The Synthesis and Physical Chemistry of Zinquin Analogues

A Thesis Submitted Towards the

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by

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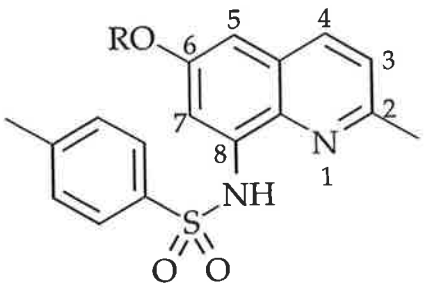
Contents

Abbreviations

ZQE	Zinquin ester.
ZQA	Zinquin Acid.
ZQB	The conjugate base of ZQA.
Zinquin	When used in the text it refers to Zinquin in a generic sense.
DMSO	Dimethyl sulfoxide.
DMF	Dimethyl formamide.
THF	Tetrahydrofuran.
EDTA	Ethylenediaminetetraacetic acid, disodium salt.
Ts	*p*-Toluene sulfonyl.

Abstract

This thesis describes the synthesis and physical chemistry of various analogues of the commercially available Zn(II) fluorophore, Zinquin (ester, **1a**; acid, **1b**). In particular the uv/visible spectra and fluorescent properties of these analogues, in the absence and presence of Zn(II), are described. All of these analogues contain a quinoline nitrogen and a sulfonamide nitrogen at the 8-position, both of which are required for binding to Zn(II) in the Zinquin series of compounds.



1a R = CH_2CO_2Et (ZQE)
1b R = CH_2CO_2H (ZQA)
1c R = CH_3

Such analogues included the alteration of the sulfonamide unit of Zinquin and the position of the alkoxy group on the quinoline ring. The group at the 2-position on Zinquin was also varied by the addition of larger alkyl and aryl substituents. In addition, an acridine and two acridone ligands were investigated as potential Zn(II) fluorophores.

The development of these analogues has led to a number of new Zn(II) fluorophores which exhibit increased fluorescence and selectivity compared with Zinquin. In particular, inclusion of electron withdrawing sulfonamides such as 2,2,2-trifluoroethyl sulfonamide and *m*-trifluoromethylbenzene sulfonamide, led to the formation of analogues that form emore fluorescent complexes with Zn(II) than the *p*-toluene sulfonamide already used in Zinquin. The 4-alkoxy isomer exhibited three

fold enhancement in fluorescence compared to the Zinquin precursor, **1c**, in the presence of Zn(II), while the 5-alkoxy isomer showed no fluorescence in the presence of Zn(II).

Increasing the size of the alkyl group at the 2-position of Zinquin was shown to both increase the fluorescence of the Zn(II)-ligand complex and increase the ligand's selectivity; shown by a reduced fluorescence of the Cd(II)-ligand complex. However, further enlargement of the group at the 2-position, such as by the inclusion of a styryl group only slightly improved the fluorescence of the Zn(II)-ligand complex compared to **1a**. The inclusion of a larger alkyl group was shown to increase the stability of the Zn(II)-ligand complex compared to the Zn(II)-ZQE complex (shown by electrospray ionisation mass spectrometry), but the larger styryl group was shown to decrease the stability of the Zn(II)-ligand complex compared to the Zn(II)-ZQE complex.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the university library, being available for loan or photocopying.

Marc C. Kimber

April, 1998

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Chapter 1 : Introduction.

1.1. Zinc (II).

1.1.1. Introduction.

Zinc is a trace element in the body and is present in the earth's crust to the extent of 0.02%[1] and it is ranked twenty third in order of abundance of all metals. Zn(II) is relatively available in sea water, compared with other divalent transition metal ions where its concentration has been calculated to be 8.1 nmol cm^{-3}.[2] Zn(II) is amongst the most available of the trace elements more so than copper, nickel, iron, cobalt and cadmium.[3] Zn(II) is essential for life in both humans and animals and this was first definitively demonstrated in the 1930's by Todd[4] and later by Bertrand and Bhattacherjee[5] with their work on mice and rats.

Zn(II) is present in all organs, tissues, fluids and secretions of the body but the distribution of the Zn(II) and the nature of its intracellular binding has, until recently, been unclear. The first demonstration of the presence of Zn(II) within cells was by Bartholomew.[6] He found that injected $^{65}Zn(II)$ was distributed within the nuclear, mitochondrial and supernatant fractions of mouse liver with the largest proportion in the supernatant fractions. Further work by Bettger and O'Dell[7] suggested that there was a fraction of intracellular Zn(II) specifically bound to membranes.

Zn(II) appears to be ubiquitously distributed within cells but the nature of its binding is much less clearly understood. The concentrations of free Zn(II) varies from 10^{-9} M in the cytoplasm to 10^{-3} M in some vesicles (Ca(II) has a similar distribution in these areas).[8] The 90% of Zn(II) within cells is known to be protein and enzyme bound with this

Zn(II) having structural and catalytic roles.[9] The remaining 10% is believed to exist as loosely bound Zn(II) or "available" Zn(II) and it is the role of this type of Zn(II) that is less well understood.

1.1.2. Clinical and biochemical manifestations of Zn(II) deficiency.

The first documented case of Zn(II) deficiency was recorded in early 1960 and involved dwarfism in adolescent Iranian males.[10] Prasad[10] noted that Zn(II) supplementation consistently shortened the period of time required to develop to sexual maturity and improve growth. Todd[11] demonstrated that rats fed on Zn(II) deficient diets exhibited growth retardation, but that this was corrected when Zn(II) was supplemented. Sadasevan[12] conducted in depth studies into Zn(II) requirements in rats showing that the effects of excess Zn(II) paralleled those of Zn(II) deficiencies. This was illustrated by reduced body fat and growth retardation in addition to an increase in nitrogen excretion.

The use of Zn(II) in medicines has been well documented. Preparations of calamine ointments have been used for irritations, abrasions and burns, for example Ebers papyrus[13] was used by the early Egyptians. Zn(II) oxides have been successfully utilised in the treatment of *staphylococci* and *streptococci* infections[14] with the Zn(II) sulfates used to treat gleet and leucorrhoea[15] which are conditions of vaginal infection.

The essential role of Zn(II) in the survival, growth and metabolism of unicellular and multicellular organisms is partially explained by the requirement of metalloenzymes, transcription factors and hormones for this element. Both corticotrophin and adrenocorticotrophic hormone extracts have been reported to contain 200μg/g Zn(II).[16] Spermatozoa have also been shown to contain very high amounts of Zn(II).[17] It is not only the

latter that is rich in Zn(II) but also the surrounding fluid, especially the fluid from the prostate.[18] Additionally, Fujii[19] in 1954 showed by histochemical methods that Zn(II) was present in the nucleus of animal cells.

Studies by Chesters[20] have shown that total DNA synthesis is reduced significantly as a consequence of Zn(II) deficiency. Zn(II) has also been linked to RNA and DNA synthesis proteins, as well as zinc-finger proteins and these all of these proteins are known to bind to promoter regions of DNA.[10] Additionally, Zn(II) has roles in both RNA and DNA metabolism with both RNA and DNA polymerases containing two bound Zn(II) ions. In RNA-polymerase, one Zn(II) ion is associated with structural proteins and the other associated with catalytic proteins.[21]

1.1.3. The value of Zn(II) in biology.

There are certain advantageous properties of metal ions, such as Zn(II), both in their binding strengths and their rates of exchange of ligands which make them of chemical and biological importance. Zn(II) has a double positive charge and a small ionic radius (0.65Å) and this results in a highly concentrated charge on the Zn(II) ion as compared with other metal ions. Zn(II) exhibits modest binding to anions such as carboxylates and phosphates and this is predominantly due to competition from water of hydration. So what makes Zn(II) unique as compared to other biologically important metal ions?

The electrostatic binding properties of Zn(II) are shared almost equally with Mg(II) and to a lesser extent Ca(II) as well as with other metal cations such as Cu(II) and Ni(II).[3] Metal ions with their high affinity for electrons, shown by the considerable energy required for conversion from the gaseous atom M to the gaseous M^{+n}, exhibit high Lewis acid capabilities differing

from metal to metal. Zn(II) differs markedly from Mg(II) and Ca(II) in terms of Lewis acid strength since its electron affinity is much higher. It is a strong Lewis acid[22] and is similar in this respect to Cu(II) and Ni(II). These latter cations along with Zn(II) bind strongly to donors such as thiolates and amines, reflecting their borderline hard Lewis acid nature in contrast to the hard acids Mg(II) and Ca(II) which do not strongly bind thiolates and amines.

Zn(II) differs from Cu(II) and Ni(II) as it does not show variable valency.[23] Variable valency or oxidation state change (redox change) can introduce the risk of potentially harmful free radical reactions. Additionally, Zn(II) has a d^{10} electronic configuration and therefore is not affected by ligand field directional bond characteristics in contrast to Ni(II) and Cu(II) whose respective d^8 and d^9 electronic configuration restricts their stereochemistry. It is probable that the widespread occurrence of Zn(II) as a metalloenzyme cation is because of the relative ease with which it can change its stereochemistry.[24] Cd(II) is very similar to Zn(II) in all the above respects, consequently Cd(II) is a competitive poison at certain Zn(II) sites[24] but is relatively rare compared to Zn(II).

The role of Ca(II) in the triggering of muscle activity is well documented.[25] This requires good binding with selective recognition but also rapid "on and off" reaction rates since there is often a need to stimulate and relax a biological system quickly. Zn(II), like Ca(II), has the required properties for a trigger or control cation due to the ability to bind strongly and exchange ligands rapidly.[8] It is used in different circumstances however since Ca(II) binds to *O*-donors only, whereas Zn(II) binds mainly to *S*- and *N*-donors. Therefore Zn(II) plays an important role in enzyme and protein binding and also in regulation and catalysis.[23]

Table 1.1. A general overview of the biological importance of Zn(II).[26]

1. Availability (> Ni(II) or proton).
2. Strongly retained (> Mn(II), Fe(II) or Mg(II)).
3. Fast ligand exchange (>Ni(II) or Mg(II)).
4. Strong borderline hard Lewis acid (only Cu(II) better).
5. No redox reactions possible (contrast with Cu(II), Fe(II) and Mn(II)).
6. Flexible coordination geometry (>Ni(II) or Mg(II)).
7. Polarises coordinated water to supply hard base, hydroxide (only Cu(II), Fe(III) and Mn(II) better).

1.1.4. Structural and catalytic proteins associated with Zn(II).

Zn(II) is the most common metal ion in the cytoplasm of cells after group IA and IIA ions. There are nearly 300 enzymes containing Zn(II) as an essential component, either for structural purposes or as part of a catalytic site.[9] Zn(II) involvement in structure ranges from filamentous (keratin) structures to the organisation of chromosomes.[23] The types of proteins that are associated with Zn(II) vary in size, form (α-helical or β-sheet), function and mobility.[26]

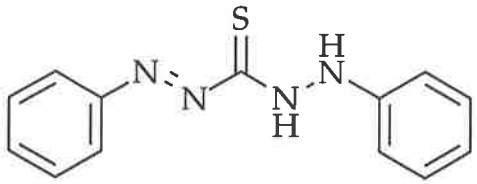
Zn(II) is a strong borderline hard Lewis acid and consequently can catalyse reactions.[22] The ability to polarise groups allows it to increase the attacking power of the bound group or increase the probability of attack on the bound group. An example of this is carbonic anhydrase (which catalyses a variety of hydration and hydrolysis reactions) where the Zn(II) acts firstly as a template to bring the two reactants together and then as a Lewis acid activating the reactants, in this case water to form hydroxide.

It is clear that intracellular Zn(II) has major roles in both structure and catalysis. The role of 90% of Zn(II) associated with structural and catalytic enzymes is known, but it is the role of the other 10% of Zn(II) which is also of major interest since it has implications in cell activation and growth. These include gene expression[27,28] neurotransmission[29,30] signal transduction[31] enzyme regulation[32,33] and apoptosis (programmed cell death).[34,35]

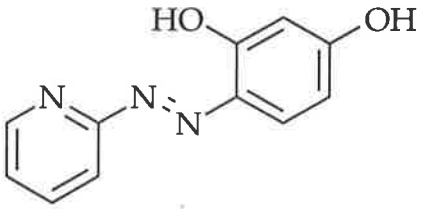
1.1.5. Current methods in Zn(II) measurements.

Previous determinations of total Zn(II) in the human body have been accomplished by using atomic absorption.[36] This method generally gives accurate measurements of total Zn(II)[26] in the body but does not differentiate between the different types of Zn(II). These determinations are generally on plasma so the distribution of the Zn(II) within the individual cells and organelles is not established.

Radioactive and stable isotopes of Zn(II) have also been utilised to measure Zn(II) in the human body. This method involves introducing the radioactive isotope into the diet of the subject and then monitoring where the Zn(II) is absorbed. ^{65}Zn(II) has been the isotope of choice due to its relatively long radioactive half-life (245 days) but ^{70}Zn(II), ^{68}Zn(II) and ^{64}Zn(II) have also been used.[37-39] Colourimetric techniques[3] have also been established. The chromophore diphenylthiocarbizone (Figure 1.1) has been employed but this method results in the destruction of the cells under investigation. 4-(2-pyridylazo)-resorcinol (Figure 1.1) has been successfully used to determine the blood serum level of Zn(II).[40]



Diphenylthiocarbizone

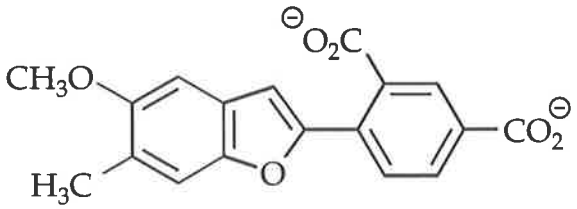


4-(2-Pyridylazo)-resorcinol (PAR)

Figure 1.1. The structures of two colourimetric probes used to detect Zn(II).

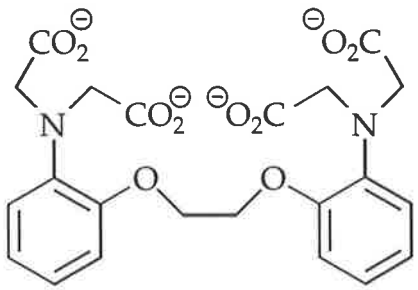
In all of these methods, the distribution of bound and available Zn(II) within the cell cannot be established. Therefore, development efficient detection system for the determination Zn(II) localisation *in vivo* and total Zn(II) concentration would be advantageous. Current methods have centred on ligand chelates especially of fluorescent chelates of sodium,[41] magnesium,[42,43] and calcium[44-46] ligands already in use.[47] Some of these chelates are outlined below in Figure 1.2.

Na(I)

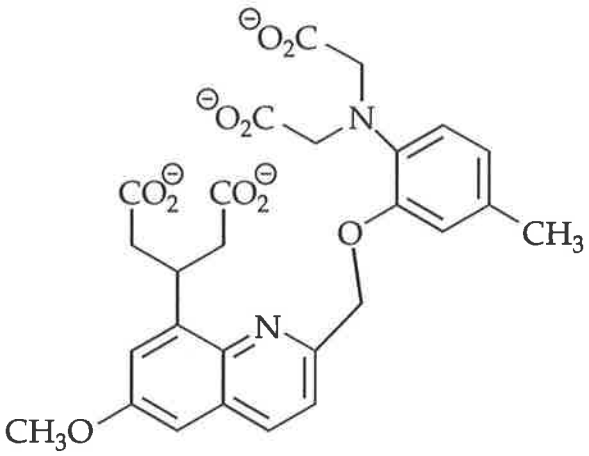


SBFI[41]

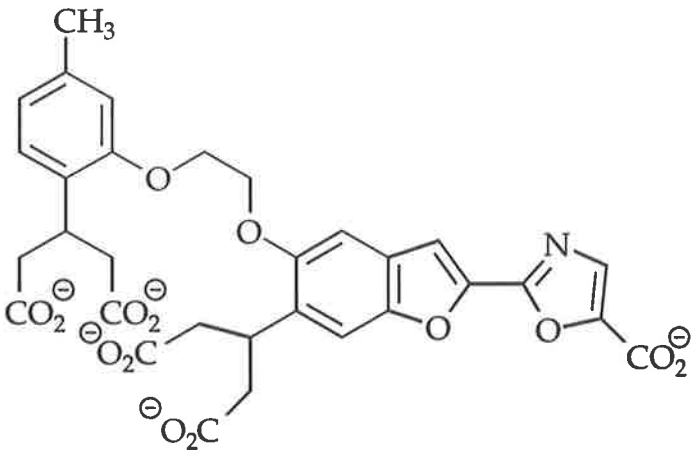
Ca(II)



BAPTA[44]

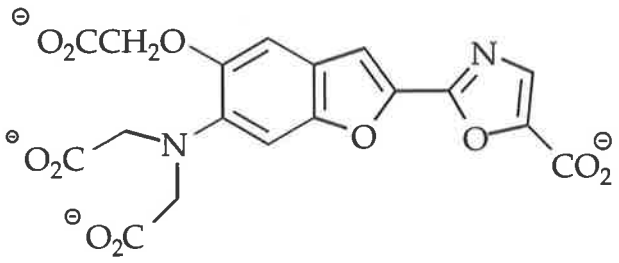


QUIN-2[42]



FURA-2[45]

Mg(II)



FURAPTRA[43]

Figure 1.2. Structures of fluorescent indicators of ion concentration. Each is drawn as if ready to bind to its target ion. The carboxylates were present as methyl esters before cleavage by cell esterases.

These ligands allow measurement of the ion activities or free concentrations *in vivo*. The added advantage of using fluorescent probes of the type used for Na(I), Ca(II) and Mg(II) is that they can be used at all levels of organisation of the cell.

1.2. Ultra-violet/visible and fluorescence spectrophotometry.

1.2.1 The nature of ultra-violet/visible and fluorescence spectra.[48-51]

(a) Absorption.

Energy absorbed by molecules from external sources such as heat, light, collisional energy, can be dissipated or emitted in a number of different ways. Examples are the breakdown of the molecule due to strain, chemical reactions due to collisions between molecules and the emission of the energy as a photon of light. The energy of a photon is quantised and this energy is given by:

$$E = h\upsilon = hc/\lambda$$

Where : h = planck's constant.

υ = frequency of the vibration of light.

c = velocity of light in a vacuum.

λ = wavelength of light.

Outer valence electrons, those involved with chemical bonding, exist only at discrete energy levels ranging from the ground state (termed S_o) to highly excited states. When a photon ($h\upsilon$) is absorbed by a molecule a valence electron is excited into a higher energy level or orbit forming an excited state, see Figure 1.3. It follows that the absorption of a photon of light by a molecule can only occur when the energy of that photon is the same as the difference between the ground electronic state (S_o) and the excited state of that valence electron. This absorption of light by molecules gives rise to characteristic excitation or absorption spectra which are measured quantitatively and qualitatively by uv/visible spectrophotometry.

The absorption spectrum of a molecule is defined by the number of energy levels accessible for the electronic state of that molecule. As a consequence, light absorption occurs only at discrete wavelengths (termed lines) of equivalent light energy equal to the separation between the quantised electronic states of the molecule. Superimposed on this absorption spectrum is a fine structure arising from vibrational and rotational molecular motion. This results in the discrete energy levels of the molecule, including the ground state (S_o), being further subdivided into sub-states of vibrational and rotational energy. As a result the number of possible energy levels is increased and this causes a broadening of the absorption spectrum as compared to a single atom. When the valence electron returns to its original lower energy orbit (usually S_o) energy is liberated. There are several pathways which the excited state electron may take to return to the ground state three of which result in the emission of light

(b) Emission.

There are three ways in which a molecule can release energy in the form of light; fluorescence, delayed fluorescence and phosphorescence. Differences in each are due to the length of time that excited valence electron(s) remain in their excited states. There are two distinct excited states, the singlet excited state, termed S_n, and the triplet state termed T_n. Fluorescence occurs when the excited electron returns to the ground state (S_o) from its singlet state, liberating the resulting energy as light ($h\upsilon$). Other types of light emission; delayed fluorescence and phosphorescence result from the excited valence electron undergoing an intersystem crossing. These types of light emission concern the electron entering a triplet state (T_n) by intersystem crossing of the excited electron from the singlet state (S_n). A triplet state occurs when the singlet excited electron has a dipole

transition. When this transfer occurs the excited electron moves from its singlet state to a triplet state of equal or less energy. As with singlet states triplet states also contain sub-states of vibrational and rotational energies.

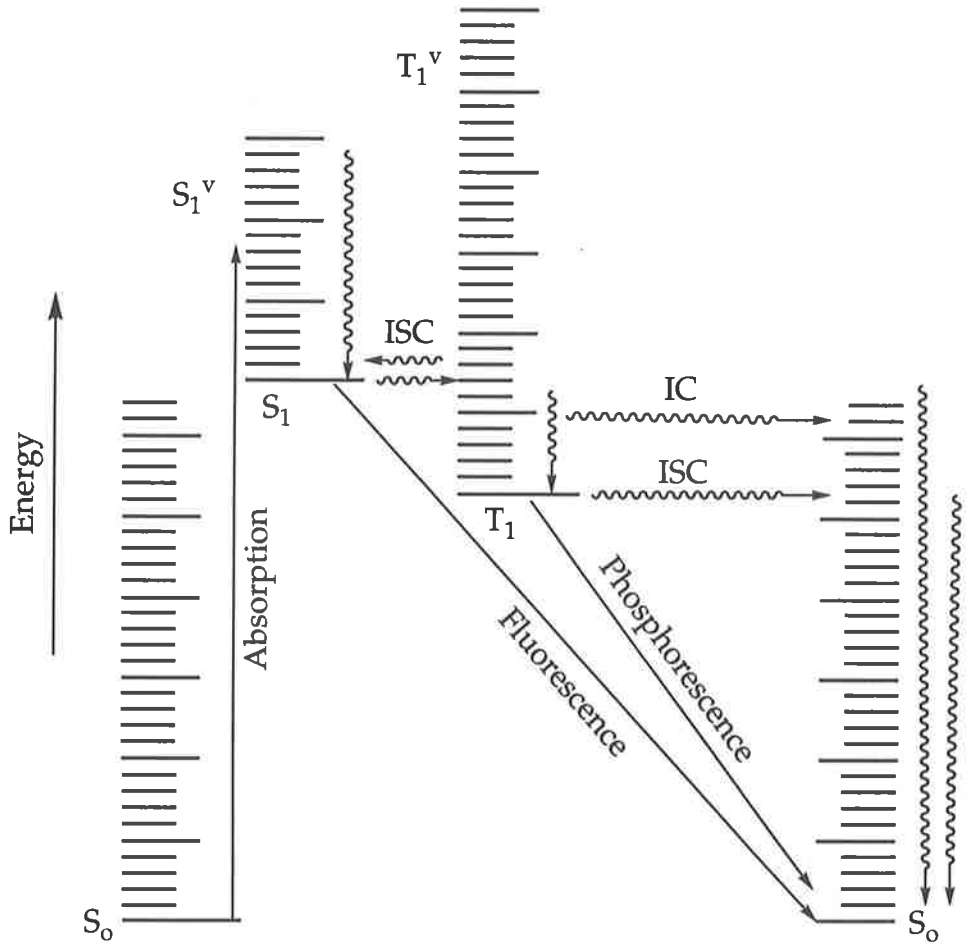


Figure 1.3. A schematic diagram showing the routes of fluorescence and phosphorescence. ISC is "intersystem crossing".

It is the lifetime of the excited state electron which differentiates fluorescence from delayed fluorescence and phosphorescence. Fluorescence radiation life-times are usually of the order of 10^{-9} seconds where the radiation life-time of delayed fluorescence transitions is of the order of 10^{-6} seconds. Phosphorescence mirrors delayed fluorescence, but when the valence electrons are excited they cross to a triplet state of lower energy than the original singlet state it occupied, consequently the radiation life-time of

the phosphorescence transitions is longer relative to fluorescence, resulting in fluorescence life-times of up to several seconds.

This de-excitation through light emission gives rise to a corresponding emission spectrum of a molecule or atom. As with absorption spectra, emission spectra of molecules are broader than emission spectra of atoms for the same reasons of vibration and rotation of nuclei around the centre of mass of the molecule. Apart from the emission of light, molecules can dissipate energy or de-excitate in a number of other ways. The energy can be dissipated by loss of energy to the medium, which can lead to chemical reactions such as the light induced reactions in photosynthesis. Additionally some fluorescent molecules can also be destroyed in the excitation process by reacting with molecular oxygen (O_2), a process known as photo-bleaching.

1.2.2. Instrumentation used in fluorimetry.[49]

As with uv/visible spectrophotometry, fluorimetry is a relative measurement of fluorescence of a given sample and therefore comparisons between samples must be made under similar conditions. The basic fluorimeter consists of a radiation source which is focused by a number of lenses where it enters an excitation wavelength sector. This isolates a desired monochromatic or narrow-band of radiation which can then be further focused toward the sample. Light emitted from the sample then enters the emission wavelength sector, which is at 90° to the radiation source, see Figure 1.4. This prevents interference of the light from the sample with light from the source. The emission sector plays a similar role to the excitation wavelength sector. The radiation hits the detector which relays the emission information to a variety of recording devices. The

amount of excitation and emission can be controlled by the slit width, which controls the amount of light hitting the detector.

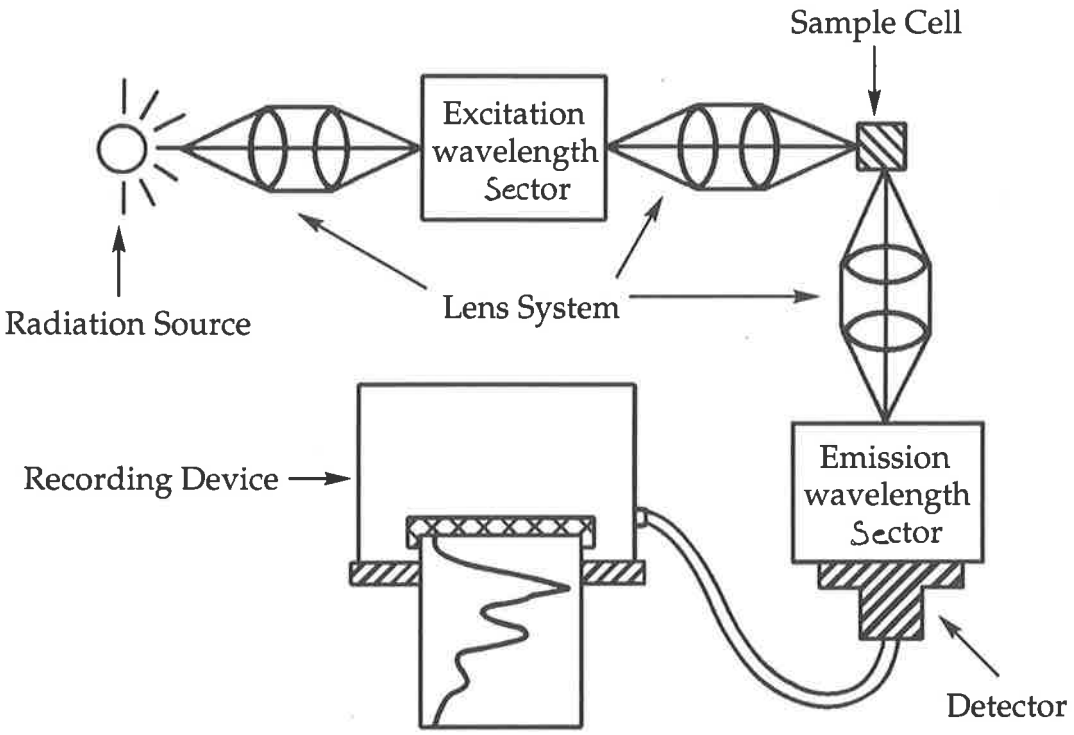


Figure 1.4. The major components of a fluorescence spectrophotometer.[51]

1.2.3. Mathematical factors involved with fluorescence.

There are three fundamental parameters that control fluorescence; the extinction coefficient, quantum yield and the fluorescence life-time.

A measure of the probability of absorption is termed the extinction or absorbance coefficient, ε. The larger the extinction coefficient the higher the probability of absorption of light by a molecule. It follows that, if there is a high probability of absorption of light exhibited by a molecule then there is a corresponding high probability of emission from that molecule. Quantum yield, ϕ, is a measure of the efficiency of fluorescence compared to the other pathways of de-excitation. Quantum yield can be best expressed as the

number of quanta emitted compared to the number of quanta absorbed. An ideal fluorescent molecule would have a quantum yield of 1, but in practice, good fluorescent compounds have quantum yields in the range of 0.1 to 1. Fluorescent life-time, τ, is the average time that a molecule remains in the excited state. As previously discussed, fluorescent molecules have life-times of the order of 10^{-9} seconds, but most useful fluorescent probes tend to have life-times of approximately 10^{-7} to 10^{-9} seconds.

These three factors can be expressed in a number of mathematical relationships;

$$\text{Optical density (OD)} = -\log I/I_o = \varepsilon cl$$

$$\therefore \varepsilon = OD/cl$$

where I is the light intensity after passing a distance l through the sample, I_o is the incident intensity, ε is the extinction coefficient (common units are $M^{-1}cm^{-1}$), and c is the concentration of the absorber. Since fluorescence intensity is also a function of the quantum yield, the product of the quantum yield and the extinction coefficient can give the fluorescence intensity of a probe;

$$I_{fluorescence} = I_o \phi \varepsilon cl$$

1.3. The development of a fluorescent ligand for Zn(II).

The development of a fluorophore for the detection of Zn(II) has the potential to increase the knowledge of the distribution of Zn(II) in the cell, in the same way that the fluorophores for Ca(II),[44-46] Mg(II)[43] and Na(I)[41] have done. The design of a good fluorescent detection agent for Zn(II) would rely on certain criteria; high specificity for the metal; fluorescence of the metal-ligand complex; the physiological status of the Zn(II).

1.3.1 High specificity for the metal.

The ligand should chelate specifically to Zn(II). Specificity of the ligand is dependant on the type of donor atoms, size of the metal ion, stability of the metal-ligand complex and the conditions employed for the complex formation. Zn(II) prefers to bind to *N*- and *S*-donors , while hard acids such as Ca(II) prefer to bind to *O*-donors. Therefore the ligand should contain donors appropriate for Zn(II).[23] The fluorophores shown in Figure 1.2, consist of either two or four donor atoms which bind to the metal ions for which they were designed for. These chelating ligands form a "bite" which is defined as the distance between the coordinated donor atoms and it is this "bite" which allows a degree of specificity for the binding of metal ions.[52] Therefore, potential ligands for Zn(II) would contain a bite-size appropriate to the size of the Zn(II) ion (0.65Å). In addition, specificity of a particular ligand is dependant upon the reaction medium and conditions,[53] so a potential ligand for Zn(II) must form stable complexes at physiological pH.

1.3.2. Fluorescence of the metal-ligand complex.

Ideally the ligand itself must be relatively non-fluorescent, yet form a highly fluorescent species in the presence of Zn(II). Alternatively the free ligand should have an emission wavelength sufficiently different to that of

the ligand-metal complex so that there is little overlap in emission wavelengths of the free ligand and ligand-Zn(II) complex.

The structural requirements of a fluorescent organic molecule are dependant on three main factors, (a) nature of the carbon skeleton (b) geometrical arrangement of the molecule and (c) type and position of any substituents;[54]

(a) Fluorescent organic compounds contain conjugated systems of double bonds, such as aromatic rings. This has a two fold effect, shifting the absorption and therefore the fluorescence wavelength towards the red end of the spectrum, since short absorption wavelengths favour predissociation rather than fluorescence[54], and enhancing the number and delocalisation of the π-electrons.[54]

(b) Planarity of the molecule is essential for fluorescence. When planarity of a system is disrupted through steric hindrance the delocalisation of the π-electrons is partially inhibited thereby lowering fluorescence.[54] Therefore it is preferable that when the metal chelates to the ligand, the ligand would become planar inducing fluorescence.

(c) Addition of substituents to conjugated systems can increase fluorescence through the introduction of electrons into an already electron rich system.[54] Electron donating substituents such as alkoxy, hydroxy and amino groups can increase fluorescence in conjugated systems compared to electron withdrawing groups such as nitro and acetamido groups.[54] Electron donating substituents can effectively extend conjugated systems by introducing π-electrons while electron withdrawing groups reduce the amount of electron density in the conjugated system therefore lowering fluorescence.[54]

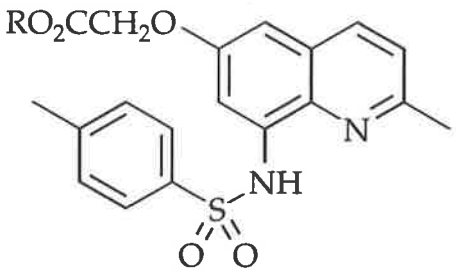
1.3.3. The physiological status of the Zn(II).

Any potential ligands should readily traverse the cell membrane, to form the fluorescent species with Zn(II) within the cell and not leak from the cell. The status of the Zn(II), and the type of Zn(II) the ligands bind to is of particular interest. Some 90% of Zn(II) is associated with metalloproteins either of a structural or catalytic nature. Our goal is the detection of the other 10% of the Zn(II) termed "available" Zn(II), since this 10% of the total Zn(II) content has particular physiological roles already discussed. What is needed is a ligand that selectively chelates to only the "available" Zn(II) without reacting with other 90% of the metalloprotein bound Zn(II). This is dependant on the stability constant of the ligand with Zn(II), if the stability constant is lower than that of the metalloprotein bound Zn(II) then the ligand will not remove this Zn(II) and theoretically bind only to the free "available" Zn(II).

1.4. Zinquin.

1.4.1. Introduction.

Ward and Lincoln have synthesised a Zn(II) ligand for use *in vivo* which meets the criteria set out in Section 1.3.[55-57] The ligand, called Zinquin (ethyl-(2-methyl-8-ρ-toluenesulfonamido-6-quinolyloxy) acetate) is based on the histochemical stain toluene sulphonamide quinoline (TSQ) **2**, (2)[58,59] . Quinoline based ligands, such as 8-hydroxyquinoline have been used for Zn(II) detection but only as histochemical stains and not *in vivo*. Zinquin differs from TSQ in two distinct ways, it contains a methyl substituent at the 2 position and an ester moiety at the 6 position on the quinoline ring.



R = Et **1a**, H **1b**

2

1.4.2. The fluorescent properties of Zinquin.

Zinquin ester (ZQE) and Zinquin acid (ZQA and its conjugate base ZQB) are all relatively non-fluorescent when not bound to Zn(II). Complexation studies employed both ZQE and ZQB forming highly fluorescent complexes with Zn(II), see Figure 1.5. The maximum excitation and emission wavelengths of these complexed species are observed at 364nm and 485nm respectively.[56,57] ZQE and ZQB also formed fluorescent species with Cd(II), but this fluorescence is weaker than the Zn(II) complex. However, Cd(II) is not a major trace metal ion in biological systems and it

should therefore not affect Zn(II) detection. Zn(II) fluorescence with ZQB has also been shown to be unaffected by either Ca(II) or Mg(II), both important biological divalent metal cations.[56] ZQB does bind to Co(II) and Cu(II) but the complexes formed are non-fluorescent because of quenching involving the unfilled d-orbital manifold.

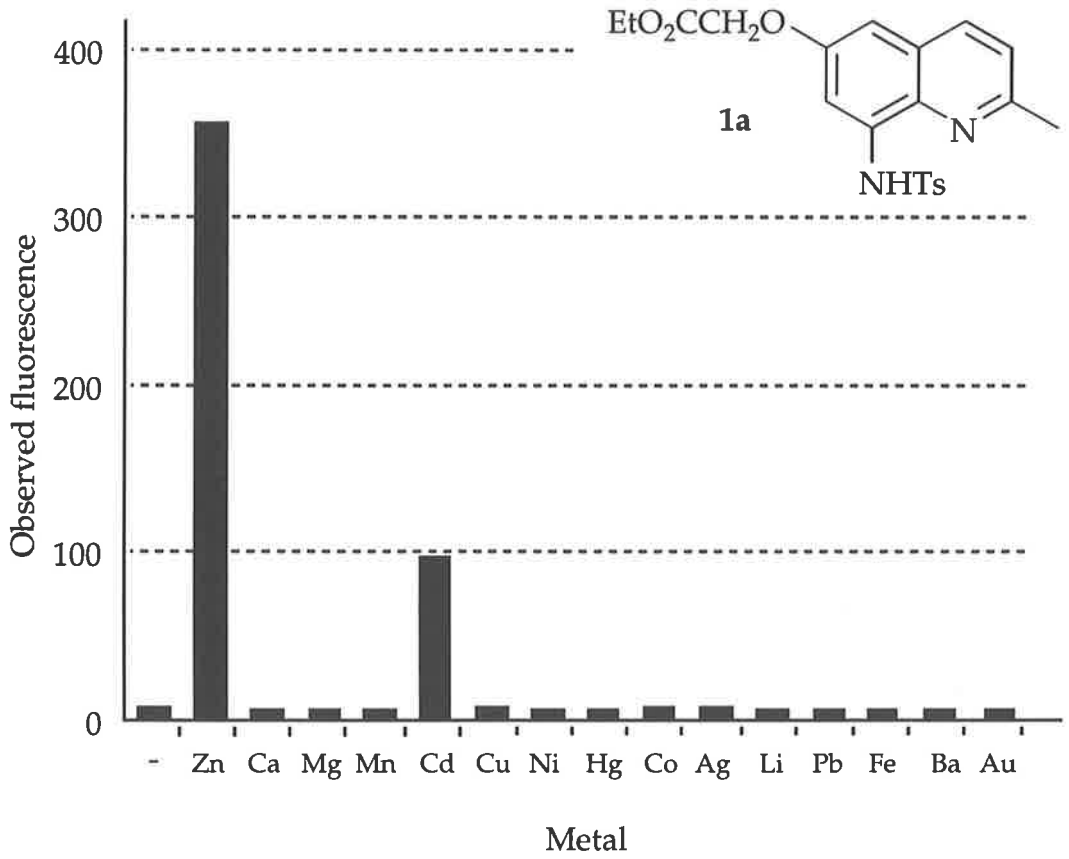


Figure 1.5. Specificity of ZQE for Zn(II).[57]

The distribution and function of the labile or "available" Zn(II) is one of the key aspects in cell growth and development. These include; gene expression[27,28], neurotransmission[29,30], signal transduction[31] and enzyme regulation as well as apoptosis (programmed cell death).[34,35] ZQB allows monitoring of the localised distribution and changes in Zn(II) flux because it can bind to the "available" Zn(II), fluoresce and remain inside the cell.

1.4.3. How Zinquin works *in vivo*.

The inclusion of a hydrolysable ester remote from the Zn(II) binding site on the Zinquin skeleton allows ZQB to remain inside the cell. The neutral ligand, ZQE, can pass into the cell through the hydrophobic bilipid membrane, where it is hydrolysed to ZQA by cell esterases. At the intracellular pH≈7 this forms a charged carboxylate anion, ZQB, which consequently cannot pass out of the cell through the bilipid cell membrane, see Figure 1.6. The charged ligand, ZQB, remains in the cell to bind to any "available" Zn(II). The rate of hydrolysis of the ester by the cell esterases is unknown but it appears from biological studies to be rapid.

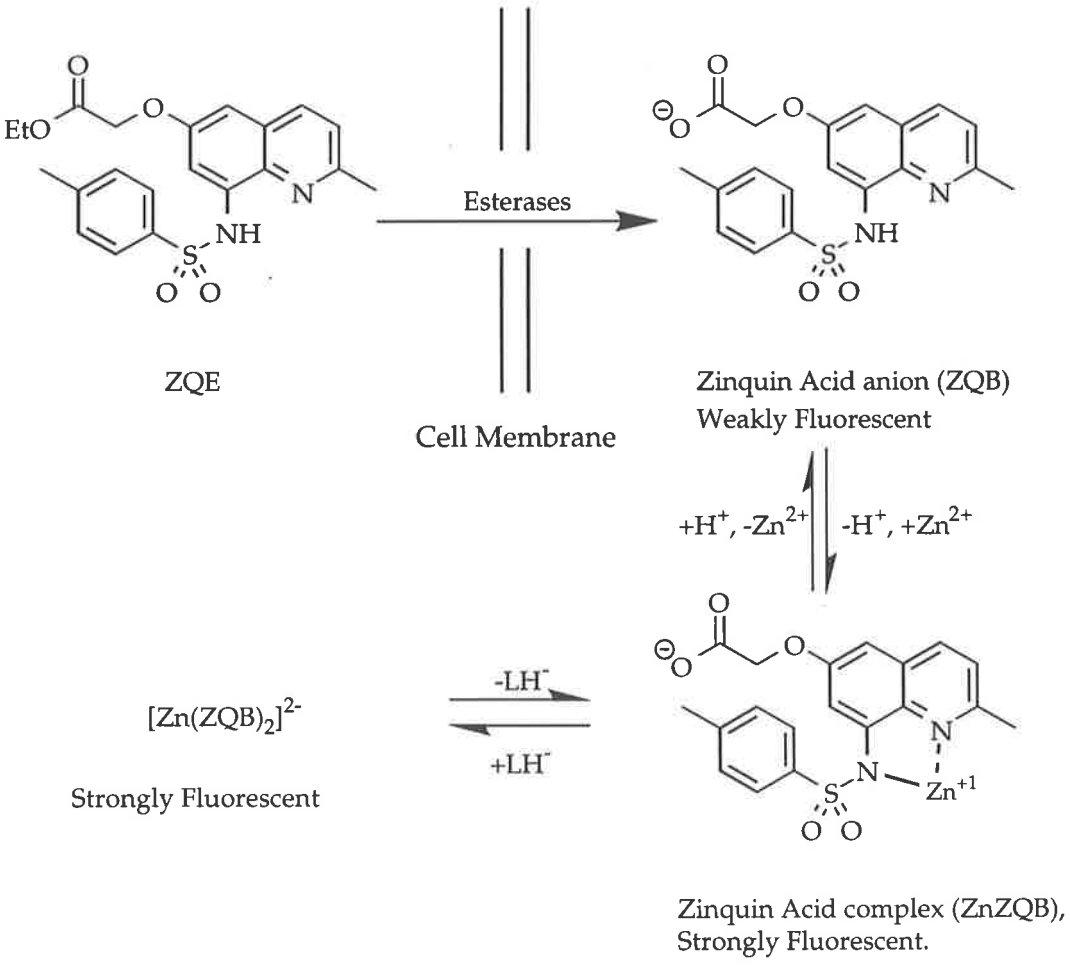


Figure 1.6. A representation of how Zinquin ester (ZQE) enters the cell, is hydrolysed by cell esterases forming a carboxylate anion (ZQB) which can not traverse the bilipid layer. This anion is then able bind to the "available" Zn(II) forming fluorescent species.

1.4.4. The stability of Zinquin with Zn(II).

ZQB forms two fluorescent species with Zn(II), namely a 1:1 and 2:1 Zn(II) complex[60], consequently it has two corresponding stability constants with Zn(II), 2.71 ± 0.41 x 10^6 dm^3 mol^{-1} and 11.7 ± 1.9 x 10^6 dm^3 mol^{-1} respectively.[56,57] Consequently, ZQB would not be expected to remove this type of Zn(II) from metalloenzyme or metalloprotein whose stability constants are in the order of 10^{12} - 10^{13} dm^3 mol^{-1}.[8]. The stability constants for Zn(II) binding to catalytic and regulatory proteins, such as those involved in cell development, are lower in magnitude. Therefore it is not known whether ZQB is removing this type of Zn(II) from its binding sites or is binding while the Zn(II) is still bound to these sites. ZQB does chelate to the "available" pool of Zn(II) as required but it may also be chelating and removing some of the less firmly held Zn(II) from enzyme sites.

1.4.5. Zinquin and its applications in the detection of Zn(II).

ZQE has been utilised in a number of Zn(II) studies of the biological role of Zn(II). Zalewski *et al*[56,61] have utilised ZQE in the determination of labile Zn(II) concentrations during phases of apoptosis (programmed cell death), see Figure 1.7. Apoptosis is a key event in cell homeostasis[56] and is distinctly different from necrosis or accidental cell death. Apoptosis involves the fragmentation of DNA, condensation of chromatin and loss of nucleoli by cellular processes as opposed to damage of the cell membrane resulting in cell lysis.[56] Studies of Zn(II) deficient animals and cells deprived of Zn(II) *in vitro*, have indicated that suppression of apoptosis is a physiological function of Zn(II).[35,34,62,63] Zalewski *et al*[56] showed through the use of fluorescence video imaging and ZQE that cells undergoing early events of apoptosis show an increase in ZQB detectable Zn(II). This increase was shown in the absence of exogenous Zn(II) and before changes in

membrane permeability, consistent with a release of Zn(II) from intracellular stores or metalloproteins.

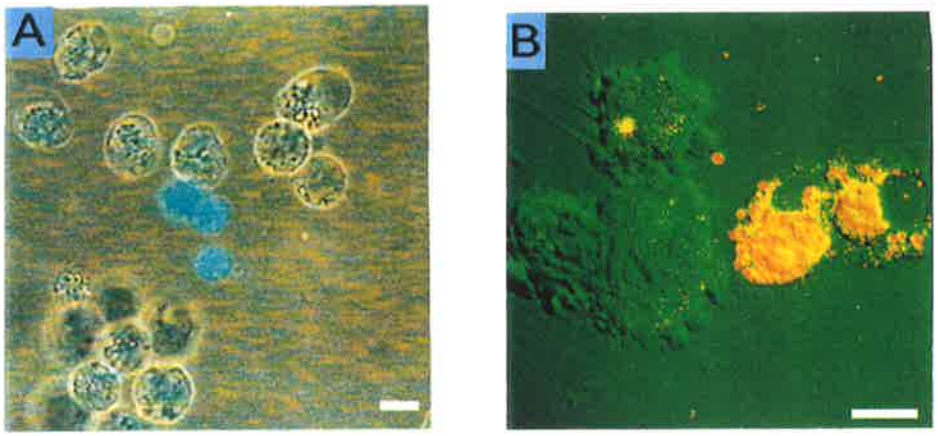


Figure 1.7. ZQE loaded apoptotic cells. Bar represents 10μm.[56]

A) ZQE loaded cells with UV fluorescence and transmitted light. Brightly fluorescent cells appear blue and have morphology of apoptotic cells.

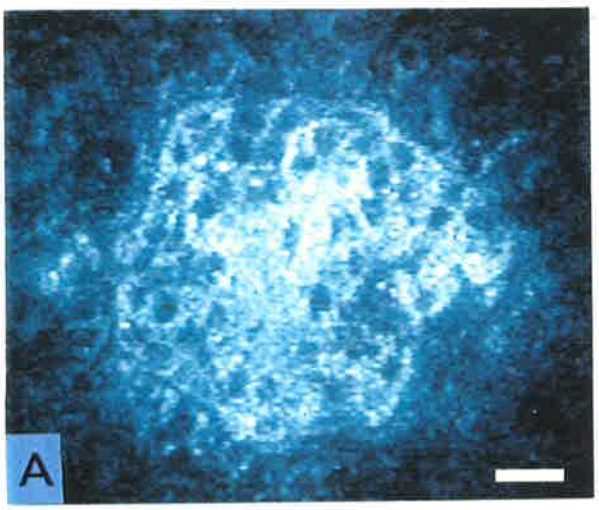
B) Confocal scanning UV-laser fluorescence microscopy of a group of three normal lymphoblastoid cells and two apoptotic brightly fluorescent cells. The figure is a pseudo-coloured superimposed image, green depicts morphology and orange-yellow shows ZQB fluorescence.

The studies described above showed that ZQB was chelating only to the available bound Zn(II) and not the tightly bound Zn(II). This available Zn(II) included both the loosely bound Zn(II) associated with regulatory enzymes and the Zn(II) associated with cellular proteins and lipids. As Figure 1.7 , indicates, ZQB did not show any Zn(II) related fluorescence in the nucleus. It is not known whether this was due to the inability of ZQB to traverse the nuclear membrane or that there is no loosely bound Zn(II) in the nucleus.[56]

The involvement of Zn(II) in insulin storage in pancreatic islet cells has been well documented.[64-70] Insulin is stored within islet cells in the

form of crystals containing hexamers of insulin with two or more Zn(II) atoms.[71] Zalewski *et al*[57] have shown, using ZQE and fluorescence video image analysis, that there is a labile pool of Zn(II) in pancreatic islet cells, which concentrates this metal for use in synthesis, storage and secretion of insulin, see Figure 1.8. Fluorescence was also seen within the granules indicating that ZQB competes with the insulin for Zn(II), since the granular Zn(II) is complexed with insulin as Zn(II)-insulin crystals. Fluorescence was not seen in the nucleus and this was attributed to the ZQB being unable to traverse the nuclear membrane due to its negative charge, a result of the hydrolysis of ZQE upon entry into the cell.

A) Frozen sections of human pancreas. Strongly fluorescent islet cells are surrounded by weakly fluorescent acinar cells.



B) Isolated marmoset islet cells showing typical intensely fluorescent islet.

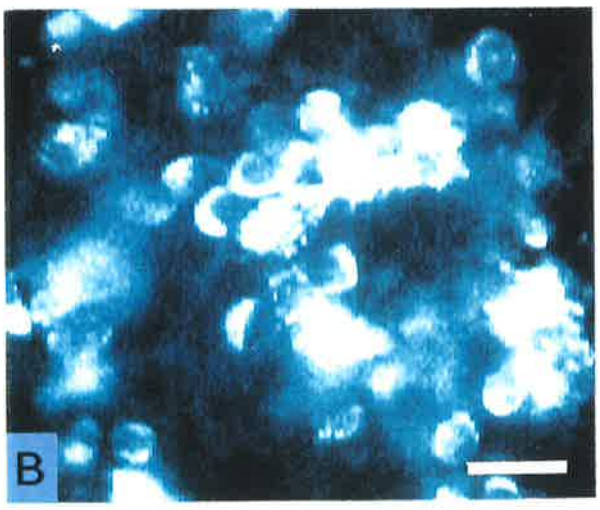


Figure 1.8. Video image analysis of Zn(II) in islets and separated islet cells. All specimens were labelled with 25μM ZQE for 30 minutes at 37°C, bar represents 20 μm.[57]

Investigations by a number of research groups have indicated that Zn(II) may be very important in the development of spermatozoa.[72,73] The effects of Zn(II) deficiency on the development and production of sperm has been well documented[72,74] as well as Zn(II) secretion in seminal fluids after ejaculation.[18,75] Introduction of ZQE into mouse spermatozoa[76] (see Figure 1.9) has shown that the regional distribution of the free or loosely bound Zn(II) is higher in the head of the spermatozoa than the tail in the early part of its development but this changes as the spermatozoa further develops. This distribution of labile Zn(II) and the high concentrations found, supported the view that this available Zn(II) is important in spermatogenesis.[77-80]

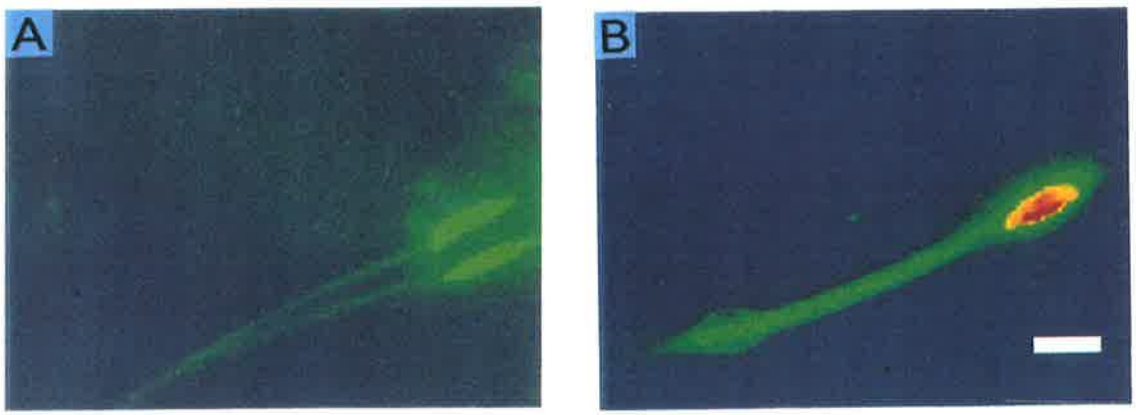


Figure 1.9. Variation of ZQB fluorescence of mouse spermatozoa from different regions of the male reproductive tract.[76] Pseudo coloured computer images of ZQB fluorescence of sperm from,

A) testes.

B) vasa differentia.

Other studies that have involved ZQE and the role of Zn(II) in the body have been in the relationship between intracellular Zn(II) and metallothionein (MT).[81] MT's role as a "metal scavenger" as well as being

the major protein associated with the intracellular pool of Zn(II) in the body, is well documented.[23,82] Coyle *et al*[81] used ZQE to examine the MT relationship between ZQB fluorescence and intracellular Zn(II) levels. The researchers found that ZQB removes Zn(II) from MT resulting in an increase in ZQB fluorescence and a corresponding decrease in Zn(II)-MT concentration. ZQB however did not remove the Zn(II) from higher molecular weight proteins. This result did support the view that Zn(II) bound to MT is part of the labile pool of Zn(II) in cells.[23]

1.5. The disadvantages of Zinquin; the aims of this thesis.

1.5.1. Electronic factors.

The alkoxy group attached to the ring of Zinquin (where "Zinquin" refers to general structure) is known to enhance fluorescence.[54] The position of the oxygen on Zinquin is at the 6-position but it is not known what effects the oxygen would have if it were at different position. Synthesis of structural isomers of Zinquin with the oxygen at differing positions would be of interest since it may lead to more highly fluorescent chelated ligands.

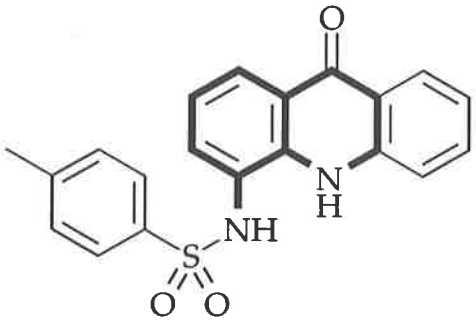
Sulfonamides are electron withdrawing, but this can be modified depending on the type of sulfonamide formed. In Zinquin the *p*-toluenesulphonamide is utilised but modification through the introduction of more or less electron withdrawing sulfonamides could lead to more highly fluorescent chelated ligands or an increase in specificity.

As already discussed, it is likely that ZQB binds to all the unbound Zn(II), the "available" Zn(II), but does not bind to the tightly bound Zn(II) in metalloenzymes. Problems arise in the definition of "available" Zn(II) in relation to the catalytic Zn(II) sites within the cell. In many cases the stability of Zn(II) in these sites is unknown and ZQB could be binding to this Zn(II) within these sites. The stability of Zn(II) in these sites could be obtained by the use of Zinquin type ligands with differing stability constants with Zn(II). An ideal way to do this would be to hinder the access of Zn(II) to the binding site of Zinquin and this can be achieved, in principle, by increasing the size of the groups attached at the 2-position. As will be discussed under the confocal laser microscope section, attachment of conjugated and nonconjugated systems could not only increase conjugation

of the ligand but could increase steric bulk in relation to the bite area of the ligand. This could produce a variety of ligands with different stability constants for Zn(II) and hence different binding powers.

1.5.2. Confocal laser microscopy (CLM).

The studies mentioned in section 1.4.5. were conducted in conjunction with ZQE and conventional microscopes. Conventional microscopy is limited because it observes fluorescence from a few layers of cells. The use of confocal laser microscopy would be of particular interest in the study of the distribution of the "available" Zn(II). The confocal laser microscopy (CLM) has the ability to focus on a planar field, that is, a 2-dimensional focal plane.[83] Problems arise though, because current commercially available CLM's are fitted with blue laser sources of 488nm wavelength. This blue light laser would seriously interfere with the emission wavelength (480nm) of the Zn(II) chelated ZQB species, via back scattering of the laser. Lower wavelength UV light source CLM's are not commercially available at this time. Consequently, it would be a distinct advantage if a Zn(II) specific fluorescent ligand could be designed which could not only be used with the commercially available blue light CLM but also be excited by the blue laser source of the CLM, that is, at 488nm. This would require that the ligand have an increase in both its excitation and emission wavelength as compared to ZQB, which could be achieved, in principle, by increasing the amount of conjugation in the ligand. Possible candidate ligands, which retain the Zinquin structural qualities, that is, the sulfonamide and quinoline nitrogens, are the acridone **3** and acridine **4** systems. Even though the ring nitrogens differ dramatically between **3** and **4** in terms of basicity both systems will be synthesised to observed the effects.



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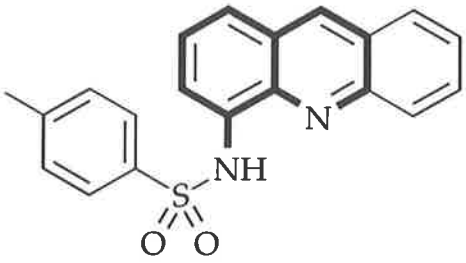

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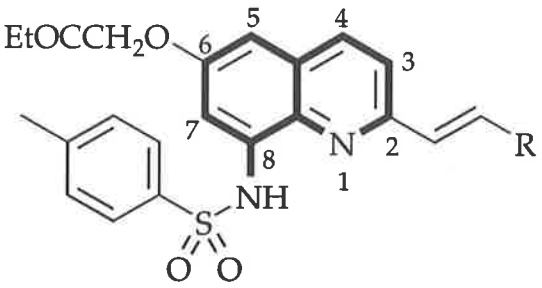
Other possible candidate ligands are conjugated ligands which incorporate the entire Zinquin skeleton but have π-donor systems at the two position **5**. Such systems could be where R is a styryl or napthyl moiety.



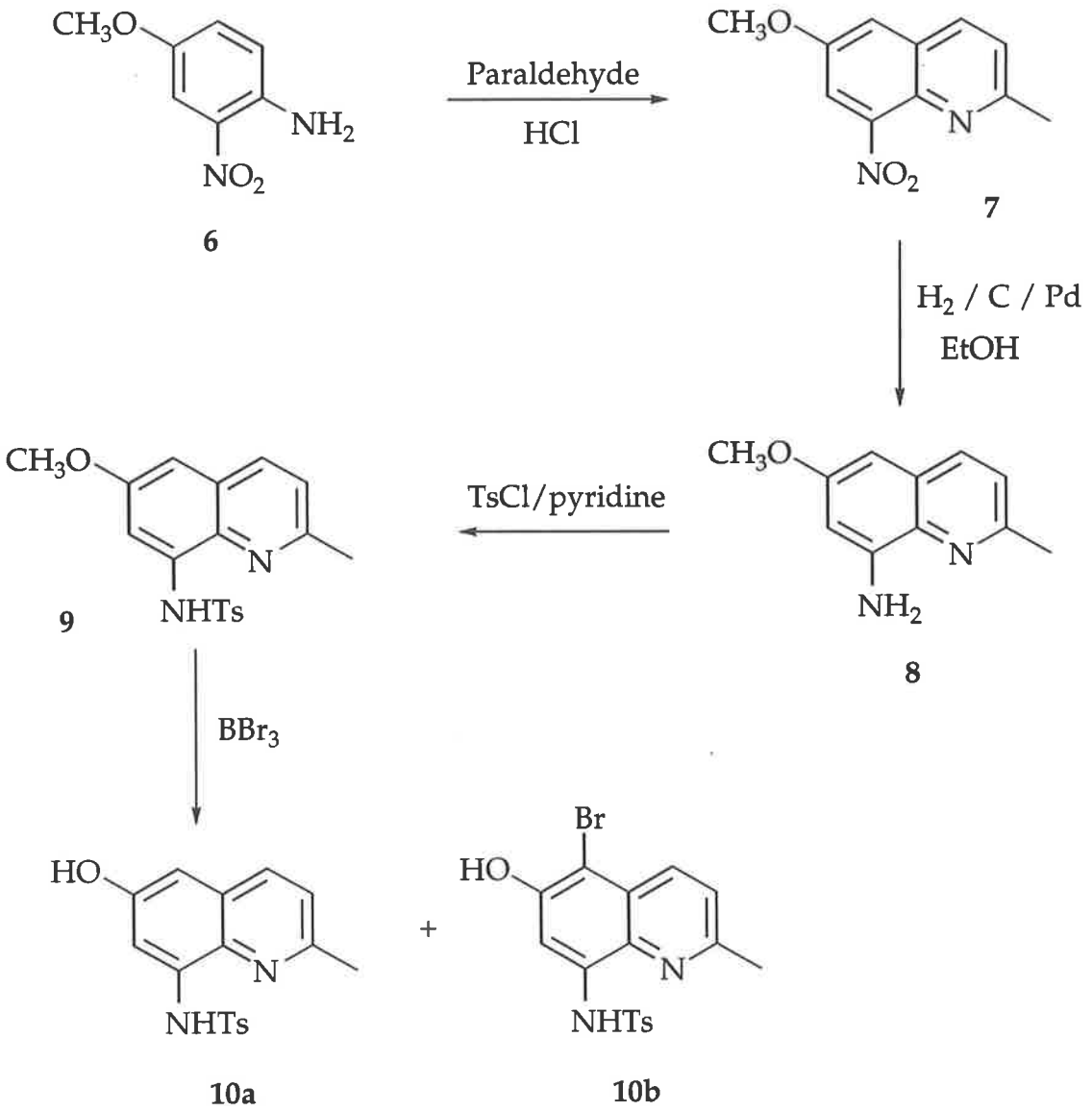
5

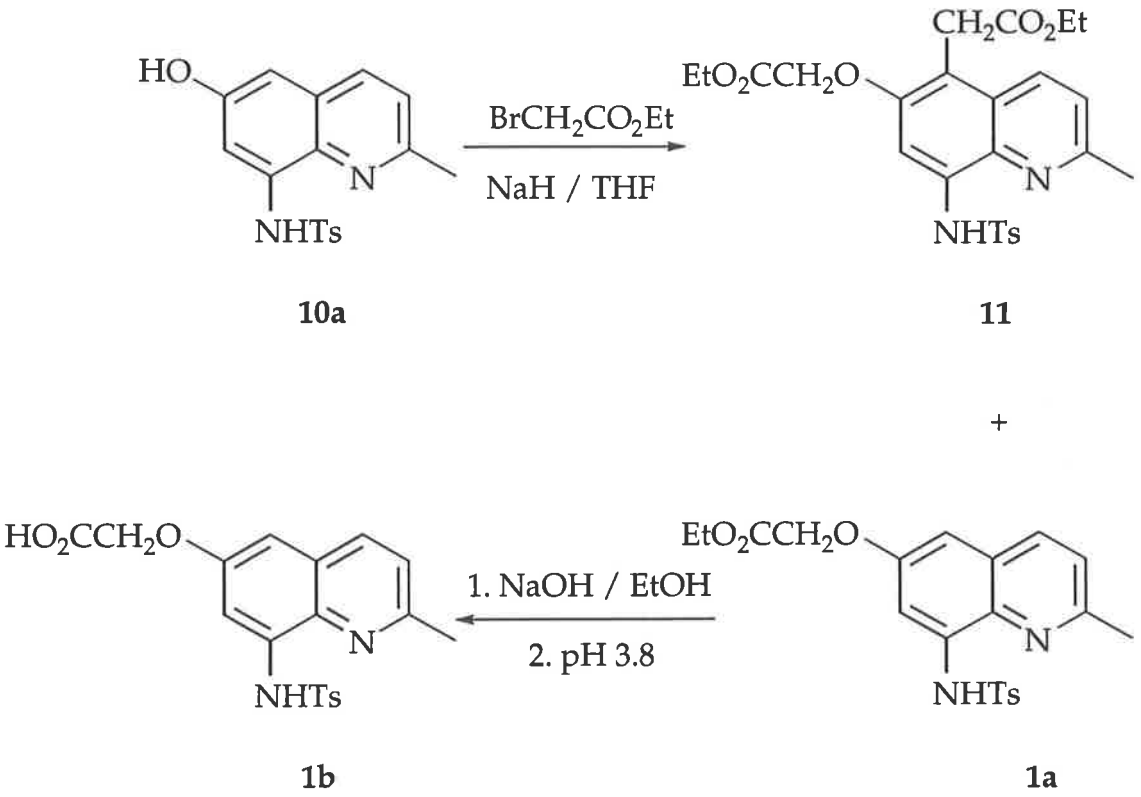
Such systems would still need a hydrolysable ester side chain so they could be used *in vivo*. Another consideration is the inherent fluorescence of such ligands since it is the Zn(II) complex which needs to be fluorescent not the unbound ligand.

Chapter 2 : The synthesis of Zinquin.

2.1. The synthesis of Zinquin, why improve it ?

The detection of available Zn(II) by Zinquin has been utilised by research groups around the world.[33,34,56,57,61,76] The supply of ZQE and ZQA has been achieved by the Adelaide group using the synthetic pathway shown in Scheme 2.1.[55]





Scheme 2.1.

A number of optimisations of the above synthetic pathway could be achieved thereby increasing the overall yield which currently stands at 4%. In particular, the steps involving the demethylation of **9** which generates the unwanted 5-bromo substituted quinoline **10b**, and the alkylation of **10a** which generates the unwanted dialkylated product **11** as well as the required *O*-alkylated product **1a**, could both be improved

Another aim in improving the Zinquin preparation is to be able to incorporate a radioactive label within ZQE, since this could increase the range of biological studies using Zinquin. The label should be incorporated as late as possible in the synthetic pathway in order to reduce exposure and the need to handle radioactive material. An obvious step in which to incorporate the label is the alkylation step. This has the added advantage that both labelled Zinquin ester and hence Zinquin acid could be prepared

through this step. In order to avoid any problems with tritium exchange a carbon 14 label of ethyl bromoacetate could be used to incorporate the label. Ethyl bromoacetate-2-^{14}C is relatively easy to handle and commercially available from Amersham. The use of C_2 labelled ethyl bromoacetate, rather than an ester label, also means that the label will remain intact upon entering the cell since it will be present before and after the hydrolysis of ZQE by cell esterases.

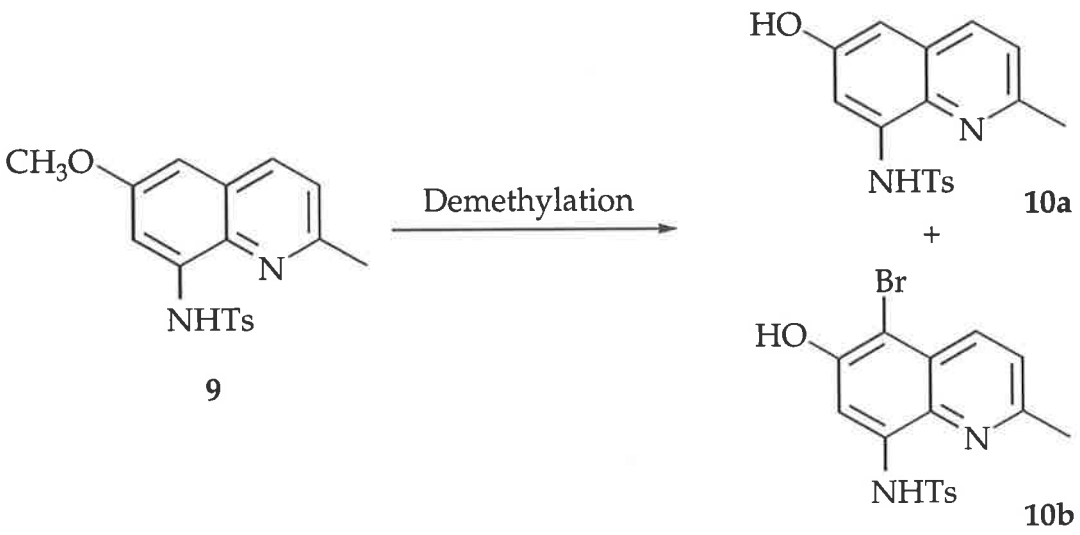
2.2. Incorporating the improvements in the Zinquin preparation.

Synthesis of ZQE begins with the condensation of 2-methoxy-4-nitroaniline **6** and paraldehyde as described by Mathur and Robinson[84] (Scheme 2.1). Their reported[84] yield of 50% was never matched and an average yield of 30-35% was attained and this low yield is attributed to difficulties in purification as well as the formation of other products. After work up of the reaction mixture by the literature method, purification consisted of fractionation by chromatography using dichloromethane, thus removing polymeric material, followed by extraction of the organic layer with 4M hydrochloric acid. The aqueous acid layer, containing the protonated quinoline, was then basified, extracted and the solid obtained upon removal of the solvent, crystallised from ethanol yielding yellow needle shaped crystals with melting point, 184-187°C, literature[84] 186-187°C. The aromatic protons in the ^{1}H n.m.r. spectrum could be readily assigned from chemical shift and coupling constant data. Both H_3 and H_4 showed an *ortho* coupling (8.5Hz) with H_4 having the higher δ value as expected.[85] H_5 and H_7 showed a coupling of 2.7Hz consistent with a *meta* coupling, with H_7 being further downfield compared to H_5 due to the electron withdrawing nature of the adjacent nitro substituent.

Reduction of the nitro group was achieved by two methods, catalytic hydrogenation and iron/acetic acid reduction.[86] Catalytic hydrogenation of **7** consistently produced yields of 75-80% where as the iron/acetic acid method[86] generated the amine **8** in near quantitative yields. The amine[87] **8**, purified by chromatography using dichloromethane, showed the expected primary amine hydrogen signals in the ^1H n.m.r. spectrum and the infra red showed absorbances of 3450, 3375 and 3325cm^{-1}, indicative of a primary amine. As expected the ^1H n.m.r. resonances of H_7 (δ6.53 ppm) and H_5 (δ6.42 ppm) of the amine **8** had shifted upfield compared to those of the nitro compound **7**.

Tosylation of **8** was achieved by standard procedures. The tosyl protons of **8** resonated at δ7.85 ppm and δ7.20 ppm and the mass spectrum showed a molecular ion at 342 and a fragmentation peak at 187, corresponding to an M-155 ($C_7H_7SO_2$) fragmentation.

Mahadevan *et al*[55] achieved the demethylation of **9** using excess boron tribromide in a dry atmosphere and obtained a mixture of the required phenol **10a** and a brominated product **10b** (Scheme 2.2).



Scheme 2.2.

The brominated product **10b** is likely to arise from electrophilic substitution by bromine at the 5-position, presumably a consequence of the generation of bromine from boron tribromide.[55] A number of different procedures were investigated to see if the demethylation of **9** could be improved and the results are summarised in Table 2.1.

Table 2.1. Results obtained from the demethylation reaction of **9**.[a]

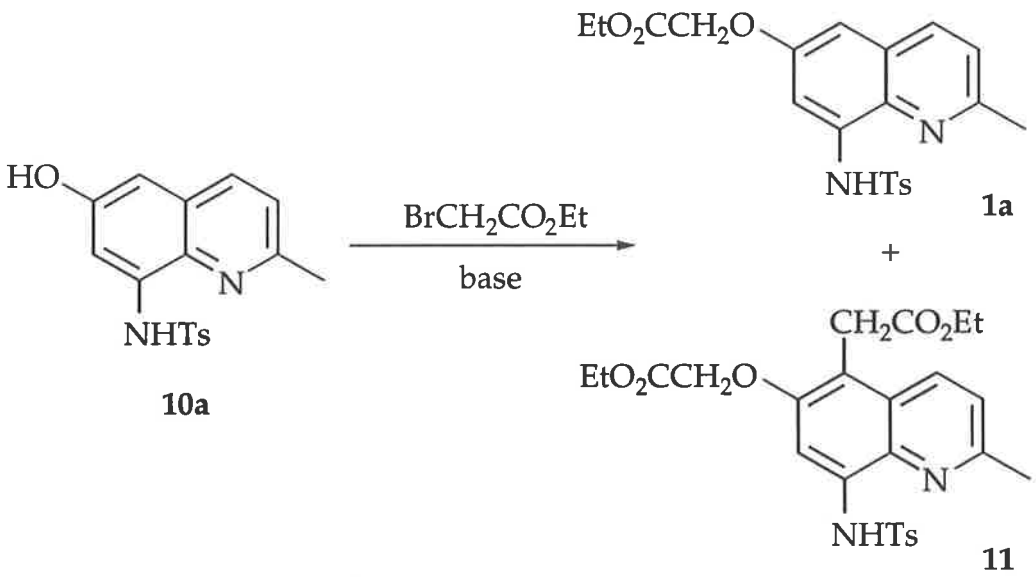
Reagent	Equivalents	Stirring	Yields		
			10a	**10b**	**9**
BBr_3[55]	8	o/n @ 25°C	4%	4%	90%
BBr_3	1	o/n @ 25°C	-	-	90%
BBr_3 / N_2	2	o/n @ 25°C	42%	>5%	50%
BBr_3 / N_2	2.5	4h Δ o/n @ 25°C	88%	-	-
BBr_3 / N_2	4	o/n @ 25°C	50%	>2%	40%
HI / P_{red}[88]	-	o/n @ 25°C	-	-	90%
HBr / NaI[89]	-	2h @ 95°C	40%	-	60%

a) The solvent used in each case was dry dichloromethane.

Two other methods apart from the boron tribromide reagent were investigated. The hydroiodic acid method[88] was unsuccessful but the hydrobromic acid procedure[89] did form some of the required phenol **10a**. Formation of the phenol was confirmed by both ^1H n.m.r. and ^{13}C n.m.r. spectra as well as by mass spectroscopy. The phenol **10a** did not show the characteristic methoxy peak seen at δ3.86 ppm in the ^1H n.m.r. spectrum and δ57.5ppm in the ^{13}C n.m.r. spectrum. In summary, the best method for the demethylation reaction in forming **10a** was to treat **9** with a 2.5 fold excess of boron tribromide. In addition, stirring for a further 12 hour at room

temperature after the initial 4h reflux period increases the yield of **10a** relative to the unwanted bromo product **10b**.

Alkylation of the phenol **10a** was accomplished using ethyl bromoacetate and a suitable base. Initially[55], sodium hydride in THF was used as the base with addition of ethyl bromoacetate after 30 minutes resulting in yields averaging 50% of Zinquin ester **1a** with the major by-product being the dialkylated product **11**, a consequence of *C*-alkylation (Scheme 2.3).

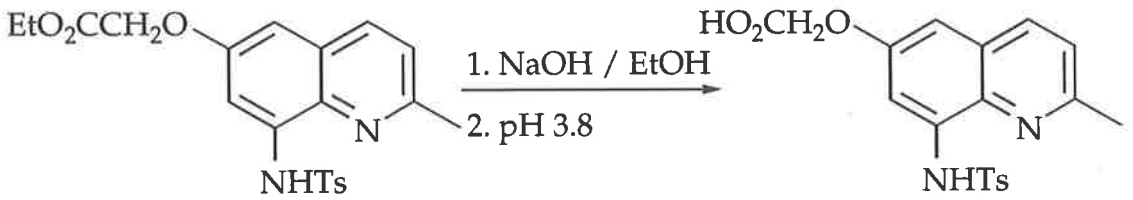


Scheme 2.3.

The dialkylated product **11** lacked the H_5 signal in the ^1H n.m.r. spectrum normally seen at δ6.64 ppm in ZQE with H_7 now appearing as a singlet. The attachment of the ester side chain to the phenol **10a** was confirmed[55] by ^1H n.m.r. with the signals for the ester occurring at δ4.30ppm and δ1.31ppm as well as a methylene signal at δ4.70ppm with the infra red also showing the presence of the ester carbonyl at 1745cm^{-1}. Changing the solvent to DMF for the alkylation did reduce the amount of *C*-alkylation but the overall yield of Zinquin ester still remained at 50%. Anhydrous

potassium carbonate was then investigated as the base with acetone as the solvent in an attempt to increase the amount of *O*-alkylation, since potassium is a softer solvation cation relative to sodium.[90] Using this method average yields of 65-70% were obtained for Zinquin ester with only small amounts of the dialkylated product being detected. The *O*-alkylated product **1a** was separated from the dialkylated product, **11** via flash chromatography. It was determined from these studies that the most viable approach to the alkylation of **10a** was to use potassium carbonate as the base and acetone as the solvent since this combination minimises that amount of *C*-alkylation.

The final step in the synthesis is the hydrolysis of ester **1a** to the free acid **1b**. This was achieved[55] by refluxing ZQE **1a** in 5% sodium hydroxide and ethanol for 3.5h followed by acidification and this afforded the required ZQA **1b** in average yields of 75% (Scheme 2.4). The formation of the acid was indicated by the disappearance of the ester signals in the ^{1}H n.m.r. spectrum and the appearance of a broad singlet at δ9.25 ppm for the carboxyl proton.

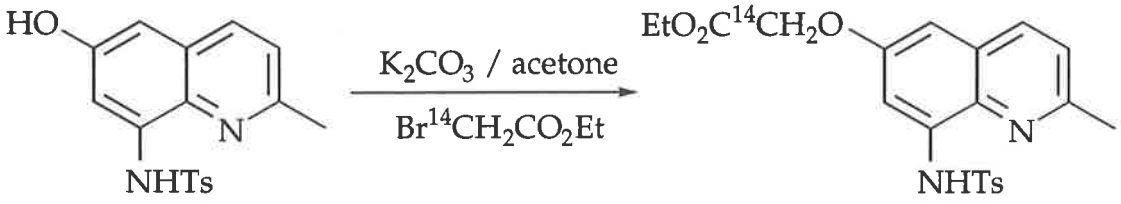


1a **1b**

Scheme 2.4.

Applying these modified procedures for the synthesis of ZQE and ZQA has enabled the overall yield of ZQE to be increased from 4% to 16%. In addition, the optimisation of the alkylation of **10a** can now allow the formation of labelled Zinquin ester with relative ease (Scheme 2.5). Ethyl

bromoacetate is replaced by commercially available ethyl bromoacetate-2-^{14}C (available from Amersham), utilising the potassium carbonate procedure. Recrystallization from dichloromethane/hexane would then afford the pure labelled ZQE.



10a **12**

Scheme 2.5.

2.3. The appearance of adventitious Zn(II) in ZQE and ZQA.

The improvements in the yield of Zinquin ester and acid provided material for use in potentiometric and fluorescence studies which require large amounts of the ligand. The fluorescence of the prepared ZQA solutions was higher than expected[57] suggesting that the ligand had reacted with some Zn(II) either during the synthesis or from the buffer used in the fluorescence studies. Stirring the ZQA solutions with EDTA, a known metal chelator, did reduce this relative fluorescence from 40 to <10 indicating that the higher than expected fluorescence values were due to adventitious Zn(II). The more likely source of the adventitious Zn(II) is the analytical grade ethanol which contains 10^{-4}M Zn(II); this ethanol is used in both the final step hydrolysis of ZQE and in the preparation of the buffer solution. The buffer solution contains a 100mM solution of sodium perchlorate, 1mM of disodium PIPES in ethanol/water (75:25 v/v). Both the disodium PIPES and the sodium perchlorate were eliminated as potential sources of this adventitious Zn(II) since this underlying fluorescence was seen in solution with and without these salts. However, this adventitious

Zn(II) is eliminated from ZQA fluorescence spectra by distilling the analytical grade ethanol.

Chapter 3 : Changing the sulfonamide unit.

3.1. The factors influencing the fluorescence in the Zinquin-Zn(II) complex.

The complexation of Zinquin to Zn(II) can be attributed to the sulfonamide and quinoline nitrogens, N(1) and N(8) respectively (in this section Zinquin is used in a generic sense).[55] Specifically, a formal bond between to N(8) and Zn(II) is formed by the deprotonation of the sulfonamide and a donor bond is formed between the Zn(II) and quinoline nitrogen, N(1), and this has been supported by X-ray studies of a Zinquin-Cu(II) complex (Figure 3.1).[91]

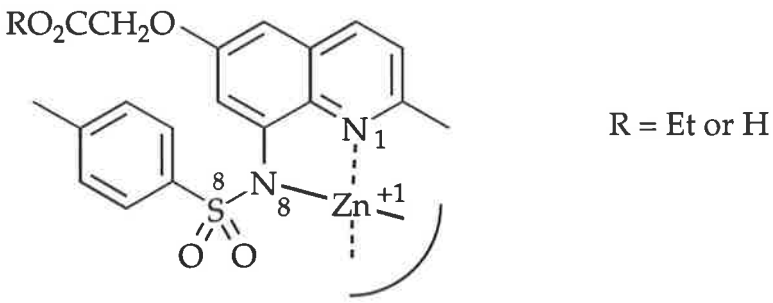
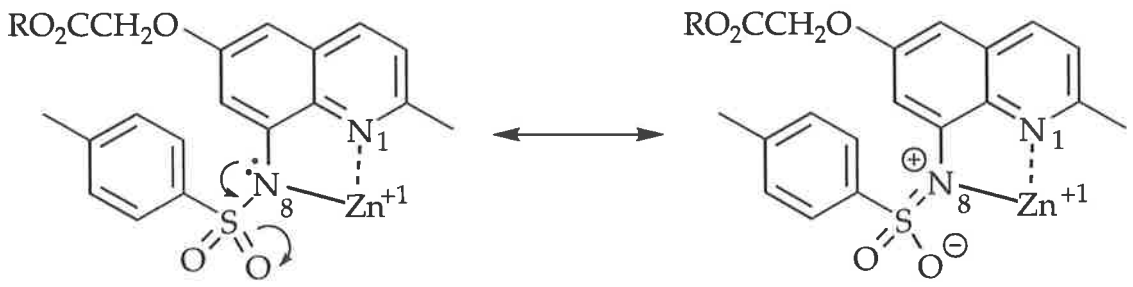


Figure 3.1. Representation of the binding of Zn(II) to Zinquin via N(1) and N(8).

The fluorescent properties of the Zinquin-Zn(II) class of complexes are thought to be due to the chelation of Zn(II) by N(1) and N(8) which can stiffen the structure of the quinoline ring therefore reducing the quenching of fluorescence by vibrational modes. The fluorescent properties are also thought be a result of the sulfonamide moiety, particularly the double bond character between the S(8) and N(8). The sulfonamide nitrogen, N(8), of the unbound Zinquin shows a distinct tetrahedral character, indicated by X-ray analysis,[92] however, the introduction of a metal cation such as Cu(II) forces N(8) into a trigonal arrangement, also indicated by X-ray analysis.[91] It is

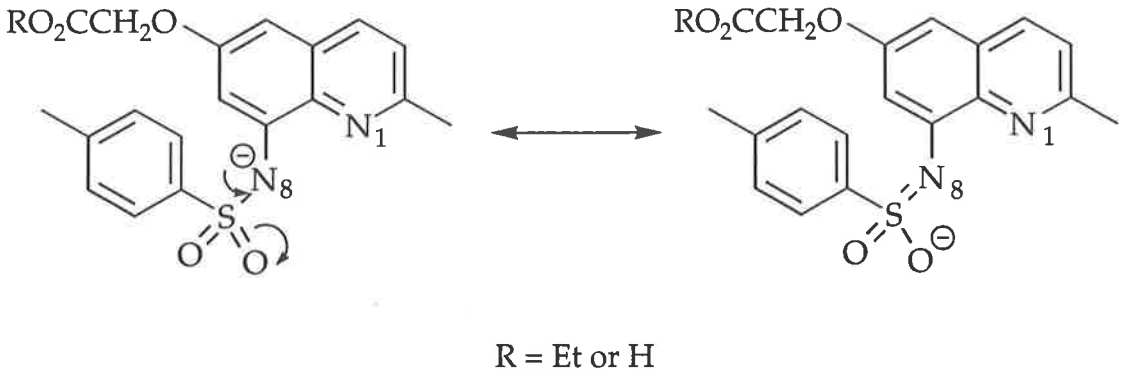
unclear why N(8) of the free sulfonamide is tetrahedral and why it is not in conjugation with the quinoline ring.

When N(8) is deprotonated in strong alkali conditions the unbound Zinquin shows fluorescence and a bathochromic shift similar to that of the complexed species, consistent with N(8) being trigonal planar. This results from deprotonation of the sulfonamide and therefore a change from sp^3 to sp^2 of N(8). This increases the "double bond" character between the N(8) and S(8) of the sulfonamide, therefore increasing conjugation and restricting the rotation of the sulfonamide relative to the quinoline, see Figure 3.2. The restriction of rotation, about N(8)-S(8), also increases the planarity of the system which is an essential element in the fluorescence of complex organic systems.[54] In contrasting, the aromatic unit attached to the sulfonamide has been shown, via an X-ray analysis of a Zinquin-Cu(II) complex, to be out of the plane of the quinoline.[91] This suggests that it is mainly the N(8)-S(8) bond which becomes conformationally constrained when complexed to Zn(II). The relative importance of the general stiffening of the Zinquin structure and the change to sp^2 hybridisation at N(8) to the increased fluorescence of Zinquin upon coordination to Zn(II) cannot be determined from present data.



R = Et or H

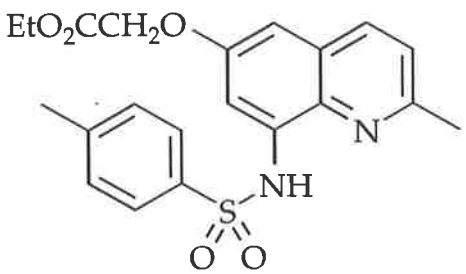
Figure 3.2(a). Zinquin upon complexing to Zn(II). The double bond nature between S(8) and N(8) results in increased planarity and conjugation in the ligand complex.



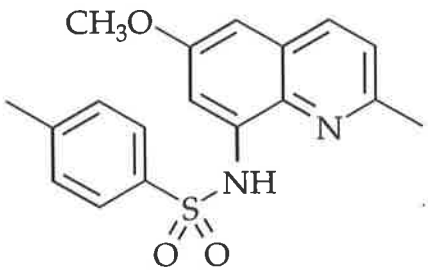
R = Et or H

Figure 3.2(b). Zinquin in basic conditions. Alkali conditions result in deprotonation of the sulfonamide and an increase in double character between S(8) and N(8).

Sulfonamides are electron withdrawing, but the extent of electron withdrawal can be modified by the type of substituent on the sulfonamide unit.[93] While Zinquin incorporates a toluene sulfonamide unit which results in excellent fluorescence when bound to Zn(II),[55,60] variation of this unit could increase the fluorescence and therefore the sensitivity of the ligand. A large range of different sulfonamides can be synthesised since the key step in their formation is the reaction of the quinaldine amine **8** with a sulfonyl chloride. Since the alkoxy group at the 6-position on **1a** is not crucial to fluorescence in the Zinquin-Zn(II) complex, it was decided that only the precursor 6-methoxy analogues of **1a** would be isolated and tested **9**.



1a



9

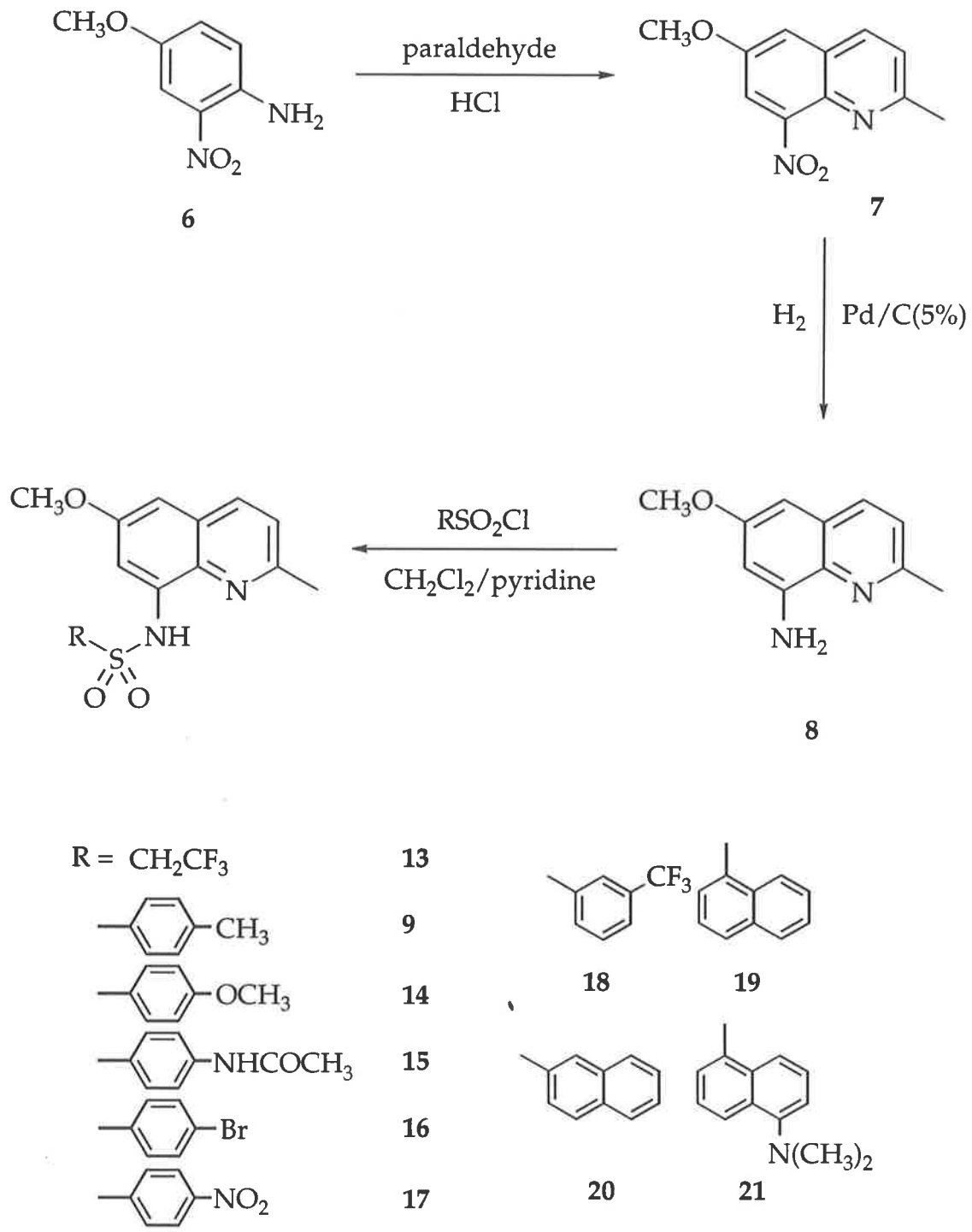
Testing of the new sulfonamide ligands would consist of; uv/visible-spectroscopy, to test for a bathochromic shift in the presence of Zn(II);

fluorimetry, to the compare the fluorescence of the new ligands relative to the 6-methoxy analogue of Zinquin, in the absence and presence of Zn(II). If a significant improvement in fluorescence is detected in any of the sulfonamide derivatives relative to the 6-methoxy Zinquin analogue then simple removal of the methoxy group and alkylation using the methods established in Chapter 2 will afford the new ligand/s for biological studies.

3.2. Synthesis of the sulfonamide derivatives.

Preparation of the sulfonamides began with the synthesis of the 8-aminoquinaldine **8** outlined in Chapter 2. The formation of each sulfonamide was by a standard literature procedure, namely stirring the amine **8** with the required sulfonyl chloride in dichloromethane and pyridine (Scheme 3.1). Both of the *p*-methoxy and *p*-acetamidobenzenesulfonyl chlorides were synthesised by standard literature methods.[94,95]

Only in one instance, **13**, did the *bis*-sulfonamide form. This was not surprising since the corresponding sulfonyl chloride, 2,2,2-trifluoroethane sulfonylchloride, is 100 times more reactive than *p*-toluenesulfonyl chloride.[93] The yield of the mono sulfonamide **13** was increased by lowering the temperature to -70°C and separation of the two compounds was accomplished by flash chromatography. The *bis*-sulfonamide was identified from the mono-sulfonamide by the ^{1}H n.m.r. spectrum which showed an absence of a characteristic sulfonamide N-H peak at approximately δ9.30 ppm.[96] Conversely, the ^{1}H n.m.r. spectrum of **13** contained a broad singlet at δ9.25 ppm and an infra red absorbance at 3250cm^{-1}, indicative of the N-H.[96,97]



Scheme 3.1.

All the sulfonamides were fully characterised by ^1H n.m.r., melting point, infra-red spectroscopy, micro analysis and mass spectroscopy. In particular; all the sulfonamides showed in the mass spectrum a peak at 187 ($M-SO_2R$) corresponding to a loss of the sulfonamide unit; infra red

spectroscopy (of all sulfonamides) showed an absorbance near 3250cm^{-1}, indicative of the NH of the sulfonamide;[97] ^{1}H n.m.r. spectrum (of all sulfonamides) contained a broad singlet in the region δ9.2-9.3 ppm, indicative of the NH of the sulfonamide.[96] The structure of **13** was confirmed by a ^{3}J, ^{1}H-^{19}F coupling constant of 8.9Hz.[96] In the ^{13}C n.m.r. spectrum two coupling constants were obtained 165.8Hz ($\mathbf{C}H_2\underline{C}F_3$) and 19.1Hz ($\underline{C}H_2\mathbf{C}F_3$), which correspond to a ^{1}J and ^{2}J, ^{13}C-^{19}F coupling, respectively (Figure 3.4).[96]

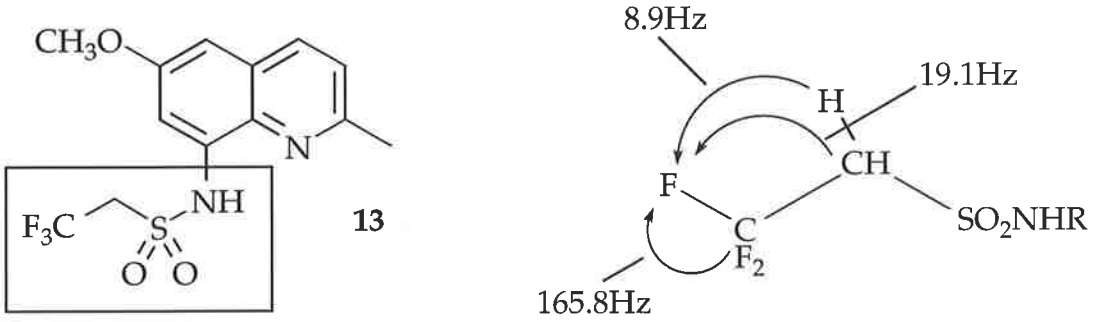


Figure 3.4. The fluorine coupling patterns observed for **13**.

3.3. The uv/visible and fluorescence spectroscopy study of the sulfonamide ligands.

The uv/visible-spectra of Zinquin when bound to Zn(II) show a characteristic bathochromic shift from 340nm to 364nm.[55,60] When the 6-methoxy Zinquin analogue **9**, is in the presence of Zn(II) a similar bathochromic shift of 337nm to 357nm is observed. The sulfonamide ligands **9**, **13-21** when bound to Zn(II) also showed a shift of approximately 20nm, with the only exception being **21**, the dansyl sulfonamide (see Figure 3.6).

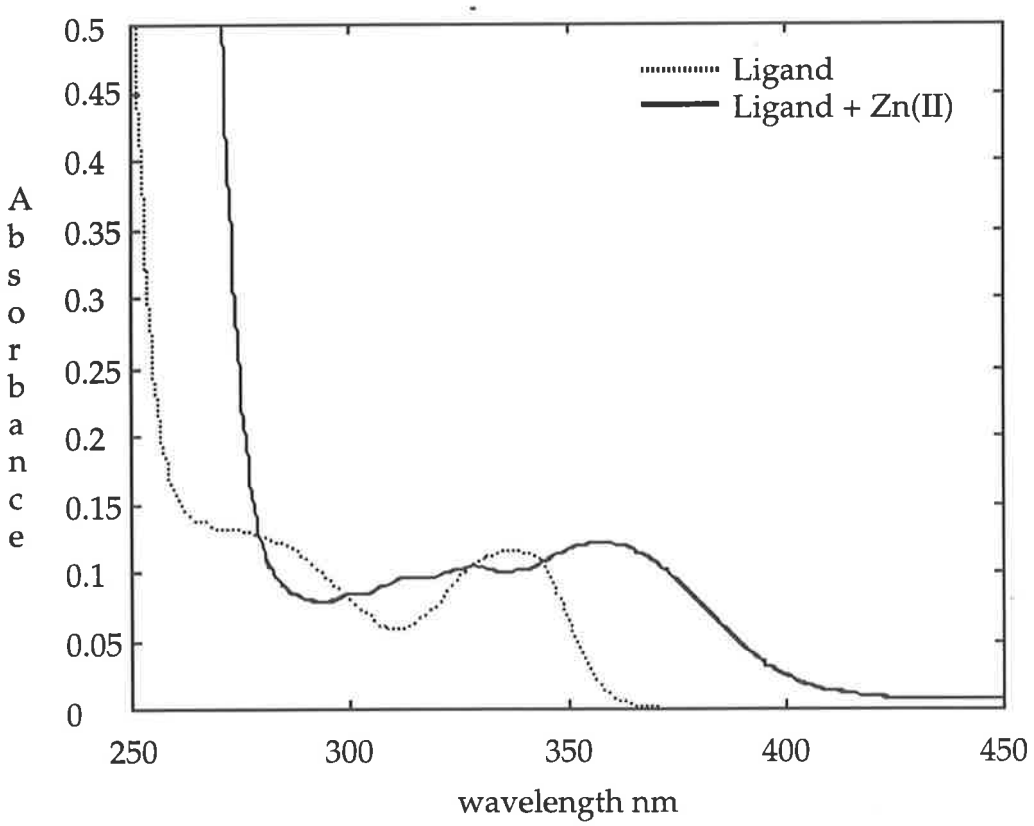


Figure 3.5. Partial uv/visible spectrum of **13** showing a strong bathochromic shift from 337nm to 357nm. Obtained from; dotted line, [L], 16.5μM, [EDTA] 165μM; solid line, [L] 16.5μM, [Zn(II)] 165μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

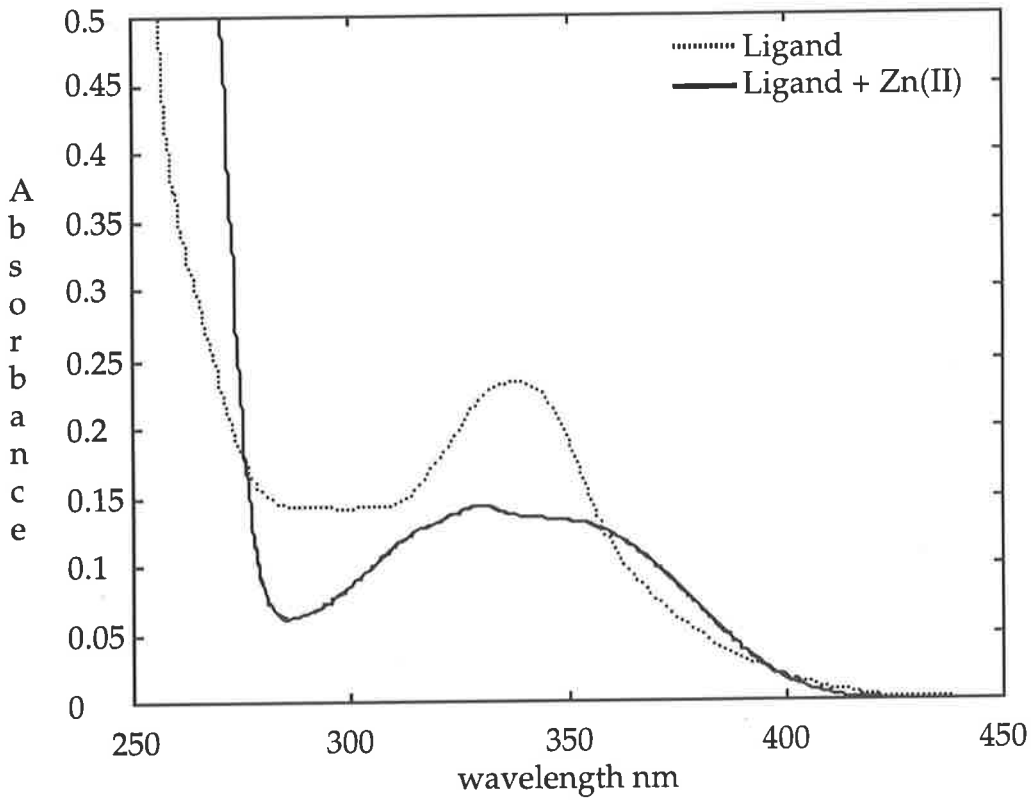


Figure 3.6. Partial uv/visible spectrum of **21** (dotted line) and the ligand in the presence of Zn(II) (solid line). Same solvent system / concentrations as in Figure 3.5.

The uv/visible spectrum of **21** in the presence of Zn(II) showed two overlapping absorptions at 330nm and 357nm. This was attributed to a bathochromic shift and a hypsochromic shift. This is likely to be a result of competitive binding of Zn(II) not only to the quinoline nitrogen, pK_a 3.72, but also the dimethyl nitrogen of the dansyl sulfonamide, which has a higher pK_a than the quinoline nitrogen (*N*,*N*-dimethyl aniline pK_a 5.15, N-methyl-α-aminonaphthlene pK_a 3.67).[60,98] Binding of Zn(II) to the dimethyl nitrogen of the dansyl group produces a structure similar in electronic terms to that resulting from the protonation of that nitrogen. This is supported by the uv/visible spectrum of **21** at pH 4, which exhibited a hypsochromic shift similar to that seen in the unbroken line of Figure 3.6. **21** exhibited high fluorescence, in the absence of Zn(II), but this fluorescence

was quenched upon addition of Zn(II). The cause of this quenching of fluorescence is not apparent and was not investigated further.

The two naphthalene sulfonamide ligands exhibited no increase in their longest wavelength of absorbance when in the presence of Zn(II). This supports the hypothesis that the aromatic unit attached to the sulfonamide plays no role in extending conjugation of the complex. Apart from **21**, the sulfonamides **9, 13-20**, all had a wavelength absorbance maxima of 357nm when bound to Zn(II). Fluorescence spectra of these sulfonamides were therefore obtained at two wavelength maxima, namely 337nm which is close to the maxima for the ligand alone and at 357nm.

Table 3.1. The fluorescence values observed when sulfonamides **9, 13-20**, were excited at 337nm.[a]

Sulfonamide.	Observed Fluorescence.
13, CH_2CF_3	-
9, *p*-C_6H_4-CH_3	31.0 ± 1.8
14, *p*-C_6H_4-OCH_3	16.0 ± 0.9
15, *p*-C_6H_4-$NHCOCH_3$	23.1 ± 1.3
16, *p*-C_6H_4-Br	13.5 ± 0.7
17, *p*-C_6H_4-NO_2	-
18, *m*-C_6H_4-CF_3	19.1 ± 1.0
19, α-naphthyl	5.2 ± 0.3
20, β-naphthyl	12.1 ± 0.7

a) Obtained from; [L] 3.4μM Ligand, [EDTA] 34μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

Table 3.2. The fluorescence values obtained when the sulfonamide ligands **9, 13-20** were excited at 357nm.[a]

Sulfonamide.	Observed Fluorescence.
13, CH_2CF_3	3.1 ± 0.2
9, *p*-C_6H_4-CH_3	16.5 ± 1.0
14, *p*-C_6H_4-OCH_3	17.0 ± 1.0
15, *p*-C_6H_4-$NHCOCH_3$	18.1 ± 1.0
16, *p*-C_6H_4-Br	10.5 ± 0.6
17, *p*-C_6H_4-NO_2	-
18, *m*-C_6H_4-CF_3	11.2 ± 0.6
19, α-naphthyl	4.9 ± 0.3
20, β-naphthyl	12.5 ± 0.7

a) Same concentrations / solvent system as in Table 3.1.

Table 3.3. The fluorescence values obtained when sulfonamides **9, 13-20** were excited at 357nm and in the presence of Zn(II).(a),(b)

Sulfonamide.	Observed Fluorescence	
13, CH_2CF_3	>1000	653.3 ± 41.8
9, *p*-C_6H_4-CH_3	601.2 ± 33.2	365.6 ± 23.2
14, *p*-C_6H_4-OCH_3	782.2 ± 42.2	
15, *p*-C_6H_4-$NHCOCH_3$	819.4 ± 42.7	
16, *p*-C_6H_4-Br	826.7 ± 41.9	
17, *p*-C_6H_4-NO_2	-	
18, *m*-C_6H_4-CF_3	845.1 ± 43.4	
19, α-naphthyl	423.9 ± 22.7	
20, β-naphthyl	725.2 ± 26.2	

a) Obtained from; [L] 3.4μM, [Zn(II)] 34μM.

b) The last column is the fluorescence of **13** and **9** obtained from; [L] 1.7μM, [Zn(II)] 17μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

The emission spectrum values in Table 3.1 and 3.2, indicate that the sulfonamides **9**, **13-20** have low fluorescence at either the excitation frequencies 337nm and 357nm, respectively. Table 3.3 shows the fluorescence values obtained for the ligands in the presence of Zn(II) at an excitation wavelength of 357nm. Six have higher relative fluorescence values than that of the sulfonamide **9**. Due to the extreme fluorescence of the tresyl sulfonamides **13** compared to the sulfonamide **9** (**13** exhibits approximately two times the fluorescence of **9**), the fluorescence data for **13** and **9** was taken at half the original concentrations in order to have both fluorescence values on scale.

As previously stated in Chapter 2, fluorescence was being seen in ZQA fluorescence spectra in the absence of Zn(II), which was being attributed to adventitious Zn(II) in the ethanol (used in both the preparation of the buffer and in the preparation of the ligand).[60] This effect of adventitious Zn(II) was also seen in the fluorescence spectra of the other sulfonamide ligands. Consequently, a ten fold excess of a chelating agent, EDTA, was added to the fluorescence solutions. This removed the adventitious Zn(II) from the sulfonamide ligands in the absence of Zn(II) and resulted in a minimal fluorescence of the ligand at the excitation wavelength, 357nm.

Any direct comparison of the fluorescence of the sulfonamides against the Zinquin analogue **9** in the presence of Zn(II) must take into consideration the relative abundances of each species: either as ligand, ligand-Zn(II) complex or/and di-ligand-Zn(II) complex.[60] The relative abundance of each of these species will be different for each of the new sulfonamides, a consequence of each ligand displaying differing stabilities in the presence of Zn(II). However conclusions have been drawn from the

containing solutions

fluorescence data since the species present in each of the sulfonamides would be expected to be similar to that of **9** in the presence of Zn(II), a consequence of distinct structural similarities between **9** and the sulfonamides **13-20**.

Attempted correlation of fluorescence of the sulfonamides bound to Zn(II) with substituent constants[99,100] failed to relate fluorescence of the sulfonamides with electronic effects of the different substituents on the sulfonamides.[99,100] It is clear though, that inductively electron withdrawing sulfonamides such as the tresyl **13**, *p*-bromobenzene **16** and *m*-trifluoromethylbenzene **18** sulfonamide, significantly increased the fluorescence compared to the tosyl sulfonamide **9**. The *p*-nitro sulfonamide **17** showed no fluorescence in the presence of Zn(II) at an excitation wavelength of 357nm. Bridges[54] has noted that highly electron withdrawing groups like nitro groups can decrease fluorescence compared to electron donating groups. In additional, nitro groups have been reported to be effective in quenching fluorescence, a process attributed to predissociation.[101]

The β-naphthyl sulfonamide **20** showed a slight increase in fluorescence compared to **9** and nearly twice the fluorescence of the corresponding α-naphthyl sulfonamide **19**, when bound to Zn(II). This is rationalised by the fact that the α-naphthyl sulfonamide **19** sterically hinders the ligand in coordinating Zn(II) to a far greater extent than the β-naphthyl sulfonamide **20**. This hindrance would force the ligand into a less planar arrangement (hence less conjugated) when bound to Zn(II) and therefore reduce fluorescence relative to that of the Zinquin analogue **9** and the β-naphthyl sulfonamide **20**.

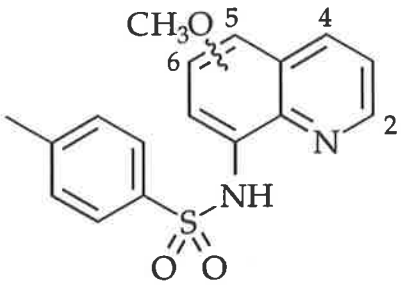
3.4. Conclusions.

The above results show that the tosyl sulfonamide used in Zinquin, is not the most fluorescent sulfonamide when the ligand is in the presence of Zn(II). The best sulfonamide ligands appear to be the tresyl sulfonamide **13** and the *m*-benzene trifluoromethyl sulfonamide **18** ligands since they increase fluorescence approximately 75% and 40%, respectively, over the tosyl sulfonamide **9**. It must be noted that the tresyl sulfonamide **13**, is chemically unstable relative to the tosyl sulfonamide **9** and this will be discussed in greater detail in Chapter 7. Removal of the 6-methoxy group from **13** and **18** and the attachment of an ester group would yield an *in vivo* ligand/s that could be compared to ZQE, the synthesis of which will be discussed in Chapter 7.

Chapter 4 : Methoxy isomers of Zinquin.

4.1. Introduction.

The second electronic factor which may control fluorescence of the Zinquin complex is the position of the electron donating alkoxy group. In Zinquin the group is at the 6-position.[55] Consequently, as stated in the aims of this thesis, the synthesis of structural isomers of Zinquin with the oxygen at differing positions would be of interest since this may lead to more highly fluorescent complexes with Zn(II). Therefore, this Chapter is concerned with the synthesis and physical chemistry of isomers of Zinquin where the methoxy group is either at the 2, 4 or 5 position.

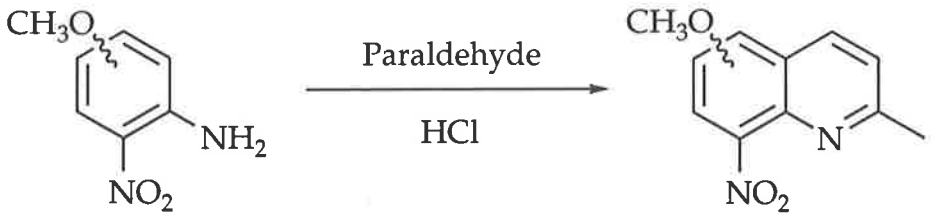


As in the previous Chapter, the ester group at the six position on ZQE **1a** is not crucial to fluorescence in the Zinquin-Zn(II) complex and it was decided that only the precursor methoxy analogues would be synthesised and tested.

4.2. The synthetic strategies toward such isomers.

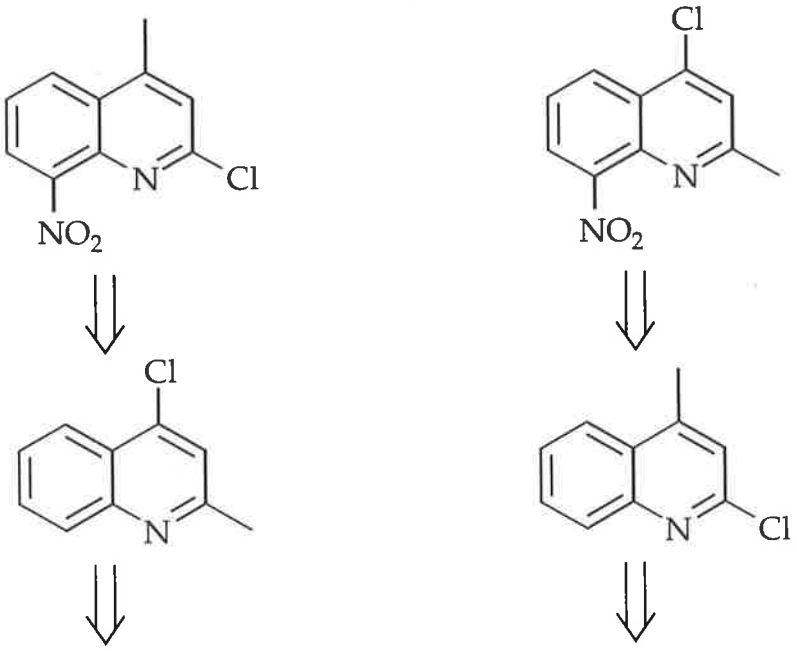
The synthesis of the 6-methoxy isomer **9**, described in Chapter 2, utilises the Skraup reaction[102] (Scheme 4.1) to form the quinoline system. The advantage of the Skraup synthesis is the wide variety of substituents that can be used on the aniline component to give the corresponding substituted quinoline. The 6-methoxy quinoline **9** uses the substituted

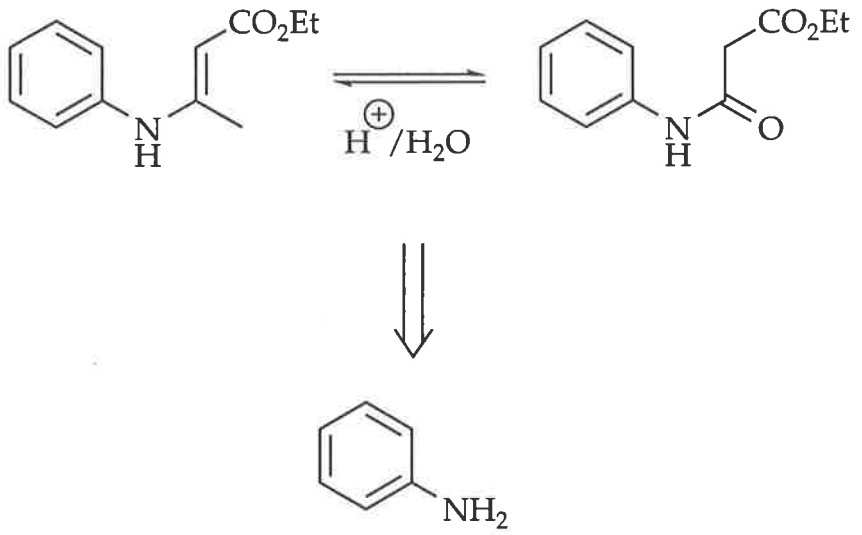
aniline, 4-methoxy-2-nitroaniline **6**, therefore the 5-methoxyquinoline could be synthesised using 5-methoxy-2-nitroaniline **25**.



Scheme 4.1.

The 2- and 4-methoxy substituted quinolines would best be synthesised from their corresponding chloro derivatives. This in turn, would require the initial synthesis of the 2- and 4-quinolone systems by the Conrad-Limpach cyclisation of aryl-amino acrylates.[102] The Conrad-Limpach cyclisation has been successfully utilised by Hauser and Reynolds[103] in their synthesis of 2- and 4-quinolones, (Scheme 4.2). All that would be required to form the 8-nitro substituted quinolines is the nitration of the 2- and 4-chloroquinolines using mild conditions, since it has been previously reported that 4-chloroquinoline has been nitrated at the 8 position.[104]



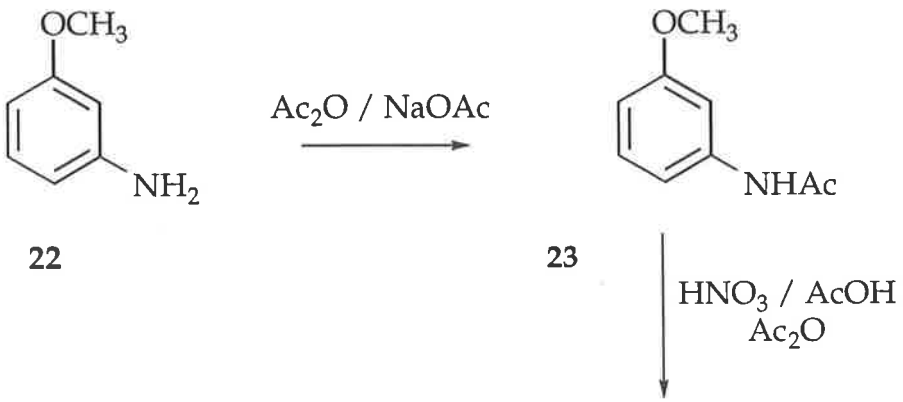


Scheme 4.2.

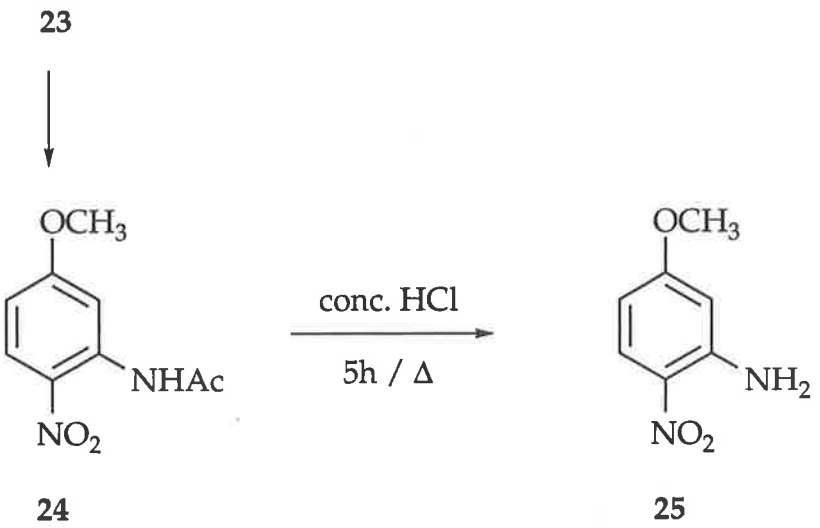
4.3. The synthesis of the 2-, 4- and 5-methoxyquinoline isomers.

(a) The 5-methoxy quinoline isomer.

This synthesis began with formation of the disubstituted aniline **25**, which was accomplished using the method of Zhang *et al*[105] in an overall yield of 21%. The synthesis involved the protection of *m*-anisidine **22** as the acetamide followed by nitration and deprotection of the acetamide (Scheme 4.3). The structure of the disubstituted aniline **25** was confirmed by comparison of the spectral data with that reported.[105]

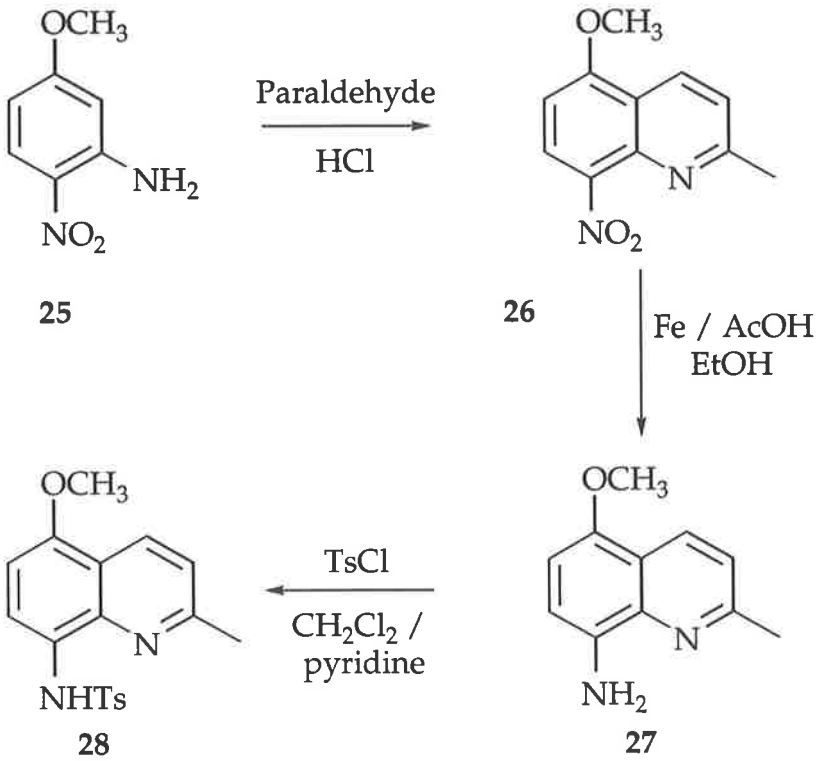


22 **23**



Scheme 4.3.

Using the synthetic protocols already established in the synthesis of the 6-methoxyquinoline isomer, namely formation of the quinaldine **26** using the Skraup reaction, reduction of the nitro group to the amine **27** followed by tosylation, the desired 5-methoxy isomer **28** was prepared in good yield (Scheme 4.4).

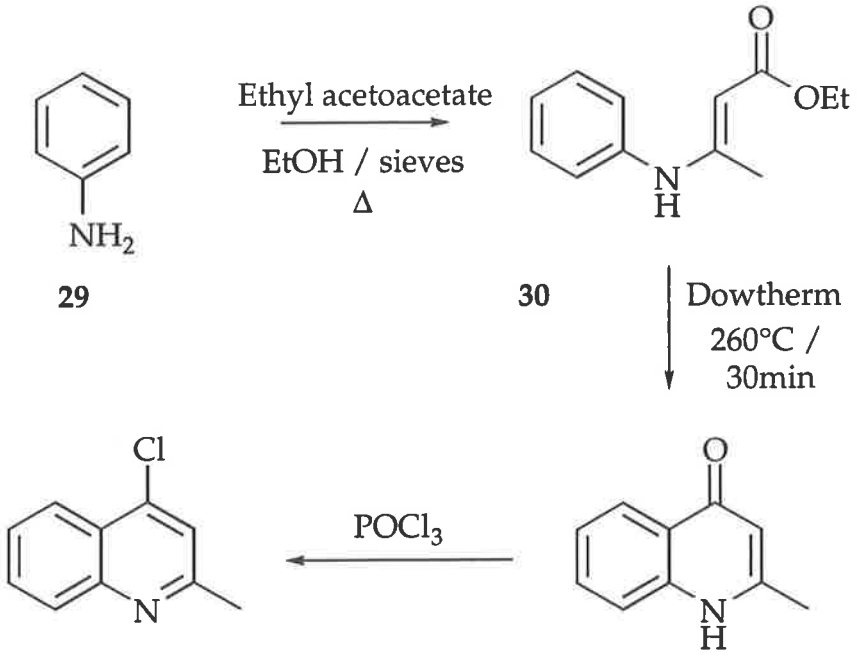


Scheme 4.4.

The nitroquinoline **26** was identified by the ^1H n.m.r. spectrum which showed the presence of two pairs of *ortho* coupled aromatic protons. H_7 and H_8 occurred at δ8.48 and δ7.37ppm, respectively, with H_7 occurring further downfield due to the electron withdrawing nitro group, and H_4 and H_3 occurred at δ7.95 and δ6.63ppm respectively. The *ortho* coupling constant values between H_7 / H_8, and H_4 / H_3 was also consistent with the values obtained for similar quinoline systems.[106] A molecular ion peak of 218 further supported the ^1H n.m.r. evidence. The aminoquinoline **27** was identified by the ^1H n.m.r. spectrum, in particular a broad singlet at δ4.60ppm and the upfield shift of H_7 and H_8 were both consistent with the presence of an amino group. A strong molecular ion at 188 confirmed the formation of the amine. Tosylation of the amine formed the sulfonamide **28** whose tosyl protons $H_{2'}$ and $H_{3'}$ resonated at δ7.72ppm and δ7.08ppm, respectively in the ^1H n.m.r. spectrum. A strong peak at 187 in the mass spectrum, resulting from the loss of $C_7H_7SO_2$ from the molecular ion together with an absorbance at 3260cm^{-1} in the infra-red spectrum further confirmed the formation of **28**.

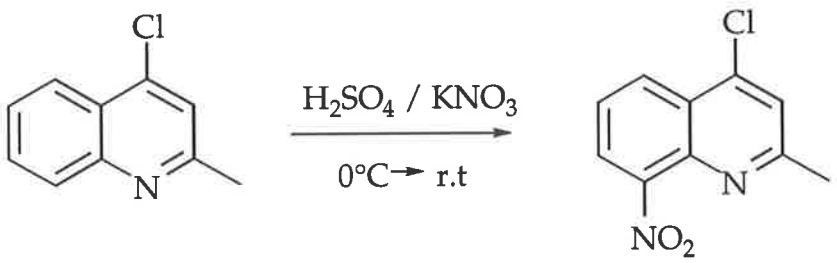
(b) The 4-methoxy quinoline isomer.

The synthesis of the 4-methoxy isomer began with formation of the 4-quinolone **31** using the method of Hauser and Reynolds.[103] 4-Chloroquinaldine **32** was formed by treatment of the quinolone **31** with phosphorous oxychloride[107] (Scheme 4.5). The identification of each product was confirmed by comparison of the spectral data with that of the literature.[103,107,108]



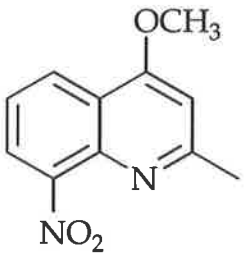
Scheme 4.5.

Nitration of the chloroquinoline **32** has been previously reported to form a mixture of nitro isomers.[104] Using the literature conditions the required 8-nitroquinoline[104] **33** was afforded in a moderate yield of 56% after chromatography (Scheme 4.6). ^{1}H n.m.r. evidence coupled with a molecular ion of 222 further confirmed the structure of the product. Other chromatographic fractions contained a mixture of the 5- and 6-nitroquinolines as indicated ^{1}H n.m.r. data.



Scheme 4.6.

Treatment[104] of **33** with sodium methoxide, produced the methoxyquinoline **34** which was then reduced using iron/acetic acid to the corresponding aminoquinoline **35** and tosylated to form the required 4-methoxy sulfonamide quinoline **36**.



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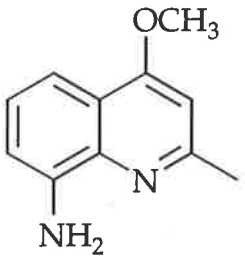

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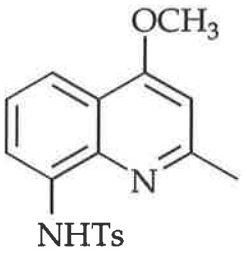
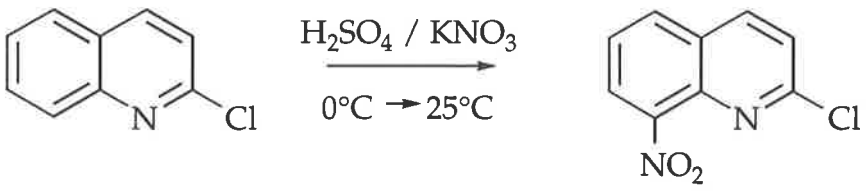


36

The methoxyquinoline structure **34** was confirmed by comparison of the spectral data with that reported.[104] In particular, a molecular ion was observed at 218 and a peak at δ4.01ppm in the ^{1}H n.m.r. spectrum showed the presence of the methoxy group. Comparison of the melting point with that of the literature[109] further confirmed the amines formation. Formation of the amine was supported with a broad singlet at δ4.75ppm in the ^{1}H n.m.r. spectrum and a strong molecular ion at 188. The corresponding sulfonamide **36** showed tosyl protons $H_{2'}$ and $H_{3'}$ at δ7.76ppm and δ7.10ppm, respectively in the ^{1}H n.m.r. spectrum, and an infra-red absorption of 3360cm^{-1} corresponding to the NH of the sulfonamide. A X-ray analysis of **36** definitively confirmed the substitution on the quinoline ring.

(c) The 2-methoxy quinoline isomer.

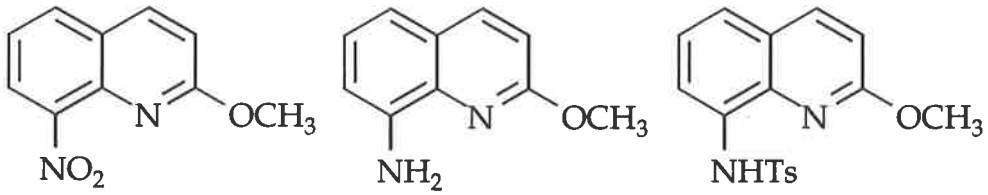
Nitration of 2-chloroquinoline **37** using the method[104] described above, yielded the required 2-chloro-8-nitroquinoline[110,111] **38** after chromatography in 49% yield (Scheme 4.7).



37 **38**

Scheme 4.7.

Chloride displacement using sodium methoxide then afforded the 2-methoxyquinoline[109] **39** in an excellent yield of 90% after chromatography. The methoxyquinoline **39** was reduced using iron/acetic acid and the resultant aminoquinoline **40** tosylated to yield the required quinolinesulfonamide **41**.



39 **40** **41**

The methoxyquinoline **39** showed a signal at δ4.03ppm due to the methoxy group in its ^1H n.m.r. spectrum along with a molecular ion at 204 in the mass spectrum. The ^1H n.m.r. spectrum of the amino quinoline **40** indicated the presence of the amine at δ4.73ppm and the mass spectrum also confirmed this with a strong molecular ion at 174. Formation of the sulfonamide **41** was supported by ^1H n.m.r., infra red and mass spectroscopy. In particular the ^1H n.m.r spectrum showed tosyl protons $H_{2'}$ and $H_{3'}$ at δ7.71ppm and δ7.10ppm, respectively, and a broad singlet at δ8.58ppm for the NH; infra red spectroscopy showed an NH absorbance at 3320cm^{-1} indicative of the sulfonamide and the mass spectrum showed a strong ion at 173 resulting from M-$C_7H_7SO_2$.

4.4. The uv/visible and fluorescence spectroscopic study of the 2-,4- and 5-methoxy Zinquin isomers.

The longest wavelength of absorbance for each of the isomers was significantly different in both the absence and presence of Zn(II) (Table 4.1). All the methoxy isomers displayed a bathochromic shift in the presence of Zn(II). However the degree of bathochromic shift observed for each isomer was also significantly different.

Table 4.1. UV/visible data for **9, 28, 36, 41** in the absence and presence of Zn(II).[a]

Ligand	Longest wavelength absorbance maximum (nm)		Bathochromic Shift (nm)
	Absence of Zn(II)	Presence of Zn(II)	
9	337	357	20
28	330	395	65
36	269	336	67
41	328	355, 321	27

a) Obtained from; [L] 16.5μM, [EDTA] 165μM ; [L] 16.5μM, [Zn(II)] 165μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).[#]

Table 4.1. shows that the 4- and 5-methoxy quinolines **28, 36** exhibited the largest bathochromic shift. The 2-methoxy isomer **41** has a similar bathochromic shift to that of the Zinquin precursor **9** although the uv/visible spectrum of **41** showed two distinct absorbances in the presence of Zn(II).

The fluorescence of the above isomers were measured at the excitation wavelength values shown in Table 4.2.

[#] The remaining uv/visible spectra are contained in Appendix A.

Table 4.2. The fluorescence of the methoxy isomers **9**, **28**, **36** and **41** .[a,b]

Isomer	Ligand		Ligand + Zn(II)		
	Excitation (nm)	Observed fluores.	Excitation (nm)	Observed fluores.	
9	337	31.0±1.8	357	601.2±33.2	365.6±23.2
28	330	5.5±0.4	395	7.6±0.4	-
36	269	40.3±2.3	336	>1000	996.3±55.1
41	328	4.5±0.3	355	181.6±10.1	-

a) Obtained from; [L] 3.4μM, [EDTA] 34μM ; [L] 3.4μM, [Zn(II)] 34μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

b) The last column is the fluorescence of **9** and **36** at half the original concentrations, obtained from; [L] 1.7μM, [Zn(II)] 17μM.

The 4-methoxy quinoline **36** shows nearly three times the relative fluorescence compared to that of the 6-methoxy quinoline **9** when in the presence of Zn(II). Conversely, the 5-methoxy quinoline **28** showed no fluorescence in either the absence or the presence of Zn(II). The 2-methoxy quinoline **41** showed limited fluorescence when compared to **9** in the presence of Zn(II) and no fluorescence in the absence of Zn(II).

4.5. Conclusions.

It is apparent from Tables 4.1 and 4.2., that there are a number of complex factors in the behaviour of the isomers in both their absorbance (Table 4.1) and when the isomers are in the excited state (Table 4.2). For example, the 4-methoxy quinoline **36** in the presence of Zn(II) exhibits a similar bathochromic shift compared to **28**, but surprisingly, the 5-methoxy quinoline **28** shows no fluorescence in the presence of Zn(II). However, the

4-methoxy isomer, **36**, does show significant improvement in fluorescence compared to the Zinquin precursor, **9**, when in the presence of Zn(II).

One particular avenue of investigation, into the behaviour of **9**, **28**, **36**, **41**, may be the determination of the pK_a values for their respective protonated quinoline nitrogens. The pK_a values of the protonated quinoline nitrogen would differ for each particular isomer, a difference which can be attributed to the amount of resonance participation the methoxy group can provide to the quinoline nitrogen. However these pK_a values and the effects which they may on the isomers (**9**, **28**, **36**, **41**, and their absorption and fluorescent behaviour in the absence and presence of Zn(II)) would need to be investigated further.

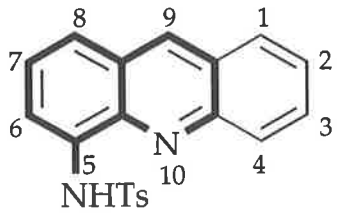
Chapter 5 : Acridine and acridone ligands.

5.1. Introduction.

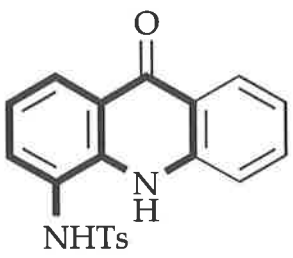
Zinquin has an excitation frequency of 364nm and a corresponding emission frequency of 485nm.[55] Zinquin fluorescence *in vivo* has been studied by a Confocal Laser Microscope (CLM, benefits are outlined in Chapter 1) but using a CLM fitted with a UV-laser, since the latter has a wavelength similar to the excitation frequency of the Zinquin-Zn(II) complex.[56] In contrast, commercially available CLM's use a blue light laser source, wavelength 488nm. Consequently a Zn(II) ligand that could be excited at 488nm would be desirable since it could be irradiated by the blue light laser. Modification strategies toward such ligands would be to increase the conjugation of Zinquin since an increase in the conjugation would result in a corresponding increase in the excitation frequency.[112]

5.2. Strategies to increase the conjugation of Zinquin.

As seen in Chapter 3, any increase in the conjugation of the sulfonamide unit of Zinquin does not affect the excitation frequency of the bound ligand. The most obvious method then is to increase the conjugation of Zinquin by the attachment of another aromatic ring to the already present quinoline system. Attachment of a benzene ring to the quinoline unit would result either in an acridine unit or the correspondingly oxidised acridone unit.

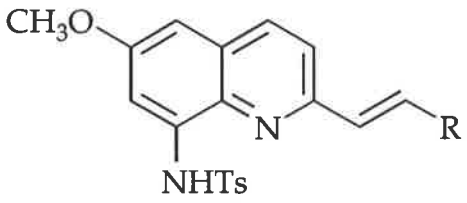


Acridine systems



Acridone systems

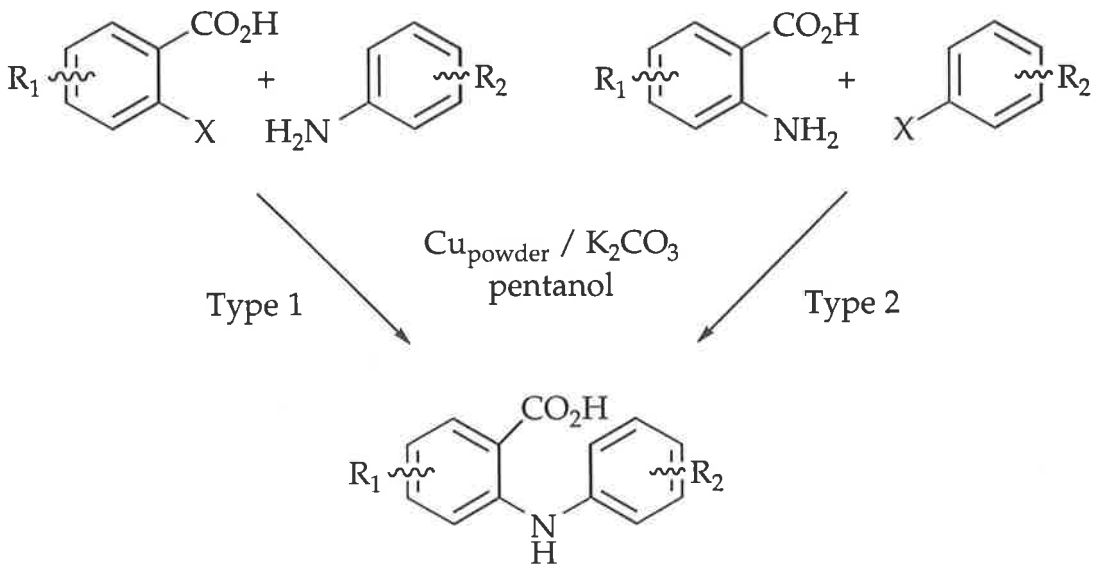
Another strategy would be the attachment of conjugated groups, such as naphthyl groups, to the 2-position of Zinquin, so that the resulting ligand would have extended conjugation.[112] Synthesis, UV and fluorescence of these types of ligands will be discussed in Chapter 6.

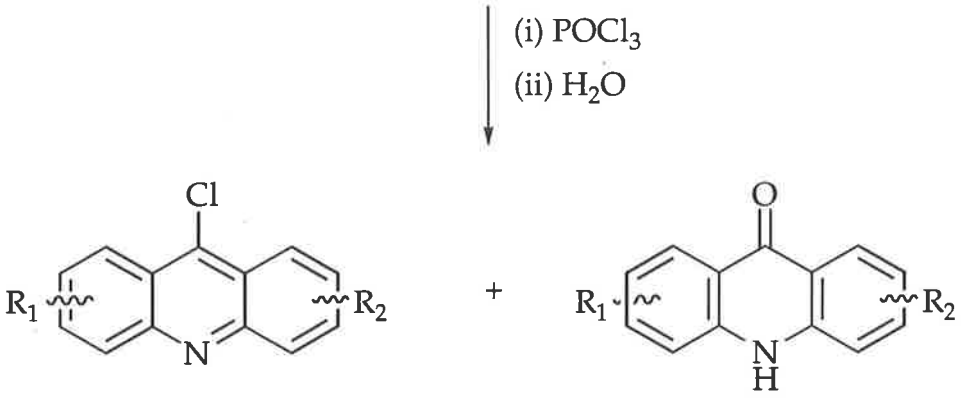


This Chapter will outline the synthesis of the acridine and acridone ligands as well as describing the uv/visible and fluorescence of such ligands.

5.3. Synthetic routes toward acridines and acridones.

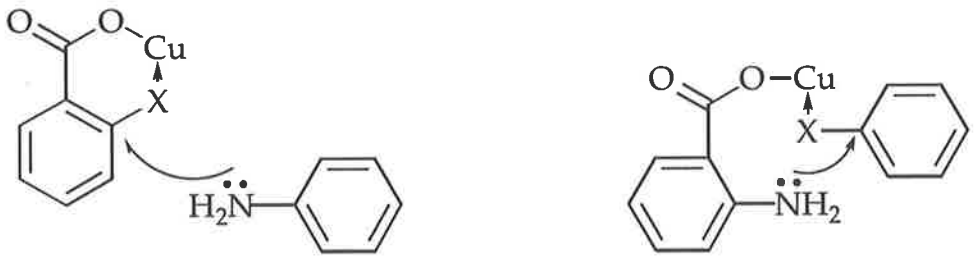
The simplest synthesis of substituted acridines and acridones is by phosphorus oxychloride induced cyclisation of the corresponding diphenylamine-2-carboxylic acid (Scheme 5.1).[113] A number of substituted diphenylamines can be synthesised with relative ease by the Ullman reaction which is based on the substitution of a halogen atom of a halobenzene by a primary or secondary phenylamine.[113]





Scheme 5.1.

The Ullman reaction utilises a copper catalyst, which aids in the polarisation of the carbon-halogen bond through coordination to the halogen atom, this then facilitates attack of the amine on the ring carbon atom (Scheme 5.2).[113] A base is also essential because it removes the hydrogen halide liberated as the Ullman reaction proceeds. If the hydrogen halide is not removed, the reaction slows and decarboxylation can occur.[113]

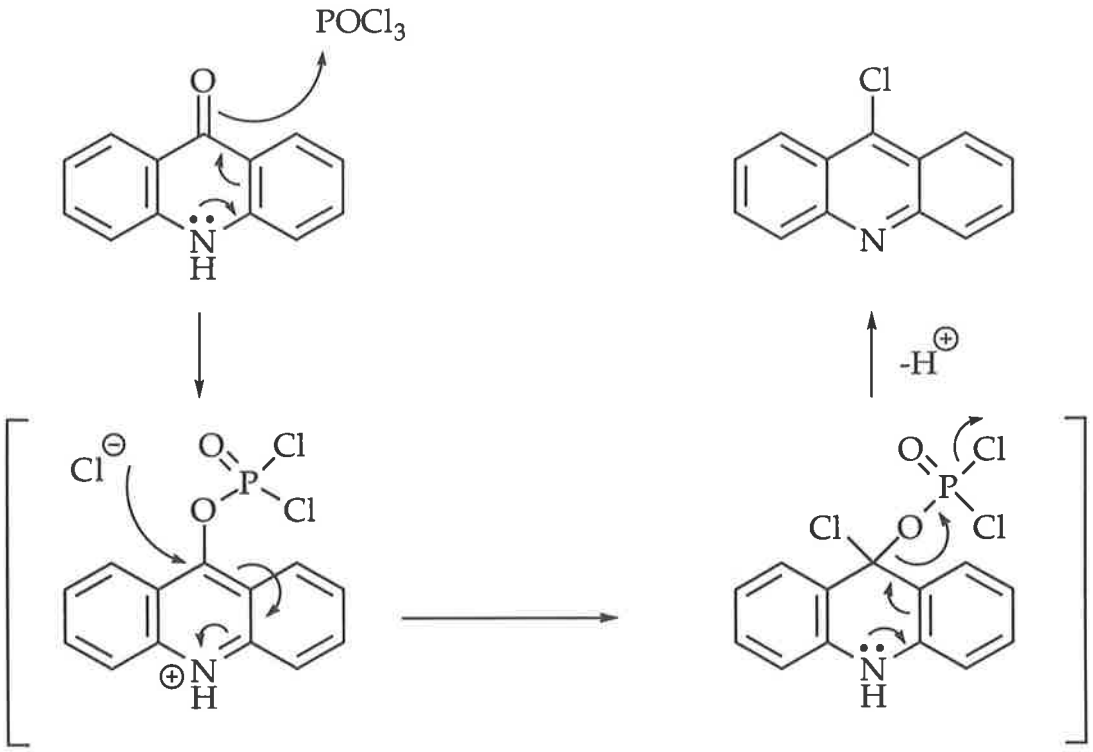


Type 1 Type 2

Scheme 5.2.

Once the diphenylamine-2-carboxylic acid is formed, cyclization with excess phosphorus oxychloride can yield both an acridone and an 9-chloroacridine. The acridone is the initial product of the reaction but can react further to form a 9-chloroacridine. The 9-chloroacridine is formed through the intermediate (Scheme 5.3) resulting from the complex formed between the acridone and excess phosphoryl chloride.[113] Commonly[114] the

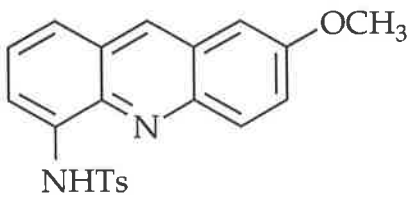
9-chloroacridine is hydrolysed to its corresponding acridone upon work up resulting in a mixture of products. 9-Chloroacridines are useful intermediates in the synthesis of acridines since the chlorine can be reductively displaced (by hydrogenolysis) or replaced by nucleophilic reagents.



Scheme 5.3.

5.4. Retrosynthetic approach toward the acridine and acridone ligands.

Initially the acridines **46**, **47** and acridones **48**, **49** were selected as synthetic targets. Both were analogues to the Zinquin structure, by having the required sulfonamide NH, and differed in the position of the methoxy group.



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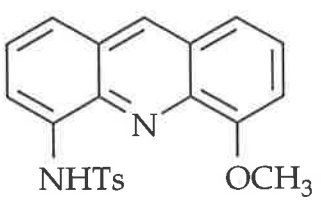
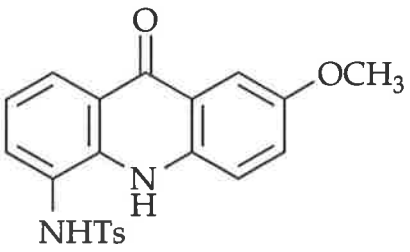
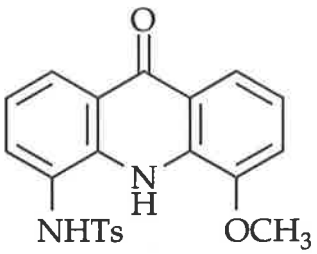


47



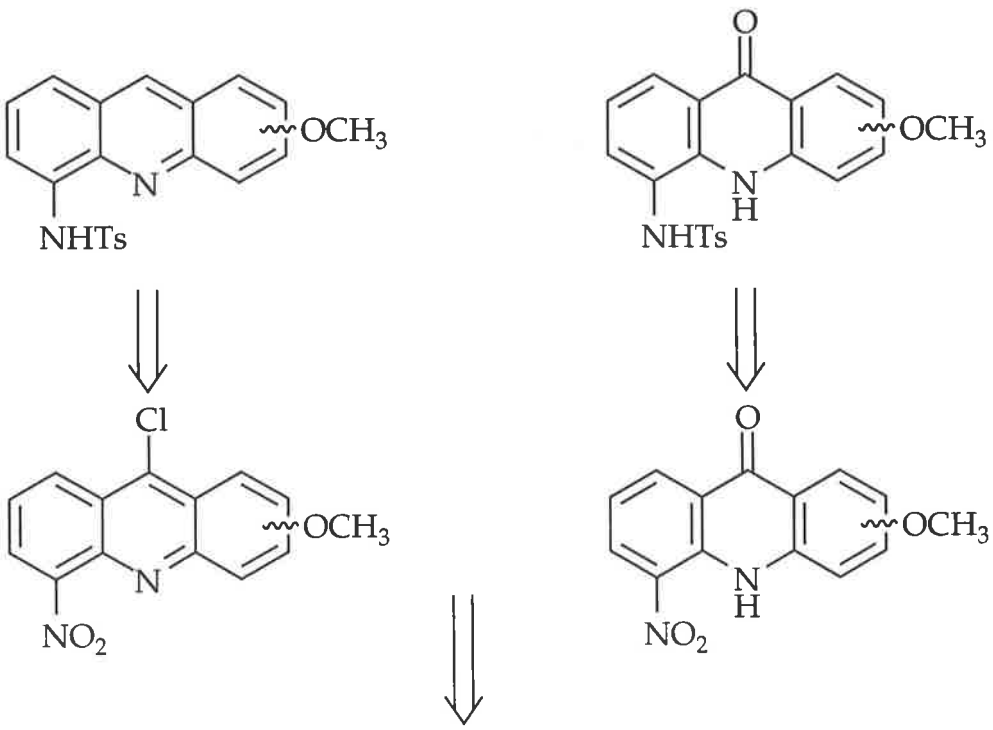
48

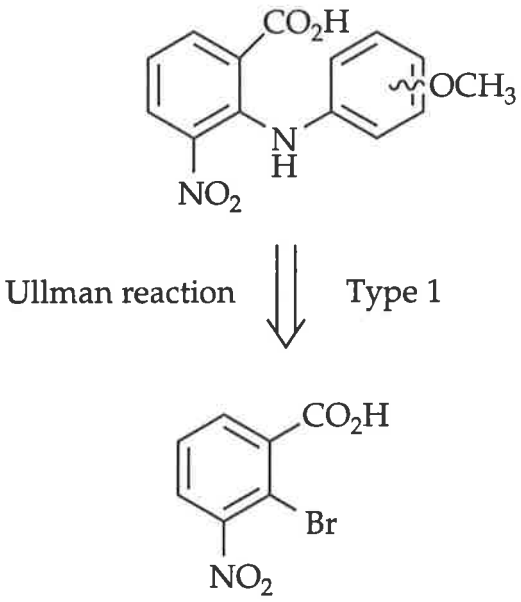


49

The methoxy group has three roles; to increase fluorescence[54]; to allow the attachment of an ester side chain; and to study the effects of a bulky group near the binding site. That is, would a bulky group, such as a methoxy group, near the binding pocket of the ligand, an example is **47**, affect the binding of Zn(II) to the ligand.

Retrosynthetically, the synthesis of the ligands must accommodate both the methoxy group and a group to attach the sulfonamide, namely a reducible nitro group.



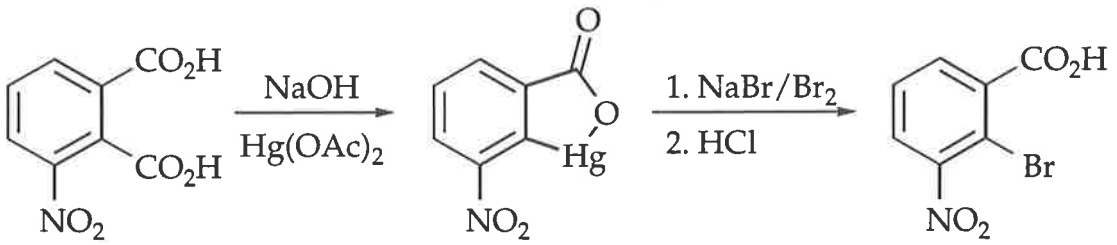


Scheme 5.4.

The retrosynthetic analysis depicted in Scheme 5.4, utilises the Ullman reaction,[113] to yield the substituted diphenylamine-2-carboxylic acids from the readily accessible 2-bromo-3-nitrobenzoic acid. Phosphorus oxychloride cyclization of the diphenylamine-2-carboxylic acids can then yield the 5-nitroacridone and 9-chloro-5-nitroacridine which can be reduced and tosylated to yield the sulfonamide acridine and acridone ligands.

5.5. The synthesis of the acridine and acridone ligands.

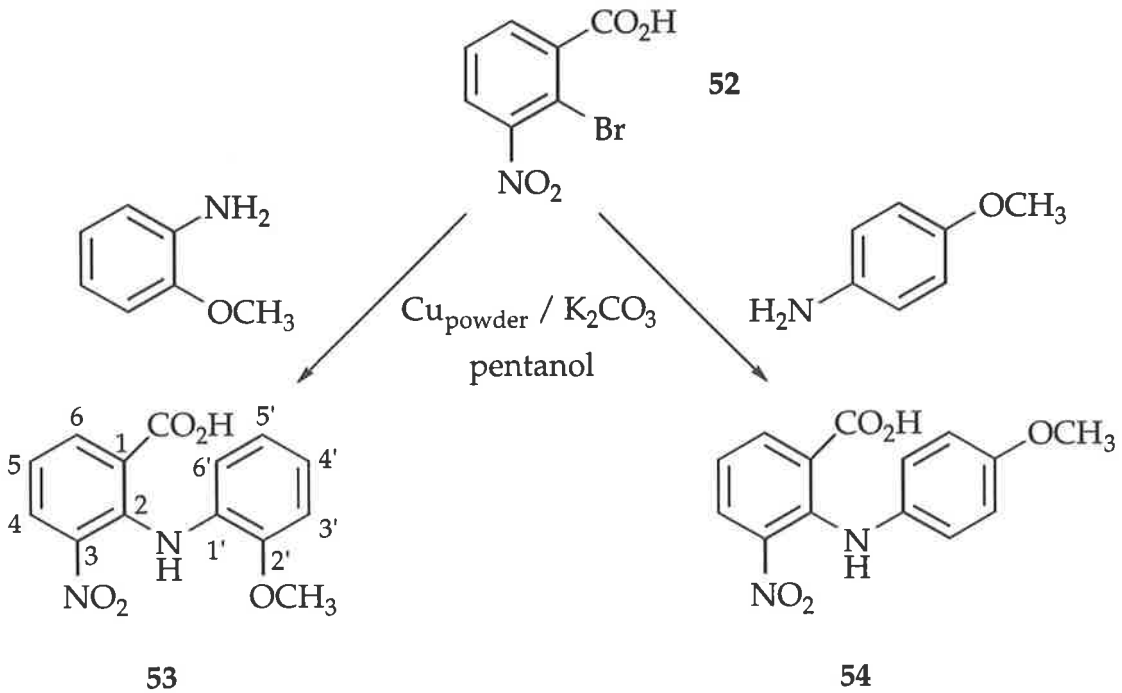
Synthesis of the acridine and acridone ligands began with the formation of the starting bromobenzoic acid **52** (Scheme 5.5). 2-Bromo-3-nitrobenzoic acid **52** was prepared in 70% yield in a two step process, with the formation of hydroxymercuric compound **51** followed by bromination.[115]



50 **51** **52**

Scheme 5.5.[115]

The aryl bromide **52** reacted readily with both *o*-anisidine and *p*-anisidine to yield the two required diphenylamine-2-carboxylic acids, **53** and **54** (Scheme 5.6). Initially the reaction was done in the melt, resulting in low yields, but changing to a solvent (pentanol) increased the yields to an average of 50-55% after recrystallization.



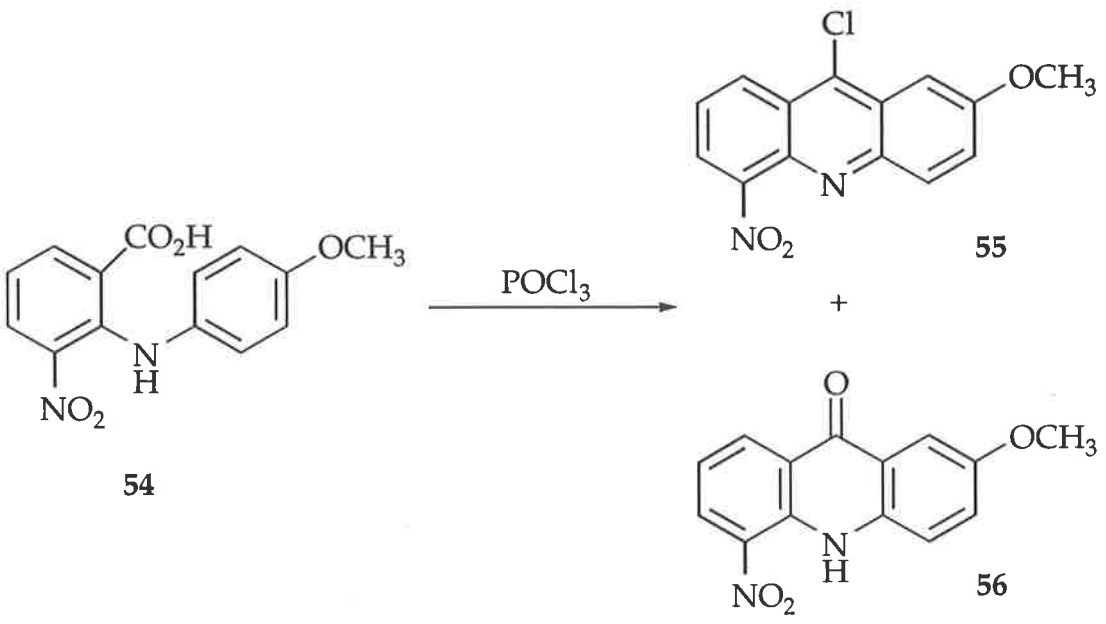
53 **54**

Scheme 5.6.[113]

Diphenylamine-2-carboxylic acids are identifiable[97] by characteristic infra-red absorbances for both the secondary amine, at 3250cm^{-1}, and the acid carbonyl, 1650cm^{-1}. The *ortho* substituted product is distinguishable

from the 4'-methoxy product by it's ^1H n.m.r. spectrum, since the latter contains a pair of chemically equivalent aromatic protons which appear as a characteristic multiplets at δ6.53ppm and δ6.43ppm, respectively.[96]

Cyclisation of **54** in excess phosphorus oxychloride afforded both the substituted 9-chloroacridine **55** and acridone **56** which were separated by flash chromatography yielding **55** in 76% and the acridone **56** in 10-15%, respectively (Scheme 5.7). The chloroacridine, **55** was stored under nitrogen since it was hydrolysed to the corresponding acridone **56** in air.[113] The mass spectrum and ^1H n.m.r. spectrum of **55** confirmed the structure of the product in particular a molecular ion at 288; *meta* coupling between H_1 and H_3 of 2.1Hz and H_3 having both *meta* and *ortho* couplings of 2.1Hz and 9.5Hz, respectively. However, the melting point of **55**, 271-274°C, did not correspond with that reported[116], 243-244°C.

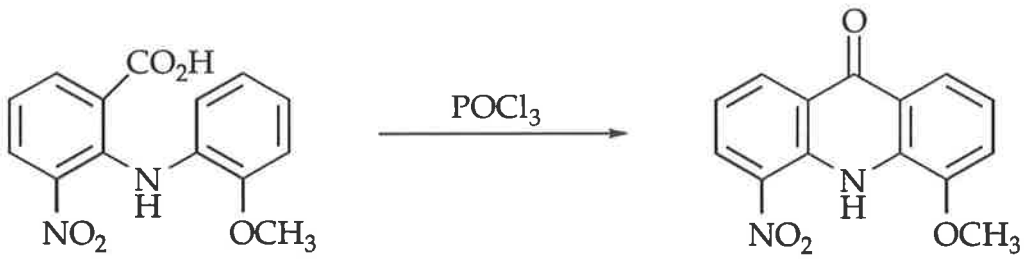


Scheme 5.7.

The acridone **56** was identified by mass, infra red and ^1H n.m.r. spectroscopy in particular a molecular ion at 270. An absorbance at 3260cm^{-1}

in the infra-red and a sharp singlet at δ7.40ppm in the ^{1}H n.m.r. spectrum were both indicative of NH in the structure of **56**. There were also inconsistencies of the melting point of the acridone **56**, 223-225°C, with that reported[116], 229-230°C.

Surprisingly, the cyclization of **53** using phosphorus oxychloride only yielded only the acridone **57** (Scheme 5.8). TLC of the reaction mixture before work up indicated the presence of another product. However, after work up this product was not seen by TLC. Refluxing **57** with excess phosphorus oxychloride also failed to yield the expected 9-chloroacridine.



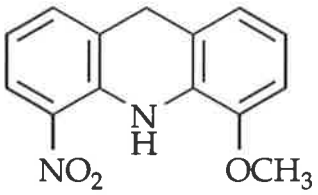
53 **57**

Scheme 5.8.

The acridone was identified by a characteristic infra red absorption at 3250cm^{-1} corresponding to the NH.[97] Once again the melting point, 255-259°C, differed from the literature[116], 246°C, but ^{1}H n.m.r. spectrum and a molecular mass of 270 supported the structure proposed.

Two alternative methods were then investigated for the synthesis of the 4-methoxy acridine **59**. Firstly, a reduction of the acridone **57** to the corresponding acridan was attempted since the acridan **58** could then be oxidised to the acridine **59**. An attempted reduction of the acridone with sodium/pentanol[117] was unsuccessful yielding a multitude of products by

TLC with the main product, the starting acridone, being isolated by flash chromatography.



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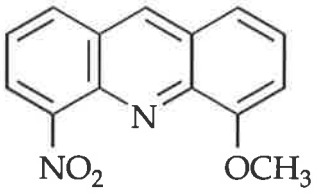
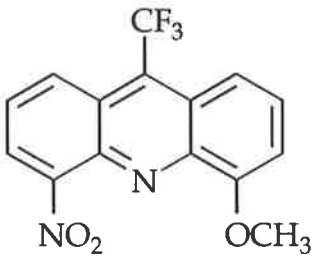


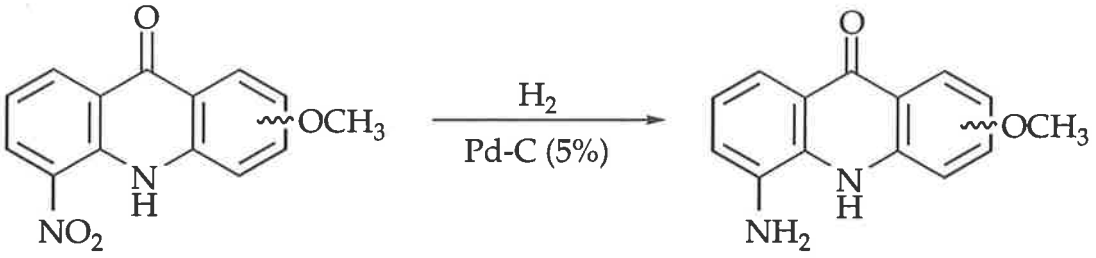
59

The second approach was conversion of the acridone to a 9-trifluoromethane derivative **60** which could then be cleaved leaving the required acridine. Treatment of **57** with triflic anhydride, using the method described by Singer and Mass[118], failed to yield the required 9-trifluoromethyl acridine **60** with only the starting acridone **57** being detected.



60

Conversion of the two methoxyacridones **56, 57** to their corresponding amino derivatives was achieved by catalytic reduction (Scheme 5.9).

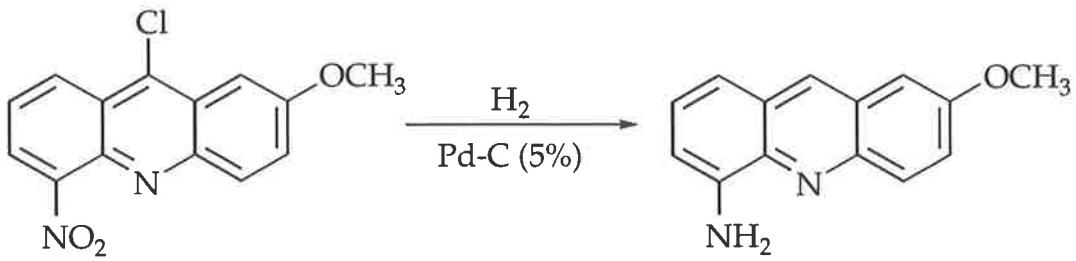


56, 57 **61, 62**

Scheme 5.9.

4-Methoxy-5-nitroacridone **57** could also be reduced using iron/acetic acid and ethanol in a good yield of 92%. The catalytic reduction methodology was higher yielding (>95%) and in all easier to perform, since the crude product required little purification. The 2-methoxyaminoacridone **62** was also isolated in excellent yield (80%). The amines were identified by both a strong molecular mass ion at 240, and their ^{1}H n.m.r. spectra.

Hydrogenolysis of 9-chloro-2-methoxy-5-nitroacridine **55** yielded the required amino compound **63** in an excellent yield of 90%. Hydrogenolysis of the chloro group was expected due to the literature precedent for such reactions.[117] Therefore, hydrogenolysis not only reduced the 5-nitro group but also cleaved the 9-chloro group leaving the parent acridine **63**, (Scheme 5.10).

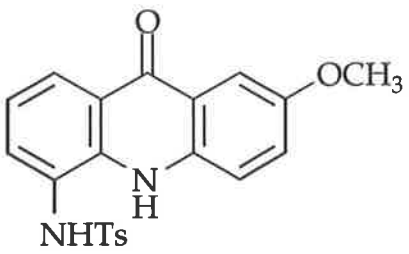


55 **63**

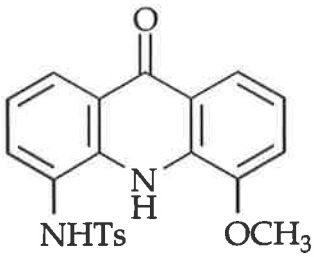
Scheme 5.10.

The disubstituted acridine **63** was identified by a molecular ion of 224 and ^1H n.m.r. spectrum which now exhibited a singlet at δ8.46ppm corresponding to H_9. Long range coupling of H_9 to H_1 or H_4 was not observed at 300MHz even though this has been reported previously for other acridine systems.[119] A broad singlet at δ5.17ppm indicated the presence of the expected primary amine at position five.

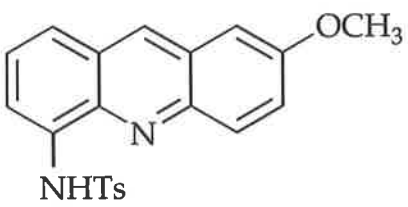
Both aminoacridones **61, 62** were tosylated to yield the required 2-methoxyacridonesulfonamide **48** in 52% yield and the 4-methoxyacridonesulfonamide **49** in 50% yield.



48



49



46

Sulfonamide formation was confirmed by infra red spectroscopy which showed absorbances at 3250cm^{-1} and 3350cm^{-1}, respectively, corresponding to the sulfonamide and acridone NH.[97] A sharp singlet at approximately δ9.80ppm characteristic of a sulfonamide NH, together with a fragmentation peak at 239 corresponding to M-$C_7H_7SO_2$ confirmed the structures **48** and **49**. TLC of both reactions indicated a higher R_f spot

consistent with the formation of the *bis*-sulfonamide and this would account for the moderate yields obtained for **48** and **49**.

The aminoacridine **63** was treated with tosyl chloride under the same conditions as above to yield the sulfonamide **46** in 67% yield. Formation of **46** was supported by the presence of a sharp singlet at δ9.50ppm in the ^{1}H n.m.r. spectrum indicative of the sulfonamide NH. X-ray analysis definitively assigned the structure of **46**.

5.6. The uv/visible and fluorescence spectroscopic study of the acridine and acridone ligands.

Zinquin exhibits a bathochromic shift from 340nm to 365nm when bound to Zn(II).[55,60] However, the acridone ligands **48**, **49** in the presence Zn(II) did not show a bathochromic shift when under the same conditions (Figures 5.1 and 5.2).

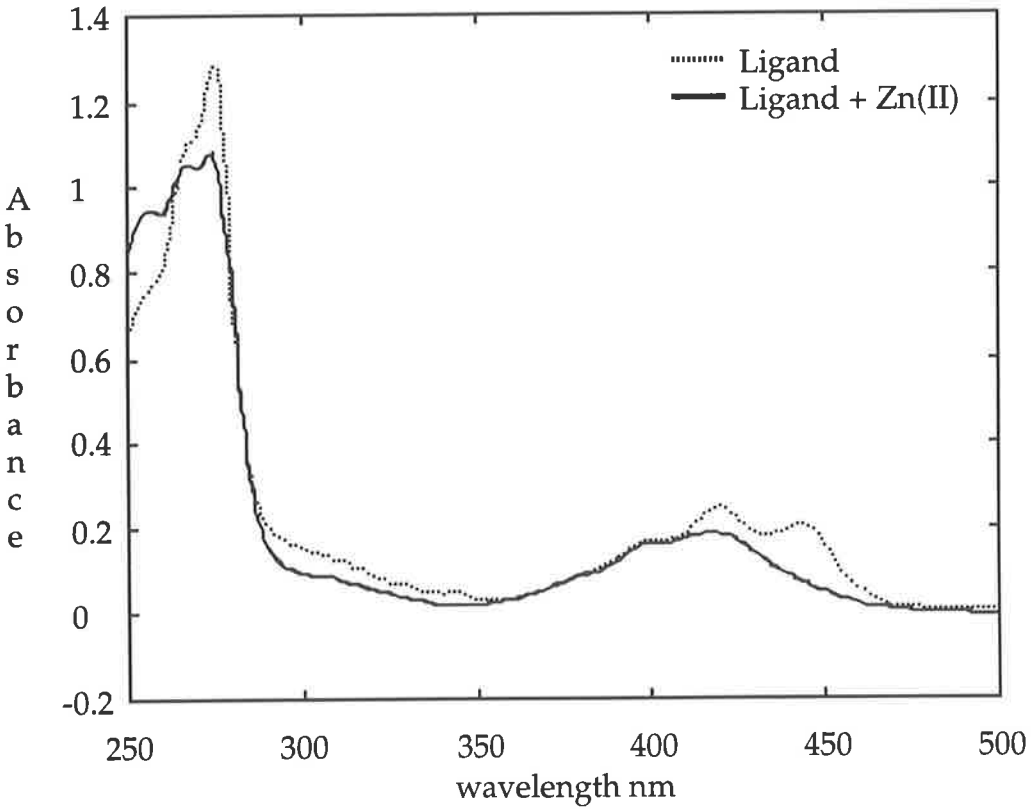


Figure 5.1. Partial UV-spectrum of **48**. Obtained from; dotted line, [L] 16.5μM, [EDTA] 165μM ; solid line, [L] 16.5μM, [Zn(II)] 165μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

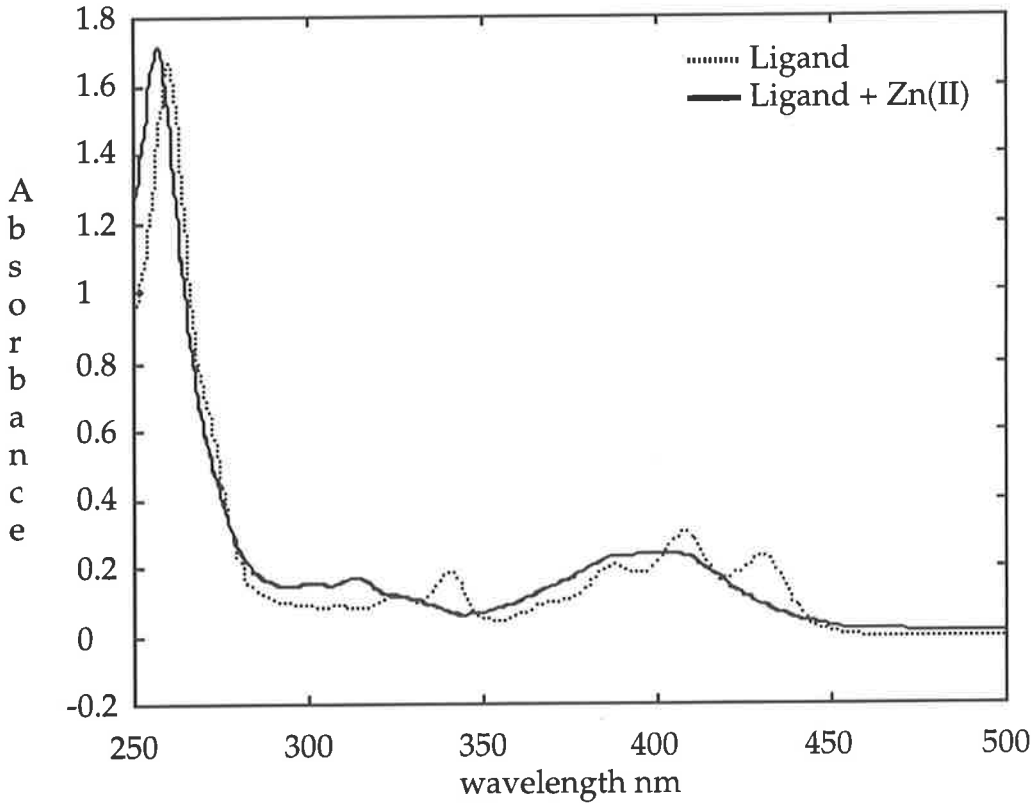
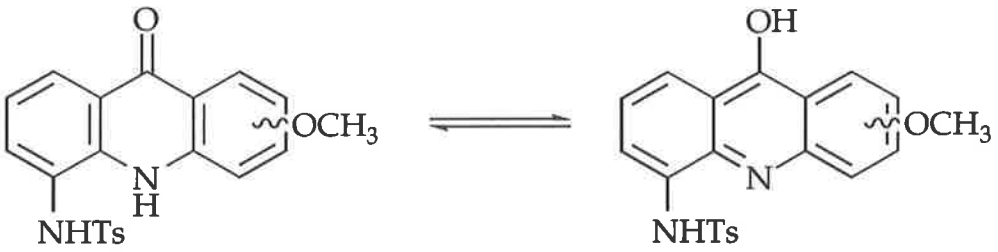


Figure 5.2. Partial UV-spectrum of **49** in the absence (dotted line) and presence (solid line) of Zn(II). Taken at the same concentration/solvent system as Figure 5.1.

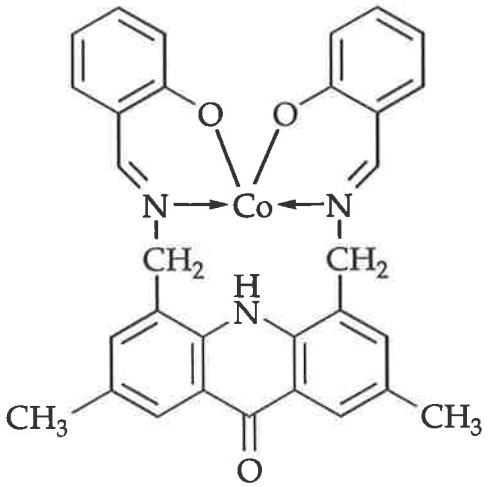
It appears from the small spectral change that the interaction of **48**, **49** with Zn(II) are weak (Figures 5.1 and 5.2). The availability of the NH lone pair of acridone is decreased through the lone pair's resonance with the acridone carbonyl. For binding to occur the lone pair on the acridone NH would need to be available, that is, the ligand would have to exist to some extent as the hydroxy tautomer (Scheme 5.11). However, the literature states that 9-hydroxyacridine has never been isolated[113] nor has it been detected in non polar solvents,[120] which are known to favour hydroxy rather than the keto tautomers.[121] Additionally, uv/visible-spectroscopy has consistently confirmed the predominance of the keto tautomer.[113]



48, 49

Scheme 5.11.

Furthermore, the acridone **64** has been shown[122] to bind to Co(II) without involving the lone pair of electrons on the acridone ring nitrogen. Consequently, acridones **48, 49** were judged to be weak due to their small spectral change in the presence of Zn(II).



64

In contrast to **48** and **49**, 4-methyl-N-(2-methoxy-5-acridinyl) benzene sulfonamide, **46**, exhibited a distinct bathochromic shift in the presence of Zn(II), see Figure 5.3.

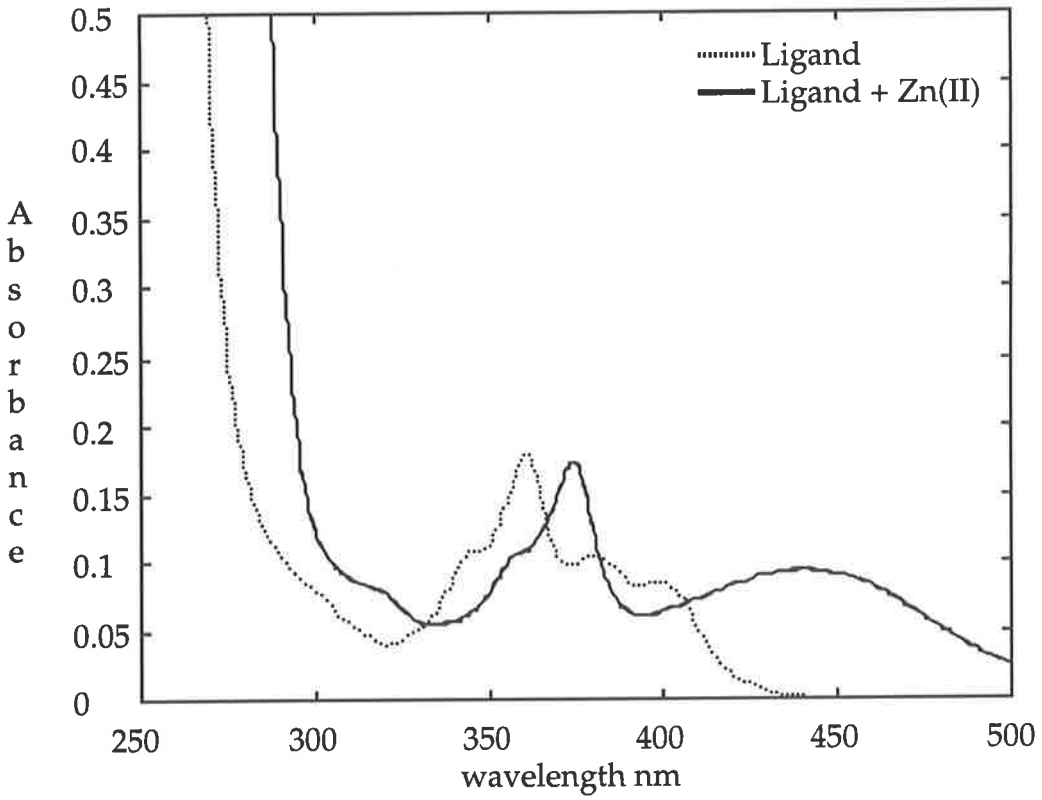


Figure 5.3. Partial UV-spectrum of **46**, in the absence (dotted line) and presence (solid line) of Zn(II). Same concentrations and solvent as Figure 5.1.

The maximum of the highest wavelength band of the acridine-Zn(II) complex was 443nm, near the required wavelength of 488nm needed for the blue laser CLM. The fluorescence of **46** was therefore determined at the wavelength of the absorption maximum for the unbound species, 399nm, and 443nm for the bound species, see Figure 5.4.

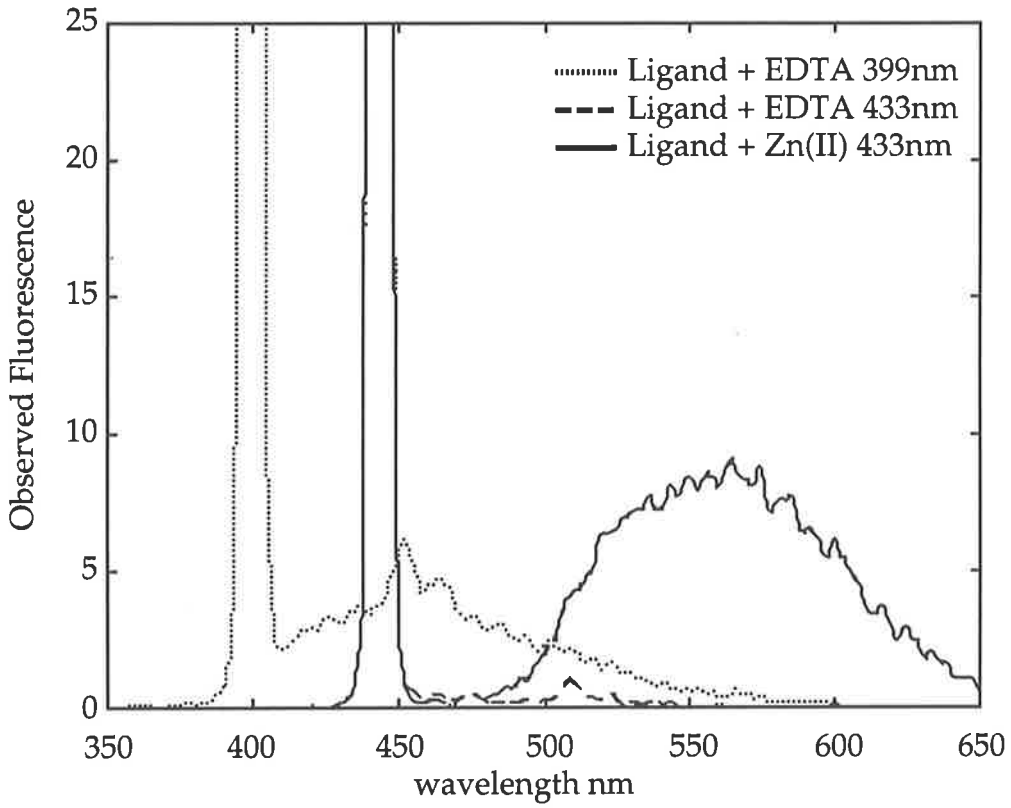
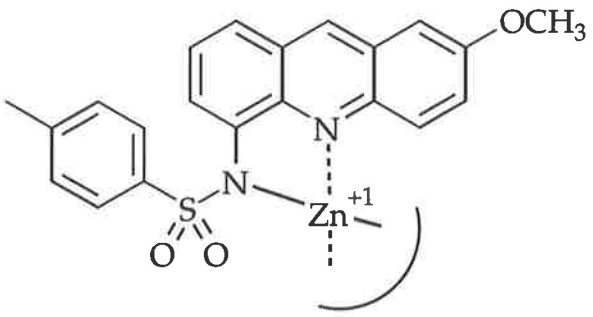


Figure 5.4. Fluorescence emission spectrum of **46**. Dotted line represents ligand excited at 399nm with EDTA, dashed line is the ligand excited at 443nm with EDTA, and solid line is the ligand excited in the presence of Zn(II) at 443nm. Obtained from; dotted/dashed line; [L] 3.4μM, [EDTA] 34μM; [L] 3.4μM, [Zn(II)] 34μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

As previously stated, adventitious Zn(II) and the background fluorescence attributed to it, has been detected in both the fluorescence spectra of ZQA[60] and in the sulfonamides discussed in Chapter 3. Therefore the fluorescence of **46** was determined for the unbound species, with EDTA present, at both 399nm and 443nm. The fluorescence of the unbound ligand at 399nm was determined to be negligible. The fluorescence of **46** in the presence of Zn(II) at 443nm was minimal compared to the fluorescence shown by the Zinquin-Zn(II) complex.

4-Aminoacridine has been reported to be non fluorescent as either the free base or in the monoanionic form.[117] This is in contrast with the 1-, 2- and 3-aminoacridines which are highly fluorescent in either their anionic form or as free molecules.[117] If Zn(II) binding to **46** is analogous to that in the Zinquin-Zn(II) complex then a complex such as the one depicted in **65** would be formed. The Zn(II) has displaced the NH proton of the sulfonamide. This is analogous to the deprotonation of 4-aminoacridine in basic conditions. Since 4-aminoacridine exhibits no fluorescence in basic conditions in ethanol[117] it is of no surprise that the ligand **46**, even though it does bind to Zn(II), does not fluoresce when complexed with Zn(II).



65

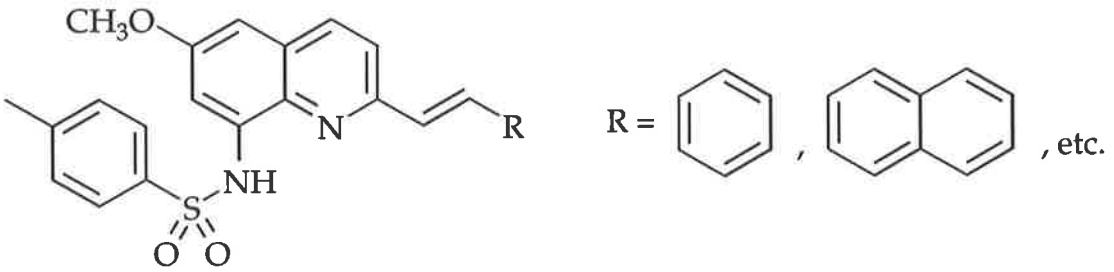
5.4. Conclusions

The acridones, **48**, **49**, can be ruled out as useful ligands because of their apparent weak binding to Zn(II). The acridine ligand, **46**, did shift the wavelength significantly compared to Zinquin, but the fact that the ligand fluoresces relatively weakly in the presence of Zn(II) excludes it from any use for fluorescent *in vivo* work.

Chapter 6 : Additions to the 2-methyl position of Zinquin.

6.1. Strategies to increase the conjugation of Zinquin.

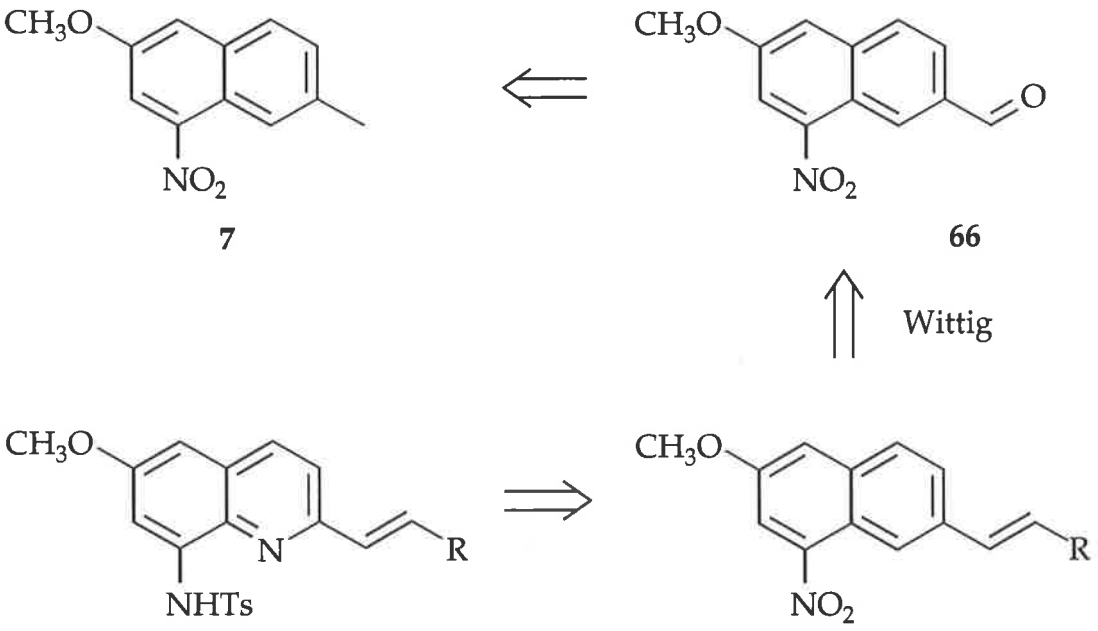
The reason for increasing the conjugation in Zinquin analogues was to enable them to be used with the blue light laser CLM. Of the strategies outlined in Chapter 5, this Chapter is concerned with the synthesis and physical chemistry of quinolines containing attached conjugated groups at the 2-position (Scheme 6.1). There are two reasons for attaching groups at the 2-position; firstly, the 2-methyl group in quinaldine is activated toward oxidation and substitution[123]; secondly, any group attached at this position may impede on the bite area of the ligand, therefore varying the ligands stability with Zn(II).



Scheme 6.1.

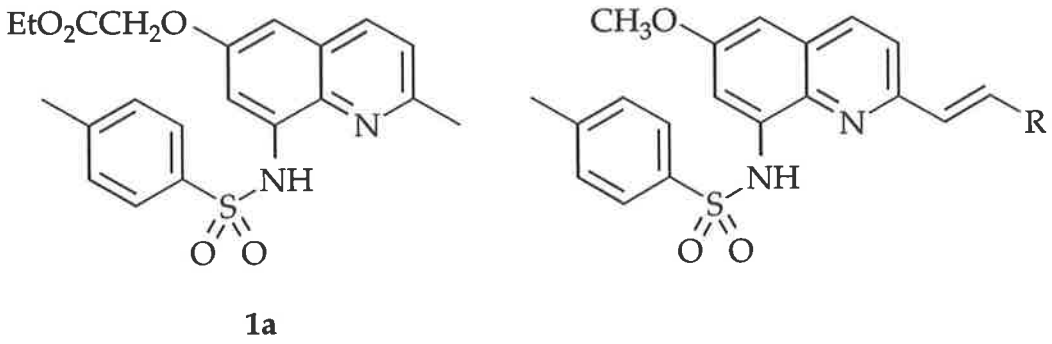
6.2. Synthetic routes toward the ligands.

The retrosynthetic analysis used toward ligands of this type is shown in Scheme 6.2. The advantages of this approach is the ease of oxidation of the starting nitroquinaldine[124,125] **7** and the reported use of Wittig reagents in the coupling of quinoline carboxaldehydes.[126-128]



Scheme 6.2.

As indicated in Chapter 3 and 4, the ester group at the six position of **1a** is not crucial to fluorescence in the Zinquin-Zn(II) complex and it was decided that only the precursor 6-methoxy analogues would be synthesised and tested (Scheme 6.3).



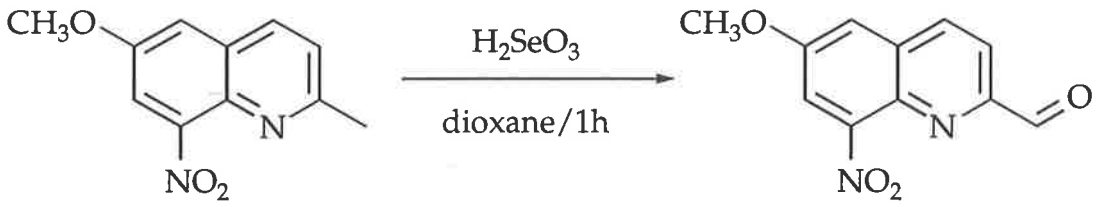
1a

Scheme 6.3.

6.3 Synthesis of the ligands.

The nitroquinoline **7** whose synthesis is outlined in Chapter 2, was oxidised to the 2-formylquinoline **66** using selenious acid (Scheme 6.4).[125] Formation of the aldehyde **66** was confirmed by the ^1H n.m.r. spectrum of

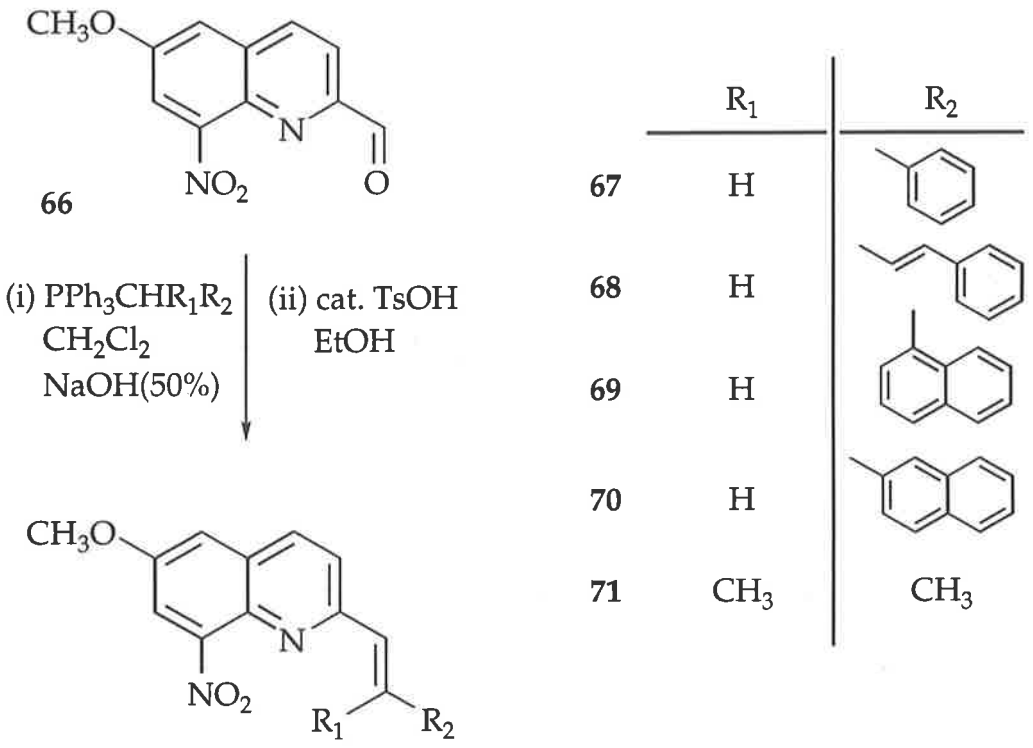
the product with the appearance of a peak at δ10.14 ppm and further supported by a molecular ion of 232.



7 **66**

Scheme 6.4.

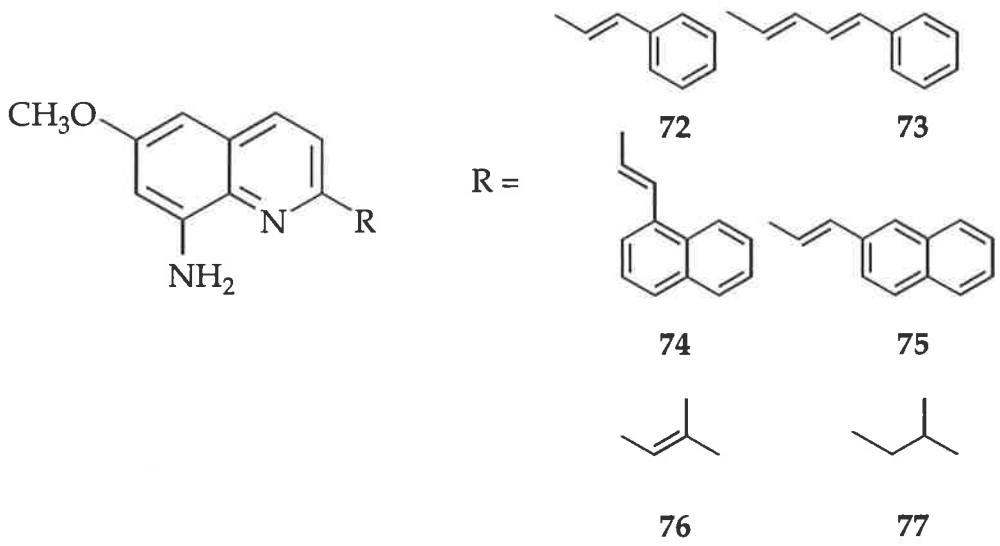
Previous investigations[129] by our group into the formation of **67** had utilised the condensation reaction between **7** and benzaldehyde in the presence of sodium hydride, but this reaction only proceeded in low yields. It was therefore of interest whether a Wittig reaction between the aldehyde **66** and appropriate phosphonium salts would lead to the desired products. The conditions chosen for the Wittig reaction were those used by Markl and Merz[130], since they were simple, mild and required non-anhydrous conditions. This procedure successfully used biphasic conditions to couple aryl aldehydes with both aryl and alkyl phosphoranes. Considering that the *cis* adduct would interfere too much with the binding pocket of the quinoline ligands it was decided that the *trans* adducts would be synthesised. Initially the Wittig adducts were isolated as a mixture of the *cis* and *trans* isomers, but refluxing the mixture in a catalytic amount of *p*-toluene sulfonic acid afforded the pure *trans* adduct (Scheme 6.5).



Scheme 6.5.

Mass spectral and ^{1}H n.m.r. data supported the formation of the Wittig adducts. In particular, the nitroquinolines **67, 69** and **70** had coupling constants between 16.2-16.4Hz in the aromatic region of their ^{1}H n.m.r. spectra consistent with a *trans* stereochemistry between the two vinyl protons.[96] An X-ray analysis of **69** definitively assigned the *trans* stereochemistry of the double bond. The cinnamyl adduct **68** exhibited a more complex ^{1}H n.m.r. spectrum with a number of peaks between δ7.05ppm and δ6.80ppm corresponding to three of the four vinyl protons, consequently the stereochemical assignment of the two double bonds was difficult. However, the coupling constants associated with H_9 and H_{10} (15.3Hz) as well as H_{11} and H_{12} (15.4Hz) were consistent with a *trans* stereochemistry around the two double bonds. Adduct **71** contained a multiplet at δ6.37ppm and 2 doublets at δ2.29ppm and δ1.99ppm indicating the presence of the isobutenyl side chain.

Reduction of the nitroquinolines to their corresponding amino derivatives was achieved by the iron/acetic acid method.[86] The nitroquinoline **71** was also reduced by catalytic reduction to the aminoalkylquinoline **77**.



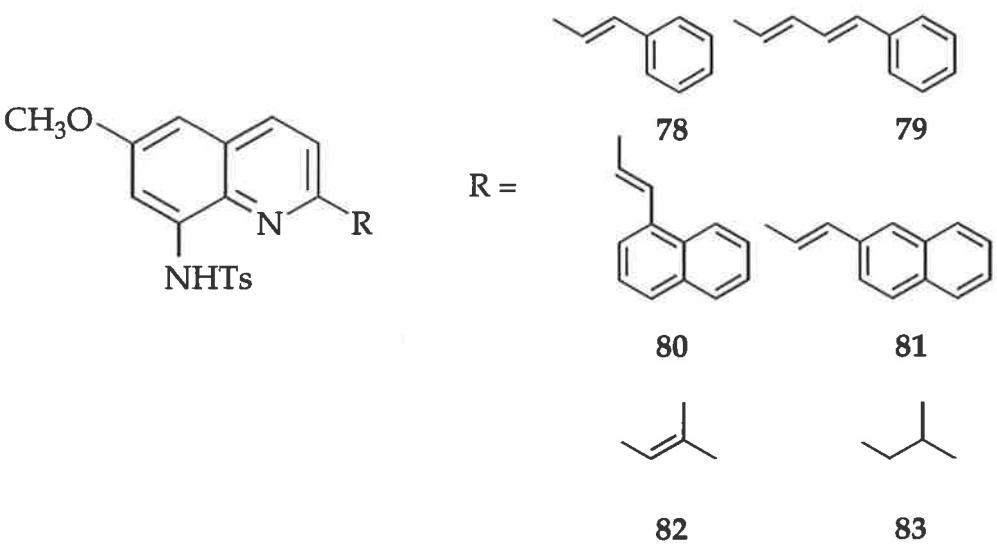
72 **73**

74 **75**

76 **77**

The structure of the aminoquinolines **72-77**, were confirmed by mass, infra-red and ^{1}H n.m.r. spectroscopy. In particular; the infra-red showed the presence of an N-H stretching at 3500 and 3380cm^{-1}, and this was supported by it's ^{1}H n.m.r. spectrum with a broad singlet at approximately δ5.0ppm. The *trans* stereochemistry of amines **72-75** was maintained using the iron reduction as indicated by their ^{1}H n.m.r. spectra coupling constants of their vinyl protons. The aminoquinoline **77** showed additional ^{1}H n.m.r. signals from the reduced alkyl chain at C_2; a 1H multiplet at δ2.21ppm and diastereotopic protons at δ2.75ppm due to the methylene group. The ^{1}H n.m.r spectrum of **72-77** also showed upfield shifts for H_5 and H_7 relative to the corresponding signals of the nitroquinolines.

The aminoquinolines were tosylated under standard conditions to yield the required sulfonamides **78-83**.



The structures of the sulfonamides were confirmed by mass, infra-red and ^1H n.m.r. spectroscopy. Ions resulting from the loss of $C_7H_7SO_2$ from the molecular ion were seen for all compounds and sulfonamide N-H absorbances at approximately 3250cm^{-1} were seen in the infra-red spectra. The *trans* stereochemistry of sulfonamides **78-81** was confirmed by ^1H n.m.r. coupling constants of the vinyl protons.

6.4. The uv/visible and fluorescence spectroscopic study of the quinolines.

All of the quinolines exhibited a bathochromic shift in the presence of Zn(II) (Figure 6.1 and 6.2). Quinolines **82** and **83** showed similar bathochromic shifts to those seen when the methoxy analogue of Zinquin **9** is in the presence of Zn(II). The cinnamyl quinoline **79** showed only a slight bathochromic shift in the presence of Zn(II)

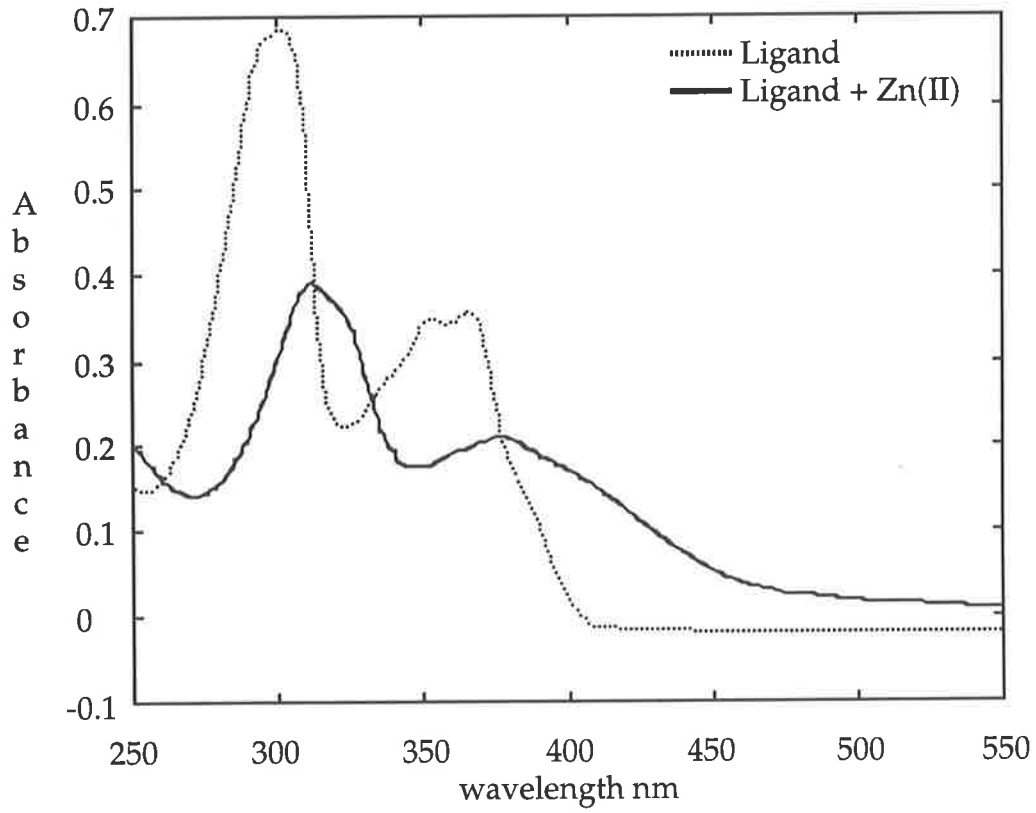


Figure 6.1. Partial uv/visible-spectrum of **78** showing a strong bathochromic shift of the longest absorbance of 363nm to 377nm in the presence of Zn(II). Obtained from; dotted line, [L] 8.3μM, [EDTA] 83μM ; solid line, [L] 8.3μM, [Zn(II)] 83μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

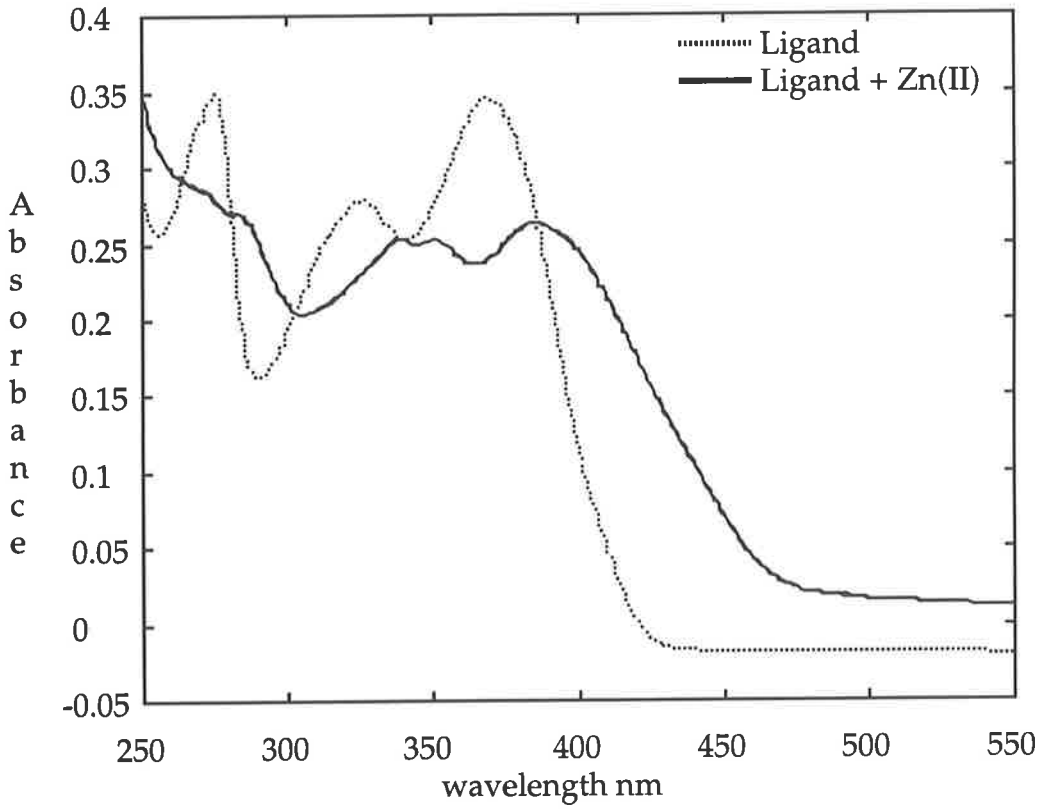


Figure 6.2. Partial uv/visible-spectrum of **80** showing a strong bathochromic shift of the longest absorbance of 369nm to 388nm in the presence of Zn(II). Same solvent system / concentrations as in Figure 6.1.#

Increases in the wavelength maxima of the ligand was observed for all the quinolines, **78-83**, over the precursor quinoline **9**. Table 6.1. indicates that the extra double bonds of **78-82** causes the absorbance maximum of the ligand to shift to a higher wavelength compared to **9** (which is a Zinquin analogue).

The remaining uv/visible spectra of **79**, **81**, **82** and **83** are contained in Appendix A.

Table 6.1. The uv/visible data of **9, 78-83** in the presence and absence of Zn(II).[a,b]

Quinoline	Ligand longest wavelength absorptions (nm)		Bathochromic shift (nm)
	Ligand	Ligand + Zn(II)	
9[a]	337	357	20
78[b]	363	377	14
79[b]	378	379	1
80[b]	369	388	19
81[b]	374	390	16
82[a]	348	382	34
83[a]	333	377	44

a) Ligand; [L] 16.5μM, [EDTA] 165μM. Ligand + Zn(II); [L] 16.5μM, [Zn(II)] 165μM.

b) Ligand; [L] 8.3μM, [EDTA] 83μM. Ligand + Zn(II); [L] 8.3μM, [Zn(II)] 83μM.

Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

The quinolines **78, 80, 81, 82, 83** all exhibited a bathochromic shift in the presence of Zn(II). The cinnamyl quinoline **79** showed only a slight bathochromic shift in the presence of Zn(II). This may suggest that the inclusion of a second double bond has increased the longest wavelength of absorption of **79** to a point where it is close to the longest wavelength of absorption of **79** in the presence of Zn(II), hence the small bathochromic shift observed. The type of aryl group appears to be a secondary factor compared to the number of double bonds when considering the amount of bathochromic shift; illustrated by comparing the styryl quinoline **78** to both the naphthyl quinolines **80, 81**. Even though the inclusion of a double bond at the 2-position has increased the absorption maxima of these ligands compared to **9** the amount of this shift is not sufficient for use in the blue light laser CLM.

The fluorescence of the quinolines **78-83** in the absence and presence of Zn(II) was measured at their longest wavelength absorbance values (Tables 6.2, 6.3).

Table 6.2. The fluorescence values obtained for the quinolines **9, 78-83.**[a]

Quinoline	Excitation wavelength (nm)	Fluorescence of the ligands	
		Emission (nm)	Value
9	337	477	31.0±1.8
78	363	410	36.0±1.8
79	378	427	157.5±7.6
80	369	428	196.1±9.2
81	374	416	131.4±6.2
82	348	488	12.1±0.7
83	333	475	9.0±0.5

a) Obtained from; [L] 3.4μM, [EDTA] 34μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

Results shown in Table 6.2. indicate that the incorporation of conjugated aromatic groups has increased the amount of fluorescence arising from the quinolines **78-81**. Quinolines **82** and **83** showed little or no natural fluorescence.

Table 6.3. The fluorescence values obtained for the quinolines **9, 78-83** in the presence of Zn(II).[a]

Quinoline	Excitation wavelength (nm)	Fluorescence of the ligand	
		Emission (nm)	Value
9	357	477	601.2±33.2
78	377	512	319.8±15.6
79	379	518	360.2±17.3
80	388	515	590.2±27.7
81	390	516	462.3±21.7
82	382	488	872.4±45.4
83	377	475	705.3±36.7

a) Obtained from; [L] 3.4μM, [Zn(II)] 34μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).

Table 6.3 shows the fluorescence values obtained for the quinolines **9, 78-83** in the presence of Zn(II). Table 6.3. indicates that quinolines **82** and **83** exhibit the highest fluorescence and that this fluorescence is higher than that of the Zinquin precursor **9**. The isobutenyl quinoline **82** showed the largest increase in fluorescence (45%) compared to the precursor of Zinquin **9**. The isobutyl quinoline **83** also showed an increased fluorescence (20%) relative to the Zinquin analogue **9**, however this fluorescence was not as much as observed for **82**.

Table 6.2 shows that significant fluorescence was exhibited by the quinoline ligands, **78-81** in the absence of Zn(II). However, the fluorescence values for the quinoline-Zn(II) complexes, **78-81**, shown in Table 6.3 are considered to arise predominantly from the quinoline-Zn(II) complexes, **78-**

81, due to the high concentration of total Zn(II) in the fluorescence solutions. The fluorescence values for quinoline-Zn(II) complexes, **78-81,** show that none are higher in fluorescence than **9**.

6.5. Conclusions.

Addition of conjugated groups to the basic quinoline structure does increase the overall longest wavelength absorbance values both in the absence and presence of Zn(II), but this has been at the cost of a decrease in fluorescence. Additionally the conjugated quinolines **79-81** exhibit a strong natural fluorescence in the absence of Zn(II) which make them less suitable for use as a Zn(II) ligand for cellular studies. The isobutenyl quinoline **82** did increase the longest wavelength absorbance value of the ligand in the presence of Zn(II) by 25nm and increases fluorescence by 45% in the presence of Zn(II), relative to the Zinquin analogue **9**. The isobutyl quinoline **83** also accomplished this, but not to the same extent, increasing the absorbance by 20nm and increasing the fluorescence by 20% in the presence of Zn(II) relative to **9**.

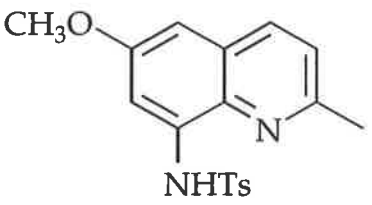
The attachment of conjugated groups has not increased the maximum wavelength of absorbance sufficiently to be used with the blue light laser CLM. However, during this research an ultra-violet CLM did become commercially available. The styryl **78**, isobutyl **82** and isobutenyl quinolines **83** do represent potentially useful ligands in their own right for the following reasons; **78** exhibits the lowest natural fluorescence out of all the conjugated quinolines; all three ligands have increased maximum absorbance wavelengths relative to **9**; and both **83** and **82** show increased fluorescence relative to **9**.

The "bite" of the three quinolines **78, 82, 83** could possibly be hindered relative to Zinquin and this may lead to a series of ligands that form Zn(II) complexes of differing stability, however this would need to be investigated in future studies. Simple removal of the methoxy group followed by alkylation with ethyl bromoacetate, using the protocols established in Chapter 2, should afford new potential fluorophores for Zn(II) *in vivo* . The synthesis of these new potential ligands will be discussed in Chapter 7.

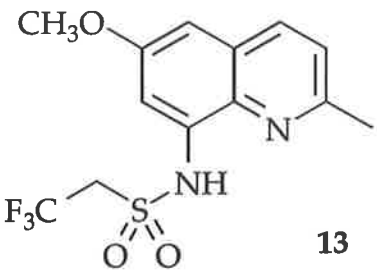
Chapter 7 : The biologically useful ligands

7.1. Introduction.

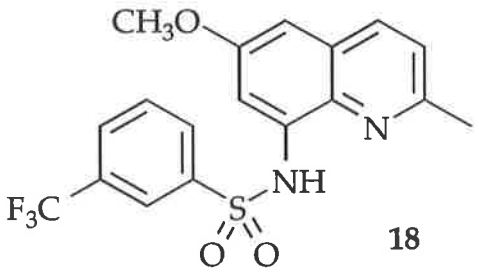
Of all the potential ligands so far described, five **13, 18, 78, 82, 83** have comparable or increased fluorescence compared to the Zinquin analogue **9**. For these ligands to be able act in a way similar to ZQE a hydrolysable ester side chain must be attached for use *in vivo*. The inclusion of a hydrolysable side chain can easily be accommodated by demethylation and alkylation of the compounds below using the Zinquin synthesis protocols established in Chapter 2.



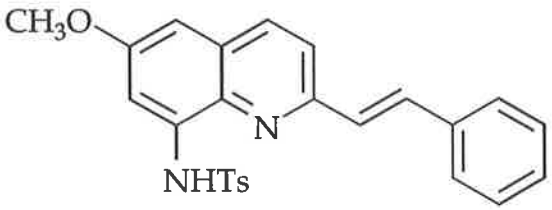
9



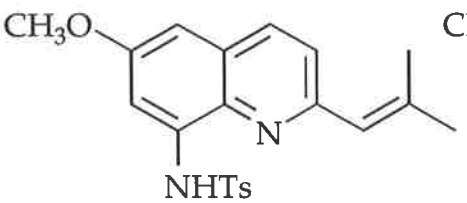
13



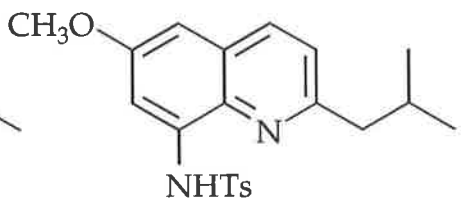
18



78



82



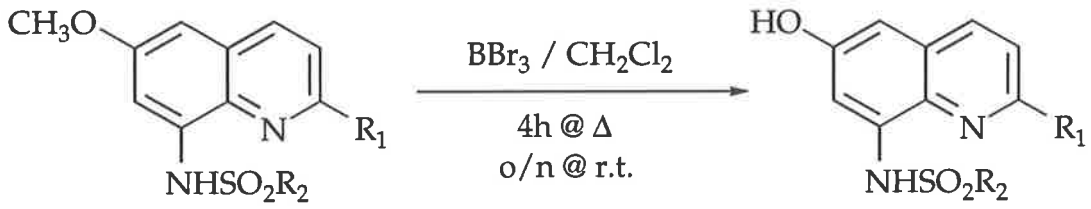
83

The above ligands were tested for their metal ion selectivity. For example their fluorescence in the presence of biologically important cations,

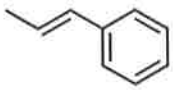
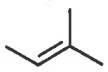
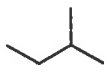
such as Ca(II), Mg(II), Fe(II) and of course Zn(II). In addition, the fluorescence of the above ligands will be compared to that of ZQE, in the presence of Zn(II).

7.2. The attachment of the ester side chain.

Compounds **13, 18, 78, 82** and **83** were subjected to the conditions used for the demethylation of **9**. Four of the five compounds were demethylated to form their hydroxy analogues with only exception being the 2,2,2-trifluoroethyl sulfonamide **13**, (Scheme 7.1).



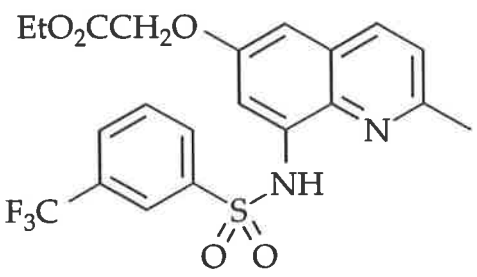
Scheme 7.1.

Compound	R_1	R_2	Hydroxy analogue.
18	CH_3	*m*-$CF_3C_6H_4$	**84**
78		*p*-$CH_3C_6H_4$	**85**
82		*p*-$CH_3C_6H_4$	**86**
83		*p*-$CH_3C_6H_4$	**87**

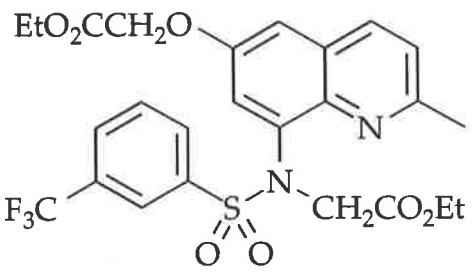
The ^1H n.m.r. spectrum from the demethylation of **13** showed the disappearance of the 2,2,2-trifluoroethyl group. It was rationalised that the conditions had cleaved both the methyl ether and the sulfonamide. Consequently, a different method was investigated in the attachment of the ester side chain to **13**, this will be discussed later in this Chapter.

The remaining hydroxy compounds were isolated in good to excellent yields and the formation of each was supported by the disappearance of the methoxy group characteristically seen at δ3.84ppm in the ^{1}H n.m.r. spectrum. This evidence was further supported by mass spectral data which illustrated abundant molecular ions corresponding to the demethylated products.

The four hydroxy compounds **84, 85, 86, 87** were alkylated with ethyl bromoacetate and a suitable base. The product obtained from the alkylation of **84** was identified as the diester **89** not the required ester **88** as shown by ^{1}H n.m.r. evidence. In particular, two methylene signals at δ4.75ppm and δ4.73ppm and two ethyl ester signals. Uv/visible spectrum of **89** showed no bathochromic shift when in the presence of Zn(II). This is consistent with the sulfonamide being alkylated and therefore being unable to participate in binding to Zn(II). Alkylation at the NH of the sulfonamide was not surprising considering that the *m*-trifluorobenzene group is electron withdrawing and therefore would increase the probability of nucleophilic attack by the nitrogen in basic conditions.



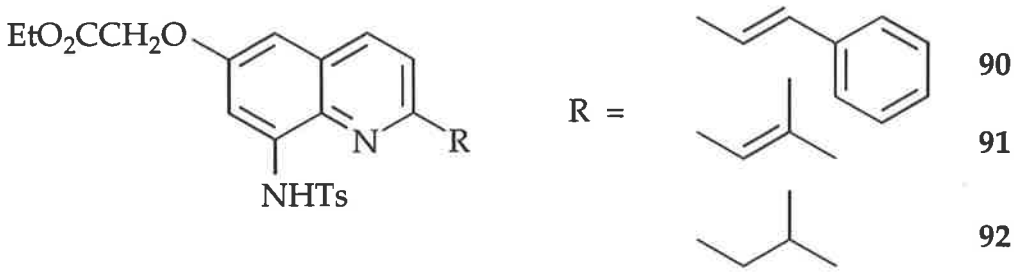
88



89

The remaining phenols **85, 86, 87** yielded the required esters **90, 91, 92** when alkylated with ethyl bromacetate. The esters were characterised by their ^{1}H n.m.r. spectra; appearance of a methylene signal at δ4.65ppm and ethyl ester signals at δ4.27ppm and δ1.29ppm. Formation of the esters was

further confirmed by infra-red spectroscopy, in particular a carbonyl absorbances at approximately 1760cm^{-1} is characteristic[97] for this type of ester.



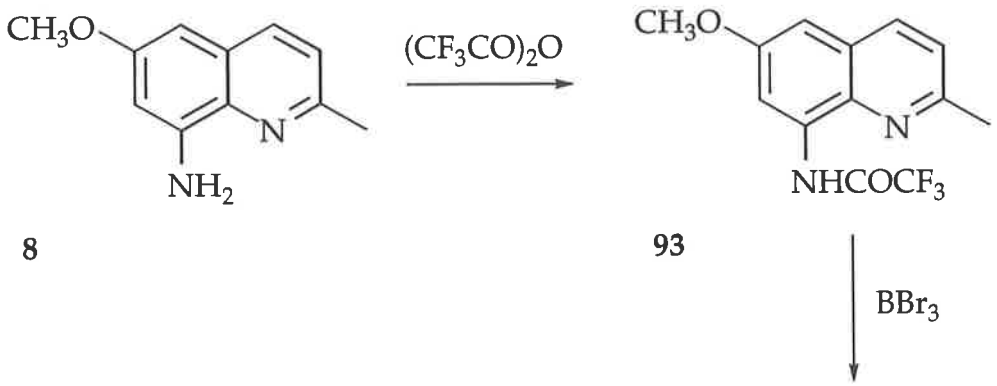
90

91

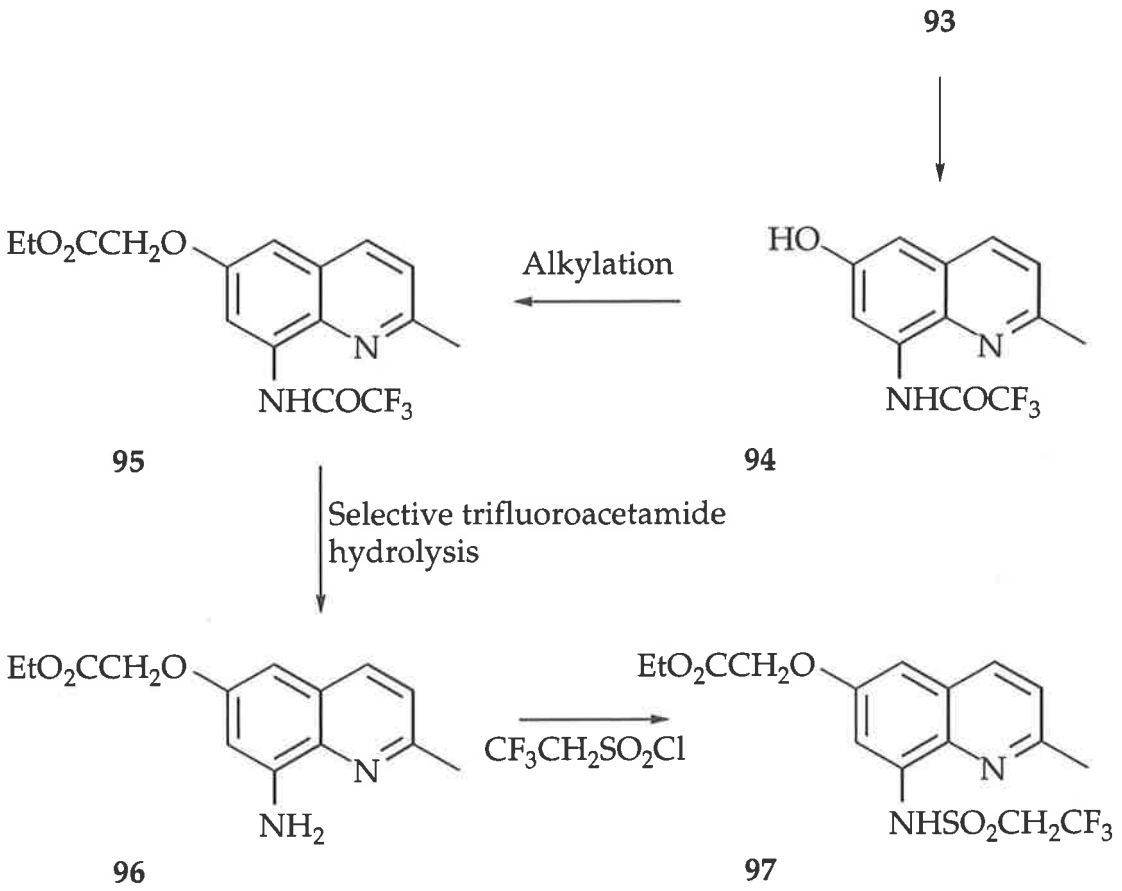
92

7.3. Formation of the 2,2,2-trifluoroethyl sulfonamide ligand, 97.

Due to the failure in the demethylation of **13**, an alternate approach to the synthesis of **97** was pursued (Scheme 7.2). The method adopted involves the initial protection of the amino group of **8** as a trifluoracetamide **93** since this group is stable to acid conditions and to Lewis acids such as boron tribromide which is used in the demethylation.[131] Demethylation of **93** would be expected to yield the phenol **94**, which when alkylated could yield the ester **95**. Deprotection of the amine under selective hydrolysis conditions followed by sulfonamide formation could then yield the required compound **97**.



8 **93**



Scheme 7.2.

The trifluoroacteamide **93** was characterised by its ^1H n.m.r. spectrum with the appearance of an acetamide NH signal at δ10.76ppm and an absorbance of 3300cm^{-1} in the infra-red spectrum. An absorbance at 1705cm^{-1}, characteristic of the acetamide carbonyl further confirmed the formation of **93**. Demethylation of **93** yielded the anticipated phenol **94** in a modest yield. The structure of the phenol was confirmed by its ^1H n.m.r. spectrum with the appearance of an hydroxy resonance at δ9.31ppm, consistent with an aromatic phenol. The phenol was then alkylated using ethyl bromoacetate with potassium carbonate as the base to yield **95**. The structure of the ester **95** was confirmed by the appearance of ^1H n.m.r. resonances at δ4.73ppm, δ4.27ppm and δ1.26ppm with the former corresponding to the methylene protons and the two latter resonances corresponding to the ester signals.

Boger and Yohannes[132] have described a method for the selective hydrolysis of trifluoroacetamide groups in the presence of a methyl ester. However, applying these conditions[132] to the ethyl ester **95**, resulted in the cleavage of both the ester and the trifluoroacetamide group. Consequently the crude product was resterified under standard conditions and this generated the desired aminoquinoline ester **96** in an excellent yield. The aminoquinoline **96** was characterised by its ^{1}H n.m.r. spectrum with a broad singlet at δ4.99ppm indicative of an amino group and ester resonances at δ4.26ppm and δ1.27ppm, respectively.

The aminoquinoline **96** was treated with 2,2,2-trifluoroethyl sulfonyl chloride yielding the desired sulfonamide **97**. The ^{1}H n.m.r. spectrum of the sulfonamide **97** showed the characteristic sulfonamide NH resonance at δ9.30ppm and this was further supported by mass spectroscopy with a fragmentation peak at 259 (M-$CF_3CH_2SO_2$). Furthermore, the ^{1}H n.m.r. spectrum of **97** showed the resonances of the 2,2,2-trifluoroethyl group, previously described in Chapter 3.

7.4. The fluorescence of ligands 90, 91, 92, 97.

A direct fluorescence comparison of ligands **90, 91, 92, 97** with ZQE **1a**, in the presence of Zn(II) is shown in Figure 7.1. The figure clearly shows that all ligands **90, 91** and **92** have increased fluorescence over ZQE **1a** in the presence of Zn(II). **97** exhibited decreased fluorescence relative to **1a**. The first five fluorescence measurements were obtained at a slit width of 5nm, but ligand **1a** ZQE and **91** were also measured at a slit width of 2.5nm. This is so that the fluorescence value of **1a** would be on scale relative to **91**.

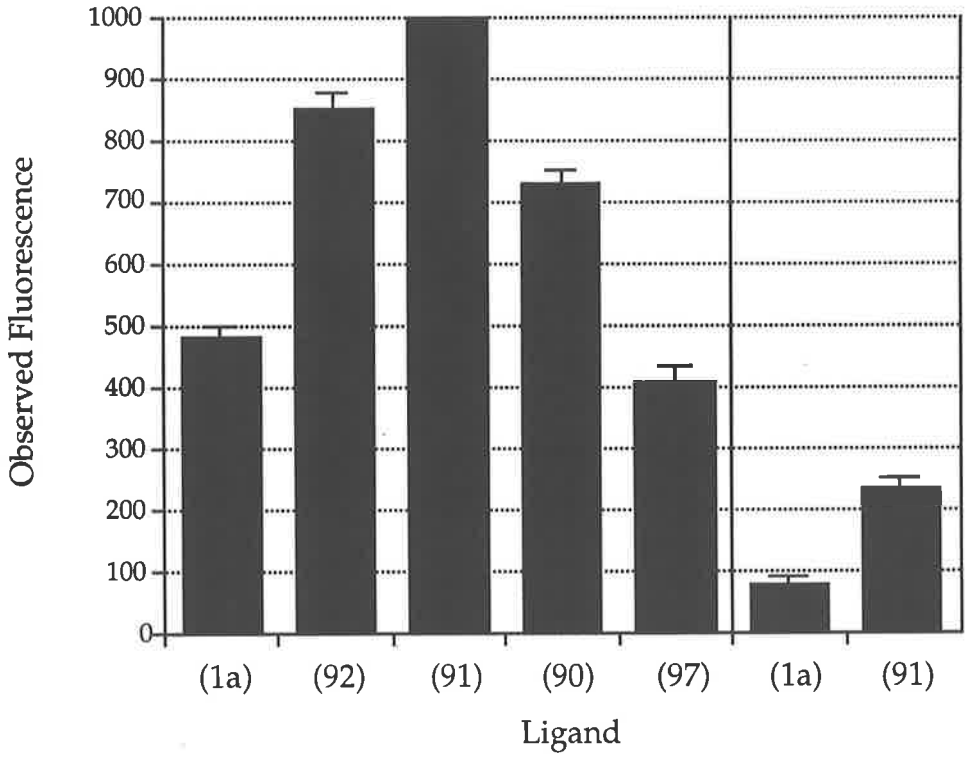


Figure 7.1. The relative fluorescence of ligands **90, 91, 92, 97** compared to ZQE **1a**. The fluorescence of ligand **91** and ZQE **1a** were take at a reduced slit width of 2.5nm since ligand **91** was off scale at a slit width of 5nm. Obtained from; [L] 2μM, [Zn(II)] 20μM. Solvent; 1mM Na PIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).#

It has been observed that the fluorescence of **97** in the presence of Zn(II) decreased over time. The initial solution of the ligands is made in

Actual fluorescence values are contained in Appendix B.

DMF and it appears that **97** maybe unstable in DMF resulting in decreased fluorescence compared to the results obtained in Chapter 3. Consequently, this may preclude **97** from being used in any cellular work.

It must be noted that a direct comparison of the fluorescence of ligands **90, 91, 92, 97** with that of ZQE, **1a**, in the presence of Zn(II) does not take into consideration the relative abundances of each species: either as ligand, ligand-Zn(II) complex or/and di-ligand-Zn(II) complex.[60] The relative abundance of each of these species will be different for each of the new potential ligands, a consequence of each ligand displaying differing stabilities in the presence of Zn(II). Therefore no conclusions have been derived from the comparisons shown in Figure 7.1. since any conclusions would be valid only for the stated concentrations. It is clear though, that the isobutyl side chain on **91** has markedly increased fluorescence compared to ZQE **1a**.

The selectivity of each of the ligands **90, 91, 92, 97** was determined by comparing their relative fluorescence values in the absence and presence of a number of metal cations; Zn(II), Cd(II), Mn(II), Ca(II), Mg(II), Ba(II), Fe(II), Fe(III), Co(II), Ni(II) and Cu(II). The fluorescence values shown in Figures 7.2., 7.3., 7.4., and 7.5. were taken at a slit width of 2.5nm;

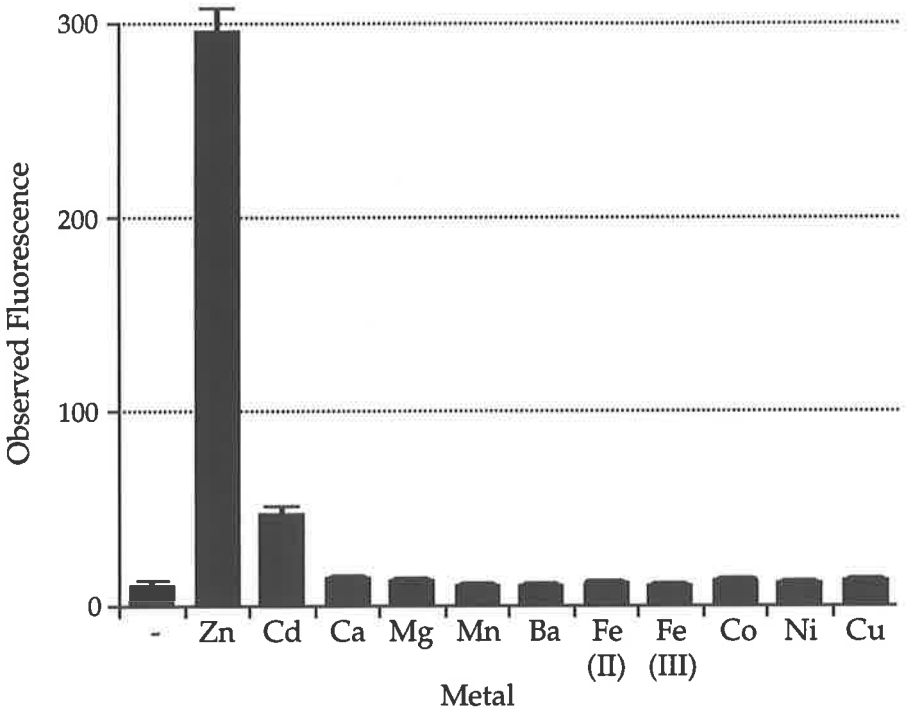


Figure 7.2. Fluorescence of **92** with a number of metal cations. Excitation wavelength 370nm, emission wavelength 478nm. Obtained from; [L] 4μM, [M] 4μM. Solvent; 1mM NaPIPES, 100mM $NaClO_4$, ethanol/water (75:25, v/v).#

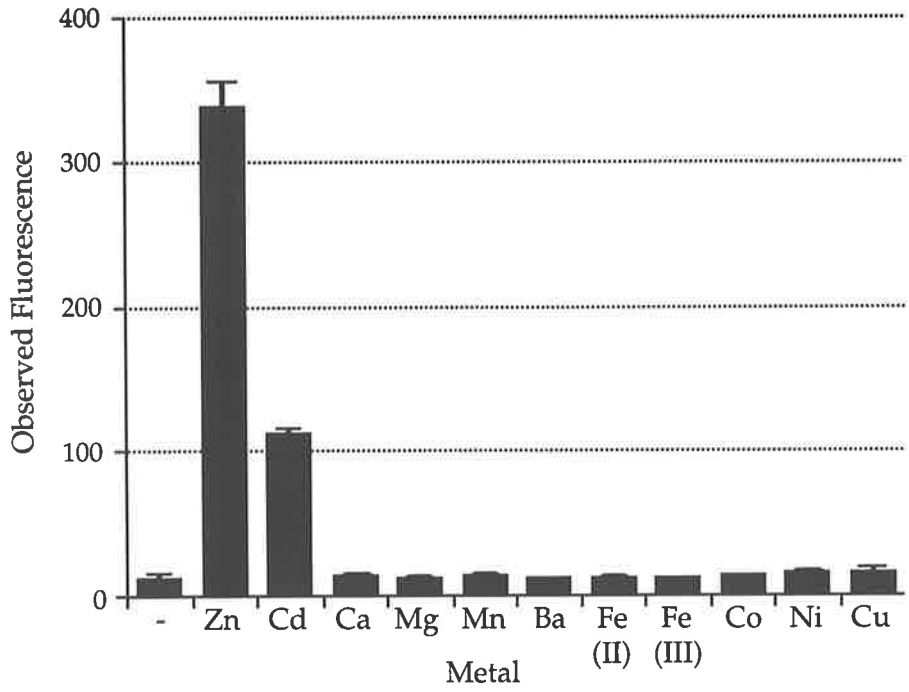


Figure 7.3. Fluorescence of **91** with a number of metal cations. Excitation wavelength 372nm, emission wavelength 487nm. Concentrations / solvent system as Figure 7.2.#

Actual fluorescence values are contained in Appendix B.

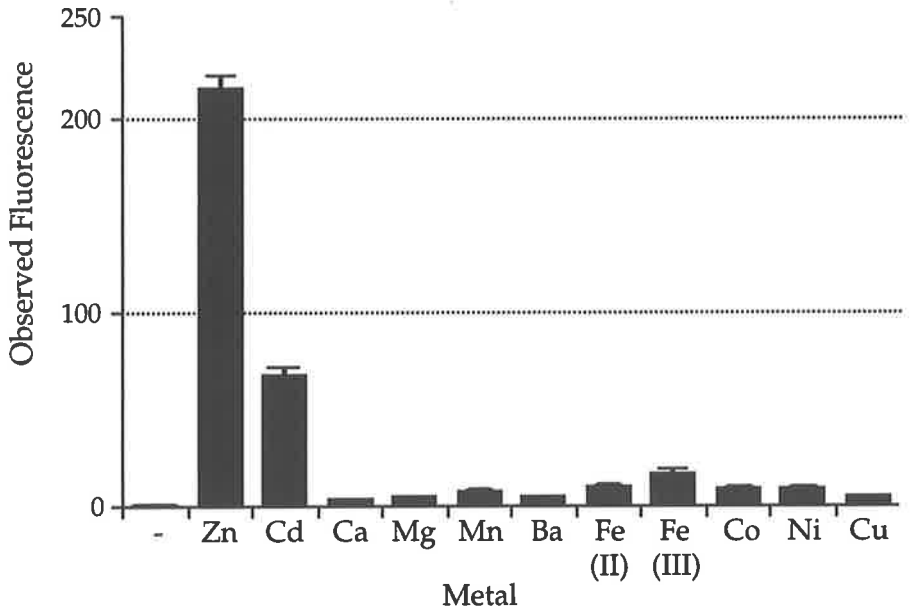


Figure 7.4. Fluorescence of **90** with a number of metal cations. Excitation wavelength 375nm, emission wavelength 516nm. Concentrations / solvent system as Figure 7.2.#

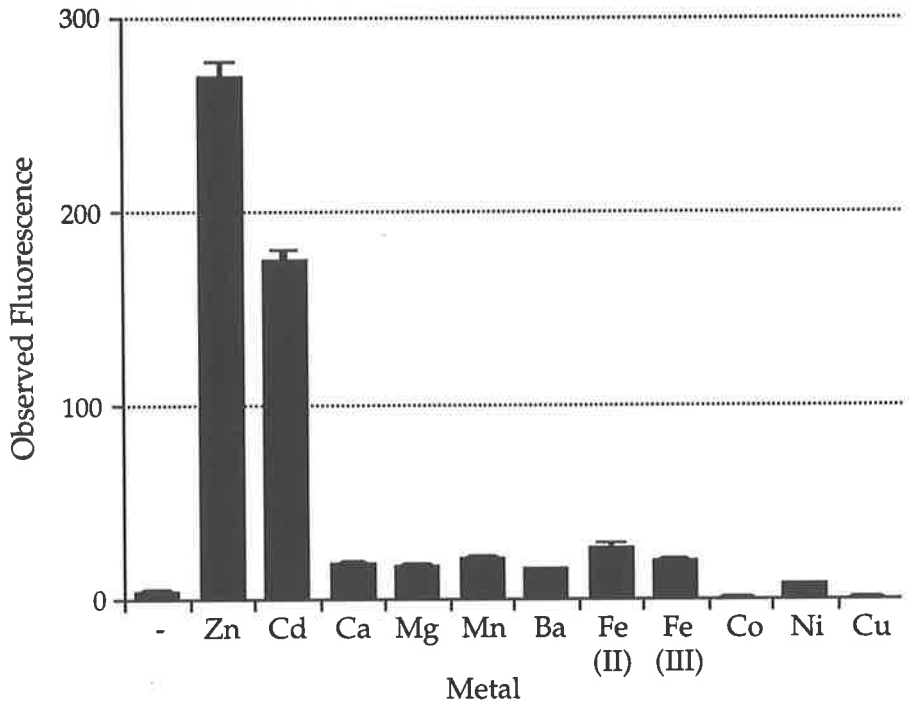


Figure 7.5. Fluorescence of **97** with a number of metal cations. Excitation wavelength 352nm, emission wavelength 467nm. Concentrations / solvent system as Figure 7.2.#

Actual fluorescence values are contained in Appendix B

All the ligands showed selectivity among the metal cations with the only exceptions being Cd(II) and Zn(II). The fluorescence that each ligand exhibited with all the metals, except Zn(II) and Cd(II), can be attributed to adventitious Zn(II), previously discussed in Chapter 2, present in the metal cation solutions. Fluorescence attributed to adventitious Zn(II) was observed in the Cd(II) fluorescence samples, however, this adventitious Zn(II) was removed by stirring a solution of ligand (dichloromethane) with EDTA, a known metal chelator. Consequently, the fluorescence results shown above of ligands (**1a, 90, 91, 92** and **97**) in the presence of Cd(II) are true fluorescence measurements.

The selectivity between the two remaining metals, Zn(II) and Cd(II), differed from ligand to ligand. The isobutyl ligand **92** exhibits the most selectivity; illustrated by the relatively low fluorescence when in the presence of Cd(II) as compared to ZQE **1a** in the presence of Cd(II). Selectivity of the isobutenyl ligand **91** was less than that of **92** but more selective than ZQE **1a** when in the presence of Cd(II).

The tresyl ligand **97** exhibited increased fluorescence relative to **90** in the presence of Zn(II) and this result is in contrast to the result shown in Figure 7.1. This can once again be explained by the instability of **97**. The results of **97** shown in Figure 7.1 were obtained from a stock solution of **97** in DMF which was 4 weeks old. Consequently, the fluorescence of **97** in the presence Zn(II) was significantly reduced compared to the results shown in Figure 7.5.

7.5 How the 90, 91, 92 and 97 performed *in vivo* .

The ligands **1a, 90, 91, 92** and **97** were used in the staining of rheumatoid arthritis synovial tissue (the staining of which was performed

by Dr W. H. Betts, at the Queen Elizabeth Hospital). The primary reason for carrying out this staining was to establish if these new potential Zn(II) ligands would be suitable for *in vivo* work.

Initially, each ligand was dissolved in ethanol to form a 1mM stock concentration which was then diluted to a 25μM concentration, however each ligand exhibited differing solubilities in ethanol. The solubilities were; **90**, poor solubility, **91** fair solubility, **92** poor solubility, and **97** very soluble. The lack of solubility of **90, 91** and **92** in ethanol could be overcome by forming the stock solutions using a dipolar aprotic solvent such as DMSO, which have been previously employed in delivering ZQE into tissue/cellular sections.[56,57,61,76]

Slides, stained with the given fluorophores, were 5μm paraffin sections of tissue taken from the synovium of an untreated rheumatoid arthritis patient. To remove the wax (paraffin), slides were heated at 60°C followed by xylene and ethanol treatments respectively. Staining was carried out overnight at 4°C, using 25μM concentrations of the fluorophores. Sections were mounted in fluorescence mounting medium, coverslips were applied, and these were then sealed with nail polish, since the mounting medium is not a permanent fixative. Each ligand exhibited Zn(II) dependant fluorescence when introduced into the tissue sample. The Pseudo coloured fluorescence images of a similarly stained area of the rheumatoid arthritis synovial tissue is shown in Figure 7.6(A-E) (yellow denotes areas of maximal intensity, followed by green and then light-blue).

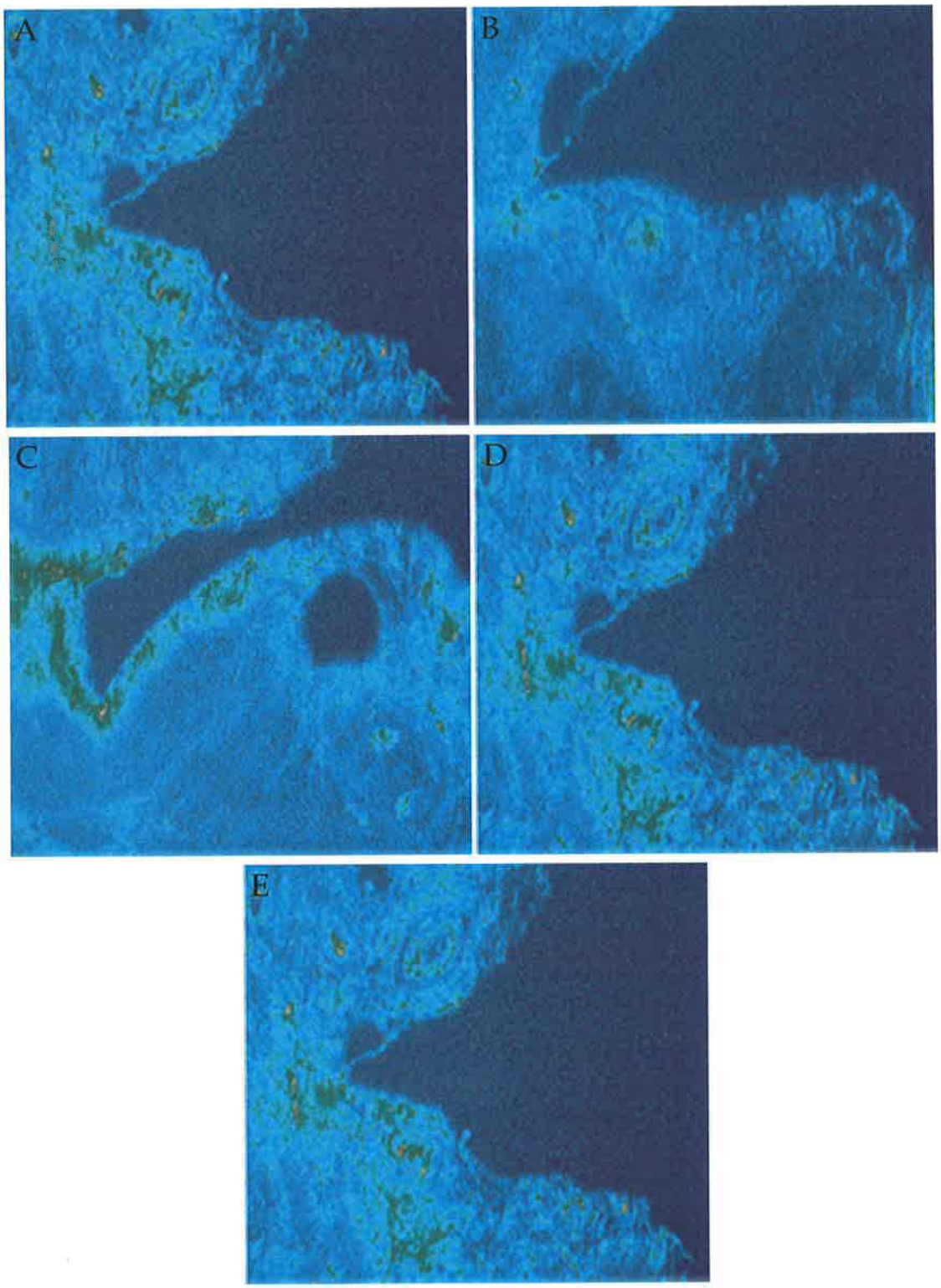


Figure 7.6. Pseudo coloured fluorescence images of similarly sections of rheumatoid arthritis synovial tissue stained with one of the five ligands (A) ZQE, **1a** (B) **92**, (C) **91**, (D) **90**, (E) **97**. The level of Zn(II) dependant fluorescence is due to the high amount of Zn(II) typically seen within tissue of this type.

7.6 Conclusions.

Both **91** and **92** represent significant improvements on ZQE; **91** exhibits a 3 fold increase in fluorescence and **92** exhibits a two fold increase in fluorescence in the presence of Zn(II), respectively, compared to ZQE,. Furthermore, both **91** and **92** showed increased selectivity toward Cd(II). This was particularly evident in the case of **92** which showed low fluorescence in the presence of Cd(II) compared to the remaining ligands, **1a**, **90**, **91** and **97** when in the presence of Cd(II). In addition, all of the ligands **90**, **91**, **92** and **97** exhibited Zn(II) dependant fluorescence when introduced into rheumatoid arthritis synovial tissue, implying that they would be suitable for *in vivo* work.

The different selectivities that each ligand exhibited for Cd(II) and Zn(II) can only be explained fully by determining the stability constants for their Cd(II) and Zn(II) complexes. An introductory investigation into the stability of these ligands by electrospray ionisation (ESI) has been done, and is described in Chapter 8. This ESI study is not quantitative but allowed a qualitative stability series of these ligands, **90**, **91**, **92**, **97** with Zn(II) and Cd(II) compared to ZQE, **1a**, to be determined.

Chapter 8 : Electrospray studies.

8.1 Introduction.

Many methods exist for the determination of the stability of organic-metal ion complexes amongst which are potentiometry, spectrophotometry, specific ion EMF measurements, NMR spectroscopy, polarography, ion exchange, colourimetry and electrospray mass spectroscopy (ESI).[52,133-136] The last method, ESI mass spectroscopy, was chosen to determine the relative stabilities of the complexes of **90**, **91**, **92** and **97** with Zn(II), relative to ZQE, **1a**, because of its simplicity and efficiency.

8.2. The ESI Technique.[137]

The ESI technique allows the direct transfer of analyte species from the condensed phase into the gas phase as isolated entities. This is accomplished by a three step process; droplet formation, droplet shrinkage and gaseous ion formation.[137] The sample solution is introduced into the tip of the electrospray capillary where it experiences a electric field associated with the maintenance of the tip at high potential, see Figure 8.1. If a positive potential is applied, then positive ions in the solution will accumulate at the surface.

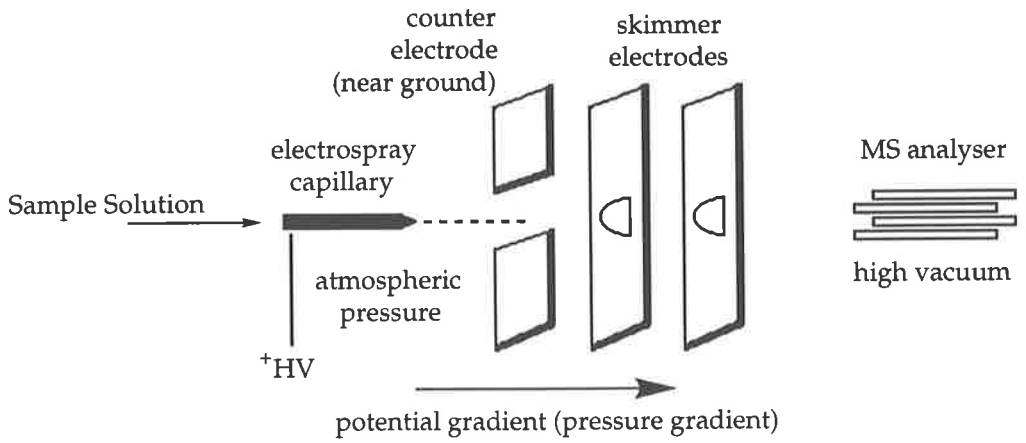


Figure 8.1. The features of an electrospray interface.[137]

The solution is then drawn out by a pressure gradient, to form a "Taylor cone", see Figure 8.2. At sufficiently high potential the cone is drawn out producing a filament which can then generate positively charged droplets, through a budding process. The diameter of the droplet is dependant on a number of parameters such as; the applied potential, the solution flow rate and solvent properties.[137]

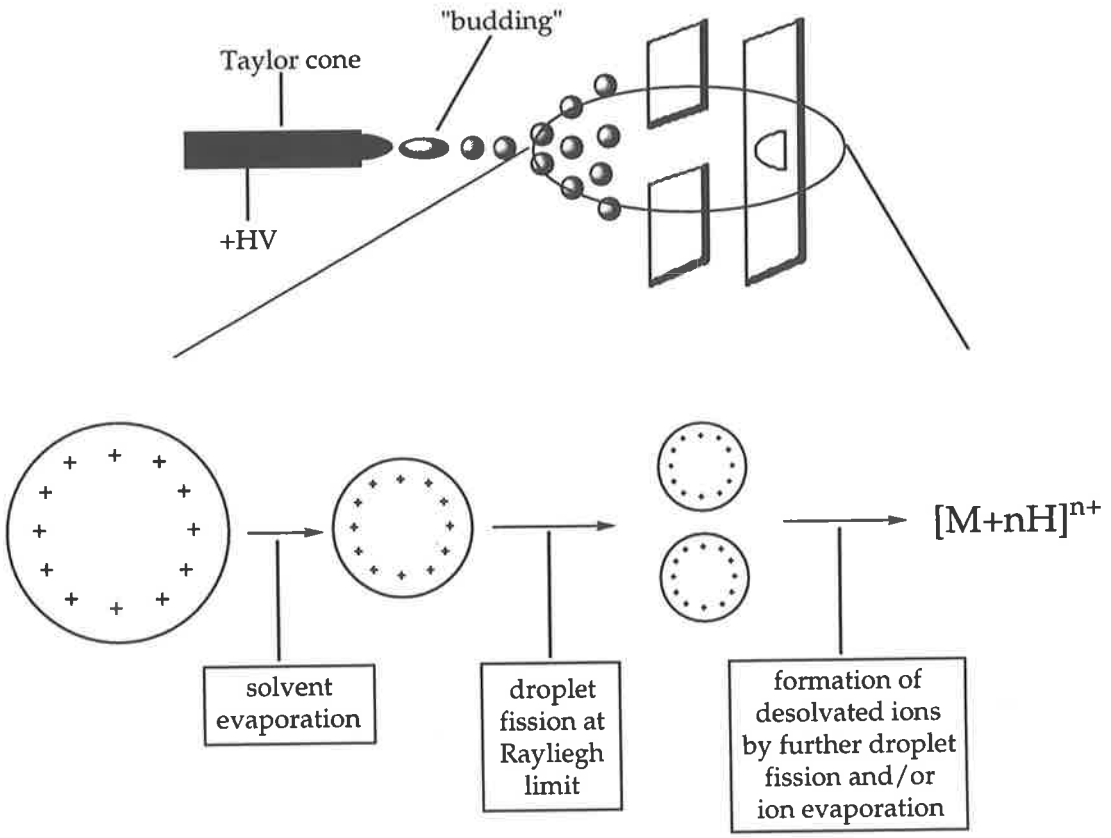


Figure 8.2. Droplet formation in the electrospray interface.[137]

Solvent evaporation from the droplets, as they traverse the pressure gradient towards the analyser of the mass spectrometer, leads to a reduction in the diameter of the droplet, Figure 8.2. Fission of the droplet occurs when the magnitude of the charge is sufficient to overcome the surface tension holding the droplet together. This is termed the "Coulombic explosion" and the point at which it occurs is called a "Rayleigh limit". Charged ions can now enter the analyser of the mass spectrometer.

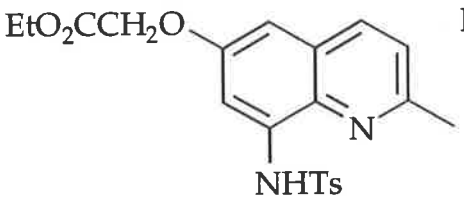
8.3. How the ESI technique can be used in determining a stability series.

The ESI technique has been shown to be a very useful tool in determining the complexing abilities of a range of crown ether complexes.[138-142] It was envisaged that a relative stability series could be determined for the ligands **1a, 90, 91, 92** and **97** as they compete for Zn(II), using the ESI technique. Since ESI allows the direct transfer of analyte species from the condensed phase into the gas phase as isolated entities, the initial formation of the complexes in solution followed by ESI investigation should lead to this relative stability series.

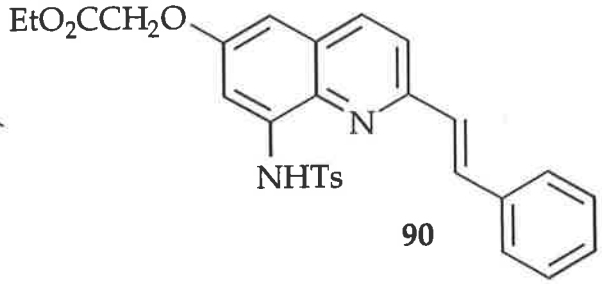
The experimental method devised to determine this series was to observe the most stable species formed when two ligands are in the presence of Zn(II). It is important to note that ZQB forms both a 1:1 and 2:1 complex with Zn(II). Therefore, any devised experimental method must take into consideration that there must be an excess of ligand concentration compared to that of Zn(II), such that the two different ligands (A and B) compete for Zn(II). For example, if ligands A, B (two equivalents each) both compete equally for Zn(II) (one equivalent) then the initial species formed ([L_2Zn(II)] since ZQB has been shown[55] preferentially to form this species in the gas phase) will be 2:1:1 distribution of [ABZn(II)]:[A_2Zn(II)]:[B_2Zn(II)]. This distribution is an example of a statistical distribution. Thus, any deviation from this distribution will reflect the relative stability of each complex. Similarly, a relative stability series for Cd(II) could be determined for these ligands, since Cd(II) also forms complexes with ligands **1a, 90, 91, 92** and **97**.

8.4. Determining a Zn(II) complex relative stability series.

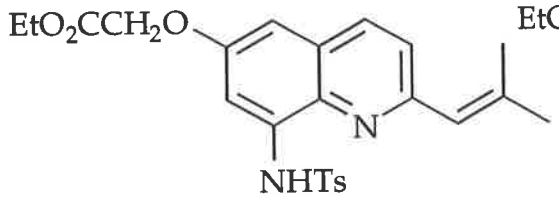
Initially, solutions containing a single ligand (four fold excess of either **1a**, **90**, **91**, **92** and **97**) and Zn(II) in ethanol were used in the positive ion ESI studies, since all may form a 1:1 and 2:1 complex with Zn(II).[60]



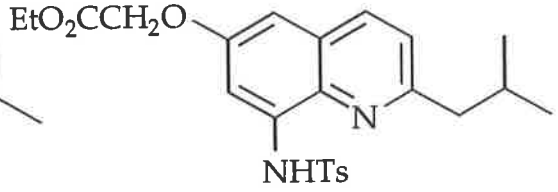
1a



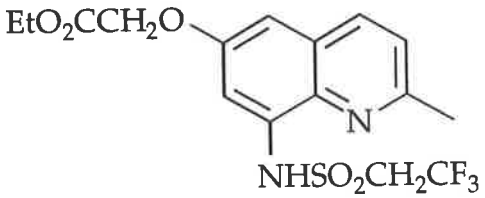
90



91



92



97

The positive ions observed for the complexes [(**1a**)$_2$Zn(II)], [(**90**)$_2$Zn(II)], [(**91**)$_2$Zn(II)], [(**92**)$_2$Zn(II)] and [(**97**)$_2$Zn(II)] are shown in Figure 8.3., where each positive ion corresponds to a [L_2Zn(II) + Na]$^+$ ion. The precise origin of the sodium is not clear, but at the low experimental ligand concentrations studied($\leq 10^{-6}$M), the abundance of sodium as a laboratory contaminant may explain its presence in the positive ions observed despite the high purity of the Zn(II) salt and ligands used. This technique

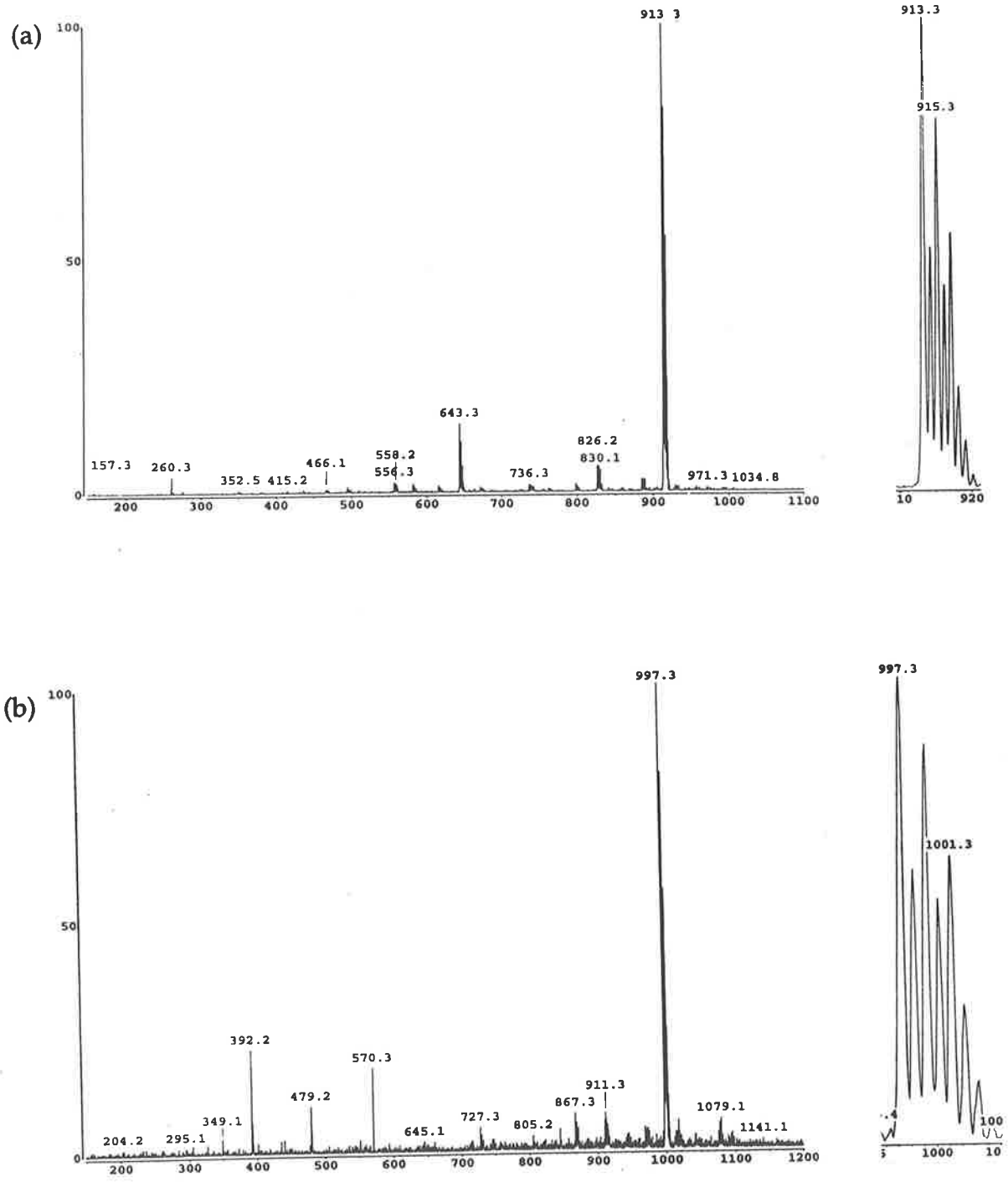
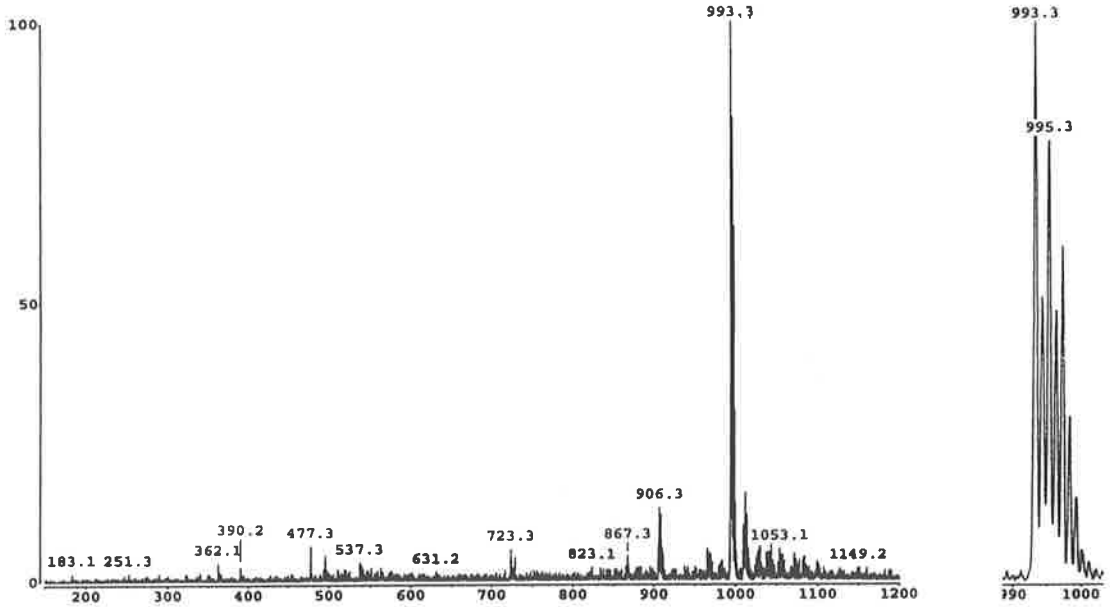
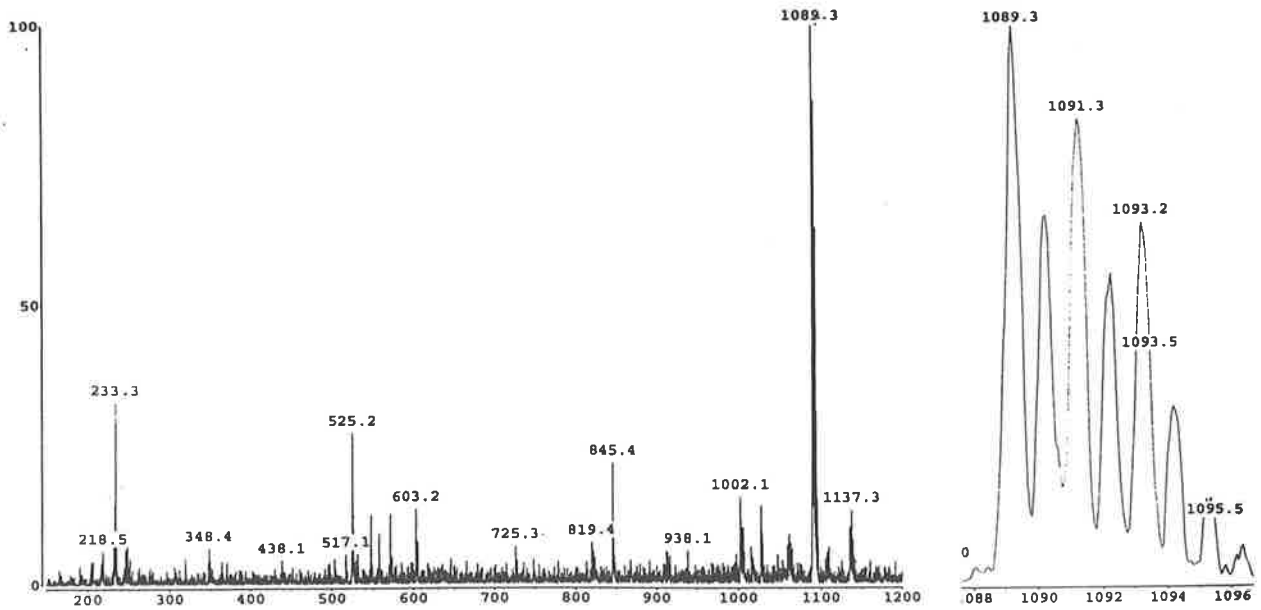


Figure 8.3. The positive ions, resulting from $[L_2Zn(II) + Na]^+$, observed for the complexes (a) $[(\mathbf{1a})_2Zn(II)]$, (b) $[(\mathbf{92})_2Zn(II)]$, (c) $[(\mathbf{91})_2Zn(II)]$, (d) $[(\mathbf{90})_2Zn(II)]$, (e) $[(\mathbf{97})_2Zn(II)]$.

(c)


100
50
0
993.3
995.3
906.3
390.2
477.3
362.1
537.3
723.3
867.3
631.2
823.1
1053.1
183.1
251.3
1149.2
200
300
400
500
600
700
800
900
1000
1100
1200
990
1000

(d)


100
50
0
1089.3
1091.3
1093.2
1093.5
1095.5
233.3
525.2
845.4
603.2
1002.1
1137.3
218.5
348.4
438.1
517.1
725.3
819.4
938.1
200
300
400
500
600
700
800
900
1000
1100
1200
1088
1090
1092
1094
1096

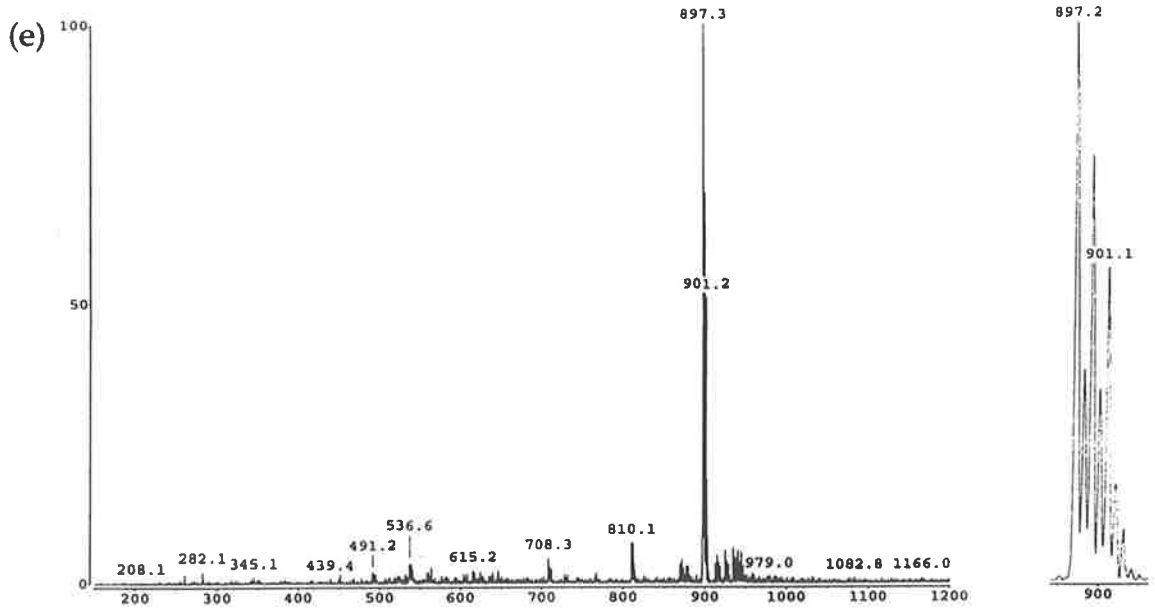


Figure 8.3. The positive ions, resulting from $[L_2Zn(II) + Na]^+$, observed for the complexes (a) $[(\mathbf{1a})_2Zn(II)]$, (b) $[(\mathbf{92})_2Zn(II)]$, (c) $[(\mathbf{91})_2Zn(II)]$, (d) $[(\mathbf{90})_2Zn(II)]$, (e) $[(\mathbf{97})_2Zn(II)]$.

consistently resulted in the formation of positive ions from sodiated adducts as opposed to an $[L_2Zn(II) + H]^+$ ion.[142] The isotopic patterns observed are typical for the ligand-Zn(II) complexes and this pattern results from the isotopic patterns of Zn(II) and the isotopic pattern exhibited by the ligand itself. For example, for the complex [**1a**$_2$Zn(II)] an isotopic pattern would result from the isotopic pattern of **1a** (a result of the distribution of naturally occurring isotopes of C, H, N, O and S) combined with the naturally occurring isotopes of Zn(II) (^{64}Zn 48.6%, ^{66}Zn 27.9%, ^{67}Zn 4.1%, ^{68}Zn 18.8% and ^{70}Zn 0.6%).

The formation of ligand-Zn(II)-ethanol complexes was observed when the capillary voltage, applied to the cone at the electrospray interface, was sufficiently low (35V), however these complexes were not observed at an increased capillary voltage (130V). The mono-complexes [(**1a**)Zn(II)]$^+$, [(**90**)Zn(II)]$^+$, [(**91**)Zn(II)]$^+$, [(**92**)Zn(II)]$^+$ and [(**97**)Zn(II)]$^+$ were not observed at either the high or low capillary voltage. The mono complex of ZQB, [LZn(II)] has been observed[60] in the condensed phase but the ESI data suggest that the mono complexes are less stable than the di-complexes in the gas phase.

A typical statistical abundance distribution of the species [(**92**)$_2$Zn(II)], [(**97**)$_2$Zn(II)] and [(**92**)(**97**)Zn(II)], resulting from the competition of ligands **92** and **97** with Zn(II), is shown in Figure 8.4. This figure illustrates that both the abundances of [(**92**)$_2$Zn(II)] and [(**97**)$_2$Zn(II)] are similar, indicating that these complexes have very similar stabilities in the gas phase. The remaining ligands pairs when placed in competition for Zn(II) exhibited a distinct non statistical abundance distribution of species, an example of which is shown in Figure 8.5. Figure 8.5 shows the distribution obtained when **91** and **97** are in competition for Zn(II). Clearly the most stable

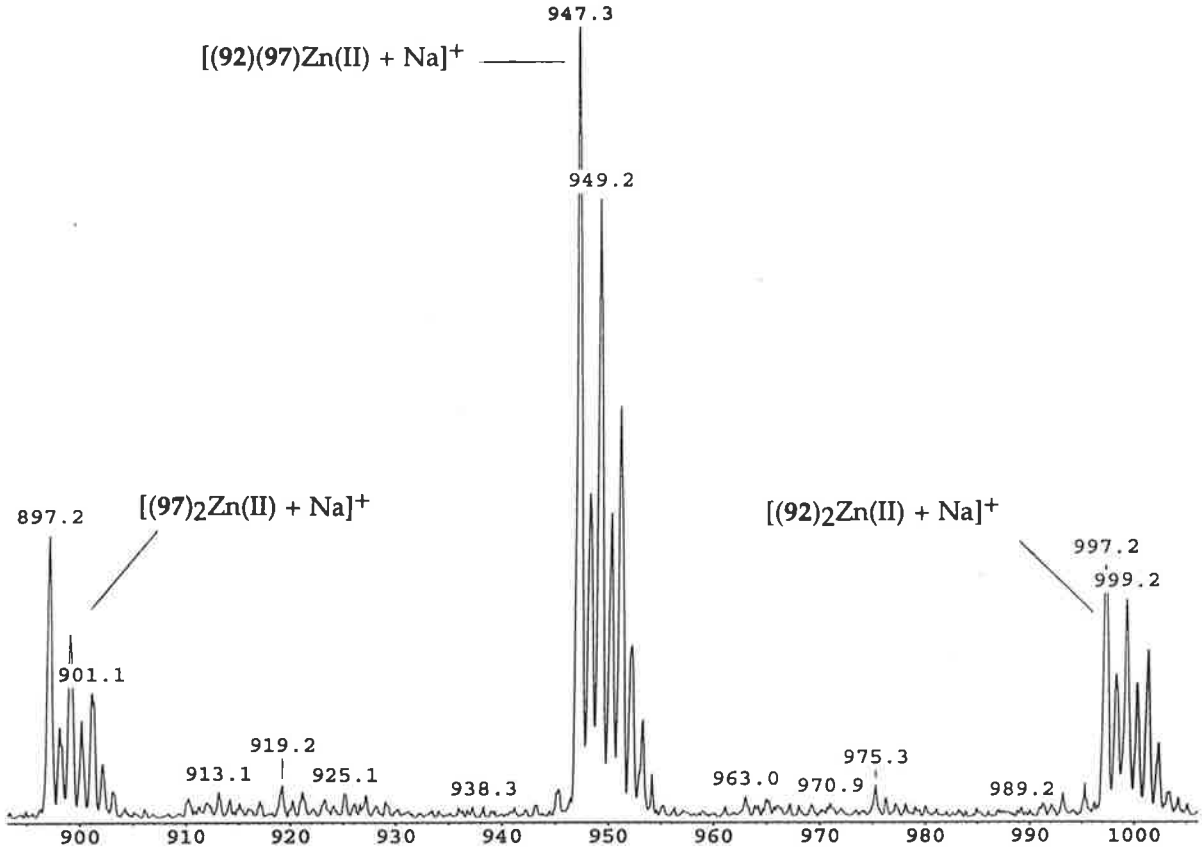


Figure 8.4. The positive ion abundance distribution, resulting from $[L_2Zn(II) + Na]^+$, observed for the complexes $[(\mathbf{92})_2Zn(II)]$, $[(\mathbf{97})_2Zn(II)]$ and $[(\mathbf{92})(\mathbf{97})Zn(II)]$.

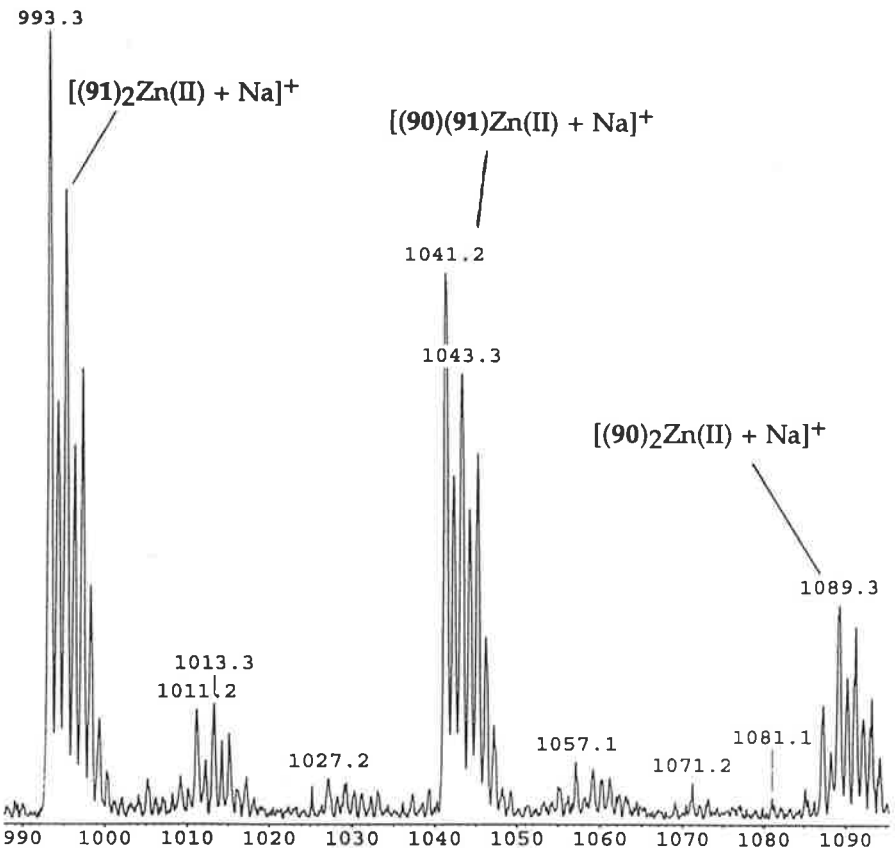


Figure 8.5. The positive ion abundance distribution, resulting from $[L_2Zn(II) + Na]^+$, observed for the complexes $[(\mathbf{90})_2Zn(II)]$, $[(\mathbf{91})_2Zn(II)]$ and $[(\mathbf{90})(\mathbf{91})Zn(II)]$.

species, by abundance of the peak, is the complex [(**91**)$_2$Zn(II)]. Table 8.1 shows a ratio of the relative abundances for each of the ligand pairs when competing for Zn(II).

Table 8.1. The ratio of species abundance obtained for the respective ligands when competing for Zn(II).[a]

Competing ligands		Ratio of species abundance		
A	B	[A$_2$Zn(II)]	[ABZn(II)]	[B$_2$Zn(II)]
1a	**90**	4.2	4.2	1
1a	**91**	1	3.1	2.1
1a	**92**	1	2.3	1.4
1a	**97**	1.1	2.4	1.2
90	**91**	1	2.5	3.6
90	**92**	1	2.4	4.2
90	**97**	1	2.4	1.7
91	**92**	-	-	-
91	**97**	1.6	2.8	1
92	**97**	1.1	2.4	1

a) Obtained from a mixture of two ligands, A and B competing for Zn(II), where [A] and [B] are 20μM, [Zn(II)] is 10μM. The ratio from the competition of **91** and **92** for Zn(II) could not be determined from the ESI spectrum.

In determining the relative stabilities of [L$_2$Zn(II)], where L=**1a**, **90**, **91**, **92** and **97** the mixed species [ABZn(II)] is unimportant. The most stable species, from the results summarised in Table 8.1, are the complexes [**91**$_2$Zn(II)] and [**92**$_2$Zn(II)]. Due to overlap of the complex signals in the ESI spectrum, because of the similar molecular weights of the two ligand, an insight into which species was the most stable could not be directly ascertained. However, this signal overlap does not occur with the

remaining [L_2Zn(II)] species and accordingly a relative stability series can still be generated. The tresyl ligand, **97**, forms a slightly stronger complex with Zn(II) compared to [**1a**$_2$Zn(II)] complex, although the [**97**$_2$Zn(II)] complex is slightly less stable than the complex that **92** forms with Zn(II). The least stable species was the styryl complex, [**90**$_2$Zn(II)].

Therefore the stability series, going from the most stable complex to the least stable complex is:

[**91**$_2$Zn(II)] >> [**92**$_2$Zn(II)] > [**97**$_2$Zn(II)] > [**1a**$_2$Zn(II)] >> [**90**$_2$Zn(II)].

The complex that **92** (contains a isobutyl group at the 2-position) forms with Zn(II) is only moderately more stable than the [**1a**$_2$Zn(II)] complex. The effect that the isobutenyl group of **91** has on the stability of the Zn(II) complex, compared with that of the methyl group contained in the [**1a**$_2$Zn(II)] complex is far more noticeable. In contrast, altering the group at the 2-position to a styryl group in **90**, noticeably decreases the stability of the Zn(II) complex.

8.5. Determining a Cd(II) complex relative stability series.

Once again solutions containing a four fold excess of the ligand/s to metal cation (this time Cd(II)) in ethanol were used in the positive ion ESI studies, since ZQE, **1a**, can form both a 1:1 and 2:1 complex with Cd(II).[60] Figure 8.6., shows the positive ions arising from ligands **1a**, **90**, **91**, **92** and **97** with Cd(II). A mixture of complexes was identified from the ESI spectra of the ligands, **1a**, **90**, **91**, **92**, **97**, with Cd(II). Each spectra showed the formation of the expected Cd(II) complexes, [(**1a**)$_2$Cd(II)], [(**90**)$_2$Cd(II)], [(**91**)$_2$Cd(II)], [(**92**)$_2$Cd(II)] and [(**97**)$_2$Cd(II)]. However, signals attributed to the ligand-Zn(II) complexes are identical to those formed in the ligand competitions described in Section 8.4, and are commented on later. Once again each of the positive

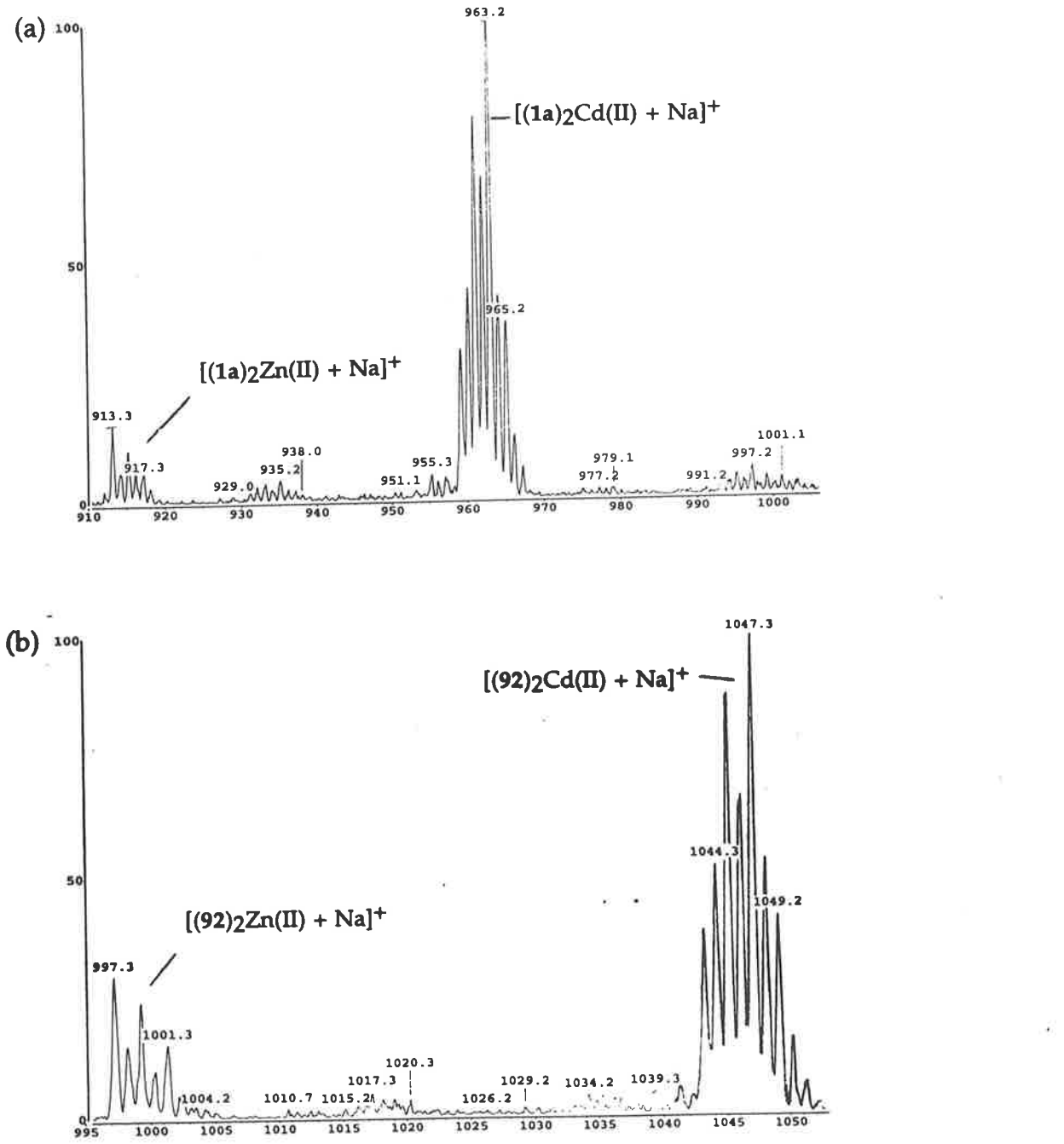
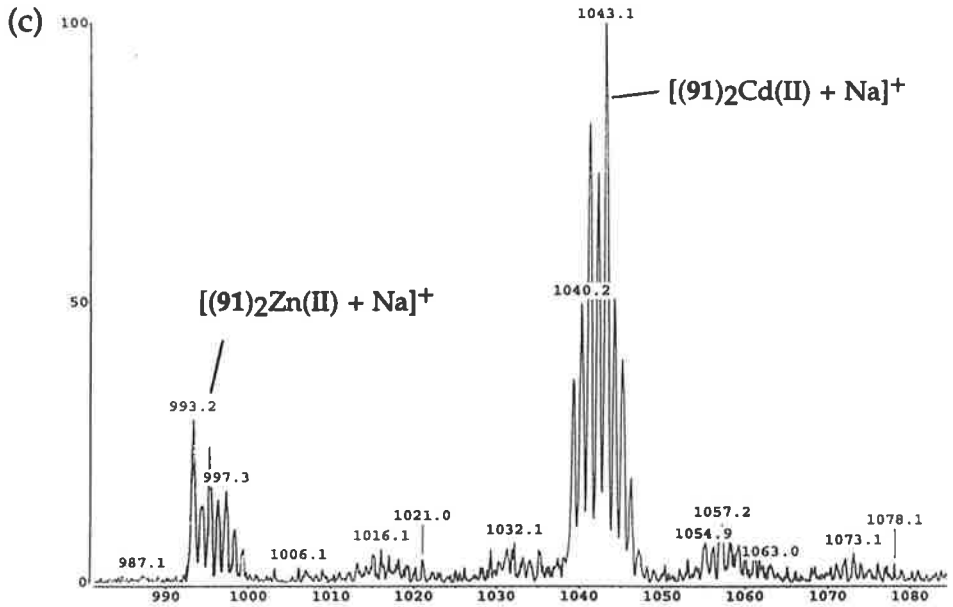
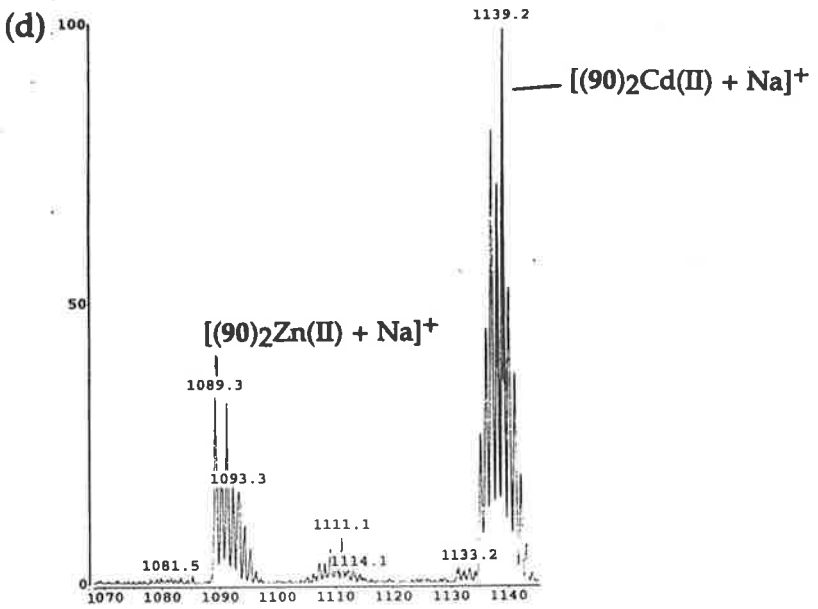


Figure 8.6. The positive ions observed for the complexes of (a) **1a**, (b) **92**, (c) **91**, (d) **90** and (e) **97** with Cd(II). In addition to the expected Cd(II) complexes, the Zn(II) complexes were also observed.


(c)
100
50
0
1043.1
[(91)2Cd(II) + Na]+
[(91)2Zn(II) + Na]+
1040.2
993.2
997.3
1021.0
1016.1
1032.1
1057.2
1054.9
1078.1
1073.1
1063.0
987.1
1006.1
990
1000
1010
1020
1030
1040
1050
1060
1070
1080


(d)
100
50
0
1139.2
[(90)2Cd(II) + Na]+
[(90)2Zn(II) + Na]+
1089.3
1093.3
1111.1
1114.1
1081.5
1133.2
1070
1080
1090
1100
1110
1120
1130
1140

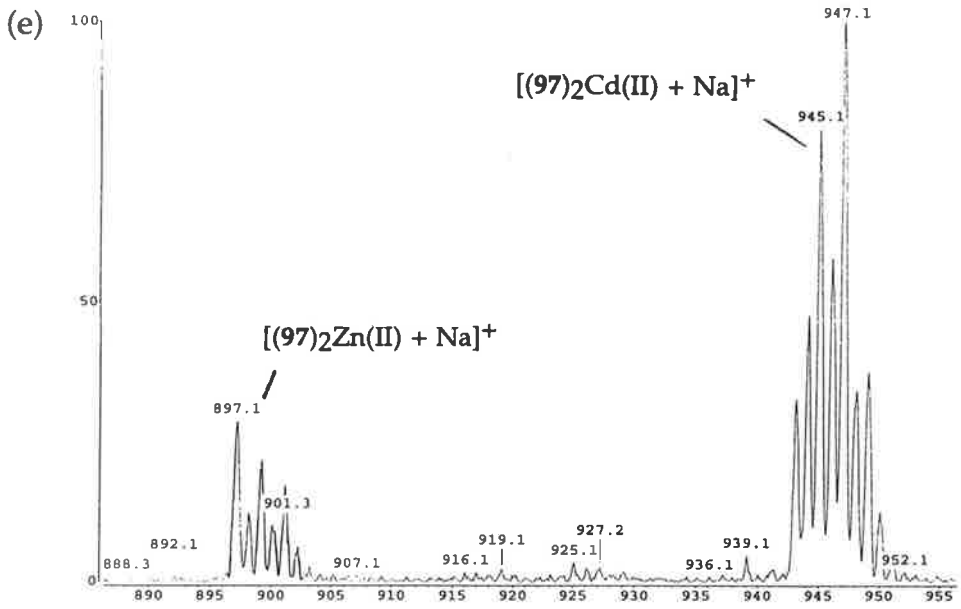


Figure 8.6. The positive ions observed for the complexes of (a) **1a**, (b) **92**, (c) **91**, (d) **90** and (e) **97** with Cd(II). In addition to the expected Cd(II) complexes, the Zn(II) complexes were also observed.

ions is a result of the sodiated adduct $[L_2M(II) + Na]^+$ where M is either Cd(II) or Zn(II). Furthermore, the mono-ligand complexes $[LCd(II)]^+$ were not observed in any of the ESI spectra, even although it has been reported in the condensed phase.[60] The isotopic patterns observed are typical of the ligand-Cd(II) complexes and this pattern results from the isotopic patterns of Cd(II) and the isotopic pattern exhibited by the ligand itself. The isotopic patterns observed are typical for the ligand-Cd(II) complexes and this pattern results from the naturally occurring isotopic patterns exhibited by the ligand combined with the naturally occurring isotopes of Cd(II) (^{106}Cd 1.2%, ^{108}Cd 0.9%, ^{110}Cd 12.4%, ^{111}Cd 12.8%, ^{112}Cd 24.0%, ^{113}Cd 12.3%, ^{114}Cd 28.8% and ^{116}Cd 7.6%).

The source of this Zn(II) contamination was identified as the ligands themselves. During the synthesis of the ligands, **1a**, **90**, **91**, **92** and **97** each had been exposed to low concentrations of Zn(II), an example of which is the amount of Zn(II) present in commercially available analytical grade ethanol (see Chapter 2, section 2.3) and consequently, each ligand had formed a small amount of its Zn(II) complex. (This contaminant, or adventitious Zn(II) has also been the cause of higher than expected fluorescence values for ZQA solutions, also discussed in Chapter 2, section 2.3). The adventitious Zn(II) was removed from one particular ligand ,**90**, by stirring a solution of ligand (dichloromethane) with EDTA, a known metal chelator.

Having two variable (two metals, Cd(II) and Zn(II) instead of one, Cd(II)) in the ligand competitions for Cd(II) complicated the results but does not affect the relative stabilities of the $[L_2Cd(II)]$ complexes. Therefore, Table 8.2 shows a ratio of the relative abundances for each of the ligand pairs when competing for Cd(II). The relative abundances of the Zn(II)

complexes, [A_2Zn(II)], [ABZn(II)] and [B_2Zn(II)] (a result of the adventitious Zn(II) previously discussed) in the ESI spectra of the Cd(II) complexes, were the same as those shown in Table 8.1.

Table 8.2. The ratio of species abundance obtained for the respective ligands when competing for Cd(II).[a]

Competing ligands		Ratio of species abundance		
A	B	[A_2Cd(II)]	[ABCd(II)]	[B_2Cd(II)]
1a	**90**	1	3.2	2.8
1a	**91**	1	3.3	3.2
1a	**92**	1	2.5	1.8
1a	**97**	1.1	2.2	1
90	**91**	1	2.1	2.3
90	**92**	1	1.6	1.2
90	**97**	1.8	2.1	1
91	**92**	-	-	-
91	**97**	2.9	2.0	1
92	**97**	1.9	2.2	1

a) Obtained from a mixture of two ligands, A and B competing for Cd(II), where [A] and [B] are 20μM, [Cd(II)] is 10μM. The ratio from the competition of **91** and **92** for Cd(II) could not be determined from the ESI spectrum.

Therefore the stability series, from the most stable complex to the least stable complex is:

$$[\mathbf{91}_2\text{Cd(II)}] > [\mathbf{92}_2\text{Cd(II)}] > [\mathbf{90}_2\text{Cd(II)}] >> [\mathbf{1a}_2\text{Cd(II)}] > [\mathbf{97}_2\text{Cd(II)}].$$

Once again the complexes [$\mathbf{91}_2$Cd(II)] and [$\mathbf{92}_2$Cd(II)] were the most stable. In contrast to the Zn(II) stability series the complex [$\mathbf{90}_2$Cd(II)] is not the least stable complex. Both [$\mathbf{1a}_2$Cd(II)] and [$\mathbf{97}_2$Cd(II)] have kept the same order of stability in the Cd(II) series but both are less stable than the complex

[$\mathbf{90}_2$Cd(II)]. This probably reflects differences in the ionic radii of Zn(II) and Cd(II) as discussed below.

8.6 Conclusions.

The results described above establish that the ESI technique represents an efficient method for determining the stability of a series of ligands relative to a metal cation. Of the ligands examined **91** and **92** were shown to form the more stable complexes with Zn(II) by comparison with those of ZQE, **1a**, in the gas phase. In particular, **91**, was shown to form the most stable complex compared to all the ligands used in the study. By contrast, **90**, which contains a styryl group at the 2-position, was shown to decrease the relative stability of the ligand-Zn(II) complex, in the gas phase.

The isobutyl and isobutenyl side chains of **91** and **92** were shown to increase the stability of the Cd(II) complex compared to the 2-methyl group of **1a**, consistent with the results obtained for the Zn(II) series, in the gas phase. However, the Cd(II) series showed that the complex [$\mathbf{90}_2$Cd(II)] was more stable than both [$\mathbf{1a}_2$Cd(II)] and [$\mathbf{97}_2$Cd(II)] and that this result is in contrast to the Zn(II) complex stability series. This change in the relative stability order between the Zn(II) and Cd(II) series may suggest that the complex formed by **90** with Cd(II) is less sterically hindered compared to its Zn(II) complex, [$\mathbf{90}_2$Zn(II)] (Zn(II) has a smaller ionic radii, 0.65Å, than that of Cd(II), 0.78Å).

The stability series of **1a, 90, 91, 92, 97** with Zn(II) and Cd(II) is based on the results obtained from the ESI technique which is a gas phase technique. Since the stability series derived from the ESI results is derived from the complexes initially formed in the condensed phase then one could tentatively say that the stability comparisons would also apply to the

condensed phase. However, any such conclusion would need to be confirmed by actual measurement of the respective stabilities of each ligand (**1a**, **90**, **91**, **92**, **97**) with Zn(II) and Cd(II), in solution by other techniques, and these stabilities will need to be investigated further.

Chapter 9 : Conclusions and future work.

9.1 Synthetic methodologies.

The synthetic methods adopted in the preparation of the various Zinquin analogues has allowed these analogues to be prepared efficiently and in good yields. In particular; the synthesis of ZQE and ZQA has been improved, firstly by optimising the boron tribromide demethylation of **9** and, secondly by increasing the yield of the alkylation of **10a**. These optimisations have enabled the overall yield of ZQE to be increased from 4% to 16% and, in addition, the optimisation of the alkylation of **10a** has allowed access to labelled Zinquin ester, **12**.

The Wittig reaction of the aldehyde **66** with various phosphoranes under the mild conditions of Markl and Merz[130] has enabled a number of quinolines with differing side chains at the 2-position to be synthesised. The Wittig coupling proved to be a higher yielding reaction than the more traditional coupling of a quinaldine with an aldehyde in the presence of sodium hydride.[129] Finally, the iron acetic acid method, compared to catalytic reduction, was shown to be an excellent method for reducing arylnitro compounds to the corresponding arylamines.

9.2 Improvements on Zinquin.

The type of sulfonamide substituent, the position of the alkoxy group on the quinoline ring and the size of the group at the 2-position on the quinoline ring can all effect the selectivity of the described Zinquin analogues for Zn(II) and the intensity of the consequent fluorescence.

The current sulfonamide used in Zinquin does not exhibit the highest amount of fluorescence when in the presence of Zn(II). By the

introduction of more electron withdrawing sulfonamides, such as tresyl and *m*-trifluoromethylbenzene sulfonamides, the Zn(II) dependant fluorescence was significantly increased. However, the most fluorescent sulfonamide, the tresyl sulfonamide, was particularly unstable (illustrated by the instability of **97** in DMF) which may preclude it from cellular work.

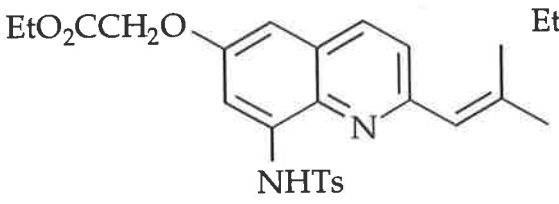
Variation of the position of the alkoxy group had a multitude of different effects. The alkoxy group at the 4-position significantly increased the Zn(II) dependant fluorescence (3 fold) but had the added effect of decreasing the maximum absorbance value of the ligand-Zn(II) complex relative to the Zinquin precursor **9**. In contrast, having the alkoxy group at the 5-position resulted in no Zn(II) dependant fluorescence, but a large increase in the maximum absorbance value of the ligand-Zn(II) complex relative to the Zinquin precursor **9**. An exact explanation on the behaviour of the isomers was not forthcoming and implies that further investigations are needed.

The primary reason for altering the size of the group at the 2-position was to increase the absorbance wavelength of the ligand-Zn(II) complex. Increases in the absorbance wavelength of the ligand-Zn(II) complex was so that the respective ligand/s could be used with the blue-light laser CLM which has a excitation wavelength of 488nm (Note, Zinquin has been used in conjunction with an uv-laser CLM, but this CLM was not commercially available at the commencement of this project). Therefore the initial proposal was the synthesis of ligands which retained the Zinquin sulfonamide and quinoline nitrogens but exhibited increased conjugation so that they could be used with the blue light CLM.

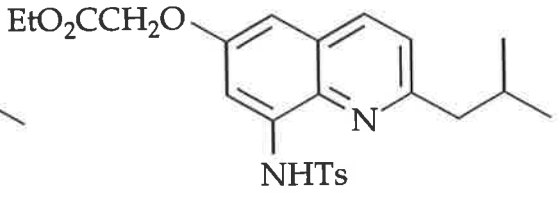
The first ligands investigated were the acridine and acridone ligands **46, 48** and **49.** The acridone ligands **48** and **49** showed little spectral change in the presence of Zn(II). The acridine ligand **46** exhibited a distinct bathochromic shift in the presence of Zn(II) but this shift was not enough as to be used with the blue light CLM. Additionally **46** also showed no fluorescence in the presence of Zn(II). Consequently, both the acridone and acridine ligands were ruled out as potential Zn(II) fluorophores.

The second approach to increasing the conjugation of Zinquin was to add conjugated groups to the 2-position. Addition of styryl, cinnamyl and naphthyl groups to the 2-position of the Zinquin precursor **9** did increase the wavelength maxima of the ligand-Zn(II) complex, but not sufficiently to be used with the blue light CLM. During this research a uv-light CLM did become commercially available but many laboratories still use the blue-light laser CLM. Therefore, the development of a Zn(II) fluorophore which could be used with the blue-light CLM would still be desirable.

The addition of an isobutyl, an isobutenyl and styryl group to the 2-position did increase the fluorescence of the ligand-Zn(II) complexes relative to ZQE, **1a.** This was particularly evident with the isobutenyl and isobutyl ligands, **91** and **92**, which exhibited approximately 300% and 180% increases in fluorescence in the presence of Zn(II), respectively, relative to **1a.** Additionally, **91** and **92,** demonstrated increased selectivity compared to **1a**, and this was shown by **91** and **92** exhibiting decreased fluorescence when in the presence of Cd(II) compared to **1a.**



91



92

Ligands **90, 91, 92** and **97** were all found to be suitable for *in vivo* work, since they all showed Zn(II) dependant fluorescence when introduced into rheumatoid arthritis tissue. However, the four candidate ligands did show varied solubility in ethanol, the solvent used to deliver the individual ligands into the tissue samples. These solubility problems could be overcome by using a dipolar aprotic solvent such as DMSO to deliver the ligands into the tissue or cellular samples. In summary, all four ligands do represent potential candidate Zn(II) specific fluorophores which could be used for tissue or cellular work. This is exemplified by **91** and **92** which ahve increased Zn(II) dependant fluorescence compared with the commercially available Zn(II) fluorophore, Zinquin, **1a**.

9.3 The ESI technique in determining complex stabilities.

Why **91** and **92** demonstrate this increase in selectivity, compared to ZQE, could be explained by a calculation of their respective stabilities with Zn(II). There was not sufficient time to determine these stabilities, but a stability series, determined by ESI mass spectrometry was determined. This series showed that the complexes [**91**$_2$Zn(II)] and [**92**$_2$Zn(II)] were more stable than the corresponding ZQE-Zn(II) complex, [**1a**$_2$Zn(II)]. The least stable Zn(II) complex was shown to be the styryl complex, [**90**$_2$Zn(II)], suggesting that the larger styryl group decreased the stability of the di-ligand-Zn(II) complex compared to **1a**. A confirmation of the stabilities of **90, 91, 92 ,97** with Zn(II) would be to quantitatively determine the stability constants with Zn(II).

A stability series of **1a**, **90**, **91**, **92** and **97** with Cd(II) was also determined. The Cd(II) ESI study did reflect the propensity of these Zinquin based ligands to pick up any adventitious Zn(II). This adventitious Zn(II) has been shown to be present in variety of sources, such as commercially available analytical grade ethanol and analytical grade drying agents. The relative stability series of **1a**, **90**, **91**, **92**, **97** with Cd(II) showed that the complex [**90**$_2$Cd(II)] was more stable than both the [**1a**$_2$Cd(II)] and [**97**$_2$Cd(II)] complexes. This result is in contrast to the Zn(II) complex stability series and suggests that the complex formed by **90** with Cd(II) is less sterically hindered compared to the complex that **90** forms with Zn(II). However this would need to be confirmed by quantitatively determining the stability constants of **1a**, **90**, **91**, **92** and **97** with Cd(II).

Experimental

General

Synthetic methods;

Melting points were determined using a Kofler hot stage equipped with a Reichert microscope and are uncorrected.

Microanalyses were performed at the Department of Chemistry, University of Otaga, Dunedin, New Zealand. Amino compounds which were unsuitable for microanalysis were analysed as their tosylsulfonamides. Remaining compounds that were unsuitable for microanalysis were analysed at the next possible intermediate within a synthetic route.

Accurate mass measurements were performed at the Department of Chemistry, University of Tasmania.

X-ray analysis was performed by Dr. E. R. Tiekink, Department of Chemistry, University of Adelaide.

Ether refers to diethyl ether. Hexane refers to the fractions of boiling range 66-68°C. Dry diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone ketyl prior to use. Dry dichloromethane was distilled from phosphorus pentoxide. All solvents/reagents were purified according to literature procedures.[143] All organic extracts were dried with anhydrous analytical grade sodium sulfate.

Flash chromatography[144] was carried out on Merck silica gel 60, particle size 0.040-0.063mm. Squat column chromatography[145] was carried out on silica

gel 60F-254 containing gypsum. Thin Layer Chromatography (TLC) was performed on aluminium backed sheets of silica gel 60F-254. TLC chromatograms were visualised using ultra-violet light (254nm).

Infra-red spectra were recorded either as Nujol mulls for solids or as liquid films; on a Hitachi 270-30 Infra-red Spectrometer. ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectra were determined in deuterochloroform solutions containing tetramethylsilane as internal standard; spectra were recorded on ACP-300MHZ spectrometer unless otherwise stated. All chemical shifts are quoted as δ in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). Where the extremities of a multiplet are well defined a range is given, otherwise a value for the centre of the signal is recorded.

Electron impact mass spectra were recorded at 70eV on a ZAB 2HF spectrometer. Only the major fragmentations are given with their relative abundances shown in parentheses.

p-Acetamidobenzenesulfonyl chloride[94] and *p*-methoxybenzenesulfonyl chloride[95] were prepared by literature procedures. Phosphonium salts were prepared by refluxing one equivalent of the corresponding alkyl bromide with one equivalent of triphenylphosphine in DMF. The salts were then collected by filtration and recrystallised from ethanol/diethyl ether.

Physical methods;

Uv/visible spectra were obtained on a Carey 2200 spectrometer; fluorescence spectra were obtained on a Perkin Elmer, LS50B luminescence spectrometer using a slit width of 2.5nm unless otherwise stated. The solutions studied were contained in 1 cm path length silica cells thermostatted at 298.2K.

Zn(II) is enviromentally ubiquitous and is present at a low level impurity in some high grade commercial chemicals as shown by atomic absorption measurements.[60] Therefore uv/visible and fluorimetric measurements of the ligands, in the absence of Zn(II), were taken in the presence of an EDTA solution. Concentrations of each species is shown under each spectra. All spectra were obtained in a buffer solution containing 100mM $NaClO_4$, 1mM NaPIPES in ethanol/water (75:25, v/v).#

Uv/visible spectra are contained in Appendix A
Fluorescene data are contained in Appendix B.

ESI Mass spectrometry methods;

A Finnigan MAT ion trap LC-Q Mass Spectrometer (Finnigan, San Jose, Ca, USA) fitted with an electrospray ionisation source was used. Samples were introduced into the LC-Q by infusion at flow rates of 10 to 20µl per minute, in positive ion mode. The samples consisted of two ligands ([L] 20µM) with $ZnSO_4$ or $CdSO_4$ ([M] 10µM) made up in distilled ethanol.

Initially the capillary temperature was maintained at 200°C, the tube lens offset was set at 55V and the capillary voltage was maintained at 35V. This yielded positive ions complexes containing ethanol. The samples were then run at a capillary temperature of 280°C, a tube lens offset of 180V and a capillary voltage of 130V. This yielded positive ions corresponding to $[L_2M+Na]^+$, where L is **1a**, **90**, **91**, **92**, **97** and M is Zn(II) or Cd(II).

Chapter 2 : The synthesis of Zinquin.

6-Methoxy-2-methyl-8-nitroquinoline 7.

The title compound was prepared using a literature procedure[84], yielding yellow-orange needle crystals (30%), m.p. 184-187°C (lit.[84], m.p. 186-187°C). υ_{max} : 1590, 1510, 1100, 1360cm^{-1}. ^{1}H n.m.r. : δ 8.00, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.62, 1H, d, $J_{7,5}$ 2.7 Hz, H_7 ; 7.34, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.24, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 3.96, 3H, s, OCH_3 ; 2.72, 3H, s, CH_3. ^{13}C n.m.r.: δ 161.20, 157.45, 150.21, 136.93, 136.72, 130.17, 125.98, 117.60, 111.42, 58.10, 27.40. Mass spectrum : *m/z* 218($M^{+\bullet}$, 100%), 188(M-NO, 11%), 172, 160.

8-Amino-6-methoxy-2-methylquinoline 8.

(a).[86] 6-Methoxy-2-methyl-8-nitroquinoline (0.6 g, 2.75 mmol), iron powder (0.55 g), glacial acetic acid (19.25 mmol, 1.16 g) and ethanol (7 ml) were refluxed for 3.5 h under nitrogen. Water (20 ml) was added and the mixture extracted with chloroform (4 x 15 ml). The organic layer was washed with saturated sodium bicarbonate solution (20 ml), dried and the solvent removed. The yellow solid was recrystallised from dichloromethane/hexane to yield 8-amino-6-methoxy-2-methylquinoline as yellow prismatic crystals (0.48 g, 94%), m.p. 103-104°C, (lit.[87] m.p. 102-103°C) (Found C, 69.9; H, 6.2; N, 15.0%. $C_{11}H_{12}N_2O$ requires C, 70.2; H, 6.5; N, 14.9%). υ_{max} : 3450, 3375, 3325, 1620, 1600cm^{-1}. ^{1}H n.m.r. : δ 7.81, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.16, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.53, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 6.42, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 4.95, 2H, bs, NH_2 ; 3.84, 3H, s, OCH_3 ; 2.64, 3H, s, CH_3. Mass spectrum : *m/z* 188($M^{+\bullet}$, 100%), 159(44%), 145(36%).

(b). The nitro compound (0.45 g, 2.06 mmol) was stirred in dry ethanol (90ml) in the presence of Pd-C (5%, 0.15g) under an atmosphere of H_2, overnight. The reaction mixture was then filtered through kenite and the solvent removed to yield a solid (0.27 g, 72%) identical with that obtained above.

4-Methyl-N-(6-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **9**.

p-Toluenesulfonyl chloride (0.56 g, 2.93 mmol), was added slowly to a cooled stirred solution of the 8-amino-6-methoxy-2-methylquinoline (0.50 g, 2.65 mmol) in pyridine (10 ml) and the mixture was stirred in an ice bath for 3 h. Ice water was added and the resulting precipitate filtered, washed well with water and dried. The solid was dissolved in dichloromethane (30 ml), washed with a saturated sodium carbonate, dried and the solvent removed under reduced pressure. Crystallisation of the residue from ethanol yielded the sulfonamide **9** as a yellow crystalline solid (0.57 g, 65%), m.p. 185-187°C (lit.[55] m.p. 185-188°C). υ_{max} : 3200, 1620, 1590, 1560, 1350, 1320cm^{-1}. ^{1}Hn.m.r : δ 9.25, 1H, bs, NH ; 7.87-7.82, 3H, m, $H_{2',4}$; 7.41, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.26-7.17, 3H, m, $H_{3',3}$; 6.67, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 3.86, 3H, s, OCH_3 ; 2.64, 3H, s, CH_3 ; 2.32, 3H, s, $ArCH_3$. ^{13}C n.m.r. : δ 159.34, 156.96, 145.70, 138.52, 137.14, 136.16, 136.06, 131.54, 129.28, 129.13, 125.20, 109.01, 111.42, 57.50, 26.70, 223.46. Mass spectrum : *m/z* 342($M^{+\bullet}$, 100%), 278(27%), 187(47%, M-C_7H_7), 172(65%).

4-Methyl-N-(6-hydroxy-2-methyl-8-quinolyl)benzenesulfonamide ***10a***.

(a). To a solution of boron tribromide (1.8 mmol, 1M, 2.5 eq.) in dry dichloromethane (30 ml) in a 100 ml 2-necked round bottom flask equipped with a reflux condenser kept under nitrogen was added dropwise a solution

of 4-methyl-N-(6-methoxy-2-methyl-8-quinolyl)benzene sulfonamide (0.300 g, 0.876 mmol) in dry dichloromethane (5 ml). The reaction mixture was refluxed for 4h and then stirred overnight at room temperature. Water was added cautiously to the mixture which was basified with saturated sodium bicarbonate (25 ml) and the aqueous layer extracted with dichloromethane (3 x 30 ml). The organic extracts were combined, dried and the solvent removed. The solid obtained was recrystallised from dichloromethane/hexane to yield yellow crystals of 4-methyl-N-(6-hydroxy-2-methyl-8-quinolyl) benzenesulfonamide (0.26 g, 88%), m.p. 179-182°C (lit.[55] m.p. 179-182°C). υ_{max} : 3350, 3200, 1610, 1590, 1580, 1360, 1170cm^{-1}. ^{1}H n.m.r : δ 7.84-7.78, 3H, m, $H_{2',4}$; 7.39, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.23-7.16, 3H, m, $H_{3',3}$; 6.72, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 2.65, 3H, s, CH_3 ; 2.32, 3H, s, $ArCH_3$. ^{13}C n.m.r. ; δ 156.95, 155.60, 145.96, 138.07, 137.32, 135.90, 135.37, 131.62, 129.41, 129.24, 125.32, 109.45, 105.87, 26.57, 23.43. Mass spectrum : *m/z* 328($M^{+\bullet}$, 96%), 264(47%), 173(100%), 144(70%), 118(19%), 91(73%).

(b).[88] The methoxy compound (5 mg, 0.015 mmol) was refluxed with red phosphorus (5 mg), acetic anhydride (1 ml) and hydroiodic acid (0.02 ml) under nitrogen for a period of 3 h. The reaction mixture was cooled and ethanol added (2 ml). The solvent was removed and the solid obtained was dissolved in ethanol, washed with sodium metabisulfite and the aqueous layer extracted with dichloromethane (3 x 5 ml). The organic extracts were then combined, dried and the solvent removed. The ^{1}H n.m.r. of the crude brown solid indicated some starting material (methoxy peak at δ 3.86 in ^{1}H n.m.r. spectrum).

(c).[89] A mixture of the methoxy compound (0.05 g, 0.146 mmol), hydrobromic acid (48%, 1.7 ml), and sodium iodide (0.025 g, 0.167 mmol) was prepared under nitrogen and then heated at 95°C for 2 h. The reaction

mixture was cooled and the contents evaporated to yield a brown solid. Comparison of the peak height integrations of the crude ^{1}H n.m.r. indicated only 40% conversion to the hydroxy compound.

Ethyl-2-(2-methyl-6-quinolyloxy-8-p-toluenesulfonamido)acetate ***1a.***

The phenol (300 mg, 0.92 mmol) in dry THF was added dropwise to a stirred suspension of sodium hydride (66 mg, 1.37 mmol., 60% suspension) in dry THF (2 ml) at 0°C under nitrogen. The reaction mixture was allowed to stir for a further 15 min. before ethyl bromoacetate (0.12 ml, 1.01 mmol) was added dropwise and the reaction mixture stirred over night. Water was added, and the aqueous layer extracted with dichloromethane (20 ml) and the organic layer washed with sodium carbonate (20 ml), dried and the solvent removed. The resultant brown oil was purified by squat column chromatography (dichloromethane). The solid obtained was recrystallised from dichloromethane/hexane to yield the ethyl ester **1a** as fine white crystals (110 mg, 30%), m.p. 110-113°C (lit.[55] m.p. 110-113°C). υ_{max} : 3200, 1745, 1610, 1595, 1580, 1360, 1320, 1150cm^{-1}. ^{1}H n.m.r : δ 9.31, 1H, bs, NH ; 7.84, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.84, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.51, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.24, 2H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 7.21, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.64, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 4.68, 2H, s, OCH_2 ; 4.30, 2H, q, *J* 7.2, CH_2CH_3 ; 2.66, 3H, s, $ArCH_3$; 2.34, 3H, s, CH_3 ; 1.31, 3H, t, *J* 7.2, CH_2CH_3. ^{13}C n.m.r. : δ 170.55, 157.60, 155.15, 145.74, 138.52, 137.22, 136.57, 136.44, 131.58, 129.25, 128.85, 125.36, 108.80, 103.28, 67.72, 63.49, 26.79, 23.46, 16.15. Mass spectrum : *m/z* 414($M^{+\bullet}$, 30%), 368(16%), 259(15%), 236(15%), 149(20%), 137(22%), 69(100%).

(2-Methyl-6-quinolyloxy-8-p-toluenesulfonamido)acetic acid ***1b***.

The Zinquin ester **1a** (200 mg, 0.481 mmol) was hydrolysed by refluxing in ethanol (4 ml) and sodium hydroxide (1.25 M, 16 ml) for 2.5 h. The reaction mixture was cooled, acidified to pH 3.5 (pH meter) with hydrochloric acid (1 M). The resultant precipitate was then extracted with ethyl acetate and the solid obtained upon removal of the solvent recrystallised from ethanol/water to yield the required compound as yellow/green crystals (140 mg, 75%), m.p. 199-201°C (lit.[55] m.p. 198-200°C). ^{1}H n.m.r : δ 7.81, 1H, d, $J_{4,3}$ 8.3Hz, H_4 ; 7.79, 2H, m, $H_{2'}$; 7.39, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.21, 1H, d, $J_{3,4}$ 8.3 Hz, H_3 ; 7.13, 2H, m, $H_{3'}$; 6.57, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 4.64, 2H, s, OCH_2 ; 2.55, 3H, s, CH_3 ; 2.32, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 368($M^{+\bullet}$, 100%), 322(40%), 231(95%), 203(25%), 145(40%), 117(10%), 91(50%), 65(20%), 32(23%).

Chapter 3 : Changing the sulfonamide.

General preparation of the sulfonamides.

All the sulfonamides were produced by stirring one equivalent of 8-amino-6-methoxy-2-methylquinoline **8** with one equivalent of the required sulfonyl chloride, in dichloromethane and pyridine (4 : 1) overnight. The reaction mixtures were then washed with 1M hydrochloric acid (3 x 20ml) followed by saturated sodium bicarbonate (20ml). The organic layers were dried, the solvent removed, the crude solid passed down a column of silica (dichloromethane followed by elution of the required compound with ethyl acetate) and then recrystallised (dichloromethane/hexane unless otherwise stated). By this means the following compounds were obtained;

2,2,2-Trifluoroethane-N-(6-methoxy-2-methyl-8-quinolyl)sulfonamide **13** as fine white needles (520mg, 72%), m.p. 123.5-125°C (Found C, 47.0%; H, 3.6; N, 8.4. $C_{13}H_{13}N_2O_3F_3S$ requires C, 46.7%; H, 3.9; N, 8.4). υ_{max} : 3250cm^{-1} , NH ; 1600 & 1630cm^{-1}, aromatics. ^{1}H n.m.r. : δ9.50, 1H, bs, NH ; 7.92, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.47, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.29, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.81, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.91-3.82, 2H, q, $J_{H,F}$ 8.9Hz, CH_2CF_3 ; 3.89, 3H, s, OCH_3 ; 2.65, 3H, s, CH_3. ^{13}C n.m.r. : δ24.7, CH_3 ; 51.0-54.0, q, $J_{C,F}$19.1Hz, CH_2CF_3 ; 55.7, OCH_3 ; 100.8 ; 107.4 ; 129.5-116.5, q, $J_{C,F}$165.8, CH_2CF_3 ; 123.7 ; 127.5 ; 133.2 ; 134.0 ; 135.3 ; 155.7 ; 157.4. Mass Spectrum : *m/z* 334($M^{+\bullet}$, 80%), 187(M-$SO_2CH_2CF_3$, 92%), 172,(M-CH_3, 100%).

4-Methoxy-N-(6-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **14** as a yellow crystalline solid (130mg, 50%), m.p. 163-165°C (lit.[146] m.p. 161-163°C). υ_{max} : 3250, 1620, 1600, 1580cm^{-1}. ^{1}H n.m.r. : δ9.23, 1H, bs, NH ; 7.87-7.83, 2H, d, $J_{2',3'}$ 9.0Hz, $H_{2'}$; 7.80, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.38, 1H, d, $J_{7,5}$ 2.5Hz, H_4 ; 7.19,

1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.83-6.80, 2H, d, $J_{3',2'}$ 9.0Hz, $H_{3'}$; 6.64, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.82, 3H, s, OCH_3 ; 3.74, 3H, s, $ArOCH_3$; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 358($M^{+\bullet}$, 55%), 187(M-$SO_2C_6H_4OCH_3$, 86%), 172(M-CH_3, 100%).

4-Acetamide-N-(6-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **15** as pale brown prismatic crystals (140mg, 46%), m.p. 215-217°C (Found C, 59.0; H, 4.8; N, 10.9%. $C_{19}H_{19}N_3O_4S$ requires C, 59.2; H, 5.0; N, 10.9%). υ_{max} : 3375, 3200, 1710, 1620, 1600, 1580cm^{-1}. ^1H n.m.r. : δ9.27, 1H, bs, $NH_{sulfonamide}$; 7.89-7.82, 4H, m, $H_{2',3'}$; 7.52, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.40, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.34, 1H, bs, NH_{amide} ; 7.22, 1H, d, $J_{3,4}$ 8.6Hz, H_3 ; 6.66, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.85, 3H, s, OCH_3 ; 2.64, 3H, s, CH_3 ; 2.15, 3H, s, $COCH_3$. Mass Spectrum : *m/z* 365($M^{+\bullet}$, 70%), 187(M-$SO_2C_6H_4NHCOCH_3$, 70%,), 172(M-CH_3, 100%).

4-Bromo-N-(6-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **16** as straw coloured needle crystals (300mg, 90%), m.p. 183-185°C (Found C, 50.3; H, 3.9; N, 6.7%. $C_{17}H_{15}N_2O_3SBr$ requires C, 50.1; H, 3.7; N, 6.9%). υ_{max} : 3200, 1620, 1600, 1570, 1340, 1170cm^{-1}. ^1H n.m.r. : δ9.26, 1H, bs, NH ; 7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.78-7.73, 2H, d, $J_{2',3'}$ 8.6Hz, $H_{2'}$; 7.50-7.46, 2H, d, $J_{3',2'}$ 8.6Hz, $H_{3'}$; 7.39, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.20, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.67, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 3.84, 3H, s, OCH_3 ; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 408($M^{+\bullet}$, 60%), 407(60%), 187(M-$SO_2C_6H_4Br$, 78%), 172(M-CH_3, 100%), 76(40%), 50(33%).

4-Nitro-N-(6-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **17** as yellow needle crystals (245mg, 82%), m.p. 227-229°C (Found C, 54.7; H, 4.0; N, 11.3%. $C_{17}H_{15}N_3O_5S$ requires C, 54.7; H, 4.1; N, 11.3%). υ_{max} : 3250, 1640, 1600, 1570, 1520, 1350cm^{-1}. ^1H n.m.r : δ9.40, 1H, bs, NH ; 8.20-8.16, 2H, d, $J_{3',2'}$ 8.8Hz, $H_{3'}$; 8.08-8.04, 2H, d, $J_{2',3'}$ 8.8Hz, $H_{2'}$; 7.84, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.4, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.22, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 6.70, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ;

3.85, 3H, s, OCH_3 ; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 373($M^{+\bullet}$, 80%), 187(M-$SO_2C_6H_4NO_2$, 100%), 173(M-CH_3, 92%).

3-Trifluoromethane-N-(6-methoxy-2-methy-8-quinolyl)benzene sulfonamide ***18*** as brown crystals (120mg, 38%), m.p. 143-145 °C (Found C, 54.5; H, 3.9; N, 7.2%. $C_{18}H_{15}F_3N_2O_3S$ requires C, 54.5; H, 3.8; N, 7.2%). υ_{max} : 3190, 1620, 1600, 1570, 1340, 1170cm^{-1}. ^1H n.m.r. : δ8.17, 1H, m, $H_{2'}$; 8.04, 1H, m, $H_{4'}$; 7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.63, 1H, m, $H_{6'}$; 7.48, 1H, t, $J_{5'6'}=J_{5'4'}$ 7.8Hz, $H_{5'}$; 7.44, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.20, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.70, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 3.84, 3H, s, OCH_3 ; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 396($M^{+\bullet}$, 100%), 377(8%), 187(M-$SO_2C_7H_4F_3$, 5%).

N-(6-Methoxy-2-methyl-8-quinolyl)-1-napthalenesulfonamide ***19*** as pale brown prismatic crystals (160mg, 47%), m.p. 190-192°C (Found C, 66.4; H, 4.9; N, 7.4%. $C_{21}H_{18}N_2O_3S$ requires C, 66.7; H, 4.8; N, 7.4%). υ_{max} : 3250, 1640, 1600, 1560cm^{-1}. ^1H n.m.r : δ9.58, 1H, bs, NH ; 8.83, 1H, m, $H_{2'}$; 8.32, 1H, m, $H_{4'}$; 7.93, 1H, m, $H_{5'}$; 7.80, 1H, m, $H_{8'}$; 7.72, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.69-7.63, 1H, m, $H_{7'}$; 7.53-7.40, 2H, m, $H_{6',8'}$; 7.33, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.11, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.56, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 3.78, 3H, s, OCH_3 ; 2.57, 3H, s, CH_3. Mass Spectrum : *m/z* 378($M^{+\bullet}$, 26%), 314(50%), 187(M-$SO_2C_{10}H_7$, 25%), 172(M-CH_3, 70%).

N-(6-Methoxy-2-methyl-8-quinolyl)-2-napthalenesulfonamide ***20*** as pale green crystals (96mg, 60%), m.p. 151-153°C (lit.[146] m.p. 147-149°C). υ_{max} : 3250, 1600, 1620, 1520cm^{-1}. ^1H n.m.r : δ9.38, 1H, bs, NH ; δ8.52, 1H, d, $J_{1',3'}$ 1.6Hz, $H_{1'}$; 7.91-7.85, 2H, m, H_{napth} ; 7.81-7.75, 2H, m, H_{napth} ; 7.77, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.57-7.49, 2H, m, H_{napth} ; 7.45, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.16, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.61, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.80, 3H, s, OCH_3 ; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 378($M^{+\bullet}$, 20%), 81(50%), 69(100%), 41(60%).

N-(6-Methoxy-2-methyl-8-quinolyl)-[(5-dimethylamino)-1-naphthyl] sulfonamide ***21*** as green fluorescent prismatic crystals (150mg, 45%), m.p. 170-172°C (Found C, 65.3; H, 5.8; N, 10.1%. $C_{23}H_{23}N_3O_4S$ requires C, 65.6; H, 5.5; N, 10.0%). υ_{max} : 3250, 1640, 1600, 1560cm^{-1}. ^{1}H n.m.r : δ9.62, 1H, bs, NH ; 8.54-8.50, 1H, m, $H_{2'}$; 8.47-8.45, 1H, m, $H_{4'}$; 8.37-8.34, 1H, dd, $J_{6'2'}$ 1.3Hz, $J_{6'3'}$ 7.4Hz, $H_{6'}$; 7.77, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.61-7.56, 1H, m, $H_{3'}$; 7.49-7.44, 1H, dd, $J_{7'6'}$ 7.4Hz, $J_{7'8'}$ 8.5Hz, $H_{7'}$; 7.37, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.17, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.13, 1H, m, $H_{8'}$; 6.61, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.82, 3H, s, OCH_3 ; 2.83, 6H, s, $N(CH_3)_2$; 2.62, 3H, s, CH_3. Mass Spectrum : *m/z* 421($M^{+\bullet}$, 100%), 357(67%), 188(M-$C_{12}H_{12}NSO_2$, 30%), 172(M-CH_3, 35%).

Chapter 4 : Methoxy isomers of Zinquin.

Preparation of 4-methyl-*N*-(5-methoxy-2-methyl-8-quinolyl)benzene sulfonamide, 28.

N-(3-Methoxyphenyl)acetamide **23**.

m-Anisidine (20g, 0.164mol) in 2M hydrochloric acid (100ml) was treated with sodium acetate solution (20%, 500ml) followed by acetic anhydride (70ml). After shaking vigorously for 20min, the mixture was cooled in an ice bath and the precipitate formed, collected washed with water and dried, yielding the title compound as colourless plates, (18.20g, 97%) m.p. 80-81°C(lit.[105] m.p. 81°C, R_f 0.35, 5% methanol/dichloromethane). ^{1}H n.m.r. : δ7.44, 1H, bs, NH ; 7.27, 1H, t, *J* 2.1Hz, H_2 ; 7.20, 1H, t, *J* 8.2Hz, H_5 ; 6.98, 1H, m, H_4 ; 6.65, 1H, dd, *J* 8.2Hz, *J* 2.1Hz, H_6 ; 3.78, 3H, s, OCH_3 ; 2.16, 3H, s, $COCH_3$. Mass Spectrum : *m/z* 165($M^{+\bullet}$, 56%), 123(M-$COCH_3$, 100%), 95(50%), 43(75%).

N-(5-Methoxy-2-nitrophenyl)acetamide **24**.

N-(5-Methoxyphenyl)acetamide (10g, 0.061mol) was dissolved in acetic anhydride (90ml) and stirred at room temperature while a mixture of nitric acid (3.4ml) and glacial acetic acid (3.4ml) was added carefully. The mixture was then stirred in ice for 1h and then at room temperature for 5h. 3M hydrochloric acid (80ml) was then added to degrade any excess acetic anhydride followed by water (280ml) and the precipitate collected by filtration. The required compound was obtained as an orange solid, (3.90g, 31%) m.p. 122-123°C (lit.[105] m.p. 121-123°C, R_f 0.40 dichloromethane). ^{1}H n.m.r. : δ10.77, 1H, bs, NH ; 8.40, 1H, d, $J_{6,4}$ 2.7Hz, H_6 ; 8.17, 1H, d, $J_{3,4}$ 9.4Hz,

H_3 ; 6.63, 1H, dd, $J_{4,6}$ 2.7Hz, $J_{4,3}$ 9.4Hz, H_4 ; 3.89, 3H, s, OCH_3 ; 2.28, 3H, $COCH_3$. Mass Spectrum : *m/z* 210($M^{+\bullet}$, 46%), 164(M-NO_2, 100%), 138(78%), 124(79%), 43(95%).

5-Methoxy-2-nitroaniline **25**.

N-(5-Methoxy-2-nitrophenyl)acetamide (3.5g, 0.017mol) was refluxed in concentrated hydrochloric acid (42ml) for 5h. The mixture was then cooled to room temperature and water added (35ml) until a precipitate formed. The precipitate was collected by Buchner filtration, dried and recrystallised from dichloromethane/hexane to yield the title compound as a yellow solid, (2.0g, 71%) m.p. 127.5-129°C (lit.[105] m.p. 126-128°C, R_f 0.50 dichloromethane). ^1H n.m.r. : δ8.05, 1H, d, $J_{2,3}$ 9.5Hz, H_2 ; 6.23, 1H, dd, $J_{3,3}$ 9.5Hz, $J_{3,6}$ 3.9Hz, H_3 ; 6.18, 2H, bs, NH_2 ; 6.12, 1H, d, $J_{6,3}$ 3.9Hz, H_6 ; 3.81, 3H, s, OCH_3. Mass Spectrum : *m/z* 168($M^{+\bullet}$, 100%), 138(M-CH_2O, 58%), 122(M-NO_2, 32%), 110(24%), 107(19%), 95(32%), 79(35%), 52(44%).

5-Methoxy-2-methyl-8-nitroquinoline **26**.

5-Methoxy-2-nitroaniline (0.50g, 2.98mmol), paraldehyde (0.80g) and concentrated hydrochloric acid (2.9ml) were refluxed for 3.5h with vigorous stirring. The mixture was then cooled, neutralised with saturated sodium hydroxide and the aqueous layer extracted with dichloromethane (100ml x 2). The organic layers were then washed with water (50ml x 2), dried and the solvent removed under vacuum. The crude mixture was then chromatographed (dichloromethane) yielding the required product as an orange solid, (0.150g, 25%) m.p. 112-114°C (R_f 0.32 dichloromethane). ^1H n.m.r. : δ8.30, 1H, d, $J_{7,6}$ 8.6Hz, H_7 ; 7.95, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.23, 1H, d, $J_{6,7}$ 8.6Hz, H_6 ; 6.63, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 3.94, 3H, s, OCH_3 ; 2.64, 3H, s, CH_3.

Mass Spectrum : *m/z* 218($M^{+\bullet}$, 88%), 188(M-CH_2O, 100%), 173(72%), 160(46%), 145(48%), 91(50%), 69(55%), 57(53%), 42(72%).

8-Amino-5-methoxy-2-methylquinoline **27**.

5-Methoxy-2-methyl-8-nitroquinoline (100mg, 0.459mmol) was reduced under iron/acetic acid conditions; Method (a) reduction of 6-methoxy-2-methyl-8-nitroquinoline **7**. The title compound was obtained as a orange brown oil (0.071g, 82%). ^{1}H n.m.r. : δ8.35, 1H, d, $J_{7,6}$ 8.5Hz, H_7 ; 7.21, 1H, d, $J_{6,7}$ 8.5Hz, H_6 ; 6.80, 1H, d, $J_{4,3}$ 8.2Hz, H_4 ; 6.62, 1H, d, $J_{3,4}$ 8.2Hz, H_3 ; 4.60, 2H, bs, NH_2 ; 3.88, 3H, s, OCH_3 ; 2.67, 3H, s, CH_3. Mass Spectrum : *m/z* 188($M^{+\bullet}$, 71%), 173(M-CH_3, 100%), 145(24%).

4-Methyl-N-(5-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **28**.

The amine (71mg, 0.378mol) was tosylated under the standard conditions used for the formation of sulfonamides **13-21**. Recystallization from dichloromethane/hexane yielded the required sulfonamide **28** as pale yellow crystals (97mg, 75%), m.p. 162-164°C (Found C, 62.9; H, 5.1; N, 8.1%. $C_{18}H_{18}N_2O_3S$ requires C, 63.1; H, 5.3; N, 8.2%). ^{1}H n.m.r. : δ8.88, 1H, bs, NH ; 8.33, 1H, d, $J_{6,7}$ 8.6Hz, H_7 ; 7.72-7.69, 3H, m, $H_{2',4}$; 7.22, 1H, d, $J_{6,7}$ 8.6, H_6 ; 7.08, 2H, d, $J_{3',2'}$ 8.4Hz, $H_{2'}$; 6.68, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 3.92, 3H, s, OCH_3 ; 2.64, 3H, s, CH_3 ; 2.26, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 342($M^{+\bullet}$, 26%), 242(30%), 187(M-$C_7H_7SO_2$, 95%), 121(68%), 105(50%), 91(32%), 65(100%).

Preparation of 4-methyl-*N*-(4-methoxy-2-methyl-8-quinolyl)benzene sulfonamide, 36.

Ethyl-β-anilinocrotonate ***30.***

The title compound was prepared via the method of Hauser and Renyolds[103] obtaining a yellow oil, (1.76g, 80%) b.p. 154-156°C @ 10mm (lit.[103] b.p. 155°C @ 10mm). ^{1}H n.m.r. : δ10.39, 1H, bs, NH ; 7.34-7,06, 5H, m, $H_{aromatics}$; 4.69, 1H, s, H_{vinyl} ; 4.18-4.11, 2H, q, *J* 7.10Hz, OCH_2CH_3 ; 1.99, 3H, s, CH_3 ; 1.30-1.26, 3H, t, *J* 7.10Hz, OCH_2CH_3. Mass Spectrum : *m/z* 205($M^{+\bullet}$, 100%), 160(68%), 144(39%), 132(72%), 118(70%), 77(40%).

4-Hydroxy-2-methylquinoline ***31.***

Also prepared by the method of Hauser and Renyolds[103] yielding a white solid, (1.5g, 39%) m.p. 232-233°C (lit.[103] m.p. 229-230°C). Mass Spectrum : *m/z* 159($M^{+\bullet}$, 47%), 130(62%), 104(20%), 74(21%), 63(33%), 52(36%), 39(100%).

4-Chloro-2-methylquinoline ***32.***

The above hydroxy quinoline (1.5g, 9.4mmol) was refluxed in phosphorous oxychloride (5ml) for 3h with vigorous stirring. The reaction was then cooled, poured onto crushed ice and neutralised with 25% sodium hydroxide. The yellow oil which formed was collected and purified by chromatography yielding the title compound, (0.90g, 81%) m.p. 40-42°C monohydrate, (lit.[108] m.p. 42-44°C). ^{1}H n.m.r. : δ8.17-8.13, 1H, dd, *J* 1.4Hz, *J* 8.4Hz, H_8 ; 8.02-7.98, 1H, m, H_5 ; 7.75-7.66, 1H, m, H_7 ; 7.59-7.50, 1H, m, H_6 ;

7.37, 1H, s, H_3 ; 2.70, 3H, s, CH_3. Mass Spectrum : *m/z* 177($M^{+\bullet}$, 30%), 140(91%), 99(41%), 74(36%), 62(40%), 49(100%), 43(82%).

4-Chloro-2-methyl-8-nitroquinoline **33**.

4-Chloro-2-methylquinoline (0.800mg, 4.52mmol) was dissolved in sulfuric acid (2.90ml) and stirred at 0°C. Potassium nitrate (0.625g, 6.17mmol) was then added gradually and the mixture stirred at room temperature overnight. The reaction was then poured onto ice and the precipitate extracted with ethyl acetate. Removal of the solvent followed by chromatography yielded the required product as a yellow solid, (0.560g, 56%) m.p. 112-114°C (lit.[104] m.p. 111-113°C, R_f 0.80 dichloromethane). ^{1}H n.m.r. : δ8.33, 1H, dd, $J_{7,6}$ 8.5Hz, $J_{7,5}$ 1.4Hz, H_7 ; 7.95, 1H, dd, $J_{5,6}$ 7.4Hz, $J_{5,7}$ 1.4Hz, H_5 ; 7.63-7.55, 1H, dd, $J_{6,7}$ 8.5Hz, $J_{6,5}$ 7.3Hz, H_6 ; 2.71, 3H, s, CH_3. Mass Spectrum : *m/z* 222($M^{+\bullet}$, 100%), 192(28%), 176(29%), 164(98%), 140(66%), 114(31%), 74(32%), 63(33%), 46(80%).

4-Methoxy-2-methyl-8-nitroquinoline **34**.

4-Chloro-2-methyl-8-nitroquinoline (0.30g, 1.33mmol) was stirred in a solution of sodium (0.06g, 2.61mmol) in methanol (3ml) and the mixture refluxed under a N_2 atmosphere for 2h. The reaction was then cooled, solvent removed and the residue dissolved in dichloromethane and purified by flash chromatography yielding the title compound as white needles, (0.20g, 68%) m.p. 130-131°C (lit.[104] m.p. 129-130°C, R_f 0.45 dichloromethane). ^{1}H n.m.r. : δ8.27, 1H, dd, $J_{7,6}$ 8.4Hz, $J_{7,5}$ 1.4Hz, H_7 ; 7.88, 1H, dd, $J_{5,6}$ 7.5Hz, $J_{5,7}$ 1.4Hz, H_5 ; 7.46-7.38, 1H, dd, $J_{6,5}$ 7.5Hz, $J_{6,7}$ 8.4Hz, H_6 ; 6.68, 1H, s, H_3 ; 4.02, 3H, s, OCH_3 ; 2.66, 3H, s, CH_3. Mass Spectrum : *m/z*

218($M^{+\bullet}$, 100%), 188(26%), 172(24%), 159(45%), 130(61%), 89(44%), 69(40%), 57(45%), 43(80%).

8-Amino-6-methoxy-2-methylquinoline **35**.

The above nitroquinoline (0.185g, 0.85mmol) was reduced under iron/acetic acid conditions; Method (a) reduction of 6-methoxy-2-methyl-8-nitroquinoline **7**. The title compound was obtained as a yellow crystalline solid, (0.153g, 96%) m.p. 113-115°C (lit.[104,109] m.p. 113-116°C). ^{1}H n.m.r. : δ7.35, 1H, dd, $J_{7,6}$ 8.4Hz, $J_{7,5}$ 1.4Hz, H_7 ; 7.12, 1H, dd, $J_{6,7}$ 8.4Hz, $J_{6,5}$ 7.5Hz, H_6 ; 6.81-6.77, 1H, dd, $J_{5,6}$ 7.5Hz, $J_{5,7}$ 1.4Hz, H_5 ; 6.47, 1H, s, H_3 ; 4.78, 2H, bs, NH_2 ; 3.87, 3H, s, OCH_3 ; 2.56, 3H, s, CH_3. Mass Spectrum : *m/z* 188($M^{+\bullet}$, 100%), 118(16%).

4-Methyl-N-(4-methoxy-2-methyl-8-quinolyl)benzenesulfonamide **36**.#

The amine (0.150g, 0.79mmol) was tosylated under the same conditions used for the formation of sulfonamide **13-21**. The required product was obtained as pale yellow prism, (0.249g, 90%) m.p. 191-193°C (Found C, 63.2; H, 5.1; N, 8.4%. $C_{18}H_{18}N_2O_3S_1$ requires C, 63.1; H, 5.3; N, 8.2%). υ_{max} : 3270, 1600, 1510, 1350, 1170cm^{-1}. ^{1}H n.m.r. : δ9.28, 1H, bs, NH ; 7.79-7.74, 2H, d, $J_{2',3'}$ 8.2Hz, $H_{2'}$; 7.22-7.65, 2H, m, $H_{5,7}$; 7.29-7.21, 1H, dd, $J_{6,5}$ 7.6Hz, $J_{6,7}$ 8.6Hz, H_6 ; 7.14-7.09, 2H, d, $J_{3',2'}$ 8.2Hz, $H_{3'}$; 5.57, 1H, s, H_3 ; 3.95, 3H, s, OCH_3 ; 2.51, 3H, s, CH_3 ; 2.27, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 342($M^{+\bullet}$, 52%), 293(70%), 278)25%), 187(M-$C_7H_7SO_2$, 100%), 137(97%), 123(33%), 91(50%), 69(40%).

X-ray crystal analysis contained in Appendix C

Preparation of 4-methyl-*N*-(2-methoxy-8-quinolyl)benzene sulfonamide, 41.

2-Chloro-8-nitroquinoline 38.

2-Chloroquinoline (1g, 0.0061mol) was dissolved in sulfuric acid (6.65g) and stirred at 0°C while potassium nitrate (0.85g, 0.0084mol) was added. The solution was then allowed to warm to room temperature and stirred overnight. The mixture was poured over ice and the aqueous layer extracted with ethyl acetate. The organic layer was then washed with sodium hydroxide (2M, 50ml), dried and the solvent removed. The product was then TLC'ed (3 : 2/hexane : ethyl acetate) and the lower R_f spot (0.5) isolated. This yielded the required nitrated isomer, indicated by ^1H n.m.r., which was then recrystallised from ethanol yielding 2-chloro-8-nitroquinoline as yellow needles, (0.620g, 49%) m.p. 148-149°C (lit.[110,111] m.p. 148-149°C). ^1H n.m.r. : δ8.17, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 8.09-8.00, 2H, m, $H_{7,5}$; 7.66-7.58, 1H, dd, $J_{6,7}$ 7.5Hz, $J_{6,5}$ 8.3Hz, H_6 ; 7.52, 1H, d, $J_{3,4}$ 8.6Hz, H_3. Mass Spectrum : *m/z* 208($M^{+\bullet}$, 100%), 192(20%), 178(53%), 156(83%), 127(72%).

2-Methoxy-8-nitroquinoline 39.

Sodium (65mg, 2.73mmol) was added to methanol (3.2ml) and stirred. 2-Chloro-8-nitroquinoline (300mg, 1.44mmol) was then added and the reaction refluxed under an atmosphere of N_2 for 2h. The reaction was then cooled solvent removed and the residue dissolved in dichloromethane. TLC (dichloromethane) indicated conversion to the required product which was passed down a small column of silica yielding the required compound as a white solid (265mg, 90%), m.p. 126.5-128°C, (lit.[109] m.p. 122-123°C). ^1H n.m.r. : δ8.00, 1H, d, $J_{4,3}$ 8.9Hz, H_4 ; 7.93, 1H, dd, $J_{7,6}$ 8.0Hz, $J_{7,5}$ 1.5Hz, H_7 ; 7.86, 1H, dd, $J_{5,6}$ 8.0Hz, $J_{5,7}$ 1.4Hz, H_5 ; 7.38, 1H, t, $J_{6,7}$=$J_{6,5}$ 8.0Hz, H_6 ; 6.96, 1H, d, $J_{3,4}$

8.9Hz, H_3 ; 4.03, 3H, s, OCH_3. Mass Spectrum : *m/z* 204($M^{+\bullet}$, 100%), 186(33%), 174(51%), 157(50%).

8-Amino-2-methoxyquinoline **40**.

2-Methoxy-8-nitroquinoline (250mg, 1.226mmol) was reduced under iron/acetic acid conditions; Method (a) reduction of 6-methoxy-2-methyl-8-nitroquinoline **7**. The required product was obtained as yellow crystals (187mg, 88%), m.p. 55-57°C, (R_f 0.45 dichloromethane). ^1H n.m.r. : δ7.93, 1H, d, $J_{4,3}$ 9.0Hz, H_4 ; 7.23-7.08, 2H, m, $H_{6,7}$; 6.94, 1H, dd, $J_{5,6}$ 7.0Hz, $J_{5,7}$ 1.8Hz, H_5 ; 6.90, 1H, d, $J_{3,4}$ 8.8Hz, H_3 ; 4.74, 2H, bs, NH_2 ; 4.07, 3H, s, OCH_3. Mass Spectrum : *m/z* 174($M^{+\bullet}$, 100%), 145(15%), 131(10%), 117(15%), 104(13%).

4-Methyl-N-(2-methoxy-8-quinolyl)benzenesulfonamide **41**.

8-Amino-2-methoxyquinoline (185mg, 1.06mmol) was tosylated under the same conditions used for the formation of the sulfonamide **13-21**. This yielded the required product as white prisms (280mg, 80%), m.p. 180.5-182°C (Found C, 61.9; H, 5.0; N, 8.6%. $C_{17}H_{16}N_2O_3S_1$ requires C, 62.2; H, 4.9; N, 8.5%). υ_{max} : 3325, 1610, 1600cm^{-1}. ^1H n.m.r. : δ8.58, 1H, bs, NH ; 7.90, 1H, d, $J_{4,3}$ 8.8Hz, H_4 ; 7.77, 1H, dd, $J_{7,5}$ 1.4Hz, $J_{7,6}$ 7.6Hz, H_7 ; 7.71, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.37, 1H, dd, $J_{5,7}$ 1.4Hz, $J_{5,6}$ 8.0Hz, H_5 ; 7.26, 1H, dd, $J_{6,7}$ 7.6Hz, $J_{6,5}$ 8.0Hz, H_6 ; 7.11, 2H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.85, 1H, d, $J_{3,4}$ 8.8Hz, H_3 ; 4.01, 3H, s, OCH_3 ; 2.27, 3H, s, CH_3. Mass Spectrum : *m/z* 328($M^{+\bullet}$, 100%), 264(17%), 173(M-$C_7H_7SO_2$, 96%), 143(25%), 91(32%), 69(28%), 41(22%).

Chapter 5 : Acridine and acridone ligands.

Preparation of 4-methyl-*N*-(2-methoxy-5-acridonyl)benzenesulfonamide, 48.

2-Bromo-3-nitrobenzoic acid **52**.

Treatment of 3-nitrophthalic acid (10.55 g, 0.045 mol) with mercuric acetate (17.5g, 0.055mol), sodium hydroxide and glacial acetic acid followed by a bromine, sodium iodide solution as described by Culhane[115] gave 2-bromo-3-nitrobenzoic acid as a white crystalline solid (8.7g, 70%), m.p. 182-184°C, (lit[115] m.p. 182-184°C). υ_{max} : 1680, 1520, 1360cm^{-1}. ^{1}H n.m.r. ; δ 8.06, 1H, dd, *J* 1.5 Hz, *J* 7.9, H_4 ; 7.92, 1H, dd, *J* 1.5 Hz *J* 7.8 Hz, H_6 ; 7.69, 1H, t, *J* 7.9 Hz, H_4. Mass spectrum : *m/z* 247($M^{+\bullet}$, 100%), 216(M-NO, 25%)

4'-Methoxy-6-nitro-diphenylamine-2-carboxcylic acid **54**.[147]

2-Bromo-3-nitrobenzoic acid (2.0 g, 8.13 mmol), *p*-anisidine (3.6 ml, 26.8 mmol), potassium carbonate (1.20 g, 8.68 mmol), copper powder (0.02 g) and 1-pentanol (10 ml) were refluxed at 140°C and stirred vigorously under a nitrogen atmosphere for a period of 3.5 h. The reaction mixture was cooled, dichloromethane added (100 ml) and the organic layer extracted with water (3 x 150ml). The combined aqueous layers was acidifed using 6M hydrochloric acid solution and the precipitate that formed collected, dried and recrystallised from ethanol/water to yield 4'-methoxy-6-nitro-diphenylamine-2-carboxcylic acid as red/orange crystals (1.40g, 60%), m.p. 178-181°C (lit.[147] m.p. 174-176°C). υ_{max} : 3250, 1650, 1570, 1520, 1510, 1340cm^{-1}. ^{1}H n.m.r. (DMSO-D_6) δ 9.77, 1H, s, CO_2H ; 7.85, 1H, dd, $J_{5,3}$ 1.6 Hz, $J_{5,4}$ 7.8 Hz, H_5 ; 7.54, 1H, dd, $J_{3,5}$ 1.4 Hz, $J_{3,4}$ 7.8 Hz, H_3 ; 6.50, 1H, t, $J_{4,3}$=$J_{4,5}$ 7.9 Hz, H_4 ; 6.53, 2H, d, $J_{6',5'}$=$J_{2',3'}$ 9.1 Hz, $H_{6'2'}$; 6.43, 2H, d, $J_{5',6'}$=$J_{3',2'}$ 9.1 Hz, $H_{5'3'}$; 3.40,

3H, s, OCH_3. Mass spectrum : *m/z* 288($M^{+\bullet}$, 100%), 105(41%), 54(43%), 42(60%)

2-Methoxy-5-nitroacridone ***56*** *and 9-chloro-2-methoxy-5-nitroacridine* ***55.***

4'-Methoxy-6-nitro-diphenylamine-2-carboxcylic acid (0.150 g, 0.520 mmol), was refluxed with phosphorous oxychloride (3 ml) for a period of 1.5-2 h. The reaction mixture was then cooled, ice water added dropwise cautiously until a precipitate formed. The aqueous layer was then extracted using dichloromethane (3 x 10ml), and the organic extracts washed with saturated sodium bicarbonate (10ml), dried and solvent removed. The product obtained was shown to be a mixture of two compounds (TLC, dichloromethane) which were separated by flash chromatography (dichloromethane);

(1) yellow needle crystals of 9-chloro-2-methoxy-5-nitroacridine, (90mg, 60%), m.p. 271-274°C, (lit.[116] m.p. 243-244°C). υ_{max} : 1620, 1590, 1530, 1320cm^{-1}. ^1H n.m.r. δ 8.63, 1H, dd, $J_{6,7}$ 8.9 Hz, $J_{6,8}$ 1.3 Hz, H_6 ; 8.15, 1H, dd, $J_{3,4}$ 9.1 Hz, $J_{3,1}$ 0.7 Hz, H_3 ; 8.05, 1H, dd, $J_{8,7}$ 7.3 Hz, $J_{8,6}$ 1.3 Hz, H_8 ; 7.66, 1H, dd, $J_{7,8}$ 7.3 Hz, $J_{7,6}$ 8.9 Hz, H_7 ; 7.55, 1H, d, $J_{1,3}$ 0.7 Hz, H_1 ; 7.51, 1H, d, $J_{4,3}$ 9.1 Hz, H_4 ; 4.07, 3H, s, OMe. Mass spectrum : *m/z* 288($M^{+\bullet}$, 42%), 242(25%), 230(30%), 164(53%), 81(46%), 69(100%), 41(57%).

(2) red crystals of 2-methoxy-5-nitroacridone, (10mg, 8%), m.p. 223-225°C, (lit.[116] m.p. 229-230°C). υ_{max} : 3250, 1620, 1590, 1550, 1530, 1330cm^{-1}. ^1H n.m.r. δ 8.87, 1H, m, H_6 ; 8.70, 1H, m, H_3 ; 7.79, 1H, m, H_1 ; 7.40-7.35, 2H, m, $H_{8,4}$; 7.31, 1H, t, $J_{7,8}$=$J_{7,6}$ 8.04 Hz, H_7 ; 3.93, 3H, s, OCH_3. Mass spectrum : *m/z* 270($M^{+\bullet}$, 69%), 240(28%), 225(21%), 181(11%), 153(14%), 129(13%), 97(19%), 55(63%), 43(100%).

2-Methoxy-5-nitroacridone **56**.

9-Chloro-2-methoxy-5-nitroacridine (100mg, 0.347mmol), was refluxed with acetic acid (80%, 5ml) for a period of 12h. The mixture was added to water and the aqueous layer extracted with dichloromethane (3 x 20ml), the organic extracts washed with sodium hydroxide (1M, 2 x 20ml) combined, dried, and the solvent removed. TLC and ^{1}H n.m.r. showed total conversion to 2-methoxy-5-nitroacridone **56** (82mg, 99%).

5-Amino-2-methoxyacridone **62**.

The acridone (100mg, 0.370mmol) was stirred in dry ethanol (30ml) in the presence of Pd-C (5%, 0.100g) under an atmosphere of H_2 for a period of 16h. The resultant brown oil was identified as 5-amino-2-methoxyacridone (71mg, 80%). ^{1}H n.m.r. δ8.06, 1H, m, H_3 ; 7.60, 1H, dd, $J_{6,7}$ 8.7Hz, $J_{6,8}$ 1.2Hz, H_6 ; 7.47, 1H, d, $J_{1,3}$ 2.7Hz, H_1 ; 7.43-7.38, 2H, m, $H_{7,4}$; 6.88, 1H, dd, $J_{8,7}$ 7.5Hz, $J_{8,6}$ 1.2Hz, H_8 ; 5.22, 2H, s, NH_2 ; 4.01, 3H, s, OCH_3. Mass Spectrum : *m/z* 240($M^{+\bullet}$, 50%), 225(49%), 197(8%), 169(16%), 77(100%)..

4-Methyl-N-(2-methoxy-5-acridonyl)benzenesulfonamide **48**.

5-Amino-2-methoxyacridone (0.071g, 0.300mmol) was dissolved in pyridine (3ml) and stirred at 0°C. Tosylchloride (0.068g, 0.355mmol) was added and the resultant mixture stirred for 20h. Water was added to the mixture and the resultant precipitate collected by suction filtration, dissolved in dichloromethane and washed with sodium bicarbonate. The solvent was removed to obtain a yellow/brown solid which was recrystallised from ethanol to yield 4-methyl-N-(2-methoxy-5-acridonyl)benzenesulfonamide as yellow-green crystals (0.06g, 52%), which decomposed at 285°C (Found C,

63.7; H, 4.9; N, 7.0%. $C_{21}H_{18}N_2O_4S$ requires C, 64.0; H, 4.6; N, 7.1%). υ_{max} : 3350, 3250, 1640, 1610, 1580, 1350, 1160cm^{-1}. ^{1}H n.m.r. (DMSO-D_6) ; δ10.85, 1H, bs, $NH_{sulfonamide}$; 9.79, 1H, bs, $NH_{acridone}$; 8.10, 1H, dd, $J_{8,7}$ 7.6Hz, $J_{8,6}$ 2.0Hz, H_8 ; 7.84, 1H, d, $J_{4,3}$ 9.2Hz, H_4 ; 7.59-7.56, 3H, m, $H_{2',1}$; 7.41, 1H, dd, $J_{3,1}$ 3.0Hz, $J_{3,4}$ 9.1Hz, H_3 ; 7.30, 2H, m, $H_{3'}$; 7.05-6.97, 2H, m, $H_{6,7}$; 3.84, 3H, s, OCH_3 ; 2.29, 3H, s, $ArCH_3$. Mass spectrum : *m/z* 394($M^{+\bullet}$, 36%), 239(M-$C_7H_7SO_2$, 100%), 224(M-CH_3, 29%), 196(20%), 168(20%).

Preparation of 4-methyl-*N*-(4-methoxy-5-acridonyl)benzenesulfonamide, 49.

2'-Methoxy-6-nitrodiphenylamine-2-carboxylic acid **53.**[147]

2-Bromo-3-nitrobenzoic acid (2.0g, 8.13mmol), *o*-anisidine (3.6ml, 26.8mmol), potassium carbonate (1.20g, 8.68mmol), copper powder (0.02g) and 1-pentanol (10ml) were refluxed at 140°C and stirred vigorously under a N_2 atmosphere for a period of 3.5h. The reaction mixture was cooled further dichloromethane (150ml) added, the organic layer extracted with water (3 x 150ml), and the combined aqueous layers acidifed using 6M hydrochloric acid solution. The precipitate formed was collected, dried and recrystallised from ethanol/water to yield 2'-methoxy-6-nitrodiphenylamine-2-carboxylic acid (1.31g, 56%) as a red/orange prisms, m.p. 241-243°C (lit.[147] m.p. 227°C). υ_{max} : 3250, 1650, 1570, 1520, 1340cm^{-1}. ^{1}H n.m.r. (DMSO-D_6) δ10.10, 1H, bd, CO_2H ; 8.20, 1H, dd, $J_{5,3}$ 1.7Hz, $J_{5,4}$ 7.8, H_5 ; 7.96, 1H, dd, $J_{3,5}$ 1.7, $J_{3,4}$ 7.8, H_3 ; 6.95-6.86, 3H, m, $H_{3',4',4}$; 6.74-6.71, 2H, m, $H_{5',6'}$; 3.76, 3H, s, OCH_3 . Mass spectrum : *m/z* 288($M^{+\bullet}$, 50%), 81(39%), 69(89%), 40(100%).

4-Methoxy-5-nitroacridone **57.**

2'-Methoxy-6-nitrodiphenylamine-2-carboxylic acid (0.350g, 1.214mmol), was refluxed with phosphorus oxychloride (7ml) for a period of 1.5-2h. The reaction mixture was cooled, ice water added dropwise cautiously until a precipitate formed. The aqueous layer was then extracted and the organic extracts washed with saturated sodium bicarbonate (10ml). The extracts were concentrated and the solid obtained recrystallised from dichloromethane/hexane to yield 4-methoxy-5-nitroacridone (0.22g, 65%) as red/orange needle crystals, m.p. 255-259°C (lit.[116] m.p. 246°C). Subsequent

runs saw an average yield of 70% attained. υ_{max} : 3250, 1630, 1600, 1580, 1550, 1330cm^{-1}. ^{1}H n.m.r. δ8.88, 1H, m, H_1 ; 8.73, 1H, dd, $J_{8,6}$ 1.2Hz, $J_{8,7}$ 8.1Hz, H_8 ; 7.99, 1H, m, H_6 ; 7.34, 1H, t, $J_{7,6}$=$J_{7,8}$ 8.0Hz, H_7 ; 7.31, 1H, t, $J_{2,1}$=$J_{2,3}$ 7.9Hz, H_2 ; 7.22, 1H, dd, $J_{3,1}$ 1.4Hz, $J_{3,2}$ 7.9Hz, H_3 ; 4.11, 3H, s, OCH_3 . Mass Spectrum : *m/z* 270($M^{+\bullet}$, 100%), 255(60%), 209(21%).

5-Amino-4-methoxyacridone ***61***.

(a). 4-Methoxy-5-nitroacridone (0.18g,), iron powder (0.14, 35mg/atom), glacial acetic acid (4.8ml) and ethanol (4.5ml) were refluxed for 3.5h under N_2. Water was added and the contents transferred to a separating funnel where the aqueous layer was extracted with chloroform (4 x 15ml). The organic layer was washed with sodium hydroxide solution (1M, 2 x 15ml), dried and the solvent removed under reduced pressure to yield 5-amino-4-methoxyacridone as a yellow solid (0.15g, 92%), m.p. 240-244°C. ^{1}H n.m.r. : δ7.65, 1H, m, H_6 ; 7.57, 1H, m, H_3 ; 6.92-6.75, 4H, m, $H_{1,2,7,8}$; 4.40, 2H, bs, NH_2 ; 3.79, 3H, s, OCH_3. Mass Spectrum : *m/z* 240($M^{+\bullet}$, 91%), 225(100%), 197(8%), 169(16%).

(b). The acridone (100mg, 0.370mmol) was stirred in dry ethanol (30ml) in the presence of Pd-C (5%, 0.100g) under an atmosphere of H_2 for a period of 16h. The resultant mixture was passed through kenite and the solvent removed to yield 4-methoxy-5-amino-acridone as a brown oil (87mg, 98%). ^{1}H n.m.r. and mass spectral data matched that of the above compound.

4-Methyl-N-(4-methoxy-5-acridonyl)benzenesulfonamide ***49***.

5-Amino-4-methoxyacridone (0.100g, 0.370mmol) was tosylated using the same conditions for the formation of **48**. Recrystallisation from ethanol

yielded the title compound as fine yellow crystals (0.070g, 50%), mp 267.5-269°C (Found C, 63.7; H, 4.6; N, 7.3%. $C_{21}H_{18}N_2O_4S$ requires C, 64.0; H, 4.6; N, 7.1%). υ_{max} 3450, 3250, 1650, 1630, 1610, 1600cm^{-1}. ^{1}H n.m.r. ; δ9.62, 1H, bs, $NH_{sulfonamide}$; 8.40, 1H, dd, $J_{8,6}$ 1.5Hz, $J_{8,7}$ 8.1Hz, H_8 ; 8.00, 1H, dd, $J_{1,3}$ 1.4Hz, $J_{1,2}$ 8.4Hz, H_1 ; 7.59, 2H, d, $J_{2',3'}$ 8.1Hz, $H_{2'}$; 7.24, 1H, m, H_2 ; 7.19, 2H, d, $J_{3',2'}$ 8.0Hz, $H_{3'}$; 7.13, 1H, dd, $J_{3,1}$ 1.4Hz, $J_{3,2}$ 7.8Hz, H_3 ; 6.97, 1H, m, H_7 ; 6.78, 1H, dd, $J_{6,8}$ 1.5Hz, $J_{6,7}$ 7.2Hz, H_6 ; 6.36, 1H, bs, $NH_{acridone}$; 4.08, 3H, s, OCH_3 ; 2.41, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 394($M^{+\bullet}$, 10%), 240(M-$C_7H_7SO_2$, 48%), 225(M-CH_3, 46%), 168(53%), 141(68%), 115(72%), 77(78%), 69(100%), 57(72%), 55(82%).

Preparation of 4-methyl-*N*-(4-methoxy-5-acridinyl)benzenesulfonamide, 46.

5-Amino-2-methoxyacridine **63**.

9-Chloro-2-methoxy-5-nitroacridine (100mg, 0.346mmol), ethanol (20ml), and Pd-C catalyst (5%, 0.100g) were stirred under an atmosphere of H_2 for 16h. The reaction mixture was filtered through kenite and the solvent removed. TLC (dichloromethane) showed two products, starting material and another lower R_f product. The mixture was separated via a squat column, with the first fraction being eluted with dichloromethane, and the second being eluted with ethyl acetate. The second fraction contained the required compound 5-amino-2-methoxyacridine **63** as a brown oil (77mg, 98%). ^{1}H n.m.r. : δ8.46, 1H, s, H_9 ; 8.06, 1H, d, $J_{4,3}$ 9.5Hz, H_4 ; 7.36, 1H, dd, $J_{3,4}$ 9.4Hz, $J_{3,1}$ 2.7Hz, H_3 ; 7.30-7.23, 2H, m, $H_{7,6}$; 7.08, 1H, d, $J_{1,3}$ 2.7Hz, H_1 ; 6.85, 1H, dd, $J_{8,7}$ 6.5Hz, $J_{8,6}$ 2.1Hz, H_8 ; 5.17, 2H, bs, NH_2 ; 3.93, 3H, s, OCH_3. Mass Spectrum : *m/z* 224.2($M^{+\bullet}$, 100%), 181(68%), 154(24%).

4-Methyl-N-(2-methoxy-5-acridinyl)benzenesulfonamide **46**.#

5-Amino-2-methoxyacridine (53mg, 0.236mmol) was tosylated using the same conditions for the formation of **48**. Recrystallization from ethanol yielded the title compound as brown crystals (40mg, 45%), mp 224-226°C (Found C, 66.3; H, 4.7; N, 7.3%. $C_{21}H_{18}N_2O_3S$ requires C, 66.7; H, 4.80; N, 7.4%). ^{1}H n.m.r. ; δ9.50, 1H, s, NH ; 8.47, 1H, s, H_9 ; 8.04, 1H, d, $J_{4,3}$ 9.5Hz, H_4 ; 7.83, 2H, d, $J_{2',3'}$ 8.5Hz, $H_{2'}$; 7.70, 1H, m, H_6 ; 7.51, 1H, m, H_8 ; 7.44, 1H, dd, $J_{3,4}$ 9.5Hz, $J_{3,1}$ 2.7Hz, H_3 ; 7.34, 1H, m, H_7 ; 7.11, 2H, d, $J_{3',2'}$ 8.5Hz, $H_{3'}$; 7.08, 1H, d, $J_{1,3}$ 2.7Hz, H_1 ; 3.94, 3H, s, OCH_3 ; 2.24, 3H, s, $ArCH_3$. Mass spectrum : *m/z* 378.6($M^{+\bullet}$, 40%), 223.4(M-$C_7H_7SO_2$, 100%), 196.4(35%).

X-ray crystal analysis contained in Appendix C

Attempted preparation of 4-methoxy-5-nitro-9-trifluoromethan sulfonyloxyacridine ***60***.[118]

4-Methoxy-5-nitroacridone (0.300g, 1.85mmol) was dissolved in dry dichloromethane (20ml) and stirred at 0°C under a N_2 atmosphere. Trifluoromethanesulfonic anhydride (0.35ml, 2.00mmol) was added dropwise and the reaction mixture stirred at 0°C for a further 1.5h. Diethyl ether (25ml) was added to the reaction mixture and the resultant precipitate collected by filtration to yield approximately 0.60g of orange solid. The solid was dissolved in acetonitrile (30ml), diisoproplyethylamine (0.4ml) added and the reaction mixture stirred for a further 1h. The solvent was removed, the solid dissolved in water (10ml), the suspension filtered and the precipitate further dissolved in diethyl ether (15ml). Any solid material remaining was removed with a final filtration and the solvent then removed. The solid obtained was identified as the starting acridone by TLC and ^{1}H n.m.r.

Attempted reduction of 4-methoxy-5-nitroacridone **57**.[117]

The acridone (50mg, 0.21mmol) was refluxed in pentanol (3.5ml) and over a period of 30min. Sodium metal (200mg) was introduced (vigorous exothermic reaction) and the reaction was then reflux for a further 10min. cooled and the pentanol distilled off. TLC (dichloromethane) showed numerous spots, with the major compound being isolated via flash chromatography. ^{1}H n.m.r. indicated starting material.

Chapter 6 : Additions to the 2-methyl position of Zinquin.

2-Formyl-6-methoxy-8-nitroquinoline ***66***.[125]

To a boiling solution of selenious acid (3.56g, 0.028mol) in dioxane (28ml) and water (2.8ml) was added 2-methyl-6-methoxy-8-nitroquinoline (4.0g, 0.018mol) in dioxane (5ml) and the reaction refluxed for 1h. The hot solution was then filtered from the selenium metal, cooled and the solvent removed under reduced pressure. The residue oil was basified with saturated sodium bicarbonate and the precipitate collected. Purification of the crude solid by flash chromatography (dichloromethane) yielded the aldehyde as a yellow solid (2.68g, 63%), m.p. 209-211°C. ^{1}H n.m.r : δ10.14, 1H, s, CHO ; 8.26, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 8.10, 1H, d, $J_{3,4}$ 8.6Hz, H_3 ; 7.77, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.33, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 4.04, 3H, s, CH_3. The spectral data matched that already reported.[125]

General procedure used for the coupling of phosphonium salts to 66 via the Wittig reaction.

The aldehyde (1 equivalent), and a phosphonium salt (1 equivalent) were stirred in a biphasic solvent system consisting of dichloromethane and sodium hydroxide (50%). The reaction was stirred and checked by TLC until the reaction had finished (approx. 5h). The aqueous layer was then extracted and the resultant organic layer dried, filtered and solvent removed under reduced pressure. TLC indicated two products, the *cis* and *trans* adducts, which upon reflux in ethanol and catalytic *p*-toluene sulfonic acid for 4-6h was converted to one product, namely the *trans* adduct. Removal of ethanol under reduced pressure followed by purification by flash chromatography (4:1/dichloromethane:hexane) yielded the adduct.

Recrystallisation, using dichloromethane/hexane then yielded the required compounds. By this means the following compounds were obtained;

6-Methoxy-8-nitro-2-[(E)2-phenyl-1-ethenyl]quinoline **67** as yellow-green prismatic crystals, (1.23g, 93%), m.p. 172-174°C. ^{1}H n.m.r. : δ8.00, 1H, d, $J_{4,3}$ 8.7Hz, H_4 ; 7.69-7.64, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.61-7.59, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.60, 1H, d, $J_{7,5}$ 2.8Hz, H_7 ; 7.57-7.56, 2H, m, H_{styryl} ; 7.40-7.29, 3H, m, H_{styryl} ; 7.28-7.22, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.17, 1H, d, $J_{5,7}$ 2.8Hz, H_5 ; 3.19, 3H, s, OCH_3. Mass Spectrum : *m/z* 306($M^{+\bullet}$, 100%), 305(M-H, 95%), 289(39%), 69(42%). ^{1}H n.m.r. and mass spectral data matched that already reported.[129]

6-Methoxy-8-nitro-2-[(1E,3E)-4-phenyl-1,3-butadienyl]quinoline **68** as yellow plates (230mg, 81%) m.p. 166-168°C (Found C, 72.2; H, 4.8 ; N, 8.2%. $C_{20}H_{16}N_2O_3$ requires C, 72.3 ; H, 4.9 ; N, 8.4%). ^{1}H n.m.r. : δ8.02-7.99, 1H, d, $J_{4,3}$ 9.0Hz, H_4 ; 7.64-7.63, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.56-7.51, 1H, d, J_{trans} 15.3Hz, H_{vinyl} ; 7.55-7.52, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.47-7.44, 2H, m, H_{phenyl} ; 7.37-7.31, 2H, m, H_{phenyl} ; 7.27-7.25, 1H, m, H_{phenyl} ; 7.21-7.20, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 7.06-6.95, 1H, m, H_{vinyl} ; 6.87-6.82, 1H, d, J_{trans} 15.6Hz, H_{vinyl} ; 6.85-6.80, 1H, d, J_{trans} 15.3Hz, H_{vinyl} ; 3.95, 3H, s, OCH_3. Mass Spectrum : *m/z* 332($M^{+\bullet}$, 38%), 284(55%), 226(60%), 196(62%), 97(48%), 69(89%), 43 (100%).

6-Methoxy-2-[(E)-2-(1-naphthy)-1-ethenyl]-8-nitroquinoline **69**[#] as yellow prisms, (240mg, 78%) m.p. 173.5-175°C (Found C, 74.0; H, 4.5 ; N, 7.9%. $C_{22}H_{16}N_2O_3$ requires C, 74.2 ; H, 4.8 ; N, 7.7%). ^{1}H n.m.r. : δ8.62-8.57, 1H, d, J_{trans} 15.9Hz, H_{vinyl} ; 8.30, 1H, m, $H_{naphthyl}$; 8.10, 1H, d, $J_{4,3}$ 8.7Hz, H_4 ; 7.90-7.84, 3H, m, $H_{naphthyl}$; 7.72, 1H, d, $J_{3,4}$ 8.7Hz, H_3 ; 7.69, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.61-7.48, 3H, m, $H_{naphthyl}$; 7.39-7.34, 1H, d, J_{trans} 15.6Hz, H_{vinyl} ; 7.25, 1H, d,

[#] X-ray crystal analysis contained in Appendix C

$J_{5,7}$ 2.7Hz, H_5 ; 3.98, 3H, s, OCH_3. Mass Spectrum : *m/z* 356($M^{+\bullet}$, 30%), 328(40%), 308(63%), 173(90%), 159(50%), 130(50%), 83 (44%), 69(97%), 57(86%), 43(100%).

6-Methoxy-2-[(E)-2-(2-naphthy)-1-ethenyl]-8-nitroquinoline **70** as yellow needles, (223mg, 73%) m.p. 195-197°C (Found C, 74.1; H, 4.6 ; N, 7.8%. $C_{22}H_{16}N_2O_3$ requires C, 74.2 ; H, 4.8 ; N, 7.7%). ^1H n.m.r. : δ8.04, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.96, 1H, m, $H_{naphthyl}$; 7.93-7.83, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.86-7.70, 4H, m, $H_{naphthyl}$; 7.67, 1H, d, $J_{3,4}$ 8.7Hz, H_3 ; 7.64, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.49-7.45, 2H, m, $H_{naphthyl}$; 7.42-7.36, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.20, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 3.94, 3H, s, OCH_3. Mass Spectrum : *m/z* 356($M^{+\bullet}$, 80%), 339(56%), 308(100%), 294(20%), 265(60%).

2-(2-Methyl-1-propenyl)-6-methoxy-8-nitroquinoline **71** as yellow needles, (580mg, 83%) m.p. 143-145°C (Found C, 64.9; H, 5.3 ; N, 10.9%. $C_{22}H_{16}N_2O_3$ requires C, 65.1 ; H, 5.5 ; N, 10.9%). ^1H n.m.r. : δ7.96, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.62, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.27, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.19, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 6.37, 1H, m, H_{vinyl} ; 3.94, 3H, s, OCH_3 ; 2.29, 3H, d, *J* 1.2Hz, CH_3 ; 1.98, 3H, d, *J* 1.2Hz, CH_3. Mass Spectrum : *m/z* 258($M^{+\bullet}$, 100%), 228(14%), 211(60%), 168(25%).

General procedure used for the reduction of the nitro Wittig adducts.

General procedure for the reduction of the nitro arenes was by the iron / acetic acid method used for the reduction of 6-methoxy-2-methyl-8-nitro quinoline **7**. 2-Isobutenyl-6-methoxy-8-nitroquinoline **71** was also reduced by hydrogenolysis over Pd-C(5%) in ethanol overnight followed by filtration and removal of the solvent. Purification of the amines was by squat

column (dichloromethane). By this means the following compounds were obtained;

8-Amino-6-methoxy-2-[(E)2-phenyl-1-ethenyl]quinoline **72** as a orange yellow solid (0.95g, 86%), m.p. 143-145°C (Found C, 78.2; H, 5.8; N, 10.1%. $C_{18}H_{16}N_2O_1$ requires C, 77.9; H, 5.8; N, 10.1%). υ_{max} : 3500, 3390, 1720, 1620, 1580cm^{-1}. ^{1}H n.m.r. : δ7.90, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.62-7.57, 3H, m, $H_{styryl,vinyl}$; 7.54-7.52, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.40-7.25, 4H, m, $H_{styryl,vinyl}$; 6.55, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 6.45, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 5.05, 2H, bs, NH_2 ; 3.86, 3H, s, OCH_3. Mass Spectrum : *m/z* 276($M^{+\bullet}$, 100%), 275(M-H, 35%), 232(20%), 69(60%), 41(75%).

8-Amino-6-methoxy-2-[(1E,3E)-4-phenyl-1,3-butadienyl]quinoline **73** as orange / yellow columns, (190mg, 91%) m.p. 165-167°C. υ_{max} : 3500, 3380, 1620, 1600, 1560cm^{-1}. ^{1}H n.m.r. : δ7.88, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.53-6.77, 12H, m, $H_{phenyl,vinyl,3}$; 6.58, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 6.46, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 5.05, 2H, bs, H_7, NH_2 ; 3.98, 3H, s, OCH_3. Mass Spectrum : *m/z* 302($M^{+\bullet}$, 100%), 225(M-C_6H_5, 83%), 182(18%), 168(12%), 128(12%), 43(28%), 41(20%).

8-Amino-6-methoxy-2-[(E)-2-(1-naphthy)-1-ethenyl]quinoline **74** as a yellow brown solid, (230mg, 93%) m.p. 156-158°C. υ_{max} : 3450, 3350, 1620, 1580, 1560cm^{-1}. ^{1}H n.m.r. : δ8.42, 1H, d, J_{trans} 16.1Hz, H_{vinyl} ; 8.31, 1H, m, $H_{naphthyl}$; 7.93, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.89-7.81, 3H, m, $H_{naphthyl}$; 7.62, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.58-7.48, 3H, m, $H_{naphthyl}$; 7.36, 1H, d, J_{trans} 16.1Hz, H_{vinyl} ; 6.58, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 6.48, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 5.09, 2H, bs, NH_2 ; 3.88, 3H, s, OCH_3. Mass Spectrum : *m/z* 326($M^{+\bullet}$, 32%), 232(21%), 189(16%), 168(12%), 129(15%), 84(52%), 71(22%), 57(100%), 43(41%).

8-Amino-6-methoxy-2-[(E)-2-(2-naphthy)-1-ethenyl] quinoline **75** as yellow needles, (187mg, 99%) m.p. 158-160°C. υ_{max} : 3480, 3375, 1630, 1600, 1580cm^{-1}. ^{1}H n.m.r. : 7.94, 1H, m, $H_{naphthyl}$; 7.92, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.86-7.81, 4H, m, $H_{naphthyl,vinyl}$; 7.77, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.58, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.48-7.40, 3H, m, $H_{naphthyl}$; 6.57, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 6.46, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 5.08, 2H, bs, NH_2 ; 3.87, 3H, s, OCH_3. Mass Spectrum : *m/z* 326($M^{+\bullet}$, 10%), 149(20%), 43(100%).

8-Amino-6-methoxy-2-(2-methyl-1-propenyl)quinoline **76** as a yellow / green oil, (163mg, 83%). υ_{max} : 3500, 3380, 1620, 1580cm^{-1}. ^{1}H n.m.r. : δ7.84, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.18, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 6.55, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 6.43-6.41, 2H, m, $H_{3,vinyl}$; 4.95, 2H, bs, NH_2 ; 3.84, 3H, s, OCH_3 ; 2.24, 3H, d, *J* 1.0Hz, CH_3 ; 1.99, 3H, d, *J* 1.0Hz, CH_3. Mass Spectrum : *m/z* 228($M^{+\bullet}$, 100%), 213(10%), 169(12%).

8-Amino-2-isobutyl-6-methoxyquinoline **77** as yellow / green oil, (345mg, 86%). ^{1}H n.m.r. : δ7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.14, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 6.55, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 6.44, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 5.01, 2H, bs, NH_2 ; 3.85, 3H, s, OCH_3 ; 2.78-2.74, 2H, m, $CH_2CH(CH_3)_2$; 2.21, 1H, m, $CH_2CH(CH_3)_2$; 0.98-0.95, 6H, m, $CH_2CH(CH_3)_2$. Mass Spectrum : *m/z* 230($M^{+\bullet}$, 58%), 215(20%), 188(100%), 173(15%), 144(10%).

General procedure used for the tosylation of the above amines.

The sulfonamides were produced by stirring one equivalent of the above amines with one equivalent of the tosylchloride, in dichloromethane and pyridine, overnight. The organic layer was then washed with 1M hydrochloric acid (3 x 20ml) followed by saturated sodium bicarbonate (20ml). The organic layers were dried, solvent removed, crude solid passed

down a column of silica (dichloromethane followed by elution of the required compound with ethyl acetate) and then recrystallised (dichloromethane/hexane unless otherwise stated). By this means the following compounds were obtained;

4-Methyl-N-(6-methoxy-2-[(E)2-phenyl-1-ethenyl]-8-quinolyl)benzenesulfonamide **78** as pale orange crystals, (1.05g, 71%) m.p. 185.5-187.5°C (Found C, 69.8; H, 5.0; N, 6.4%. $C_{25}H_{22}N_2O_3S$ requires C, 69.8 ; H, 5.2 ; N, 6.5%). ^{1}H n.m.r. : δ9.24, 1H, bs, NH ; 7.90, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.84-7.81, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{3'}$; 7.64-7.61, 2H, m, H_{styryl} ; 7.59-7.56, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.57-7.52, 1H, d, J_{trans} 16.4Hz, H_{vinyl} ; 7.44-7.30, 4H, m, $H_{7,\ styryl}$; 7.31-7.26, 1H, d, J_{trans} 16.4Hz, H_{vinyl} ; 7.18-7.15, 2H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.67, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.85, 3H, s, OCH_3 ; 2.28, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 430($M^{+\bullet}$, 30%), 275(M-$C_7H_7SO_2$, 25%), 91(33%), 69(100%), 41(83%).

4-Methyl-N-(6-methoxy-2-[(1E,3E)-4-phenyl-1,3-butadienyl]-8-quinolyl)benzenesulfonamide **79** as yellow crystals, (208mg, 89%) m.p. 213-215°C (Found *m/z* 456.152. $C_{27}H_{24}N_2O_3S_1$ requires *m/z* 456.151). υ_{max} : 3275, 1630, 1590, 1570cm^{-1}. ^{1}H n.m.r. : δ9.21, 1H, bs, NH ; 7.86, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.84-7.81, 2H, d, $J_{2',3'}$ 8.1Hz, $H_{2'}$; 7.51-7.25, 6H, m, H_{phenyl} ; 7.46-7.41, 1H, d, J_{trans} 16Hz, H_{vinyl} ; 7.46, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.18-7.15, 2H, d, $J_{3',2'}$ 8.1Hz, $H_{3'}$; 7.10-6.99, 1H, m, H_{vinyl} ; 6.90-6.85, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 6.85-6.80, 1H, d, J_{trans} 15.9Hz, H_{vinyl} ; 6.65, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 3.84, 3H, s, OCH_3 ; 2.92, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 457($M^{+\bullet}$, 10%), 447(8%), 302(M-$C_7H_7SO_2$, 30%), 57(15%).

4-Methyl-N-(6-methoxy-2-[(E)-2-(1-naphthy)-1-ethenyl]-8-quinolyl)benzenesulfonamide **80** as yellow prisms, (267mg, 93%) m.p. 166-168°C (Found C, 72.5; H, 5.2; N, 5.9%; *m/z* 480.151. $C_{29}H_{24}N_2O_3S_1$ requires C,

72.5; H, 5.0; N, 5.8%; *m/z* 480.151). υ_{max} : 3225, 1640, 1600, 1570cm^{-1}. ^{1}H n.m.r. : δ9.26, 1H, bs, NH ; 8.33, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 8.26, 2H, d, $J_{2',3'}$ 8.1Hz, $H_{2'}$; 7.94, 1H, d, $J_{4,3}$ 8.7Hz, H_4 ; 7.91-7.83, 4H, m, $H_{naphthyl}$; 7.68, 1H, d, $H_{3,4}$ 8.7Hz, H_3 ; 7.62-7.51, 3H, m, $H_{naphthyl}$; 7.46, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 7.36, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.17, 2H, d, $J_{3',2'}$ 8,1Hz, $H_{3'}$; 6.69, 1H, d, $H_{5,7}$ 2.4Hz, H_5 ; 3.87, 3H, s, OCH_3 ; 2.29, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 481($M^{+\bullet}$, 10%), 480(20%), 325(M-$C_7H_7SO_2$, 40%), 43(100%), 41(82%).

4-Methyl-N-(6-methoxy-2-[(E)-2-(2-naphthy)-1-ethenyl]-8-quinolyl)benzenesulfonamide ***81*** as yellow prisms, (204mg, 85%) m.p. 185-187°C (Found *m/z* 480.151. $C_{29}H_{24}N_2O_3S$ requires *m/z* 480.151). υ_{max} : 3275, 1640, 1610, 1570cm^{-1}. ^{1}H n.m.r. : δ9.28, 1H, bs, NH ; 7.99, 1H, m, $H_{naphthyl}$; 7.91, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.87-7.82, 6H, m, $H_{naphthyl,2'}$; 7.73, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.61, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.51-7.47, 2H, m, $H_{naphthyl}$; 7.43, 1H, d, $J_{7,5}$ 2.5Hz, H_7 ; 7.39, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.16, 2H, d, $J_{3',2'}$ 8.1Hz, $H_{3'}$; 6.67, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 3.85, 3H, s, OCH_3 ; 2.29, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 481($M^{+\bullet}$, 10%), 447(99%), 325(M-$C_7H_7SO_2$, 20%), 168(26%), 141(30%), 115(31%), 84(100%), 49(75%).

4-Methyl-N-(6-methoxy-2-(2-methyl-1-propenyl)-8-quinolyl)benzenesulfonamide ***82*** as white prisms, (244mg, 91%) m.p. 158-160°C (Found C, 65.7; H, 5.8; N, 7.2%. $C_{21}H_{22}N_2O_3S_1$ requires C, 66.0; H, 5.8; N, 7.3%). ^{1}H n.m.r. : δ9.13, 1H, bs, NH ; 7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.77, 1H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.24, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 7.20, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.13, 1H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.63, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 6.36, 1H, m, H_{vinyl} ; 3.83, 3H, s, OCH_3 ; 2.27, 3H, s, $ArCH_3$; 2.19, 3H, m, CH_3 ; 2.00, 3H, m, CH_3. Mass Spectrum : *m/z* 382($M^{+\bullet}$, 50%), 447(99%), 227(M-$C_7H_7SO_2$, 100%).

4-Methyl-N-(2-isobutyl-6-methoxy-8-quinolyl)benzenesulfonamide **83** as white needles, (490mg, 85%) m.p. 154-156°C (Found C, 65.9; H, 6.5; N, 7.3%. $C_{21}H_{24}N_2O_3S_1$ requires C, 65.6; H, 6.3; N, 7.3%). υ_{max} : 3260, 1620, 1600, 1570cm^{-1}. ^{1}H n.m.r. : δ9.25, 1H, bs, NH ; 7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.76, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.43, 1H. d. $J_{7,5}$ 2.7Hz, H_7 ; 7.16, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.12, 1H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.66, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 3.84, 3H, s, OCH_3 ; 2.72, 2H, m, $CH_2CH(CH_3)_2$; 2.27, 3H, s, $ArCH_3$; 2.13, 1H, m, $CH_2CH(CH_3)_2$; 0.91, 6H, m, $CH_2CH(CH_3)_2$. Mass Spectrum : *m/z* 384($M^{+\bullet}$, 33%), 447(99%), 229(M-$C_7H_7SO_2$, 30%), 187(100%), 129(16%), 91(38%), 69(100%).

Chapter 7 : The biologically useful ligands.

General procedure for the demethylation of 78, 82 and 83.

General procedure for the demethylation of the methoxy compounds was the same as for the method (A) preparation of 4-Methyl-N-(6-hydroxy-2-methyl-8-quinolyl) benzene sulfonamide **10a**. By this means the following compounds were obtained;

4-Methyl-N-(6-hydroxy-2-[(E)-2-phenyl-1-ethenyl]-8-quinolyl) benzenesulfonamide ***85*** as an orange crystalline solid, (0.85g, 84%), m.p. 242-245°C. ^{1}H n.m.r. : δ9.31, 1H, bs, NH ; 7.51-7.47, 1H, d, $J_{4,3}$ 8.7Hz, H_4 ; 7.45-7.42, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.29-7.16, 5H, m, $H_{styryl,vinyl,3}$; 7.07-6.98, 3H, m, H_{styryl} ; 7.06, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 6.95-6.84, 1H, d, J_{trans} 16.5Hz, H_{vinyl} ; 7.84-6.81, 2H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.36, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 1.93, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 416($M^{+\bullet}$, 100%), 261(M-$C_7H_7SO_2$, 90%), 91(55%), 77(30%).

4-Methyl-N-[6-hydroxy-2-(2-methyl-1-propenyl)-8-quinolyl] benzenesulfonamide ***86*** as a yellow oil, (0.22g, 98%). ^{1}H n.m.r. : δ9.28, 1H, bs, NH ; 7.76-7.72, 2H, d, $J_{2',3'}$ 8.4Hz, $H_{2'}$; 7.70-7.66, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.49, 1H, m, H_7 ; 7.13-7.09, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.05-7.01, 2H, d, $J_{3',2'}$ 8.4Hz, $H_{3'}$; 6.72, 1H, m, H_5 ; 6.32, 1H, m, H_{vinyl} ; 2.16, 3H, s, CH_3 ; 2.15, 3H, s, CH_3 ; 1.98, 3H, s, $ArCH_3$. Mass Spectrum : *m/z* 369($M^{+\bullet}$, 29%), 213(M-$C_7H_7SO_2$, 100%), 198(20%), 91(32%).

4-Methyl-N-(2-isobutyl-6-hydroxy-8-quinolyl) benzenesulfonamide ***87*** as orange needles, (0.37g, 87%) m.p. 147-149°C. ^{1}H n.m.r. : δ9.25, 1H, bs, NH ; 7.80-7.77, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.76-7.74, 2H, d, $J_{2',3'}$ 8.5Hz, $H_{2'}$; 7.42, 1H, d,

$J_{7,5}$ 2.5Hz, H_7 ; 7.17-7.13, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 7.13-7.10, 2H, d, $J_{3',2'}$ 8.4Hz, $H_{3'}$; 6.71, 1H, d, $J_{5,7}$ 2.5Hz, H_5 ; 2.71, 2H, d, J 7.2Hz, $CH_2CH(CH_3)_2$; 2.27, 3H, s, $ArCH_3$; 2.10, 1H, m, $CH_2CH(CH_3)_2$; 0.91-0.88, 6H, m, $CH_2CH(CH_3)_2$.

Ethyl-2-(2-[(E)-2-phenyl-1-ethenyl]-6-quinolyloxy-8-p-toluenesulfonamido) acetate ***90.***

To a suspension of sodium hydride (0.097g, 2.22mmol) in DMF (10ml) at 0°C was added a solution of the phenol (0.84g, 2.01mmol) in DMF (20ml) and the reaction mixture stirred for 20 minutes. Ethyl bromoacetate (0.225ml, 2.01mmol) was then added, the reaction mixture allowed to warm up to room temperature and then stirred overnight. The DMF was then removed, the residue dissolved in dichloromethane and washed with sodium bicarbonate. Purification by flash chromatography (dichloromethane) followed by recrystallisation from dichloromethane / hexane, yielded the title compound as white needles (0.30g, 30%), m.p. 178-179.5°C (Found C, 66.8; H, 5.4; N, 5.7%. $C_{28}H_{26}N_2O_5S_1$ requires C, 66.9; H, 5.2; N, 5.6%). υ_{max} : 3325, 1760, 1610, 1600cm^{-1}. ^1H n.m.r. : δ9.25, 1H, bs, NH ; 7.88, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.84-7.81, 2H, d, $J_{2',3'}$ 8.4Hz, $H_{2'}$; 7.64-7.62, 2H, m, H_{styryl} ; 7.58, 1H, d, $J_{3,4}$ 8.6Hz, H_3 ; 7.58-7.51, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.51, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.44-7.33, 3H, m, H_{styryl} ; 7.31-7.26, 1H, d, J_{trans} 16.2Hz, H_{vinyl} ; 7.18-7.16, 2H, d, $J_{3',2'}$ 8.4Hz, $H_{3'}$; 6.62, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 4.67, 2H, s, OCH_2 ; 4.31-4.23, 2H, q, J 7.2Hz, CH_2CH_3 ; 2.29, 3H, s, $ArCH_3$; 1.31-1.26, 3H, t, J 7.2Hz, CH_2CH_3. Mass Spectrum : *m/z* 502($M^{+\bullet}$, 100%), 347(M-$C_7H_7SO_2$, 45%), 319(15%), 232(25%), 91(24%).

Ethyl-2-[2-(2-methyl-1-propenyl)-6-quinolyloxy-8-p-toluenesulfonamido] acetate ***91***.

The phenol (0.215g, 0.583mmol), ethyl bromoacetate (65µl, 0.59mmol), and potassium carbonate (0.081g, 0.59mmol) were refluxed in dry acetone (15ml) for 4h under a N_2 atmosphere. The solvent was then removed, the residue dissolved in dichloromethane and washed with sodium bicarbonate. The crude product was purified by flash chromatography and then recrystallised from dichloromethane / hexane yielding the title compound as white needle crystals (0.182g, 67%), m.p. 149-151°C (Found C, 63.1; H, 5.8; N, 6.3%. $C_{24}H_{26}N_2O_5S_1$ requires C, 63.4; H, 5.8; N, 6.2%). ^{1}H n.m.r. : δ9.15, 1H, bs, NH ; 7.82, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.79-7.76, 2H, d, $J_{2',3'}$ 8.3Hz, $H_{2'}$; 7.51, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.22, 1H, d, $J_{3,4}$ 8.6Hz, H_3 ; 7.17-7.14, 2H, d, $J_{3',2'}$ 8.3Hz, $H_{3'}$; 6.61, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 6.38-6.36, 1H, t, J 1.4Hz, C**H**C$(CH_3)_2$; 4.66, 2H, s, OCH_2 ; 4.30-4.23, 2H, q, J 6.9Hz, C**H**$_2$CH_3 ; 2.29, 3H, s, ArCH_3 ; 2.19, 3H, d, J 1.4Hz, CHC(C**H**$_3)_2$; 2.01, 3H, d, J 1.4Hz, CHC(C**H**$_3)_2$; 1.31-1.26, 3H, t, J 6.9Hz, CH_2C**H**$_3$. Mass Spectrum : *m/z* 454($M^{+\bullet}$, 52%), 299(M-$C_7H_7SO_2$, 100%), 184(20%), 91(21%), 69(19%).

Ethyl-2-(2-isobutyl-6-quinolyloxy-8-p-toluenesulfonamido) acetate ***92***.

The quinoline phenol (0.334g, 0.90mmol) was refluxed with ethyl bromoacetate (0.100ml, 0.90mmol) and potassium carbonate (0.124g, 0.90mol) in dry acetone (15ml) for 4h under a N_2 atmosphere. The solvent was then removed, the crude solid dissolved in dichloromethane and washed with sodium bicarbonate. Purification by flash chromatography followed by recrystallisation from dichloromethane / hexane yielded the required compound as a white solid, (0.375g, 90%), m.p. 146.5-148.5°C (Found C, 63.0; H, 6.3; N, 6.2%. $C_{24}H_{28}N_2O_5S_1$ requires C, 63.1; H, 6.2; N,

6.2%). υ_{max} : 3260, 1760, 1630, 1610, 1590cm^{-1}. ^{1}H n.m.r. : δ9.26, 1H, bs, NH ; 7.82, 1H, d, $J_{4,3}$ 8.3Hz, H_4 ; 7.78-7.75, 2H, d, $J_{2',3'}$ 8.5Hz, $H_{2'}$; 7.54, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.17, 1H, d, $J_{3,4}$ 8.3Hz, H_3 ; 7.14-7.11, 2H, d, $J_{3',2'}$ 8.5Hz, $H_{3'}$; 6.63, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 4.66, 2H, s, OCH_2 ; 4.30-4.23, 2H, q, J 7.2Hz, $\mathbf{CH_2}CH_3$; 2.72, 2H, d, J 6.9Hz, $\mathbf{CH_2}CH(CH_3)_2$; 2.28, 3H, s, $ArCH_3$; 2.11, 1H, m, $CH_2\mathbf{CH}(CH_3)_2$; 1.31-1.26, 3H, t, J 7.2Hz, $CH_2\mathbf{CH_3}$; 0.90, 6H, m, $CH_2CH(\mathbf{CH_3})$. Mass Spectrum : *m/z* 456($M^{+\bullet}$, 77%), 414(20%), 301(M-$C_7H_7SO_2$, 32%), 259(100%), 155(19%), 91(55%).

Preparation of Ethyl-2-(2-methyl-6-quinolyloxy-8-(2,2,2-trifluoroethyl sulfonamido) acetate.

N-(6-Methoxy-2-methyl-8-quinolyl)trifluoroacetamide **93**.

To a stirring solution of the quinoline amine (0.50g, 2.66mmol) in dichloromethane (20ml) and pyridine (0.43ml) at 0°C, was added trifluoroacetic anhydride (0.395ml, 2.79mmol) and the reaction mixture stirred overnight. After removal of the solvent the reaction mixture was purified by flash chromatography (dichloromethane) and recrystallised from dichloromethane / hexane yielding the title compound as pale blue needles (0.58g, 78%), m.p. 163.5-165°C (R_f 0.77, dichloromethane). υ_{max} : 3295, 1710, 1630, 1600cm^{-1}. ^{1}H n.m.r. : δ10.76, 1H, bs, NH ; 8.38, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.94, 1H, d, $J_{4,3}$ 8.3Hz, H_4 ; 7.30, 1H, d, $J_{3,4}$ 8.3Hz, H_3 ; 6.86, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 3.91, 3H, s, OCH_3 ; 2.69, 3H, s, CH_3. Mass Spectrum : *m/z* 284($M^{+\bullet}$, 60%), 215(M-CF_3, 100%), 172(M-$NHCOCF_3$, 42%), 144(75%), 69(24%).

N-(6-Hydroxy-2-methyl-8-quinolyl)trifluoroacetamide **94**.

Using the method (A) procedure as for the preparation of 4-Methyl-*N*-(6-hydroxy-2-methyl-8-quinolyl) benzene sulfonamide **10a**, the title compound was obtained as a pale orange solid, (0.265g, 62%), m.p. 230-233°C (Found C, 53.5; H, 3.4; N, 10.3%. $C_{12}H_9F_3N_2O_2$ requires C, 53.3; H, 3.4; N, 10.4%). υ_{max} : 3340, 3275, 1705, 1640, 1610cm^{-1}. ^{1}H n.m.r. : δ10.54, 1H, bs, NH ; 9.31, 1H, s, OH ; 8.08, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 7.64, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.04, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 6.66, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 2.42, 3H, s, CH_3. Mass Spectrum : *m/z* 270($M^{+\bullet}$, 13%), 201(M-CF_3, 100%), 145(8%), 69(33%).

Ethyl-2-(2-methyl-6-quinolyloxy-8-trifluoroacetamido)acetate **95**.

The hydroxy quinoline (0.265g, 0.98mmol), ethyl bromoacetate (109µl, 0.98mmol) and potassium carbonate (0.136g, 0.98mmol) were refluxed in acetone (30ml) for 5h under a N_2 atmosphere. The crude solid obtained after the removal of the solvent was dissolved in dichloromethane and washed with sodium bicarbonate and purified by flash chromatography. Recrystallisation from dichloromethane / hexane yielded the required compound as white needles, (0.244g, 70%), m.p. 137-140°C (Found C, 54.2; H, 4.3; N, 8.1%. $C_{16}H_{15}F_3N_2O_3$ requires C, 53.9; H, 4.2; N, 7.9%). υ_{max} : 3310, 1760, 1740, 1640, 1600cm^{-1}. ^{1}H n.m.r. : δ10.76, 1H, bs, NH ; 8.46, 1H, d, $J_{7,5}$ 2.7Hz, H_7 ; 7.93, 1H, d, $J_{4,3}$ 8.5Hz, H_4 ; 7.32, 1H, d, $J_{3,4}$ 8.5Hz, H_3 ; 6.84, 1H, d, $J_{5,7}$ 2.7Hz, H_5 ; 4.73, 2H, s, OCH_2 ; 4.31-4.24, 2H, q, J 7.2Hz, CH_2CH_3 ; 2.69, 3H, s, CH_3 ; 1.31-1.23, 3H, t, J 7.2Hz, CH_2CH_3. Mass Spectrum : *m/z* 356($M^{+\bullet}$, 17%), 287(M-CF_3, 100%), 184(13%), 144(22%), 41(65%).

Ethyl-2-(8-amino-2-methyl-6-quinolyl)acetate **96**.

The trilfuoroacetamide quinoline (0.244g, 0.69mmol) was stirred in a 10% ethanol / water (5:2) potassium carbonate solution (70ml) for 3h at room temperature. TLC analysis after this period indicated no starting material so the reaction was evaporated to dryness. Infra-red analysis of the crude solid indicated cleavage of the trifluoroacetamide and hydrolysis of the ester so the crude product was re-esterified by refluxing in ethanol and catalytic *p*-toluene sulfonic acid. The crude solid obtained by removal of the solvent was dissolved in dichloromethane, washed with sodium bicarbonate and purified by flash chromatography yielding the title compound as a yellow crystalline solid (0.170g, 96%), m.p. 110-113°C. ^{1}H n.m.r. : δ7.78, 1H, d, $J_{4,3}$ 8.2Hz, H_4 ; 7.15, 1H, d, $J_{3,4}$ 8.2Hz, H_3 ; 6.61, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 6.33, 1H, d,

$J_{5,7}$ 2.6Hz, H_5 ; 4.99, 2H, bs, NH_2 ; 4.65, 2H, s, OCH_2 ; 4.31-4.22, 2H, q, J 7.1Hz, CH_2CH_3 ; 2.63, 3H, s, CH_3 ; 1.31-1.24, 3H, t, J 7.1Hz, CH_2CH_3. Mass Spectrum : *m/z* 260($M^{+\bullet}$, 100%), 159(60%), 145(61%).

Ethyl-2-(2-methyl-6-quinolyloxy-8-(2,2,2-trifluoroethylsulfonamido)acetate ***97.***

To the amine (0.175g, 0.672mmol) in dichloromethane (10ml) at -78°C was added pyridine (82µl, 0.100mmol) and tresyl chloride (79µl, 0.707mmol). The solution turned milky green and was stirred for a further 6h at -78°C under a N_2 atmosphere. The reaction was then allowed to warm to room temperature where it turned to a straw colour. The reaction was then washed with 1M hydrochloric acid and then with sodium bicarbonate. The crude solid obtained upon drying of the organic layer, filtration and removal of the solvent, was purified by flash chromatography (dichloromethane elution of the required band with dichloromethane / ethyl acetate). Recrystallisation from dichloromethane / hexane yielded the title compound as white plates, (0.214g, 78%), m.p. 112-114°C (Found C, 47.6; H, 4.2; N, 6.8%. $C_{16}H_{17}N_2O_5S_1F_3$ requires C, 47.3; H, 4.2; N, 6.9%). ^{1}H n.m.r. : δ9.35, 1H, bs, NH ; 7.92, 1H, d, $J_{4,3}$ 8.3Hz, H_4 ; 7.56, 1H, d, $J_{7,5}$ 2.6Hz, H_7 ; 7.30, 1H, d, $J_{3,4}$ 8.3Hz, H_3 ; 6.75, 1H, d, $J_{5,7}$ 2.6Hz, H_5 ; 4.71, 2H, s, OCH_2 ; 4.34-4.23, 2H, q, J 7.1Hz, CH_2CH_3 ; 3.95-3.82, 2H, q, $J_{H,F}$ 8.8Hz, CH_2CF_3 ; 2.66, 3H, s, CH_3 ; 1.34-1.27, 3H, t, J 7.1Hz, CH_2CH_3. Mass Spectrum : *m/z* 406($M^{+\bullet}$, 100%), 259(M-$CF_3CH_2SO_2$, 42%), 197(43%), 119(44%), 69(37%).

Attempted preparation of Ethyl-2-(2-methyl-6-quinolyloxy-8-(*m*-trifluoromethyl sulfonamido) acetate.

3-Trifluoromethane-N-(6-methoxy-2-methy-8-quinolyl)benzenesulfonamide **84**.

Using the method (A) procedure as for the preparation of 4-Methyl-*N*-(6-hydroxy-2-methyl-8-quinolyl) benzene sulfonamide **10a**, the title compound was obtained as a yellow oil (0.260g, 84%). υ_{max} : 3350, 3250, 1630, 1610, 1570cm^{-1}. ^{1}H n.m.r. : 8.11, 1H, m, $H_{2'}$; 8.04, 1H, m, $H_{4'}$; 7.86, 1H, d, $J_{4,3}$ 8.6Hz, H_4 ; 7.63, 1H, m, $H_{6'}$; 7.49-7.40, 1H, m, $H_{5'}$; 7.41, 1H, d, $J_{7,5}$ 2.4Hz, H_7 ; 7.20, 1H, d, $J_{3,4}$ 8.6Hz, H_3 ; 6.92, 1H, d, $J_{5,7}$ 2.4Hz, H_5 ; 2.20, 3H, s, CH_3. Mass Spectrum : *m/z* 382($M^{+\bullet}$, 56%), 173(M-$C_7H_4SO_2F_3$, 67%), 145($C_7H_4F_3$, 100%), 43(53%).

Attempted preparation of Ethyl-2-[2-methyl-6-quinolyloxy-8-(m-trifluoromethylsulfonamido)]acetate **88**.

The above phenol (0.523g, 1.36mmol), ethyl bromoacetate (159µl, 1.4mmol) and potassium carbonate (0.198g, 1.40mmol) were refluxed in acetone (20ml) for 5h under a N_2 atmosphere. The crude solid obtained upon removal of the solvent was dissolved in dichloromethane, washed with sodium bicarbonate and purified by flash chromatography (dichloromethane). Recrystallisation from dichloromethane / hexane then afforded a white crystalline solid, identified as ethyl-2-([6-(2-ethoxy-2-oxoethoxy)-2-methyl-8-quinolyl]*m*-trifluoromethylbenzene sulfonamido) acetate **89**, (0.480g, 63%), m.p. 140-142°C (Found C, 54.2; H, 4.7; N, 5.2%. $C_{25}H_{25}N_2O_7S_1F_3$ requires C, 54.2; H, 4.5; N, 5.1%). ^{1}H n.m.r. : δ7.85, 1H, d, $J_{7.5}$ 2.8Hz, H_7 ; 7.82, 1H, d, $J_{4,3}$ 8.4Hz, H_4 ; 7.76-7.40, 4H, m, H_{phenyl} ; 7.05, 1H, d, $J_{3,4}$ 8.4Hz, H_3 ; 7.04, 1H, d,

$J_{5,7}$ 2.8Hz, H_5 ; 4.75, 2H, s, NCH_2 ; 4.72, 2H, s, OCH_2 ; 4.28, 2H, q, J 7.2Hz, $OCH_2CO_2CH_2CH_3$; 4.14, 2H, q, J 7.1Hz, $NCH_2CO_2CH_2CH_3$; 2.17, 3H, s, CH_3 ; 1.54-1.20, 6H, m, 2 x CH_2CH_3. Mass Spectrum : *m/z* 555($M^{+\bullet}$, 10%), 345(M-$CF_3C_7H_4SO_2$, 100%), 271(12%), 245(11%), 145(27%).

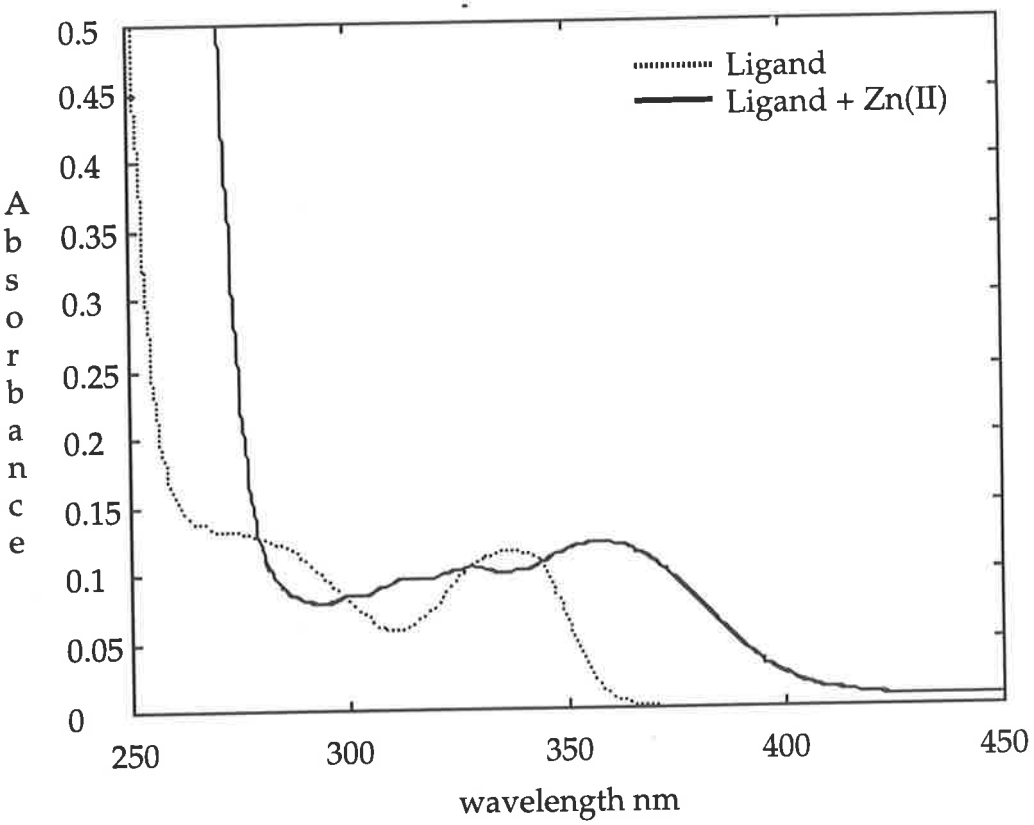
Appendix A - Uv/visible spectra

Uv/visible spectra were obtained on a Varian, Carey 2200 spectrophotometer at either the two following concentrations;

(A) Ligand; [L] 16.5μM, [EDTA] 165μM in 100mM $NaClO_4$, 1mM Na PIPES, ethanol/water (75:25, v/v).
Ligand + Zn(II); [L] 16.5μM, [Zn(II)] 165μM in 100mM $NaClO_4$, 1mM Na PIPES, ethanol/water (75:25, v/v).

(B) Ligand; [L] 8.3μM, [EDTA] 83μM in 100mM $NaClO_4$, 1mM NaPIPES, ethanol/water (75:25, v/v).
Ligand + Zn(II); [L] 8.3μM, [Zn(II)] 83μM in 100mM $NaClO_4$, 1mM NaPIPES, ethanol/water (75:25, v/v).

13A


Ligand
Ligand + Zn(II)
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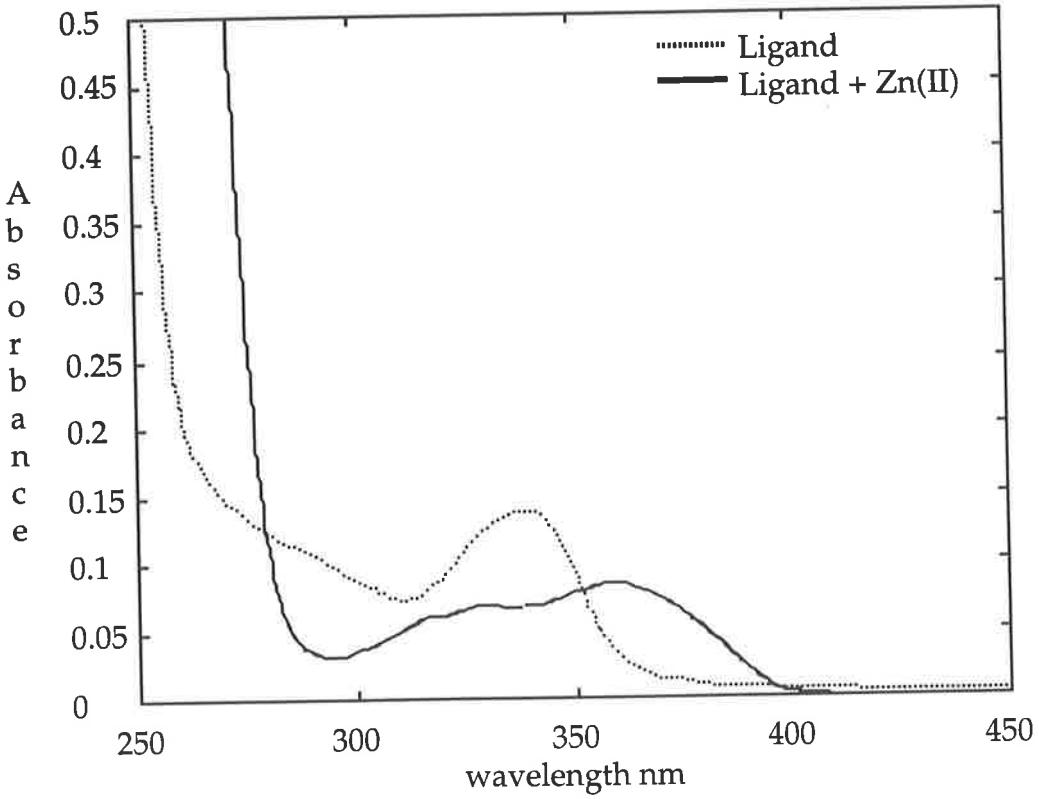
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

9A


Ligand
Ligand + Zn(II)
Absorbance
0.5
0.45
0.4
0.35
0.3
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0.2
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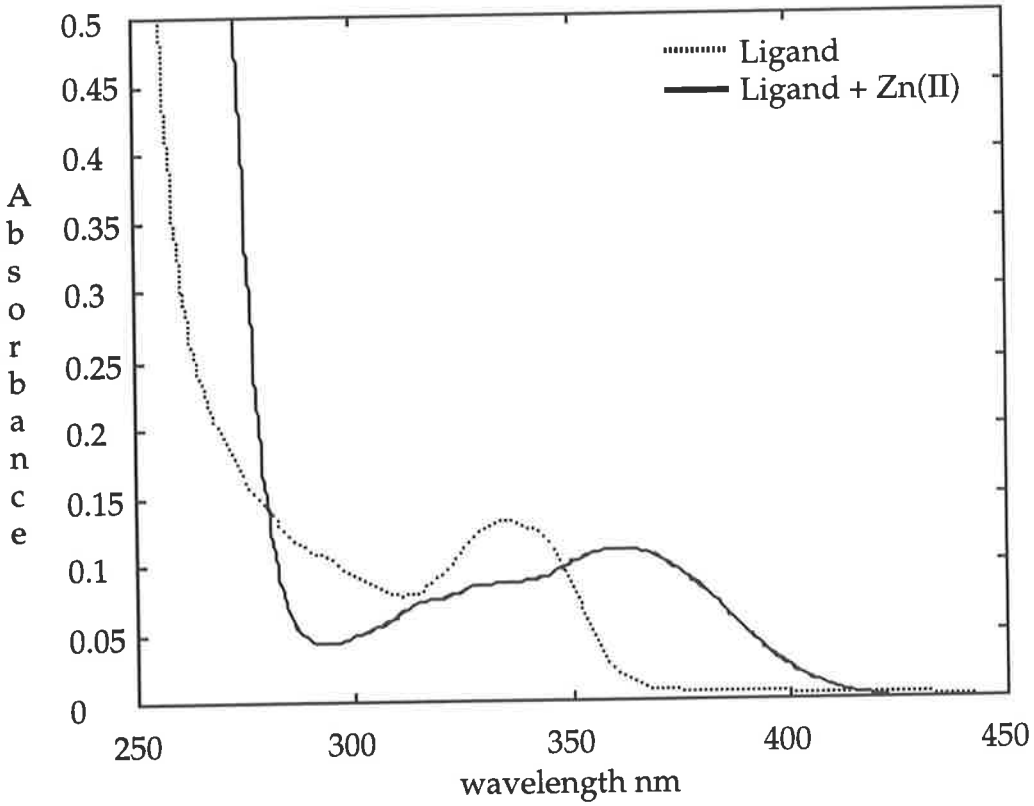
0.1
0.05
0
250
300
350
400
450
wavelength nm

14[A]


Ligand
Ligand + Zn(II)
Absorbance
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0.45
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0.35
0.3
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0.2
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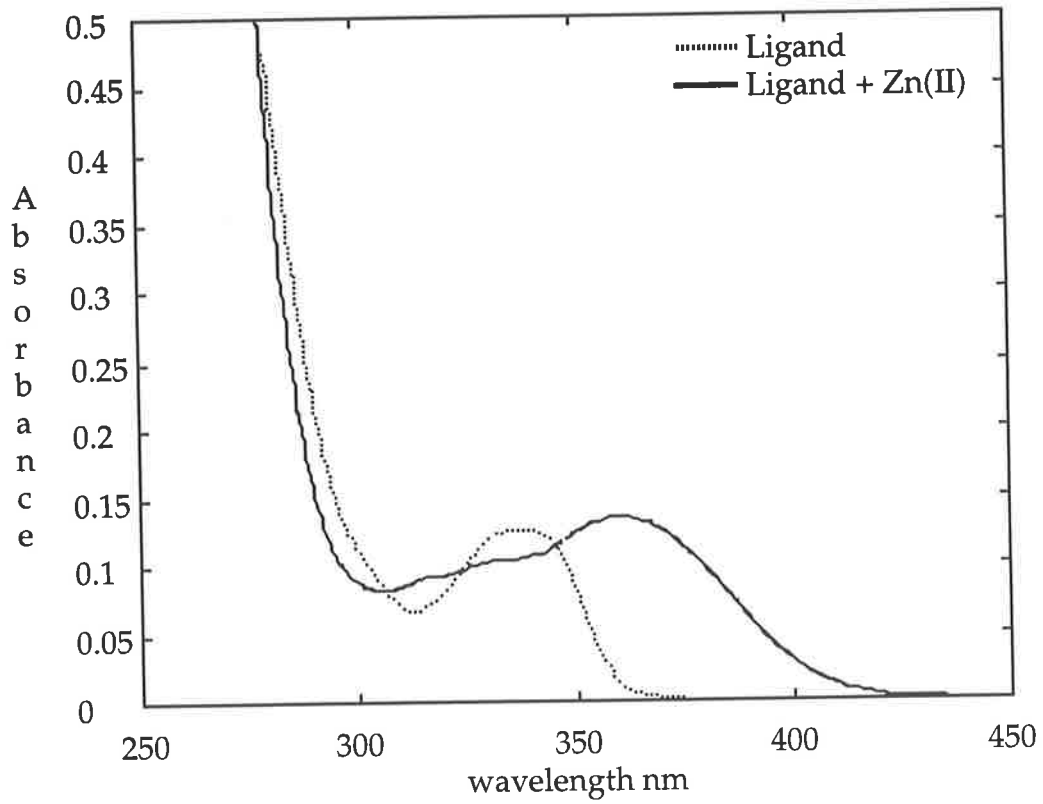
0.1
0.05
0
250
300
350
400
450
wavelength nm

15[A]


Ligand
Ligand + Zn(II)
Absorbance
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0.45
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0.3
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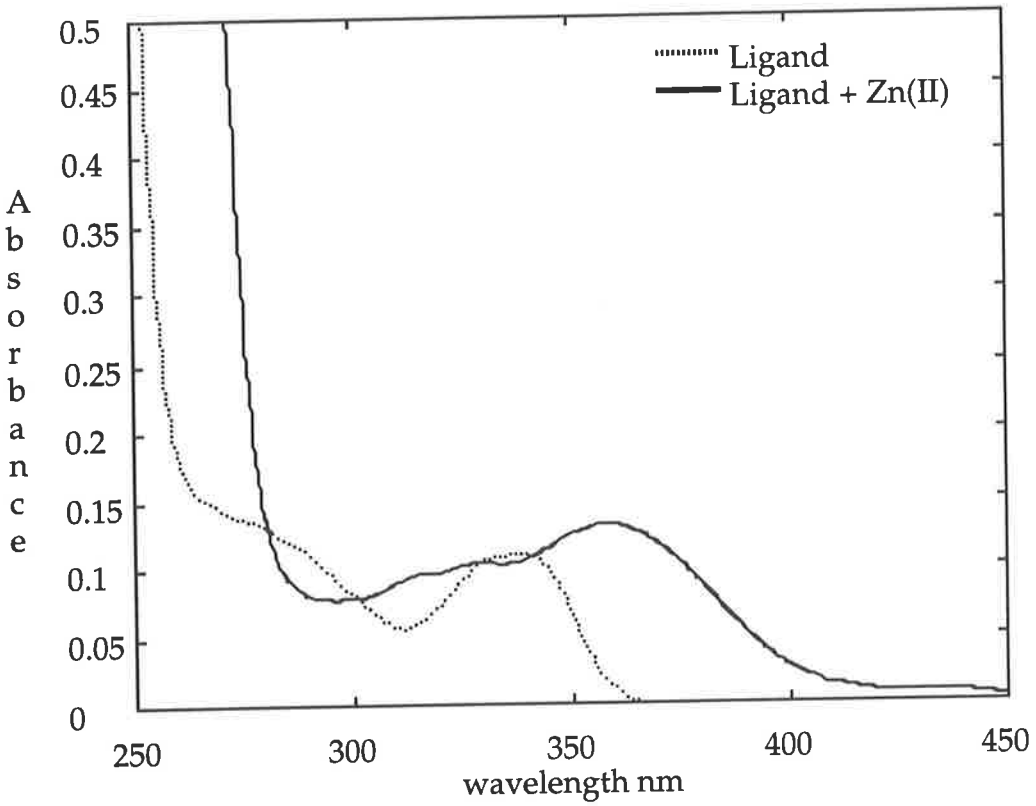
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

16A


Ligand
Ligand + Zn(II)
Absorbance
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0.05
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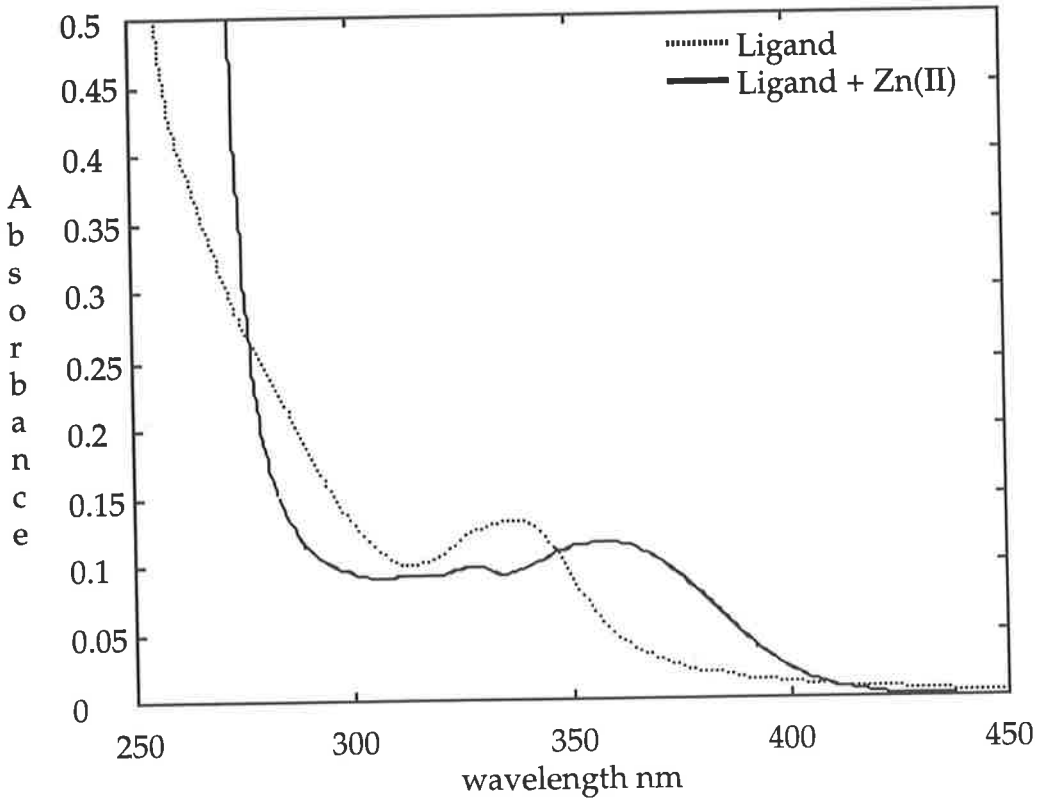
250
300
350
400
450
wavelength nm

17A


Ligand
Ligand + Zn(II)
Absorbance
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0.45
0.4
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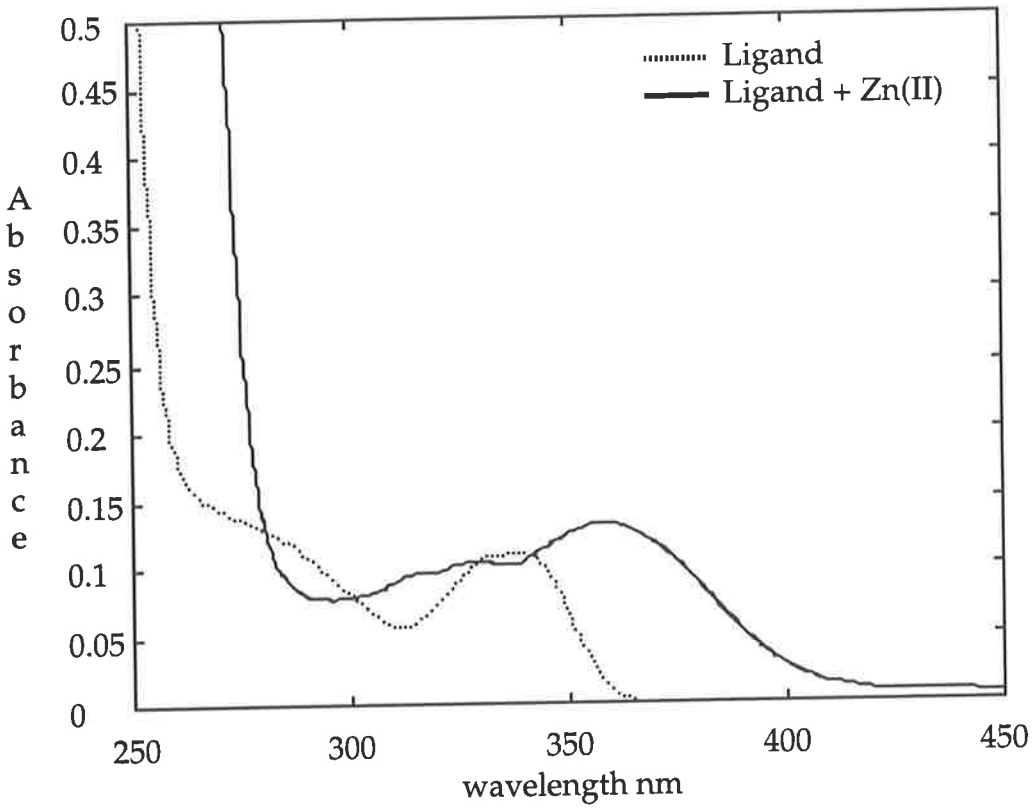
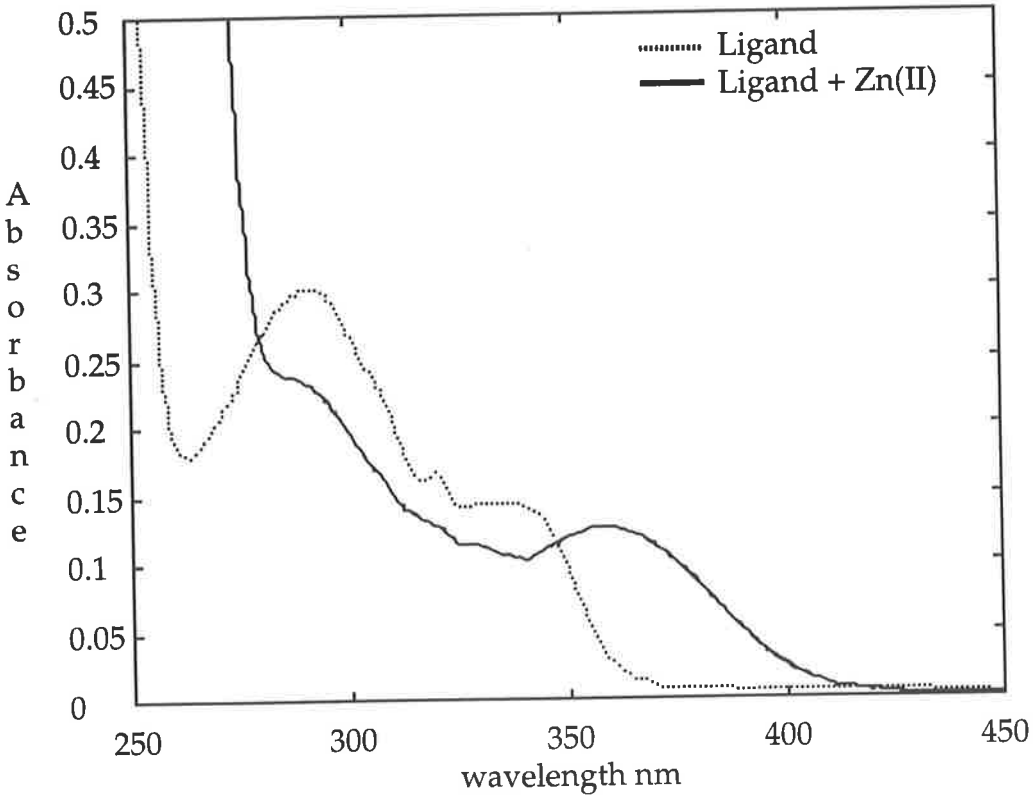
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

18A


Ligand
Ligand + Zn(II)
Absorbance
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

19A


Ligand
Ligand + Zn(II)
Absorbance
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0.45
0.4
0.35
0.3
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0.2
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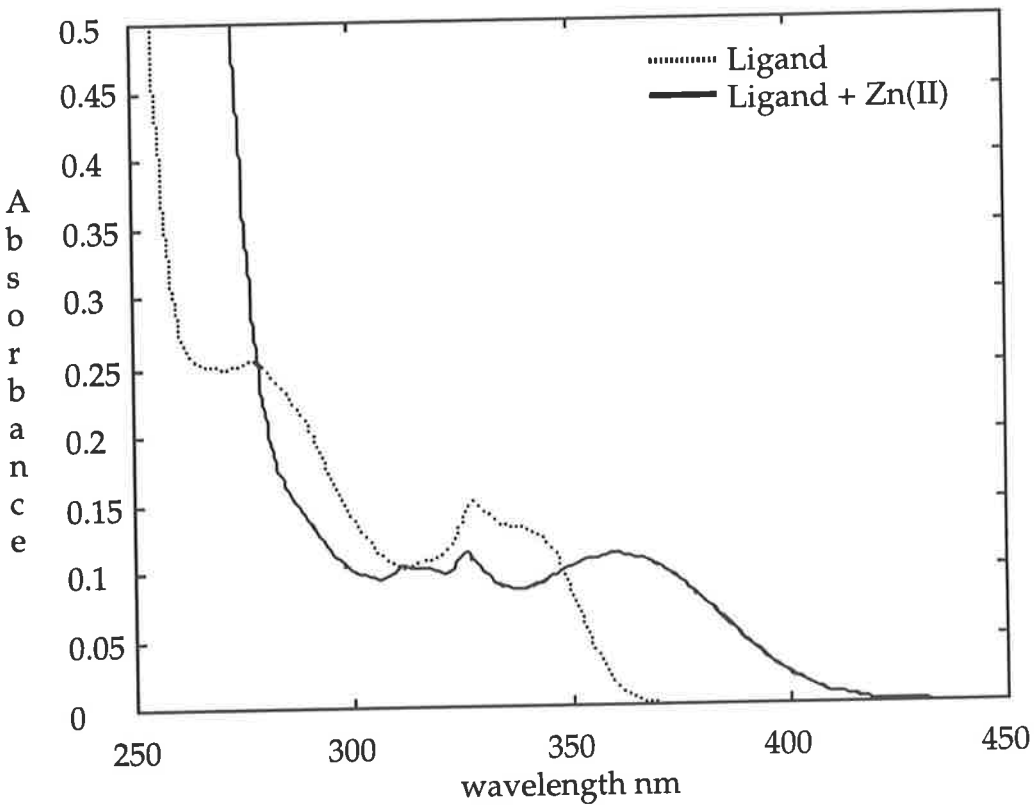
0.1
0.05
0
250
300
350
400
450
wavelength nm

20[A]


Ligand
Ligand + Zn(II)
Absorbance
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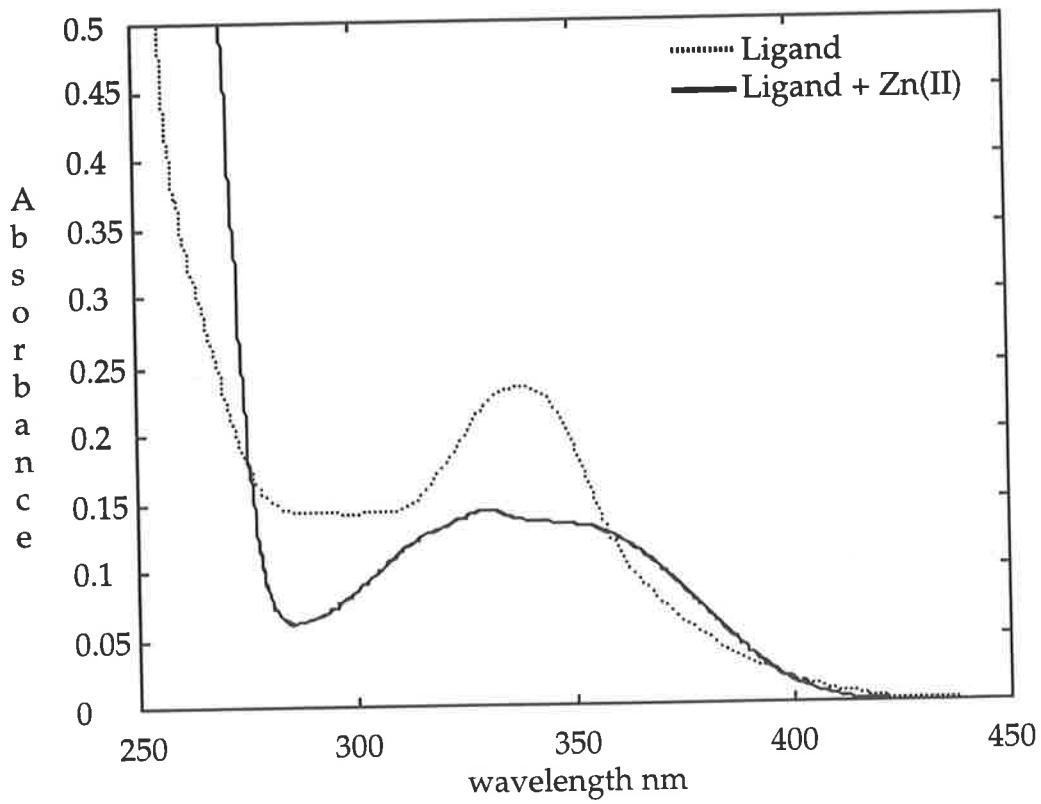
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

21[A]


Ligand
Ligand + Zn(II)
Absorbance
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0.35
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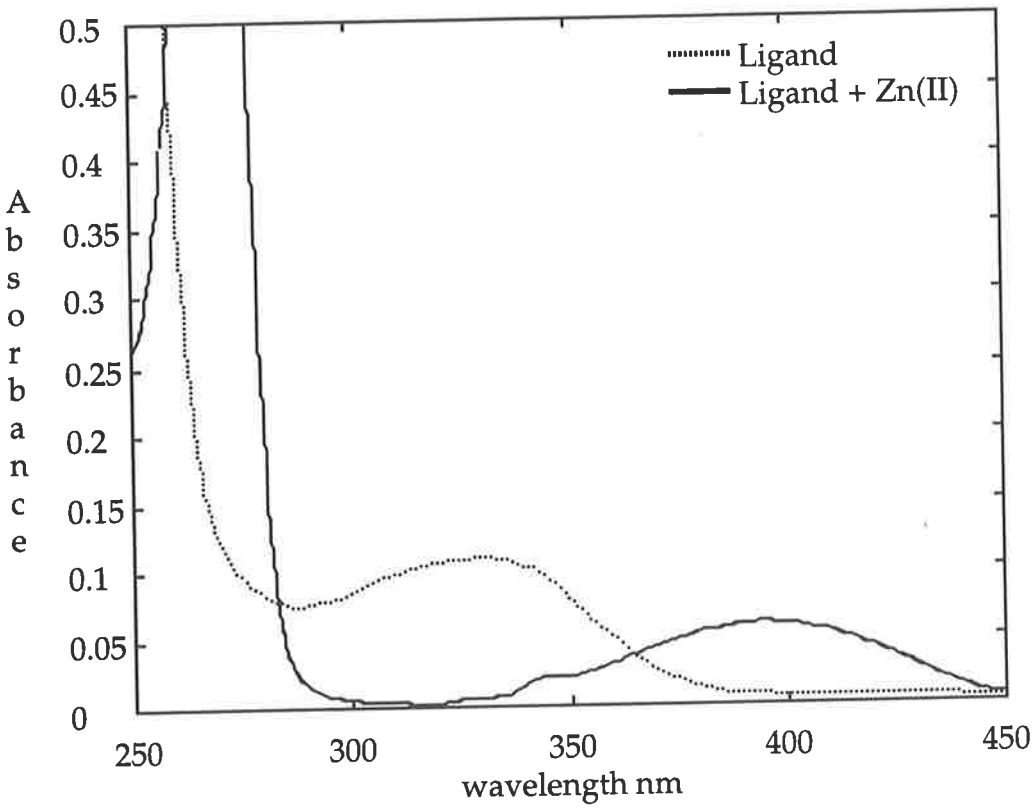
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

28[A]


Ligand
Ligand + Zn(II)
Absorbance
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0.3
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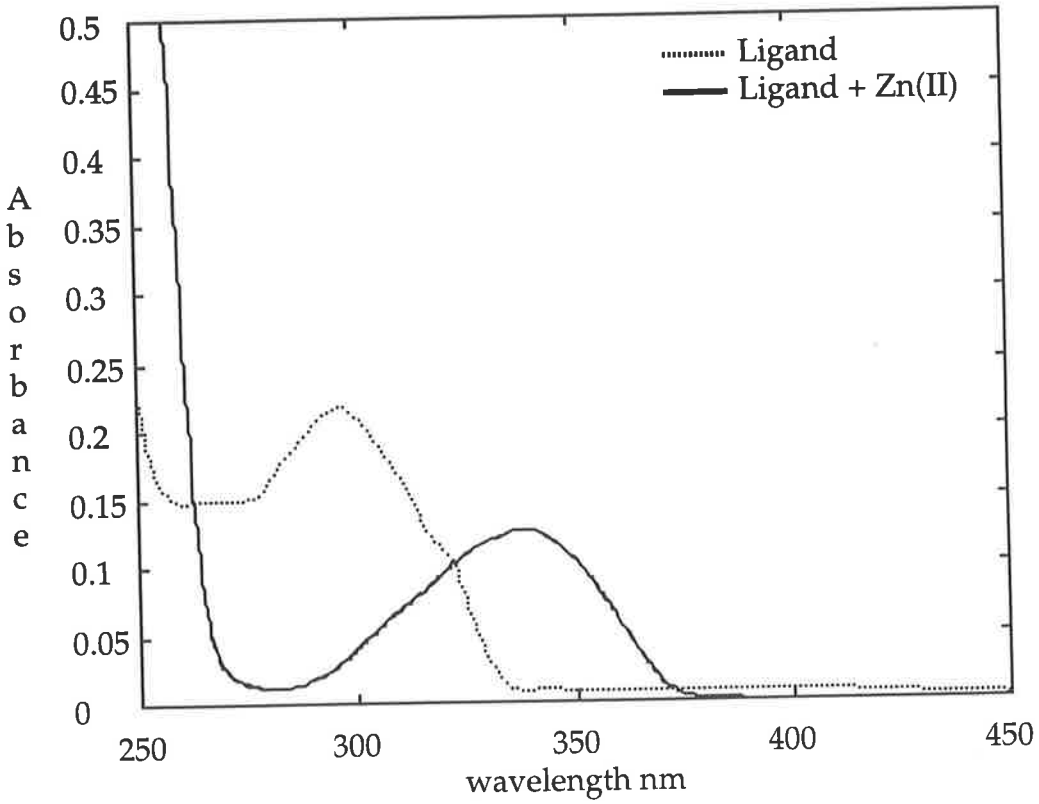
0.2
0.15
0.1
0.05
0
250
300
350
400
450
wavelength nm

36[A]


Ligand
Ligand + Zn(II)
Absorbance
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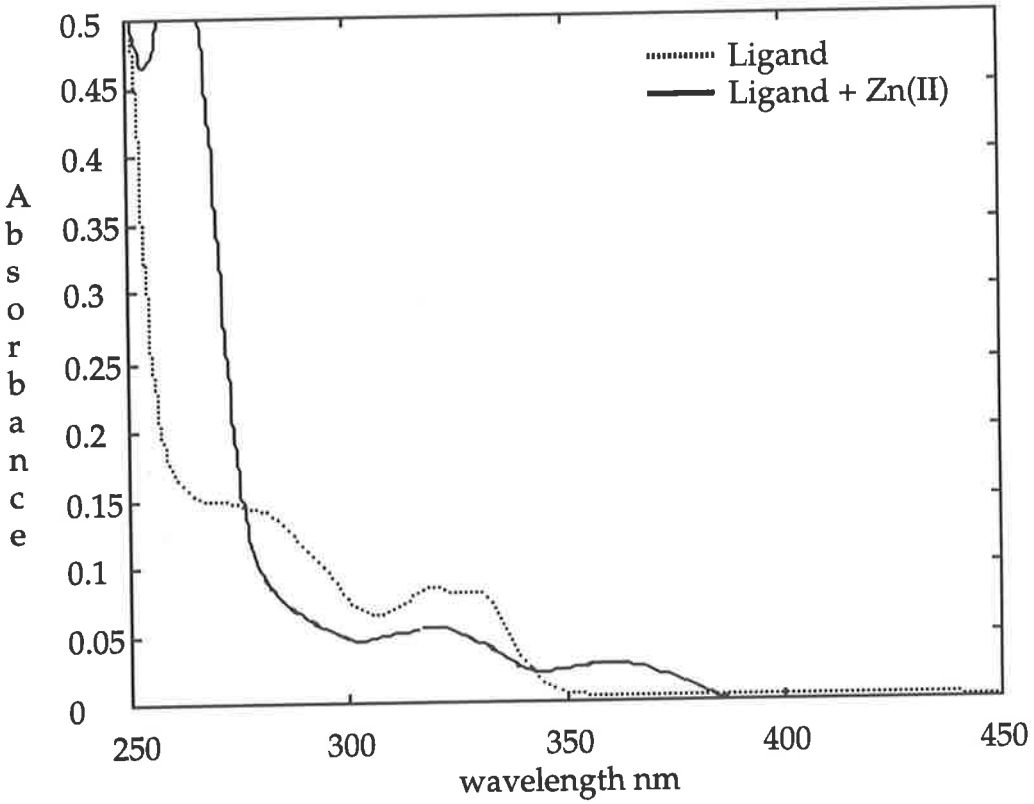
0.1
0.05
0
250
300
350
400
450
wavelength nm

41A


Ligand
Ligand + Zn(II)
Absorbance
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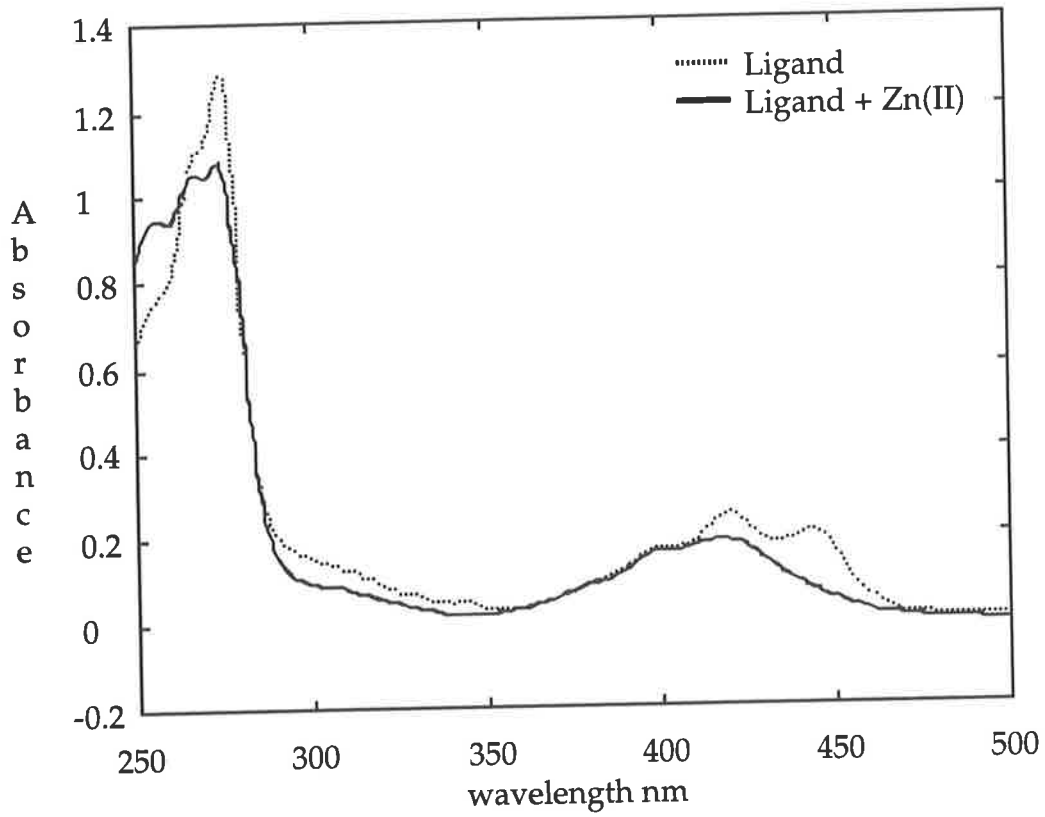
0.1
0.05
0
250
300
350
400
450
wavelength nm

48[A]


Ligand
Ligand + Zn(II)
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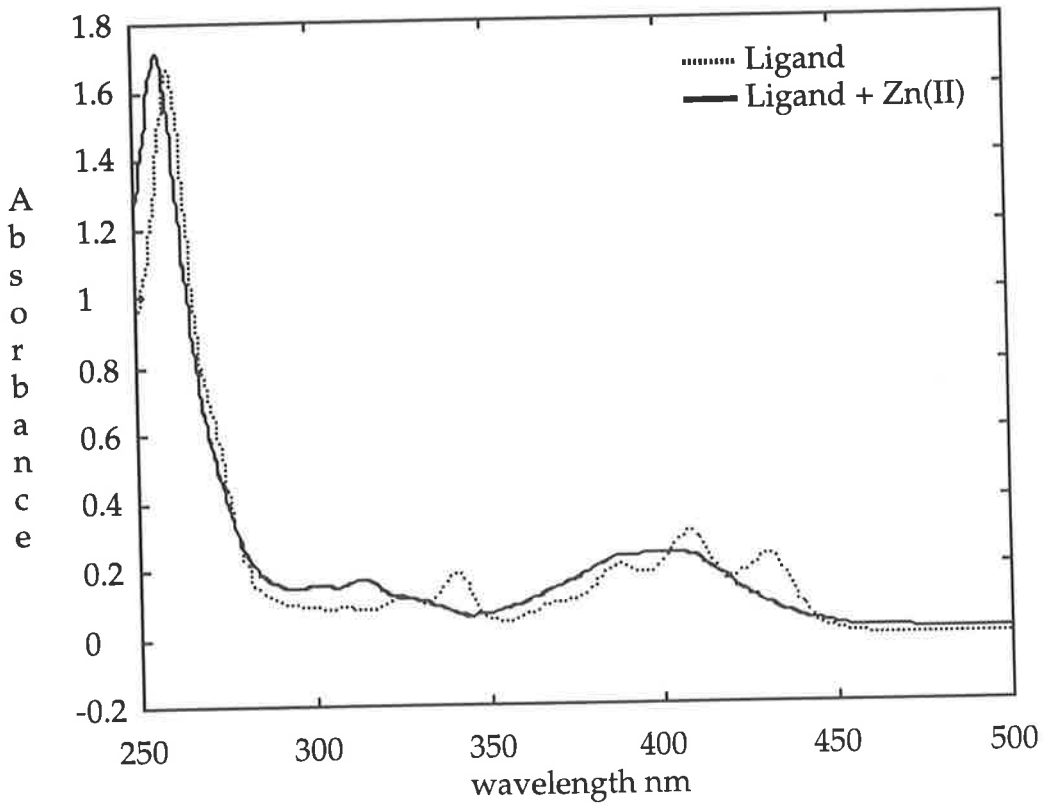
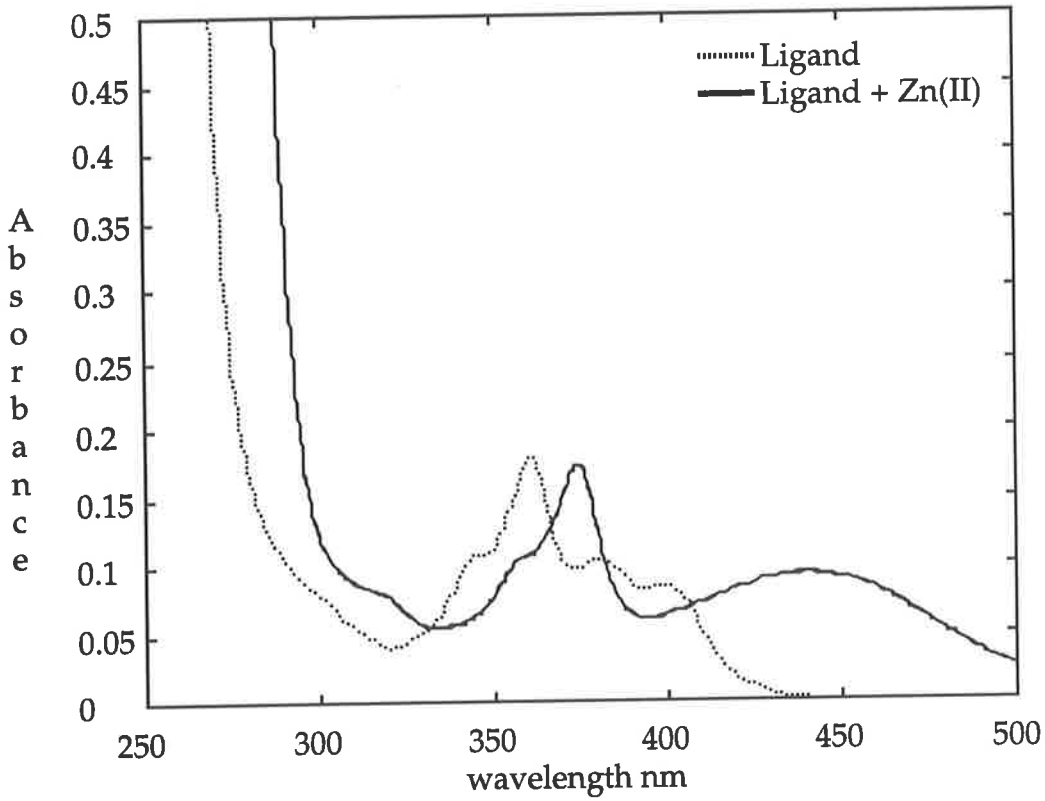
0.6
0.4
0.2
0
-0.2
250
300
350
400
450
500
wavelength nm

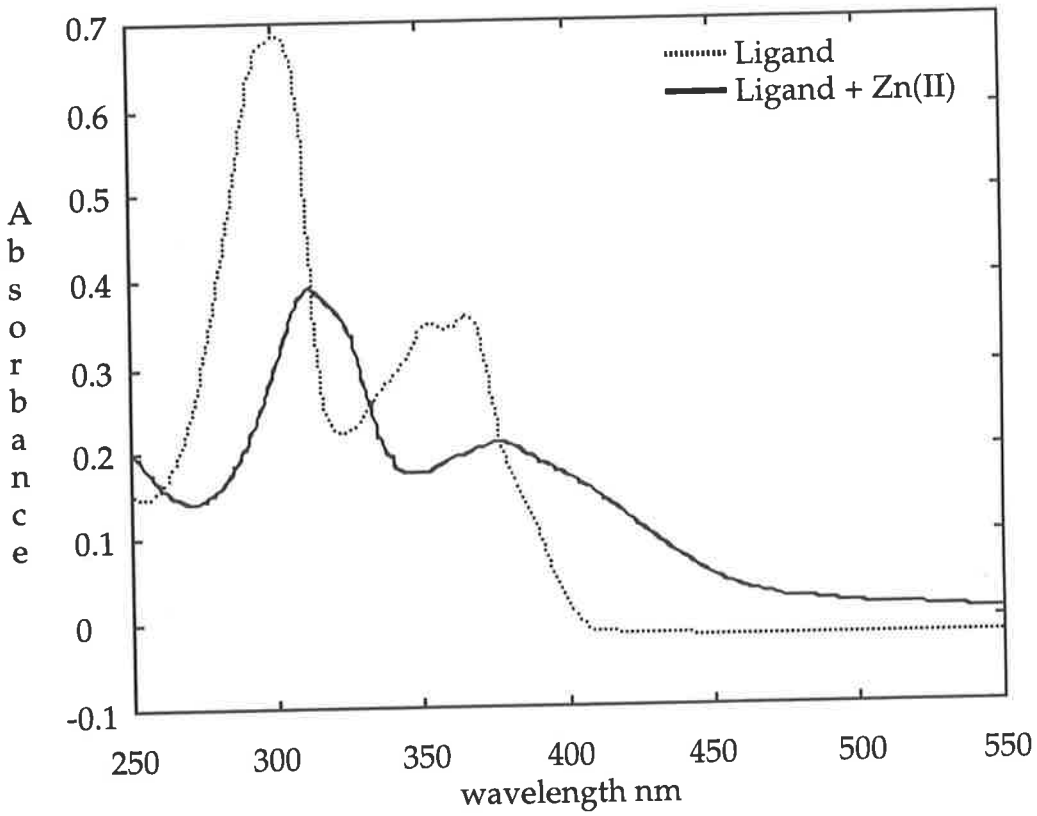
49[A]


Ligand
Ligand + Zn(II)
Absorbance
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
250
300
350
400
450
500
wavelength nm

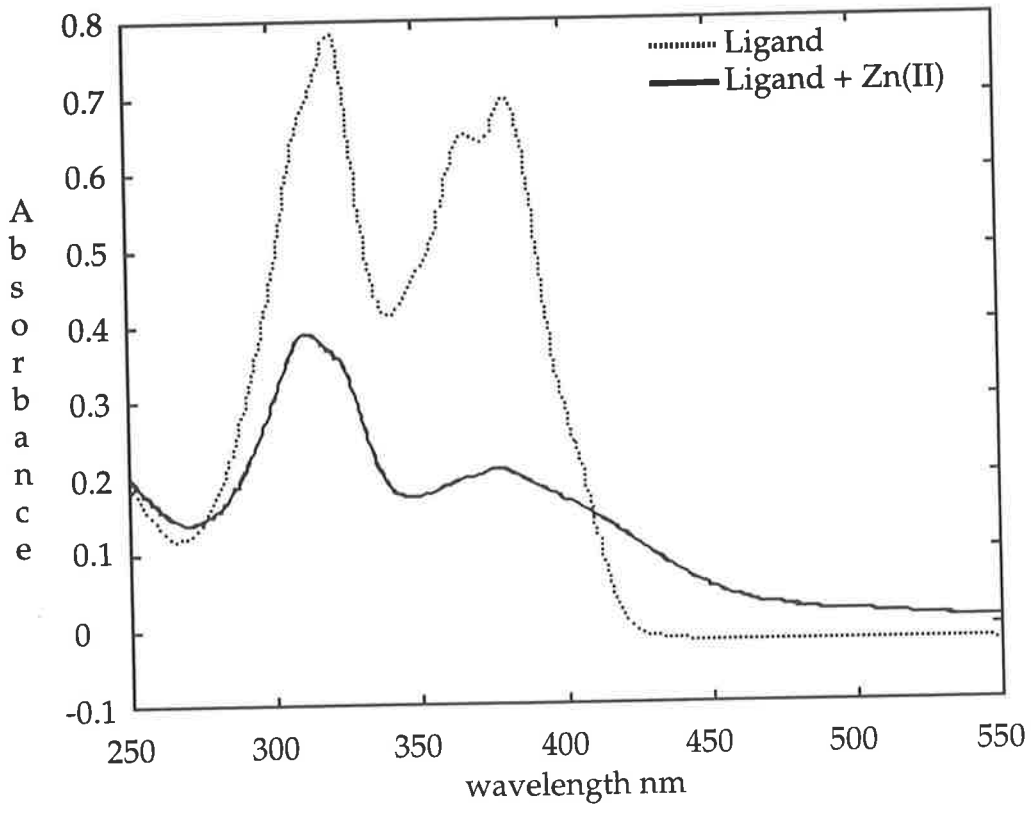
46[A]


Ligand
Ligand + Zn(II)
Absorbance
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
500
wavelength nm

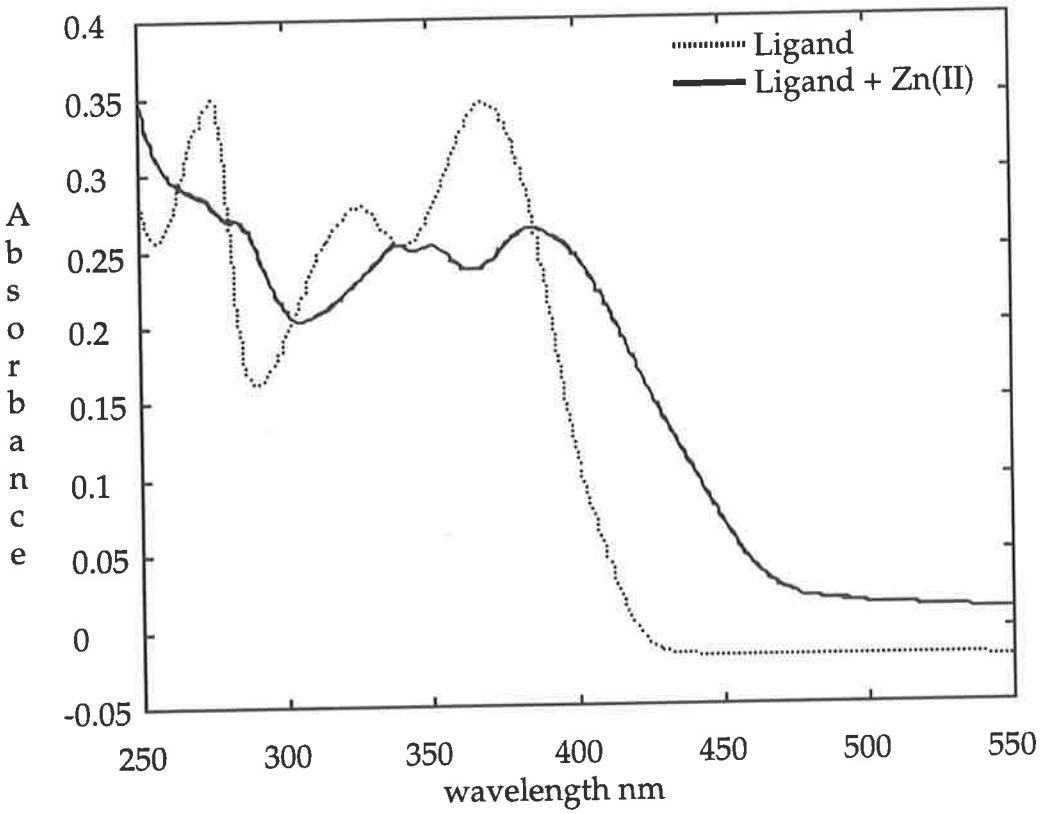
78B


Ligand
Ligand + Zn(II)
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
250
300
350
400
450
500
550
wavelength nm

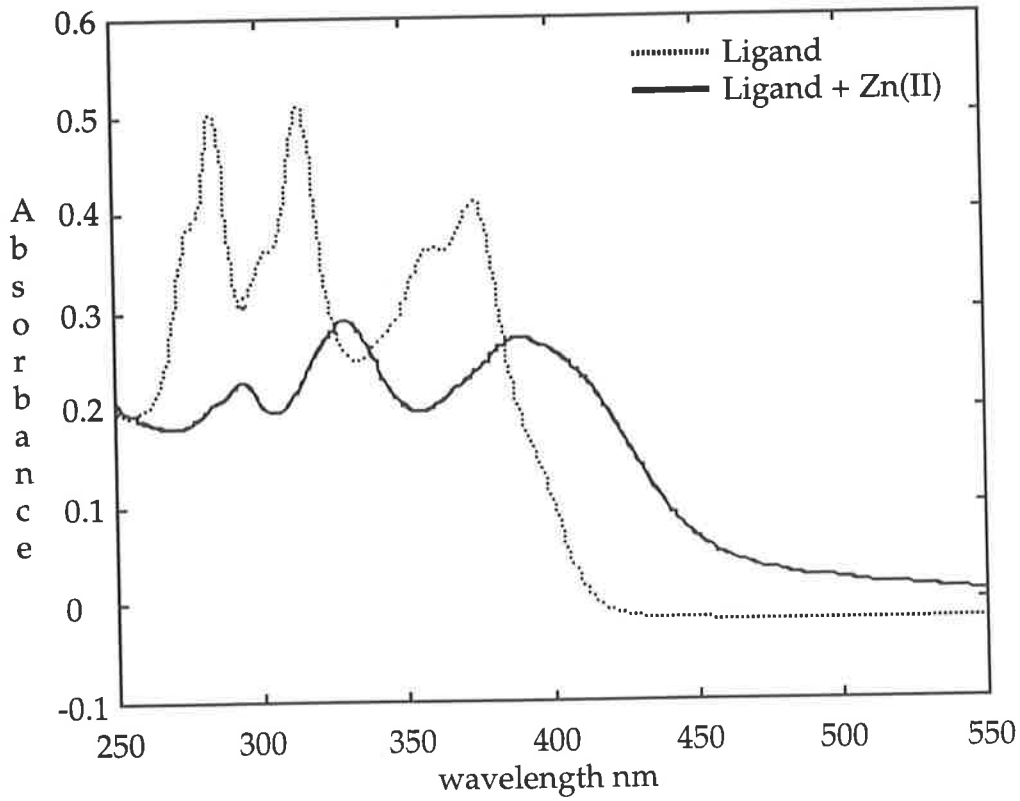
79B


Ligand
Ligand + Zn(II)
Absorbance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
250
300
350
400
450
500
550
wavelength nm

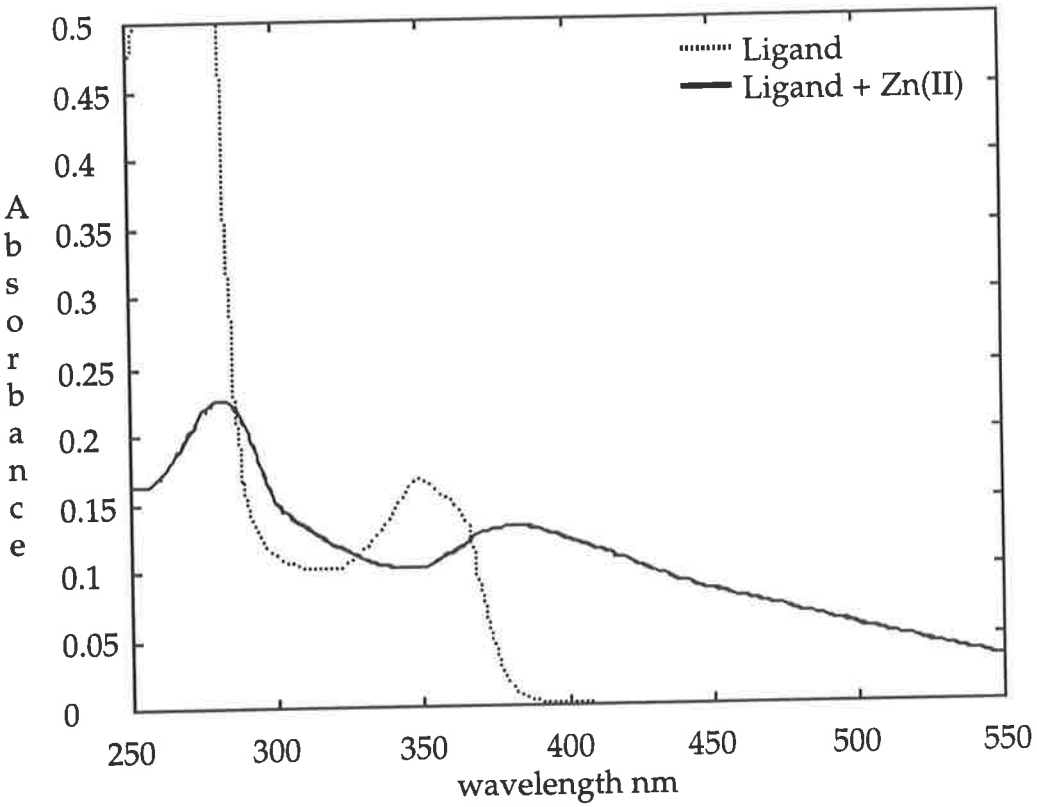
80^B


Ligand
Ligand + Zn(II)
Absorbance
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
250
300
350
400
450
500
550
wavelength nm

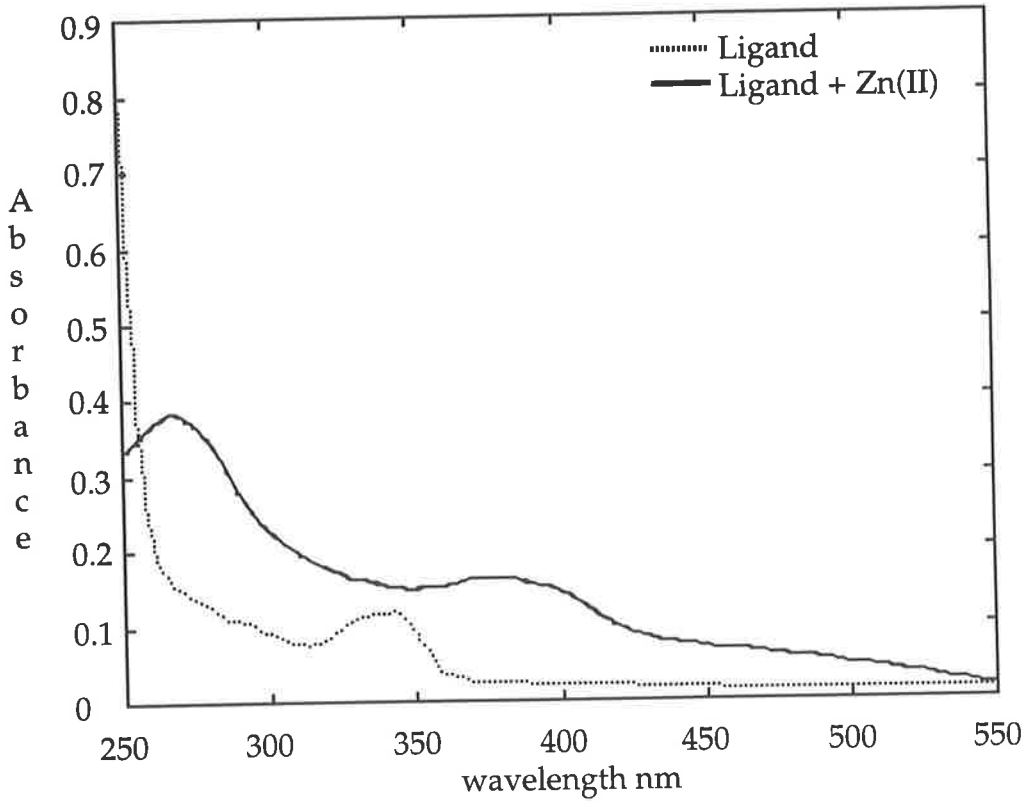
81^B


Ligand
Ligand + Zn(II)
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
250
300
350
400
450
500
550
wavelength nm

82[A]


Ligand
Ligand + Zn(II)
Absorbance
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
250
300
350
400
450
500
550
wavelength nm

83[A]


Ligand
Ligand + Zn(II)
Absorbance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250
300
350
400
450
500
550
wavelength nm

Appendix B - Fluorescence data

Table 1. Observed fluorescence of ligands **90, 91, 92, 97** with a number of Metal cations.[a]

Metal	**92**	**91**	**90**	**97**
-	10.5±0.6	11.8±0.7	1.5±0.1	3.6±0.3
Zn(II)	295.6±15.9	339.0±18.3	216.1±11.3	269.9±15.3
Cd(II)	47.0±2.6	113±6.1	69.0±3.6	174.8±9.9
Ca(II)	14.2±0.8	14.1±0.8	4.1±0.3	17.8±1.1
Mg(II)	13.3±0.8	13.1±0.8	5.6±0.3	16.8±1.0
Mn(II)	10.5±0.5	13.9±0.8	7.8±0.5	21.6±1.3
Ba(II)	10.6±0.6	12.0±0.7	4.9±0.3	15.6±0.9
Fe(II)	11.3±0.7	11.9±0.7	10.1±0.6	26.6±1.6
Fe(III)	10.6±0.6	12.0±0.7	17.1±0.9	19.4±1.2
Co(II)	12.6±0.7	14.1±0.8	9.8±0.5	0.6±0.5
Ni(II)	11.9±0.6	15.0±0.9	8.9±0.5	8.0±0.5
Cu(II)	12.7±0.7	16.1±0.9	5.8±0.4	0±0.5

a) Obtained from; [L] 4μM, [M] 4μM in 100mM $NaClO_4$, 1mM Na PIPES, ethanol/water (75:25, v/v).

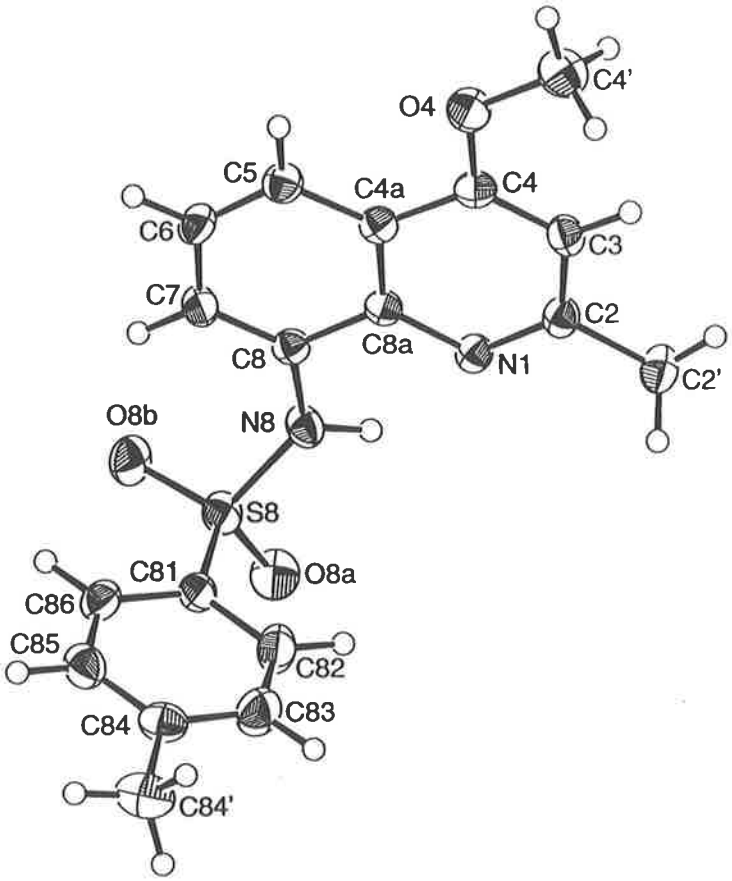
Table 2. Observed fluorescence of ligands **1a, 90, 91, 92** and **97** in the presence of Zn(II).[a,b]

Ligand (ex., em. in nm)	Relative Fluorescence	
	Slit width 5nm	Slit width 2.5nm
1a (ex. 364, em. 485)	481.4±21.3	79.0±3.5
92 (ex. 370, em. 478)	850.2±36.6	-
91 (ex. 372, em. 487)	>1000	236.7±10.2
90 (ex. 375, em. 516)	730.3±30.7	-
90 (ex. 403, em. 516)	587.3±24.7	-
97 (ex. 352, em. 467)	411.4±16.7	-

a) Obtained from; [L] 2μM, [Zn(II)] 2μM in 100mM $NaClO_4$, 1mM NaPIPES, ethanol/water (75:25, v/v). Slit width of 5nm.

b) Last column was taken at a slit width of 2.5nm.

Appendix C - X-Ray Crystal Analysis data



4-Methyl-N-(4-methoxy-2-methyl-8-quinolyl)benzene sulfonamide **36**

Intramolecular Distances Involving the Nonhydrogen Atoms

atom	atom	distance	atom	atom	distance
S(8)	O(8b)	1.429(3)	C(4a)	C(5)	1.407(5)
S(8)	O(8a)	1.432(3)	C(4a)	C(8a)	1.411(5)
S(8)	N(8)	1.627(3)	C(5)	C(6)	1.363(5)
S(8)	C(81)	1.763(4)	C(6)	C(7)	1.401(5)
O(4)	C(4)	1.353(4)	C(7)	C(8)	1.371(5)
O(4)	C(4')	1.429(4)	C(8)	C(8a)	1.421(5)
N(1)	C(2)	1.315(4)	C(81)	C(82)	1.384(5)
N(1)	C(8a)	1.372(4)	C(81)	C(86)	1.378(5)
N(8)	C(8)	1.409(4)	C(82)	C(83)	1.378(5)
C(2)	C(2')	1.509(5)	C(83)	C(84)	1.376(6)
C(2)	C(3)	1.403(5)	C(84)	C(84')	1.513(6)
C(3)	C(4)	1.365(5)	C(84)	C(85)	1.375(6)
C(4)	C(4a)	1.423(5)	C(85)	C(86)	1.382(5)

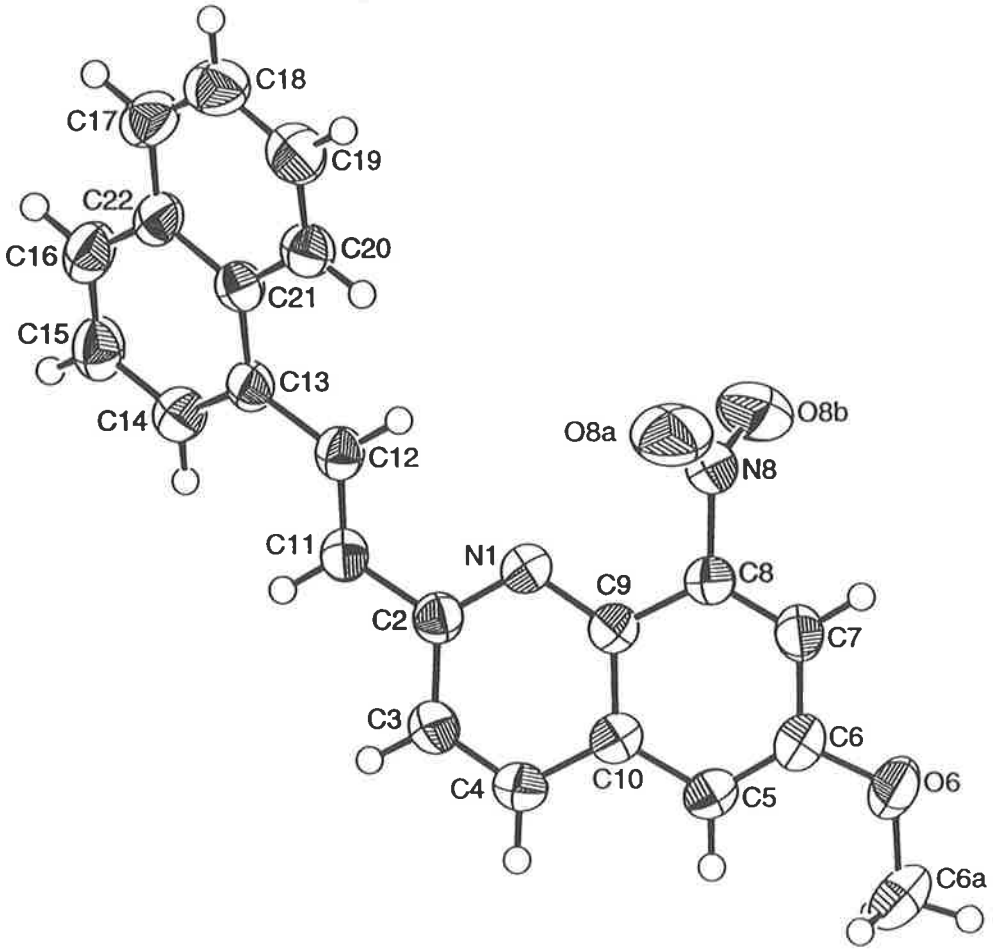
Intramolecular Bond Angles Involving the Nonhydrogen Atoms

atom	atom	atom	angle	atom	atom	atom	angle
O(8b)	S(8)	O(8a)	119.8(2)	C(4a)	C(5)	C(6)	119.4(3)
O(8b)	S(8)	N(8)	108.9(2)	C(5)	C(6)	C(7)	121.9(3)
O(8b)	S(8)	C(81)	108.3(2)	C(6)	C(7)	C(8)	119.6(3)
O(8a)	S(8)	N(8)	104.3(2)	N(8)	C(8)	C(7)	125.4(3)
O(8a)	S(8)	C(81)	108.7(2)	N(8)	C(8)	C(8a)	114.4(3)
N(8)	S(8)	C(81)	106.1(2)	C(7)	C(8)	C(8a)	120.2(3)
C(4)	O(4)	C(4')	118.1(3)	N(1)	C(8a)	C(4a)	124.2(3)
C(2)	N(1)	C(8a)	116.8(3)	N(1)	C(8a)	C(8)	116.8(3)
S(8)	N(8)	C(8)	127.3(2)	C(4a)	C(8a)	C(8)	119.1(3)
N(1)	C(2)	C(2')	116.7(3)	S(8)	C(81)	C(82)	118.3(3)
N(1)	C(2)	C(3)	124.1(3)	S(8)	C(81)	C(86)	121.4(3)
C(2')	C(2)	C(3)	119.1(3)	C(82)	C(81)	C(86)	120.2(4)
C(2)	C(3)	C(4)	119.0(3)	C(81)	C(82)	C(83)	119.3(4)
O(4)	C(4)	C(3)	125.1(3)	C(82)	C(83)	C(84)	121.4(4)
O(4)	C(4)	C(4a)	114.9(3)	C(83)	C(84)	C(84')	120.2(4)
C(3)	C(4)	C(4a)	120.0(3)	C(83)	C(84)	C(85)	118.3(4)
C(4)	C(4a)	C(5)	124.3(3)	C(84')	C(84)	C(85)	121.5(4)
C(4)	C(4a)	C(8a)	115.8(3)	C(84)	C(85)	C(86)	121.7(4)
C(5)	C(4a)	C(8a)	119.8(3)	C(81)	C(86)	C(85)	119.0(4)

Torsion or Conformation Angles

(1)	(2)	(3)	(4)	angle
S(8)	N(8)	C(8)	C(7)	-22.1(5)
S(8)	N(8)	C(8)	C(8a)	159.0(3)
S(8)	C(81)	C(82)	C(83)	-178.7(3)
S(8)	C(81)	C(86)	C(85)	177.8(3)
O(4)	C(4)	C(3)	C(2)	180.0(3)
O(4)	C(4)	C(4a)	C(5)	0.0(5)
O(4)	C(4)	C(4a)	C(8a)	-180.0(3)
O(8b)	S(8)	N(8)	C(8)	50.8(3)
O(8b)	S(8)	C(81)	C(82)	-178.3(3)
O(8b)	S(8)	C(81)	C(86)	3.2(3)
O(8a)	S(8)	N(8)	C(8)	179.8(3)
O(8a)	S(8)	C(81)	C(82)	50.0(3)
O(8a)	S(8)	C(81)	C(86)	-128.4(3)
N(1)	C(2)	C(3)	C(4)	-0.2(6)
N(1)	C(8a)	C(4a)	C(4)	0.1(5)
N(1)	C(8a)	C(4a)	C(5)	-179.8(3)
N(1)	C(8a)	C(8)	N(8)	-1.9(4)
N(1)	C(8a)	C(8)	C(7)	179.2(3)
N(8)	S(8)	C(81)	C(82)	-61.6(3)
N(8)	S(8)	C(81)	C(86)	119.9(3)
N(8)	C(8)	C(7)	C(6)	-177.9(3)
N(8)	C(8)	C(8a)	C(4a)	177.8(3)
C(2)	N(1)	C(8a)	C(4a)	0.0(5)
C(2)	N(1)	C(8a)	C(8)	179.7(3)
C(2)	C(3)	C(4)	C(4a)	0.4(5)

(1)	(2)	(3)	(4)	angle
C(2')	C(2)	N(1)	C(8a)	178.9(3)
C(2')	C(2)	C(3)	C(4)	-179.1(3)
C(3)	C(2)	N(1)	C(8a)	0.0(5)
C(3)	C(4)	O(4)	C(4')	-2.4(5)
C(3)	C(4)	C(4a)	C(5)	179.6(4)
C(3)	C(4)	C(4a)	C(8a)	-0.4(5)
C(4)	C(4a)	C(5)	C(6)	-179.6(3)
C(4)	C(4a)	C(8a)	C(8)	-179.6(3)
C(4a)	C(4)	O(4)	C(4')	177.2(3)
C(4a)	C(5)	C(6)	C(7)	-0.6(6)
C(4a)	C(8a)	C(8)	C(7)	-1.1(5)
C(5)	C(4a)	C(8a)	C(8)	0.5(5)
C(5)	C(6)	C(7)	C(8)	-0.1(6)
C(6)	C(5)	C(4a)	C(8a)	0.4(5)
C(6)	C(7)	C(8)	C(8a)	0.9(5)
C(8)	N(8)	S(8)	C(81)	-65.5(3)
C(81)	C(82)	C(83)	C(84)	1.1(7)
C(81)	C(86)	C(85)	C(84)	0.7(6)
C(82)	C(81)	C(86)	C(85)	-0.6(6)
C(82)	C(83)	C(84)	C(84'	177.5(4)
C(82)	C(83)	C(84)	C(85)	-1.1(6)
C(83)	C(82)	C(81)	C(86)	-0.2(6)
C(83)	C(84)	C(85)	C(86)	0.2(6)
C(84'	C(84)	C(85)	C(86)	-178.4(4)



6-Methoxy-2-[(E)-2-(1-naphthy)-1-ethenyl]-8-nitroquinoline **69**

Intramolecular Distances Involving the Nonhydrogen Atoms

atom	atom	distance	atom	atom	distance
O(6)	C(6)	1.366(3)	C(8)	C(9)	1.415(3)
O(6)	C(6a)	1.424(3)	C(9)	C(10)	1.416(3)
O(8a)	N(8)	1.205(3)	C(11)	C(12)	1.328(3)
O(8b)	N(8)	1.208(3)	C(12)	C(13)	1.473(3)
N(1)	C(2)	1.333(3)	C(13)	C(14)	1.378(3)
N(1)	C(9)	1.363(3)	C(13)	C(21)	1.424(3)
N(8)	C(8)	1.472(3)	C(14)	C(15)	1.401(3)
C(2)	C(3)	1.412(3)	C(15)	C(16)	1.358(3)
C(2)	C(11)	1.464(3)	C(16)	C(22)	1.408(3)
C(3)	C(4)	1.354(3)	C(17)	C(18)	1.346(4)
C(4)	C(10)	1.411(3)	C(17)	C(22)	1.416(3)
C(5)	C(6)	1.363(3)	C(18)	C(19)	1.394(4)
C(5)	C(10)	1.410(3)	C(19)	C(20)	1.357(3)
C(6)	C(7)	1.407(3)	C(20)	C(21)	1.418(3)
C(7)	C(8)	1.349(3)	C(21)	C(22)	1.424(3)

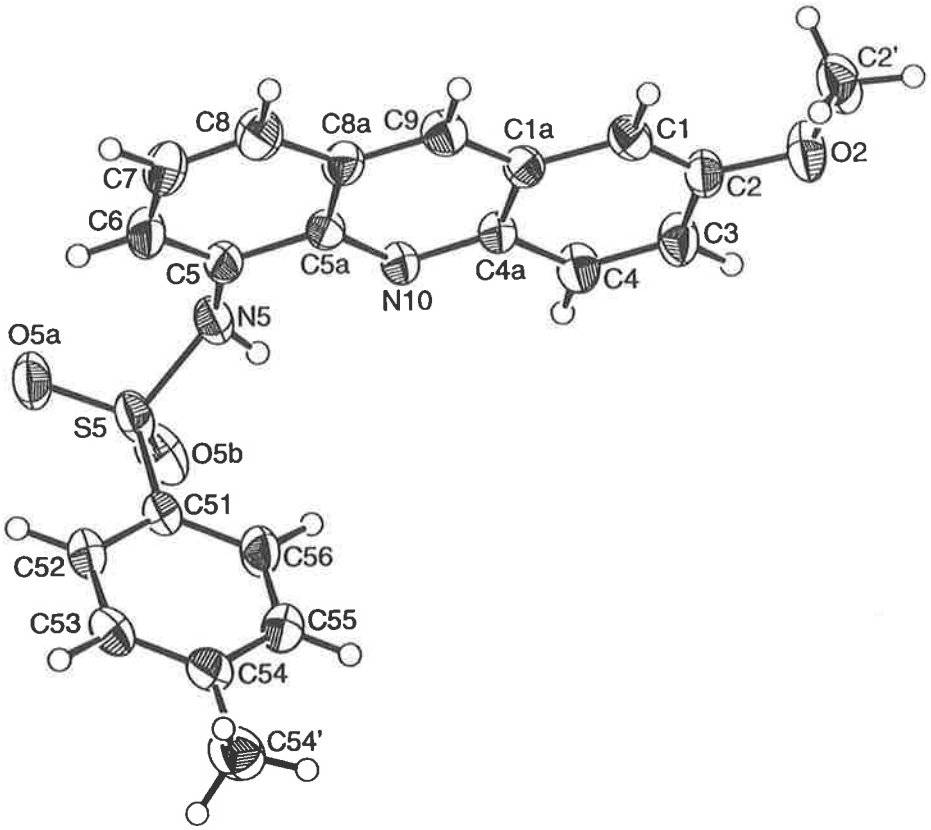
Intramolecular Bond Angles Involving the Nonhydrogen Atoms

atom	atom	atom	angle	atom	atom	atom	angle
C(6)	O(6)	C(6a)	117.0(2)	C(4)	C(10)	C(5)	123.1(2)
C(2)	N(1)	C(9)	117.4(2)	C(4)	C(10)	C(9)	116.3(2)
O(8a)	N(8)	O(8b)	123.3(2)	C(5)	C(10)	C(9)	120.5(2)
O(8a)	N(8)	C(8)	118.9(2)	C(2)	C(11)	C(12)	124.6(2)
O(8b)	N(8)	C(8)	117.9(2)	C(11)	C(12)	C(13)	125.5(2)
N(1)	C(2)	C(3)	121.9(2)	C(12)	C(13)	C(14)	120.3(2)
N(1)	C(2)	C(11)	118.3(2)	C(12)	C(13)	C(21)	120.9(2)
C(3)	C(2)	C(11)	119.8(2)	C(14)	C(13)	C(21)	118.6(2)
C(2)	C(3)	C(4)	120.8(2)	C(13)	C(14)	C(15)	122.2(2)
C(3)	C(4)	C(10)	119.4(2)	C(14)	C(15)	C(16)	119.7(2)
C(6)	C(5)	C(10)	120.4(2)	C(15)	C(16)	C(22)	120.9(2)
O(6)	C(6)	C(5)	125.6(2)	C(18)	C(17)	C(22)	121.1(2)
O(6)	C(6)	C(7)	114.2(2)	C(17)	C(18)	C(19)	120.3(3)
C(5)	C(6)	C(7)	120.3(2)	C(18)	C(19)	C(20)	121.0(3)
C(6)	C(7)	C(8)	119.2(2)	C(19)	C(20)	C(21)	120.9(2)
N(8)	C(8)	C(7)	118.2(2)	C(13)	C(21)	C(20)	123.0(2)
N(8)	C(8)	C(9)	118.0(2)	C(13)	C(21)	C(22)	119.1(2)
C(7)	C(8)	C(9)	123.7(2)	C(20)	C(21)	C(22)	117.8(2)
N(1)	C(9)	C(8)	120.0(2)	C(16)	C(22)	C(17)	121.6(2)
N(1)	C(9)	C(10)	124.1(2)	C(16)	C(22)	C(21)	119.4(2)
C(8)	C(9)	C(10)	115.9(2)	C(17)	C(22)	C(21)	119.0(2)

Torsion or Conformation Angles

(1)	(2)	(3)	(4)	angle
O(6)	C(6)	C(5)	C(10)	179.5(2)
O(6)	C(6)	C(7)	C(8)	-179.0(2)
O(8a)	N(8)	C(8)	C(7)	-113.5(3)
O(8a)	N(8)	C(8)	C(9)	65.5(3)
O(8b)	N(8)	C(8)	C(7)	65.6(3)
O(8b)	N(8)	C(8)	C(9)	-115.4(2)
N(1)	C(2)	C(3)	C(4)	-1.0(3)
N(1)	C(2)	C(11)	C(12)	-5.4(3)
N(1)	C(9)	C(8)	N(8)	3.2(3)
N(1)	C(9)	C(8)	C(7)	-177.8(2)
N(1)	C(9)	C(10)	C(4)	-2.5(3)
N(1)	C(9)	C(10)	C(5)	178.3(2)
N(8)	C(8)	C(7)	C(6)	178.4(2)
N(8)	C(8)	C(9)	C(10)	-178.6(2)
C(2)	N(1)	C(9)	C(8)	179.7(2)
C(2)	N(1)	C(9)	C(10)	1.7(3)
C(2)	C(3)	C(4)	C(10)	0.1(4)
C(2)	C(11)	C(12)	C(13)	171.5(2)
C(3)	C(2)	N(1)	C(9)	0.1(3)
C(3)	C(2)	C(11)	C(12)	176.8(2)
C(3)	C(4)	C(10)	C(5)	-179.3(2)
C(3)	C(4)	C(10)	C(9)	1.5(3)
C(4)	C(3)	C(2)	C(11)	176.8(2)
C(4)	C(10)	C(5)	C(6)	-179.7(2)
C(4)	C(10)	C(9)	C(8)	179.5(2)
C(5)	C(6)	C(7)	C(8)	0.2(4)
C(5)	C(10)	C(9)	C(8)	0.2(3)
C(6)	C(5)	C(10)	C(9)	-0.5(3)
C(6)	C(7)	C(8)	C(9)	-0.6(4)
C(6a)	O(6)	C(6)	C(7)	180.0(2)
C(7)	C(6)	C(5)	C(10)	0.3(4)
C(7)	C(8)	C(9)	C(10)	0.3(3)
C(9)	N(1)	C(2)	C(11)	-177.7(2)
C(11)	C(12)	C(13)	C(14)	-24.9(3)
C(11)	C(12)	C(13)	C(21)	160.1(2)
C(12)	C(13)	C(14)	C(15)	-174.0(2)
C(12)	C(13)	C(21)	C(20)	-5.9(3)
C(12)	C(13)	C(21)	C(22)	173.6(2)
C(13)	C(14)	C(15)	C(16)	0.4(4)
C(13)	C(21)	C(20)	C(19)	178.1(2)
C(13)	C(21)	C(22)	C(16)	0.4(3)
C(13)	C(21)	C(22)	C(17)	-178.7(2)
C(14)	C(13)	C(21)	C(20)	179.0(2)
C(14)	C(13)	C(21)	C(22)	-1.5(3)
C(14)	C(15)	C(16)	C(22)	-1.5(4)
C(15)	C(14)	C(13)	C(21)	1.2(3)
C(15)	C(16)	C(22)	C(17)	-179.8(2)
C(15)	C(16)	C(22)	C(21)	1.1(3)
C(16)	C(22)	C(17)	C(18)	-178.5(2)
C(16)	C(22)	C(21)	C(20)	180.0(2)

C(5) C(6) O(6) C(6a)	0.8(3)	C(17)C(18)C(19)C(20)	0.9(4)
C(17)C(22)C(21)C(20)	0.8(3)		
C(18)C(17)C(22)C(21)	0.7(3)		
C(18)C(19)C(20)C(21)	0.6(4)		
C(19)C(18)C(17)C(22)	-1.6(4)		
C(19)C(20)C(21)C(22)	-1.4(3)		



4-Methyl-N-(2-methoxy-5-acridinyl)benzene sulfonamide **46**

Intramolecular Distances Involving the Nonhydrogen Atoms

atom	atom	distance	atom	atom	distance
S(5)	O(5a)	1.423(2)	C(4a)	C(4)	1.419(3)
S(5)	O(5b)	1.431(2)	C(5a)	C(5)	1.436(3)
S(5)	N(5)	1.640(2)	C(5a)	C(8a)	1.426(3)
S(5)	C(51)	1.753(3)	C(5)	C(6)	1.357(4)
O(2)	C(2)	1.361(3)	C(6)	C(7)	1.414(4)
O(2)	C(2')	1.415(3)	C(7)	C(8)	1.352(4)
N(5)	C(5)	1.399(3)	C(8a)	C(8)	1.421(4)
N(10)	C(4a)	1.344(3)	C(8a)	C(9)	1.382(3)
N(10)	C(5a)	1.340(3)	C(51)	C(52)	1.383(3)
C(1)	C(1a)	1.428(3)	C(51)	C(56)	1.384(3)
C(1)	C(2)	1.348(3)	C(52)	C(53)	1.383(4)
C(1a)	C(4a)	1.418(3)	C(53)	C(54)	1.376(4)
C(1a)	C(9)	1.393(3)	C(54')	C(54)	1.500(4)
C(2)	C(3)	1.414(4)	C(54)	C(55)	1.385(4)
C(3)	C(4)	1.352(3)	C(55)	C(56)	1.365(4)

Intramolecular Bond Angles Involving the Nonhydrogen Atoms

atom	atom	atom	angle	atom	atom	atom	angle
O(5a)	S(5)	O(5b)	119.8(1)	N(10)	C(5a)	C(8a)	123.6(2)
O(5a)	S(5)	N(5)	108.6(1)	C(5)	C(5a)	C(8a)	118.8(2)
O(5a)	S(5)	C(51)	108.1(1)	N(5)	C(5)	C(5a)	114.2(2)
O(5b)	S(5)	N(5)	104.1(1)	N(5)	C(5)	C(6)	125.0(2)
O(5b)	S(5)	C(51)	108.8(1)	C(5a)	C(5)	C(6)	120.7(3)
N(5)	S(5)	C(51)	106.8(1)	C(5)	C(6)	C(7)	119.6(2)
C(2)	O(2)	C(2')	117.6(2)	C(6)	C(7)	C(8)	121.8(2)
S(5)	N(5)	C(5)	126.4(2)	C(5a)	C(8a)	C(8)	118.6(2)
C(4a)	N(10)	C(5a)	117.7(2)	C(5a)	C(8a)	C(9)	117.0(2)
C(1a)	C(1)	C(2)	119.8(2)	C(8)	C(8a)	C(9)	124.4(3)
C(1)	C(1a)	C(4a)	120.0(2)	C(7)	C(8)	C(8a)	120.4(3)
C(1)	C(1a)	C(9)	122.9(2)	C(1a)	C(9)	C(8a)	121.1(2)
C(4a)	C(1a)	C(9)	117.1(2)	S(5)	C(51)	C(52)	121.0(2)
O(2)	C(2)	C(1)	125.7(2)	S(5)	C(51)	C(56)	119.0(2)
O(2)	C(2)	C(3)	113.8(2)	C(52)	C(51)	C(56)	120.0(2)
C(1)	C(2)	C(3)	120.5(2)	C(51)	C(52)	C(53)	119.0(2)
C(2)	C(3)	C(4)	121.0(2)	C(52)	C(53)	C(54)	121.7(2)
N(10)	C(4a)	C(1a)	123.5(2)	C(53)	C(54)	C(54')	121.5(3)
N(10)	C(4a)	C(4)	118.7(2)	C(53)	C(54)	C(55)	118.0(3)
C(1a)	C(4a)	C(4)	117.8(2)	C(54')	C(54)	C(55)	120.6(3)
C(3)	C(4)	C(4a)	120.8(2)	C(54)	C(55)	C(56)	121.6(2)
N(10)	C(5a)	C(5)	117.6(2)	C(51)	C(56)	C(55)	119.8(2)

Torsion or Conformation Angles

(1)	(2)	(3)	(4)	angle
S(5)	N(5)	C(5)	C(5a)	145.3(2)
S(5)	N(5)	C(5)	C(6)	-37.9(4)
S(5)	C(51)	C(52)	C(53)	178.6(2)
S(5)	C(51)	C(56)	C(55)	-178.6(2)
O(2)	C(2)	C(1)	C(1a)	179.6(2)
O(2)	C(2)	C(3)	C(4)	-179.6(3)
O(5a)	S(5)	N(5)	C(5)	56.4(2)
O(5a)	S(5)	C(51)	C(52)	9.9(3)
O(5a)	S(5)	C(51)	C(56)	-171.9(2)
O(5b)	S(5)	N(5)	C(5)	-175.0(2)
O(5b)	S(5)	C(51)	C(52)	-121.7(2)
O(5b)	S(5)	C(51)	C(56)	56.6(2)
N(5)	S(5)	C(51)	C(52)	126.6(2)
N(5)	S(5)	C(51)	C(56)	-55.2(2)
N(5)	C(5)	C(5a)	N(10)	-4.6(3)
N(5)	C(5)	C(5a)	C(8a)	175.2(2)
N(5)	C(5)	C(6)	C(7)	-175.7(3)
N(10)	C(4a)	C(1a)	C(1)	179.7(2)
N(10)	C(4a)	C(1a)	C(9)	0.0(4)
N(10)	C(4a)	C(4)	C(3)	-179.7(2)
N(10)	C(5a)	C(5)	C(6)	178.4(2)
N(10)	C(5a)	C(8a)	C(8)	-179.1(2)
N(10)	C(5a)	C(8a)	C(9)	0.6(4)
C(1)	C(1a)	C(4a)	C(4)	0.1(3)
C(1)	C(1a)	C(9)	C(8a)	-179.4(2)
C(1)	C(2)	C(3)	C(4)	0.3(4)
C(1a)	C(1)	C(2)	C(3)	-0.2(4)
C(1a)	C(4a)	N(10)	C(5a)	0.0(3)
C(1a)	C(4a)	C(4)	C(3)	-0.1(4)
C(1a)	C(9)	C(8a)	C(5a)	-0.6(4)
C(1a)	C(9)	C(8a)	C(8)	179.1(3)
C(2)	C(1)	C(1a)	C(4a)	0.0(4)
C(2)	C(1)	C(1a)	C(9)	179.7(2)
C(2)	C(3)	C(4)	C(4a)	-0.1(4)
C(2')	O(2)	C(2)	C(3)	178.3(2)
C(4a)	N(10)	C(5a)	C(5)	179.5(2)
C(4a)	N(10)	C(5a)	C(8a)	-0.3(3)
C(4a)	C(1a)	C(9)	C(8a)	0.3(4)
C(4)	C(4a)	N(10)	C(5a)	179.6(2)
C(4)	C(4a)	C(1a)	C(9)	-179.6(2)
C(5a)	C(5)	C(6)	C(7)	1.0(4)
C(5a)	C(8a)	C(8)	C(7)	0.3(4)
C(5)	N(5)	S(5)	C(51)	-59.9(2)
C(5)	C(5a)	C(8a)	C(8)	1.1(4)
C(5)	C(5a)	C(8a)	C(9)	-179.2(2)
C(5)	C(6)	C(7)	C(8)	0.5(5)
C(6)	C(5)	C(5a)	C(8a)	-1.8(4)
C(6)	C(7)	C(8)	C(8a)	-1.2(5)
C(7)	C(8)	C(8a)	C(9)	-179.4(3)
C(51)	C(52)	C(53)	C(54)	0.4(4)

C(1) C(2) O(2) C(2')	-1.6(4)	C(51)C(56)C(55)C(54)	-0.5(4)
C(52)C(51)C(56)C(55)	-0.3(4)		
C(52)C(53)C(54)C(54'	179.0(3)		
C(52)C(53)C(54)C(55)	-1.1(4)		
C(53)C(52)C(51)C(56)	0.4(4)		
C(53)C(54)C(55)C(56)	1.2(4)		
C(54'C(54)C(55)C(56)	-178.9(3)		

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