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SCHIFF BASE METAL COMPLEXES OF ISATIN: SYNTHESIS, CHARACTERISATION AND DNA-BINDING STUDIES

SEBASTIAN HOFFMEISTER[1]*, DARRAGH HENNESSY[2], VALENTINA VITALE[3]

Affiliation

[1]Department of Medicinal Chemistry, Fichtelgebirge Centre for Drug Research, Hof, Germany
[2]Department of Pharmaceutical Chemistry, Connacht Institute of Pharmacy Practice, Castlebar, Ireland
[3]Department of Medicinal Chemistry, Cilento Institute of Pharmaceutical Sciences, Salerno, Italy

*Corresponding author: Sebastian Hoffmeister, s.hoffmeister@fcdr-hof.de
ORCID iD: Sebastian Hoffmeister 0000-0001-3503-9077; Darragh Hennessy 0000-0001-1127-2405; Valentina Vitale 0000-0001-4684-141X

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Abstract

Isatin is an endogenous indole-2,3-dione whose reactive C-3 carbonyl condenses readily with primary amines to give Schiff bases that bind transition metal ions. This narrative review summarises published work on how such ligands and their copper, nickel, cobalt, zinc and ruthenium complexes are prepared, how their structures are assigned from spectroscopic and magnetic data, and how their interaction with DNA is studied by absorption, fluorescence, viscosity and cleavage experiments. Reported binding is mostly modest and groove or partial intercalative in character. The literature is limited by reliance on calf thymus DNA, unstandardised conditions and little cellular follow-up. This review reports no new experimental data.

1. INTRODUCTION

Isatin (1H-indole-2,3-dione) was first obtained in the nineteenth century by oxidation of indigo, and it has stayed in the chemical literature ever since [1, 2]. It is an orange-red solid that also occurs in mammals, where it has been identified as an endogenous inhibitor of monoamine oxidase and is present in tissue and body fluids at low concentrations [3, 4]. Its derivatives have been reported to show antibacterial, antiviral, anticonvulsant and cytotoxic activity, and the 2005 and 2009 reviews of this activity are still much cited [5, 6]. A good part of the interest comes from the fact that the molecule is small, inexpensive and reactive at more than one position.

The C-3 carbonyl is the most electrophilic centre. It reacts with primary aromatic and aliphatic amines, hydrazines and thiosemicarbazides to give 3-imino derivatives, which are Schiff bases in the sense first described by Hugo Schiff in 1864 [2, 7]. The azomethine nitrogen has a lone pair that is available for coordination, and when the amine carries an additional donor group, such as a phenolic hydroxyl, a thione sulphur or a heterocyclic nitrogen, the ligand becomes bidentate or tridentate. Metal chelation then changes the geometry, lipophilicity and redox behaviour of the compound [8, 9].

Why combine a Schiff base with a metal at all? Three arguments are usually offered. Coordination can lock a flexible ligand into a rigid, more planar shape, which favours stacking against nucleobases. It can raise lipophilicity by neutralising polar donor groups, helping the compound cross membranes. And it can introduce a redox-active centre that the organic ligand lacks, as with copper(II) and the Cu(II)/Cu(I) couple [10, 11, 12]. All three arguments are reasonable, and none is guaranteed to hold for a given compound, which is why the evidence has to be examined case by case. Non-platinum metal drugs have been reviewed repeatedly for exactly this reason, with ruthenium, gold, copper and others offering mechanisms distinct from cisplatin [12, 13, 14].

This review concentrates on the synthesis and structural characterisation of metal complexes of isatin-derived Schiff bases, and on the studies that examine their binding to DNA, which is the most commonly used model for interaction with a biological target. Section headings follow the order in which the work is normally done. Representative findings are collected in table 1, and the spread of published studies across the main topics is shown in fig. 1. The aim is to judge what the evidence actually shows rather than to catalogue every compound, and no new experiments are reported [5, 15, 16].

2. SYNTHESIS

The standard procedure for the ligands is short. Isatin and the amine, in equimolar amounts, are dissolved in hot ethanol or methanol, a few drops of glacial acetic acid are added, and the mixture is refluxed for between two and ten hours. The Schiff base usually separates as a coloured solid on cooling and is recrystallised from ethanol or a mixture with dimethylformamide [2, 5]. Microwave irradiation and solvent-free grinding have been described as quicker alternatives. Water is lost in the condensation, so the reaction is reversible, and the imine bond in these compounds can hydrolyse during recrystallisation if wet solvents are used, a point sometimes overlooked in the literature [2, 9].

Complexation is equally simple in outline. A hot ethanolic solution of the ligand is mixed with a metal chloride, nitrate, acetate or perchlorate in a ligand-to-metal ratio of 1:1 or, more usually, 2:1, and the solution is refluxed for several hours. A base such as sodium acetate or triethylamine is added when the ligand has to be deprotonated, for example when a phenolic or amide proton is lost on binding. The solid complex is filtered, washed with ethanol and ether, and dried in a desiccator [16, 17]. Yields are commonly moderate to good. Counter-ions can be coordinated or free, and deciding which is the case is a major part of the characterisation.

The choice of the amine component largely decides the denticity. Aniline gives a ligand that can only bind through the azomethine nitrogen and possibly the C-2 carbonyl oxygen, forming a five-membered chelate ring. 2-Aminophenol, 2-aminothiophenol and thiosemicarbazide introduce a third donor atom, and many of the complexes studied for biological activity use this strategy [9, 14, 16]. Isatin itself is also ambident: the lactam N-H can be deprotonated, and N-substituted isatins such as N-methyl or N-benzyl derivatives are used when coordination through the ring nitrogen has to be suppressed. Each of these options leads to a different shape and charge, which in turn affects how the complex later interacts with DNA [8, 15].

Tautomerism complicates matters when the amine part is a thiosemicarbazide or a hydrazide. Such ligands can exist as thione or thiol forms (and amide or iminol forms), and

on coordination the ligand often reacts as the deprotonated thiolate, binding through the azomethine nitrogen and the sulphur. Infrared spectra support the assignment when the thioamide N-H and C=S bands change or disappear and a new C-S band appears. The consequence is that the formula written for the ligand is not always the formula of the bound species, a detail that can cause confusion when comparing papers from different groups [9, 16, 18]. Careful authors write the deprotonated form explicitly and give the charge balance.

Table 1: Representative findings on isatin Schiff bases and their metal complexes

Topic|system	Reported finding or feature	Ref.
Ligand preparation acid-catalysed condensation	Isatin condenses with primary amines in refluxing ethanol with a few drops of acetic acid to give 3-imino derivatives.	[2, 5, 7]
Complex preparation first-row metals	Ethanolic metal salts react with ligand at 1:1 or 2:1 ratios, with base added when deprotonation is needed.	[16, 17]
Conductivity DMF solution	Low molar conductivity in DMF indicates neutral complexes with coordinated anions.	[19]
Infrared spectra azomethine band	Shift of the C=N stretch to lower frequency signals coordination through the imine nitrogen.	[16, 18]
Magnetic moments geometry	Moments and d-d bands suggest square planar, square pyramidal or octahedral geometries.	[20, 21]
Absorption titration calf thymus DNA	Hypochromism or hyperchromism with K_b commonly of the order of 10^4 to 10^6 M^{-1}.	[15, 22]
Ethidium displacement fluorescence	Quenching of the DNA-ethidium emission is taken as evidence of competitive binding.	[15, 23]
Plasmid cleavage copper complexes	Conversion of supercoiled to nicked DNA, often with oxidant and reactive oxygen species involved.	[11, 24]
Antimicrobial tests chelation theory	Complexes often outperform free ligands, explained by increased lipophilicity.	[9, 17, 25]
Cytotoxicity cancer cell lines	Micromolar IC_{50} values reported, generally weaker than cisplatin.	[6, 26, 27]

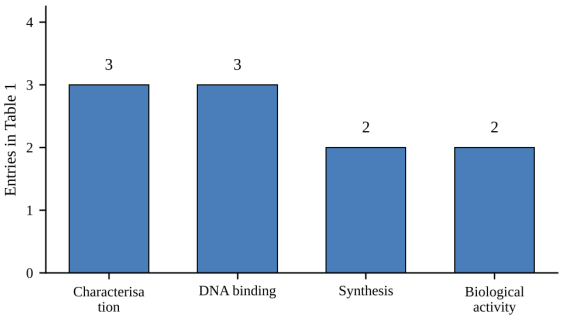


Fig. 1: Number of entries in table 1 by topic area

Some groups prepare mixed-ligand complexes, in which a second chelating ligand such as 1,10-phenanthroline or 2,2'-bipyridine occupies part of the coordination sphere. The reason is largely practical. Planar heteroaromatic co-ligands favour intercalation between base pairs, as shown by the classical work on ruthenium polypyridyl complexes, and so mixed-ligand designs aim to add an intercalating surface to a Schiff base that is itself only weakly planar [28, 29, 30]. Whether the strategy works for isatin-based compounds depends on how much of the binding is attributable to the phenanthroline unit alone, a control experiment that is not always run.

3. STRUCTURAL CHARACTERISATION

Elemental analysis and molar conductivity come first. Conductivity measured in dimethylformamide or dimethyl sulphoxide at about 10^{-3} M concentration distinguishes neutral complexes, which show low values, from 1:1 or 1:2 electrolytes using ranges tabulated by Geary [19]. Many reported isatin Schiff base complexes behave as non-electrolytes, which is taken to mean that chloride or acetate ions are coordinated. Measurements in coordinating solvents have to be handled carefully, because ligand exchange can occur over hours, and a value taken immediately after dissolution may not match one measured the next day [19, 20].

Infrared spectra provide the clearest evidence of coordination. The azomethine stretch of the

free Schiff base lies near 1600 to 1620 cm^{-1} and typically moves by 10 to 25 cm^{-1} in the complexes, usually to lower frequency, indicating donation from the imine nitrogen [16, 18]. The lactam carbonyl band near 1730 cm^{-1} also shifts if the oxygen is involved, and a broad phenolic O-H band disappears when the oxygen is deprotonated. New weak bands in the far infrared, roughly 400 to 600 cm^{-1}, are assigned to metal-nitrogen and metal-oxygen stretches. These assignments rest on comparison with reference compounds and are best treated as supporting rather than decisive evidence [18].

Ultraviolet and visible spectra of the ligands show intense bands below 350 nm assigned to $\pi \rightarrow \pi^*$ transitions in the aromatic and isatin rings, and weaker $n \rightarrow \pi^*$ bands of the carbonyl and azomethine groups at longer wavelength. On complexation these bands shift, and new bands from ligand-to-metal charge transfer may appear around 380 to 450 nm, giving the complexes their typical orange, brown or green colours. The weak d-d bands are found at lower energy and have low extinction coefficients, which makes them easy to miss in dilute solutions [19, 20, 21]. Solid-state diffuse reflectance spectra are sometimes used for this reason and avoid solvent effects on the coordination sphere.

Electronic spectra and magnetic moments are used to propose a geometry. Copper(II) complexes show a broad d-d band in the visible region, together with a magnetic moment close to the spin-only value for one unpaired electron, and the band position is read as indicating square planar, square pyramidal or distorted octahedral coordination [21]. Nickel(II) complexes with moments near 3 BM are usually assigned as octahedral and those that are diamagnetic as square planar. Cobalt(II) values above 4 BM suggest high-spin octahedral or tetrahedral species, and zinc(II) complexes, being d^{10}, are diamagnetic and colourless apart from ligand bands [20, 21]. Electron paramagnetic resonance spectra, when recorded, add detail on the copper environment.

The weakness of this proposal-by-spectroscopy approach is that several geometries can give similar data. A band that suits an octahedral nickel(II) ion may also be consistent with a tetrahedral arrangement in solution, and the tabulated ranges used for assignment overlap [20, 21]. Single-crystal X-ray structures settle the question but are available for only a minority of the complexes in the literature. Where crystals have been obtained, they often reveal features missed by indirect methods, such as bridging chlorides, solvent molecules in the coordination sphere or dimers. Readers should therefore view geometries based on spectra alone as working hypotheses [18, 21].

Thermal analysis, mass spectrometry and, for diamagnetic compounds, nuclear magnetic resonance provide further clues. Thermogravimetric curves can show loss of lattice or coordinated water at different temperatures, which helps to decide how water is held, and mass spectra can confirm the metal-to-ligand ratio. In the proton NMR spectra of zinc complexes, the azomethine and aromatic signals commonly move downfield on coordination, and the lactam N-H signal near 11 ppm disappears if the nitrogen is deprotonated [16, 18]. Magnetic susceptibility is measured by the Gouy or Evans method, and corrections for diamagnetism of the ligands are applied before the effective moment is quoted. The size of this correction is not always given.

4. DNA-BINDING STUDIES

Why DNA? Several clinically used metal and organic agents act on it, from cisplatin to doxorubicin and bleomycin, and a molecule that can bind or cleave DNA is a plausible lead for anticancer or antimicrobial work [31, 32]. Metal complexes interact in three main ways: electrostatic binding to the phosphate backbone, groove binding, and intercalation of a planar aromatic unit between adjacent base pairs, with covalent coordination to a base nitrogen as a fourth route for labile complexes [15, 30]. Most isatin Schiff base complexes are cationic and moderately planar, so groove binding and partial intercalation are the most frequently proposed modes.

Electronic absorption titration is the entry-level experiment. A fixed concentration of complex is titrated with increasing amounts of calf thymus DNA, and changes in

the intraligand band are followed. Hypochromism with a small red shift is generally read as intercalation or strong stacking interaction, whereas hyperchromism is attributed to groove binding or partial unwinding of the helix. An intrinsic binding constant (K_b) is then calculated from the decrease in absorbance using the Wolfe-Shimer-Meehan equation [15, 22]. Published values for isatin-based compounds commonly fall in the range of 10^4 to 10^6 M^{-1}, which is lower than for classical intercalators such as ethidium bromide and the ruthenium polypyridyl complexes [15, 22, 28, 29].

Fluorescence quenching adds a competitive element. Ethidium bromide fluoresces strongly when intercalated in DNA, and a complex that displaces it or interacts with the DNA-dye adduct reduces the emission. Stern-Volmer plots give a quenching constant, which is often presented as supporting intercalation [15, 23]. The inference is not watertight, since quenching can also occur by direct interaction between the complex and the dye or by changes in the local environment of the DNA without true displacement. Viscosity measurements are therefore regarded as a more discriminating test, because intercalation lengthens the helix and raises the relative viscosity of a sonicated DNA solution, whereas groove binders cause little change [15, 30].

Cleavage experiments ask a different question: can the complex damage DNA? Supercoiled plasmid DNA, most often pBR322 or pUC19, is incubated with the complex, with or without an oxidant such as hydrogen peroxide or a reductant such as ascorbate, and the conversion of supercoiled to nicked and linear forms is followed on an agarose gel. Copper complexes in particular have been reported to cleave DNA through reactive oxygen species, and scavenger experiments with DMSO, azide or superoxide dismutase are used to identify the species involved [11, 24, 33]. Chemical nucleases of this kind are of interest for therapeutic design, but cleavage at micromolar concentration in a test tube does not predict what would happen inside a cell [15, 24].

Thermal denaturation and circular dichroism provide additional checks that are used less often. Intercalators stabilise the double helix and raise its melting temperature by several degrees, while groove binders and electrostatic binders have a smaller effect. Circular dichroism spectra of DNA, with a positive band near 275 nm and a negative band near 245 nm, change in intensity when a ligand perturbs base stacking or helicity, and induced bands appear when an achiral complex binds in a chiral environment [24, 30]. Measuring these properties alongside absorption titration helps to separate genuine intercalation from weaker association, and reports that include them are more persuasive than those that rely on a single method [24, 34].

These four experiments give a consistent picture for the better-characterised complexes: moderate affinity, a mix of groove and partial intercalative binding, and oxidative cleavage when a redox-active copper centre is present, as summarised in table 1 [15, 22, 24]. Several practical cautions apply. Experiments are normally performed in Tris buffer at low ionic strength, which favours electrostatic binding. Calf thymus DNA is a mixture of sequences and cannot reveal any base-pair selectivity. The purity of the DNA, assessed by the ratio of absorbance at 260 and 280 nm, should exceed about 1.8 for protein-free material, but this check is not always described [33, 34].

5. BIOLOGICAL ACTIVITY

Antimicrobial testing is the most common biological application. Isatin Schiff bases and their complexes have been tested by disc diffusion or broth dilution against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*, with the complexes frequently reported as more active than the free ligands [9, 14, 17]. The explanation usually offered is Tweedy's chelation theory, which proposes that coordination reduces the polarity of the metal ion through partial sharing of its charge with the donor atoms, increasing lipophilicity and permeation through the lipid membrane of the organism [17, 25]. The theory is appealing, although it has been tested directly in very few studies.

Cytotoxicity against cancer cell lines is the second major theme. Isatin derivatives themselves inhibit kinases, tubulin polymerisation and caspase pathways, and metal complexation is expected to add DNA-directed or redox effects [6, 26]. Copper complexes are favoured because copper is an essential metal with its own cellular handling, and a number of copper compounds can generate reactive oxygen species through Fenton-type chemistry [11, 26]. Reported cell data for isatin-derived complexes include IC_{50} values in the micromolar range against breast, cervical and liver lines, usually higher than cisplatin, with the exact figures depending on the assay [6, 27].

Does DNA binding explain the cell data? Sometimes it fits, but the link is rarely tested. A complex with high affinity for DNA in a cell-free assay may be excluded by the cell membrane, bound by serum albumin, reduced inside the cell, or exchanged for other ligands in the medium. In the opposite direction, complexes with weak DNA binding may kill cells through mitochondrial damage or protein targets [11, 27, 32]. A convincing programme would therefore combine uptake measurements, subcellular distribution, and mechanistic assays for apoptosis, rather than assume that the in vitro binding constant is predictive [26, 27].

Selectivity and tolerability are the questions that decide whether a cytotoxic complex ever progresses. Copper is tightly regulated in mammalian cells by chaperones and transporters, and an excess of redox-active copper can harm healthy tissue as well as tumour cells. Ruthenium complexes have been proposed to be better tolerated because they mimic iron in transferrin binding, though this remains a hypothesis supported by a limited set of clinical candidates [12, 13, 31]. For isatin-derived compounds, very few studies measure activity in non-malignant cells, so statements of selectivity are mostly speculative [6, 14].

6. LIMITATIONS AND FUTURE DIRECTIONS

The main limitation is methodological. Published studies use different buffers, DNA sources, concentrations and ways of calculating binding constants, so values cannot be compared between laboratories with confidence. Many structures are inferred rather than determined, and purity data for the complexes are limited to elemental analysis. Controls are inconsistent: free ligand, free metal salt and an established intercalator should be tested in parallel, yet the first two are often missing [15, 20, 22]. Stability under assay conditions deserves more attention, because Schiff base complexes may hydrolyse in aqueous buffer, giving rise to isatin and the free amine as the actual active species [2, 9].

Several improvements could make the field more useful. Crystal structures, or at least solution evidence of stability such as spectra recorded over 24 hours, should accompany geometry claims. Binding should be examined with defined oligonucleotides, including G-quadruplex and mismatched sequences, and with circular dichroism and isothermal titration calorimetry in addition to absorbance [15, 30]. Cell work should include non-cancerous lines to give selectivity indices, and cellular copper or ruthenium measurements should be reported where metal uptake is part of the argument. These steps are routine in the better inorganic medicinal chemistry laboratories and would place isatin-based work on firmer footing [11, 26, 27].

Reporting standards could be tightened at modest cost. A checklist might include the ligand-to-metal ratio and its evidence, the nature of counter-ions, the purity of the DNA, the buffer, the final percentage of organic solvent, and the stability of the complex in the assay medium. Raw titration curves should be shown in supplementary material, with the equation used to derive binding constants stated explicitly [15, 22, 34]. Such details seem trivial, yet their absence is the main reason that published constants cannot be compared. Journals and reviewers are well placed to ask for them [15, 20].

7. CONCLUSION

The literature on isatin Schiff base complexes shows a reliable chemistry and a patchier biology. The ligands are made in a single

step, the complexes by direct reaction with metal salts, and the spectroscopic toolkit of infrared, electronic, conductivity and magnetic measurements gives a coherent if sometimes provisional picture of structure [2, 16, 18, 19, 20]. DNA-binding experiments, particularly absorption titration, ethidium displacement, viscosity and plasmid cleavage, are well established and widely reproduced in method [15, 22, 23].

What they tell us is more limited than the papers often suggest. Reported affinities are modest, binding modes are inferred from indirect data, and the cellular relevance of cell-free binding is seldom tested [11, 15, 26]. Progress will depend on better controls, crystal structures where possible, evidence of stability in buffer, and cell data that go beyond a single IC_{50} value. Researchers in this area who adopt those habits will produce results that others can use and compare [27, 30, 32].

8. ABBREVIATIONS

DMF-Dimethylformamide, DMSO-Dimethyl sulphoxide, K_b-Intrinsic DNA-binding constant, IC_{50}-Half-maximal inhibitory concentration, NMR-Nuclear magnetic resonance, MAO-Monoamine oxidase, HIV-Human immunodeficiency virus, BM-Bohr magneton, pBR322-Bacterial plasmid used in cleavage assays

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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