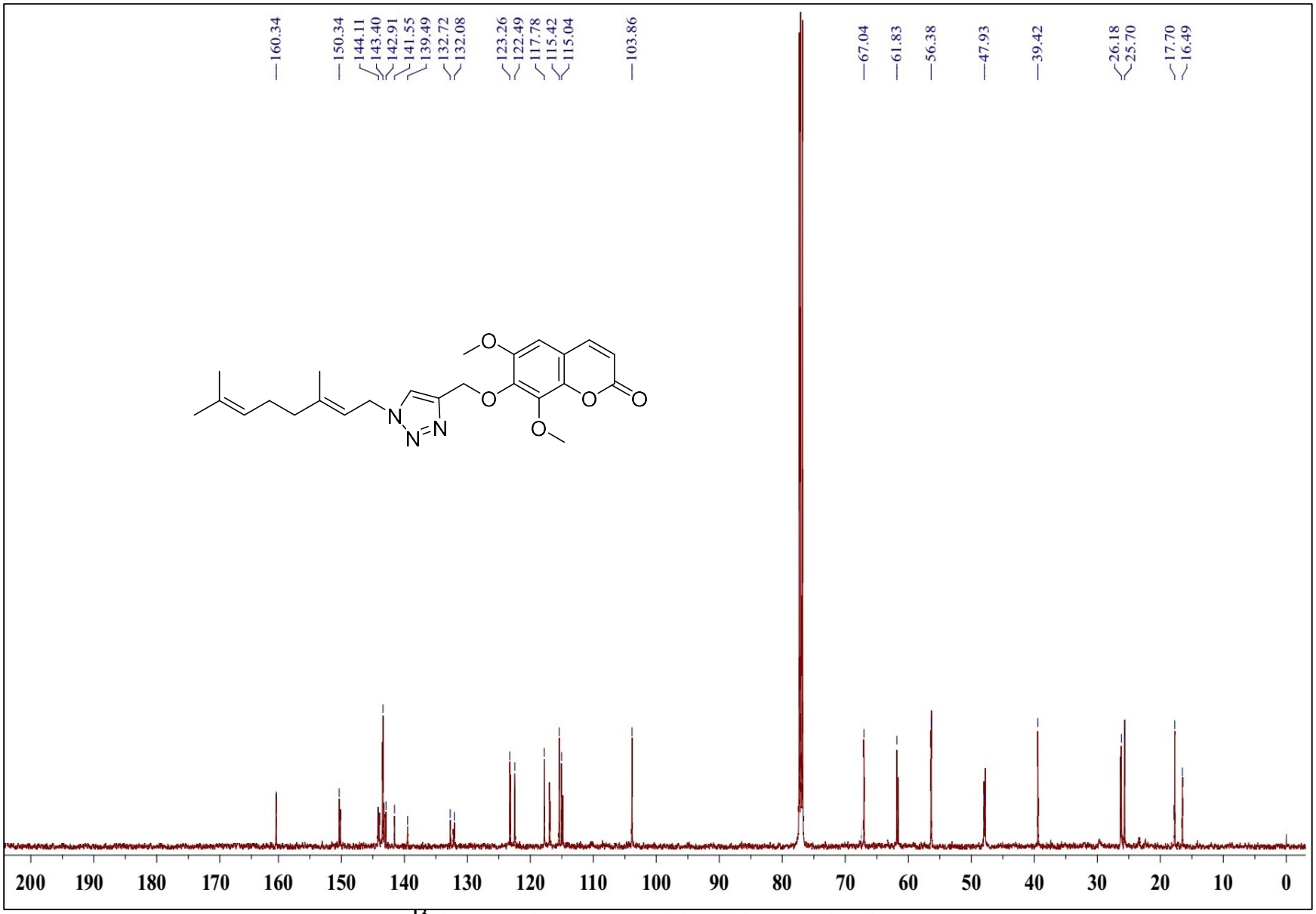

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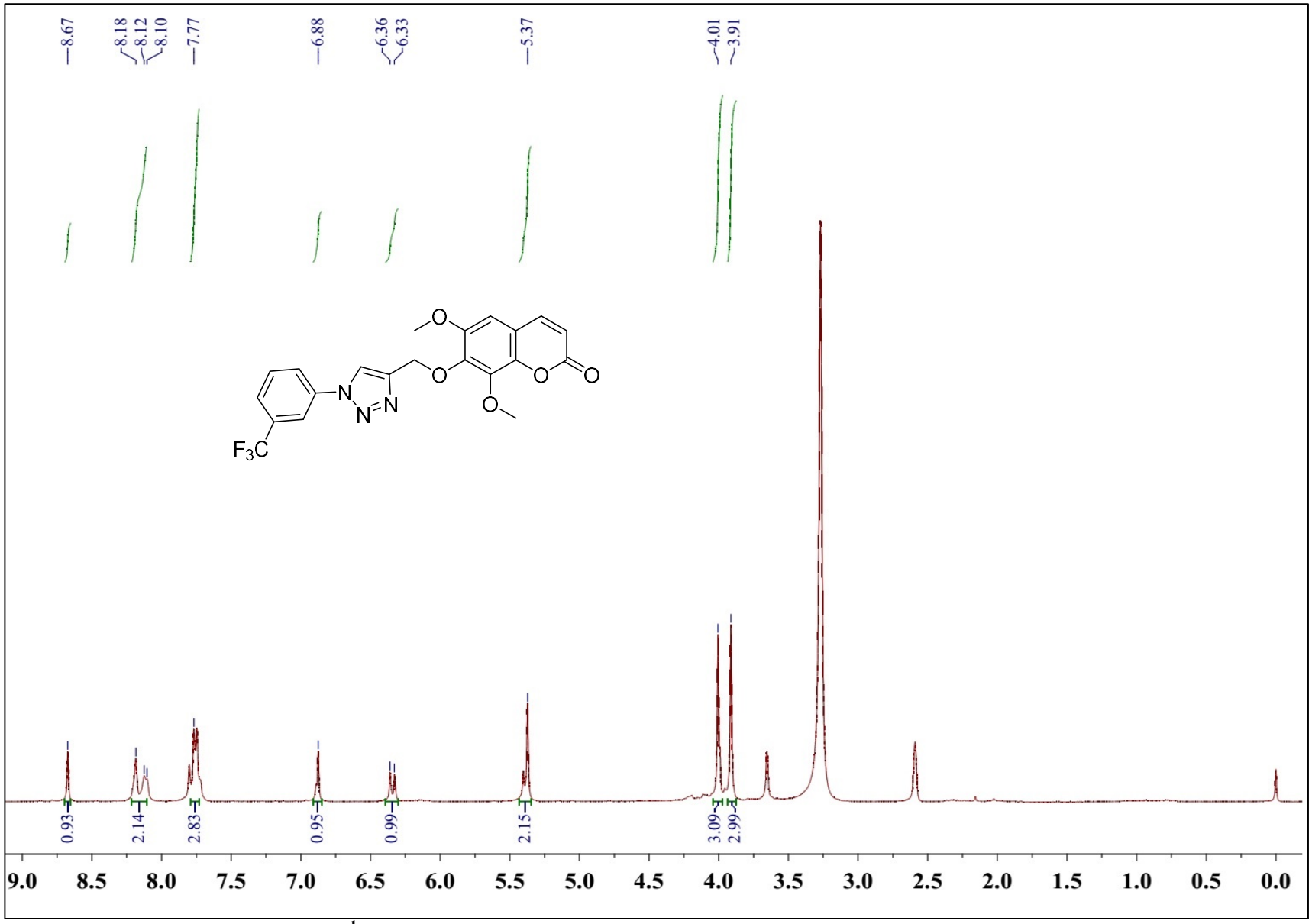

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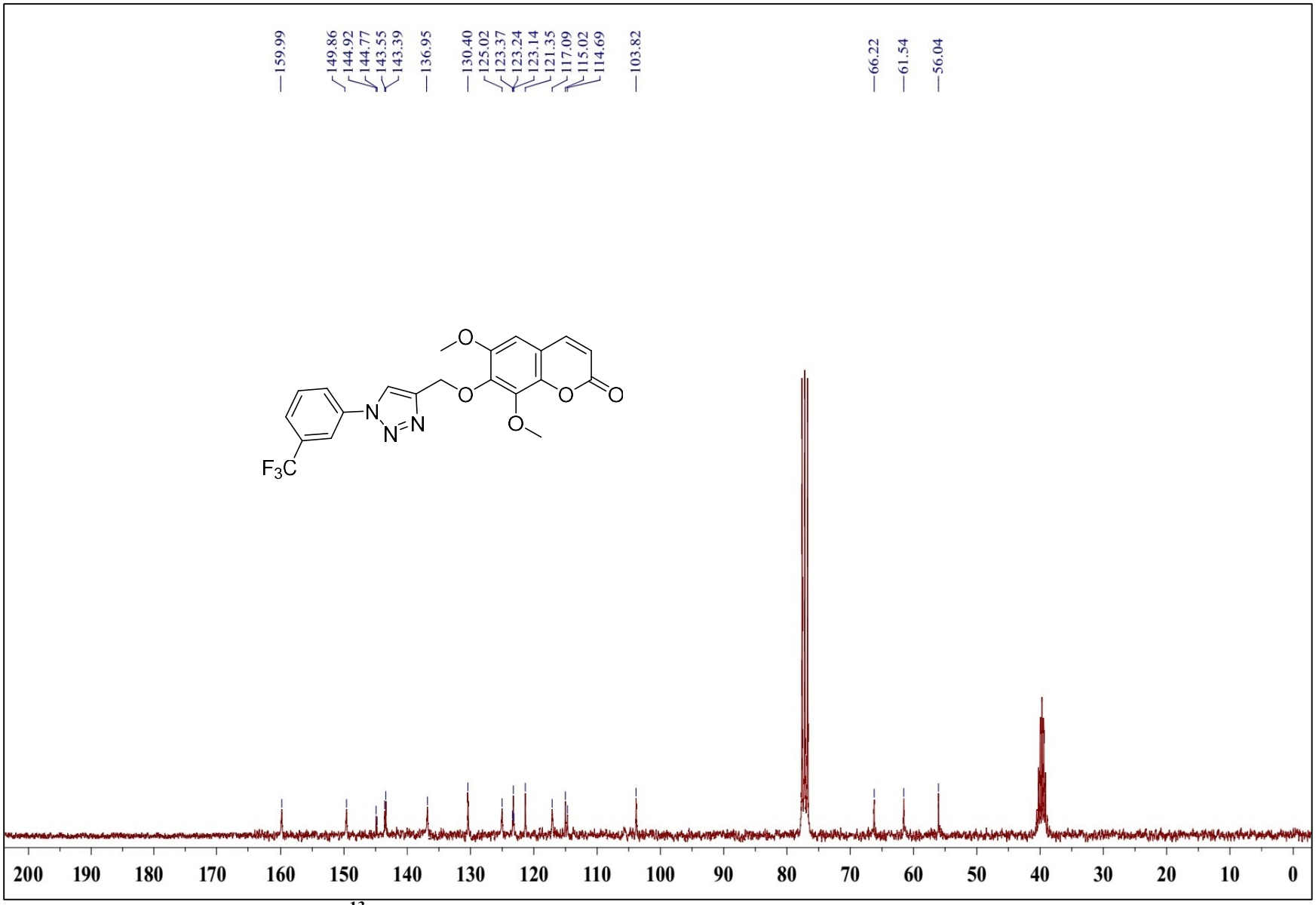

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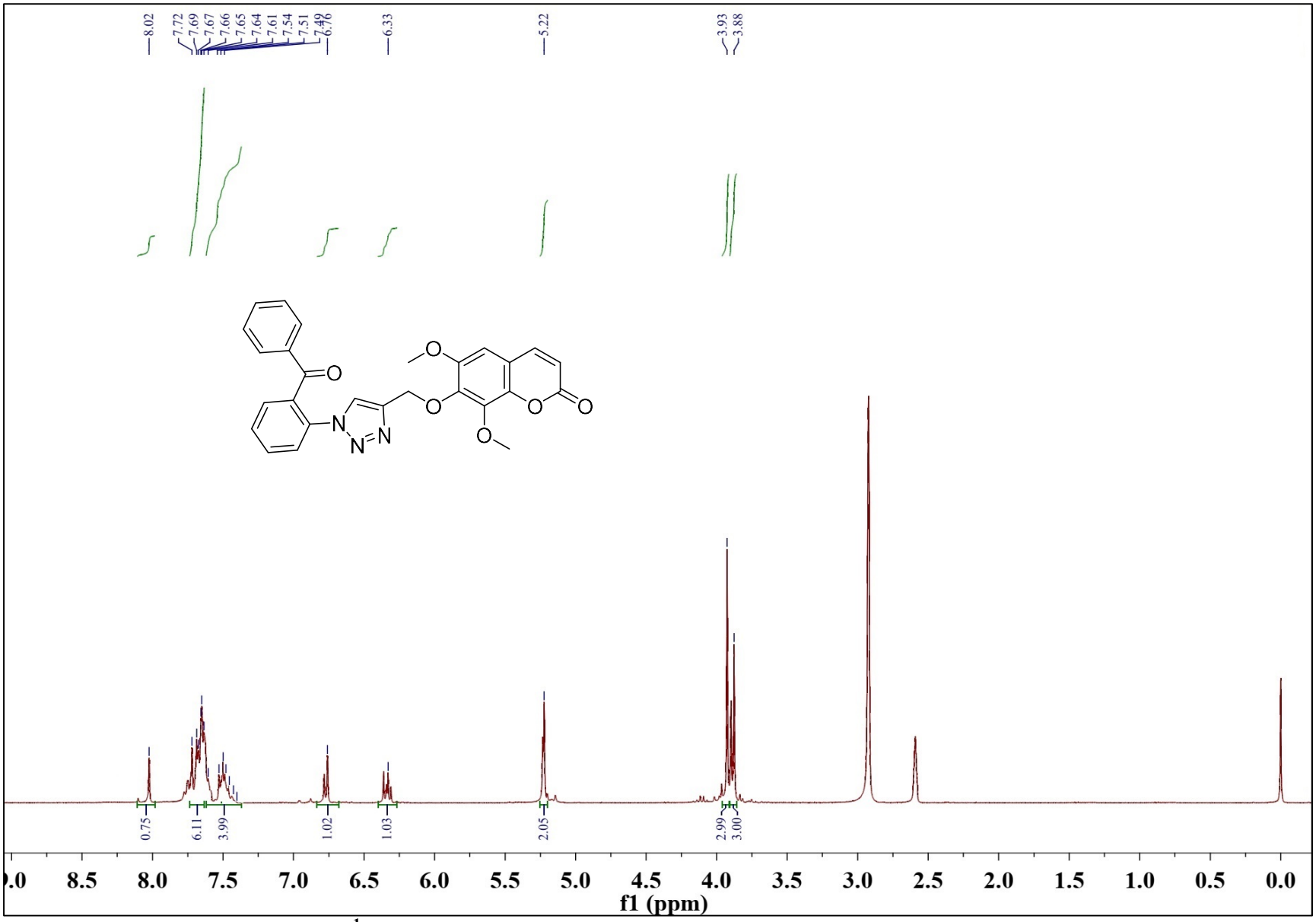

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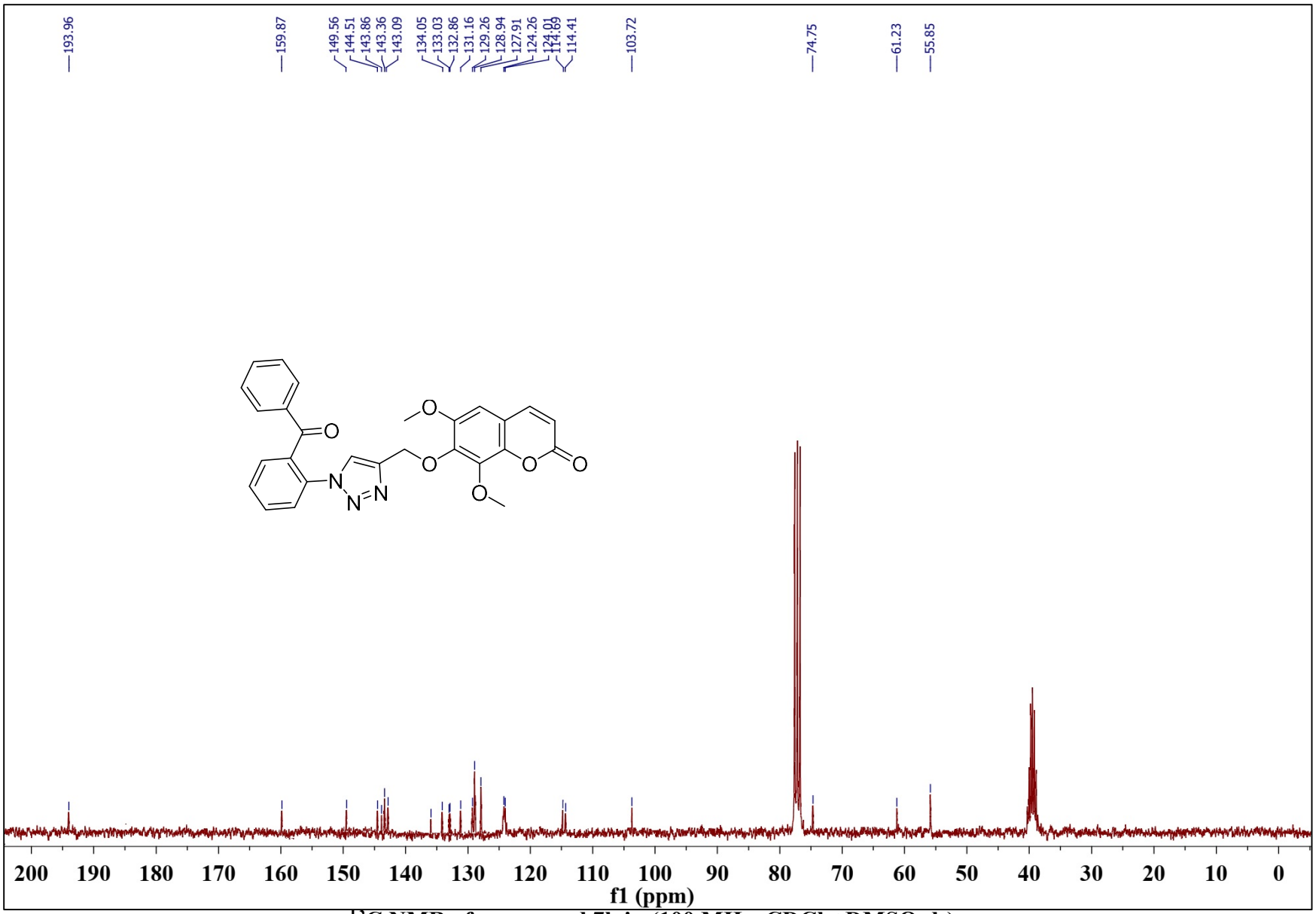

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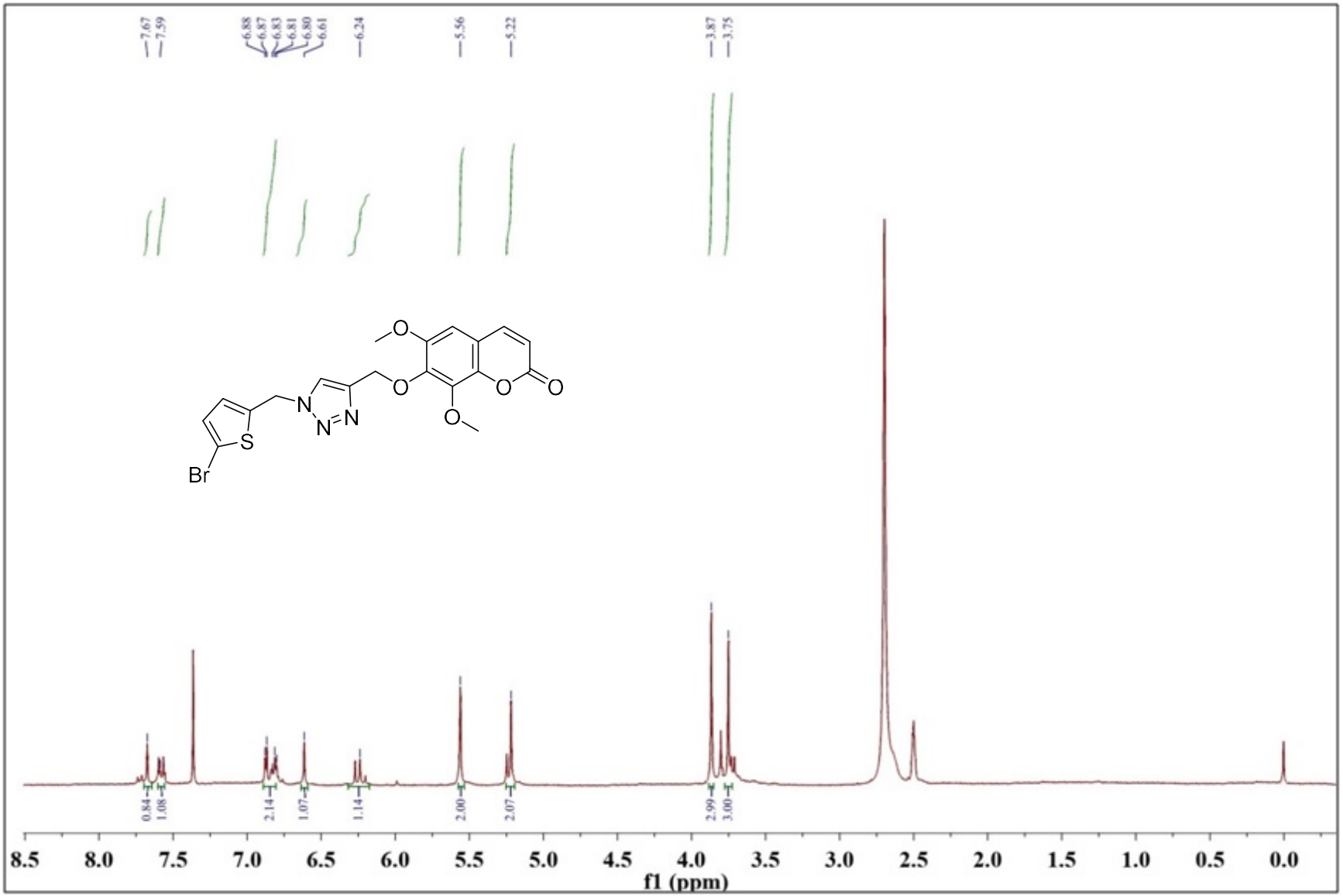

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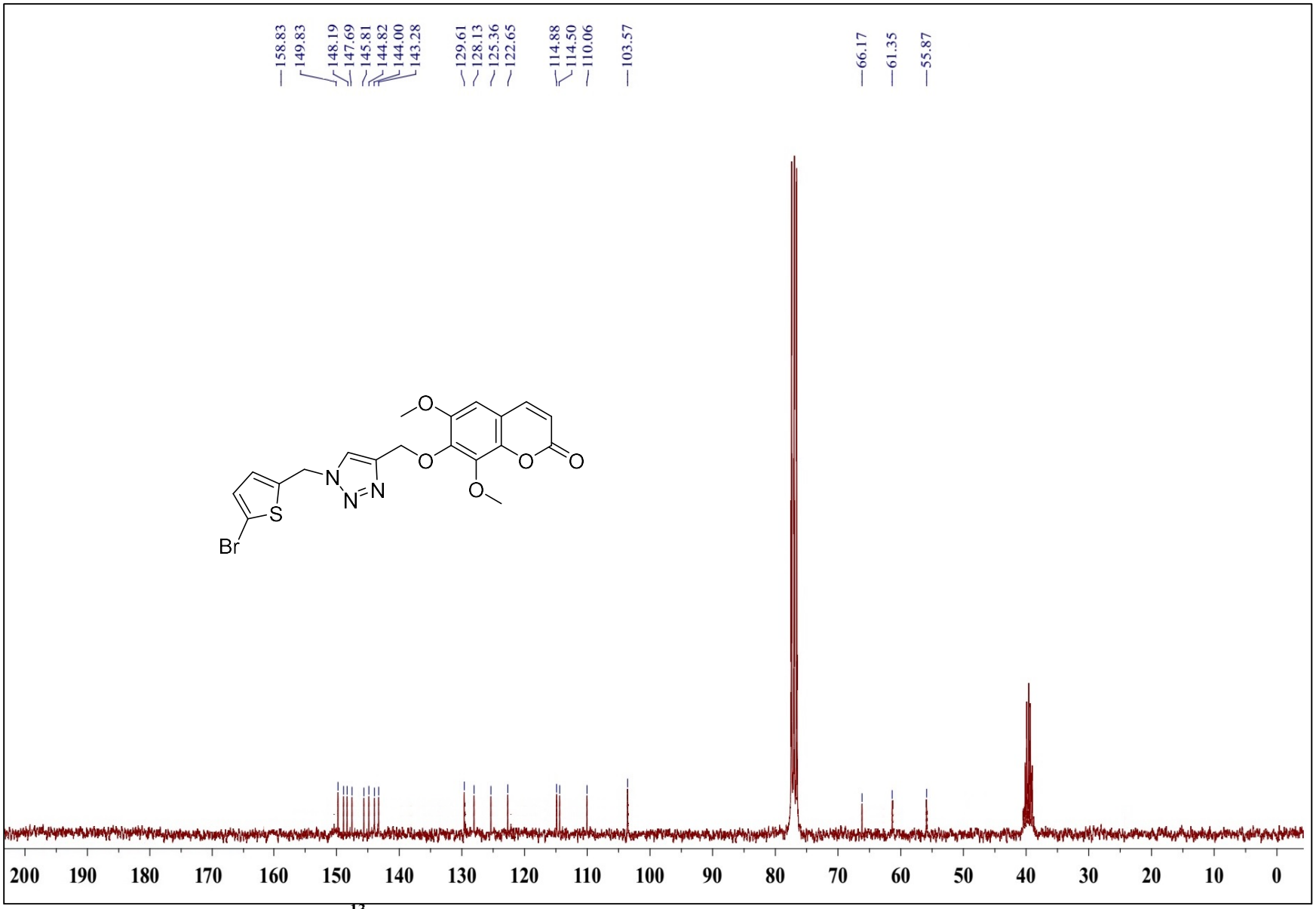

^{13}C NMR of compound 7l in (100 MHz, $CDCl_3$+ DMSO-d_6)

Molecular Docking Studies:

As part of our study to explore the anti-inflammatory activity of all the compounds **7a - 7l**, we have carried out the molecular docking studies by the x-ray crystal structures of COX-2 enzyme co-crystallized with Flurbiprofen and DprE1 enzyme of Mycobacterium tuberculosis downloaded from the RCS Protein Data with PDB ID-3PGH and 4FDO respectively.

The active sites of COX-2 enzyme revealed three different regions, a hydrophobic pocket beneath the heme group, defined by the residues Phe381, Tyr385, Tyr387, Phe518, Ala201, Tyr248 and Lue352. The mouth of the active site has three hydrophobic residues Arg120, Glu524 and Tyr355. A large side pocket has His90, His / Arg513 and Ile / Val523. Most of the synthesized compounds shown good binding interactions and binding affinities (range of -7.083 Kcal / mol to -9.870 Kcal / mol) with Tyr355 and Arg120. The compounds **7d** and **7j** were shown nearby binding affinities -8.806 Kcal / mol and -8.642 Kcal / mol respectively with the standard Flurbiprofen, -9.870 Kcal / mol. The various interactive residues with type of interactions and their docking scores were shown in the table-1.

Table-S1: Interactive residues, type of interactions and docking score of 1,2,3-triazole-Isofroxadin molecules 7a - 7l with 3PGH.

Compound	Interactive Residues with (COX-2)	Type of interactions	Docking Score (Kcal/mol)
7a	Tyr355(triazole ring), Arg120 (lactone ring)	pi-pi, pi-cation	-7.083
7b	Tyr355 (N-triazole ring), Tyr355 (triazole ring), Arg120 (triazole ring)	H-bond, pi-pi pi-cation	-8.328
7c	Tyr355 (triazole ring)	pi-pi	-7.369
7d	Tyr355 (N-triazole ring), Tyr355 (triazole ring)	H-bond, pi-pi	-8.806
7e	Tyr355 (O-ether linkage), Arg120 (triazole ring)	H-bond, pi-cation	-7.439
7f	Tyr355 (N-triazole ring), Tyr355 (O-ether linkage), Tyr355 (triazole ring)	H-bond, H-bond pi-cation	-7.298
7g	Tyr355 (O-ether linkage), Arg120 (triazole ring)	H-bond, pi-cation	-8.009
7h	Tyr355 (N-triazole ring), Tyr355 (O-ether linkage), Tyr115 (Ar-ring), Arg120 (triazole ring)	H-bond, H-bond pi-pi, pi-cation	-7.910
7i	Tyr355 (N-triazole ring), Tyr355 (triazole ring), Arg120 (lactone ring), Arg120 (Ar-ring)	H-bond, pi-pi, pi-cation, pi-cation	-7.202
7j	Tyr355 (N-triazole ring), Tyr355 (triazole ring)	H-bond, pi-pi	-8.642
7k	Tyr355 (triazole ring)	pi-pi	-8.358
7l	Tyr115 (lactone ring)	pi-pi	-7.408
Flubriprofen	Tyr355 (C=O)	H-bond	-9.870

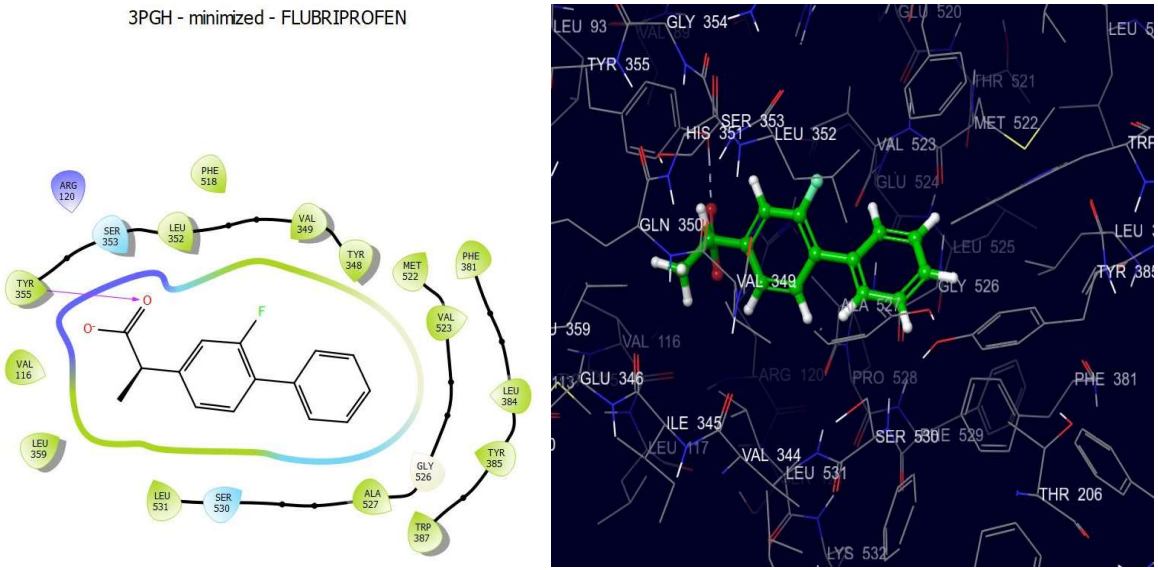

Figure-3: 2D and 3D Binding interactions of Flurbiprofen with 3PGH

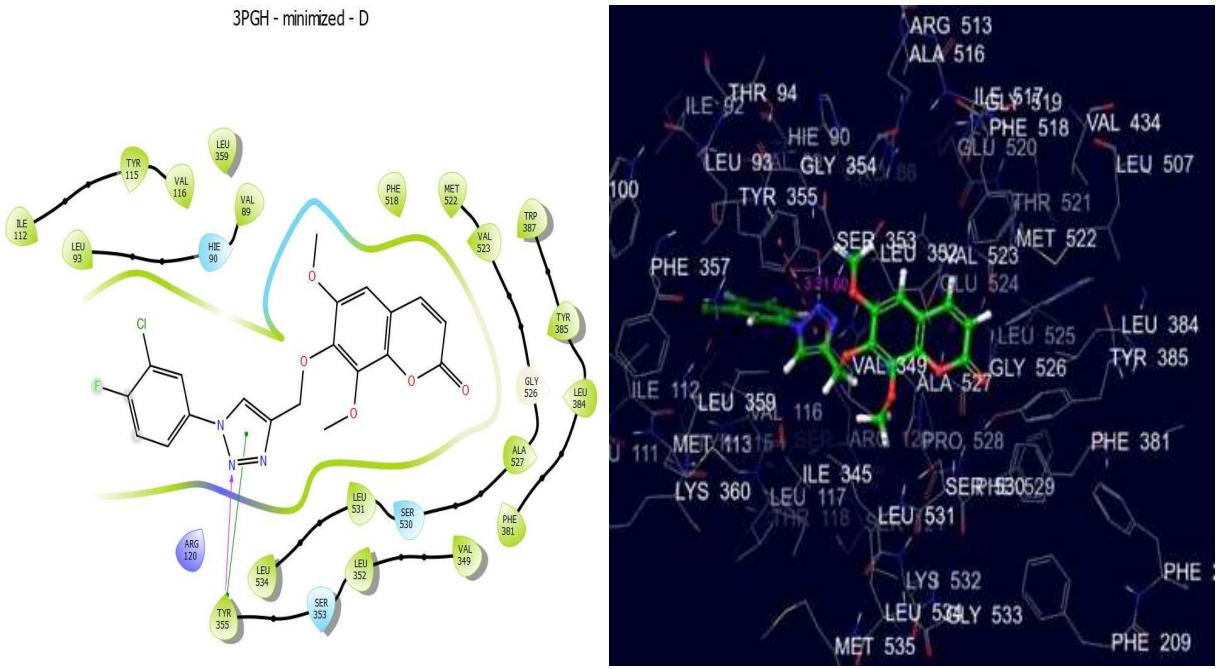

Figure-4: 2D and 3D Binding interactions of 7d with 3PGH

Many, 1, 2, 3-triazole derivatives have been reported to inhibit DprE1 (decaprenylphosphoryl-b-D-ribose-20-epimerase) enzyme of MTB. DprE1 is involved in the biosynthesis of decaprenyl phosphoryl-*D*-arabinose (DPA), which is an essential component of the mycobacterium cell wall. Molecular docking study was performed using the Glide (grid-based ligand docking with energetics) program incorporated in the Schrodinger molecular modeling package. Analysis of the docking poses shows that, all the inhibitors snugly fit into the active site of DprE1 in positions very close to that of native ligand in the crystal structure of its complex with DprE1. All the derivatives have shown very good affinity (range of -8.777 Kcal / mol to -6.415 Kcal / mol) toward DprE1 with a very similar topology of binding. The enzyme–inhibitor complex was found to be stabilized by strong H-bonding interaction observed with the amino acid residues Lys418, Tyr115, Ser228, His132 and Tyr60 in the enzyme active site. The binding mode of the most active compound **7j** (-8.777 Kcal / mol) in the active site of DprE1 enzyme (PDB ID:4FDO) obtained by molecular docking is shown in the Figure-6 and the interactive residues with type of interactions and their docking scores are shown in table-2.

Table-S2: Interactive residues, type of interactions and docking score of 1,2,3-triazole-Isofroxadin hybrids 7a - 7l with 4FDO.

Compound	Interactive Residues with (4FDO)	Type of interactions	Docking Score (Kcal/ mol)
7a	Gly117 (N-triazole ring), Lys418 (triazole ring), His132 (lactone ring)	H-bond pi-cation, pi-pi	-7.402
7b	Lys418 (O-ether linkage), H_2O (triazole ring)	H-bond	-7.472
7c	Lys418 (O-ether linkage), Tyr60 (C=O)	H-bond	-7.854
7d	Lys418 (O-ether linkage), Tyr60 (C=O), Cys387 (C=O),	H-bond	-8.156
7e	H_2O (6-OMe)	H-bond	-8.602
7f	Lys418 (N-triazole ring), Asn385 (C=O), Tyr60 (triazole ring),	H-bond, H-bond pi-cation	-7.199
7g	H_2O (6-OMe)	H-bond	-8.493
7h	H_2O (6-OMe), Lys418 (triazole ring)	H-bond, pi-cation	-8.653
7i	Lys418 (C=O), Lys418 (triazole ring)	H-bond, pi-cation	-6.415
7j	Lys418 (O-ether linkage), Tyr60 (C=O), H_2O (C=O)	H-bond	-8.777
7k	Lys418 (C=O), Lys418 (bPhC=O), Gly117 (N-triazole ring), Tyr60 (Ar-ring), Lys418 (triazole ring)	H-bond pi-pi, pi-cation	-8.263
7l	Lys418 (Ar-ring)	pi-cation	-7.247
Ligand-OT5	Lys418 (C=O), His132 (NO_2), Asn385(NO_2)	H-bond	-8.317

Molecular Properties:

Molecular properties of **(7a-7l)** were predicted by using *QikProp* software (product of Schrodinger Inc.) to investigate the possible considerations of their stability to develop them as an oral drug candidates based on the Lipinski's rule of five and Jorgensen's rule of three and all the results were shown in the table-3 & table-4**.** While considering the Lipinski's rule of five, most of the compounds have molecular weight less than 500, all the compounds had log *P* values less than 5, positive PSA values, DHB values < 5 and AHB values < 10. Meanwhile on considering the Jorgensen's rule of three, all the compounds had PCaco values > 22, all the compounds had PM values and log *S* values between 3-6 and smaller than -5.7 respectively except the compound 7i. The log BB values of all the compounds are predicted to find their access into the central nervous system (CNS) and were found in recommended range of -3 to 1.2 for all the compounds. Based on this most of the compounds were found to have good pharmacokinetic profile and high blood-brain barrier (BBB) penetration which enhances the biological activity of the compounds in treating certain CNS relevant diseases.

Table-S3: Calculated ADME parameters of compounds (7a-7l).

Product	MW	RB	DM	MV	DHB	AHB	log *P*	log *S*
7a	404.381	6	5.048	1212.51	0	8.25	2.184	-5.021
7b	505.268	5	11.635	1212.55	0	6.75	3.788	-5.299
7c	407.425	5	4.424	1253.93	0	6.75	3.611	-5.279
7d	431.807	5	4.315	1204.68	0	6.75	3.73	-5.329
7e	397.362	5	11.547	1165.52	0	6.75	3.355	-4.65
7f	409.398	6	10.426	1222.1	0	7.5	3.16	-4.323
7g	413.816	5	11.666	1193.54	0	6.75	3.62	-5.043
7h	447.37	5	14.37	1227.22	0	6.75	3.985	-5.271
7i	439.51	10	3.387	1448.03	0	7.25	4.542	-6.252
7j	447.37	5	1.899	1242.65	0	6.75	4.019	-5.721
7k	483.479	7	7.188	1385.4	0	8.75	3.583	-4.374
7l	478.316	7	1.769	1237.49	0	7.25	3.71	-5.215

MW: molecular weight; RB: number of rotatable bonds (recommended value: 0-15); DM: computed dipole moment (recommended value: 1-12.5); MV: total solvent-accessible volume (recommended value: 500-2000); DHB: estimated number of hydrogen bond donors (recommended value: 0-6); AHB: estimated number of hydrogen bond acceptors (recommended value: 2-20); log *P*: Predicted octanol / water partition coefficient (recommended value: -2-6.5); log *S*: predicted aqueous solubility (recommended value: -6.5-0.5).

Table-S4: Calculated ADME parameters of compounds (7a-7l).

Product	log *S*	PCaco	logBB	PMDCK	logKp	PM	%HOA	PSA	VRF
7a	-5.021	132.507	-1.88	55.667	-3.49	4	77.72	120.766	0
7b	-5.299	780.942	-0.752	1086.89	-2.112	4	87.941	94.342	1
7c	-5.279	640.739	-1.062	305.77	-2.476	6	100	94.97	0
7d	-5.329	640.306	-0.777	1223.64	-2.416	4	100	94.981	0
7e	-4.65	781.03	-0.814	684.844	-2.074	4	100	94.344	0
7f	-4.323	783.716	-0.993	380.144	-2.037	5	100	103.364	0
7g	-5.043	780.983	-0.769	934.33	-2.108	4	100	94.343	0
7h	-5.271	986.674	-0.595	1532.77	-1.918	5	100	89.176	0
7i	-6.252	642.77	-1.49	306.818	-2.274	10	100	94.832	0
7j	-5.721	640.703	-0.777	1339.52	-2.372	5	100	94.972	0
7k	-4.374	452.035	-1.258	209.716	-1.981	4	95.448	116.178	0
7l	-5.215	672.392	-0.894	1425.78	-2.291	6	100	93.811	0

PCaco: Predicted apparent Caco-2 cell permeability (recommended value: <25 poor, >500 great); log BB: predicted brain/blood partition coefficient (recommended value: -3-1.2); PMDCK: predicted apparent MDCK cell permeability (recommended value: <25 poor, >500 great); PM: number of likely metabolic reactions (recommended range value: 1-8); %HOA: predicted human oral absorption percent (recommended value: >80 high, <25 poor); PSA: Vander Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (recommended value: 7-200); VRF: number of violations of Lipinski's rule of five. The rules are: MW < 500, log *P*> 5, DHB ≤ 5, AHB ≤ 10, and a positive PSA value.

Computational study:

***Molecular docking*:** The molecular docking studies were performed by using the computer software's Glide-Maestro (Schrodinger, LLC, USA).[30-32] The protein preparation wizard integrated in the package was used to clean and optimize the protein-ligand crystal structure. The protein structure was preprocessed by removing all the crystallographic water molecules (water without H-bonds) since no water molecule was found to be conserved, rectifying the mistakes in PDB file and optimizing the hydrogen bonds. The hydrogen atoms were added to the protein structure corresponding to the physiological pH 7.0 considering the appropriate ionization states for the acidic as well as basic amino acids. After assigning charge and protonation state finally, energy minimization with root-mean-square deviation (RMSD) value of 0.30 A^o was carried out using optimized potentials for liquid simulations (OPLS-2005) force field. Thereafter, the 3D geometries of the ligands were optimized using the Schrodinger LigPrep utility (Schrodinger, LLC, USA). This utility generates a number of low energy 3D structures, with various ionization states, tautomers, stereo chemistries and ring conformations, from each molecule input. Finally, partial charges were ascribed to these geometry-optimized ligands by using the OPLS-2005 force field. The active site of 3PGH and 4FDO was defined by a bounding box (grid) size of X-25.8 Y-22.45 Z-15.12 A^o and X-38.47 Y-12.74 Z-12.36 A^o that was centered on the native ligand in the crystal complex. Extra precision glide docking (Glide XP) which docks ligands flexibly was used to rank the docking poses and to gauze the binding affinity of these ligands toward the protein.