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Full Paper

Bis-indolylmethane Derivatives as A Potential Colorimetric Sensor for Chromium Ion Detection in Water

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Abstract

Contamination of water with chromium ions is a significant concern due to its potential adverse effects on human health and the environment. In addition, it is also hazardous to biological systems and can cause a variety of human diseases. Hence, an efficient and reliable detection method is needed to overcome this issue. In this study, a colorimetric sensor method based on bis-indolylmethane (BIM) was developed to evaluate its ability as a heavy metal ionophore with the existence of H-bonding interactions between the indolic N–H groups and the chromium ion. In the study, two bis-indolylmethane derivatives compounds were synthesised using acetic acid as the catalyst via the condensation reaction between 5-bromoindole and benzaldehyde derivatives. The synthesised compounds were then characterised using three types of spectroscopic analysis, which are 1H and 13C NMR, FT-IR and UV-Vis to determine the structural information and to confirm the formation of the bis-indolylmethane derivatives produced. Finally, colourimetric sensor development was conducted involving metal screening with Cr3+ ion by colourimetric sensing analysis, and compound 1 was shown to have the most intense colour change, hence having the highest possibility of interaction or bonding with the Cr3+ ion. A further binding interaction study was conducted using UV-Vis titration analysis between compound 1 and Cr3+, proving the binding ability and capability of the synthesised BIMs as a heavy metal ion sensor. The successful development of this sensor will have wide-ranging applications in environmental monitoring, water treatment, and public health. Additionally, it can be employed by regulatory agencies, industries, and researchers, especially to ensure the safety and quality of water resources. By enabling quick detection of chromium ions, this sensor will contribute to the preservation of human health and the environment.

Keywords: - Bis-indolylmethane, colourimetric sensor, chromium

1. Introduction

Heavy metals are known to be essential for the continual functioning of many biochemical and physiological processes in living things. They become major environmental pollutants when their concentration exceeds the legal limit, and their toxic effect on the environment and human health (Dahiya, 2022). One of the common heavy metals, chromium, is employed widely in the steel, pigment, alloy, leather tanning, and electroplating industries. However, excessive chromium consumption has caused water pollution issues (Sahin et al., 2020).

Industrial discharges contaminate water with Cr(III), which is also eventually genotoxic to living things. Additionally, it is also carcinogenic and harmful to humans and aquatic life when it is present in water bodies. Anorexia, brain damage, and other mental illnesses can result from consuming water polluted with chromium (Sahu et al., 2022). The International Agency for Research on Cancer and the United States Environment Protection Agency have both classified it as a group III carcinogen (Shahim et al., 2019).

Indole and its derivatives are the most important and commonly utilised heterocyclic molecules in chemical and pharmaceutical chemistry due to their unique structures and broad variety of biological and pharmacological effects (Chen et al., 2022). They are widely used as anti-inflammatory, antibacterial, antiviral, anti-cancer, anti-rheumatoidal, anti-HIV, and anti-tumor medications (Sarkar et al., 2022). Besides, due to its structural characteristics and biological significance, indole derivatives have recently received significant attention and are widely utilised in biosensing and sensing applications (Ozcan et al., 2019).

Bis-indolylmethane (BIM) is an important indole derivative consisting of two units of indole linked by a single methylene bridge (Santos et al., 2024) as shown in Fig. 1. BIM has demonstrated numerous interesting biological functions, including anti-inflammatory, anticonvulsant, antipyretic, and anti-fungal properties. It is also important in numerous natural products, agrochemicals, fine chemicals, and functional materials (Jat et al., 2022).

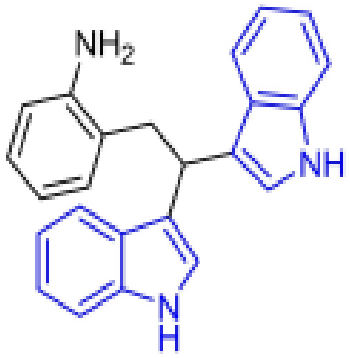


Fig. 1. Example of bis-indolylmethane derivative (Santos et al., 2024)

Besides, BIM is one of the indole derivatives that have been reported in Cu2+ colorimetric sensing applications (Kandasamy et al., 2015). BIM derivatives are commonly used as colorimetric sensor due to their structure that can function as a binding affinity control group with an acidic H-bond donor moiety and a basic H-bond acceptor moiety (He et al., 2006). These functions improve their sensitivity and efficiency in sensing applications.

In this study, two different BIM derivatives have been synthesised and characterised. Their potential as colorimetric sensors for detection of chromium ions have also been evaluated using two primary analytical methods via naked eye study (Yang & Ge, 2023) and UV-Vis titration analysis in colorimetric sensing analysis and binding study respectively.

2. Materials and Methods

2.1 Chemicals

Acetic acid and acetonitrile were purchased from Fisher Scientific, Massachusetts, USA. 5-bromoindole, 4-hydroxybenzaldehyde, 4-nitrobenzaldeyde, dimethyl sulfoxide-d6 (DMSO-d6), diethyl ether and chromium (III) nitrate nonahydrate were purchased from Sigma-Aldrich, Darmstadt, Germany. Ethyl acetate was purchased from Merck KGaA, Darmstadt, Germany. Petroleum ether was purchased from R&M Chemicals, Semenyih, Selangor. All chemicals were used without further purification

2.2 Synthesis of Bis-indolylmethane (BIM) Derivatives

5-bromoindole (2 mmol) and substituted benzaldehydes (1 mmol) were added into a round bottom flask containing 5 mL of acetic acid and was stirred vigorously using a stirring heating mantle at room temperature for 7 hours. The reaction mixture was then transferred into a beaker containing crushed ice to allow precipitation. The precipitate formed was filtered and washed with diethyl ether to afford a pure product. The pure product was allowed to dry at room temperature and characterised using 1H and 13C NMR, FT-IR, and UV-V spectroscopy.

2.3 Colorimetric Sensing Analysis

Stock solutions with concentration of 1×10−3 M for ligands bis-indolylmethane (BIM) derivatives and Cr3+ were prepared in acetonitrile and deionised water respectively. The colour changes for 1 mL of the ligands (1×10−3 M) were observed instantaneously after mixing with 1 mL of Cr3+ stock solution in a small vial at room temperature and were recorded using a smartphone camera (Li et al., 2019).

2.4 UV-Vis Titration Analysis

Solutions with a concentration of 3×10^{-4} M for the analysed compound (BIM) and 3×10^{-5} M for $Cr(NO_3)_3$ were prepared, respectively. Next, 3 mL of the analysed compound solution was transferred directly into a quartz cuvette and the spectrum of the solution was recorded using UV-V is method after each drop of the metal cation solution was added and uniformly mixed using a micropipette. All experiments were conducted at room temperature.

The Benesi-Hildebrand equation (1) was used to obtain the association constant (K_a). The following equation was used to calculate K_a:

$$\frac{1}{A-A_o}=\frac{1}{\{K(A_{max}-A_o)[M]\}}+\frac{1}{A_{max}-A_o} \quad (1)$$

A_o is the receptor's absorbance in the absence of a guest, A is the absorbance measured in the presence of an additional guest, A_{max} is the absorbance measured in the presence of an additional $[M]_{max}$, and K_a is the association constant, where [M] is $[Cr3^+]$.

3. Result and Discussion

3.1 Synthesis of Bis-indolylmethane (BIM) Derivatives

All the bis-indolylmethane (BIM) derivatives synthesis processes were conducted in one pot reaction involving reaction between 5-bromoindole and three substituted benzaldehydes to produce two BIM derivatives (1 & 2) as shown in Fig. 2. Two different methods were applied in the process: non-reflux method (stirring at room temperature) and reflux method that required stirring at higher temperature respectively to compare the product yield for each method. Acetic acid was used as the catalyst during the synthesis process, and it also helped to accelerate the electrophilic substitution reaction between indole and aromatic aldehyde (El-Sayed et al., 2014).

Based on the result, it is shown that the non-reflux method was able to produce a higher yield of product range between 45%-78% (Table 1) in a shorter time compared to the reflux method that produced only 32% yield in a longer reaction time. Since a significant percentage of products were produced at lower temperatures, it is proposed that this synthesis reaction might be carried out using an exothermic process (Lu et al., 2006). However, the result of the reflux method will not be included in this study as the product yield is too minimal to proceed with the characterisation and preliminary study on sensor development.

In the characterisation study of the synthesised products, a singlet peak displayed in 1H NMR at around 7 ppm representing the methine group (C-H) and the disappearance of C=O peak in 13C NMR and FT-IR spectra respectively confirmed the formation of the final BIM compound.

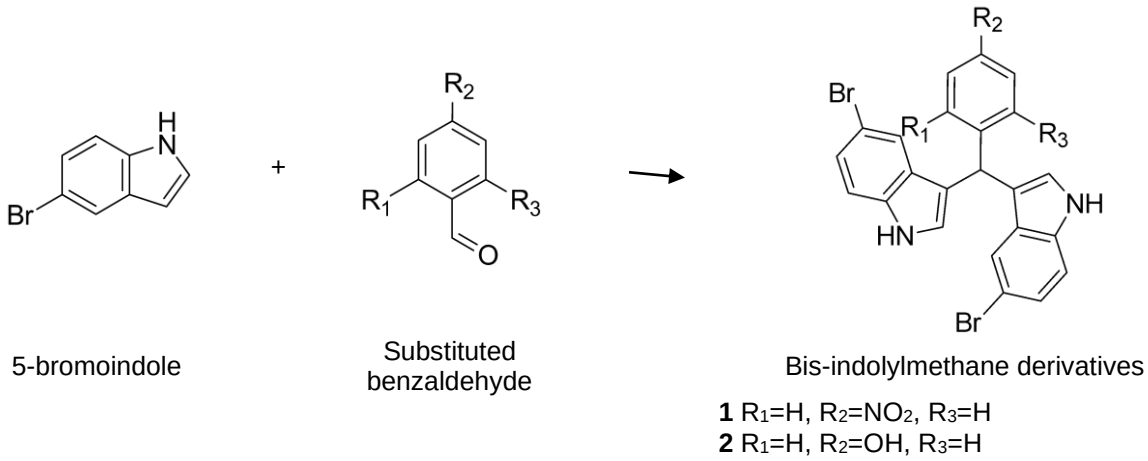


Fig. 2. General reaction of 5-bromoindole with substituted benzaldehyde to produce bis-indolylmethane derivatives 1 & 2

3.2. Colorimetric Sensing Analysis

Using naked eye study, the colorimetric sensitivity of bis-indolylmethane (BIM) compounds 1 and 2 to Cr3+ was evaluated in this study (Fig. 3). The colour changes showed the highest ability of BIM derivatives to interact or bind with the chromium ion (Tarasi et al., 2021). Based on the result obtained, ligand 1 was reported to display the most intense colour change when its grey colour solution was turned into light purple after adding grey colour of Cr3+ solution compared to ligand 2, which display no colour change. Hence, ligand 1 is shown to be more selective and have the highest potential to interact with Cr3+ (Choi et al., 2015).

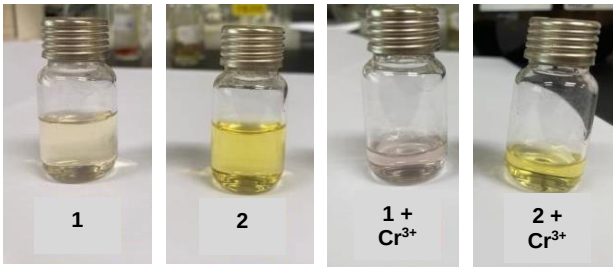


Fig. 3. Colour changes of ligand 1 and 2 with Cr^{3+} tested at a concentration of 1×10^{-3} M

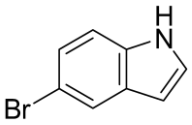
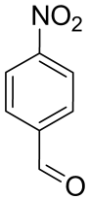
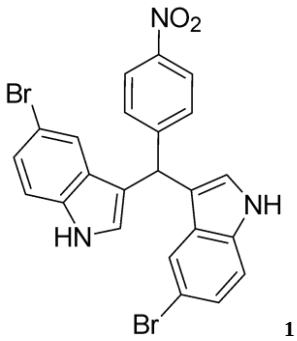
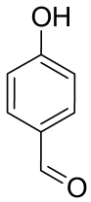
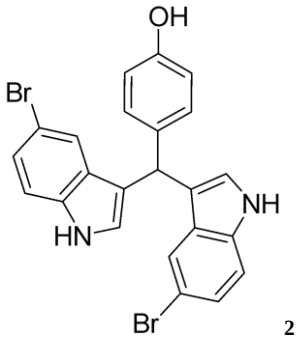
3.3. UV-Vis Titration Analysis

A further UV-Vis titration analysis was conducted on ligand 1 due to its properties that showed the ability to make interaction with Cr3+. The UV-Vis spectra of the free ligand for compound 1 (Fig. 4) exhibits two bands at 235 nm and 285 nm respectively. The strong absorption band at 235 nm is due to the conjugated aromatic system π→π^* electron transitions while the medium band at 285 nm is likely due to the result of n→π^* electron transitions involving lone pairs of the nitrogen atoms near the edge of the ligand (Zhang et al., 2017).

The titration study between ligand 1 and Cr^{3+} has displayed a new absorbance peak formation, indicating an interaction between them (Fig. 5). Different shifts were observed in the d-d band of the chromium ion and the formation of a new peak at around 510 nm was known as d-d orbital transition of Cr^{3+} (Zhang et al., 2022). Following 40 times of titration, the interaction between compound 1 and $Cr3^{+}$ was shown to have reached a plateau in the rise of the UV-Vis titration. The plateau indicated a formation of a complex between the compound and the chromium ion (Fig. 6).

The analysis was continued with the determination of stoichiometric binding between the ligand 1 and Cr^{3+} ion based on the association constant (K_a) calculated using the Benesi-Hildebrand equation. In the UV-Vis titration plot of the data of $1/(A-A_o)$ against $1/[Cr^{3+}]$, the ligand's stoichiometry for the ratio of Cr^{3+} ion is 1:1, as demonstrated by the linear relationship with R_2 value equal to 0.9338. Based on the *y*-intercept value, the value of K_a for ligand **1** interacting with Cr^{3+} ion has been calculated to be 5.7×10^3 M^{-1}. This value is considered a good value as it is comparable with the previous study that reported K_a values within the range $10^3 – 10^5$ M^{-1} (Alraqa et al., 2021; Iqbal et al., 2020).

Table 1. List of bis-indolylmethane derivatives with their percentage yield

Indole	Benzaldehyde	Compound	Percentage of yield
		 **1**	78% (Ke et al., 2005)
		 **2**	45% (Sharma et al., 2014)

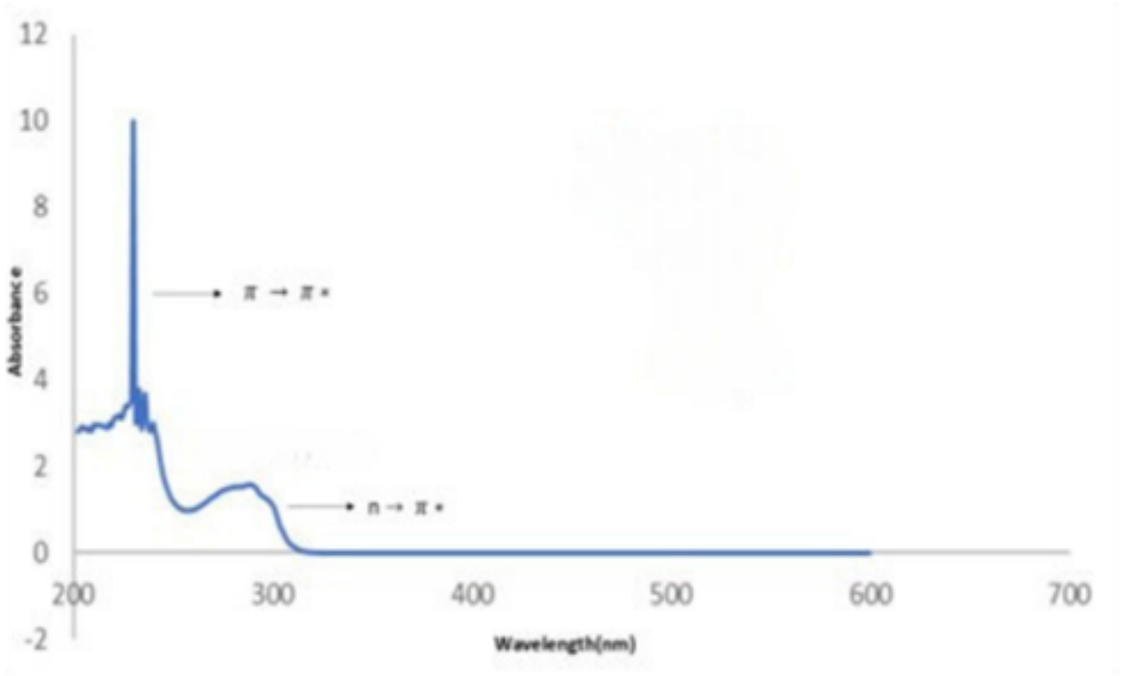


Fig. 4. UV-Vis spectra of free ligand 1

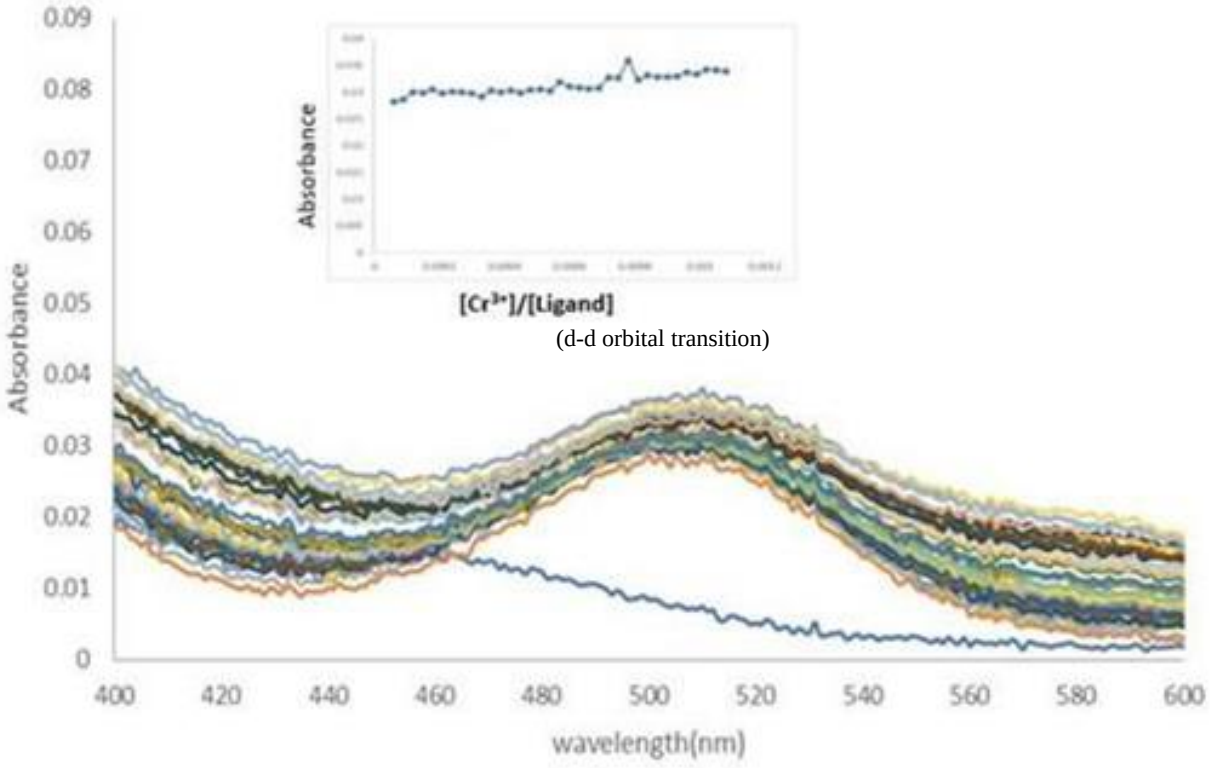


Fig. 5. UV-Vis spectra generated from the titration of ligand 1 with Cr^{3+}

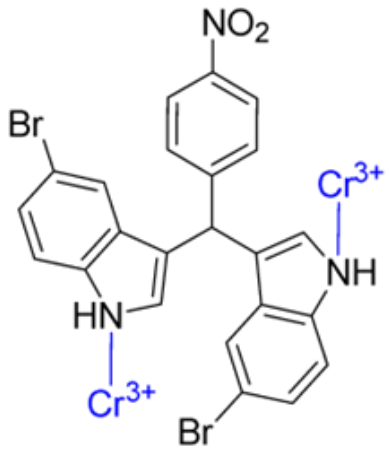


Fig. 6. Formation of complex between BIM derivative and Cr^{3+}

The analysis was continued with the determination of stoichiometric binding between the ligand 1 and Cr3+ ion based on the association constant (Ka) calculated using the Benesi-Hildebrand equation. In the UV-Vis titration plot of the data of 1/(A-Ao) against 1/[Cr3+], the ligand's stoichiometry for the ratio of Cr3+ ion is 1:1, as demonstrated by the linear relationship with R2 value equal to 0.9338. Based on the y-intercept value, the value of Ka for ligand 1 interacting with Cr3+ ion has been calculated to be 5.7 ×103 M−1. This value is considered as a good value as it is comparable with the previous study that reported Ka values within the range 103 – 105 M−1 (Alraqa et al., 2021; Iqbal et al., 2020).

4. Conclusion

From the study, it can be concluded that all the bis-indolylmethane (BIM) compounds have been successfully synthesised using a convenient room temperature stir method compared to the conventional reflux method. This easy and straightforward method is environmentally friendly and can produce a higher yield of products in a shorter time. The colour change observed from the colourimetric sensing analysis also shows that the ligand can interact with Cr3+. In the UV-Vis titration analysis, the formation of a new absorbance peak known as the d-d peak and the calculated association constant (Ka) value also have proved the ability of the ligand to interact with the Cr3+ and act as a potential heavy metal ion sensor. Hence, the potential of BIM derivatives can be explored further for the future development of heavy metal ion sensors.

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