

## Supporting Information

# BODIPY-Pyridylhydrazone Probe for Fluorescence Turn-on Detection of Fe<sup>3+</sup> and Its Bioimaging Application

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### 1. <sup>1</sup>H and <sup>13</sup>C Nuclear Magnetic resonance (NMR) spectra

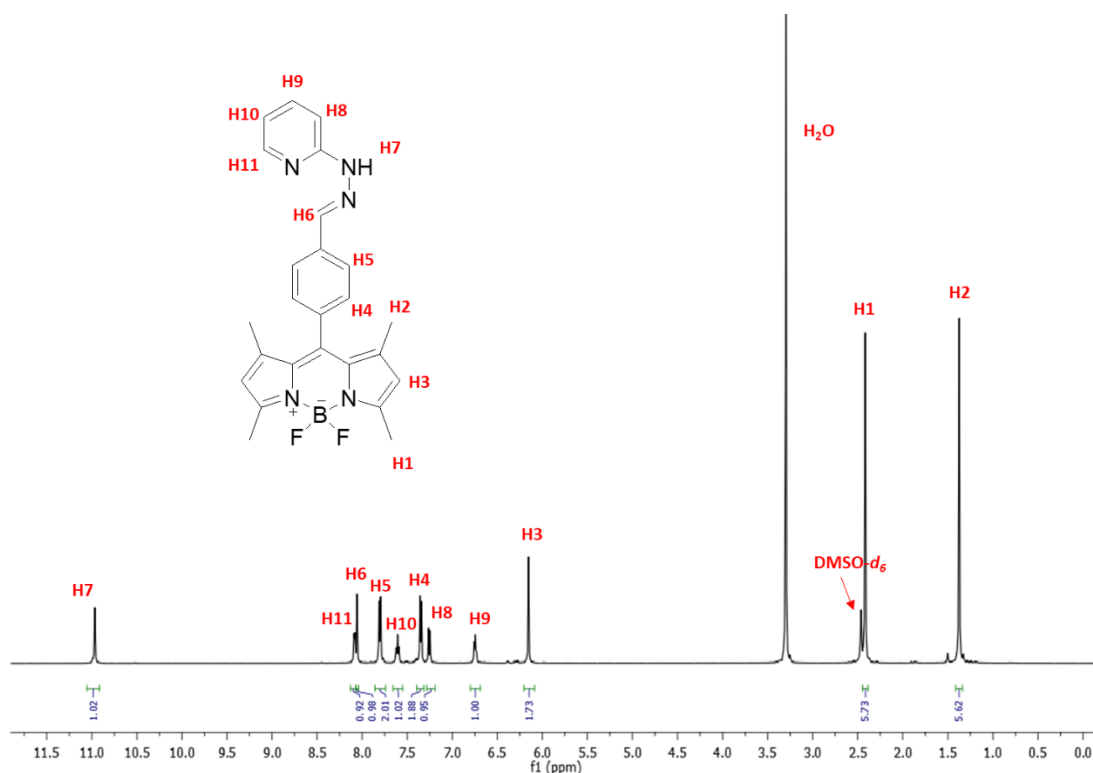


Figure S1. <sup>1</sup>H NMR spectra of **BODIPY-PH**.

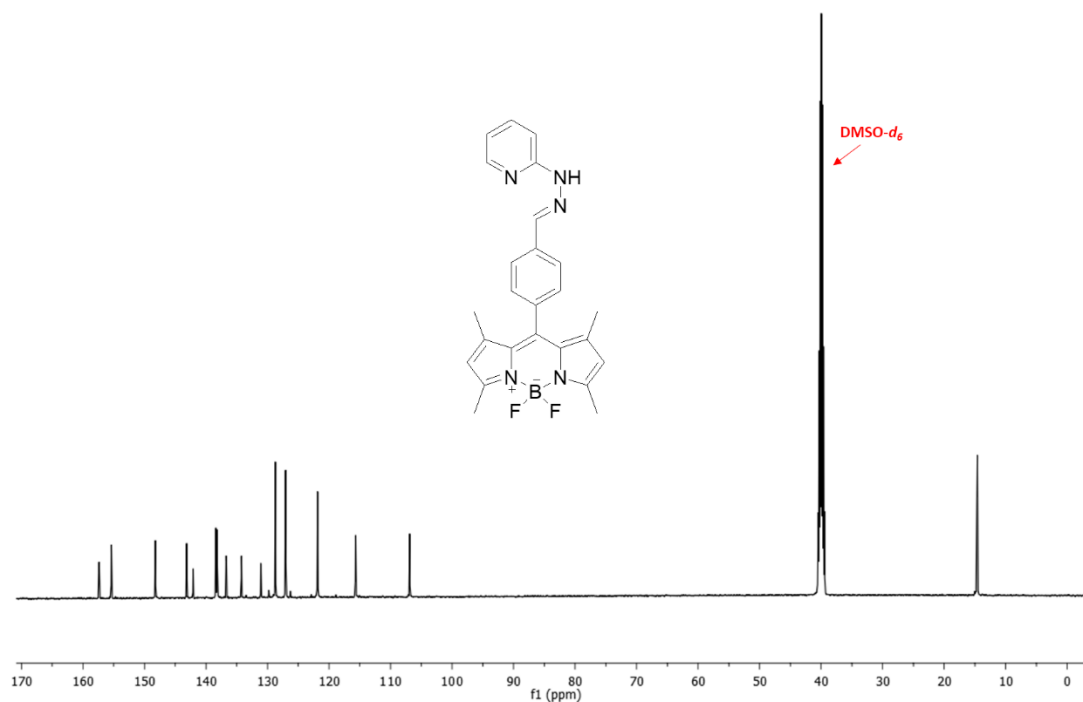


Figure S2. <sup>13</sup>C NMR spectra of BODIPY-PH.

## 2. Mass spectra BODIPY-PH compound and its Fe<sup>3+</sup> complexes

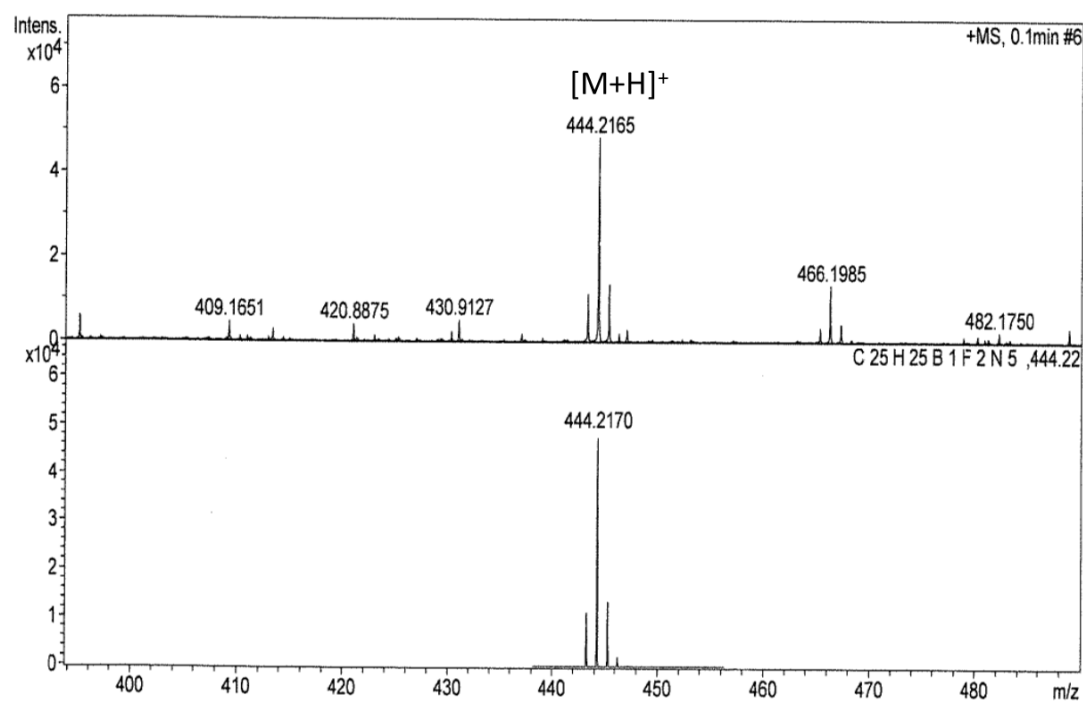
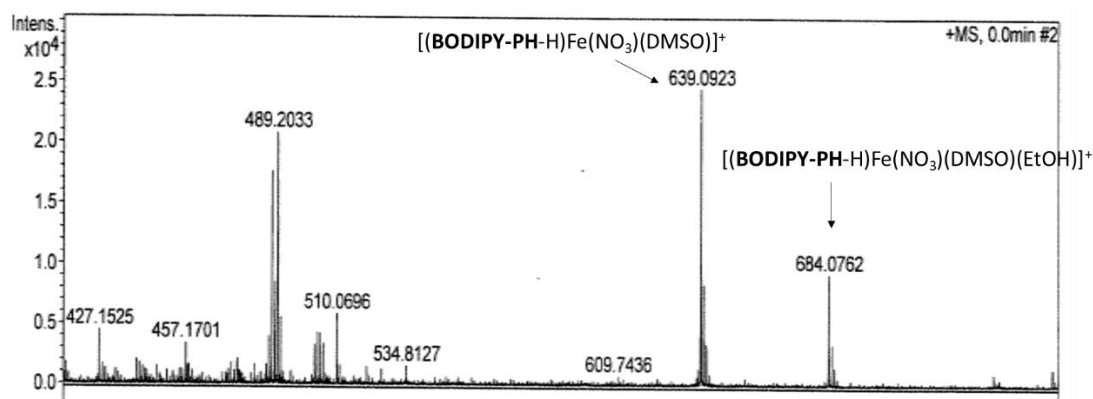


Figure S3. Mass spectra of BODIPY-PH.



**Figure S4.** Mass spectra of BODIPY-PH-Fe<sup>3+</sup> complexes.

### 3. Crystal data

**Table S1.** Crystal data and structure refinement for BODIPY-PH.

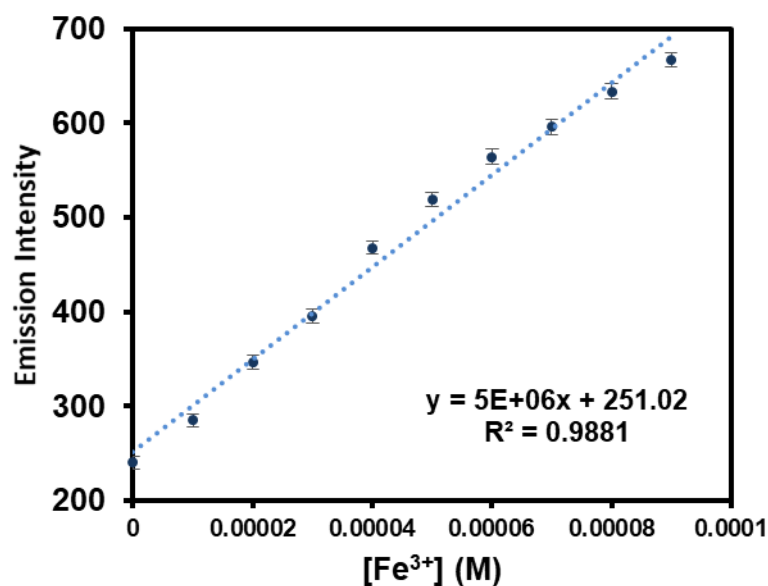
Compound	BODIPY-PH
Empirical formula	C <sub>25</sub> H <sub>24</sub> BF <sub>2</sub> N <sub>5</sub>
Formula weight	443.30
Temperature/K	298.0
Crystal system	Triclinic
Space group	$P\bar{1}$
$a/\text{\AA}$	10.9769 (7)
$b/\text{\AA}$	11.0720 (7)
$c/\text{\AA}$	13.0490 (8)
$\alpha/^\circ$	85.470 (2)
$\beta/^\circ$	78.635 (2)
$\gamma/^\circ$	60.491 (2)
Volume/ $\text{\AA}^3$	1352.83 (15)
$Z$	2
$\rho_{\text{calc}}/\text{g/cm}^3$	1.088
$\mu/\text{mm}^{-1}$	0.616
$F(000)$	464.0
Crystal size/ $\text{mm}^3$	$0.4 \times 0.33 \times 0.07$
Radiation	CuK $\alpha$ ( $\lambda = 1.54178$ )
$2\theta$ range for data collection/ $^\circ$	9.41 to 136.46
Index ranges	$-13 \leq h \leq 13, -13 \leq k \leq 13, -15 \leq l \leq 14$
Reflections collected	20374
Independent reflections	4790 [ $R_{\text{int}} = 0.0202, R_{\text{sigma}} = 0.0202$ ]
Data/restraints/parameters	4790/0/306
Goodness-of-fit on $F^2$	1.738
Final R indexes [ $I \geq 2\sigma(I)$ ]	$R_1 = 0.0965, wR_2 = 0.3336$
Final R indexes [all data]	$R_1 = 0.1025, wR_2 = 0.3518$
Largest diff. peak/hole / $e \text{\AA}^{-3}$	0.53/-0.53

**Table S2.** Geometric parameters and symmetry operations for intermolecular H-bond interactions ( $\text{\AA}, ^\circ$ ).

D—H $\cdots$ A	$d(\text{D—H})/\text{\AA}$	$d(\text{H}\cdots\text{A})/\text{\AA}$	$d(\text{D}\cdots\text{A})/\text{\AA}$	D—H $\cdots$ A/ $^\circ$
N4—H4 $\cdots$ N5 <sup>i</sup>	0.81(4)	2.00(2)	2.891(2)	177(2)
C15—H15 $\cdots$ F1 <sup>ii</sup>	0.93	2.45	3.280(2)	148
C25—H25 $\cdots$ F2 <sup>iii</sup>	0.93	2.47	3.225(4)	139

Symmetry codes: (i)  $-x, 2-y, -z$ ; (ii)  $1-x, -y, 1-z$ ; (iii)  $-1+x, 1+y, -1+z$ .

### 4. Standard calibration curve of BODIPY-PH vs. Fe<sup>3+</sup> concentrations



**Figure S5.** Standard calibration curve demonstrating the fluorescence intensity increments at 518 nm ( $n = 3$ ) as a function of  $\text{Fe}^{3+}$  concentrations incubated with **BODIPY-PH** (10  $\mu\text{M}$ ) in acetonitrile-water (1:9 v/v).

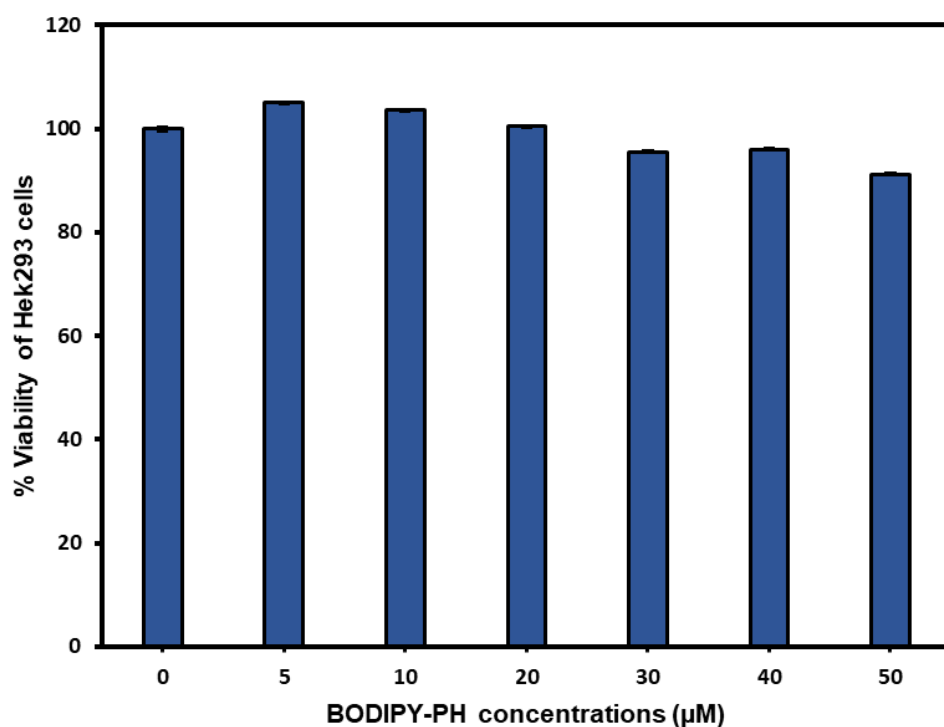
## 5. Investigation of the binding and complexation between BODIPY-PH ligand and $\text{Fe}^{3+}$ ion by X-ray absorption spectroscopy

**Table S3.** EXAFS fitting data for investigating the structural information of the complexation between BODIPY-PH ligand and  $\text{Fe}^{3+}$  ion.

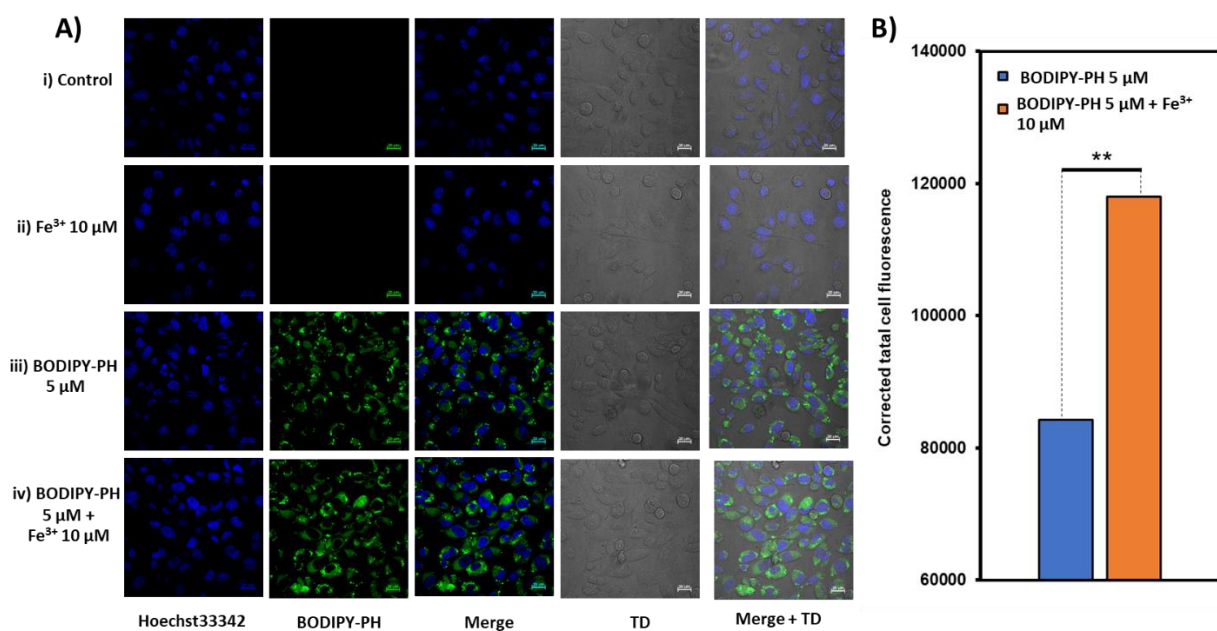
EXAFS fitting parameters		Fitting value	
<b>R-factor</b>		<b>0.00482</b>	
Reduced chi-square		51.2883	
$\Delta E_0$ (eV)		1.4547	
$S_0^2$		0.9582	
Scattering path (local structure)	N	$\sigma^2$ ( $\text{\AA}^2$ )	refined R ( $\text{\AA}$ )
<b>First shell</b>			
Fe—O ( $\text{H}_2\text{O}$ )	4	0.00192	2.01300
Fe—N5 (BODIPY-PH)	1	0.00110	1.99489
Fe—N3 (BODIPY-PH)	1	0.00110	2.26568
<b>Second shell / Multiple scattering</b>			
Fe—C21 (BODIPY-PH)	1	0.01106	2.86696
Fe—C25 (BODIPY-PH)	1	0.01106	2.92637
Fe—N4 (BODIPY-PH)	1	0.00110	2.96870
Fe—C20 (BODIPY-PH)	1	0.01106	3.22192
Fe—N3—C20 (BODIPY-PH)	2	0.00664	3.37143
<b>Third shell / Multiple scattering</b>			
Fe—C22 or Fe—C24 (BODIPY-PH)	2	0.01106	4.19386
Fe—C21—C22 (BODIPY-PH)	4	0.01659	4.22603

Fe—N5—C25 (BODIPY-PH)	4	0.00664	4.26876
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## 5. Cell viability and imaging data

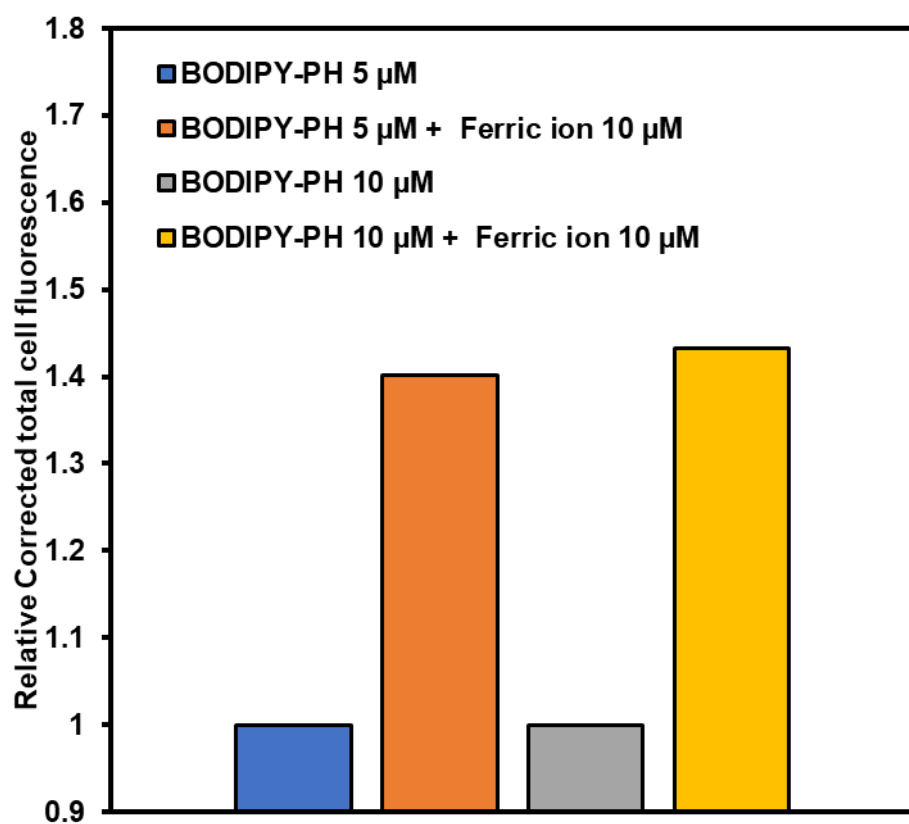


**Figure S6.** Percent cell viability estimated by MTT proliferation test versus incubation concentrations of **BODIPY-PH**. HEK-293 cells were cultured in the presence of **BODIPY-PH** (0-50 μM) at 37 °C for 24 h.



**Figure S7.** A) Confocal laser scanning microscopy (CLSM) images of HEK-293 cells. Row i, control HEK-293 cells with no treatment. Row ii, HEK-293 cells incubated with Fe<sup>3+</sup> (10 μM) for 30 min. Row iii, HEK-293 cells incubated with **BODIPY-PH** (5 μM) for 30 min. Row iv, HEK-293 cells incubated with Fe<sup>3+</sup> (10 μM) for 30 min and subsequently incubated with **BODIPY-PH** (5 μM) for another 30 min (For **BODIPY-PH**:  $\lambda_{ex}$  = 488 nm,  $\lambda_{em}$  = 509 nm, scale bar = 20 μm, TD = transmitted

light differential interference contrast image). All images were collected at the same microscopy settings. B) Bar chart showing corrected total cell fluorescence (CTCF) of HEK-293 cells incubated with **BODIPY-PH** (5  $\mu$ M) before and after the addition of  $\text{Fe}^{3+}$  (10  $\mu$ M). Statistical analysis is based on student's two tailed t-test (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).



**Figure S8.** Bar chart showing the relative corrected total cell fluorescence (CTCF) of HEK-293 cells incubated with **BODIPY-PH** (5 and 10  $\mu$ M) comparing between before and after the addition of  $\text{Fe}^{3+}$  (10 and 20  $\mu$ M).