Fluorescence Sensing Properties of Thiazolobenzo-crown Ether Incorporating Coumarin[†]

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A new coumarin-thiazolobenzo-crown ether based fluorogenic chemosensor BTC (**1**) was reported. The ion-selective binding properties of **1** with different alkali, alkaline earth metals and transitional metals were investigated in an ethanol-DMSO system. BTC (**1**) showed the highest binding constant toward Hg^{2+} over Ag^+, Pb^{2+} and Cu^{2+}.

Key Words: Thiazolobenzo-crown ether, Coumarin, Fluorescence sensor, Mercury ion

Introduction

A macrocycle that possesses ionophoric ability has been a subject of extensive investigation not only for its synthetic protocol but also for its potential applications since its discovery by Pedersen.[1] There is a growing interest in synthesizing crown ether derivatives with fluorescence properties showing marked changes upon metal complexation, aiming to develop ion-selective and sensitive material.[2] Crown ethers that contain thiazole moieties have been reported to exhibit a large degree of ammonium ion selectivity.[3] Recently thiazolobenzo-crown ether incorporated with a naphthalene moiety has been used for selective detection of Ag^+ over Hg^{2+}, Pb^{2+} and Cu^{2+}.[4]

Coumarins are relatively photo-stable and their excitation and emission maxima, in many cases, are long enough to minimize the so-called "background" fluorescence of cellular components, tissues and biological fluids.[5] Therefore, coumarins are used in the fields of biology, medicine, perfumes, cosmetics and laser dyes.[6] They exhibit Stokes shifts large enough to avoid significant overlap of the excitation and emission spectra and the fluorescence quantum yields allow for ion detection at low indicator concentrations.[7] By the integration of coumarin chromophore in crown ethers, coumarin and its analogues have been extensively used in metal ion detection.[8-10]

Fluorescent sensors for the detection of heavy metals such as mercury, cadmium and lead are of growing interest due to their bioaccumulation, biomagnification and persistence in the environment. An especially active area of current growth is the detection of mercury ions with optical probes.[11] Bacteria-mediated organomercury accumulates in higher organisms and has been implicated as a cause of prenatal brain damage,[12] various cognitive and motion disorders,[13] or Minamata disease.[14] Therefore, it is of considerable importance to sense the presence of lethal mercury ions with a cost-effective, facile and biologically applicable detection method. Some Hg^{2+} sensors were reported that were based on moieties of azo-derivatives,[15,16] N-dansylcarboxamide,[17] thiocoumarin,[18] calixarene,[19] thiocarbamate,[20] rhodamine,[11d,21] anthraquinone,[22] porphyrin[23] and fluorescein.[24] Accordingly, macrocycles bearing suitable light sensitive moi-eties such as coumarin may undergo intermolecular changes at the electronic level upon following the cation dipole interactions of the oxygen donors. Therefore the alterations in the fluorescence spectra of fluorogenic macrocycles in the presence of ions would be a good measure of ion dipole interactions.[25-27]

This paper is the account of the synthesis of a coumarin-thiazolobenzo-crown ether based mercury selective chemosensor that was developed by introducing a coumarin moiety as a potential fluorophore near the binding site of the thiazolobenzo-crown ether moiety. The absorption and emission properties of the sensor were investigated with different cations in ethanol:DMSO (v/v, 9:1). The importance of the close ring structure of coumarin-thiazolobenzo-crown ether in the sensing of Hg^{2+} was also studied.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using Me₄Si as the internal standard. UV-vis absorption spectra were determined on a Shimadzu UV-1650PC spectrophotometer. Fluorescence spectra were measured on a Shimadzu RF-5301 fluorescence spectrometer equipped with a xenon discharge lamp, 1 cm quartz cells. All of the measurements were carried out at 298 K.

Analytical grade absolute ethanol and DMSO were purchased from Merck. All other materials used for the synthesis were purchased from Aldrich Chemical Co. and were used without further purification. 6,7-Dimethoxycoumarin[28] **3** and 2,3-bis [2(4-chloromethyl)thiazolyl]-methyloxybenzene **4** and [2(4-chloromethyl)thiazolyl)]methyloxybenzene **5** were prepared according to the cited procedure.[3] The solutions of metal ions were prepared from their perchlorate salts of analytical grade and then subsequently diluted to prepare working solutions.

Synthesis of BTC (1). A mixture of esculetin (163 mg, 0.914 mmol) and K_2CO_3 (283 mg, 2.04 mmol) in 2-butanone (50 mL) was heated at 60 °C for 30 min. A solution of **4** (300 mg, 0.747 mmol) and NaI (246 mg, 1.60 mmol) in 2-butanone (5 mL) was added to the resulting mixture and refluxed for 12 h. The solvent was removed under reduced pressure and the residue was ex-

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tracted with EtOAc. The organic layer was washed, dried over anhydrous Na$_2$SO$_4$ and concentrated. The residue was purified by SiO$_2$ column chromatography (EtOAc:hexane = 2:1) to give **1** in a 70% yield. mp 249 - 250 ^{o}C (CH$_2$Cl$_2$ - hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.07 (s, 2H), 5.15 (s, 2H), 5.21 (s, 2H), 5.23 (s, 2H), 6.31 (d, *J* = 9.4 Hz, 1H), 6.97-6.99 (m, 2H), 7.14-7.24 (m, 2H), 7.24 (s, 1H), 7.41 (s, 1H), 7.82 (s, 1H), 7.83 (s, 1H), 7.93 (d, *J* = 9.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 66.1, 66.2, 66.4, 66.6, 101.7, 110.7, 112.2, 113.7, 114.2, 114.3, 122.2, 122.3, 122.4, 122.8, 145.2, 145.8, 148.4, 148.5, 150.4, 152.0, 152.4, 152.5, 161.5, 164.3, 164.5; Anal. Calcd for: C$_{25}$-H$_{18}$N$_2$O$_6$S$_2$: C, 59.28; H, 3.58; N, 5.53; S, 12.66; Found C, 59.22; H, 3.77; N, 5.12; S, 12.83.

Synthesis of OTC (2). This compound was obtained from the reaction of esculetin (178 mg, 0.56 mmol) with **5** (296 mg, 1.23 mmol) and NaI (221 mg, 1.48 mmol) in the presence of K$_2$CO$_3$ (170 mg, 1.25 mmol) in 2-butanone (50 mL). After work up, the crude product was purified by SiO$_2$ column chromatography (EtOAc:hexane = 1:3) to give **2** in a 68% yield. mp 172 ^{o}C (CH$_2$Cl$_2$ -hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.20 (s, 2H), 5.31 (s, 2H), 5.21 (s, 2H), 5.40 (d, *J* = 2.2 Hz, 4H), 6.30 (d, *J* = 9.4 Hz, 1H), 6.95-6.99 (m, 2H), 7.03 (d, *J* = 8.1 Hz, 4H), 7.27-7.30 (m, 5H), 7.44 (s, 1H), 7.77 (s, 1H), 7.84 (s, 1H), 7.91 (d, *J* = 9.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 60.0, 66.5, 66.8, 66.9, 102.1, 112.0, 112.1, 113.4, 115.2, 115.8, 119.7, 120.0, 120.3, 120.5, 121.8, 130.0, 144.5, 145.0, 149.88, 151.14, 151.7, 151.8, 157.9, 160.8, 167.2, 167.4; Anal. Calcd for: C$_{31}$-H$_{24}$N$_2$O$_6$S$_2$: C, 63.68; H, 4.14; N, 4.79; S, 10.97; Found C, 63.65; H, 4.46; N, 4.49; S, 10.67.

Results and Discussion

The chemosensors BTC (**1**) and OTC (**2**) were prepared in good yields by reacting **4** and **5** respectively with esculetin in 2-butanone and **3** was prepared by simple methylation of esculetin.[29] The structures of **1**, **2** and **3** were confirmed by ^{1}H NMR, ^{13}C NMR and the elemental analyses data.

The cation-binding properties of BTC (**1**) were investigated by observing the changes in its absorption and emission spectrum, in a mixed ethanol and DMSO (v/v, 9:1) system. An initial UV-vis study of **1** showed that it produced a peak at 340 nm with a high molar absorption co-efficient (ε >10^4 cm^{-1}M^{-1}) indicating that the absorption band was due to π-π* transitions, on the slow addition of Hg^{2+} there was a blue shift of 9 nm (Figure 2).

This blue shift was only noticed in Hg^{2+} and a little in Ag$^+$ (6 nm), none of the other ions including Cd^{2+} and Pb^{2+} revealed such a blue shift (Figure 3).
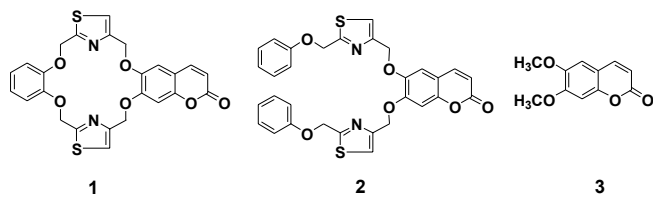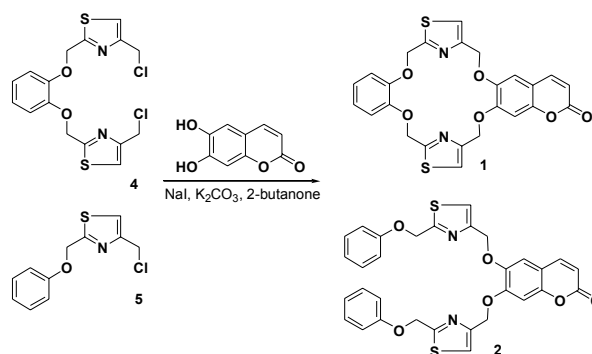

Figure 1. Structure of BTC (**1**), OTC (**2**), and dimethoxycoumarin (**3**).

The sensor **1** produced an emission spectrum at 428 nm on excitation at 329 nm due to the intramolecular charge transfer (ICT) from the oxygen donor of the thiazolobenzo-crown ether to the carbonyl group of the coumarin moiety (Table1). The binding of Hg^{2+} with the oxygen of the crown ether reduces the

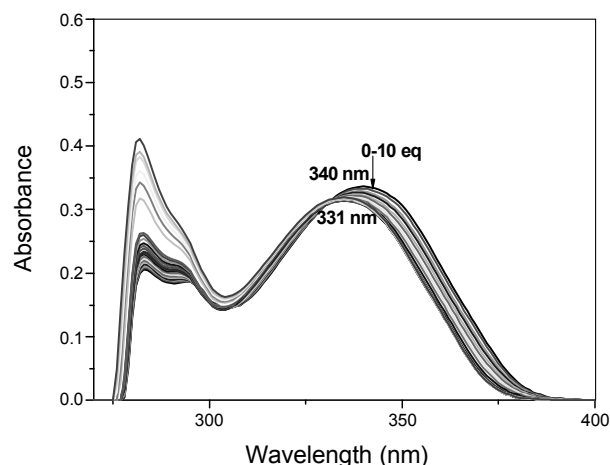**Scheme 1.** Synthesis of BTC (**1**) and OTC (**2**).

Figure 2. Changes in UV-vis spectra of **1** (30 μM) upon the addition of Hg(ClO$_4$)$_2$ in EtOH:DMSO (v/v, 9:1).
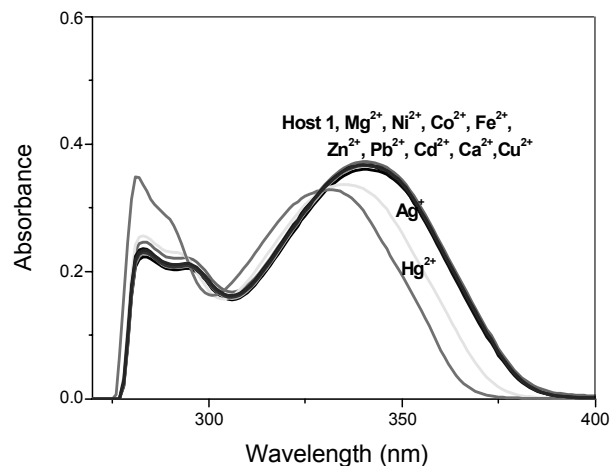

Figure 3. Changes in UV-vis spectra of **1** (30 μM) upon the addition of 10 equiv of different ions in EtOH:DMSO (v/v, 9:1).
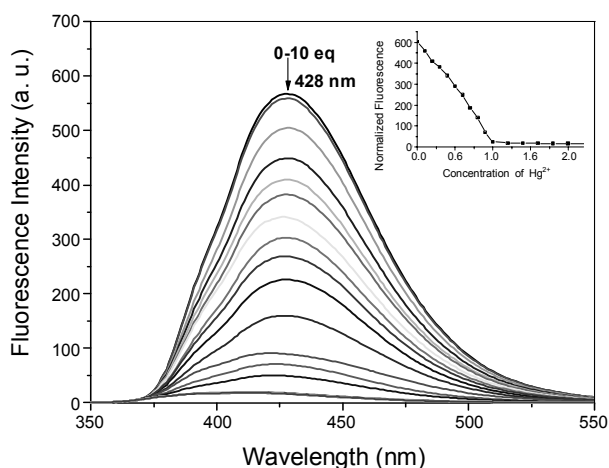
Figure 4. Changes in fluorescence spectra of **1** (30 μM) upon the addition of Hg(ClO₄)₂ in EtOH:DMSO (v/v, 9:1). λ_{ex} = 329 nm. Inset: mol ratio plot of emission band at 428 nm.
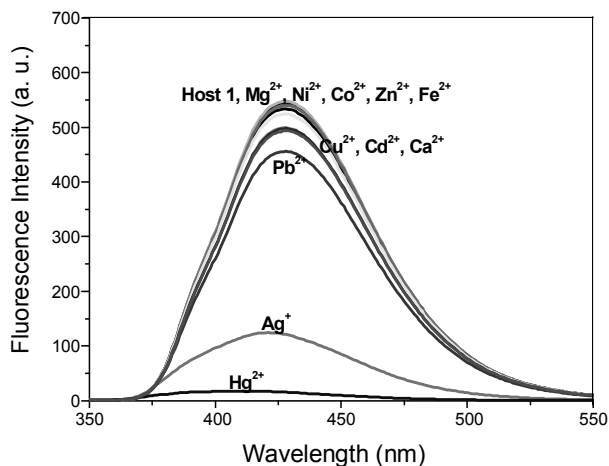

Figure 5. Fluorescence spectra of **1** (30 μM) in EtOH:H₂O (v/v, 9:1) upon addition of various metal ions (each concentration was 300 μM) with an excitation wavelength of 329 nm.
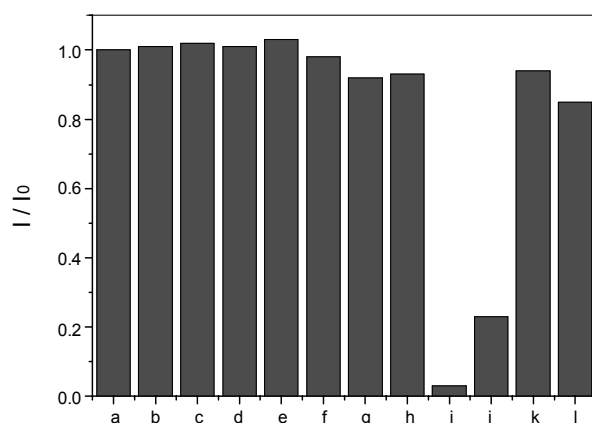

Figure 6. Quenching of fluorescence intensity of **1** (30 μM) in EtOH: DMSO (v/v, 9:1) upon the addition of 10 equiv of respective metal ions. (a) **1** only, (b) **1** + Mg²⁺, (c) **1** + Ni²⁺, (d) **1**+ Zn²⁺, (e) **1** + Co²⁺, (f) **1** + Fe²⁺, (g) **1** + Ca²⁺, (h) **1** + Cd²⁺, (i) **1**+Hg²⁺, (j) **1** + Ag²⁺, (k) **1** + Cu²⁺ and (l) **1**+ Pb²⁺.

Table 1. Photophysical properties of compounds **1**, **2** and **3** in EtOH: DMSO (v/v, 9:1).

Sensors	Absorption Max. nm (log ε)		Δλ (nm)	Emission Max. nm, (I/I_0)	
	Free ligand	Complex with Hg²⁺		Free ligand	Complex with Hg²⁺
1	340 (4.09)	331 (4.04)	9	428, (1.0)	413, (0.02)
2	339 (4.10)	337 (4.10)	2	428, (1.0)	429, (0.59)
3	345 (4.11)	345 (4.11)	0	432, (1.0)	432, (0.97)

Table 2. Fluorescence-based binding constants of sensors **1** and **2**.

Sensors	Hg²⁺	Ag⁺	Pb²⁺	Cu²⁺
1	3.71×10^4	1.52×10^4	2.07×10^3	8.12×10
2	3.73×10^3	4.57×10^3	-	-

electron donating character of this group disrupting the ICT from the oxygen to the coumarin fluorophore. This results in a blue-shift in absorption and a very fast and efficient nonradiative decay of the excited state that leads to fluorescence quenching.

In terms of electronic interaction, the cationic ion-dipole interactions of coumarin-thiazolobenzo-crown ether caused polarizations at the electronic level, which altered the fluorescence quantum yields significantly upon complex formation. Complexing of such compounds, therefore, showed remarkable changes in the emission intensity and excitation maxima, which were dependent on the cationic radii and the size of the macrocycle.[30,2c] The binding mode of BTC (**1**) with Hg²⁺ from the results of fluorescence titration spectra (Figure 4 inset) are shown to be 1:1 with a binding constant of $3.7 \times 10^4 \text{M}^{-1}$ (Error estimated to be ≤10%).[31]

The selectivity and tolerance of **1** for Hg²⁺ over other metal ions was investigated by adding 10 equiv of metal ions to a 30 μM solution of **1**. In the case of Hg²⁺ the molecular fluorescence

was quenched to the maximum level followed by Ag⁺ which was only partially quenched. Cu²⁺, Cd²⁺, Ca²⁺ and Pb²⁺ show little quenching but there was no quenching in any other metal ions as shown in Figure 5.

The plot of fluorescence intensity ratio shows that maximum quenching took place in the case of Hg²⁺, followed by Ag⁺ (Figure 6), there was little decrease in fluorescence intensity in the case of Cu²⁺, Cd²⁺, Ca²⁺ and Pb²⁺ and they were negligible in comparison to Hg²⁺. Table 2 shows that the binding was maximum with Hg²⁺ followed by Ag⁺. Also, when irradiated with a UV lamp at 365 nm, Hg²⁺ produced full quenching while in the case of other cations fluorescence was not quenched as show in Figure 9.

To gain more insight into the chemo-sensing properties and mechanism of BTC (**1**) toward Hg²⁺ ions, UV-vis absorption and fluorescence emission studies of OTC (**2**) with Hg(ClO₄)₂ and AgClO₄ in EtOH:DMSO (v/v, 9:1) were carried out. The UV-vis data in Figure 7a and 7b shows that upon the addition of 10
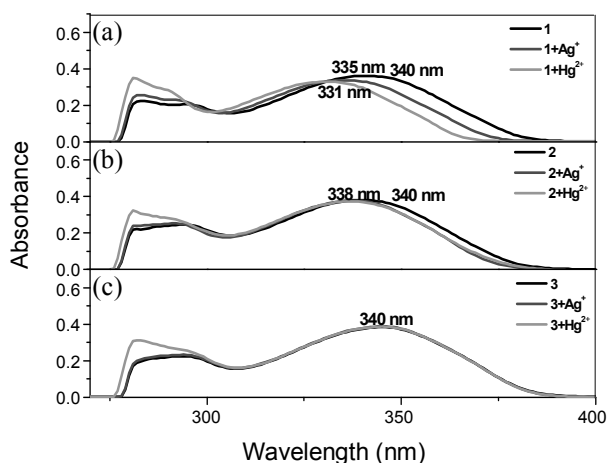
Figure 7. UV-vis spectra of (a) **1**, (b) **2** and (c) **3** (30 μM) in EtOH: DMSO (v/v, 9:1) in the presence of 10 equiv of Hg²⁺ and Ag⁺.
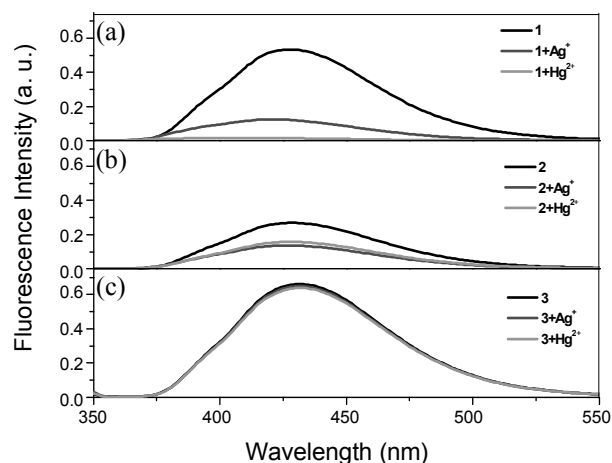

Figure 8. Fluorescence spectra of (a) **1**, (b) **2** and (c) **3** (30 μM) in EtOH:DMSO (v/v, 9:1) upon the addition of 10 equiv of Hg²⁺ and Ag⁺. λ_{ex} = 329, 337, and 345 nm, respectively.

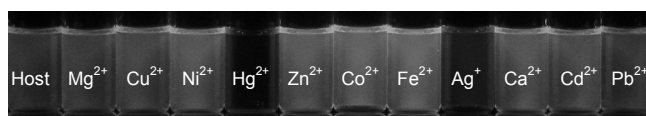**Figure 9.** Fluorescence changes of **1** (30 μM) upon addition of **1** only, **1** + Mg²⁺, **1** + Cu²⁺, **1** + Ni²⁺, **1** + Hg²⁺, **1** + Zn²⁺, **1** + Co²⁺, **1** + Fe²⁺, **1** + Ag²⁺, **1** + Ca²⁺, **1** + Cd²⁺ and **1** + Pb²⁺. Addition of 10 equiv of ions in EtOH:DMSO (v/v, 9:1).

equiv of Hg²⁺ to both BTC (**1**) and OTC (**2**), only BTC (**1**) showed 9 nm-blue shifts due to binding, while with OTC (**2**) the shift was negligible (Table 1).

   In the fluorescence spectrum (Figure 8a and 8b) the sensor BTC (**1**) also underwent full quenching with Hg²⁺ while with OTC (**2**) the quenching was partial. These results indicated that the presence of the crown ether ring in BTC (**1**) produced stronger binding (Table 1).

   It is known that coumarin itself can bind to a cation.[32] In order to investigate the significance of the thiazolo-benzocrown ether, 5,6-dimethoxycoumarin **3** was prepared. The UV-vis spectrum of **3** showed no shift upon the addition of 10 equiv of Hg²⁺ (Figure 7c). The emission spectrum of **3** also showed no change on addition of Hg²⁺ (Figure 8c), which established the fact that the presence of the thiazolobenzo-crown ether acted as the main binding site with coumarin as the fluorophore.

   To gain further insight into the nature of the Hg²⁺ complexation, cation recognition was evaluated using ¹H NMR in DMSO-d_6. The partial ¹H NMR spectra of **1**, upon the addition of Hg²⁺ ions, is shown in Figure 10. Notably, when 1 equiv of Hg²⁺ was added, the signals of H_c (Δδ, 0.19) and H_d (Δδ, 0.29) of the coumarin ring, H_b (Δδ, 0.45) of the thiazole ring, the oxo-methylene protons of the crown ether H_h (Δδ, 0.63), H_i (Δδ, 0.45), H_j (Δδ,

0.38), and H_e (Δδ, 0.38), H_f (Δδ, 0.63) of the benzene ring were shifted downfield due to the deshielding effect created by binding with the metal ion.

Conclusion

   In summary a new coumarin-thiazolobenzo-crown ether based chemosensor BTC (**1**) was developed for mercury ions that utilized the strong coordination of mercury ions on the crown oxygen and thiazole nitrogen. The complexation of Hg²⁺ disrupted the ICT from the oxygen donor to the coumarin fluorophore resulting in blue-shift in absorption and quenching of the fluorescence.
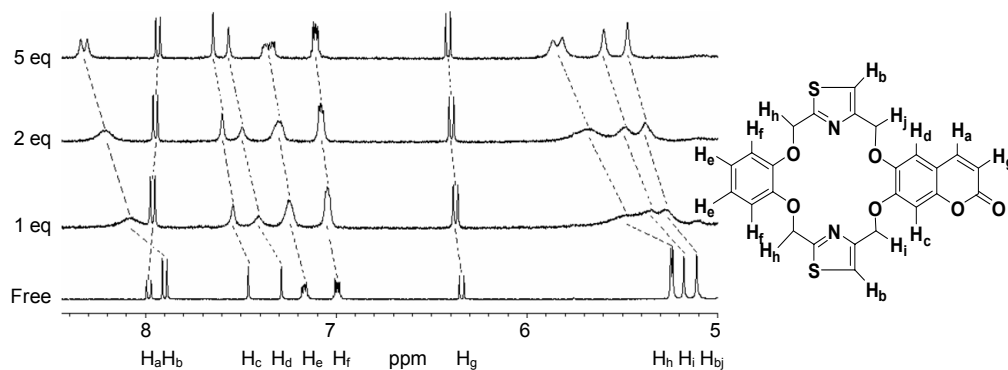

Figure 10. Partial ¹H NMR spectra of **1**, with increasing the Hg(ClO₄)₂ concentration in DMSO-d_6.

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