Electronic Supplementary Material (ESI) for RSC Advances

Chemical tweaking of non-fluorescent GFP chromophore to highly fluorescent coumarinic fluorophore: application towards photo-uncaging and stem cell imaging

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S1: Materials, methods, and Instrumentation

Materials and methods:

Reagents (4-(Diethylamino)salicylaldehyde, N-Acetyl glycine, Sodium acetate, Sodium carbonate from Sigma-aldrich; glycine methyl ester hydrochloride, ethyl acetimidate hydrochloride, methylamine , 2-Nitrobenzylbromide from Acros organics, Boron tribromide and Methyl iodide from spectrochem. and oligo chemicals respectively.) were procured and used without further purification. Solvents used for spectroscopic measurements were of spectroscopic grade and were procured from Spectrochem. Thin layer chromatography (TLC) analysis were performed on Merck Kieselgel 60 F_{254} plate using 100-200 mesh size silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ in Bruker AVANCE III 500 (500MHz) and in Bruker AVANCE III 500 (125MHz) spectrometer respectively. Chemical shift δ (in ppm) are reported relative to tetramethylsilane (^{1}H NMR) as internal standard to residual signal of the solvent ($CDCl_3$, 7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). IR spectra were recorded on a Perkin Elmer (model – spectrum RX-1) FT-IR spectrometer with the KBr pellets. Mass spectra (TOF MS ES^{+}) were taken in a QTOF Micromass system.

Optical measurement:

(a) Steady state measurements:

Steady state absorption and corrected emission spectra were taken in a U-4100 Hitachi spectrophotometer and Fluoromax-3, Horiba Jobin Yvon spectrofluorimeter respectively. Molar extinction coefficients of all dyes in different solvents were calculated by using Lambert-Beer law: $A = \varepsilon C l$ (where A is the absorbance, ε is the molar extinction coefficient, C is the concentration and l is the path length). Different concentrated solutions of OHIM, OMIM, OMBO and cOHBO were prepared in toluene, DCM and Methanol and their Absorbance values were measured by spectrophotometer. The molar extinction co-efficient values were calculated from the slope by plotting Absorbance (A) along the Y-axis and Concentration (C) along the X-axis. Quantum Yields determination was accomplished by comparison of the wavelength integrated intensity of the unknown to that of the standard. Fluorescence quantum yields were calculated with solutions having OD less than 0.05 to avoid inner filter effect. To measure the Q.Y of cOHBO, quinine sulphate; for OMBO, OHIM and OMIM 4NBD and for ONBYOHBO Rhodamine 6G were used as the reference compound. For cOHBO, both the compound and the reference were excited at 366 nm for OMBO, OHIM and OMIM both the compounds and the reference were excited at 450 nm and for ONBYOHBO both the compound and the reference were excited at 488 nm. Quantum Yield of all the compounds was calculated using the following equation:

$$Q = Q(Ref)\frac{OD\ (Ref)}{OD}\frac{I}{I(Ref)}\frac{n^2}{n^2(ref)} \qquad \text{---- eq—(1)}$$

Where, Q, I and n stands for Quantum yield, Integrated intensity and refractive index of the solvents respectively.

(b) Time resolved measurements:

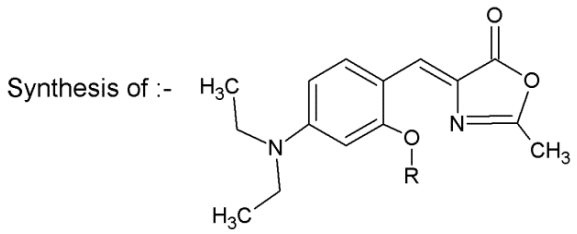
(i) Picosecond TCSPC measurement:

Fluorescence lifetime measurement at ps-ns time domain were carried out using a time correlated single photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH). Diode laser with λ_{ex} = 377 nm and 402 nm were used as the excitation source and an MCP photomultiplier tube (PMT) (Hamamatsu R3809U-50 series) as the detector. The width of the instrument response function (IRF), which was limited by the fwhm of the exciting pulse, was less than 100 ps for 377 nm and 402 nm excitation source. IRF was recorded using a scatterer (dilute solution of ludox in water). Nonlinear least squares iterative reconvolution procedure using IBH DAS6 (Version 2.2) was employed to fit the fluorescence decay curve using a single exponential decay equation. The quality of the fit was assessed from the χ^2 values and the distribution of the residuals.

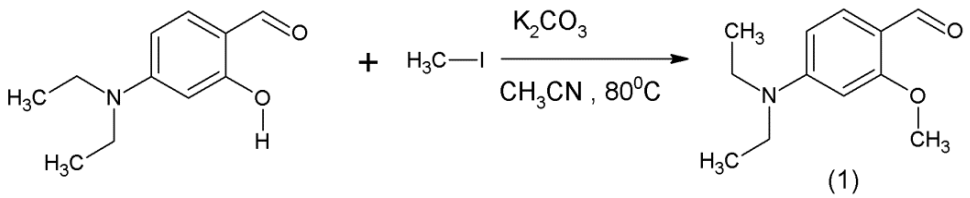
(ii) Femtosecond Fluorescence Up-conversion Measurement:

Femtosecond fluorescence transients were collected using Fluorescence Up-conversion technique in femtosecond up-conversion setup (FOG 100, CDP, Russia). The second harmonic (400 nm) of a mode locked Ti-sapphire laser (Tsunami, Spectra physics) were used as the excitation source for the samples. The fundamental beam (800 and 790 nm) was frequency doubled in nonlinear crystal (1 mm BBO, $\Theta = 25^0$,$\phi = 90^0$) and used for the excitation . The sample was placed inside a 1 mm thick rotating quartz cell. The fluorescence emitted from the sample was up-converted in a nonlinear crystal (0.5 mm BBO, $\Theta = 38^0$, $\phi = 90^0$) using the fundamental beam as the gate pulse. The Up-converted light is dispersed in a monochromator and detected using photon counting electronics. The instrument response function of the apparatus is 300 fs. The decays were deconvoluted using a Gaussian shape of the exciting pulse using commercial software (IGOR-Pro, Wavemetrics). All the experiments were performed at 20^0C. Femtosecond up conversion measurement was performed for three molecules in three solvents of different polarity.

S2: Syntheses and characterisation:



Step:- 1



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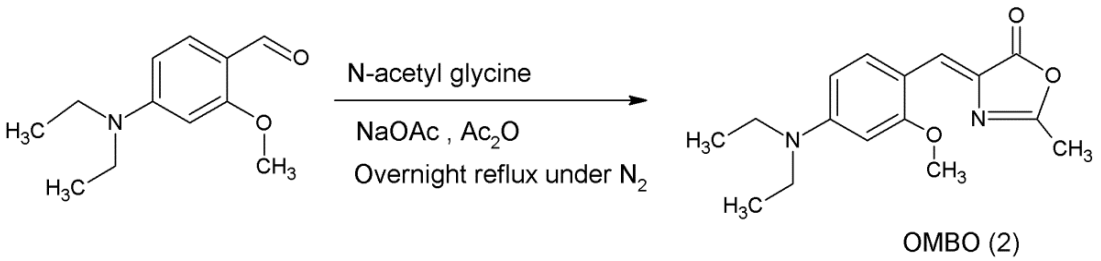

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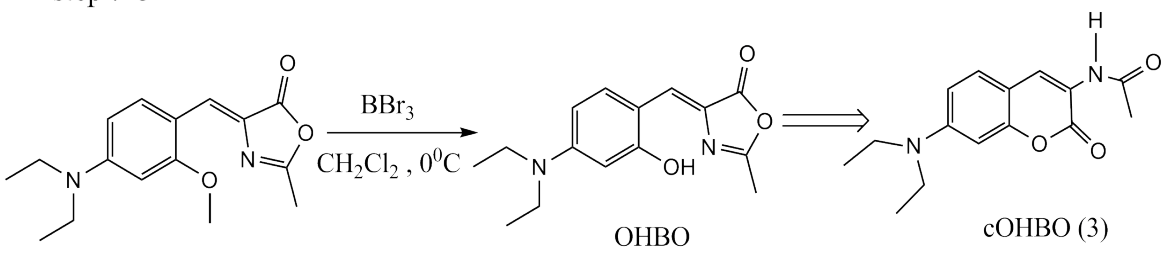


Preparation of 4-Diethylamino-2-methoxy benzaldehyde (1):

The compound was prepared according to the literature procedure.[1] A mixture containing 4-diethylamino-2-hydroxy benzaldehyde (5.8 g, 30 mol), KOH (2 g, 35 mol), K_2CO_3 (8.3 g, 60 mol) in acetonitrile (30 ml) was heated for 10 min at 80^0C. In this alkaline solution MeI (3.5 ml, 56 mol) was added. The stirring was continued for another 2h at 80^0C. The reaction mixture was then cooled to room temperature followed by vacuum filtration. The filtrate was concentrated under reduced pressure and chromatographed on silica gel column (mesh size – 120-200) eluting with EtOAc / hexane in 1:3 ratio to get pure compound as yellow solid (yield = 85%.).

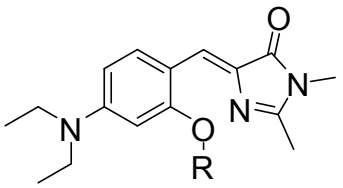
Preparation of (*Z*)-4-(4-(diethylamino)-2-methoxybenzylidene)-2-methyloxazol-5(4*H*)-one (2):

This compound was prepared with slight modification of the literature report.[2] 4-diethylamino-2-methoxybenzaldehyde (1.5g, 7.24 mol), N-acetylglycine (0.84855g, 7.24 mol), anhydrous sodium acetate(0.593g, 7.24 mol), and acetic anhydride (5 ml) were stirred under nitrogen at 60^0C for 1h and then it was refluxed under positive pressure of nitrogen overnight. After cooling, the whole mixture turned into a dark brown solid and it was directly loaded on silica gel coloum and was eluted by EtOAc/hexane system (1:9) using flash column chromatography. After purification 300 mg compound was obtained as a orange solid (yield 15%). It was then recrystallised twice from methanol. ^{1}H NMR (Bruker 500 MHz, $CDCl_3$) δ 1.223 (t , J = 7.1Hz , 6H) δ 2.342 (s , 3H) δ 3.44(q , J = 7.1Hz , 4H) δ 3.861 (s , 3H) δ 6.05 (d , 2.4 Hz ,1H) δ 6.36 (dd , J = 9.1 Hz, 2.4 Hz , 1H) δ 7.68 (s , 1H) δ 8.60 (d , J = 9.1Hz , 1H) ^{13}C NMR (Bruker 125 MHz , $CDCl_3$) : 12.703 , 15.524 , 44.770 , 55.296 , 92.806 , 105.160 , 110.752 , 125.979 , 126.925 , 134.264 , 151.949 , 161.611 , 161.756, 169.093 . FT-IR (neat) - 2977 , 2927,1756 , 1641, 1611 , 1570 ,1409 , 1279 , 1258, 1225 , 1111 ,1073 ,1030 . MS calculated for $C_{16}H_{20}N_2O_3$: 288.2 , Found: 288.2

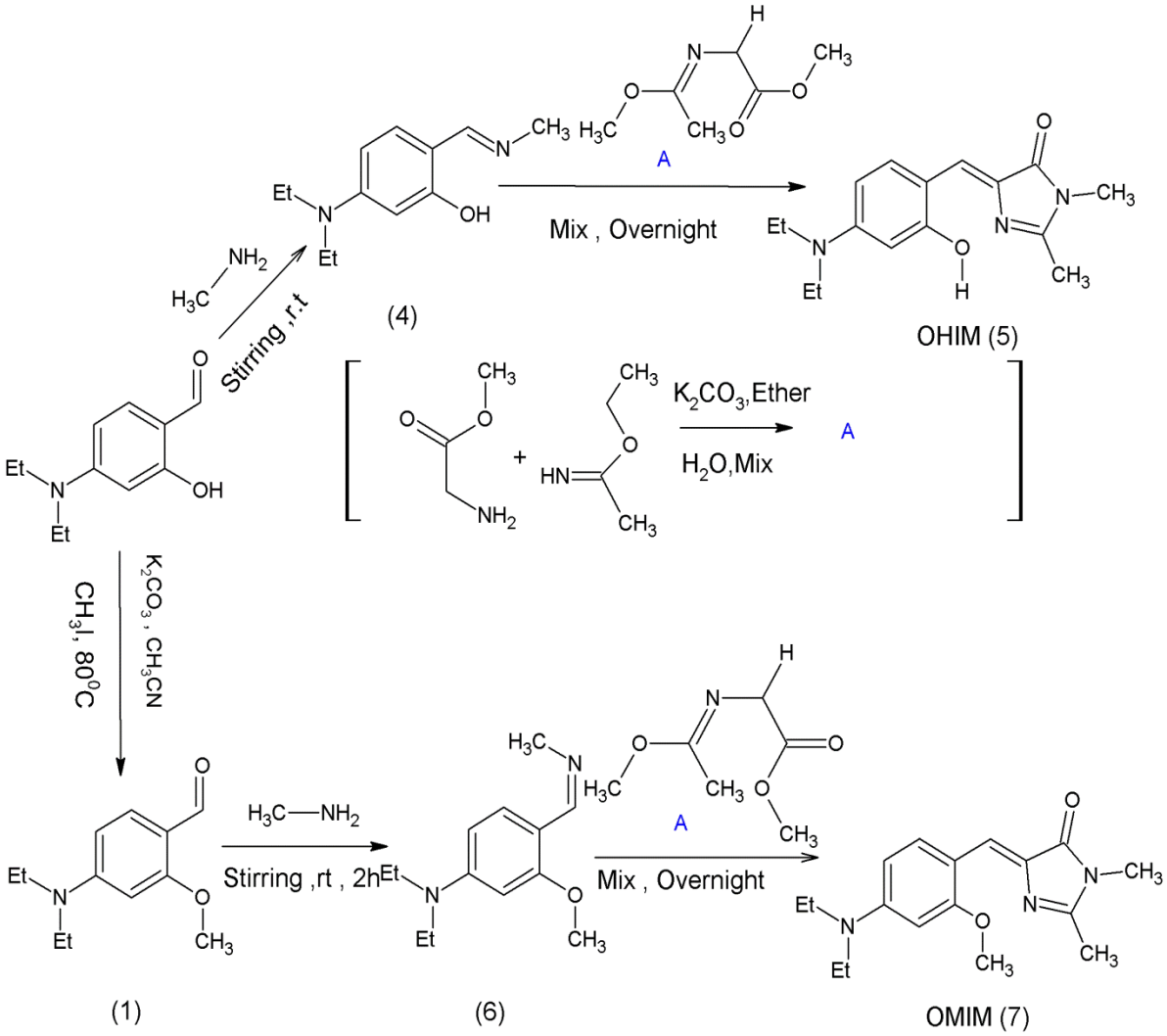
***N*-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acetamide [cOHBO] (3):**

(*Z*)-4-(4-(diethylamino)-2-methoxybenzylidene)-2-methyloxazol-5(4*H*)-one (**2**) (250 mg, 0.8680 mol) was dissolved in dry dichloromethane (10 ml) in a round bottom flask and the flask was cooled to 0^0C by keeping it on ice bath. To this ice cold solution of **2**, BBr_3 solution (in DCM 2.2 ml, 2.2 mol) was added drop wise with proper care. The reaction was continued for 5h. The reaction mixture was then hydrolyzed and extracted two to three times taking 10 ml of DCM in each time. The combined organic phase was then dried over magnesium sulphate. The compound was purified by column chromatography (with silica gel and DCM as eluent) (yield = 30% (75 mg)). ^{1}H NMR (Bruker 500MHz , $CDCl_3$) δ1.19 (t , J = 7Hz , 6H) δ 2.19 (s , 3H) δ 3.40 (q , J = 7Hz, 4H) δ 6.49 (d , J =2.5 Hz ,1H) δ 6.61 (dd , J = 2.5 Hz , 8.8Hz, 1H) δ 7.29 (d , J = 9Hz , 1H) δ 7.88 (broad s , 1H) δ 8.56 (s , 1H) . ^{13}C NMR (Bruker 125 MHz , $CDCl_3$) : 12.433 , 24.639, 44.725 , 97.385 , 108.262 , 109.634 , 118.958 , 125.581 , 128.680 , 149.401 , 152.615 , 159.650 , 168.873 . FT-IR (neat) – 3328 ,2971 , 2927 , 1705 , 1673 , 1622 , 1602 ,1530 ,1408 , 1264 ,1246 , 1190 , 1131 . MS calculated for $C_{15}H_{18}N_2O_3$: 274.1 , Found: 274.1.

Synthesis of :-



Here , R=CH_3 / H



Synthesis of 5-(diethylamino)-2-((methylimino)methyl)phenol (4):

The Schiff base (4) was prepared by mixing 4-(Diethylamino)-2-hydroxybenzaldehyde (2 g, 10.362 mol) with methylamine (in absolute ethanol 1.6 ml, 10.362 mol) in ethanol (25ml) and stirring the mixture at room temperature for 1h.[3] The yield of the reaction was 99%. So after removing ethanol in a rotavapour, the compound was used directly for the next step.

Synthesis of Methyl 2-(1-ethoxyethylidene) aminoethanoate (A):

The compound (**A**) was prepared according to Bazureau report.[4] A suspension was formed when K_2CO_3 (6.9 g, 50 mol) was mixed with methyl glycinate hydrochloride (6.28 g , 50 mol) in diethylether (150 ml). This was followed by the addition of ethyl acetimidate hydrochloride(6.18 g, 50 mol). The mixture was shaken for 10 min. The ether layer was decanted off. An additional amount of diethylether (75 ml) was

added. Again the mixture was shaken for 10 min and the ether was decanted. Then the combined organic portion was dried over anhydrous $MgSO_4$ and the solvent was removed under vacuum. The imidate thus obtained (yield = 52%) was used for the next step directly due to its instability.

Synthesis of (*Z*) -4-(4-(diethylamino)-2-hydroxybenzilidene)-1,2-dimethyl-1H-imidazol-5(4*H*)-one (5):

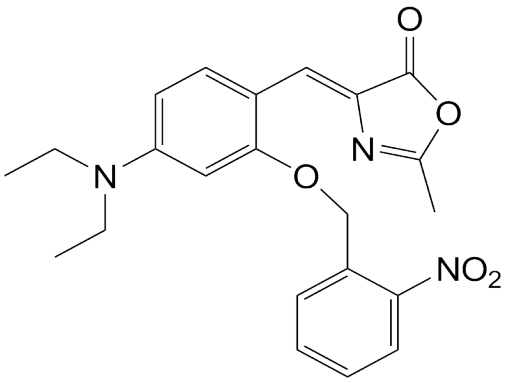
The compound (5) was synthesized according to a literature procedure[1] by mixing the Schiff base (1) (2g, 9.69 mol) and the imidate (A) (2.60g, 16.35 mol) in ethanol (13ml) and stirring the mixture overnight at ambient condition. The product got precipitated out and it was then washed sequentially with diethyl ether (15 ml) and ethanol (10 ml) to get the pure product as a yellow powder (yield = 85%). It was then recrystallised twice from methanol. : ^{1}H NMR (Bruker-500 MHz , $CDCl_3$) δ 1.20 (t , J= 7.1 Hz , 6H) δ 2.34 (s, 3H) δ 3.213(s , 3H) δ 3.398 (q , J=7.1 Hz , 4H) δ 6.167(d , J= 2.5Hz, 1H) δ 6.23(dd , J=2.58 Hz , 8.8 Hz, 1H) δ 7.11((d(mixed up with δ7.13 peak), J =9.02 Hz, 1H)) δ 7.13(s, 1H) δ14.28 (b s ,1H) . ^{13}C NMR (125 MHz, $CDCl_3$) : 12.77 , 14.90 , 26.68 , 44.60 , 99.31 , 104.59 , 109.45 , 127.69 , 131.61 , 138.71 , 152.40 , 153.04 , 160.94 , 167.83.
FT-IR (neat) – 3436 , 2971 , 2929 , 1697 ,1610 , 1518 , 1395 , 1357,1305 ,1242 , 1141, 1078 ,1030 . MS calculated for $C_{16}H_{21}N_3O_2$:287.2 , Found : 287.2 .

Synthesis of N,N-diethyl-3-methoxy-4-((methylimino)methyl)aniline (6):

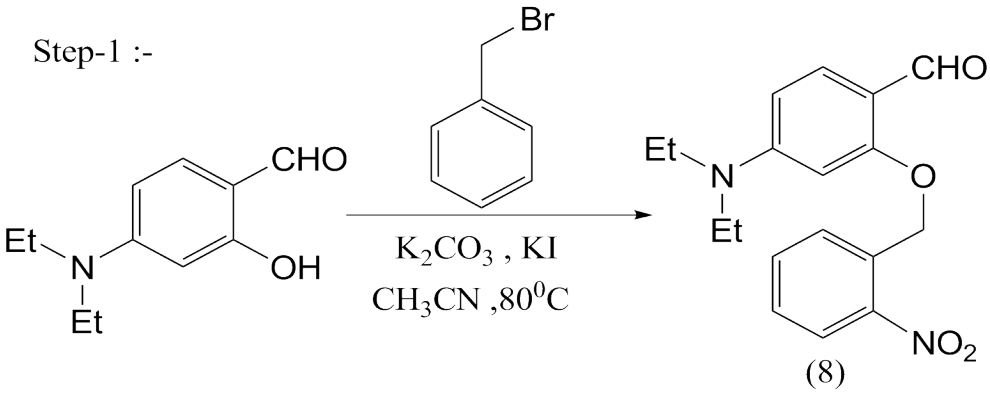
The Schiff base (**6**) was prepared following a literature procedure[3] by mixing 4-(diethylamino)-2-methoxybenzaldehyde (1) (2 g, 9.66 mol) and methylamine (1.6 ml, 10.362 mol) in ethanol (25 ml) and stirring the reaction mixture at r.t for 2h. The yield of the reaction was almost quantitative. So after evaporating ethanol in a rotavapour, the compound was used for the next step without purification.

Synthesis of (*Z*) -4-(4-(diethylamino)-2-methoxybenzilidene)-1,2-dimethyl-1H-imidazol-5(4*H*)-one (7):
The compound (**7**) was synthesized by mixing the Schiff base (6) (1g, 4.5 mol) and the imidate (A) (0.795g, 5 mol) in ethanol (10ml) and overnight stirring of the mixture at ambient condition. The product got precipitated out and it was then washed sequentially with diethyl ether (15 ml) and ethanol (10 ml) to get the pure product as a yellow powder (yield = 62%). It was then recrystallised from methanol. ^{1}H NMR (Bruker 500 MHz , $CDCl_3$) δ 1.20(t , J=7.10 Hz , 6H) δ 2.33(s , 3H) δ 3.168(s , 3H) δ 3.41(q , J=7.10 Hz, 4H) δ 3.85 (s , 3H) δ 6.06 (d , J=2.37 Hz , 1H) δ 6.35 (dd, J= 9.05Hz , 2.42 Hz , 1H) δ 7.65(s, 1H) δ 8.71 (d , j= 9.02 Hz , 1H) . ^{13}C NMR (Bruker 125 MHz, $CDCl_3$) : 12.725 , 15.552 , 26.513 , 44.663 , 55.259 , 93.085 , 105.021 , 111.410 , 122.944 , 133.543 , 134.319 , 151.034 , 157.715 , 161.362 , 170.701 . FT-IR (neat)- 2976 , 2940 , 1686 , 1597 , 1556 ,1405 ,1393 , 1360 , 1305 , 1272 , 1224 , 1105 , 1076 , 1022 . MS calculated for $C_{17}H_{23}N_3O_2$: 301.2 , Found : 301.2.

Synthesis of :-

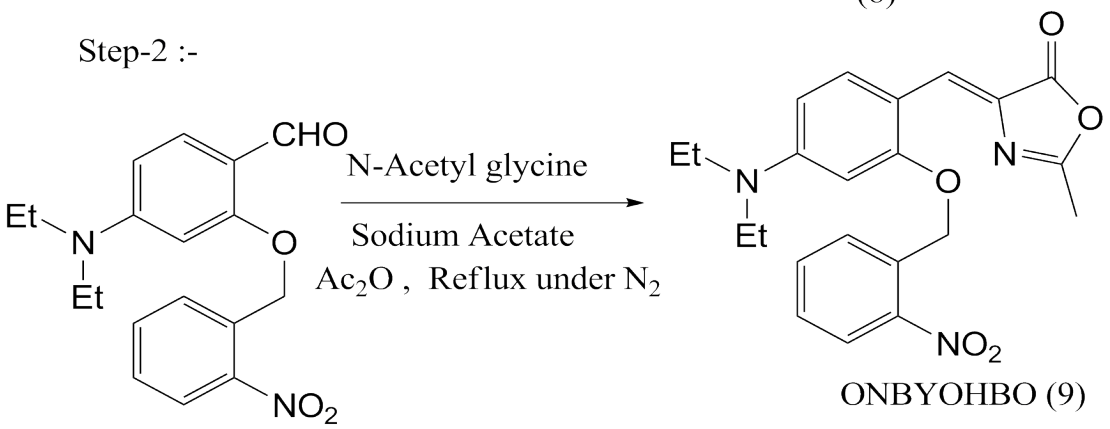


Step-1 :-



(8)

Step-2 :-



ONBYOHBO (9)

Synthesis of 4-(diethylamino)-2-(2-nitrobenzyloxy)benzaldehyde (8) :

A mixture containing 4-diethylamino-2-hydroxy benzaldehyde (500 mg, 2.59 mol), KOH (0.3g , 5.35 mol) , K_2CO_3 (0.72g , 5.21 mol) in acetonitrile (15 ml) was heated for 10 min at 80^0C. In this alkaline solution a mixture of O-nitrobenzylbromide (1.23 gm, 5.18 mol) and KI (900 mg , 5.18 mol) in CH_3CN (10ml) was added. The stirring was continued for another 3h at 80^0C. The reaction mixture was then cooled to room temperature in a ice bath and upon cooling a yellow precipitate formed and that was separated by vacuum filtration. The precipitate was chromatographed on silica gel column (mesh size – 120-200) eluting with EtOAc / hexane in 1:4 ratio to get pure compound as yellow solid (yield 450 mg. 54%).

^{1}H NMR ($CDCl_3$, Bruker-500 MHz) δ 1.15 (t , J=7.12 Hz , 6H) δ 3.38 (q , J=7.12 Hz, 4H) δ 5.5 (s , 2H) δ 6.05 (d , J= 2.2Hz, 1H) δ 6.31 (dd , J=2.1 Hz, 8.9Hz, 1H) δ 7.50 (dt , J=1.1Hz, 8.2Hz, 1H) δ 7.71 (d, J=8.9Hz, 1H) δ 7.73 (dd ,J =1.2 Hz, 8.2Hz, 1H) δ 8.0 (dd , J=0.6 Hz, 7.8Hz, 1H) δ 8.16 (dd , J= 1.2 Hz, 8.2 Hz , 1H) δ 10.23 (s , 1H)

^{13}C NMR ($CDCl_3$, Bruker 125 MHz, $CDCl_3$) : 12.38 , 44.78 , 66.63 , 93.92 , 104.83 , 114.29 , 124.84 , 128.41, 128.48 , 131.39 , 133.27 , 134.20 , 146.69 , 153.72 , 162.23 , 186.52 .

Synthesis of 4-(4-(diethylamino)-2-(2-nitrobenzyloxy)benzylidene)-2-methyloxazol-5(4H)-one (9) : The compound was prepared as follows. 4-(diethylamino)-2-(2-nitrobenzyloxy)benzaldehyde (432 mg, 1.32 mol), N-acetylglycine (0.150 mg , 1.32 mol) , anhydrous sodium acetate(108 mg, 1.32 mol), and acetic anhydride (8 ml) were stirred under nitrogen at 60^0C for 1h and then it was refluxed under positive pressure of nitrogen overnight. After cooling the whole mixture turned into a dark brown solid and it was directly loaded on silica gel coloum and was eluted by EtOAc/hexane system (1:8) using flash chromatography. After purification, 300 mg target compound was obtained as a orange solid (yield- 55%). It was then recrystallised twice from methanol.

^{1}H NMR (Bruker-500 MHz , $CDCl_3$) δ 1.13(t , J=7.10 Hz , 6H) δ 2.36 (s , 3H) δ 3.36 (q , J=7.12 Hz, 4H) δ 5.6 (s , 2H) δ 6.03 (d , J= 2.4Hz ,1H) δ 6.39 (dd , J=2.4 Hz ,9.1Hz 1H) δ 7.50 (dt, J=1.1Hz, 8.2 Hz, 1H) δ 7.73 (dt , J= 1.2 Hz , 7.8 Hz , 1H) δ 7.76 (s , 1H) δ 7.90 (dd , J= 0.7Hz , 7.8 Hz , 1H) δ 8.16 (dd , J= 1.2 Hz , 8.2 Hz , 1H) δ 8.67 (d , J= 9.1 Hz , 1H)

^{13}C NMR (Bruker-125 MHz , $CDCl_3$) : 12.55 , 15.53 , 44.84 , 66.78 , 94.61, 105.85 , 110.86 , 124.94 , 126.25 , 126.52 , 128.45 , 128.49 , 133.44 , 134.36 , 134.48 , 146.87 , 151.75 , 159.80 , 162.16 , 168.95

FT-IR(neat) - 2978 , 2931 , 1769 , 1640 , 1613 , 1571 , 1520 , 1412 , 1339 , 1266 , 1220 , 1173 , 1107 , 1034 , 900 , 861 , 809 , 788 , 723

MS calculated for [$C_{22}H_{23}N_3O_5$ + H]$^{+}$: 410.2 , Found : 410.2

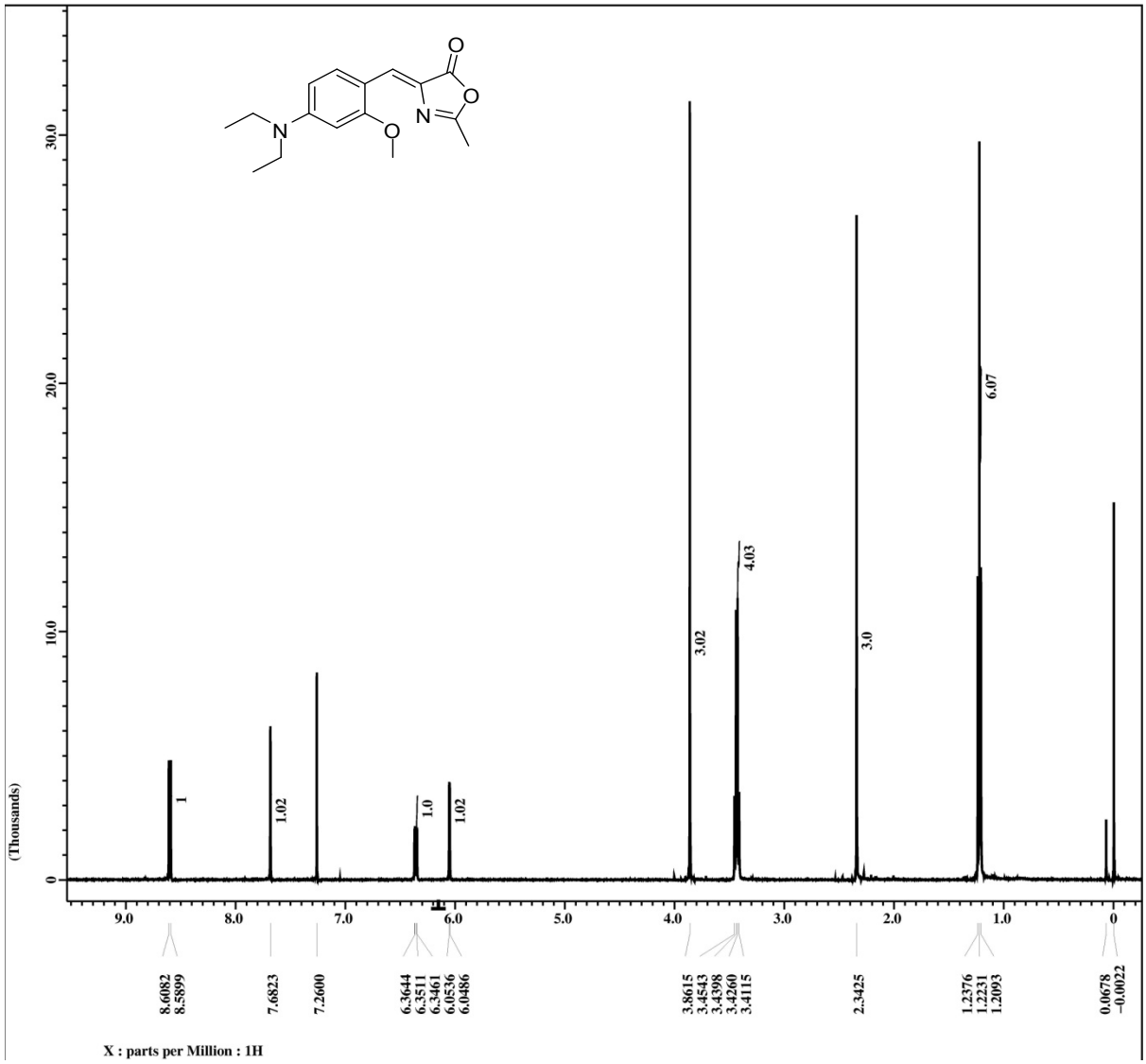


Figure 1: ^{1}H NMR spectrum of OMBO.

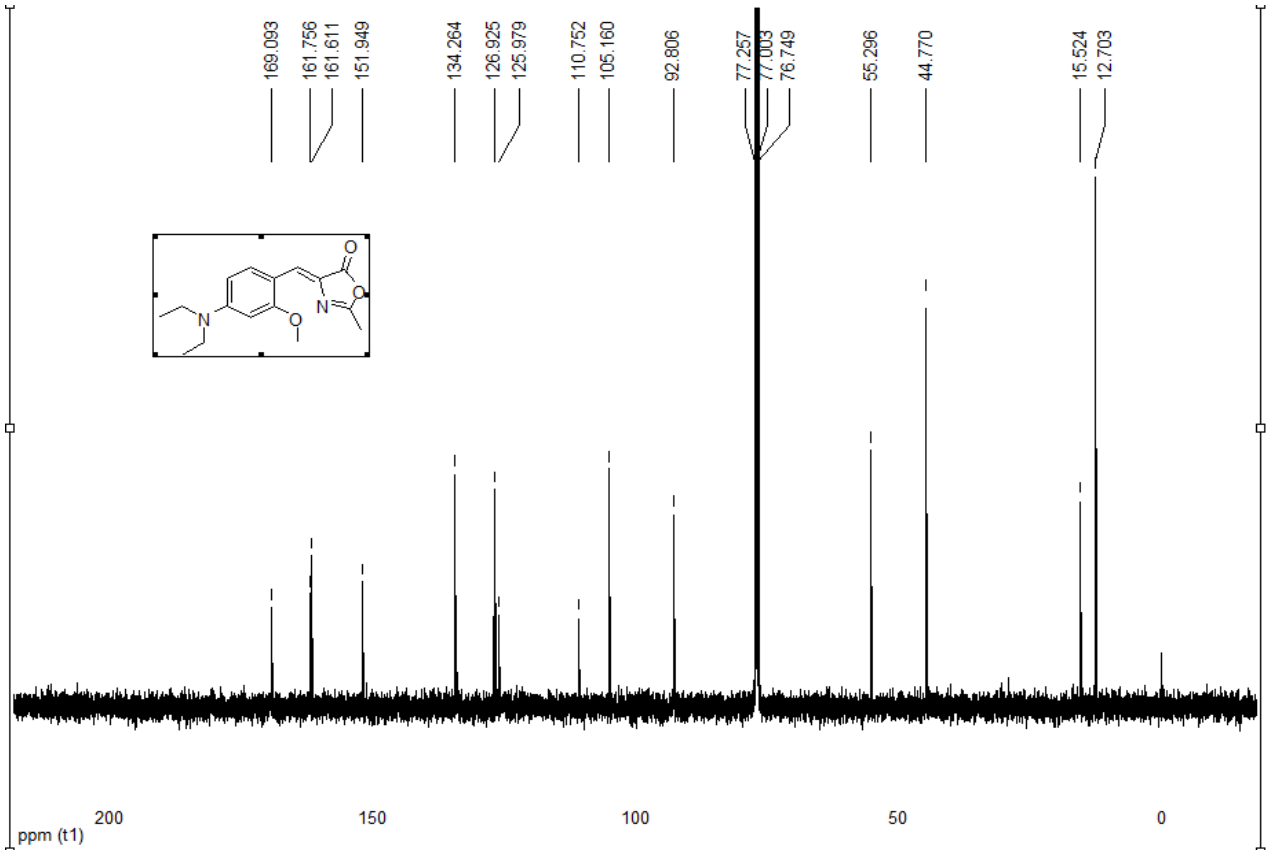


Figure 2: ^{13}C NMR spectrum of OMBO.

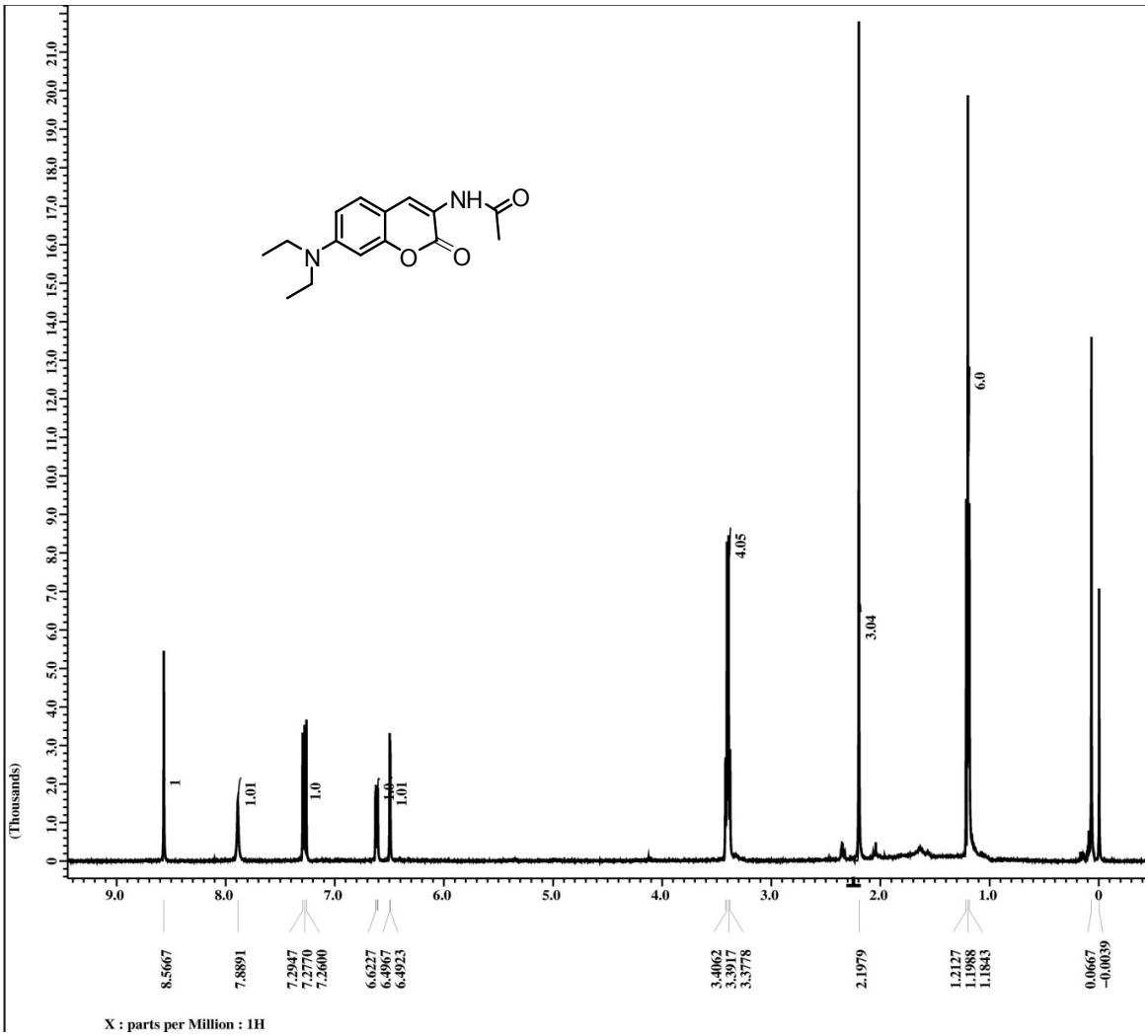


Figure 3: ^{1}H NMR spectrum of cOHBO.

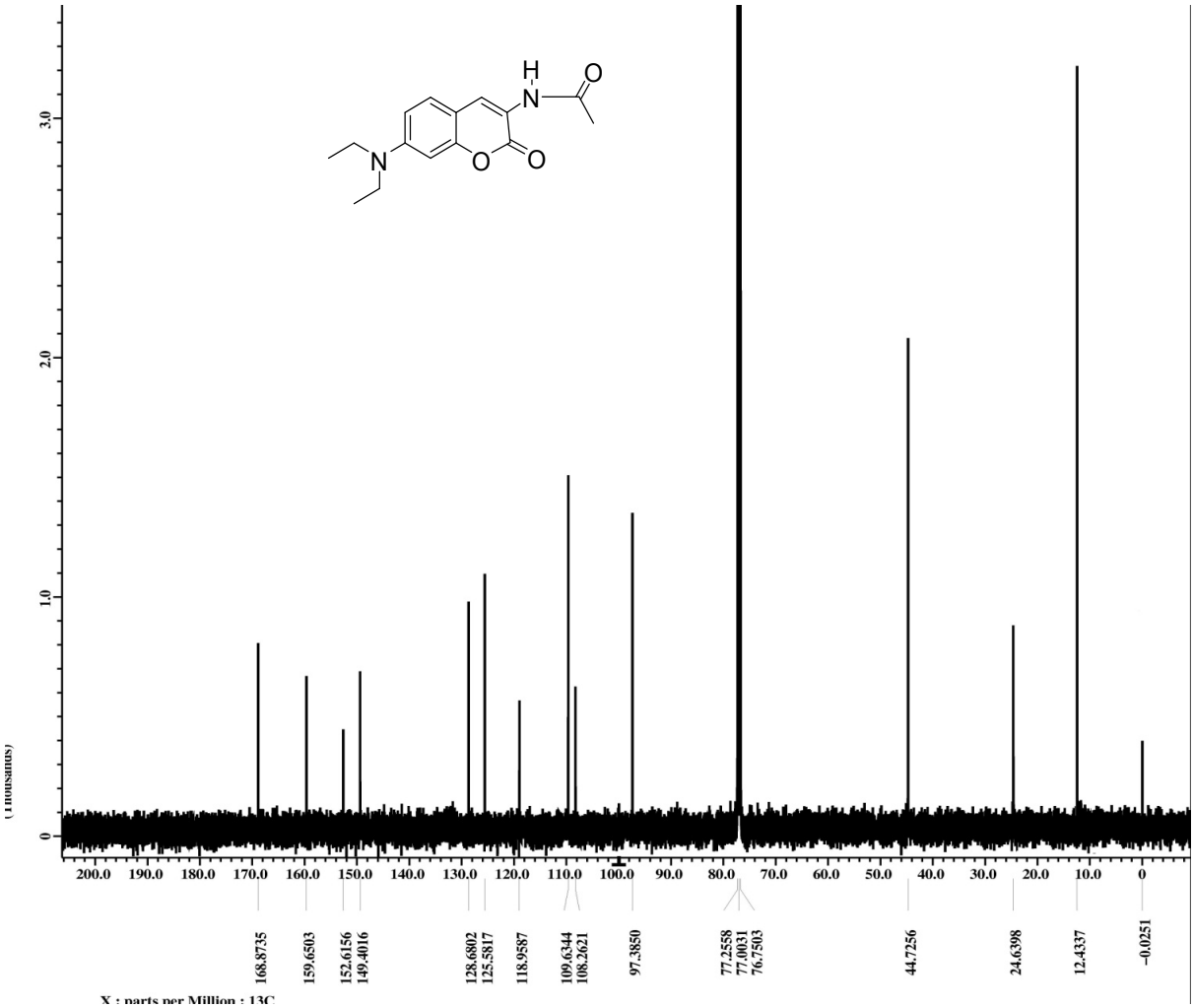


Figure 4: ^{13}C NMR spectrum of cOHBO.

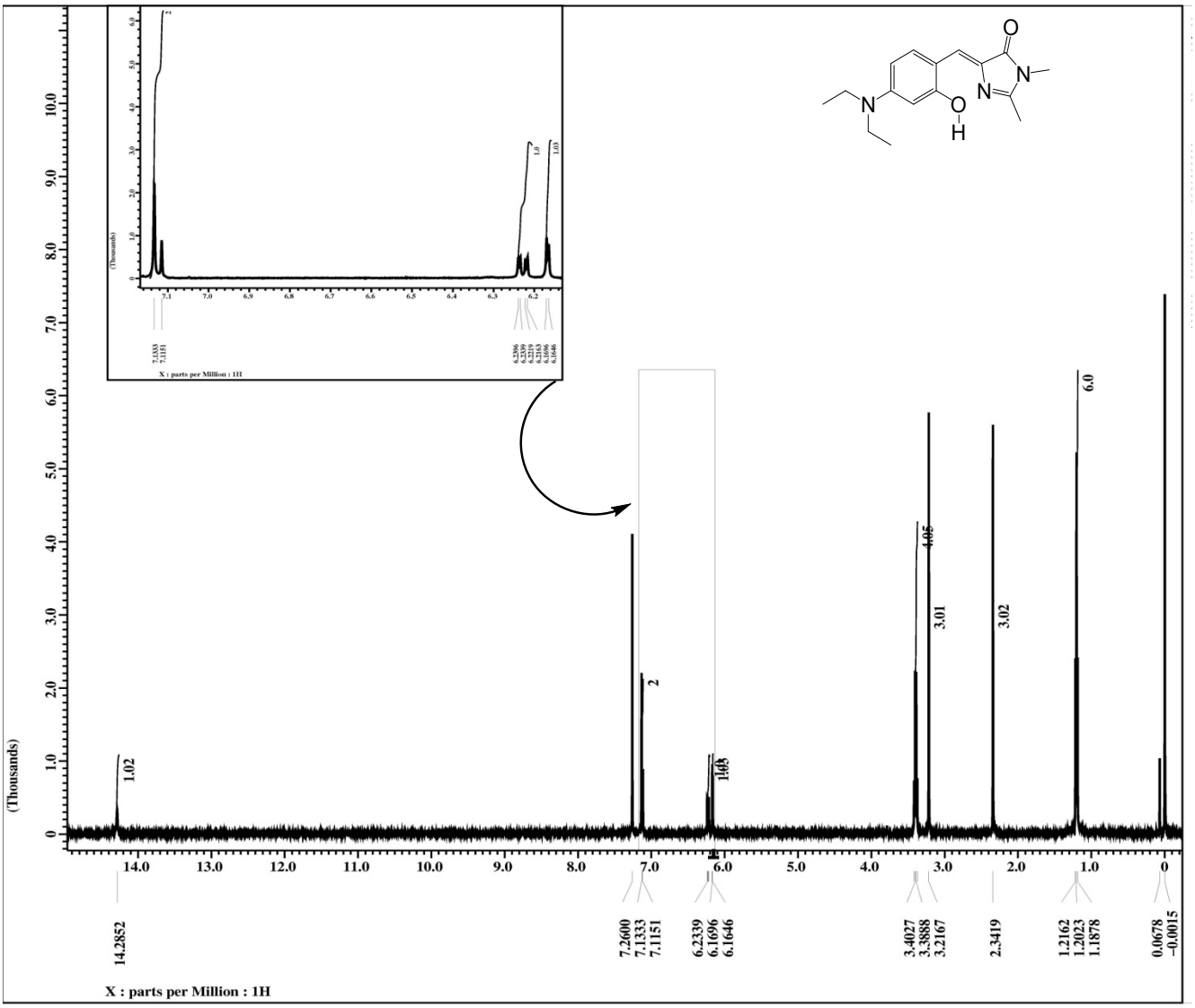


Figure 5: ^{1}H NMR spectrum of OHIM.

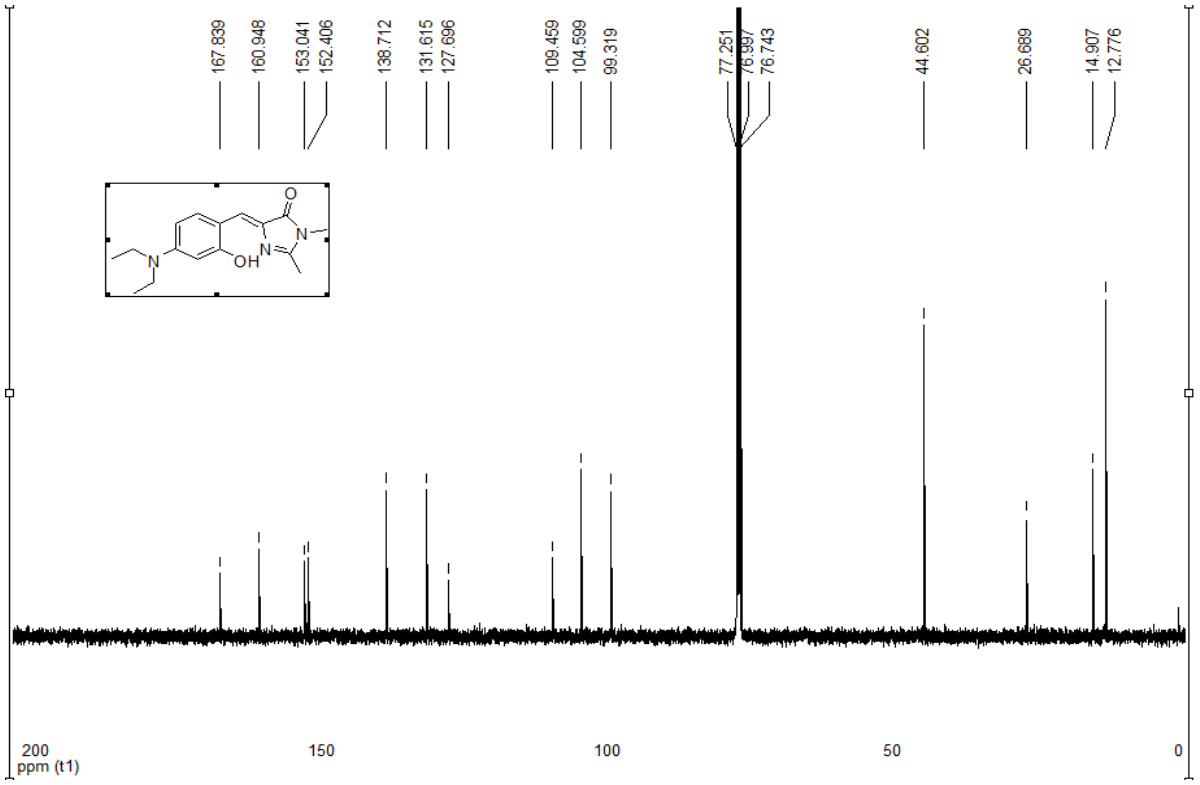


Figure 6: ^{13}C NMR spectrum of OHIM.

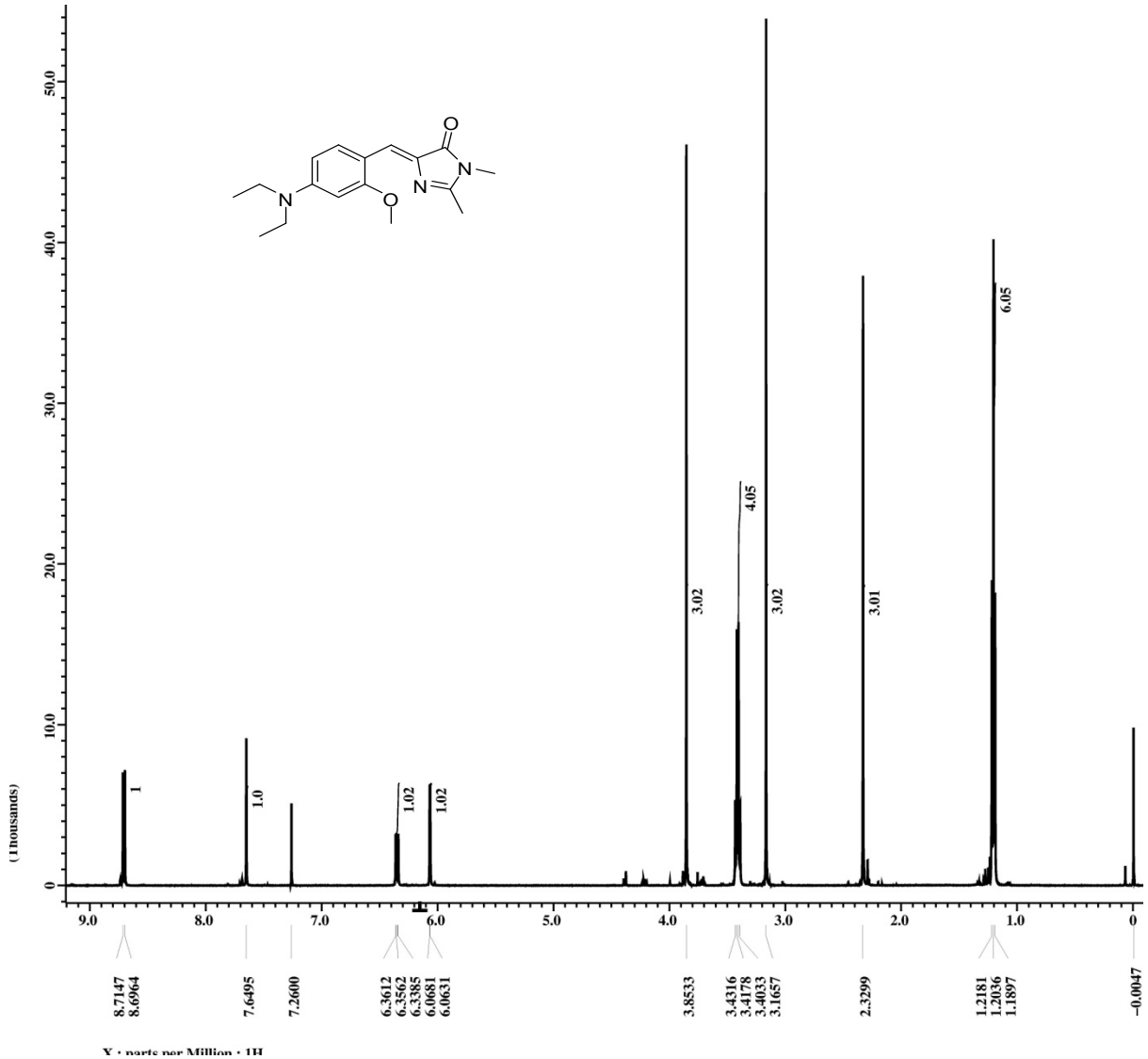


Figure 7: ^{1}H NMR spectrum of OMIM.

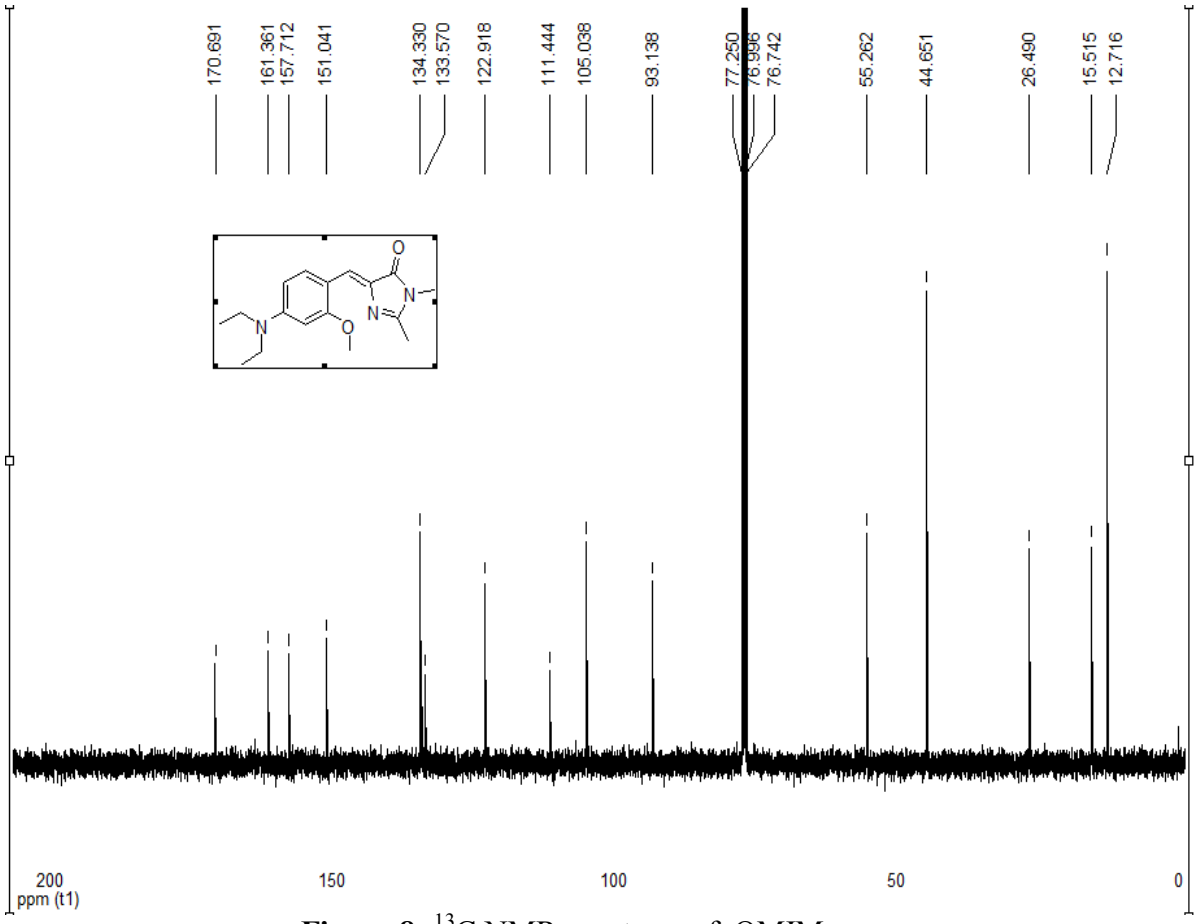


Figure 8: ^{13}C NMR spectrum of OMIM.

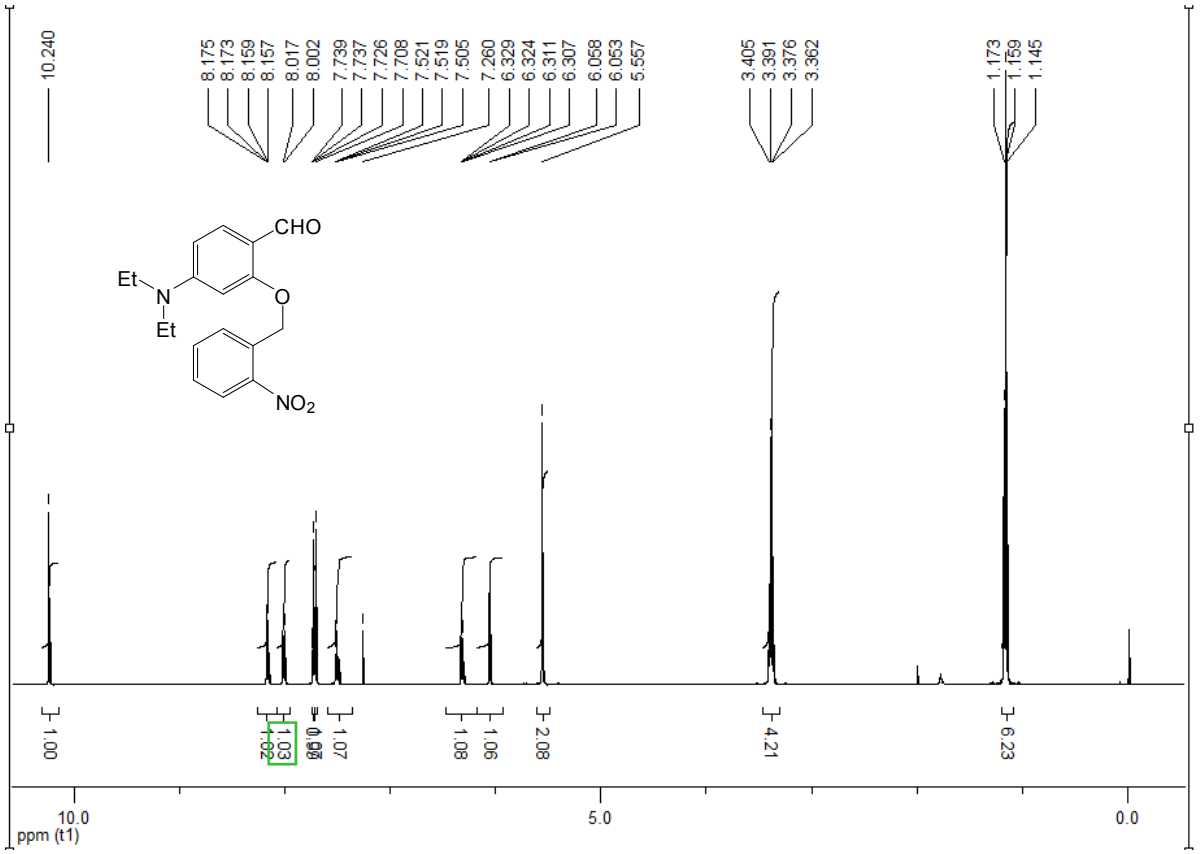


Figure 9: ^{1}H NMR spectrum of **ONBY- PROTECTED ALDEHYDE**

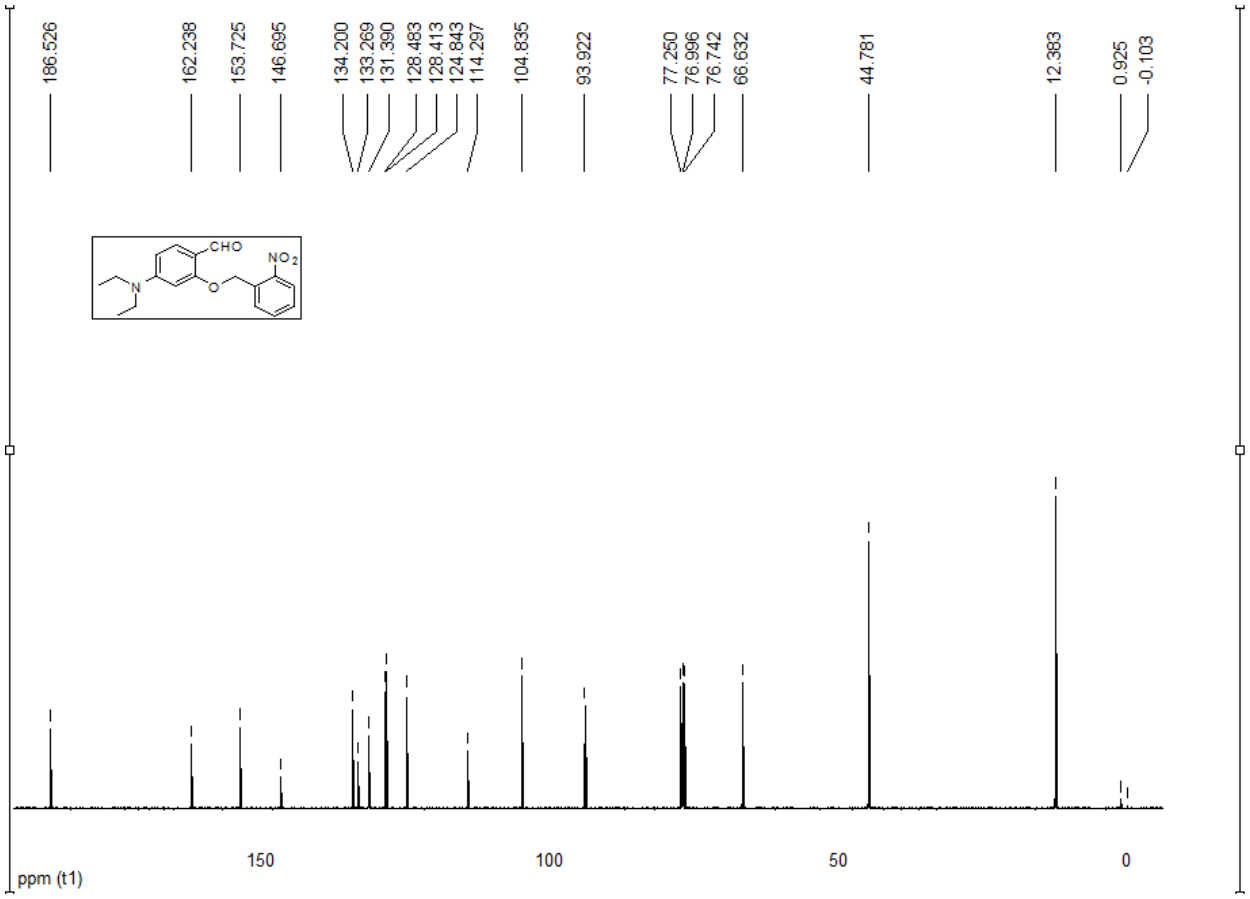


Figure 10: ^{13}C NMR spectrum of **ONBY- protected aldehyde**

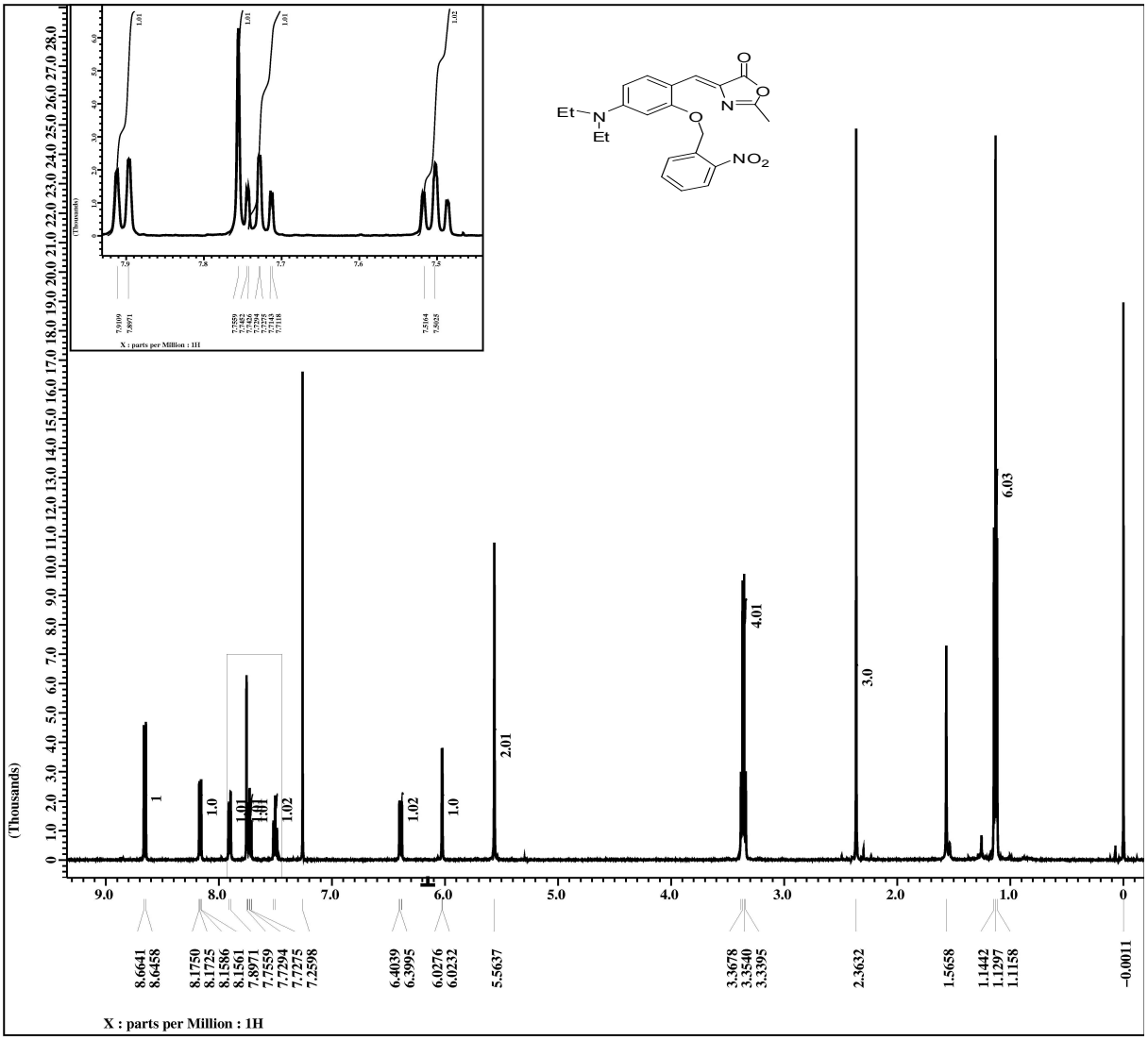


Figure 11: ^{1}H NMR spectrum of ONBYOHBO.

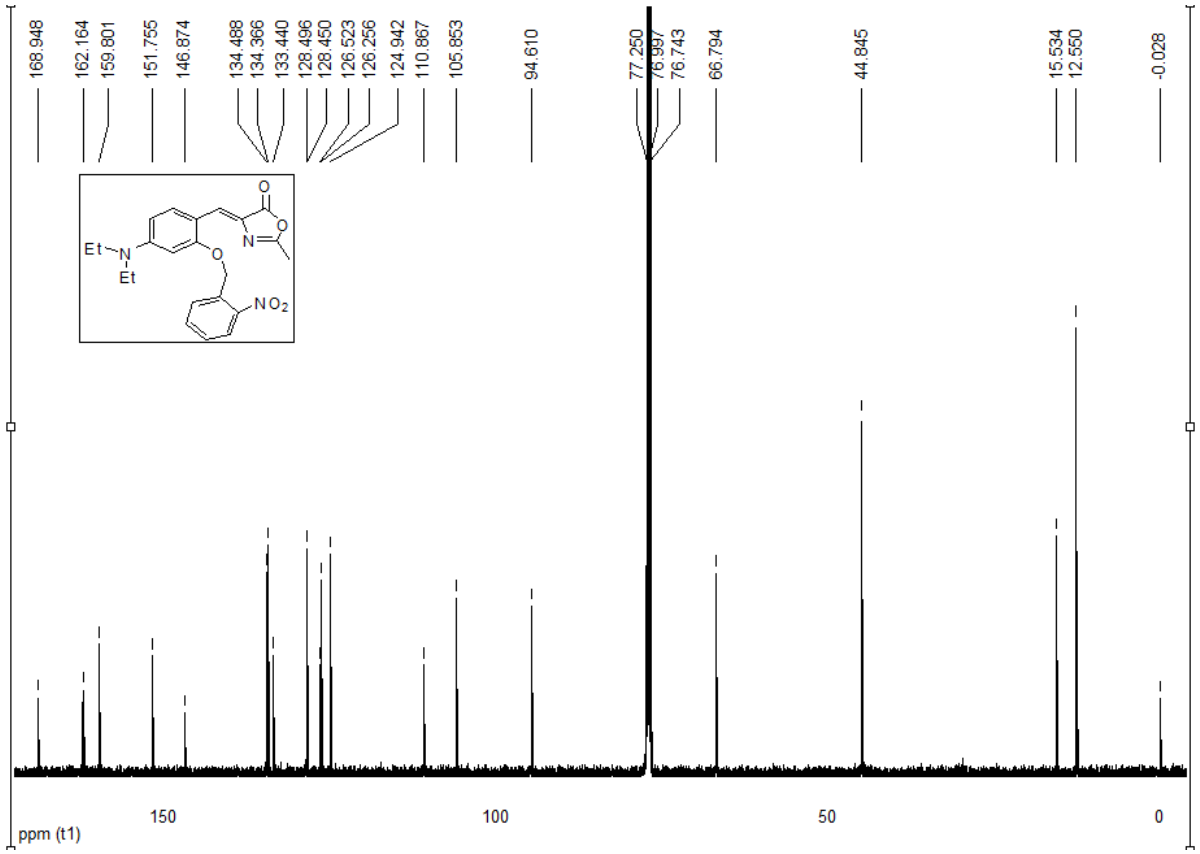


Figure 12: ^{13}C NMR spectrum of ONBYOHBO

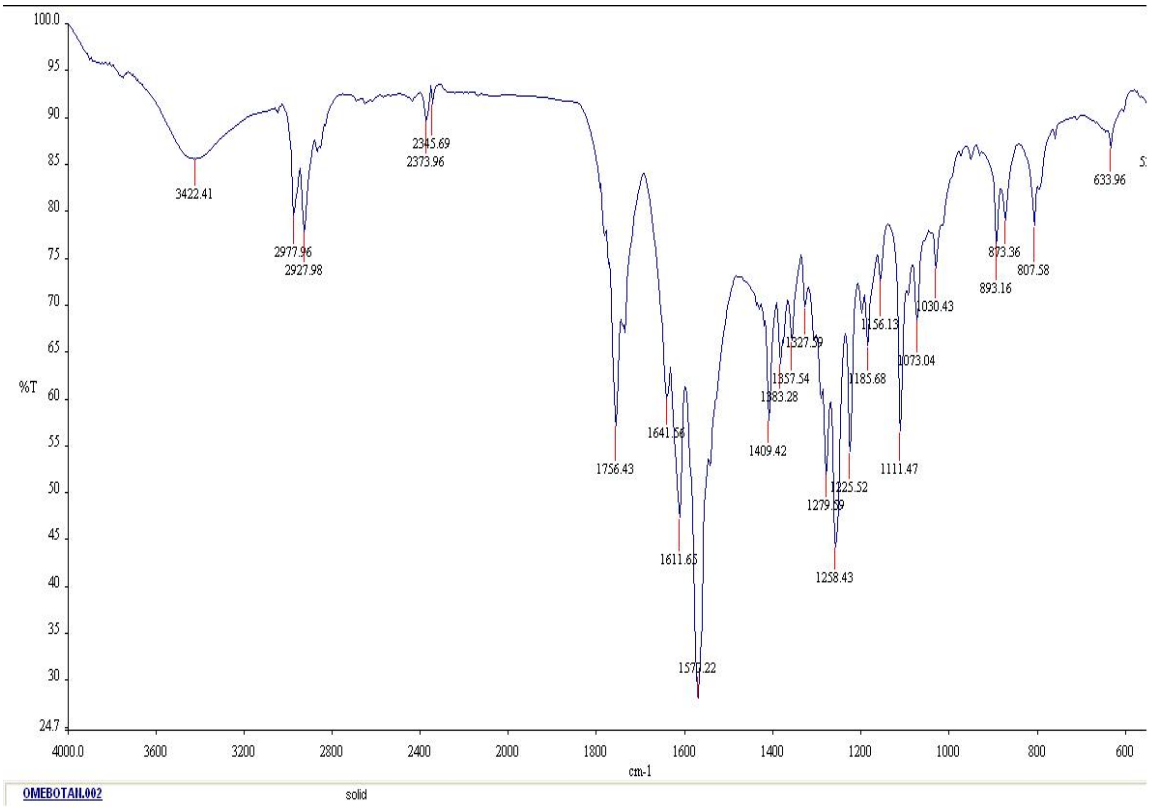


Figure 13: IR spectrum of OMBO.

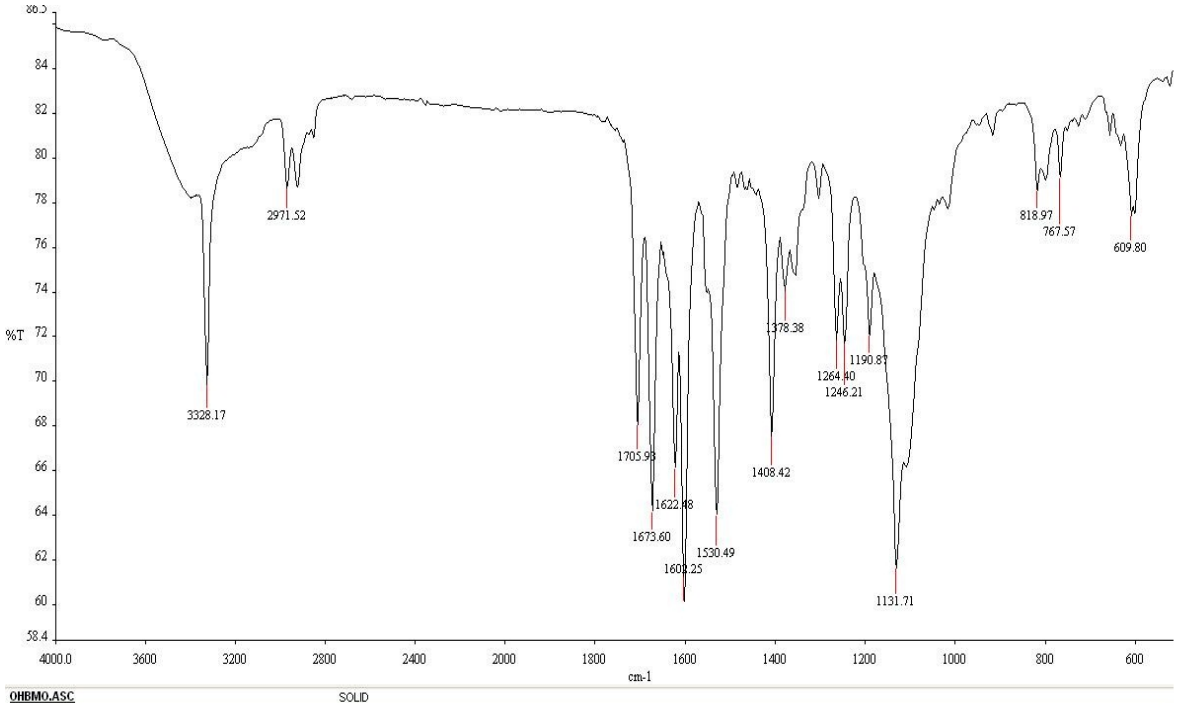


Figure 14: IR spectrum of cOHBO.

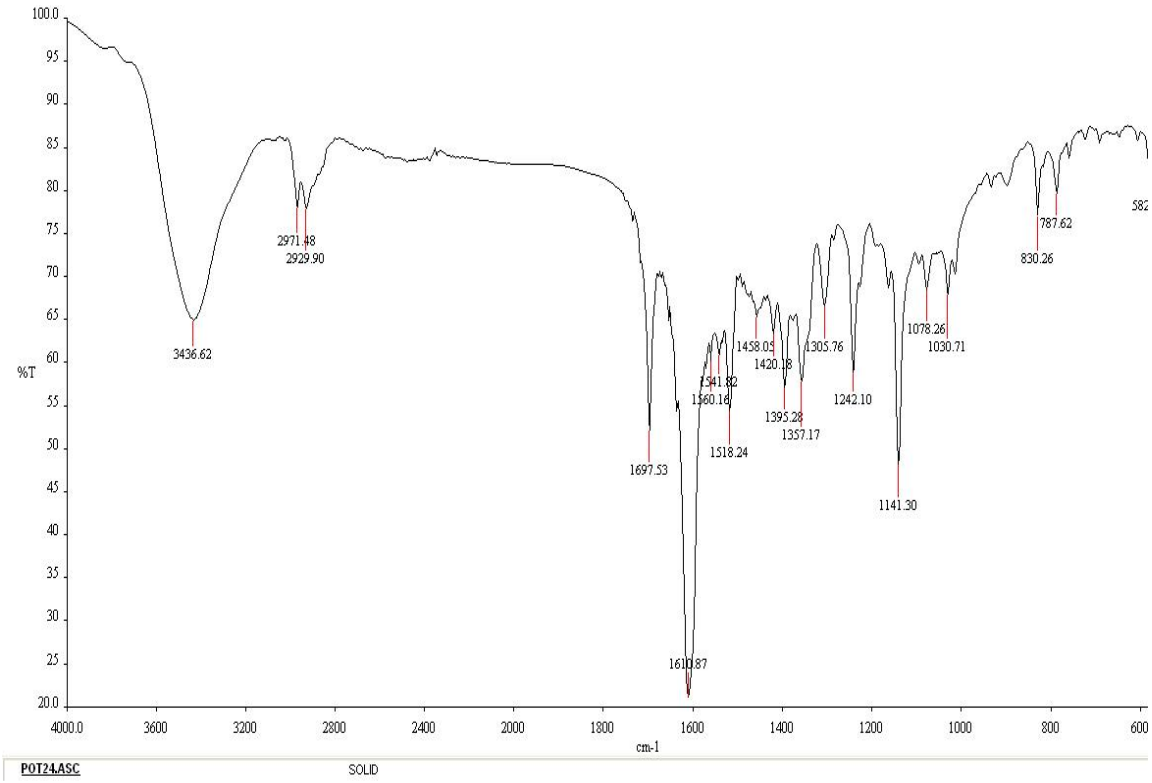


Figure 15: IR spectrum of OHIM.

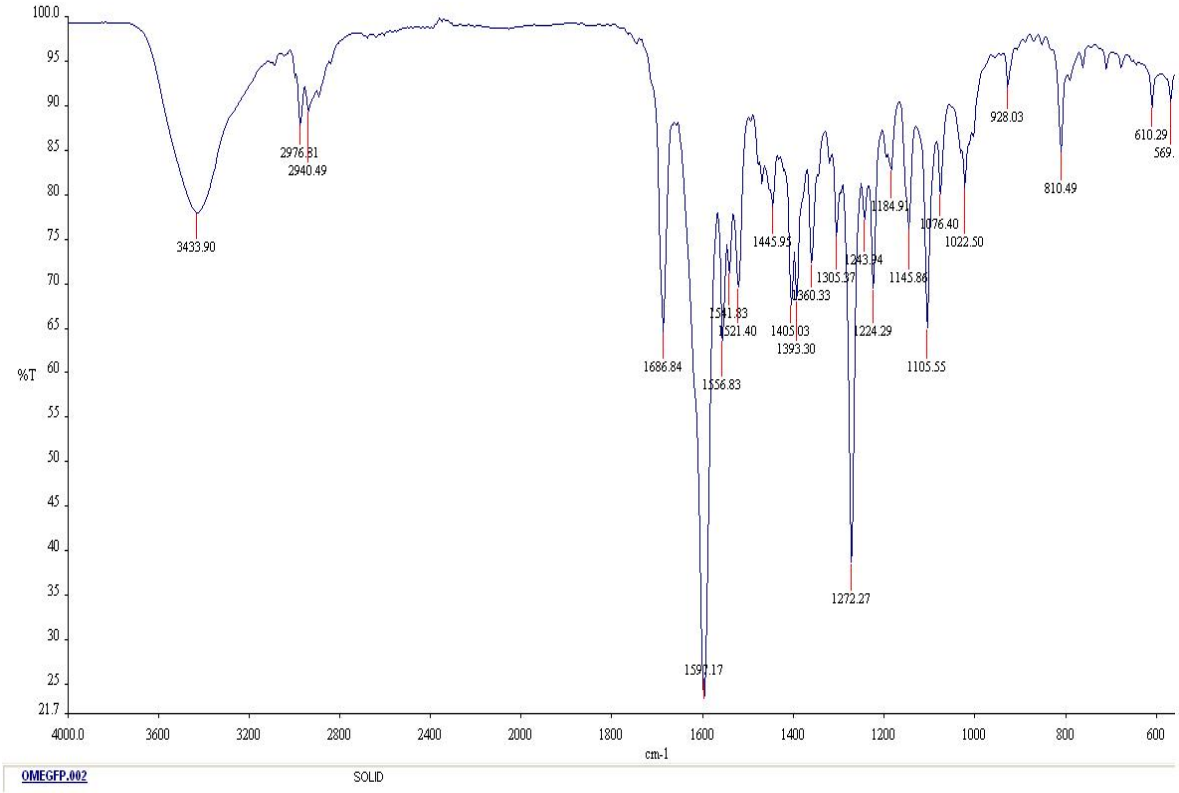


Figure 16: IR Spectrum of OMIM.

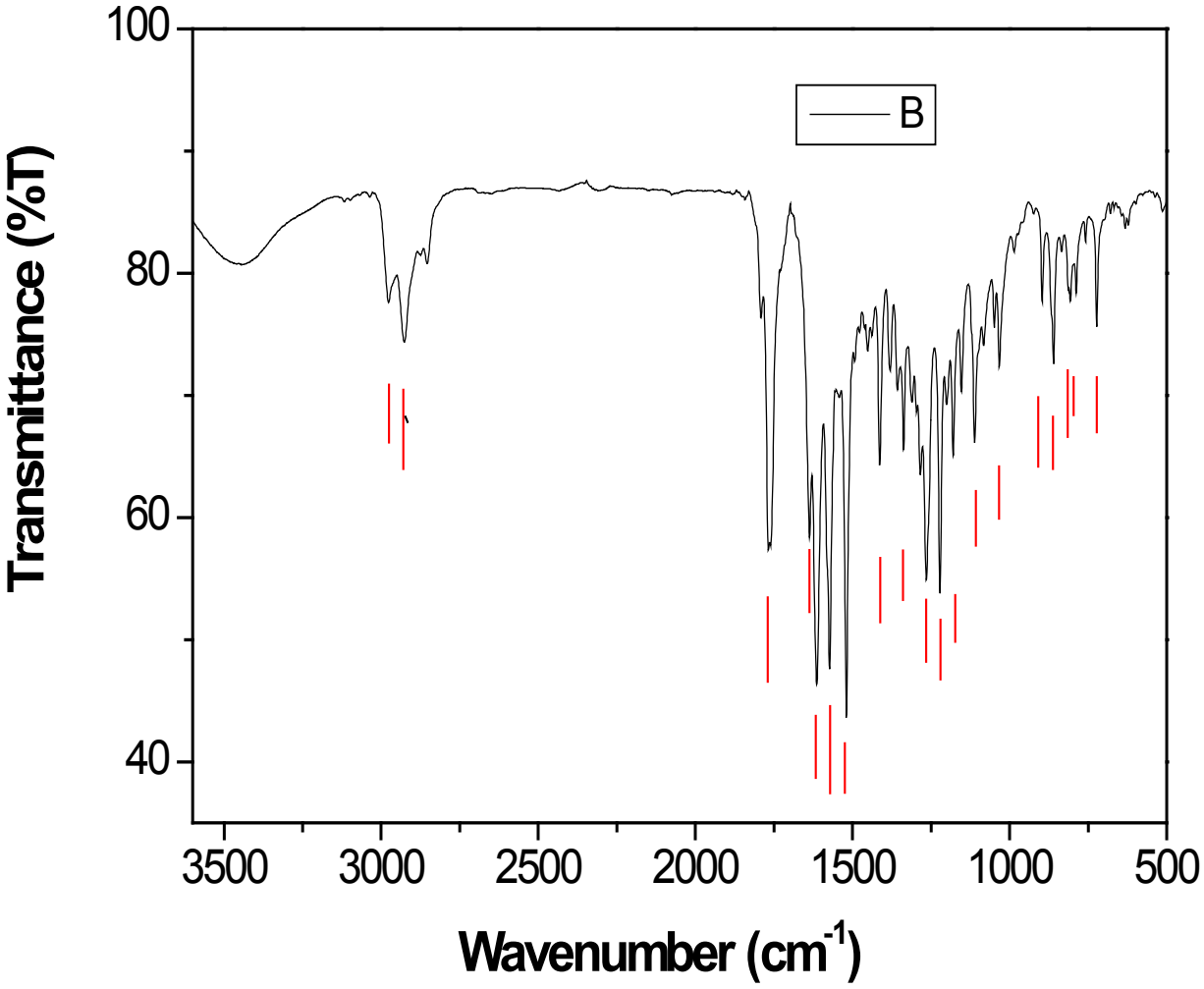


Figure 17: IR Spectrum of ONBYOHBO

S3: Normalised absorption and emission spectra of OHIM, cOHBO, OMIM, and OMBO:

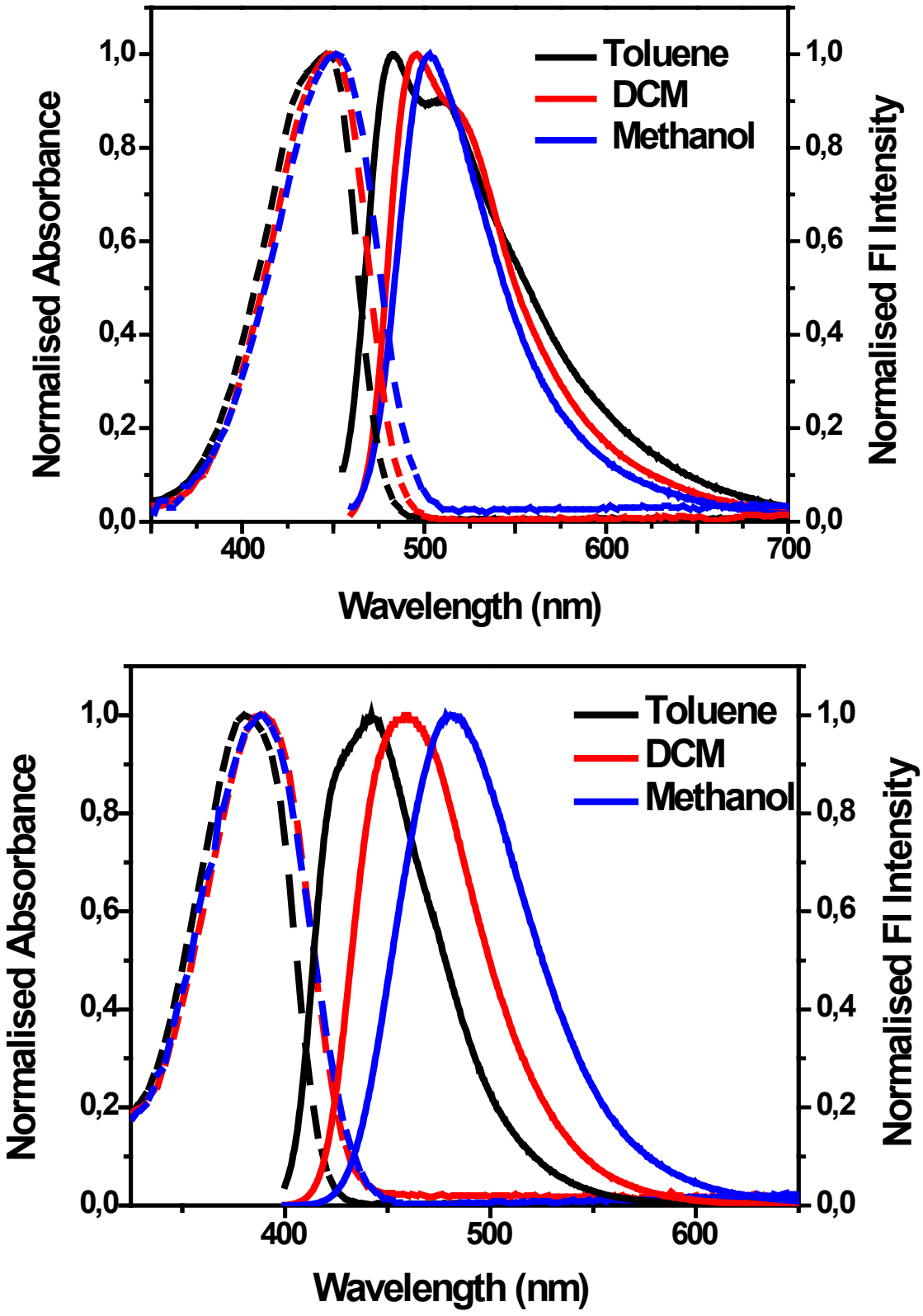


Figure 18: Normalized absorption (---) and fluorescence (–) spectra of OHIM (above) and cOHBO (below) in different solvents.

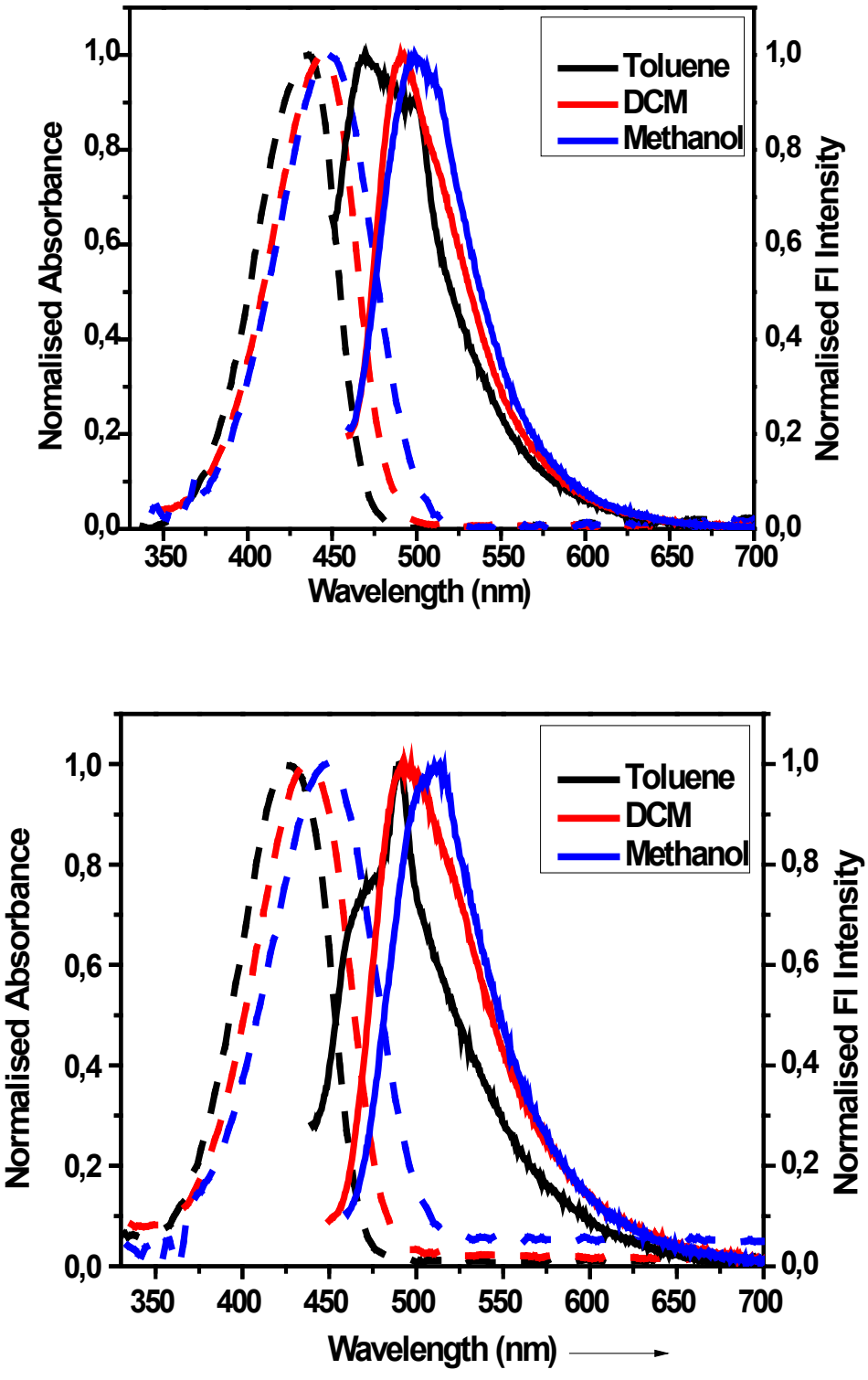
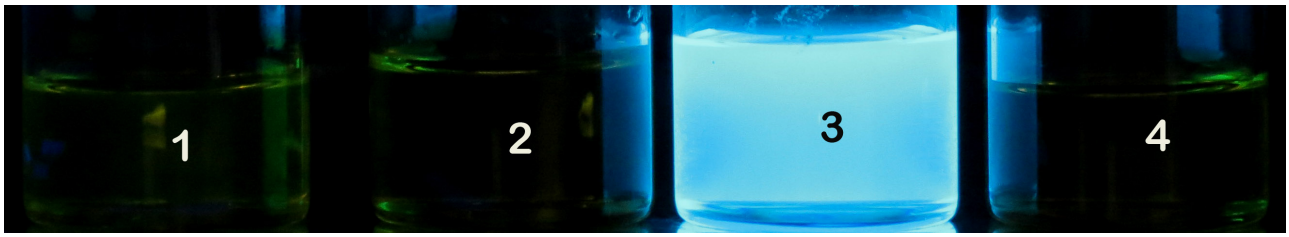


Figure 19: Normalized absorption (---) and fluorescence (–)spectra of OMBO (above) OMIM (below) in different solvents.

S4: Naked eye visualization of enhanced fluorescence of cOHBO (3) in comparison to OHIM (1), OMIM (2), and OMBO (4).



S5: Fluorescence decay curves of OHIM, cOHBO, OMIM, and OMBO

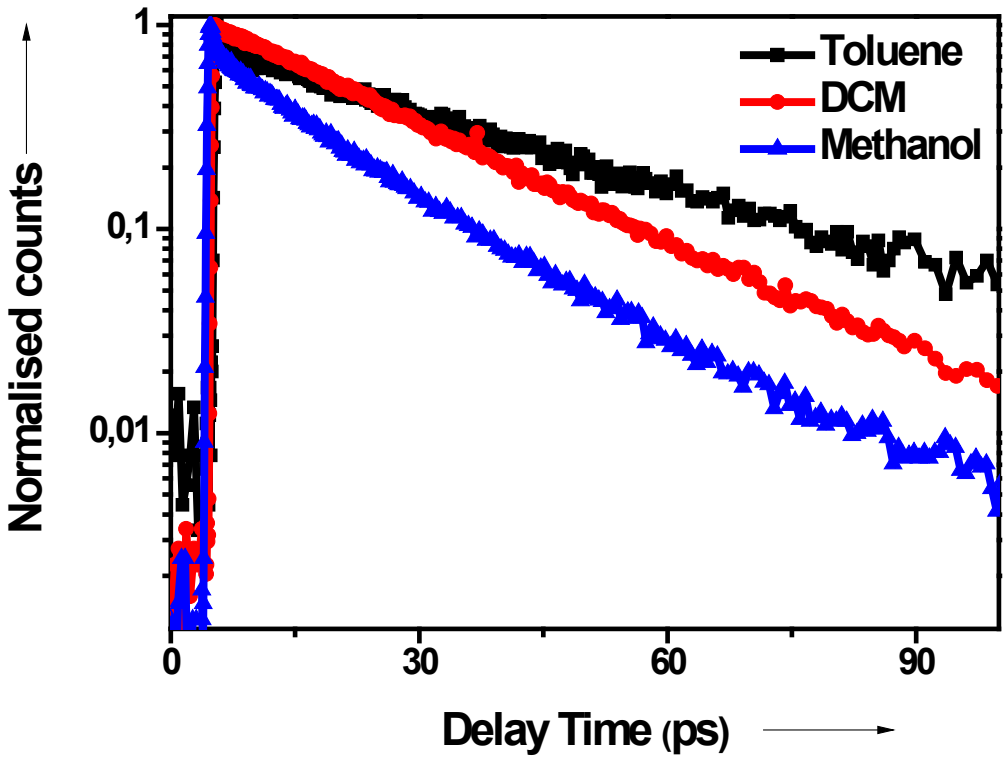


Figure 20: Fluorescence upconversion decay of OHIM in three solvents.

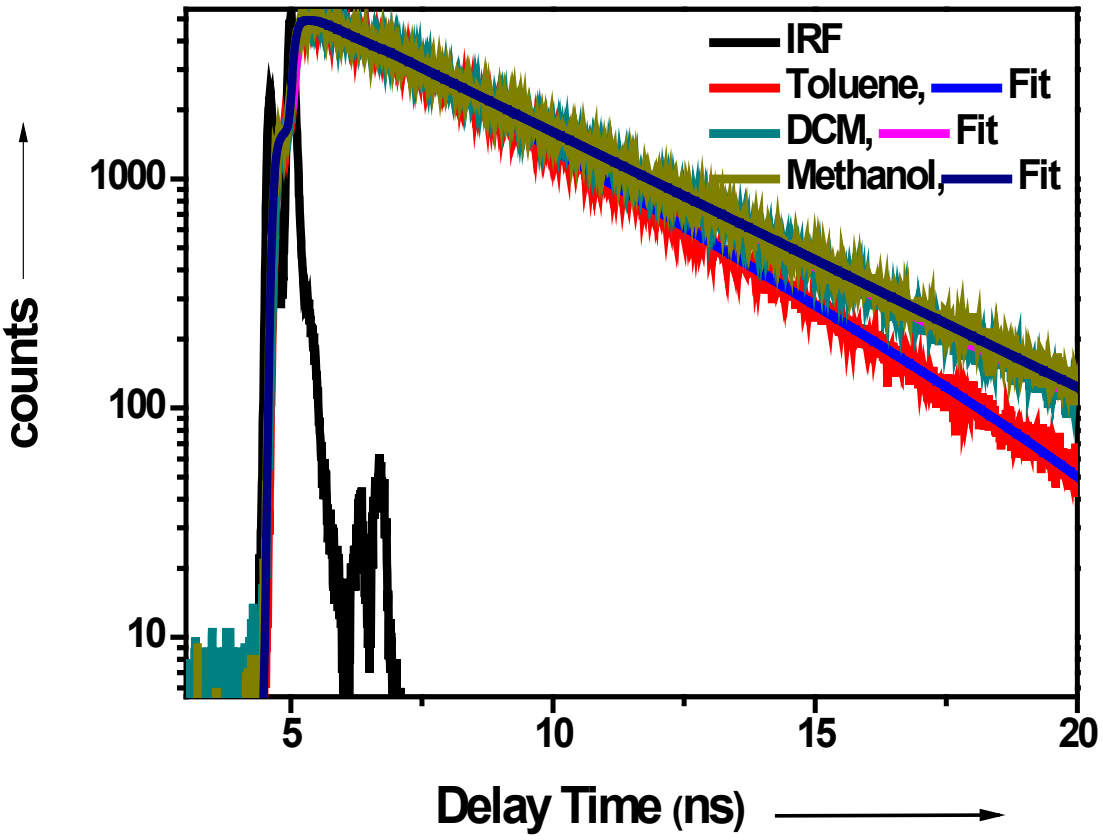


Figure 21: Fluorescence (TCSPC) decay curves of cOHBO in three solvents.

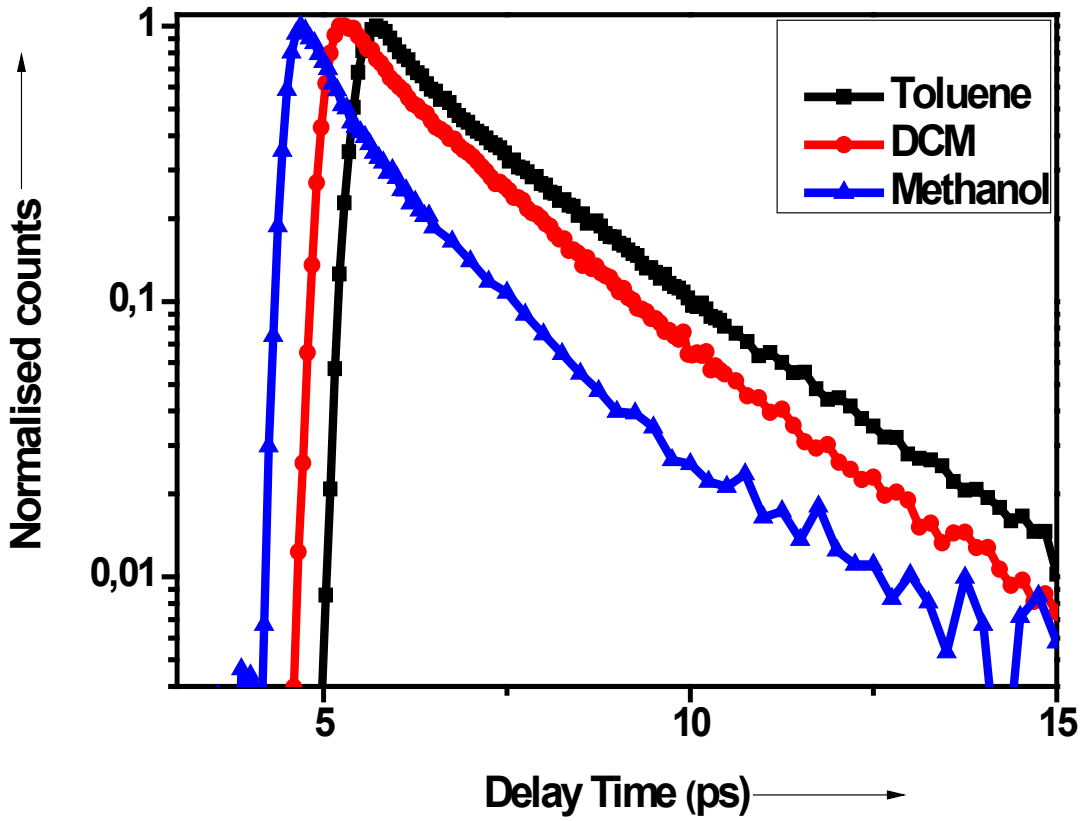


Figure 22: Fluorescence upconversion decay of OMIM in three solvents.

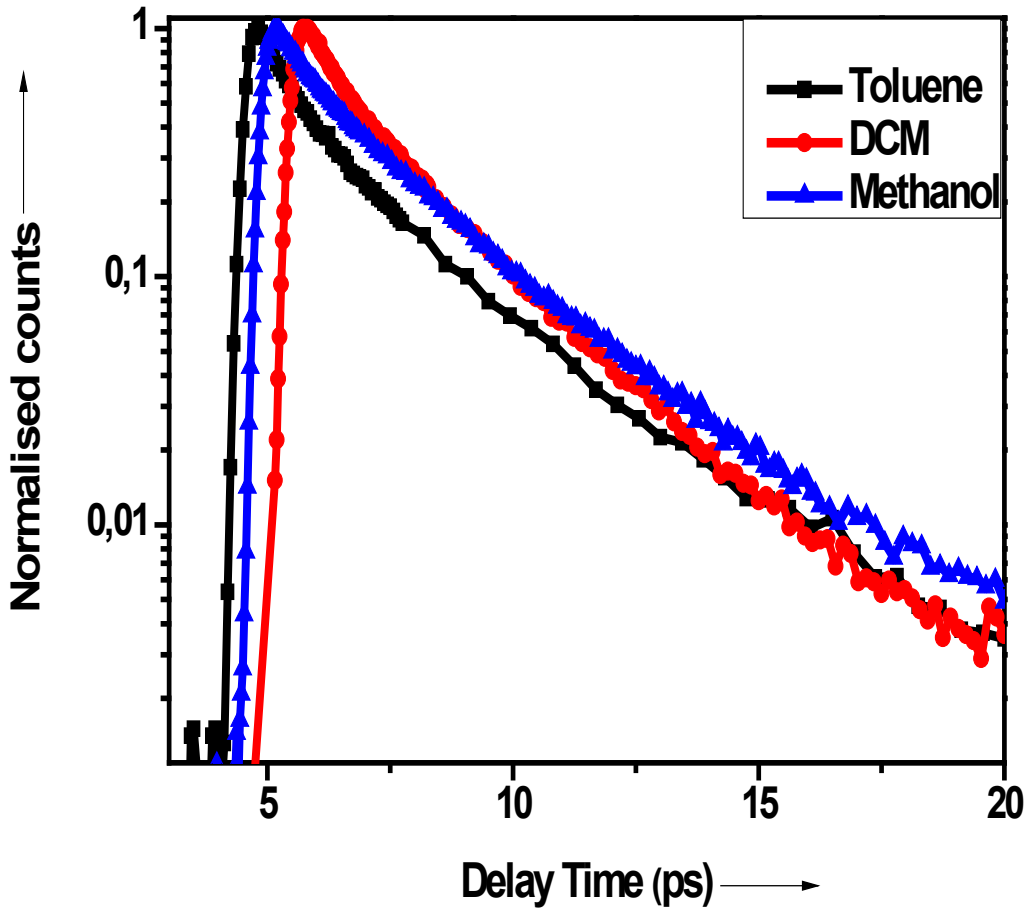


Figure 23: Fluorescence upconversion decay of OMBO in three solvents.

S6: Detailed fluorescence decay parameters of OHIM, OMIM, OMBO, and cOHBO:

compound	Solvent	λ_{ex} (nm)	λ_{em} (nm)	τ_1 (ps)	B_1	τ_2 (ps)	B_2
OHIM	Toluene	400	485	0.76	25.48	32.8	74.52
	DCM		495	0.42	0.048	22.20	99.95
	Methanol		510	0.73	35.47	13.90	64.53
OMIM	Toluene	400	485	0.96	44.82	2.35	55.18
	DCM		495	0.83	39.5	2.02	60.5
	Methanol		510	0.43	39.21	1.70	60.79
OMBO	Toluene	400	475	0.89	46.5	2.45	53.5
	DCM		495	0.86	45.4	2.74	54.6
	Methanol		500	0.650	56.53	2.52	43.47

Compound	Solvent	E^T_N	λ_{ex}(nm)	λ_{em}(nm)	τ (ns)	CHISQ
cOHBO	Toluene	0.09	377 402	445	2.63 2.99	1.10 1.12
	DCM	0.306	377 402	462	3.74 3.60	1.00 1.08
	Methanol	0.76	377 402	485	3.88 3.93	1.09 1.08

S7: Details of single crystal X-ray measurement and ORTEP diagram of cOHBO and ONBYOHBO:

The sample was transferred to a 10 ml conical flask followed by addition of pure methanol . The suspension was heated until a clear solution is obtaind . The resulting mixture was boiled for 10 min before being filtered into a fresh conical flask . The filtrate was left to evaporate slowly at ambient conditions . The single crystals suitable for X-Ray diffraction were obtained in a single day .The crystals were mounted on a glass pip . Intensity data were collected on a Brukar KAPPA APEX II CCD Duo system with graphite monochromatic Mo Kα radiation (λ = 0.71073 Å) . The data were collected at 100 K temperature for cOHBO and ONBYOHBO . Data reduction was performed using Bruker SAINT software.[5] Crystal structures were solved by direct methods using SHELEXL-97 and refined by full matrix least squares on F2 with anisotropic displacement parameters for non-H atoms using SHELXL-97.[6] Hydrogen atoms associated with carbon atoms were fixed in a geometrically constrained positions . Structure graphics shown in the figures were created using the X-Seed software package version 2.0.[7] Assymetric unit of ONBYOHBO contains one molecule and cOHBO contains two essentially identical molecule, only one of which is shown in figure1.

	cOHBO	**ONBYOHBO**
chemical formula	C15 H18 N2 O3	C22 H23 N3 O5
formula weight	274.31	409.44
cryst. system	monoclinic	Triclinic
space group	$P2_1/n$	P-1
a (Å)	12.521(2)	7.9078(8)
b (Å)	15.687(3)	10.8875(12)
c (Å)	14.616(2)	13.1152(14)
α (°)	90	72.008(2)
β (°)	100.589(4)	78.518(2)
γ (°)	90	80.023(2)
vol (Å^3)	2822.0(8)	1044.87(19)
D_{calcd} (g/cm^3)	1.291	1.301
μ (mm^{-1})	0.091	0.094
θ range (°)	2.36-20.06	2.92-29.09
Z	8	2
range h	–15 to +15	–4 to+10
range k	–20 to +20	–13 to +13
range l	–18 to +17	-16 to +16

reflns collected	34391	16944
independent reflns	5653	4565
obsd reflns	2508	3358
T (K)	100(2)	100(2)
*R*1	0.0652	0.0493
*wR*2	0.1454	0.1321
GOF	1.016	0.840
CCDC No.	CCDC 923722	CCDC 923721

S8: Fluorescence spectrum of ONBYOHBO, photo-irradiated product, and cOHBO:

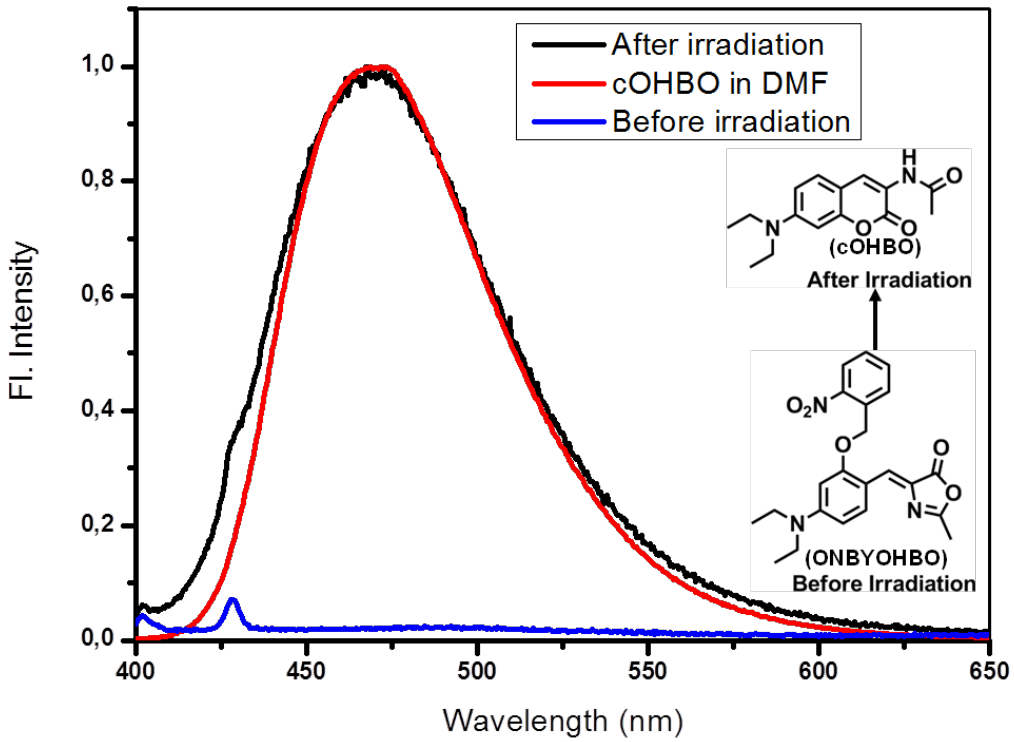


Fig. 24: Fluorescence emission spectra of substrate before irradiation (ONBYOHBO, fluorescence quantum yield = 0.0007) and photo-irradiation product (cOHBO). Fluorescence spectrum of cOHBO is also shown for comparison.

S9: Fluorescence decay parameters of photoactivation compounds:

Compounds	Solvent	Fluorescence lifetime
ONBYOHBO (i.e. compound before irradiation)	DMF	4.47 ps (84.24), 660 ps (8.16), 3.50 ns (7.60)
cOHBO (i.e. compound after irradiation)	DMF	3.45 ns
cOHBO	DMF	3.50 ns

S10: Live Stem cell imaging experimental details:

Live cell imaging was performed in human umbilical cord derived mesenchymal stem cells.* These cells were grown in Knock-out Dulbecco's modified Eagle's medium (DMEM-KO, Invitrogen, CA, USA) supplemented with 10% foetal bovine serum (FBS, Hyclone, Victoria, Australia), 2mM L-glutamine (Invitrogen) and 0.5x Antibiotic-Antimycotic (Invitrogen). All cells were incubated at 37^0C with 5% humidified CO_2. Prior to measurements cells were seeded on sterile fluorodish at 50,000 cells/dish. Cells were then incubated with dyes (concentration ~ 10 μM) in 1% DMSO concentration (v/v) in DMEM culture medium for about two hr. Then the cells were washed once with DPBS and stored with DMEM-KO culture medium prior to imaging.

The mesenchymal stem cells were imaged using confocal microscope (Carl Zeiss , CLSM-710) , with 405 nm Diode laser used as excitation source, capturing the emission bandwidth (421 nm-562 nm) using appropriate 405 MBS (main beam splitter), with plan-Apochromat 40X, N.A 1.3 oil immersion objective; by simultaneous imaging and captured on a PMT. Size of images is whole image 512 pixel x 512 pixel (212.13 μm * 212.13 μm). The images were of 8 bit depth taken using a 29 μm pinhole and 2 frame average. DIC image was also captured alongside the fluorescence signal on the T-PMT (Transmitted light PMT).

* Human umbilical cords were collected from full-term births after either caesarean section or normal vaginal delivery with informed consent using the guidelines approved by the Institutional Ethics Committee (IEC) at the IISER, Kolkata, India. Authors thank Dr. Malancha Ta (IISER-Kolkata) for getting the necessary ethical clearance for live stem cell imaging.

References:

[1] Y. M. Cheng, S. C. Pu, Y. C. Yu, P.T. Chou, C. H. Huang, C. T. Chen, T. H. Li, W. P.Hu**,** *J. Phys .Chem. A,* 2005**, 109**, 11696-11706.
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[5] SAINT *Plus* (version 6.45); Bruker AXS Inc.: Madison, WI, 2003.
[6] SMART (version 5.625) and SHELX-TL (version 6.12); Bruker AXS Inc.: Madison, WI, 2000.
[7] Barbour, L. J. *X-Seed, Graphical Interface to SHELX-97 and POV-Ray*; University of Missouri; Columbia: Columbia, MO, 1999.