

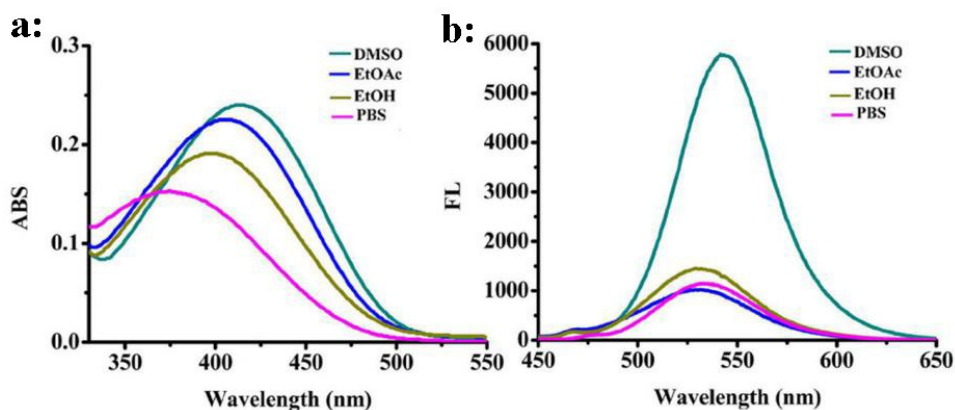
## Supporting information

### Experiment details of synthesis:

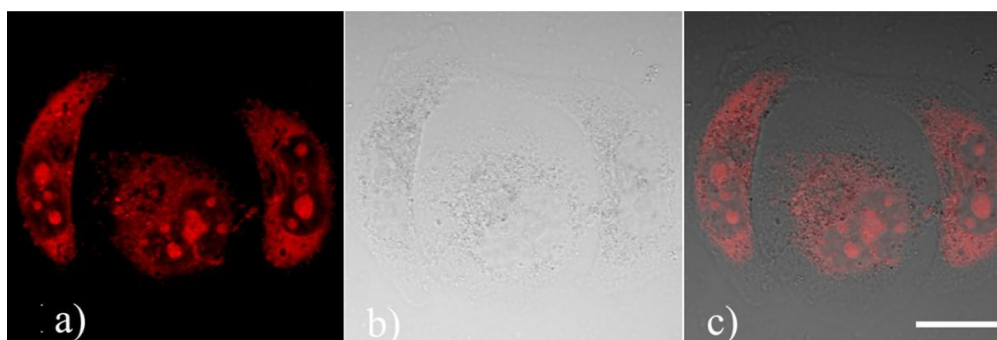
#### *N*-Methyl -2-methylbenzimidazole iodine (1) :

(1): 0.05 mol of 2-Methylbenzimidazole (6.61 g) and 0.11 mol of 2-iodomethane (16.6 g) were dissolved in toluene and stirred for 6 hours, thereafter refluxed 30 min. After cooling and filtrating, the resulting solid were washed with ether. White powder (*N*-Methyl -2-methylbenzimidazole iodine) was obtained.

### Spectrum spectrogram



**Fig. S1** The UV-vis absorption (a) and one-photon excited fluorescence (b) spectra of **DMI** in DMSO (green), EtOAc (blue), EtOH (brown) and PBS (pink). Compound concentration:  $2 \times 10^{-6}$  mol/L.



**Fig. S2** Confocal fluorescence images of fixed HeLa cells stained with the **DMI** ( $5 \mu\text{M}$ , 30 min). a, Fluorescent image. b, DIC picture. c, Overlay image of a and b.  $\lambda_{\text{ex}} = 488 \text{ nm}$ ,  $\lambda_{\text{em}} > 565 \text{ nm}$ . Bar = 20  $\mu\text{m}$ .

**Table S1** Cytotoxicity Data (HeLa,  $5 \mu\text{M}$ )<sup>a</sup> of **DMI** .

| Incubate time (h)     | 6    | 12   | 24   |
|-----------------------|------|------|------|
| DMI (% cell survival) | 92±1 | 79±3 | 63±5 |

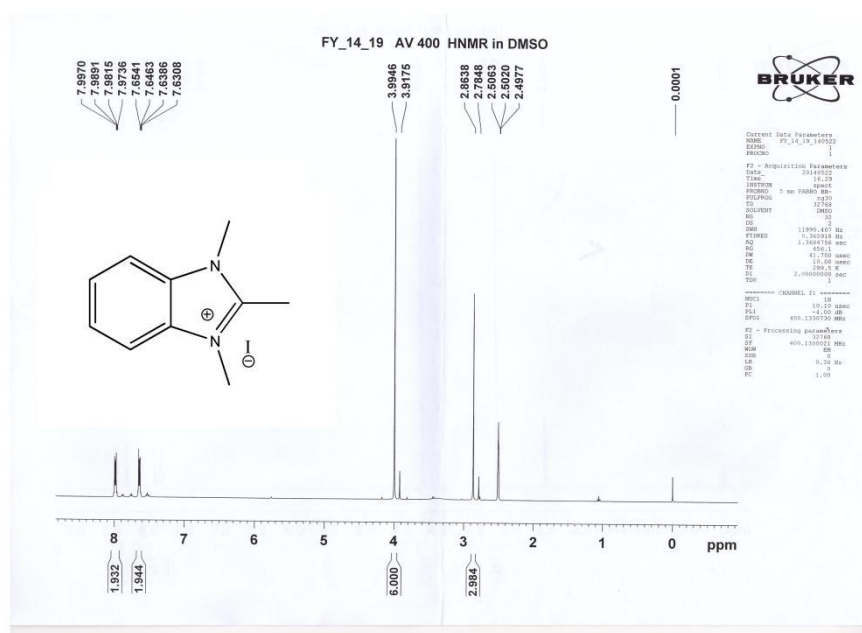
<sup>a</sup> Cell viability was quantified by the MTT assay (mean±SD)

**Table S2** Cytotoxicity Data (HeLa, 24h)<sup>a</sup> of DMI .

| Incubate concentration ( $\mu\text{M}$ ) | 2          | 5          | 10         | 20         |
|------------------------------------------|------------|------------|------------|------------|
| DMI (% cell survival)                    | 79 $\pm$ 1 | 67 $\pm$ 3 | 60 $\pm$ 3 | 52 $\pm$ 5 |

<sup>a</sup> Cell viability was quantified by the MTT assay (mean $\pm$ SD)

The characterization of (1) and DMI, including their <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS spectra.



**Fig. S3** <sup>1</sup>H NMR spectrum of (1) in DMSO.

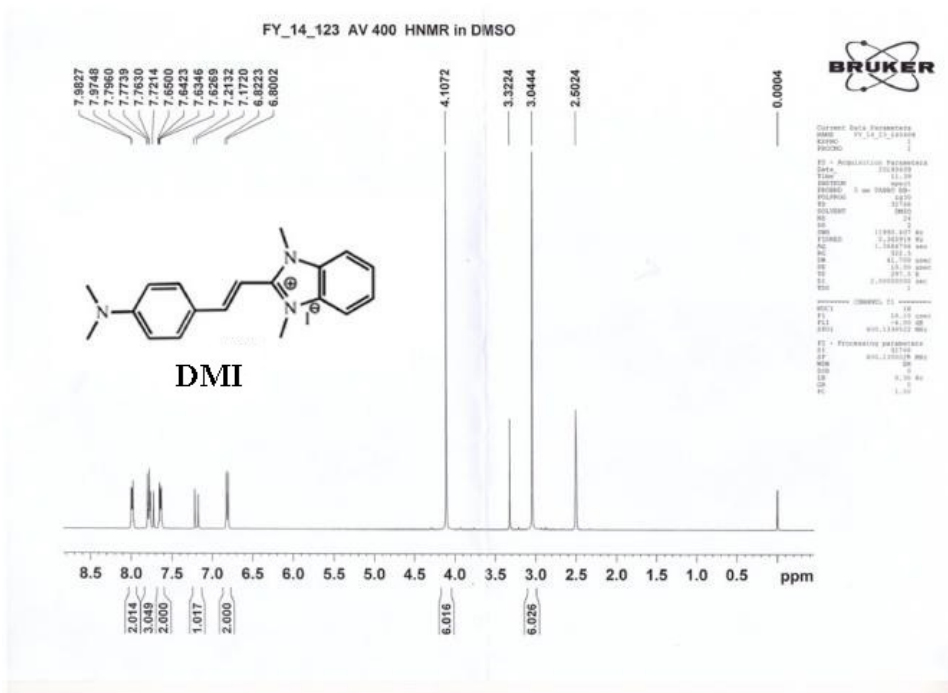


Fig. S4  $^1\text{H}$  NMR spectrum of DMI in DMSO.

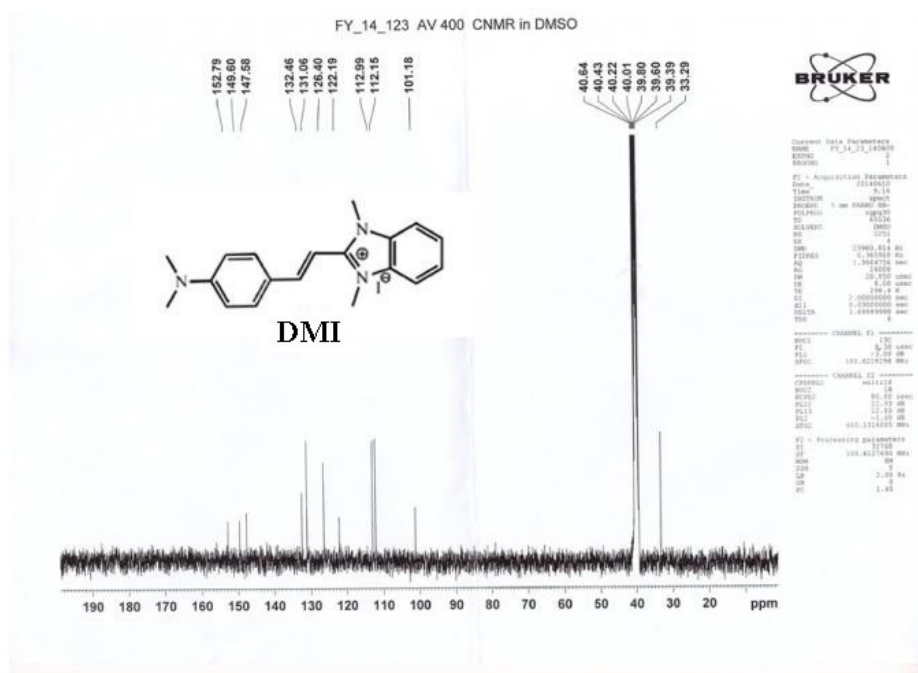
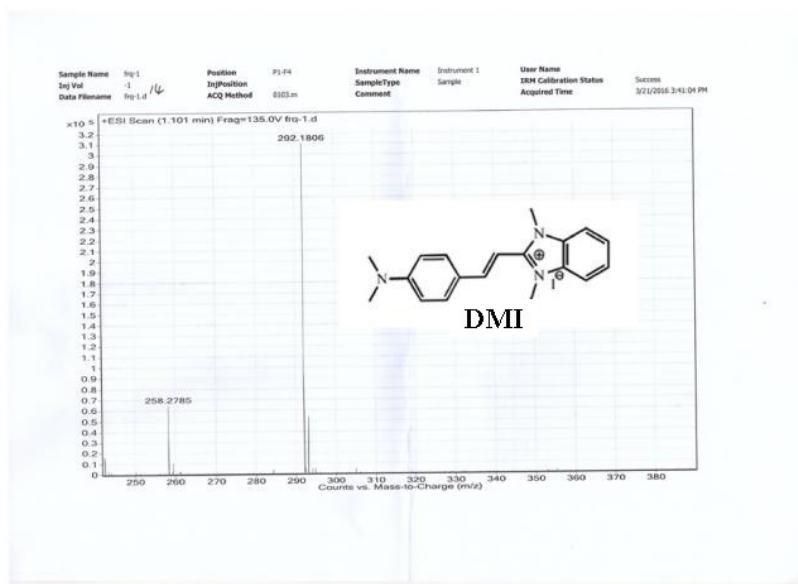


Fig. S5  $^{13}\text{C}$  NMR spectrum of DMI in DMSO.



**Fig. S6** HRMS spectra of DMI.