2-Hydroxy-1-((2-(pyridin-2-yl)hydrazono)methyl)tryptanthrin as a Fluorescent Chemosensor for Metal Ions

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2-Hydroxy-1-((2-(pyridin-2-yl)hydrazono)methyl)tryptanthrin (**T2OH1CH=N-NH-Pyri**) was synthesized and applied as a fluorescent chemosensor for metal ions. We investigated the metal-ion recognition of **T2OH1CH=N-NH-Pyri** in separate Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Ag^+ , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} acetonitrile solutions. Li^+ , Na^+ , K^+ , Ca^{2+} , Ba^{2+} , and Ag^+ had no effect on the shape and intensity of the fluorescence spectrum of the chemosensor. **T2OH1CH=N-NH-Pyri** formed a 1:1 complex with Mg^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} . The fluorescence intensity of the sensor increased for Mg^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} , and decreased for Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , and Cu^{2+} .

Key words: tryptanthrin, fluorescent chemosensor, metal ion

1. INTRODUCTION

The synthesis and application of fluorescent tryptanthrin derivatives are generating widespread research interest [1–5]. These compounds absorb and emit radiation at long wavelengths and are used in a diverse range of fields and for a variety of functions, including as labeling reagents, chemosensors, laser dyes, photosensitizers, and in the manufacture of fluorescent organic devices. However, our research is concerned with the use of organic fluorescent dyes as imaging probes in biological research. The near-infrared (NIR) region (650–900 nm) is referred to as the “optical window” for cells and tissues because of the lack of efficient endogenous absorbers in this spectral range. Consequently, NIR radiation has a high penetration depth (of the order of a few millimeters) for most biological tissues [6]. Organic fluorescent dyes that emit NIR fluorescence are preferable for applications in biological systems because their use ameliorates problems associated with auto-fluorescence and photodamage to living cells.

2-Hydroxytryptanthrin (**T2OH**, Fig. 1a) has been applied as a NIR dye for fluorescent imaging. The absorption maxima ($\lambda_{a, \text{max}}$) of **T2OH** in the pH ranges 1.3–7.2 and 8.5–10.6 are *ca.* 410 and 495 nm, respectively, and its fluorescence maximum ($\lambda_{f, \text{max}}$) is *ca.* 660 nm, regardless of pH. The sodium salt of **T2OH** (sodium tryptanthrin-2-olate, **T2ONa**, Fig. 1b) is water soluble and its $\lambda_{f, \text{max}}$ is *ca.* 660 nm in both aprotic and protic solvents [5].

The metal-ion recognition capabilities of **T2OH** and its derivative 1-formyl-2-hydroxytryptanthrin (**T2OH1CHO**, Fig. 1c) have been investigated [7]. **T2OH** was demonstrated to be a fluorescent chemosensor for Cu^{2+} and Al^{3+} in acetonitrile solutions, and was also applied as an NIR fluorescent ($\lambda_{f, \text{max}} > 650 \text{ nm}$) labeling reagent. However, it was unable to detect changes in an aqueous (DMSO/H₂O = 1/9, v/v) environment. Conversely, the fluorescence of an acetonitrile solution of **T2OH1CHO** at *ca.* 590 and 520 were shown to decrease and increase, respectively, upon addition of Al^{3+} . Furthermore, **T2OH1CHO** selectively detects Al^{3+} in aqueous solutions. Thus, **T2OH1CHO** is a good fluorescent chemosensor for

Al^{3+} in both acetonitrile and aqueous solutions.

In the current study, the novel 2-hydroxytryptanthrin derivative 2-hydroxy-1-((2-(pyridin-2-yl)hydrazono)methyl)tryptanthrin (**T2OH1CH=N-NH-Pyri**, Fig. 1d) was synthesized and its metal-ion recognition properties were investigated using acetonitrile solutions of Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Ag^+ , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} .

Fig.1 Structures of (a) **T2OH**; (b) **T2ONa**; (c) **T2OH1CHO**; and (d) **T2OH1CH=N-NH-Pyri**.

2. EXPERIMENTAL

2.1 Synthesis

T2OH1CH=N-NH-Pyri was synthesized according to Scheme 1. The synthesis of 1-formyl-2-hydroxytryptanthrin (**T2OH1CHO**) was performed using the Duff reaction [8]. The synthesis of **T2OH1CH=N-NH-Pyri** was performed according to a previously reported method [9] with some slight modifications.

Hexamethylenetetramine (HMTA) (501 mg, 3.57 mmol) was added to a solution of **T2OH** [5] (633 mg, 2.40 mmol) in 20 mL of trifluoroacetic acid (TFA). The solution was refluxed for 24 h protected from light. After cooling the

Scheme 1 Synthesis of **T2OH1CH=N-NH-Pyri**.

solution to room temperature, 20 mL of water was added, and the solution was refluxed for a further 90 min. The solution was then cooled to room temperature followed by cooling in a refrigerator. The reaction mixture was suction filtered, and the solid yellow residue was thoroughly washed with water. Pure **T2OH1CHO** (584 mg) was obtained in 83% yield. Next, **T2OH1CHO** (58.5 mg, 0.201 mmol) was dissolved in 10 mL ethanol and 2-hydrazinylpyridine (21.6 mg, 0.198 mmol) was added to the solution. The mixture was stirred at room temperature for 24 h. The solvent was then removed by evaporation, and the red residue was washed with hexane and ethanol to yield **T2OH1CH=N-NH-Pyri** (62.0 mg) in 81% yield. The electron-spray ionization (ESI) mass spectra of **T2OH1CHO** and **T2OH1CH=N-NH-Pyri** showed their corresponding molecular ion peaks, and the ^1H NMR spectra confirmed that the compounds were appropriately synthesized.

T2OH1CHO: ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.51 (1H, td, $J = 7.5$ Hz, 0.8 Hz), 7.56 (1H, d, $J = 8.9$ Hz), 7.88 (2H, m), 8.05 (1H, d, $J = 8.9$ Hz), 8.47 (1H, d, $J = 8.0$ Hz), 11.03 (1H, s), 11.91 (1H, s); ESI-MS m/z ($M+H$) calcd 293.0562, found 263.0576.

T2OH1CH=N-NH-Pyri: ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 6.88 (1H, s), 7.40–7.80 (9H, m), 8.52 (1H, d, $J = 7.5$ Hz), 10.11 (1H, s), 11.65 (1H, s); ESI-MS m/z ($M+H$) calcd 384.1097, found 384.0903.

2.2 Measurements

Stock solutions of **T2OH1CH=N-NH-Pyri** were prepared by dissolving a weighed amount of the compound in acetonitrile. The metal ions ($[\text{Metal ions}] = 1\text{--}100\ \mu\text{M}$) were titrated against **T2OH1CH=N-NH-Pyri** solutions ($[\text{T2OH1CH=N-NH-Pyri}] = 10\ \mu\text{M}$) in a spectrophotometric cell with a path length of 1 cm. UV-Vis spectra (250–800 nm) of the solutions were recorded at room temperature using a JASCO V-670 spectrophotometer after the separate addition of each of the 16 metal salts LiClO_4 , NaClO_4 , KClO_4 , $\text{Mg}(\text{ClO}_4)_2$, $\text{Ca}(\text{ClO}_4)_2$, $\text{Ba}(\text{ClO}_4)_2$, $\text{Fe}(\text{ClO}_4)_2$, $\text{Fe}(\text{ClO}_4)_3$, $\text{Co}(\text{ClO}_4)_2$, $\text{Ni}(\text{ClO}_4)_2$, $\text{Cu}(\text{ClO}_4)_2$, AgClO_4 , $\text{Zn}(\text{ClO}_4)_2$, $\text{Cd}(\text{ClO}_4)_2$, $\text{Al}(\text{ClO}_4)_3$, and $\text{Pb}(\text{ClO}_4)_2$. The same titrations were also performed using fluorescence spectra measurements. The fluorescence spectra were measured between 400 and 900 nm with a Hitachi F-4500 fluorometer. ^1H NMR spectra were recorded on a JEOL

JNM-ECA 500 spectrometer in deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) with tetramethylsilane as an internal standard. High-resolution mass spectra were obtained using ESI on a HITACHI NanoFrontier LD spectrometer.

3. RESULTS AND DISCUSSION

The UV-Vis absorption and fluorescence of an acetonitrile solution of **T2OH1CH=N-NH-Pyri** were measured at room temperature. To this solution, 16 different metal salts were separately added at concentrations up to 10-times that of the sample (10 μM). When Li^+ , Na^+ , K^+ , Ca^{2+} , Ba^{2+} , and Ag^+ were added to the solution, the shape and intensity of the fluorescence spectra did not change. Figure 2 shows the absorption and fluorescence spectra of **T2OH1CH=N-NH-Pyri** with and without K^+ as a typical example. The collision quenching between **T2OH1CH=N-NH-Pyri** and the metal ions resulted in slight changes in the fluorescence spectra.

Fig.2 Spectral changes in the (a) UV-Vis and (b) fluorescence spectra of **T2OH1CH=N-NH-Pyri** (10 μM) with K^+ in an acetonitrile solution; $[\text{K}^+]/[\text{T2OH1CH=N-NH-Pyri}] = 0\text{--}10$.

From the absorbance titration curves, it was found that **T2OH1CH=N-NH-Pyri** forms a 1:1 complex with Mg^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} . Figure 3 shows the titration curve for **T2OH1CH=N-NH-Pyri** with Mg^{2+} as a typical example. The titration curve monitored by absorbance at 510 nm shows a sharp endpoint at a ligand/ion ratio of 1:1 for Mg^{2+} .

In polar solvents, **T2OH1CH=N-NH-Pyri** chelates metal ions via the olate anion of the hydroxy group and the nitrogen atoms (inset of Fig. 3). The fluorescence intensity increased for Mg^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} , and decreased for Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , and Cu^{2+} . Figures 4 and 5 shows the absorption and fluorescence spectra of **T2OH1CH=N-NH-Pyri** with and without Mg^{2+} and Ni^{2+}

Fig.3 Absorbance at 510 nm for **T2OH1CH=N-NH-Pyri** titrated with Mg^{2+} in an acetonitrile solution.

Fig.4 Spectral changes in the (a) UV-Vis and (b) fluorescence spectra of **T2OH1CH=N-NH-Pyri** (10 μM) with Mg^{2+} in an acetonitrile solution. $[\text{Mg}^{2+}]/[\text{T2OH1CH=N-NH-Pyri}] = 0-10$.

as typical examples.

The $\lambda_{f, \text{max}}$ values for Mg^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} , i.e., where fluorescence increases were observed, were 659, 645, 627, 594, and 630 nm, respectively. Thus, for Mg^{2+} , $\lambda_{f, \text{max}}$ was observed in the NIR region (650–900 nm). Fluorescence increases would be due to suppression of molecular motion by formation of metal complexes. That is, thermal deactivation due to internal conversion was reduced. When Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , and Cu^{2+} were individually added to the solution, the fluorescence at 606 nm was quenched. Transition metal ions such as Cu^{2+} usually behave as fluorescence quenchers via electron transfer from the excited-state fluorophore to the empty d -orbital [10]. Therefore, several fluorescent dyes show fluorescence-quenched responses upon the addition of transition metal salts.

Fig.5 Spectral changes in the (a) UV-Vis and (b) fluorescence spectra of **T2OH1CH=N-NH-Pyri** (10 μM) with Ni^{2+} in an acetonitrile solution. $[\text{Ni}^{2+}]/[\text{T2OH1CH=N-NH-Pyri}] = 0-10$.

We calculated the association constants (K) for Mg^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , and Pb^{2+} . Even though the solvent participates in the association interaction, the solvent concentration is virtually unaffected. Therefore, we can express the interaction of the metal salts in terms of the equilibrium [11]:

where L is **T2OH1CH=N-NH-Pyri** and M is Mg^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , or Pb^{2+} .

Furthermore, the association constants (K) can be expressed as follows:

$$\begin{aligned} K &= [\text{LM}]/[\text{L}][\text{M}] \\ &= [\text{LM}]/\{([\text{L}]_0 - [\text{LM}])([\text{M}]_0 - [\text{LM}])\} \\ &= [\text{LM}]/\{([\text{L}]_0 - [\text{LM}])([\text{M}]_0)\} \end{aligned} \quad (2)$$

$$[\text{LM}] = \{(I_a - I_{a,0})/(I_{a,\text{max}} - I_{a,0})\}[\text{L}]_0 \quad (3)$$

and from Eqs. 2 and 3, the following equation can be derived:

$$1/((I_a - I_{a,0}) = 1/\{(I_{a,\text{max}} - I_{a,0})K[\text{M}]_0\} + 1/I_{a,\text{max}} - I_{a,0} \quad (4)$$

where $[\text{L}]_0$ and $[\text{M}]_0$ are the initial concentrations of **T2OH1CH=N-NH-Pyri** and the metal ion, respectively, I_a and $I_{a,0}$ are the observed absorbances of **T2OH1CH=N-NH-Pyri** with and without the metal ion, respectively, and $I_{a,\text{max}}$ is the observed absorbance of the complex of **T2OH1CH=N-NH-Pyri** and the metal ion. The association

constants were determined from the absorbance changes using Eq. 4. The values of $\log K$ for **T2OH1CH=N-NH-Pyri** with the metal ions are reported in Table I.

Table I Association constants (K) of **T2OH1CH=N-NH-Pyri** with metal ions in acetonitrile solutions.

$\log K$										
Mg ²⁺	Fe ²⁺	Fe ³⁺	Co ²⁺	Ni ²⁺	Cu ²⁺	Zn ²⁺	Cd ²⁺	Al ³⁺	Pb ²⁺	
5.8	6.1	6.3	6.5	6.0	6.3	5.7	5.4	4.5	4.8	

Thus, the stability constant of the transition metal ions (Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, and Cu²⁺) are larger than those of the other metal ions (Mg²⁺, Zn²⁺, Cd²⁺, Al³⁺, and Pb²⁺).

4. CONCLUSIONS

We investigated the metal-ion recognition properties of **T2OH1CH=N-NH-Pyri** by adding Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Ag⁺, Zn²⁺, Cd²⁺, Al³⁺, and Pb²⁺ to acetonitrile solutions of the chemosensor. When Li⁺, Na⁺, K⁺, Ca²⁺, Ba²⁺, or Ag⁺ were added to the solution, the shape and intensity of the fluorescence spectra did not change. **T2OH1CH=N-NH-Pyri** forms a 1:1 complex with Mg²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Al³⁺, and Pb²⁺. The fluorescence intensity increases for Mg²⁺, Zn²⁺, Cd²⁺, Al³⁺, and Pb²⁺, and decreases for Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, and Cu²⁺. Furthermore, for Mg²⁺, $\lambda_{f, \max}$ is observed in the NIR region (650–900 nm).

Mg²⁺ is the fourth most abundant metal in our body and plays crucial roles in several physiological functions, such as maintaining membrane integrity, aiding nervous tissue conduction, improving neuromuscular excitability, muscle contraction, increasing hormone secretion, and assisting in intermediary metabolism [12]. Therefore, development of a fluorescent chemosensor for Mg²⁺ is important. We thus propose **T2OH1CH=N-NH-Pyri** as a fluorescent chemosensor for this purpose.

5. ACKNOWLEDGEMENTS

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