SUPPORTING INFORMATION

CONFORMATIONAL STABILIZATION OF ISATIN SCHIFF BASES – BIOLOGICALLY ACTIVE CHEMICAL PROBES

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TABLE OF CONTENTS

1. METHODS

XRD. Crystal structures of **I** – **VI** were determined by the means of single crystal X-ray diffraction analysis. Crystal of **I** and **IV** were fixed on a micro mounts, placed on a Rigaku Oxford Diffraction Supernova Atlas diffractometer and measured at a temperature of 100K using microfocused monochromated Cu*Kα* radiation. Crystal of **V** was fixed on a micro mount, placed on a Rigaku Oxford Diffraction Supernova Atlas diffractometer and measured at a temperature of 100K using microfocused monochromated Mo*Kα* radiation. Crystals of **II**, **III** and **VI** were fixed on a micro mounts, placed on a Rigaku Oxford Diffraction Excalibur Eos diffractometer and measured at a temperature of 100K using monochromated Mo*Kα* radiation. The unit cell parameters and refinement characteristics for the crystal structures of **I** – **VI** are given in the Table S1. Empirical absorption correction for **I** – **VI** was applied in CrysAlisPro[1] program complex using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. The structures were solved by direct methods and refined using the *SHELXL* program[2] incorporated in the *OLEX2* program package[3]. Twin refinement of the crystal structure **VI** by means of the *SHELXL* HKLF 5 instruction, which precludes merging of the data as part of the refinement process, was used. The final models included coordinates and anisotropic displacement parameters for all non-hydrogen atoms. The carbon-bound H atoms were placed in calculated positions and were included in the refinement in the 'riding' model approximation, with U_{iso}(H) set to 1.5U_{eq}(C) and C–H 0.96 Å for the CH_3 groups, U_{iso}(H) set to 1.2U_{eq}(C) and C–H 0.98 Å for the tertiary CH groups and U_{iso}(H) set to 1.2U_{eq}(C) and C–H 0.93 Å for the CH groups in cyclic fragments. Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic Data Centre (CCDC 1505866, 1505868, 1505867, 1505870, 1506944, and 1505869 for **I** – **VI**, respectively) and can be obtained *via* www.ccdc.cam.ac.uk/data_request/cif. X-ray powder diffraction spectra were recorded on a Bruker D2 Phaser diffractometer. The **FT-IR** spectra were recorded using pellets with KBr in the range 4000–400 cm^{-1} on a Shimadzu IR-Affinity-1 spectrometer. **UV-Vis spectra** were recorded in 1 cm quartz cuvette on a Shimadzu UV-1800 spectrophotometer on 1 nm resolution in the range from 190 to 1100 nm. **NMR.** All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer at 400.13 and 100.61 MHz. **MS-spectra** were recorded in a positive mode in the range of 100-1000 m/z with ESI ionization type with on a Shimadzu Maxima-Resonance spectrometer. **DFT calculations**. Were performed using Gaussian 09 software package[4] with CAM-B3LYP and cc-PVTZ basis set. The calculation of vibrational frequencies was performed to confirm that all optimized structures are in the local minima on the potential energy surface.

2. SYNTHESIS

All necessary chemicals were of analytical grade (Sigma) and were used without further purification, unless otherwise stated. 4,7-dimethyl isatin was purchased from LifeChemicals. Molecular sieves 3Å were dried under vacuum at 250°C for 20 hours. Ethanol was stored with freshly dried molecular sieves. DMF distilled over $MgSO_4$ *in vacuo*.

1-methylisatin

To a solution of 50 g isatin (340 mmol) in anhydrous DMF (500 mL) was added during 1.5 h portion-wise 60% suspension of 15 g (370 mmol) of sodium hydride in mineral oil. After 45 min exposition at 20°C to the reaction mixture 23.25 mL (370 mmol) of methyl iodide was dropwise (~ 2 h) added. The reaction was performed at 4°C during the addition of sodium hydride and methyl iodide. Reaction mixture was stirred at RT overnight, and then DMF was evaporated *in vacuo*. The residue obtained was worked up with water (200 ml), formed precipitate was filtered off and recrystallized from ethanol to give 37 g (230 mmol) of red needle-like crystals. Yield 67%. M.p. 132-134 °C. ^{1}H NMR, δ, ppm: 7.60-7.54 (m, 2H), 7.12-7.08 (m, 1H), 6.88 (d, 1H), 3.22 (s, 3H).

1,4,7-trimethylisatin was prepared by the analogy with 1-methylisatin. M.p. 169-172 °C. ^{1}H NMR, δ, ppm: 7.17-7.15 (d, 1H), 6.77-6.75 (d, 1H), 3.49 (s, 3H), 2.51 (s, 6H).

1-methyl-3(phenylimino)indolinone-2-one (I)

In a round bottom flask equipped with Dean-Stark receiver the solution of 3.0 g (18.5 mmol) N-methyl-isatin, 2.2 mL (25 mmol) aniline and catalytic amount of PTSA in 60 mL of toluene was refluxed for 6 h. The reaction mixture was cooled, washed with water, dried over $MgSO_4$ and solvent evaporated in vacuum. The product was eluted on silica gel with chloroform and chloroform-methanol 9/1. The eluent was evaporated. The precipitate was crystallized first time from ethanol. Yield ~80 %. Prismatic orange crystals suitable for the X-ray diffraction analysis were obtained after crystallization from ethanol at 4 °C. M.p. 148-149 °C. Elemental analysis: calculated for $C_{15}H_{12}N_2O$, C 76.25, H 5.12, N 11.86; estimated C 76.23, H 4.64, N 11.72. IR (KBr) ν, cm^{-1}: 1734 ($\nu_{C=O}$), 1653 ($\nu_{C=N}$), 1605, 1587, 1483, 1468, 1421, 1371, 1333, 1258, 1227, 1159, 1121, 1098, 1072, 1055, 1024, 980, 918, 880, 862, 783, 752, 721, 698, 590, 534, 515, 465. ^{1}H NMR, δ, ppm: 7.44-7.40 (t, 2H), 7.37-7.34 (t, 1H), 7.26-7.22 (t, 1H), 7.01-6.99 (d, 2H) 6.86-6.84 (d, 1H), 6.67-6.63 (t, 1H) 6.62-6.60 (d, 1H), 3.30 (s, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 163.2, 154.4, 150.3, 148.0, 134.2, 133.9, 129.4, 128.5, 126.1, 125.3, 125.1, 123.2, 123.0, 122.7, 118.9, 117.8, 115.6, 110.0, 109.3, 108.7, 26.3, 25.8. MS (ESI), m/z: 237.102, 238.106, 239.109, 417.207, 495.180.

1,4,6-trimethyl-3(phenylimino)indolinone-2-one (II)

In the round bottom flask equipped with Soxlett adaptor filled with 3Å Molecular Sieves the mixture composed of 1,4,7-trimethylindoline-2,3-dione 0.5 g (2.6 mmol), aniline 0.28 g (2.9 mmol) and catalytic amount of PTSA in 40 mL of ethanol was refluxed for 3 h. The reaction mixture was cooled, solvent was evaporated. The residue was subjected to column chromatography using DCM as eluent. Finally, 0.25 g (35%) of title compound was isolated as orange solid. M.p. 140-143°C. Elemental analysis: calculated for $C_{17}H_{16}N_2O$, C 77.25, H 6.10, N 10.60; estimated 77.04, H 6.04, N 10.30. IR (KBr) ν, cm^{-1}: 1715, 1700, 1696, 1689, 1685, 1593, 1576, 1558. ^{1}H NMR, δ, ppm: 7.40-7.36 (t, 2H), 7.18-7.16 (m, 1H), 7.08-7.06 (d, 1H), 6.96-6.94 (m, 2H), 6.83-6.81 (d, 1H), 3.41 (s, 3H), 2.61 (s, 3H), 2.53 (s, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 18.9, 19.3, 29.2, 117.7, 118.1, 119.0, 124.4, 126.0, 128.6, 136.9, 144.5, 150.5, 153.4, 158.1. MS (ESI), m/z: $[M+Na]^+$ 212.070.

1,4,6-trimethyl-3(2,6-diisopropyl-phenylimino)indolinone-2-one (III)

In the round bottom flask equipped with Dean-Stark receiver the solution of 3.0 g (18.5 mmol) N-methyl-isatin, 2.2 mL (25 mmol) of 2,6-diisopropylaniline and catalytic amount of PTSA in 60 mL of ethanol was refluxed for 6 h. The reaction mixture was cooled, washed with water, dried over $MgSO_4$ and solvent evaporated in vacuum. The product was eluted on silica gel with chloroform and chloroform-methanol 9/1. The eluent was evaporated. The precipitate was crystallized first time from ethanol at 4 °C. Yield ~80 %. M.p. 205-207 °C. Elemental analysis: calculated for $C_{23}H_{28}N_2O$, C 79.27, H 8.10, N 8.04; estimated C 79.33, H 8.08, N 8.24. IR (KBr) ν, cm^{-1}: 1734 ($\nu_{C=O}$), 1653 ($\nu_{C=N}$), 1605, 1587, 1483, 1468, 1421, 1371, 1333, 1258, 1227, 1159, 1121, 1098, 1072, 1055, 1024, 980, 918, 880, 862, 783, 752, 721, 698, 590, 534, 515, 465. ^{1}H NMR, δ, ppm: 7.18-7.16 (m, 2H), 7.12-7.08 (m, 2H), 6.88-6.86 (d, 1H), 3.41 (s, 3H), 2.73 (sep, 2H), 2.68 (s, 3H), 2.56 (s, 3H), 1.17 (d, 6H), 1.12 (d, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 157.5, 154.5, 147.1, 145.0, 136.9, 136.6, 133.5, 125.9, 123.7, 122.8, 118.5, 117.8, 29.3, 28.5, 23.1, 19.4, 18.9. MS (ESI), m/z: $[M+Na]^+$ 371.211.

[Cd(L)]Br_2 (V)

First, 100 mg (0.42 mmol) of **I** was dissolved in 5 mL of ethanol. Then gradually during 60 min 1.5 mL of ethanoic solution containing 58 mg (0.21 mmol) of $CdBr_2$ was added to **I**. After the final aliquot addition, the resulting solution was filtered and left overnight at ambient temperature to form needle-like crystals. Yield 66 %. Decomposition above 250 °C. Elemental analysis: calculated for $C_{15}H_{12}Br_2CdN_2O$, C 35.43, H 2.38, N 5.51; estimated C 36.16, H 2.40, N 5.44. IR (KBr) ν, cm^{-1}: 1707, 1607, 1485, 1469, 1448, 1382, 1339, 1128, 1109, 890, 768, 698. MS (ESI), m/z: $[Cd(L)Br]^+$ 428.92, $[Cd(L)_2Br]^+$ 665.01.

[Hg(L)]Br_2 (VI)

Compound **VI** was prepared by the analogy with **V** using $HgBr_2$ as a metal source. Yield 60 %. M.p. 178−181 °C. Elemental analysis: calculated for $C_{15}H_{12}Br_2HgN_2O$, C 30.19, H 2.03, N 4.70; estimated C 30.38, H 1.75, N 4.60. IR (KBr) ν, cm^{-1}: 1708, 1655, 1607, 1485, 1469, 1448, 1423, 1377, 1337, 1227, 1068, 1024, 1003, 885, 756, 792, 781. ^{1}H NMR ($CDCl_3$), δ, ppm: 3.35 (s, 3H), 6.74-6.76 (d, 1H), 6.83-6.87 (t, 1H), 6.92-6.94 (d, 1H), 7.18-7.20 (d, 2H), 7.35-7.39 (t, 2H), 7.44-7.52 (m, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 27.1, 110.5, 115.5, 119.1, 124.0, 127.0, 127.4, 130.1, 135.9, 146.8, 148.4, 154.0, 164.0. MS (ESI), m/z: $[Hg(L)Br]^+$ 516.98, $[Hg(L)_2Br]^+$ 753.08, $[Hg(L)Br]^+$ 516.98.

3. X-RAY

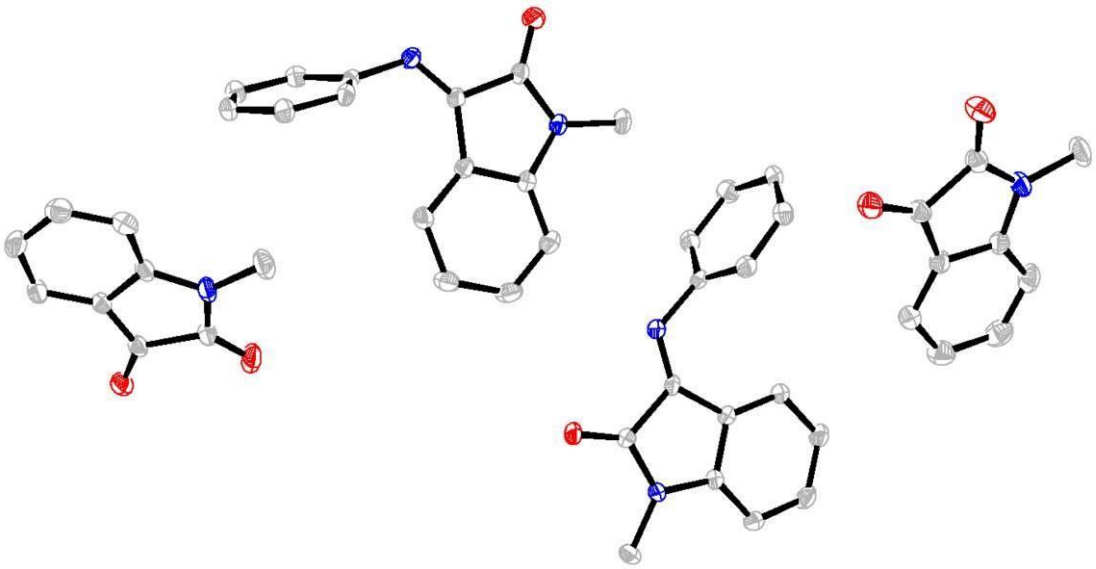

Figure S1 ORTEP representation of mixed crystal structure (**IV)**

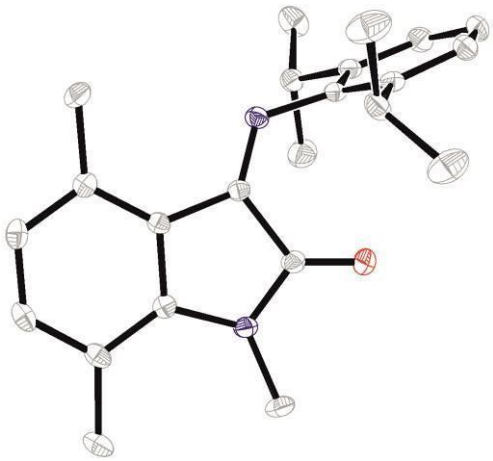

Figure S2 ORTEP representation of X-ray resolved compound **III** molecular structure

Table S1 Crystallographic and structure refinement data

Compound	**I**	**II**	**III**	**IV**	**V**	**VI**
CCDC #	1505866	1505868	1505867	1505870	1506944	1505869
Formula	$C_{15}H_{12}N_2O$	$C_{17}H_{16}N_2O$	$C_{23}H_{28}N_2O$	$C_{15}H_{12}N_2O{\cdot}C_9H_7NO_2$	$C_{15}H_{12}Br_2CdN_2O$	$C_{30}H_{24}Br_4Hg_2N_4O_2$
Crystal System	Monoclinic	Monoclinic	Monoclinic	Triclinic	Triclinic	Monoclinic
a (Å)	9.4554(3)	10.2483(3)	8.5182(6)	7.8377 (2)	7.2618(3)	23.6723(11)
b (Å)	11.8767(3)	8.1749(2)	29.4795(14)	8.33995(19)	11.2036(10)	19.9737(5)
c (Å)	10.4401(2)	16.2529(6)	8.4782(5)	31.7116(6)	20.2141(11)	14.0418(4)
α (°)	90	90	90	85.8492(17)	93.876(6)	90
β (°)	91.739(2)	102.182(3)	115.150(8)	85.7112(18)	98.487(4)	101.024(3)
γ (°)	90	90	90	69.989(2)	107.037(6)	90
V (Å^3)	1171.88(5)	1330.99(8)	1927.1(2)	1939.83(8)	1544.46(18)	6516.8(4)
Molecular weight	236.27	264.32	348.47	397.42	508.49	1193.35
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$	P-1	P-1	$P2_1/c$
μ (mm^{-1})	0.685	0.083	0.073	0.743	6.587	14.351
Temperature (K)	100(2)	100(2)	100(2)	100	100	100
Z	4	4	4	4	4	8
D_{calc} (g/cm^3)	1.339	1.319	1.201	1.361	2.187	2.433
Crystal size (mm^3)	0.35×0.28×0.21	0.18×0.10×0.06	0.19×0.12×0.04	0.19×0.14×0.09	0.35×0.21×0.05	0.21×0.13×0.06
Radiation	CuKα	MoKα	MoKα	CuKα	MoKα	MoKα
Total reflections	5796	11162	15518	46094	11894	35683
Unique reflections	2277	3053	4310	7703	11894	13989
Angle range 2θ (°)	9.36-145.00	5.60-55.00	5.28-55.00	5.60-145.00	5.96-50.00	11.14-54.00
Reflections with $\|F_o\| \geq 4\sigma_F$	2018	2689	3571	7226	9405	9725
R_{int}	0.0196	0.0266	0.0284	0.0546	merged	0.0713
R_σ	0.0194	0.0248	0.0307	0.0281	0.0507	0.1113
R_1 ($\|F_o\| \geq 4\sigma_F$)	0.0370	0.0377	0.0470	0.0608	0.0685	0.0676
wR_2 ($\|F_o\| \geq 4\sigma_F$)	0.0929	0.0924	0.0994	0.1443	0.1938	0.1179
R_1 (all data)	0.0421	0.0434	0.0598	0.0637	0.0824	0.1071
wR_2 (all data)	0.0970	0.0965	0.1053	0.1456	0.2022	0.1325
S	1.051	1.037	1.070	1.203	1.039	1.127
ρ_{min}, ρ_{max}, e/Å^3	–0.162, 0.251	–0.218, 0.305	–0.204, 0.299	–0.273, 0.292	–1.914, 3.394	–1.708, 2.940

$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$; $w = 1/[\sigma^2(F_o^2)+(aP)^2+bP]$, where $P = (F_o^2+2F_c^2)/3$; $s = \{\Sigma[w(F_o^2 - F_c^2)]/(n-p)\}^{1/2}$ where n is the number of reflections and p is the number of refinement parameters.

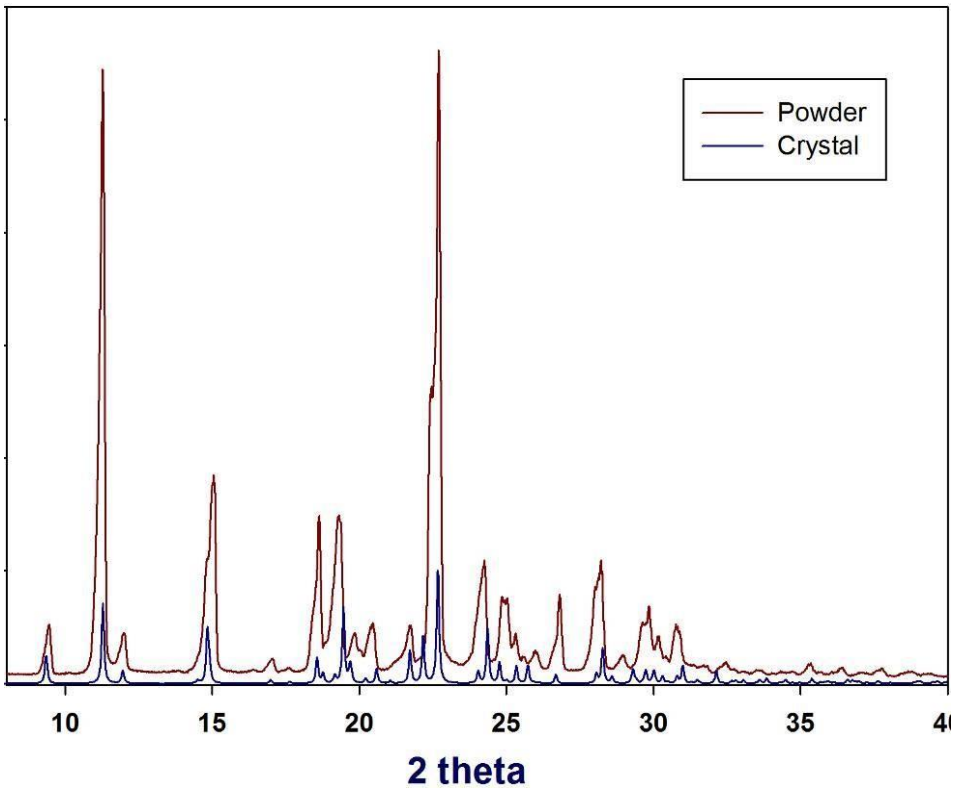

Figure S3 Superimposed X-ray diffraction patterns for crystalline and powder compound **I**

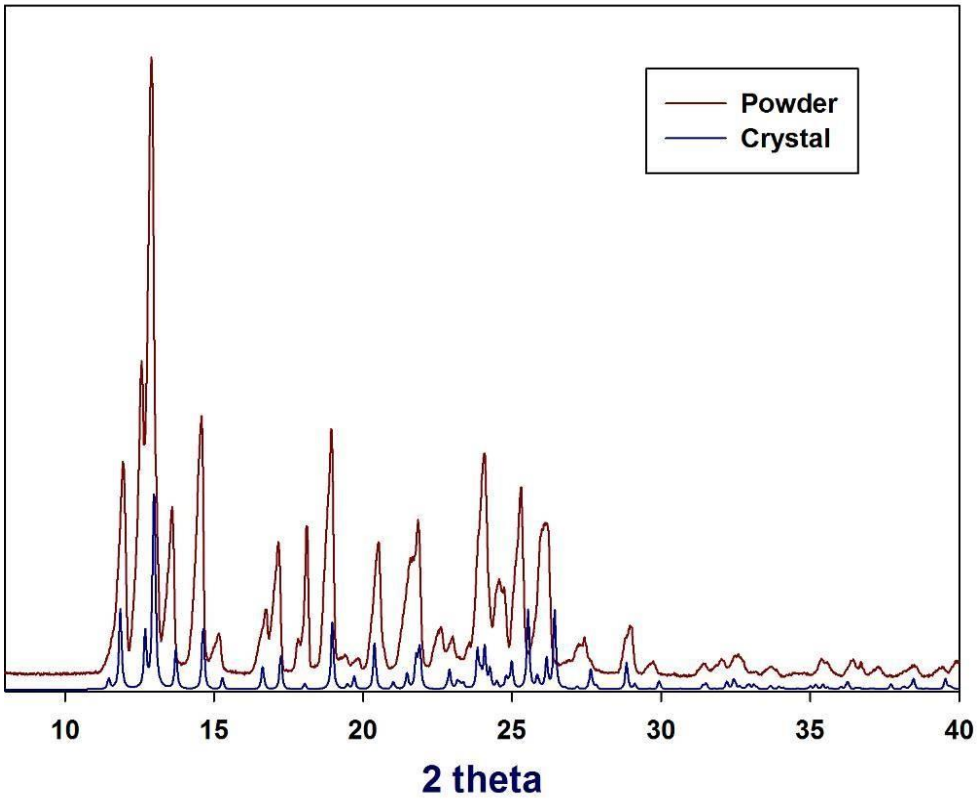

Figure S4 Superimposed X-ray diffraction patterns for crystalline and powder compound **III**

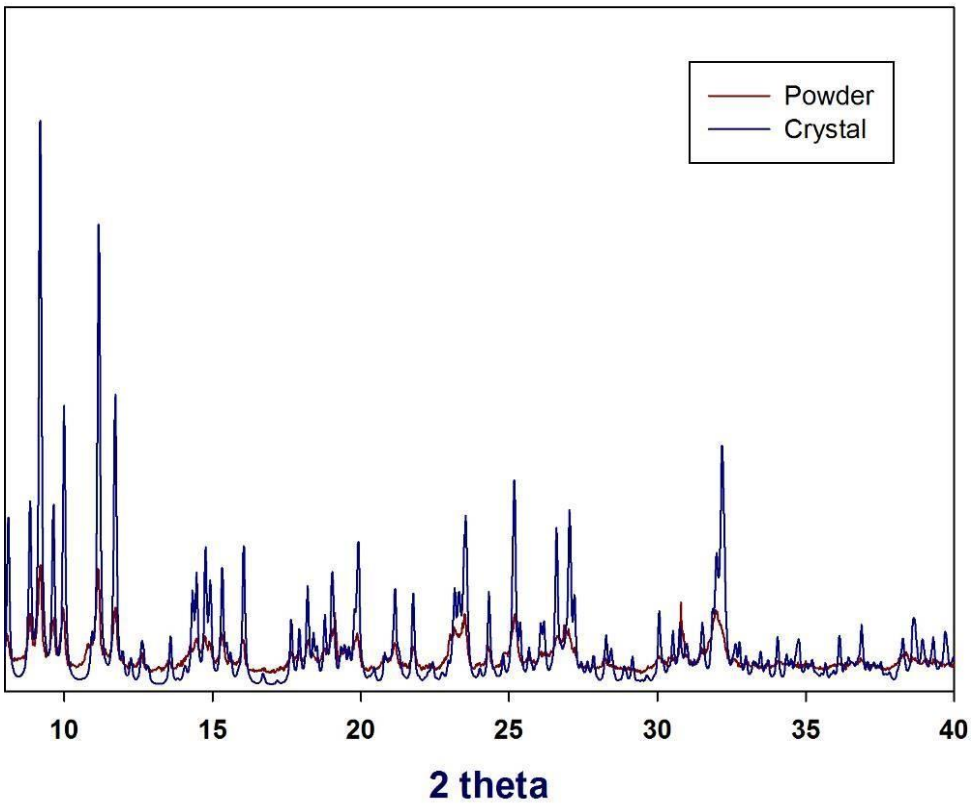

Figure S5 Superimposed X-ray diffraction patterns for crystalline and powder compound **VI**

4. NMR SPECTRA

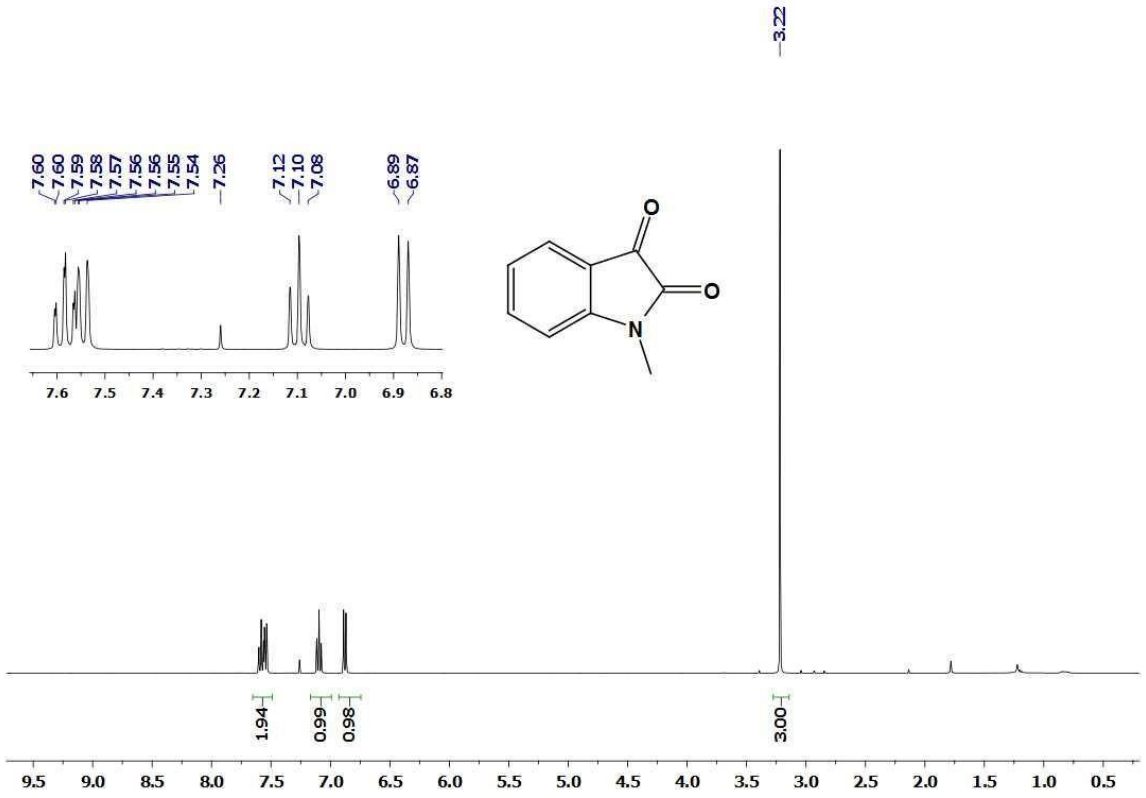

Figure S6 1-methyl-isatin ^{1}H NMR spectrum in $CDCl_3$

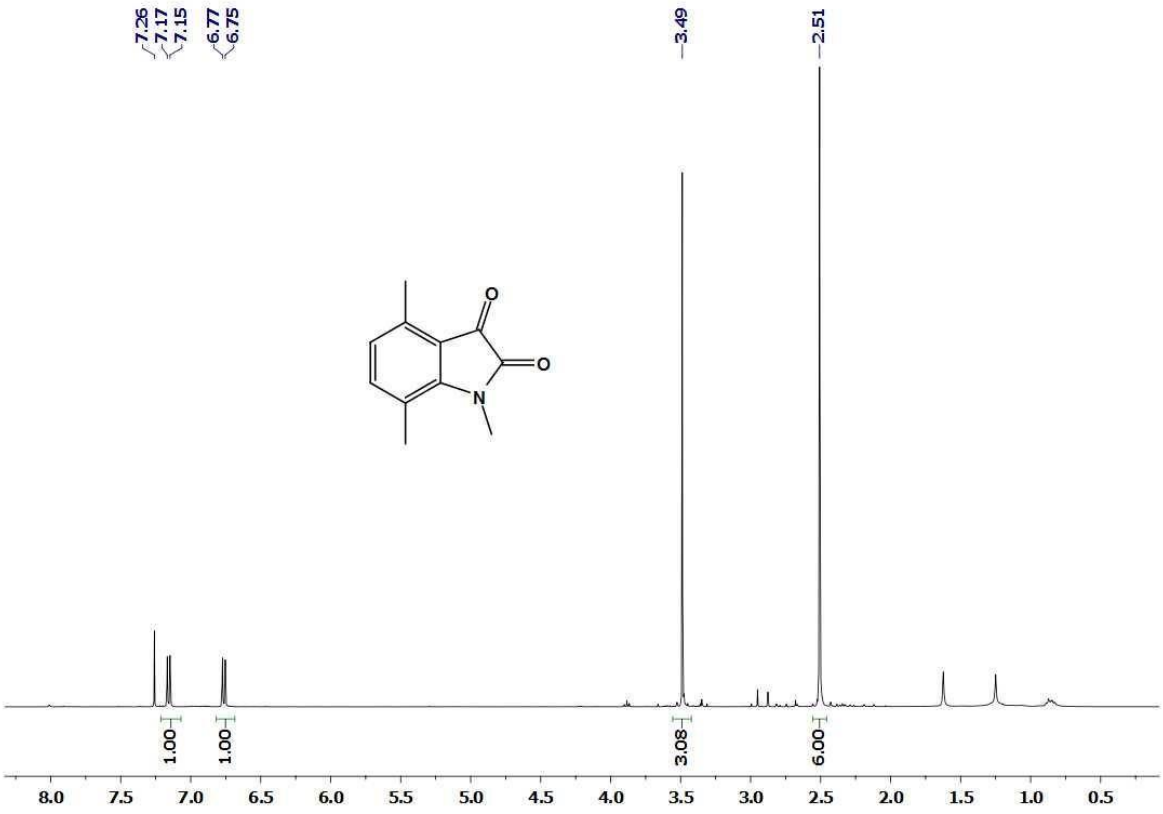

Figure S7 ^{1}H NMR spectrum of 1,4,7-methyl-isatin in $CDCl_3$

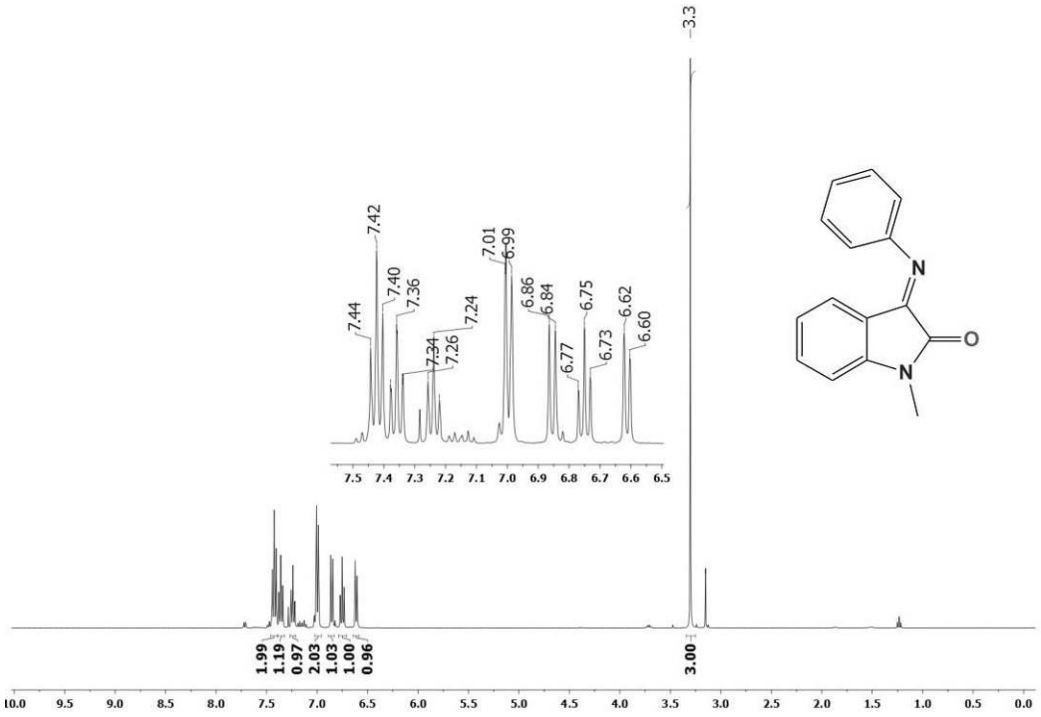

Figure S8 ^{1}H NMR spectrum of **I** in $CDCl_3$

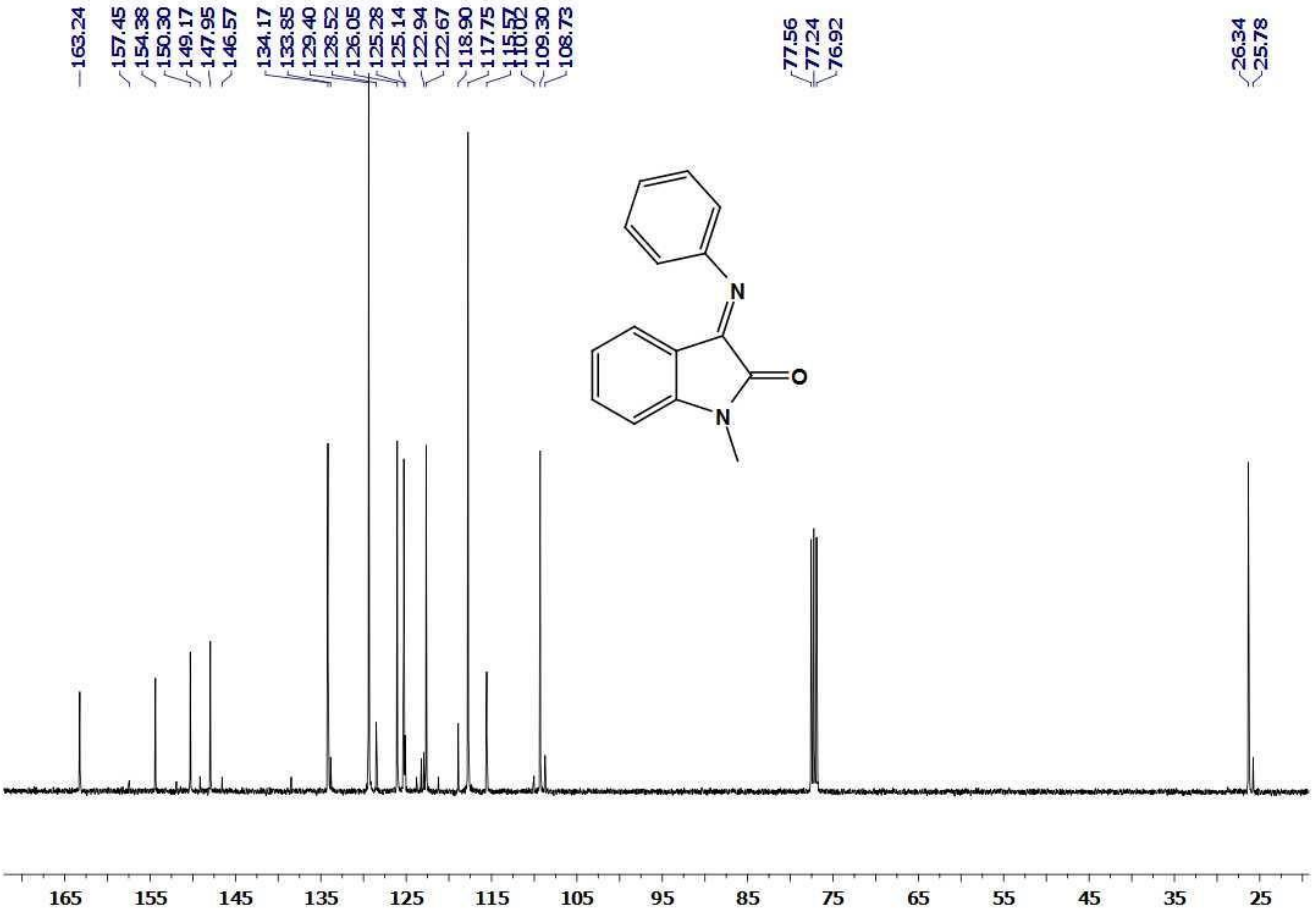

Figure S9 ^{13}C NMR spectrum of **I** in $CDCl_3$

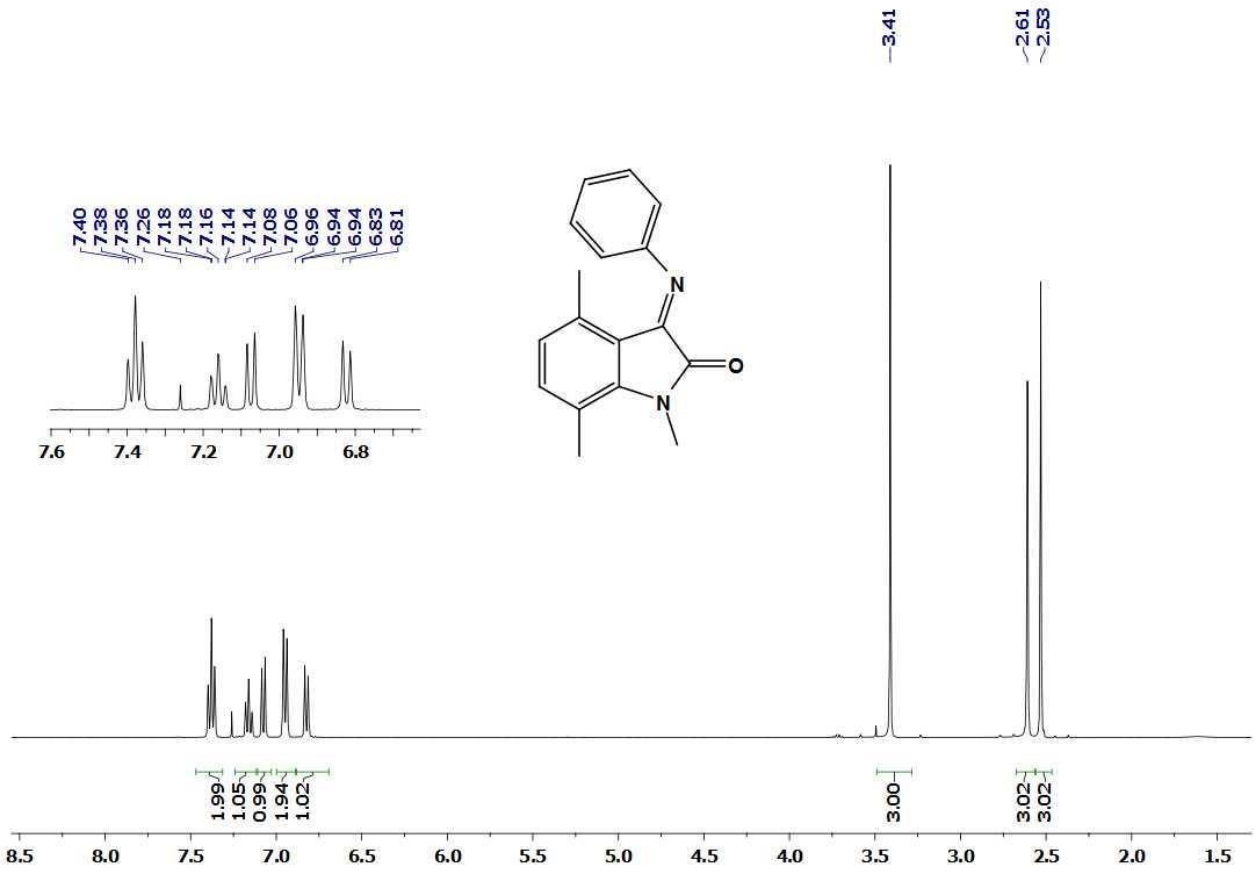

Figure S10 ^{1}H NMR spectrum of **II** in $CDCl_3$

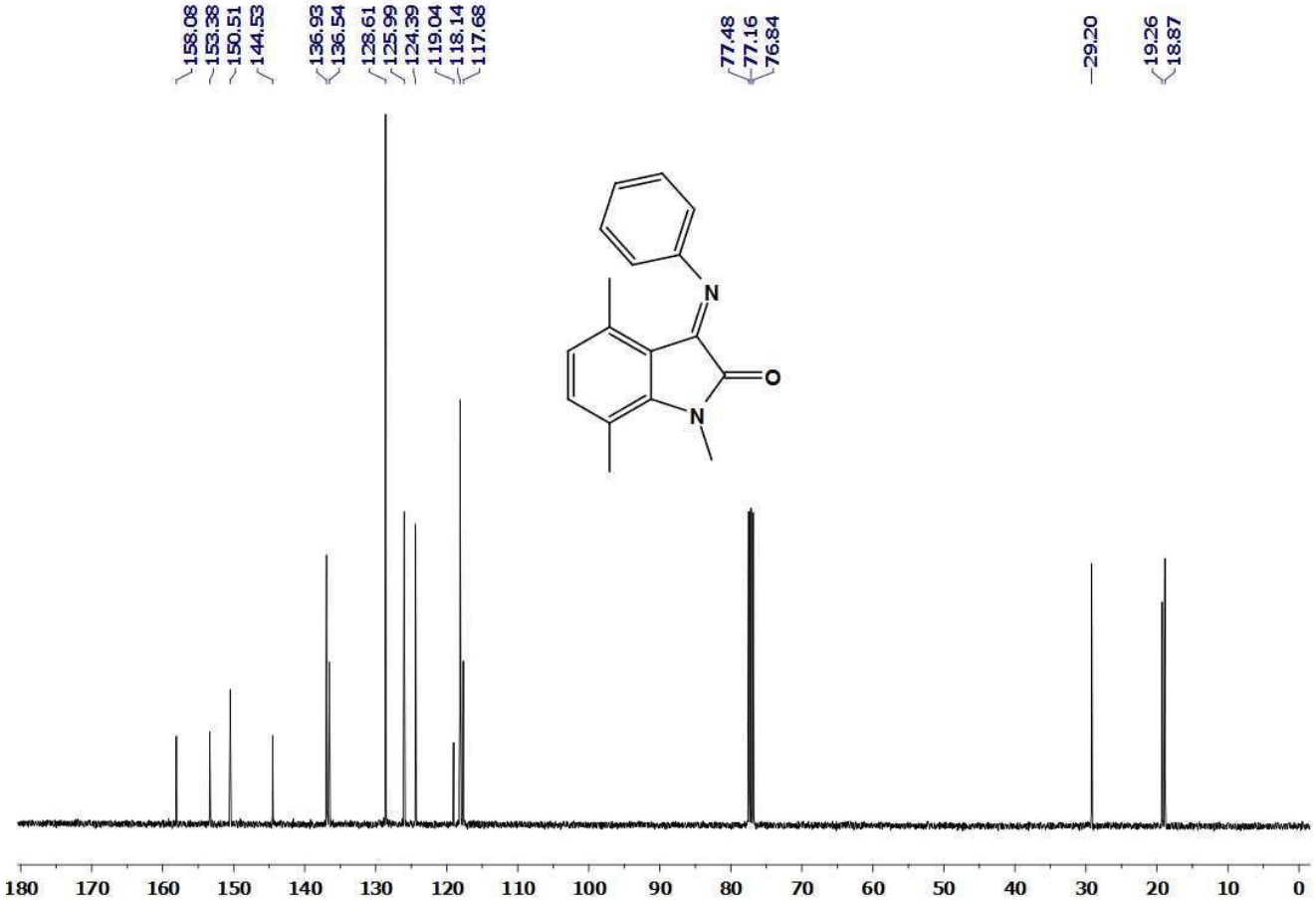

Figure S11 ^{13}C NMR spectrum of **II** in $CDCl_3$

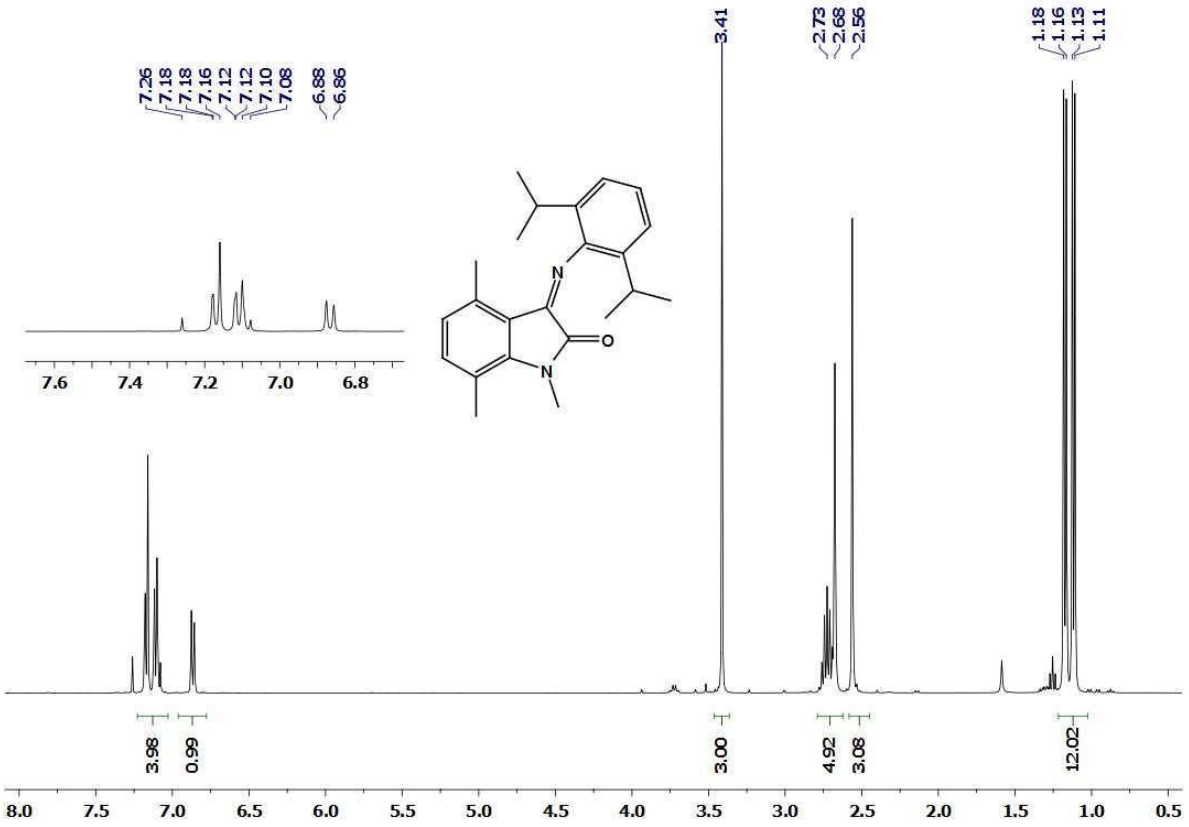

Figure S12 ^{1}H NMR spectrum of **III** in $CDCl_3$

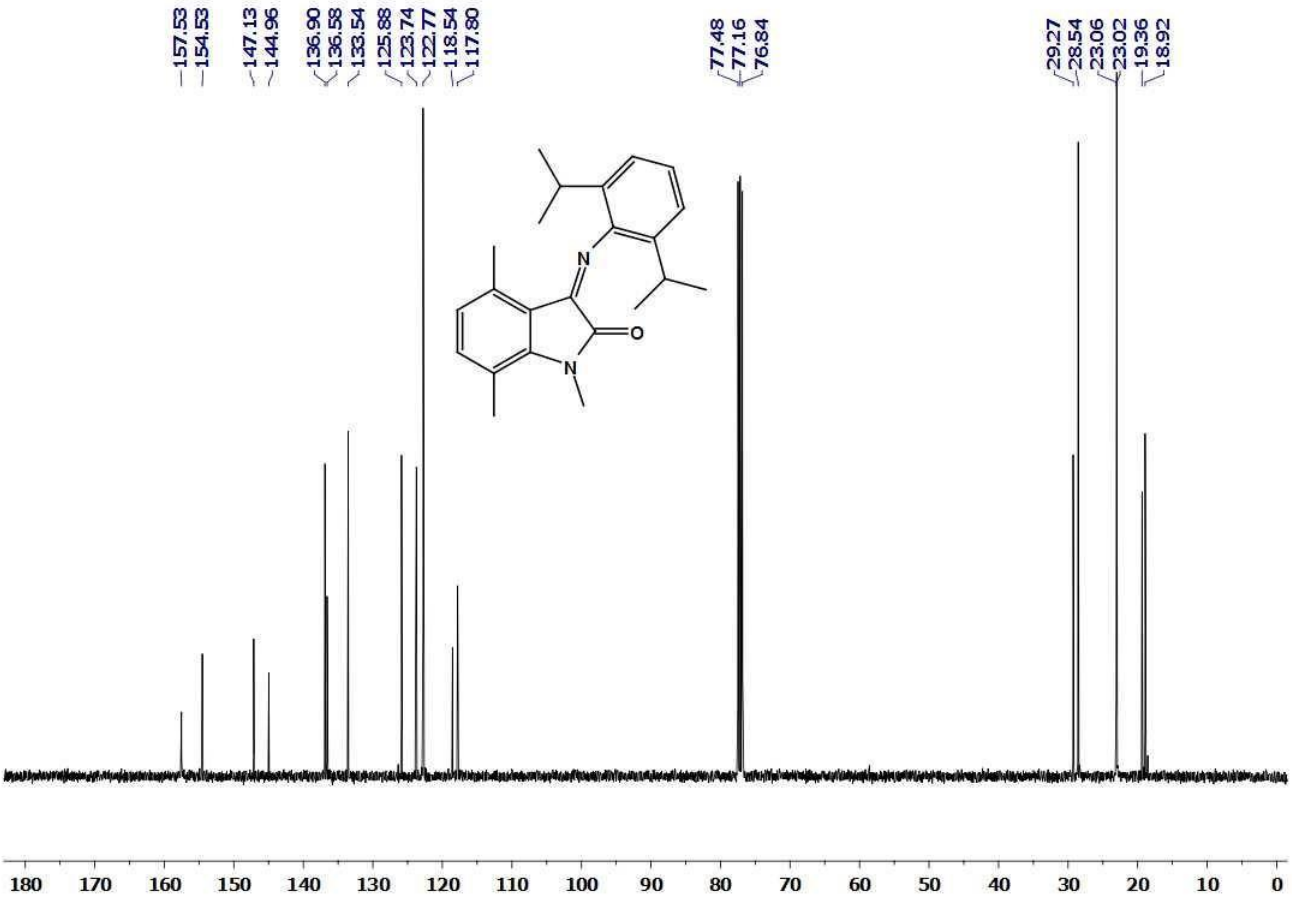

Figure S13 ^{13}C NMR spectrum of **III** in $CDCl_3$

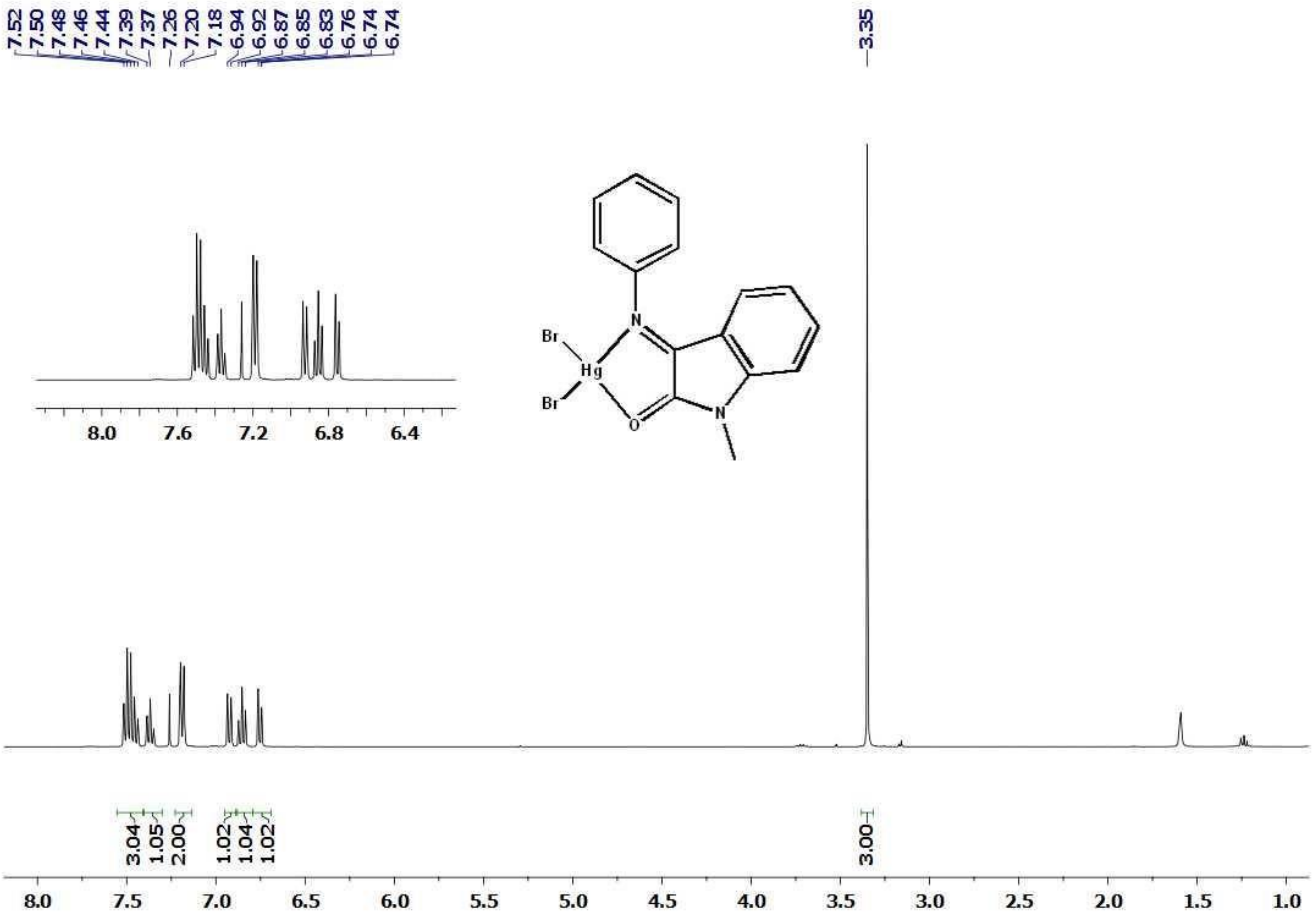

Figure S14 ^{1}H NMR spectrum of **VI** in $CDCl_3$

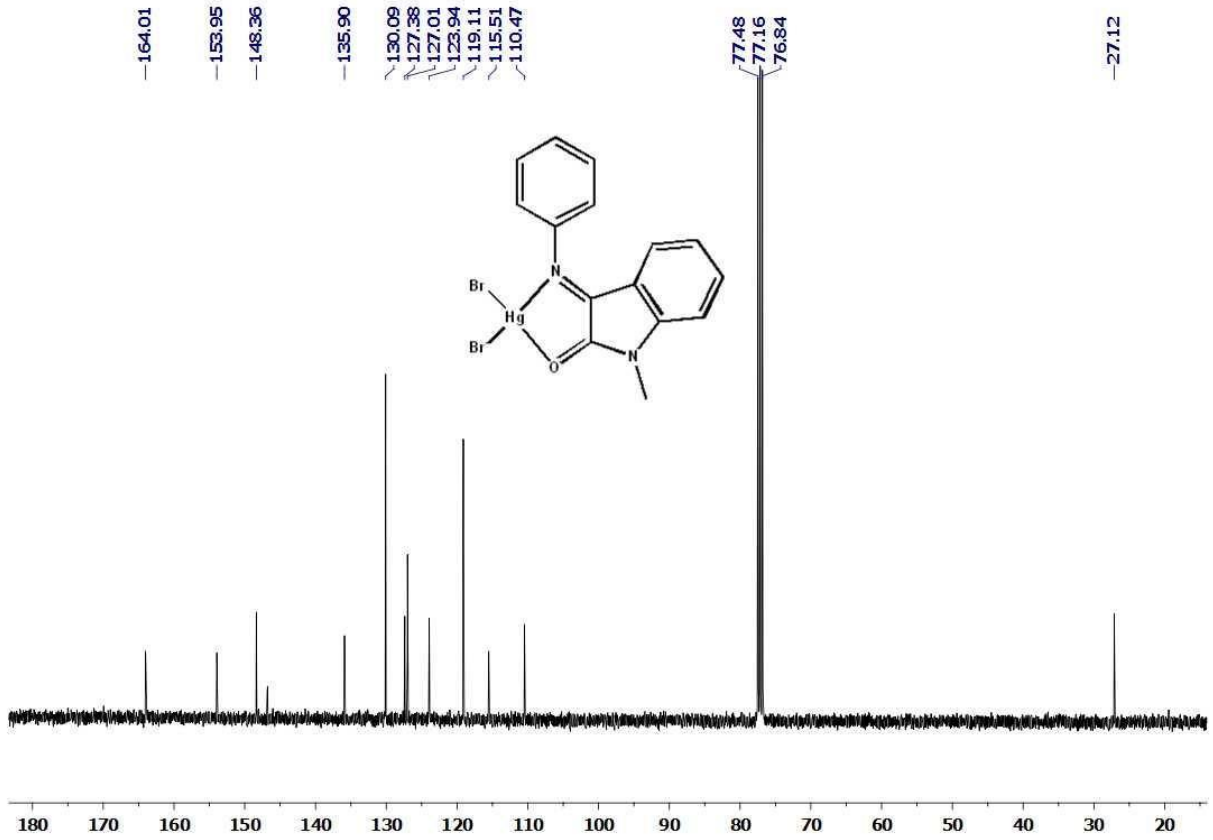

Figure S15 ^{13}C NMR spectrum of **VI** in $CDCl_3$

5. MS SPECTRA

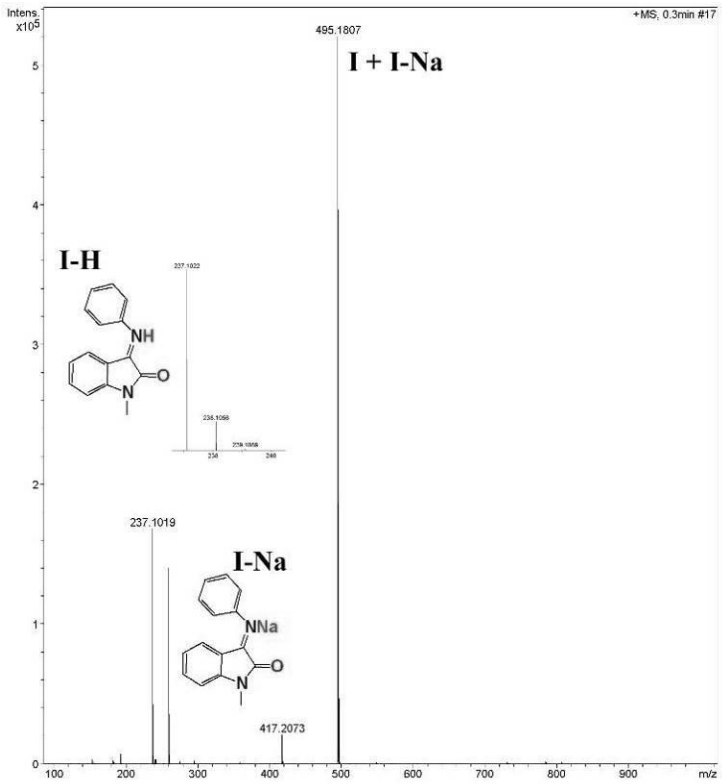

Figure S16 MS spectrum of **I** (MW = 236.27)

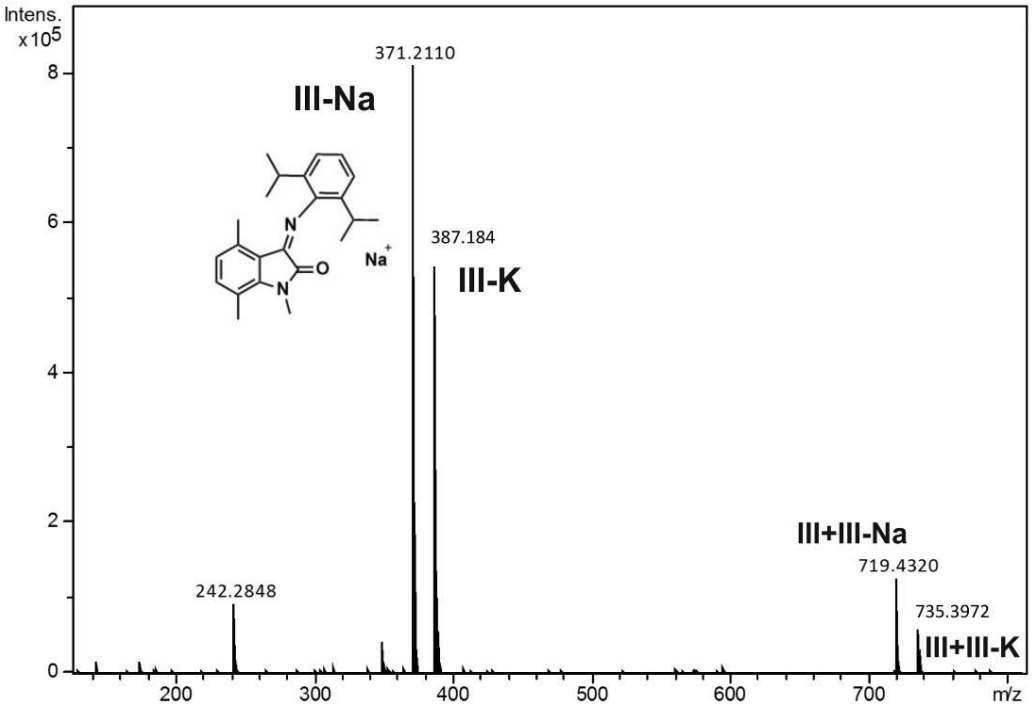

Figure S17 MS spectrum of **III** (MW = 348.22)

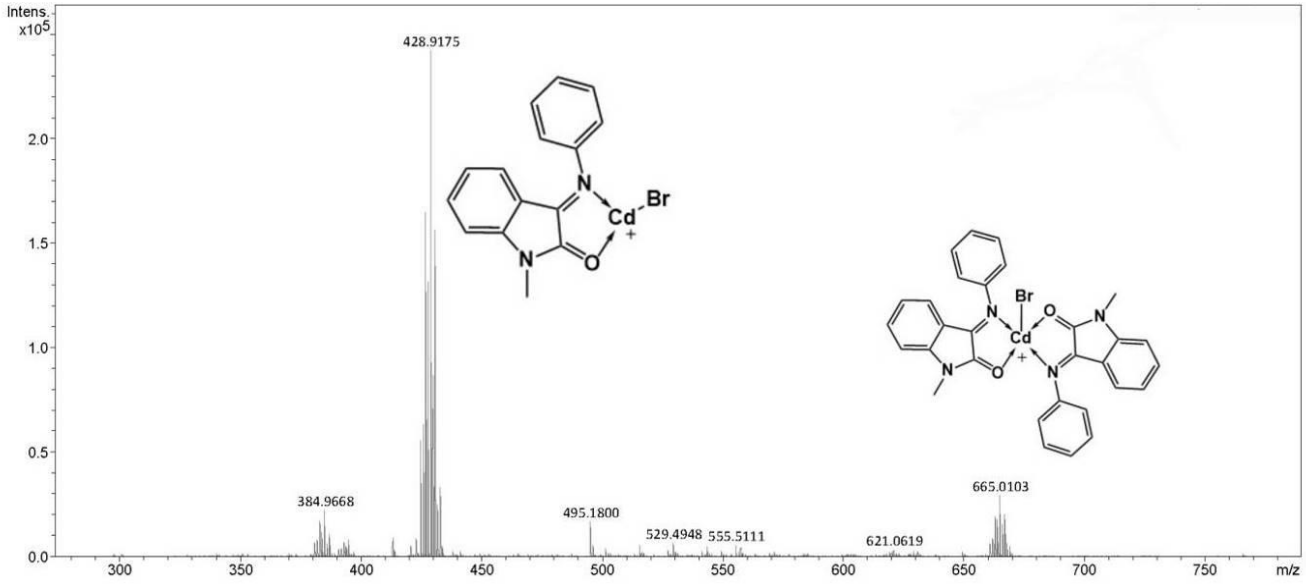

Figure S18 MS spectra of **V** from MeOH/DSMO mixture

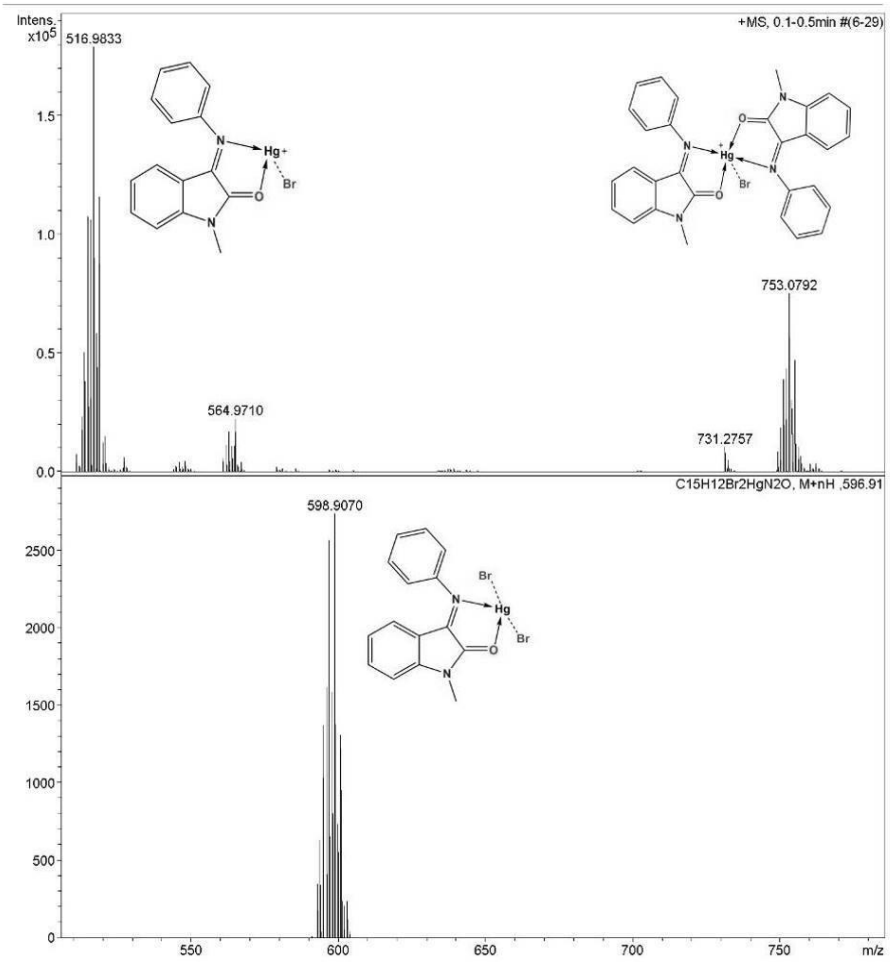

Figure S19 MS spectra of **VI** (MW = 596.67)

6. REFERENCES

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