SYNTHESIS, CHARACTERIZATION AND PHOTOPHYSICAL STUDIES ON CHALCONE-BASED CHEMOSENSORS FOR THE DETECTION OF ALUMINIUM AND FLUORIDE IONS

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SYNTHESIS, CHARACTERIZATION AND PHOTOPHYSICAL STUDIES ON CHALCONE-BASED CHEMOSENSORS FOR THE DETECTION OF ALUMINIUM AND FLUORIDE IONS

by

CHAN YI HUAN

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<tr>
<td>a.u.</td>
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<tr>
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<td>Degree Celcius</td>
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<tr>
<td>D₂O</td>
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<tr>
<td>λ</td>
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<td>μM</td>
<td>Micromolar</td>
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<tr>
<td>π</td>
<td>Pi</td>
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<td>σ</td>
<td>Sigma</td>
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<tr>
<td>δ</td>
<td>Delta</td>
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<td>$K_a$</td>
<td>Association constant</td>
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<td>LOD</td>
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<tr>
<td>MOPS</td>
<td>3-(N-morpholino)propanesulfonic acid</td>
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SINTESIS, PENCIRIAN DAN KAJIAN FOTOFIZIKAL ATAS KEMOSENSOR YANG BERASASKAN KALKON BAGI PENGESANAN ION ALUMINIUM DAN FLUORIDA

ABSTRAK

Dalam tesis ini, sintesis dan kajian tiga kemosensor berasaskan kalkon yang berbeza untuk mengesan ion Al\(^{3+}\) dan F\(^-\) dengan kaedah mata kasar dan fluorimetrik telah diterangkan. Pencirian struktur bagi sebatian utama telah dijalankan dengan teknik spektroskopi Fourier Transform Infraerah (FT-IR), spektroskopi Proton dan Karbon Resonans Magnetik Nuklear (1H- dan 13C-NMR). Respons molekul penerima terhadap pelbagai ion logam dan anion telah diselidiki dengan kaedah UV-Vis dan spektroskopi pendarfluor dan melalui pemerhatian mata kasar. Kajian mengenai tingkah laku pengikatan kemosensor (1-[3-(2-Hidroksi-fenil)-3-oxo-propenil]-naftalena-2-iloksi)-asid asetik (NAC) yang disintesis dengan spektrofotometer UV-Vis menunjukkan kepilihan yang sangat baik dan respons yang pantas terhadap ion Al\(^{3+}\). Peningkatan pendarfluor yang ketara semasa penambahan ion Al\(^{3+}\) berbanding dengan ion logam lain menjadikannya kemosensor terpilih yang sesuai bagi ion Al\(^{3+}\).

Penerima NAC memaparkan perubahan penyerapan dari 399 nm kepada 416 nm kerana pengikatan NAC dengan Al\(^{3+}\) mencetuskan peningkatan ketegaran sistem melalui operasi pendarfluoran pengkelatan dipertingkat (CHEF) yang tidak berlaku pada kation logam lain. Stoikiometri penerima NAC dan ion Al\(^{3+}\) didapati sebagai 2:1 dengan menggunakan kaedah Job dengan pemalar pengikatan \(K_a = 0.21 \times 10^2 \text{ M}^{-2}\).

Anjakan dalam data spektrum FT-NMR mendedahkan bahawa dua atom O kumpulan karboksil dan hidroksil serta atom oksigen kumpulan oksimetilena terlibat dalam koordinasi. Penerima (1-[3-Oxo-3-(3-oxo-3H-benzo[f]kromen-2-il)- propenil]-
naftalena-2-iloksi)-asid asetik (CAC) mengesan ion Al$^{3+}$ dalam medium air 100% di mana perubahan warna dari kuning ke sian dapat dilihat melalui mata kasar. Selepas penambahan ion Al$^{3+}$, mekanisme pemindahan cas dalaman (ICT) dalam kromofor kumarin ditingkatkan dan ia dapat dikenal pasti dengan mata kasar. Stoikiometri 2:1 bagi penerima CAC:ion Al$^{3+}$ telah diselidiki oleh plot Job dan pemalar pengikatan didapati sebagai $K_a = 5.91 \text{ M}^{-2}$. Tingkah laku penderiaan anion 7-Dietilamino-3-[3-(2-hidroksi-naftalena-1-il) -akrilokil]-kromen-2-on (DNAC) telah dikajikan dengan teknik UV-Vis. Pembentukan ikatan hidrogen antara pengesan DNAC dan ion F$^-$ menimbulkan peningkatan intensiti pelepasan pengesan pada 524 nm yang boleh dikaitkan dengan pemindahan cas dalaman (ICT) yang dibangunkan oleh deprotonasi OH dari unit naftol ke moieti kalkon dalam pengesan DNAC. Tidak terdapat sebarang gangguan oleh anion lain dalam intensiti pendarfluor kompleks semasa pengesanan ion F$^-$ dalam CH$_3$CN. Plot Job mencadangkan nisbah DNAC:ion F$^-$ sebagai 1:1 dengan pemalar pengikatan $K_a = 3.33\times10^{-5} \text{ M}^{-1}$. 
SYNTHESIS, CHARACTERIZATION AND PHOTOPHYSICAL STUDIES ON CHALCONE-BASED CHEMOSENSORS FOR THE DETECTION OF ALUMINIUM AND FLUORIDE IONS

ABSTRACT

In this thesis, the syntheses and studies of three different chalcone-based chemosensors for the detection of \( \text{Al}^{3+} \) and \( \text{F}^- \) ion by naked eye and fluorimetric method have been described. The structure elucidation for the ultimate compounds have been characterized by spectroscopic techniques (FT-IR, \(^1\text{H}-\) and \(^{13}\text{C}-\text{NMR})\). The response of the receptor molecules to various metal ions and anions were investigated by UV-Vis and fluorescence spectroscopy methods and to some extend through naked-eye observations. Study on binding behavior of the synthesized chemosensor (1-[3-(2-Hydroxy-phenyl)-3-oxo-propenyl]-naphthalen-2-yloxy)-acetic acid (\textbf{NAC}) by UV-Vis spectrophotometer has shown excellent selectivity and rapid response toward \( \text{Al}^{3+} \) ion. The obvious fluorescence increment upon the addition of \( \text{Al}^{3+} \) ion over other metal ions made it a suitable selective chemosensor for \( \text{Al}^{3+} \) ion. Receptor \textbf{NAC} displays a change in absorption from 399 nm to 416 nm as the binding of \textbf{NAC} with \( \text{Al}^{3+} \) triggered the improvement in the rigidity of the system through the operation of chelation enhanced fluorescence (CHEF) which did not happen to other metal cations. The stoichiometry of receptor \textbf{NAC} and \( \text{Al}^{3+} \) ion was found to be 2:1 using Job’s method with an association constant of \( K_a = 0.21 \times 10^2 \text{ M}^{-2} \). The shift in FT-NMR spectral data reveals that two \( \text{O} \) atoms of carboxyl and hydroxyl groups and the oxygen atom of the oxymethylene group involved in coordination. The receptor (1-[3-Oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propenyl] -naphthalen-2-yloxy)-acetic acid (\textbf{CAC}) detect \( \text{Al}^{3+} \) ion in 100% water medium in which a change in colour from yellow to
cyan can be observed by naked eye. After the addition of Al$^{3+}$ ion, the internal charge transfer (ICT) mechanism in coumarin chromophore was enhanced that can clearly be identified by naked eye. The 2:1 stoichiometry of receptor CAC:Al$^{3+}$ ion was investigated by Job’s plot and the association constant was found to be $K_a = 5.91 \text{ M}^{-2}$.

The anion sensing behaviours of 7-Diethylamino-3-[3-(2-hydroxy-naphthalen-1-yl)-acryloyl]-chromen-2-one (DNAC) were studied by UV-Vis technique. The formation of hydrogen bonding between the probe DNAC and F$^-$ ion gave rise to the increase in the emission intensity of probe at 524 nm which can be attributed to the internal charge transfer (ICT) developed by the deprotonation of OH from napthol unit to the chalcone moiety in the probe DNAC. There was no interference by other anions in the fluorescence intensity of the complex during the F$^-$ ion detection in CH$_3$CN. Job’s plot suggests the ratio of DNAC: F$^-$ ion as 1:1 with an association constant of $K_a=3.33\times10^{-5} \text{ M}^{-1}$. 
CHAPTER 1

INTRODUCTION

Cations and anions play an indispensable role in physiology, catalysis, medical diagnostics, chemical, environmental and biological processes such as an ion transport through membranes, enzyme cofactor, maintenance of cell shape and signal transduction [Christianson & Lipscomb 1989, Gloe et al. 1998, Valeur & Leray 2000, Vázquez et al. 2004]. These heavy metals are widely used in mining, extraction, chemical and pharmaceutical industries in a range of different forms. As a result, public health problems such as Parkinson, Alzheimer and Wilson’s diseases arose [Bonda et al. 2001, Perl et al. 1982, Pithadia et al. 2012]. Although most of the metals are essential elements for living systems at varying amounts but they are the major pollutants in our environment and have entered into our human body through drinking water and food intake which might cause serious illness at excessive accumulation amount. Not only cations, anions are also highly health hazardous and toxic in nature.

Despite having many methods for the cations and anions detection such as mass spectrometry (MS) [Kriegeskotte et al. 2009], atomic absorption spectrometry (AAS) [Frankowski et al. 2010, Pourreza & Hoveizavi 2005], inductively coupled plasma mass spectroscopy (ICP-MS) [del Castillo Busto et al. 2005] and others, but they do not allow continuous monitoring and expensive. In recent years, the development of chemosensors for the detection of various cations and anions have attracted significant attention because of ease to operate, high sensitivity and selectivity and observed by naked eye without the use of sophisticated instruments.

The recognition of metal ions with non-covalent interactions has reminded us of a Nobel Prize in 1987 which was shared by triad of the scientists – Pedersen, Cram and Lehn for the development of host-guest in the field of supramolecular chemistry.
[Pedersen 1967]. A supramolecule consists of two parts, a host and a guest species that interact with one another in a weaker and reversible non-covalent manner such as Van der Waals forces, hydrogen bonding, $\pi-\pi$ interactions, metal coordination, hydrophobic forces and electrostatic forces [Gellman 1997]. This chapter deals about the general overview on different type of chemosensors and their advantages, heavy metals and their properties, a short literature review on the mode of sensing and their examples and finally the challenges faced and the objectives of the present research.

1.1 Sensor

Nowadays, sensors are everywhere in our life. A sensor is a device that detects some type of input from the physical environment and produces a human-readable signal in response. For example, thermometers, smoke alarms, pH meter are very common sensors in our daily life. A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal [Hulanicki et al. 1991].

Basically, there are three components that constitute a sensor: A receptor, spacer and an active unit (Figure 1.1). A receptor is responsible for the selective analyte binding. In another word, the selective receptor is capable of identifying analyte of interest. A spacer is a device that is able to convert the energy carrying the chemical information about the recognition element into a measurable signal. As for the active unit, its properties should change upon analyte binding and responsible for reporting the recognition event [Martínez-Máñez & Sancenón 2003].
In general, the chemosensors can be divided into three types:

- Colorimetric chemosensors
- Fluorometric chemosensors
- Electrochemical chemosensors

A colorimetric chemosensor is a technique in which a change in color occurs due to the interaction between analyte and sensor. For example, litmus paper, where there is a color change with H⁺ ion. In fluorescent chemosensor, there is a difference in emission intensity upon analyte binding to the sensor. In electrochemical chemosensor, there is a change in oxidation/reduction potential when analyte bound to the sensor unit. In this research, only colorimetric and fluorometric chemosensors for the detection of cations and anions will be discussed.

1.2 Colorimetric Chemosensors

A colorimetric chemosensor is a method in which a selectively change in color occurs upon interaction between sensor and a particular target analyte, due to their change in absorbance. For examples, litmus paper, where there is a color change with H⁺ ions; and phenolphthalein, an acid-base indicator, which is colorless in acidic condition and magenta in alkaline solution.
Colorimetric determination of both cations and anions based on supramolecular ideas had gained the popularity since a long time ago. Among the techniques available for the detection of ions, colorimetric method is the easiest to operate and the cheapest way to offer the qualitative and quantitative information. Cheng and his coworkers had designed a receptor with azobenzene moiety for the recognition of Hg$^{2+}$ ions based on Internal Charge Transfer (ICT) mechanism from light yellow to deep red [Cheng et al. 2011]. An aldazine-based colorimetric chemosensor was reported by Narayanaswamy et al. to detect Cu$^{2+}$ and Fe$^{3+}$ from pale yellow to purple color [Narayanaswamy & Govindaraju 2012]. Color change visible by naked eye allows an immediate indication of the presence of the particular analyte without employing any spectroscopic device.

1.3 Fluorometric Chemosensors

The fluorescence detection of specific molecules and ions has attracted considerable interest because it is cheap, highly sensitive, easy to operate and also due to its importance in the field of medicine, analytical, industrial and environmental chemistry.

In a fluorescence sensing approach, usually a fluorophore is non-fluorescent and acts as a signal transducer that converts the information into an optical signal when the analyte molecule is bound to it [Arimori et al. 2002, Guliyev et al. 2009]. For the recognition moiety or ionophore, it determines mainly the interaction selectivity and sensitivity with analytes based on the non-covalent bonding such as hydrogen bonding, metal complexation, π-π stacking, hydrophobic forces and electrostatic interactions. Numerous chemical and biochemical analytes can be detected by fluorescence methods: cations, anions, neutral molecules and gases.
1.4 Importance of Cations and Anions for Detection

Alkali and alkaline earth metal ions are found in the human body and have a great importance owing to their involvement in wide environmental and biological processes. For example, potassium and sodium are two of the most important ions in our human nervous system. As an electrolyte, potassium and sodium transport and maintain electrical impulses of human cells which are vital for human blood, hydration and help to ensure other important body functions [Beer et al. 1997].

The d-block transition metals are found everywhere on earth in various amounts and also found in our bodies. In today’s society, transition metals are in high demand and are extensively used in industrial and construction purposes. Besides, many of the transition elements are essential to our human body and play important role in numerous biological processes. In hemoglobin, Fe$^{3+}$ iron is used to transport oxygen throughout the human body in blood. Cu$^{2+}$ copper is used to protect the body from free radicals damage and also work with iron to help the body form red blood cells. However, transition metal ions can become toxic at high concentration. Accumulation of transition metals in human body can cause Alzheimer’s disease, infant liver damage and gastrointestinal problems [Bush 2003, Uauy et al. 1998].

Toxic heavy metals such as lead, mercury and cadmium, are highly health hazardous and require sensitive detection in the environment. Lead enters the environment and human body through smoking, automobile emission, mining and pesticide which will cause mental retardation, liver and kidney damage and anemia [Rifai et al. 1993]. Mercury is poisonous in any form and poisoning happen from mercury vapor inhalation, ingestion, injection and absorption through skin [Davidson et al. 1998, Hennrich et al. 1999]. Therefore, selective and sensitive recognition of these metal ions are of great interest to many scientists.
Anions play many roles in chemical and biological processes such as transfer catalysts, redox agents, nucleophiles or bases [Christianson et al. 1989]. Chloride ions are essential element for human health by maintaining metabolism and the acid-base balance in body. Fluoride is used in dental care and for the treatment of osteoporosis but can lead to fluorosis upon excessive exposure [Ayoob & Gupta 2006]. Cyanide ion is highly hazardous to living organisms in any type of release but still in high demand in industrial processes such as gold mining, electroplating and production of polymers. The selective and sensitive detection of all of these ions are very important to ensure human can in a healthy environment.

1.5 **Mechanisms of Sensing**

There is a variety of different signalling principles that are utilized in fluorescent chemosensors design such as electron transfer, charge transfer, energy transfer, and formation of excimer. The fluorophore that experiences this perturbation of photoinduced processes converts the recognition event into the photophysical changes, such as spectra, fluorescence quantum yield and lifetime.

1.6 **Photoinduced Internal Charge Transfer (PICT)**

When a sensor design incorporated an electron-donating group and an electron-withdrawing group into the molecule in a manner which allows direct electronic communication between the two components, it undergoes photo-induced internal charge transfer (PICT) by excitation of light as shown in Figure 1.2. Upon excitation of a fluorophore, a redistribution of electron density in both donor and acceptor groups occurs which will then be accompanied by an instantaneous change
in the dipole moment of the fluorophore. As such, it results in a stroke shift in wavelength [Valeur & Leray 2000].

If a cation interacting with the acceptor group, the electron withdrawing nature of that group enhanced resulting in a red shift in absorption spectrum. In contrast, when a cation bound to the donor moiety conjugated to the fluorophore, the electron donating capability of the moiety reduced and a blue shift in absorption spectrum will be observed owing to the reduction in the conjugation of the π system [Loehr & Voegtle 1985].

Figure 1.2  Schematic representation of PICT chemosensor in sensing process [Liu et al. 2013].
1.7 Photoinduced Electron Transfer (PET)

Photoinduced electron transfer (PET) is one of the most important mechanisms for fluorescent chemosensors. A numerous fluorescent sensors for cations and anions had been developed based on this principle [Baki & Akkaya 2001, Czarnik 1993, de Silva et al. 1991]. However, in PICT-type chemosensors, a PET system design must incorporate a spacer unit between receptor and fluorophore groups. Upon exposure of fluorophore with light, an electron in highest occupied molecular orbital (HOMO) was excited to lowest unoccupied molecular orbital (LUMO). Before relaxation occurs, a PET take place from an external orbital of this fluorophore with energy level between HOMO and LUMO of the fluorophore to the HOMO of the fluorophore resulting in fluorescence quenching [Martínez-Máñez & Sancenón 2003]. However, when binding with a cation, the energy level of external orbital is reduced to lower than that of HOMO of the fluorophore and hence the electron transition become unfavourable which leads to fluorescence enhancement as displayed in Figure 1.3.

Figure 1.3 Molecular orbital diagram and schematic representation of photoinduced electron transfer process (PET) [Culzoni et al. 2013].
1.8 Fluorescence Resonance Energy Transfer (FRET)

Fluorescence resonance energy transfer (FRET) is another signaling mechanism that can be relied on in fluorometric chemosensor design. It is a non-radiative process in which the energy of an excited state donor (D) is transferred to an adjacent ground state acceptor (A) that allows fluorescence of latter is observed. In other words, energy of the donor (D) at its excited state is used to excite the acceptor (A) through energy transfer as shown in Figure 1.4. The efficiency of energy transfer depends on the distance between D and A, the concentration of A, and the extent of spectral overlap [Harekrushna 2011]. The difference between PET and FRET is that the net result of FRET is a transfer of the excitation energy from a donor to an acceptor, whereas PET took place from the ground state of the donor which initially excites the acceptor and donor is never converted to an excited state.

![Figure 1.4](image-url)

Figure 1.4 Schematic representation of FRET chemosensor in sensing process [Tabassum et al. 2012].
1.9 Chemistry of Chalcone

Chalcone is a natural compound bearing the 1,3-diphenyl-2-propen-1-one framework and is one of the most abundant groups of natural products [Tomazela et al. 2000]. In the past few years, chalcone had been recognized as one of the groups that showed prominent result in antimicrobial, antitumor, anti-inflammatory and anticancer activities due to the presence of enone functionality in chalcone moiety [Das & Manna 2016, Fang et al. 2014, Nowakowska 2007, Saydam et al. 2003]. Chalcone was claimed to possess excellent blue light transmittance and remarkable nonlinear optical (NLO) property, which is an essential element for optical application and it has also been served as starting material for the synthesis of a variety of heterocyclic compounds [Denis et al. 1988, Devarajegowda et al. 2001, Zhao et al. 2000].

1.10 Chalcone In Chemosensor Applications

The chalcones and their derivatives are important intermediates in organic synthesis. They possess keto functionality which may interact with cation of particular interest. In addition, the hydroxychalcone derivative can be a promising candidate for anion chemosensor because of its ability for proton donating in hydrogen bonding interaction and its conjugation system through phenol ring and enone group.

Fitriana et al. (2016) studied a colorimetric detection of F⁻, CO₃²⁻, CN⁻, and SO₄²⁻ anions in DMSO system using a chalcone-based receptor 1. The decrease of absorption band at 367 nm with the appearance of new broad peak at 507 nm was due to the breaking of O-H bond and the formation of deprotonated sensor, which is responsible for the colorimetric signalling. A detectable colour change from yellow to
red was observed upon addition of $F^-$, $CO_3^{2-}$ and $CN^-$ to sensor and it became orange in the presence of $SO_4^{2-}$.

A ratiometric chalcone-based receptor 2 appended by anthracene unit as the fluorophore was designed and developed by Velmurugan et al. (2015) to selectively discriminate $Ag^+$ ion over other metal ions tested. The fluorescence intensity of emission band at 415 nm was enhanced by addition of $Ag^+$ ion in the aqueous system with a prominent blue shift of the emission maximum of about 10 nm from 415 nm to 405 nm. This is due to the hindrance of photoinduced electron transfer along with the intramolecular charge transfer mechanism.

Prabhu et al. (2017) had reported a ratiometric fluorescent chemosensor 3 based on pyrene-conjugated pyridine. Pyrene was chosen as a reporter unit due to its ideal monomer and excimer emissions. Pyridine that behave as a good coordinating ability for metal ion binding sites is covalently linked with $\pi$-conjugated C=C bridge. A new intense peak with maximum absorption at 456 nm was observed upon addition of $Ni^{2+}$ to a solution of 3 corresponding to the formation of an excimer. The binding
ratio of receptor 3 with Ni$^{2+}$ ion of 2:1 was further confirmed by Benesi-Hildebrand plot.

Recently, Don et al. (2017) have reported a furan-pyrene conjugated chalcone receptor 4 for selective detection of Al$^{3+}$ and HSO$_3^-$ in aqueous acetonitrile. Addition of Al$^{3+}$ ion to receptor produces a highly intense fluorescence band at 388 nm. The enhancement of emission intensity can be attributed to the suppression of PET process. Reversible binding of receptor 4 with Al$^{3+}$ ion was also examined by addition of EDTA to Al$^{3+}$ bound receptor 4 resulting a decrease in the fluorescence intensity at 388 nm. Interestingly, addition of HSO$_3^-$ caused fluorescence enhancement with a notable red shift from 388 to 394 nm owing to the intramolecular charge transfer process from furan to pyrene rings.
1.11 Chemosensors Design for Ions

Metals are found naturally in the earth’s crust and have been used extensively in many different areas for thousands of years owing to their high electrical conductivity, malleability and luster. Toxic metals and anions are well known environmental pollutants and they enter the environment by natural and anthropogenic activities such as mining, industrial discharge, pest control, power generation and a number of others. Accumulation of these hazardous ions in human body can cause serious illness. Therefore, there has been increasing concern about exposures and intakes of toxic ions by humans nowadays. Implementation of restrictive regulations on drinking water was conducted to limit the concentration of toxic ions to enter human body. In order to create a useful chemosensor, the detection limit of a sensor toward ions should be lower than the desirable limit provided in drinking water quality standard as displayed in Table 1.1.

Table 1.1 Specifications of allowed ion concentration in drinking water.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Desirable Limit (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ministry of Health (MOH)</td>
</tr>
<tr>
<td>1</td>
<td>Nickel</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>Copper</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>Iron</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>Aluminium</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>Sulphate</td>
<td>250</td>
</tr>
<tr>
<td>6</td>
<td>Zinc</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Chloride</td>
<td>250</td>
</tr>
<tr>
<td>8</td>
<td>Fluoride</td>
<td>0.4 - 0.6</td>
</tr>
<tr>
<td>9</td>
<td>Manganese</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>Cadmium</td>
<td>0.003</td>
</tr>
<tr>
<td>11</td>
<td>Lead</td>
<td>0.01</td>
</tr>
<tr>
<td>12</td>
<td>Arsenic</td>
<td>0.01</td>
</tr>
<tr>
<td>13</td>
<td>Mercury</td>
<td>0.001</td>
</tr>
<tr>
<td>14</td>
<td>Nitrate</td>
<td>10</td>
</tr>
</tbody>
</table>
1.11.1 Chemosensors Design for Al\textsuperscript{3+} Ion Detection

Among all the metals as listed in Table 1.1, aluminium is the most abundant metal and the third most abundant element found in the earth’s crust after oxygen and silicon. The properties of aluminium include high thermal conductivity, lightweight, high strength, easy to recycle and excellent corrosion resistance that make it attractive to be applied in the textile, transportation, packaging and aerospace industries. Aluminium also can be found in numerous consumer items such as pharmaceutical drugs, cosmetics, food additives, cooking utensils, antiperspirant and water [Jung et al. 2012, Maity & Govindaraju 2010].

Besides, aluminium is a well known non-essential trace element in human body. The World Health Organization (WHO) report had summarized that the maximum intake of aluminum has been prescribed to be around 3-10 mg per day [Lu et al. 2011]. Excessive accumulation of aluminium in body might damage kidney and nervous system causing aluminium-induced disease such as Alzheimer’s disease and Parkinson’s disease [Ambreen et al. 2017, Chin-Chan et al. 2015, Iglesias-González et al. 2017]. It also impedes plant growth and harm fish in acidified water. Owing to the potential impact of Al\textsuperscript{3+} ion on human and environment, highly sensitive and selective chemosensors is highly required. It is a challenging task to design an aluminium recognition chemosensor because of aluminium’s poor coordination ability and lack of spectroscopic properties [Soroka et al. 1987].

Lu et al. (2011) reported a 1,2-dihydroxyanthraquinone based chemosensor that was designed and synthesized based on PET mechanism. Receptor 5 specifically recognized Al\textsuperscript{3+} ions in aqueous solution by coordinating with S\textsubscript{2}N podand moiety and one OH group from 1,2-dihydroxyanthraquinone fluorophore unit that cause a significant enhancement of the emission intensity at 603 nm in the fluorescence
spectrum. This observation can be ascribed to the inhibition of PET processes from both the sulphur and nitrogen donors to the fluorophore.

Schiff base colorimetric and fluorescent receptor 6 was designed by combining ethanolamine with 8-hydroxyjulolidine-9-carboxaldehyde and used for studying chemosensing properties Kim et al. (2014) for various metal ions. Upon complexation of Al$^{3+}$ ion with receptor unit induced a red shift of 46 nm in absorption as well as emission spectrum and recorded in DMF/HEPES buffer, thus changing colorless to yellow followed by blue emission. This is attributed to the formation of rigid complex after binding of 6 with Al$^{3+}$ causing the chelation-enhanced fluorescent (CHEF) effect and inhibiting the C=N isomerization.

A water-soluble carboxylic functionalized chemosensor 7 reported by Lee et al. (2015) was developed for selective recognition of Al$^{3+}$ ion based on CHEF mechanism. In this process, the Al$^{3+}$ ion was incorporated by coordinating to two OH groups from both julolidine and carboxylate groups and imine nitrogen. It exhibited a highly sensitive and selective “turn-on” fluorescent response toward Al$^{3+}$ ion even at
24 nm level. It was claimed that the presence of carboxylate group as a hard base in a chemosensor make it more selective towards the hard acid, Al$^{3+}$ and increase its water solubility.

Dhara et al. (2013) had developed a new receptor 8 for Al$^{3+}$ ions. The sensing mechanism of receptor based on Al$^{3+}$-induced reversible ring-opening mechanism of rhodamine spirolactam form. The receptor 8 was designed by combination of 2,7-dimethoxy-9H-fluoren-9-one with rhodamine B. It exhibited a highly sensitive and selective “turn-on” bright orange fluorescent and colorimetric response towards Al$^{3+}$ ion from colourless to purple. Further, sensing ability of receptor was observed to be 2.4 μM level in the fluorescence method. Herein, spirolactam moiety of 8 acted as a signal switcher which was envisioned to turn on when the cation was bound to the rhodamine molecule.
1.11.2 Chemosensors Design for F⁻ Ion Detection

Being the first anion and the most electronegative element in the periodic table, fluoride ion plays an important role in chemical and biological processes such as treatment of osteoporosis and skeleton fluorosis [Riggs et al. 1990]. It is also able to form hydrogen bond with hydrogen-bond donor. Fluoride released into environment naturally through volcanoes eruption and in marine aerosols. Human activities such as coal combustion and waste from various industrial processes also lead to the release of fluoride into the environment. Besides that, fluoride also present in drinking water for the prevention of dental caries.

However, at high exposure level, intake of fluoride causes skeleton fluorosis, memory loss, kidney failure, gastrointestinal, neurological and urinary problem [Lantz et al. 1987]. The WHO recommended allowed fluoride level in drinking water should not be more than 1.5 μg/L which considered as a limit of health-risk to human being [Kanduti et al. 2016]. Owing to these reasons, selective recognition of fluorine ion is significance. The four main interaction modes between receptor and fluoride ion are as follow:

1. anion-π interactions,
2. anion sensing through hydrogen bonds between CH, -NH and -OH groups,
3. Lewis acid-base interactions and
4. anion induced chemical transformation reactions.

A coumarin-based with Schiff-base as bridge receptor 9 was developed by Zhuang et al. (2011). A high selectivity and sensitivity for F⁻ ion over other tested anions was rendered by receptor 9 in acetonitrile medium. Addition of F⁻ ion induced
red shift of absorption spectrum attributed to the intramolecular charge transfer in the whole system via deprotonated form of -NH group in receptor unit. The receptor exhibited a large color changes from yellow to blue and can be clearly visualized by naked eye.

![Diagram](image1)

(9)

Devaraj et al. (2007) have reported anthraquinone-based receptor 10 as a highly colorimetric and selective fluorescent probe for F⁻ ion. Receptor 10 possessing a phenolic OH group able to bind fluoride via hydrogen bond interactions produces a dramatic colour changes from light pink to brown. Addition of successive amounts of F⁻ ion results in a reduction of the intensity of the fluorescence emission maximum of receptor 10.

![Diagram](image2)

(10)

Yang et al. (2011) had synthesized isoxazole derivative composed of pyrrole receptor 11 for F⁻ ions in CH₃CN medium. Receptor 11 operates via a two-stage deprotonating process in pyrrole moiety leading to a new absorption peak at 375 nm with a clear isosbestic point at 352 nm. The binding behavior of receptor 11 with F⁻
was further confirmed by $^1$H-NMR titration with the disappearance of the pyrrole NH proton. With the increasing concentration of fluoride, the emission maximum was red-shifted and the emission intensity was quenched when $F^-$ ion was added.

A novel 1,3,4-oxadiazole based receptor 12 was developed by Ma et al. (2013) containing phenol hydroxyl as the hydrogen-bond donor. Receptor 12 underwent stepwise deprotonating by the influence of $F^-$ ion addition, which gave rise to a blue shift of absorption peak from 294 to 272 nm and a new broad absorption band formed at 423 nm. A distinct colour change occurs from colourless to yellow can be clearly identified by naked eye. Fluorescence enhancement and fluorescence colour change from pale blue to green was observed after the addition of $F^-$ ion.
1.12 Problem Statements

Although an enormous work on chemosensor had been reported by researchers all over the world since the last two decades, however, there is a limited work focusing on the chalcone-based chemosensors. In addition, the water soluble chalcone-based chemosensor remains rare because of its hydrophobic properties. Hence, the current investigation will place the emphasis on the synthesis and characterization of new chalcone-based chemosensor workable for the selective detection of cations or anions in the aqueous medium through naked eye and the increment of fluorescence intensity. Several chalcone-based molecules incorporated with carboxyl group as chelating units which is hydrophilic in nature will be employed for selective detection of specific cations or anions in water medium.

1.13 Objectives of Present Research Project

The objectives of this research project are:

(a) To synthesize new chalcone derivatives capable to exhibit chemosensing properties particularly for selective detection of cations and anions in the aqueous medium detected by naked eye.

(b) To characterize the synthesized chemosensors in solid state via various spectroscopic techniques.

(c) To investigate the chemosensing behaviour of the synthesized chemosensors by the UV-Vis and fluorescence measurements.

(d) To identify whether a chalcone moiety could be connected to a suitable chelating unit bearing N, O soft donor atoms at various positions and hence induce the chemosensing behaviour of the synthesized receptor.
CHAPTER 2
EXPERIMENTAL WORK

This chapter describes experimental details for the preparation of three different receptor compounds and the recognition studies.

2.1 Chemicals

All the intermediary and the title compounds in this project have been synthesized by using chemicals listed below. These chemicals and solvents were used directly without further purification.

2-hydroxy-1-napthaldehyde, 4-(diethylamino)salicylaldehyde, 2'-hydroxy-1’-acetonaphthone, ethyl acetoacetate, piperidine, 3-(N-morpholino)propanesulfonic acid (MOPS) and the perchlorate salts of all cations used (Ag\(^+\), Al\(^{3+}\), Ca\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), Hg\(^{2+}\), K\(^+\), Mn\(^{2+}\), Na\(^+\), Ni\(^{2+}\), Pb\(^{2+}\) and Zn\(^{2+}\)) were obtained from Sigma-Aldrich. Ethyl chloroacetate was purchased from Merck. 2'-hydroxyacetophenone and tetrabutylammonium salts of all anions (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\) and OH\(^-\)) used were obtained from Fluka. Potassium carbonate anhydrous was obtained from R & M Chemical. Potassium iodide, sodium hydroxide pellets, sodium sulfate anhydrous, acetone, methanol and ethanol were supplied by QRec. The title compounds were prepared following the procedure as described in section 2.2.
2.2 Synthesis of Intermediary and Title Compounds

2.2.1 Synthetic scheme of receptor \{1-[3-(2-Hydroxy-phenyl)-3-oxo-propenyl]-naphthalen-2-yloxy\}-acetic acid (NAC)

The synthetic route towards the synthesis of receptor NAC is shown in Figure 2.1. Firstly, 2-hydroxy-1-napthaldehyde was alkylated with ethyl chloroacetate which was then subjected to hydrolysis and finally reacted with 2’-hydroxyacetophenone through aldol condensation in a single step to obtain the target receptor NAC.

2.2.1(a) Synthesis of (1-Formyl-naphthalen-2-yloxy)-acetic acid ethyl ester (NAEE)

To a solution of 2-hydroxy-1-napthaldehyde (1.0 equiv.) and ethyl chloroacetate (1.0 equiv.) in acetone, potassium carbonate anhydrous (4.0 equiv.) was added followed by a pinch of potassium iodide. After 24 hours reflux, the reaction mixture was filtered off to remove K₂CO₃ and the solvent was left to evaporate. The reaction mixture was treated with water and extracted with diethyl ether and the organic layer was dried on anhydrous Na₂SO₄. The crude product was washed with ethanol to afford a beige solid. Percent yield is 53%.

2.2.1(b) Synthesis of \{1-[3-(2-Hydroxy-phenyl)-3-oxo-propenyl]-naphthalen-2-yloxy\}-acetic acid (NAC)

Compound NAEE (1.0 equiv.) was added to a mixture of 2’-hydroxyacetophenone (1.0 equiv.) in ethanol and stirred for 15 mins. A 10% sodium hydroxide solution was added and stirred for overnight. The precipitate thus formed was filtered and dried. The product was washed with acetonitrile to get a yellow solid, NAC. Percent yield is 78%.
2.2 Synthetic scheme of receptor \{1-[3-Oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propenyl]-naphthalen-2-yloxy\}-acetic acid (CAC)

The general synthetic route towards the formation of chalcone-based chemosensor CAC is summarized in Figure 2.2. Firstly, 2-hydroxy-1-napthaldehyde was alkylated with ethyl chloroacetate in base medium to form the intermediate 1 with 61% yield. At the same time, 2-hydroxy-1-napthaldehyde underwent condensation with ethyl acetoacetate to form intermediate 2 (75% yield). Finally, both intermediates were reacted and underwent hydrolysis and aldol condensation in a single step to form the target receptor CAC with 89% yield.

2.2.2(a) Synthesis of (1-Formyl-naphthalen-2-yloxy)-acetic acid ethyl ester (NAEE)

To a solution of 2-hydroxy-1-napthaldehyde (1.0 equiv.) and ethyl chloroacetate (1.0 equiv.) in acetone, potassium carbonate anhydrous (4.0 equiv.) was
added followed by a pinch of potassium iodide. After 24 hours reflux, the reaction mixture was filtered off to remove K₂CO₃ and the solvent was left to evaporate. The reaction mixture was treated with water and extracted with diethyl ether and the organic layer was dried on anhydrous Na₂SO₄. The crude product was washed with ethanol to afford a beige solid. Percent yield is 53%.

2.2.2(b) Synthesis of 2-Acetyl-benzo[f]chromen-3-one (ABCM)

To a solution of 2-hydroxy-1-napthaldehyde (1.0 equiv.) and ethyl acetoacetate (1.0 equiv.) in ethanol, 4 drops of piperidine were added. After refluxed for 3 hours, the reaction mixture was filtered and washed with diethyl ether to get a yellow solid. Percent yield is 85%.

2.2.2(c) Synthesis of {1-[3-Oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propenyl]-naphthalen-2-yloxy}-acetic acid (CAC)

Compound NAEE (1.0 equiv.) was added to a mixture of compound ABCM (1.0 equiv.) in ethanol and stirred for 15 mins. A 10% sodium hydroxide solution was added and stirred for overnight. The precipitate thus formed was filtered and dried. The product was washed with acetonitrile to get a dark red solid, CAC. Percent yield is 81%.
2.2.3 Synthetic scheme of receptor 7-Diethylamino-3-[3-(2-hydroxy-naphthalen-1-yl)-acryloyl]-chromen-2-one (DNAC)

The general synthetic route towards the formation of chalcone-based chemosensor DNAC is summarized in Figure 2.3. Firstly, 4-(diethylamino)salicylaldehyde underwent condensation with ethyl acetoacetate to form intermediate 1 and finally reacted with 2-hydroxy-1-naphthaldehyde through aldol condensation to lead to the formation of desirable receptor DNAC with 37% yield.

2.2.3(a) Synthesis of 3-acetyl-7-(diethylamino)-2H-chromen-2-one (ADCM)

In a two-neck round bottom flask, 4-(diethylamino)salicylaldehyde (1.0 equiv.) and ethylacetoacetate (1.2 equiv.) were dissolved in absolute methanol (15 mL). A
few drops of piperidine were then added as catalyst. The mixture was stirred at room temperature for 24 hrs to obtain a bright yellow precipitates by filtration and washed with cold absolute ethanol (5 mL). Recrystallization from absolute ethanol gave compound ADCM as a bright yellow solid. Percent yield is 83%.

2.2.3(b) Synthesis of 7-Diethylamino-3-[3-(2-hydroxy-naphthalen-1-yl)-acyroyl]-chromen-2-one (DNAC)

3-acetyl-7-(diethylamino)-2H-chromen-2-one (1.0 equiv.) was added to 2-hydroxynapthaldehyde (1.0 equiv.) in a round bottom flask with 3 drops of piperidine as a base. The reaction mixture was heated to reflux for 48 hrs in absolute ethanol. The crude mixture was poured into the ice-cold water whereupon the fine precipitate was formed immediately. The product was filtered and recrystallized from ethanol. It was purified further using column chromatography to afford compound DNAC as a brown solid. Percent yield is 37%.

![Figure 2.3 Synthesis of DNAC.](image-url)
2.3 Characterization

All the receptors molecules were characterized to study the structural and chemosensing behaviour using various experimental techniques as listed below:

2.3.1 Carbon, Hydrogen and Nitrogen microanalysis (CHN)

The percentage composition of carbon, hydrogen and nitrogen atoms for all receptor compounds were determined by using a Perkin Elmer 2400 LS Series CHNS/O analyzer at the School of Chemical Sciences, USM.

2.3.2 Fourier transform-infrared spectroscopy (FT-IR)

Fourier transformed infrared spectroscopy (FT-IR) is an analytical tool to determine the functional groups present in organic and inorganic materials. An IR spectrum can be divided into two regions. The region of 4000–400 cm\(^{-1}\) is called as a functional group region, in which most of the functional group demonstrates absorption bands. The region of 1000–400 cm\(^{-1}\) is called a fingerprint region. About 5 mg of the samples were ground with the dry KBr (1:10, w/w) into fine powder and then pressed into pellet form. The sample pellets were then analyzed and all the spectra were recorded in the frequency range of 400–4000 cm\(^{-1}\) by using a Perkin Elmer 2000-FT-IR spectrophotometer at the School of Chemical Sciences, Universiti Sains Malaysia (USM).

2.3.3 Fourier transform nuclear magnetic resonance spectroscopy (FT-NMR)

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to the chemical shift and Zeeman Effect on the resonance frequencies of the nuclei. High-resolution
\(^1\)H-NMR spectra were recorded using Bruker-Avance 500MHz Ultrashield spectrometers at the School of Chemical Sciences, USM. The samples were dissolved in deuterated chloroform (CDCl\(_3\)) with tetramethylsilane (TMS) as the internal standard and deuterated oxide (D\(_2\)O). Approximately 10 mg of each sample was dissolved in 1 mL of the deuterated solvents in the NMR tube.

### 2.3.4 UV-Visible Spectrophotometer

UV-Vis spectroscopy has been used to determine the amount of substance present in the sample as the absorbance is directly proportional to the concentration of the absorbing species. Interaction of the receptor molecules with cations/anions may lead to a change in the molecules’ absorbance and thus in their UV-Vis spectra.

UV-Vis titration was performed by gradually increasing the amount of ions added to examine the sensor properties of the receptor molecule by observing the absorbance pattern of the compound. The presence of isobestic points indicates the formation of receptor-ion complex. All the UV-Vis absorption spectra of the targeted compounds were obtained on a Shimadzu model UV-2600 UV-Vis spectrophotometer at the School of Chemical Sciences, USM. The stock solutions for metal ion (1 mM) and title compound (1 mM) were prepared and operated in distilled water or acetonitrile for the spectral measurements.

### 2.3.5 Fluorescence Spectrophotometer

When a molecule absorbs light energy to form an excited state, the excited state can lose its acquired energy with an emission of radiation termed as luminescence. Fluorescence is a luminescence pathway involving energy singlet state of absorbing molecules. The existence of complexation can also be detected by monitoring the
changes in fluorescence intensity of the receptor upon addition of cations/anions. The fluorescence spectra of the samples were recorded on a Perkin Elmer model LS55 fluorescence spectrometer at the School of Chemical Sciences, USM. The band passes were 5 nm for both excitation and emission slit widths. All the stock solutions for metal ion (1 mM) and title compound (1 mM) were prepared and operated in distilled water or acetonitrile for the spectral measurements.

2.4 Details of the measurement procedures

2.4.1 Details of metal titrations

For both NAC and CAC, the titrations were carried out using 1×10^{-4} M solutions in distilled water with pH 7. However, DNAC is only modestly water-soluble, and thus titrations were carried out using 2.0×10^{-5} M solutions in 1:1 acetonitrile/pH 6 aqueous MOPS. Cations and anions was added as a 1×10^{-3} M solution of metal perchlorate salt and tetrabutylammonium salts respectively in distilled water via micropipette to fluorophore solution in a quartz cuvette. The solutions were mixed well prior to acquiring absorption and fluorescence spectra.

2.4.2 Details of selectivity study

The selectivity of probe was studied by adding 1×10^{-3} M of each cations (solution of metal perchlorate salt) and anions (solution of tetrabutylammonium salts) via micropipette to blank chemosensor solution in a quartz cuvette. The solutions were mixed well prior to acquiring absorption and fluorescence spectra. A solution of Al^{3+} or F^- ions in 1×10^{-3} M was then added into the mixture with well mixed prior to obtaining absorption and fluorescence spectra.
2.4.3 Determination of association constants

The association constants $K_a$ for the interaction of NAC and CAC with Al$^{3+}$ ions were determined by Benesi-Hilderbrand plot analysis, using the following equation:

$$\frac{1}{(A-A_o)} = \frac{1}{K_a(A_{\text{max}}-A_o)} [\text{Al}^{3+}]^{0.5} + \frac{1}{[A_{\text{max}}-A_o]}$$

Where, $A$ and $A_o$ are the absorbance of NAC and CAC solution in the presence and absence of Al$^{3+}$ ions; $A_{\text{max}}$ is the saturated absorbance of NAC and CAC in the presence of excess amounts of Al$^{3+}$ and $[\text{Al}^{3+}]$ is the concentration of Al$^{3+}$ ions added (mol L$^{-1}$). The titrations were carried out in a fluorescence cuvette by adding aliquots of cations solutions via micropipette to NAC and CAC fluorophore solution of known concentration in distilled water. The solutions were mixed well prior to acquiring the absorption spectra.

For the interaction of DNAC with F$^-$ ions, the association constants $K_a$ was determined by Benesi-Hilderbrand plot analysis, using the following equation:

$$\frac{1}{(I-I_o)} = \frac{1}{K_a(I_{\text{max}}-I_o)} [\text{F}^-] + \frac{1}{[I_{\text{max}}-I_o]}$$

Where, $I$ and $I_o$ are the fluorescence intensity of DNAC solution in the presence and absence of F$^-$ ions; $I_{\text{max}}$ is the saturated intensity of DNAC in the presence of excess amounts of F$^-$ and $[\text{F}^-]$ is the concentration of F$^-$ ions added (mol L$^{-1}$). The titrations were carried out in a fluorescence cuvette by adding aliquots of cations solutions via micropipette to DNAC fluorophore solution of known concentration in 1:1 acetonitrile/pH 6 aqueous MOPS. The solutions were mixed well prior to acquiring the absorption spectra.
2.4.4 Determination of Stoichiometry

To determine binding stoichiometry, method of continuous variation (Job’s method) was used. Different volumes of Al\(^{3+}\) including 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 mL were prepared by taking the required amounts from the standard stock solution of Al\(^{3+}\) \((1\times10^{-3} \text{ M})\) and transferring to 5 mL volumetric flasks each. In addition, different volumes of NAC including 1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, 0.2 mL of the standard stock solution of NAC \((1\times10^{-4} \text{ M})\) were added into each of the volumetric flasks respectively in the way where titrations were performed holding the total concentration of Al\(^{3+}\) and NAC constant while varying the mole fraction of both. The resulting solutions were prepared to be measured on a UV-vis spectrophotometer. Same method was used in all cases.

2.4.5 Determination of limit of detection

To determine the sensitivity of probe NAC towards Al\(^{3+}\) ions, a linear calibration curve was plotted between the relative fluorescence intensity and the concentration of Al\(^{3+}\) ion. The detection limit (LOD) was calculated based on fluorescence titration. According to the IUPAC definition, the detection limit was calculated using the relationship as follow:

\[
\text{LOD} = 3\sigma, \text{ where } \sigma = \text{ standard deviation of blank samples/slope.}
\]

To calculate the relative standard deviation, the emission measurements of ten blank samples of NAC were recorded. The calibration curves (fluorescence intensity vs [Al\(^{3+}\)]) was plotted and then the obtained slope was used to calculate the LOD using the 3\(\sigma\) method. Same method was used in all cases.
CHAPTER 3
RESULTS AND DISCUSSION

In this chapter, the characterization of intermediate compounds and chemosensing receptor compounds using FT-IR, $^1$H-NMR and $^{13}$C-NMR spectroscopy will be discussed. The spectra for all the intermediate compounds and chemosensing receptor compounds were reported in detail. Accompanied with this spectral characterization, chemosensing behaviour was examined by UV-Vis and fluorescence measurements.

3.1 Characterization of Key Intermediates and Receptor Compounds by Spectroscopic Techniques

All the title compounds were characterized via infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (FT-NMR) and CHN microanalysis. The empirical formula, molecular weights of compounds and percentages of C, H and N as well as percentage of yield are shown in Table 3.1. According to the data in the table, the percentage of nitrogen is negligible in both NAC and CAC compounds because there is no nitrogen element included.
Table 3.1  Empirical formula, molecular weights (MW), percentage yields (%) and CHN microanalytical data of all title compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Formulas</th>
<th>MW (g/mol)</th>
<th>% Yield</th>
<th>% C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% H&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% N&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>348.35</td>
<td>78</td>
<td>74.82 (72.41)</td>
<td>4.50 (4.63)</td>
<td>0.33 (0)</td>
</tr>
<tr>
<td>CAC</td>
<td>C&lt;sub&gt;28&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>450.44</td>
<td>81</td>
<td>75.02 (74.66)</td>
<td>4.27 (4.03)</td>
<td>0.11 (0)</td>
</tr>
<tr>
<td>DNAC</td>
<td>C&lt;sub&gt;26&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;NO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>413.47</td>
<td>37</td>
<td>76.13 (75.53)</td>
<td>5.49 (5.61)</td>
<td>3.57 (3.39)</td>
</tr>
</tbody>
</table>

Note:  <sup>a</sup>Calculated values in parentheses.
3.1.1 Fourier transform infrared spectroscopy (FT-IR)

The functional groups of all the title compounds were determined by using infrared spectroscopy. The spectral data are summarized in Table 3.2. Based on the spectra, two medium absorptions observed in high frequency region of spectra which are 3616–3385 cm\(^{-1}\) and 3373–3200 cm\(^{-1}\) can be attributed to aromatic hydroxyl group and carboxyl group, respectively. A weak absorption band around 3114–2913 cm\(^{-1}\) corresponds to the symmetry and asymmetry stretching of -CH- group. The chalcone C=O group gives rise to an absorption band with strong intensity around 1622–1603 cm\(^{-1}\). Another strong absorption band around 1511–1451 cm\(^{-1}\) was attributed to the aromatic C=C group. The FT-IR spectra of all the receptor molecules are shown in Figures 3.1–3.3.
Table 3.2  FT-IR absorption frequencies (v/cm\(^{-1}\)) of selected functional groups and relative intensities for all title compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>v (O-H)</th>
<th>v (O-H) acid</th>
<th>v (C-H) aliphatic</th>
<th>v (C=O) chalcone</th>
<th>v(C=C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>3616(m)</td>
<td>3373(m)</td>
<td>3011, 2913(w)</td>
<td>1622(s)</td>
<td>1511, 1460(s)</td>
</tr>
<tr>
<td>CAC</td>
<td>3559(m)</td>
<td>3200(m)</td>
<td>3059, 2915(w)</td>
<td>1603(s)</td>
<td>1510, 1467(s)</td>
</tr>
<tr>
<td>DNAC</td>
<td>3385(m)</td>
<td>–</td>
<td>3114, 2981(w)</td>
<td>1614(s)</td>
<td>1506, 1451(s)</td>
</tr>
</tbody>
</table>

Abbreviations: s, strong; m, medium; w, weak
Figure 3.1  FT-IR spectrum of receptor NAC
Figure 3.2 FT-IR spectrum of receptor CAC
Figure 3.3 FT-IR spectrum of receptor DNAC

- $v (\text{O-H})$ at 3385 cm$^{-1}$
- $v (\text{C-H})$ aliphatic at 2981 cm$^{-1}$
- $v (\text{C} = \text{O})$ chalcone at 1614 cm$^{-1}$
- $v (\text{C} = \text{C})$ at 1508 cm$^{-1}$
- $v (\text{C}-\text{H})$ aliphatic at 3114 cm$^{-1}$
3.1.2 $^1$H-NMR Spectroscopy

The title compounds were analyzed with $^1$H-NMR spectroscopy to further elucidate the molecular structures of the compounds in order to substantiate the FT-IR spectroscopic results. The protons in the same environment resonate at the same field strength while for the protons in different environment resonate at different field strength. The $^1$H chemical shifts data of the intermediate and title compounds are tabulated in Tables 3.3 and 3.4, respectively. $^1$H spectra of intermediate and receptor compounds are given in Figures 3.4–3.9.

In the $^1$H-NMR spectrum of intermediate NAEE, the singlet at $\delta = 11.00$ ppm was attributed to the presence of aldehydic proton which was observed as a singlet because there was no adjacent proton for coupling. Aromatic protons gave rise to six different sets of signals at the low field region within $\delta = 7.13–9.29$ ppm. The oxymethylene protons (O-CH$_2$) attached to naphthalene having a more down-field shift value at $\delta = 4.87$ ppm with a singlet signal owing to the deshielding effect by the electron-withdrawing oxygen atom and this signal confirmed that the Williamson etherification reaction has occurred [Wiley & Crawford 1965]. Another oxymethylene protons from the ethyl chloroacetate gave rise to a set of multiplet at $\delta = 4.26–4.30$ ppm. A triplet was observed at $\delta = 1.28–1.31$ ppm which can be attributed to methylene protons of the ethyl chloroacetate moiety.

In the $^1$H-NMR spectrum of intermediate ABCM, the C=CH proton from the coumarin moiety gave rise to a singlet at $\delta = 9.32$ ppm. This signal indicated that the condensation reaction has occurred. Aromatic protons gave rise to six different sets of signals at the low field region within $\delta = 7.47–8.38$ ppm. A singlet was observed at $\delta = 2.78$ ppm which can be attributed to three carbonyl protons.
Inspection on the $^1$H-NMR of the intermediate ADCM shows a singlet at chemical shift $\delta = 8.43$ ppm corresponding to the C=CH proton at the 4-position of coumarin ring. This signal showed that the condensation reaction has occurred. Aromatic protons gave rise to three different sets of signals at the low field region within $\delta = 6.47$–7.41 ppm. The N-alkylated methylene protons having a more down-field shift value at $\delta = 3.45$–3.47 ppm due to the deshielding effect by the electron-withdrawing nitrogen atom. A singlet was observed at $\delta = 2.68$ ppm which can be attributed to three carbonyl protons. A triplet was observed at $\delta = 1.23$–1.26 ppm which can be attributed to methylene protons of the 4-(diethylamino) salicylaldehyde moiety.

The absence of the carboxylic acid and hydroxyl protons in the receptors NAC and CAC was due to the hydrogen-deuterium exchange in D$_2$O solvent. Based on the $^1$H-NMR spectrum of receptor NAC, two doublets at $\delta = 7.17$–7.19 ppm and $\delta = 8.31$–8.32 ppm indicating the formation of $\alpha, \beta$-unsaturated carbonyl linkage of the chalcone system. An oxymethylene protons having a more down-field shift value at $\delta = 4.67$ ppm with a singlet signal.

In the $^1$H-NMR spectrum of receptor CAC, formation of $\alpha, \beta$-unsaturated carbonyl linkage of the chalcone moiety was confirmed by the doublets appeared at $\delta = 6.82$–6.84 ppm and $\delta = 7.62$–7.64 ppm. For receptor DNAC, a singlet appeared at $\delta = 10.21$ ppm was attributed to the hydroxyl proton in deuterated chloroform solvent. The two doublets that proved the formation of $\alpha, \beta$-unsaturated carbonyl linkage of the chalcone group appeared at respective $\delta = 6.97$–6.98 ppm and 8.02–8.05 ppm.
Table 3.3  $^1$H-NMR chemical shifts (δ / ppm) of the intermediate compounds (multiplicity in parentheses).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ (C-H alkyl)</td>
</tr>
<tr>
<td>NAEE</td>
<td>1.28–1.31 (1t), 3H</td>
</tr>
<tr>
<td>ABCM</td>
<td>–</td>
</tr>
<tr>
<td>ADCM</td>
<td>1.23–1.26 (1t), 6H</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, t = triplet, m = multiplet.
Table 3.4  
\(^1\)H-NMR chemical shifts (\(\delta / \text{ppm}\)) of the receptor compounds (multiplicity in parentheses).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
<th>Chemical shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta ) (C-H alkyl)</td>
<td>(\delta ) (N-CH(_2))</td>
<td>(\delta ) (O-CH(_2))</td>
<td>(\delta ) (C=CH, (\alpha) chalcone)</td>
<td>(\delta ) (C=CH, (\beta) chalcone)</td>
<td>(\delta ) (Ar-H)</td>
<td>(\delta ) (C=CH coumarin)</td>
</tr>
<tr>
<td>NAC</td>
<td>–</td>
<td>–</td>
<td>4.67 (1s), 2H</td>
<td>7.17–7.19 (1d), 1H</td>
<td>8.31–8.32 (1d), 1H</td>
<td>6.67–8.25 (6d, 4t), 10H</td>
<td>–</td>
</tr>
<tr>
<td>CAC</td>
<td>–</td>
<td>–</td>
<td>4.08 (1s), 2H</td>
<td>6.82–6.84 (1d), 1H</td>
<td>7.62–7.64 (1d), 1H</td>
<td>6.49–7.59 (8d, 4t), 12H</td>
<td>8.00 (1s), 1H</td>
</tr>
<tr>
<td>DNAC</td>
<td>1.11–1.15 (1t), 6H</td>
<td>3.41–3.49 (1m), 4H</td>
<td>–</td>
<td>6.97–6.98 (1d), 1H</td>
<td>8.02–8.05 (1d), 1H</td>
<td>6.58–7.96 (1s, 6d, 2t), 9H</td>
<td>8.55 (1s), 1H</td>
</tr>
</tbody>
</table>

\(s = \text{singlet, } d = \text{doublet, } t = \text{triplet, } m = \text{multiplet.}\)
Figure 3.4  $^1$H-NMR spectrum of compound NAEE.
Figure 3.5  $^1$H-NMR spectrum of compound ABCM.
Figure 3.6 \(^1\)H-NMR spectrum of compound ADCM.
Figure 3.7  
$^1$H-NMR spectrum of compound NAC.
Figure 3.8  $^1$H-NMR spectrum of compound CAC.
Figure 3.9  $^1$H-NMR spectrum of compound DNAC.
3.1.3 $^{13}$C-NMR Spectroscopy

The $^{13}$C-NMR spectroscopic method was carried out to further substantiate the molecular structure of the receptor compounds. $^{13}$C-NMR gives information about the number of magnetically distinct carbon atoms present in the compound. As expected, the signals observed in the $^{13}$C-NMR spectra were found to be consistent with the proposed structure.

In $^{13}$C-NMR analysis, peak ascribable to the oxymethylene carbon was observed at around $\delta = 67.34$–$67.74$ ppm. The signals associated with the aromatic carbons were resonated in the range of $\delta = 96.58$–$157.56$ ppm. The quaternary aromatic carbon singly bonded to an oxygen atom has been assigned at around $\delta = 155.59$–$165.77$ ppm. The signal around $\delta = 149.92$ ppm corresponded to quaternary carbon bonded to the hydroxyl group. The carbonyl carbon in the acid and chalcone groups gave rise to a peak at $\delta = 176.54$–$176.58$ ppm and $186.23$–$200.10$ ppm, respectively.

In the $^{13}$C-NMR spectrum of receptor DNAC, the signal resonates around $45.10$ ppm corresponds to N-alkylated carbon while the signal appears at around $12.77$ ppm was attributed to aliphatic carbon. The peaks at around $\delta = 158.62$–$168.35$ ppm and $\delta = 160.30$–$174.23$ ppm can be attributed to the C=O carbonyl carbon and the C=C carbon at the 4-position of coumarin ring in receptor CAC and DNAC, which confirmed the formation of coumarin [Khan et al. 2017]. The $^{13}$C-NMR spectra of compounds shown in Figures 3.10–3.12 and their chemical shift values are presented in Tables 3.5.
Table 3.5  $^{13}$C-NMR chemical shifts (δ / ppm) of the receptor compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aliphatic (-CH$_3$)</th>
<th>N-CH$_2$</th>
<th>O-CH$_2$</th>
<th>C$_{aromatic}$</th>
<th>C$_{aromatic}$-O</th>
<th>C$_{aromatic}$-OH</th>
<th>Coumarin (C=C)</th>
<th>Coumarin (C=O)</th>
<th>Acid (C=O)</th>
<th>Chalcone (C=O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>–</td>
<td>–</td>
<td>67.69</td>
<td>136.19, 136.04, 135.08, 132.22, 132.01, 131.85, 130.65, 128.92, 128.68, 127.63, 124.21, 123.56, 122.56, 117.21, 114.02, 113.76, 155.07, 139.67, 137.85, 137.03, 133.90, 131.68, 131.37, 128.41, 127.37, 126.29, 125.81, 124.55, 123.89, 123.09, 122.78, 120.65, 116.34, 115.62, 114.88, 113.31</td>
<td>155.59</td>
<td>169.46</td>
<td>–</td>
<td>–</td>
<td>176.78</td>
<td>198.48</td>
</tr>
<tr>
<td>CAC</td>
<td>–</td>
<td>–</td>
<td>67.34</td>
<td>165.77</td>
<td>–</td>
<td>168.35</td>
<td>174.23</td>
<td>176.54</td>
<td>200.10</td>
<td></td>
</tr>
<tr>
<td>DNAC</td>
<td>12.77</td>
<td>45.10</td>
<td>–</td>
<td>–</td>
<td>153.32</td>
<td>158.62</td>
<td>160.30</td>
<td>–</td>
<td>186.23</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.10  $^{13}$C-NMR spectrum of compound NAC.
Figure 3.11  $^{13}$C-NMR spectrum of compound CAC.
Figure 3.12  $^{13}$C-NMR spectrum of compound DNAC.
3.2 Spectral and Chemosensing Studies of Chalcone-based Receptor (NAC) for Al$^{3+}$ ion

The NAC chalcone-based azobenzene chemosensor was designed and developed for the selective sensing of Al$^{3+}$ ions in water medium with pH 7. The synthesized receptor NAC was composed of a carboxyl group for selective recognition of metal ions and the chalcone unit is responsible for signal transduction during spectroscopic studies. The hydrophilic nature of carboxyl moiety has enabled NAC to be completely dissolved in water phase and its hard base properties increased the selectivity of receptor NAC towards hard acid, Al$^{3+}$. This novel chemosensing receptor NAC exhibited completely water solubility and showed highly selective fluorometric response towards Al$^{3+}$ only in water without utilizing any organic solvent.

3.2.1 Photophysical studies of NAC

The cation recognition ability of receptor NAC was investigated by adding various metal ions (Ag$^+$, Al$^{3+}$, Ca$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, K$^+$, Mn$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$) in the form of their perchlorate salts to the solution of receptor NAC in 100% H$_2$O. The chemosensing behavior of NAC was initially studied by UV-vis analysis. As shown in Figure 3.13, the free receptor NAC exhibited a maximum absorbance at 399 nm which was assigned to $\pi \rightarrow \pi^*$ transition of the chalcone unit. A red shift to 416 nm occurred upon addition of Al$^{3+}$ ions owing to the binding of aluminium with the receptor. However, the other cations Ag$^+$, Ca$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, K$^+$, Mn$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$ with NAC did not induce any significant color change. Addition of other cation such as Cu$^{2+}$ brought to a slight red shift as compared with Al$^{3+}$ which might be due to the minor interaction with receptor NAC. Hence, it implies that Al$^{3+}$ can easily been identified as compared to all other cations.
An absorption titration experiments (Figure 3.14) was carried out in order to investigate the binding properties of receptor NAC towards Al\(^{3+}\). It can be found that upon treating NAC with increasing concentration of Al\(^{3+}\) ions, the absorbance band has gradually red-shifted. The existence of an isosbestic point at 435 nm clearly supports the conversion of receptor NAC to the NAC-Al\(^{3+}\) complex.

Figure 3.15 exhibits the studies of the fluorescent response behaviors of NAC towards different metal ions in water. The receptor NAC alone exhibited negligible fluorescent emission upon excitation. By adding the Al\(^{3+}\) ion, the receptor NAC showed a fluorescent emission at 575 nm. Although the increment in fluorescence intensity after the formation of NAC-Al\(^{3+}\) complex was little (from 0 to 33 a.u.), but fluorescence enhancement can be clearly seen for Al\(^{3+}\) only indicating that sensor NAC shows high selectivity towards Al\(^{3+}\) over other metal ions.
Figure 3.13  UV-vis absorption spectra of chemosensor NAC (100 μM) in the absence and presence of various metal ions (100μM) in water.
Figure 3.14  Absorption spectral changes of NAC (100 μM) in the presence of different concentrations of Al$^{3+}$ ions in water.
Figure 3.15  Fluorescence emission spectra of sensor **NAC** (100 μM) in water upon addition of metal perchlorate salts (1 equiv.) such as Ag$^{+}$, Al$^{3+}$, Ca$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, K$^{+}$, Mn$^{2+}$, Na$^{+}$, Ni$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$. 
Further investigation on the binding affinity of sensor NAC with Al\(^{3+}\) was performed by fluorescent titration experiments. Figure 3.16 exhibits a plot of change in the fluorescent intensity at 575 nm as a function of Al\(^{3+}\) ion concentration. This can be explained by the activation of chelation enhanced fluorescence (CHEF) as the complexation of NAC with Al\(^{3+}\) brings rigidity to the system [Sen et al. 2012].

From the absorbance titration results, the binding constant for receptor NAC with Al\(^{3+}\) ion in water was found to be \(K_a = 0.21 \times 10^2\) M\(^{-2}\) by a Benesi-Hildebrand plot using the following equation 3.1 [Zhu et al. 2017].

\[
\frac{1}{(A-A_o)} = \frac{1}{K_a(A_{\text{max}}-A_o)[Al^{3+}]^{0.5}} + \frac{1}{A_{\text{max}}-A_o} \tag{3.1}
\]

where \((A-A_o)\) is the absorbance difference between the apparent metal complex and the free ligand\((A_{\text{max}}-A_o)\) is the absorption difference at saturation. The association constant value, \(K_a\) was evaluated graphically by plotting \(1/(A- A_o)\) against \(1/[Al^{3+}]^{0.5}\). Plot gave a straight line, and the \(K_a\) value was obtained from the slope and intercept of this line as shown in Figure 3.17.

To determine the sensitivity of probe NAC towards Al\(^{3+}\) ions, a linear calibration curve was plotted in Figure 3.18 between the relative fluorescence intensity and the concentration of Al\(^{3+}\) ion from \(6 \times 10^{-4}\) M to \(1.5 \times 10^{-3}\) M. The detection limit was calculated based on fluorescence titration and the lowest concentration of Al\(^{3+}\) that can be detected by receptor NAC is about \(5.66 \times 10^{-8}\) M which is 0.002 mg/L. The equation of the detection limit as follows [Das et al. 2015]:

\[
\text{Limit of detection (LOD)} = 3\sigma \tag{3.2}
\]

Where \(\sigma\) is the division of standard deviation of the blank solution of probe NAC by the slope of the calibration curve.
Figure 3.16  Fluorescence titration spectra of chemosensor NAC (100 μM) upon addition of different concentrations of Al$^{3+}$ ions in water.
Figure 3.17  A Benesi-Hildebrand plot based on absorption intensity of NAC with added Al$^{3+}$ concentrations in water.
Figure 3.18  A plot between the relative fluorescence intensity and the concentration of Al$^{3+}$ ion to determine the detection limit of probe NAC towards Al$^{3+}$ ions.

$y = 24478x - 7.283$

$R^2 = 0.994$
3.2.2 Competition with other cations

In order to examine the selectivity of NAC towards the detection of Al\(^{3+}\), competition experiment with other cations was performed. From the bar chart showed in Figure 3.19, no significant interference was observed in the presence of other competing metal ions. Interestingly, all the metal ions including Cu\(^{2+}\) and Fe\(^{2+}\) which are known as fluorescence quenchers were also unable to diminish the fluorescence intensity of the NAC- Al\(^{3+}\) complex and the fluorescence enhancement induced by Al\(^{3+}\) was retained [He et al. 2010]. Therefore, there is no metal ion that able to alter the fluorescence response of NAC towards Al\(^{3+}\).

3.2.3 Determination of stoichiometry between NAC and Al\(^{3+}\) ions

The binding stoichiometry of chemosensor NAC with Al\(^{3+}\) was indicated by Job’s plot method. In the Job’s plot method [MacCarthy 1978], the total molar concentration of the NAC and Al\(^{3+}\) ion are held constant, but their mole fractions are varied. The presence of a maximum absorbance at mole fraction 0.6 suggests the formation of a hexa-coordinated complex of two molecules of NAC with one molecule of Al\(^{3+}\) ion. The Job’s plot and binding mode was proposed as shown in Figure 3.20.

3.2.4 \(^1\)H-NMR spectral studies of NAC-Al\(^{3+}\) complex

The interaction between NAC and Al\(^{3+}\) ion was investigated by \(^1\)H-NMR titration experiments (Figure 3.21) in D\(_2\)O. The hydrogen-deuterium exchange in D\(_2\)O brings to the absence of the protons of carboxyl and hydroxyl groups. It can be deduced from the \(^1\)H-NMR spectra that the coordination bond between receptor NAC and Al\(^{3+}\) led to resonance signals shift and peaks broadening as the electron density in the aromatic rings were changed. The oxymethylene protons were shifted upfield by
0.11 ppm from 4.56 to 4.67 ppm. These results suggested that the two oxygen atoms of carboxyl and hydroxyl groups and the oxygen atom of the oxymethylene group involved in coordination. The binding mode was proposed as shown in Figure 3.22.

It can be seen in Figure 3.22 that two molecules of NAC in ionic forms were coordinated to a central Al$^{3+}$ ion via the oxygen atoms of carboxyl and hydroxyl groups along with the oxygen atom of the oxymethylene group.
Figure 3.19  Competitive selectivity of NAC towards Al\textsuperscript{3+} in the presence of other metal ions (1 equiv.) in water.
Figure 3.20  Job’s plot for the interaction of NAC with Al\(^{3+}\) in water indicating 2:1 stoichiometry for NAC-Al\(^{3+}\) complex.
Figure 3.21 $^1$H-NMR titration of NAC with Al$^{3+}$ ions in D$_2$O.
Figure 3.22  The binding structure after the interaction between NAC and Al$^{3+}$ ions.
3.3 Spectral and Chemosensing Studies of Chalcone-based Receptor (CAC) for Al$^{3+}$ ion

In previous set of studies, the synthesized chalcone-based chemosensor has some disadvantages like poor color change and weak fluorescent enhancement. To overcome these issues, a new receptor has been designed and synthesized by coupling of chalcone chemosensor with electron rich coumarin unit to enhance its chemosensing property with Al$^{3+}$ ion which is elucidated by naked eye detection method in 100% water with pH 7.

3.3.1 Photophysical studies of CAC

The binding behaviour of receptor CAC was investigated against different metal ions like Ag$^+$, Al$^{3+}$, Ca$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, K$^+$, Mn$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$ using UV-Vis spectrophotometer both in separate and combined manners. According to Figure 3.23, a remarkable formation of a new hump has been observed at 610.2 nm upon addition of Al$^{3+}$ ions only to the probe of CAC. The appearance of this new hump suggests the binding of aluminium with receptor CAC which brings to the color change from yellow to cyan. However, the other metal ions do not induce any spectral changes upon addition and hence the color changes are negligible.

To acquire more information on the complexation behaviour of receptor CAC with Al$^{3+}$ ion, UV-vis titration experiments (Figure 3.24) were carried out. In this experiment Al(ClO$_4$)$_3$ as aluminium source and it was gradually added to a solution of receptor CAC. A gradual increase in absorption intensity at 610.2 nm upon treating of CAC with increasing concentration of aluminium indicates the conversion to CAC-Al$^{3+}$ complex from CAC.
The detection limit for the recognition of aluminium was determined based on the UV titration (Figure 3.25) with the concentration of aluminium range from $1 \times 10^{-4}$ M to $7 \times 10^{-4}$ M. It was calculated from 3σ method and the value is $2.21 \times 10^{-5}$ M which is 0.78 mg/L. Figure 3.26 depicts the Benesi-Hildebrand plot where the concentration of the ligand was kept constant with increasing amount of metal ions. The $K_a$ was then calculated from the plot using the equation 3.1 to give a value of $5.91 \text{ M}^{-2}$.
Figure 3.23  UV-vis absorption spectra of chemosensor CAC (100 μM) in the absence and presence of various metal ions (100μM) such as Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Cu²⁺, Fe²⁺, Hg²⁺, K⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺ and Zn²⁺ in water.
Figure 3.24  Absorption spectral changes of CAC (100 μM) in the presence of different concentrations of Al$^{3+}$ ions in water.
Figure 3.25  A plot between the relative absorption intensity and the concentration of Al\(^{3+}\) ion to determine the detection limit of probe CAC towards Al\(^{3+}\) ions.
Figure 3.26  Benesi-Hildebrant plot based on absorption intensity of CAC with added Al$^{3+}$ ions.

\[ y = 0.9787x - 33.565 \]

\[ R^2 = 0.9297 \]
3.3.2 Competition with other cations

Competition experiment was then carried out to check for the selectivity of receptor CAC towards Al\(^{3+}\). From the bar chart shown in Figure 3.27, there is no significant interference caused by other competing metal ions. The color change induced by aluminum was retained. Therefore, it can be concluded that probe CAC is highly selective toward Al\(^{3+}\).

3.3.3 Determination of stoichiometry between CAC and Al\(^{3+}\) ions

The binding stoichiometry between the probe CAC and Al\(^{3+}\) was determined by UV-Vis spectrophotometer. The total concentration of CAC and Al\(^{3+}\) ion was held constant while the mole fraction of Al\(^{3+}\) ions changed and the absorbance value plotted against mole fraction as displayed in Figure 3.28. Upon addition of increasing concentration of Al\(^{3+}\), the variation in absorbance indicated the formation of 2:1 stoichiometry CAC-Al\(^{3+}\) complex with maximum absorbance value occurred at mole fraction of 0.6.

3.3.4 \(^1H\)-NMR spectral studies of CAC-Al\(^{3+}\) complex

To get more insight into complexation behavior of CAC with Al\(^{3+}\), \(^1H\)-NMR studies were carried out by adding different equivalent of Al\(^{3+}\) into CAC.

Figure 3.29 displayed the stacked plots of \(^1H\)-NMR spectra before and after the addition of Al\(^{3+}\). The protons of carboxyl and hydroxyl groups were absent due to the hydrogen-deuterium exchange in D\(_2\)O. Upon coordination between CAC and aluminium, most of the aromatic protons peaks had shifted due to the change in electron density of the aromatic rings. The oxymethylene protons were shifted downfield from 4.11 to 4.16 ppm. Further addition of Al\(^{3+}\) ions into the solution of
CAC induce peak broadening due to the paramagnetic nature of Al\(^{3+}\) ion. The binding mode was proposed as shown in Figure 3.30. It can be seen in Figure 3.30 that the complex consists of two probes coordinated to a central Al\(^{3+}\) ion via the oxygen atoms of carboxyl and hydroxyl groups along with the oxygen atom of the oxymethylene group.
Figure 3.27  Competitive selectivity of CAC towards Al$^{3+}$ in the presence of other metal ions (1 equiv.) in water.
Figure 3.28  Job’s plot for the interaction of CAC with Al$^{3+}$ in water indicating 2:1 stoichiometry for CAC-Al$^{3+}$ complex.
Figure 3.29  Stacked $^1$H-NMR spectra of CAC before and after the addition of Al$^{3+}$ in D$_2$O.
Figure 3.30  Proposed structure of probe CAC before and after the complexation with Al$^{3+}$ ions.
3.4 Spectral and Chemosensing Studies of Chalcone-based Receptor (DNAC) for F⁻ ion

Synthetic anion receptor chemistry is in its infancy in comparison with the more well-established field of cation recognition. Lately, fluorescence recognition of anions as well as colorimetric method has drawn the awareness from research community [Kao et al. 2017]. In the present work, a novel chalcone-based chemosensor armed by a coumarin which holding a strong flurogenic behaviour and a naphthol in order to enhance the selectivity of probe toward anions was synthesized.

3.4.1 Photophysical studies of DNAC

The stock solutions for probe DNAC at concentration of 2.0×10⁻⁵ M solutions in 1:1 acetonitrile/pH 6 aqueous MOPS and anions at concentration of 1.0×10⁻³ M were prepared. Anion binding abilities of receptor DNAC were observed by introducing the various anions (F⁻, Cl⁻, Br⁻, I⁻ and OH⁻) in CH₃CN using absorption and fluorescence spectroscopy at excitation wavelength of 420 nm as evidenced in Figure 3.31. In UV-vis titration study, probe DNAC did not show any significant changes in colour as well as in the absorption spectrum after the addition of F⁻ ions. In the absence of anion, the fluorescence spectrum of receptor DNAC showed an emission band at 524 nm. By adding the excess tetrabutylammonium salts of anions such as F⁻, Cl⁻, Br⁻, I⁻ and OH⁻ to acetonitrile solution of probe DNAC, an enhancement in the fluorescence intensity with fluoride ion was observed while other anions does not display such changes but only an insignificant increase in fluorescence intensity.

This observation can be attributed to the formation of hydrogen bonding between the receptor DNAC and F⁻ ions and subsequently lead to the formation of
HF$_2^-$ species such changes could be ascertained in Figure 3.32. The strong interaction of the F$^-$ ions with the probe DNAC was due to their high electronegativity and smallest in size as compared to the other halides.

An increase in the emission intensity of probe DNAC at 524 nm was induced when the probe DNAC is titrated with increasing concentration of F$^-$ (Figure 3.33). The probable reason can be credited to the ICT developed by the deprotonation of OH from napthol unit to the chalcone moiety in the probe DNAC and formation of HF$_2^-$ complex as reported in azo-based phenyl hydrazone and thiosemicarbozone [Li et al. 2010, Shao et al. 2009]. The occurrence of the deprotonation is depicted in Figure 3.32.

The donor–acceptor–donor architecture (D–A–D) structure in chalcone as shown in Figure 3.34 brings about an efficient ICT especially when the electron density in the naphthol unit is increased by excessive F$^-$ ions, in which the C=C–C=O group behaves as the acceptor while the naphthol moiety serves as donor. In such case, the deprotonation will take place afterwards which influences the efficient electron transfer from donor to acceptor and in turn increases the fluorescence intensity.

The sensitivity of chemosensor DNAC towards F$^-$ ions was determined by plotting a linear calibration curve between the relative fluorescence intensity and the concentration of F$^-$ ions from 5×10$^{-4}$ M to 1.5×10$^{-3}$ M as displayed in Figure 3.35. Based on the fluorescence titration, the detection limit was calculated from 3σ method and the lowest concentration of F$^-$ ions that can be detected by probe DNAC is 8.72×10$^{-8}$ M which is 0.0031 mg/L.
Figure 3.31  Fluorescence spectra of probe DNAC ($2.0 \times 10^{-5}$ M) with different anions (2 eq.).
Figure 3.32  Proposed structures relating to the deprotonation and formation of HF$_2^-$ species.
Figure 3.33  Fluorescence titration spectra of probe DNAC (2.0×10^{-5} M) with F^{-} ions from 0 to 5 eq.
Figure 3.34 Proposed intramolecular charge transfer from naphthol to chalcone core.
Figure 3.35 A plot between the relative fluorescence intensity and the concentration of F\(^{-}\) ions to determine the detection limit of DNAC towards F\(^{-}\) ions.

\[
y = 33727x - 15.629 \\
R^2 = 0.9896
\]
3.4.2 Competition with other anions

Competitive studies were carried out to check the binding stability of the probe \textbf{DNAC}. Both of the stock solution of probe \textbf{DNAC} and various anions were prepared in a standard flask separately in CH$_3$CN with a similar molar concentration of 50 μM. Each of the remaining anion Cl$^-$, Br$^-$, I$^-$ and OH$^-$ was added in the respective cuvette after the addition of two equivalents of F$^-$ ion to the receptor \textbf{DNAC}. The solution underwent a mixing for a few seconds before the fluorescence measurement at room temperature. As displayed in Figure 3.36, there was no change in the fluorescence intensity of the \textbf{DNAC}-F$^-$ complex after the addition of other anions which verified that during the F$^-$ ion detection in CH$_3$CN, there was no interference by other anions. Therefore, a conclusion can be drawn is the present chalcone-based sensor can be a suitable candidate for F$^-$ anion detection despite having other competing anions in the same mixture.

3.4.3 Determination of stoichiometry between DNAC and F$^-$ ions

The stoichiometry of the complex formation between the probe \textbf{DNAC} and fluoride ions was studied by changes in fluorescence intensity of the complex at 524 nm and plotted against the molar fraction of the F$^-$ ion. A solutions containing probe \textbf{DNAC} and F$^-$ were prepared in CH$_3$CN such that the sum of the total concentration of anion and \textbf{DNAC} remains constant as 50 μM. The mole fraction (X) of F$^-$ was varied from 0.1 to 1.0. In Figure 3.37, the change in fluorescence intensity with varying concentration of F$^-$ ions indicating the formation of 1:1 complex with K$_a$ of 3.33×10$^{-5}$ M.
Figure 3.36  Fluorescence response of probe **DNAC** with and without competing anions.
Figure 3.37  Fluorescence Job’s plot for probe DNAC with F\(^-\) in acetonitrile.
CHAPTER 4
CONCLUSION

In this thesis, three novel chalcone-based derivatives were synthesized by reacting the corresponding aldehydes and ketones in the base medium to act as colorimetric and fluorometric highly selective chemosensors for the detection of Al$^{3+}$ and F$^{-}$ ions respectively. The synthesized compounds were subjected to various spectroscopic studies after purification. The chemical structure of all the compounds was acknowledged by FT-IR spectroscopy. In all the receptor compounds, C=O group in chalcone moiety absorbs around 1603 cm$^{-1}$ to 1622 cm$^{-1}$ and a broad stretching band at around 3385 cm$^{-1}$ to 3616 cm$^{-1}$ was attributed to hydroxyl group as one of the binding unit.

$^1$H-NMR spectra of all the receptors was elucidated in Chapter 3, a doublet corresponds to alpha, $\alpha$ proton of carbonyl group in the chalcone unit resonates around 6.82 ppm to 7.19 ppm. Another doublet at 7.62 ppm to 8.32 ppm ascribed to the beta, $\beta$ proton of carbonyl group in chalcone unit which thereby confirms the formation of chalcone moiety in receptors NAC, CAC and DNAC.

$^{13}$C-NMR spectra of all the receptors exhibit carbonyl carbon peak resonates 186.23 ppm to 200.10 ppm suggest the presence of chalcone group. On the other side of the receptor DNAC, the signal resonates around 45.10 ppm corresponds to N-alkylated carbon and the signal at around 12.77 ppm resonate aliphatic carbon. As expected, the number of peaks observed in the $^{13}$C-NMR spectra was found in agreement with the proposed structure.
After characterization, receptors NAC and CAC were brought in contact with metal ions (Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Cu²⁺, Fe²⁺, Hg²⁺, K⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺ and Zn²⁺), while receptor DNAC was tested with the anions (F⁻, Cl⁻, Br⁻, I⁻ and OH⁻).

The formation of host-ion complex was detected by observing the color changes of host solutions by naked eye, by UV-Vis titrations and by observing the intensity changes in hosts’ fluorescence spectra.

NAC receptor was synthesised by condensation of 2-hydroxy-1-napthaldehyde derivative with 2’-hydroxyacetophenone unit. The binding properties of NAC were determined through the titration of receptor with various metal ions and examined in fluorescence spectroscopic studies. This novel water soluble probe displayed highly selective fluorometric enhancement towards Al³⁺ ions over other cations. Enhanced fluorescence can be ascribed to the improved rigidity in the system through the activation of CHEF after complexation. Upon addition of Al³⁺, the receptor NAC exhibited a fluorescent emission at 575 nm with 2:1 complex between NAC and Al³⁺ ion. The association constant, Kₐ was determined as 0.21×10² M⁻² by a Benesi-Hildebrant plot indicates that receptor NAC have a good binding affinity with Al³⁺ ion. In ¹H-NMR titration experiment, the oxymethylene protons were shifted upfield by 0.11 ppm from 4.56 to 4.67 ppm reveal that the two oxygen atoms of carboxyl and hydroxyl groups and the oxygen atom of the oxymethylene group took part in coordination.

CAC receptor has been successfully synthesized by constructing coumarin unit appended on the 2-hydroxy-1-napthaldehyde derivative. The chemosensing properties of CAC were examined against various metal ions in purely water medium
and showed excellent naked eye selectivity towards Al\(^{3+}\) ion with difference from yellow to cyan. The appearance of a new hump in the absorption spectrum after the addition of aluminium ions at 610.2 nm implies the increment in internal charge transfer mechanism in coumarin chromophore which can clearly be identified by naked eye. An association constant of \(K_a = 5.91 \text{ M}^{-2}\) was measured from Benesi-Hildebrand linear regression with 2:1 coordination between receptor CAC and Al\(^{3+}\) ion from Job’s plot study. Besides that, the resonating signal shift was observed in \(^1\)H-NMR from 4.11 ppm to 4.16 ppm for the oxymethylene proton suggests the change in electron density in the CAC receptor system during the complexation process.

Receptor DNAC has been successfully synthesized by condensing 2-hydroxy-1-napthaldehyde derivative with coumarin derivative. The chemosensing properties of DNAC were identified through the conduction of fluorescence titration with various anions and monitored using fluorescence spectrophotometer. Free receptor DNAC displays an emission band at 524 nm in the fluorescence spectrum. Addition of F\(^-\) ion shows an enhancement in the fluorescence intensity which can be ascribed to the formation of hydrogen bonding between the probe DNAC and F\(^-\) ions. Increasing concentration of F\(^-\) ions added to the solution of probe DNAC gave rise to the heightening of the emission intensity of probe at 524 nm due to the ICT developed by the fluoride induced deprotonation in napthol unit and formation of HF\(_2^+\) complex. Job’s plot revealed that the 1:1 coordination between DNAC and F\(^-\) ion with an association constant of \(K_a=3.33\times10^5\) M.
Finally, it can be concluded that chemosensing behaviours of the chalcone-based receptors may be used in environmental and biological applications for the recognition of the targeted ions of Al$^{3+}$ and F$^-$.  

4.1 Scope of future work

The chalcone-based chemosensors for the selective detection of cations or anions as reported in current work can be suitably functionalized with different chelating units. However, in order to apply such chemosensors in a real time analysis, it is essential to incorporate the water soluble chromophore and fluorophore into the molecules. Besides, through the comparison with the naked-eye chemosensor, the fluorescence chemosensor will be given greater attention owing to its inclination to lower molar detection limit.
REFERENCES


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LIST OF PUBLICATION

International Journals

