Optical and Electrochemical Sensors for the Detection of Metal Ions and Nerve Gas Mimics via Fluorescein and Coumarin Derivatives

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OPTICAL AND ELECTROCHEMICAL SENSORS FOR THE DETECTION OF METAL IONS AND NERVE GAS MIMICS VIA FLUORESCEIN AND COUMARIN DERIVATIVES

by

Fasil Adefris Abebe

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the requirement for the Degree of Doctor of Philosophy
Department of Chemistry
Advisor: Ekkehard Sinn, Ph.D.

Western Michigan University
Kalamazoo, Michigan
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WE HEREBY APPROVE THE DISSERTATION SUBMITTED BY

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AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

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Trace element detection of metals, for example, iron, Copper and zinc, are important in biological systems and in the environment. Many of us are aware that our cells contain metal ions that are “tied” up in proteins. However, chelatable or “free” trace elements can also be found in small quantities, and can have a negative impact on our bodies. Despite the increasing surge in developing sensors for metals and nerve gas agents, efficient detection is still remains a challenge. Among the various sensors developed so far, fluorescence sensors play an important role due to their simplicity. I design fluorescein-based chemosensors for \( \text{Co}^{2+} \), \( \text{Ni}^{2+} \) and \( \text{Cu}^{2+} \), which exhibit highly selective “off-on” behavior in both absorption and emission, attributed to the transformation of a colorless, nonfluorescent spirolactam form to its yellow, fluorescent, ring-open amide equivalent. This study finds the reversibility of the sensors that bind to the ions, as indicated by the bleaching of color when the experiment extracts the metals with EDTA. Given the difficulty of designing enhanced fluorescent sensors for paramagnetic \( \text{Co}^{2+} \) and \( \text{Ni}^{2+} \) ions, the fluorescein compounds may inspire the further development of more sophisticated sensing constructs for the detection of these transition metal ions. Optical measurements such as UV-Vis, Fluorescence, and Electrochemical
measurements such as differential Pulse Voltammetry and impedance are carried out for characterization.

Chemical warfare agents are highly toxic and lethal even at low concentrations. Novel turn-off fluorescent coumarin-functionalized sensors for nerve gas agents in acetonitrile are designed and synthesized. These compounds selectively recognize the nerve gas mimic (diethylchlorophosphate, DCP). Addition of DCP to an acetonitrile solution of each sensor results in the quenching of the fluorescence intensity at 500nm, which provides an optical detection for DCP. There are other coumarin based molecules that exhibit high selectivity for Zn$^{2+}$, Cr$^{3+}$, Fe$^{3+}$ and Pt$^{2+}$ in acetonitrile and the selectivity is not affected by the presence of other alkalis, alkaline earths, transition metal ions or organophosphorus compounds.
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CHAPTER I

GENERAL INTRODUCTION

This work deals with detection/determination of toxins that may be accidentally or maliciously introduced into the environment.

A few decades ago, there was a general feeling among many people that nature could effectively handle hazardous substances. Although, nowadays human beings are more concerned of their sensitive natural environment, pollution is still a problem. Experts estimate that industrial processes introduce up to a million different pollutants into the atmosphere and the aquatic ecosystem.\(^1\) Heavy metals are one group of these substances, although not all of them are considered harmful to humans.

1.1 Heavy Metals in the Environment

Heavy metals are defined as metals of a density higher than 5 g/cm\(^3\). They occur as pure elements, as ions and complexes. Heavy metals were brought into the environment by human activities, which has influenced and modified natural cycles. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu), iron (Fe), and the platinum group elements.\(^1\) Human activities started more than 4000 years ago with metal mining. Unprecedented pollution came up with the industrialization and its consumption of energy. The combustion of
fossil fuels introduces a large amount of heavy metals into the atmosphere and the aquatic environment. Crude oil, for example, contains 3.4 ppm mercury and the firing of coal causes the worldwide emission of $2.4 \times 10^4$ t of lead per year.\(^1\) Additionally, heavy metals are released to the ecosystem with the exponential growth of metal mining, the following processes and their industrial use.

Metal ion poisoning through polluted water supplies is a potentially deadly problem causing neurological dysfunction and in some cases cancer.\(^1\) Metal ions are cited as the second most abundant water contaminant in the United States with the most prevalent ion being mercury.\(^1\) The Environmental Protections Agency (EPA) reported to Congress in October 2007 that 47% of assessed lakes, ponds, and reservoirs, totaling approximately seven million acres, in the US are impaired. “Impaired” is the most severe category of contamination given by the EPA and corresponds to water that cannot support one or more of its intended uses such as swimming or drinking.\(^1\)

The EPA cites mercury as a leading contaminant in US water, but arsenic, lead, and chromium are also in abundant supply in America and abroad. Arsenic is another metal present in the earth’s crust. As (III) is the most toxic form of the element and has a high occurrence in fish.\(^2\) Although the EPA sets a limit of 50 μg/l, 13 million Americans, mostly in western states are exposed to drinking water with twice this level, at 0.1 mg/l.\(^2\) Ingestion of arsenic contaminated water has been linked to increased risk of lung, liver, bladder, and kidney cancers.\(^3\)

The International Agency for Research on Cancer and The US Toxicology Program cite hexavalent chromium as a pulmonary carcinogen.\(^4\) Less toxic trivalent chromium and toxic hexavelant chromium are present in US drinking water at mean levels of 1.8 μg/l.\(^4\)
Cr(VI) is most commonly found in shallow groundwater with pH 6-8. At low levels, the human body can reduce Cr(VI) to the less toxic Cr(III), but levels higher than 0.012 μg/l have shown an increased risk of lung cancer, as well as allergic reactions in the skin and lungs.\textsuperscript{4,5}

The National Institute of Environmental Health Sciences (NIEHS) states that lead poisoning is one of the most persistent metal contamination issues. This problem is difficult to combat because it occurs in low income homes where aging paint exists and eroding lead pipes are carrying the household’s water supply.\textsuperscript{5} These contaminants are difficult to locate because there is not a single contained source, but many individual sources. Lead poisoning is known to cause nervous and reproductive system damage and is especially harmful to fetuses and young children. For these reasons it is especially important to implement a sensor system to detect levels of lead, and other harmful metals in drinking water throughout the world.

1.2 Biochemistry and Bio-Toxicity of Heavy Metals

Recently, the term “heavy metals” has became widely used in biology and environmental studies related to their potential toxicity and ecotoxicity.\textsuperscript{6} Human exposure to heavy metals is through the food chain, air, water chain, industry products and also occupational exposure. The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, toxic metal ions (e.g., Zn\textsuperscript{2+}, Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, As\textsuperscript{2+}, As\textsuperscript{3+}, Hg\textsuperscript{2+} and Cu\textsuperscript{2+}) are converted to their stable oxidation states and combine with the body’s biomolecules (e.g., proteins and enzymes) to form strong and stable chemical bonds. The
hydrogen atoms or the metal groups in the bio-molecules are replaced by the metals and the enzyme is thus inhibited from functioning.\textsuperscript{7,8}

The toxicity of heavy metal ions depends on the type of metal, its biological role and the type of organisms that are exposed to it. Some heavy metals are essential to maintain the metabolism of the human body at trace concentrations, such as Cu, Fe, Mg, Mn, and Zn, though they can be toxic in excess.\textsuperscript{9} Some are considered to be both very toxic above recommended allowable levels and also relatively accessible, such as As, Cd, Hg, Pb and Sn.\textsuperscript{10} Inhalation of heavy metals inhaled in vapor form can cause humans to exhibit the following symptoms: gastrointestinal (GI) disorders, diarrhoea, tremor, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia.\textsuperscript{9,10}

Heavy metals show a high tendency to form complexes, especially with nitrogen, sulphur- and oxygen-containing ligands of biological matter.\textsuperscript{11} The toxicological effects can be explained by this interaction. As a result, changes in the molecular structure of proteins, breaking of hydrogen bonds or inhibition of enzymes can occur. Chronic toxicity is much more relevant and caused by repeated exposure over long periods of time. Mutagenic, carcinogenic or teratogenic effects have been described for some heavy metals.

Besides the fact that mercury, cadmium and arsenic are highly toxic, some heavy metals such as iron, copper, zinc, manganese, cobalt, nickel, tin, and selenium are essential to many organisms. These elements, along with amino and fatty acids and vitamins are required for normal biochemical processes such as respiration, biosynthesis and metabolism.\textsuperscript{11} An undersupply of these so called trace metals leads to deficiency, while oversupply results in toxic effects.\textsuperscript{11}
1.3 Nerve Agents

Nerve agents are a class of phosphorus-containing organic chemicals (organophosphates) that disrupt the mechanism by which nerves transfer messages to organs. The disruption is caused by blocking acetylcholinesterase, an enzyme that normally relaxes the activity of acetylcholine, a neurotransmitter.

Poisoning by a nerve agent leads to contraction of pupils, profuse salivation, convulsions, involuntary urination and defecation, and eventually death by asphyxiation as control is lost over respiratory muscles. Some nerve agents are readily vaporized or aerosolized and the primary portal of entry into the body is the respiratory system. Nerve agents can also be absorbed through the skin, requiring that those likely to be subjected to such agents wear a full body suit in addition to a respirator.

1.4 Sensor Classification

Sensors can be classified in many ways (figures 1.1). They may be classified according to the principle of operation of transducer in two main groups as “physical” and “chemical” sensors. They also can be divided in to sub groups as optical, electrochemical, electrical, mass sensitive, magnetic and thermometric devices. They can also be classified as direct and indirect sensors or as reversible or non-reversible and respect of their applications or sizes16.
1.5 Optical Chemical Sensors

An optical sensor device consists of the following components: (a) the recognition unit, where specific interaction and identification of the analyte takes place; (b) the transducer unit that converts the recognition process into a measurable optical signal; (c) an optical device (process unit) which consists of at least a light source and finally (d) a detector (in the simplest form a photodiode), which detects and converts the change of optical properties, after amplification of the primary signal, into a unit readout. The optical properties measured can be absorbance, reflectance, luminescence, light polarization, Raman and other.$^{17}$

Optical sensors have found many applications in various fields, including biomedical, clinical, and environmental monitoring and process controlling. They are an attractive analytical tool, whenever continuous monitoring and real-time information is desired. They can track sources of contamination in an industrial process, follow the
formation and movement of environmental pollutants and can raise the alarm when a toxic species exceed an expected level of exposure.

1.6 Optical Chemical Sensors for the Determination of Heavy Metal ions

In recent years, chemical sensors for heavy metal ions have seen an increasing interest. A ‘metal sensor’ is described as a device which is capable of responding to the presence of a heavy metal ion in a reversible and continuous manner.

1.6.1 Sensors based on chromophores

The majority of heavy metal ion sensors are based on the use of an indicator dye which undergoes a binding reaction with the ions. This reaction is accompanied by a change in the absorbance or fluorescence of such chelates. In other words, an indicator acts as a transducer for the chemical species that cannot be determined directly by optical means.

Many indicators cannot be used in optical sensors because of unfavorable analytical wavelengths, poor photostability, low molar absorptivity or the need for additional reagents. Most of them bind with the metal ion irreversibly or only at low or high pH so they cannot be used for continuous sensing at near neutral pH. Upon binding with the metal ion, most indicators undergo a change in color, with one band appearing as another disappears, rather than an intensity change of one single band.

1.6.2 Sensors based on fluorophores

In contrast to chromogenic reagents, fluorescent indicators are of the on/off type in that only one of the species (the complexed or the noncomplexed) is fluorescent. Fluorescent indicators frequently provide improved sensitivity (which is important in
miniature sensors) and also selectivity because it is unlikely that an interfering species would have the same absorbance and emission as the analyte complex\textsuperscript{18}.

Fluorimetry (and luminescence spectrometry in a wider sense) also offers a broad variety of spectroscopic techniques including the measurement of lifetime, polarization and energy transfer. An important group of indicators is based on quenching of luminescence by heavy metals and transition metals. In the case of static quenching, the quencher interacts with the fluorophore in its ground state. In dynamic (collisional) quenching, the interaction between the metal ion (quencher) and the fluorophore occurs in the excited state only and leads to a reduction in both the emission intensity and the decay time. The photophysical process of dynamic quenching is fully reversible, that is, the indicator is not consumed. Hence, the quenching efficiencies of many transition metals, in particular Fe(III), Co(II) and Ni(II), are thought to be due to their numerous unpaired spins\textsuperscript{19,20}.

1.7 Electrochemical Sensors

A steady effort has been made on the development of efficient and easy-use electrochemical sensors. Electrochemical sensors with rapid and highly sensitive detection capabilities of various bio/chemical species are in great demand in many areas of science. Developing easy-to-use electrochemical sensors for detecting the concentration and activities of the various species therefore become very important. Hand-held electrochemical devices with accuracy and sensitivity similar to that of bench-top analyzers have already been developed for certain applications. Although still at basic research stage, many new applications are yet to be discovered. Improved modern fabrication techniques play a major role in developing these miniaturized devices.
Therefore, the innovation of miniaturized, rapid, portable and cost-effective dual-detection sensor systems is vital towards progression in electro-optical sensor technology.

1.8 Aim of the Thesis

The accurate and on-site detection of low concentration of toxic heavy metal ions and nerve gas agents in the environment is essential because of its lethal effects on the environment and living organisms. Conventional methods require high cost analytical instruments and complicated samples preparations. Nowadays, the development of portable, low cost, simple-to-use and accurate optical chemical and electrochemical sensors for determination of heavy metal ions and nerve gas agents is a great challenge. The goal of this thesis is to develop novel optical and electrochemical sensing materials for use in portable, sensitive, selective, low cost and low toxicity optical and electrochemical chemical sensors for the determination of toxic heavy metal ions and nerve gas agents in the environment.
CHAPTER II

A “TURN-ON” FLUORESCENT SENSOR FOR THE SELECTIVE DETECTION OF COBALT AND NICKEL IONS IN AQUEOUS MEDIA

2.1 Importance of Detecting Co$^{2+}$ and Ni$^{2+}$

Cobalt and nickel are essential micronutrients for animals and plants. Cobalt is an essential element as the metal cofactor of Vitamin B$_{12}$ and other cobalamins. Animals deprived of cobalt show retarded growth; anemia, loss of appetite, and decreased lactation.$^{12}$ In large doses cobalt and its salts can be toxic. Occupational exposure (>0.05 mg/m$^3$) causes irritant and allergic effects.$^{13}$ Cobalt is mainly used in steel and other alloys, abrasion-resistant glasses, ceramics, paints and batteries. Nickel is also an essential element, and loss of nickel homoeostasis is harmful to prokaryotic and eukaryotic organisms alike.$^{14}$ In excess it is associated with acute pneumonitis, dermatitis, asthma, cancer of the lung and sinus, adverse effects on blood and kidneys along with other disorders of the respiratory and central nervous systems.$^{15}$ Selective monitoring of Co$^{2+}$ and Ni$^{2+}$ in industrial, environmental, and food samples is, therefore, important.

Recently, the development of molecular sensing systems for transition metal ions has attracted intense attention.$^{15-21}$ Although great success has been achieved in this field, most fluorescence probes exhibit a quenching response upon binding with paramagnetic transition metal ions.$^{16-18}$ Many fluorescent sensors display amplification for transition
metal ions such as Zn$^{2+}$, $^{19,20}$ Cu$^{2+}$, $^{21,22}$ Fe$^{3+}$, $^{23,24}$ but fluorescence molecules suitable for use with typical transition metal fluorescence quenchers viz Ni$^{2+}$ and Co$^{2+}$, are scarce. Bharadwaj$^{25}$ and Qian$^{26}$ reported two sensors that provided an enhanced fluorescence response toward Ni$^{2+}$ and Co$^{2+}$ in the absence of oxidizing agents. However subsequently, de Silva et al,$^{27}$ indicated that these results must be treated with caution, as the onset of fluorescence is most likely attributable to the protonation of an amino receptor. Hence, there is still a need to develop readily available fluorescent sensors which display an enhanced fluorescence due to coordination with Co$^{2+}$ or Ni$^{2+}$ ions.

Several attempts have been made to develop turn-on sensors for Co$^{2+}$ and Ni$^{2+}$. Recently Weiying et al$^{35}$ have developed coumarin – quinoline based sensors for paramagnetic Co$^{2+}$ and Ni$^{2+}$. A readily available coumarin – quinoline is employed as a novel fluorescent probe for the ions (figure 2.1). NMR and IR indicated that the enhanced fluorescence response is attributable to coordination of the paramagnetic ions with the coumarin – quinoline compound. Fluorescence enhancement is more likely due to the binding of the metal ions than due to the protonation of the quinoline receptor by the coordinated acidic water molecules. Given the difficulty of designing enhanced fluorescent probes for paramagnetic Co$^{2+}$ and Ni$^{2+}$ ions, the coumarin – quinoline compound inspired the further development of more sophisticated sensing constructs for the fluorescence detection of these transition metal ions.
Recently, Kaur et al have developed anthracene-9,10-dione based sensors for Ni$^{2+}$, Cu$^{2+}$ and Co$^{2+}$ (Figure 2.2). Sensor A produced two different bathochromic shifts upon binding with Cu$^{2+}$ and Ni$^{2+}$ allowing the selective identification of the two metal ions. In the continuation of their work on metal sensing, they have synthesized B which allows selective identification of Co$^{2+}$ and Cu$^{2+}$ or Co$^{2+}$ and Ni$^{2+}$. They observed color changes upon binding with metals and they were able to detect them in concentrations as low as 3 μM.$^{36}$

Recently, Dodani et al have developed a fluorescent Ni$^{2+}$ probe with a BODIPY dye with a N/O/S receptor to bind with the metal (Figure 2.3).$^{38}$ They observed 25-fold fluorescence enhancement with 50 equivalents of Ni$^{2+}$. The sensor is used to detect low Ni$^{2+}$ levels in live cells. Live cells loaded with 1 mM NiCl$_2$ in the growth medium were
stained with the probe after 18 h and increased fluorescence was observed. Interestingly, this level of Ni$^{2+}$ is not lethal to lung carcinoma cells but 2 – 10 mM Ni$^{2+}$ does cause cell death.

![Figure 2.3 Structure of the fluorescent Ni$^{2+}$ sensor](image)

Figure 2.3 Structure of the fluorescent Ni$^{2+}$ sensor$^{38}$.

2.2 Fluorescein Compounds as Chemosensors

We chose fluorescein dyes to design fluorescent probes because of their excellent spectroscopic properties, such as long absorption and emission wavelength, high fluorescence quantum yield, large extinction coefficient and high stability to light.$^{28-30}$ In addition, they can interconvert between a non-fluorescent ring-closed spirolactam form and by fluorescent ring-open spirolactam form which makes them excellent candidates for chemosensors. We have recently developed fluorescein-based fluorescent and colorimetric chemosensors for copper in aqueous media.$^{34}$ Several other fluorescein and rhodamine-based sensors also have been reported for metal ions including Fe, Hg, and Pb. In all cases the mechanism involves the formation of ring-open spirolactum triggers by metal ions. Generally the ring-open form of fluorescein derivatives is yellow in color.
Therefore, we decided to use fluorescein-based compounds to detect Co\textsuperscript{2+} and Ni\textsuperscript{2+}. We designed sensors 1, 2, 4, and 5 for the detection of these paramagnetic ions in aqueous media. They function via conversion of the weakly fluorescent form to the ring–open fluorescent amide form.\textsuperscript{28-33} All the Compound were designed to bind metal ions via the carbonyl O and inamine N groups as donors. All function as excellent Co\textsuperscript{2+} and Ni\textsuperscript{2+} sensors while other common metals, particularly the alkalis, alkaline earths and transition metals, produce little or minimal spectral change. We will discuss the synthesis, characterization and the optical properties of them as the discussion progresses.
Scheme 2.1 Synthetic route of chemosensors 1 and 2.
Scheme 2.2 Synthetic route of chemosensors 4 and 5.

**Compound 3**: To a suspension of fluorescein (6 g, 18.1 mmol) in 50 mL methanol, excess hydrazine hydrate (24 mL; hydrazine content >80 mass%) was added. The reaction mixture was heated to reflux for 7 h with stirring, during which time the suspended particles were consumed and a clear solution was obtained. The ensuing solution was allowed to cool and poured into 400 mL H$_2$O at which time a yellow precipitate formed, which was allowed to settle for 2 h. The aqueous suspension was filtered, washed with water until the filtrate was colorless, then the filtrate was washed, 3 x 10 mL with cold absolute ethanol. The crude product was purified by recrystallization from ethanol to give 3.59 g of 3 as an off-white solid (57%). Melting point: 263°C, $^1$H NMR (400 MHz, DMSO-d6), δ (ppm): 4.38 (s, 2H), 6.42 (m, 4H), 6.59 (s, 2H), 6.97 (m, 1H), 7.48 (m, 2H), 7.76 (m, 1H), 9.81 (s, 2H). $^{13}$C NMR (400 MHz,
Compound 1: In a 25 mL flask, 3 (0.2 g, 1mmol) and thiazole-2-carbaldehyde (0.06 g, 1mmol) were suspended in 20mL ethanol. The mixture was refluxed for 12hr with stirring, during which time a gray precipitate formed. The precipitated was separated by filtration and washed with 3x 10 mL ethanol. After drying, a gray solid in 75% yield was obtained. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)), δ (ppm): 6.48 (dd, 8.8Hz, 2.3Hz, 2H), 6.52 (d, 8.8Hz, 2H), 6.67 (s, 2H), 7.1 (d, 8.8Hz, 1H), 7.71 (m, 2H), 7.73 (d, 7.3Hz, 1H), 7.82 (d, 2H), 7.95 (d, 6.96Hz, 1H), 8.68 (s, 1H), 10.00 (s, 2H). \(^{13}\)C NMR (400 MHz, DMSO-d\(_6\)), δ (ppm): 65.71, 103.1, 109.7, 113.19, 122.78, 124.09, 124.43, 128.30, 128.53, 129.88, 135.26, 140.66, 144.61, 151.36, 152.52, 159.45, 164.59, 165.12.  

Elemental analysis Calcd for \(\text{C}_24\text{H}_{15}\text{N}_3\text{O}_4\text{S}_1\): C, 65.30; H, 3.42; N, 9.52, O, 14.50; S, 7.26 Found: C, 65.43; H, 3.40; N, 9.42; S, 7.12; O, 14.63. ESI-MS. 442.1[M-H]^+

Compound 2: In a 25 mL flask, 3 (0.2 g, 1mmol) and chromene-3-carbaldehyde (0.1 g, 1mmol) were suspended in 25mL ethanol. The mixture was refluxed for overnight with stirring, to form a clear solution. After the mixture was allowed to cool to room temperature, a white precipitate formed. The precipitated was separated by filtration and washed with 3x 10 mL ethanol. 72% yield was obtained. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)), δ (ppm): 6.46 (dd, 8.8Hz, 2.3Hz, 2H), 6.47 (d, 8.8Hz, 2H), 6.67 (s, 2H), 7.10 (d, 7.36Hz, 1H), 7.49 (t, 1H), 7.63 (m, 3H), 7.65 (t, 1H), 7.94 (d, 1H), 8.00 (s, 2H), 10.7 (d, 1H), 8.38 (s, 1H), 8.70(s, 1H), 9.95(s, 2H). \(^{13}\)C NMR (400 MHz, DMSO-d\(_6\)), δ (ppm): 65.42, 103.22, 110.05, 113.04, 119.04, 119.20, 123.78, 123.89, 125.70, 126.61, 128.36, 128.53, 129.6, 134.8, 135.18, 140.23, 151.64, 152.50, 154.46, 156.20, 159.23, 164.44,
175.24. Elemental Analysis Calcd for 2. C₃₀H₁₈N₂O₆. C₃₂H₂₄N₂O₇: C, 70.07; H, 4.41; N, 5.11; O, 20.42 Found: C, 70.31; H, 4.54; N, 5.36; O, 19.79. ESI-MS. 503.1[M-H]⁺

**Compound 4:** In a 25 mL flask, 3 (0.2 g, 1mmol) and pyridine-2, 6-dicarbaldehyde (0.04 g, 1mmol) were suspended in 25mL ethanol. The mixture was refluxed for overnight with stirring, to form a clear solution. After the mixture was allowed to cool to room temperature, a white precipitate formed. The precipitated was separated by filtration and washed with 10 mL ethanol. 71% yield was obtained. ¹H NMR (400 MHz, DMSO-d₆), δ (ppm): 6.44 (dd, 2H), 6.49 (d, 2H), 6.66 (s, 2H), 7.14 (d, 1H), 7.48 (d, 1H), 7.60 (m, 2H), 7.65 (t, 1H), 7.95 (d, 1H), 9.94 (s, 2H). 13C NMR (400 MHz, DMSO-d₆), δ (ppm): 19.13, 56.59, 65.87, 103.13, 110.31, 113.01, 120.14, 124.01, 124.43, 128.52, 128.85, 129.79, 135.02, 138.31, 147.84, 151.21, 152.64, 153.85, 159.24, 164.66.

**Compound 5:** In a 25 mL flask, 3 (0.2 g, 1mmol) and isophthalaldehyde (0.04 g, 1mmol) were suspended in 25mL ethanol. The mixture was refluxed for overnight with stirring, to form a clear solution. After the mixture was allowed to cool to room temperature, a white precipitate formed. The precipitated was separated by filtration and washed with 3x 10 mL ethanol. 65% yield was obtained. ¹H NMR (400 MHz, DMSO-d₆), δ (ppm): 6.47(dd, 4H), 6.72 (s, 2H), 7.14 (d, 1H), 7.41 (d, 2H), 7.62 (m, 2H), 7.92 (d, 1H), 8.82 (s, 1H), 9.93 (s, 2H). ¹³C NMR (400 MHz, DMSO-d₆), δ (ppm): 19.11, 56.60, 65.78, 103.20, 110.49, 112.91, 123.85, 124.37, 128.46, 129.25, 130.01, 134.73, 135.54, 147.82, 151.07, 152.77, 159.20, 164.29.
2.3 Solution preparations for UV Absorption and Emission Studies

The stock solutions of metal ions (5mM) were prepared using nitrates [Cr(NO$_3$)$_3$, Zn(NO$_3$)$_2$, Ni(NO$_3$)$_2$, Cd(NO$_3$)$_2$, Hg(NO$_3$)$_2$, NaNO$_3$, KNO$_3$] or chlorides [CuCl$_2$, CoCl$_2$, FeCl$_2$, FeCl$_3$, PbCl$_2$, CaCl$_2$]. The stock solutions of compounds 1-5 (3 mM) were also prepared in 50% CH$_3$CN, 50% 0.01 M Tris HCl buffer (pH = 7.5). Fluorescence and UV absorption studies were performed using a 10 µM solution of the compounds and appropriate amounts of analytes. The solutions were shaken for half minute after each addition and allowed to stand 3 minutes before measuring the fluorescence. The fluorescence measurements were performed with 470 nm excitation. Both excitation and emission slit widths were 3 nm.

2.4 Synthesis

Scheme 2.1 outlines the syntheses of 1 and 2 by preparing 3 and reacting it in ethanol with thiazole-2-carbaldehyde and chromene-3-carbaldehyde, respectively. Both 1 and 2 were obtained in high yield, 75% and 72% respectively. Both formed precipitates upon cooling down to room temperature and were separated by filtration. Single crystals of 1 and 2 were grown in ethanol. Both have three coordination sites O/N/N and O/N/O respectively. Compound 4 and 5 were synthesized in a similar way by reacting 3 with the corresponding ligands (Scheme 2.2). The structures were confirmed using $^1$H NMR, $^{13}$C NMR, mass spectrometry, elemental analysis and X-ray crystallography.

2.5 Crystal Structure and Spectroscopic Studies

The molecular structures of 1 and 2 are given in Fig. 2.4 and 2.5. Hydrogen bonding plays an important role in the supramolecular structure, binding these molecules in 2D chains and 3D lattices with further solid state features involving solvent molecules
(Figure 2.6). In 1, the network of hydrogen bonding is likely the predominant factor orienting the thiazole ring which is rotated 18.75 ° from the carbonyl. The sulfur atom points in the same general direction as the carbonyl group and cation binding pocket. However, since nitrogen is a much harder base compared to sulfur, it is postulated in Scheme 1 that rotation occurs about the C21-C22 bond. This yields a pocket of hard donors, the carbonyl oxygen and imine nitrogen, which are more favorable ligands for the hard Ni$^{2+}$ and Co$^{2+}$ ions. The Pearson hard and soft acid base concept (HSAB) is widely used in chemistry for explaining stability of compounds, reaction mechanisms and pathways. It assigns the terms 'hard' or 'soft', and 'acid' or 'base' to chemical species. 'Hard' applies to species which are small, have high charge states, and are weakly polarizable. 'Soft' applies to species which are big, have low charge states and are strongly polarizable.

Both structures show the juction of mean planes of atoms at the point of C1 and at an angle of 88.38° for 1 and 87.94° for 2. The carbonyl group from the 5-membered ring participates in H-bonding with a hydroxyl group of the fluorescein moiety in a neighboring molecule in both compounds.

The structure of compound 2 also contains 4 hydrogen bonds per molecule. Each carbonyl oxygen is H-bonded (avg. 2.862 Å) to a hydroxyl group of the neighboring molecule in order to form chains rather than associated dimers. Each H-bonded chain is closely associated due to the aromatic stacking interactions between the fluorescein moieties with an interplane separation of 3.616 Å. The packing of these cross-shaped molecules also provides large pockets occupied by disordered THF molecules (removed for clarity) (Figure 2.35 and 2.36).
Figure 2.4. X-ray crystal structure of sensor 1. Hydrogen removed for clarity and Structures shown with 50% ellipsoids.

Figure 2.5. X-ray crystal structure of sensor 2. Hydrogen removed for clarity and Structures shown with 50% ellipsoids.
In Compound 1, one hydroxyl group of the fluorescein moiety hydroden bonds with the carbonyl oxygen of the neighboring molecule related by a 2-fold screw axis about [010] and at a donor-acceptor distance of 2.7045(15) Å. The other hydroxyl group begins a network of three H-bonds generated through both ethanol oxygens to the nitrogen atom within the thiazole ring.

Upon the addition of Co$^{2+}$ and Ni$^{2+}$ to a colorless solution of compound 1 and 2, both a yellow color and the characteristic fluorescence of fluorescein appear (Fig.2.7). The large fluorescence enhancement as well as the colorimetric change can be attributed to spirolactam ring opening induced by the complexation of Co$^{2+}$. 

Figure 2.6 ORTEP diagram of the hydrogen bond interaction in Compound 1. Hydrogens removed for clarity.
Scheme 2.3. Proposed mechanism for the fluorescence changes of 1 upon addition of Co\textsuperscript{2+} or Ni\textsuperscript{2+}.

Chlorides of Ca\textsuperscript{2+}, K\textsuperscript{+}, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, Pb\textsuperscript{2+}, Co\textsuperscript{2+} and nitrates of Zn\textsuperscript{2+}, Cd\textsuperscript{2+}, Cr\textsuperscript{3+}, Hg\textsuperscript{2+} and Ni\textsuperscript{2+} ions were evaluated for their metal-binding properties with compounds 1 and 2 (10 µM) in DMSO-water. The resulting solution was shaken well before recording the absorption and emission spectra. The fluorescence spectra were obtained by excitation of the fluorescein fluorophore at 470nm. All spectroscopic studies were performed in 2% DMSO in aqueous solution in which the sensors formed colorless solutions that are stable for more than a month and titration experiments were carried out at ambient temperature.

Figure 2.7 Compound 1(10 µM) with different metal ions (20 µM).
The compounds are very weakly fluorescent in solution and nearly devoid of absorption in the visible range, due to the predominance of the ring-closed spirolactam form. This was further confirmed by $^{13}$C NMR signals at 65.71 ppm and 65.42 ppm for compound 1 and 2 respectively. All the sensing and optical measurements were performed in 10 mM Tris-HCl buffer solution with a pH of 7.5 to keep the dye molecules in their ring closed form. The absorption spectrum of 1 and 2 (10 µM) in DMSO-water exhibited only a very weak band above 400 nm, ascribed to a trace of the ring-open form of the compounds, but addition of 1 equivalent Co$^{2+}$ and Ni$^{2+}$ showed a new absorbance peak at 500 nm with an immediate color change visible to naked eye (Fig. 2.8 and 2.9). Interestingly, both ions showed the highest absorbance enhancement (18 fold) while other metals showed no significant interference. This shows the excellent selectivity of 1 and 2 towards Co$^{2+}$ and Ni$^{2+}$. Co$^{2+}$ and Ni$^{2+}$ can form either strong tetrahedral or octahedral complexes with three donor atoms from 1 and 2 participating in the complexation. In the case of octahedral complex formation, other three coordination sites can be occupied by nitrate anions. On the other hand, both octahedral and tetrahedral complexes of Co$^{2+}$ and Ni$^{2+}$ are paramagnetic which explain the reason behind the selectivity of the sensor to these metal ions.
Figure 2.8 UV-vis absorption spectra of 1 (10µM) upon addition of respective metal ions (1 equiv.) in DMSO-water (2%DMSO) at pH 7.5 in aqueous solution.

Figure 2.9 UV-vis absorption spectra of 2 (10µM) upon addition of respective metal ions (1 equiv.) in DMSO-water (2%DMSO) at pH 7.5 in aqueous solution.
Figure 2.10 UV-vis absorption changes of 1 (10µM) upon titration of Co$^{2+}$ ions in 2% DMSO-water buffered at pH 7.5. Co$^{2+}$ ion concentration (µmol L$^{-1}$): 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15.

Figure 2.11 UV-vis absorption changes of 2 (10µM) upon titration of Co$^{2+}$ ions in 2% DMSO-water buffered at pH 7.5. Co$^{2+}$ ion concentration (µmol L$^{-1}$): 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15.
Figure 2.12 UV-vis absorption changes of 2 (10µM) upon addition of different concentration of Ni$^{2+}$ in DMSO-water (2%DMSO) at pH 7.5. Ni$^{2+}$ ion concentration (µmolL$^{-1}$): 0, 1.5, 3, 4.5, 6, 7.5.

The 1:1 stoichiometry of the sensor was confirmed by the Job’s plot of the fluorescence changes (Fig. 2.13) From a fluorescence titration, the association constant of 1 with Co$^{2+}$ and Ni$^{2+}$ were observed to be 2.1 x 10$^4$ and 4.5 x 10$^3$ M$^{-1}$ respectively. When the colored 1-Co$^{2+}$ solution was treated with 1.0 mmol L$^{-1}$ EDTA (1 equivalent), the yellow color almost disappeared, indicating that the coordination of the sensor with Co$^{2+}$ is chemically reversible. Additional EDTA eliminated the color completely, indicating complete shift of the equilibrium to the right.

$$1\text{-Co}^{2+} + \text{ETDA} \rightleftharpoons 1\text{-EDTA} + \text{Co}^{2+}$$
Figure 2.13 Job’s plot of sensor 1 (20uL) with Co$^{2+}$ (20 uL) in DMSO-water (2%DMSO) at pH7.5.

Figure 2.14 Fluorescence emission changes in 1 (10 μM) respectively upon addition of different metal ions (1 equiv.) in 2% DMSO-water at pH 7.5 (excitation at 470 nm).
Figure 2.15 Relative fluorescence intensities of 1 (10 µM) in the presence of various metal ions.

Figure 2.16 Fluorescence emission changes in 2 (10 µM) respectively upon addition of different metal ions (1 equiv.) in 2% DMSO-water at pH 7.5 (excitation at 470 nm).
Fluorescence intensity changes of 1 and 2 (10 μM) upon the addition of metal ions (10 μM, 1 equiv.) in aqueous solution showed a remarkable sensitivity and selectivity towards Co\textsuperscript{2+} and Ni\textsuperscript{2+} (Figure 2.14 and 2.16). The observed fluorescence enhancement at 515 nm ($\lambda_{ex} = 470$ nm) was over 50-fold, which is extremely high compared to other metals (Fig. 2.14 and 2.16). The emission maximum for 1-Co\textsuperscript{2+} at 515 nm is hypsochromically shifted about 15 nm compared with that of pure 1. Continuous addition of Co\textsuperscript{2+} resulted in an increase in the fluorescent intensity, which became saturated with 100 μM of Co\textsuperscript{2+} (Fig. 2.17). Interestingly, the 2-Ni\textsuperscript{2+} complex was not fluorescent at all. However, the absorption enhancement and visible color change make it a selective sensor for Ni\textsuperscript{2+}.

Figure 2.17 Fluorescence changes of 1 (10 μM) with Co\textsuperscript{2+} (0 – 100 μM). Insets: fluorescence enhancement at 515 nm as a function of [Co\textsuperscript{2+}].
Figure 2.18 Fluorescence changes of 2 (10 µM) with Co$^{2+}$ (0-120 µM). Insets: fluorescence enhancement at 515 nm as a function of [Co$^{2+}$].

Figure 2.19 Fluorescence changes of 1 (10 µM) with Ni$^{2+}$ (0 – 100 µM). Inset: fluorescence enhancement factor F at 515 nm as a function of Ni (II) concentration.
To explore the utility of 1 and 2 as a cation–selective chromogenic chemosensor, a competition experiment was carried out by adding 20 µM Co^{2+} to a solution of 1 and 2 in the presence of selected other cations including Ca^{2+}, K^{+}, Fe^{3+}, Fe^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Hg^{2+}, Zn^{2+} and Cd^{2+} (each at 20 µM). The competing cations alone did not lead to significant absorption changes of 1. However, additions of two equiv. of Co^{2+} to the solution that contain 1 and the other metal ions produced the spectral and color changes characteristic of Co^{2+}.

{superscript}1H NMR titration in DMSO was carried out in order to clarify the binding mechanism of Co^{2+} with Sensor 1 (Figure 2.20). Addition of different equivalent of Co^{2+} resulted in slight shifting and broadening of the peak at δ 10, corresponding to the inamine hydrogen, to further down field. This indicates a decrease in electron density at this nitrogen, resulting from direct coordination with Co^{2+}.

![Figure 2.20 1H NMR (DMSO) spectra of 1 with Co^{2+} (0, 0.75, 1.5, 2 equiv. from bottom to top).]
2.6 Electrochemical Detection of Co$^{2+}$

Molecules that provide optical and electrochemical signals are ideal for exploitation in developing sensors that offer dual signal transductions in addition to the color change. Differential pulse voltammetry (DPV) was carried out with a CHI440A model potentiostat and utilizing a three electrode configuration at room temperature. Changes in the electrochemical signals of 1 with 1 equiv of the Co$^{2+}$ were measured as shown in Fig. 2.21 and 2.22. The 1-Co$^{2+}$ complex formed had significantly different redox characteristics relative to 1. The DPV of 1 displays two reductions couples (-0.75V and -1.61V) and two oxidation couples (0.14 V and 0.35V). There is shift in the reduction and oxidation potential in the presence of Co$^{2+}$. For reduction, the shift is from -1.61V to -1.17V with a difference of 440 mV; for oxidation it shifts from 0.14V to 0.176V with a difference of 36mV. The current flow in the 1-Co$^{2+}$ complex is higher than the current flow in the free compound and a shift of the peaks towards more positive values is observed for compound 1 in the presence of Co$^{2+}$. The changes in electrochemical signal confirm the binding of Co$^{2+}$ to the molecule.
Figure 2.21 Differential pulse voltammograms of 1.

Figure 2.22 Differential pulse voltammograms of 1 after the addition of Co$^{2+}$. 
The need for cost-effective miniaturized devices that provide rapid, automated, and dual detection signals are paramount to advancement in electro-optical sensor technology\textsuperscript{39}. The unique properties of these sensors such as high sensitivity and selectivity along with very low detection limits are extremely promising for toxic metal detection applications. A sensor device with gold (Au) interdigitated electrodes on a glass substrate was used for the electrochemical detection of metal ions. Pico molar amounts of Co\textsuperscript{2+} were detected by electrical impedance spectroscopy using 1 and 2 as sensors.

Figure 2.23 and 2.24 below shows the impedance response of the electrochemical sensor towards different concentrations of Co\textsuperscript{2+} in the presence of 1. A distinguishable and better signal to noise ratio was achieved from 100 Hz to 500 Hz. As an example, for measurements at operating frequency of 200 Hz, the impedance response decreased from 39 kΩ to 35 kΩ to 29 kΩ to 16 kΩ to 9 kΩ as concentrations of Co\textsuperscript{2+} increased from 100 pM to 1 nM to 100 µM to 1 mM to 100 mM, respectively. This response showed that the impedance percentage change achieved with respect to 1 at 200 Hz were 50 \%, 55 \%, 63 \%, 80 \% and 89 \% as the concentration of Co\textsuperscript{2+} was varied from 100 pM to 1 nM to 100 µM to 1 mM to 100 mM, respectively. The electrochemical impedance spectroscopy sensing mechanism is based on the disturbance of charge transfer dynamics between metal electrodes\textsuperscript{39} and 1 at its surface which binds to Co\textsuperscript{2+}.

The sensor response and impedance percentage change at the Sensor 2 – Co\textsuperscript{2+} interface when compared to a solution of 2 is shown in Figure below 2.26. Here, it was observed that a distinctive and better signal to noise ratio was achieved from 500 Hz to 1 kHz. The impedance percentage change achieved with respect to 2 at 500 Hz for Co\textsuperscript{2+}
were 12%, 21%, 39%, 52%, 73% and 84% as the concentrations were varied from 1 pM to 100 nM to 1 µM to 100 µM to 1 mM to 100 mM, respectively.

Figure 2.23 Impedance response of Compound 1 with Co²⁺ ion.
Figure 2.24  Impedance response of sensor 1 towards different concentrations of Co$^{2+}$, at applied potential of 1 mV.
Figure 2.25 Impedance response of Compound 2 with Co ion.

Figure 2.26 Impedance response of sensor 2 towards different concentrations of Co\(^{2+}\), at applied potential of 1 mV.
2.7 Spectroscopic Study of Compound 4 and 5

The absorption spectrum of 4 (10 µM) in DMSO-water buffer system had no peak above 400nm, but showed a new peak at 500nm with a shoulder at 475nm upon addition of 1 equivalents of Co\(^{2+}\), suggesting the formation of the ring-open form of 4 (Fig.2.27). The addition of Ni\(^{2+}\) also gave a new peak at 500 nm in the absorption spectra with 60-fold enhancement which is quite low compared with that observed for Co\(^{2+}\). This resulted in an immediate color change from colorless to yellow. The absorption titration is carried out using 10 µM of 4 in DMSO-water at pH 7.5 in which Co\(^{2+}\) clearly produced a continuous absorption enhancement (Fig. 2.28).

![UV-vis absorption spectra](image)

Figure 2.27 UV- vis absorption spectra of 4 (10 µM) upon addition of different metal ions (1 equiv.) in DMSO-water (2%DMSO) at pH 7.5 in aqueous solution.
Figure 2.28 UV-vis absorption changes of 4 (10 µM) upon titration of Co$^{2+}$ ions in 2% DMSO-water buffered at pH 7.5. Co$^{2+}$ ion concentration (µmol L$^{-1}$): 0 – 10.

The fluorescence spectrum of compound 4 (10 µM) in DMSO-water showed a peak at 530 nm upon the addition of 10 equivalent of Co$^{2+}$ due to the delocalization in the xanthene moiety of fluorescein (Fig.2.29). The emission enhancement was large compared with that for the other metal ions tested in this experiment. The fluorescence enhancement is much weaker than that of absorbance. In addition, there is a considerable blue shift in the emission maxima of 4-Co$^{2+}$, 4-Cu$^{2+}$ and 4-Ni$^{2+}$ (15 nm). Interestingly, Cu$^{2+}$ registered a slight fluorescence enhancement while other metals showed no enhancement. The emission enhancement of 4 with Ni$^{2+}$ is negligible compared to that of its absorption: it gives the second highest absorption enhancement of all the metals tested. This behavior allows us to detect and identify Co$^{2+}$ independently from Ni$^{2+}$ and Cu$^{2+}$. 
Figure 2.29 Fluorescence emission changes in 4 (10 µM) respectively upon addition of different metal ions (1 equiv.) in 2% DMSO-water at pH 7.5 (excitation at 470 nm).

Figure 2.30 Fluorescence changes of 4 (10 µM) with Co$^{2+}$ (0-100 µM). Insets: fluorescence enhancement at 530 nm as a function of [Co$^{2+}$].
The fluorescence and absorption properties of 5 were also tested with metal ions in DMSO-Water. It forms a colorless aqueous solution which is strongly fluorescent upon excitation at 470 nm. The fluorescence profile is very similar to that of sensor 4: again Co\textsuperscript{2+} registers the highest fluorescence enhancement with some fluorescence interference from Cu\textsuperscript{2+} and Ni\textsuperscript{2+}, while other metals show no significant enhancement (Fig.2.33). Compound 4 and 5 have pockets to bind Co\textsuperscript{2+} and Ni\textsuperscript{2+} with two similar five and six membered rings (Scheme 2.2). Both are very selective and sensitive sensors for Co\textsuperscript{2+} and the nitrogen and carbonyl oxygen (N, N, O, O system) in the pocket are important in the binding. The absorption data showed a similar trend like sensor 4 and showed no absorbance band above 400nm in DMSO-Water at pH 7.5. The addition of one equivalent of Co\textsuperscript{2+} and Ni\textsuperscript{2+} give absorption enhancement at 500nm (Fig 2.31 and 2.32). In addition, both Co\textsuperscript{2+} and Ni\textsuperscript{2+} showed an instantaneous color change with 5.

![Figure 2.31](image-url)  

**Figure 2.31** UV- vis absorption spectra of 5 (10 \textmu M) upon addition of respective metal ions (1 equiv.) in DMSO-water (2\%DMSO) at pH 7.5 in aqueous solution.
Figure 2.32 UV-vis absorption changes of 5 (10 µM) upon titration of Co²⁺ ions in 2% DMSO-water buffered at pH 7.5. Co²⁺ ion concentration (μmol L⁻¹): 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.

Figure 2.33 Fluorescence emission changes in 5 (10 µM) respectively upon addition of different metal ions (1 equiv.) in 2% DMSO-water at pH 7.5 (excitation at 470 nm).
In conclusion, we have synthesized four new fluorescent chemosensors that are very stable in aqueous solution for more six a month. Sensor 1 showed high sensitivity and selectivity towards Co\textsuperscript{2+} and Ni\textsuperscript{2+} over other interference cations. Sensor 2 is also very selective for Co\textsuperscript{2+} and Ni\textsuperscript{2+}. Even though it is non-fluorescent, it is highly colored and the high absorption enhancement enables the selective identification of Ni\textsuperscript{2+} over other metal ions. We were able to use the ring opening mechanism of the new fluorescein derivatives to develop these new sensors for Co\textsuperscript{2+} and Ni\textsuperscript{2+}. Given the difficulty of designing enhanced fluorescent sensors for paramagnetic Co\textsuperscript{2+} and Ni\textsuperscript{2+} ions, the fluorescein compounds may inspire the further development of more sophisticated sensing constructs for the detection of these transition metal ions.
The fluorescence spectrum of compound 4 and 5 (10 µM) in DMSO-water showed a peak at 530 nm upon the addition of 10 equivalent of Co$^{2+}$ due to the delocalization in the xanthene moiety of fluorescien. The emission enhancement was large compared with that for the other metal ions tested in this experiment. The fluorescence enhancement is much weaker than that of absorbance. The absorption spectrum of 4 and 5 (10 µM) in DMSO-water buffer system had no peak above 400nm, but showed a new peak at 500nm with a shoulder at 475nm upon addition of 1 equivalents of Co$^{2+}$, suggesting the formation of the ring-open form of 4 and 5.

In this work we have successfully employed electrochemical spectroscopy for the detection of Co$^{2+}$ in acetonitrile solution. Varying concentration of the Co$^{2+}$ in the presence of 1 and 2 were quantified by using a highly sensitive electrochemical sensor device. The response of the electrochemical sensor device displayed an impedance percentage change of 50 %, 55 %, 63 %, 80 % and 89 % with respect to 1 at 200 Hz as the concentration of Co$^{2+}$ was varied from 100 pM to 1 nM to 100 µM to 1 mM to 100 mM, respectively.
3.1 Importance of Copper

Copper is the third most abundant heavy metal (after Fe\(^{3+}\) and Zn\(^{2+}\)) in humans and is essential as a catalytic cofactor in metalloenzymes like superoxide dismutase, cytochrome c oxidase and tryosinase. Toxic in excess, copper can also cause neurodegenerative diseases, like Alzheimer’s and Wilson’s disease, probably by promotion of reactive oxygen species.\(^{40,41}\) Given the intense interest in heavy metal sensors,\(^{42,43}\) much effort has been devoted to developing copper-selective fluorescent chemosensors. Various Cu\(^{2+}\) sensors have been proposed, mostly with a turn-off (quenching) response,\(^{43}\) because Cu\(^{2+}\) usually acts as a quencher via energy or electron transfer.\(^{43}\) Recently, “turn-on” sensors have also been reported,\(^{44,45,46}\) but few of them work in aqueous media, which greatly limits their analytical applications.

We chose fluorescein dyes to design fluorescent probes because of their excellent spectroscopic properties, such as long absorption and emission wavelength, high fluorescence quantum yield, large extinction coefficient and high stability to light.\(^{45,46,47,48}\) In recent years, several rhodamine-based chemosensors and chemodosimeters for Cu\(^{2+}\), Hg\(^{2+}\), Hg\(^{2+}\) and Fe\(^{3+}\) have been studied, but fluorescein-based sensors have received comparatively little attention.\(^{47}\) Fluorescein-derived colorimetric sensors, 6, 7 and 8, synthesized by preparing 3 and combining it
with (2-hydroxynaphthaldehyde, 2-hydroxybenzaldehyde and 5-tert-butylhydroxylisophthalaldehyde), Scheme 3.1. The sensors 6 and 7 are rapid and selective, and capable of detecting Cu$^{2+}$ in aqueous media.

Rhodamine-B derivatives and their ring-open reaction have received greater attention following the report of a rhodamine-B hydrazine sensor for Cu$^{2+}$ by Czarnik et al$^{56}$ (Figure 3.1). The rate at which the hydrolysis takes place by Cu$^{2+}$ is much greater than it is for other metal ions.$^{57}$ This work triggered a whole new generation of metal ion sensors. Interestingly, compound c has been used widely as the starting material for most of the metal ion sensors.

![Figure 3.1 Mechanism of Cu$^{2+}$ binding with C.](image)

Xi et al reported fluorescent sensor based on Rhodamine$^{70}$. The sensor, D, has a spirolactam ring formation, show a reversible, selective, and sensitive fluorescence enhancement response to Cu (II) at biological pH value for practical use. Also, the selectivity of this system for Cu (II) over other metal ions is remarkably high, and its sensitivity is below 2ppb in methanol solutions. Furthermore, its high cell permeability grants the access to employ the sensor as Cu (II) detector in living cells$^{70}$.  

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Recently Weerasinghe et al.\textsuperscript{71} showed Rhodamine-based dual sensor selectively detects Cu\textsuperscript{2+} and Fe\textsuperscript{3+}. Complexation with Fe\textsuperscript{3+} triggers the formation of the highly fluorescent ring-open form, while Cu\textsuperscript{2+} forms a nonfluorescent complex detectible by its UV-Vis spectrum. Both Cu\textsuperscript{2+} and Fe\textsuperscript{3+} give an immediate color change and the sensing mechanism is reversible, as indicated by disappearance of the color with the addition of excess EDTA (Figure 3.3).

Recently, Sheng et al reported a coumarin-based colorimetric chemosensor\textsuperscript{72}. It exhibits good sensitivity and selectivity for the copper cation over other cations in aqueous solution on paper-made test kits. Diaminomaleonirile is coupled with coumarin at the 3-position carbonyl to form a Schiff base derivative. An intramolecular charge transfer (ICT) is enhanced as a result of the extended π-conjugation and the stronger
electron withdrawing ability of the nitrile group. The formed imine and the rest of the amine group in the chemosensor, F act as chelating sites of metallic cations.

![Figure 3.4 Chemosensor F for Cu²⁺.](image)

Jung et al also reported coumarin based fluorogenic sensor bearing a 2-picoly unit. This sensor is highly fluorescent with high selectivity and suitable affinity in biological systems toward Cu²⁺ over other cations tested. The fluorescence on–off mechanism was studied by femtosecond time-resolved fluorescence (TRF) upconversion technique. The receptor can be applied to the monitoring of Cu²⁺ ion in aqueous solution with a pH span 4-10. To confirm the suitability of G for biological applications, they also employed it for the fluorescence detection of changes of intracellular Cu²⁺ concentration in cultured cells. The results indicate that G is useful for the fluorescence microscopic imaging and the study on the biological functions of Cu²⁺.

![Figure 3.5 Mechanism of Cu²⁺ binding with G.](image)

Recently Kim et al developed a coumarin derivative-based off-on catalytic chemodosimeter, H, for Cu²⁺ ions. This is a highly effective turn-on fluorescent sensor that is catalytically hydrolyzed by Cu²⁺, and the catalytic process induces a large increase
in the fluorescence intensity, due to amplification of the fluorescence signal. When the
catalytic hydrolysis of the hydrazone occurs in the presence of Cu$^{2+}$ ions, the regeneration
of coumarin from the hydrazone derivative induces a fluorescence change. The
hydrazone derivative used in this study is synthesized from coumarin and a hydrazine.
The detection limit of the sensor, H for Cu$^{2+}$ is estimated to be $8.7 \times 10^{-8}$ M and this
detection limit is acceptable within the US EPA limit (20 µM) for the detection of Cu$^{2+}$ in
drinking water.

![Figure 3.6 Cu$^{2+}$- induced catalytic sensing cycle of H$^{74}$.](image)

3.2 Fluorescein for the Detection of Cu$^{2+}$

After a thorough investigation of the current Cu$^{2+}$ sensors, we decided to design
fluorescein-based sensors with an aim to improved features. These are shown in the
synthesis scheme 3.1. As shown, these sensors contain different ligand units and a
fluorescein moiety connected through Schiff base linkages and the synthesis is less
complicated compared to other synthesis methodologies.

Our considerations for synthesizing 6, 7 and 8 are as follows:
(a) the ligands that contain hydroxyl and carbonyl functional groups are known to have a high chelating ability with common metal ions. Introducing this kind of moiety can enhance the chelating ability of the compounds towards some metal ions;

(b) when the coordination takes place, the spirolactam of the fluorescein moiety can be opened, at the same time obvious color and optical change can be observed. Thus, sensors 6, 7 and 8 were synthesized via a simple two-step reaction with a reasonable yield, and confirmed by $^1$H NMR, $^{13}$C NMR, elemental analysis and mass spectroscopy.

3.3 Materials and Instruments

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Fluorescence spectra were recorded on an Edinburgh FS920 fluorimeter at room temperature, on 10 µM solutions of 6, 7 and 8 in DMSO-water with appropriate amounts of metal salts. UV absorbance spectra were recorded on a Shimadzu UV-2101PC. $^1$H NMR and $^{13}$C NMR spectra were obtained on a JEOL eclipse (400 MHz) instrument.

All the measurements were taken at room temperature, about 298 K. After 2 min of mixing of metal ions with the compounds, UV-vis absorption spectra or fluorescence emission spectra were measured, unless otherwise indicated. For all fluorescent tests, the excitation wavelength was 470 nm, with excitation and emission slit widths both 3 nm.

3.4 Association Constant

The Benesi-Hildebrand equation was used to calculate the binding constants:

$$(F_0 / F - F_0) = (a / b - a) (1 / K [M] + 1)$$

where $F_0$ is the fluorescence intensity of the sensor at 518 nm in the absence of Cu$^{2+}$, $F$ is the fluorescence intensity upon the addition of Cu$^{2+}$ at 518 nm, while $a$ and $b$ are
constants. [M] is the concentration of Cu$^{2+}$, the ($F_0 / F - F_0$) was plotted against $1 / [M]$ and the association constant (K) was obtained by the ratio intercept/slope.

Scheme 3.1 Synthetic route of chemosensors 6, 7 and 8.

3.5 Synthesis

**Compound 3**: To a suspension of fluorescein (6 g, 18.1 mmol) in 50 mL methanol, excess hydrazine hydrate (24 mL; hydrazine content >80 mass%) was added. The reaction mixture was heated to reflux for 7 h with stirring, during which time the suspended particles were consumed and a clear solution was obtained. The ensuing
solution was allowed to cool and poured into 400 mL H$_2$O at which time a yellow precipitated formed. The aqueous suspension was filtered, and the filtrate was washed with water until it was colorless, then washed again 3 x 10 mL with cold absolute ethanol. The crude product was purified by recrystallization from ethanol to give 3.59 g of 3 as an off-white solid (57%). Melting point: 263 °C, $^1$H NMR (400 MHz, DMSO-d$_6$), δ (ppm): 4.38 (s, 2H), 6.42 (m, 4H), 6.59 (s, 2H), 6.97 (m, 1H), 7.48 (m, 2H), 7.76 (m, 1H), 9.81 (s, 2H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 64.6, 102.3, 109.9, 111.9, 122.3, 127.9, 128.4, 129.3, 132.6, 151.5, 152.4, 158.1, 165.4.

**Compound 6:** In a 25 mL flask, 3 (0.2g, 1mmol) and naphthaldehyde (0.099g, 1mmol) were suspended in 20mL ethanol. The mixture was refluxed for 12 hr with stirring, during which time a yellow precipitate formed. The precipitate was separated by filtration and washed with 3 x 10 mL ethanol. After drying, a bright yellow solid in 80% yield was obtained. $^1$H NMR (400 MHz, DMSO-d$_6$), δ (ppm): 6.50 (dd, 8.8Hz, 2.3Hz, 2H), 6.59 (d, 8.8Hz, 2H), 6.68 (s, 2H), 7.1 (d, 8.8Hz, 1H), 7.20 (d, 6.96Hz, 1H), 7.34 (t, 7.52Hz, 1H), 7.46 (t, 7.32Hz, 1H), 7.66 (p, 7.32Hz, 2H), 7.82 (m, 2H), 7.97 (d, 6.96Hz, 2H), 9.8 (s, 1H), 10.02 (s, 2H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 65.76, 103.01, 109.49, 109.82, 113.29, 119.11, 121.71, 124.13, 128.41, 128.98, 129.89, 131.85, 133.84, 134.79, 149.19, 151.01, 152.76, 158.32, 159.38, 163.96 Elemental Analysis calcd for C$_{31}$H$_{20}$N$_2$O$_5$. 0.7 C$_2$H$_5$OH: C, 73.05; H, 4.58; N, 5.35; O, 17.12. Found C, 73.03; H, 4.66; N, 5.48 O, 16.83. ESI-MS. 501.2[M-H]$^+$

**Compound 7:** In a 25 mL flask, 3 (0.2g, 2mmol) and 5-tert-butyl-hydroxy isophthaldehyde (0.058g, 1mmol) were suspended in 30mL ethanol. The mixture was refluxed overnight with stirring, to form a clear solution. Following the reaction, the
mixture was allowed to cool to room temperature. The pale yellow precipitated that formed was separated by filtration and washed with 3x 10 mL ethanol. 70% yield was obtained. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)), \(\delta\) (ppm): 6.43 (s, 2H), 6.48 (s, 2H), 6.69 (s, 2H), 7.18 (d, 7.36Hz, 1H), 7.44 (s, 1H), 7.66 (m, 2H), 7.93 (d, 7.32Hz, 1H), 9.19 (s, 1H), 9.96 (s, 2H), 10.7 (s, 1H). \(^{13}\)C NMR (400 MHz, DMSO-d\(_6\)), \(\delta\) (ppm): 66.04, 103.09, 110.23, 112.94, 120.28, 123.85, 124.57, 127.78, 128.56, 129.8, 129.9, 134.76, 142.3, 148.81, 150.33, 153.06, 154.75, 159.27, 163.79. Elemental Analysis calcd for C\(_{52}\)H\(_{38}\)N\(_4\)O\(_9\). C\(_{2}\)H\(_5\)OH: C, 71.35; H, 4.88; N, 6.32; O, 17.60. Found C, 71.12; H, 5.08; N, 6.16; O, 17.64. ESI-MS. 863.2 [M-H]\

**Compound 8:** In a 25 mL flask, 3 (0.2g, 1mmol) 2-hydroxybenzaldehyde (0.07g, 1mmol) were suspended in 30mL ethanol. The mixture was refluxed overnight with stirring, to form a clear solution. Following the reaction, the mixture was allowed to cool to room temperature. The precipitate that formed was separated by filtration and washed with 3x 10 mL ethanol. 75% yield was obtained. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)), \(\delta\) (ppm): 6.5 (m, 4H), 6.64 (s, 2H), 6.80 (m, 2H), 7.14 (d, 1H), 7.31 (t, 1H), 7.33 (d, 1H), 7.64 (m, 2H), 7.9 (d, 1H), 9.16 (s, 1H), 10.25 (s, 2H).

3.6 Synthesis and Characterization

Compound 3 was synthesized according to a literature procedure. Compound 6, 7 and 8 are obtained by reacting 3 with the corresponding ligands in the presence of ethanol. The structures of 6, 7 and 8 were confirmed by \(^1\)H NMR, \(^{13}\)C NMR, ESI-MS, elemental analysis and X-ray crystallography (Fig 3.7 and 3.8).
All the spectroscopic studies were performed in DMSO-Water at pH 7 in which all compounds formed a colorless solution. Compound 6 and 7 showed similar emission bands at 518 nm.

Figure 3.7 X-ray crystal structure of 3. Hydrogen removed for clarity and structures shown with 50% ellipsoids.
3.7 Spectroscopic Studies of 6, 7 and 8

Compound 6 and 7 are fluorescein-based chemosensors for Cu$^{2+}$ with highly selective “off-on” behavior, two of them working in both absorption and emission, the other only in absorption, 7. Binding to Cu$^{2+}$ binding is reversible, as indicated by the bleaching of the color when the metal is extracted with excess EDTA. The compounds are the basis for rapid, selective and sensitive Cu$^{2+}$ chemosensors in aqueous media.

The compounds function via conversion of the weakly fluorescent ring-closed form to the ring-open fluorescent amide form, Scheme 3.1 and 3.2. They are designed to bind metal ions via the carbonyl O, inamine N, and hydroxyl groups as donors. Compound 6 has a pocket to bind Cu(II) with 5- and 6-membered rings, Scheme 3.1 and 3.2, while 7 has two similar pockets. The structures were confirmed using $^1$H NMR, $^{13}$C NMR, mass spectrometry and elemental analysis.
Minimal fluorescence  
highly fluorescent

Scheme 3.2. Proposed mechanism for the fluorescence changes of 6 (and 7) with Cu$^{2+}$.

All spectroscopic studies were performed in 2% DMSO in aqueous solution in which the sensors formed colorless solutions that are stable for more than six month. The compounds are very weakly fluorescent in solution and nearly devoid of absorption in the visible, due to the predominance of the ring-closed spirolactam form. This was further confirmed by $^{13}$C NMR signals at δ 65.76 and δ 66.04 for compounds 6 and 7 respectively.

Figure 3.9 UV- vis absorption spectra of 6 (10µM) upon addition of respective metal ions (20 µM) in DMSO-water (2%DMSO) at pH 7.0 aqueous solution.
High selectivity is an important characteristic of an ion-selective chemosensor. Therefore the chlorides of Ca\(^{2+}\), K\(^{+}\), Fe\(^{3+}\), Fe\(^{2+}\), Pb\(^{2+}\), Co\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\) and nitrates of Cr\(^{3+}\), Hg\(^{2+}\) and Ni\(^{2+}\) ions were evaluated for their metal-binding properties (10 µM) in DMSO-water (Fig. 3.9), and these showed no significant UV-vis absorption changes for 6 and 7. Under the same conditions, Cu\(^{2+}\) produces an immediate and obvious change, which indicates that chemosensor 6 has much higher binding affinity toward Cu\(^{2+}\) than to the other metal ions surveyed. The color change can be attributed to the resonance stabilization of the carbocation moiety formed upon Cu(II) complexation.

![Figure 3.10 UV-vis absorption changes of 6 (10 µM) upon addition of different concentration of Cu\(^{2+}\) ions in DMSO-water (2% DMSO) at pH7.0 aqueous solution. Cu\(^{2+}\) ion concentration (µmolL\(^{-1}\)]: 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15, 16.5, 18, 19.5, 21.](image)

The absorption spectrum of 6 (10 µM) in DMSO-water (10mM Tris-HCl buffer, pH7.0) exhibited only a very weak band above 400nm, ascribed to a trace of the ring-open form of 6, but addition of Cu\(^{2+}\) produced a new peak at 500 nm with a shoulder
at 475 nm (Fig. 3.9). The change is visible to the naked eye: Cu$^{2+}$ binding instantly turns the solution yellow (Fig. 3.12). The anion has no effect: 6 has the same UV-vis absorption spectra with Cu(AcO)$_2$, CuSO$_4$, Cu(NO$_3$)$_2$ and CuCl$_2$. Upon coordination, the absorption band at 375nm decreases in intensity while that at 325nm increases (Fig. 3.10). Isosbestic points at 345 nm and 400 nm indicate a new metal complex in equilibrium with the free ligand 6.

The Job’s plot indicates a 1:1 binding between 6 and Cu$^{2+}$ with an association constant of $3.7 \times 10^4$. When the 6-Cu$^{2+}$ solution is treated with 1.0 mmol L$^{-1}$ EDTA, the yellow color almost disappears, indicating that the coordination of 6 with Cu$^{2+}$ is chemically reversible. Additional EDTA eliminates the color completely.

Figure 3.11: Job’s plot of 6 (10 µM) with Cu$^{2+}$ (10 µM) in DMSO-Water at pH 7.5.
To explore the utility of 6 as a cation–selective chromogenic chemosensor, a competition experiment was carried out by adding 20 µM Cu$^{2+}$ to a solution of 6 (10 µM) in the presence of selected other cations including Ca$^{2+}$, K$^+$, Fe$^{3+}$, Fe$^{2+}$, Pb$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Cr$^{3+}$, Hg$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$ (each at 20 µM). The competing cations alone did not lead to significant absorption changes of 6. However, additions of two equivalents of Cu$^{2+}$ to the solution produced the spectral and color changes characteristic of Cu$^{2+}$. Similar results were found for 7, which indicate that both 6 and 7 have good selectivity toward Cu$^{2+}$ over other competitive cations, and further, that the detection of Cu$^{2+}$ is little affected by the presence of these common metal ions.

The fluorescence spectra were obtained by excitation of the fluorescein fluorophore at 470 nm. Among the metal ions tested, compound 6 showed significant fluorescence enhancements with Cu$^{2+}$ only (Figs. 3.13), identifying it as an excellent turn-on sensor for Cu$^{2+}$. The emission maximum for 6-Cu$^{2+}$ at 518 nm is red-shifted by about 7nm compared with that of pure 6. In contrast to 6, compound 7 shows no significant fluorescence with Cu (II) nor any of the other cations (Figure 3.18). However, 7 shows remarkably high absorption in the presence of Cu(II) over other competitive cations, with a new peak at 500 nm and a shoulder at 475 nm (Figure 3.16 and 3.17).
Figure 3.13  Fluorescence emission changes in 6 (10 µM) upon addition of different metal ions (1 equiv.) in DMSO-Water (2% DMSO) at pH7.0 (excitation at 470 nm).

Figure 3.14 Relative fluorescence intensities of compound 6 with different metal ions.
The fluorescence titration was also carried out using 6 (10 µM) in buffered DMSO-water at pH 7.0. To get a view of the practical application potential, we examined the fluorescence sensing behavior of 6 for Cu(II) in buffered water solution (with 2% DMSO). Continuous fluorescence enhancement and a red shift of the emission peak from 511 to 518 nm were observed upon addition of 20 equiv of Cu(II) (Figure 3.15).

Figure 3.15 Fluorescence changes of 6 (10 µM) with Cu$^{2+}$ (0–180 µM). Inset: fluorescence enhancement factor $F$ at 518 nm as a function of Cu (II) concentration.
Figure 3.16 UV-vis absorption spectra of 7 (10 µM) upon addition of respective metal ions (20 µM) in DMSO-water (2%DMSO) at pH 7.0 aqueous solution.

Figure 3.17 UV-vis absorption changes of 7 (10 µM) upon addition of different concentration of Cu^{2+} ions in DMSO-water (2%DMSO) at pH 7.0 aqueous solution.
Figure 3.18 Fluorescence emission changes of 7 (10 µM) upon addition of different metal ions (1 equiv.) in DMSO-Water (6/94, v/v) at pH 7.0 (excitation at 470 nm).

Sensor 6 has a two-component decay lifetime, a fast component of 0.931 ns and a slower one of 3.308 ns. On adding Cu\(^{2+}\) (10 µM), the faster component changes to 0.376 ns and the slower one to 3.791 ns, evidence of increased relaxation processes. The simultaneous non-radiative processes have slightly longer lifetimes (Figure 3.19).
In conclusion, we used the ring-opening of the new fluorescein derivatives to develop two new Cu$^{2+}$ chemosensors, 6 and 7. These display reversible absorption and fluorescence-enhanced responses to Cu(II) via a 1:1 complex. Addition of Cu$^{2+}$ to an aqueous solution of 6 or 7 results in obvious changes from colorless to deep yellow. Their selectivity toward Cu(II) is very high: there is little interference from the presence of other commonly coexistent metal ions. These features indicate that the sensors are good candidates for rapid, selective and sensitive detecting Cu$^{2+}$ in aqueous media.
CHAPTER IV

FLUORESCENCE AND ELECTROCHEMICAL SENSING OF Zn$^{2+}$, Fe$^{3+}$ and Cr$^{3+}$
BASED ON COUMARIN DERIVATIVES

4.1 Luminescence

Luminescence is the emission of light that occurs at low temperatures from a photochemically generated excited electronic state of any substance. The singlet ground, first and second electronic states are depicted by $S_0$, $S_1$ and $S_2$ respectively. At each of these electronic energy levels the fluorophores can exist in a number of vibrational energy levels, denoted by 0, 1, 2, etc. Absorption of a photon of appropriate energy results in the promotion of an electron to an orbital of higher energy $S_n$ ($n=1, 2, \ldots$). This process is known as excitation and has an instantaneous nature ($10^{-15}$ s, depicted as vertical lines on the diagram)\(^7\). The lowest energy and the most important electronic transitions from an organic perspective are between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the ground state configuration; $n-\pi^*$ and $\pi-\pi^*$ transition are of particular importance.

Following light absorption, several processes usually occur: a fluorophore is excited to a higher vibrational level of $S_1$ ($S_2$ followed by internal conversion (IC) to $S_1$) then relaxes to the lowest vibrational level of $S_1$. This process occurs in $10^{-12}$ s or less. Intersystem crossing (ISC) takes place if the singlet excited state undergoes a change in spin multiplicity. Emission from the singlet state is defined as fluorescence. It typically
occurs in $10^9$ s. The emitted light is always of longer wavelength than the incident light, a characteristic of fluorescence emission known as Stokes shift. Emission from triple state is called phosphorescence. The spin-forbidden process of ISC and formation of the triplet excited state is facilitated by the presence of $n\pi^*$ states or heavy atoms.

Scheme 4.1 Jablonski diagram.

4.2 Fluorescent Chemosensors

There are several reasons for which fluorescence may be identified as the optimal signaling in potential sensing application. Fluorescence is an enormously sensitive technique. Fluorescence signaling permits the monitoring of both excitation and emission wavelengths. The emission signal may be observed by intensity, intensity ratio or life time measurement. Fluorescence is usually nondestructive.

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The most common type of fluorescent chemosensors is ion (cation or anion) selective chemosensors that are capable of reporting the ion coordination process. In the concept for ion recognition binding sites can be coupled to certain groups that are capable of “reporting” the ion coordination process. In this case the binding process is transduced into a signaling event. One basic principle in this multi-component system is that the sensing event has to be related with an easy-to-measure signal.

Scheme 4.2. Principle of fluorescent chemosensors.

4.3. Fluorescent Signaling Mechanisms

4.3.1. Photoinduces Electron Transfer (PET)

Photoinduced Electron Transfer (PET) is one of the most important mechanisms for fluorescent chemosensors that has been intensively studied and widely used for sensing purposes of cations and anions. The thermodynamic basis for PET has been described for intermolecular systems by the pioneering work of Weller. It is well known, that fluorescence in a molecule occurs when an excited electron in the lowest unoccupied molecular orbital (LUMO of the ground state $S_0$) goes to the highest occupied molecular orbital (HOMO of the ground state $S_0$), releasing the excess energy.
as light. If an orbital from another part of the same molecule or from another molecular entity has energy between that of the HOMO and that of the LUMO and if this orbital if occupied (e.g. if we have a donor group “D” (Step 1)), a PET from this full orbital to the HOMO of the fluorophore can take place (Step 2). A further electron transfer from the LUMO of the fluorophores to the external orbital retrieves the stable ground state (Step 3) (Scheme 4.3).

Scheme 4.3. Frontier energy diagram illustrating PET and reverse back ET.

If the donor and acceptor are to be fixed within the same molecular framework, then they must be separated by a spacer that is short enough for efficient electron transfer, but long enough to minimize the extent of electronic delocalization between the partners. The design of fluorescent chemosensors should take advantage of PET effects in such a way that the presence of an ion should induce or suppress PET, leading to quenching or enhancement of fluorescence intensity.
One of the first specially designed chemosensors for cation detection was the system in Figure 4.1. Chelation-enhanced fluorescence (CHEF) on ZnCl$_2$ addition is a result of metal ion complexation of the amine lone pair, which decreases amine oxidizability such that it can not reduce the anthracene S$_1$ excited state.$^{79}$

### 4.3.2. Photoinduced charge transfer

When a fluorophores contains an electron-donating group (often an amino group) conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer (ICT) from the donor to the acceptor upon excitation by light. The consequent change in dipole moment results in a Stokes shift that depends of the microenvironment of the fluorophore.$^{80}$ Usually the donor and acceptor groups are conjugated in the ground state and undergo significant charge transfer in the excited state. If the two groups are able to adopt an orthogonal geometry, then full charge separation occurs, producing a twisted intramolecular charge transfer (TICT) state. It can be expected that cations in close interaction with the donor or the acceptor moiety will change the photophysical
properties of the fluorophores because the complexed cation affects the efficiency of the ICT.

Scheme 4.4. Spectral displacement of PCT sensor resulting from interaction of a bound cation with an electron-donating or electron-withdrawing group. When an electron donating group (like an amino group) within the fluorophores interacts with a cation, its electron-donating character is reduced. What can be expected is a blue shift in the absorption spectra. Conversely, a cation interacting with the acceptor group enhances its electron-withdrawing character. This leads to the red shift in absorption spectra and the molar absorption coefficient is increased. The fluorescence spectra are in principle shifted in the same direction. These shifts are usually accompanied by changes in quantum yields and lifetime. All these photophysical effects depend on the charge and the size of the cation as well as on the nature of the receptor (Scheme 4.4). Most chemosensors of this type contain an azacrown as the cation receptor, which is conjugated to an electron-withdrawing group. For instance (4-
dicyanomethylene-2-methyl-[6-p-(dimethylamino)styryl]-4H-pyran, the so-called DCM-crown 9 (Figure 4.2).

![Figure 4.2](image_url) Azacrown electron donor group incorporating a cation.

Upon complexation with alkaline-earth metal cations, it undergoes dramatic changes in absorption spectrum (hypsochromic shift and hypochromic effect) and fluorescence quantum yield (quenching), whereas the emission spectrum is only slightly blue-shifted and fluorescence lifetime is almost unchanged. In this DCM derivative the nitrogen atom belongs to the crown, and therefore, complexation by cation reduces its donor character which in turn hinders the charge transfer depending on the nature of the cation\(^8\).

While most examples of fluorescent sensing in ICT systems rely on coordination of the analyte near the donor, a few examples exist where the electron-accepting region participates in the binding event. In coumarin derivatives linked to crown the cation interacts directly with the electron-withdrawing group, i.e. the carbonyl group. Both absorption and emission spectra are red shifted upon binding (Figure 4.3)\(^8\).
4.4 Importance of Detection of Zn$^{2+}$

There is significant interest in the design and synthesis of fluorescent sensors for the detection of physiologically important ions and molecules \(^{83}\), and monitoring of harmful pollutants in the environment. The best chemosensors are designed to be highly selective, sensitive and simple \(^{84}\). Chemosensors for Zn$^{2+}$ ion have received considerable attention\(^{85-89, 90}\). As the second most abundant transition metal in the human body, zinc plays a critical role in many biological activities such as gene expression, metalloenzyme catalysis and neurotransmission\(^{85, 86}\). Therefore, the design and development of efficient, fluorescent chemosensors selective to Zn ions are of interest. Although several fluorescent-based chemosensors for Zn$^{2+}$ have been developed, some of them have shortcomings for practical application, such as sensitivity toward other metal ions, susceptibility to pH and difficult syntheses\(^{91}\). It is therefore necessary to develop new chemosensors for Zn$^{2+}$ with high selectivity and sensitivity at physiological pH.

Recently Su et al\(^{85}\) have reported a coumarin-based fluorescent chemosensor for Zn$^{2+}$ in aqueous ethanol media. The sensor exhibits lower background fluorescence due to intramolecular photoinduced electron transfer. However, upon mixing with Zn$^{2+}$ in 30% (v/v) aqueous ethanol, a “turn-on” fluorescence emission is observed. The fluorescence emission increase linearly with Zn$^{2+}$ concentration in the range 0.5 – 10 µmol
L$^{-1}$ with a detection limit of 0.29 µmol L$^{-1}$. The proposed chemosensor was applied to the determination of Zn$^{2+}$ in water samples with satisfactory results.$^{85}$

![Chemical structure diagram]

Figure 4.4 The proposed binding model of the sensor$^{85}$ with Zn$^{2+}$.

Lim et al.$^{88}$ have also reported coumarin-based sensor that incorporate the dipicolylamine (DPA) chelating unit. Variations of the nature of the chelating unit, positions of the attachment point of the chelating unit, and nature of the 7-substituent on the coumarin play a crucial role in whether, and to what extent, a CHEF-type or ratiometric response of the chemosensor is observed. Solvent effects are also discussed. The chemosensors were shown to be competent for detecting Zinc pools in cultural hepatoma cell lines.$^{88}$

![Chemical structure diagram]

Figure 4.5 Binding model of the sensor$^{14}$ with Zn$^{2+}$.

4.5 Importance of Detection of Fe$^{3+}$ and Cr$^{3+}$

The detection of various metal ions has developed quickly$^{114}$, because of their importance in many biological and environmental processes. As an essential element for
life, Fe$^{3+}$ provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain, and both its deficiency and excess can induce a variety of diseases $^{115}$. This makes it very important to detect iron ions. Several techniques such as atomic absorption spectroscopy, spectrophotometry and voltametry have been used for iron assay. Some of them are complicated, and not suitable for quick and online monitoring. In recent years, fluorescent sensors have attracted more and more attention due to their advantages over other techniques, including ease of detection, sensitivity, and instantaneous response. Many metal ions have been detected by this method $^{115}$. However, the examples of Fe$^{3+}$ selective fluorescent probes are still scarce $^{116}$ owing to ready interference by other transition-metal ions such as Cu$^{2+}$ and Co$^{2+}$.

The development of Fe$^{3+}$ sensors using coumarin derivatives has increased in the recent past. Yao et al.$^{118}$ have developed a Fe$^{3+}$-selective sensor based on coumarin derivative. The sensor showed an excellent selectivity towards Fe$^{3+}$ (figure 4.6) and was not affected by the presence of other potentially interfering metals.
Figure 4.6 Proposed binding mode of sensor with Fe$^{3+}$, $x =$ solvent molecule$^{118}$.

Trivalent Chromium is also an essential trace element in the human body as a part of the glucose tolerance factor and also plays an important role in lipid and protein metabolism$^{107}$. Chromium deficiency can increase the risk of diabetes and cardiovascular diseases$^{107,108}$. On the other hand, chromium is an environmental pollutant and its build-up due to various industrial and agricultural activities is a matter of concern$^{107,108}$. Thus, there is an urgent need to develop chemical sensors that are capable of detecting the presence of chromium ions in biological and environmental samples. Extant Cr$^{3+}$ detection uses traditional methods such as electrochemical$^{111}$ and potentiometric$^{112}$. In addition there are few reports on fluorometric detection$^{109}$ of Cr$^{3+}$ which has a greater impact as it is simple and rapid. However, in most cases the selectivity for Cr$^{3+}$ is low.
Due to the paramagnetic nature of Cr\(^{3+}\), the development of turn-on sensors still remains challenging. Figure 4.7 shows the fluorescent turn-on sensor developed by Sarkar et al\(^{113}\). This sensor exploits the guest-induced inhibition of the photoinduced electron transfer signaling mechanism. The system shows an approximately 17-fold Cr(III)-selective chelation-enhanced fluorescence response in tetrahydrofuran and the system is highly selective against the background of environmentally and biologically relevant metal ions.

![Figure 4.7 Chemosensor\(^{113}\) for Cr\(^{3+}\).]

4.6 Coumarin Derivatives as Chemosensors for Zn\(^{2+}\)

Coumarin-based fluorescent chemosensors have received increasing interest in recent years\(^{85, 86, 90, 91}\) by virtue of their low toxicity, excellent photophysical properties such as high absorption coefficient, high fluorescent quantum yield and ease of modification. Direct interaction with the carbonyl group of coumarin is frequently exploited as a good model for chemo-sensor design, because the spectroscopic response is fast and efficient when guests such as metals ions are introduced. This phenomenon gives them an excellent potential for the development of turn-on fluorescent sensors. In all these sensors, the mechanism involves metal chelation and stops photoinduced C=N isomerization of the sensors, resulting in fluorescence enhancement in the visible region (460-600 nm).
Herein, reported new coumarin chemosensors, 10-14 (Scheme 4.5), which exhibit a high selectivity toward Zn\(^{2+}\) ions via a chelation-enhanced fluorescence mechanism. In free Schiff-base derivatives of coumarin, the photoinduced C=N isomerization quenches the excited state emission and decreases the fluorescence intensity of the compounds. However, metal binding could stop isomerization, thereby greatly enhancing the fluorescence. In this design, therefore, we expect that the newly designed chromogenic chemosensors will show good selectivity for Zn\(^{2+}\) and other metal ions. Also, a large shift in absorption would be expected due to the significant enhancement of the intramolecular charge transfer from the donating moiety (diethylamino) to the electron withdrawing moieties (carbonyl and imine) generated by the binding of a metal ion. Our interest in coumarin derivatives as fluorescence signaling systems stems from the fact that they have a large Stokes shift upon metal binding.

Research has shown the use of direct electrochemical methods along with electrical impedance spectroscopy (EIS) for the detection of toxic heavy metals. Sensing devices that integrate the dual use of fluorescent and electrochemical detection techniques reduces the possibility of false positives. Therefore the novel approach of using rapid, efficient, portable and miniaturized dual detection sensing systems is crucial to the advancement of opto-electrical sensor technology. The high sensitivity of EIS-based electrochemical sensing devices along with research on the integration of these sensors into hand-held portable devices for in-situ detection of heavy metals is important for applications in the medical and environmental agencies. An EIS-based efficient sensor device is used for the electrochemical detection of Zn\(^{2+}\). The device is fabricated using photolithography technique, which employs gold (Au) interdigitated electrodes (IDE) on a glass substrate.
The EIS and fluorescence based response of the electrochemical sensor towards Zn$^{2+}$ using the coumarin-based chemosensors 10 and 11 is demonstrated.

4.7 General Procedure for UV-Vis, Fluorescence and Electrochemical Impedance Studies of Compound 10-19

Fluorescence spectra were recorded on an Edinburgh FS920 fluorimeter at room temperature, on 10 µM solutions of 10-19 in acetonitrile with appropriate amounts of metal salts. UV absorbance spectra were recorded on a Shimadzu UV-2101PC. $^1$H NMR and $^{13}$C NMR spectra were obtained on a JEOL eclipse (400 MHz) instrument.

Stock solutions (5 mM) of chloride and nitrate salts were prepared in CH$_3$CN. Stock solutions of free 10–19 (10 µM) were prepared in CH$_3$CN. Excitations were carried out at 370 nm (for 10-14) and 390 nm (for 15-19) with all excitation slit widths is 3 nm that of emission is 3 nm. For the Job plot experiment, 15 (10.0 µM) in CH$_3$CN and ferric chloride (10.0 µM) in CH$_3$CN were prepared as stock solutions. The concentration of each CH$_3$CN solution was varied, but the total volume was fixed at 3.0 mL. After the mixture was shaken, the absorbance intensity at 355 nm was recorded.

Electrical impedance spectroscopy: The design and fabrication process of the electrochemical sensor device used in this work has been comprehensively described in a previously published study. The sensor was rinsed with acetone and then blow dried with pressurized air before use. All measurements were conducted in room temperature. Initially, 10 µL of the 10 was placed on the sensor to establish a reference signal, then it was washed with acetone and dried in a stream of pressurized air. Then 10 µL of varying concentrations of 10 - Zn$^{2+}$ (100 pM, 100 nM, 1 µM and 1 mM) and 11 - Zn (100 pM, 100 nM, 1 µM, 1 mM and 100 mM) were pipetted, in individual tests, onto the sensor.
The impedance measurements were performed using an Agilent E4980A precision LCR meter, connected to the sensor via small outline integrated circuit (SOIC) test clips, at frequency ranges between 20 Hz to 2 KHz with a 1 mV voltage excitation.

4.8 Synthesis of Compounds 10-19

Chemosensors 10 - 14 were synthesized in good yields from readily available starting materials. 7-Diethylaminocoumarin-3-aldehyde was synthesized via a series of reactions, and then reacted in ethanol at reflux with an equimolar amount of the corresponding ligand to afford the compounds in very high yields. The structures were characterized using $^1$H NMR, $^{13}$C NMR, elemental analysis and x-ray crystallography and are shown in Fig 4.8. Single crystals for x-ray analysis were grown by slow evaporation of a CHCl$_3$ and CH$_3$OH mixture. Chemosensors 15-19 also synthesized via a one step Schiff base reaction and the compounds characterized by $^1$H NMR, $^{13}$C NMR and elemental analysis.
Figure 4.8 X-ray crystal structure of sensor 10 and the intermediate aldehyde, 9.
Scheme 4.5 Synthetic route of sensor 10–14.
Synthesis of 7-diethylaminocoumarin

4-Diethylaminosalicylaldehyde (1.93g, 10mmol), diethylmalonate (3.2g, 20mmol) and piperidine (1 mL) were combined in absolute ethanol (30 mL) and stirred for 16hr under reflux conditions. Ethanol was evaporated under reduced pressure, then concentrated HCl (20 mL) and glacial acetic acid (20 mL) were added to hydrolyze the reaction mixture with stirring for another 16 hours. The solution was cooled to room temperature and poured into 100mL ice water. NaOH solution (40%) was added dropwise to modulate the pH of the solution to 5.0, and a pale precipitate formed immediately. After stirring for 30min., the mixture was filtered, washed with water, dried, then recrystallized with toluene to give 1 as gray powder (1.73g, 8.0mmol) in 80% yield. $^1$H-NMR (CDCl$_3$) δ 7.53 (d,1H), 7.20 (d,1H), 6.48 (d,1H), 6.40 (s,1H), 6.01(d,1H), 3.40 (m,4H), 1.20 (t,6H).

Synthesis of 7-diethylaminocoumarin-3-aldehyde, 9

Freshly distilled DMF (2 mL) was added dropwise to POCl$_3$ (2 mL) at 20-50°C in a N$_2$ atmosphere and stirred for 30 minutes to yield a red solution. This solution was combined with a portion of 1 (1.50g, 6.91 mmol, dissolved in 10 mL DMF) to yield a scarlet suspension. The mixture was stirred at 60°C for 12 hours and then poured into 100mL of ice water. NaOH solution (20%) was added to adjust the pH of the mixture to yield large amount of precipitate. The crude product was filtered, thoroughly washed with water, air dried and recrystallized in absolute ethanol to give the product (1.20g, 4.89mmol) in 70.8% yield. $^1$H NMR (CDCl$_3$) δ 10.15 (s, 1H), 8.24 (s, 1H), 7.40 (d, 1H), 6.40 (s, 1H), 6.01(d,1H), 6.63 (d, 1H), 6.47 (s, 1H), 3.47 (m, 4H), 1.21 (t, 6H).
Synthesis of compound 10

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (1mmol) and 2-amino-5-methyl phenol (1mmol) were suspended in 20 mL ethanol. The mixture was refluxed for 12 hr with stirring, during which time an orange precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, an orange solid in 85% yield was obtained. $^1$H NMR (400 MHz, CDCl$_3$-d$_6$), δ (ppm) 8.89 (s, 1H), 8.44 (s, 1H), 7.42 (dd, 1H), 7.25 (dd, 1H), 6.81 (s, 1H), 6.64 (dd, 1H), 6.64 (dd, 1H), 6.50 (s, 1H), 3.46 (m, 4H), 2.32 (s, 3H), 1.24 (t, 6H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 12.58, 21.58, 45.17, 97.30, 108.92, 109.81, 115.24, 115.40, 115.81, 121.09, 130.96, 133.21, 139.34, 140.55, 150.03, 152.06, 152.18, 157.56, 162.41. Elemental Analysis Calcd for C$_{21}$H$_{22}$N$_2$O$_3$: C, 71.98; H, 6.33 N, 7.99; O, 13.70 Found: C, 71.58; H, 6.67; N, 8.08; O, 13.67.

Synthesis of compound 11

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (1mmol) and 4-amino-3-hydroxy benzoic acid (1mmol) were suspended in 20 mL ethanol. The mixture was refluxed for 12 hr with stirring, during which time an orange precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, an orange solid in 80% yield was obtained. $^1$H NMR (400 MHz, CDCl$_3$-d$_6$), δ (ppm) 9.40 (s, 1H), 8.79 (s, 1H), 8.64 (s, 1H), 7.65 (dd, 1H), 7.63 (s, 1H), 7.44 (dd, 1H), 7.18 (dd, 1H), 6.83 (dd, 1H), 6.6 (s, 1H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 0.68, 12.96, 40.49, 97.03, 108.71, 110.65, 114.24, 117.17, 120.14, 121.65, 132.18, 142.87, 151.11, 152.70, 155.54, 157.94, 161.80, 167.62. Elemental analysis Calcd for
C\textsubscript{21}H\textsubscript{20}N\textsubscript{2}O\textsubscript{5}: C, 66.31; H, 5.30; N, 7.36; O, 21.03 Found : C, 67.14; H, 4.50; N, 8.25; O, 20.11.

**Synthesis of Compound 14**

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (1mmol) and 3-amino-2H-chromeen-2-one (1mmol) were suspended in 20mL ethanol. The mixture was refluxed for 12hr with stirring, during which time a brown red precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, a brownish red solid in 87% yield was obtained. \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}-d6), δ (ppm) 8.94 (s, 1H), 8.61 (s, 1H), 7.81 (s, 1H), 7.72 (d, 1H), 7.69 (d, 1H), 7.52 (t, 1H), 7.42 (d, 1H), 7.36 (t, 1H), 6.83 (d, 1H), 6.62 (s, 1H), 3.50 (q, 4H), 1.15 (t, 6H). \(^1^3\)C NMR (400 MHz, CHCl\textsubscript{3}-d6) 12.58, 45.36, 97.23, 108.89, 109.99, 114.34, 116.21, 116.49, 120.04, 124.74, 127.58, 127.74, 130.48, 132.60, 138.27, 142.83, 145.46, 152.55, 158.07, 159.06, 162.16.

Elemental analysis Calcd for C\textsubscript{23}H\textsubscript{20}N\textsubscript{2}O\textsubscript{4}: C, 71.12; H, 5.19; N, 7.21; O, 16.48 Found : C, 71.16; H, 5.47; N, 7.20; O, 16.17.
Compound 15: A portion of chromene-3-carbaldehyde (0.15 g, 1 mmol) and 7-amino-4-(trifluoromethyl) coumarin (0.2 g, 1 mmol) were combined in hot absolute ethanol (30.0 mL) for 20 h to obtain the yellow solid of 15 in 82% yield. $^1$H NMR (400 MHz, CDCl$_3$-d6), δ (ppm) 5.81 (s, 1H), 6.67 (s, 1H), 7.02 (t, 2H), 7.09 (s, 1H), 7.12 (d, 1H), 7.47 (t, 2H), 7.69 (d, 1H), 7.95 (d, 2H). $^{13}$C NMR (400 MHz, CDCl$_3$-d6), δ (ppm): 15.17, 64.23, 100.19, 103.14, 106.81, 109.29, 114.00, 118.20, 122.39, 126.43, 126.61, 131.17, 134.05, 134.96. 

Scheme 4.6 Synthetic route of sensor 15 – 19.
Compound 16: 3-hydroxy-2-naphthaldehyde (0.15 g, 1 mmol) and 7-amino-4-(trifluoromethyl) coumarin (0.2 g, 1 mmol) were combined in hot absolute ethanol (40.0 mL) for 10 h to obtain the orange solid of 16 in 88 % yield. $^1$H NMR (400 MHz, CDCl$_3$-d6), δ (ppm) 6.98 (d, 1H), 7.04 (s, 1H), 7.38 (t, 1H), 7.57 (t, 1H), 7.64 (d, 1H), 7.79 (dd, 2H), 7.98 (d, 1H), 8.02 (s, 1H), 8.60 (d, 1H), 9.71 (s, 1H). Elemental Analysis Calcd for C$_{20}$H$_{10}$F$_3$NO$_4$: C, 62.35; H, 2.62 N, 3.64 Found: C, 60.90; H, 4.31; N, 3.45.

ESI-MS. 384.1[M-H]$^+$

Compound 17: A portion of salicylaldehyde (0.58g) and 7-amino-4-(trifluoromethyl) coumarin (1g) were combined in hot absolute ethanol (50.0 mL) for 5h to obtain the orange solid of 17 in 85 % yield. $^1$H NMR (400 MHz, CDCl$_3$-d6), δ (ppm) 6.78 (s, 1H), 6.99 (t, 1H), 7.06 (d, 1H), 7.27 (m, 2H), 7.45 (d, 2H), 7.76 (d, 1H), 8.65 (s, 1H).

Compound 18: 7-diethylaminocoumarin-3-aldehyde, 9 (0.21 g, 1 mmol) and 7-amino-4-(trifluoromethyl) coumarin (0.2 g, 1 mmol) were combined in hot absolute ethanol (30.0 mL) and refluxed for 10 hr. The reaction mixture was allowed to cool down to room temperature and evaporated to dryness. The crude product was purified on a silica gel column using EtOAc/Hexane (1:1) as the mobile phase to get 18 as a brownish red (93 mg, 47%). $^1$H NMR (400 MHz, DMSO-d$_6$), δ (ppm) 1.15 (t, 6H), 3.50 (q, 4H), 6.44 (s, 1H), 6.50 (d, 1H), 6.53 (s, 2H), 6.61 (s, 1H), 6.61 (d, 1H), 7.35 (d, 1H), 7.69 (d, 1H), 8.41 (s, 1H), 9.89 (s, 1H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 12.92, 40.46, 96.94, 99.51, 102.30, 108.25, 111.07, 112.77, 113.7, 126.35, 133.66, 146.77,
Elemental Analysis Calcd for C_{24}H_{19}F_{3}N_{2}O_{4}: C, 63.16; H, 4.20 N, 6.14 Found: C, 62.90; H, 4.31; N; 6.00.

Compound 19: 8-hydroxy quinoline-2-carbaldehyde (0.19 g, 1mmol) and 7-amino-4-methyl-chromene-2-one (0.20 g, 1mmol) were combined in hot absolute ethanol (30.0 mL) and refluxed for 10h. The white solid which resulted was filtered out and washed with ethanol to obtain 19 in 78 % yields. ^1H NMR (400 MHz, DMSO-d$_6$), δ (ppm) 2.42 (s, 3H), 5.76 (s, 1H), 6.05 (s, 1H), 6.25 (d, 1H), 6.93 (d, 1H), 7.07 (d, 2H), 7.09 (d, 1H), 7.18 (dd, 1H), 7.46 (dd, 1H), 7.51 (t, 1H), 7.63 (d, 1H), 7.74 (d, 1H), 8.13 (d, 1H), 8.45 (d, 1H). ^13C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 15.89, 18.62, 58.60, 83.26, 99.64, 109.48, 112.27, 118.19, 121.22, 126.74, 137.04, 138.01, 150.69, 153.77, 154.30, 155.71, 161.15. Elemental Analysis Calcd for C_{20}H_{14}N_{2}O_{3}: C, 72.72; H, 4.27 N, 8.48 Found: C, 72.74; H, 4.24; N; 14.52. ESI-MS. 331.2[M-H]^+ 

4.9 Absorbance Study of Compounds 10 - 13

Compounds 10 - 13 were designed to bind metal ions via the carbonyl O, imine N, and the –OH group (Scheme 4.5). The absorption spectrum showed a new absorbance at 525 nm in the visible region due the binding with Zn$^{2+}$. As the concentration of Zn ion increases, the absorption intensity of the compound 10 gradually increases at 500 nm and decreases at 537 nm forming an isosbestic point at 520nm (Figure 4.10).
Figure 4.9 The absorbance of compounds 10-13 (3 $\mu$M) in CH$_3$CN and their complex after addition of Zn(NO$_3$)$_2$.6H$_2$O.

Figure 4.10 UV-vis absorbance titration of Compound 10 with different concentration of Zn$^{2+}$ in CH$_3$CN.
The selectivity of 10 and 11 toward Zn$^{2+}$ was investigated by treating both compounds with other metal ions such as Ni$^{2+}$, Co$^{2+}$, Fe$^{3+}$, Fe$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Cu$^{2+}$, Na$^+$, and Ca$^{2+}$ in CH$_3$CN solution. The absorption maximum shows no obvious change upon addition of the metal ions Fe$^{2+}$, Fe$^{3+}$, Pb$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Na$^+$, and Ca$^{2+}$ but with Hg$^{2+}$, Co$^{2+}$ and Ni$^{2+}$, a single new absorption at 530, 515 and 518 nm respectively, was observed (Figure 4.11).

![UV absorption of compound 10 with addition of metal ions Zn$^{2+}$, Hg$^{2+}$, Co$^{2+}$ and Ni$^{2+}$ (10 uM).](image)

**Figure 4.11** UV absorption of compound 10 with addition of metal ions Zn$^{2+}$, Hg$^{2+}$, Co$^{2+}$ and Ni$^{2+}$ (10 uM).

4.10 Fluorescence Study of Compound 10 and 11

When excited at 370 nm, the solutions of compounds 10-13 were weakly fluorescent due to isomerization of the C=N bond. Wu et al. recently reported that C=N isomerization was the predominant decay process of the excited states for compounds with unbridged C=N structures often causing these compounds to be nonfluorescent$^{92}$. 

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Compound 10 showed very weak emission changes when coordinating with some metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}) and showed a small emission enhancement with Hg^{2+} (Fig 4.12 and 4.13). By contrast, the addition of Zn^{2+} to solutions of 10 results in a drastic fluorescence emission change and bathochromic shift. The fluorescence peak at 475 nm and red shifts to 550 nm with a marked fluorescence intensity enhancement. The fluorescent quantum yield of the coumarin derivative, compound 10, increases about 100-fold due to inhibition of the C=N isomerization process upon Zn ion binding at the NOO site (Scheme 4.5). The fluorescent enhancement of sensor 10 peaks at 550nm with Zn^{2+} and at 556 nm with Hg^{2+}. This indicates a red shift for Hg^{2+} of 6 nm from Zn^{2+} (Fig 4.12 and 4.13). All these observations indicate that sensors 10 and 11 have high sensitivity and selectivity towards Zn^{2+} over other metal ions tested. The selectivity of 10 and 11 for zinc can be explained, in part, due to fact that the close-shelled orbitals of Zn do not provide a nonradiative pathway for the excited state electron or energy transfer occurring within the associated complex. The binding of the sensor 10 and 11 with Zn also showed a color change which is unique and advantageous for direct observation.
Figure 4.12 Fluorescence intensity of sensor 10 (3 µM) in the presence of Na⁺, Ca²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Fe²⁺ and Zn²⁺ (10 µM) in CH₃CN (excitation 370 nm).

Figure 4.13 Relative fluorescence intensities of 10 (3 µM in CH₃CN) in the presence of various metal ions.
The fluorescent titration spectrum shows the fluorescence “turn on” characteristic of the compound 10 by Zn$^{2+}$ (Fig 4.14) and determines the association constant $^{93-104}$ to be $2.4 \times 10^4$ M$^{-1}$. The reversibility and binding activity of the sensor with Zn$^{2+}$ was also investigated. Ethylenediaminetetraacetic acid (0.02 M EDTA), a very strong cation chelating agent, was added to the solution of 10-Zn$^{2+}$ and it was observed that the fluorescence intensity decreased near to the intensity of the free compound. The fluorescence spectrum of compound 11 indicates that its fluorescence intensity is less than that of compound 10 (Figure 4.15). The observed fluorescence intensity difference between 10 and 11 after interaction with Zn$^{2+}$ is due to the high electron withdrawing power of the -COOH group on the phenyl ring.

![Fluorescence spectrum](image)

Figure 4.14 Fluorescence changes if 10 (3 µM) with [Zn$^{2+}$] (0 - 30µM) in CH$_3$CN (excitation 370 nm).
To evaluate the detection limit of Zn\(^{2+}\) by the chromophore in solution, the fluorescence changes were measured by increasing the amounts of Zn\(^{2+}\). The fluorescence intensity of compound 10 increased almost tenfold in the presence of 3.0 µM of Zn\(^{2+}\), indicating the concentration limit of detection (LOD). From the inset in Fig 4.12 LOD was calculated to be 1.109 µM.

4.11 Impedance Spectroscopy Study of Compound 10 and 11

Figures 4.15 show the impedance response of the electrochemical sensor towards different concentrations of 10- Zn\(^{2+}\). A distinguishable and better signal to noise ratio was achieved from 100 Hz to 400 Hz, as shown in the inset. As an example, for measurements at operating frequency of 200 Hz, the impedance response decreased from 34 kΩ to 32 kΩ to 31 kΩ to 25 kΩ as concentrations of 10- Zn\(^{2+}\) increased from 100 pM to 100 nM to 1 µM to 1 mM, respectively. This response showed that the impedance percentage change achieved with respect to the free 10 at 100 Hz were 38 %, 33 %, 28 % and 4 % as the concentration of 10 – Zn\(^{2+}\) was varied from 100 pM to 100 nM to 1 µM to 1 mM, respectively.

The sensor response and impedance percentage change at the 11 – Zn\(^{2+}\) - sensor interface when compared to a solution of Free Sample is shown in Fig. 4.16. Here, it was observed that a distinctive and better signal to noise ratio was achieved from 200 Hz to 500 Hz. The impedance percentage change achieved with respect to acetonitrile at 200 Hz for 11 – Zn\(^{2+}\) were 32 %, 34 %, 43 %, 45 %, 97 % and 69 % as the concentrations were varied from 100 pM to 100 nM to 1 µM to 1 mM to 100 mM, respectively.
The change in measured impedance response of the electrochemical sensor is due to the change in charge transfer dynamics between the metal electrodes and varying concentrations of the Zn\(^{2+}\) ions\(^{122, 123}\). The impedance responses displayed the capability of the sensor device to detect Zn\(^{2+}\) concentrations as low as 100 pM and the ability of the sensor to distinguish among a wide range (micro, nano and pico level concentrations) of Zn\(^{2+}\). It is worth noting that the approved level of zinc by the US Food and Drug Administration (USFDA) is 0.2 mM\(^{101}\).
Figure 4.15 (a) Sensor response towards different concentrations of Zn$^{2+}$ in 10, at applied potential of 1 mV (Inset shows impedance response at 100 Hz, 200 Hz, 300 Hz and 400 Hz) and (b) Impedance percentage change at 100 Hz, 200 Hz, 300 Hz and 400 Hz.
Figure 4.16 (a) Sensor response towards different concentrations of Zn$^{2+}$ in 11, at applied potential of 1 mV (Inset shows impedance response at 100 Hz, 200 Hz, 300 Hz and 400 Hz) and (b) Impedance percentage change at 200 Hz, 300 Hz, 400 Hz and 500 Hz.
4.12 UV-Vis and Fluorescence Study of Compound 15 – 19

Compound 15 and 16 shows a characteristics UV-vis absorbance band centered at 400 nm in acetonitrile (CH$_3$CN). In the presence of the Fe$^{3+}$, the absorption band at 400 nm shifted to 360 nm and 390 nm respectively (Figure 4.17 and 4.18). In the absorption spectra of compounds 15 and 16, the absorption intensities of the n-$
\pi^*$ transitions increased upon addition of Fe$^{3+}$, which was attributed to the complexation of Fe$^{3+}$ with inamine N and carbonyl group of 15 and hydroxyl group of 16.

![Figure 4.17 Absorbance of compound 15(5 µM) in the presence of Na$^+$, Ca$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Fe$^{3+}$ and Zn$^{2+}$(10 µM) in CH$_3$CN.](image-url)
Figure 4.18 Absorbance of compound 16 (5 µM) in the presence of Na⁺, Ca²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Ce³⁺ and Zn²⁺ (10 µM) in CH₃CN.

Figure 4.19 The absorbance of 15 (5 µM) in CH₃CN after addition of different concentration FeCl₃ (5 mM).
Figure 4.20 The absorbance of 16 (5 µM) in CH$_3$CN after addition of different concentration FeCl$_3$ (5 mM).

More interestingly, observed remarkable fluorescent increments of 15 and 16 at 470 nm upon addition of Fe$^{3+}$ to a solution of CH$_3$CN, whereas no meaningful fluorescence changes were noticed with other metal ions. In order to investigate the effect of other metal ions on the fluorescence spectra of 15 and 16, Na$^+$, Mg$^{2+}$, Ca$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Fe$^{2+}$ and Cr$^{3+}$ were used to evaluate the metal ion binding properties of the compounds. Figure 4.21 shows the changes in fluorescence spectra of 15 upon addition of various metal ions. The fluorescence spectra were observed by excitation at 390 nm. As shown in Figure 4.21 and 4.24, 15 and 16 had a large fluorescence effect only with Fe$^{3+}$ among the metal ions examined. Other metal ions, gave no distinct response to 15 and 16 in fluorescence spectra. This interesting feature reveals that 15 and 16 can serve as a selective fluorescent chemosensor for Fe$^{3+}$, which was introduced by the cooperative metal ion recognition. The fluorescence titration
spectra of 15 and 16 with Fe\textsuperscript{3+} at 470 nm show a 20 and 18-fold fluorescence enhancement respectively (Figure 4.22 and 4.24). The off-on fluorescence mechanism of 15 and 16 are explained by the widely accepted photo-induced electron transfer (PET) mechanism.

Figure 4.21 Relative fluorescence intensity of sensor 15(5 \mu M) in the presence of Na\textsuperscript{+}, Ca\textsuperscript{2+}, Fe\textsuperscript{3+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Cu\textsuperscript{2+}, Cd\textsuperscript{2+}, Hg\textsuperscript{2+}, Pb\textsuperscript{2+}, Fe\textsuperscript{2+} and Zn\textsuperscript{2+}(10 \mu M) in CH\textsubscript{3}CN (excitation 390 nm).
Figure 4.22 Fluorescence changes of 15 (5µM) with [Fe$^{3+}$] (0 – 60 µM) in CH$_3$CN (excitation 390 nm).

Figure 4.23 Relative fluorescence intensity of sensor 16 (5 µM) in the presence of Na$^+$, Ca$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ (10 µM) in CH$_3$CN (excitation 390 nm).
Figure 4. Fluorescence changes if 16 (5 µM) with [Fe$^{3+}$] (0 - 60µM) in CH$_3$CN (excitation 390 nm).

Achieving high selectivity for the analyte of interest over a complete background of potential competing species is a challenging task in sensor development. The selectivity of the sensors towards Fe$^{3+}$ was confirmed by the following experiments. First, 40 equivalents of alkali, alkaline earth, and other transition-metal ions were added to the solution of 15 (10 µM). No significant changes in the fluorescent spectra of 15 were observed. Second, 40 equivalent of Fe$^{3+}$ was added to the solution containing 15 and other metal ions (40 equivalent). The fluorescence changes were almost the same as that using 40 equivalent of Fe$^{3+}$ alone. The results indicate that the selectivity of 15 towards Fe$^{3+}$ is hardly affected by these commonly coexistent ions.
Figure 4.25  Fluorescence decay traces for sensor 16 in presence of Fe$^{3+}$.

4.13 Electrochemical Detection (differential pulse voltammetry) of Fe$^{3+}$

Voltammetry of the compounds 15, 16, and 18 and with Fe$^{3+}$ was carried out in de-aerated DMSO containing TBAP. Figure 4.26 shows the differential pulse voltammogram (DPV) of compound 18 (1mM) in DMSO/TBAP. Compounds 15 and 16 display three reduction couples and two oxidation couples respectively, whereas compound 18 shows three reduction and oxidation couples. The current density that flows through the adduct Fe$^{3+}$–18 is higher that of the free compound 18. This change in current density and shift in redox potentials can be attributed the binding of Fe$^{3+}$ with the free compounds. The redox couples of the compounds 15, 16 and 18 and their Fe$^{3+}$ complexes are summarized in the following table.
Table 4.1 Oxidation and Reduction potentials of compound 15, 16 and 18 and their Fe$^{3+}$ complexes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Red$_1$</th>
<th>Red$_2$</th>
<th>Red$_3$</th>
<th>Ox$_1$</th>
<th>Ox$_2$</th>
<th>Ox$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 15</td>
<td>-1.168</td>
<td>-1.366</td>
<td>-1.937</td>
<td>0.422</td>
<td>0.937</td>
<td></td>
</tr>
<tr>
<td>15 + Fe$^{3+}$</td>
<td>-1.159</td>
<td>-1.358</td>
<td>-1.897</td>
<td>0.433</td>
<td>0.940</td>
<td></td>
</tr>
<tr>
<td>Compound 16</td>
<td>-1.040</td>
<td>-1.302</td>
<td>-1.738</td>
<td>0.472</td>
<td>0.940</td>
<td></td>
</tr>
<tr>
<td>16 + Fe$^{3+}$</td>
<td>-1.032</td>
<td>-1.300</td>
<td>-1.732</td>
<td>0.480</td>
<td>0.932</td>
<td></td>
</tr>
<tr>
<td>Compound 18</td>
<td>-0.798</td>
<td>-1.253</td>
<td>-1.644</td>
<td>1.113</td>
<td>0.936</td>
<td>0.574</td>
</tr>
<tr>
<td>18 + Fe$^{3+}$</td>
<td>-0.796</td>
<td>-1.251</td>
<td>-1.522</td>
<td>1.121</td>
<td>0.938</td>
<td>0.580</td>
</tr>
</tbody>
</table>
Figure 4.26 (a) Differential Pulse Voltammograms of 18 (1 mM) in the absence and (b) presence of Fe$^{3+}$ (340 µM).

4.14 Steady State Spectroscopy of Compound 14 and 19

The recognition between compound 14 and 19 and different metal ions were investigated by UV-vis and fluorescence in the solution CH$_3$CN. The stock solution of ligand 14 and 19 were 3x10$^{-6}$ mol/L, while the metal ions stock solutions were 1.0x10$^{-3}$ mol/L. From the absorption spectrum of compound 14 in acetonitrile solution, it was found that an intense absorption band appeared in visible region at 460nm, which could be assigned to the charge transfer absorbance. The absorption spectrum showed a new peak at 550 nm in the visible region due the binding with Cr$^{3+}$. As the concentration of Cr ion increases, the absorption intensity of the compound gradually increases at 550 nm and decreases at 460nm forming an isosbestic point at 498 nm (Figure 4.28). An isosbestic point in the titration curves indicates a new species, which could be assigned to the Cr$^{3+}$
complex of 14 (Figure 4.28 and 4.29). A simultaneous marked color change from orange to pink is observable by naked eye. These results indicate that 14 have high binding affinity toward Cr$^{3+}$ ions. The UV titration method was employed to investigate the interaction between 14 and Cr$^{3+}$.

Compound 14 showed very weak emission change with some metal ions (Fe$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Pb$^{2+}$) and showed a small emission enhancement with Hg$^{2+}$ (<50 fold) (Figure 4.29). By contrast, the addition of 1.0 equiv Cr$^{3+}$ to the solution of 14 results in a drastic fluorescence emission change and a bathochromic shift. This indicates that compound 14 is an excellent turn-on sensor for Cr$^{3+}$. It shows a peak at 565nm. The fluorescent quantum yield of the coumarin derivative, compound 14, increases about (>800-fold) due to inhibition of the C=N isomerization process upon Cr ion binding at the ONO site (Scheme 4.5).

![Absorbance of 14 (3 µM) in CH$_3$CN after addition of some naturally abundant metal ions.](image)
Figure 4.28 The absorbance of 14 (3 µM) in CH$_3$CN after addition different concentration of Cr(NO$_3$)$_2$.6H$_2$O.

Figure 4.29 Fluorescence intensity of compound 14(3 µM) in the presence of Na$^+$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Fe$^{2+}$ and Cr$^{3+}$ (10 µM) in CH$_3$CN (excitation 490 nm).
The fluorescent titration spectrum shows the fluorescence “turn on” characteristic of the compound by Cr$^{3+}$ (Figure 4.31) and determines the association constant to be $3.4 \times 10^6$ M$^{-1}$. The reversibility and binding activity of the sensor with Cr$^{3+}$ was also investigated and also found the detection limit of 14 for Cr$^{3+}$ is 0.039µM. Ethylenediaminetetraacetic acid (0.03M EDTA), a very strong cation chelating agent, was added to the solution of 14-Cr$^{3+}$ and it was observed that the fluorescence intensity decreased close to that of the free compound. The pink color of the complex also disappeared with the addition of excess EDTA.

![Fluorescence Intensity](image)

Figure 4.30 Relative fluorescence change of Compound 14 with different metal ions.
Figure 4.31 Fluorescence changes if 14 (3 µM) with Cr$^{3+}$ (0 - 28 µM) in CH$_3$CN (excitation 490 nm).

Compound 14 has a two component life time, a fast component of 1.274 ns and longer component of 2.5 ns. On adding Cr$^{3+}$ (10 µM) the longer component changes to 117.47 ns and the faster component changes to 1.837 ns; slowing down the relaxation processes. The non-radiative processes taking place have longer life times.
Compound 19 was designed to bind metal ions via imine N, -OH and pyridine N (Scheme 4.6). The absorption spectrum showed a new absorbance peak at 400 nm in the visible region due to binding with Cr$^{3+}$. As the concentration of Cr$^{3+}$ increases, the absorption intensity of the complex gradually increases at 300 nm and 400 nm (Figure 4.34). The selectivity of 19 toward Cr$^{3+}$ was investigated by treating both compounds with other metal ions such as Ni$^{2+}$, Co$^{2+}$, Fe$^{3+}$, Fe$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Cu$^{2+}$, Na$^+$, and Ca$^{2+}$ in CH$_3$CN solution. The absorption maximum shows no obvious change upon addition of the metal ions Fe$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Na$^+$, and Ca$^{2+}$ but with Cr$^{3+}$, Hg$^{2+}$, Cu$^{2+}$ and Fe$^{3+}$, a single new absorption at 400 nm was observed (Figure 4.33).
Figure 4.33 The absorbance of 19 (3 µM) in CH$_3$CN after addition of some metal ions.

Compound 19 showed very weak emission changes when coordinating with some metal ions (Hg$^{2+}$ and Fe$^{3+}$) and showed a strong emission enhancement with Cr$^{3+}$ (Fig 4.35 and 4.36) at 420 nm. The fluorescence intensity of the compound increases with incremental amounts of Cr$^{3+}$. 
Figure 4.34 The absorbance of 19 (3 µM) in CH$_3$CN after addition various concentrations of Cr(NO$_3$)$_2$.6H$_2$O.

Figure 4.35 Fluorescence intensity of compound 19 (3 µM) in the presence of Na$^+$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Fe$^{2+}$ and Cr$^{3+}$ (10 µM) in CH$_3$CN (excitation 370 nm).
Figure 4.36 Relative fluorescence change of Compound 19 with different metal ions.

Figure 4.37 Fluorescence changes if 19 (3 µM) with Cr$^{3+}$ (0 - 33 µM) in CH$_3$CN (excitation 370 nm).
In this work, new chromogenic chemosensors based on coumarin Schiff-base derivatives were developed. Compound 10-13 shows a good selectivity for Zn$^{2+}$ over other metal cations. An obvious color change from light green to yellowish was observed by the naked eye. The sensor showed a “turn on” fluorescent response as the emission red-shifted from 475 nm to 550 nm. The high selectivity of 10 and 12 for Zn$^{2+}$ is evidenced by its exceptional fluorescence enhancement compared to other metal ions. Signaling systems that employ the C=N isomerization continue to be a part of our efforts to explore new sensing mechanisms and increase selectivity for other toxic metals.

Quantitative electrochemical detection of Zn$^{2+}$ ions was made possible by using a highly sensitive sensor device. The response of the electrochemical sensor device towards varying concentrations of Zn$^{2+}$ ions displayed an impedance percentage change of 38%.
and 32 % for 10–Zn$^{2+}$ and 11–Zn$^{2+}$, respectively for the 100 pM concentration when compared to Free Sample at 200 Hz operating frequency and 1 mV excitation.

We synthesized new fluorescent sensors 15-19 for Fe$^{3+}$ using coumarin as a fluorophore. These compounds showed highly selective Fe$^{3+}$–amplified fluorescence emission in actonitrile. Commonly coexistent metal ions displayed little interference.
CHAPTER V

NOVEL TURN-OFF /ON NERVE GAS SENSORS BASED ON COUMARIN SCHIFF BASE AND AZINE DERIVATIVES

5.1 Nerve Gas Agents

The organophosphorus cholinesterase inhibitors commonly known as nerve agents (NA) and these compounds are structurally related to highly toxic phosphoric acid esters. Organophosphorus compounds are closely related to insecticides and pesticides. The first nerve agent was developed in 1936 by German scientist Dr. Gerhard Schroeder. Nerve gas agents can be classified according to the following criteria:

1) Boiling point: Nerve agents can be classified into two categories namely volatile and non volatile.

2) Country of origin: Agents originally developed in Germany were designated as “G series” agents; these include Sarin (GB), Tabun (GA) and Soman (GD).

3) Toxicity: “V series” agents, V stand for venomous and include VE, VG, and VX.

G-series of nerve agents Sarin (GB), Tabun (GA) and Soman (GD) are inhibitors of serine proteases and acetylcholinesterase, the enzyme responsible for transmitting nerve impulses across synaptic junctions \(^{124, 125}\). All nerve agents are odorless and colorless organophosphonates (OP) and are among the most toxic substances known \(^{126}\). Related OP derivatives, parathion, ethion, malathion, fenthion, and diazinon, are used in
pesticides around the world. These OP compounds are structurally similar to nerve gases and are also acetylcholinesterase inhibitors. They differ mainly in having the P=O bond replaced with P=S. Related OP compounds, diisopropyl-fluorophosphate (DFP) and diethylchlorophosphate (DCP), have similar reactivity to typical nerve agents, but of lower toxicity and hence are good model compounds for the design of nerve gas sensors.

Detection methods for OP nerve agents have been developed based on surface acoustic wave devices, interferometry, electrochemistry, and a variety of other analytical approaches including enzymetic assays. These systems suffer from such limitations as slow response time, lack of specificity, low sensitivity, operational complexity, and limited portability. The ease of production and extreme toxicity of OP nerve agents underscores the need to rapidly detect and ideally, deactivate them.
5.2 Mechanism of Action of Nerve Agents on Human Beings

Acetylcholine (Ach) is a central neurotransmitter and hydrolysis of Ach is the vital mode of regulation of the neural response system. The enzyme Acetylcholinesterase (AChE) hydrolyzes approximately 10,000 acetylenecholine (ACh) molecules per second into inactive products like acetic acid and choline, and also maintains the concentration of neurotransmitter Ach within the synaptic cleft of the nervous system. Nerve agents are organophosphate compounds that inhibit the activity of Acetylcholine Esterase enzyme. The phosphorus atom of the nerve agent covalently binds to the serine hydroxyl group in the catalytic site (esteratic site) of acetylcholine esterase and forms a phosphate ester bond. This bonding blocks the enzyme from interaction with
its normal substrate Ach and causes accumulation of Ach rather than the normal breakdown. If nerve agents are not removed from AChE (by treating with Oxime) within a short time after exposure, AChE will undergo an aging process where AChE becomes resistant to hydrolysis and is considered as irreversibly bound to the nerve agents. The inhibition of AChE by nerve gases leads to the headache, nausea, vomiting, diarrhea, bradycardia, respiratory failure and sometimes paralysis and death depending on the dose. The interaction and mechanism of the nerve agent with the enzyme can be seen below in scheme 5.1.

![Scheme 5.1 Interaction of a nerve agent with AChE](image)

Scheme 5.1 Interaction of a nerve agent with AChE.
5.3 Fluorescent Sensors for Nerve Gas Agents

The development towards the detection of nerve agents is very important because of their high toxicity and use as chemical weapons for terrorists or war actions. A lot of research is being devoted towards the development of new and improved methods for the detection of highly toxic nerve agents. There are several types of detection methods for nerve agents based on different techniques, including surface acoustic wave detectors, enzymatic assays, mass spectrometry, interferometry and colorimetric detectors.

All the above detection methods have limitations such as weaker response, limited selectivity, false positives, low sensitivity, cost and real-time recognition. Optical or chemosensors are simple and inexpensive and overcome all the above-mentioned limitations, sometimes detection can even be performed with the naked eye. Fluorescent sensors are designed to undergo change in the absorptive or emissive behavior in presence of target analytes. There are many different analytical techniques developed based on changes in the fluorescence properties of a molecule in different environments. These fluorescence changes include quenching, Förster resonance energy transfer, photo-induced electron transfer (PET) and surface modified fluorescence.

Fluorescence sensors towards the detection of nerve agents gained attention because of their ease of operation and high response rate. Detection of nerve agents by using chromo-fluorogenic sensors, first described by Schonemann in 1944, was based on the oxidation of amines in presence of organophosphorus compounds. The mechanism of action is based in the formation of a peracid from the organophosphorus compound which is then involved in the oxidation of an amine to give a color change.
Recently, Zhang et al have developed a fluorescence-based sensor for the detection of nerve agents\textsuperscript{125}. Nerve agents react with hydroxyl group of acetylcholine esterase enzyme and form the phosphodiester bond. Based on this observation, Zhang developed the compounds which react with nerve agents and form the phosphodiester bond. The hydroxyl group was converted into a phosphate ester, a good leaving group, then intramolecular cyclization occurs, yielding rigid planar highly delocalized systems with a different emission.

Scheme 5.2: Intra-molecular cyclization which leads to the fluorescence change upon binding with DCP\textsuperscript{125}.

A photo-induced electron transfer (PET) based fluorescent sensor was developed by Dale et al\textsuperscript{131} in which a primary alcohol is used to detect the nerve agents. This primary alcohol is attached to a tertiary amine. The primary alcohol is acylated with
nerve agents to produce a quaternary ammonium salt through an intramolecular N-alkylation reaction (Figure 5.3). A fluorophore (pyrene) was attached to the amine with a spacer (methylene) and then exposed to nerve agents. Reaction with the nerve agents causes quenching of the fluorophore near the amine via PET resulting in increase in the emission intensity.\textsuperscript{131}

Scheme 5.3 Fluorescent (PET) sensors for nerve gas by Dale\textsuperscript{131}.

Nagale et.al developed a fluorescent sensor based on microbeads. They coated microbeads with fluoresceinamine (FLA) dye and poly (2-vinylpyridine) and then reacted the beads with the nerve agent simulant, DCP. FLA’s amine group quantum yield increases upon reaction with the phosphoryl group of nerve agents (Scheme 5.4).

Scheme 5.4 Fluorescent sensor for nerve gas agents (DCP) by Walt\textsuperscript{151}.
5. 4 Coumarin Derivatives for OP Sensor

The very high molar extinction coefficients and high fluorescent quantum yields of coumarin-based compounds make them especially useful as chemosensors. Derivatives of 7-diethylamino coumarin are widely employed as molecular platforms for chemo sensors. OP-binding may affect intramolecular charge transfer and the consequent changes in absorbance and emission make them potential OP sensors. In the present work, a new chromogenic chemosensor was designed and synthesized based on a 7-diethylaminocoumarin derivative with intramolecular charge transfer (ICT) character. Structurally, the coumarin chromophore contains an imine bond and carbonyls group both capable of acting as chelate donor atoms. In this design, we expect chromogenic chemosensors that show good selectivity for DCP and most importantly, a large absorption shift due to the significant enhancement of the intramolecular charge transfer from the donating moiety (diethylamino) to the electronic withdrawing moiety (carbonyl and C = N bond) induced by the binding of DCP.

Rhodamine- and fluorescein-based compounds have been widely used as chemo sensors due to their remarkable spectroscopic properties including high absorption coefficients, high fluorescent quantum yields, and excitation and emission within the visible wavelength region. In addition, they can interconvert between a non-fluorescent ring-closed spirolactam form and a high fluorescent ring-open spirolactam form which makes them excellent candidates for chemo sensors. Here we are also utilizing this mechanism in conjugation with coumarin dyes for the detection of a nerve gas simulant (DCP).
In order to avoid the interference from inorganic acids such as HCl or HBr, we decided to use a buffer system to study the binding of nerve gas mimics. Nerve gas agents, such as sarin, VX, soman, and mustard gas hydrolyze in aqueous systems with half-lives varying from minutes to days. For example, the half-life of sarin is 2340 minutes at pH 7.5 while soman has an even longer half-life. As sarin and soman are the closest analogs to DCP, we can assume that DCP does not readily undergo hydrolysis in buffer systems. In addition, sarin and soman generate HF upon hydrolysis. Therefore all the compounds were tested with HCl to show that sensors should interact with the nerve gas and not the nerve gas degradation products. The compounds were also tested with DMMP to study the importance of the leaving group (Cl) of DCP in binding with sensors.

5.5 Instrumentation

Fluorescence spectra were recorded on an Edinburgh FS920 fluorimeter at room temperature. Measurements were taken with 1 µM solution of 20 - 23 in CH₃CN, excited at 430 nm with appropriate amounts of OP compounds. UV-Vis Absorbance Spectra were recorded on a Shimadzu UV-2101PC at room temperature with 1 µ M solution of 20 – 23 in CH₃CN with appropriate amounts of OP compounds.

¹H and ¹³C NMR: The ¹H NMR (400MHz) and ¹³C (100MHz) spectra were obtained on a JEOL eclipse spectrometer in CDCl₃. Single Crystal Xray diffraction intensities were measured on a Bruker SMART APEX II CCD system equipped with a graphite monochromator and a MoKα fine-focus tube (λ = 0.71073 Å).

Electrochemistry: Differential pulse voltammetry (DPV) was carried out with a CHI440A model potentiostat controlled by an external PC and utilizing a three electrode
configuration at room temperature. The working electrode was a Pt disc electrode with a
diameter of 2 mm. A Pt wire served as the counter electrode. A saturated calomel
electrode was employed as reference electrode. The supporting electrolyte was
electrochemical grade tetrabutylammonium perchlorate in pure DMSO at a concentration
of 0.1mol dm$^{-3}$. Aliquots of 1 mM solution of the compounds were used in the
experiments. High purity argon was used to deoxygenate the solution for 30 min prior to
each run and to maintain an argon blanket during the measurements. The working
electrode was polished with 0.05μm Al$_2$O$_3$ slurry and cleaned electrochemically by
potential cycling in 0.1 M H$_2$SO$_4$ solution. DPV measurements were carried out with a
scan rate of 25 mVs$^{-1}$, and quiet time of 5 s.

5.6 Synthesis

Chemosensors 20 – 23 were synthesized in good yields from readily available
starting materials. 7-diethylaminocoumarin-3-aldehyde, 9, was synthesized via a series of
reactions, shown in Scheme 5.5. As indicated in Scheme 5.5, 7-diethylaminocoumarin-3-
aldehyde was reacted in ethanol at reflux with an equimolar amount of the corresponding
ligands to afford the given compounds in very high yields. The structures were
characterized using $^1$H NMR, $^{13}$C NMR, mass spectrometry and for the intermediate, X-
ray crystallography. Compound 26 and 28 were also synthesized by reacting rhodamine
and fluorescein derivatives with 7-diethylaminocoumarin-3-aldehyde in the presence of
ethanol and the compounds are characterized using $^1$H NMR, $^{13}$C NMR, and mass
spectrometry.
Scheme 5.5 Synthetic route for compound 20 – 23.
Scheme 5.6 Synthetic route for compound 24.

**Synthesis of 7-diethylaminocoumarin**

4-Diethylaminosalicylaldehyde (1.93g, 10mmol), diethylmalonate (3.2g, 20mmol) and piperidine (1 mL) were combined in absolute ethanol (30 mL) and stirred for 16hr under reflux conditions. Ethanol was evaporated under reduced pressure, then concentrated HCl (20 mL) and glacial acetic acid (20 mL) were added to hydrolyze the reaction mixture and the mixture was stirred for another 16 hours. The solution was cooled to room temperature and poured into 100mL ice water. NaOH solution (40%) was added drop wise to modulate the pH of the solution to 5.0, and a pale precipitate formed immediately. After stirring for 30 min., the mixture was filtered, washed with water, dried, then recrystallized with toluene to give 1 as a gray powder (1.73g, 8.0 mmol) in
80% yield. $^1$H-NMR (CDCl$_3$) $\delta$ 7.53 (d,1H), 7.20 (d,1H), 6.48 (d,1H), 6.40 (s,1H), 6.01(d,1H), 3.40 (m,4H), 1.20 (t,6H).

**Synthesis of 7-diethylaminocoumarin-3-aldehyde, 9**

Freshly distilled DMF (2 mL) was added dropwise to POCl$_3$ (2 mL) at 20-50°C in a N$_2$ atmosphere and stirred for 30 minutes to yield a red solution. This solution was combined with a portion of 1 (1.50g, 6.91 mmol, dissolved in 10 mL DMF) to yield a scarlet suspension. The mixture was stirred at 60°C for 12 hours and then poured into 100 mL of ice water. NaOH solution (20%) was added to adjust the pH of the mixture yielding a large amount of precipitate. The crude product was filtered, thoroughly washed with water, air dried and recrystallized from absolute ethanol to give the product (1.20g, 4.89mmol) in 70.8% yield. $^1$H NMR (CDCl$_3$) $\delta$ 10.15 (s, 1H), 8.24 (s, 1H), 7.40 (d, 1H), 6.63 (d, 1H), 6.47 (s, 1H), 3.47 (m, 4H), 1.21 (t, 6H).

**Synthesis of compound 20**

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde, 9 (0.05, 2mmol) and 3,4-diaminobenzonitrile (0.01g, 1mmol) were suspended in 20mL ethanol. The mixture was refluxed for 7 hr with stirring, during which time a yellow precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, the product was obtained as a yellow solid in 85% yield. $^1$H NMR (400 MHz, CDCl$_3$-d6), $\delta$ (ppm): 8.92 (dd, 2H), 8.04 (s, 1H), 7.76 (m, 1H), 7.75 (s, 1H), 7.51 (dd, 2H), 7.46 (d, 2H), 6.71 (d, 2H), 6.56 (s, 2H), 3.47 (m, 8H), 1.24 (t, 12H). $^{13}$C NMR (400 MHz, CDCl$_3$-d6), $\delta$ (ppm): 12.53, 45.28, 97.01, 108.80, 110.46, 112.13, 115.89, 119.51, 123.62, 126.27, 130.89, 143.96, 144.15, 151.01, 152.12, 156.03. Elemental Analysis
Calcd for C_{35}H_{36}N_{4}O_{6}: C, 71.53; H, 5.66; N, 11.92; O, 10.89. Found: C, 71.49; H, 5.59; N, 11.93; O, 10.99.

**Synthesis of compound 21**

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (0.05, 2mmol) and 4-methylbenzene (0.01g, 1mmol) were suspended in 20 mL ethanol. The mixture was refluxed for 7 hr with stirring, during which time an orange precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, an orange solid in 75% yield was obtained. \(^1\)H NMR (400 MHz, CDCl$_3$-d6) \(\delta\) (ppm): 8.90 (s, 2H), 7.64 (s, 1H), 7.46 (m, 1H), 7.51 (dd, 2H), 7.25 (d, 2H), 7.09 (d, 2H), 6.67 (dd, 2H), 6.55 (d, 1H), 3.46 (m, 8H), 2.16 (s, 3H), 1.24 (t, 12H). Elemental Analysis Cald for C$_{35}$H$_{36}$N$_{4}$O$_{4}$: C, 72.90; H, 6.29; N, 9.72; O, 11.10 Found: C, 72.70; H, 6.22; N, 9.90; O, 11.18.

**Synthesis of compound 22**

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (0.05, 2mmol) and methyl 3,4-diaminobenzoate (0.02 g, 1mmol) were suspended in 30mL ethanol. The mixture was refluxed for 12 hr with stirring, during which time a yellow precipitate formed. The precipitated was separated by filtration and washed with 3 x 10 mL ethanol. After drying, the product was obtained as a yellow solid in 82% yield. \(^1\)H NMR (400 MHz, CDCl$_3$-d6), \(\delta\) (ppm): 8.94 (s, 2H), 8.47 (s, 1H), 8.22 (s, 1H), 7.99 (d, 2H), 7.73 (d, 2H), 7.51 (s, 1H), 7.49 (dd, 2H), 6.70 (d, 1H), 6.56 (s, 1H), 3.94 (s, 3H), 3.48 (m, 8H), 1.25 (t, 12H). 12.54, 45.23, 52.16, 97.03, 110.30, 112.07, 118.26, 122.90, 130.08, 142.65, 153.09, 157.44. Elemental Analysis Cald for C$_{36}$H$_{36}$N$_{4}$O$_{6}$: C, 69.66; H, 5.85; N, 9.03; O, 15.47 Found: C, 69.69; H, 5.88; N, 9.01; O, 15.42.
**Synthesis of compound 23**

In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde (0.05, 2 mmol) and methyl 3,4-diaminobenzoic acid (0.02 g, 1 mmol) were suspended in 30 mL ethanol. The mixture was refluxed for 12 hr with stirring, during which time a brown precipitate formed. The precipitated was separated by filtration and washed with 3 x 10 mL ethanol. After drying, the product was obtained as a brown solid in 70% yield. $^1$H NMR (400 MHz, DMSO-d$_6$), δ (ppm): 8.99 (dd, 2H), 8.27 (s, 1H), 8.17 (s, 1H), 7.75 (dd, 2H), 7.73 (dd, 2H), 7.68 (d, 1H), 7.63 (d, 1H), 6.85 (s, 2H), 6.68 (s, 2H), 3.47 (m, 8H), 1.24 (t, 12H). $^{13}$C NMR (400 MHz, CDCl$_3$-d6), δ (ppm): 12.93, 40.48, 96.77, 108.70, 110.64, 112.66, 115.01, 117.88, 120.16, 123.76, 131.68, 139.01, 144.47, 152.50, 158.27, 160.74, 168.49. Elemental Analysis Calcd for C$_{35}$H$_{34}$N$_4$O$_6$: C, 69.29; H, 5.65; N, 9.24; O, 15.82 Found: C, 69.26; H, 5.68; N, 9.27; 15.79.

**Synthesis of Compound 24**

7-Diaminocoumarin-3-aldehyde (250mg, 1 mmol) in 20ml ethanol, hydrazine monohydride (80% aqueous solution) (30mg, 0.5 mmol) in 2 ml ethanol was added dropwise, then the reaction was kept at room temperature for 15 hr, and the precipitate formed was collected and washed with ethanol. The product was dark-red solid obtained in analytic pure form (Yield: 80%). $^1$H NMR (400 MHz, CDCl$_3$-d$_6$), δ (ppm): 1.29 (t, J=7.0Hz, 6H), 3.49 (m, J=7.1 Hz, 4H), 6.52 (s, 1H), 6.64 (d, J=7.6 Hz, 1H), 7.45 (d, J=8.9Hz, 1H), 8.26 (s, 1H), 8.93 (s, 1H).
Synthesis of Compound 26

Compound 25 was synthesized based on a literature procedure. A solution of 25 (0.250 g, 1 mmol) and 7-diethylaminocoumarin-3-aldehyde (0.134 g, 1 mmol) were mixed in 25 ml of ethanol. The reaction mixture was refluxed overnight. The solvent was
evaporated in vacuo and the crude product was recrystallized from ethanol/DMSO (3:1) to get the product 26 (0.220g). $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.33 (s, 1H), 8.19 (s, 1H), 7.98 (d, 1H), 7.44 (t, 2H), 7.27 (d, 2H), 7.11 (d, 1H), 6.51 (d, 3H), 6.46 (s, 2H), 6.20 (d, 2H), 3.31 (q, 12H), 1.36 (t, 18H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 12.72, 41.10, 44.40, 44.93, 65.83, 97.13, 98.29, 105.47, 108.08, 108.87, 109.19, 114.97, 123.49, 123.75, 127.82, 128.19, 128.42, 130.24, 133.49, 138.38, 141.10, 149.03, 151.07, 152.71, 153.00, 156.90, 161.42, 165.21. ESI-MS. 684.35[M-H]$^+$

**Synthesis of Compound 28**

Compound 27 was synthesized based on a literature procedure.$^{158}$ A solution of 27 (0.250 g, 1 mmol) and 7-diethylaminocoumarin-3-aldehyde, 9 (0.134 g, 1 mmol) was prepared in 25 ml of ethanol. The mixture was refluxed for 12 hr with stirring, during which time an orange precipitate formed. The precipitate was separated by filtration and washed with 3 x 10 mL ethanol. After drying, the product was obtained as an orange solid in 82% yield. $^1$H NMR (400 MHz, DMSO) δ: 9.94 (s, 2H), 8.56 (s, 1H), 7.96 (s, 1H), 7.91 (d, 1H), 7.53 (m, 3H), 7.03 (d, 1H), 6.65 (d, 3H), 6.49 (d, 3H), 6.46 (d, 2H), 3.41 (q, 4H), 1.09 (t, 6H).

5.7 Optical Spectroscopy of Compounds 20-23

Novel turn-off fluorescent coumarin-functionalized sensors for the nerve gas simulant diethylchlorophosphate (DCP) were designed and synthesized as described above. The compounds exhibit fluorescence around 500nm which is quenched by DCP. This results from the DCP-induced increase in charge density in the carbonyl O and imine N of the coumarin moiety and the rigidity of the sensor molecules. $^1$H NMR results confirm the binding mode to be via the imine nitrogen. These coumarin derivatives are
the basis for rapid, selective and sensitive colorimetric DCP chemosensors in acetonitrile (Figure 5.1).

Figure 5.1 Fluorescent and colorimetric sensor for DCP.

The four diethylcoumarin-derived sensor candidates were designed with a cavity having an electron donating or withdrawing group at the 3rd position of a phenyl ring, which enables them to bind DCP via hydrogen bonding interactions and bonding with the inamine nitrogen. They were also tested with close OP relatives of DCP: parathion, malathion, ethion, fenthion and diazinon. The compounds were also tested with DMMP to study the importance of the leaving group (Cl) of DCP in binding with sensors.

Figure 5.2 X-ray crystal structure of the intermediate compound.
All the compounds are highly selective towards DCP alone, and showed no response to the other OP compounds. All the spectroscopic studies were performed in 50% CH$_3$CN, 50% 0.01M phosphate buffer (pH=7.0) medium and the binding constants with DCP were calculated using the Benesi-Hildebrand equation. In order to avoid the interference from inorganic acids such as HCl or HBr, a buffer system was used to study the binding of nerve gas mimics.

The chemical interaction was confirmed by UV-Vis spectroscopy. Upon addition of one molar equivalent DCP to 23 (1µM in CH$_3$CN), the absorption maximum at 440 nm decreased while a new maximum appeared around 480 nm with a pseudo-isosbestic point at 460 nm (Figure 5.4). All the four compounds exhibit the same isosbestic point. These spectral changes were also observed with the naked eye. The light green color of 23 in CH$_3$CN changed to pink as soon as DCP was added. This corresponds to a bathochromic shift in the UV-vis absorbance from 440 to 480 nm, which was promoted by DCP. Meanwhile the other species tested produced no significant changes under these conditions.
Figure 5.3 Changes in UV-vis spectra for 20, 21 and 22, (1 µM) in CH$_3$CN upon the addition of DCP.

Figure 5.4 Changes in UV-vis spectra for 23 (1 µM) in CH$_3$CN upon the addition of DCP.
The sensing phenomenon was also monitored by fluorescence spectroscopy. As DCP was added to \(23\) (1 \(\mu\)M in CH\(_3\)CN), the fluorescence emission intensity of \(23\) quenched more than twenty fold and was saturated at 1 equivalent of DCP. However, other compounds such as malathion, parathion, DMMP, fenthion, ethion, and diazinon did not cause any significant changes in the fluorescence emission intensity of \(23\), even at 10 equivalents (Figure 5.5). The fluorescence profiles at 500 nm showed a higher selectivity for DCP over the various other organophosphorus compounds (Figure 5.5 and 5.6) and this is due to the formation of a DCP- sensor complex. During the titration of compound \(23\) with DCP there were no other observable changes in the emission spectra (Figure 5.7).

![Fluorescence spectra of compound 23 (1 \(\mu\)M) in CH\(_3\)CN after the addition of 10 equiv of organophosphorus compounds (Ex 430 nm).](image)
Figure 5.6 Relative fluorescence intensities of 23 (1 µM in CH$_3$CN) in the presence of various organophosphorus compounds.

Figure 5.7 Fluorescence emission ($\lambda_{max}$= 500 nm) of compound 23 (1 µM) upon the addition of DCP (340 µM) in CH$_3$CN (Excitation at 430 nm). Inset: fluorescence quenching at 500 nm as a function of DCP concentration.
The addition of DCP to the solution of compound 20 and 22 in CH$_3$CN resulted in a minor change in the UV-vis spectrum and did not result in any new peaks at longer wavelengths. Compounds 20 and 23 showed considerable fluorescence quenching (> 10-fold) with DCP (Fig. 5.8), while that for 22 was much less, giving a weak 22-DCP binding constant (K= 1.37 x 10$^{2}$ M$^{-1}$). This can be attributed to the weaker electron withdrawing power of the -COOCH$_3$ group than those of –CN and -COOH. By contrast, the compounds 20 and 23 with strong electron withdrawing groups at the 3$^{\text{rd}}$ position have high binding, 3.5 x 10$^{3}$ M$^{-1}$ and 3. 3 x 10$^{3}$ M$^{-1}$ respectively. The fluorescence changes of 21 upon addition of DCP were significant, but small compared to those for 20 and 23. This is reflected in the binding constant values, 1.7 x 10$^{3}$ M$^{-1}$ which was measured by following the change in fluorescence as a function of the concentration of DCP $^{142-148}$.

To evaluate the detection limit of DCP by the chromophore in solution, the fluorescence changes were measured with increasing amounts of DCP. The fluorescence intensity of compound 23 decreased almost fivefold in the presence of 3.0 µM of DCP, indicating the concentration limit of detection (LOD). From the inset in Figure 5.7, LOD was calculated to be 0.175 µM.

All four coumarin derivatives were reacted individually with DCP. Efforts to obtain crystals of these suitable for X-ray structure determination were not successful. Accordingly, we employed $^1$HNMR and TLC techniques to further elucidate the coordination mode between the compounds and DCP. Changes in the $^1$HNMR spectra of sensor 23 before and after the addition of DCP are shown in Figure 5.9. In general, DCP induced a slight shift in the well-resolve resonance signals of the sensor molecule and the
disappearance of other peaks. For instance, the resonance signal corresponding to the – N=CH proton was shifted downfield from 7.8 to 7.9 ppm and the phenyl proton resonance peaks at 8.2 ppm disappeared upon addition of DCP. The doublet proton resonance peak at 9ppm from phenyl ring also changed to a singlet which display the effects of binding on the imine nitrogen and these changes indicated that the sensor interacted with DCP at this nitrogen. As 23 did not interact with DMMP, it is clear that the chlorine leaving group of DCP plays a key role in binding with sensor 23 to trigger the color change.

Figure 5.8 Relative fluorescence intensities of the compounds 20 – 23 (1 µM in CH₃CN) in the presence of DCP (340 µM).
Figure 5.9 Partial $^1$H NMR (400MHz) Spectra of Compound 23 in the absence (a, bottom) and presence (b, top) of DCP.

5.8 Electrochemical Measurements

Voltammetric measurements of 20 and 21 were carried out in order to compare the redox behavior with DCP complexation. Figs 5.11 and 5.12 indicate the differential pulse voltammograms (DPV) of compounds 20 and 21 respectively. The DPV of 20 displays two reductions couples (-0.76V and -1.43V) and one oxidation couple (1.02 V). There is a shift in the oxidation potential in the presence of DCP. It changes from 1.02 V to 1.19 V with a difference of 170 mV. The current flow in the DCP complex is higher than the current flow in the free compound and a shift of the peaks is observed in the presence of DCP for compound 20 towards more positive values. For compound 21 there is a large change in current density with a 20 mV shift in the oxidation potential towards more negative values (opposite to compound 20). These results confirm that there is a strong binding between DCP and compounds 20 and 21, and that compound 20 is the most sensitive. This correlates with the fluorescence measurements. A similar trend is observed in the case of the reduction potential. This trend could be due to the strong electron withdrawing nature of the -CN substituent in compound 20. In the case of both compounds we do not observe the oxidation potential peak of free DCP (0.62V, Table 1).
Figure 5.10  Differential Pulse Voltammograms of DCP (1mM).

Figure 5.11 Differential Pulse Voltammograms of 20 (1mM) in the presence of DCP (340 µM).
Figure 5.12  Differential Pulse Voltammograms of 21 (1 mM) in the presence of DCP (340 µM).

Table 5.1  Oxidation and Reduction potentials of DCP, compound 20 and compound 21.

<table>
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<th>Sample</th>
<th>Ox 1</th>
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<th>Red 2</th>
<th>Red 3</th>
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<td>-</td>
</tr>
<tr>
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<td>Compound 21 with DCP</td>
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<td>-0.42</td>
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<td>-1.55</td>
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</tbody>
</table>

5.9 Sensing Activity of Compound 24

The diethylamino coumarin derivative with its intramolecular charge transfer (ICT) character\textsuperscript{149, 150} is selected as a chromophore, and an azine and the carbonyl group in coumarin act as the binding site for DCP in compound 24. In this design, we can expect
the constructed chromogenic chemosensors to show a good selectivity for DCP and more importantly, a large shift of absorption spectrum could also be expected due to the significant enhancement of the intramolecular charge transfer from the donating part (diethyl amino) to the electronic withdrawing part (carbonyl and azine) induced by the binding of DCP.

The recognition between compound 24 and different organophosphate compounds was investigated by UV-vis spectroscopy in acetonitrile solution. From the absorption spectrum of compound 24 in acetonitrile, it was found that an intensive absorption band in the visible region peaked at 495 nm, which could be assigned to the charge transfer (CT) absorbance, as observed in other compounds with intramolecular charge transfer (ICT) character. Variation of absorption of compound 24 upon addition of different organophosphates including parathion, ethion, malathion, fenthion, DMMP and DCP, is shown in Figure 5.13a. It was found that the maximum absorption of compound 24 shifts from 495 nm to 590 nm upon addition of DCP whereas other organophosphates tested did not show any significant absorption change. Actually, an obvious color change from orange to purple was observed by naked eye, as shown in Figure 5.13b. These results indicate that compound 24 has high – binding affinity towards DCP.
The UV titration method was employed to investigate the interaction between compound 24 and DCP. The dependence of absorption spectroscopy of compound 24 in the solution on the concentration of DCP was investigated. With the addition of DCP, the intensity of the maximum at 495 nm decreased, accompanied by an increase of the wavelength at 590 nm. An isosbestic point in the titration curves indicates that new
specie appeared upon the addition of DCP, which could be assigned to the binding of DCP with 24 (DCP-24) (Figure 5.14)

![Figure 5.14 Changes in UV-vis spectra for 24 (10 µM) in CH3CN upon the addition of DCP.](image)

The fluorescence spectrum of 24 showed a peak at 600 nm after addition of 20 equivalents of DCP; this is red shifted from 565 nm for the free compound 24. There was a significant fluorescent intensity quenching as the solution turn to purple. The compound was tested with possible interferences including dimethyl methyl phosphonate (DMMP) but none of them show any fluorescence change with the sensor, 24 (Figure 5.15). This clearly shows that compound 24 is highly selective towards DCP and the leaving group (chloride ion) of DCP plays a key role in binding which triggers a color change.
Figure 5.15 Fluorescence spectra of compound 24 (1 µM) in CH$_3$CN after the addition of 20 equiv of organophosphorus compounds (Ex 470 nm).

5.10 Ring – Opening of Rhodamine Coumarin Conjugation System for DCP Detection

Stock solutions of metal ions, DCP, DMMP and other OP compounds (0.034 M) were prepared in acetonitrile. The stock solutions of compounds 26 and 28 (3.1 mM) were also prepared in 50% CH$_3$CN, 50% 0.01 M Tris HCl buffer (pH = 7.0). Fluorescence and UV absorption studies were performed using a 10 µM solution of the compounds and appropriate amounts of the analytes. The fluorescence measurements were performed with 530 nm excitation. Both excitation and emission slit widths were 3 nm.

I have synthesized two new rhodamine B and fluorescein derivatives conjugated with an electron-rich diethylcoumarin moiety (Scheme 5.7). We expected DCP to bind with sensors via the carbonyl O, and imine N similar to what Kang et al$^{159}$ suggested.
This design is used in order to better understand the binding mechanism between the sensors and nerve gas mimics. The colorless solutions rhodamine and fluorescein were very weakly fluorescent and showed no absorption above 400 nm, properties that are characteristic of the ring-closed spirolactam. The predominance of the spirolactam form was further confirmed by observation of the characteristic carbon resonance near 65.83 ppm for each of the compounds. Initially, compound 26 showed an absorption band at 450 nm which is from the coumarin moiety, but a new absorption band around 560 nm appeared upon the addition of DCP (Figure 5.16). This new absorption band is due to the ring opening of the rhodamine dye and electron transfer between the two dyes.

![Figure 5.16: UV-Vis spectra of compounds 26 (10 μM) with DCP, metal ions and other OP (340 μM) in 50% CH₃CN, 50% 0.01 M Tris HCl buffer (pH = 7.0).](image)

Figure 5.16: UV-Vis spectra of compounds 26 (10 μM) with DCP, metal ions and other OP (340 μM) in 50% CH₃CN, 50% 0.01 M Tris HCl buffer (pH = 7.0).
Compound 26 showed the highest emission intensity with DCP among all the potential interferences tested during the study (Figure 5.18). Only Cr$^{3+}$ showed a slight emission enhancement among the metals and other analytes used in the experiment (Figure 5.18). The fluorescence spectrum of 26 has a maximum at 580 nm after the addition of 20 equivalents of DCP, corresponding to delocalization in the xanthene moiety of rhodamine. There was a significant fluorescent intensity enhancement (>80 fold, Figure 5.18 and 5.19) as the solution turned pink, a color change clearly visible to the naked eye. As 26 did not give any response to DMMP, it is clear that the leaving group (chloride ion) of DCP plays a key role in binding with sensor 26 to trigger the color change. Continuous addition of DCP resulted in increased fluorescence as shown in Figure 5.19. The linearity of the $F_0/(F-F_0)$ vs $1/[DCP]$ plot confirms the formation of a 1:1 complex between 26 and DCP. The binding constant was calculated using the Benesi-Hildebrand method and found to be $2.4 \times 10^3$ M$^{-1}$. The detection limit of the sensor, 26
for DCP is estimated to be 2.3 μM. The fluorescence studies of sensor 28 were also performed in acetonitrile buffer system. There was a very small absorption and emission intensity change (Figure 5.20) with 1.0 equivalent of DCP and this is probably due to the instability the carbocation resonance structure.

Figure 5.18: Fluorescence spectra of compound 26 (10 μM) with DCP, DMMP, HCl and metals (340 μM) in 50% CH₃CN, 50% 0.01 M Tris HCl buffer (pH = 7.0) (λₑₓ = 530 nm).
Figure 5.19: Compound 26 (10 μM) with DCP (0 - 1.4 mM) in CH$_3$CN ($\lambda_{ex} = 530$ nm).

Figure 5.20: Fluorescence spectra of compound 28 (10 μM) with DCP, DMMP, HCl and metals (340 μM) in 50% CH$_3$CN, 50% 0.01 M Tris HCl buffer (pH = 7.0) ($\lambda_{ex} = 530$ nm).
A $^1$H NMR titration was performed to elucidate the binding mechanism of DCP with compound 26 (Figure 5.21). Continuous addition of DCP resulted in a shortening and broadening of the imine hydrogen peak at δ 8.33. Interestingly, the peaks corresponding to the coumarin hydrogens did not show any significant change with the addition of DCP, while the intensities of some of the xanthene ring hydrogens decreased and broadened. DCP is expected to trigger the formation of the highly fluorescent ring-open form involving the carbonyl oxygen and imine nitrogen as Kang et al.\textsuperscript{159} postulated for the protonation of rhodamine hydrazides. As 26 did not interact with DMMP, it is clear that the chlorine leaving group of DCP plays a key role in binding with sensor 26 to trigger the color change (Figure 5.22).

![Figure 5.21: $^1$H NMR (CHCl$_3$) spectra of 26 with DCP (0, 0.75, 1.5, 3, 4, 6 equiv. from bottom to top).](image)
Figure 5.22: The possible DCP binding mechanism that triggers the formation of the colored ring-open form.

Figure 5.23: Fluorescence spectra of compound 26 (10 μM) with DCP, DMMP, HCl and metals (340 μM) in 50% CH₃CN, 50% 0.01 M Tris HCl buffer (pH = 7.0) (λ_ex = 430 nm).
Figure 5.24: Compound **26** (10 μM) with DCP (0 - 1.4 mM) in CH$_3$CN ($\lambda_{\text{ex}}$ = 430 nm).

5.11 Electrochemical Detection of Nerve Gas Mimic

A sensor device with gold (Au) interdigitated electrodes on a glass substrate was used for the electrochemical detection of DCP. Picomolar amounts of DCP were detected by electrical impedance spectroscopy using **26** as a sensor. Figure 5.25 shows the typical response of the electrochemical sensor towards varying concentration of DCP in 10μM of **26**. The electrochemical impedance spectroscopy sensing mechanism is based on the disturbance of charge transfer dynamics between metal electrodes and **26** at its surface which binds to DCP. Differential pulse voltametry also confirmed the binding of DCP with the sensor (Figure 5.26).
Figure 5.25 Electrochemical Impedance detection of DCP with Compound 26.
In conclusion, we have synthesized four new coumarin-based compounds in high yields of which 20, 21, and 23 are the best sensors for DCP. The effect of electron withdrawing and donating groups in the phenyl ring in DCP binding is very important. These compounds also show very high selectivity towards DCP over the other organophosphorus compounds. $^1$HNMR data provided evidence that the imine N and phenyl protons are the key sites in DCP binding. Combining the phenomenon of fluorescence quenching followed by a change in color visible to the naked eye renders a faster way to detect DCP. As these molecules are redox active this will help reduce the number of false positives considerably. Earlier when discussing the electrochemical nature of these molecules we have seen that on binding to DCP these compounds show a drastic change in current density. The results lead the way for device development which is being pursued.
A coumarin azine derivative 24 also selectively detects DCP from other organophosphates and metal ions that tested. The absorption maximum of compound 24 shows a large red shift from 495 nm to 590 nm in presence of DCP. The change in color is very easily observed by the naked eye, while other OP such as parathion, malathion, ethion, fenthion and DMMP do not induce such a change. Compound 26 was synthesized for the detection of DCP and the $^1$H NMR titration confirmed that imine nitrogen is important in DCP binding. The ring opening of the rhodamine that conjugated with coumarin results the naked eye detection of DCP. This conjugated system enhances the sensitivity of the sensor. Picomolar amounts of DCP were detected by electrical impedance spectroscopy using 26 as a sensor.
6.1 Importance of Detecting Platinum

The detection of platinum ion has attracted the interest of analysts and has developed rapidly because this metal ion is valuable and rare, yet also very important for many industrial processes and products. For example, platinium-containing drinking water and skin care products are sold for their potential benefits to human health\textsuperscript{160, 161}. Cisplatin and its analogues are widely used as anticancer drugs, and additional platinium-based compounds are emerging\textsuperscript{160, 161}. Despite the importance of this metal in such fields, a major problem is the subsequent pollution of the environment that comes with its frequent use. In addition, this metal ion plays important roles in medicine. Accordingly, the recognition and sensing of these metal ions has also been an especially active research area.

Platinum is a widely used precious metal in various materials including commercial drinking water, anticancer drugs, catalytic converters, fuel cells, and jewelry. From the 1990s, Pt(II) complexes were extensively investigated due to their unique luminescent properties, photocatalysis and biological activities, and covalent binding to biomolecules with potential applications\textsuperscript{160}. Although platinum-based chemotherapy is crucial for the treatment of many types of cancer, it is considered to be potentially hazardous to human health. Platinum salts can cause DNA alteration, cancers,
autoimmune disorders, respiratory and hearing problems, and damage to organs, such as the intestines, kidneys, and bone marrow\textsuperscript{161}. Reliable and efficient analytical methods are required for the detection of this metal ion in a wide variety of biological and environmental matrices. Fluorescent sensors are powerful tools for monitoring biologically relevant species \textit{in vitro} and/or \textit{in vivo}, because of their simplicity and high sensitivity. Hence, the development of fluorescent and colorimetric chemosensors for the detection of platinum ion has attracted significant attention.

6.2 Colorimetric and Fluorescent Methods of Detecting Platinum Ions

6.2.1 Based on the Tsuji–Trost allylic oxidative insertion mechanism

Koide \textit{et al.} developed a fluorescein derivative X bearing a terminal allylic group for palladium and platinum ions detection based on the Tsuji–Trost allylic oxidative insertion mechanism (Scheme 6.1). In this mechanism, \( M^0 \) (\( M = Pt, Pd \)) oxidatively inserts into the allylic C–O bond of the nonfluorescent allylic ether X to form the putative complex Y. This complex then reacts with a nucleophile to form the fluorescent compound Z and an allylated nucleophile. The conversion of X to Y can be catalyzed by Pt\(^0\) but not by Pt\(^{2+}\) in aqueous media. The reaction with PtCl\(_2\) was driven to completion by the addition of a reducing agent such as Ph\(_3\)P or NaBH\(_4\), indicating that this method can be applied to the detection of the total platinum concentration upon its \textit{in situ} reduction. This method allows the detection of the total quantities of platinum ions at low nanomolar levels. This method was also used to detect platinum ions in heterogeneous samples such as human serum\textsuperscript{162-165}. 
6.2.2 Based on rhodamine spirolactam ring-opening processes

Tae et al. utilized a rhodamine-6G triazole\textsuperscript{166} as a fluorescent chemosensor for Pt\textsuperscript{2+} in aqueous solution (Scheme 6.2)\textsuperscript{166}. The dual binding unit composed of a hydroxamate and a triazole showed high selectivity and high sensitivity toward Pt\textsuperscript{2+} over a series of other metal ions in water. Probe shows neither color nor fluorescence in H\textsubscript{2}O (DMSO 1% v/v), indicating that it exists predominantly in the spirocyclic form, as expected. On treatment with 5.0 equivalents of Pt\textsuperscript{2+} ions, the probe (5 μM) exerts strong fluorescence at 562 nm in H\textsubscript{2}O (DMSO 1% v/v). In addition, the solution changes from colorless to a pink-red color. This suggests that the triazole group accelerates the ring-opening of spirolactam, thereby allowing it to play an important role as a Pt\textsuperscript{2+} sensor. This Probe could monitor Pt\textsuperscript{2+} (K\textsubscript{2}PtCl\textsubscript{4}, Pt(COD)Cl\textsubscript{2}, PtCl\textsubscript{2}, and cisplatin) ions in the PH range of 5-9.
After thorough investigation of the current Pt$^{2+}$ sensors, I decided to design a heterocyclic coumarin based sensors. Introduction of thiazole moiety into the coumarin could provide a well organized coordination platform for metal ions (Scheme 6.3). As shown in Scheme 6.3, a series of Schiff bases of aminothiazolyl coumarin were synthesized by treating 2’-amino-4’-(3-coumarinyl) thiazole with aldehydes. The structures of the synthesized compounds were characterized on the basis of $^1$H NMR, $^{13}$C NMR and elemental analysis. All the spectroscopic studies were performed in acetonitrile in which all formed colored solutions.
Scheme 6.3 Synthesis of compounds 31-36.
Synthesis of Compound 30

A suspension of compound 29 (2.7 g, 0.01 mole) in 5 ml of hot ethanol was treated with thiourea (1.6 g, 0.021 mole) a mild exothermic reaction took place, giving a clear solution that soon deposited as crystals. The deposit was removed, washed with ethanol and then boiled with water containing sodium acetate which yielded 2.2g (70%) of 2’-amino-4’-(3-coumarinyl) thiazole, 30 and the product obtained was recrystallized with absolute ethanol. M.P 220°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.14 (s, 2H), 7.40 (s, 2H), 7.51 (s, 2H), 7.79 (s, 1H), 8.51 (s, 1H).

Synthesis of Compound 31

A mixture of 0.001 mol of 30 and hydroxyl benzaldehyde (0.001 mol) was refluxed in ethanol containing catalytic amount of piperidine for 4 hours. The reaction mixture was cooled and the separated solid was filtered and crystallized from dichloromethane to yield compound 31(81%) in pure form. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 7.02 (d, 1H), 7.41 (t, 1H), 7.49 (m, 1H), 7.66 (t, 1H), 7.92 (d, 1H), 7.99 (d, 1H), 8.37 (s, 1H), 8.88 (s, 1H), 9.37 (s, 1H), 11.53 (s, 1H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), $\delta$ (ppm): 116.50, 117.49, 119.74, 120.13, 120.37, 120.61, 125.40, 129.76, 131.64, 132.69, 135.77, 140.43, 146.31, 153.15, 159.42, 160.92, 164.90, 171.05. Elemental Analysis Calcd for C$_{19}$H$_{12}$N$_2$O$_3$S : C, 65.51; H, 3.47; N, 8.04; S, 9.20 Found: C, 65.52; H, 3.46; N, 8.02; S, 9.22.

Synthesis of Compound 32

A mixture of 0.001 mol of 30 and hydroxyl 2-methoxybenzaldehyde (0.001mol) was refluxed in ethanol containing catalytic amounts of piperidine for 4 hours. The reaction mixture was cooled and the separated solid was filtered and crystallized from
dichloromethane to yield the compound 32(74%) in pure form. $^1$H NMR (400 MHz, CDCl$_3$) δ: 6.96 (t, 1H), 3.94 (s, 3H), 7.05 (d, 1H), 7.14 (d, 1H), 7.33 (t, 1H), 7.39 (d, 1H), 7.61 (t, 1H), 7.72 (d, 1H), 8.41 (s, 1H), 8.76 (s, 1H), 9.25 (s, 1H), 12.50 (s, 1H). $^{13}$C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 56.10, 115.0, 116.1, 116.6, 119.5, 120.9, 124.1, 124.4, 127.9, 128.3, 129.4, 146.1, 149.0, 149.4, 150.1, 153.0, 160.0, 161.9, 171.8.

Elemental Analysis Calcd for C$_{20}$H$_{14}$N$_2$O$_4$S: C, 63.48; H, 3.73; N, 7.40; S, 8.47 Found: C, 63.49; H, 3.71; N, 7.41; S, 8.49.

Synthesis of Compound 34

A mixture of 0.001 mol of 30 and 3-formylchromone (0.00 mol) was refluxed in ethanol containing catalytic amount of piperidine for 2 hours. The solution was poured into cold water under thorough stirring. The solid that separated was filtered, washed with water and recrystallized from aqueous ethanol. Orange product with 81% yield. M.P 183°C; $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.42 (d, 2H), 7.47 (t, 1H), 7.50 (s, 1H), 7.55 (s, 1H), 7.56 (t, 1H), 7.58 (s, 1H), 7.65 (t, 1H), 7.84 (d, 1H), 8.05 (s, 1H), 8.08 (s, 1H), 8.44 (s, 1H). Elemental Analysis Calcd for C$_{22}$H$_{12}$N$_3$O$_4$S: C, 65.99; H, 3.03; N, 7.00. Found: C, 65.74; H, 3.02; N, 6.93.

Synthesis of Compound 35

A mixture of 0.001mol of 30 and appropriate pyridine-2-carboxaldehyde (0.001mol) was refluxed in ethanol containing catalytic amount of piperidine for 4 hours. The solution was poured into cold water under thorough stirring. The solid that separated was filtered, washed with water and recrystallized from aqueous ethanol. The light brown product resulted in 70% yield. M.P 200°C; $^1$H NMR (400 MHz, CDCl$_3$) δ: 6.21 (d, 1H), 7.38 (m, 2H), 7.49 (d, 1H), 7.51 (s, 1H), 7.64 (s, 2H), 7.86 (d, 2H), 8.50 (d, 1H), 8.55 (s,
1H), 8.65 (d, 1H). Elemental Analysis Cald for C_{18}H_{11}N_{3}O_{2}S: C, 64.85; H, 3.33; N, 12.61
Found: C, 64.56; H, 3.30; N, 12.49.

Synthesis of Compound 36

A mixture of 0.001 mol of 30 and appropriate thiophene-2-carboxaldehyde (0.001 mol) was refluxed in ethanol containing catalytic amount of piperidine for 4 hours. The solution was poured into cold water under thorough stirring. The solid that separated was filtered, washed with water and recrystallized from aqueous ethanol. Bright yellow product formed in 80% yield. M.P 205°C; ^1^H NMR (400 MHz, CDCl$_3$) δ: 7.14 (s, 2H), 7.40 (s, 2H), 7.51 (s, 2H), 7.79 (s, 1H), 8.51 (s, 1H). Elemental Analysis Cald for C$_{17}$H$_{10}$N$_2$O$_2$S: C, 60.33; H, 2.99; N, 8.28 Found: C, 60.12; H, 2.85; N, 8.19.

Synthesis of Compound 37

A mixture of 0.001 mol of 30 and furan-2-carboxaldehyde (0.001 mol) was refluxed in ethanol containing catalytic amount of piperidine for 4 hours. The solution was poured into cold water under thorough stirring. The solid that separated was filtered, washed with water and recrystallized from aqueous ethanol. Bright yellow product formed in 75% yield. ^1^H NMR (400 MHz, DMSO) δ: 6.83 (d, 1H), 7.47 (m, 1H), 7.49 (d, 2H), 7.51 (t, 1H), 7.91 (d, 1H), 8.13 (s, 1H), 8.34 (s, 1H), 8.86 (s, 1H), 8.99 (s, 1H). ^13^C NMR (400 MHz, DMSO-d$_6$), δ (ppm): 112.6, 116.1, 118.9, 120.9, 124.1, 125.4, 127.9, 128.3, 129.4, 144.4, 146.1, 146.4, 149.4, 150.4, 153.0, 161.9, 171.8. Elemental Analysis Cald for C$_{17}$H$_{10}$N$_2$O$_3$S: C, 63.34; H, 3.13; N, 8.69 Found: C, 63.00; H, 3.17; N, 8.70.
6.4 Spectroscopic Studies of Compounds 31-37

In this study, linear optical properties of new donor-acceptor coumarin derivatives are investigated with an emphasis to explore the fluorescence sensing of metal ions. The coumarin derivatives that have been studied are 31, 32, 33, 34, 35, 36 and 37. Different derivatives are chosen such that they represent varying degrees of donating and accepting abilities. The dye molecules have reasonable fluorescence quantum yield (Table 1). However, addition of metal ion complexing groups reduces the quantum yield both because of charge transfer as well as conformational relaxation.

The electronic absorption spectra of all the compounds in acetonitrile solutions exhibited intense absorption bands at 360 – 430 nm. The compounds shows two absorption bands; the band at 290 nm is due to $\pi \rightarrow \pi^*$ transitions and the absorption at 350 nm is from ligand-to-ligand charge transfer (LLCT) transition. Most of these compounds did not show any significant absorption band change with metal ions including Pt$^{2+}$. 
Table 6.1 Optical values of the compounds 31-37

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{abs(max)}}$ (nm)</th>
<th>$\lambda_{\text{em(max)}}$ (nm)</th>
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<th>Stokes shift</th>
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<tr>
<td>32(Hex)</td>
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<td>418</td>
<td>-</td>
<td>3474</td>
</tr>
<tr>
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Figure 6.1 UV-vis absorption bands of 31-35.
The fluorescence intensity changes of **31-37** (10 µM) upon the addition of metal ions (10 µM, 1 equiv.) in acetonitrile showed a remarkable sensitivity and selectivity towards Pt\(^{2+}\). The observed fluorescence enhancement at 445 nm (\(\lambda_{ex} = 365\) nm) was over 300-fold, which is extremely high compared to that of the other metals. In addition to fluorescence enhancements, the emission shifted towards lower wavelength (hypsochromic) in the presence of Pt\(^{2+}\). In order to demonstrate the high selectivity of these sensors, we tested all compounds with other common metal ions including Na\(^+\), K\(^+\), Fe\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Mn\(^{2+}\), Pt\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), Cr\(^{3+}\) and Hg\(^{2+}\), and found either no fluorescence enhancement or very slight enhancement (Cr\(^{3+}\) and Zn\(^{2+}\)), which is negligible compared to the huge value observed for Pt\(^{2+}\).

![Figure 6.2 Fluorescence emissions of compound 31.](image-url)
The emission spectrum of compound 31 was recorded in CH$_3$CN (Fig. 6.2) and upon excitation at 365 nm showed an emission at 495 nm. The fluorescence change were recorded upon the addition of Na$^+$, K$^+$, Fe$^{2+}$, Fe$^{3+}$, Pt$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Cr$^{3+}$ and Hg$^{2+}$ to determine the sensing activity of 31 and which more selective for Pt$^{2+}$ (Figure 6.3). The Pt$^{2+}$ concentrations used in the experiment ranged from 0 to 6 x 10$^{-5}$ M. The fluorescence spectrum recorded upon the addition of Pt$^{2+}$ showed an increase in emission and hypsochromic shift (50nm) in emission maxima indicating the shift of the MLCT state to the higher energy (Figure 6.3 and 6.4).

![Fluorescence emission changes in 31 (10 µM) upon addition of different metal ions (10 µM) in acetonitrile (excitation at 365 nm).](image_url)

Figure 6.3  Fluorescence emission changes in 31 (10 µM) upon addition of different metal ions (10 µM) in acetonitrile (excitation at 365 nm).
Figure 6.4 Fluorescence changes of 31 (10 µM) with Pt\(^{2+}\) (0 – 60 µM). Insets: fluorescence enhancement at 445 nm as a function of [Pt\(^{2+}\)].

Figure 6.4 shows the emission of compound 31 [10x10\(^{-6}\) M] with variable Pt\(^{2+}\) concentrations, excited at 365 nm. Initial emission was at 495 nm, when 10 µM Pt\(^{2+}\) was added the emission enhanced and continued with increased concentrations and hypsochromic shift was observed. The above emission enhancement was attributed to adduct formation between compound 31 and Pt\(^{2+}\). The emission was quantified by calculating the association constant, which is a very small value (1.3 x 10\(^3\) M\(^{-1}\)). Unfortunately the changes in the absorbance are too small to actually determine the ratio of compound 31 to Pt\(^{2+}\) using conventional methods such as Job’s plot. Addition of ethylenediamine to the mixture of 31 and Pt\(^{2+}\) decreases the fluorescence intensity of the solution, which implies the reversible binding between 31 and Pt\(^{2+}\), and the fluorescence titration of Pt\(^{2+}\) at 10 µM concentrations of 31 demonstrates that the detection of Pt\(^{2+}\) is possible 118 nM levels.
The fluorescence responses of 34 and 37 to other biologically relevant metal ions in CH₃CN were examined (Figure 6.5 and 6.8). Upon additions of 2.0 equiv of metal ions (Na⁺, K⁺, Fe²⁺, Fe³⁺, Pt²⁺, Pb²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Mn²⁺, Cr³⁺ and Hg²⁺), only Pt²⁺ leads to a dramatic enhancement in fluorescence intensity and hypsochromic shift in CH₃CN solution. Other metal ions develop no significant fluorescence intensity changes but there is some interference from Cr³⁺.

Figure 6.5  Fluorescence emission changes in 34 (10 µM) upon addition of different metal ions (10 µM) in acetonitrile (excitation at 375 nm).
Figure 6.6 Fluorescence changes of 34 (10 µM) with Pt$^{2+}$ (0 – 75 µM). Insets: fluorescence enhancement at 445 nm as a function of [Pt$^{2+}$].

Figure 6.7 Fluorescence emissions of compound 37.
Figure 6.8  Fluorescence emission changes in 37 (10 μM) upon addition of different metal ions (10 μM) in acetonitrile (excitation at 375 nm).

Figure 6.9  Fluorescence changes of 37 (10 μM) with Pt$^{2+}$ (0 – 83 μM). Insets: fluorescence enhancement at 445 nm as a function of [Pt$^{2+}$].
In conclusion, we have described a highly selective and sensitive fluorescent chemosensor for the detection of Pt$^{2+}$ in acetonitrile solutions. This method allows for the detection of platinum at low nanomolar levels. The binding platform of a thiazole moiety into the coumarin displays selective complexations with Pt$^{2+}$. In addition fluorescent enhancement there is hypsochromic shift of all the sensors in the presence of Pt$^{2+}$. This fluorometric detection method may find broad applications in materials and human health.

Figure 6.10 Fluorescence life time measurements of compounds 31, 33 and 37.
Table 6.2 Summary of sensing activity of all compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Selectivity for M^{ii+} and DCP</th>
<th>Binding constant (M^{-1})</th>
<th>Sensitivity (µM)</th>
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<td>19</td>
<td>Cr^{3+}</td>
<td>2.2 x 10^5</td>
<td>1.5</td>
</tr>
<tr>
<td>20</td>
<td>DCP</td>
<td>3.5 x 10^4</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>DCP</td>
<td>1.7 x 10^4</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>DCP</td>
<td>1.4 x 10^4</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>DCP</td>
<td>3.3 x 10^4</td>
<td>0.18</td>
</tr>
<tr>
<td>24</td>
<td>DCP</td>
<td>1.5 x 10^4</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>DCP</td>
<td>2.4 x 10^4</td>
<td>2.3</td>
</tr>
<tr>
<td>28</td>
<td>DCP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Pt^{2+}</td>
<td>1.3 x 10^5</td>
<td>0.12</td>
</tr>
</tbody>
</table>
REFERENCES

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Appendix A

X-ray crystallography, 1H NMR, 13C NMR and ESI-MS data of fluorescein derivatives in chapter II
Crystal Structure data Data of Compound 1 and 2

The X-ray intensity data was measured on a Bruker SMART APEX II CCD system equipped with a graphite monochromator and a MoK\(_\alpha\) fine-focus tube (\(\lambda = 0.71073\) Å). Deposited X-ray crystallography data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB21EZ, UK; fax (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

Compound 1: CCDC 832829

Sample and crystal data for 1.

Chemical formula \(\text{C}_{24}\text{H}_{15}\text{N}_{3}\text{O}_{4}\text{S} + 2 \text{C}_{2}\text{H}_{6}\text{O}\)
Formula weight 533.59
Temperature 100(2) K
Wavelength 0.71073 Å
Crystal size 0.15 x 0.31 x 0.32 mm
Crystal habit clear colourless rectangular prisms
Crystal system orthorhombic
Space group \(\text{P 2}_1\text{ 2}_1\text{ 2}_1\)
Unit cell dimensions \(a = 8.76080(10)\) Å \(\alpha = 90^\circ\)
\(b = 14.39650(10)\) Å \(\beta = 90^\circ\)
\(c = 20.4864(2)\) Å \(\gamma = 90^\circ\)
Volume 2583.84(4) Å\(^3\)

Data collection and structure refinement for 1.

Theta range for data collection 1.73 to 30.03°
Index ranges -11\(\leq h \leq 12\), -20\(\leq k \leq 20\), -28\(\leq l \leq 27\)

\(^{188}\)}
Reflections collected 73741
Independent reflections 7557 [R(int) = 0.0478]
Coverage of independent reflections 100.0%
Absorption correction multi-scan
Max. and min. transmission 0.9737 and 0.9464
Structure solution technique direct methods
Structure solution program SHELXS-97 (Sheldrick, 2008)
Refinement method Full-matrix least-squares on F^2
Refinement program SHELXL-97 (Sheldrick, 2008)
Function minimized Σ w(F_o^2 - F_c^2)^2
Data / restraints / parameters 7557 / 0 / 413
Goodness-of-fit on F^2 0.843
Δ/σ_max 0.001
Final R indices 6696 data; R1 = 0.0356, wR2 = 0.1026
I>2σ(I)  R1 = 0.0433, wR2 = 0.1106
all data  
Weighting scheme w=1/[σ^2(F_o^2)+(0.1000P)^2+0.0000P]
where P=(F_o^2+2F_c^2)/3
Absolute structure parameter -0.1(1)
Largest diff. peak and hole 0.278 and -0.235 eÅ^-3
R.M.S. deviation from mean 0.047 eÅ^-3

Hydrogen bond distances(Å) and angles(°) for 1.

<table>
<thead>
<tr>
<th>Donor-H</th>
<th>Acceptor-H</th>
<th>Donor-Acceptor</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2-H2O-O5#1 0.91(2)</td>
<td>1.72(3)</td>
<td>2.6327(18)</td>
<td>174.0</td>
</tr>
<tr>
<td>O3-H3O-O4#3 0.94(3)</td>
<td>1.82(3)</td>
<td>2.7045(15)</td>
<td>156.0</td>
</tr>
<tr>
<td>O5-H5O-O6#4 0.84(3)</td>
<td>1.88(3)</td>
<td>2.7185(19)</td>
<td>172.0</td>
</tr>
<tr>
<td>O6-H6O-N3#2 0.94(3)</td>
<td>1.88(3)</td>
<td>2.7786(18)</td>
<td>159.0</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:
#1 x, y+1, z  #3 -x, y+1/2, -z+1/2
#2 x+1, y-1, z  #4 x-1/2, -y+1/2, -z
Selected bond distances (Å), torsions(°) and angles(°) for 1.

<table>
<thead>
<tr>
<th>Bond Sequence</th>
<th>Distance (Å)</th>
<th>Bond Type</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2-C21-C22-S1</td>
<td>17.20(19)</td>
<td>C1-N1</td>
<td>1.5089(17)</td>
</tr>
<tr>
<td>C2-C1-C14-C15</td>
<td>63.41(19)</td>
<td>N1-N2</td>
<td>1.3567(15)</td>
</tr>
<tr>
<td>O4-C20-N1-N2</td>
<td>0.2(2)</td>
<td>C20-N1</td>
<td>1.3877(18)</td>
</tr>
</tbody>
</table>
**Figure 2.35** Packing diagrams for Compound 1 showing the hydrogen bonding framework along all three axes. Fluorescein molecule in red and green; ethanol in purple; H-bonds shown as blue, dashed lines.

**Compound 2: CCDC 832830**

**Sample and crystal data for 2.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C₃₄H₂₆N₂O₇</td>
</tr>
<tr>
<td>Formula weight</td>
<td>574.57</td>
</tr>
<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.10 x 0.22 x 0.35 mm</td>
</tr>
<tr>
<td>Space group</td>
<td>P -1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 10.7817(4) Å  α = 95.381(2)°</td>
</tr>
<tr>
<td></td>
<td>b = 11.1833(4) Å  β = 111.757(2)°</td>
</tr>
<tr>
<td></td>
<td>c = 12.6293(5) Å  γ = 100.662(2)°</td>
</tr>
<tr>
<td>Volume</td>
<td>1367.72(9) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.395 Mg/cm³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.099 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>600</td>
</tr>
</tbody>
</table>
Data collection and structure refinement for 2.

Theta range for data collection
1.76 to 25.68°

Index ranges
-13 ≤ h ≤ 13, -13 ≤ k ≤ 13, -15 ≤ l ≤ 15

Reflections collected
39045

Independent reflections
5200 [R(int) = 0.0569]

Coverage of independent reflections
99.9%

Absorption correction
numerical

Max. and min. transmission
0.9905 and 0.9659

Structure solution technique
direct methods

Structure solution program
SHELXS-97 (Sheldrick, 2008)

Refinement method
Full-matrix least-squares on F²

Refinement program
SHELXL-97 (Sheldrick, 2008)

Function minimized
Σ w(F_o² - F_c²)

Data / restraints / parameters
5200 / 0 / 359

Goodness-of-fit on F²
0.932

Δ/σ_max
0.002

Final R indices
3123 data; I>2σ(I) R1 = 0.0510, wR2 = 0.1459
all data R1 = 0.0889, wR2 = 0.1652

Weighting scheme
w=1/[σ²(F_o²)+(0.1000P)²+0.0000P] where P=(F_o²+2F_c²)/3

Largest diff. peak and hole
0.197 and -0.202 eÅ⁻³

R.M.S. deviation from mean
0.038 eÅ⁻³

Hydrogen bond distances (Å) and angles (°) for 2.

<table>
<thead>
<tr>
<th>Donor-H</th>
<th>Acceptor-H</th>
<th>Donor-Acceptor</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1-H1-O5#1</td>
<td>0.91(4)</td>
<td>2.06(4)</td>
<td>2.955(3)</td>
</tr>
<tr>
<td>O3-H2-O4#2</td>
<td>0.78(3)</td>
<td>1.99(3)</td>
<td>2.769(2)</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:

#1 -x, -y+1, -z
#2 -x+1, -y+2, -z+1
**Selected bond distances (Å), torsions (°) and angles(°) for 2.**

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Torsion (°)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2-C21-C22-C30</td>
<td>-174.5(2)</td>
<td>C1-N1</td>
<td>1.512(3)</td>
</tr>
<tr>
<td>O4-C20-N1-N2</td>
<td>5.6(4)</td>
<td>N1-N2</td>
<td>1.381(2)</td>
</tr>
<tr>
<td>C2-C1-C14-C15</td>
<td>58.6(3)</td>
<td>C20-N1</td>
<td>1.373(3)</td>
</tr>
</tbody>
</table>

**Figure 2.36** ORTEP packing diagram for Compound 2. THF molecules present in the interstitial spaces are removed for clarity.
Figure 2.37 2-dimensional H-bonded chains in Compound 2.

$^1$H NMR (400 MHz, DMSO-d6) of compound 1
$^{13}$C NMR (400 MHz) of Compound 1

Mass spectrum (High resolution ESI) for Compound 1
$^1$H NMR (400 MHz, DMSO-d6) of compound 2

$^{13}$C NMR (400 MHz) of Compound 2
Mass Spectrum (High resolution ESI) for Compound 2

\(^1\text{H} \text{ NMR (400 MHz, DMSO-d6) of compound 4}\)
$^{13}$C NMR (400 MHz) of Compound 4

Mass Spectrum (High resolution ESI) for Compound 4
$^1$H NMR (400 MHz, DMSO-d6) of compound 5

$^{13}$C NMR (400 MHz) of Compound 5
Appendix B

X-ray crystallography, 1H NMR, 13C NMR and ESI-MS data of fluorescein derivatives in chapter III
### Crystal Data for compound 3

<table>
<thead>
<tr>
<th>Property</th>
<th>Value/Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C(<em>{20})H(</em>{16})N(_2)O(_5)</td>
</tr>
<tr>
<td>Formula weight</td>
<td>364.35</td>
</tr>
<tr>
<td>Temperature</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.11 x 0.32 x 0.32 mm</td>
</tr>
<tr>
<td>Crystal habit</td>
<td>translucent light colourless-brown rhombus</td>
</tr>
<tr>
<td>Crystal system</td>
<td>triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P -1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 8.0017(10) Å, (\alpha = 103.5950(10))°, (\beta = 110.7620(10))°, (c = 10.9155(10)) Å, (\gamma = 100.3110(10))°</td>
</tr>
<tr>
<td>Volume</td>
<td>806.78(15) Å(^3)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.500 Mg/cm(^3)</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.109 mm(^{-1})</td>
</tr>
<tr>
<td>(F(000))</td>
<td>380</td>
</tr>
</tbody>
</table>

Data collection and structure refinement for the compound 3.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value/Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theta range for data collection</td>
<td>2.06 to 26.02°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-9 (\leq) h (\leq) 9, -13 (\leq) k (\leq) 13, -13 (\leq) l (\leq) 13</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>8611</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>3165 ([R(int) = 0.0257])</td>
</tr>
<tr>
<td>Coverage of independent reflections</td>
<td>99.7%</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>numerical</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9881 and 0.9657</td>
</tr>
<tr>
<td>Structure solution technique</td>
<td>direct methods</td>
</tr>
<tr>
<td>Structure solution program</td>
<td>SHELXS-97 (Sheldrick, 2008)</td>
</tr>
</tbody>
</table>
Refinement method | Full-matrix least-squares on $F^2$
---|---
Refinement program | SHELXL-97 (Sheldrick, 2008)
Function minimized | $\Sigma w(F_o^2 - F_c^2)^2$
Data / restraints / parameters | 3165 / 0 / 304
Goodness-of-fit on $F^2$ | 1.242
Final R indices | 2538 data; $I>2\sigma(I)$
 | R1 = 0.0493, wR2 = 0.1546
 | all data
 | R1 = 0.0629, wR2 = 0.1703
Weighting scheme | $w=1/[\sigma^2(F_o^2)+ (0.1000P)^2 + 0.0000P]$ where $P=(F_o^2 + 2F_c^2)/3$
Largest diff. peak and hole | 0.582 and -0.684 eÅ$^{-3}$
R.M.S. deviation from mean | 0.055 eÅ$^{-3}$
Hydrogen bond distances (Å) and angles (°) for the Compound 3.

<table>
<thead>
<tr>
<th>Donor-H</th>
<th>Acceptor-H</th>
<th>Donor-Acceptor</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2-H2O</td>
<td>O1S#3</td>
<td>1.00(3)</td>
<td>1.76(3)</td>
</tr>
<tr>
<td>O3-H3O</td>
<td>O1S#1</td>
<td>1.01(4)</td>
<td>1.81(4)</td>
</tr>
<tr>
<td>N2-H2N</td>
<td>N2#4</td>
<td>0.85(5)</td>
<td>2.61(5)</td>
</tr>
<tr>
<td>O1S-H1S</td>
<td>O4#2</td>
<td>0.86(4)</td>
<td>1.89(4)</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:
#1  x-1, y, z
#2  x, y-1, z-1
#3  x, y+1, z
#4  -x, -y+2, -z+2
Crystal data for Compound 8

**Chemical formula** \( C_{28}H_{22}N_{2}O_{6} \)

**Formula weight** 482.48

**Temperature** 100(2) K

**Wavelength** 0.71073 Å

**Crystal size** 0.09 x 0.15 x 0.18 mm

**Crystal habit** translucent light colourless-bronze rhombus

**Crystal system** Triclinic

**Space group** P -1

**Unit cell dimensions**
- \( a = 10.4382(7) \) Å \( \alpha = 96.2670(10)^\circ \)
- \( b = 10.6433(7) \) Å \( \beta = 96.0730(10)^\circ \)
- \( c = 11.5078(14) \) Å \( \gamma = 117.1990(10)^\circ \)

**Volume** 1112.65(17) Å\(^3\)

**Z** 2

**Density (calculated)** 1.440 Mg/cm\(^3\)

**Absorption coefficient** 0.102 mm\(^{-1}\)

**\(F(000)\)** 504

Data collection and structure refinement for FA04

**Theta range for data collection** 1.81 to 29.45°

**Index ranges** -14<=h<=14, -14<=k<=14, -15<=l<=15

**Reflections collected** 20331

**Independent reflections** 5725 [R(int) = 0.0572]

**Coverage of independent reflections** 92.6%

**Absorption correction** numerical

**Max. and min. transmission** 0.9910 and 0.9822

**Structure solution technique** direct methods

**Structure solution program** SHELXS-97 (Sheldrick, 2008)

**Refinement method** Full-matrix least-squares on \( F^2 \)

**Refinement program** SHELXL-97 (Sheldrick, 2008)

**Function minimized** \( \Sigma w(F_o^2 - F_c^2)^2 \)

**Data / restraints /** 5725 / 0 / 330
parameters
Goodness-of-fit on $F^2$ 0.941
$\Delta/\sigma_{\text{max}}$ 0.001

Final R indices
3686 data; $R_1 = 0.0511$, $wR_2 = 0.1223$
$I>2\sigma(I)$
all data $R_1 = 0.0898$, $wR_2 = 0.1475$

Weighting scheme
$w=1/[\sigma^2(F_o^2)+(0.0722P)^2+0.3962P]$ where $P=(F_o^2+2F_c^2)/3$

Largest diff. peak and hole 0.318 and -0.333 e\text{Å}^{-3}

R.M.S. deviation from mean 0.062 e\text{Å}^{-3}

Hydrogen bond distances (Å) and angles (°) for Compound 8.

<table>
<thead>
<tr>
<th>Donor-</th>
<th>Acceptor-</th>
<th>Donor-Acceptor</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>O3-H3A··O4#3</td>
<td>0.84</td>
<td>1.91</td>
<td>2.7538(18)</td>
</tr>
<tr>
<td>O2-H2··O6#1</td>
<td>0.84</td>
<td>1.92</td>
<td>2.722(2)</td>
</tr>
<tr>
<td>O5-H5··N2</td>
<td>0.84</td>
<td>1.92</td>
<td>2.650(2)</td>
</tr>
<tr>
<td>O6-H6A··O5#2</td>
<td>0.84</td>
<td>1.97</td>
<td>2.807(2)</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:
#1 $x, y, z+1$
#2 $-x, -y, -z+1$
#3 $-x+1, -y, -z+1$
\textsuperscript{1}H NMR for compound 6 in DMSO

\textsuperscript{13}C NMR for compound 6 in DMSO
ESI-MS spectra for compound 6

$^1$H NMR for Compound 7 in DMSO
\(^{13}\)C NMR spectra for Compound 7 in DMSO
ESI-MS spectra for Compound 7

$^1$H NMR for Compound 8 in DMSO
Appendix C

X-ray crystallography, 1H NMR, 13C NMR, and ESI-MS data of coumarin derivatives in chapter IV
Crystal structure Data for 9

Chemical formula \( C_{14}H_{15}NO_3 \)
Formula weight 245.27
Temperature 100(2) K
Wavelength 0.71073 Å
Crystal size 0.22 x 0.44 x 0.51 mm
Crystal habit lustrous intense red cut needle burst
Crystal system triclinic
Space group \( P -1 \)
Unit cell dimensions
\[
a = 7.05130(10) \, \text{Å} \quad \alpha = 87.4490(10)^\circ \\
b = 7.29230(10) \, \text{Å} \quad \beta = 86.0690(10)^\circ \\
c = 12.51790(10) \, \text{Å} \quad \gamma = 66.8290(10)^\circ 
\]
Volume 590.251(13) Å³
Z 2
Density (calculated) 1.380 Mg/cm³
Absorption coefficient 0.097 mm⁻¹

Data / restraints / parameters 3428 / 0 / 223
Goodness-of-fit on \( F^2 \) 1.281
Final R indices
\[
3057 \text{ data; } \quad R_1 = 0.0385, \quad wR_2 = 0.1390 \\
l > 2\sigma(I) \quad R_1 = 0.0427, \quad wR_2 = 0.1484
\]

Crystal Structure data for compound 10

Formula weight 350.41
Temperature 100(2) K
Wavelength 0.71073 Å
Crystal size 0.21 x 0.23 x 0.47 mm
Crystal habit translucent intense orange-red cut rhombus
Crystal system monoclinic
Space group \( P 1 21/n 1 \)
Unit cell dimensions
\[
a = 11.81950(10) \, \text{Å} \quad \alpha = 90^\circ \\
b = 6.73660(10) \, \text{Å} \quad \beta = 103.5860(10)^\circ \\
c = 22.0350(2) \, \text{Å} \quad \gamma = 90^\circ 
\]
Volume 1705.40(3) Å³
Z 4
Density (calculated) 1.365 Mg/cm³
Absorption coefficient 0.092 mm⁻¹
F(000) 744

¹H - NMR of Compound 10 (d-DMSO)
$^{13}$C-NMR for Compound 10 in CDCl$_3$

1H-NMR for Compound 11 (d-DMSO)
ESI-MS spectrum for compound 16
Appendix D

1H NMR, 13C NMR, and ESI-MS data of coumarin derivatives in chapter V
$^1$H NMR (400 MHz, DMSO-d6) of compound 23

$^1$H NMR (400 MHz, CDCl$_3$-d6) of compound 22
1H-NMR for Compound 26 (d-CDCl₃)
$^{13}$C-NMR for Compound 26 (d-CDCl$_3$)
ESI-MS spectrum for compound 26
Appendix E

$^1$H NMR and $^{13}$C NMR data of hetroyclic coumarin derivatives in chapter VI
$^1$H NMR for compound 31
$^{13}$C NMR for compound 31
\(^1\text{H} \text{NMR for compound 32}\)
$^1$H NMR for compound 35