A molecular tweezers-like calix[4]arene based alkaline earth metal cation (Ca$^{2+}$, Sr$^{2+}$ & Ba$^{2+}$) chemosensor and its imaging in living cells and zebrafish

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Figure S1. $^1$H NMR spectrum of sensor L (400MHz, DMSO-$d_6$/D$_2$O = 9/1, 293 K).

Figure S2. $^{13}$C NMR spectrum of sensor L (100MHz, DMSO-$d_6$/D$_2$O = 9/1, 293 K).
**Figure S3.** ESI-HRMS spectrum of sensor L.
Figure S4. The max absorbance (a) and max fluorescence emission (b) of sensor L (10 μM) with or without 20 equiv. Ca$^{2+}$, Sr$^{2+}$ or Ba$^{2+}$ cations at different water content. The max absorbance (c) and max fluorescence emission (d) of sensor L (10 μM) with or without 20 equiv. Ca$^{2+}$, Sr$^{2+}$ or Ba$^{2+}$ cations under different pH.

Figure S5. The Job's plot data of sensor L with Ca$^{2+}$ (a), Sr$^{2+}$ (b) and Ba$^{2+}$ (c).
**Figure S6.** Absorption spectra (a) or fluorescence spectra (b, $\lambda_{ex} = 405$ nm) changes of sensor L (10 $\mu$M, DMF/H$_2$O = 9/1, pH = 8.5) solution upon addition of Sr$^{2+}$ (0 ~ 3 equiv.). Inset: the plot of absorption or fluorescence intensity of sensor L as a function of Sr$^{2+}$ concentration.

**Figure S7.** Absorption spectra (a) or fluorescence spectra (b, $\lambda_{ex} = 405$ nm) changes of sensor L (10 $\mu$M, DMF/H$_2$O = 9/1, pH = 8.5) solution upon addition of Ba$^{2+}$ (0 ~ 3 equiv.). Inset: the plot of absorption or fluorescence intensity of sensor L as a function of Ba$^{2+}$ concentration.
Figure S8. Absorption response of (a) sensor $L + \text{Ca}^{2+}$ at 560 nm, (b) $L + \text{Sr}^{2+}$ at 570 nm and (c) $L + \text{Ba}^{2+}$ at 580 nm with competing cations. Blue bars: The absorbance of sensor $L$ on addition of the respective cations (20 equiv.). Red bars: The absorbance of $L \cdot \text{Ca}^{2+}$, $L \cdot \text{Sr}^{2+}$ and $L \cdot \text{Ba}^{2+}$ complex on addition of the respective competing cations (20 equiv.).
Figure S9. Fluorescence response of (a) sensor L + Ca$^{2+}$, (b) L + Sr$^{2+}$ and (c) L + Ba$^{2+}$ with competing cations. Red bars: The fluorescence intensity of sensor L on addition of the respective cations (20 equiv.). Blue bars: The fluorescence intensity of L$\cdot$Ca$^{2+}$, L$\cdot$Sr$^{2+}$ and L$\cdot$Ba$^{2+}$ complex on addition of the respective competing cations (20 equiv.). $\lambda_{ex}/\lambda_{em} = 405 \text{ nm}/510 \text{ nm}$. 
Figure S10. (a) Absorbance calibration curve of sensor L (10 μM) at 560 nm as a function of Ca$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 1.33 × 10$^{-7}$ μM; (b) Absorbance calibration curve of sensor L (10 μM) at 570 nm as a function of Sr$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 1.53 × 10$^{-7}$ μM; (c) Absorbance calibration curve of sensor L (10 μM) at 580 nm as a function of Ba$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 2.23 × 10$^{-7}$ μM.
Figure S11. (a) Fluorescence calibration curve of sensor L (10 μM) at 560 nm as a function of Ca$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 7.51 × 10^{-8} μM; (b) Fluorescence calibration curve of sensor L (10 μM) at 570 nm as a function of Sr$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 9.08 × 10^{-8} μM; (c) Fluorescence calibration curve of sensor L (10 μM) at 580 nm as a function of Ba$^{2+}$ concentration in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution. LOD = 1.44 × 10^{-7} μM.
Figure S12. (a) Non-linear plot of sensor \( L \) (10 μM) assuming a 1:1 stoichiometry for association between sensor \( L \) and \( \text{Ca}^{2+} \) in DMF/H\(_2\)O (v/v, 9/1, pH = 8.5) solution by absorption spectroscopy, \( \lambda_{\text{max}} = 560 \) nm; (b) Non-linear plot of sensor \( L \) (10 μM) assuming a 1:1 stoichiometry for association between sensor \( L \) and \( \text{Sr}^{2+} \) in DMF/H\(_2\)O (v/v, 9/1, pH = 8.5) solution by absorption spectroscopy, \( \lambda_{\text{max}} = 570 \) nm; (c) Non-linear plot of sensor \( L \) (10 μM) assuming a 1:1 stoichiometry for association between probe \( L \) and \( \text{Ba}^{2+} \) in DMF/H\(_2\)O (v/v, 9/1, pH = 8.5) solution by absorption spectroscopy, \( \lambda_{\text{max}} = 580 \) nm.\(^1\)
Figure S13. (a) Non-linear plot of sensor L (10 μM) assuming a 1:1 stoichiometry for association between sensor L and Ca$^{2+}$ in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution by fluorescence spectroscopy; (b) Non-linear plot of sensor L (10 μM) assuming a 1:1 stoichiometry for association between sensor L and Sr$^{2+}$ in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution by fluorescence spectroscopy; (c) Non-linear plot of sensor L (10 μM) assuming a 1:1 stoichiometry for association between probe L and Ba$^{2+}$ in DMF/H$_2$O (v/v, 9/1, pH = 8.5) solution by fluorescence spectroscopy.$^1$
Figure S14. Partial $^1$H NMR spectra of sensor L and increasing concentrations (0 ~ 2.0 equiv.) of Ca$^{2+}$ in DMSO-$d_6$/D$_2$O = 9/1 at 298K.

Figure S15. Partial $^1$H NMR spectra of sensor L and increasing concentrations (0 ~ 2.0 equiv.) of Tris in DMSO-$d_6$/D$_2$O = 9/1 at 298K.
Figure S16. Partial $^1$H NMR spectra of sensor L with 2.0 equiv. Tris and increasing concentrations (0 ~ 2.5 equiv.) of Ca$^{2+}$ in DMSO-$d_6$/D$_2$O = 9/1 at 298K. Blue peaks assigned to sensor L; Red peaks assigned to sensor L after complex; Green peaks assigned to Tris.

Figure S17. Partial $^1$H NMR spectra of sensor L with 3.0 equiv. Tris and increasing concentrations (0 ~ 10.0 equiv.) of Sr$^{2+}$ in DMSO-$d_6$/D$_2$O = 9/1 at 298K. Blue peaks assigned to sensor L; Red peaks assigned to sensor L after complex; Green peaks assigned to Tris.
Figure S18. Partial $^1$H NMR spectra of sensor $L$ with 3.0 equiv. Tris and increasing concentrations (0 ~ 3.0 equiv.) of Ba$^{2+}$ in DMSO-$d_6$/$D_2O = 9/1$ at 298K. Blue peaks assigned to sensor $L$; Red peaks assigned to sensor $L$ after complex; Green peaks assigned to Tris.

Figure S19. Fluorescence microscopy images of Hela cells treated with 0 μM, 10 μM, 25 μM, 50 μM, 100 μM and 200 μM sensor $L$ under Bright field and Green channel, respectively.
Figure S20. Assessment of cytotoxicity of different concentrations of sensor L for 24 h Hela cells using the cytotoxicity assay (CCK-8 assay). Error bars stand for the mean ± SD of at least triplicate experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

Reference: