Electronic Supplementary Material (ESI) for Journal of Materials Chemistry C.

Supporting Information

Deciphering the proficiency of aliphatic/aromatic functionality on heteroatom embedded planar polycyclic core: towards an advanced onsite detection of Tetracycline and Oxytetracycline

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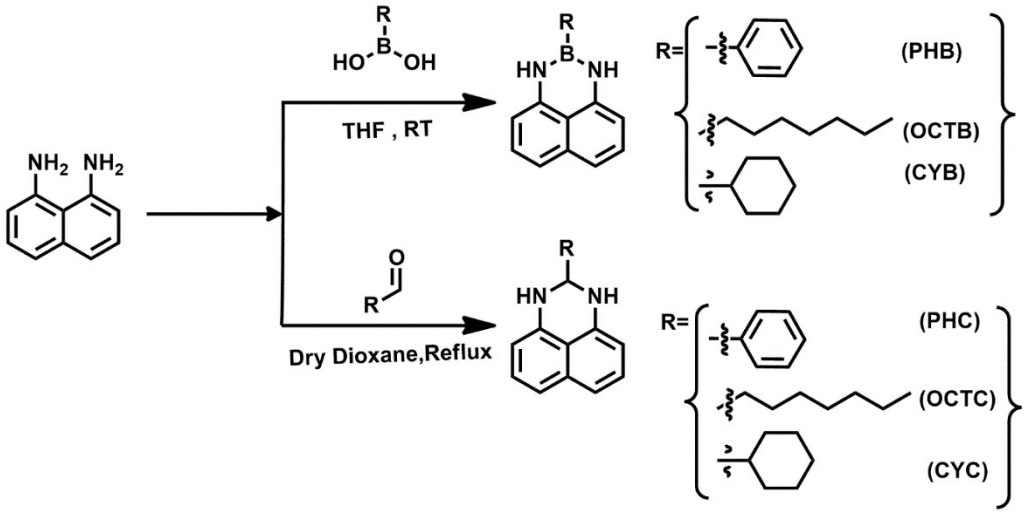
1. Experimental Section

1.1. Materials and methods

All the required precursor materials and other reagents (1,8-Diaminonaphthalene, phenylboronic acid, octyl boronic acid, cyclohexyl boronic acid, benzaldehyde, Nonanal, cyclohexanecarbaldehyde, Tetracycline hydrochloride, Oxytetracycline hydrochloride) were purchased from Sigma Aldrich (INDIA). HPLC grade solvents were purchased from Zenith India and Northeast Chemicals. NMR (^{1}H, ^{13}C) spectra were recorded with a Varian-AS400 NMR spectrometer or Bruker Avance 600 MHz spectrometer. All solutions for ^{1}H and ^{13}C spectra were obtained taking residual solvent signal as internal reference. Electro spray ionization mass (ESI-MS) spectra were recorded on a Waters (Micro mass MS-Technologies) Q-Tof MS Analyzer spectrometer. Microbalance (± 0.1mg) and volumetric glassware were used for the preparation of solutions. UV/vis and PL spectra were recorded on a Perkin-Elmer Model Lambda-750 spectrophotometer and a Horiba Fluoromax-4 spectrofluorometer respectively using 4 mm quartz cuvettes at 298 K.

1.2. Syntheses of Compounds[1]



Scheme 1 Synthesis procedure for all the congeners

1.2.1. Synthesis of Compounds PHB, OCTB and CYB

At room temperature naphthalene-1,8-diamine (100 mg, 0.633 m.mol) and phenylboronic acid (78 mg, 0.633 m.mol for **PHB**) was taken in 50 mL round bottom flask and 10 mL THF was added as a solvent. Then the mixture was stirred for 15 hours at room temperature. After the reaction was over, the mixture was cooled to room temperature, solvent evaporated using rotatory evaporator under low pressure. The mixture was washed with water and extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulphate and dried using rotatory evaporator. The residue was purified by column chromatography over silica gel using 2% ethyl acetate in hexane to obtain the pure product. Same procedure was followed with Octyl boronic acid (for **OCTB**) and cyclohexyl boronic acid (for **CYB**)

Characterization Data of PHB[1]: Grey colored solid (105 mg, 68.08% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.1 Hz, 2H), 7.50 – 7.44 (m, 3H), 7.16 (t, *J* = 7.7 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.43 (d, *J* = 7.3 Hz, 2H), 6.04 (s, 2H). 13C NMR (150 MHz, $CDCl_3$) δ 141.2, 136.5, 131.6, 130.4, 128.4, 127.8, 120.0, 118.0, 106.2. HRMS (+ ESI): Calculated for $C_{16}H_{13}BN_2$, 244.1182 [M] +.

Characterization Data of OCTB: Light grey colored solid (138 mg, 77.98% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09 (t, *J* = 8.1 Hz, 2H), 6.99 (d, *J* = 8.1 Hz, 2H), 6.29 (d, *J* = 7.3 Hz, 2H), 5.60 (s, 2H), 1.45 – 1.40 (m, 2H), 1.35 – 1.24 (m, 10H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.87 – 0.83 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 141.4, 136.5, 127.7, 119.7, 117.4, 105.5, 32.7, 32.1, 29.6, 29.4, 24.9, 22.8, 14.3. HRMS (+ESI): Calculated for $C_{18}H_{25}BN_2$, Found 281.2203 [M+H]+

Characterization Data of CYB: Dark grey colored solid (102 mg, 64.55% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (t, *J* = 8.1 Hz, 2H), 6.99 (d, *J* = 8.1 Hz, 2H), 6.29 (d, *J* = 7.3 Hz, 2H), 5.59 (s, 2H), 1.78 – 1.70 (m, 5H), 1.32 – 1.21 (m, 5H), 0.99 – 0.93 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 141.4, 136.5, 127.7, 119.7, 117.4, 105.6, 30.5, 29.0, 27.6, 26.9. HRMS (+ESI): Calculated for $C_{15}H_{19}BN_2$, Found 251.1735 [M+H]+.

1.2.2. Syntheses of Compounds PHC, OCTC, and CYC

Naphthalene-1,8-diamine (100 mg, 0.633 m.mol) and benzaldehyde (193 µL, 0.633 for **PHC**) was taken in 50 mL round bottom flask and 10 mL dry dioxane was added as a solvent under inert condition. The reaction mixture was stirred for 24h at 90 °C temperature, followed by evaporation using rotatory evaporator under low pressure. The mixture was washed with water and extracted with ethyl acetate and the organic phase was dried over anhydrous sodium sulphate and evaporated using rotatory evaporator. The residue was purified by column chromatography over silica gel using 3% ethyl acetate in hexane to obtain the pure product. Same procedure was followed with Nonanal acid (for **OCTC**) and Cyclohexanecarboxaldehyde (for **CYC**)

Characterization Data of PHC[1]: Light yellow powder (70 mg, 44.96% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 6.4, 2.8 Hz, 2H), 7.38 (dd, *J* = 4.8, 1.6 Hz, 3H), 7.20 – 7.15 (m, 4H), 6.46 (dd, *J* = 7.1, 0.7 Hz, 2H), 5.40 (s, 1H), 4.51 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 142.2, 140.1, 135.0, 129.8, 129.0, 128.1, 127.0, 118.1, 113.6, 106.0, 68.6. HRMS (+ESI): Calculated for $C_{17}H_{14}N_2$, Found 247.1230 [M+H]+.

Characterization Data of OCTC: : Light yellow powder (83mg, 46.57% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (t, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 6.51 (d, *J* = 7.2 Hz, 2H), 4.49 (t, *J* = 5.7 Hz, 1H), 4.36 (s, 2H), 1.78 – 1.74 (m, 2H), 1.53 – 1.48

(m, 2H), 1.44 – 1.22 (m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 142.1, 135.0, 127.0, 117.8, 114.2, 106.0, 65.00, 36.08, 32.0, 29.7, 29.6, 29.3, 24.6, 22.81, 14.3. HRMS (+ESI): Calculated for $C_{19}H_{26}N_2$, Found 283.2192 [M+H]+.

Characterization Data of CYC: : Pale yellow powder (78 mg, 48.97% yield), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (t, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 6.49 (d, *J* = 7.3 Hz, 2H), 4.41 (s, 2H), 4.27 (d, *J* = 5.4 Hz, 1H), 1.93 – 1.90 (m, 2H), 1.86 – 1.80 (m, 2H), 1.75 – 1.72 (m, 1H), 1.67 – 1.62 (m, 1H), 1.32 – 1.26 (m, 2H), 1.24 – 1.16 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 142.1, 135.0, 127.0, 117.4, 113.9, 105.8, 69.1, 42.3, 28.0, 26.5, 26.1. HRMS (+ESI): Calculated for $C_{17}H_{20}N_2$, Found 253.1709 [M+H]+.

1.3. Fluorescence quantum yield calculation:

Quinine sulphate (Φ_r = 0.52 in 0.1N H_2SO_4) was used as a reference to record the fluorescence quantum yield of all the probes in solution state (methanol) and aggregate state (water). Quantum yield values were calculated using below equation.

$$\Phi_s = \Phi_r(A_r E_s / A_s E_r)(\eta_s^2/\eta_r^2)$$

Here, ‘s’ and ‘r’ mentioned in the subscript, denote the sample and reference fluorophore respectively. A and E represent Absorbance and Integrated fluorescence emission intensity. η signifies refractive index of the solvent.

1.4. Sensing Studies

10 mM stock solution of OCTB was prepared by using methanol as a good solvent. Next stock solution was diluted into 20μM nano-aggregate solution using HEPES buffer as a bad solvent which further used for the sensing studies. The emission spectra of OCTB nano-aggregates was recorded under 330 nm excitation at slit 3 in

a 3ml cuvette. After this, the change in the fluorescence intensity was recorded with instant addition of the tetracycline and oxytetracycline stock (similarly prepared in water using their hydrochloride salt) up to 260μM. The same procedure has been implied for the interference study. Later, the decrement in the fluorescence intensity was further quantified using smartphone application named “Color Picker” under 365nm light which provide a prototype platform for the rapid detection of these antibiotics.

2. Theoretical Data

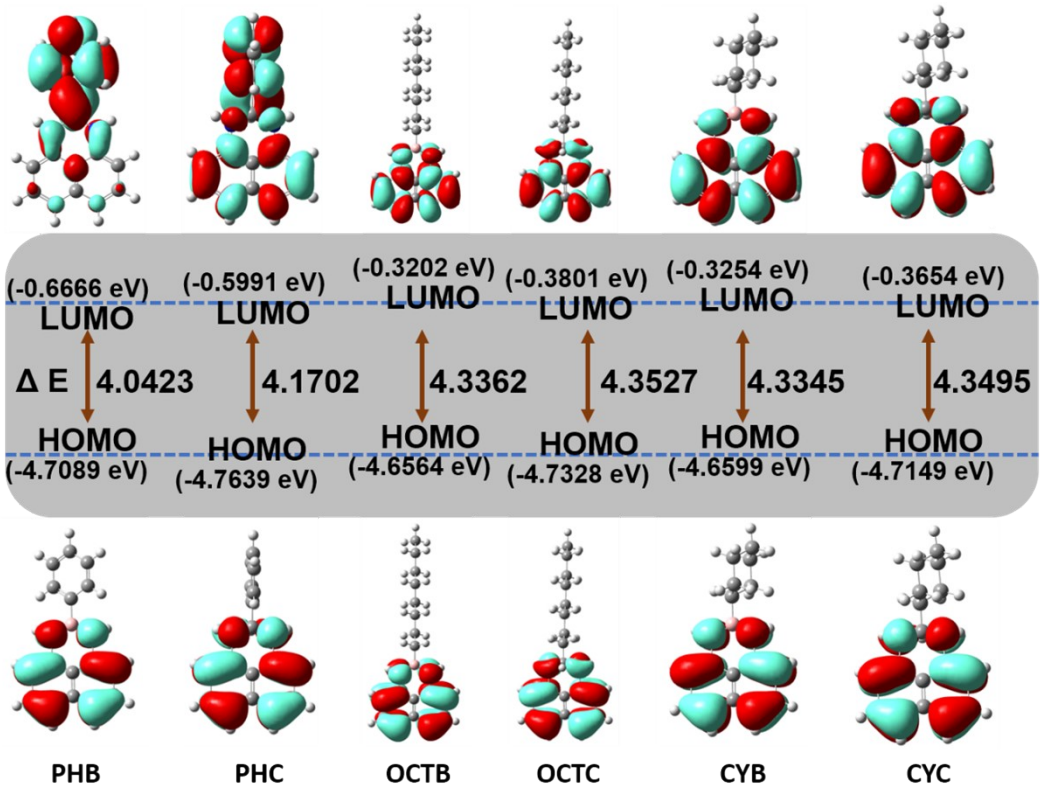


Figure S1 Calculated ground state HOMO-LUMO energy profile and distribution of electronic density in gaseous phase for all the probes using DFT with B3LYP/6-31G set in in the Gaussian 09 package.

3. Experimental Data

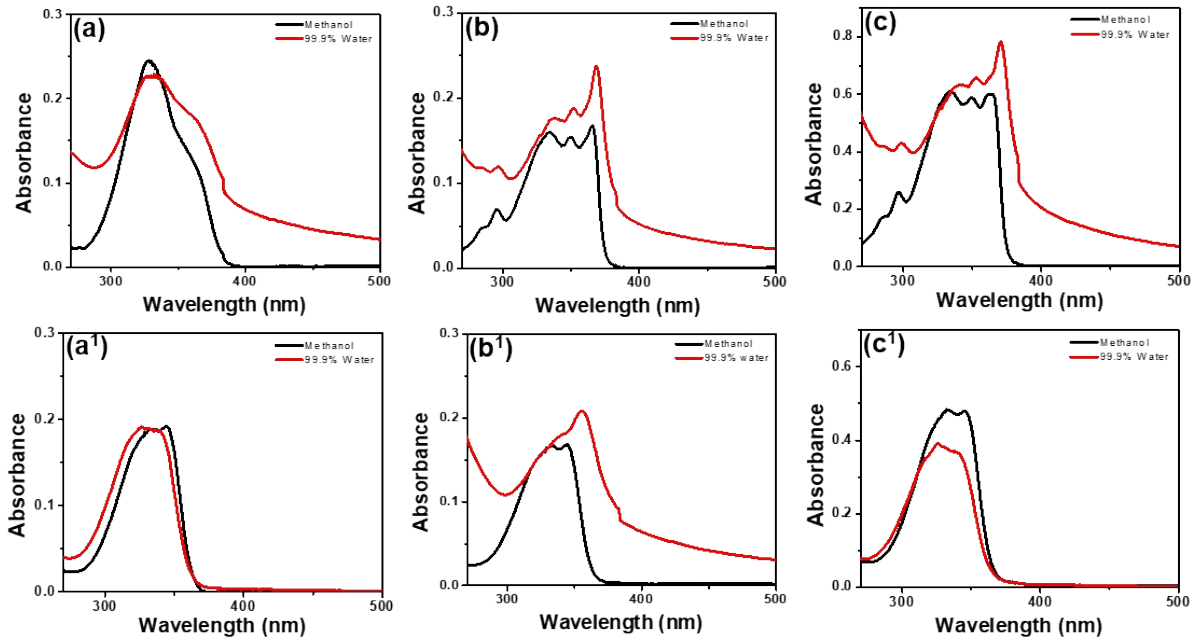


Figure S2 UV/visible spectra of (a)**PHB**, (b)**OCTB** (c)**CYB** (a^1) **PHC** (b^1) **OCTC** (c^1)**CYC** in MeOH (black) and 99.9% water fraction (red)

Table S1 Summarization of optical properties

Name	Excitation (λ_{ex}) (nm)	Emission in solution (λ_{em1}) (nm)	Emission in Aggregate(λ_{em2}) (nm)	QY (solution) (%)	QY (aggregate) (%)
PHB	**330**	**500**	**450**	**0.124**	**0.885**
OCTB	**330**	**390**	**425**	**34.664**	**11.402**
CYB	**330**	**389**	**420**	**25.992**	**8.515**
PHC	**330**	**390**	**410**	**8.632**	**3.135**
OCTC	**345**	**390**	**410**	**9.539**	**1.804**
CYC	**344**	**392**	**412**	**8.116**	**11.335**

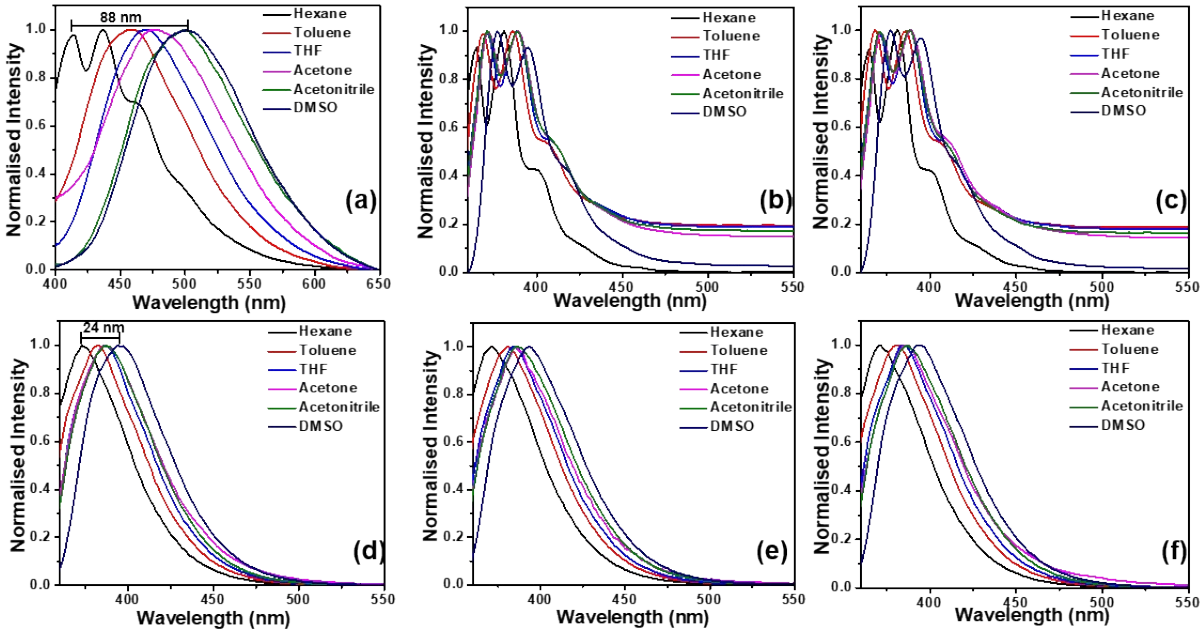


Figure S3 Normalized emission spectra of (a)PHB, (b)OCTB (c)CYB (d)PHC (e)OCTC (f)CYC in various solvents with different polarity

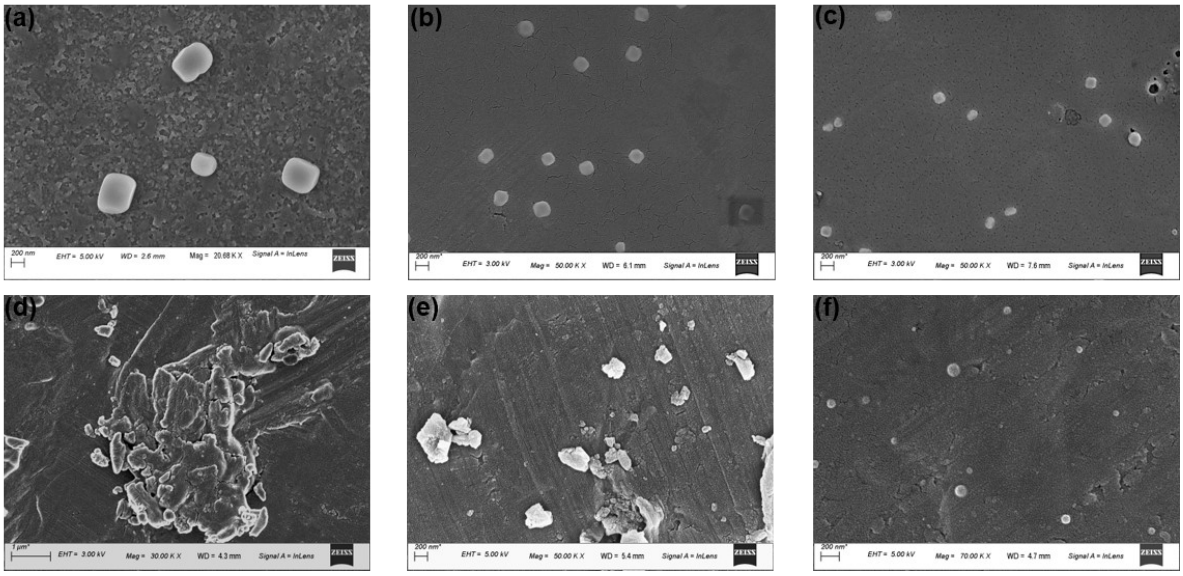


Figure S4. FESEM image of (a)**PHB**, (b)**OCTB** (c)**CYB** (d)**PHC** (e)**OCTC** (f)**CYC** nano-aggregate prepared using drop casting method on aluminum foil/ silicon wafer in the 99.9% water fraction (f_w) in methanol.

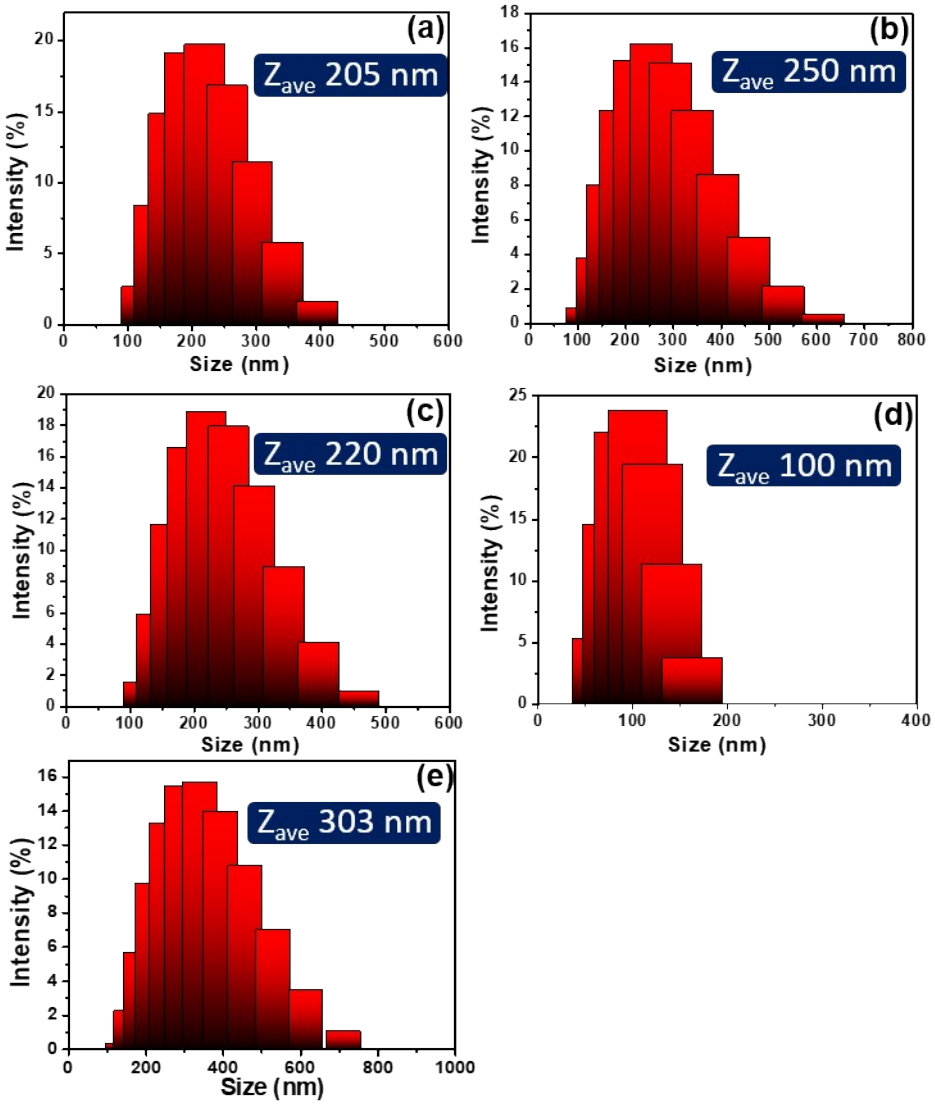


Figure S5 Size distribution of nanoaggregates (a)**OCTB** (b)**OCTC** (c)**CYB** (d)**CYC** (e)**PHB** measured by dynamic light scattering in 99.9% water fraction at 25 °C

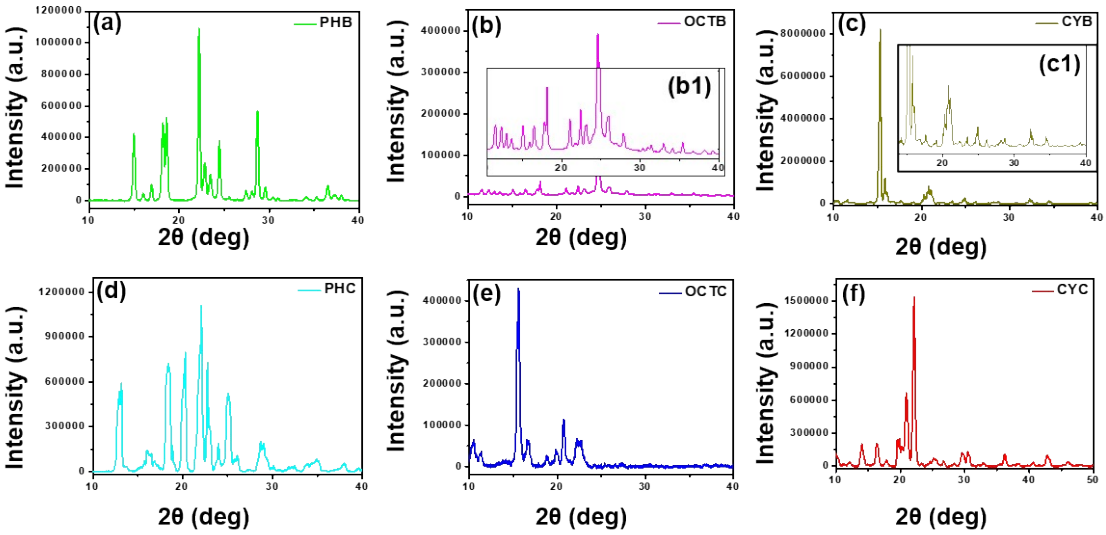


Figure S6 Powder XRD pattern of all the probes. Inset images are the magnified representation of the corresponding spectra.

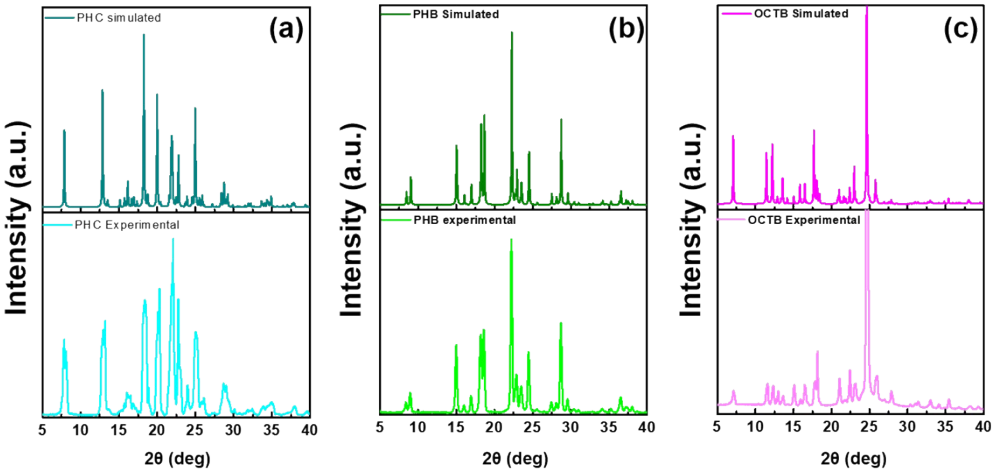


Figure S7 Comparison of simulated and experimental Powder XRD pattern for (a) PHC, (b) PHB, (c) OCTB.

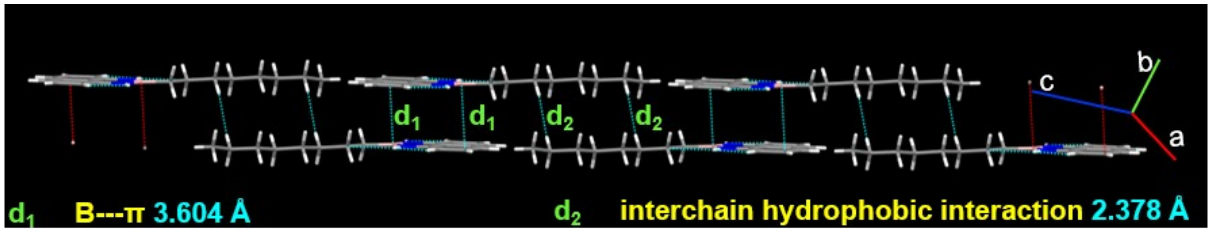


Figure S8 Intermolecular packing arrangements for OCTB along c axis

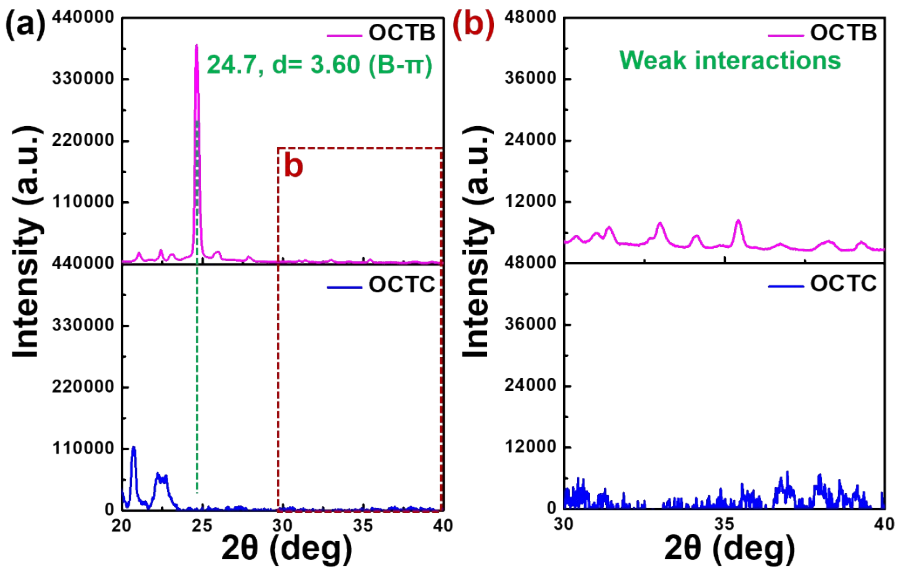


Figure S9 (a) Comparison of Powder XRD pattern between OCTB and OCTC. (b) magnified PXRD pattern from 2θ value 30-40.

Table S2. Single Crystal Data and Parameters of all the obtained crystals

Compound code	**OCTB**	**PHC**	**PHB**
CCDC	2210041	2210045	2210065
Empirical Formula	C18 H25 B N2	C17 H14 N2	C32 H26 B2 N4
Formula Weight	280.21	246.30	488.19
Temperature	296 K	273K	296 K
Wavelength	0.71073	0.71073	0.71073
Crystal System	triclinic	monoclinic	monoclinic
Space Group	P -1	P 21/c	P 21
Unit Cell Dimension	a=7.740(2) b=8.555(3) c=12.499(4) alpha=93.164(8)	a=12.619(3) b=8.688(2) c=13.129(4) alpha=90	a=11.0213(11) b=5.4398(5) c=11.7722(11) alpha=90

	beta=90.771(8) gamma=93.010(9)	beta=116.872(4) gamma=90	beta=117.600(2) gamma=90
Volume	825.1(4)	1284.0(6)	625.47(10)
Z	2	4	1
Absorption coefficient	0.065	0.076	0.076
F (000)	304.0	520.0	256.0
Theta range for data collection	1.632-24.983	1.809- 27.434	1.952-24.996
Index Range	-9<=h<=9, -10<=k<=10, -14<=l<=14	-16<=h<=16, -11<=k<=11, -16<=l<=16	-13<=h<=13, -6<=k<=6, -13<=l<=13
Reflections Collected/unique	23148/2890 (R_{int} = 0.0290)	17884/2914 (R_{int} = 0.0722)	25719/2208 (R_{int} = 0.0419)
Goodness-of-fit on F2	1.084	0.995	0.632
Final R indices [I>2σ(I)]	R1 = 0.0527, ωR2 = 0.1438	R1 = 0.0585, ωR2 = 0.1269	R1 = 0.0482, ωR2 = 0.1100
R indices (all data)	R1 = 0.0737, ωR2 = 0.1679	R1 = 0.1490, ωR2 = 0.1651	R1 = 0.0559, ωR2 = 0.1247
Largest difference peak and hole (e $Å^{-3}$)	0.132, -0.153	0.258, -0.216	0.243, -0.333

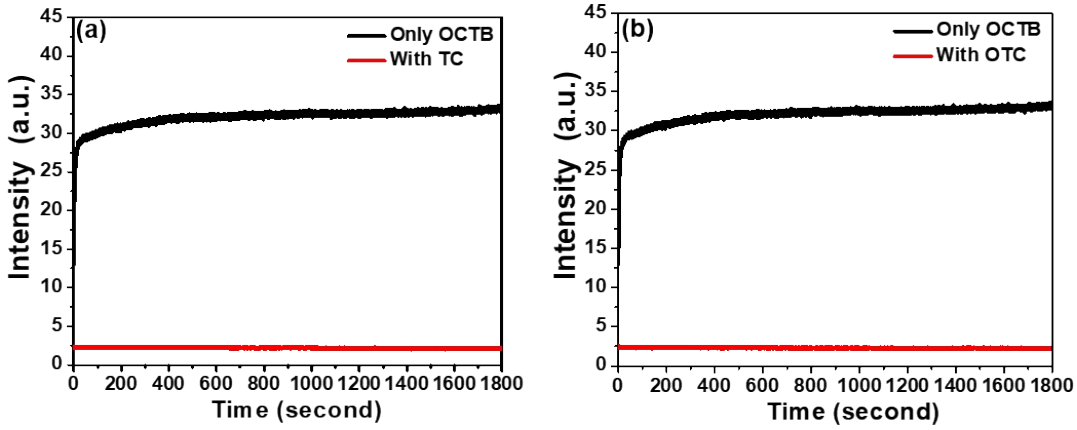


Figure S10 Fluorescence kinetics spectra of OCTB (20μM) in the absence (black line) and presence (red line) of antibiotic analyte (260 μM) recorded up to 30 minutes

Calculation of limit of Detection[2]

The fluorescence response was recorded at low concentration of analytes. The plot of fluorescence intensity vs analyte concentration produces a liner fit curve. The slop of this graph used to determine the limit of detection (LOD) using the equation 3σ/K, where σ refers to the standard deviation in the fluorescence intensity without addition of any analyte and K denotes the slop of the fluorescence intensity vs analyte concentration plot.

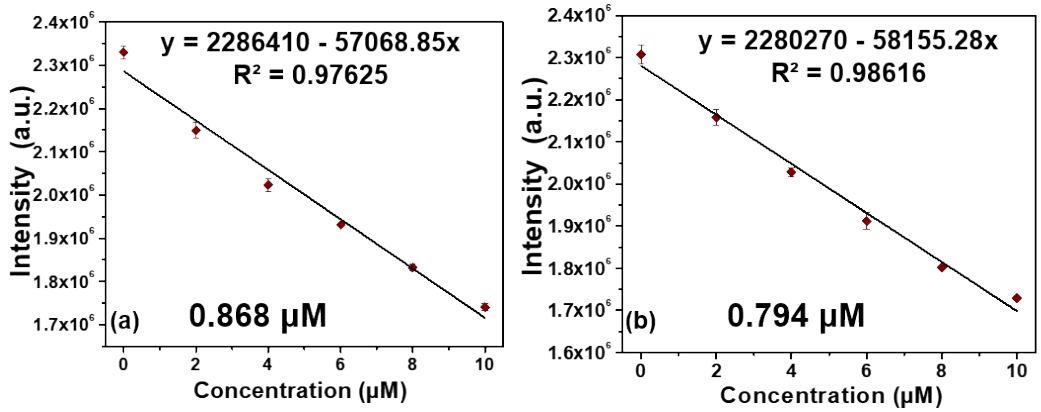


Figure S11 Fluorescence response of OCTB (20μM) in presence of tetracycline(left) and oxytetracycline (right) used for limit of detection calculation.

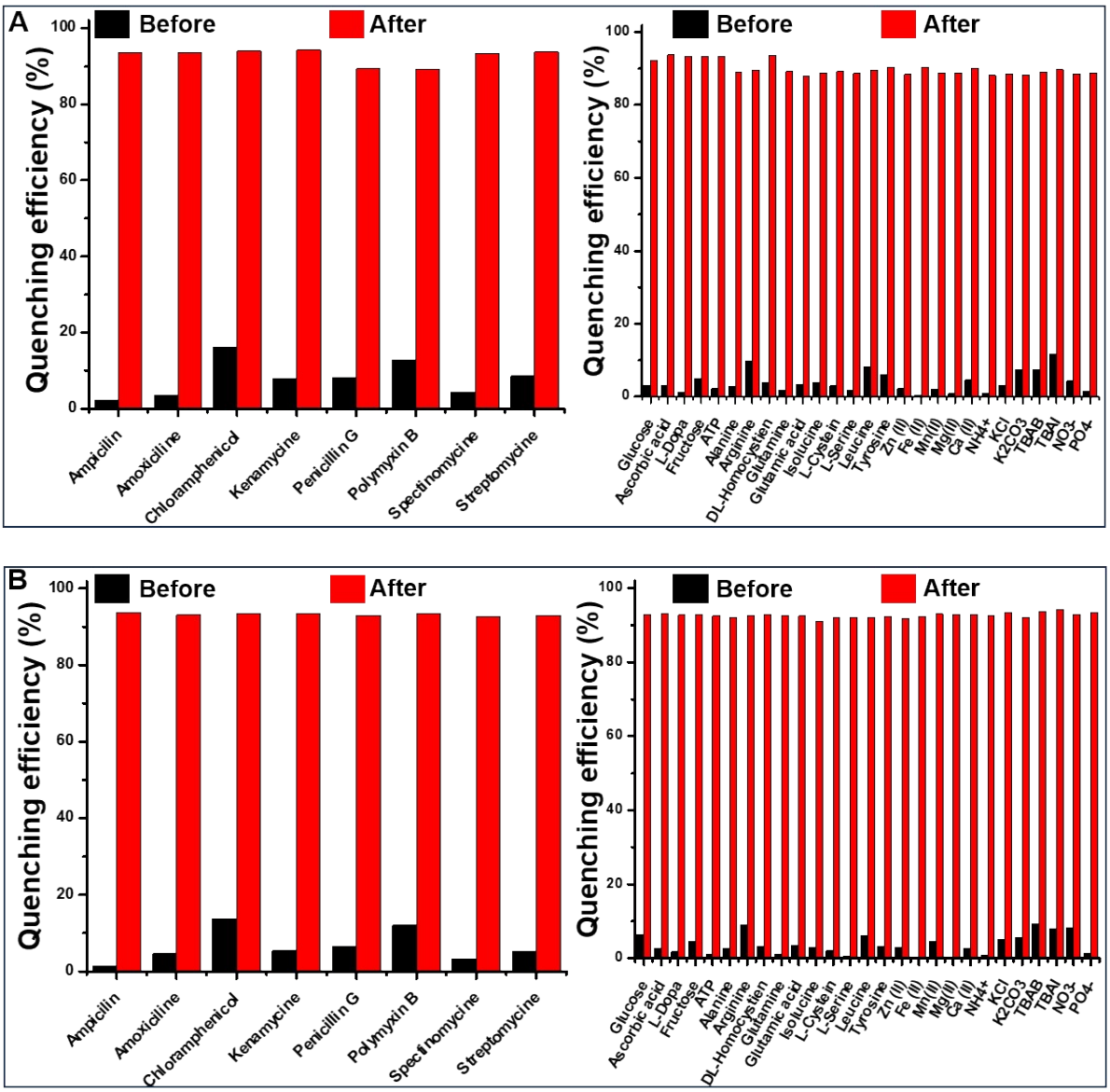


Figure S12 Bar diagram depicts the competitive quenching efficiency of the OCTB probe towards similar antibiotics, and other interfering/competing elements before (black bar) and after (red bar) the addition of (A) Tc and (B) OTc.

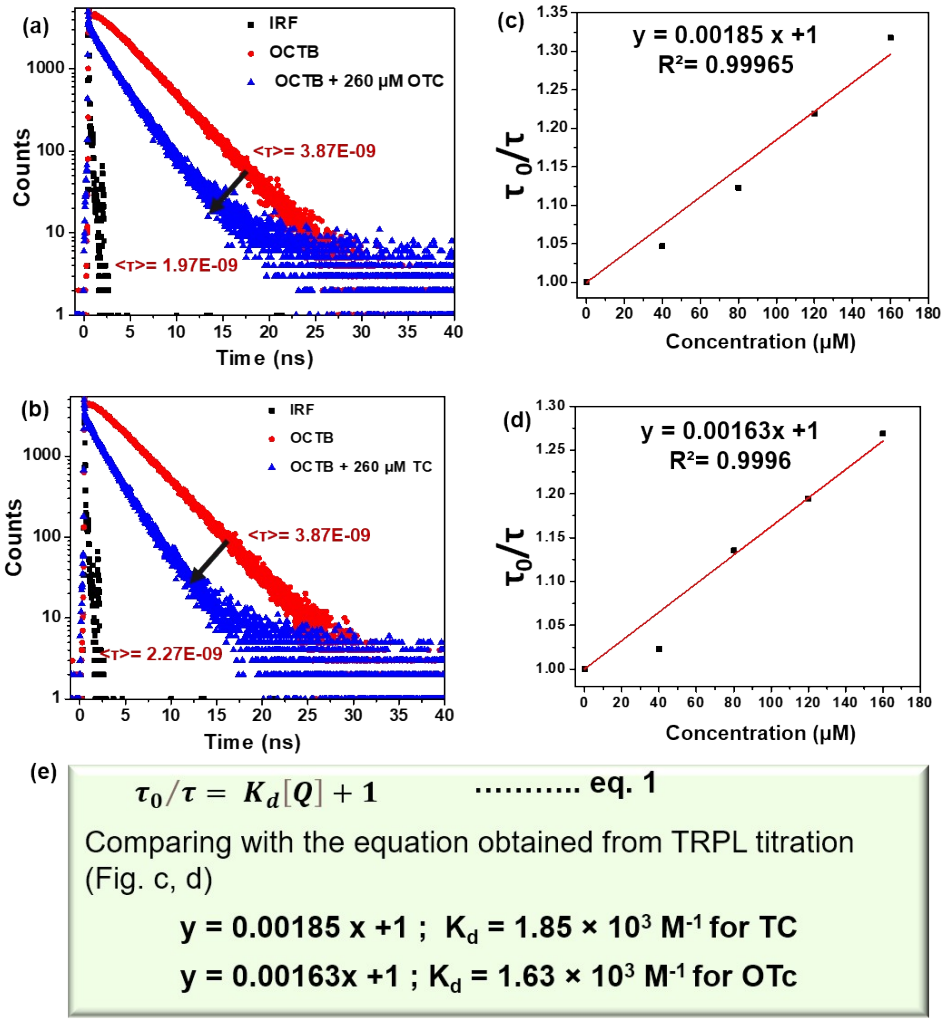


Figure S13 Change in the Time-resolved photoluminescence (TRPL) spectra of OCTB (20µM) with the addition of (a) Tc and (b) OTc. (c) and (d) represents a linear regression curve for the change in fluorescence lifetime against the variation of Tc and OTc concentration respectively. (e) demonstrates the equation and calculation for the dynamic quenching constant (K_d)

FRET Parameters:

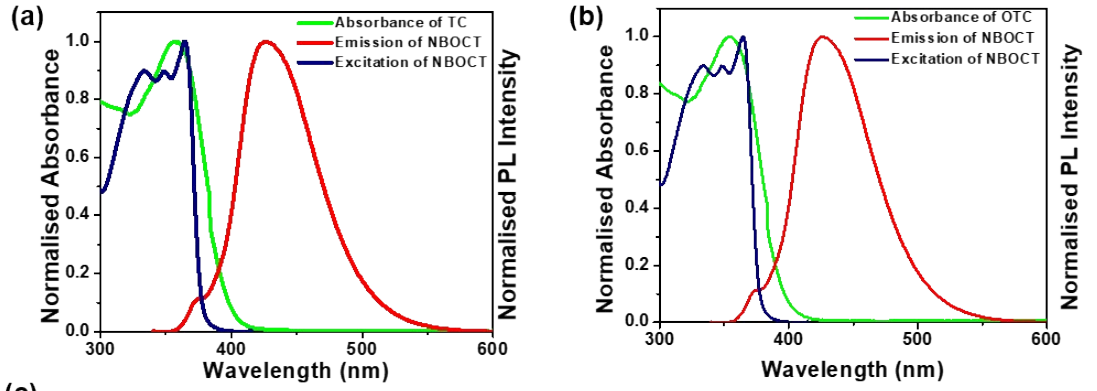
All the parameters were calculated using the below equations.

(1) **Overlap Integral *[J(λ)]* =** $\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$

(2) **Forster distance (R_0) = $0.211[(J)Q(\eta^{-4})(k^2)]^{1/6}$**

***(3)* RET Efficiency (E%) = 1- (t_{DA}/t_D)**

Equation (1) J denotes overlap integral value, $F_D(\lambda)$ represents the corrected fluorescence intensity from λ to Δλ with total fluorescence intensity for OCTB normalized to unity, and $\varepsilon_A(\lambda)$ represents molar absorptivity of acceptor (analyte) at λ in M^{-1} cm^{-1}. Equation (2) R_0 represents Förster distance, Q denotes the fluorescence quantum yield OCTB without any analyte, η represents refractive index of the medium, and k^2 signifies dipole orientation factor of donor and acceptor which generally assumed to be equal to 0. 667.Equation (3) E% denotes RET efficiency where $\mathbf{t}_{DA}$ and $\mathbf{t}_D$ signifies the average fluorescence lifetime in presence and absence of analyte.



(c)

	Tc	OTc
[J(λ)] =	1.58×10^{13} M^{-1} cm^{-1} nm^4	1.37×10^{13} M^{-1} cm^{-1} nm^4
(R_0) =	17.98 Å	17.54 Å
(E%) =	49.09 %	41.34 %

Figure S14 Indicates the spectral overlap between the absorbance of analyte and the emission of OCTB for (a) Tc and (b) OTc, respectively. (c) illustrates the FRET Parameters calculated from the above-mentioned equation for both the analytes.

Inner filter effect (IFE):

IFE has been calculate using the below equation[3, 4]

$$\frac{I_{corr}}{I_{obs}} = \frac{2.3dA_{ex}}{1-10^{-dA_{ex}}} 10^{gA_{em}} \frac{2.3sA_{em}}{1-10^{-sA_{em}}} \quad \ldots \text{eqn S2}$$

Here, I_{obs},I_{obs} signify the measured and IFE corrected maximum fluorescence intensity value; A_{ex} and A_{em} denotes the absorbance and emission maxima value at wavelength 330nm and 426nm respectively; s represents thickness of excitation beam (0.10 cm), g is the distance between the edge of the excitation beam and the edge of the cuvette (0.40 cm) and d is the width of the cuvette (1.00 cm).

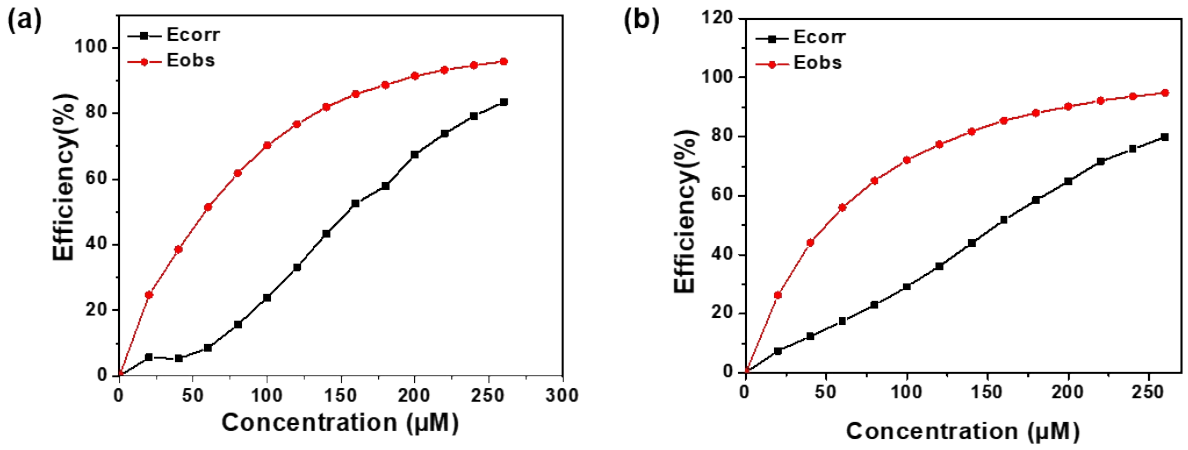


Figure S15 The Observed and corrected quenching efficiencies (%) for the quenching of OCTB with the addition of (b)Tc and (c) OTc.

Table S3 Calculations for IFE corrections for fluorescence quenching Of OCTB in presence of Tc

Concentration of Tc (μM)	**A_{ex}**	**A_{em}**	**I_{obs}**	**I_{corr}**	**I_{corr}/I_{obs}**	**E_{obs}**	**E_{corr}**
0	0.1633	0.0267	2.33E+06	2.87E+06	1.23E+00	0.00E+00	0.00E+00
20	0.378	0.0308	1.76E+06	2.71E+06	1.54E+00	2.47E+01	5.57E+00
40	0.5911	0.0372	1.43E+06	2.72E+06	1.90E+00	3.86E+01	5.23E+00
60	0.8154	0.0454	1.13E+06	2.63E+06	2.32E+00	5.14E+01	8.45E+00
80	1.0118	0.0523	890202.2	2.42E+06	2.72E+00	6.18E+01	1.56E+01
100	1.2133	0.0574	692561.2	2.18E+06	3.15E+00	7.03E+01	2.39E+01
120	1.3818	0.0614	543253.1	1.92E+06	3.53E+00	7.67E+01	3.31E+01
140	1.5243	0.0641	421264	1.63E+06	3.86E+00	8.19E+01	4.33E+01
160	1.6476	0.0668	327639.8	1.36E+06	4.15E+00	8.59E+01	5.26E+01
180	1.8315	0.0694	263673.3	1.21E+06	4.59E+00	8.87E+01	5.78E+01
200	1.8658	0.072	199607.8	9.35E+05	4.68E+00	9.14E+01	6.74E+01
220	1.8829	0.0746	158351.8	7.50E+05	4.74E+00	9.32E+01	7.39E+01
240	1.914	0.0768	123657.7	5.96E+05	4.82E+00	9.47E+01	7.92E+01
260	1.923	0.0784	97456.92	4.73E+05	4.85E+00	9.58E+01	8.35E+01

Table S4 Calculations for IFE corrections for fluorescence quenching of OCTB in presence of OTc

Concentration of OTc (μM)	**A_{ex}**	**A_{em}**	**I_{obs}**	**I_{corr}**	**I_{corr}/I_{obs}**	**E_{obs}**	**E_{corr}**
0	0.1735	0.0417	2.35E+06	2.98E+06	1.26E+00	0.00E+00	0.00E+00
20	0.393	0.044	1.74E+06	2.76E+06	1.59E+00	2.63E+01	7.39E+00
40	0.6247	0.0495	1.32E+06	2.61E+06	1.98E+00	4.41E+01	1.23E+01
60	0.8333	0.0517	1.04E+06	2.46E+06	2.37E+00	5.59E+01	1.74E+01
80	1.0432	0.0547	822743.3	2.29E+06	2.79E+00	6.51E+01	2.29E+01
100	1.2398	0.0565	658519.1	2.11E+06	3.20E+00	7.20E+01	2.91E+01
120	1.402	0.0586	533832.5	1.90E+06	3.56E+00	7.73E+01	3.61E+01
140	1.5382	0.0608	430659.3	1.67E+06	3.88E+00	8.17E+01	4.39E+01
160	1.6761	0.0627	342040.1	1.44E+06	4.20E+00	8.55E+01	5.18E+01
180	1.7564	0.0634	282089.4	1.24E+06	4.39E+00	8.80E+01	5.84E+01
200	1.8029	0.0667	231760.4	1.05E+06	4.51E+00	9.02E+01	6.49E+01
220	1.8424	0.0655	183807.6	8.45E+05	4.60E+00	9.22E+01	7.16E+01
240	1.9295	0.0672	150156.1	7.22E+05	4.81E+00	9.36E+01	7.57E+01
260	1.9575	0.0685	122191.3	5.96E+05	4.88E+00	9.48E+01	8.00E+01

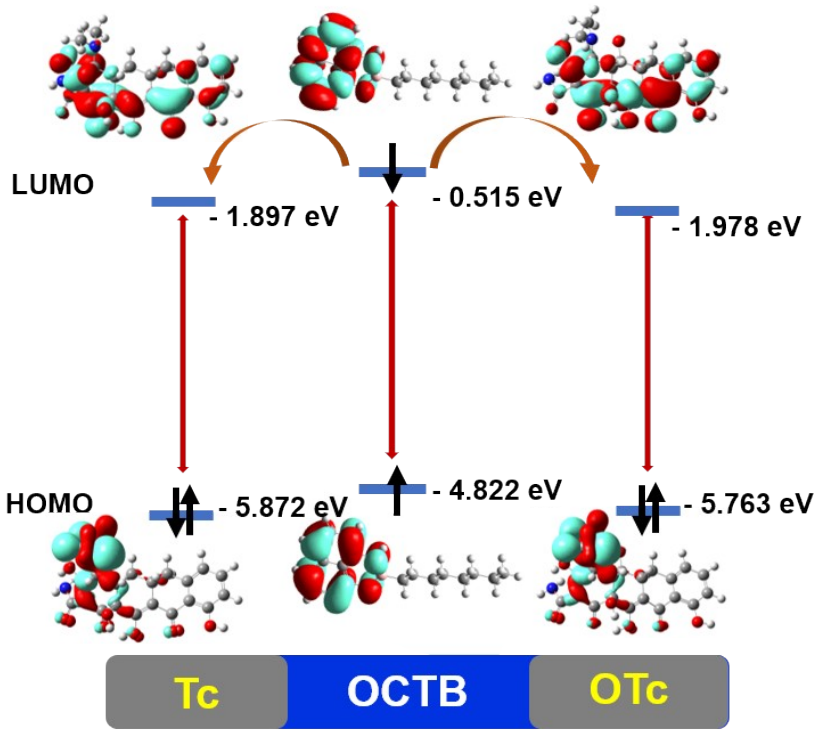
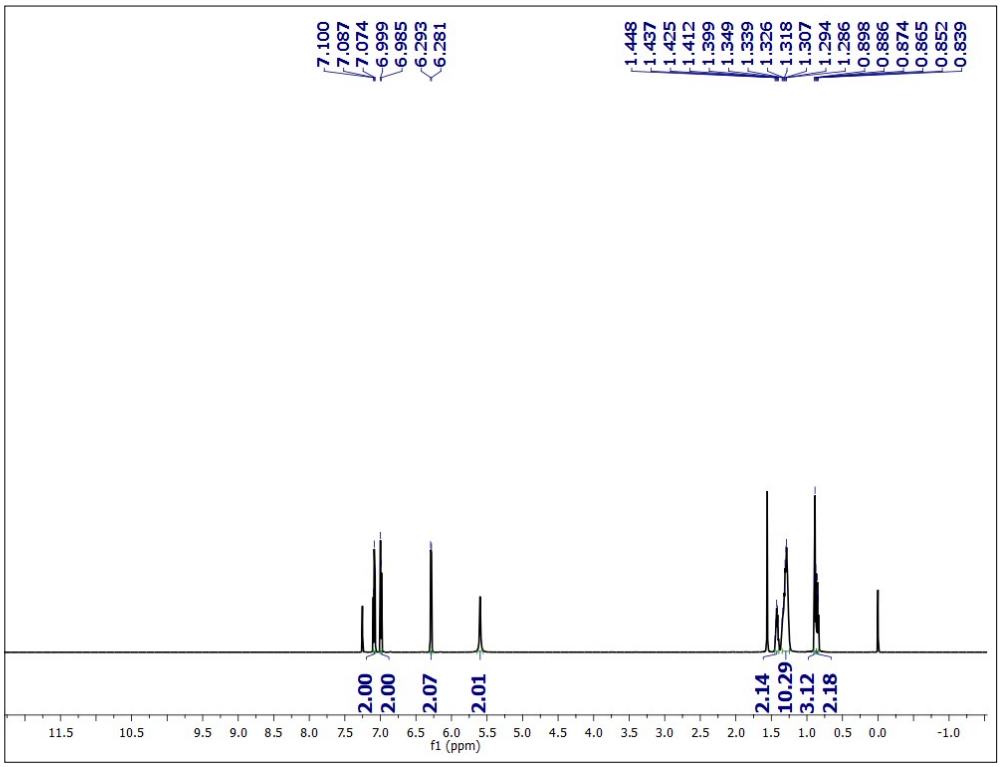
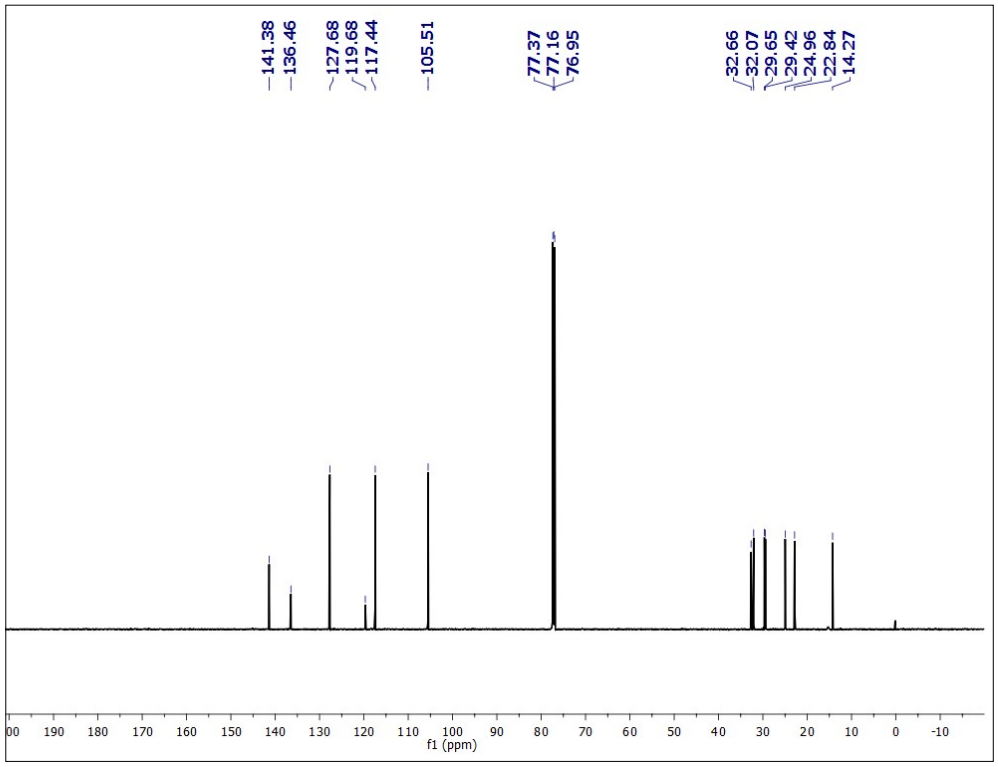


Figure S16: DFT optimised HOMO-LUMO energy band profile to understand for the possibility of d-PET mechanism triggering the quenching phenomena.

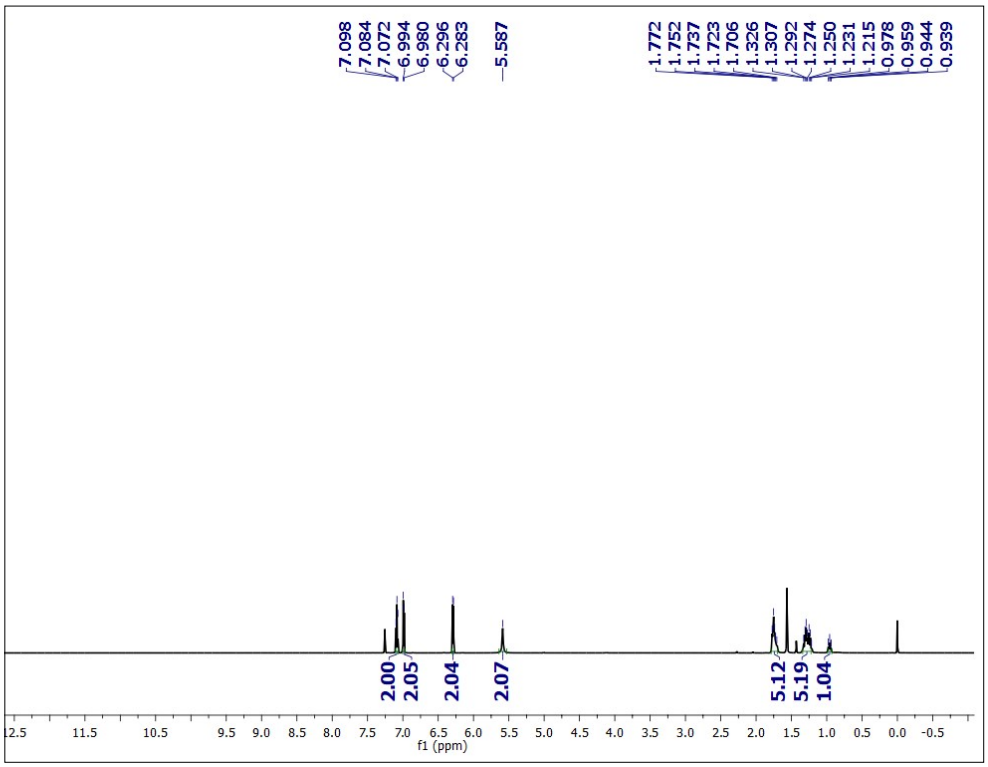
^{1}H-NMR, ^{13}C-NMR, and Mass Spectra



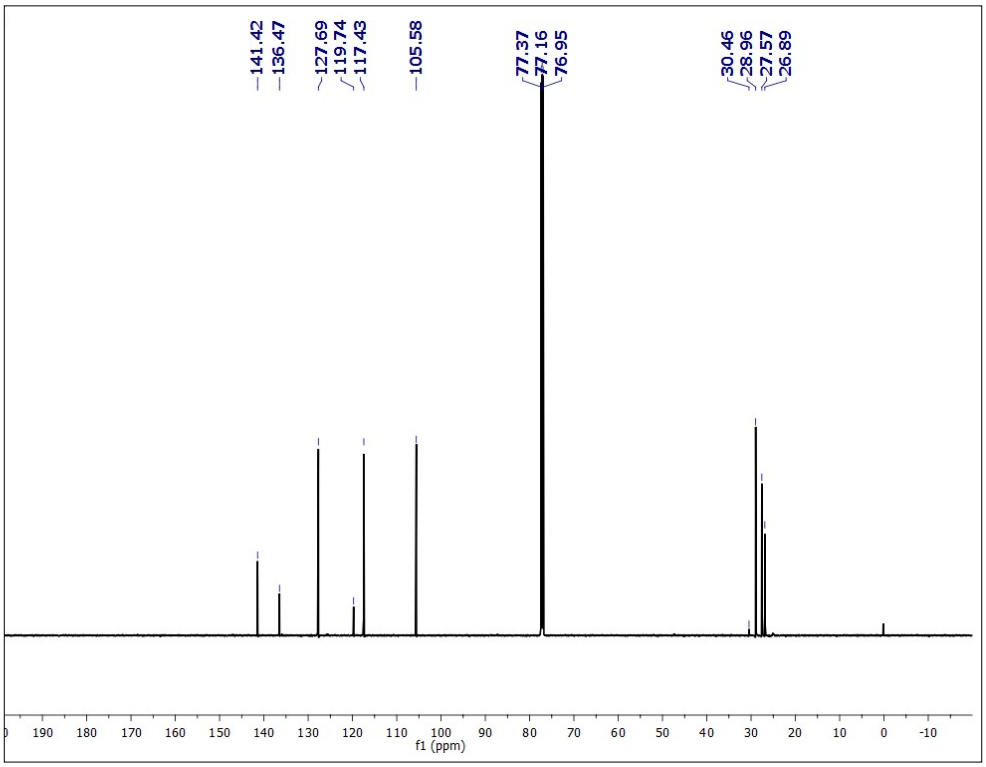
^{1}H-NMR of OCTB



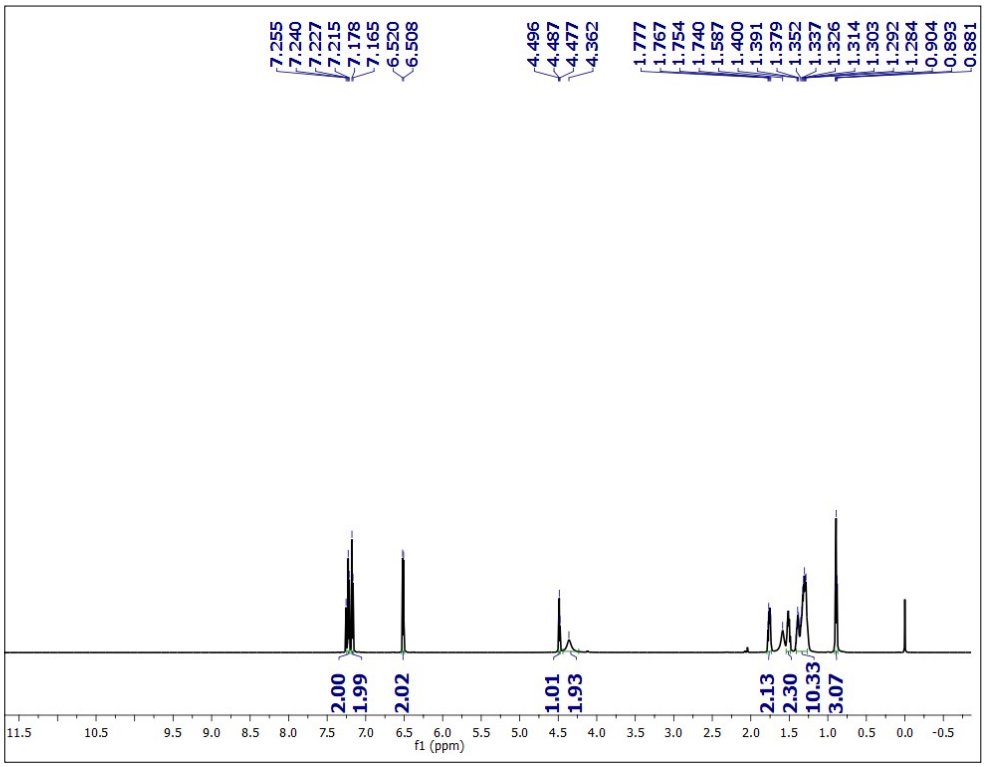
^{13}C-NMR of OCTB



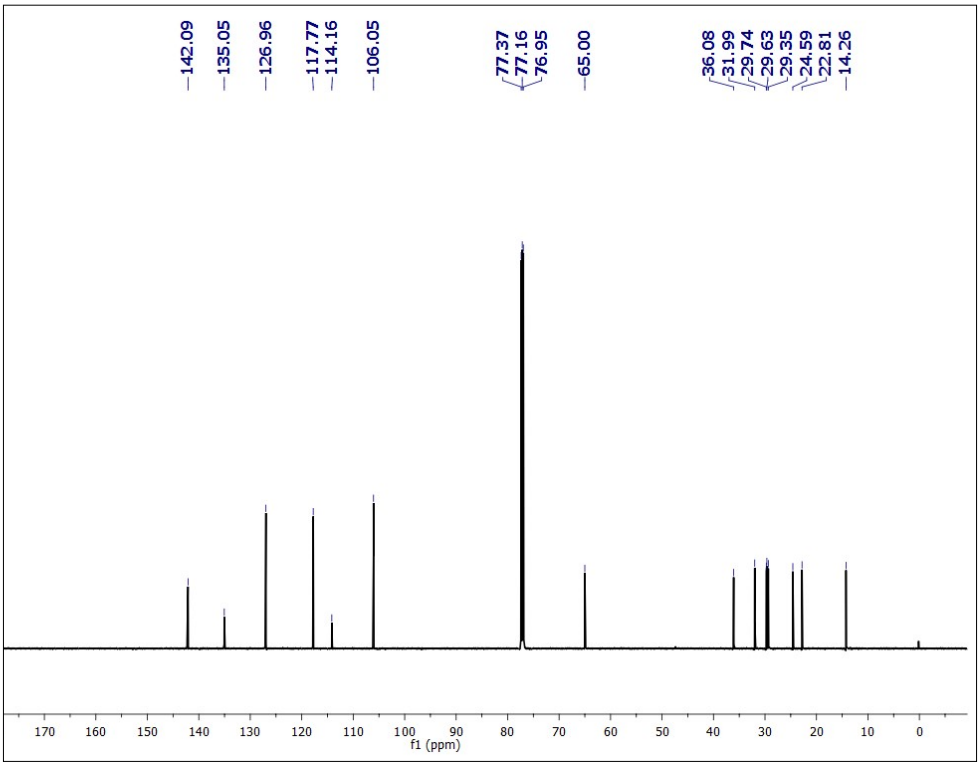
^{1}H-NMR of CYB



^{13}C-NMR of CYB



^{1}H-NMR of OCTC



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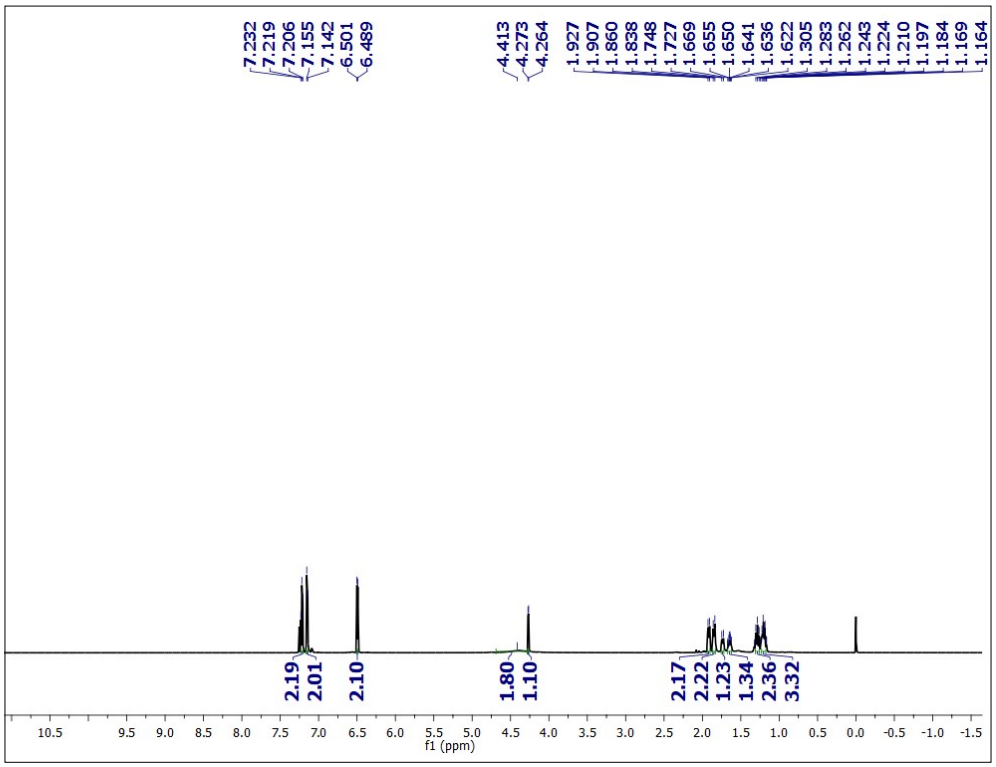

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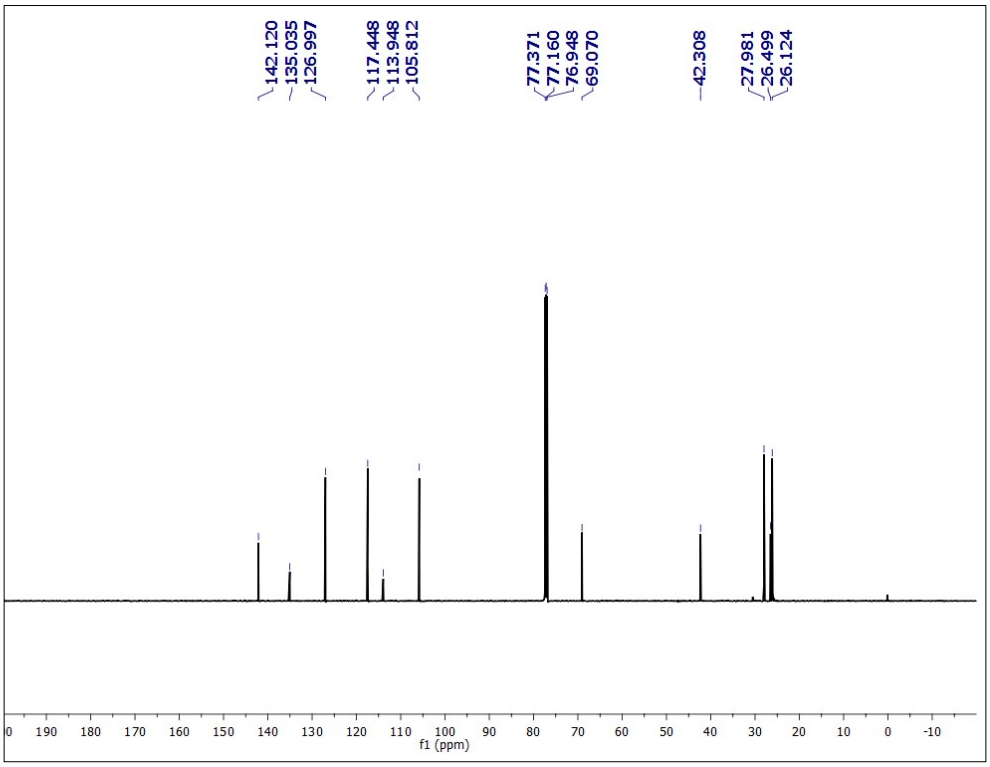

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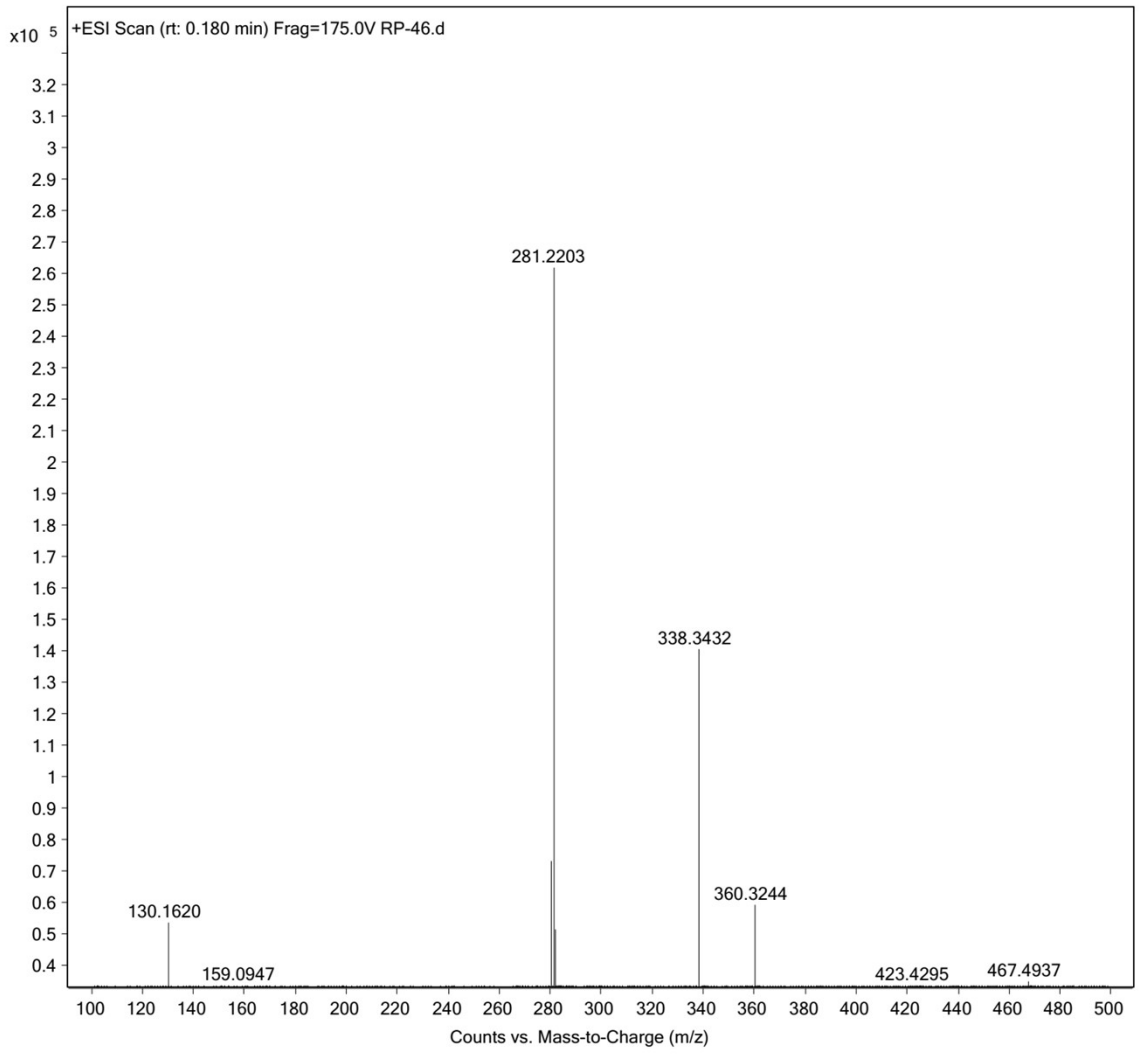
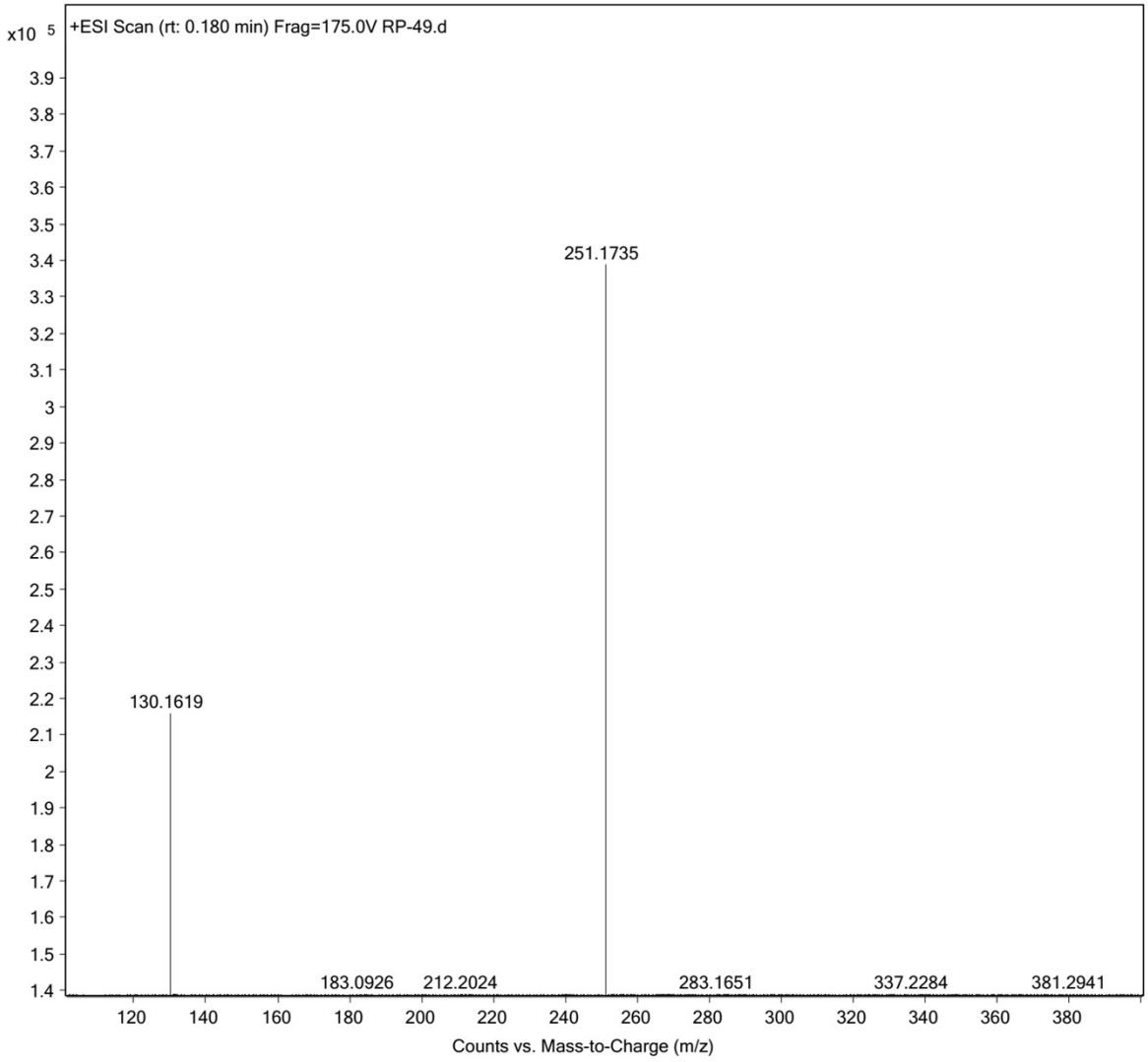
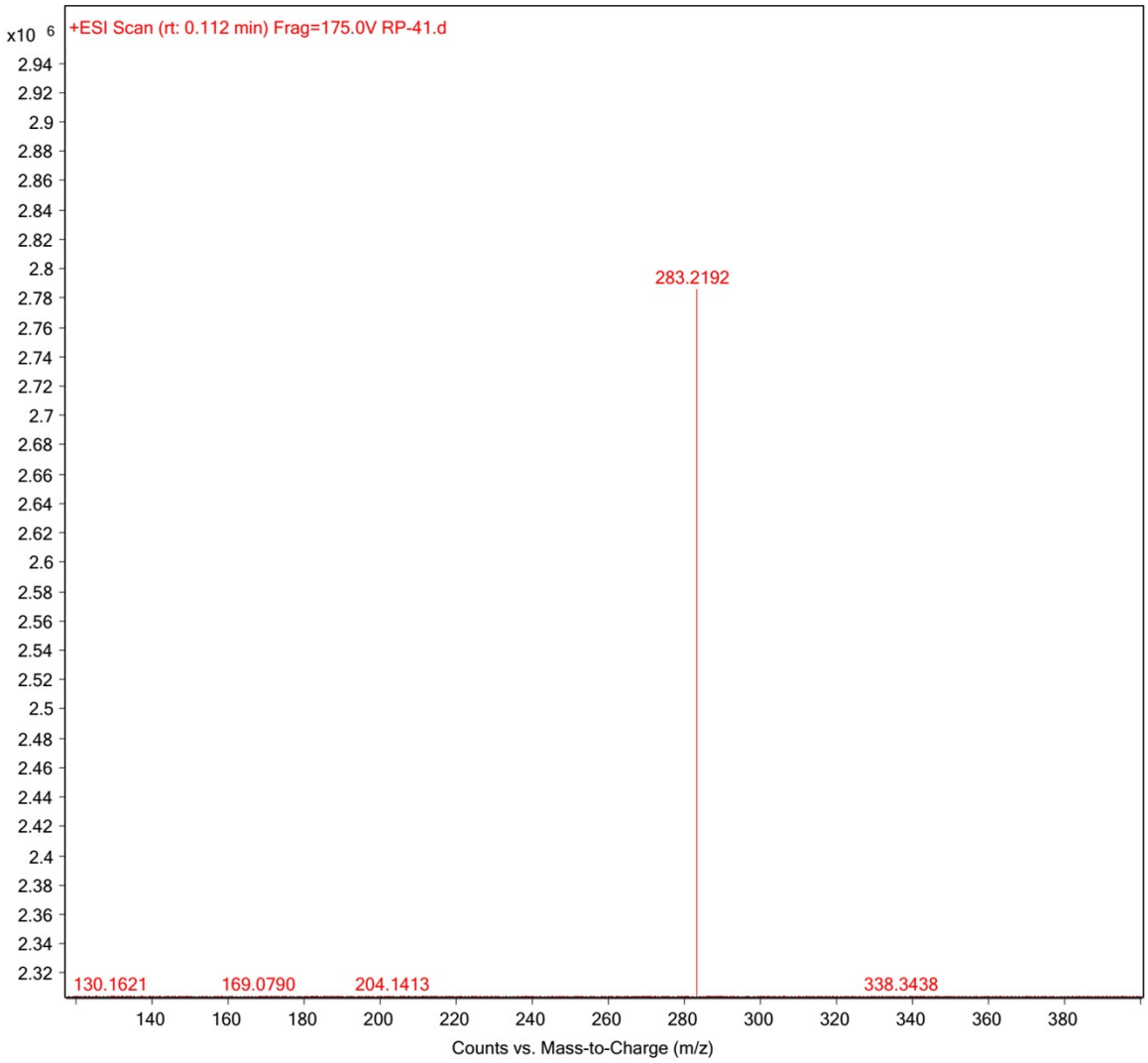


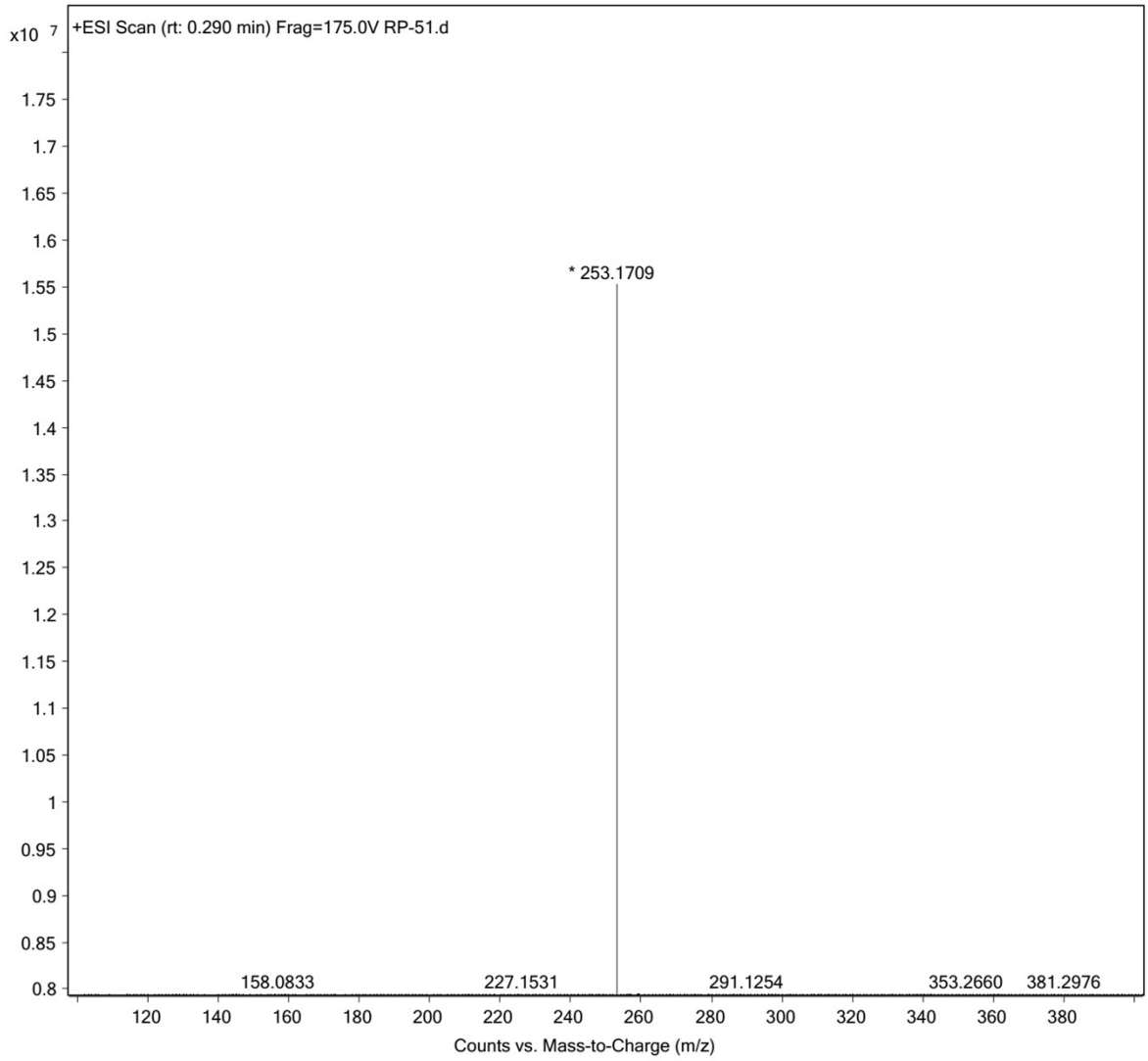
Mass spectra of OCTB



Mass spectra of CYB



Mass spectra of OCTC



Mass spectra of CYC

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