



Communication

Red-emitting salicylaldehyde Schiff base with AIE behaviour and large Stokes shift

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ABSTRACT

Three salicylaldehyde Schiff base (SSB), *iso*-PBP, PBP and EPB, were facilely synthesized and exhibited aggregation-induced emission. The introduction of C=N=N=C moiety in these SSB dyes largely extend the conjugation system and push their emission to yellow to red spectral region. These SSB dyes were negligibly fluorescent in dilute THF solution. In THF/water mixtures with high water fractions, they displayed strong yellow to red fluorescence (up to 617 nm) and large Stokes shifts (up to 152 nm). Single crystal analysis on EPB showed the longer emission of in aggregated state was attributed to the molecular packing effect as compared with that in dilute solution. The bio-imaging application indicated EPB could specifically accumulate in lipid droplets in living cells.

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Red-emitting fluorophores such as rhodamines, BODIPY derivatives and some coumarins have been widely applied as bio-imaging agents, photo-sensitizers and optical materials [1–5]. Usually, these dyes are strongly fluorescent in dilute solutions, however, are weakly fluorescent in high concentration and/or in solid state due to the aggregation-caused quenching (ACQ) effect [6–8]. In 2001, multiphenylsiloles had been discovered to display strong emission in high aggregation and solid state and are weakly fluorescent in dilute solutions, which is called aggregation-induced emission (AIE) [9,10]. Later on, more materials have been found to exhibit AIE properties with different features and functions [11–19]. The typical prototypical AIE luminogens (AIEgens) such as 1-methyl-1,2,3,4,5-pentaphenylsilole and tetraphenylethylene (TPE) emit in a short-wavelength spectral region [20,21]. To date, not many AIEgens with simple structures have exhibited emissions in red/NIR spectral region. It is well known that fluorophores with long-wavelength emissions are more favorable because long-wavelength photos have deeper tissue penetration, less photodamage to the biological tissue, and less interference from auto fluorescence in organism. Therefore, it is very meaningful to develop new red-emitting AIEgens.

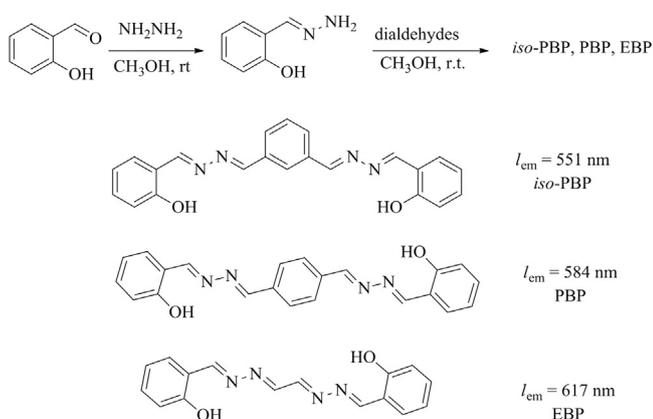
Most of the reported red-emitting AIEgens are subjected to complicated preparation [22]. Salicylaldehyde Schiff base (SSB) dyes could be easily obtained and were reported as AIEgens by Tong's group in 2009, which displayed a large Stokes shift due to the excited state intramolecular proton transfer (ESIPT) process [23]. Later on, more SSB dyes have been investigated [24–32]. However, so far SSB dyes emitting in red/NIR spectral region (above 600 nm) are still rare.

SSB dyes belong to donor-acceptor dyes and undergo an intramolecular charge transfer (ICT) process upon excited. It is known that long-wavelength emission can be obtained by enhancing ICT effect in SSB dyes through two approaches: 1) using strong electron donors such as dialkylamino groups and/or strong electron acceptors such as nitro group [33–36]; 2) extending conjugation system to significantly narrow the energy gap between ground state and first singlet excited-state [37].

Herein, we reported the design and facile synthesis of three new SSB dyes, *iso*-PBP, PBP and EPB, in which C=N=N=C segments were readily introduced through the condensation reaction between hydrazine and aldehydes to extend their conjugation system and thereby to shift their emissions to red spectral region (Scheme 1). It is noteworthy that the ESIPT process in these SSB dyes would provide a large Stokes shift which is beneficial for practical applications.

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Scheme 1. Synthetic route and chemical structures of *iso*-PBP, PBP and EBP.

The synthetic routes of *iso*-PBP, PBP and EBP were shown in Scheme 1. First, salicylaldehyde reacted with hydrazine in methanol at room temperature to afford salicylaldehyde hydrazone with a 75% yield. Next, the hydrazone reacted with dialdehydes: glyoxal, terephthalaldehyde and isophthalaldehyde to yield *iso*-PBP, PBP and EBP, with 88%, 77% and 90% yields, respectively. Pleasantly, all the starting materials are commercially available and low-cost; the reaction conditions are mild; especially the purification of these products can be realized by simple recrystallization. All the new compounds were fully characterized by NMR and high-resolution mass spectral analysis.

We further investigated their optical behavior in solid form. *iso*-PBP, PBP and EBP were light yellow powder and were strongly fluorescent in solid-state. The photoluminescence (PL) spectra of these SSB dyes in solid state were shown in Fig. 1. *iso*-PBP, PBP and EPB emitted yellow ($\lambda_{\max}^{\text{Em}} = 551 \text{ nm}$), orange ($\lambda_{\max}^{\text{Em}} = 584 \text{ nm}$) and red ($\lambda_{\max}^{\text{Em}} = 617 \text{ nm}$) fluorescence, respectively. All the SSB dyes exhibited large Stokes shifts (up to 152 nm for EBP) due to the ESIPT process. The absolute fluorescent quantum yields for *iso*-PBP, PBP and EBP in solid-state (Φ_{ss}) are 7.21%, 4.60% and 2.00%, respectively.

iso-PBP, PBP and EBP were soluble in common organic solvents such as hexane, toluene, dichloromethane, THF, ethyl acetate and DMF. As expected, *iso*-PBP, PBP and EBP were weakly fluorescent in these organic solvents due to the rotation of N–N single bonds, as shown in Fig. S1 (Supporting information). Their quantum yields of *iso*-PBP, PBP and EBP in dilute THF solutions were 0.31%, 0.11% and 0.03%, respectively.

Next, their AIE properties were investigated by measuring their PL spectra in THF-water mixtures with different water fractions (f_w) (Fig. 2). For EPB, the fluorescence started to occur when the water fraction was increased to 60% and the fluorescence enhancement was up to 70-fold when the f_w reached 90%. The absolute fluorescent quantum yields (Φ_{aggr}) of *iso*-PBP, PBP and

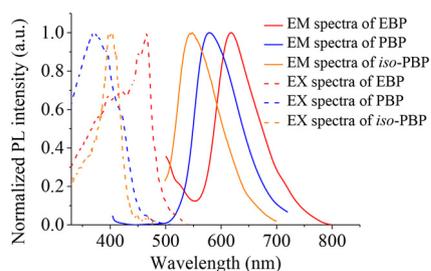


Fig. 1. Normalized emission (solid) and excitation (dash) spectra of *iso*-PBP, PBP and EBP in solid state.

EBP in the mixture of THF-water with $f_w = 90\%$ were 21.00%, 13.12% and 5.10%, following the same order in solid state. Dynamic light scattering (DLS) analysis was performed to determine the particle size of EBP, PBP and *iso*-PBP in the mixture of THF-water with $f_w = 90\%$, which were 171.59 nm, 393.81 nm and 300.35 nm, respectively (Fig. S2 in Supporting information). These results indicated that the fluorescence enhancement of these SSB dyes was ascribed to the restriction of intramolecular rotation in aggregation state (Scheme S1 in Supporting information). The AIE activity of them is 170.0, 119.3 and 67.7 (as shown in Table S2 in Supporting information). In the mixture of THF/water with small f_w , the dyes did not aggregate and the ESIPT process was inhibited to some extent due to the formation of intermolecular hydrogen bonds between dye molecules and the solvent. In contrast, dyes aggregated in the mixture of THF/water with high f_w , which prevented the formation of intermolecular hydrogen bonds between dye molecules and recovered intramolecular hydrogen bonds between dye molecules to favor the ESIPT process [13]. It was noted that a blue-shift in the absorption spectra was observed for PBP when the solvent changed from pure THF to the mixture of THF/water with $f_w = 90\%$. In contrast, EBP displayed a red-shift in its absorption spectra when making the same solvent change (Fig. S3 in Supporting information).

To provide further mechanistic insight, we performed density function theoretical (DFT) calculations on these SSB dyes (Fig. S4 in Supporting information). It was found that they all had a highly coplanar geometry. The energy gaps between HOMO and LUMO of *iso*-PBP, PBP and EPB were 3.54 eV, 3.20 eV and 3.21 eV, which well agreed with their absorption spectra in THF.

In order to gain profound information about the AIE behavior of these SSB dyes, we managed to obtain the single crystal structure of EBP, as shown in Fig. S5 (Supporting information). The distance of O–H...N was 1.84 Å, indicating the formation of strong intramolecular hydrogen bonding which facilitated the ESIPT process in EPB. EBP had a completely co-planar conformation and the intermolecular distance C(1)...N(2) and C(2)...N(1) was 3.43 Å, which implied the strong intermolecular interaction. The strong dipole coupling between two neighbouring molecules of EPB enhanced the ICT process in EPB to induce the bathochromic shift, which gave a good explanation of red-shift in the emission of EPB in aggregated state as compared with that in THF solution [13]. Since the single crystal of PBP was not obtained, we did DFT calculation on PBP and EPB to obtain their electrostatic potential surfaces. As shown in Fig. S6 (Supporting information), the negative charge on nitrogen atoms in PBP were localized by the middle benzene ring, which result in a loose packing in aggregation form or solid state and weak intermolecular dipole interactions between neighbouring molecules. As a result, the less conjugated EBP showed a longer emission compared to PBP.

Moreover, since C=N bond is usually unstable in strong acidic condition, the stability of EBP and PBP should be concerned. It was investigated at different pH values by monitoring their fluorescence behavior (Fig. S7 in Supporting information). In acidic media, the emission spectra of EBP and PBP hardly changed. However, in basic media a large blue-shift in their absorption spectra and a serious fluorescence quench was observed for EPB and PBP. This result was ascribed to the prohibition of ESIPT process and was consisted with the previous report on SSB dye [30]. ^1H NMR spectral analysis showed that the signal of the active proton of hydroxyl group in PBP disappeared when NaOH was added (Fig. S8 in Supporting information). The optical spectral change of EPB in acidic and basic media was reversible for many cycles, indicating the good stability of these SSB dyes (Fig. S9 in Supporting information).

Finally, we used EPB to perform bio-imaging experiments in living HeLa cells (Fig. 3). When cells were incubated with 0.02

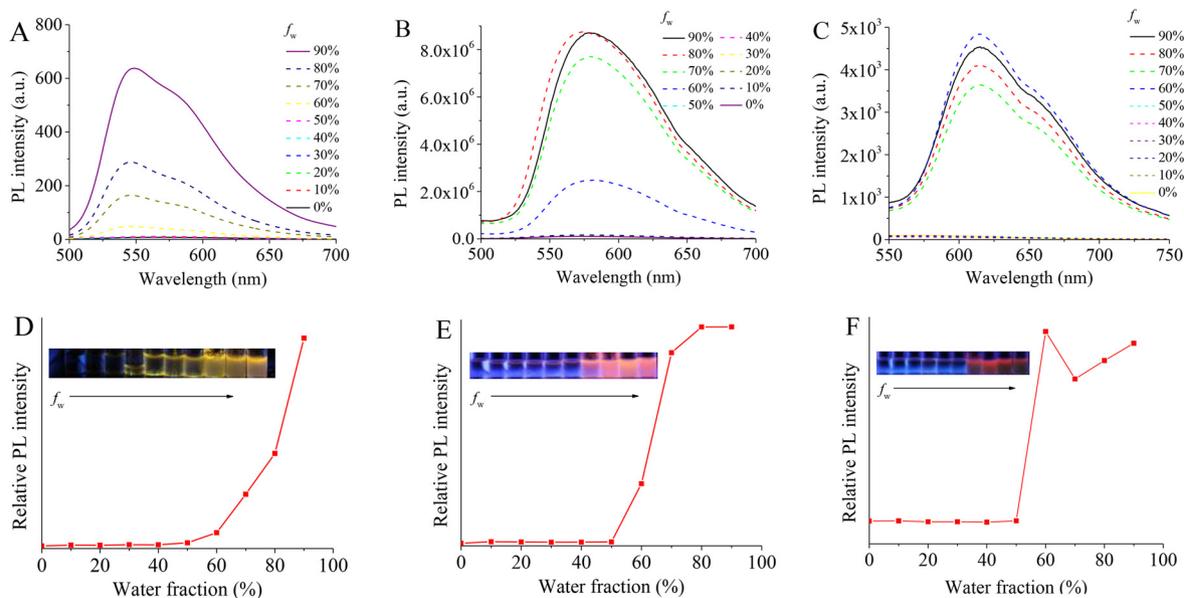


Fig. 2. Fluorescence spectra of *iso*-PBP (A), PBP (B) and EBP (C) in THF/water mixtures. Fluorescence intensity of *iso*-PBP (D), PBP (E) and EBP (F) in THF/water mixtures as a function of the water fraction. Inset: fluorescence images of *iso*-PBP, PBP and EBP in THF/water mixture under a 365 nm light irradiation.

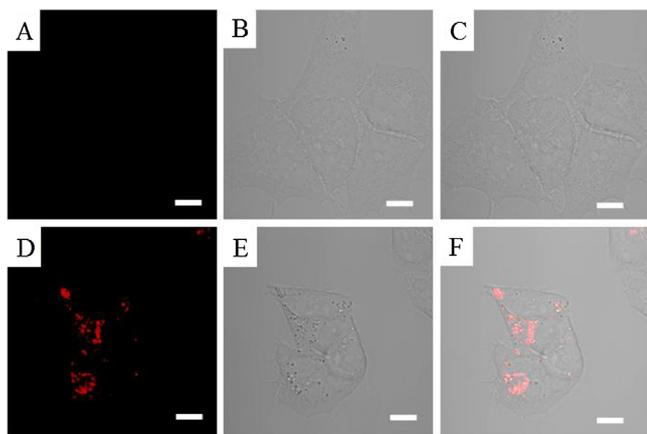


Fig. 3. Fluorescence (A, D), bright-field (B, E) and merged (C, F) images of HeLa cells. Top row (A, B, C): cells without the treatment of EPB; bottom row (D, E, F): cells incubated with EBP (20 $\mu\text{mol/L}$) at 37 $^{\circ}\text{C}$ for 1 h. Scale bar: 10 μm .

mmol/L EBP at 37 $^{\circ}\text{C}$ for 1 h, a strong red fluorescence was observed. It is noted that EBP selectively accumulated in lipid droplets (LDs) of cells. LDs, as important subcellular organelles, are intimately involved in many diseases and are indicators for cell's living status [38,39]. However, there were few red-emitting AIEgens that could specifically accumulate in LDs [30,40,41]. Thus, EBP was a promising AIEgen for specific imaging LDs in living cells.

In conclusion, we developed a new type of SSB dyes exhibiting AIE behavior with long-wavelength emissions, large Stokes shifts and LDs-targeting ability in living cells. Particularly, the facile preparation of these SSB dyes and the low cost of starting materials made them have a good potential for the future application.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ccl.2018.05.043>.

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