

*Supplementary Material for*

Endoplasmic Reticulum-Targeting Two-Photon Fluorescent Probe for CYP1A  
Activity and Its Imaging Application in Endoplasmic Reticulum Stress

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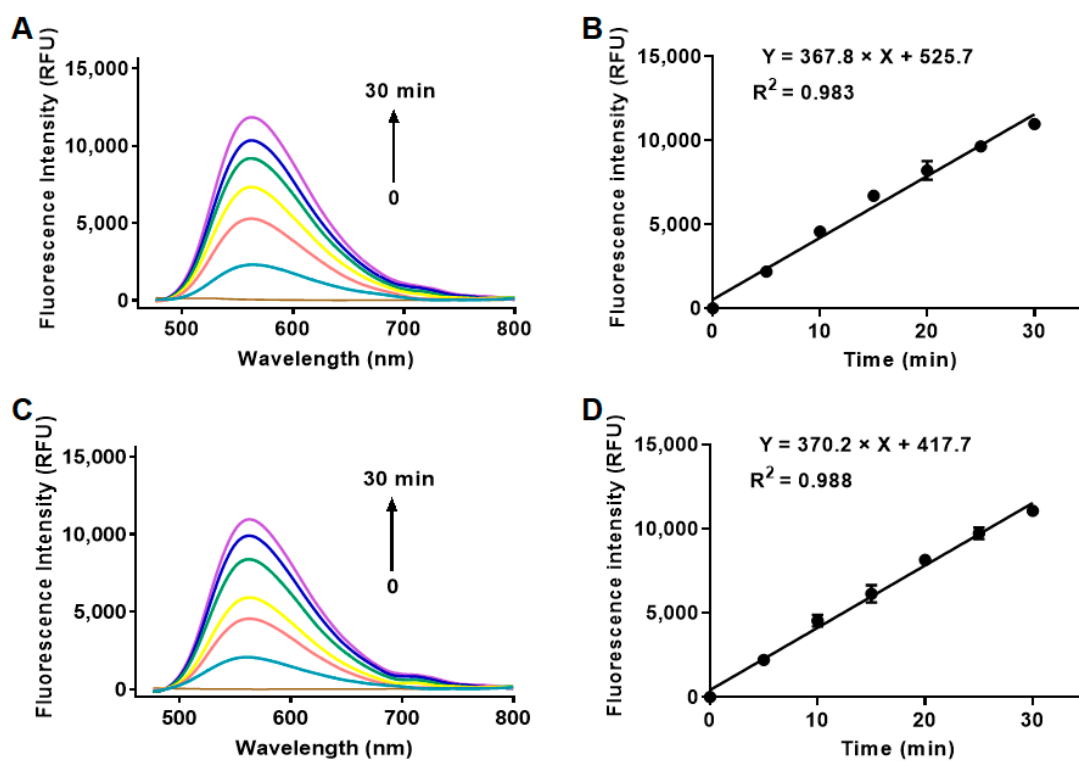
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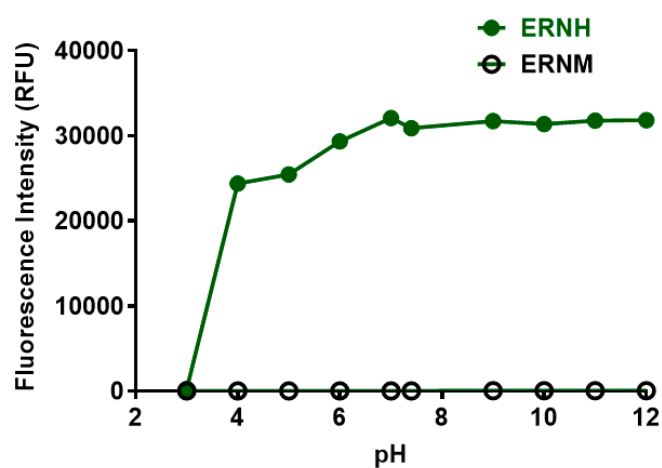
<sup>†</sup>These authors made equal contributions to this work.

## Table of Contents

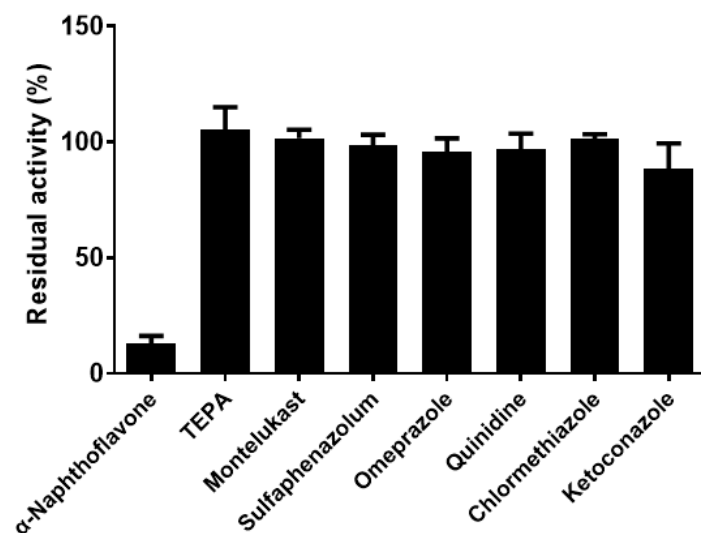
Supplementary Figure S1. ....	1
Supplementary Figure S2. ....	1
Supplementary Figure S3. ....	2
Supplementary Figure S4. ....	2
Supplementary Figure S5. ....	3
Supplementary Table S1. ....	3
NMR and MS Spectra. ....	4



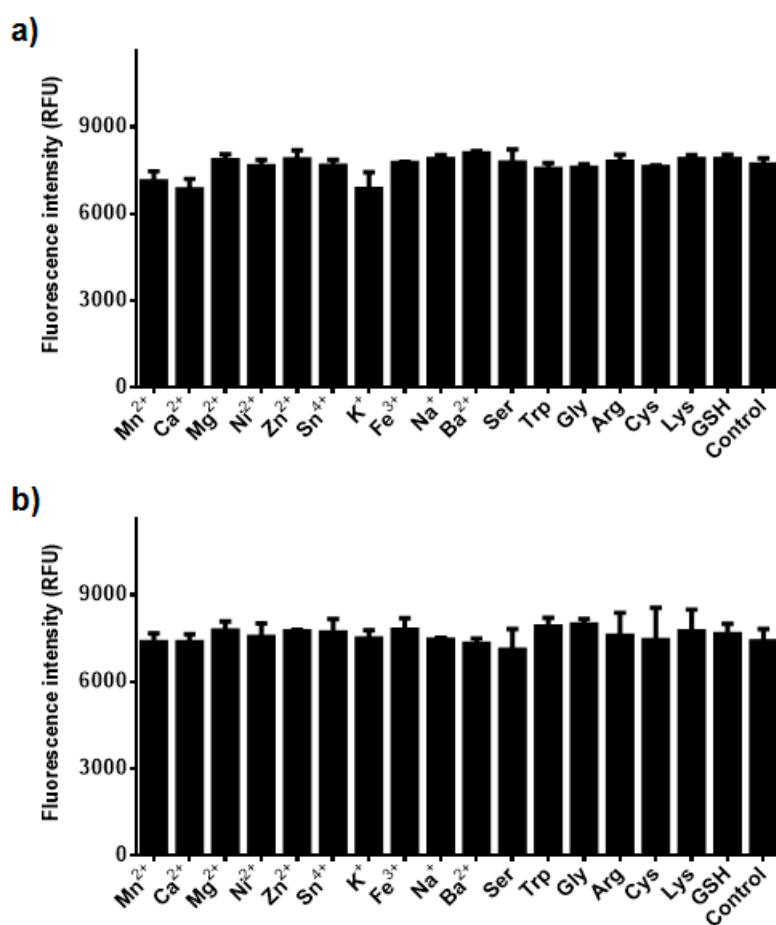
**Supplementary Figure S1.** Fluorescence spectra of **ERNM** that incubated with 7.5 nM CYP1A1 (A) and 7.5 nM CYP1A2 (C) with the prolonging of time, respectively. Fluorescence intensity of **ERNM** at 558 nm that incubated with CYP1A1 (B) and CYP1A2 (D) for different duration.  $\lambda_{\text{ex}} = 450$  nm. Data in Figure B and D are shown as the mean  $\pm$  S.D.



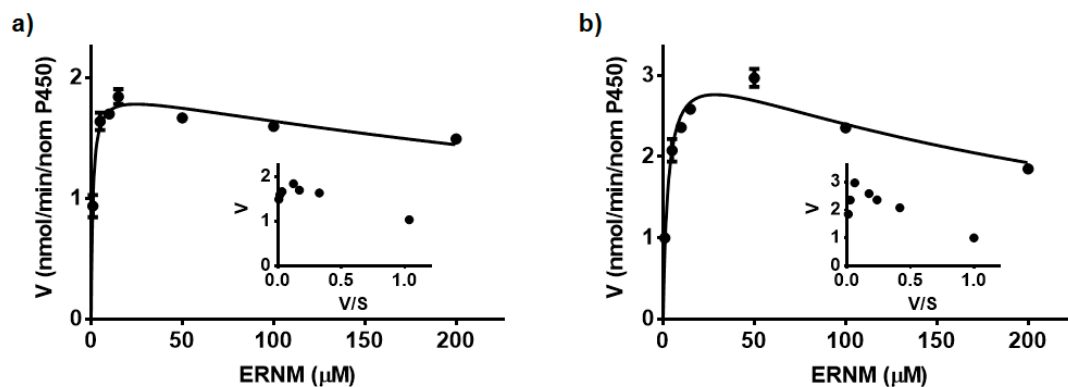
**Supplementary Figure S2.** The effects of pH values on the fluorescence intensity of **ERNM** and its metabolite **ERNH** (2.5  $\mu$ M).



**Supplementary Figure S3.** Inhibitory effects of selective CYP inhibitors on **ERNM** *O*-demethylation in human liver microsomes. TEPA, N,N',N''-triethylene thiophosphoramide.



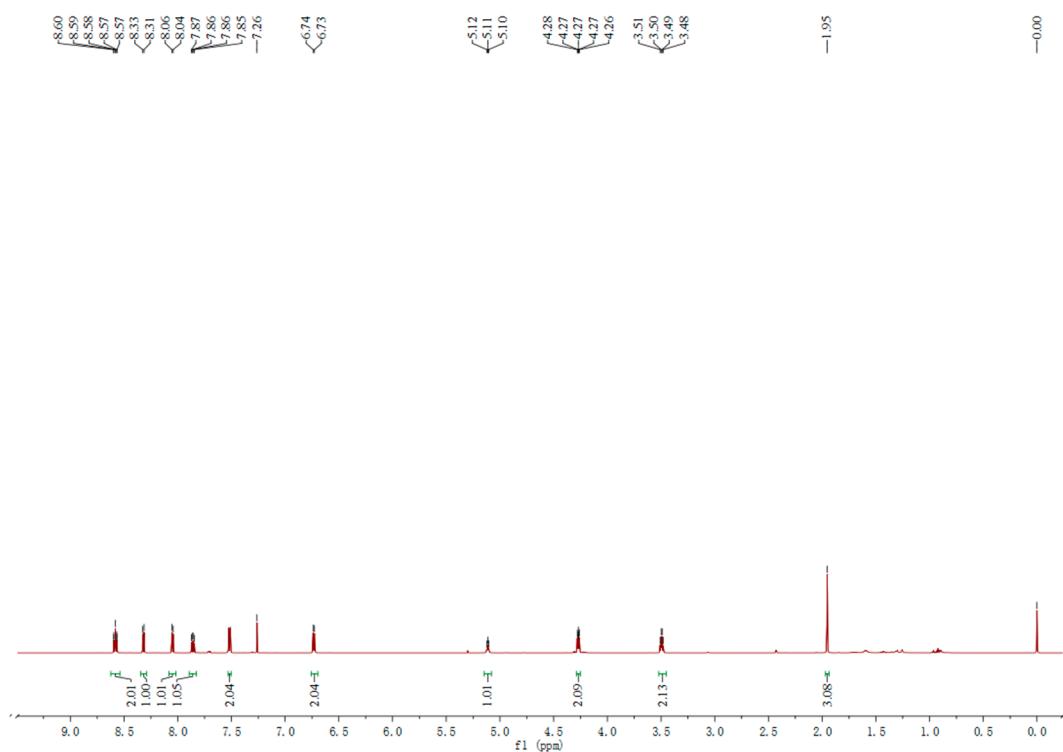
**Supplementary Figure S4.** Fluorescence responses of **ERNM** towards CYP1A1 (a) and CYP1A2 (b) in the presence of various analytes.



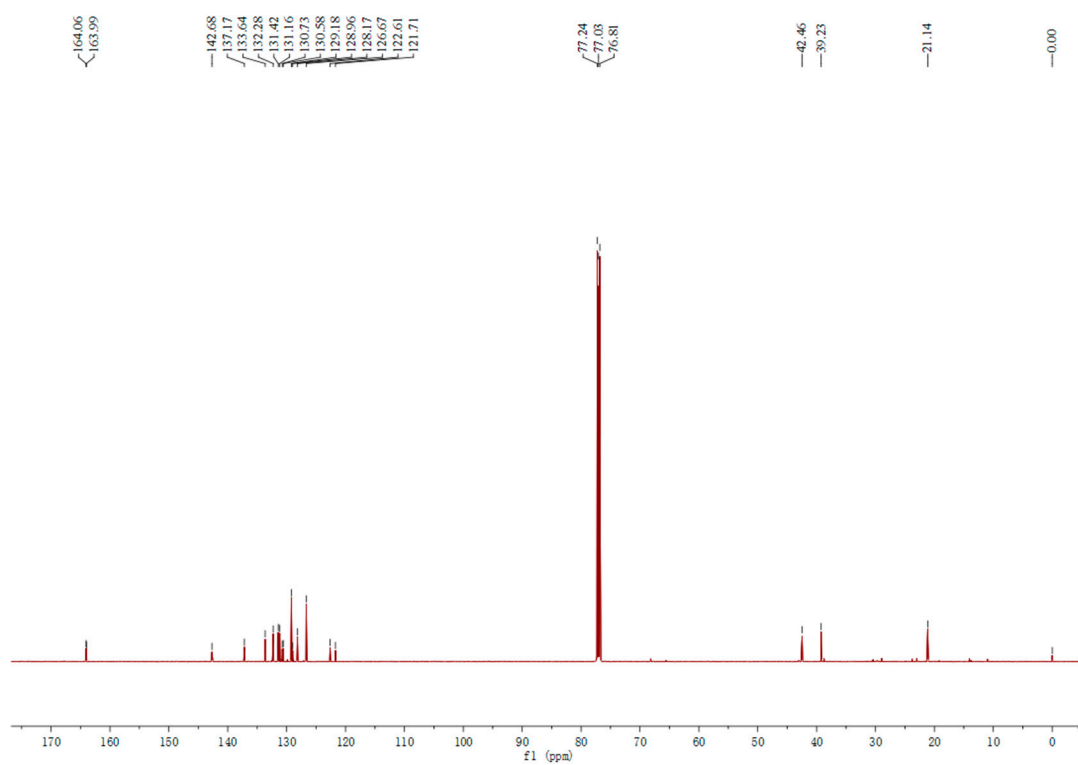
**Supplementary Figure S5.** Kinetic plots of **ERNM** *O*-demethylation in CYP1A1 (a) and CYP1A2 (b). The corresponding Eadie-Hofstee plot is shown as an inset.

**Table S1.** Kinetic parameters of **ERNM** *O*-demethylation determined in CYP1A1 and CYP1A2.

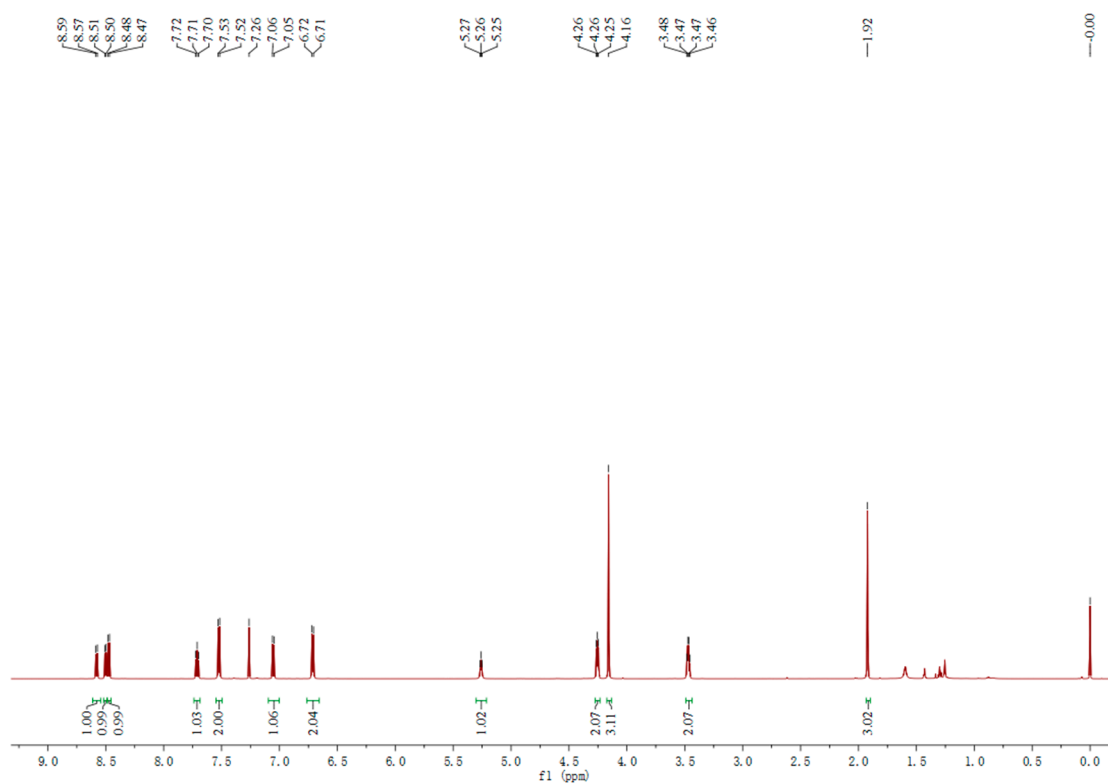
Enzyme sources	$V_{\max}$ (nmol/min/nmol p450)	$K_m$ ( $\mu\text{M}$ )	$K_{si}$ ( $\mu\text{M}$ )
CYP1A1	$1.92 \pm 0.04$	$1.0 \pm 0.1$	$611.3 \pm 93.8$
CYP1A2	$3.32 \pm 0.15$	$2.9 \pm 0.5$	$281.2 \pm 50.2$



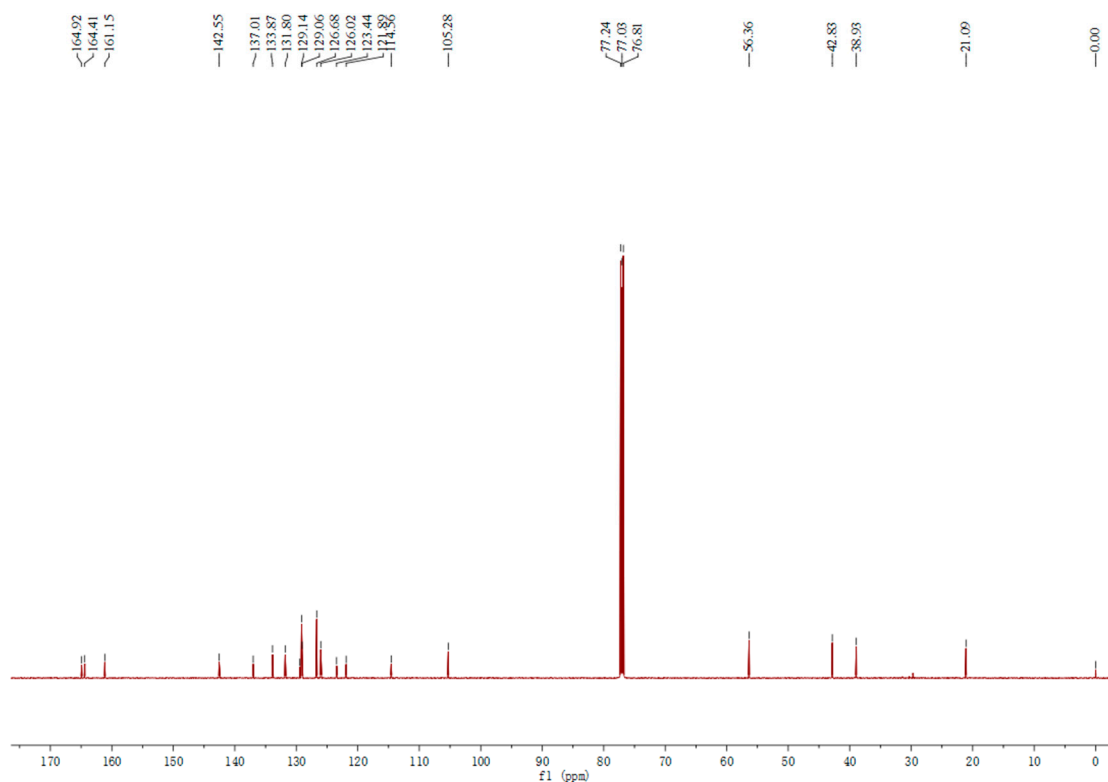
Supplementary Figure S6. <sup>1</sup>H NMR of ERNBr



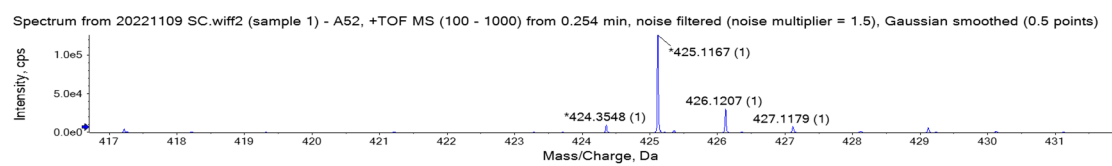
Supplementary Figure S7. <sup>13</sup>C NMR of ERNBr



Supplementary Figure S8. <sup>1</sup>H NMR of ERNM



Supplementary Figure S9. <sup>13</sup>C NMR of ERNM



**Supplementary Figure S10. HRMS spectrum of ERNM**