Electronic Supplementary Material (ESI) for Analytical Methods.


# *Supporting Information*

# *for*

## *Development of a reaction based turn-on fluorosensor and biomarker selective for hypochlorite ion in aqueous medium*

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**Materials and general methods**

All chemicals herein were collected from commercial suppliers (Aldrich and merck companies) and used without further purification. *3-benzotriazol-2-yl-2-hydroxy-5-methyl-benzaldehyde* was prepared by the literature method [32]. Elemental analyses (C, H and N) were carried out on a Perkin Elmer 2400 CHN elemental analyzer. $^{1}H$ and $^{13}C$ NMR spectra were measured on a Bruker Avance 400 MHz spectrometer using TMS as the internal standard and micrOTOF-Q mass spectrometer was used to record the electrospray ionization (ESI) mass spectra. Systronics digital pH meter (model 335) was used for pH study and the adjustment of pH was done using either 50 mM HCl or NaOH solution. Absorption spectra were measured using a Shimadzu -2450 UV-vis spectrophotometer. Fluorescence measurements were performed with a Hitachi-4500 spectrofluorimeter (All emission spectra were collected with the emission slits set at 5 nm.). Time-resolved fluorescence lifetime measurements were carried out using a HORIBA JOBIN Yvon picosecond pulsed diode laser-based time-correlated single-photon counting (TCSPC) spectrometer from IBH (UK) at $\lambda_{ex}$= 440 nm and MCP-PMT as a detector.

The selectivity study was carried out by the sodium/potassium salts of hypochlorite, chlorate, thiocyanate, cyanide, azide, bicarbonate, nitrate, arsenite, arsenate, monohydrogen phosphate, phosphate, sulphate, sulphide and Tetrabutylammonium salts of halides ($F^-$, $Cl^-$, $Br^-$, $I^-$), acetate, dihydrogen phosphate etc in 10 mM HEPES buffer, pH 7.4, DMSO/$H_2O$ (1:19, v/v) solution with an LH concentration of 10 μM solution. In the study of selectivity, the amount of the anions was a 10 equivalent greater than that of the probe used.

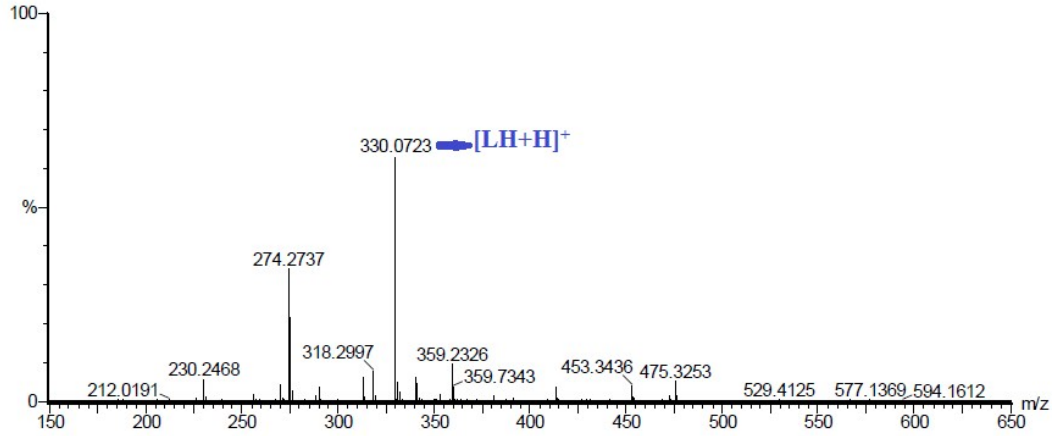


**Fig. S1.** Mass spectrum of **LH**

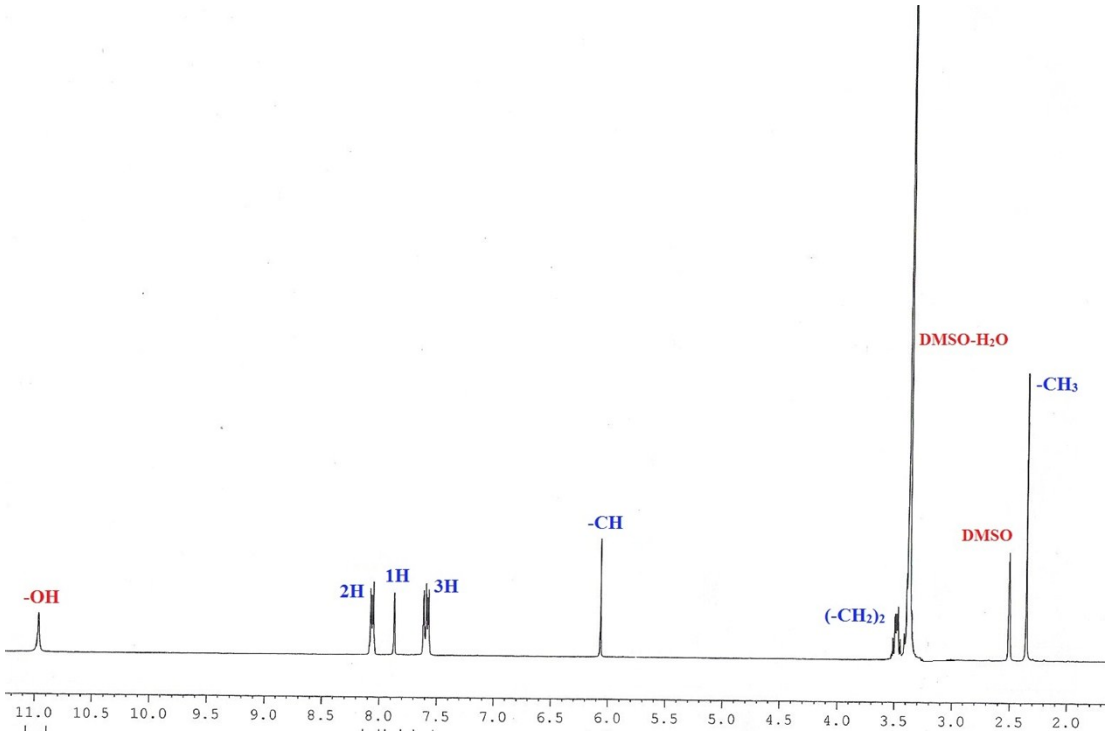


**Fig. S2.** $^{1}$H NMR spectrum of **LH** in DMSO-$d_6$

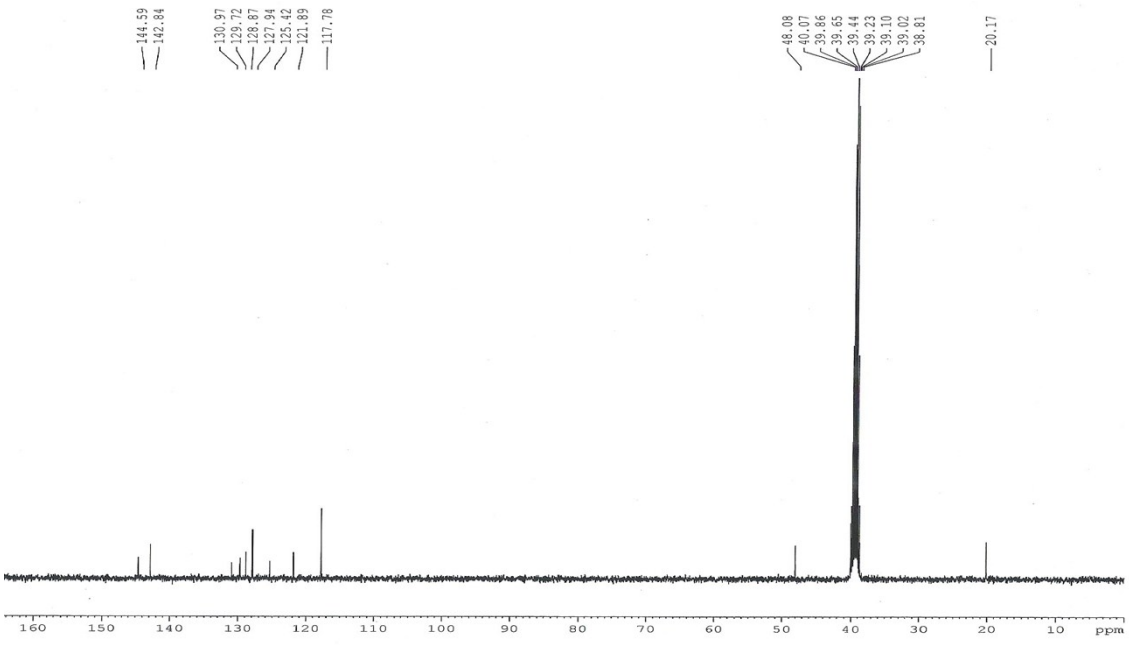


**Fig. S3.** $^{13}C$ NMR spectrum of **LH** in DMSO-$d_6$

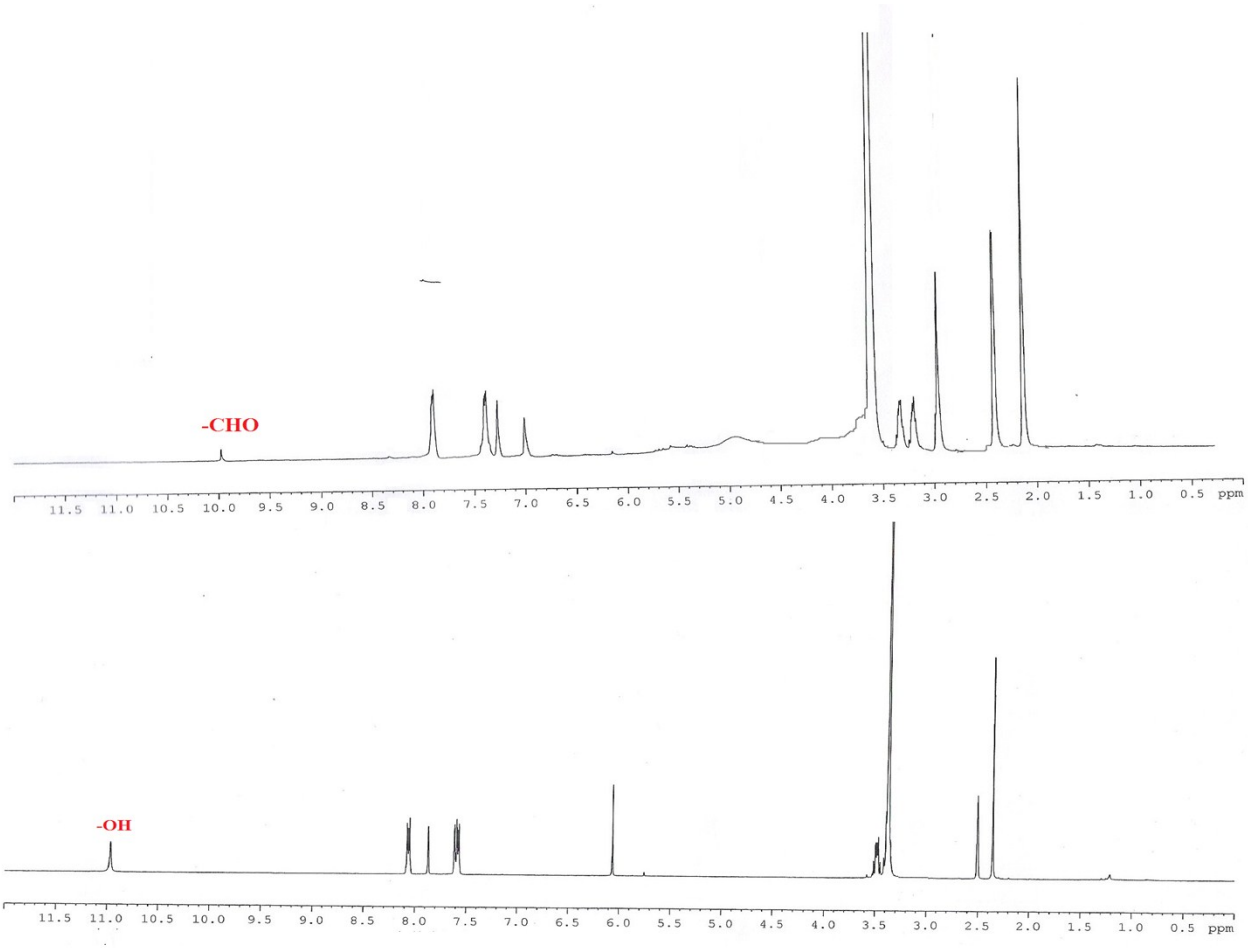


**Fig. S4.** $^{1}$HNMR titration of **LH** with $ClO^{-}$ ions

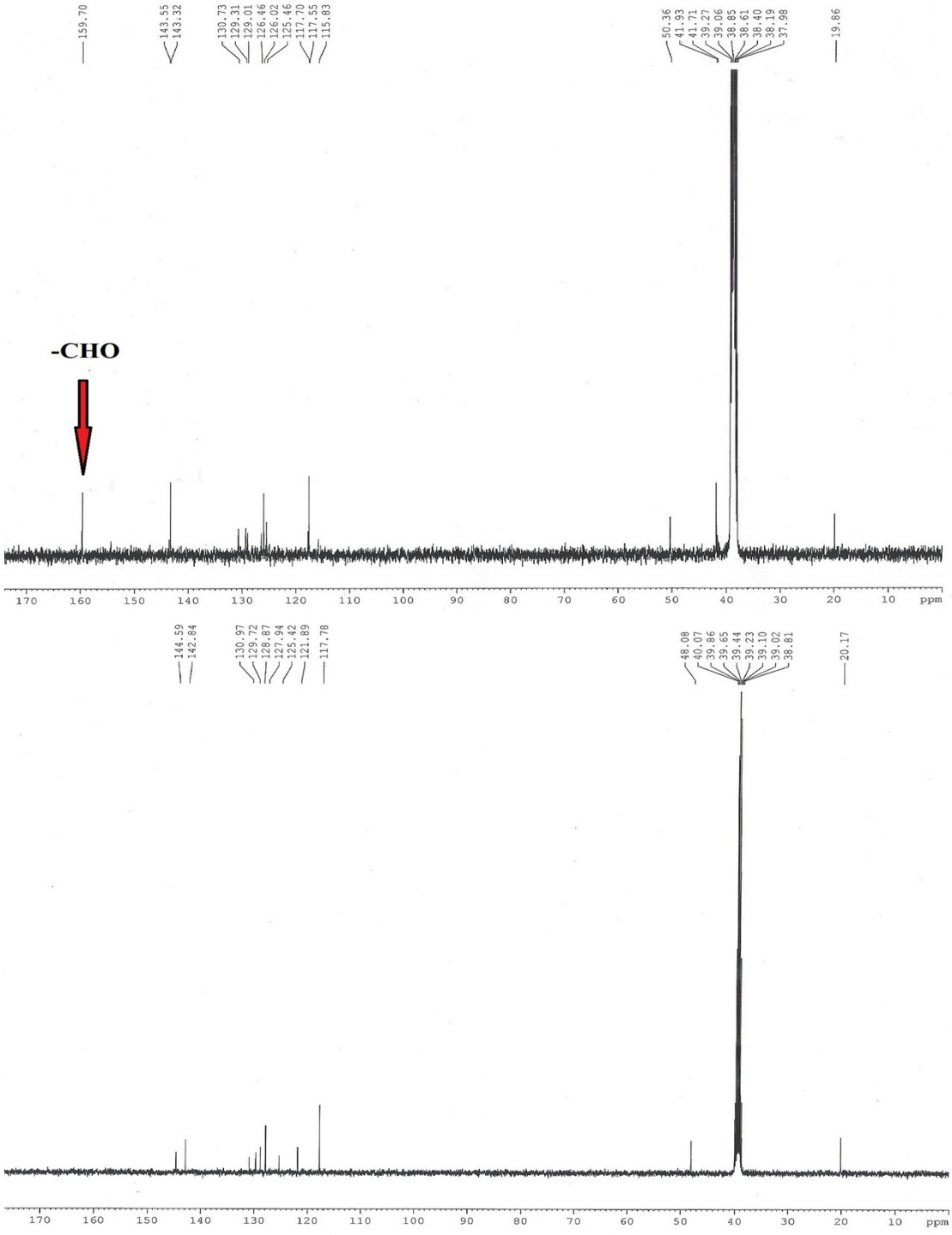


**Fig. S5.** $^{13}C$ NMR titration of **LH** with $ClO^-$ ions

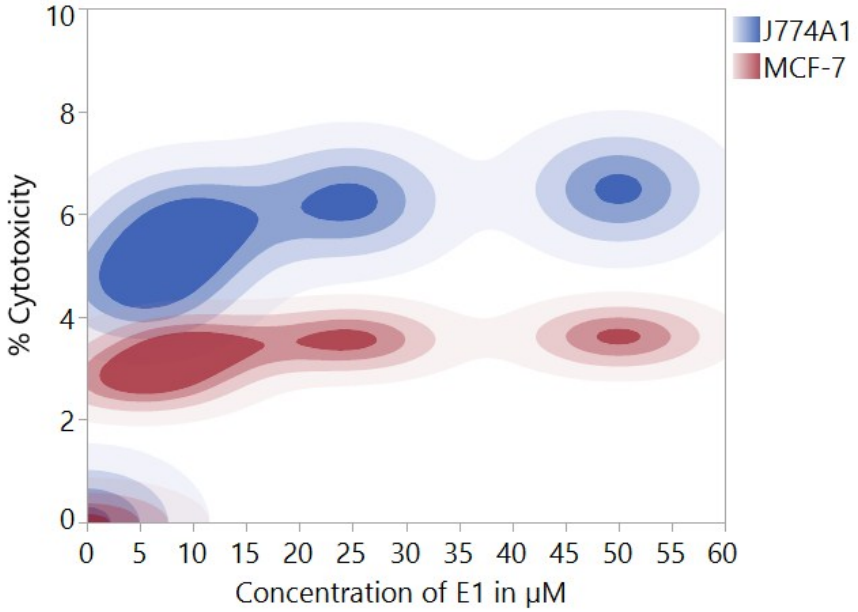


**Fig. S6.** Concentration dependent in vitro cytotoxicity of E1 against MCF-7 and J774A1.

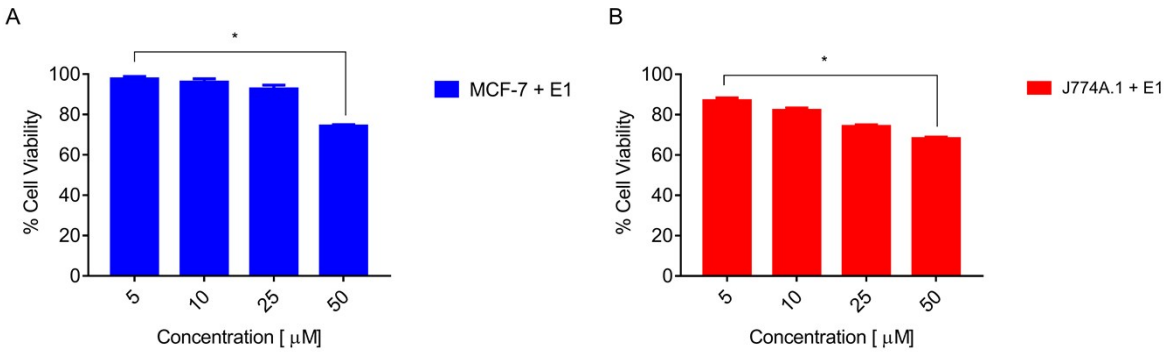


**Fig. S7.** Cell viability in MCF-7 (A) and J774A1 (B) cells in the presence of free probe E1.

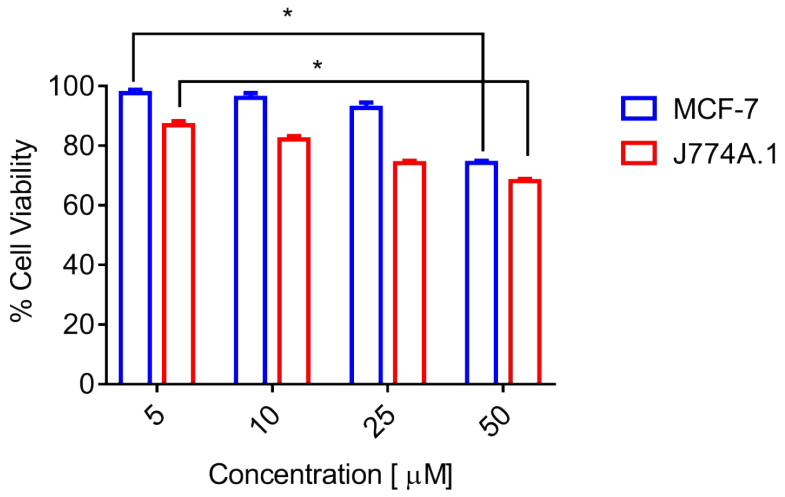


**Fig. S8.** Cell viability in MCF-7 and J774A1 cells in the presence of exogenous $ClO^-$ ions.

**Table S1** Crystallographic data and details of refinements for **LH**.

| | |
|---|---|
| Empirical Formula | $C_{16}H_{15}N_3OS_2$ |
| Formula Weight | 328.44 |
| Crystal system | monoclinic |
| Space group | ***C 2/c*** |
| *a* (Å) | 16.1495(7) |
| *b* (Å) | 12.7748(5) |
| *c* (Å) | 16.1233(7) |
| α | 90.00° |
| *β* | 111.003(2)° |
| γ | 90.00° |
| Volume (Å$^3$) | 3105.4(2) |
| *Z* | 8 |
| $\rho_{calc}$ (g/cm$^3$); μ (mm$^{-1}$) | 1.409; 0.347 |
| θ range (deg) | 2.09- 28.36° |
| Reflections collected | 22185 |
| Reflections independent | 3879 |
| Final R indices [$I > 2\sigma(I)$] | R = 0.0579, wR2 0.1717 |

**Table S2** Selected bond distances (Å) and bond angles (°) for **LH**.

| **Bond length (Å)** | | | |
|---|---|---|---|
| S1 - C14 | 1.795(3) | N2 C4 | 1.425(2) |
| S1 - C13 | 1.841(2) | N1 - C8 | 1.349(3) |
| S2 - C13 | 1.797(2) | N3 - C7 | 1.350(3) |
| S2 - C15 | 1.782(3) | O1 - C3 | 1.357(2) |
| N2 - N1 | 1.329(2) | C5 - C4 | 1.391(2) |
| N2 - N3 | 1.332(2) | | |
| **Bond angles (°)** | | | |
| C14- S1 -C13 | 97.93(14) | N2- N3- C7 | 103.38(17) |
| C14- S1- C13 | 97.93(14) | C6- C5- C4 | 120.61(16) |
| C15 -S2 -C13 | 94.96(13) | C5 -C4- N2 | 119.08(15) |
| N1- N2- N3 | 116.21(16) | C3- C4- N2 | 120.09(15) |
| N1- N2 -C4 | 122.15(15) | N3 -C7- C8 | 108.24(18) |
| N3- N2- C4 | 121.60(15) | N3 -C7- C12 | 130.2(2) |
| N2 -N1- C8 | 102.86(16) | | |

**Table S3** Life time detail of **LH** in absence and presence of $ClO^-$ ions

| | $B_1$ | $B_2$ | $B_3$ | $\tau_1$(ns) | $\tau_2$(ns) | $\tau_3$(ns) | $\tau_{av}$(ns) | $\chi^2$ | φ | $K_r$ | $K_{nr}$ | $K_r$ / $K_{nr}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LH | 0.2400 | 0.0698 | 0.0013 | 0.04 | 1.50 | 5.53 | 1.63 | 0.94 | 0.0028 | 0.0017 | 0.6116 | 0.002 |
| LH + $ClO^-$ | 0.0112 | 0.0252 | 0.510 | 5.54 | 0.37 | 0.02 | 4.04 | 1.27 | 0.048 | .0118 | 0.2356 | 0.050 |