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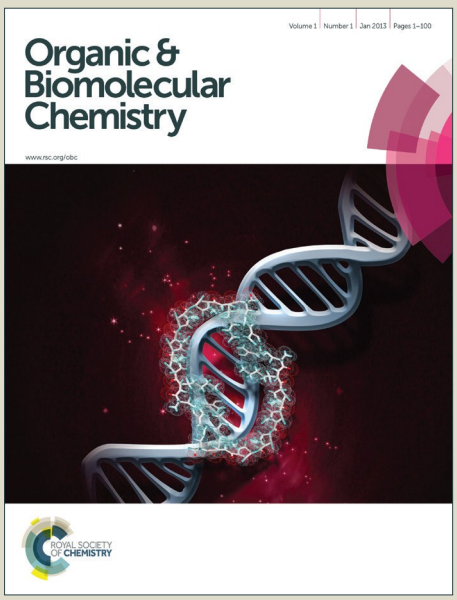

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ARTICLE

Colorimetric and fluorescence probe for detection of nano molar lysine in aqueous medium

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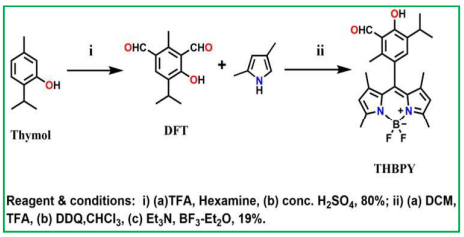
Single crystal X-ray structurally characterized BODIPY based probe **THBPY**, derived from 4-hydroxy-5-isopropyl-2 methyl-isophthalaldehyde detects nano molar lysine in aqueous medium. In presence of lysine, **THBPY** visibly changes its colour and fluorescence profile due to formation of a stable imine bond. Distinctive color change provides facile discrimination over other amino acids in a wide range of concentrations of lysine. The detection limit for lysine is 0.001 μM by fluorescence method and 0.01 μM by colorimetric method. The probe shows good reversibility for multiple use and cleanly discriminates lysine from other amino acids. Density functional theoretical studies closely resembles to the experimental facts.

Introduction

Lysine (Lys), closely involved in Krebs–Henseleit cycle and polyamine synthesis,[1] undergoes catabolism in the liver of mammals *via* saccharopine pathway to produce glutamate and α-amino adipate that subsequently undergoes deamination and oxidation.[2] As human body cannot produce Lys, appropriate amount is essential in the diet for normal metabolic functions and weight gain.[3] However, high level Lys in plasma and urine indicates congenital metabolic disorders like cystinuria or hyperlysinemia.[4] Lys plays an important role in neurotoxicity of αβ40 and αβ42 oligomers in Alzheimer's disease.[5] Thus, monitoring of Lysine is very demanding. Chromatography and capillary electrophoresis, [6] two major techniques for Lys determination suffer from tedious separation step[7] for large sample volume. Lys oxidase, widely used Lys sensing suffers interference from ascorbic acid.[8] Among several methods for Lys determination in aqueous solution,[9] fluorescence and/ or colorimetric probe is highly desirable.[10] Among them, pyrene based colorimetric Lys sensor [11] and triphenylamine based fluorescent probes[12] offers no interference from histidine and arginine.[13] Our previous reports on pyrene based ratiometric Lys probe,[14] function through monomer-excimer conversion. However, all these pyrene and its derivatives are metabolized in our body to form toxic substances[15] that can bind DNA forming pyrene-DNA adducts to alter DNA replication with increased risk of cancer.[16]

On the other hand, remarkable photo physical properties *viz.* sharp absorption and emission peaks, high fluorescence quantum yield and stability and, non-toxic nature of BODIPY unit insist us to include the unit for design and building a fluorescence probe selective for Lys.[17] 4-Hydroxy-5-isopropyl-2-methyl isophthalaldehyde (DFT)[18] is synthesized from thymol. Thymol and its derivatives being well tolerable in mammalian systems,[18, 19] its small size and lipophilic character allows easy absorption and distribution within the sub cellular organelles.[20] In view of the above discussion, herein we report a simple BODIPY based colorimetric and fluorescence probe, **THBPY** which is highly selective for Lys at neutral conditions.



Scheme 1 Synthetic route for **THBPY**

Results and discussion

THBPY is synthesized from 4-hydroxy-5-isopropyl-2-methyl isophthalaldehyde (DFT)[18] in three steps with 25% yield (Scheme 1). The structures of DFT and **THBPY** are characterized by ^{1}H NMR, ^{13}C NMR and mass spectra (Figure S-1 to S-3 and Figure S-4 to S-6, ESI†). Moreover, the structure

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†Electronic Supplementary Information (ESI) available: [^{1}H, ^{13}C, ESI-MS, UV-Vis absorption spectra, cell imaging, DFT studies and additional spectroscopic data]. See DOI: 10.1039/x0xx00000x

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of **THBPY** is confirmed by single crystal X-ray diffraction analysis (Figure 1, Table S-1, ESI†).

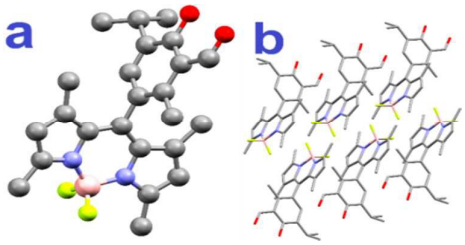

Fig. 1 (a) Single crystal X-ray structure and (b) packing diagram of **THBPY.**

Photo physical studies

Figure 2a reveals that the sharp band at 503 nm of **THBPY** is slightly blue shifted to 497 nm upon addition of Lys (DMSO–HEPES buffer, 1: 9, v/v, 0.01 M, pH 7.4). However, a broad band at 550 nm appears that increases with increasing Lys (up to 100 equiv.).

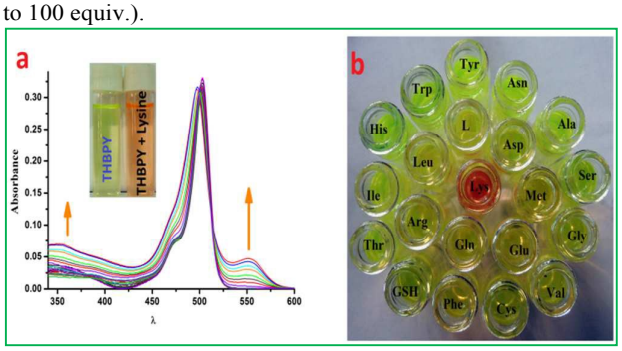

Fig. 2 Changes in the (a) UV-Vis spectra of **THBPY** (10 μM, DMSO–HEPES buffer, 1: 9, v/v, 0.01 M, pH 7.4) upon addition of Lys (up to 100 equiv., left) and (b) colour of **THBPY** solution in presence of different amino acids (100 equiv.).

Consequently, the color of the solution changes from greenish yellow to light orange. Different colors of **THBPY** in presence of various amino acids are shown in Figure 2b. The fluorescence selectivity of **THBPY** for Lys is presented Figure 3a. **THBPY** shows strong green fluorescence (λ_{em} = 515 nm) with high quantum yield (Φ_{fl} = 0.80, ESI†). The strong emission intensity of free **THBPY** at 515 nm (λ_{ex}, 371 nm) in the same solvent quenches upon addition of Lys (0-3000 μM, Figure 3b and 3d).

Figure 3c shows the plot of emission intensity of **THBPY** vs. Lys concentration, the linear region (up to 15 μM Lys, inset) is useful for unknown Lys determination. The disappearance of strong green fluorescence of **THBPY** upon addition of Lys is presented in Figure 4. Other naturally occurring amino acids fail to cause any significant change of fluorescence and absorbance of **THBPY**. Cysteine forms a faint red colour after ~2h. Even though cysteine forms 5-membered substituted aliphatic ring with active aldehyde group[21] but salicylaldehyde or its derivatives with a phenolic OH function group adjacent to the aldehyde group have not been reported so far. **THBPY** is also a modified salicylaldehyde moiety and therefore it is expected to react very slowly with cysteine. Theoretical calculation also shows that the HOMO-LUMO energy gap of resulting complexes of **THBPY** with cysteine and homocysteine are higher than that observed in case of Lys (ESI†).

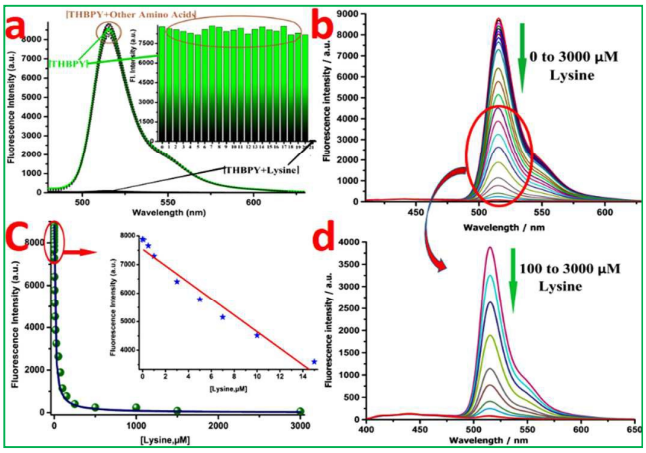

Fig. 3 (a) Emission spectra and intensities (inset) of **THBPY** in presence of various amino acids. (b) changes in emission intensities of THBPY with increasing Lys concentration. (λ_{ex} = 371 nm). (c) Plot of emission intensity *vs.* Lys concentration (linear region, up to 15 μM Lys, inset). (d) Expanded view of 3b.

UV and visible light exposed colours of **THBPY** with increasing Lys concentration are shown in Figure S-7 (ESI†). Lys quenches florescence of **THBPY** ~110 fold 515 nm (λ_{em} = 515 nm, λ_{ex} = 371 nm). Interestingly, at higher Lys concentration (≥ 50 μM), a new relatively weak emission band at 582 nm is observed (λ_{ex} = 503 nm, Figure 5) associated with a clear iso-emissive point at 545 nm.

Fig. 4 UV light exposed colours of **THBPY** (10 μM) in presence of different amino acids (50 μM) in DMSO–HEPES buffer (1: 9, v/v, 0.01 M, pH 7.4).

Effect of pH

Effect of pH (pH, 2.0-10.0) on the emission properties of THBPY in absence and presence of Lys is thoroughly investigated (Figure S-8, ESI†) and found that **THBPY** is stable at biological pH range and effective for detection of Lys in biological samples.

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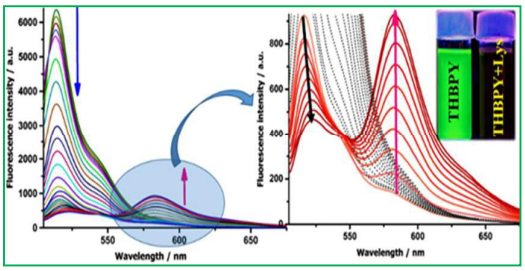


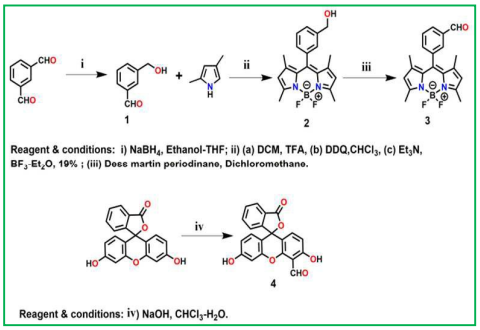
Fig. 5 Emission profile of **THBPY** (10 μM) at 582 nm (λ_{ex} = 503 nm) upon addition of Lys from 50 to 3000 μM (red, left). Right figure shows the expanded view of the blue shed.

Recognition mechanism

To unfold the colorimetric and fluorescence recognition of Lys by **THBPY**, several model compounds are synthesised, characterised and studied their interaction with Lys. Moreover, interactions of **THBPY** with several molecules resembling Lys have also been thoroughly studied to identify any potential interference. Although the possibility of existence of these molecules in biological system where Lys recognition aimed for is rare, it is of academic interest. Our objective is to facile and selective colorimetric recognition of Lys where other naturally occurring amino acids do not interfere and this recognition process may be corroborated by the fluorescence method. In this context, butylamine instead of Lys is employed to unveil the origin of changes of the absorption and emission profile of **THBPY**. Interestingly, butylamine imparts similar absorption profile to that of Lys (Fig. S-9, ESI†), however, the reaction proceeds at much slower rate with generation of brown color. On the other hand, interactions of **THBPY** with molecules resembling Lys, *viz.* 6-amino hexanoic acid and 1, 5-diamino pentane and ethylenediamine are different (Fig. S-10, ESI†) where ~550 nm absorption band is absent, unlike Lys. Corresponding changes in the emission profile are presented in Fig.S-11 (ESI†). Therefore, it may be concluded that butylamine part of Lys is responsible to interact with **THBPY**. Further, the reversibility of imine formation between **THBPY** and Lys (Figure S-12 and S-13, ESI†) is tested with H_2O_2 and found positive (Scheme S-2, ESI†) as supported by the mass spectroscopy (Figure S-14, ESI†). For deeper understanding of the sensing mechanism, two model compounds (compound **3** and **4**) have been synthesised (Scheme 2) and tested for Lys selectivity. Compound **3** is synthesised by partial reduction[22,23] of isophthalaldehyde with $NaBH_4$ in mixed THF-ethanol media to yield compound **1** in good yield. Reaction of **1** with 2, 4-dimethyl pyrrole following the procedure of **THBPY** synthesis yields **2**. Oxidation of **2** following procedure of Dess martin periodinane gives the BODIPY derivative **3**.

On the other hand, modified Reimer–Tiemann formylation of fluorescein gives compound **4** in good yield.[24] The structures of all intermediates and model compounds are characterized by ^{1}H NMR, ^{13}C NMR and mass spectra (Figure S-15 to S-22, ESI†) analyses.

Like **THBPY,** the BODIPY derivative **3** possesses an aldehyde group with emission maximum at 530 nm (λ_{ex}, 425 nm). Upon addition of Lys (50 μM), no significant change of its absorption (λ_{max}, 501 nm, Fig.S-23, ESI) and emission (Fig.S-24, ESI) spectra are observed.



Scheme 2 Synthetic route for model compounds

Like **THBPY**, fluorescence of **4** (10 μM, λ_{ex}, 425 nm, λ_{em}, 470) is quenched in presence of Lys (50 μM, Fig.S-25, ESI†). However, the absorption maximum of **4** is red shifted from 456 nm to 464 nm upon addition of Lys (Fig. S-26, ESI†). While 1, 5-diaminopentane causes similar changes to **4** as that of Lys, 6-aminohexanoic acid remains spectator. Thus it may be concluded that presence of OH group next to aldehyde functionality have specific role to stabilize the adduct of **THBPY** and Lys.

^{1}H NMR titration

In order to strengthen the above mechanism, ^{1}H NMR titration has been performed by adding lysine to the DMSO-d_6 solution of **THBPY**. Significant spectral change has been observed upon addition of lysine. The bare eye red colour is attributed to the formation of a Schiff's base between Lysine and **THBPY**, corroborated from ^{1}H titration (Figure 6).

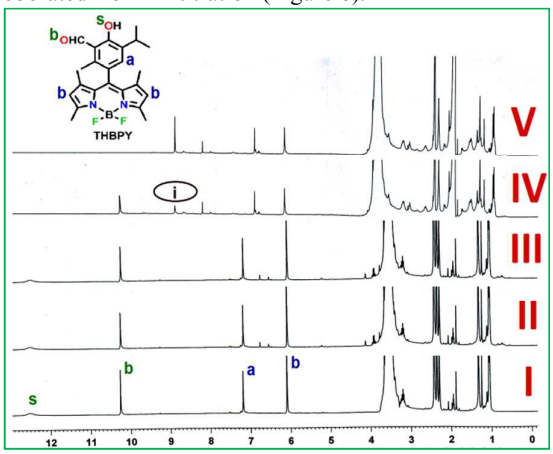


Fig. 6 (I) ^{1}H NMR spectra of **THBPY** in DMSO-d_6; upon addition of (II) 0.5 equiv. Lys in D_2O, (III) 1.0 equiv. Lys in D_2O, (IV) 3.0 equiv. Lys in D_2O and (V) 5.0 equiv. Lys in D_2O.

Upon addition of Lys, the aldehyde proton (b) of **THBPY** at 10.26 ppm disappears with the appearance of the new imine proton (i) in the adduct product at 8.72 ppm. Moreover, aromatic protons of both sub-units are up field shifted. The formation of imine bond is further supported from the mass

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spectrum of the **THBPY**-Lys adduct (Scheme S-1, ESI†) that shows m/z = 553.3160, attributed to the fragment [**THBPY**-lysine] (Figure S-27, ESI†).

DFT calculations

To have deeper understanding of the sensing mechanism, time-dependent density functional theory (TDDFT) calculations have been performed using (B3LYP) and a 6-31G basis set with Gaussian 09 program.[25] The energy optimized structure of **THBPY** is used to calculate the energetics of imine bond formation involving Lys as both free base (neutral form) and cation form. The energy optimized geometries with HOMO-LUMO of THBPY (Table S-2, ESI†) and its Lys adducts (Table S-3 and S-4, ESI†) have been generated. The HOMO-LUMO energy gap of THBPY-Lys adduct is lower than that of free **THBPY**. The individual energy levels of HOMO and LUMO of the adduct product are also lower in energy.

The energy optimized structure of **THBPY**-Lys (neutral form) shows H-bonding interaction between the imine nitrogen and adjacent OH of **THBPY**. The calculated O-H distances of free **THBPY** and that of the imine adduct are approximately 1.00 and 1.053Å respectively. Thus, H-bonding may be responsible for the enhanced stability of **THBPY**-Lys (neutral form) adduct. In case of **THBPY**-Lys (Lys in cationic form), H-bond exists between OH of **THBPY** with amine group of Lys. The calculated O-H bond distance is 1.327Å, well within the recognized H-bond distance. Thus Lys in both forms can interact with **THBPY**. The HOMO-LUMO energy gaps of **THBPY** and **THBPY**-Lys adduct in both forms are shown in Figure 7. The energy gap between HOMO-LUMO in **THBPY**-Lys (neutral) is lowest. The simulated spectrum of the gas phase structure of **THBPY** indicates the dominant absorption band having an oscillator strength, f = 0.6202 a. u. is observed at 500.8 nm. More importantly, the adduct formation between **THBPY** and neutral Lys leads to decrease in the oscillator strength from f = 0.6202 a.u. to f = 0.5512 a.u. as well as red-shift of the absorption band from 500.8 nm to 550.5 nm. The results are qualitatively in line with the experimental spectra. It is interesting to note that the TD-DFT absorption spectra of Lys (cation) have different characteristics of the dominant band when compared with the neutral form. Therefore, the observed change in absorbance is attributed to the imine bond formation between **THBPY** and Lys.

The HOMO-LUMO energy gap of resulting complexes of **THBPY** with cysteine and homocysteine are higher than that observed in case of Lys (Figure S-27, ESI†) and thus support a very very slow reaction between them

very slow reaction between them.

***In vitro* cell imaging**

Intracellular Lys imaging in MDA-MB 231 cells using **THBPY** has been investigated. The cells are incubated with **THBPY** (10 μM) for 1h and washed thrice with PBS buffer. Lys (50 μM) is added to the medium followed by further incubation for 30 min. Figure 8 reveals that **THBPY** is cell permeable and its fluorescence is quenched upon addition of Lys within MDA-MB 231cells when observed under fluorescence microscope.

The cytotoxicity of **THBPY** on MDA-MB 231 cells is determined by MTT assay (Fig. S-29, ESI†). Upon exposure of 10 μM **THBPY** to MDA-MB 231 cells for 12h, ~90% cells remain alive. This nullifies the possibility of significant cytotoxic influence of **THBPY** on MDA-MB 231 cells.

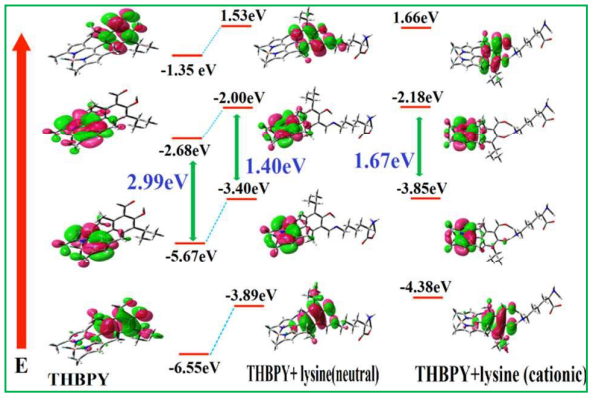

Fig. 7 Selected MOs of **THBPY**, [**THBPY**-Lys (neutral)], [**THBPY**-Lys (cationic)] (not to scale; isovalue = 0.02).

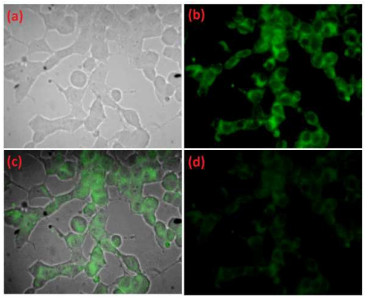

Fig. 8 Fluorescence images of **THBPY** treated MDA-MB 231 cells: (a) bright field images of free cells; (b) fluorescence image of cells after incubation with **THBPY**; (c) overlay image of a and b; (d) fluorescence image of **THBPY** treated cells in presence of Lys. The images are captured using blue filter. **THBPY** is prepared in ~0.3% DMSO-water (v/v).

Conclusion

In summary, we have designed and synthesized BODIPY based excellent colorimetric and fluorescence probe for selective detection of Lys. The new probe, **THBPY** shows high sensitivity as well as visual response towards Lys. **THBPY** shows reversibility in presence of peroxide. Formation of Schiff's base between **THBPY** and Lys results change of colour from yellow-green to orange, observed by bare eye. So, the present demonstration is a new addendum for Lys sensing in the horizon of BODIPY based receptors.

Experimental

Materials and equipment

All metal salts were used as either their nitrate or their chloride salts. All the reagents were of analytical reagent grade and used without further purification. ^{1}H NMR spectra were recorded at 300 and 75 MHz for ^{13}C NMR using solvent peak as internal reference at 25°C. Thin layer chromatography (TLC) was carried out on 0.25 mm thick pre-coated silica plates, and spots were visualized under UV light. Milli-Q Millipore 18.2 MΩ

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cm−1 water was used whenever required. A Shimadzu Multi Spec 2450 spectrophotometer was used for recording UV−vis spectra. FTIR spectra were recorded on a Shimadzu FTIR (model IR Prestige 21 CE) spectrophotometer. Mass spectra were recorded using a QTOF XEVO-G2 mass spectrometer in ESI positive mode. The steady state emission and excitation spectra were recorded with a Hitachi F-4500 spectrofluorimeter. A Systronics digital pH meter (model 335) was used for pH measurement. Fluorescence microscope (Dewinter, Italy) was used for capturing images and processed with Bio-wizard 4.2 software.

Synthesis

3-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo [1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-6-hydroxy-5-isopropyl-2-methylbenzaldehyde (THBPY)

2, 4-Dimethylpyrrole (380 mg, 4 mmol) and 3-hydroxy-2-isopropyl-5-methylterephthalaldehyde (412.48 mg, 2 mmol) were dissolved in dry CH_2Cl_2 (150 mL) under argon atmosphere. 50 µL of trifluoroacetic acid (TFA) was added in dark, and the solution was purged with argon for 10 min. Then the reaction mixture was stirred for 4h at ambient temperature at dark. Then, 2, 3-dichloro-5, 6-dicyanoquinone (DDQ, 442 mg, 2 mmol) was added prior to addition of 10 mL dry $CHCl_3$, and the mixture was stirred for another 4h. Then, triethylamine (3 mL) was added to the reaction mixture and stirred for 5 min. Further, boron trifluoride etherate (5 mL) was added and the mixture was stirred for another 40 min. The dark brown solution was formed which was partitioned with water (3 X 20 mL) and brine (30 mL), dried over anhydrous magnesium sulfate and concentrated at reduced pressure. The crude product was purified by silica-gel flash column chromatography (EtOAc–petroleum ether, 15%, v /v) and recrystallized from $CHCl_3$–hexane to give **THBPY** as red crystals (150 mg, yield, 19%) M.P.> 200°C. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 1.19-1.24 (6H, 2s), 1.44 (3H, s), 1.56 (3H, s), 2.44 (3H, s), 2.46 (6H, 2s), 3.39 (1H, m), 6.01 (2H, s), 7.23 (1H, s), 10.39 (1H, s), 12.58 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$), 195.70, 161.46, 155.94, 142.49, 140.01, 137.32, 136.50, 133.78, 131.46, 125.74, 121.36, 118.11, 26.02, 22.34, 14.60, 14.38, 14.12. QTOF mass, m/z $(M+H)^+$ calcd. for $C_{24}H_{28}BF_2N_2O_2^+$: 425.2206, found, 425.2212 and m/z $(M+Na)^+$ calcd. for $C_{24}H_{27}BF_2N_2NaO_2^+$: 447.2026, found, 447.2067.

3-(Hydroxymethyl)benzaldehyde (1)

To a solution of isophthalaldehyde (22.5 g, 0. 125 mol) in a mixture of 95% EtOH (250 mL) and THF (350 mL), $NaBH_4$ (1.2 g, 26.11 mmol) was added at –15 °C with continuous stirring for 30 min. Then the mixture was stirred for 6h at -15 °C and neutralized with 2M HCl to pH 5. The solvent was removed and the residue was partitioned with EtOAc. The organic extract was dried with anhydrous Na_2SO_4 and the solvent was removed. The yield of crude product 2 was 15.9g (95%). The crude product was purified by column chromatography using an EtOAc-hexane (1: 1, v/v) mixture. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 9.899-9.878 (d, 1H), 7.803 (s, 1H), 7.737-7.699 (m, 1H), 7.593-7.568 (d, 1H), 7.485-7.417 (m, 1H), 4.700-4.683 (d, 2H). ^{13}C NMR, (75 MHz, $CDCl_3$) 192.61, 192.53, 141.94, 136.11, 132.80, 132.72, 132.65, 128.93, 128.86, 128.79, 128.74, 128.67, 128.60, 127.59, 127.51, 127.44, 63.77, 63.70, 63.62; QTOF-MS ES$^+$ calcd. for $C_6H_9O_2$ $(M+H)^+$, 137.0602: found, 137.0602.

(3-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4, 5l4-dipyrrolo [1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)methanol (2)

2, 4-Dimethylpyrrole (1g, 10.51 mmol) and 3-(hydroxymethyl) benzaldehyde (680 mg, 5 mmol) were dissolved in dry CH_2Cl_2 (150 mL) under argon atmosphere. Two drops of trifluoroacetic acid (TFA) were added in dark, and the solution was purged with argon for 10 min. Then the reaction mixture was stirred for 4h at ambient temperature at dark. Then 2, 3-dichloro-5, 6-dicyanoquinone (DDQ, 1.13 g, 2 mmol) was added prior to addition of 10 mL dry $CHCl_3$ and the mixture was stirred for another 4h. Then triethylamine (7 mL) was added to the reaction mixture and stirred for 5 min. Then, boron trifluoride etherate (7 mL) was added and the mixture was stirred for another 40 min. The dark brown solution was partitioned with water (3 X 20 mL) and brine (30 mL), dried over anhydrous magnesium sulfate and concentrated at reduced pressure. The crude product was purified by silica-gel flash column chromatography (EtOAc–petroleum ether, 20%, v/ v) and recrystallized from $CHCl_3$–hexane to give **THBPY** as red crystal (177 mg, yield, 10%) M. P.> 200°C. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 7.468-7.448 (m, 2H), 7.266-7.242 (d, 1H), 7.190-7.186 (d, 1H), 5.953 (s, 2H), 4.727 (s, 2H), 2.527 (s, 6H), 1.350 (s, 6H). ^{13}C NMR (Figure S8) (75 MHz, $CDCl_3$), δ (ppm), 155.49, 143.02, 142.08, 141.50, 135.19, 131.37, 129.32, 127.24, 127.16, 126.39, 121.22, 64.71, 30.88, 29.23, 14.56, 14.43; QTOF-MS ES$^+$, calcd. for $C_{40}H_{43}N_4O_4$ $(M+H)^+$ 643.3278: found, 643.3276. QTOF-MS ES$^+$ calcd. for $C_{20}H_{20}BF_2N_2O$ $(M+H)^+$, 355.1793, found, 355.1793.

3-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)benzaldehyde (3)

Compound **2** (150 mg, 0.423 mmol) was dissolved in dry CH_2Cl_2 under argon atmosphere and stirred at 0°C for 15 min and then Dess-Martin periodinane (179.61 mg, 0.423 mmol) was added. The reaction mixture was stirred at r.t. for 30 min. After completion of the reaction (checked by TLC), the solvent was removed under reduced pressure. Organic residue was extracted with EtOAc and dried over anhydrous sodium sulphate. Removal of solvent gave brown oil. It was purified by column chromatography using 40% EtOAc in petroleum ether to give 135 mg red solid (yield, 93%) having R_f = 0.35 in 10% EtOAc in petroleum ether. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 10.073 (s, 1H), 8.043-8.013 (m, 1H), 7.852-7.848 (d, 1H), 7.731-7.680 (t, 1H), 7.608-7.578 (m, 1H), 6.004 (s, 2H), 2.567 (s, 6H), 1.341 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm), 191.30, 156.16, 142.66, 139.45, 137.10, 136.14, 134.08, 131.17, 129.97, 129.52, 121.60, 29.64, 14.64, 14.58; QTOF-MS ES$^+$ calcd. for $C_{20}H_{20}BF_2N_2O$ $(M+H)^+$, 353.1636, found, 353.1636.

3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene] -4'- carbaldehyde (4)[24]

Fluorescein (2.5 g, 7.75 mmol) was dissolved in MeOH (3 mL) in a 100 mL three neck round bottom flask. Next, 10g of 50% NaOH solution, 2.42 mL (30 mmol) of $CHCl_3$ and 0.03 mL of 15-crown-5 were carefully added maintaining the reaction

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temperature at 55°C. The mixture was stirred at 55°C for 7h. After cooling, the mixture was acidified with 10M H_2SO_4 while precipitate appeared. The solid was filtered and dried in vacuum overnight. Silica gel chromatography using EtOAc: DCM (15:85, v/v) gave a light yellow solid with 34.1% yield (952 mg). R_f= 0.28 (EtOAc: DCM, 15:85, v/v). ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm), 10.589-10.580 (d, 1H), 10.241-10.231 (d, 1H), 7.963 (s, 1H), 7.766 (s, 1H), 7.688 (s, 1H), 7.284-7.259 (d, 1H), 6.926-6.886 (t, 1H), 6.804 (s, 1H), 6.683-6.653 (d, 1H), 6.569 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6), δ (ppm), 193.31, 169.00, 163.38, 160.07, 152.83, 152.62, 151.31, 136.97, 136.25, 130.78, 129.45, 126.36, 125.25, 125.09, 124.44, 113.98, 113.83, 110.14, 109.66, 109.59, 103.10, 82.26, 70.26, 29.43.

Cell imaging studies

Human breast cancer cell line MDA-MB 231 were incubated with **THBPY** (50 μM) for 2h followed by addition of Lys (5 μM). Then the cells were washed with PBS buffer and observed under fluorescence microscope with 10 X 40x magnification using blue filter. Only **THBPY** treated cells were used as reference.

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