A Practical Hydrazine-Carbothioamide-Based Fluorescent Probe for the Detection of Zn\(^{2+}\): Applications to Paper Strip, Zebrafish and Water Samples

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**Abstract:** A practical hydrazine-carbothioamide-based fluorescent chemosensor TCC (N-(4-chlorophenyl)-2-(thiophene-2-carbonyl)hydrazine-1-carbothioamide) was applied for Zn\(^{2+}\) detection. TCC exhibited selective fluorescence emission for Zn\(^{2+}\) and did not show any interference with other metal ions. In particular, TCC was utilized for the detection of Zn\(^{2+}\) in paper strips, zebrafish and real water samples. TCC could detect Zn\(^{2+}\) down to 0.39 \(\mu\)M in the solution phase and 51.13 \(\mu\)M in zebrafish. The association ratio between TCC and Zn\(^{2+}\) was determined to be 2:1 by ESI-mass and Job plot. The sensing mechanism of TCC for Zn\(^{2+}\) was illustrated to be a chelation-enhanced fluorescence process through spectroscopic experiments and theoretical calculations.

**Keywords:** zinc ion; hydrazine; chemosensor; carbothioamide; zebrafish; test-strip

1. **Introduction**

Zinc is a crucial trace nutrient for organisms and the second-most plentiful transition metal in the body [1–4]. For decades, zinc has been noted for its pivotal roles involved in biological processes, such as the growth of living organisms, neural signal transmission and gene transcription [5–8]. Due to the various functions of zinc in biological processes, however, an unbalance of zinc has been associated with various pathological troubles [9–11].

Particularly, zinc deficiency in the human body results in a severe effect on impaired taste, depressed immunity, delayed sexual maturation and growth defects [12]. In contrast, too much zinc can lead to neurodegenerative damage, including infantile diarrhea, Alzheimer’s disease, diabetes and Parkinson’s disease [13,14]. Thus, there is an imperative need to develop tools that can prevent undue exposure to zinc in living organisms.

The zinc detection methods reported thus far include atomic absorption spectrometry, electrochemistry, potentiometry and fluorescence spectroscopy [15–22]. Among them, chemosensors based on fluorescence spectroscopy have been a useful method for sensing of Zn\(^{2+}\) due to the fast response, high selectivity and sensitivity, ease of manipulation and bioimaging ability [23–26].

Hitherto, several studies have reported that fluorescent probes based on naphthalene, coumarin, phenanthrene, anthracene, rhodamine, antipyrine and triazole have been applied to the sensing of Zn\(^{2+}\) [27–33]. However, there are still many disadvantages, such as complex synthesis processes and difficulty in bioimaging. Thus, it is necessary to develop an easily accessible fluorescent chemosensor for detecting zinc in biological systems.

Thiourea has attracted attention for its capability to bind to metals [34,35]. In particular, the sulfur atom in thiourea prefers to chelate with soft metal ions, such as Zn\(^{2+}\) and Hg\(^{2+}\), through the hard-soft acid base theory [36–38]. In order to selectively detect only Zn\(^{2+}\) with
the thiourea moiety, we intended to endow a hard character to the thiourea by combination
with hydrazine with hard base nitrogen atoms [39,40].

Moreover, hydrazine has a water-soluble character [41]. To keep these properties
in mind, we designed and found the compound TCC, including the thiourea and hy-
drazine moieties, as reported in the literature [42,43]. We applied TCC as a sensor with
the expectation that it could coordinate well to zinc ion and might be soluble in water for
biological applications.

Herein, we address a practical hydrazine-carbothioamide-based fluorescent sensor
TCC for detecting Zn\(^{2+}\). TCC exhibited selective fluorescence emission for only Zn\(^{2+}\)
and all the other cations did not interfere with the fluorescence emission of TCC to Zn\(^{2+}\).
Significantly, TCC was a suitable chemosensor capable of detecting Zn\(^{2+}\) with practical
applications, such as real water samples, paper-strips and zebrafish. TCC could detect
down to 0.39 \(\mu\)M of Zn\(^{2+}\) in the solution phase and 51.13 \(\mu\)M of Zn\(^{2+}\) in zebrafish. The
sensing interaction of TCC for Zn\(^{2+}\) was demonstrated by ESI-mass, \(^1\)H NMR titration,
calculations, fluorescent experiments and UV-vis titration.

2. Experiments

2.1. Materials and Equipment

All the chemicals were supplied by Sigma–Aldrich (Burlington, MA, USA). A Var-
ian spectrometer was employed to obtain \(^1\)H NMR and \(^13\)C NMR. Perkin Elmer model
spectrometers were employed to obtain the absorption and fluorescent spectra. ESI-mass
measurements were conducted using a Thermo MAX instrument (Molecular Devices,
San Jose, CA, USA).

2.2. Synthesis of TCC (N-(4-Chlorophenyl)-2-(thiophene-2-carbonyl)hydrazine-1-carbothioamide)

The compound TCC reported in the literature [42,43] was synthesized in reaction solvent
acetonitrile as follows (Scheme 1). Thiophene-2-carbohydrazide (128 mg, \(9.0 \times 10^{-4}\) mol)
and 1-chloro-4-isothiocyanatobenzene (170 mg, \(1.0 \times 10^{-3}\) mol) were dissolved in 5.0 mL
acetonitrile. The resulting solution was shaken for 2 h at room temperature. The white
powder produced was collected by filtration, washed with diethyl ether and dried at 60 \(^\circ\)C
for 4 h (yield: 75%).

\[
\begin{align*}
\text{HN-} \xrightarrow{\text{MeCN}} \text{HNN} \quad \text{S} & \quad \text{Cl} \\
& \quad \text{N} \quad \text{Cl}
\end{align*}
\]

\[ \text{TCC} \]

Scheme 1. The synthesis of TCC.

TCC was affirmed by \(^1\)H, \(^13\)C NMR and ESI-MS (Figures S1–S3). \(^1\)H NMR (DMSO-\(d_6\)):
10.60 (s, 1H), 9.95 (s, 1H), 9.92 (s, 1H), 7.87 (d, 2H), 7.60 (s, 1H), 7.47 (d, 1H), 7.35 (t, 1H), 7.21
d (2H). \(^13\)C NMR (DMSO-\(d_6\)): 140.73 (1C), 137.33 (1C), 131.73 (2C), 129.55 (3C), 127.99 (2C)
and 124.73 (3C). ESI-MS for [\(\text{TCC} + \text{H}^+ + \text{H}_2\text{O}\)]\(^+\), calcd, 330.01 (m/z); found, 330.08. Water
solubility of TCC: 0.11 g/L (Figure S4).

2.3. Fluorescent and UV-Vis Titrations

TCC (3.1 mg, \(2.0 \times 10^{-5}\) mol) was dissolved in 1.0 mL DMF to make a stock (\(2.0 \times 10^{-2}\) M).
We added 6 \(\mu\)L of the TCC stock to 2.990 mL bis-tris buffer (\(1 \times 10^{-2}\) M, pH 7.0) to make
40 \(\mu\)M. Zn(NO\(_3\))\(_2\) (15.2 mg, \(5 \times 10^{-5}\) mol) was dissolved in 5 mL buffer to make a Zn\(^{2+}\) stock
(1.0 × 10^{-2} \text{ M}). We added 1.2–20.4 \mu L of the Zn^{2+} stock to TCC (40 \mu M). After blending them for 5 s, their fluorescent and UV-vis data were obtained.

2.4. Job Plot

Two stock solutions, TCC (2.0 × 10^{-2} \text{ M}) and Zn^{2+} (1.0 × 10^{-2} \text{ M}), were prepared as described in titration section. We diluted 100 \mu L of the TCC stock in 49.9 mL buffer to give 4 × 10^{-5} \text{ M}, and 200 \mu L of the Zn^{2+} stock was diluted to 49.9 mL buffer to afford 4 × 10^{-5} \text{ M}. We delivered 0.3–2.7 mL of the diluted TCC to the UV-vis cell. The diluted Zn^{2+} was delivered to the cells to provide 3 mL. After blending them for 5 s, fluorescent data were obtained.

2.5. Competitive Tests

The TCC (40 \mu M) solution was prepared as mentioned in the titration section. To provide metal stocks (1.0 × 10^{-2} \text{ M}), 5x10^{-5} \text{ mol of various cations (Zn}^{2+}, K^{+}, Pb^{2+}, Na^{+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+}, Ga^{3+}, Cr^{3+}, Fe^{3+}, Co^{3+}, In^{3+}, and Al^{3+}) was dissolved separately in 5 mL of buffer. We added 19.2 \mu L of each metal stock (1.0 × 10^{-2} \text{ M}) into TCC (40 \mu M). Then, 19.2 \mu L of Zn(NO_3)_2 stock (1.0 × 10^{-2} \text{ M}) was delivered to the mixed solution of TCC and each metal. Fluorescent data were obtained after blending them for 5 s.

2.6. $^1$H NMR Titration

Four NMR glass tubes of TCC (3.1 mg, 1.0 × 10^{-5} \text{ mol}) dissolved in deuterated DMF (1.0 mL) were prepared. We added 0–20 \mu L (0–2.0 equiv) of Zn^{2+} dissolved in deuterated DMF to the TCC. After blending these for 5 s, their $^1$H NMR spectra were obtained.

2.7. pH Test

A diverse pH range (6–9) of buffer solutions was prepared by mixing KOH and HCl in Tris-HCl buffer and bis-tris buffer. We placed 6 \mu L of TCC (2.0 × 10^{-2} \text{ M}) stock into 2.99 mL buffer solutions to produce 4.0 × 10^{-5} \text{ M}. We added 19.2 \mu L of a Zn^{2+} solution (1.0 × 10^{-2} \text{ M}) to each TCC solution (4.0 × 10^{-5} \text{ M}). After blending them for 5 s, fluorescent data were obtained.

2.8. Water Sample

To analyze the utilization of TCC for Zn^{2+} in real water samples, tap and drinking water were prepared in our laboratory. A TCC stock (2.0 × 10^{-2} \text{ M}) was prepared as described in titration section. We added 6 \mu L of the TCC stock to a 2.99 mL water sample containing Zn^{2+} (8.00 \mu M). After blending for 5 s, fluorescent data were obtained.

2.9. Fluorescent Paper-Strips

The TCC-paper strips were provided by soaking the filter papers in TCC (2 × 10^{-2} \text{ M}, DMF) and drying them. TCC-paper strips were added to 1 mM of metal ions in buffer. After drying, their photographs were taken.

2.10. Zebrafish Imaging

The 6-day-old zebrafish were reared under our former conditions [44]. Before proceeding with the imaging experiment, we prepared a TCC stock (2.0 × 10^{-2} \text{ M}) and a Zn^{2+} stock (1.0 × 10^{-2} \text{ M}). We added 50 \mu L of the TCC stock to 19.95 mL E2 media. The zebrafish were incubated with TCC (50 \mu M) in E2 media with 0.3% DMSO for 15 min and then washed with E2 media.

The zebrafish were separated into four groups. One was a control group, and the other groups were further treated with 150, 250 or 500 \mu M of Zn^{2+} for 15 min. The zebrafish were anesthetized by ethyl-3-aminobenzoate methanesulfonate. A few seconds later, we conducted all the imaging experiments using a fluorescence microscope. With Icy software, the mean fluorescence intensity of the images was analyzed.
2.11. Theoretical Studies

Theoretical calculations for TCC and TCC-Zn\(^{2+}\) were studied using the Gaussian 16 program [45]. The DFT method was employed for geometry optimizations [46,47]. The B3LYP and 6–31G(d,p) basis set was employed for all atoms except Zn\(^{2+}\) [48,49]. In the case of TCC-Zn\(^{2+}\), the LANL2DZ basis set was applied to Zn\(^{2+}\) [50–52]. None of the imaginary frequency appeared in the optimized-patterns and local minima of TCC and TCC-Zn\(^{2+}\) were verified. The solvent effect of water was dealt with IEFPCM [53]. The thirty probable UV-vis transition states were calculated with the TD-DFT method based on the energy-optimized patterns of TCC and TCC-Zn\(^{2+}\).

3. Results and Discussion

3.1. Fluorescence Investigation of TCC to Zn\(^{2+}\)

To identify the selectivity of TCC toward various cations (Zn\(^{2+}\), K\(^{+}\), Pb\(^{2+}\), Na\(^{+}\), Cu\(^{2+}\), Hg\(^{2+}\), Fe\(^{2+}\), Cd\(^{2+}\), Mn\(^{2+}\), Mg\(^{2+}\), Ca\(^{2+}\), Ni\(^{2+}\), Ga\(^{3+}\), Cr\(^{3+}\), Fe\(^{3+}\), Co\(^{3+}\), In\(^{3+}\) and Al\(^{3+}\)) the fluorescent response was tested in bis-tris buffer (Figure 1). With excitation at 320 nm, TCC displayed no fluorescence around 450 nm (\(\lambda_{\text{ex}} = 320 \text{ nm}, \Phi = 0.0258\)).

![Figure 1. Fluorescence spectral response of TCC (4.0 \times 10^{-5} \text{ M}) toward diverse metal ions (Zn\(^{2+}\), K\(^{+}\), Pb\(^{2+}\), Na\(^{+}\), Cu\(^{2+}\), Hg\(^{2+}\), Fe\(^{2+}\), Cd\(^{2+}\), Mn\(^{2+}\), Mg\(^{2+}\), Ca\(^{2+}\), Ni\(^{2+}\), Ga\(^{3+}\), Cr\(^{3+}\), Fe\(^{3+}\), Co\(^{3+}\), In\(^{3+}\) and Al\(^{3+}\); \(\lambda_{\text{ex}} = 320 \text{ nm})\). Inset: Fluorescent pictures of TCC (4.0 \times 10^{-5} \text{ M}) and TCC (4.0 \times 10^{-5} \text{ M}) with Zn\(^{2+}\) (1.6 equiv).](image)

When each cation (1.6 equiv) was added to TCC, only Zn\(^{2+}\) rapidly induced remarkable fluorescence emission at 450 nm (\(\Phi = 0.1255\)). There was no fluorescence emission with the other analytes, indicating that TCC may work as a selective fluorescent probe for detecting Zn\(^{2+}\). On the other hand, the quenching effect of S\(^{2-}\) and pyrophosphate (PPi) to TCC-Zn\(^{2+}\) was examined, but no fluorescence change occurred.

Fluorescence and UV-vis titrations were conducted to examine the sensing property of TCC for Zn\(^{2+}\) (Figures 2 and 3). As different concentrations of Zn\(^{2+}\) (0–1.7 equiv) were added to TCC, the fluorescence emission at 450 nm constantly increased until 1.6 equiv of Zn\(^{2+}\) was added. UV-vis titration was also performed under the same condition. Upon addition of Zn\(^{2+}\) into TCC, the absorbance of 340 nm consistently increased and that of 270 nm decreased until Zn\(^{2+}\) reached at 1.6 equiv. There was an evident isosbestic point at 288 nm, which signifies that the interaction of TCC and Zn\(^{2+}\) provided a product.
Figure 2. Fluorescence spectral response of TCC (4.0 × 10⁻⁵ M) with varied concentrations of Zn²⁺ (λₑₓ = 320 nm). The arrow from bottom to top represents that fluorescence emission increased with the increasing Zn²⁺ (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64 and 68 µM).

Figure 3. Absorption variations of TCC (4.0 × 10⁻⁵ M) with varied concentrations of Zn²⁺. As indicated by the arrow, the absorption of 270 nm gradually decreased with the increasing Zn²⁺ (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64 and 68 µM), while the absorption of 340 nm increased.

A Job plot was employed to apprehend the association ratio of TCC for Zn²⁺ (Figure 4). The greatest fluorescence emission at 450 nm appeared at a molar fraction of 0.7, which means that TCC and Zn²⁺ formed a complex with a 2:1 association ratio. The ratio was also proven by ESI-MS (Figure S5). The peak of 725.82 (m/z) corresponded to [2·TCC − H⁺ + Zn²⁺ + MeCN]⁺ (calculated m/z = 725.94) in the positive-ion spectrum.
Figure 4. A Job plot for TCC with Zn$^{2+}$ ($\lambda_{ex} = 320$ nm).

From the definition of IUPAC ($C_{DL} = 3\sigma / k$) [54], the detection limit for Zn$^{2+}$ was calculated to be 0.39 $\mu$M (Figure 5). This was much lower than the drinking water standard (76 $\mu$M) stipulated by the World Health Organization (WHO) [55]. More importantly, the value is the lowest among those formerly addressed for hydrazine-carbothioamide-based fluorescent Zn$^{2+}$ chemosensors (Table S1) [34,39,56–58]. The association constant ($K$) of TCC-Zn$^{2+}$ was given as $2 \times 10^8$ M$^{-2}$ from Li’s equation (Figure S6).

Figure 5. The detection limit for Zn$^{2+}$ by TCC ($4.0 \times 10^{-5}$ M) based on the fluorescence emission at 450 nm ($\lambda_{ex} = 320$ nm).

To determine an appropriate sensing mechanism between TCC and Zn$^{2+}$, $^1$H NMR titrations were conducted (Figure S7). When 0.5 equiv of Zn$^{2+}$ was added to TCC, the peak of thiourea protons (H$_4$, H$_5$ and H$_6$) shifted downfield. Upon the addition of Zn$^{2+}$ up to 2.0 equiv, the integral value of H$_4$ decreased to half, indicating that the proton H$_4$ of one
of two TCC molecules was deprotonated by binding with Zn\(^{2+}\). Thus, we predicted that both the nitrogen of amide and the sulfur of thiourea would bind to Zn\(^{2+}\). Based on the results of the ESI-mass, Job plot and \(^1\)H NMR titration, a proper structure of Zn\(^{2+}\)-2-TCC was suggested (Scheme 2).

![Scheme 2. The proposed response mechanism of TCC for Zn\(^{2+}\).](image)

A competition test was performed to understand a probing ability of TCC toward Zn\(^{2+}\). The fluorescent spectra of TCC were recorded in the presence of Zn\(^{2+}\) along with other cations (Figure 6). There was no interference in the fluorescent spectra of TCC for detecting Zn\(^{2+}\), indicating that TCC was an excellent sensor to detect Zn\(^{2+}\) without interference from other cations. The pH test of TCC and Zn\(^{2+}\)-2-TCC was conducted in different pH conditions (pH 6–9) (Figure S8). For TCC, there was no fluorescence emission from pH 6 to 9. Meanwhile, the fluorescence intensity of Zn\(^{2+}\)-2-TCC was prominently increased between pH 7 and 9. This outcome signified that TCC may be utilized for sensing Zn\(^{2+}\) at pH 7–9.

![Figure 6. The fluorescence intensity for the reaction of TCC (4.0 \times 10^{-5} \text{ M}) at 450 nm with the addition of Zn\(^{2+}\) (1.6 equiv) with/without other metal ions (1.6 equiv; \(\lambda_{\text{ex}} = 320 \text{ nm}\)).](image)

To ensure the practical availability of TCC, a fluorescent paper-strip application was performed under fluorescence lamp (\(\lambda_{\text{ex}} = 365 \text{ nm}\)) (Figure 7). Among the various metals, TCC could detect only Zn\(^{2+}\) with definite fluorescent emission. The results suggested that TCC was able to detect Zn\(^{2+}\) in the paper-applied phase. The application of TCC
in real samples was conducted to inspect the practical utility of TCC (Table 1). Reliable recoveries and R.S.D. values were observed in both drinking and tap water samples, meaning that TCC has a great potential to be employed as a reliable tool for monitoring Zn\(^{2+}\) in real samples.

![Figure 7](image_url)

**Figure 7.** Photographs of TCC-paper strips dipped in varied metal ions.

**Table 1.** The determination of Zn\(^{2+}\).  

| Sample          | Zn\(^{2+}\) Added (µM) | Zn\(^{2+}\) Found (µM) | Recovery (%) | R.S.D. (n = 3) (%) |
|-----------------|------------------------|------------------------|--------------|-------------------|
| Drinking water  | 0.0                    | *n.d.                  | 8.25         | 103.12            | 0.94              |
|                 | 8.00 \(^{b}\)          |                        | 8.25         |                   |                   |
| Tap water       | 0.0                    | *n.d.                  | 7.90         | 98.75             | 0.19              |
|                 | 8.00 \(^{b}\)          |                        | 7.90         |                   |                   |

\(^{a}\) Conditions: [TCC] = 40 µM in buffer. \(^{b}\) 8 µM of Zn\(^{2+}\) was artificially added. *n.d.: Not detected.

3.2. Imaging in Zebrafish

To identify the biological applications of TCC for Zn\(^{2+}\), imaging experiments were achieved with zebrafish (Figure 8). When the zebrafish were treated with TCC (50 µM) for 15 min, there was no fluorescence in the swim bladder (Figure 8(a)). However, as the amounts of Zn\(^{2+}\) increased to 150, 250 and 500 µM (Figure 8(b–d)), the fluorescence in the swim bladder gradually increased. In the swim bladder, the detection limit for Zn\(^{2+}\) was analyzed to be 51.13 µM with the Icy software (Figure S9). These results illustrate that TCC may be applied to trace Zn\(^{2+}\) in live organisms.

3.3. Calculations

Optimized patterns of TCC and Zn\(^{2+}\)-2-TCC were investigated according to the analyses of the ESI-mass and Job plot. As shown in Figure 9, TCC had a twist structure with a dihedral angle of −101.27° for 1C, 2N, 3N and 4C, whereas the coordination of Zn\(^{2+}\) to two TCC molecules displayed a more rigid tetrahedral structure (dihedral angle = 175.18°). The bond distances related to coordination of Zn\(^{2+}\) to TCC were calculated to be 1.992 Å for 2N-Zn\(^{2+}\) and 2.341 Å for 5S-Zn\(^{2+}\), which are in the range of the general bond distances for binding with Zn\(^{2+}\) [59,60].

TD-DFT calculations were achieved based on energy-optimized patterns of TCC and Zn\(^{2+}\)-2-TCC complex. The leading absorption of TCC at 259.1 nm was caused from the HOMO-3 → LUMO (61%), HOMO-4 → LUMO (17%) and HOMO-6 → LUMO (13%) transitions, which are related to the π → π* transition (Figures S10 and S11). For the Zn\(^{2+}\)-2-TCC complex, an absorption band related to the red-shift originated from the HOMO → LUMO+2 (96%) transition (319.9 nm, Figures S11 and S12) and exhibited a π → π* transition. The red-shift recorded in the UV-vis spectra corresponded well with the calculated transition states.

Both TCC and its complex state showed similar transition characters, and the rigidity in the complex state of TCC increased. Thus, fluorescent ‘turn-on’ sensing would be caused by chelation-enhanced fluorescence process [61]. When TCC was converted into the complex state with Zn\(^{2+}\), the reduction of nonradiative transitions, such as rotations and vibrations, would lead to the enhancement of radiative transitions, like fluorescence.
Referring to various spectroscopic experiments and theoretical calculations, we present a plausible sensing model of Zn$^{2+}$ by TCC (Scheme 2).

Figure 8. Fluorescence images of 6-day-old zebrafish exposed to TCC followed by the addition of Zn$^{2+}$. (a1–a3): TCC only; (b1–b3): TCC with 150 µM Zn$^{2+}$; (c1–c3): TCC with 250 µM Zn$^{2+}$; and (d1–d3): TCC with 500 µM Zn$^{2+}$. [TCC] = 50 µM. Scale bar: 2.00 mm.

Figure 9. Energy-optimized patterns of (a) TCC and (b) Zn$^{2+}$-2·TCC.

4. Conclusions

We presented a practical hydrazine-carbothioamide-based fluorescent chemosensor TCC that could effectively detect Zn$^{2+}$ in aqueous media. Probe TCC could detect Zn$^{2+}$ among the other metal ions through selective fluorescence emission. In addition, TCC
could clearly recognize Zn\(^{2+}\) with competition from metal ions. Particularly, TCC could be used as a practical probe capable of detecting Zn\(^{2+}\) in paper-strip, zebrafish and real water samples.

The detection limit of TCC for Zn\(^{2+}\) was calculated to be 0.39 \(\mu\)M in the solution phase and 51.13 \(\mu\)M in zebrafish. Importantly, the value in the solution phase is the lowest among those formerly addressed for hydrazine-carbothioamide-based fluorescent Zn\(^{2+}\) chemosensors. The binding mode of TCC for Zn\(^{2+}\) was revealed to be a 2:1 by the Job plot and ESI-mass. The detecting mechanism of TCC toward Zn\(^{2+}\) was described as the chelation-enhanced fluorescence process based on the results of spectroscopic studies and theoretical calculations.

Future study will focus on the development of hydrazine-carbothioamide-based chemosensors, which may operate at long excitation wavelengths for fluorescence bio-imaging. In addition, we will consider the development of an integrated system with portable fluorescent recognition or smartphone-based sensors [62,63].

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors10010032/s1, Table S1: Examples of hydrazine-carbothioamide-based fluorescence chemosensors for detecting Zn\(^{2+}\). Figure S1: \(^1\)H NMR spectrum of TCC. Figure S2: \(^{13}\)C NMR spectrum of TCC. Figure S3: Positive-ion ESI-mass spectrum of TCC (100 \(\mu\)M). Figure S4: Solubility of TCC in distilled water based on the absorbance at 320 nm. Solubility was calculated to the TCC-saturated solution with linear fitting curve of TCC (0, 40, 80, 120, 160 and 200 \(\mu\)M). Figure S5: Positive-ion ESI-mass spectrum of TCC (100 \(\mu\)M) upon the addition of Zn\(^{2+}\) (1 equiv). Figure S6: Li’s equation plot (at 450 nm) of TCC (40 \(\mu\)M) based on fluorescence titration, assuming 2:1 stoichiometry for association between TCC and Zn\(^{2+}\). Figure S7: \(^1\)H NMR titration of TCC (10 mM) upon the addition of different amounts of Zn\(^{2+}\) (0–2.0 equiv). Figure S8: Fluorescence intensity of TCC and TCC-Zn\(^{2+}\) at a pH range of 6 to 9. Figure S9: Quantification of the mean fluorescence intensity in Figure S8 at pH 6. Figure S10: (a) The theoretical excitation energies and the experimental UV-vis spectrum of TCC. (b) The major electronic transition energies and molecular orbital contributions of TCC. Figure S11: The major molecular orbital transitions and excitation energies of TCC and the Zn\(^{2+}\)-2-TCC complex. Figure S12: (a) The theoretical excitation energies and the experimental UV-vis spectrum of the Zn\(^{2+}\)-2-TCC complex. (b) The major electronic transition energies and molecular orbital contributions of the Zn\(^{2+}\)-2-TCC complex.

**Author Contributions:** Conceptualization, B.S. and C.K.; formal analysis, B.S.; D.G. and S.Y.; data curation, B.S. and D.G.; writing—original draft preparation, B.S. and D.G.; writing—review and editing, B.S. and C.K.; supervision, C.K. and K.-T.K.; funding acquisition, C.K.

**Funding:** The National Research Foundation of Korea (2018R1A2B6001686) is gratefully.

**Institutional Review Board Statement:** The maintenance of zebrafish was approved by the Institutional Animal Care and Use Committees at the Seoul National University of Science and Technology.

**Ethical Review and Approval:** Ethical review and approval were waived for this study because early-life stages of zebrafish were used as a practical probe capable of detecting Zn\(^{2+}\) in paper-strip, zebrafish and real water samples. Particularly, TCC could be used as a practical probe capable of detecting Zn\(^{2+}\) in paper-strip, zebrafish and real water samples. Particularly, TCC could be used as a practical probe capable of detecting Zn\(^{2+}\) in paper-strip, zebrafish and real water samples.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Yun, D.; Chae, J.B.; So, H.; Lee, H.; Kim, K.T.; Kim, C. Sensing of zinc ions and sulfide using a highly practical and water-soluble fluorescent sensor: Applications in test kits and zebrafish. *New J. Chem.* 2019, 44, 442–449. [CrossRef]

2. Qu, W.J.; Guan, J.; Wei, T.B.; Yan, G.T.; Lin, Q.; Zhang, Y.M. A turn-on fluorescent sensor for relay recognition of two ions: From a F-­selective sensor to highly Zn\(^{2+}\)-selective sensor by tuning electronic effects. *RSC Adv.* 2016, 6, 35804–35808. [CrossRef]

3. Gilbert, R.; Peto, T.; Lengyel, I.; Emri, E. Zinc Nutrition and Inflammation in the Aging Retina. *Mol. Nutr. Food Res.* 2019, 63, e1801049. [CrossRef] [PubMed]

4. Kim, M.J.; Kaur, K.; Singh, N.; Jang, D.O. Benzimidazole-based receptor for Zn\(^{2+}\) recognition in a biological system: A chemosensor operated by retarding the excited state proton transfer. *Tetrahedron* 2012, 68, 5429–5433. [CrossRef]
5. Berg, J.M.; Shi, Y. The galvanization of biology: A growing appreciation for the roles of zinc. *Science* **1996**, *271*, 1081–1085. [CrossRef]

6. Zhang, C.; Liu, Z.; Li, Y.; He, W.; Gao, X.; Guo, Z. In vitro and in vivo imaging application of a 1,8-naphthalimide-derived Zn$^{2+}$ fluorescent sensor with nuclear envelope penetrability. *Chem. Commun.* **2013**, *49*, 11430–11432. [CrossRef]

7. Maity, D.; Govindaraju, T. A differentially selective sensor with fluorescence turn-on response to Zn$^{2+}$ and dual-mode ratiometric response to Al$^{3+}$ in aqueous media. *Chem. Commun.* **2012**, *48*, 1039–1041. [CrossRef]

8. Helal, A.; Rashid, M.H.O.; Choi, C.H.; Kim, H.S. New regioisomeric naphthol-substituted thiazole based ratiometric fluorescence sensor for Zn$^{2+}$ with a remarkable red shift in emission spectra. *Tetrahedron* **2012**, *68*, 647–653. [CrossRef]

9. Valkée, B.L.; Auld, D.S. Zinc Coordination, Function, and Structure of Zinc Enzymes and Other Proteins. *Biochemistry* **1990**, *29*, 5647–5659. [CrossRef]

10. Quang, D.T.; Kim, J.S. Fluoro- and chromogenic chemodosimeters for heavy metal ion detection in solution and biospecimens. *Chem. Rev.* **2010**, *110*, 6280–6301. [CrossRef]

11. Yun, J.Y.; Jo, T.G.; Han, J.; Jang, H.J.; Lim, M.H.; Kim, C. A highly sensitive and selective fluorescent chemosensor for the sequential recognition of Zn$^{2+}$ and S$^{2−}$ in living cells and aqueous media. *Sens. Actuators B Chem.* **2018**, *255*, 3108–3116. [CrossRef]

12. Ploysangam, A.; Falciiglia, G.A.; Brehm, B.J. Effect of marginal zinc deficiency on human growth and development. *J. Trop. Pediatr.* **1997**, *43*, 192–198. [CrossRef]

13. Kim, S.; Lee, H.; So, H.; Lee, H.; Kim, K.T.; Kim, C. A benzyl carbazate-based fluorescent chemosensor for detecting Zn$^{2+}$: Application to zebrafish. *Spectrochim. Acta Part A* **2020**, *228*, 117787–117793. [CrossRef]

14. Chan, W.C.; Saad, H.M.; Sim, K.S.; Lee, V.S.; Ang, C.W.; Yeong, K.Y.; Tan, K.W. A rhodamine based chemosensor for solvent dependent chromogenic sensing of cobalt (II) and copper (II) ions with good selectivity and sensitivity: Synthesis, filter paper test strip, DFT calculations and cytotoxicity. *Spectrochim. Acta Part A* **2021**, *262*, 120099–120112. [CrossRef]

15. Sturgeon, R.E.; Berman, S.S.; Desauhiers, A.; Russell, D.S. Reply to Comments on Determination of Iron, Manganese, and Zinc by Graphite Furnace Atomic Absorption Spectrometry Using a Microemulsion Preparation Method. *Energy Fuels* **2017**, *31*, 2949–2950. [CrossRef]

16. Srivastava, S.K.; Gupta, V.K.; Jain, S. PVC-based 2,2,2-cryptand sensor for zinc ions. *Anal. Chem.* **1996**, *68*, 1272–1275. [CrossRef] [PubMed]

17. Chaiyo, S.; Mehmeti, E.; Žagar, K.; Sjiangroh, W.; Chailapakul, O.; Kalcher, K. Electrochemical sensors for the simultaneous determination of zinc, cadmium and lead using a Nafion/ionic liquid/graphene composite modified screen-printed carbon electrode. *Anal. Chim. Acta* **2016**, *918*, 26–34. [CrossRef] [PubMed]

18. Ansari, R.; Delavar, A.F.; Mohammad-Khah, A. Solid-state ion selective electrode based on polypyrrole conducting polymer nanofilm as a new potentiometric sensor for Zn$^{2+}$ ion. *J. Solid State Electrochem.* **2012**, *16*, 3315–3322. [CrossRef]

19. Shamsipour, M.; Rouhani, S.; Ganjali, M.R.; Sharghi, H.; Eshghi, H. Zinc-selective membrane potentiometric sensor based on a recently synthesized benzo-substituted macrocyclic diamide. *Sens. Actuators B Chem.* **1999**, *50*, 30–34. [CrossRef]

20. Goswami, S.; Paul, S.; Manna, A. A differentially selective chemosensor for a ratiometric response to Zn$^{2+}$ and Al$^{3+}$ in aqueous media with applications for molecular switches. *RSC Adv.* **2013**, *3*, 25079–25085. [CrossRef]

21. Narayanawaswamy, N.; Maity, D.; Govindaraju, T. Reversible fluorescence sensing of Zn$^{2+}$ based on pyridine-constrained bis(triazole-linked hydroxyquinoline) sensor. *Sens. Actuators B Chem.* **2011**, *155*, 23, 703–709. [CrossRef]

22. Lee, D.Y.; Singh, N.; Kim, M.J.; Jang, D.O. Ratiometric fluorescent determination of Zn(II): A new class of tripodal receptor using mixed imine and amide linkages. *Tetrahedron* **2010**, *66*, 7965–7969. [CrossRef]

23. Helal, A.; Kim, S.H.; Kim, H. Thiazole sulfonamide based ratiometric fluorescent chemosensor with a large spectral shift for zinc sensing. *Tetrahedron* **2010**, *66*, 9925–9933. [CrossRef]

24. Wei, T.B.; Zhang, P.; Shi, B.B.; Chen, P.; Lin, Q.; Liu, J.; Zhang, Y.M. A highly selective chemosensor for colorimetric detection of Fe$^{3+}$ and fluorescence turn-on response of Zn$^{2+}$ ions. *D. Pigm. 2013*, *97*, 297–302. [CrossRef]

25. Cheah, P.W.; Heng, M.P.; Saad, H.M.; Sim, K.S.; Tan, K.W. Specific detection of Cu$^{2+}$ by a pH-independent colorimetric rhodamine based chemosensor. *Opt. Mater.* **2021**, *114*, 110990–110997. [CrossRef]

26. Darjee, S.M.; Modi, K.M.; Panchal, U.; Patel, C.; Jain, V.K. Highly selective and sensitive fluorescent sensor: Thiacalix[4]arene-1-naphthalene carboxylate for Zn$^{2+}$ ions. *J. Mol. Struct.* **2017**, *1133*, 1–8. [CrossRef]

27. Bhattacharyya, A.; Ghosh, S.; Makhal, S.C.; Guchhait, N. Hydrazine bridged coumarin-pyrimidine conjugate as a highly selective and sensitive Zn$^{2+}$ sensor: Spectroscopic unraveling of sensing mechanism with practical application. *Spectrochim. Acta Part A* **2017**, *183*, 306–311. [CrossRef]

28. Hwang, S.M.; Kim, C. Fluorescent detection of Zn$^{2+}$ and Cu$^{2+}$ by a phenanthrene-based multifunctional chemosensor that acts as a basic pH indicator. *Inorg. Chem. Acta* **2018**, *482*, 375–383. [CrossRef]

29. Kim, J.H.; Noh, J.Y.; Hwang, I.H.; Kang, J.; Kim, J.; Kim, C. An anthracene-based fluorescent chemosensor for Zn$^{2+}$. *Tetrahedron Lett.* **2013**, *54*, 2415–2418. [CrossRef]

30. Wechakorn, K.; Suksen, K.; Piyachaturawat, P.; Kongsaeree, P. Rhodamine-based fluorescent and colorimetric sensor for zinc and its application in bioimaging. *Sens. Actuators B Chem.* **2016**, *228*, 270–277. [CrossRef]
32. Gupta, V.K.; Singh, A.K.; Kumawat, L.K. A turn-on fluorescent chemosensor for Zn$^{2+}$ ions based on antipyrene Schiff base. Sens. Actuators B Chem. 2014, 204, 507–514. [CrossRef]
33. Gusev, A.N.; Shul’Gin, V.F.; Meshkova, S.B.; Smola, S.S.; Linert, W. A novel triazole-based fluorescent chemosensor for Zinc ions. J. Lumin. 2014, 155, 311–316. [CrossRef]
34. So, H.; Cho, H.; Lee, H.; Tran, M.C.; Kim, K.T.; Kim, C. Detection of zinc (II) and hypochlorite by a thiourea-based chemosensor via two emission channels and its application in vivo. Microchem. J. 2020, 155, 104788–104795. [CrossRef]
35. Francl, M.M.; Pietro, W.J.; Hehre, W.J.; Binkley, J.S.; Gordon, M.S.; DeFrees, D.J.; Pople, J.A. Self-consistent molecular orbital core orbitals. J. Chem. Phys. 1982, 77, 3244–3253. [CrossRef]
36. Zhu, H.; Fan, J.; Zhang, S.; Cao, J.; Song, K.; Ge, D.; Dong, H.; Wang, J.; Peng, X. Ratiometric fluorescence imaging of lysosomal Zn$^{2+}$ release under oxidative stress in neural stem cells. Biomat. Sci. 2014, 2, 89–97. [CrossRef]
37. Seo, Y.; Park, S.; Kim, G.; Lee, M.; Kim, C. A naphthyl thiourea-based effective chemosensor for fluorescence detection of Ag$^+$ and Zn$^{2+}$. Luminescence 2021, 36, 1725–1732. [CrossRef]
38. Chen, Z.E.; Zhang, H.; Iqbal, Z. A new thiosemicarbazone fluorescent probe based on 9,9’-bianthracene for Hg$^{2+}$ and Ag$^+$. Spectrochim. Acta Part A 2019, 215, 34–40. [CrossRef]
39. Li, Z.; Xiang, Y.; Tong, A. Ratiometric chemosensor for fluorescent determination of Zn$^{2+}$ in aqueous ethanol. Anal. Chim. Acta 2008, 619, 75–80. [CrossRef]
40. Farr, R.G.; Pearson, R.G. Absolute Hardness: Companion Parameter to Absolute Electronegativity. J. Am. Chem. Soc. 1983, 105, 7512–7516. [CrossRef]
41. Von Burg, R. Toxicology Update. J. Appl. Toxicol. 1973, 12, 73–74. [CrossRef]
42. Dzitko, K.; Paneth, A.; Plech, T.; Paweleczky, J.; Stączek, P.; Stefańska, J.; Paneth, P. 1,4-Disubstituted Thiosemicarbazide Derivatives Are Potent Inhibitors of Toxoplasma Gondii Proliferation. Molecules 2014, 19, 9926–9943. [CrossRef] [PubMed]
43. Al-Wahabi, L.H.; Rahul, B.; Mohamed, A.A.B.; Abdelbaky, M.S.M.; Garcia-Granda, S.; El-Emam, A.A.; Percino, M.J.; Thamotharan, S. Supramolecular Self-Assembly Built by Weak Hydrogen, Chalcogen, and Unorthodox Nonbonded Motifs in 4-(4-Chlorophenyl)-3-[(4-fluorobenzyl)sulfanyl]-5-(thiophen-2-yl)-4 H-1,2,4-triazole, a Selective COX-2 Inhibitor: Insights from X-ray and Theoretical Studi. ACS Omega 2021, 6, 6996–7007. [CrossRef]
44. Kang, J.H.; Han, J.; Lee, H.; Lim, M.H.; Kim, K.T.; Kim, C. A water-soluble fluorescence chemosensor for the sequential detection of Zn$^{2+}$ and pyrophosphate in living cells and zebrafish. Dye. Pigm. 2018, 152, 131–138. [CrossRef]
45. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; et al. Gaussian 16 Revision C.01; Gaussian, Inc.: Wallingford, UK, 2016.
46. Becke, A.D. Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys. 1993, 98, 5648–5652. [CrossRef]
47. Lee, C.; Yang, W.; Parr, R.G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B 1988, 37, 785–789. [CrossRef] [PubMed]
48. Hariharan, P.C.; Pople, J.A. The influence of polarization functions on molecular orbital hydrogenation energies. Theor. Chim. Acta 1973, 28, 213–222. [CrossRef]
49. Franci, M.M.; Pietro, W.J.; Hehre, W.J.; Binkley, J.S.; Gordon, M.S.; DeFrees, D.J.; Pople, J.A. Self-consistent molecular orbital methods. XXIII. A polarization-type basis set for second-row elements. J. Chem. Phys. 1982, 77, 3654–3665. [CrossRef]
50. Hay, P.J.; Wadt, W.R. Ab initio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. J. Chem. Phys. 1985, 82, 270–283. [CrossRef]
51. Wadt, W.R.; Hay, P.J. Ab initio effective core potentials for molecular calculations. Potentials for main group elements Na to Bi. J. Chem. Phys. 1985, 82, 284–298. [CrossRef]
52. Hay, P.J.; Wadt, W.R. Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. J. Chem. Phys. 1985, 82, 299–310. [CrossRef]
53. Klamt, A.; Moya, C.; Palomar, J. A Comprehensive Comparison of the IEPCCM and SS(V)PE Continuum Solution Methods with the COSMO Approach. J. Chem. Theory Comput. 2015, 11, 4220–4225. [CrossRef] [PubMed]
54. Goswami, S.; Aich, K.; Das, S.; Das Mukhopadhyay, C.; Sarkar, D.; Mondal, T.K. A new visible-light-excitable ICT-CHEF-mediated fluorescence “turn-on” probe for the selective detection of Cd$^{2+}$ in a mixed aqueous system with live-cell imaging. Dalton Trans. 2015, 44, 5763–5770. [CrossRef] [PubMed]
55. World Health Organization. WHO Guidelines for Drinking-Water Quality, 2nd ed.; World Health Organization: Geneva, Switzerland, 1998; Volume 1.
56. Ji, Z.J.; Wu, Y.M.; Wu, F.Y. A ratiometric fluorescence sensor for zinc in neutral solution based on thiourea receptor. Chem. Lett. 2006, 35, 950–951. [CrossRef]
57. Samanta, S.; Manna, U.; Ray, T.; Das, G. An aggregation-induced emission (AIE) active probe for multiple targets: A fluorescent sensor for Zn$^{2+}$ and Al$^{3+}$ & a colorimetric sensor for Cu$^{2+}$ and F$^-$. Dalt. Trans. 2015, 44, 18902–18910. [CrossRef]
58. Zhang, C.; Pu, S.; Sun, Z.; Fan, C.; Liu, G. Highly Sensitive and Selective Fluorescent Sensor for Zinc Ion Based on a New Diarylthene with a Thiocarbamide Unit. J. Phys. Chem. B 2015, 119, 4673–4682. [CrossRef]
59. Gui, Z.; Green, A.R.; Ksrais, M.; Michael Bancroft, G.; Stillman, M.J. Sulfur K-Edge EXAFS Studies of Cadmium-, Zinc-, Copper-, and Silver-Rabbit Liver Metallothioneins. Inorg. Chem. 1996, 35, 6520–6529. [CrossRef]
60. Deerfield, D.W.; Carter, C.W.; Pedersen, L.G. Models for protein-zinc ion binding sites. II. The catalytic sites. Int. J. Quantum Chem. 2001, 83, 150–165. [CrossRef]
61. Kshirsagar, N.; Sonawane, R.; Patil, P.; Nandre, J.; Sultan, P.; Sehlangia, S.; Pradeep, C.P.; Wang, Y.; Chen, L.; Sahoo, S.K. Inorganica Chimica Acta Fluorescent chemosensor for Al (III) based on chelation-induced fluorescence enhancement and its application in live cells imaging. *Inorg. Chim. Acta* 2020, 511, 119805–119810. [CrossRef]

62. Liu, L.; Shan, D.; Zhou, X.; Shi, H.; Song, B.; Falke, F.; Leinse, A.; Heideman, R. TriPleX™ waveguide-based fluorescence biosensor for multichannel environmental contaminants detection. *Biosens. Bioelectr.* 2018, 106, 117–121. [CrossRef]

63. Xing, Y.; Zhu, Q.; Zhou, X.; Qi, P. A dual-functional smartphone-based sensor for colorimetric and chemiluminescent detection: A case study for fluoride concentration mapping. *Sens. Actuators B Chem.* 2020, 319, 128254–128261. [CrossRef]