Design, Synthesis, and Anti-inflammatory Evaluation of New Isatin Azo Disperse Dyes: Computational studies, Molecular Docking, and dyeing performance on Polyester Fabrics

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Running title: Isatin-based azo dyes and their applications.

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Chemicals and Reagents

All reagents and starting material (1) were supplied by Sigma-Aldrich. For printing on polyester fabrics, an anionic acrylic polymer, specifically thickener EM-630, sourced from Guangdong EM Chemicals Technology Co., Ltd., served as the dye carrier. The pH of the printing paste was adjusted to 6 using acetic acid. Additionally, levegal MDL, an anionic dispersing agent from TANATEX Chemicals, was employed. El-Mahalla Company for Spinning and Weaving in El-Mahalla, Egypt, kindly provided 100% plain-weave polyester fabric (150 g/m², 56 ends/cm, 34 picks/cm). Furthermore, the fabric was treated for 30 minutes at 80°C in an aqueous solution containing two grams per liter of nonionic detergent (Sera Wash M-RK, DyStar, Egypt) at a 50% liquor-to-goods ratio, followed by washing and air drying.

Instruments and Methods

All ^{1}H- and ^{13}C-NMR spectra were recorded on a *JEOL* ECA500 FT NMR spectrometer at 500 MHz and 125 MHz, respectively, at the Spectral Analyses Unit, National Research Center, Dokki, Egypt. Dimethyl sulfoxide (DMSO-d6) was used as a solvent. Chemical shifts were expressed in δ ppm relative to the position with respect to the solvent. Fourier transform infrared (FT-IR) spectra were recorded using a Thermo Fisher Nicolet IS10 at Spectral Analyses Unit, National Research Center, Dokki, Egypt. Electron impact ionization mass spectra (EI-MS) were obtained on a Thermos Scientific Trace 1310 Gas Chromatography-Mass Spectrometry at Cairo University, Egypt. UV/Vis absorption spectra for azo compounds were measured by a Hunter Lab Ultra Scan PRO spectrophotometer. The in vitro anti-inflammatory screening was carried out at Micro Analytical Center, National Research Centre, Dokki, Egypt. Melting points (m.p.) were measured and recorded by a Stuart Scientific melting point apparatus and were uncorrected. All synthesized products were detected by thin-layer chromatography (TLC) on Kiesel gel F254 precoated plates (Merck).

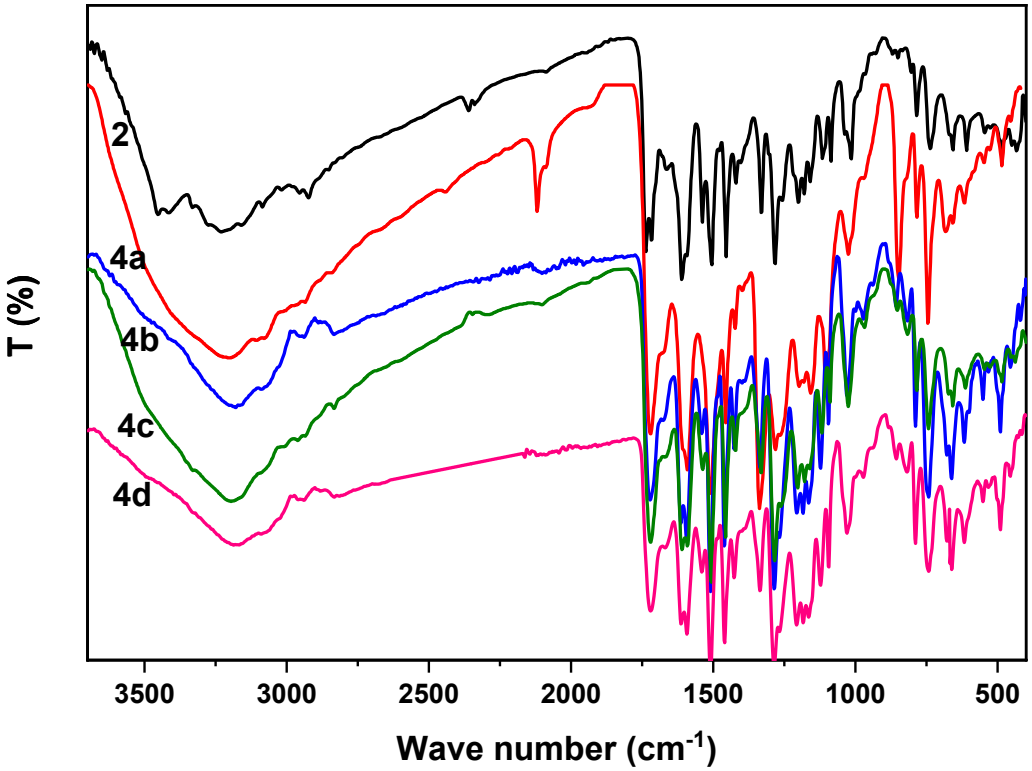

Fig.S1. FT-IR spectra of compounds **2** and **4a-d**.

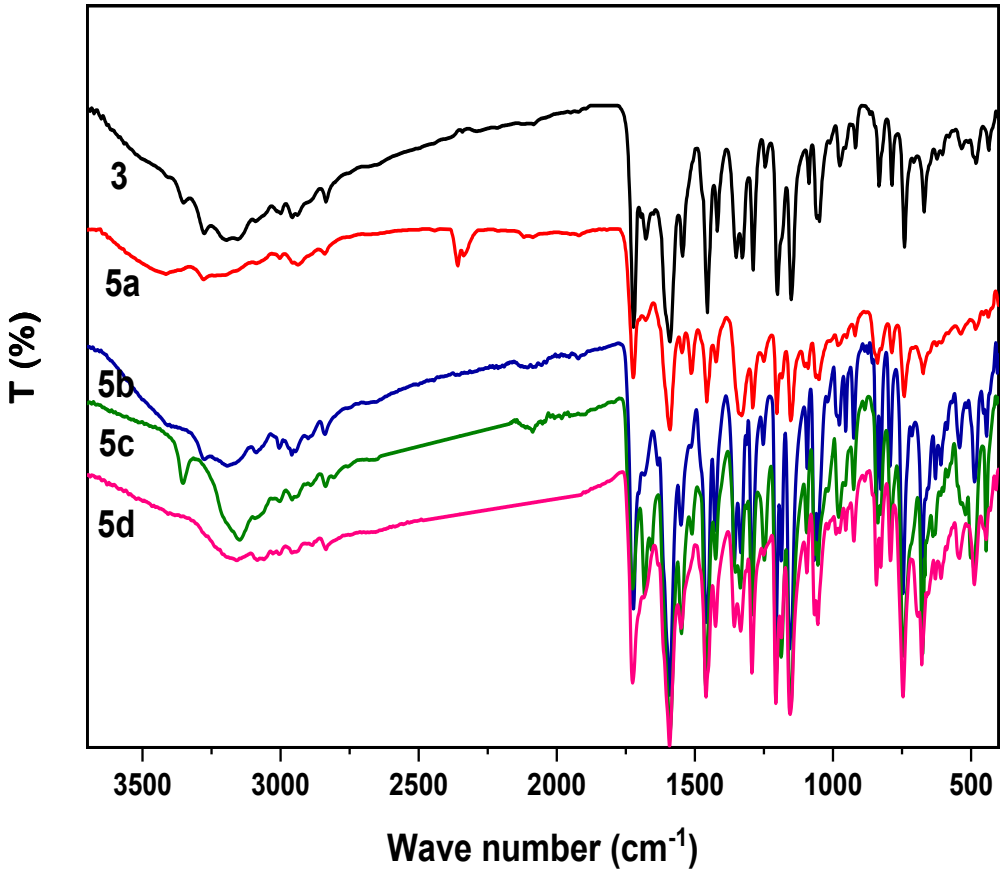

Fig.S2. FT-IR spectra of compounds **3** and **5a-d**.

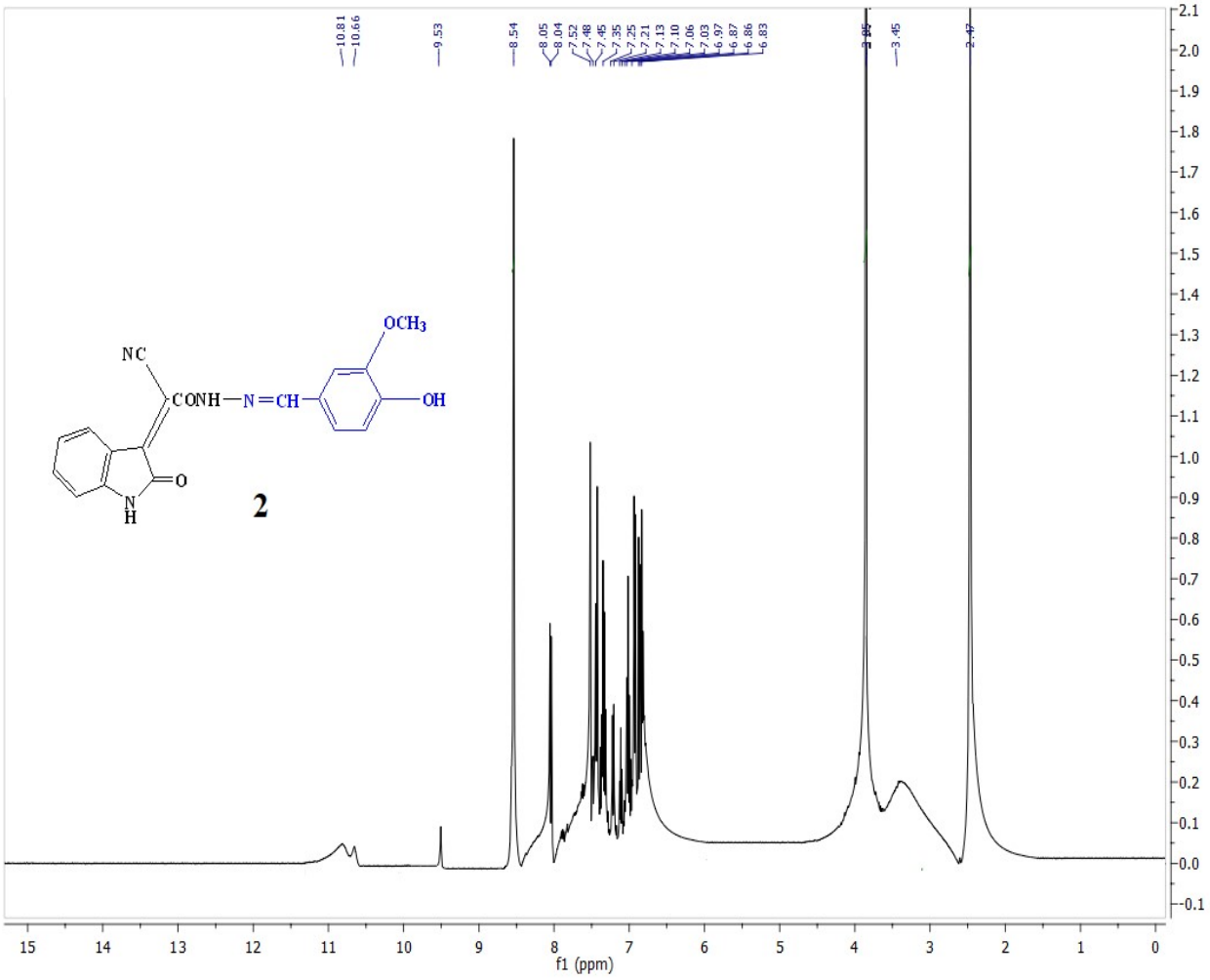

Fig.S3. ^{1}H-NMR spectra of compound **2.**

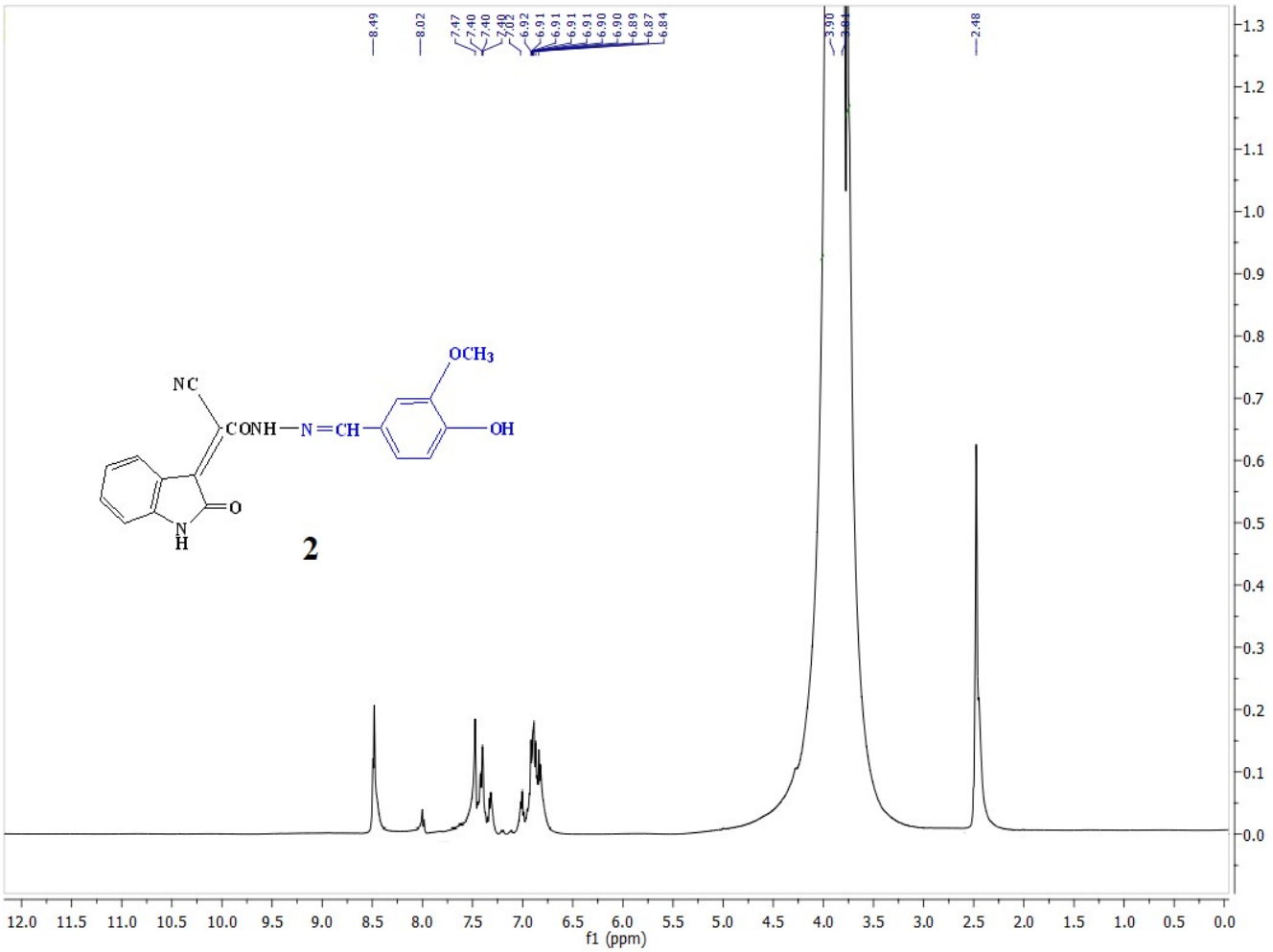

Fig.S4. D_2O-^{1}H-NMR spectra of compound **2.**

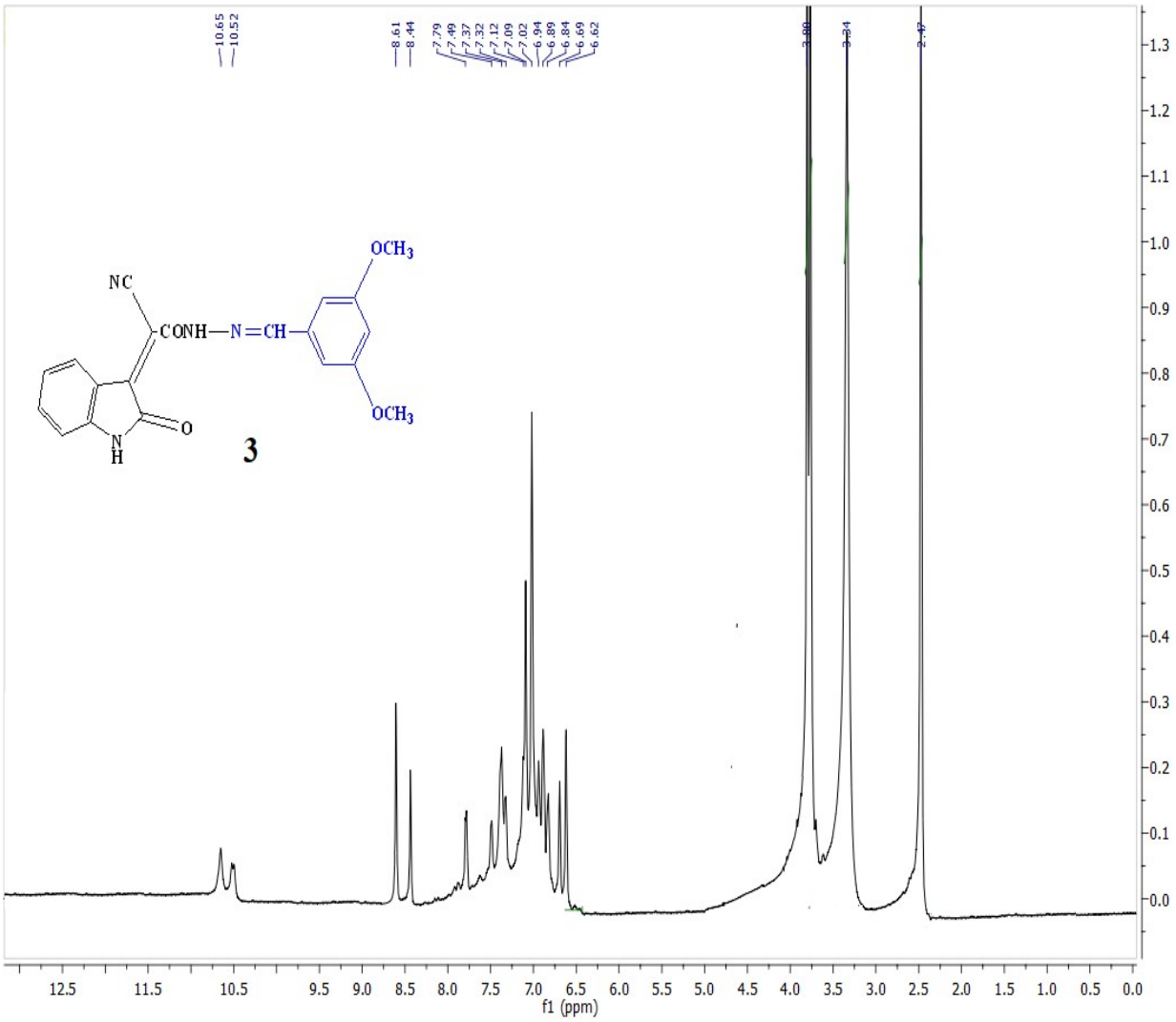

Fig.S5. ^{1}H-NMR spectra of compound **3.**

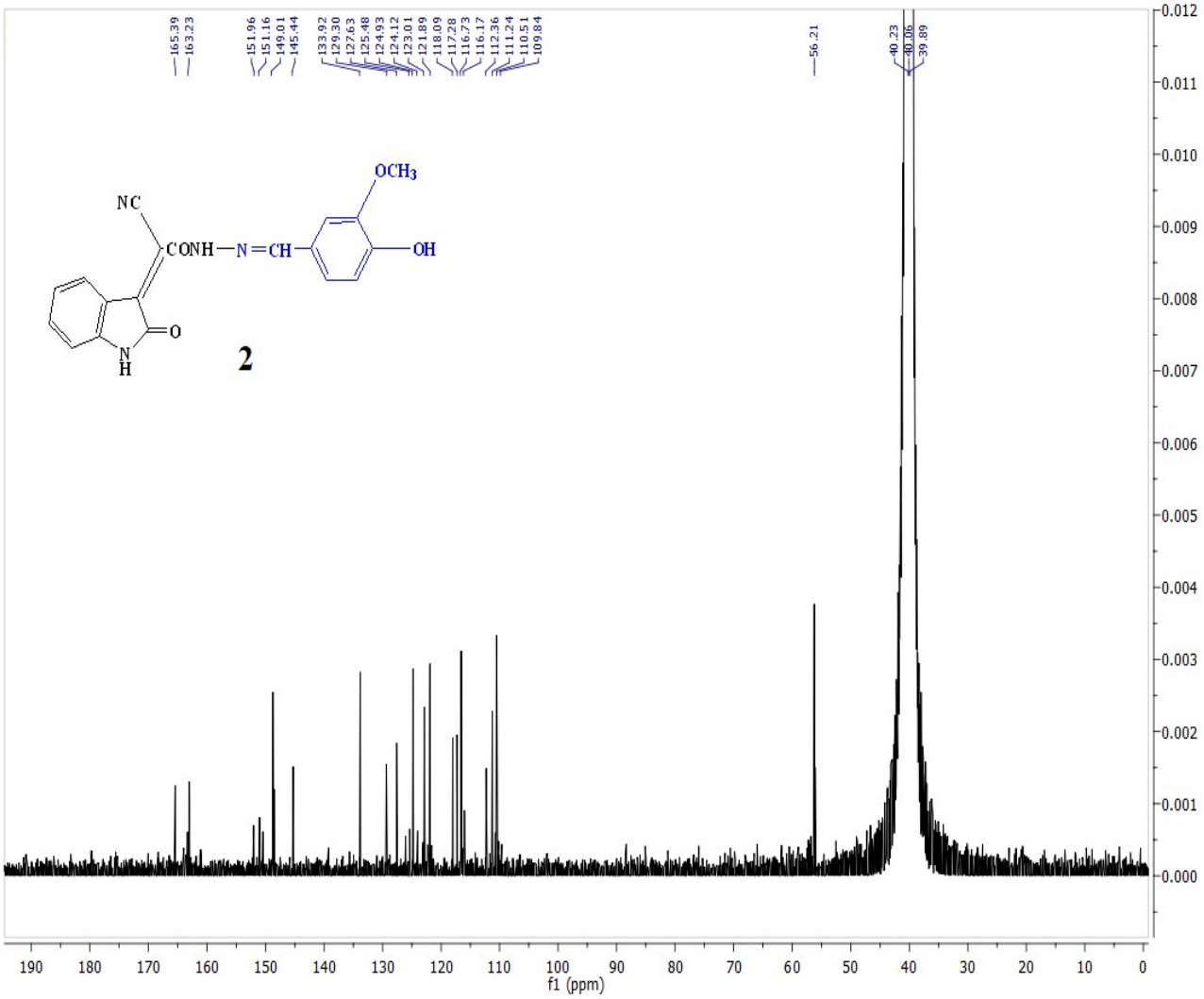

Fig.S6. ^{13}C-NMR spectra of compound **2.**

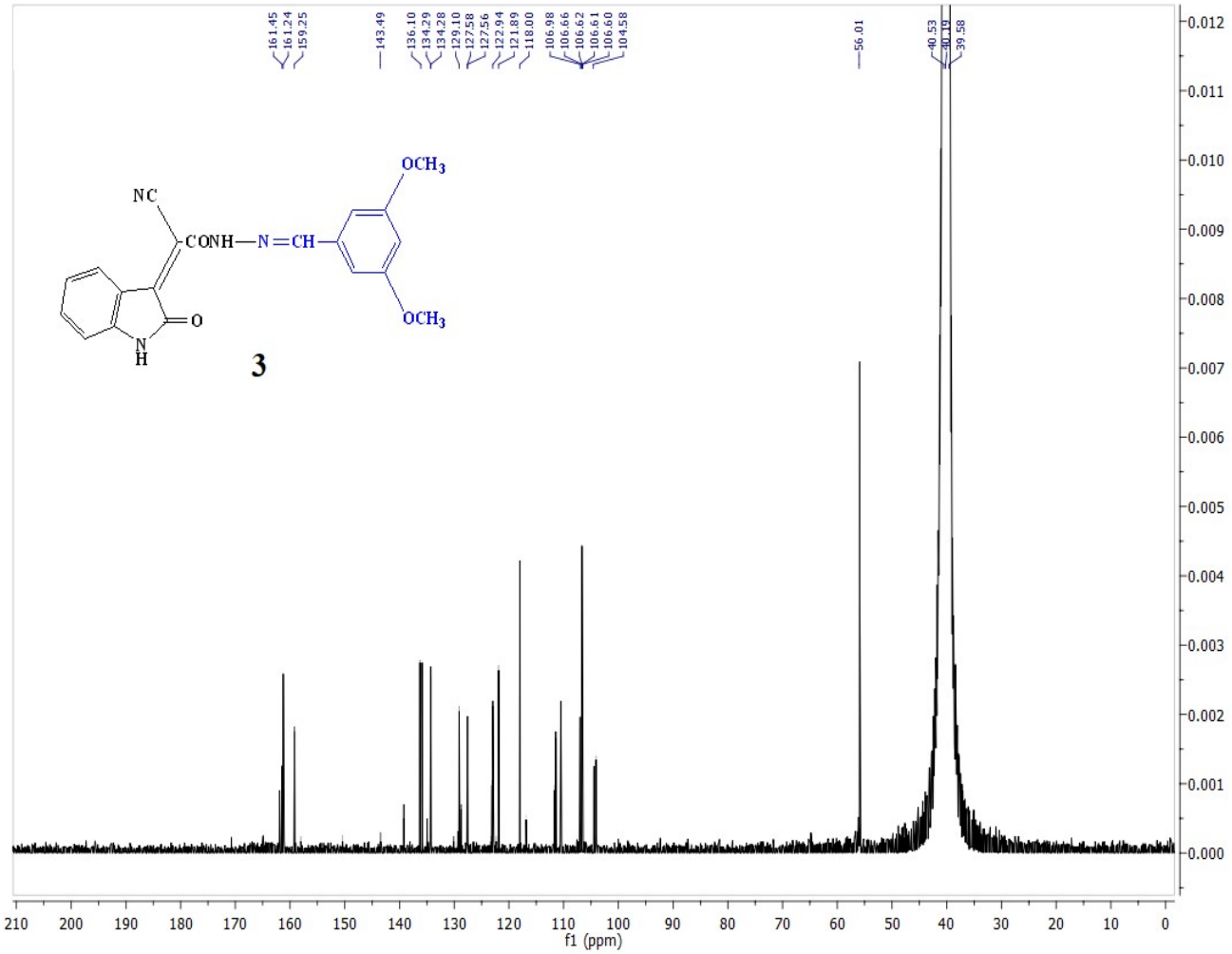

Fig.S7. ^{13}C-NMR spectra of compound **3.**

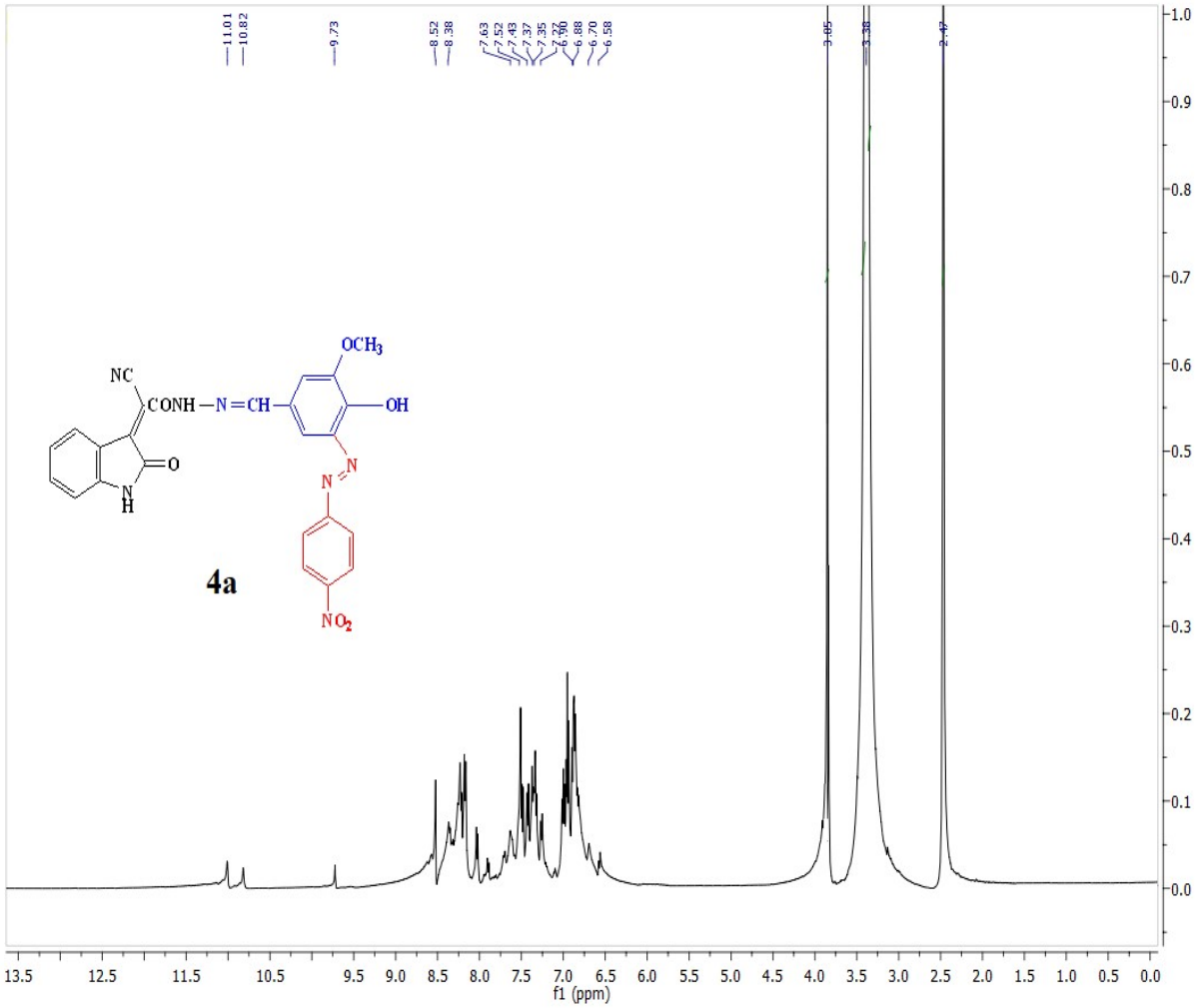

Fig.S8. ^{1}H-NMR spectra of compound **4a.**

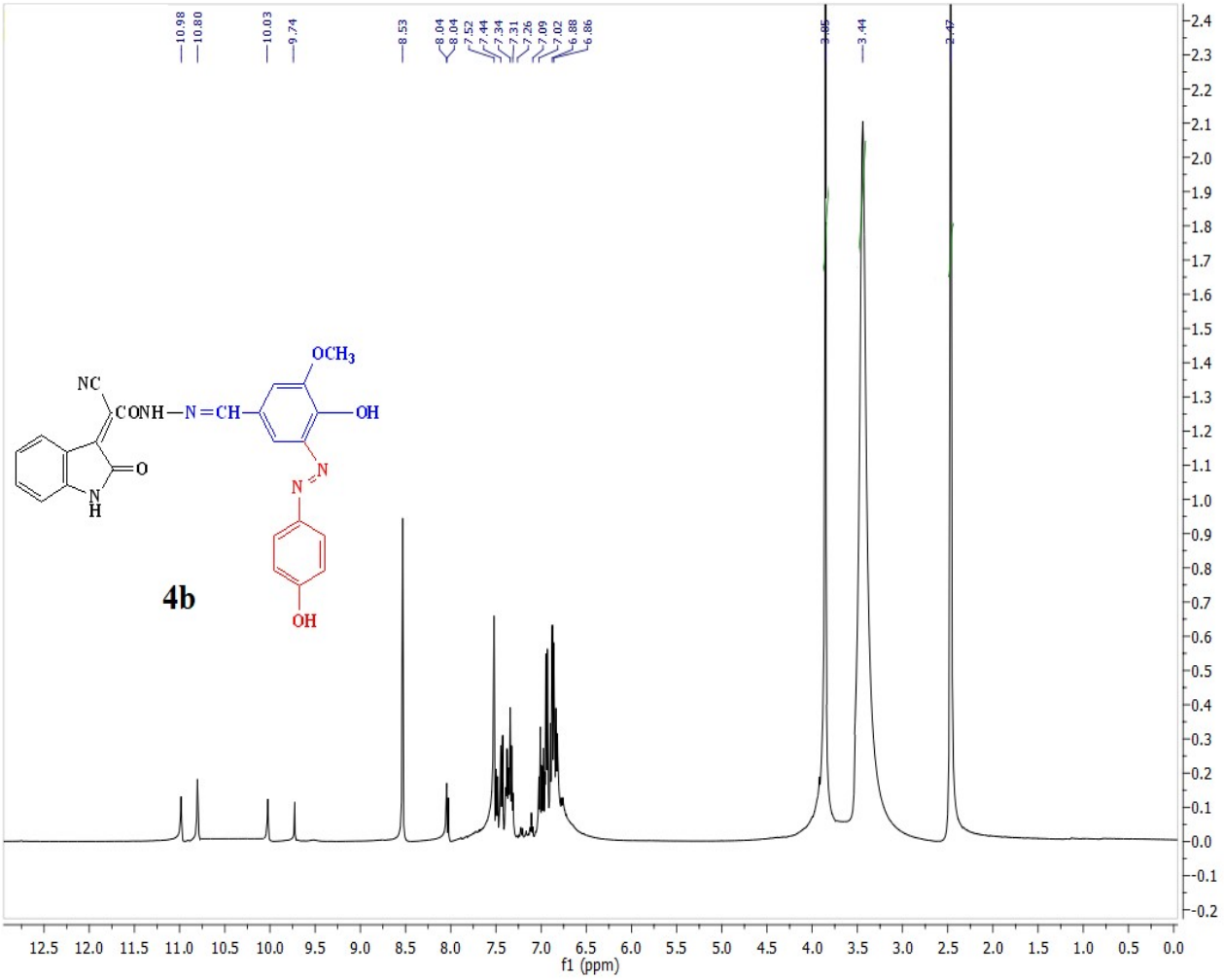

Fig.S9. ^{1}H-NMR spectra of compound **4b.**

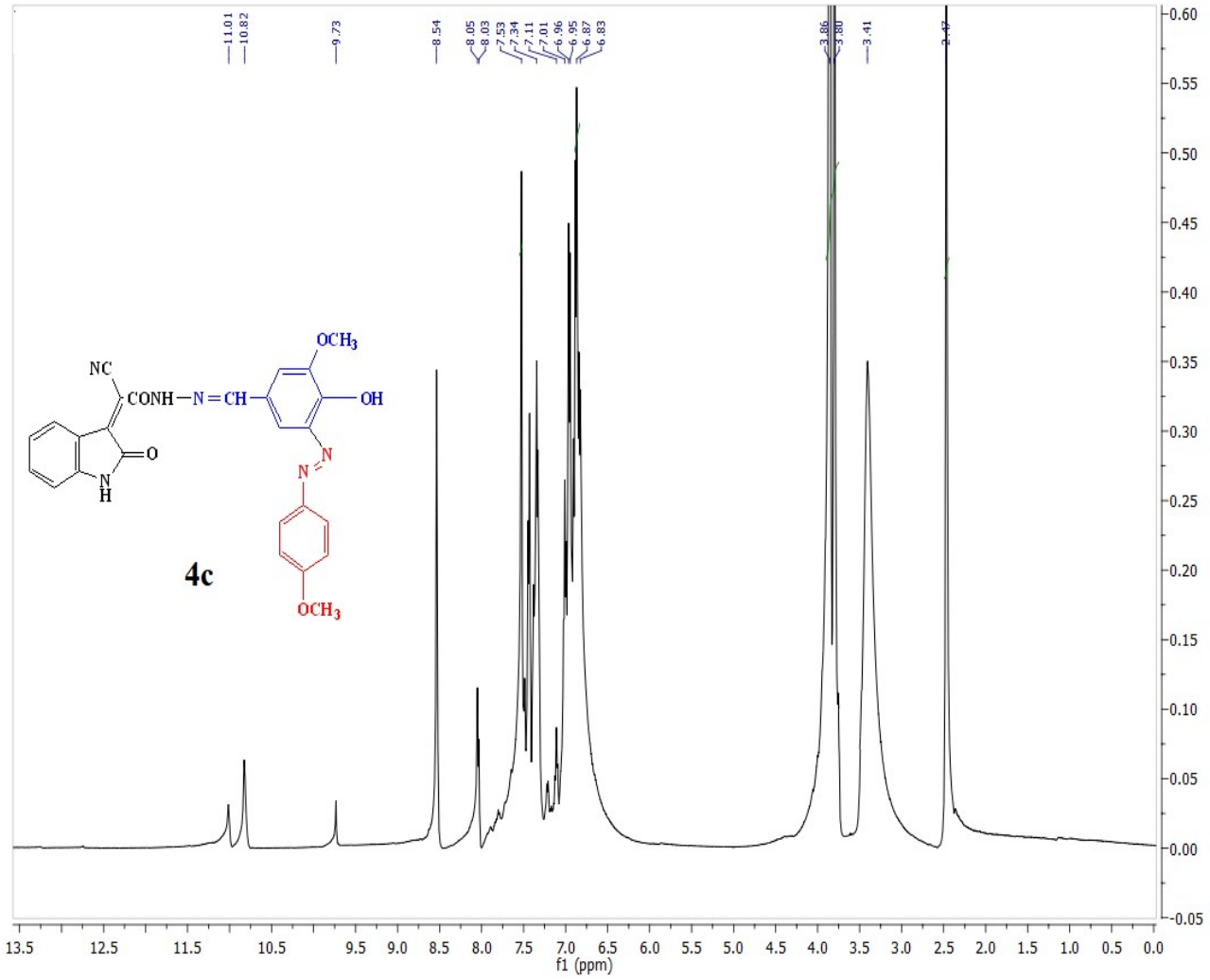

Fig.S10. ^{1}H-NMR spectra of compound **4c.**

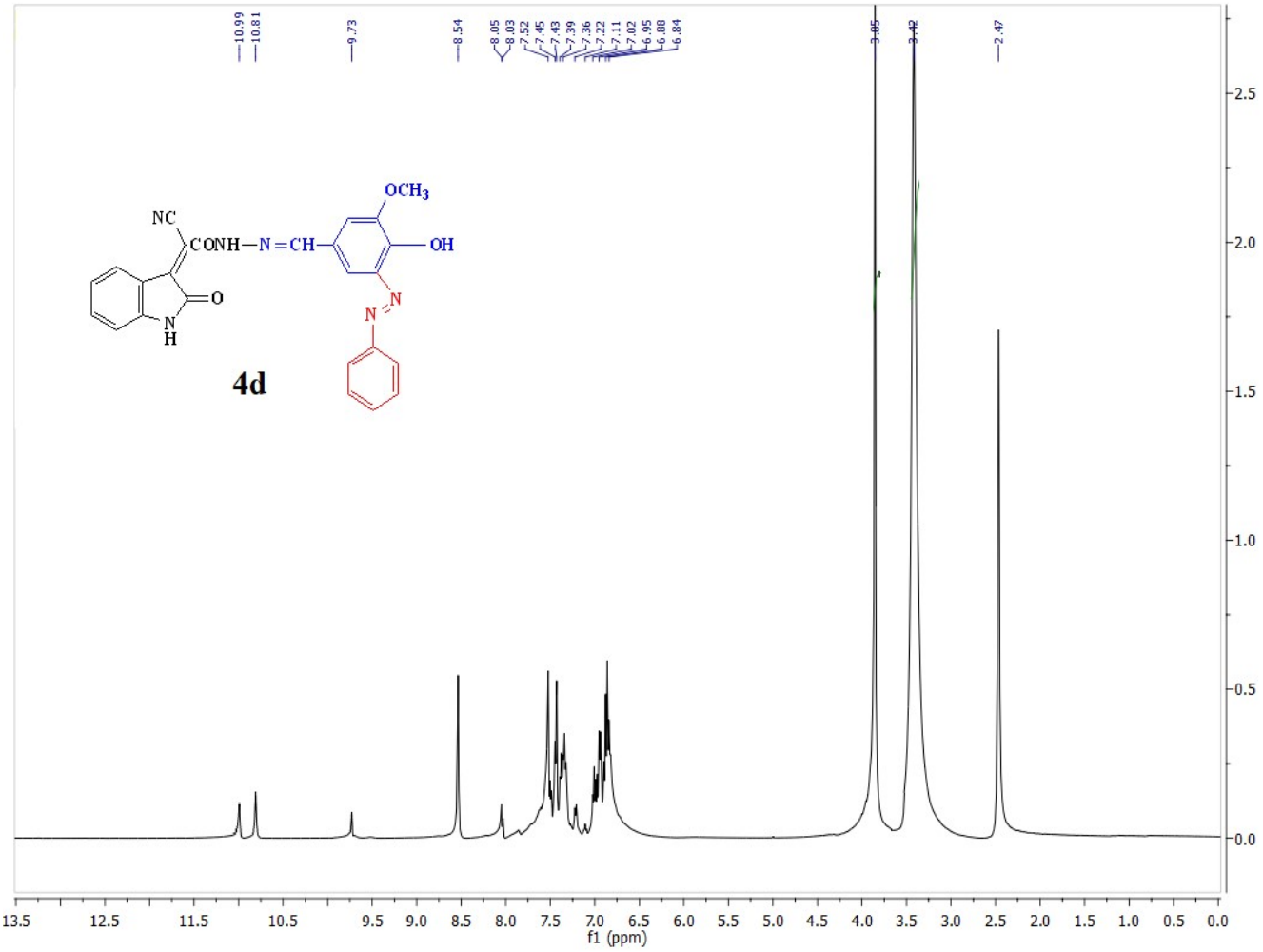

Fig.S11. ^{1}H-NMR spectra of compound **4d.**

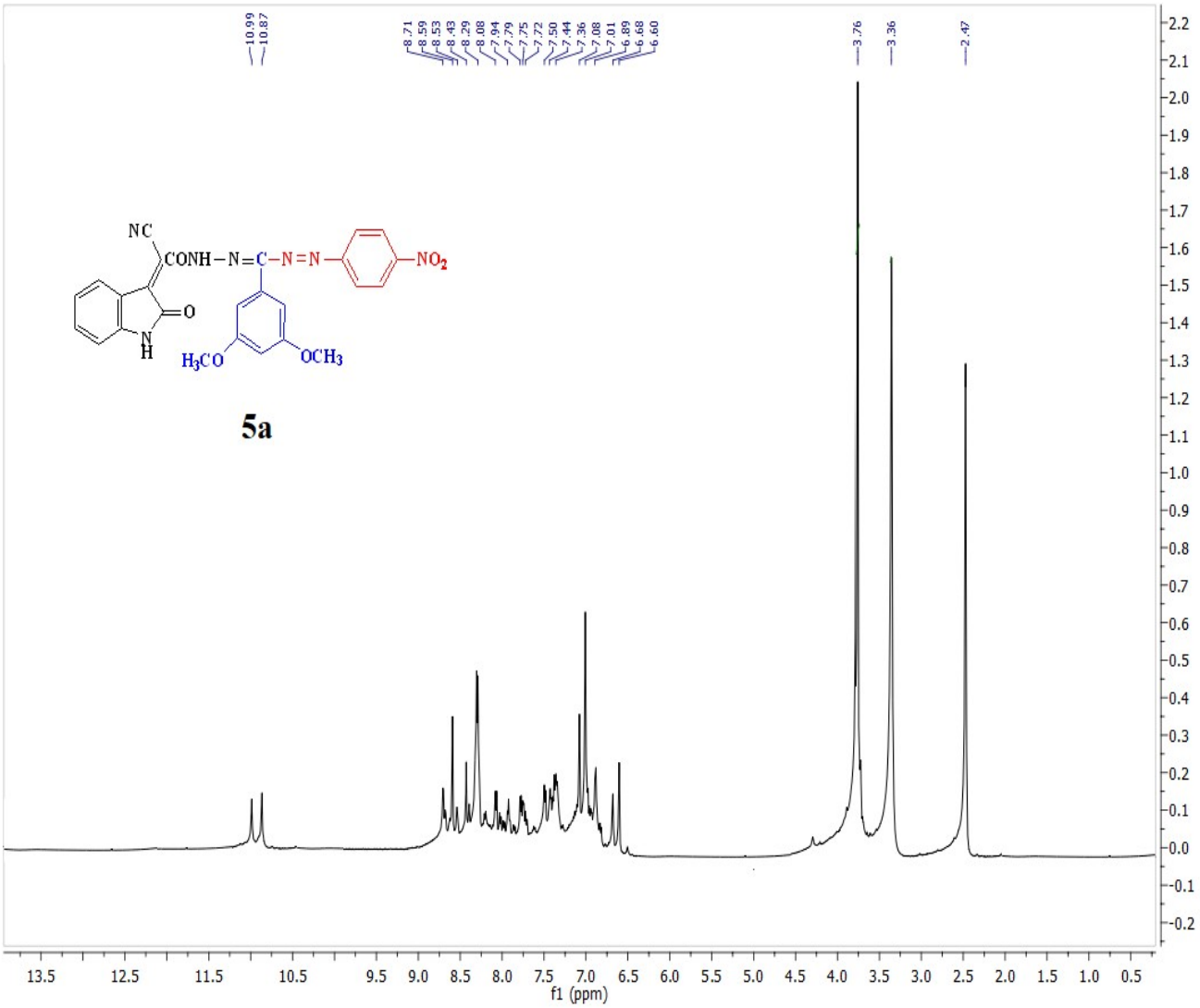

Fig.S12. ^{1}H-NMR spectra of compound **5a.**

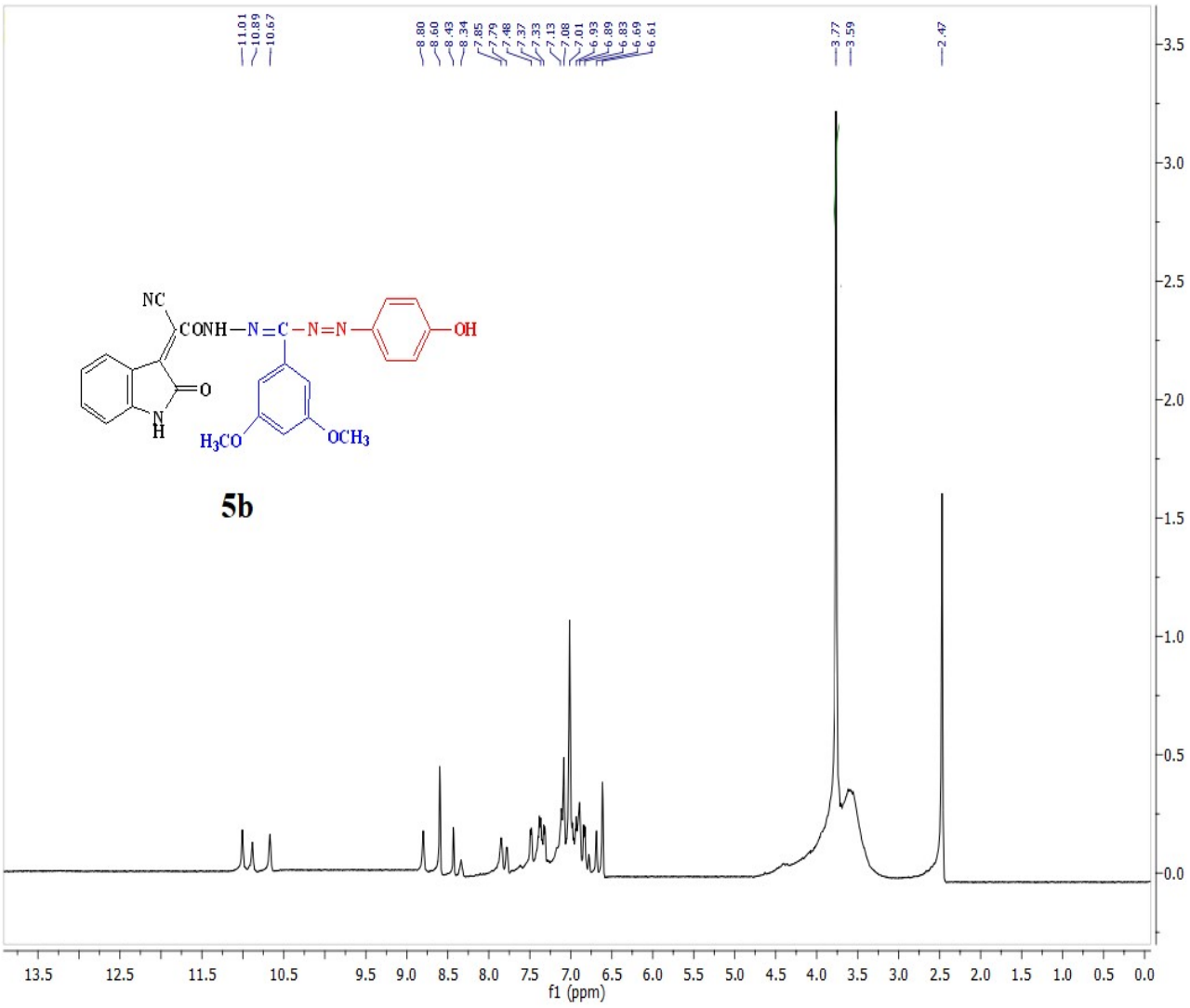

Fig.S13. ^{1}H-NMR spectra of compound **5b.**

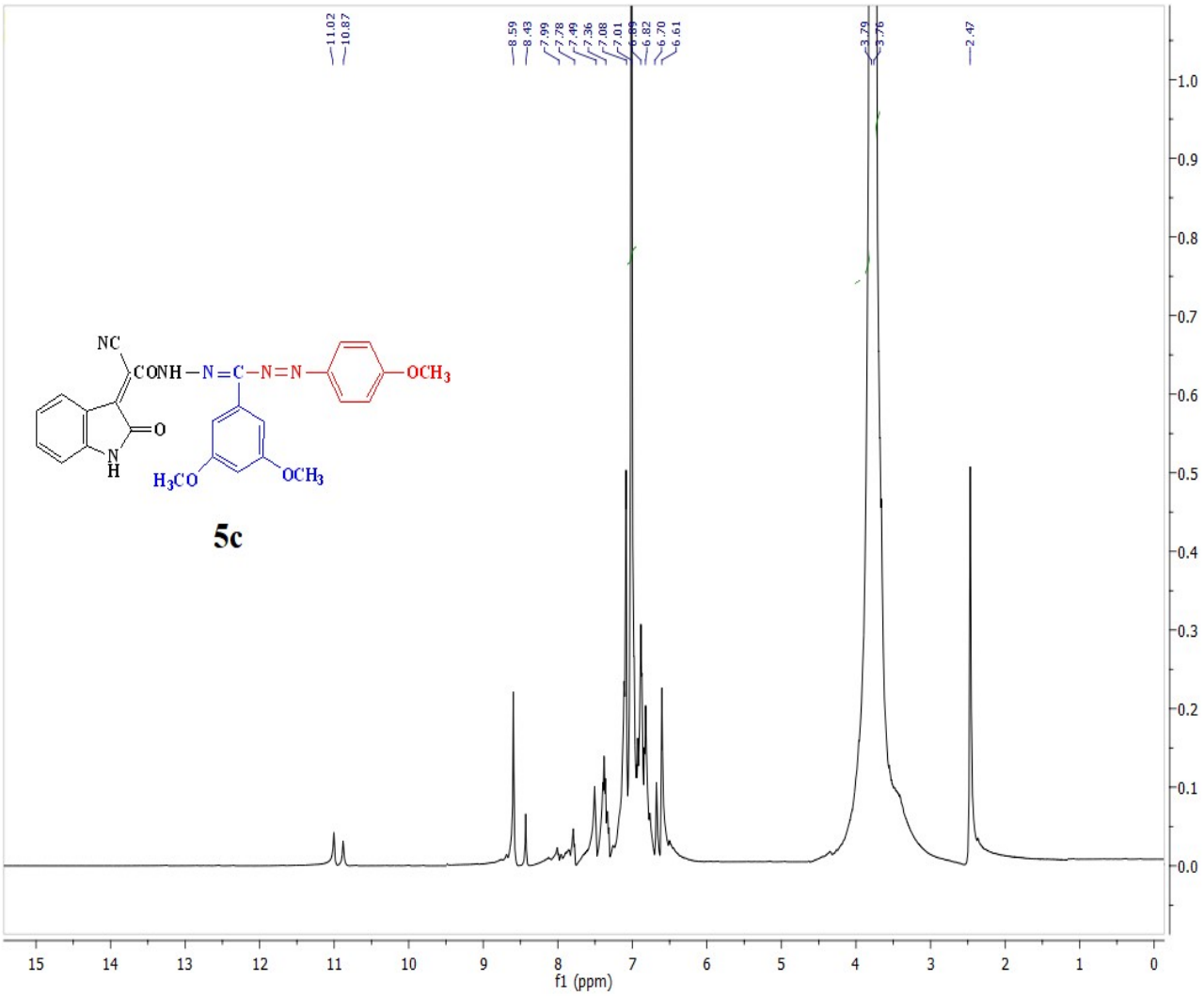

Fig.S14. ^{1}H-NMR spectra of compound **5c.**

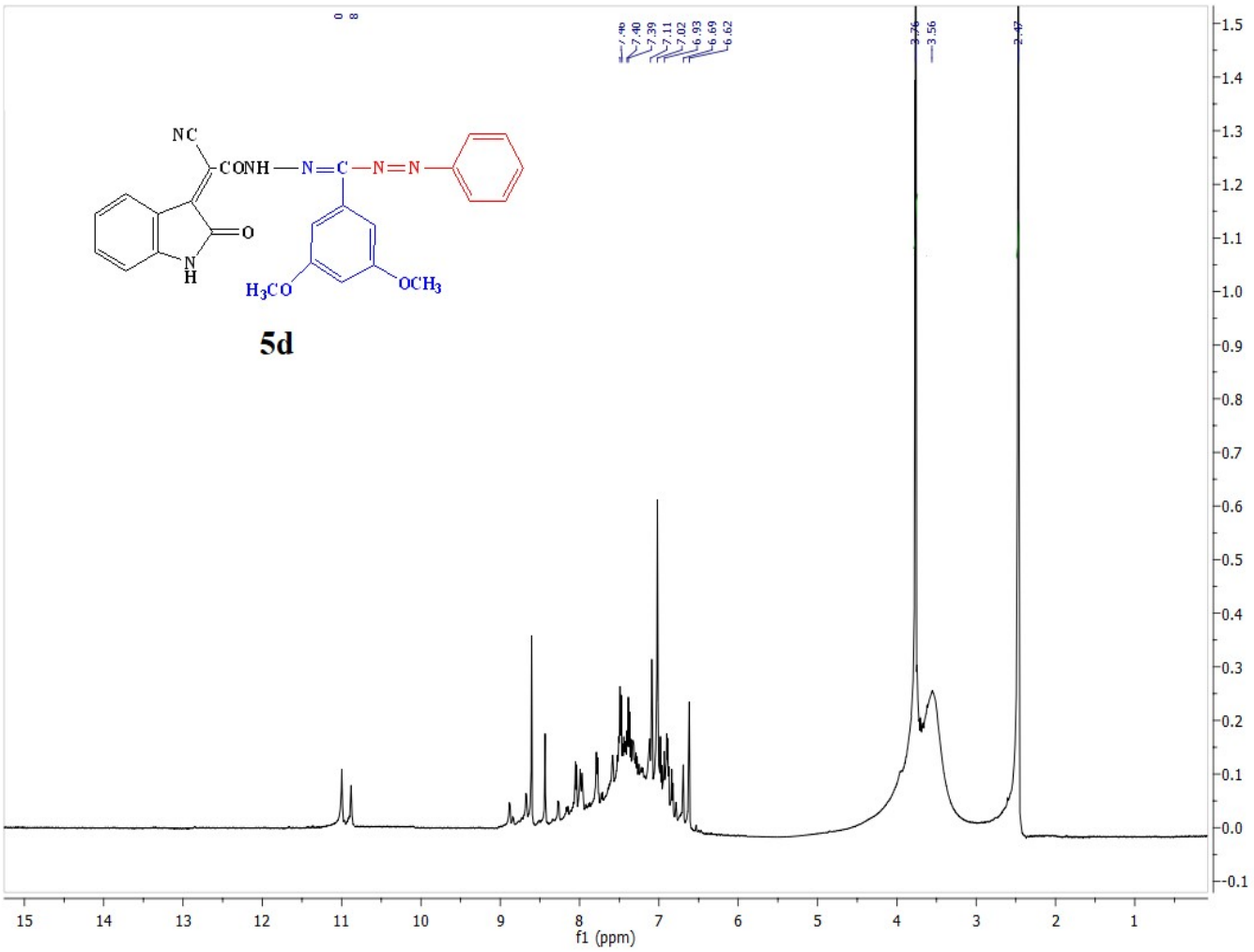

Fig.S15. ^{1}H-NMR spectra of compound **5d.**

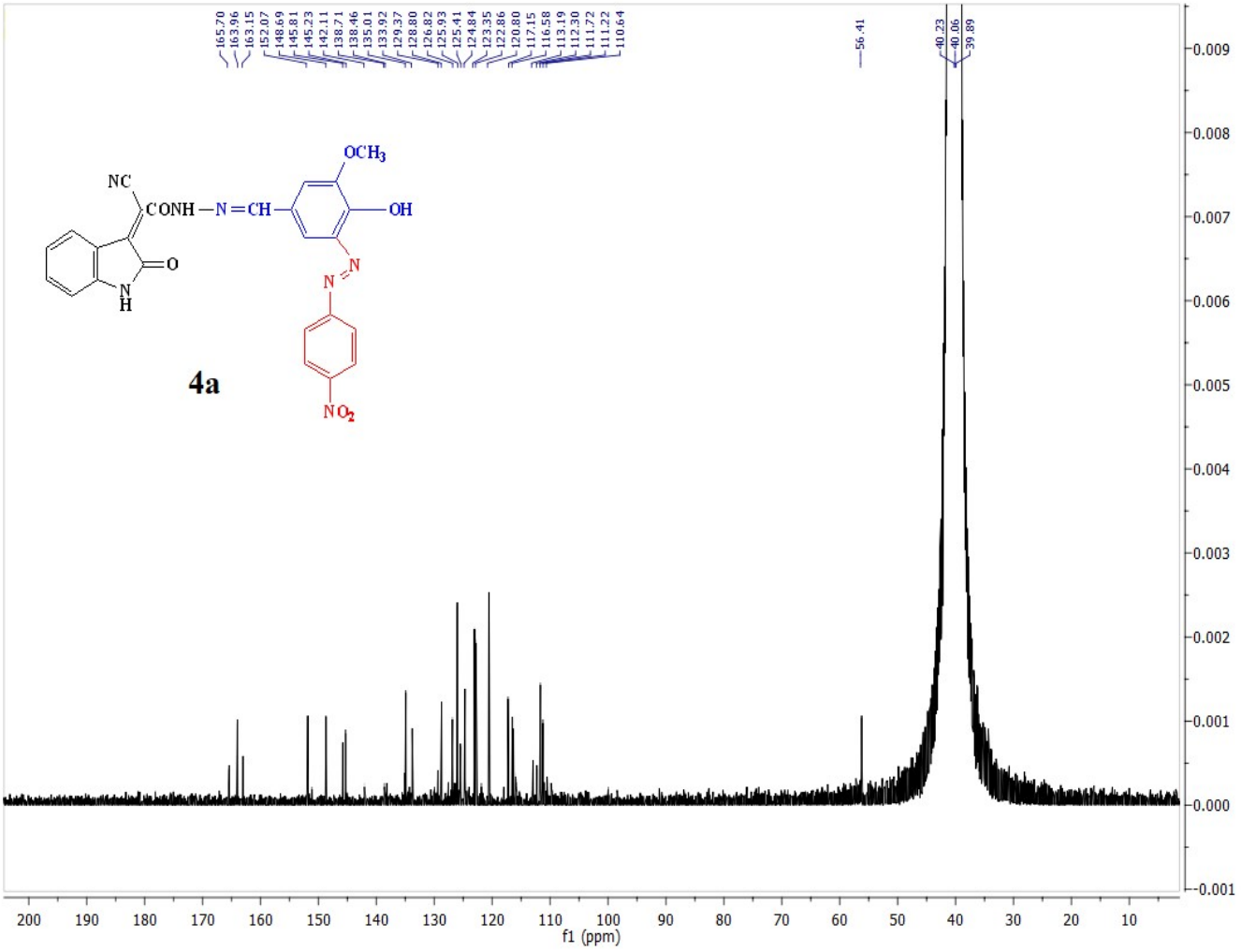

Fig.S16. ^{13}C-NMR spectra of compound **4a.**

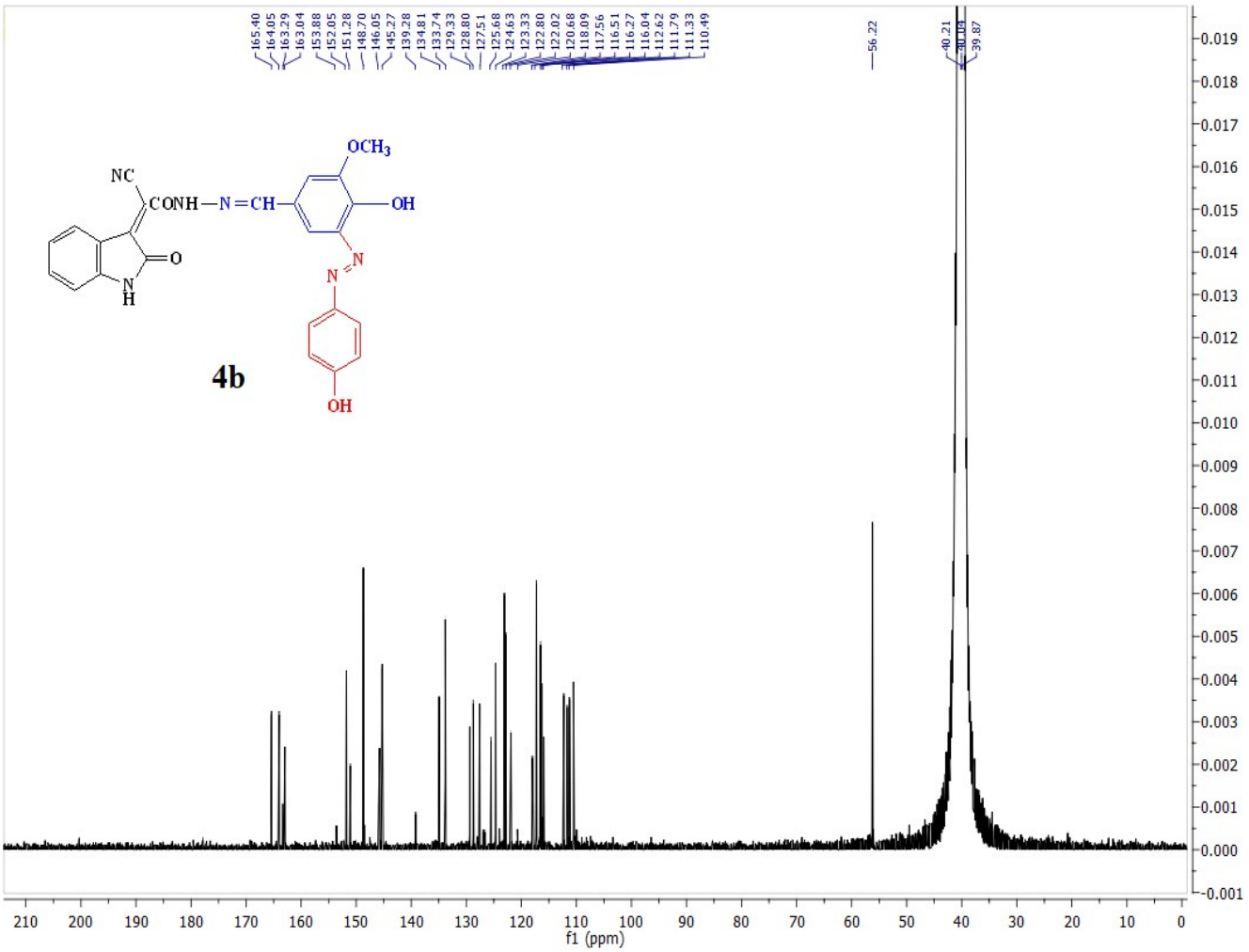

Fig.S17. ^{13}C-NMR spectra of compound **4b.**

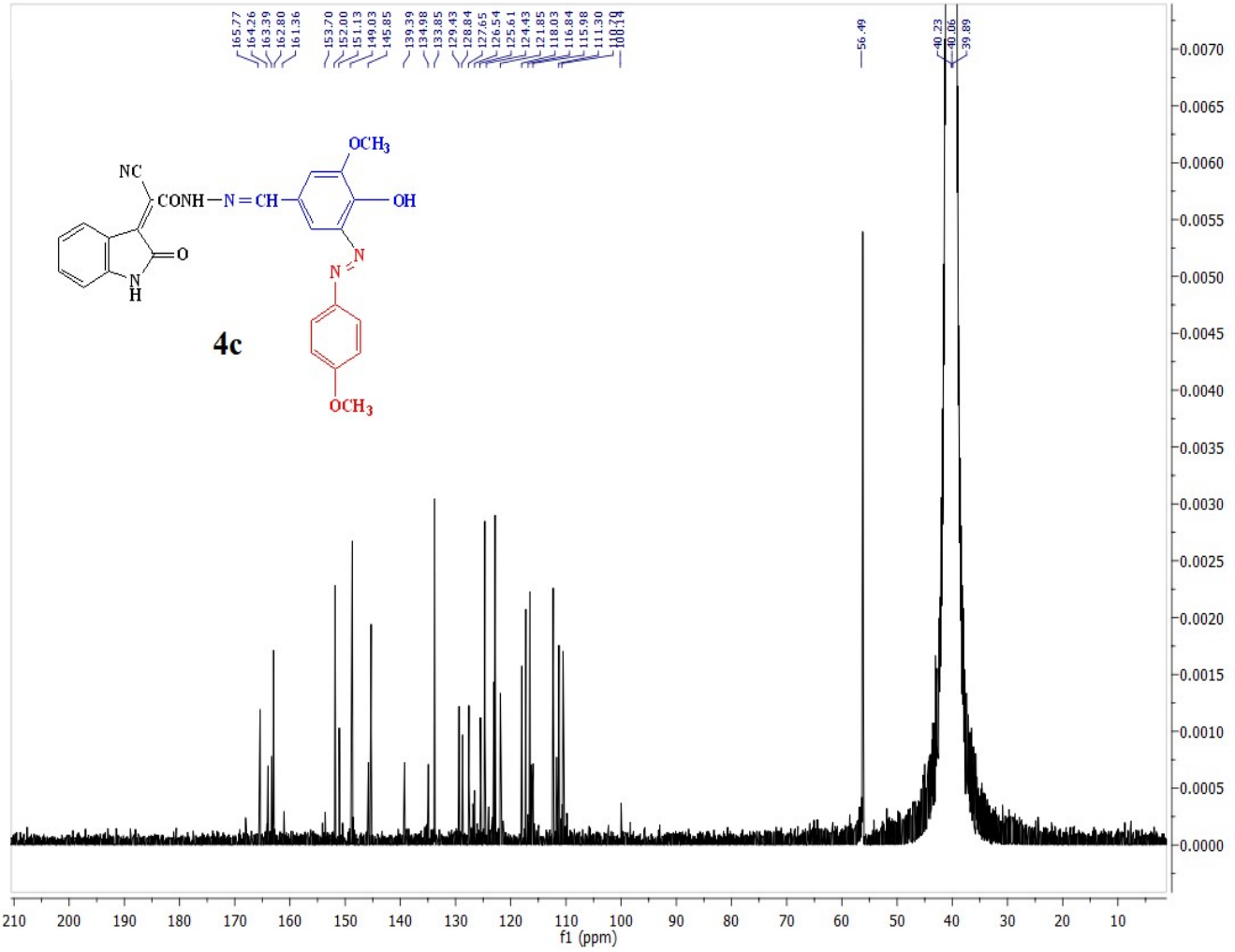

Fig.S18. ^{13}C-NMR spectra of compound **4c.**

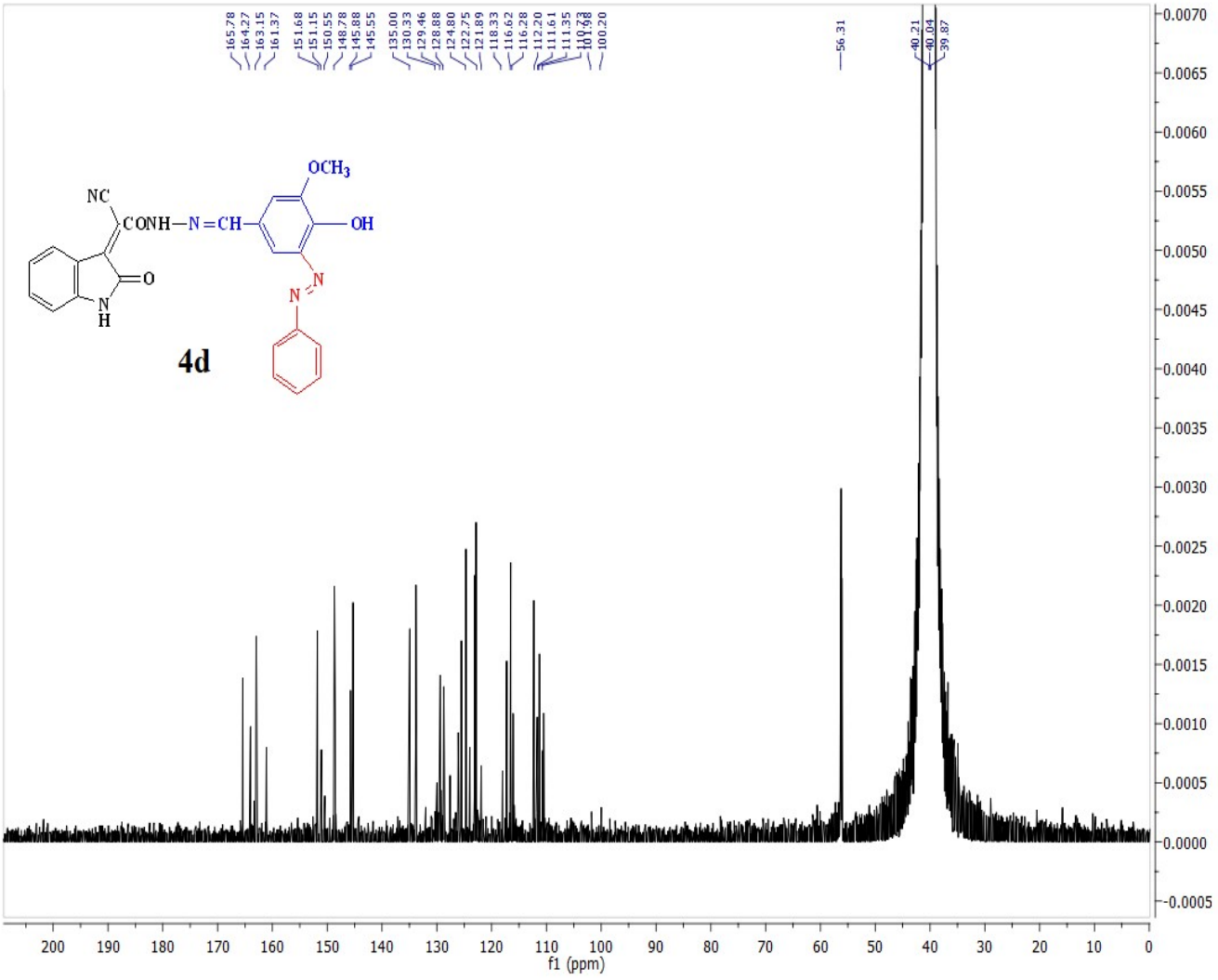

Fig.S19. ^{13}C-NMR spectra of compound **4d.**

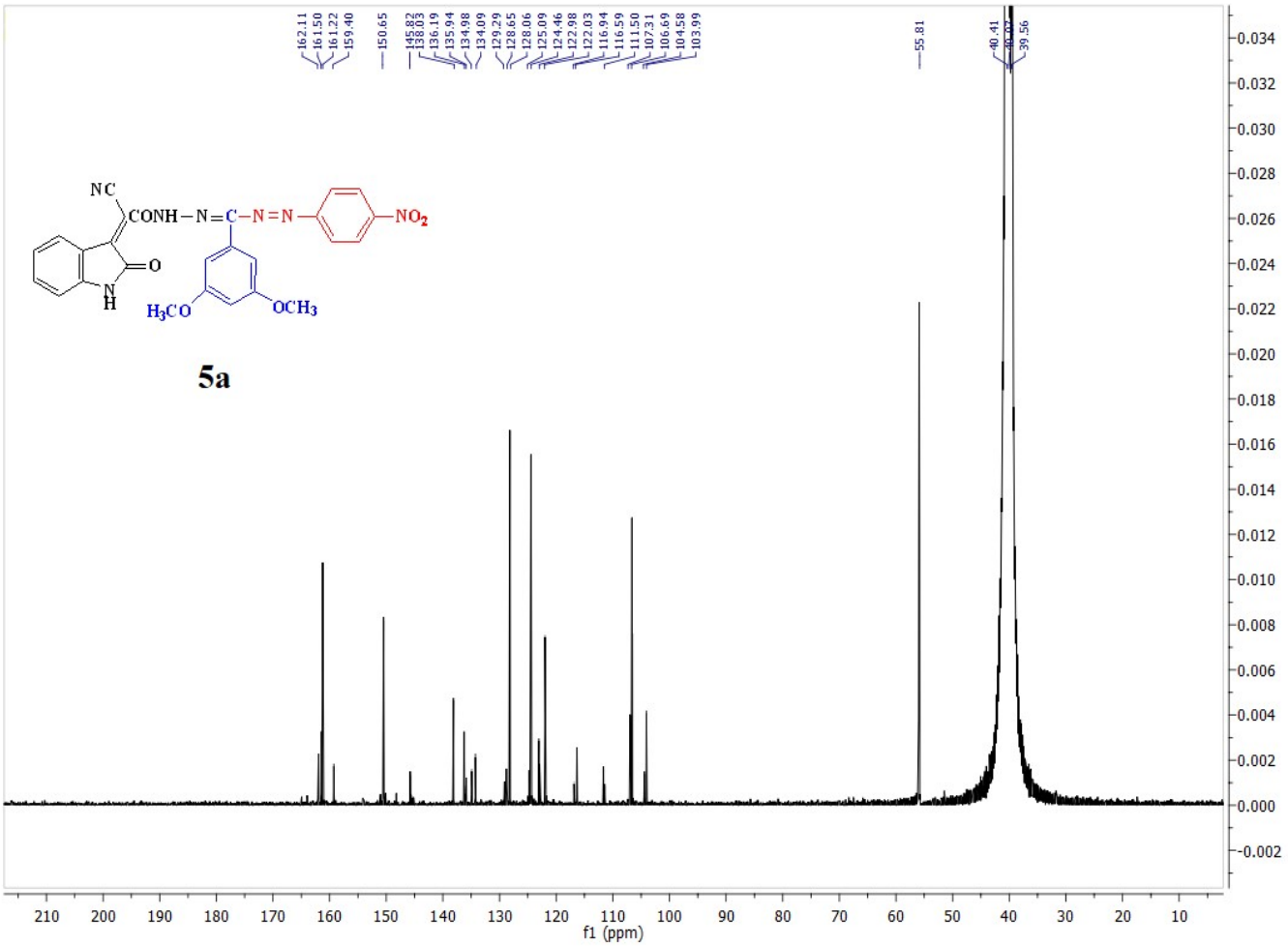

Fig.S20. ^{13}C-NMR spectra of compound **5a.**

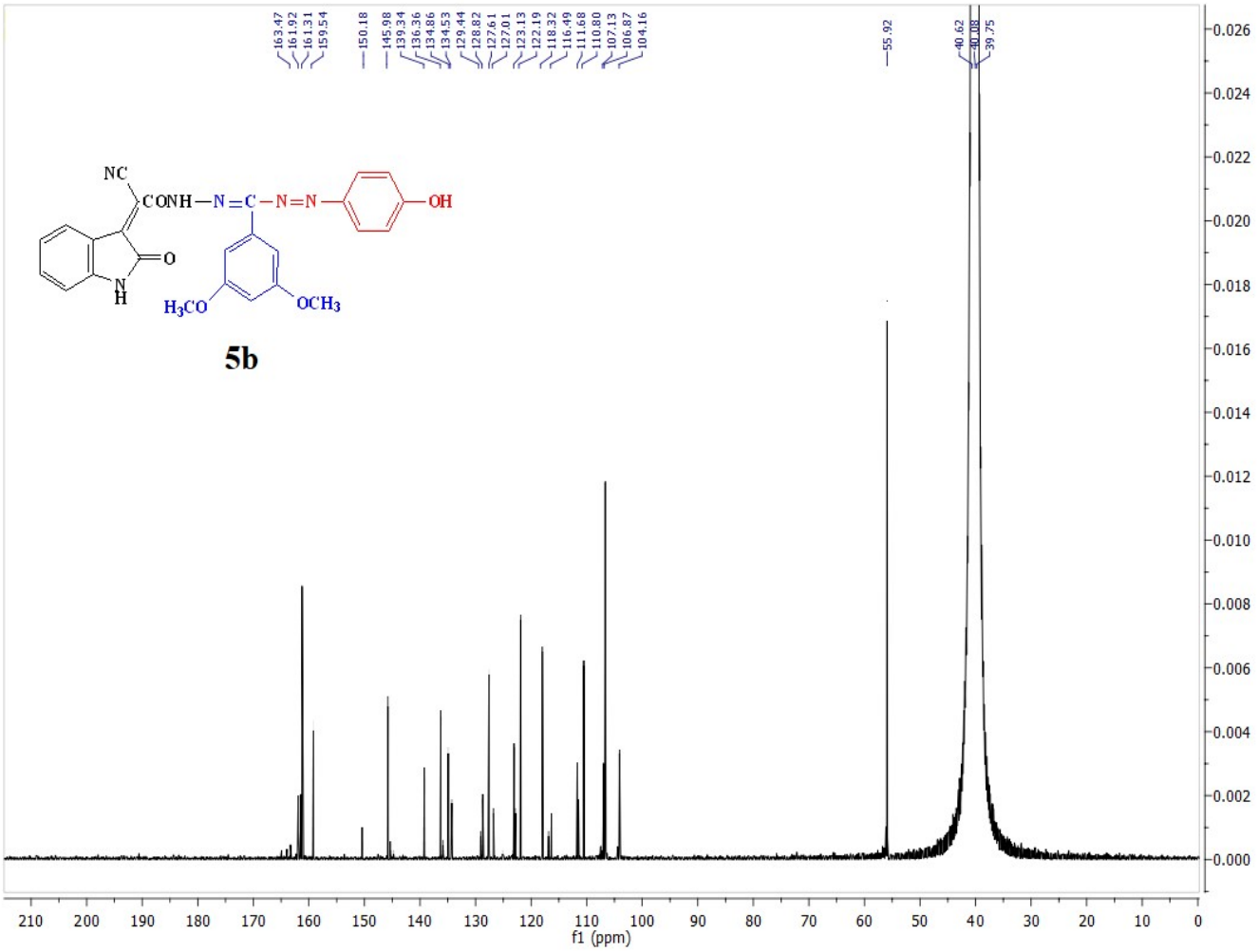

Fig.S21. ^{13}C-NMR spectra of compound **5b.**

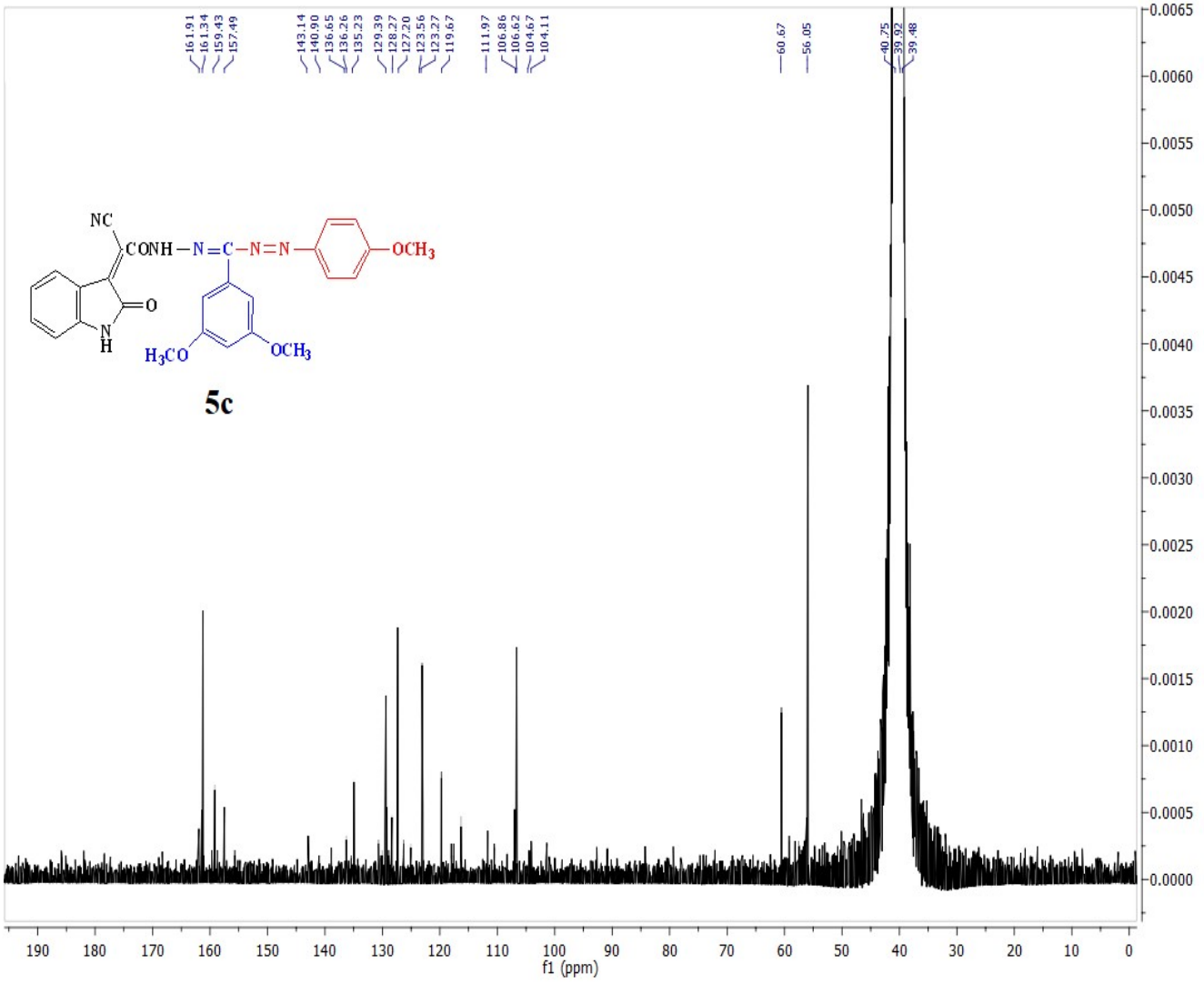

Fig.S22. ^{13}C-NMR spectra of compound **5c.**

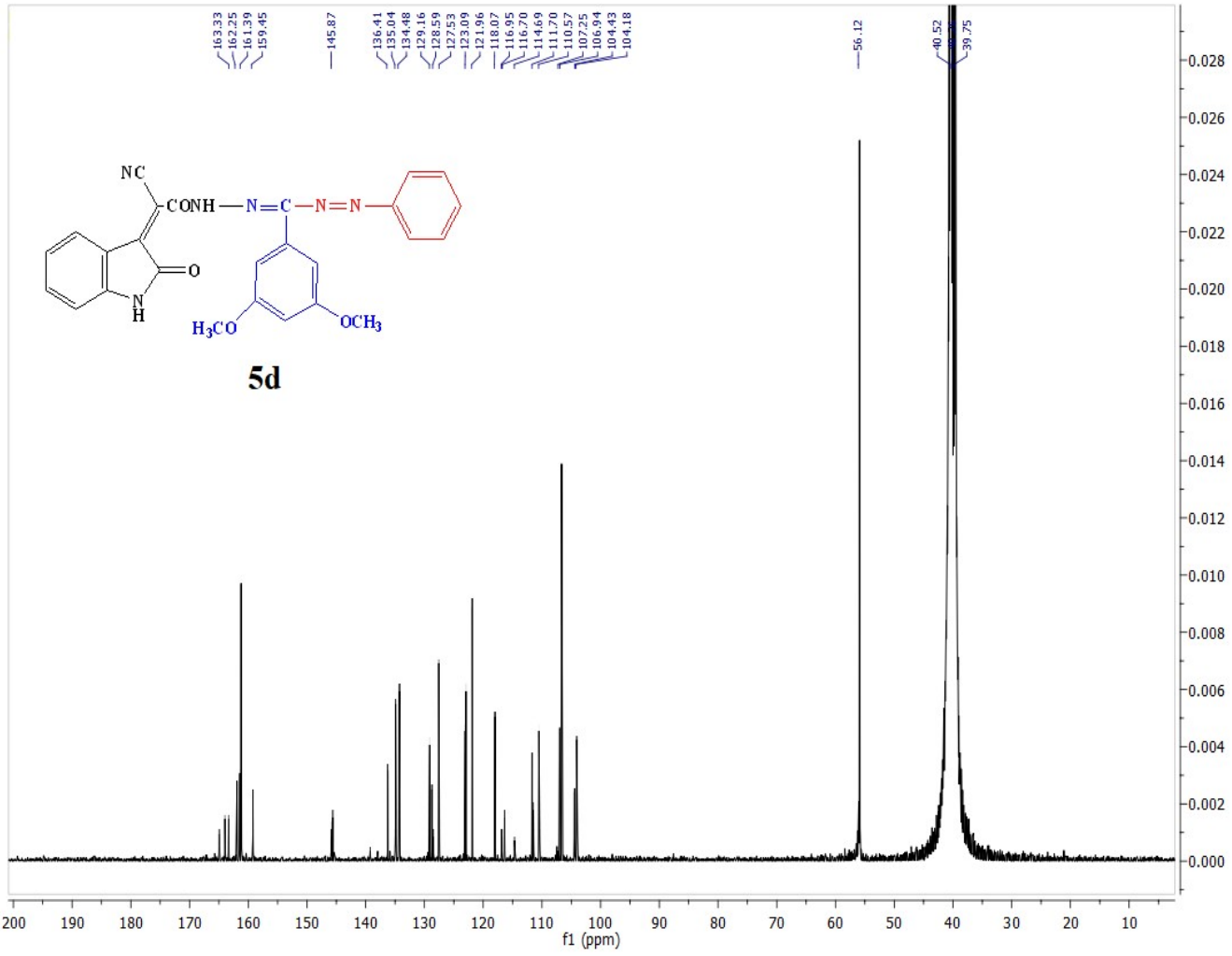

Fig.S23. ^{13}C-NMR spectra of compound **5d.**

Table S1. IC_{50} values of Isatin derivatives on Nitric Oxide inhibition and viability of Raw-macrophages:

compound	**Cytotoxicity IC_{50}(µg\ml)**	**Nitric Oxide IC_{50}(µg\ml)**
2	593.1±0.1	2.103±0.1
4a	171.4±0.06	10.83±0.06
4b	50.94±0.3	2.936±0.3
4c	214.3±0.1	19.56±0.1
4d	1578±0.05	55.27±0.05
3	3939±0.2	2.909±0.2
5a	2330±0.2	3.243±0.2
5b	15010±0.05	11.07±0.05
5c	89.76±0.15	6.924±0.2
5d	1048±0.3	1.27±0.3
Celecoxib	1043±0.3	1.12±0.1

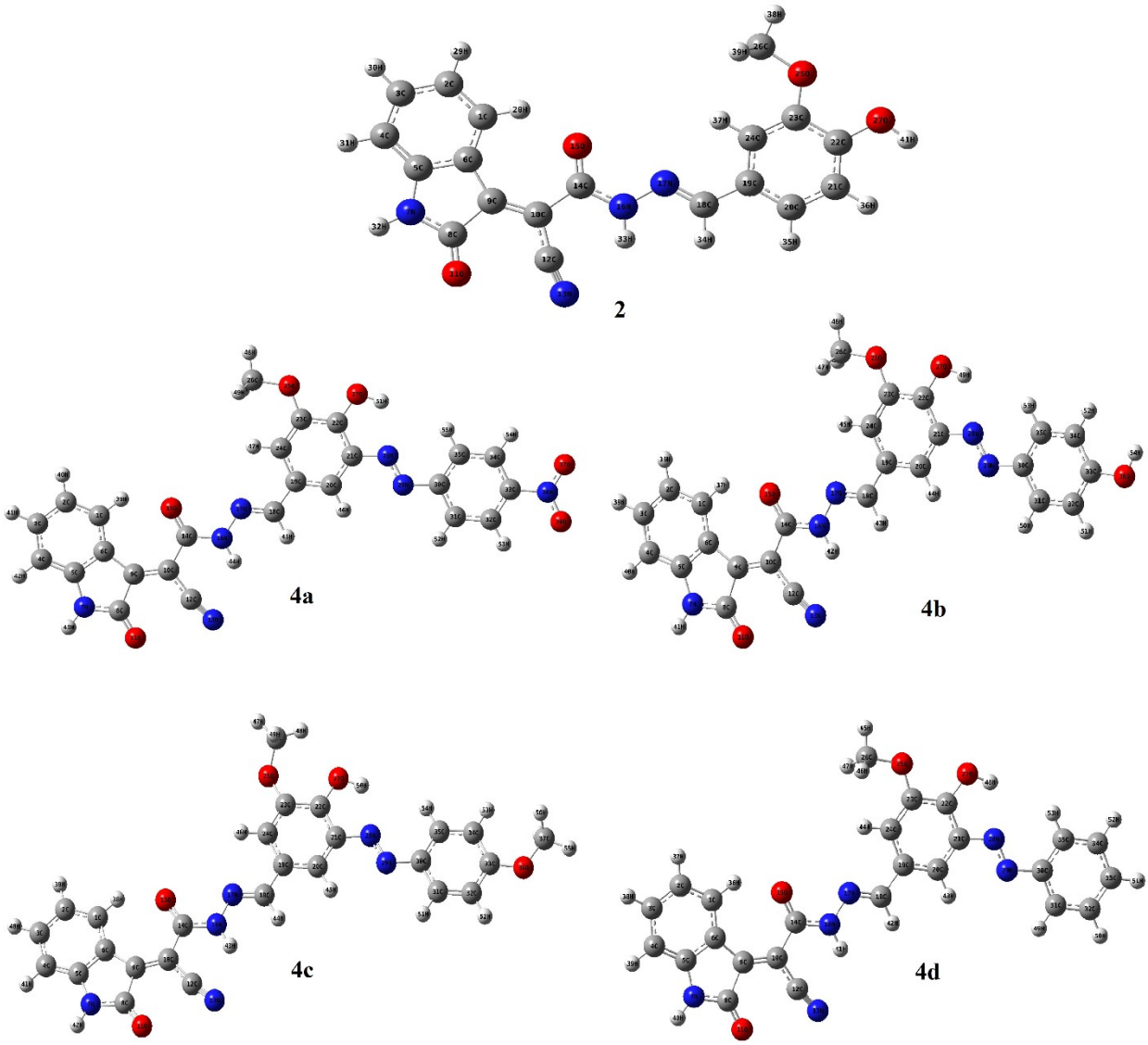

Fig.S24. Optimized isatin dye structures **2, 4a-d**.

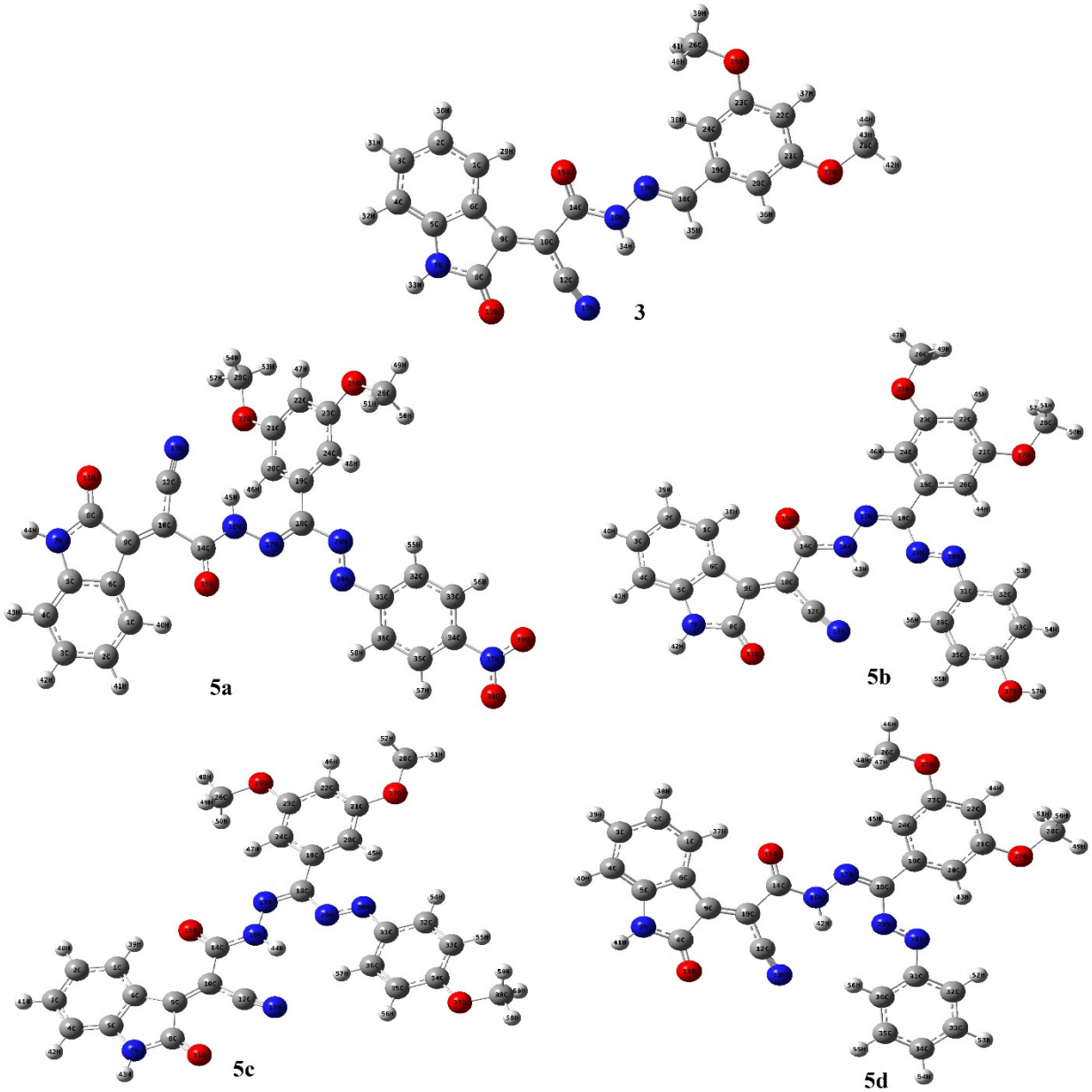

Fig.S25. Optimized isatin dye structures **3, 5a-d**.

Table S2. calculated parameters for the isatin compounds **2, 3, 4a-d,** and **5a-d** that were obtained using DFT/B3LYP/6-311G++(d, p).

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (E_{LUMO}-E_{HOMO}) (eV)	Dipole moment DM (D)	Ionization potential IP (eV)	Electron affinity EA (eV)	Hardness η (eV)	Softness σ (eV^{-1})	Chemical potential μ (eV)	Electronegati-vity χ (eV)	Electroph-ilicity ω	ΔN_{max}
2	-6.0442	-3.54973	2.494469	2.8176	6.044196	3.549727	1.247235	0.801774	-4.79696	4.796962	9.224745	3.846078
4a	-6.42026	-3.833	2.58726	9.9897	6.420258	3.832998	1.29363	0.773019	-5.12663	5.126628	10.15836	3.962979
4b	-5.98705	-3.55735	2.429706	3.1473	5.987052	3.557346	1.214853	0.823145	-4.7722	4.772199	9.373104	3.928211
4c	-6.04311	-3.50156	2.541545	4.008	6.043108	3.501563	1.270772	0.786923	-4.77234	4.772335	8.961158	3.75546
4d	-6.0812	-3.58374	2.497462	2.5668	6.081204	3.583741	1.248731	0.800813	-4.83247	4.832473	9.350608	3.869906
3	-6.29264	-3.58946	2.70318	4.6211	6.292636	3.589456	1.35159	0.739869	-4.94105	4.941046	9.03156	3.655728
5a	-6.57672	-3.87654	2.700187	5.7359	6.576723	3.876536	1.350094	0.740689	-5.22663	5.22663	10.11695	3.871309
5b	-5.8812	-3.52986	2.351337	2.7877	5.8812	3.529863	1.175669	0.85058	-4.70553	4.705531	9.416781	4.00243
5c	-5.92909	-3.57014	2.358956	0.3559	5.929092	3.570136	1.179478	0.847833	-4.74961	4.749614	9.563056	4.026877
5d	-6.01045	-3.6583	2.352153	2.7992	6.010454	3.658301	1.176077	0.850285	-4.83438	4.834377	9.936088	4.110597

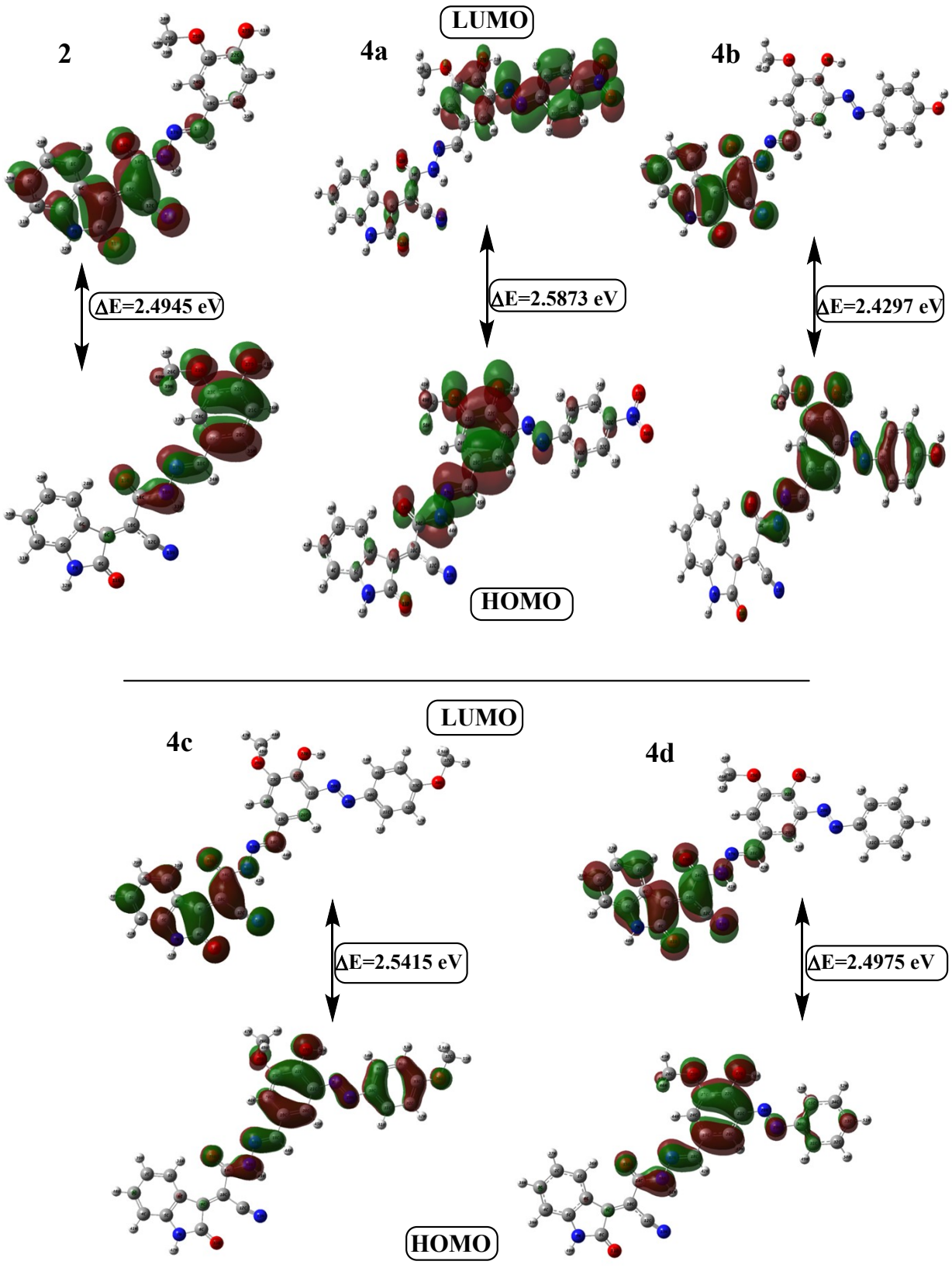

Fig. S26. The calculated HOMO and LUMO of compounds **2, 4a-d**.

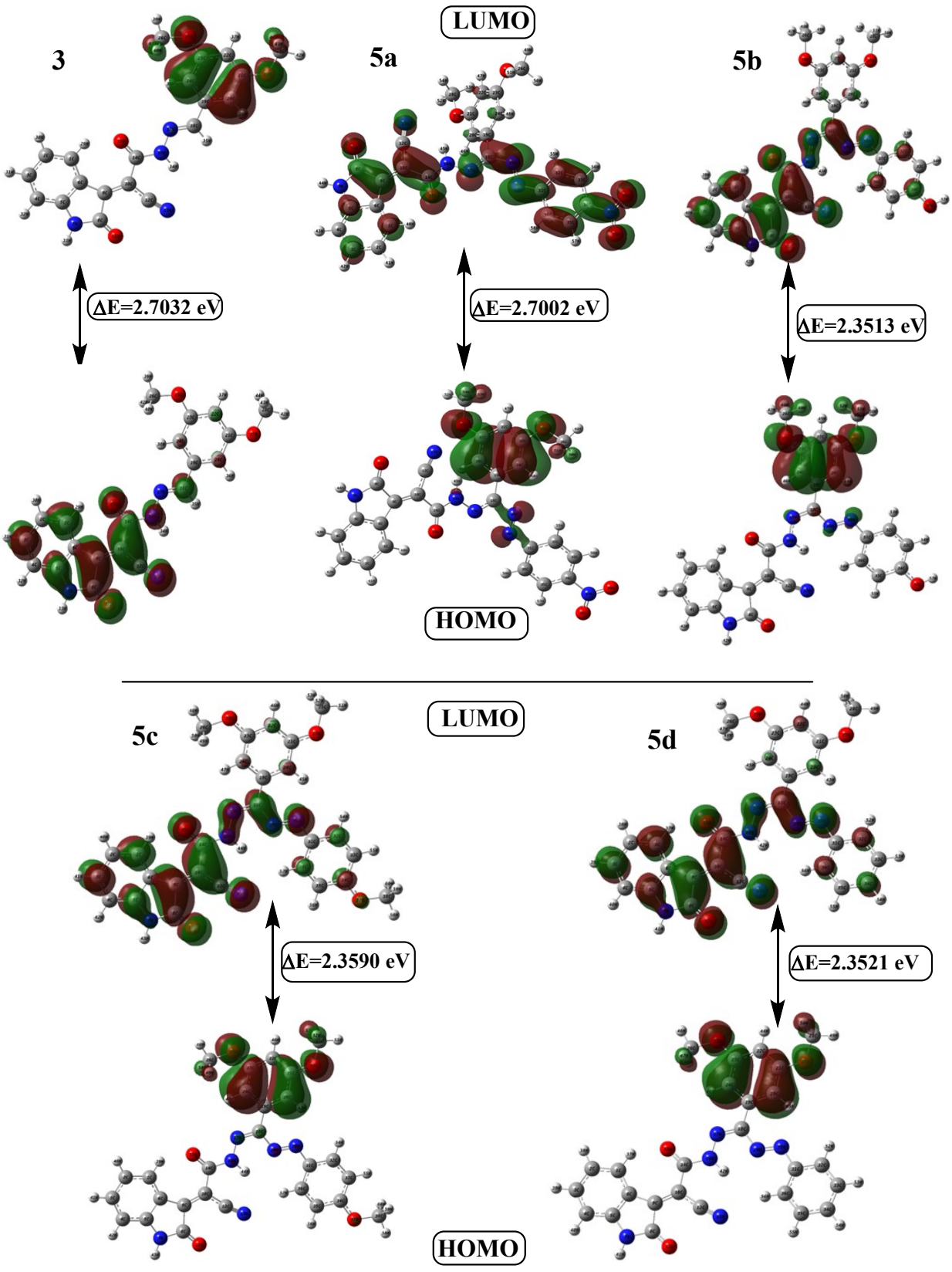

Fig. S27. The calculated HOMO, and LUMO of compounds **3, 5a-d**.

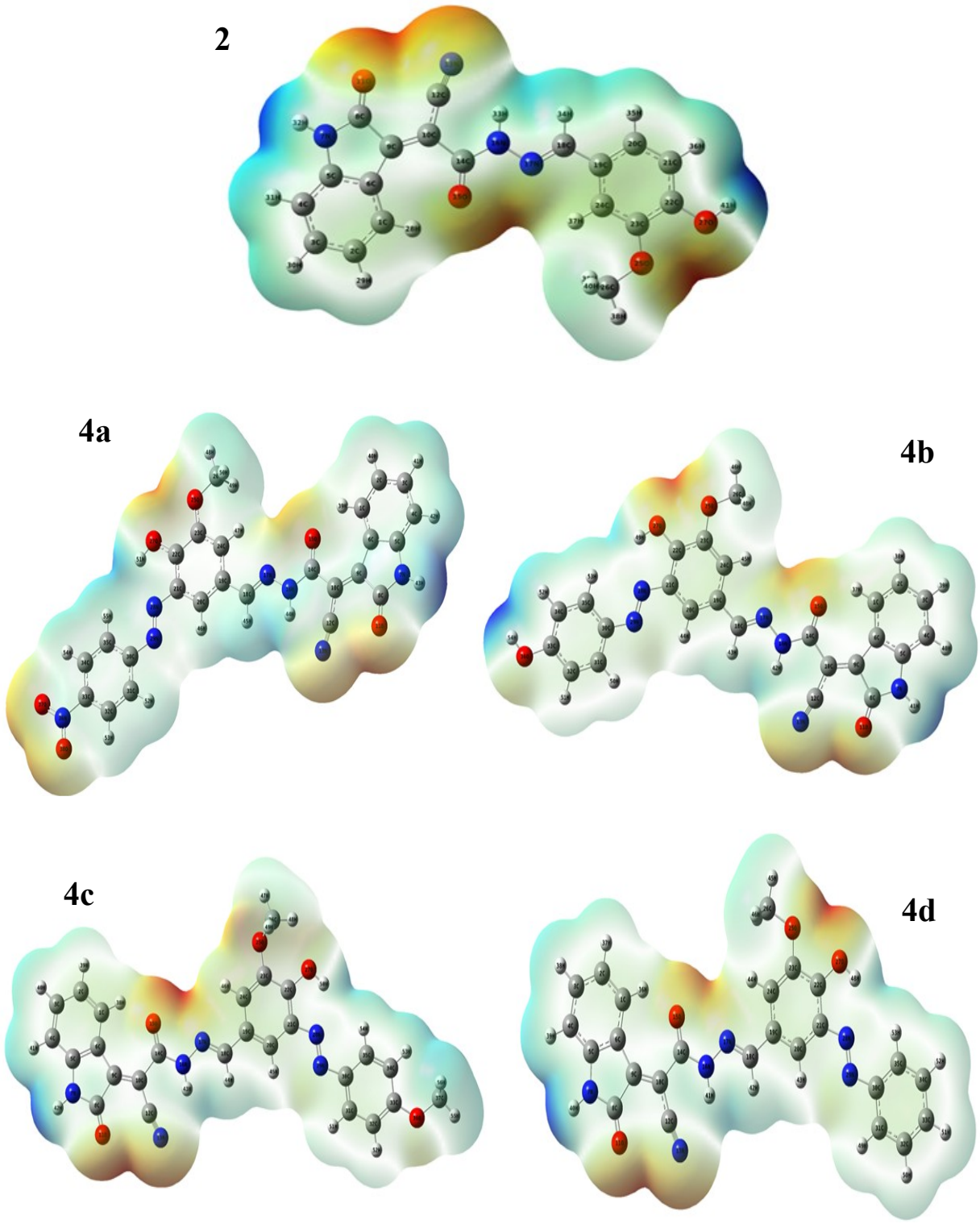

Fig. S28. MEP of Isatin compounds **2** and **4a-d**.

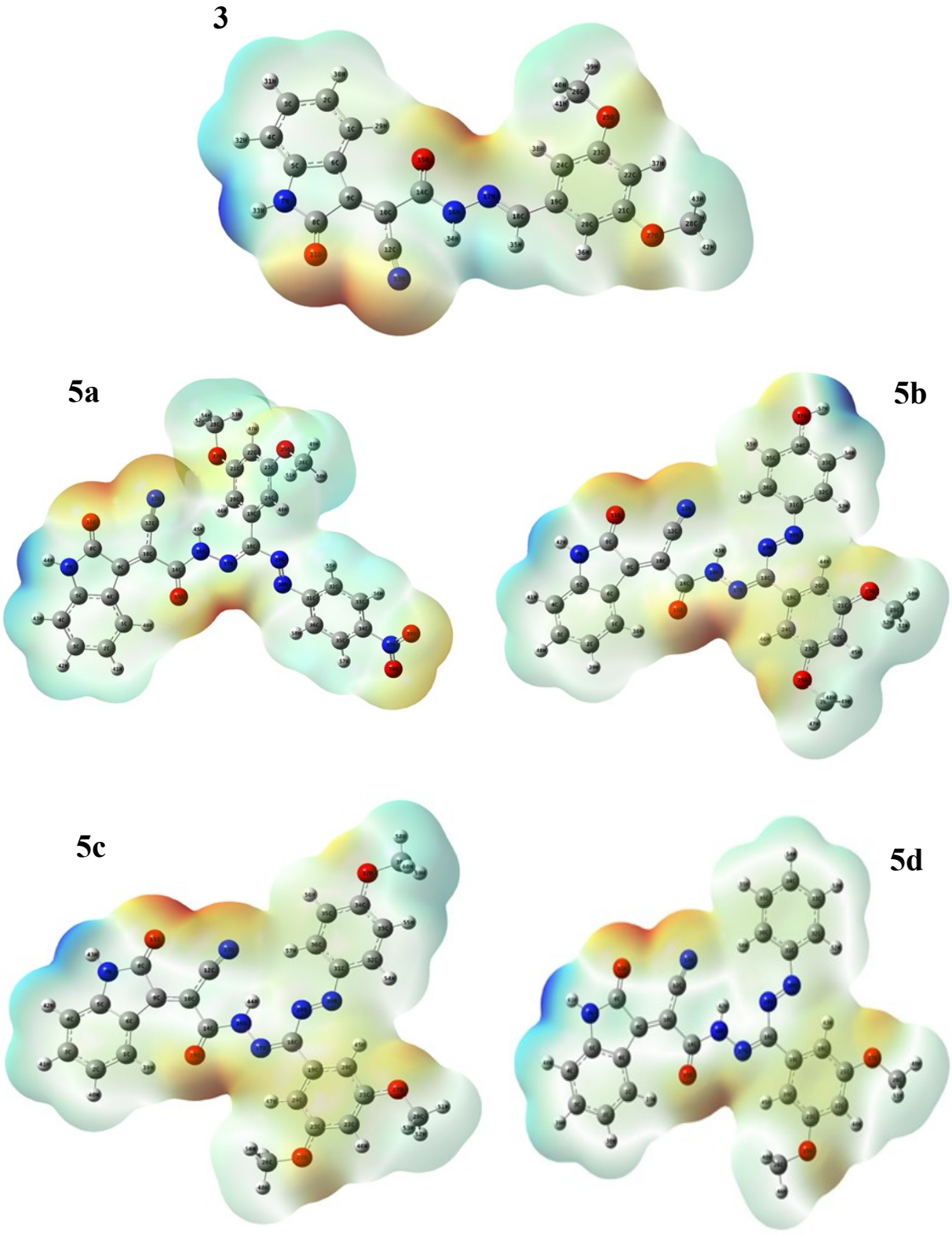

Fig. S29. MEP of Isatin compounds **3** and **5a-d**.

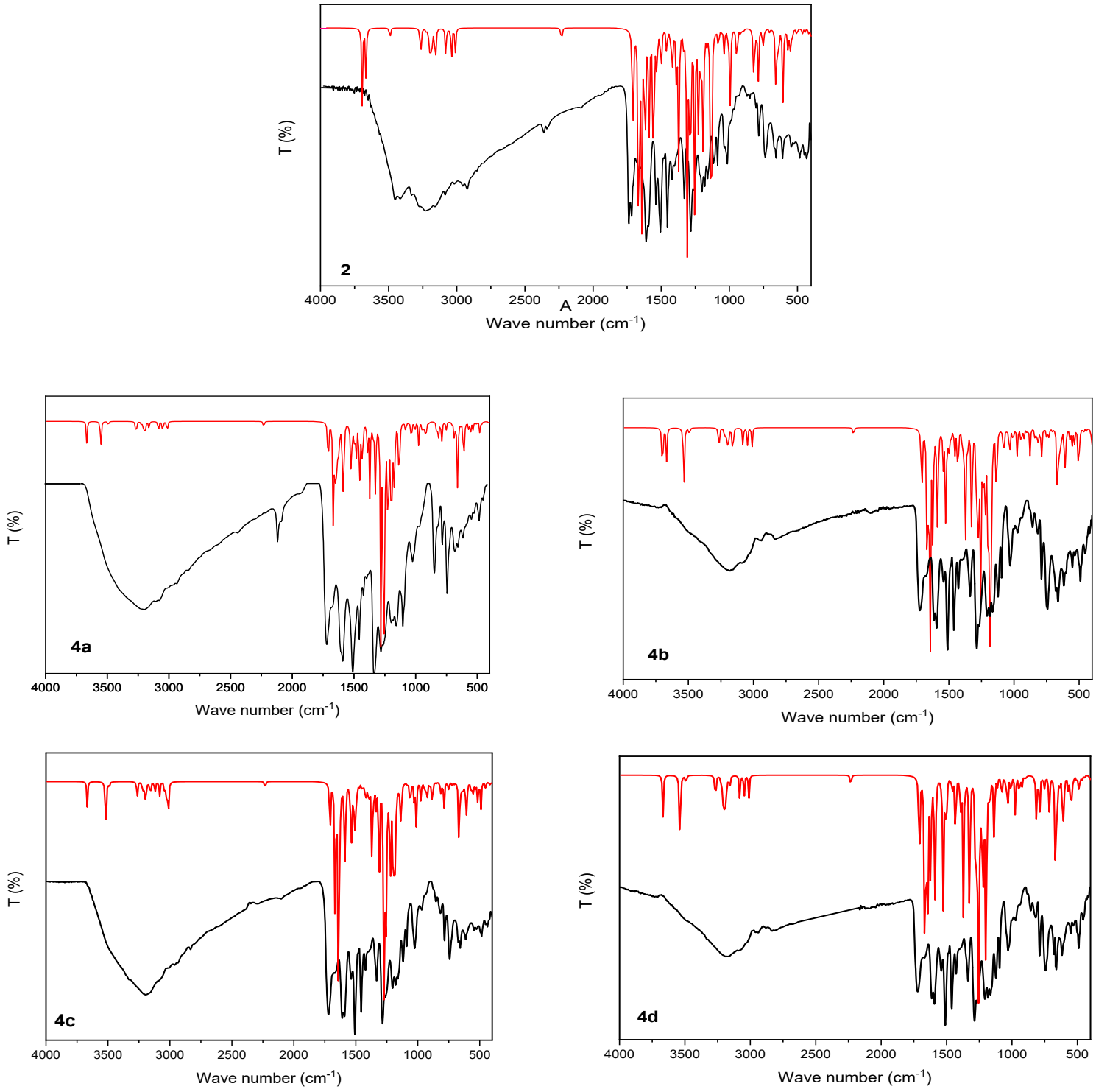

Fig. S30. Comparison of the computed (red color) and experimental (black color) FT-IR spectra of Isatin compounds **2**, and **4a-d**.

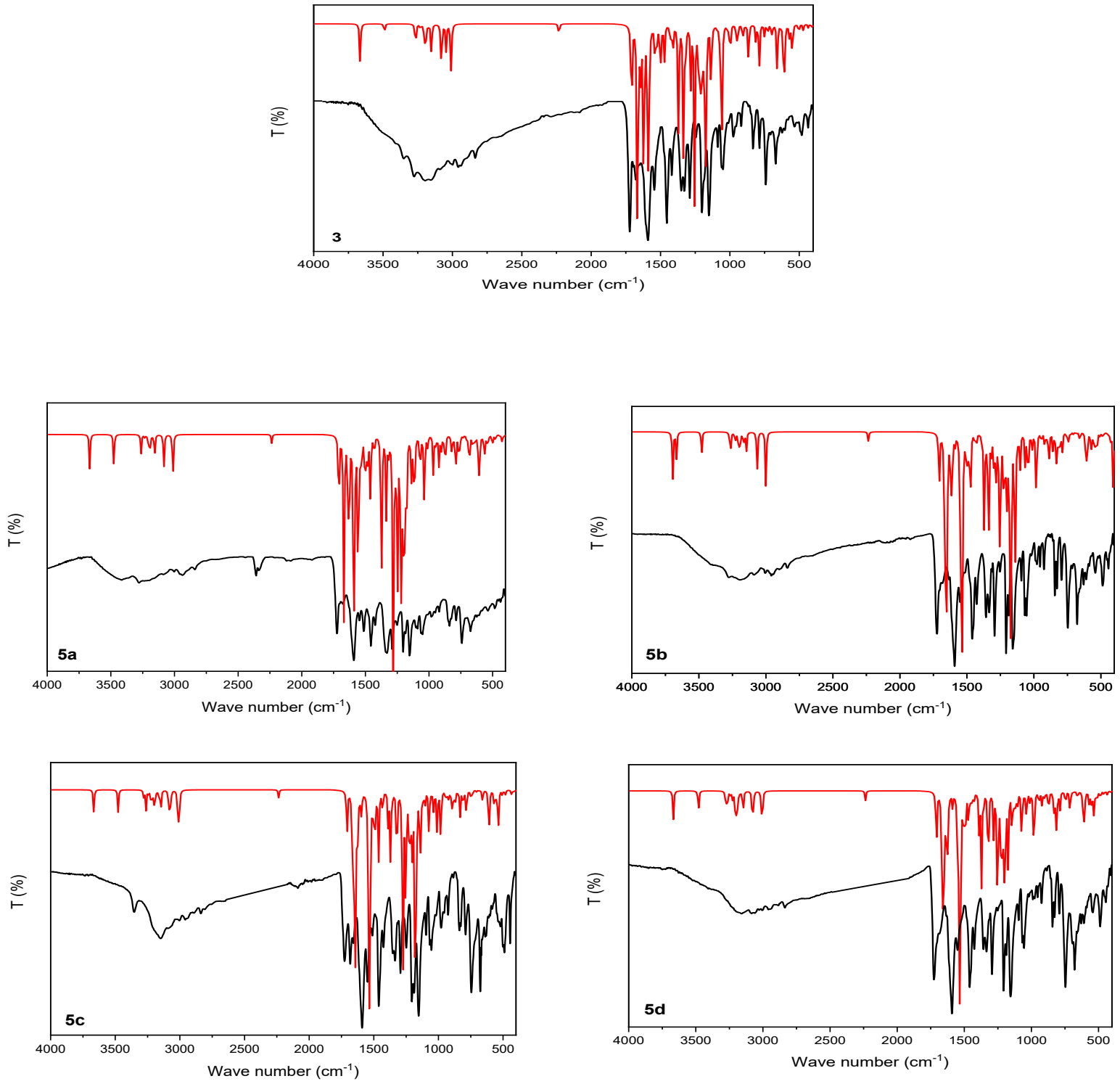

Fig. S31. Comparison of the computed (red color) and experimental (black color) FT-IR spectra of Isatin compounds **3**, and **5a-d**.

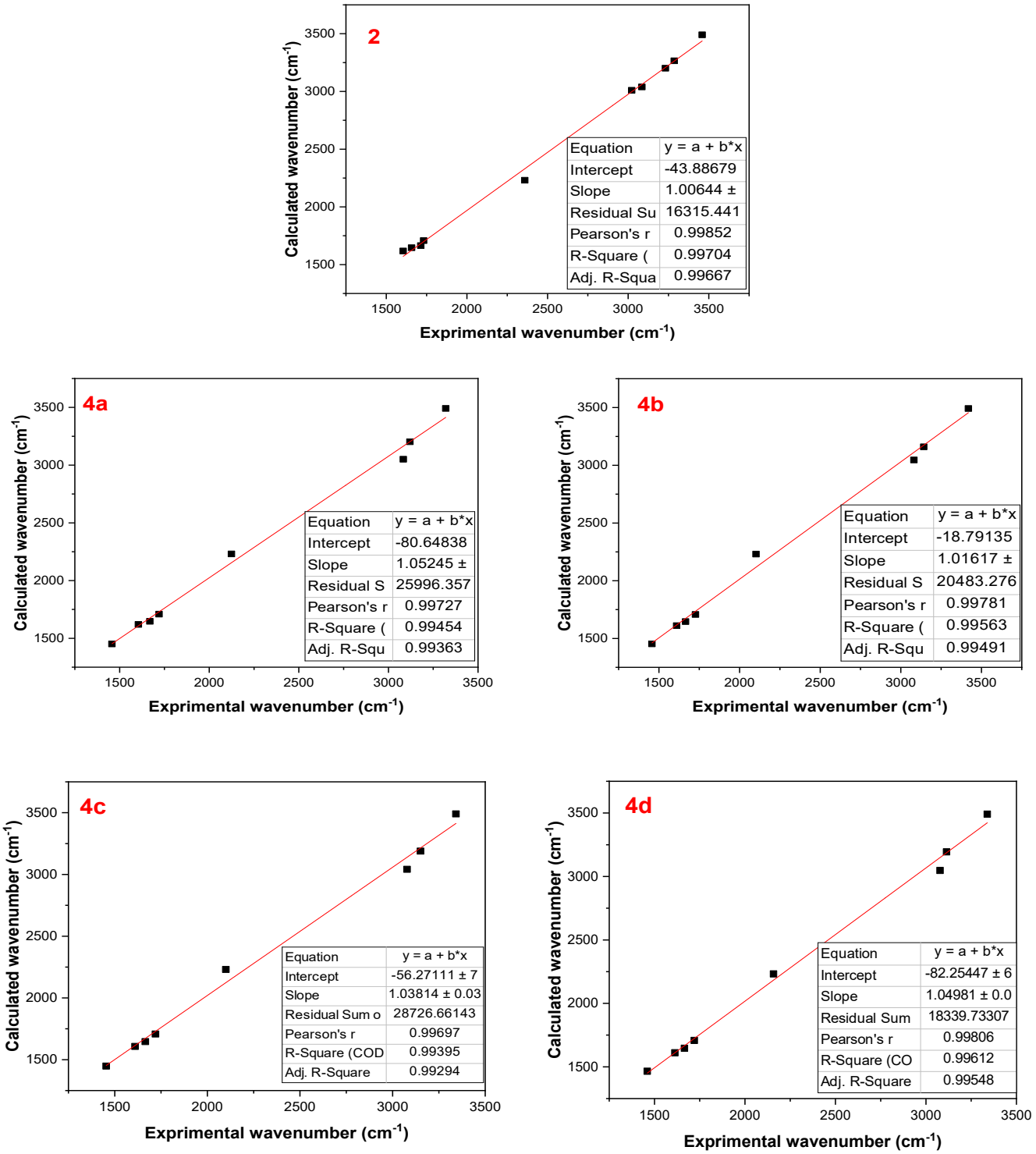

Fig. S32. Correlation between FT-IR spectra (calculated and observed) of Isatin compounds **2**, and **4a-d**.

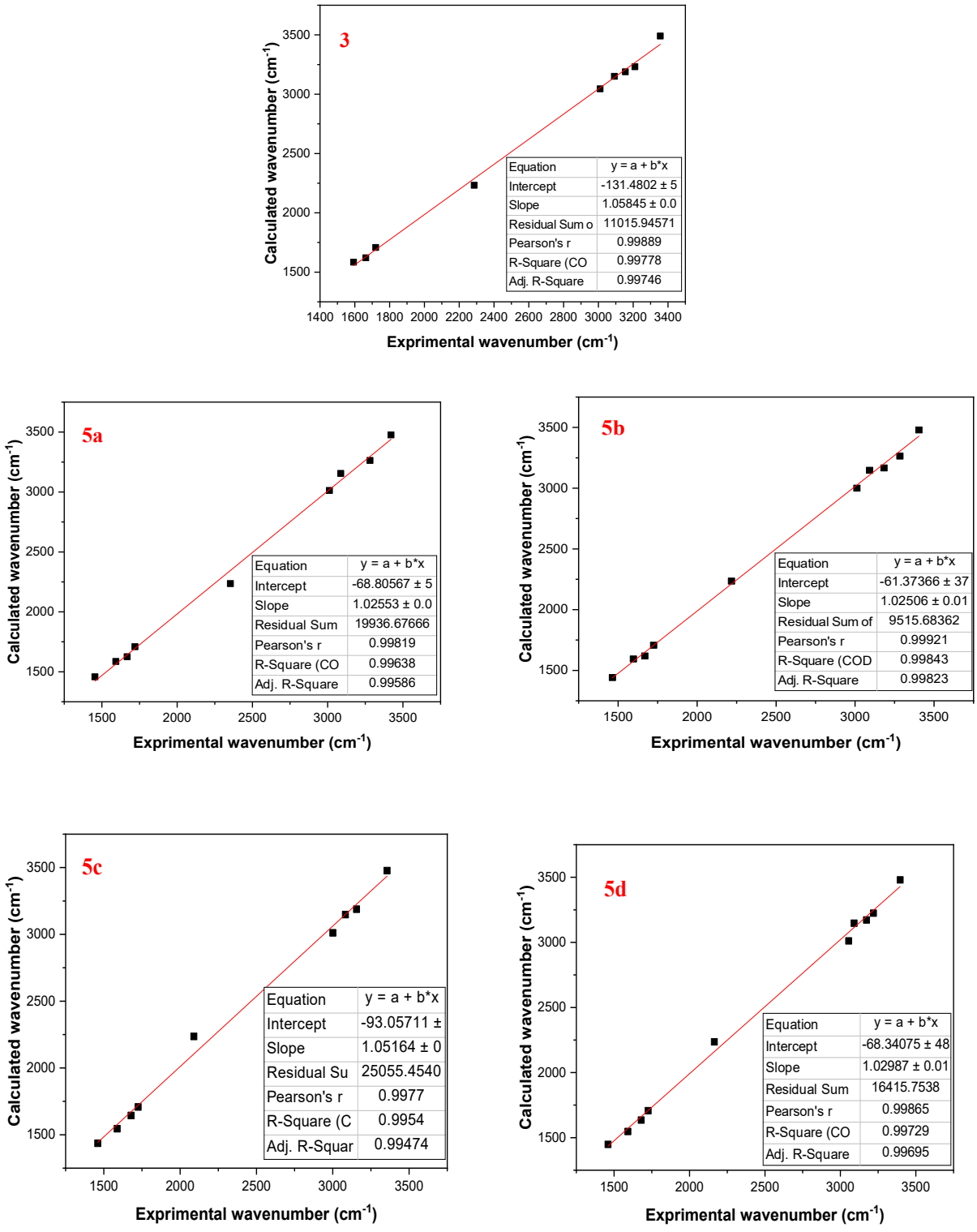

Fig. S33. Correlation between FT-IR spectra (calculated and observed) of Isatin compounds **3**, and **5a-d**.

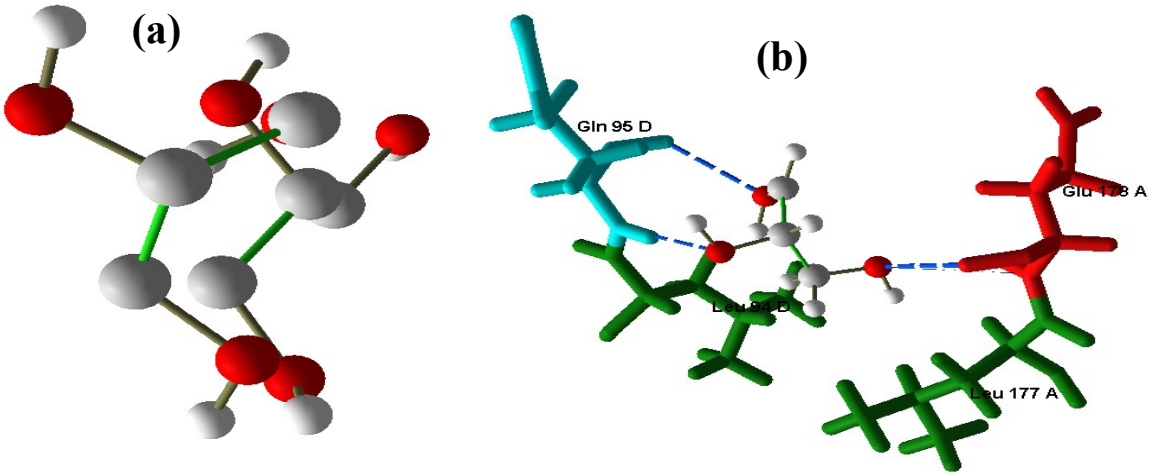

Fig. S34. (a) The co-crystallized and docked ligand structures in the TNF active site (PDB ID: 4RSU); (b) The native ligand's hydrogen-bonding contacts with important residues in the TNF binding site (PDB ID: 4RSU). A ball-and-stick depiction of the ligand is displayed. Hydrogen bonds formed with Gln95(D), Leu94(D), and Glu178(A) are indicated by blue dashed lines.

Table S3. Comparison of the experimental and calculated vibrational frequencies (cm^{-1}) of Isatin dyes **4a-d**.

Band assignment (cm^{-1})	**NH/OH Stretch**		**CH Stretch Aromatic**		**CH Stretch Aliphatic**		**C≡N Stretch**		**C=O Stretch**		**C=N Stretch**		**C=C Stretch**		**N=N Stretch**	
Compound	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**
2	3459.47	3666.12 3490.43	3286.31	3265.20 3201.52	3085.80	3155.21 3039.31	2359.49	2231.24	1732.73	1708.22	1657.20	1646.41	1605.14	1618.61	1541.34	1590.72
4a	3320.66	3552.40 3491.89	3120.15	3266.61 3237.94	3083.70	3161.05 3051.12	2124.00	2231.80	1720.81	1709.24	1668.93	1647.12	1605.84	1620.00	1457.91	1451.31
4b	3419.51	3667.17 3492.12	3142.12	3265.45 3224.82	3081.59	3156.88 3046.86	2102.90	2231.97	1727.13	1708.39	1665.43	1646.49	1610.04	1622.18	1457.91	1453.12
4c	3342.39	3517.37	3151.01	3264.54 3220.86	3078.09	3157.28 3120.81	2100.79	2231.87	1720.81	1708.25	1665.43	1646.62	1610.75	1607.63	1454.41	1448.80
4d	3339.59	3490.25	3114.57	3266.38 3218.64	3078.09	3157.38 3047.53	2158.98	2232.04	1720.81	1708.45	1666.13	1645.75	1613.55	1621.90	1460.72	1465.42

Table S4. Comparison of the experimental and calculated vibrational frequencies (cm^{-1}) of Isatin dyes **5a-d**.

Band assignment (cm^{-1})	**NH/OH Stretch**		**CH Stretch Aromatic**		**CH Stretch Aliphatic**		**C≡N Stretch**		**C=O Stretch**		**C=N Stretch**		**C=C Stretch**		**N=N Stretch**	
Compound	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**	**Exp.**	**Cal.**
3	3356.42	3665.77 3490.49	3210.59	3266.01 3202.02	3092.11	3151.63 3045.72	2286.58	2232.25	1720.82	1708.99	1663.33	1621.08	1593.92	1611.49	….	….
5a	3421.61	3665.41 3476.35	3281.40	3262.93 3202.81	3087.21	3157.60 3013.27	2354.59	2235.84	1720.82	1709.15	1668.93	1586.36	1593.92	1611.74	1454.41	1458.87
5b	3404.09	3667.59 3479.59	3284.20	3264.83 3201.74	3092.81	3147.01 3000.23	2218.06	2234.89	1725.58	1707.41	1597.54	1594.23	1669.76	1611.58	1463.74	1440.80
5c	3356.42	3665.91 3477.71	3155.91	3262.57 3201.59	3082.99	3148.12 3014.70	2089.58	2236.03	1725.02	1707.56	1588.32	1544.92	1679.45	1611.73	1460.72	1436.36
5d	3396.37	3667.50 3480.24	3219.00	3266.68 3196.41	3091.41	3147.48 3011.51	2164.70	2236.04	1725.58	1707.87	1592.61	1547.61	1680.43	1611.59	1460.46	1448.19