

# A Novel Sensitive Fluorescent Coumarin-Based Chemosensor for Detection of Copper Ion

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## Abstract

A sensitive fluorescent chemosensor was developed for detecting Cu<sup>II</sup> through microwave irradiation by using diethylamino-3-carboxamide coumarin. This receptor was employed as fluorescent probe for Cu<sup>II</sup> with selectivity over other cations in aqueous solution. The fluorescence intensity quenched when 7-diethylamino-N-[2-(dimethylamino)ethyl]-2-oxo-2H-chromene-3-carboxamide (3) used as a receptor in the presence of very low concentration of Cu<sup>II</sup> with an excitation at 360 nm.

**Keywords:** Fluorescent chemosensor; Cation detection; Coumarin-based fluorescent probe

## Introduction

Coumarins (benzopyran-2-ones) as oxygenated heterocycles with natural base possess several biological activity consist of anticancer, antioxidant, anti-inflammatory, MAO inhibitory, antiviral, antimicrobial and antihyperlipidemic activity [1]. Several methods for synthesis of coumarins have been developed in recent years. Some of the most common synthetic reactions for oxygenated heterocycles are Pechmann and Duisberg [2], Perkin and Henry [3], Brafolo et al. [4], Claisen et al. [5], Shringer [6], Heck, and 1,3-dipolar cycloaddition reactions [7].

Coumarin scaffold is a privileged structure as fluorescent chemosensor [8]. In particular, for a selective measurement of heavy metal ions such as mercury, lead and copper in trace amounts [9,10].

The copper plays an important role in various biological processes [10]. However, copper is released into the bloodstream and is deposited in the kidneys, cornea, and particularly, the brain [11,12]. Therefore, exposure to higher levels of copper can cause rheumatoid arthritis, gastrointestinal disturbances, and Wilson's disease.

Copper ion has an essential role in various enzymes involve cytochrome oxidase, superoxide dismutase, dopamine-hydroxylase and catalysis [13]. Moreover, anticancer activity and DNA-binding [14] of the Cu<sup>II</sup> has been investigated by coumarin derivatives [15].

Despite these functional roles, over load of this metal have toxic challenges in human health which have been visible in Huntington's disease [16], Alzheimer's disease (AD) [17] and Parkinson's disease (PD) [18].

The average diet provides substantial amounts of copper, and the recommended intake is 0.9 mg/day. Also, the average concentration of blood copper in the normal people is 100-150 µg/dL. Thus, detection and elimination of this ion is important. In this regard, many analytical techniques are available for the determination of species. Among them, substantial attention has been paid to fluorometry technique because of its high sensitivity [19-22].

Therefore, we designed a novel derived coumarin fluorescent sensor for selective detection of Cu<sup>II</sup> through solvent-free condition.

## Experimental Section

<sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded using a Bruker (400 MHz) Avance (III) spectrometer. Chemical shifts (δ) were reported in ppm downfield from the internal standard tetramethylsilane (TMS). The fluorescence emission spectra were obtained using a Jasco FP-200 spectrofluorometer. The Perkin-Elmer lambda-EZ 201 was used to record UV-Vis spectra.

The FT-IR Perkin-Elmer spectrometer was employed to record Infrared (IR) spectra in cm<sup>-1</sup>. Microwave irradiation reactions were carried out on a Milestone Micro-SYNTH apparatus. Internal temperatures were measured with fiber-optic sensor in conjunction with Milestone immersion well. Electrothermal-9200 melting point apparatus was used to measure melting point in open capillary tubes.

1-(7-diethyl)-N-(2-(dimethylamino)ethyl)-2-oxo-2H-chromene-3-carboxamide Coumarin ester derivative was prepared through the condensation of 4-(Diethylamino)salicylaldehyde and malonic ester under microwave irradiation. The prepared ester was hydrolyzed to produce the related carboxylic acid. Consequently, 3-carboxamide coumarin, as a receptor, was easily made through reaction with N,N'-dimethylethylenediamine. Fluorescence spectra was investigated in the presence of low concentration of cations, including Na<sup>I</sup>, K<sup>I</sup>, Cu<sup>II</sup>, Pb<sup>II</sup>, Hg<sup>II</sup>, Co<sup>II</sup>, Zn<sup>II</sup>, Fe<sup>II</sup>, Cd<sup>II</sup>, Al<sup>III</sup> and Cr<sup>III</sup>. Compound 1 was formed by the reaction of 4-(Diethylamino)salicylaldehyde and diethyl malonate in the presence of piperidine and glacial acetic acid under solvent-free conditions (Table 1, Scheme 1). The related carboxylic acid 2 was produced by hydrolysis of 1 in sodium hydroxide solution.

In order to produce the desired carboxamide 3, dicyclohexylcarbodiimide (DCC), N,N'-dimethylethylenediamine, and 4-(dimethylamino) pyridine (DMAP) as a catalyst were added

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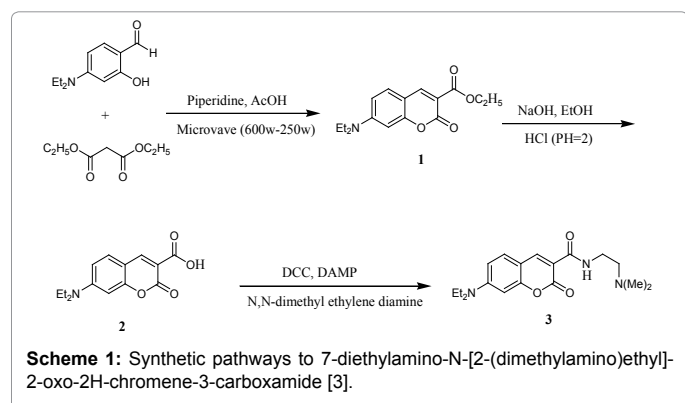
Received January 21, 2016; Accepted February 02, 2016; Published February 05, 2016

Citation: Bekhradnia A, Ghanbarimasir Z (2016) A Novel Sensitive Fluorescent Coumarin-Based Chemosensor for Detection of Copper Ion. Pharm Anal Acta 7: 461. doi:10.4172/2153-2435.1000461

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Step	Time	Temperature (T <sub>1</sub> )	Temperature (T <sub>2</sub> )	Max Power
1	15 min	Ramp to 110°C	85	800 W
2	17 min	110°C	85	700 W
3	7 min	Ramp to 135°C	105	700 W
4	6 min	135°C	105	700 W

Table 1: Microwave settings for compound [1].



to a mixture of 2 dissolved in chloroform. After filtration of N,N'-dicyclohexylurea (DCU) precipitate, the carboxamide 3 was obtained after purification in mixture of ethanol and water. Subsequently, spectrofluorometry was used to assessment of the cations chelating on receptor 3 in (HEPES:DMSO) 9:1, v/v.

The selected physical and spectral data for synthetic compounds are as follows: (1): mp: 195–199°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.1(Me, t, 6H, J=7.2), 1.5(Me, t, 3H, J=7.0), 3.2 (CH<sub>2</sub>, 4H, q, J=7.2), 4.3 (CH<sub>2</sub>, q, 2H, J=7.0), 6.0 (Ar, d, 1H), 6.1 (Ar, dd, 1H), 7.1 (Ar, d, 1H), 9.3 (Ar, s, 1H). IR (KBr): 1685 (ester C=O), 1769 (lacton C=O) cm<sup>-1</sup>. (2): m.p. 201–205°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.1(Me, t, 6H, J=7.2), 3.2 (CH<sub>2</sub>, 4H, q, J=7.2), 6.1 (Ar, d, 1H), 6.3(Ar, dd, 1H), 7.3 (Ar, d, 1H), 9.3 (Ar, s, 1H), 11.3 (OH, s, 1H). IR (KBr): 3445 (OH), 1960 (acid C=O), 1786 (lacton C=O) cm<sup>-1</sup>. (3): m.p. 212–215°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.1(Me, t, 6H, J=7.2), 2.4 (N(Me)<sub>2</sub>, s, 6H), 3.2 (CH<sub>2</sub>, 4H, q, J=7.2), 3.8–4.1 (HNCH<sub>2</sub>, m, 2H, J<sub>CH<sub>2</sub>-CH<sub>2</sub></sub>=7.1), 6.1 (Ar, d, 1H), 6.3 (Ar, dd, 1H), 6.8 (NH, 1H), 7.3 (Ar, d, 1H), 9.3 (Ar, s, 1H).

IR (KBr): 3425 (amide NH), 1652 (amide NH), 1610 (amide C=O) cm<sup>-1</sup>. <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ: 12.1, 37.1, 43.2, 45.2, 46.8, 57.2, 115.1, 116.7, 118.2, 121.0, 129.2, 135.2, 140.1, 155.1, 161.2, 163.1.

## Results

UV–Vis spectra show hypochromic effects in absorbance for all the cations studied in this research. Nevertheless, it was difficult to detect specific cations because of the no selectivity related to any cations. Thus, spectrofluorometry was used to investigate of the Cu<sup>II</sup> chelating on fluorophore 3 in the presence of other cations.

The fluorescence spectra for complex 7-diethyl-N-(2-(dimethylamino) ethyl)-2-oxo-2H-chromene-3-carboxamide and cation, 3-M<sup>n+</sup>, was studied and given in Figure 1 in aqueous solution (HEPES:DMSO) 9:1, v/v). The fluorescence spectra 3-M<sup>n+</sup> was recorded at 360 nm with an emission at 470 nm. Also, Figure 2 shows the fluorescence spectra of 7-(N,N-diethyl)-N-(2-(dimethylamino)ethyl)-2-oxo-2H-chromene-3-carboxamide upon the addition of Cu<sup>II</sup> in the presence of various mixture of cations with an excitation at 360 nm.

To investigate of stoichiometry between the sensor and Cu<sup>II</sup>, spectroscopic changes generated upon titration experiments were done using 0.6 μmol of 3 in solution (HEPES:DMSO) 9:1 with changing concentrations of metal salts (0–4.5 μmol) by means of UV-visible spectroscopy (Figure 3).

Furthermore, full geometry optimization were performed by means of Hartree–Fock (HF) and 6-31G\* basis set employing the Gaussian 03 code [23,24]. To assess the performance of this approach, the 3-Cu<sup>II</sup> structure was proposed and computed at abinitio method (Figure 4) [25].

## Discussion

Coumarin-based derivatives are important group of fluorescent heterocycles that can be used as fluorescent probes [26–29]. Recently, we have synthesized nitro-3-carboxamide coumarin derivative, proposed as novel fluorescent chemosensor and has been employed as fluorescent probe for Cu<sup>II</sup> [30]. That receptor played a role for complexation of heavy toxic metals and exhibit enhanced fluorescence in the presence of Cu<sup>II</sup>. In this research, we prepared and studied a new fluorescent chemosensor that Cu<sup>II</sup> over other ions was detected by fluorescence quenching technique. In fact, the fluorescence intensity decreased in the presence of Cu<sup>II</sup> in comparison of other metal ions (Figure 1). Thus, fluorescence quenching was occurred when Cu<sup>II</sup> gradually added (Figure 2).

Figure 3 shows the Uv-Vis titration of complex sensor-Cu<sup>II</sup> using 0.6 μmol of 3 in solution (HEPES:DMSO) 9:1 with changing concentrations of Cu<sup>II</sup> salts (0–4.5 μmol). The mild negative slope of the plotted line during the initial phase indicates a low concentration of 3 in the vessel and forming 3-Cu<sup>II</sup> complex. Increasing of Cu<sup>II</sup> concentration, the slope became linear, representing the highest concentration of 3-Cu<sup>II</sup> complex and the ligand 3 was consumed. It shows 1:1 stoichiometry between the sensor and Cu<sup>II</sup>.

To find reliable results for investigation of binding modes between fluorophore and its Cu<sup>II</sup>, ab initio calculations was done using HF/6-31G\*. The optimized model of 3-Cu<sup>II</sup> complex is revealed that Cu<sup>II</sup> binds with an oxygen atom, two nitrogen atoms of an amide and an amine, as well as two oxygen atoms of an anion (Figure 4).

As a result, the *ab initio* calculation outcomes confirm the relationship between absorbance and a mole ratio of 1:1 stoichiometry.

The association constant (K<sub>a</sub>) of complex sensor-Cu<sup>II</sup> was found using the Benesi–Hildebrand equation [30] as follows:

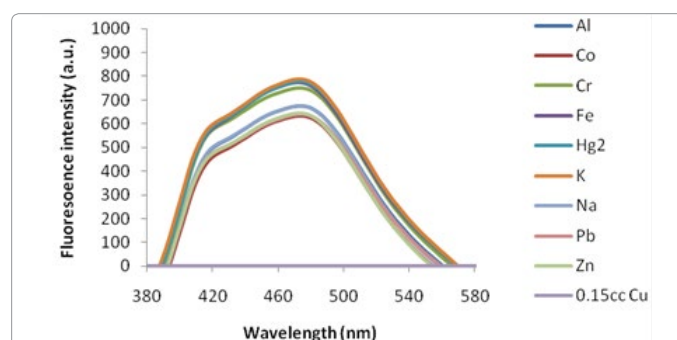
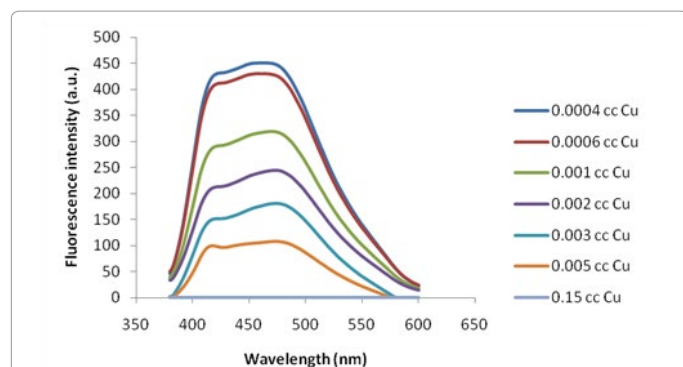
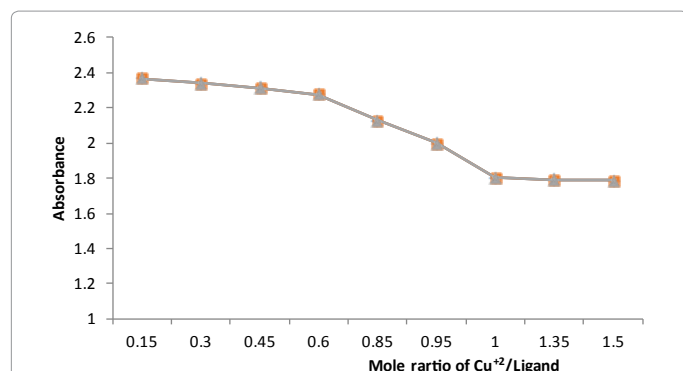


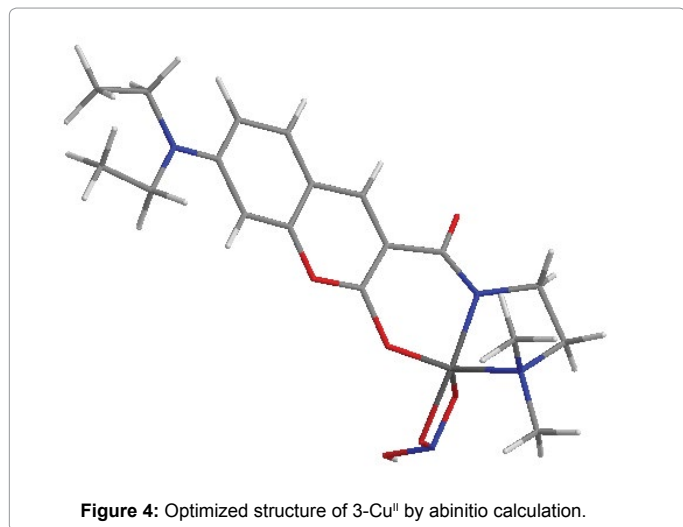
Figure 1: Relationship between the fluorescence intensity and additional concentration of cations (M<sup>n+</sup>). The fluorescence intensity of 7-diethyl-N-(2-(dimethylamino) ethyl)-2-oxo-2H-chromene-3-carboxamide decreased when Cu<sup>II</sup> was added in additional concentration.



**Figure 2:** Fluorescence spectra of 7-diethyl-N-(2-(dimethylamino) ethyl)-2-oxo-2H-chromene-3-carboxamide in the presence of various concentration of Cu<sup>II</sup>.



**Figure 3:** UV-Vis titration of complex sensor-Cu<sup>II</sup> using 0.6 μmol of 3 in solution (HEPES:DMSO) 9:1 with changing concentrations of Cu<sup>II</sup> salts (0-4.5 μmol).



**Figure 4:** Optimized structure of 3-Cu<sup>II</sup> by abinitio calculation.

$$\frac{1}{F - F_{\min}} = \frac{1}{k_a (F_{\max} - F_{\min}) [Cu^{2+}]} + \frac{1}{(F_{\max} - F_{\min})}$$

F and F<sub>min</sub> represent the fluorescent intensity of the ligand 3 at moderate concentration and in the presence of excess amount of Cu<sup>II</sup>, respectively. F<sub>max</sub> is the saturated fluorescent intensity of free ligand 3.

Regarding to Benesi-Hildebrand evaluation, the association constant for complex sensor-Cu<sup>II</sup> was calculated from the spectrofluorometric titration data and was found to be 7.2 × 10<sup>4</sup> M<sup>-1</sup>.

These results led us to the agreement of the Benesi-Hildebrand evaluation method and abinitio study for the determination of association constant with 1:1 stoichiometry between 3 and Cu<sup>II</sup>.

## Conclusion

Here, a new coumarin-based fluorescent chemosensor through microwave irradiation as fluorescent probe for Cu<sup>II</sup> ion was designed and synthesized. Dimethylamino-N-[2-(dimethylamino)ethyl]-2-oxo-2H-chromene-3-carboxamide (3) is a suitable fluorophore because of its large Stokes shift and visible excitation and emission wavelengths. The receptor exhibits quenched fluorescence in the presence of Cu<sup>II</sup> with selectivity over other metal ions in aqueous solution. The lowest fluorescence intensity for diethylamino-3-carboxamide coumarin was observed in the presence of Cu<sup>II</sup> in preference to a variety of other common heavy and toxic metal ions. Therefore, we synthesized a new diethylamino-3-carboxamide coumarin as a receptor, which employed for detection of Cu<sup>II</sup> with excitation at 360 nm.

## Acknowledgement

The authors gratefully acknowledge the financial support for this work received from the Mazandaran University of Medical Sciences "Professor's Projects Funds".

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