Supporting Information for

Combinatorial Strategy to Identify Fluorescent Probes for Biothiol and Thiophenol Based on Diversified Pyrimidine Moieties and Their Biological Applications

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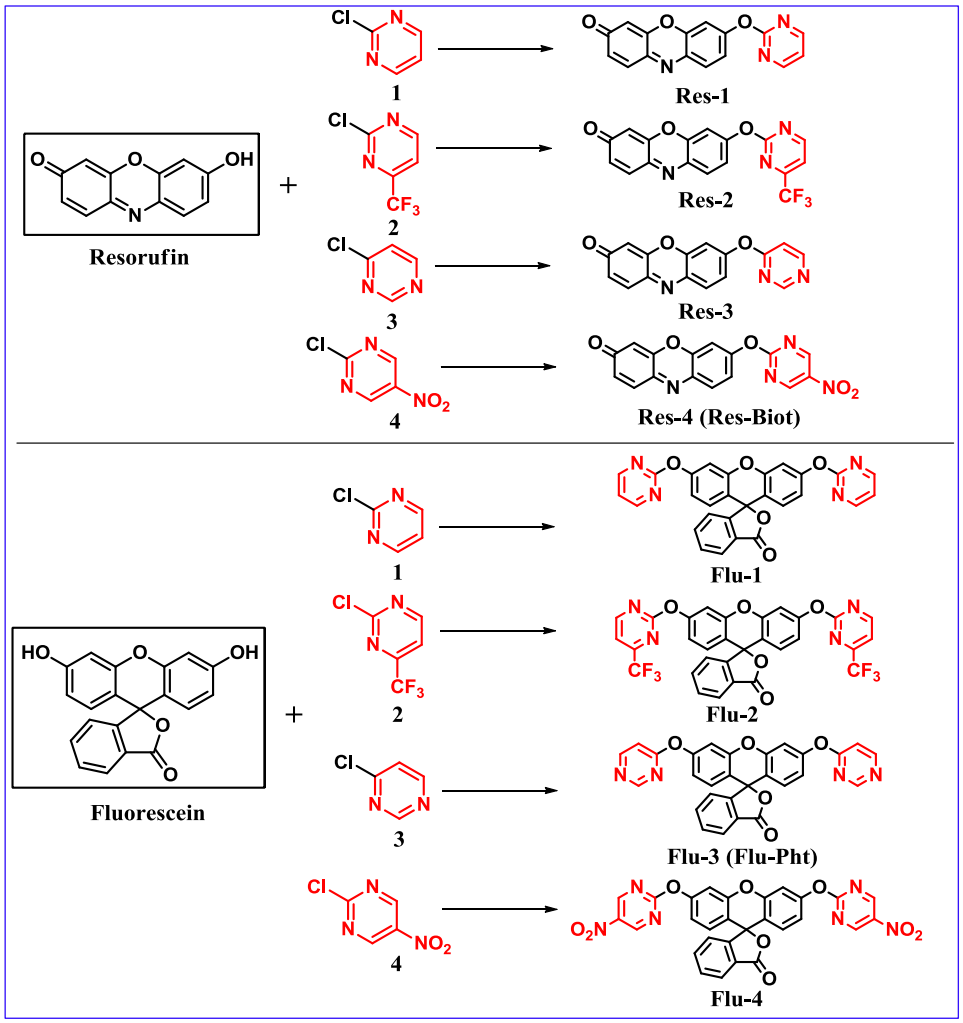
Contents:

1. Preparation of ROS and RNS

Hydrogen peroxide (H_2O_2) and hypochlorite (ClO^-) were delivered from the corresponding commercial aqueous solutions. Hydroxyl radicals ($^{\bullet}OH$) was generated in situ from reaction of Fe^{2+} and H_2O_2. Singlet oxygen ($^1O_2$) was generated in situ from ClO^-/H_2O_2 system. Nitric oxide (NO) was used from a stock solution prepared by sodium nitroprusside.

2. Parallel synthesis of the fluorescent probe library



Scheme S1. Parallel synthesis of fluorescent probe candidates

3. Rapid screening of probe candidates

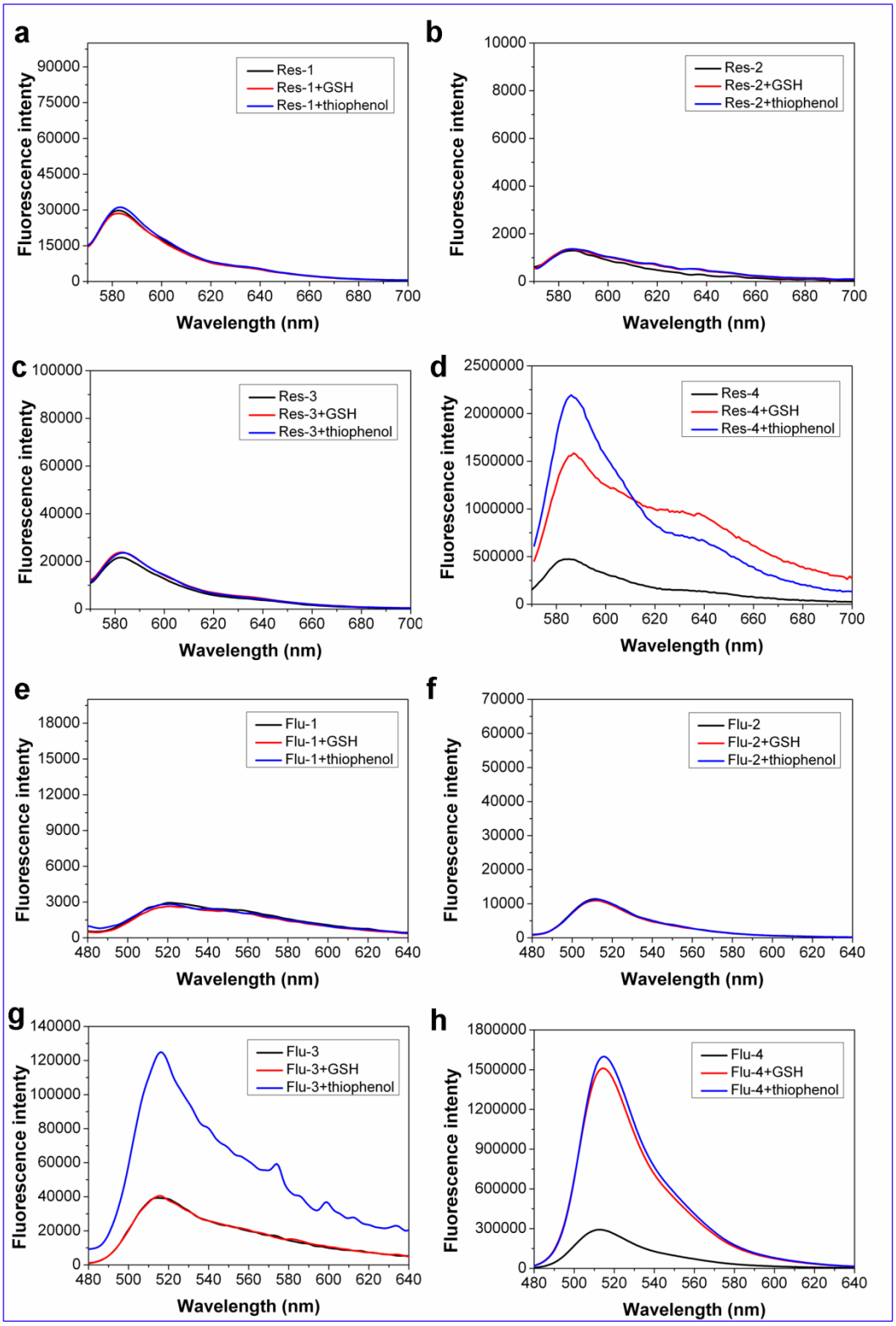


Figure S1. Fluorescence responses of probe candidates (40 μM) toward 500 μM GSH and 500 μM thiophenol at 37 ℃ for 60 min. (a) Res-1, (b) Res-2, (c) Res-3, (d) Res-4, (e) Flu-1, (f) Flu-2, (g) Flu-3, and (h) Flu-4.

4. Verification of the reaction mechanism

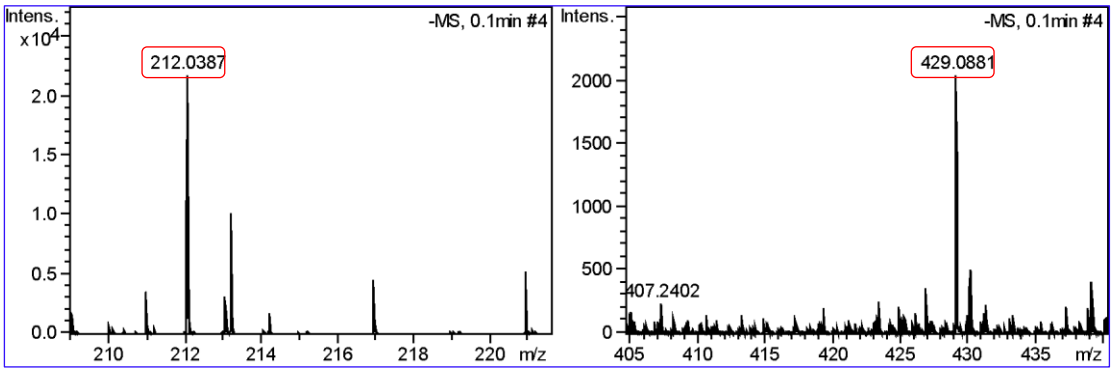


Figure S2. HRMS spectral analysis of reaction between Res-Biot and GSH. Res-Biot (100 μM) was reacted with GSH (1 mM) in HEPES buffer (20 mM, pH 7.4) at 37 ℃ for 30 min, and then characterized by HRMS spectrometry.

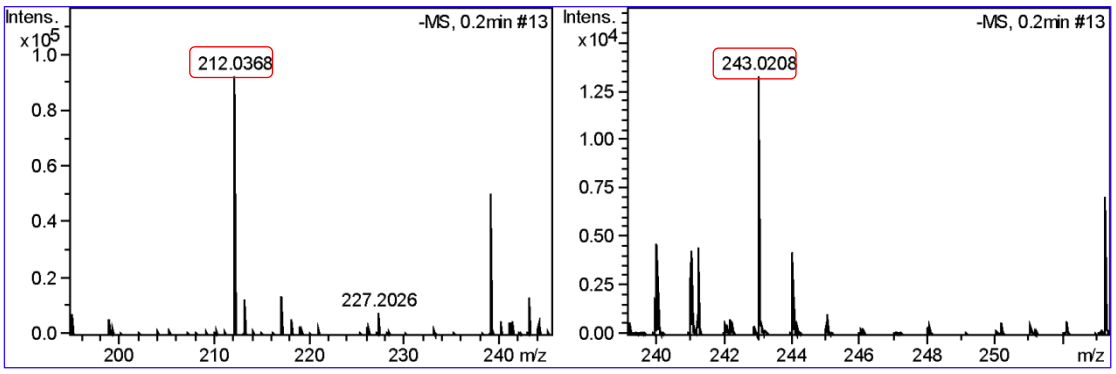


Figure S3. HRMS spectral analysis of reaction between Res-Biot and Cys. Res-Biot (100 μM) was reacted with Cys (1 mM) in HEPES buffer (20 mM, pH 7.4) at 37 ℃ for 30 min, and then characterized by HRMS spectrometry.

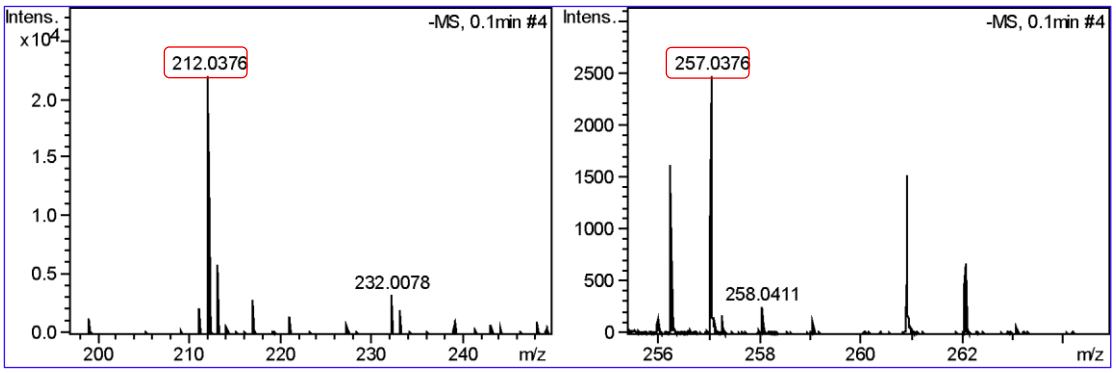
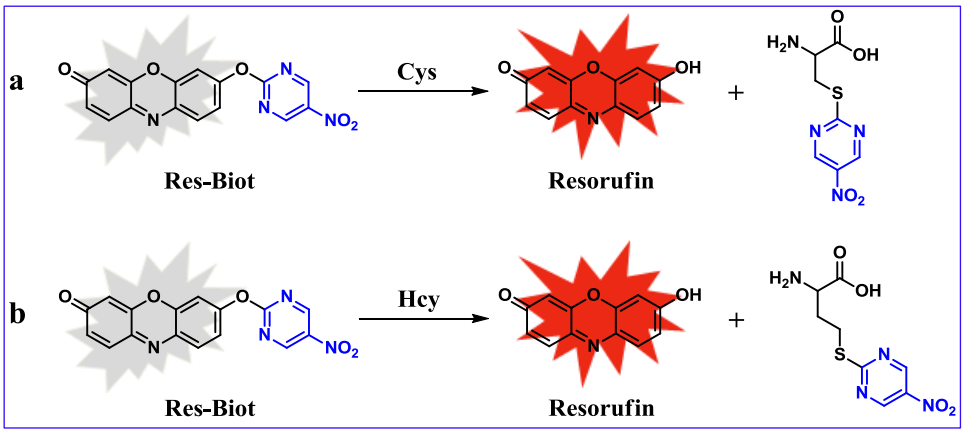


Figure S4. HRMS spectral analysis of reaction between Res-Biot and Hcy. Res-Biot (100 μM) was reacted with Hcy (1 mM) in HEPES buffer (20 mM, pH 7.4) at 37 ℃ for 30 min, and then characterized by HRMS spectrometry.



Scheme S2. Proposed reaction mechanism of Res-Biot toward (a) Cys and (b) Hcy.

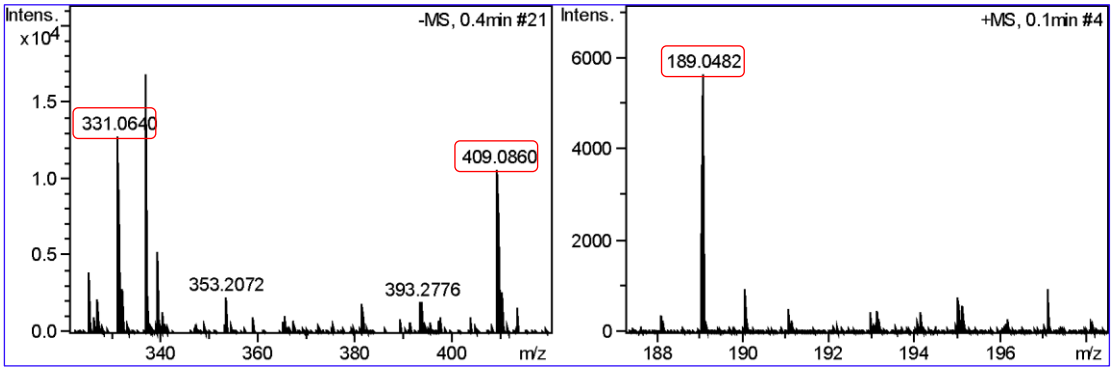


Figure S5. HRMS spectral analysis of reaction between Flu-Pht and thiophenol. Flu-Pht (100 μM) was reacted with thiophenol (2 mM) in PBS buffer (50 mM, pH 7.4) at 37 ℃ for 120 min, and then characterized by HRMS spectrometry.

5. Effect of pH values

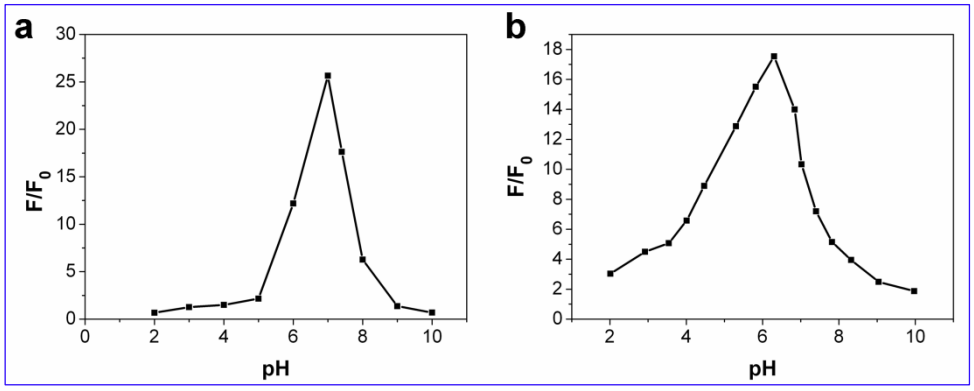


Figure S6. (a) Fluorescence increments of Res-Biot (10 μM) toward GSH (100 μM) at various pH values at 37 ℃. (b) Fluorescence increments of Flu-Pht (10 μM) toward thiophenol (100 μM) at various pH values at 37 ℃.

6. MTT assay

HepG2 cells were seeded into a 96-well microtiter plate at 37 ℃ in a 5% CO_2/95% air incubator for 24 h. The cells were incubated for an additional 24 h with different concentrations of tested probe (0, 10, 20, 50, 100, and 200 μM), respectively. Then the cells were washed with PBS three times. Subsequently, MTT (200 μL, 0.5 mg/mL) was added to each well and the cells were incubated at 37 ℃. After 4 h, the remaining MTT solution was removed, and the formazan crystals were dissolved in 200 μL of DMSO with gentle agitation for 5 min. The absorbance at 490 nm was measured using a TRITURUS microplate reader.

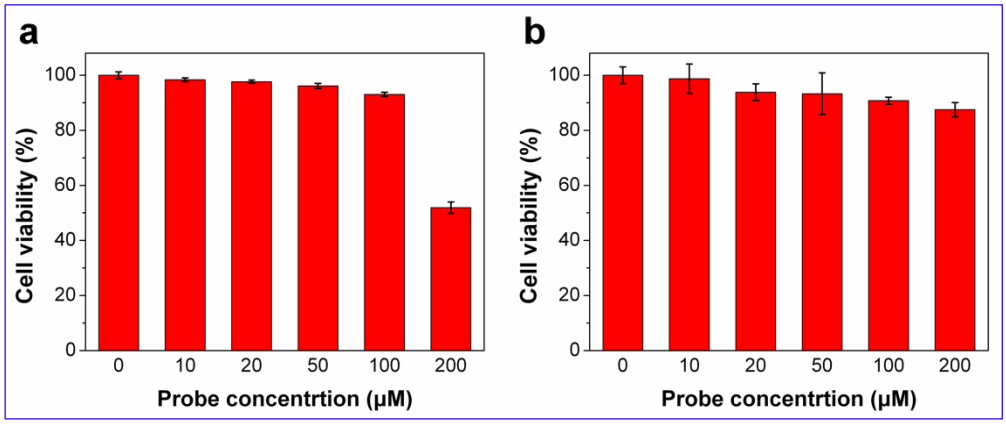


Figure S7. MTT assay of HepG2 cells with different concentrations of (a) Res-Biot or (b) Flu-Pht.

7. Cell confocal imaging experiment

HepG2 and Hela cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were seeded at a density of 1×10^6 cells mL^{-1} in high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin. Cultures were maintained in a humidified incubator MCO-5AC (Panasonic, Japan) at 37 ℃, in 5% CO_2/95% air. One day before imaging, cells were passed and plated on 18-mm glass coverslips in a culture dish. Prior to imaging, the medium was removed and cells were washed with PBS three times.

8. Animal models and in vivo imaging

Nude mice were purchased from the School of Medicine at Shandong University.

All animal experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee. Mice were divided into four groups and pretreated with (a) vehicle, (b) NEM (1 mM, 200 μL, 30 min), (c) LPS (1 mg/mL, 200 μL, 4 h), or (d) BSO (1 mM, 200 μL, 1 h), and then injected with Res-Biot (100 μM, 200 μL). After 20 min, the mice were anesthetized with 1.5% pelltobarbitalum natricum (150 μL) by intraperitoneal injection. Whole body images of the mice were then acquired using an IVIS Lumina III system with 540-nm excitation and 570-nm emission channel.

9. NMR and HRMS spectra

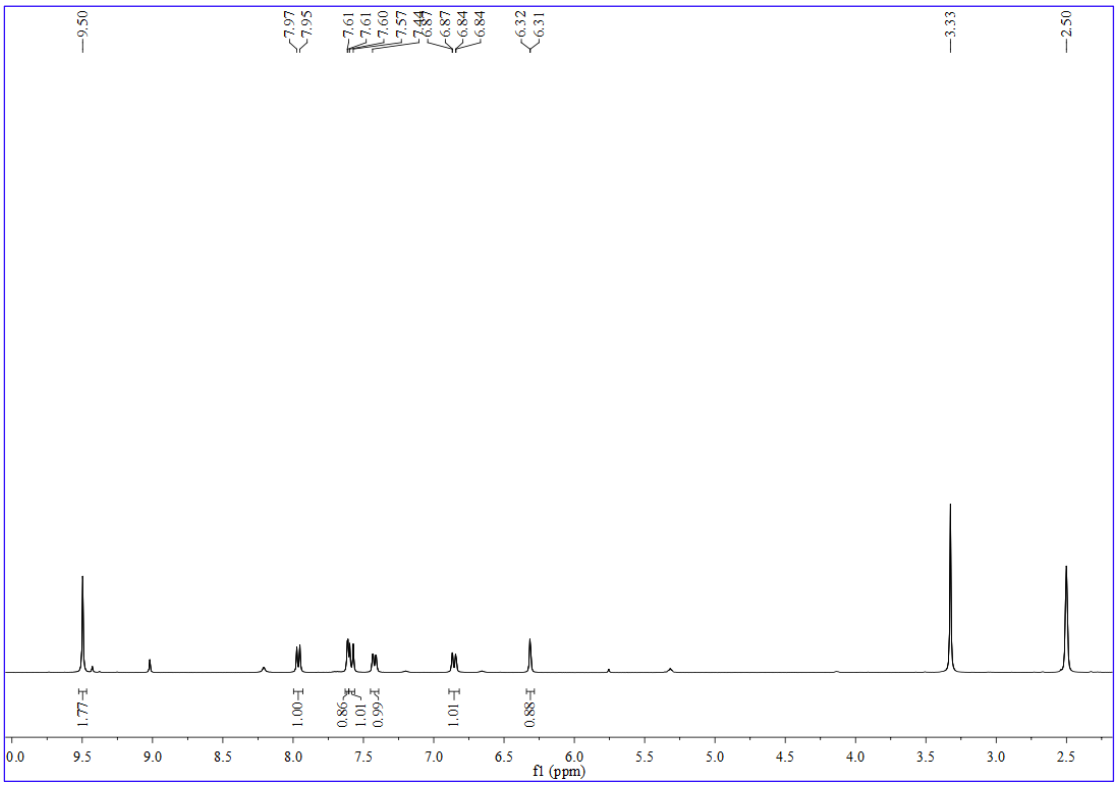


Figure S8. ^{1}H NMR spectrum of Res-Biot

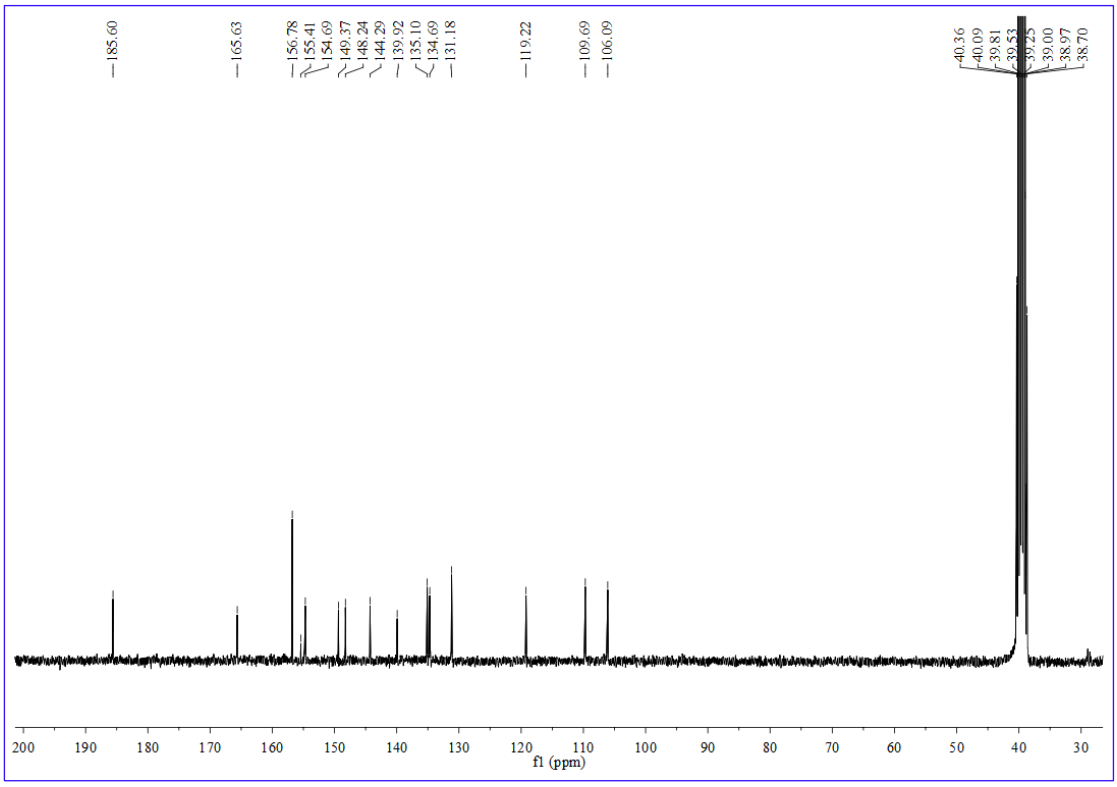


Figure S9. ^{13}C NMR spectrum of Res-Biot

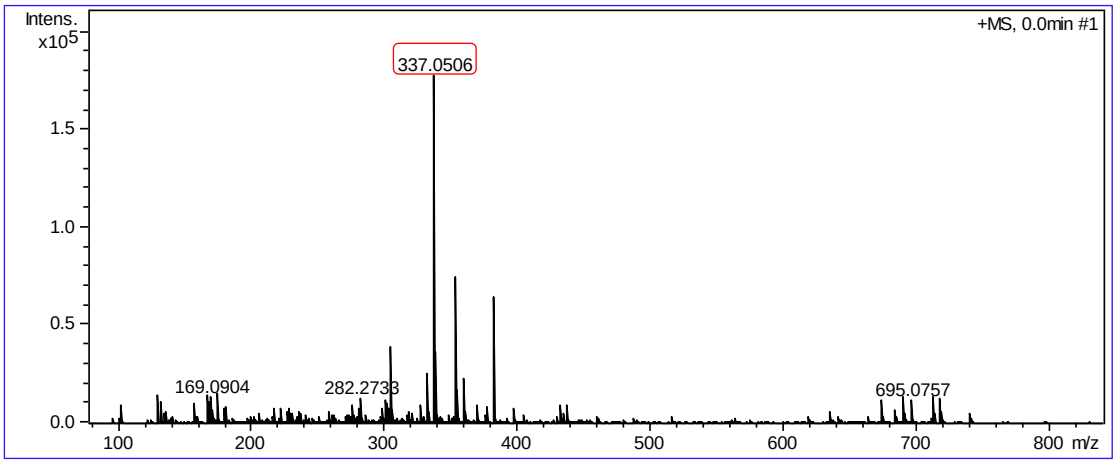


Figure S10. HRMS spectrum of Res-Biot

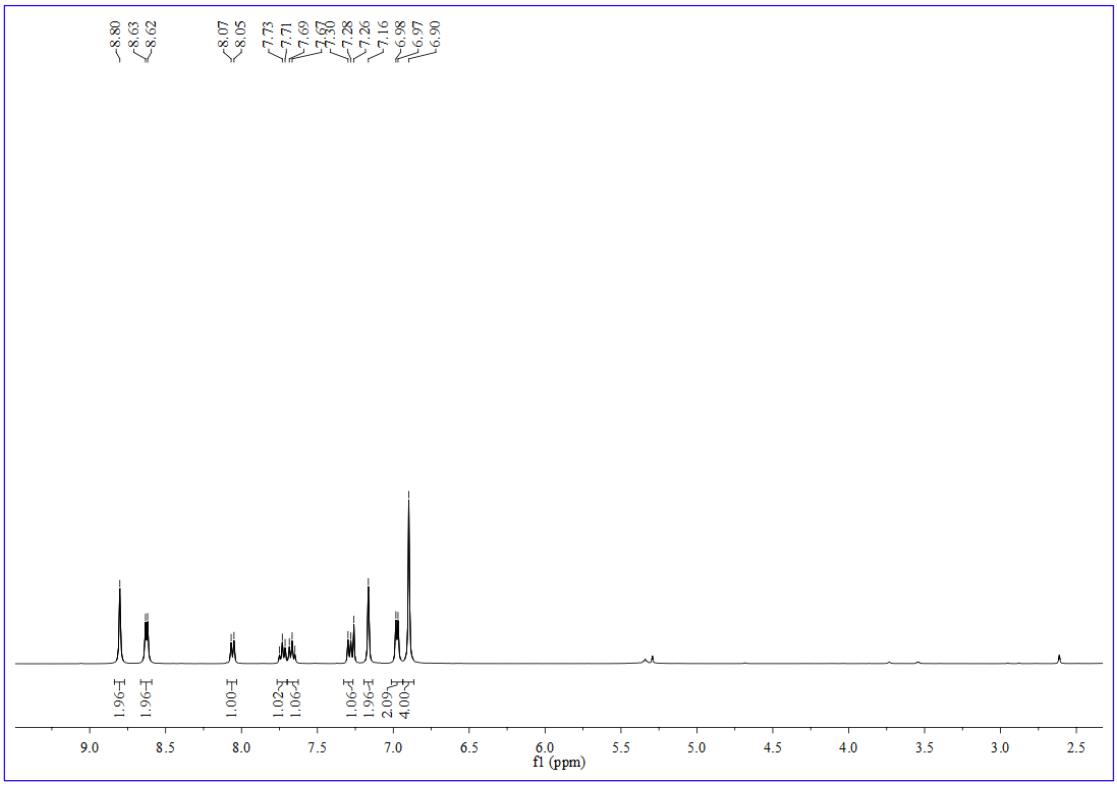


Figure S11. ^{1}H NMR spectrum of Flu-Pht

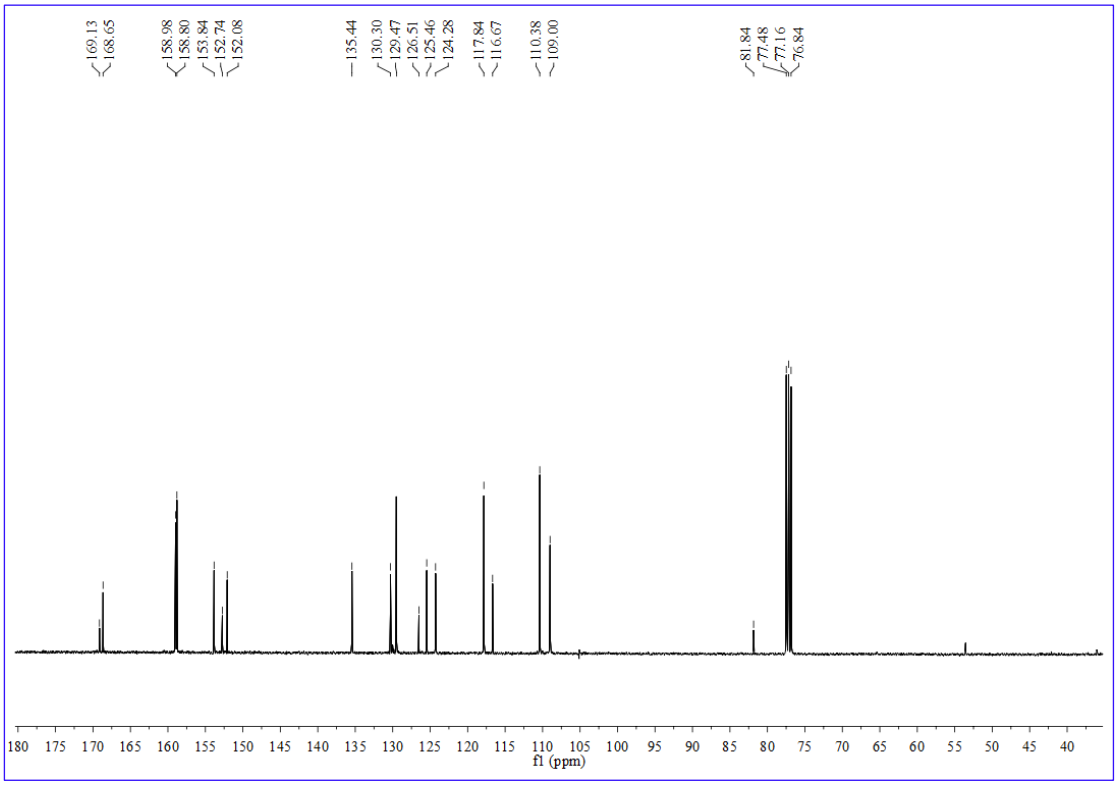


Figure S12. ^{13}C NMR spectrum of Flu-Pht

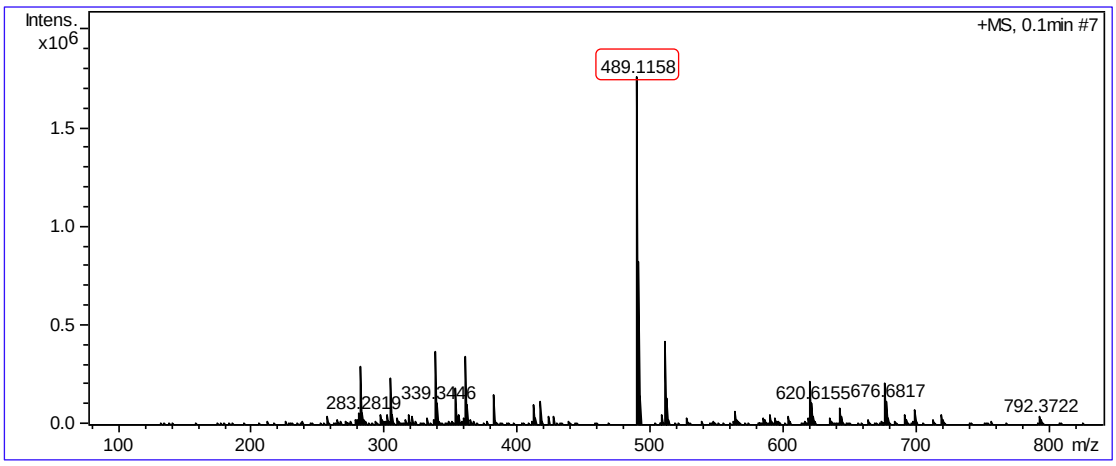


Figure S13. HRMS spectrum of Flu-Pht