The Crystal Structure of 2-Amino-4-(2,3-Dichlorophenyl)-6-Methoxy-4*H*-Benzo[*h*]chromene-3-Carbonitrile: Antitumor and Tyrosine Kinase Receptor Inhibition Mechanism Studies

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3.1. Materials and Equipment's

All chemicals were purchased from Sigma-Aldrich Chemical Co. (Sigma-Aldrich Corp., St. Louis, MO, USA). All melting points were measured with a Stuart Scientific Co. Ltd apparatus are uncorrected. The IR spectra were recorded on a KBr disc on a Jasco FT/IR 460 plus spectrophotometer. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra was measured on BRUKER AV 500 MHz spectrometer in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts were expressed as δ (ppm). The Microwave apparatus used is Milestone Sr1, Microsynth. The mass spectrum was determined on a Shimadzu GC/MS-QP5050A spectrometer. Elemental analysis was carried out at the Regional Centre for Mycology and Biotechnology (RCMP), Al-Azhar University, Cairo, Egypt, and the results were within ± 0.25 %. Reaction courses and product mixtures were routinely monitored by thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates.

3.2. Synthesis of 2-Amino-4-(2,3-dichlorophenyl)-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (4)

The two minutes at 140 °C are the optimum microwave irradiation condition which was used reacted of 4-methoxynaphthalenol (**1**;0.01 mol), 2,3-dichlorobenzaldehyde (**2**; 0.01 mol) in presence of malononitrile (**3**;0.01 mol) with catalytic amount of piperidine (0.5 ml) in ethanol (25 ml. After completion of the reaction, the reaction mixture was cooled to room temperature and the precipitated solid was filtered off, washed with methanol, and was recrystallized from ethanol to give compound **4** as colorless crystals; yield 92 %; m.p. 287-288 ^{o}C; (Literature procedure, reflux condition, yield 84%; m.p. 285–286 °C [52]).

3.3. Biological Screening

3.3.1. Cell Culture

The tumor cell lines MCF-7, HepG-2, and PC-3 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10 % inactivated fetal calf serum and 50 μg/ml gentamycin. The cells were maintained at 37 ^{o}C in a humidified atmosphere with 5 % CO_2 and were subculture two to three times a week.

3.3.2. Cytotoxicity Evaluation Using Viability Assay

The cytotoxic activity was appraised, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, as reported previously [51].

3.3.3. In Vitro Tyrosine Kinases Inhibition

3.3.3.1. EGFR Inhibition Activities

Target compounds were measured using the EGFR kinase assay kit (Cat. #40321, BPS bioscience). The detection kit is a luminescence kinase detection approach that detects the amount of ADP produced by the kinase reaction. After ADP is converted into ATP, ATP can be used as the substrate of the luciferase-catalyzed reaction to generate an optical signal, which is positively correlated with kinase activity.

3.3.3.2. VEGFR-2 Inhibition Activities

Target compounds were used for evaluating their VEGFR-2 inhibition activities as mentioned above using VEGF-2 Receptor 2Kinase Assay Kit (Cat. #7788, Cell Signaling Technology).

Figure S1: ^{1}H NMR of compound 4

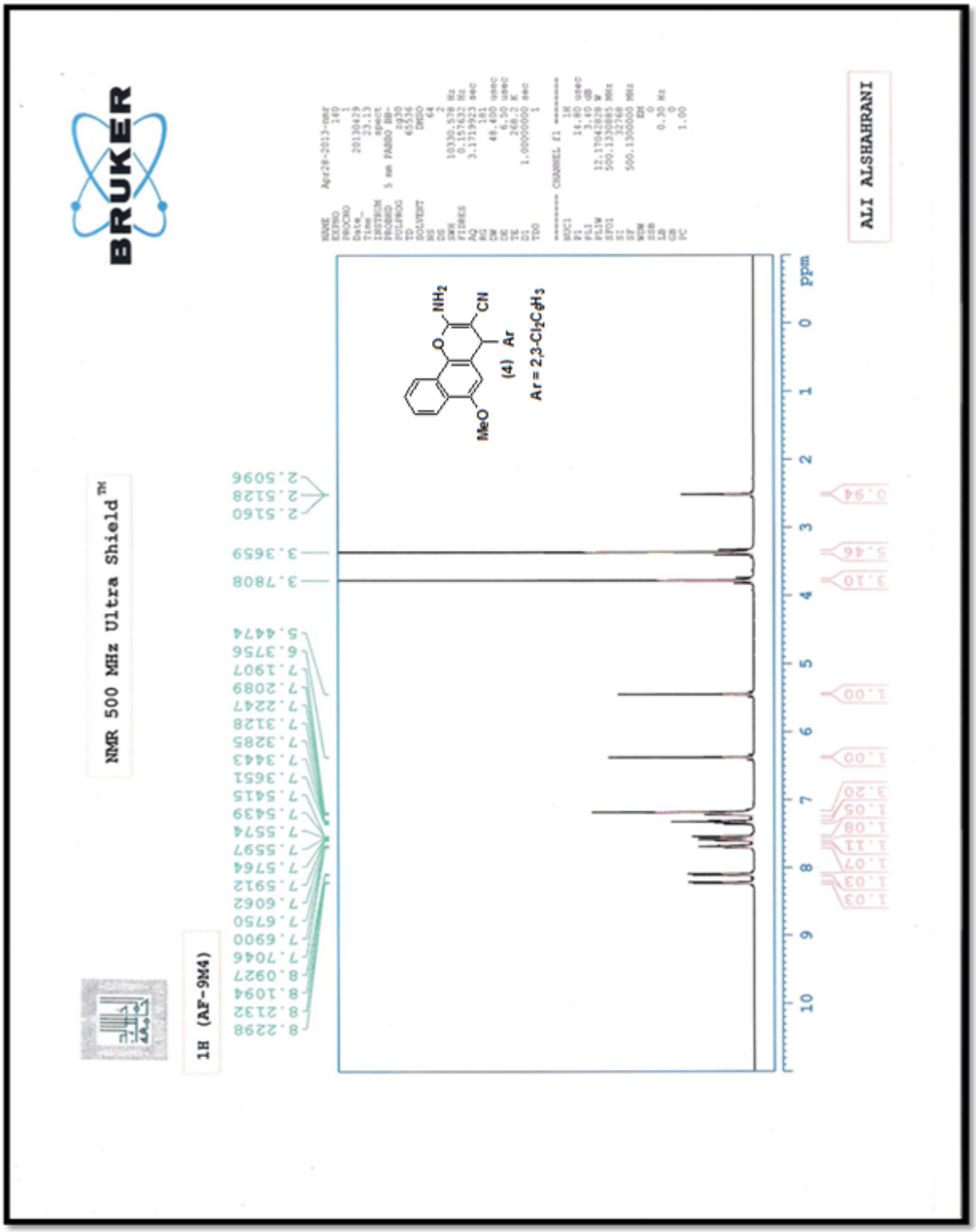


Figure S2: ^{1}H NMR 8-6 ppm of compound 4

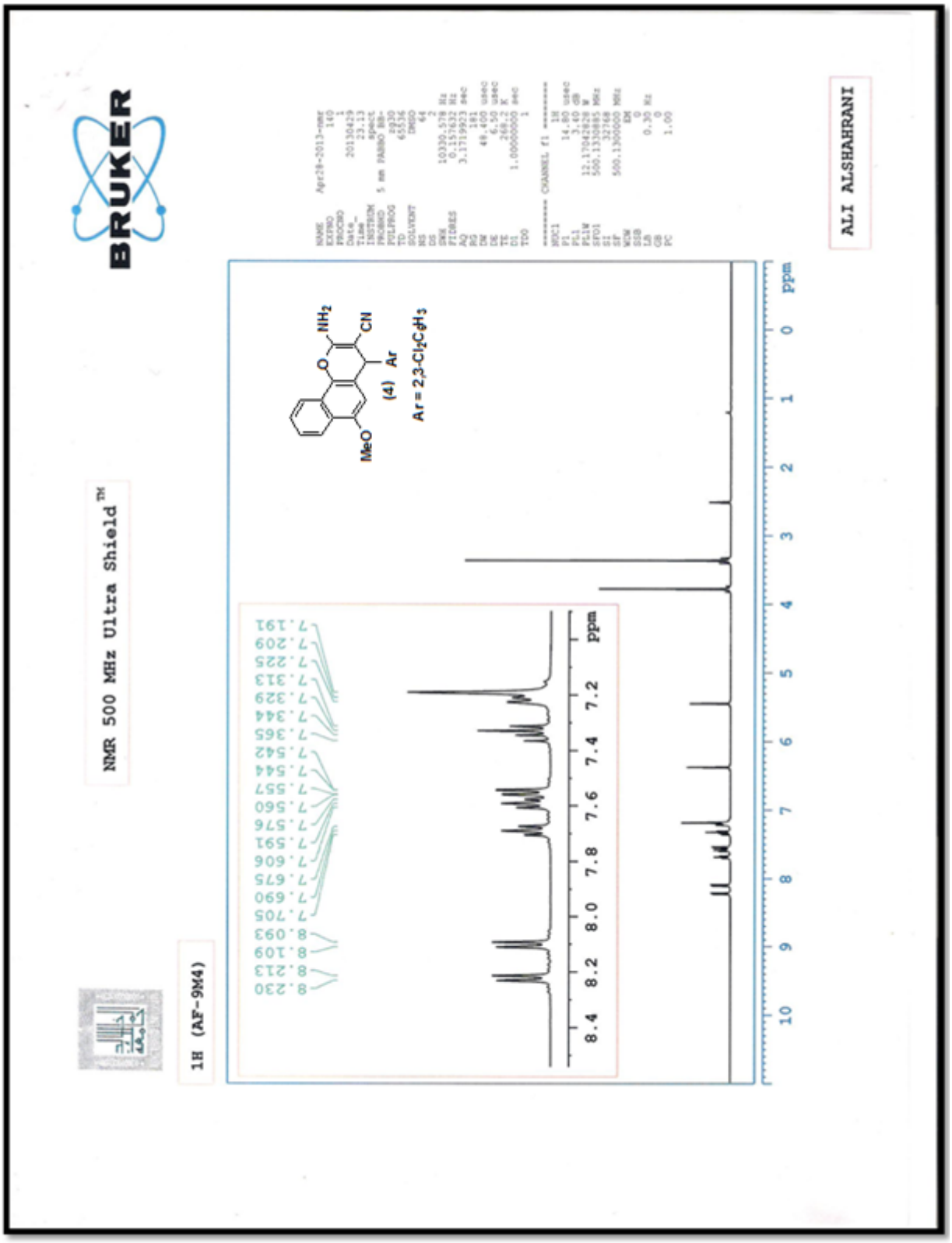


Figure S3: ^{13}C NMR of compound 4

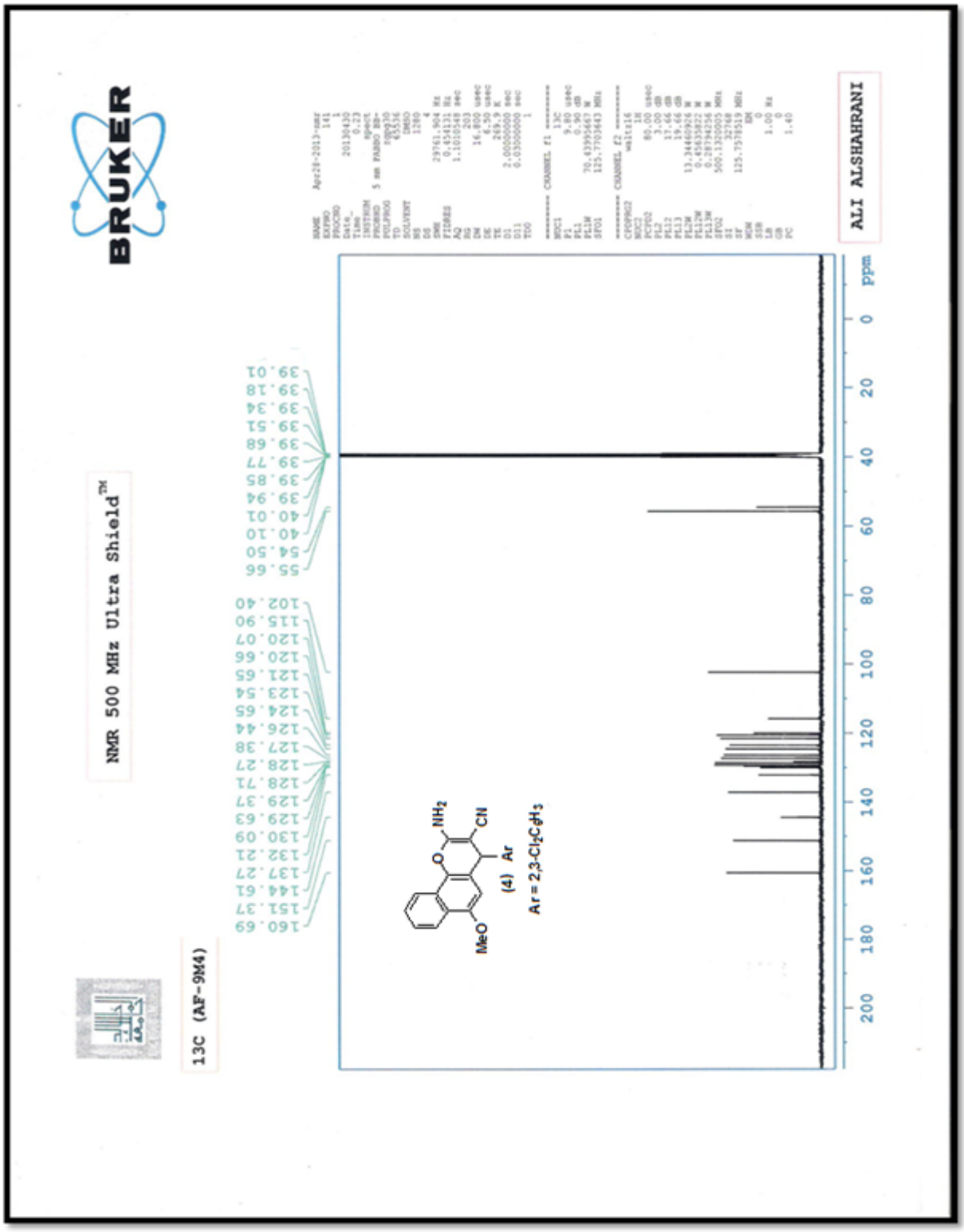


Figure S4: ^{13}CNMR-DPT 45 of compound 4

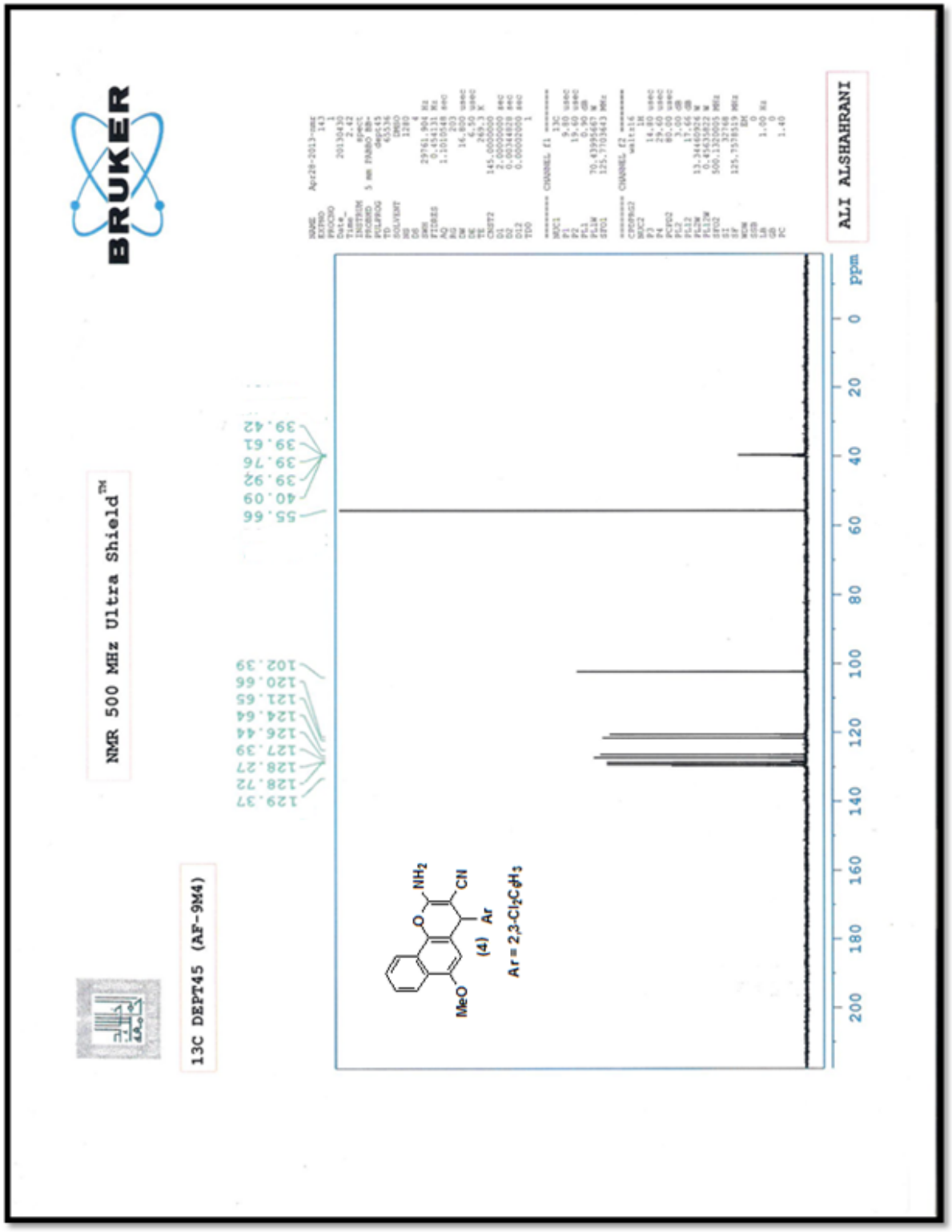


Figure S5: ^{13}CNMR-DPT 90 of compound 4

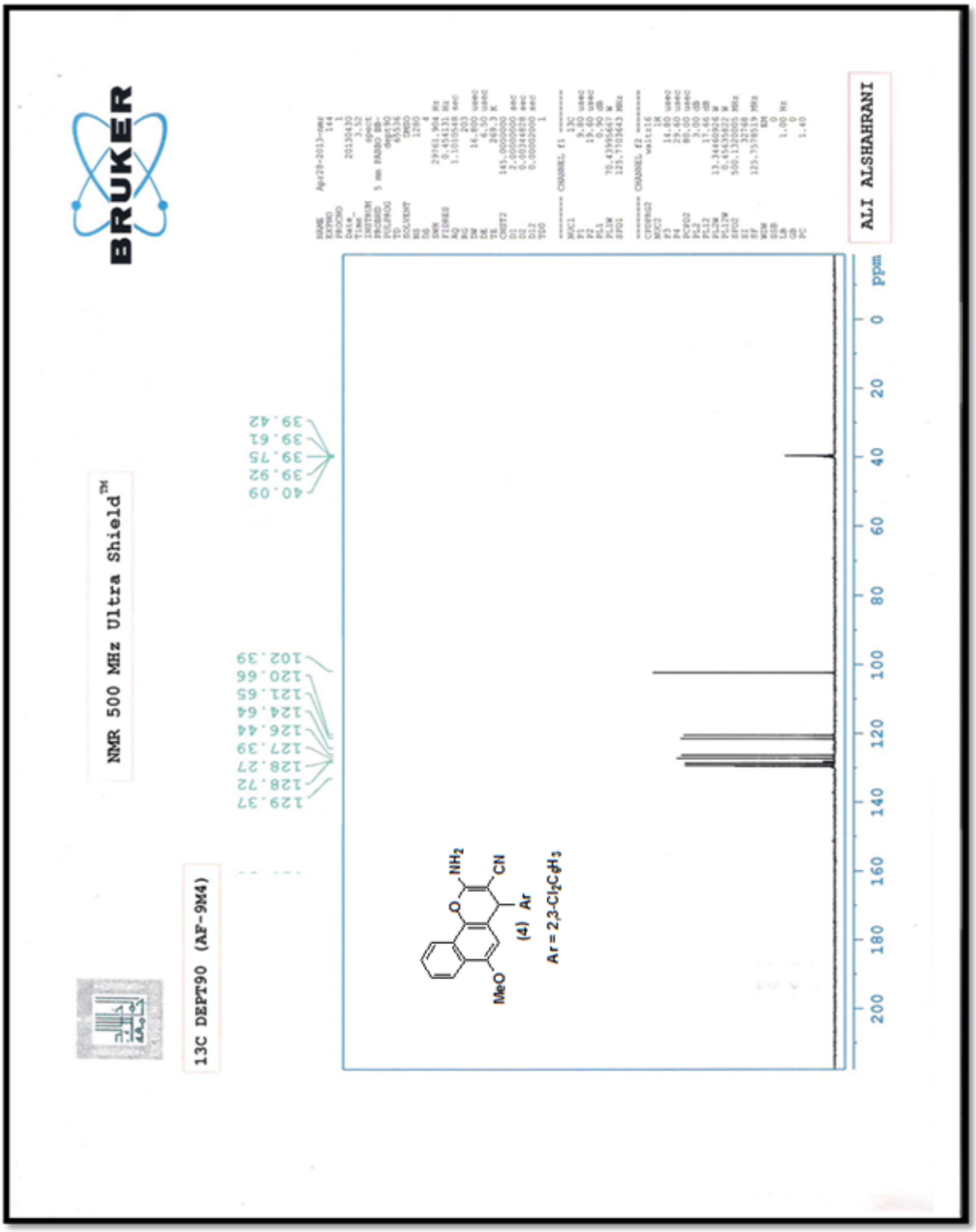


Figure S6: ^{13}CNMR-DPT 135 of compound 4

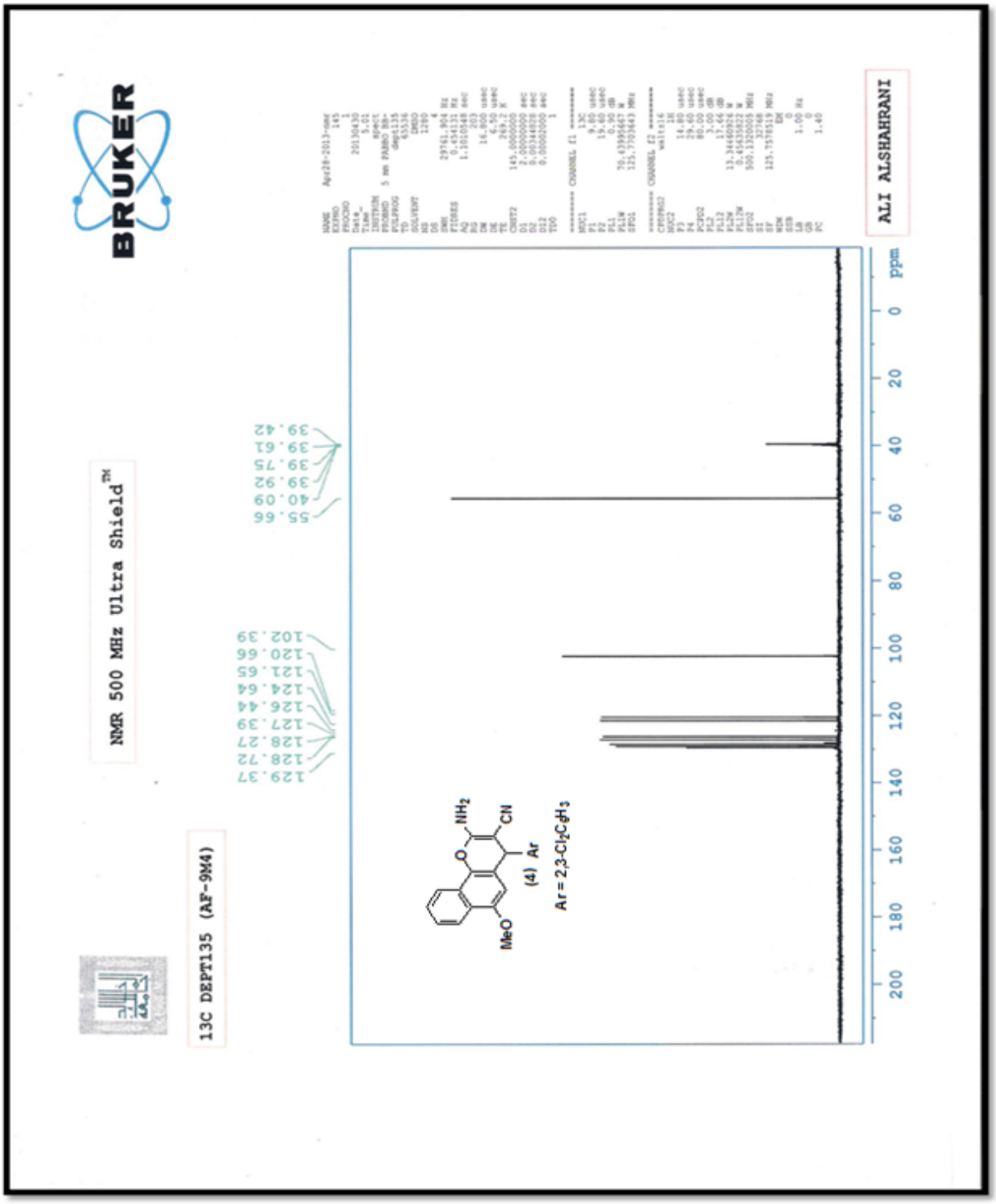
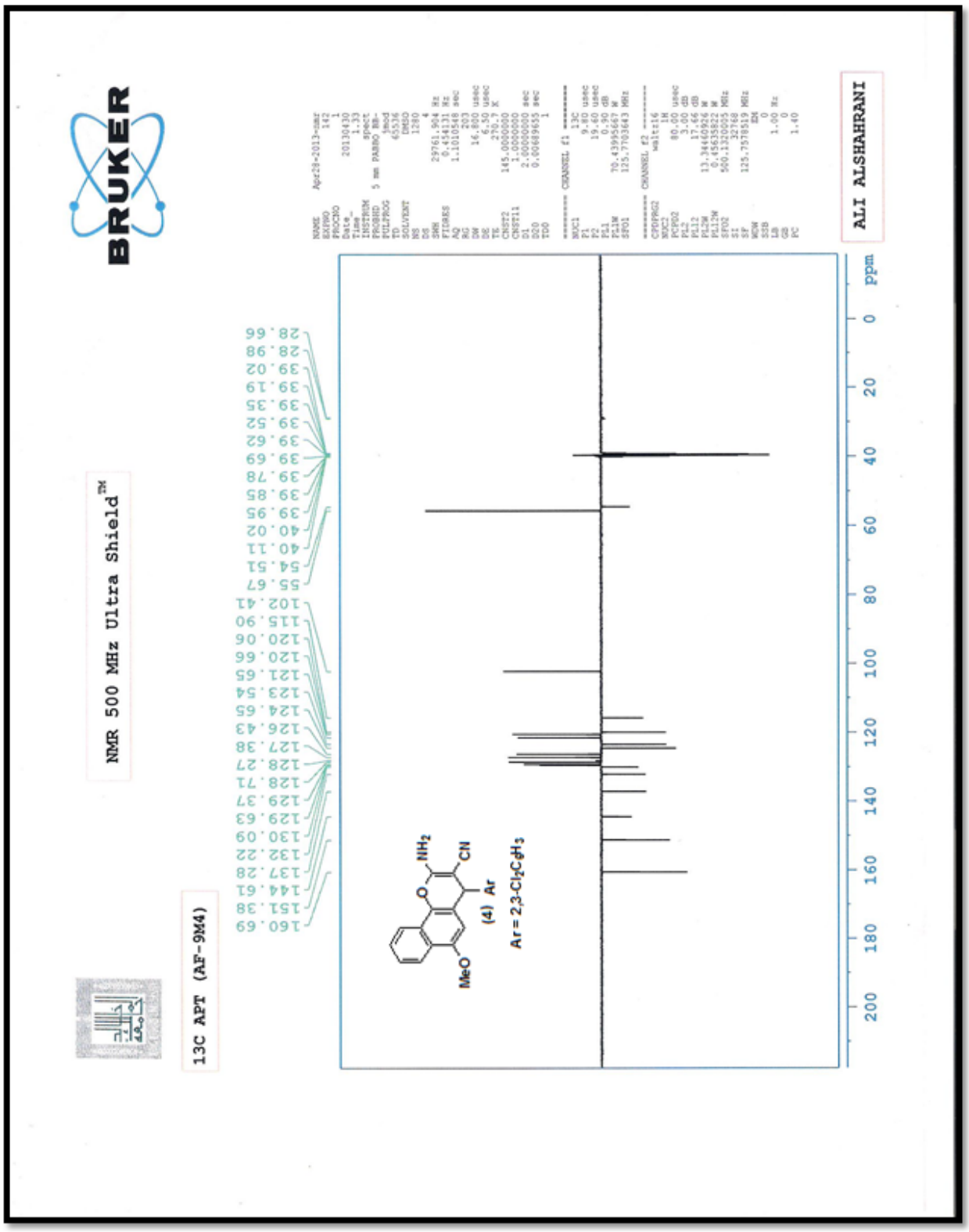


Figure S7: ^{13}CNMR-APT of compound 4



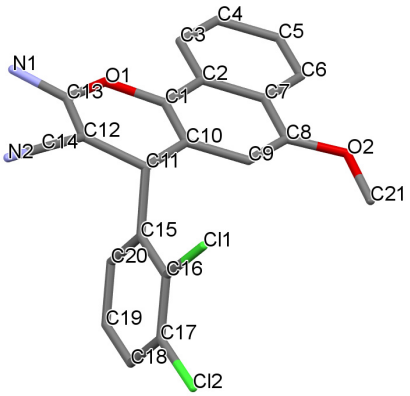


Figure S8: The number of atoms for compound **4** viewed along the *a*-axis direction.

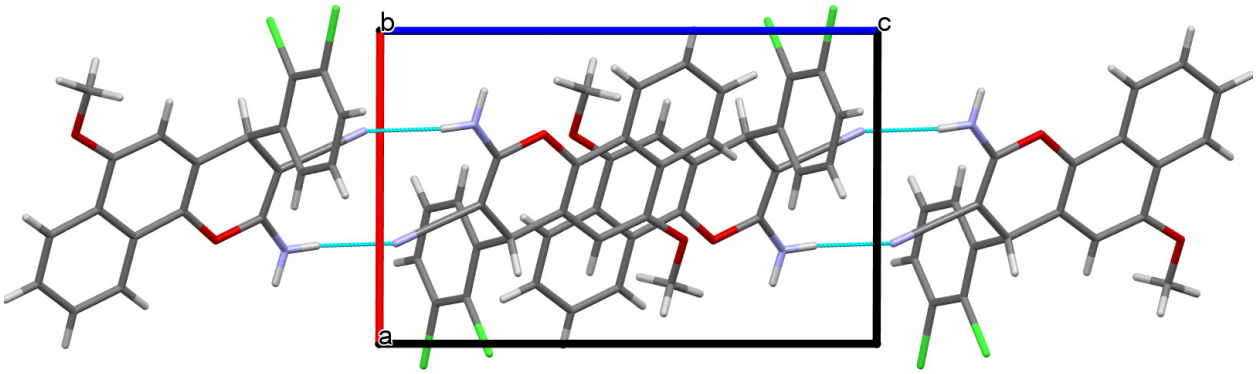


Figure S9: Packing viewed along the *b*-axis direction with C—H···N hydrogen bonds shown as light purple dashed lines.

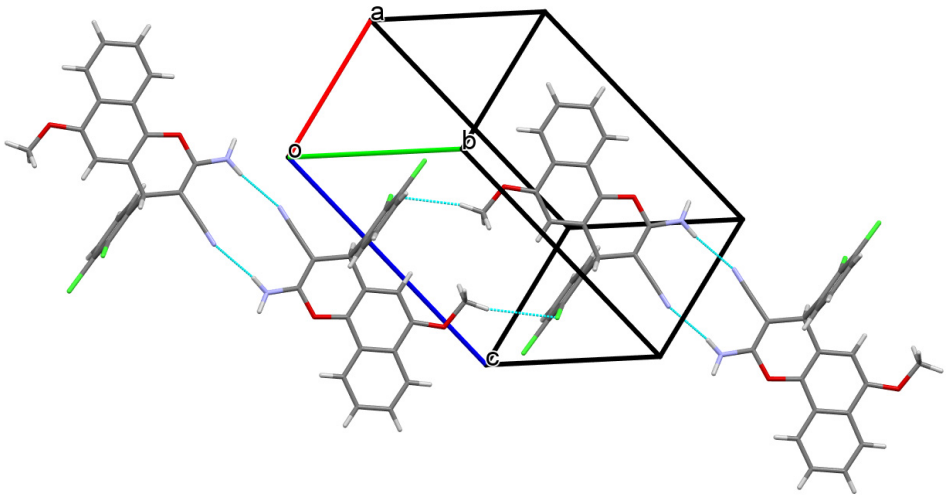


Figure S10: Detail of the intermolecular interactions forming one chain viewed along the b-axis direction. N—H•••N and Cl•••H-O hydrogen bonds are shown by blue dashed lines.

Table S1: The selected experimental and theoretical geometrical parameters for compound **4**.

Atoms	**Å**		**Atoms**	**Angle (°)**	
C(30)-C(32)	1.374	0.93	C(19)-C(21)-C(7)	118.883	118.857
C(29)-C(30)	1.374	1.374	C(21)-C(19)-C(18)	120.607	120.662
C(28)-C(29)	1.404	1.404	C(21)-C(7)-O(3)	123.193	119.709
C(27)-C(28)	1.401	1.399	C(22)-C(21)-C(19)	118.876	118.914
C(27)-C(34)	1.4	1.401	C(22)-C(21)-C(7)	122.241	122.23
C(24)-C(26)	1.411	1.411	C(24)-C(22)-C(21)	109.524	109.474
C(24)-C(25)	1.342	1.341	C(24)-C(25)-N(5)	127.779	127.792
C(22)-C(27)	1.525	1.521	C(24)-C(25)-O(3)	122.538	122.503
C(21)-C(22)	1.511	1.511	C(24)-C(26)-N(6)	179.106	179.229
C(19)-C(21)	1.421	0.929	C(25)-C(24)-C(22)	123.675	123.746
C(18)-C(19)	1.362	1.362	C(26)-C(24)-C(22)	118.176	118.447
C(17)-C(18)	1.425	1.426	C(26)-C(24)-C(25)	118.1	118.082
C(8)-C(17)	1.421	1.421	C(27)-C(22)-C(21)	110.754	110.762
C(8)-C(9)	1.395	1.395	C(27)-C(22)-C(24)	111.556	111.561
C(7)-C(8)	1.422	1.351	C(27)-C(28)-Cl(1)	120.216	120.203
C(7)-C(21)	1.35	1.422	C(28)-C(27)-C(22)	122.458	122.422
N(6)-C(26)	1.146	1.147	C(28)-C(27)-C(34)	118.125	120.216
N(5)-C(25)	1.34	1.34	C(28)-C(29)-Cl(2)	120.238	119.836
O(4)-C(36)	1.378	1.378	C(29)-C(28)-C(27)	119.865	119.951
O(4)-C(18)	1.37	1.369	C(29)-C(28)-Cl(1)	119.909	120.879
O(3)-C(7)	1.396	1.367	C(30)-C(29)-C(28)	120.911	118.884
O(3)-C(25)	1.367	1.396	C(30)-C(29)-Cl(2)	118.831	119.245
Cl(2)-C(29)	1.729	1.728	C(32)-C(30)-C(29)	119.207	120.424
Cl(1)-C(28)	1.728	1.727	C(32)-C(34)-C(27)	120.684	120.678
Atoms	Angle (°)		C(34)-C(27)-C(22)	119.401	119.41
C(11)-C(9)-C(8)	121.312	121.37	C(34)-C(32)-C(30)	121.192	118.15
C(13)-C(11)-C(9)	119.564	119.582	C(8)-C(7)-C(21)	123.015	121.195
C(15)-C(13)-C(11)	120.298	120.281	C(8)-C(7)-O(3)	113.791	119.003
C(15)-C(17)-C(8)	117.789	117.825	C(9)-C(8)-C(7)	123.157	113.824
C(17)-C(15)-C(13)	121.888	121.877	N(5)-C(25)-O(3)	109.683	123.201
C(17)-C(18)-O(4)	113.923	113.948			
C(17)-C(8)-C(7)	117.691	117.731			
C(17)-C(8)-C(9)	119.138	119.055			
C(18)-C(17)-C(15)	123.483	123.473			
C(18)-C(17)-C(8)	118.727	118.701			
C(19)-C(18)-C(17)	121.05	121.032			

Table S2: Topological parameters at BCP of compound **4** [Electron density(ϱ (r)), Laplacian of electron density ($\nabla^2\varrho$ (r)), Ellipticity (ε), Hamiltonian form of Kinetic energy density (K(r)) and distance (D, in A^0) of Bond Path Length (BPL) from the nuclearattractors].

	ρ(r)a.u	*∇²ρ(r)a.u*	*ε*	*K.a.u*	*D(Å)*
N1 - H2	0.327489	-1.52709	0.085791	0.455229	0.002522
N1 - H3	0.327556	-1.50409	0.087249	0.451174	0.002453
N1 - C4	0.288882	-0.0066	0.228124	0.41235	0.007355
C4 - O5	0.263211	0.036117	0.111542	0.337036	0.004804
C4 - C6	0.298624	-0.20094	2.708324	0.428247	0.00057
O5 - C7	0.237524	0.250112	0.309196	0.285876	0.005693
C7 - C11	0.309223	-0.79706	0.552241	0.383797	0.00163
C8 - C11	0.249069	-0.67658	0.039228	0.236678	0.000262
C8 - H12	0.271667	-0.70883	0.00492	0.242775	0.000123
C6 - C8	0.243256	-0.6368	0.105404	0.227134	0.000393
C11 - C17	0.281558	-0.77386	0.277561	0.295408	0.000056
C6 - C9	0.262557	-0.32318	0.970567	0.333775	0.00008
H12 - Cl26	0.014806	0.075518	0.748092	-0.00329	0.061882
C7 - C10	0.281406	-0.77548	0.243039	0.299279	0.000468
C8 - C13	0.243369	-0.63344	0.016977	0.227247	0.000087
C9 - N14	0.403717	0.928625	0.106799	0.735645	0.000048
C10 - C15	0.292662	-0.82335	0.267002	0.320268	0.000081
C10 - C16	0.284261	-0.7799	0.292049	0.298748	0.000147
C16 - C23	0.280857	-0.78329	0.231475	0.29465	0.000319
C23 - O32	0.257647	0.152711	0.167536	0.327188	0.006952
C16 - C22	0.285631	-0.79706	0.246656	0.303183	0.000074
C17 - C23	0.303374	-0.75103	0.616144	0.375308	0.001177
C13 - C18	0.285999	-0.7357	0.36126	0.32286	0.001577
C13 - C19	0.290738	-0.7984	0.32124	0.312705	0.000054
C15 - C21	0.304232	-0.84497	0.424738	0.337062	0.000046
C15 - H20	0.265264	-0.67403	0.037604	0.236648	0.00003
C22 - C29	0.315311	-0.89518	0.437489	0.35911	0.000103
C21 - C29	0.28966	-0.81781	0.256048	0.309278	0.000084
C22 - H31	0.265992	-0.68314	0.029201	0.236771	0.000036
O32 - C37	0.255098	0.087935	0.024696	0.319135	0.006701
C17 - H24	0.264453	-0.65809	0.06862	0.236102	0.000023
C18 - C25	0.28683	-0.76983	0.424642	0.303376	0.004138
C18 - Cl26	0.187235	-0.29691	0.064237	0.145481	0.000026
C19 - C27	0.299031	-0.83889	0.348092	0.327069	0.000061
C19 - H28	0.264993	-0.67568	0.035934	0.23616	0.000031
C21 - H30	0.264287	-0.67137	0.029659	0.236282	0.000005
C25 - C33	0.297704	-0.73854	0.411689	0.370277	0.001104
C27 - C33	0.301742	-0.85438	0.334388	0.335049	0.000097
C25 - Cl34	0.187407	-0.29996	0.056832	0.145693	0.000587
C27 - H35	0.265315	-0.68117	0.027416	0.236477	0.000007

C29 - H36	0.26457	-0.6702	0.032483	0.236688	0.000016
C33 - H38	0.2655	-0.68713	0.0358	0.235391	0.000059
C37 - H39	0.269005	-0.71392	0.043523	0.240042	0.000277
C37 - H40	0.269382	-0.69482	0.045878	0.24134	0.000479
C37 - H41	0.269455	-0.69635	0.046146	0.241269	0.000338

Table S3. Crystal data and structure refinement parameters for compound **4**.

Crystal data	
Chemical formula	$C_{21}H_{14}Cl_2N_2O_2$
Formula weight	397.24
Crystal system, space group	Triclinic, P -1 (2)
Temperature (K)	293
a, b, c (Å)	7.8561(4),9.2701(5),12.8343(7)
V (Å3)	910.31(9)
Z	2
Radiation type	Mo Kα
μ (mm−1)	3.368
Crystal size (mm)	0.556 × 0.143 × 0.974
Data collection	
Diffractometer	BrukerAPEX-II D8 venture diffractometer
Absorption correction	Multi-scan, SADABSBruker 2018
Tmin, Tmax	0.417,0.501
No. of measured, independent and observed [I> 2σ(I)] reflections	1647, 2993, 3181
Rint	0.049
Refinement	
R[F2 > 2σ(F2)], wR(F2), S	0.0752, 0.2346, 1.075
No. of reflections	3025
No. of parameters	253
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement