Electronic Supplementary Material (ESI) for Analytical Methods.

A novel highly selective near-infrared and naked-eye fluorescence probe for imaging peroxynitrite

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Contents

1. Generation processes of analytes

H_2O_2:

H_2O_2 was diluted from a 30% solution in water.

TBHP:

Tert-butyl hydroperoxide（TBHP）was diluted from a 70% solution in water.

ClO^-:

HOCl was obtained by dilution of 5% of the solution in water.

$O_2^{\bullet-}$:

Solid potassium superoxide was added to dry DMSO and stirring vigorously for 10 min.

•OH:

Hydroxyl radical (•OH) was generated by the Fenton reaction. To generate •OH, iron(II) chloride was added in the presence of 10 equiv of H_2O_2. The concentration of •OH was equal to the Fe(II) concentration.

NO•:

Nitric oxide was generated from SNP (sodium nitroferricyanide (III) dehydrate). Experiments were performed under anaerobic conditions. The deionized water was degassed with Ar for 30 min and then SNP was added into it under an Ar atmosphere and was stirred for 30 min at room temperature. The probe solution was also degassed before the reaction with NO•.

$ONOO^-$:

A mixture of sodium nitrite (0.6 M) and hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M). Sodium hydroxide (1.5 M) was added within 1-2 s to make the solution alkaline. The resulting solution was stored at a temperature of −18 °C or lower. The solution was thawed immediately before use. The concentration of the stock solution was determined in 0.1 M NaOH by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}cm^{-1}$.

2. Effect of reaction time and pH value

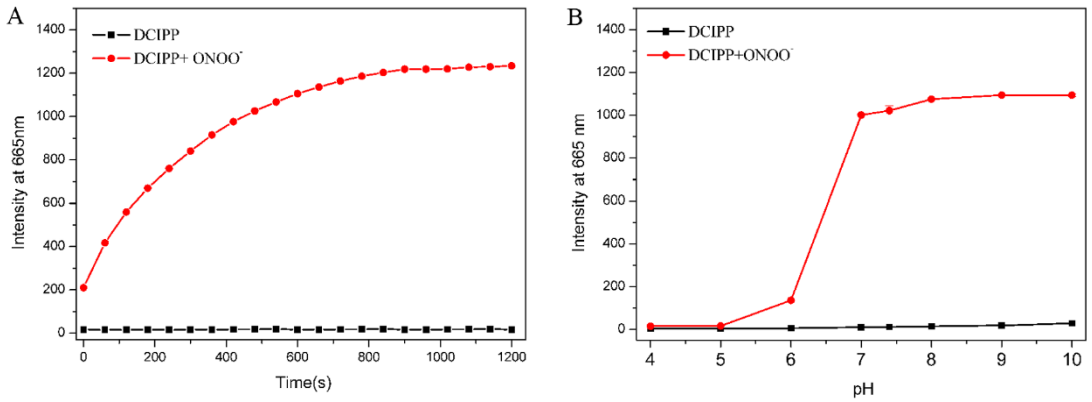


Fig. S1 (A) Time and (B) pH-dependent fluorescence changes of probe (5 μM) upon addition of $ONOO^-$ (10 μM) in H_2O/DMSO solution (1: 1, v/v, 10 mM PBS), λ_{ex} = 527 nm, λ_{em} = 665 nm.

3. UV spectra titration

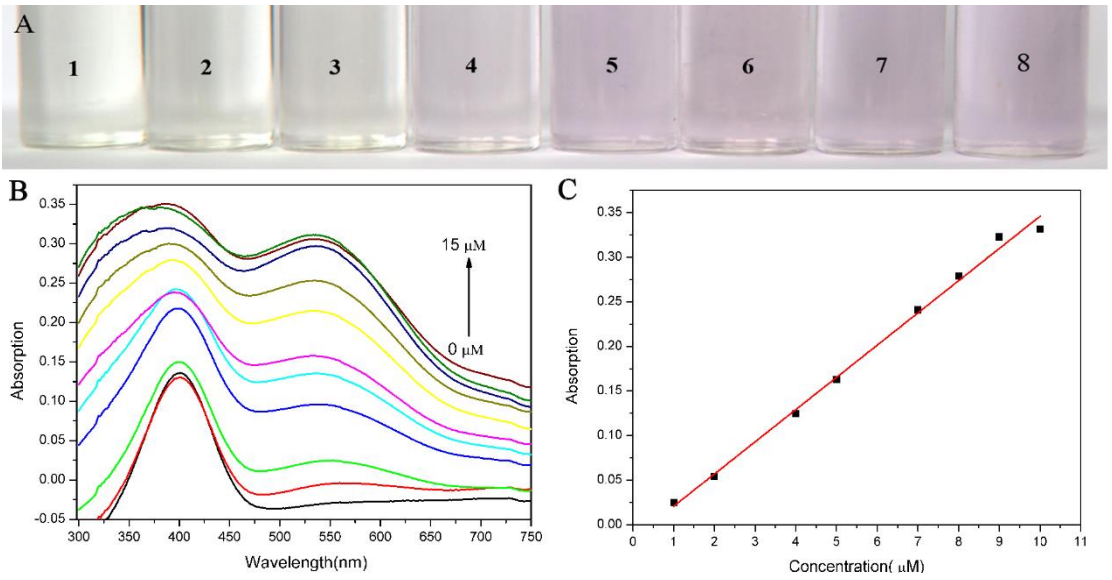


Fig. S2 (A) The color change of **DCIPP** (5 μM) upon addition of $ONOO^-$ (1-8: 1.0, 2.0, 4.0, 5.0, 7.0, 8.0, 9.0, 10.0 μM) in H_2O/DMSO solution (1: 1, v/v, 10 mM PBS, pH = 7.4). (B) UV titration of **DCIPP** (5 μM) upon addition of $ONOO^-$ (0-15.0 μM). (C) The linear relationship between the absorbance at 550 nm and the concentrations of $ONOO^-$.

4. Specificity of the probe DCIPP

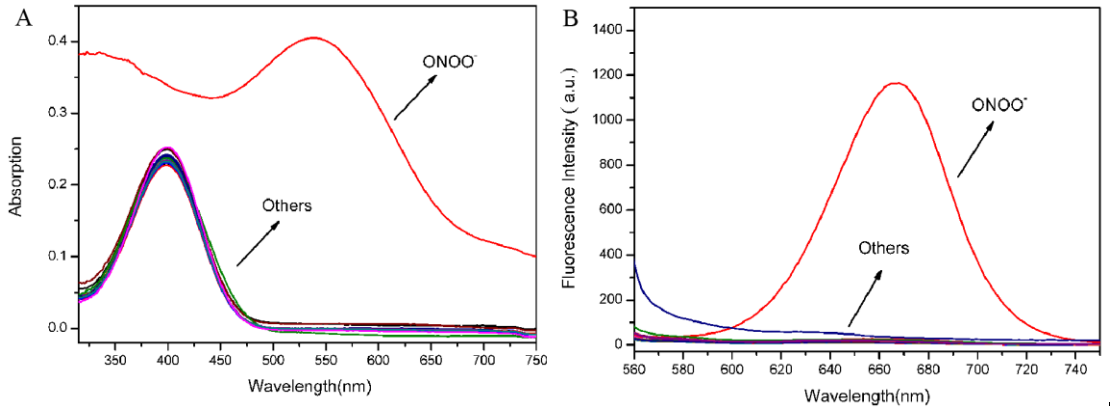


Fig. S3 (A) UV and (B) fluorescence response of **DCIPP** (5 μM) to various analytes ($ONOO^-$, Na^+, Mg^{2+}, Fe^{3+}, Co^{2+}, NO_2^-, NO_3^-, F^-, CH_3COO^-, $S_2O_4^{2-}$, $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, PO_4^{3-}, Glu, Tyr, Glu, Gln, Ala, Cys, GSH, H_2O_2, OCl^-, •OH, TBHP, O_2^-, HNO), each spectrum was recorded at 20 min after addition of the analytes ($ONOO^-$ 10 μM, others 100 μM).

5. Study on reaction mechanism

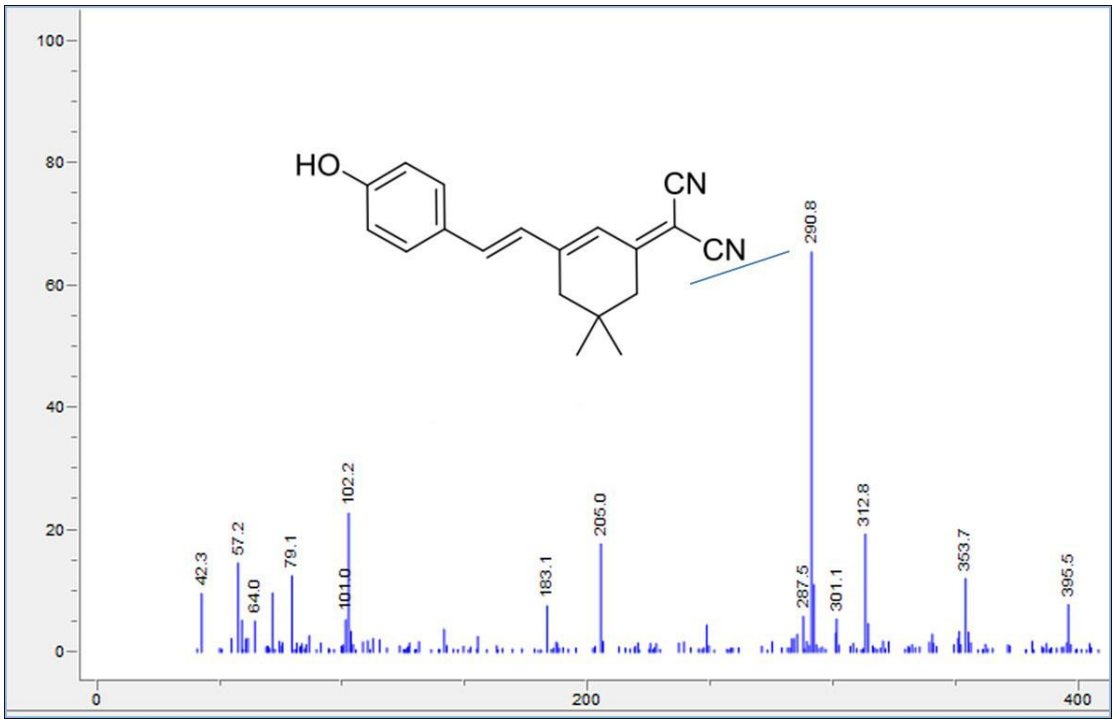


Fig. S4 The LC-MS spectrum (ESI) of **DCIPP** treated with $ONOO^-$ (2.0 equiv).

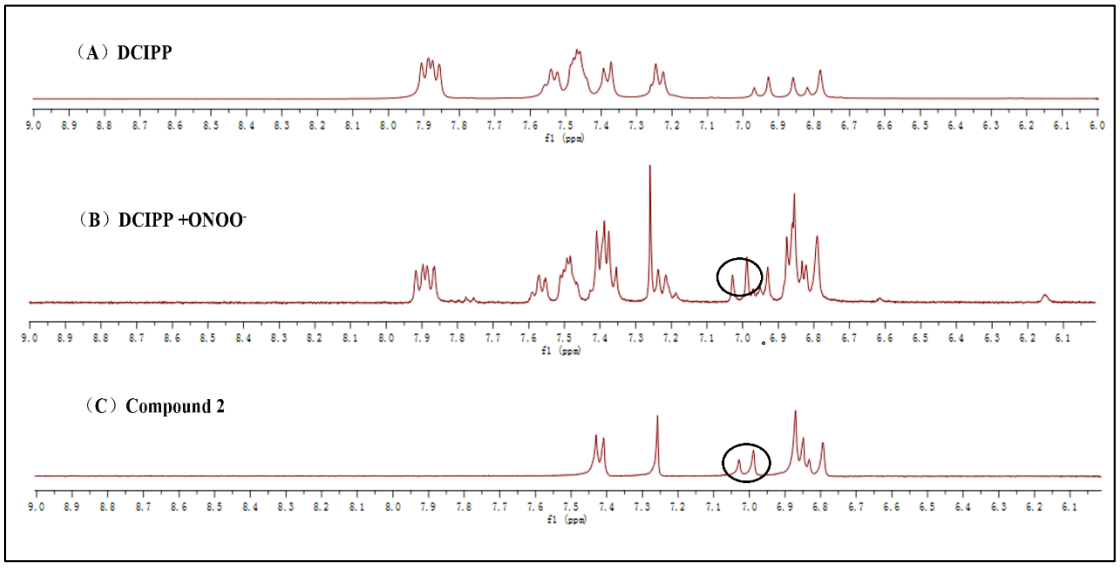


Fig. S5 ^1H NMR spectrum of (A) **DCIPP** (B) **DCIPP** treated with $ONOO^-$. (C) compound **2**

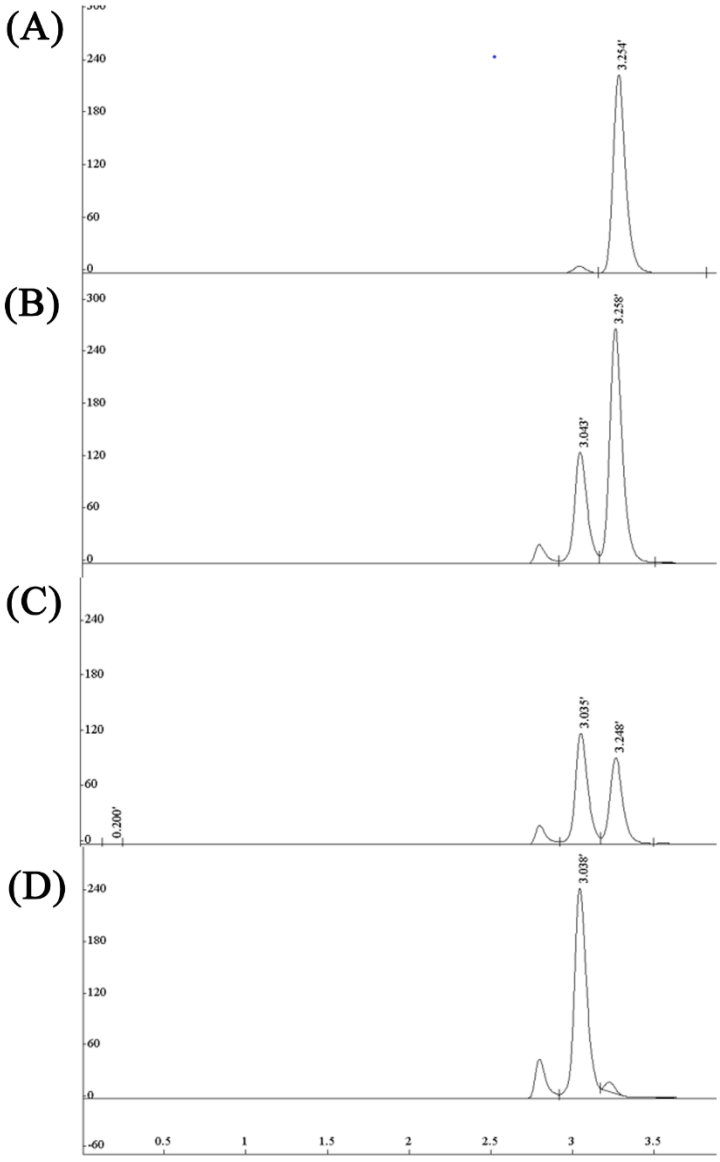


Fig. S6 HPLC chromatograms of (A) **DCIPP**, (B) **DCIPP** with 0.5 equiv of $ONOO^-$, and (C) **DCIPP** incubated with 1.5 equiv of $ONOO^-$, (D) compound **2**. HPLC analysis was carried out on a Lab Alliance system equipped with Model 500 detector.

6. Cells cytotoxicity of probe DCIPP

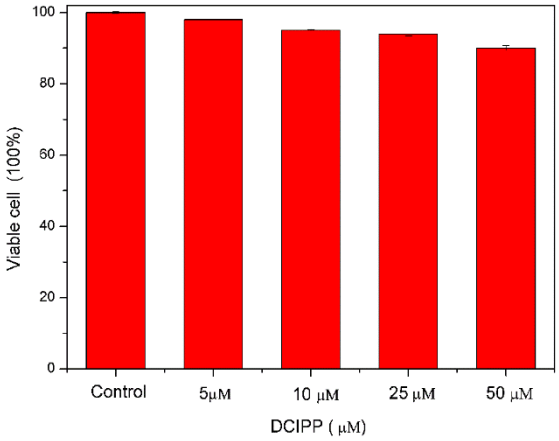
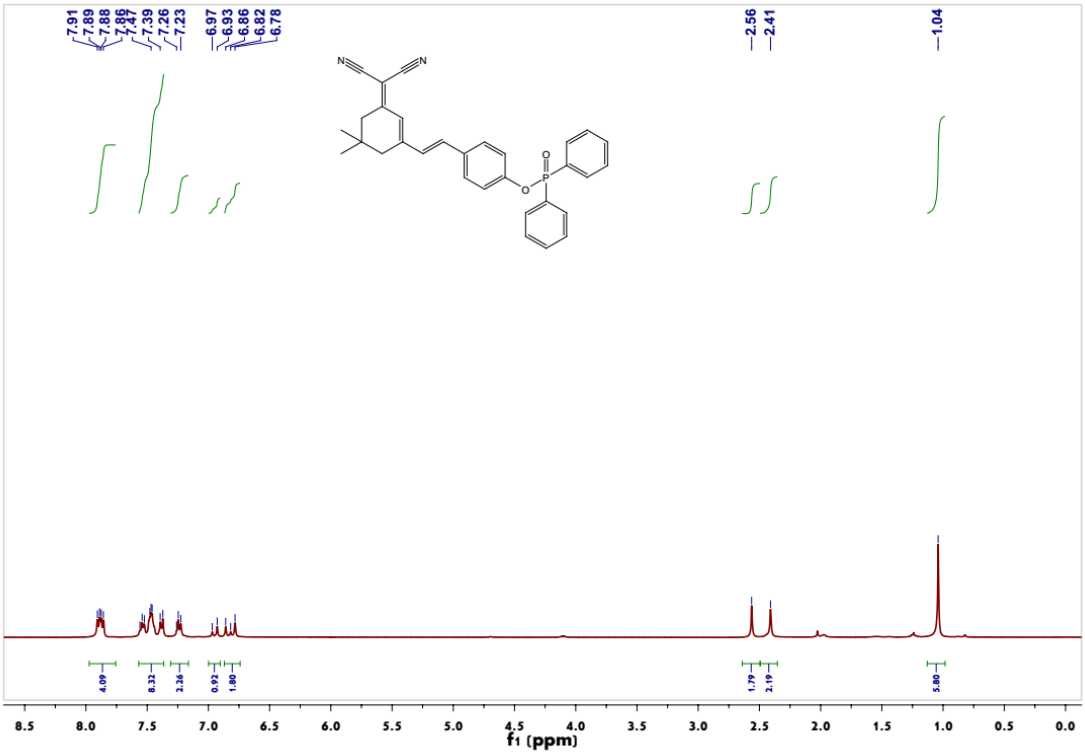
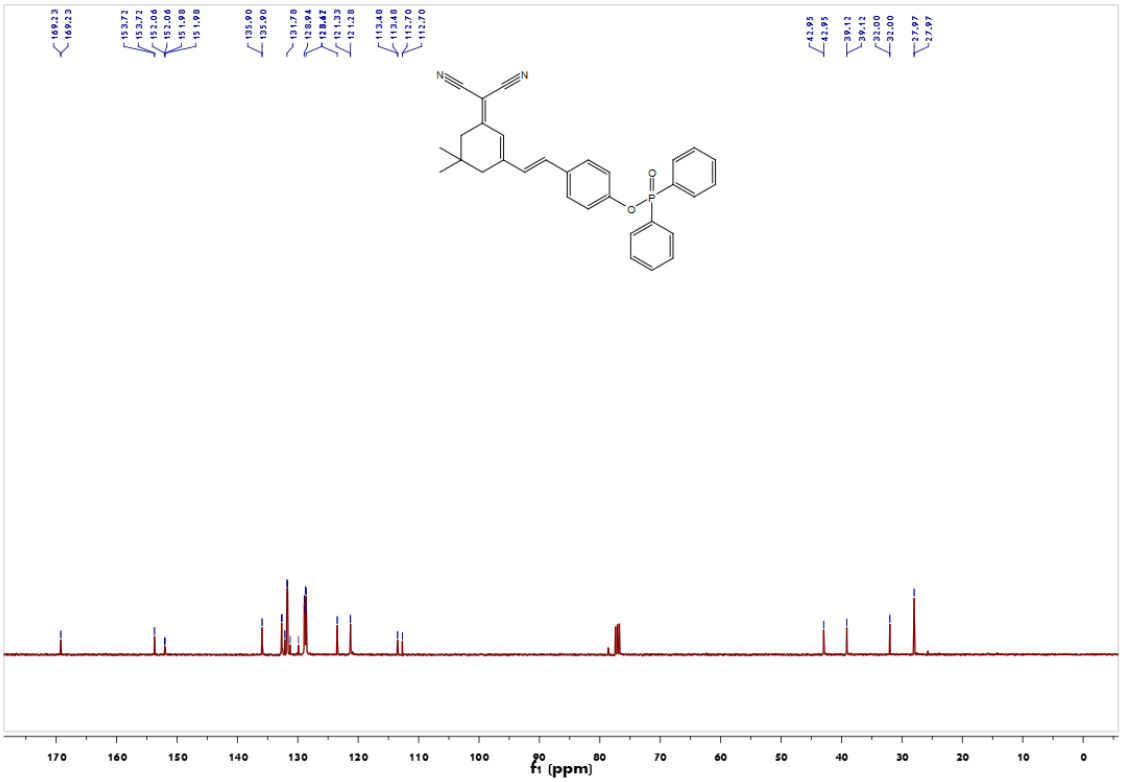


Fig. S7 MTT assay for the survival rate of HeLa cells treated with different concentrations of **DCIPP** for 24 h.

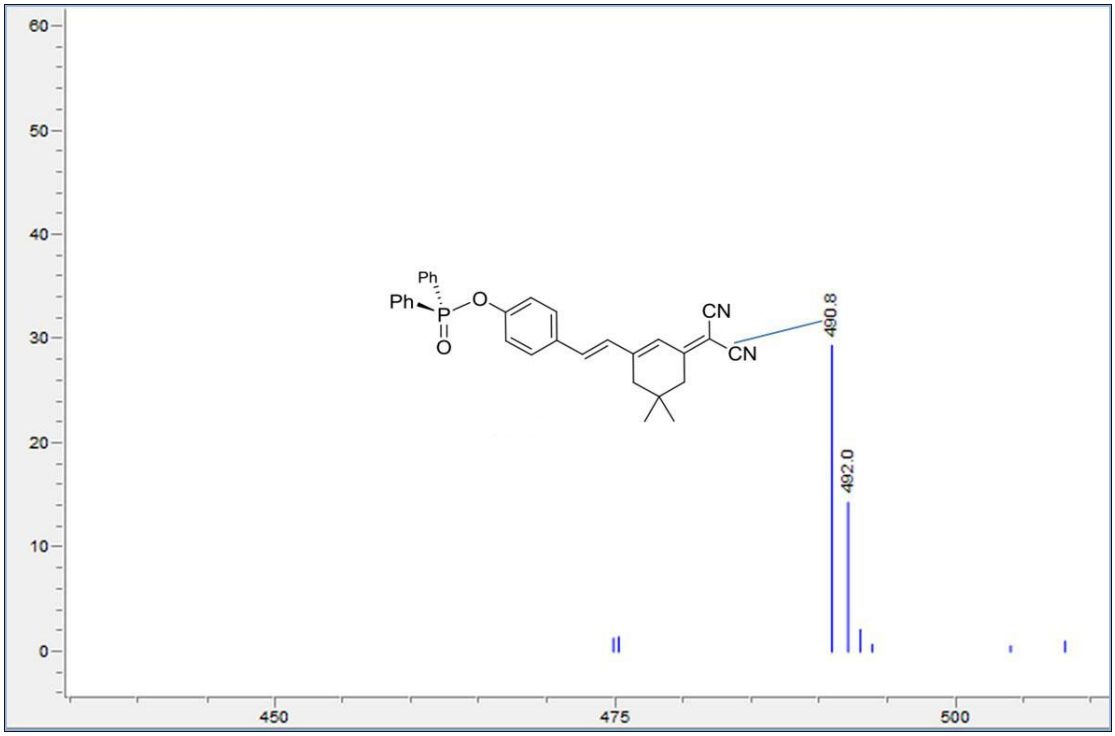
7. NMR, HRMS and LC-MS spectrum data



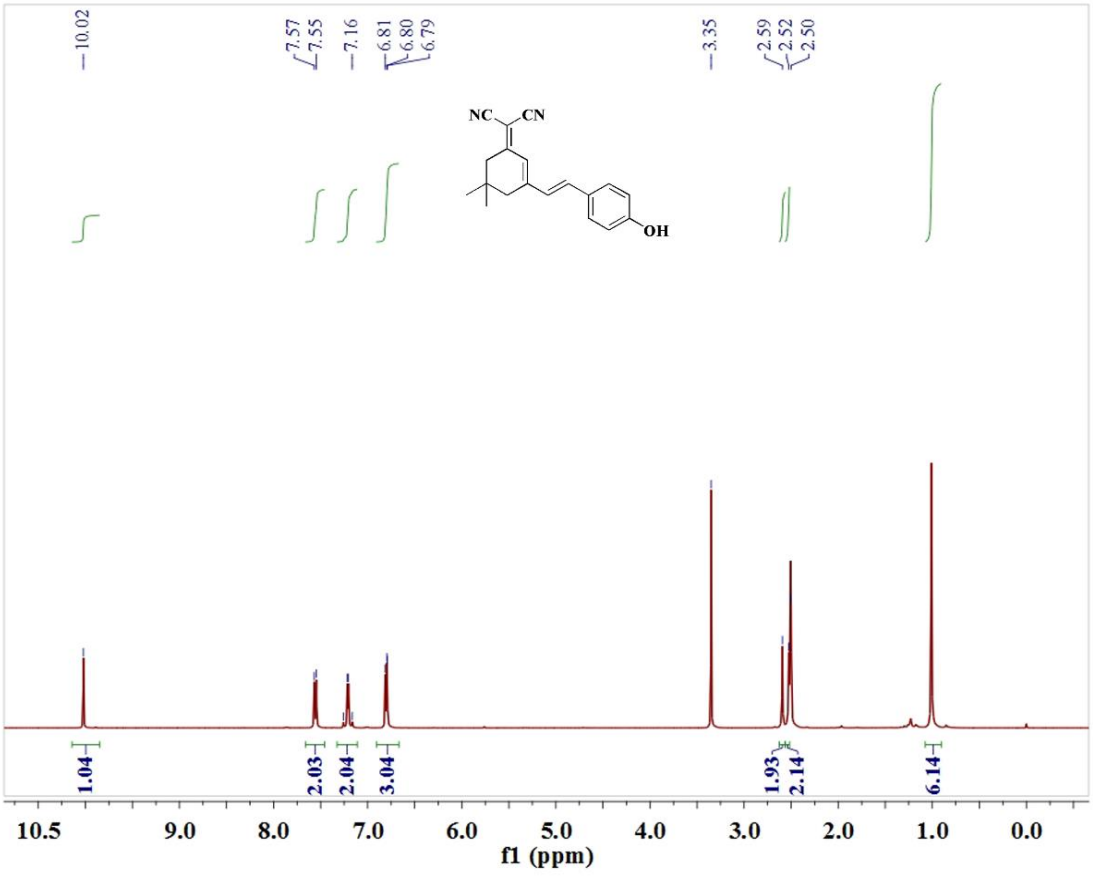
^{1}H NMR spectrum of **DCIPP**.



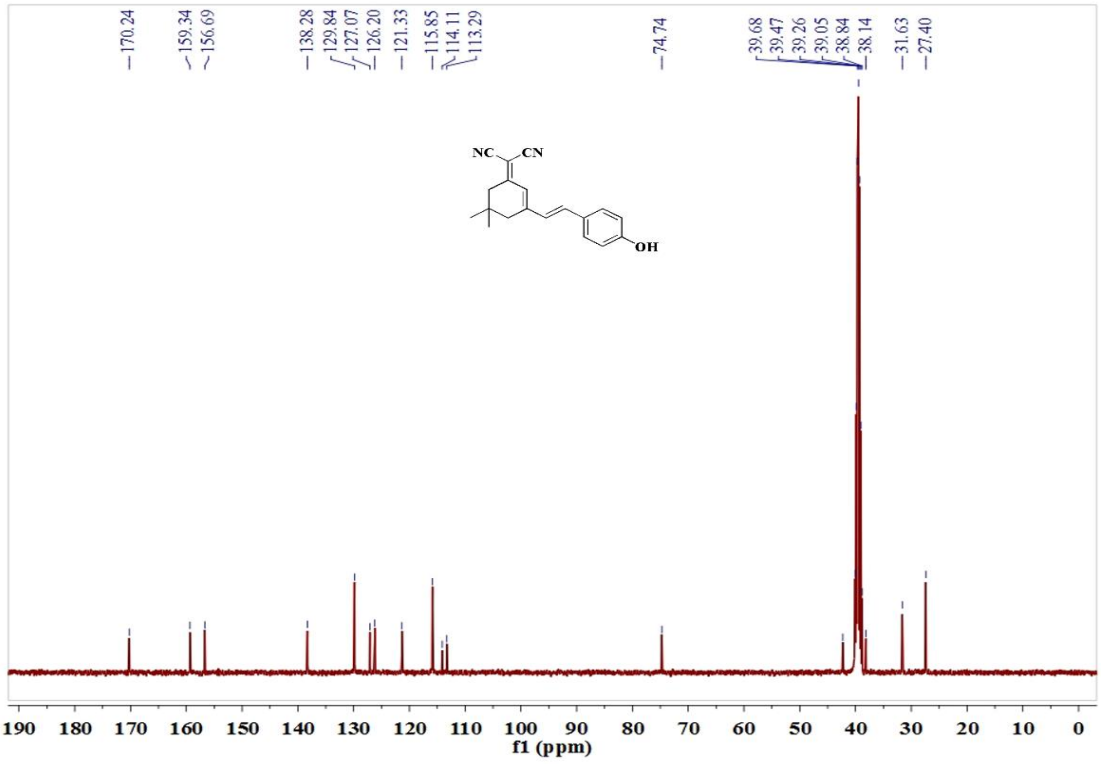
^{13}C NMR spectrum of **DCIPP**.



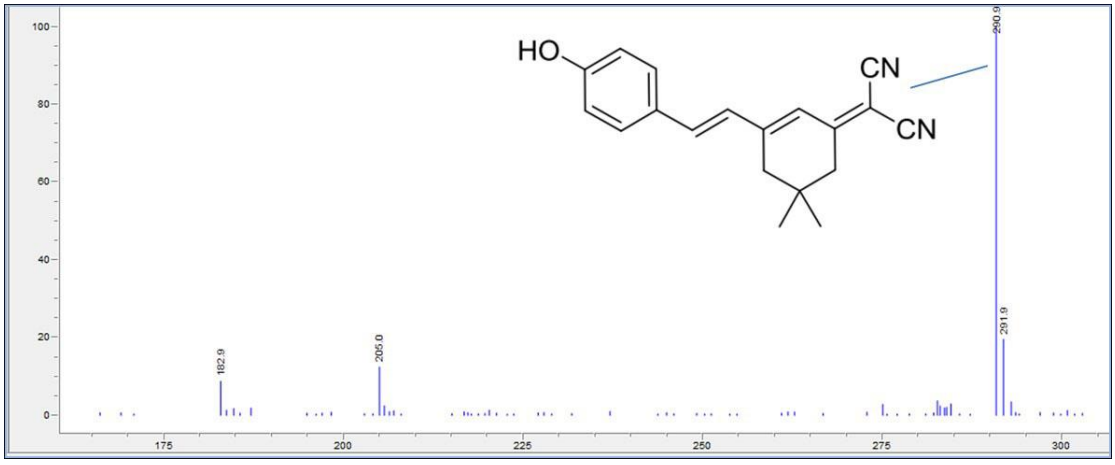
The LC-MS spectrum (ESI) of **DCIPP**.



^{1}H NMR spectrum of compound **2**.



^{13}C NMR spectrum of compound **2**.



The LC-MS spectrum (ESI) of compound **2**.