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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ISATIN DERIVATIVES OF Cu (II), Co(II), Cd(II) AND Au(III) COMPLEXES ARE POTENTIAL ANTICANCER DRUGS

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ABSTRACT

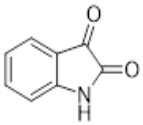
Bio-inorganic chemistry is a thriving field of drug research for cancer treatment. Transitional metal complexes coordinated to essential biological scaffolds represent a highly promising class of compounds for design of novel target specific therapeutics1. The biological evaluation of novel Isatin Anthranilic acid schiff base**(amphoteric)** derivatives and its metal complexes in several tumor cell lines by assessing their effects on cellular metabolism, real-time cell proliferation and induction of apoptosis is reported in this study3,4. These derivatives demonstrate a diverse array of biological and pharmacological activities including anti-bacterial, anti-fungal, and anti-cancer properties. This broad spectrum of biochemical targets has been facilitated by the synthetic versatility of isatin, which has allowed the generation of a large number of structurally diverse derivatives from nitrogen and C_2 / C_3 carbonyl moieties. The C_3 isatin anthranilic acid schiff base derivatives are a multitargeted receptor tyrosine kinase inhibitor. The main objective of the study was to synthesize a novel series of 3-substituted isatin anthranilic acid metal complexes by reacting suitable compounds and systematically evaluate the biological activities such as cytotoxic and anticancer properties of various substituted analogs of Cu(II), Co(II), Cd(II) and Au(III) complexes of isatin against various cancer cell lines have been performed. The purity was checked by TLC by using silica gel-Gas stationary phase. The structure of synthesized compounds were elucidated by using Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400MHz) FT- NMR using DMSO- d6 as a solvent and TMS as an internal standard. Some isatin derivatives of Au(III)complexes are isoelectronic with platinum. These findings are a promising class of cytotoxic agents in the cancer treatment and mode of action studies in medicinal chemistry.

KEYWORDS: Isatin anthranilic acid schiff base, organometallic, cytotoxic, anticancer.

INTRODUCTION

Isatin is found in the plant of Genus Isatis(phytochemical) and in humans as a metabolic derivative of adrenaline. In cancer treatment metal chelates are more potent than the chelating agents.[1,2] The isatin molecule or 1H-indole-2,3-dione is an indole derivative containing the keto group at position 2 and 3 of the ring. It is a versatile chemical building block, able to form a large number of heterocyclic molecules.

Discovery of metal complexes as anticancer such as cisplatin6, led to a dramatic shift of focus in organometallic compounds and their chelates as novel compounds for drug discovery and development. Enormous interest has emerged for the development of various platinum-based analogs, since the serendipitous discovery of cisplatin



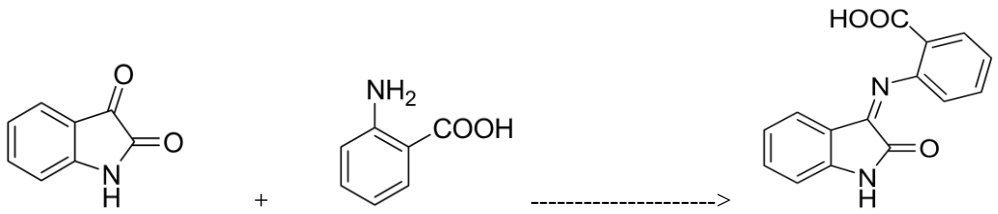
structure of Isatin(1H-Indole 2,3 dione).

Isatin is able to participate in a broad range of synthetic reactions, leading to its extensive use as a precursor molecule in medicinal chemistry.[1] The keto group at position C $_2$ and particularly little variation at position C_3 is nucleophile attacking site, it enters into a condensation reaction and produces different degrees of biological activities[5,6], like antimicrobial, antioxidant, anticancer activity.[2] This large number of heterocyclic compounds are raw material for drug synthesis. Some new isatin derivatives have been found to block cell cycle progression in a variety of malignant cell lines including those derived from the prostate, brain, breast, pancreas and colon. Some indole or new isatin derivatives like Vincristine and Vinblastine are mainly useful for treating Hodgkin's disease[3], lymphocytic lymphoma, histiocytic lymphoma, advanced testicular cancer, advanced breast cancer and exhibit a broad spectrum of biological activity. They are active against variola, influenza viruses and some of these derivatives exhibit antioxidant, anticancer, antibacterial, and anti-tuberculous activity.

METHODOLOGY

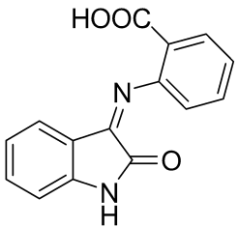
All the reagents used in the preparation of ligands and their metal complexes were of reagent grade(Merck). The solvents used for the synthesis of ligands and metal complexes were distilled before use. All other chemicals were of AR grade and used without further purification.

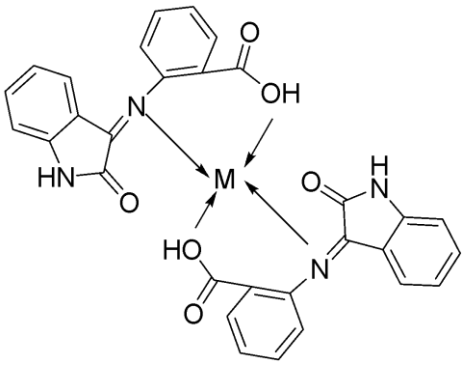
a) Synthesis of isatin ligands: A $1x10^{-3}$M solution was prepared by dissolving appropriate amounts of Anthranilic acid(amphoteric)in 50mL.methanol and 2mL.of glacial acetic acid was added drop wise to a $5x10^{-2}$M solution of Isatin in 50mL. Methanol while stirring and refluxed for 1 hour and the product that separated was recrystallized in methanol. Identification of the product was based on elemental analysis, viz FT-IR, H NMR, UV methods.



Isatin + Anthranilic acid ----------> Ligand

b) Synthesis of isatin metal complexes: To 50mL.of metal Au(III)/Cu(II) solution($5x10^{-2}$M) in methanol was added($1x10^{-2}$M) ligand in methanol and the mixture refluxed for about 1hour in a separate reflux arrangement. The solid that separated was filtered and washed with water and recrystallized with methanol. Identification of the product was based on elemental analysis, viz FT-IR, H NMR methods.





Ligand + $AuCl_3$ / $CuSO_4$ ----------> Isatin Metal complex

H+ / reflux

1hr

RESULTS AND DISCUSSION

To reduce the potential side effect and maximize efficiency, it is important to retain the target specificity of small molecule therapeutics. In the last decade, the Ubiquitin-proteasome system(UPS) and its components have emerged as key targets for the development of novel small molecule therapeutics. The drug discovery research that targets E_3 Ubiquitin ligases, enzymes conferring the substrate specificity to the UPS, gains a lot of interest due to the paramount role of these enzymes in a wide range of biological processes and human diseases, including cancer and auto-immune disorders. There are numerous examples of Cu(II), Pt(II), and Au(III)complexes as well as Isatin derivatives acting as anti-tumor therapeutics that target various components of UPS, mainly the proteasome and E_3 Ubiquitin ligases.

Biological Activity of Isatin Metal Complex (IMC) derivatives

(a) IMC as antioxidant activity: A new group of Isatin schiff bases synthesized by Isatin and anthranilic acid are evaluated for antioxidant activity. The synthesized compounds showed most effective antioxidant and antimicrobial activity against DPPH and H_2O_2 scavenging activity. The total antioxidant capacity by a phosphomolybdenum assay and their ability to chelate ferrous iron. The derivatives were found to exhibit antioxidant activity.

(b) IMC as antitubercular agents: The reported group of Isatin derivatives are a versatile lead molecule for designing of potential antitubercular agents6. Some of these derivatives are natural products. The Isatin schiff base was investigated against four mycobacterium strains: Mycobacterium intercellulari, Mycobacterium Xenopi, Mycobacterium Cheleneo and Mycobacterium Smegmatis. Modest anti-TB activity was observed with the investigated compounds.

(c) IMC as antibacterial and antifungal agents: The antimicrobial activity of the synthesized compounds was evaluated by tube dilution method5. The synthesized compounds showed better antibacterial activity than the reference drugs. These compounds have been screened for their antibacterial activity against the Gram-positive and Gram-negative bacteria**.** These schiff bases have also been reported to possess antibacterial activity. In this study, we use twenty eight bacteria of clinical interest. The isatin gold complex had notably more results.

(d) IMC as anticancer agents: The group of compounds showed antioxidant activity and was cytotoxic to the HL60 cells due to induction of apoptosis11. The isatin can be used as a prophylactic agent to prevent the free radical induced cancer and as a chemotherapeutic agent to kill the cancer cells. Some of the synthesized compounds was assessed in the MCF-7 human cancer cell line.

In my study, it is clear that the Au(III)complex of Isatin anthranilic acid exhibited comparatively more cytotoxic activity against the two cell lines reports. It is more susceptible with a CTC 50 value of 49.2 - 56.5 mg/mL of solution. The other compounds showed less activity, these isatin derivatives are cytotoxic in nature and may possess antitumor activity.

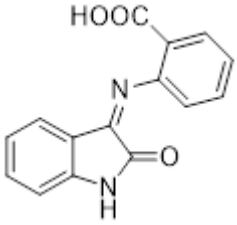
(e) IMC as anticonvulsants: Almost all isatin compounds have been reported to possess anticonvulsant activity8,9. isatin Schiff bases were prepared by reacting Isatin with Anthranilic acid. The metal complexes of isatin evaluated for anticonvulsant and neurotoxic properties. The Gold(III) complex of isatin is the most active analogue showing better activity than others. Three compounds of the group exhibited significant anticonvulsant activity.

Physical properties of Ligand, metal complexes of Isatin.

Sl.NO	Compound	Colour	M.P^{O}C	Yield %	Spectra UV- RANGE
1	Isatin -I	Orange	194	78	421
2	N-MethylIsatin -Me	Yellow	232	76	416
3	N-BenzylIsatin -B	Yellow	245	71	407
4	I Anthranilic acid -A	Yellow	249	74	412
5	IA_2 Cu	Green	275	85	429
6	IA_2 Cd	Yellow	248	82	422
7	IA_2 Zn	Yellow	254	96	288
8	IA Ni	Yellow	275	85	408
9	IA Co	Brown	262	85	407
10	IA_2 Au	Green	278	81	410

IR(KBr, Vmax Cm-1) Spectral analysis for ligand

3190(N-H), 1740&1620(C=O), stretching frequencies and Isatin Anthranilic acid schiff base have shown IR spectral frequencies at 3473Cm-1(C-OH), 3373(N-H), 1728&1616(C=O) and 1604(C=N)

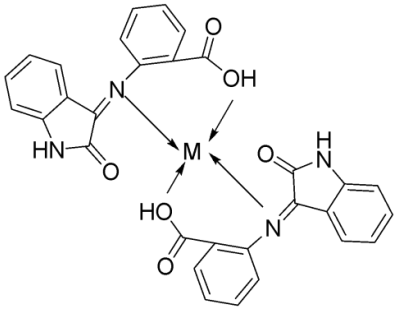


structure of ligand(IA)

IR(KBr,Vmax Cm-1) Spectral analysis for metal complexes:

In this analysis, 3450-3055(N-H), 1249-1242(C=O), 547-501(M-N) the decrease in the -NH2 frequency of anisidine and disappearance of Isatin -NH frequencies indicated that they may involved in bonding with metal atom. The bonding was proved by M-N stretching.

IR spectral values range from 3394-3271Cm-1(C-OH), 1581-1458Cm-1(C=N). The decrease in the C-OH frequency and C=N frequencies indicated that they may be involved in bonding with metal atoms. We observed that irrespective of the anionic counterpart of metal salts both the complexes involved the bonding of C-OH group, nitrogen atom of C=N with metal atom. Further the bonding was proved by M-O stretching frequency range within 455-424Cm-1 and M-N stretching frequency range from 524-478Cm-1. Hence the complex may have the structure



IR Spectral data of Isatin-Anthranilic acid Schiff base and metal complexes.

Sl.No	Ligand/complexes	Vmax,cm-1 (C-OH)	Vmax,cm-1(N-H)	Vmax,cm-1(C=O)	Vmax,cm-1(C=N)	Vmax,cm-1(M-O)	Vmax,cm-1(M-N)
1	Isatin I	--	3300	1674	1510	--	--
2	N-Me Isatin Me	--	3245	1672	1508	--	--
3	N-B Isatin B	--	3222	1655	1541	--	--
4	IA	3473.2	3373.0	1722(W) 1620(b)	1604	--	--
5	IA_2Cu	3270.5	3114.2	1722(W) 1611(b)	1579.2	422.80	477.80
6	IA Cu	3272.4	3114.0	1708(W) 1606(b)	1548.0	422.3	477.80
7	IA_2Zn	3303.2	3112.0	1601(W) 1592(b)	1456.9	418.2	518.2
8	IA Zn	3303.2	3178.0	1722(W) 1620(b)	1541.4	418.6	458.8
9	IA Ni	3301.4	3204.4	1722(W) 1606(b)	1540.2	456.0	477.0
10	IA Co	3384.0	3102	1722(W) 1606(b)	1540.4	455.0	476.0
11	IA Cd	3303.2	3178.0	1708(W) 1620(b)	1541	420.6	518.0
12	IA_2Au	3268.2	3108.2	1684(W) 1608(b)	1536	418.2	468.2

Methodology - General preparation for in vitro cytotoxic activity:

Each synthesized compounds were separately dissolved in distilled dimethyl sulphoxide and the volume was made upto 10mL with Dulbecco's Modified Eagle's medium(DMEM), pH 7.4 Hep-2(caucasian male larynx epithelium carcinoma) cell line was obtained from the laila impex, vijayawada. Dalton's lymphoma ascites(DLA) cells were obtained from Visakha Institute of Medical Science, visakhapatnam were propagated in DMEM, pH 7.4 supplemented with 10% inactivated new born calf serum, penicillin(100 IU/mL), Streptomycin(100 µg/mL) and amphotericin B (5µg/mL) to maintained in a humidified atmosphere of 5% CO_2 at 37 o C until confluent. The cells were dissociated with 0,2% Trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock was grown in 25 cm2 tissue culture flasks and all cytotoxicity experiments were carried out in 60 well microtiter plates. DLA cells used were propagated and maintained in the peritoneal cavity of swiss albino rats. Cell lines in exponential growth phase were washed, trypsinized and resuspended DMEM medium with 10% inactivated newborn calf serum. Cells were plated at 10000cells/well in 60 well microtiter plates and incubated for 24 hour at 37^{o}C, 5% CO_2 in a humidified atmosphere during which period a partial monolayer was formed. The cells were then exposed to different concentrations (1000 - 15.6µg/mL) prepared by serial dilution using maintenance medium from the stock solution. Control wells received only maintenance medium, the cells were incubated at 37oC in a humidified incubator with 5% CO_2 for a period of 72 hours. Morphological changes of cell cultures were examined using an inverted tissue culture microscope at 24 hours time intervals and compared with the control. At the end of 72 hours cellular viability was determined using standard MTT and SRB assays. The CTC50 value was calculated.

Cytotoxic Activity of Isatin derivatives on different cell lines by MTT and SRB assays

Compound	CTC 50 (µg/mL)			
	Hep -2		DLA	
	MTT	SRB	MTT	SRB
Isatin	494.17 ± 14.63	446.27 ± 11.42	494.17 ± 14.63	450.56 ± 2.67
IA	242.44 ± 5.20	438.40 ± 14.20	242.44 ± 5.20	512.00 ± 12.42
IA_2Cu	142.44 ± 4.42	426.44 ± 8.24	140.22 ± 4.42	247.44 ± 6.88
IA_2Zn	494.74 ± 12.26	424.24 ± 8.64	494.74 ± 6.24	446.24 ± 2.64
IANi	142.44 ± 4.48	224.62 ± 6.24	142.44 ± 4.48	242.28 ± 8.24

IACo	138.22 ± 2.24	234.42 ± 6.44	138.2 ± 2.24	232.48 ± 6.44
IA_2Cd	152.28 ± 4.82	246.42 ± 8.58	152.28 ± 4.82	244.24 ± 8.54
IA_2Au	49.8 ± 5.22	121.14 ± 6.42	122.46 ± 2.84	218.20 ± 4.42

Average of all independent determination, values are ± SEM.

Antimicrobial Activity of Isatin A schiff base metal complexes

slno	Organisms	Isatin ligand Metal complex zone of Inhibition(mm)							
		IA_2Cd	IA_2Cu	IACu	IA_2Zn	IAZn	IANi	IACo	IA_2Au
1	Staphylococcus aureus	A	A	NA	A	NA	NA	A	A
2	Bacillus subtilis	NA	NA	NA	NA	NA	NA	NA	NA
3	Escherichia coli	NA	A	NA	NA	A	A	NA	A
4	Pseudomonas aeruginosa	A	NA	NA	NA	NA	NA	NA	NA
5	Salmonella typhi "H"	NA	NA	NA	NA	NA	NA	NA	NA
6	Micrococcus SP	A	A	NA	A	NA	NA	NA	A

CONCLUSION

Isatin anthranilic acid derivatives of metal complexes showed diverse pharmacological activities including anticonvulsant, anticancer, antiviral, antibacterial, antifungal, antitubercular, antioxidant etc., of the all synthesized complexes, the Au(III)complex of Isatin anthranilic acid exhibited comparatively more cytotoxic activity against the two cell lines reports.It is more susceptible with a CTC 50 value of 49.2 - 56.5 mg/mL of solution. The other compounds showed less activity, these Isatin derivatives are cytotoxic in nature and may possess antitumor activity.

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