ORIGINAL ARTICLE

Synthesis and Evaluation of Coumarin Metal Complexes for their Potential Activity against Lung Cancer

P. M. Ronad, A. A. Ankalikar, Akshata Patil, Thehasis Sheikh

Department of Pharm-Chemistry, KLE College of Pharmacy, Hubli, Karnataka, India

Abstract

Background: The most prevalent and deadliest cancers around world are lung cancer, primarily due to tobacco smoking, exposure to radiations, air pollution, and genetic predisposition. The classic compounds available for its treatment include platinum-based compounds, folic acid analogues, microtubule inhibitors, high molecular weight monoclonal antibodies, and aromatic heterocycles. However, resistance development, severe toxicity, poor tumor selectivity, and hypersensitivity reactions account for some of the major drawback of these classes of anti-cancer drugs. **Objectives:** Designing safer and novel Schiff base calcium metal complexes of coumarin quantify their cytotoxic property. **Methodology:** Five calcium metal complexes of schiff bases of coumarin derivatives were synthesized and they were screened for their *in vitro* biological activities. Using methods such as elemental analysis and spectroscopy, the structures of the synthesized compounds were confirmed. These novel compounds were evaluated for their antineoplastic potency against the recognized gold standard, cisplatin. The *in vitro* cytotoxicity of the complexes against the lung cancer cell line A549 was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test. **Results and Conclusion:** The metal complex MC1 demonstrated excellent cytotoxicity on A549, IC_{50} value of 73.14 µg/mL was seen. Further subjecting this compound to *in vivo* studies could lead us to establishing newer anti-cancer agents defying the drawbacks of the existing ones.

Keywords: Calcium complexes, coumarin derivatives, lung cancer, metal complexes, Schiff bases

INTRODUCTION

In the past few decades, coumarins, a naturally occurring molecule, have been the subject of much phytochemical and pharmacological research. Naturally occurring coumarins can be found in a wide variety of plants, with *Coumarouna odorata* (Tonka bean) (Fabaceae/Leguminosae) having a particularly high concentration. Coumarin was first isolated from tonka beans in 1820 by H. A. Vogel and it was in 1868 W. M. Perkin first time synthesized this class of compound.[1]

The coumarin nucleus is common in synthetic as well as natural compounds showing a broad range of pharmacological activities, including anticoagulant, anti-inflammatory, antioxidant, antiviral, antibacterial, and anticancer medicines, as well as enzyme inhibitors. Contrarily, it has been claimed that adding metal ion to coumarin derivatives can make these complexes more active when compared to coumarin-based ligands, as shown in Figure 1. Consequently, it has been discovered that several of them have promising antioxidant, anticancer, or antibacterial activity.[2]

Chelating compounds with a range of metals in different oxidation states is possible with the numerous pharmacophores referred to as Schiff bases. The significance of metal complexes, in which the metal is coordinated to different ligands, has increased in medicinal chemistry due to their capacity to stabilize the metal and change its chemical and pharmacological properties.[3]

Schiff base complexes are used for designing metal complexes relevant to synthetic and biological oxygen carriers, which can act as stereospecific catalysts for different types of

Address for correspondence:
A. A. Ankalikar, Department of Pharm-Chemistry,
KLE College of Pharmacy, Hubli, Karnataka, India.
Phone: +91-9986659791.
E-mail: aa.ankalikar@gmail.com

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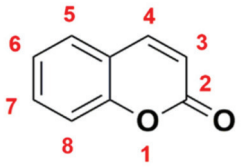


Figure 1: Structure and numbering of coumarin nucleus

reactions such as hydrolysis, oxidation, and reduction, and biological activity. In comparison to molecules with a –C=N– coordinating moiety, organic compounds having –C=N– along other functional groups form more stable complexes. Since coumarin derivatives both natural and synthetic display promising activity, they have drawn a lot of attention. Coumarin containing subunits have shown several biological activities such as molluscicides, anthelmintics, hypnotics, insecticides, anticoagulants, and fluorescent brighteners. Hence, it is anticipated that combining these coumarins with a Schiff bases will have improved biological effects, such as anticancer effects. It is well known that the presence of the active pharmacophore (–CONH–N=C–) is the component that gives hydrazone compounds their biological activity. According to the literature, numerous hydrazone compounds that contained this active moiety had good anticancer bioactivities.[4]

Lung cancer one of the most common and deadliest cancers whose cause is mainly exposure to tobacco smoking exposure to radon and predisposing genetic factors. Lung cancer typically originates as an uncontrolled division of cell tissue presenting squamous cell carcinoma and small cell carcinoma which metasizes aggressively and rapidly. Classic chemical compounds existing for the treatment of lung carcinoma include platinum-based compounds such as cisplatin, heterocyclic analogues which inhibit thymidylate synthase enzyme, and polycyclic diterpenes like paclitaxel. However, severe toxicity, nephrotoxicity, ototoxicity, neurotoxicity development of resistance, and poor tumor selectivity are some of the drawbacks of the existing treatment regimen. Keeping this background in mind the study has been designed to develop novel potent anti-cancer agents.[5-15]

MATERIALS AND METHODS[16-19]

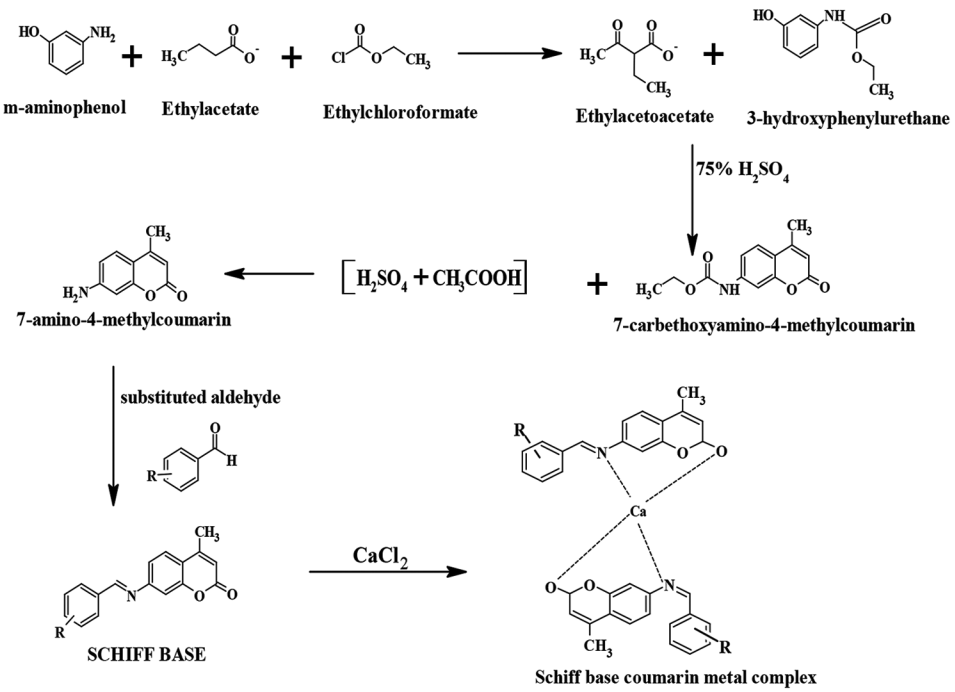
- Chemicals used: Meta-aminophenol, ethyl acetate, ethyl chloroformate, ethanol (99%), conc. sulfuric acid, glacial acetic acid, ethyl acetoacetate, diluted ammonia solution, sodium hydroxide pellets, calcium chloride, and the aromatic aldehydes were obtained from S.D. Fine Chem. Ltd., Mumbai and Sigma Aldrich Co.
- Measurements: Fourier-transform infrared spectra on a Shimadzu spectrophotometer and nuclear magnetic resonance (NMR) spectra on a 400 MHz FT NMR spectrometer. Analytical thin-layer chromatography (TLC) was performed on silica gel while the visualization of TLC plates was performed using a UV lamp.

General procedure for the synthesis of ligands and calcium complexes

The 7-hydroxy-4-methyl-coumarin was produced by the Pechmann condensation technique for which sulfuric acid is imperative to combine m-aminophenol and ethyl acetoacetate which will give 3-hydroxyphenylurethane, m-aminophenol was first treated with ethyl acetate while it was combined with ethyl chloroformate. In the presence of 75% sulfuric acid, this compound was added to with ethyl acetoacetate to generate 7-carbethoxyamino-4-methylcoumarin, as shown in the Scheme 1. To create 7-amino-4-methylcoumarin, the 7-carbethoxyamino-4-methylcoumarin was additionally treated with an equal volume of sulfuric acid and glacial acetic acid. Sodium hydroxide and sodium carbonate were then used to make the mixture alkaline. The 7-amino-4-methylcoumarin was stirred with substituted aldehydes dissolved in 100% alcohol in the presence of acetic anhydride to create the Schiff bases. Calcium chloride can be used to convert the 7-amino-4-methylcoumarin Schiff bases into the appropriate metal complexes.

In vitro anticancer activity (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] assay)

The cells were trypsinized and aspirated into a 15 mL centrifuge tube. To obtain the cell pellet, 300× g of centrifugation was used. The cell count was altered using (Dulbecco's Modified Eagle media) media so that 200 µL of suspension contained roughly 10,000 cells. The 96-well microtiter plate was filled with 200 µL of the cell suspension, and the plate was then incubated for 24 h at 37°C with 5% CO_2. Twenty-four hours later, the used medium was aspirated. The appropriate wells received 200 µL of various test drug concentrations (20, 40, 60, 80, and 100 µg/mL for sample from stock). Following that, the plate was incubated for 24 h at 37°C and 5% CO_2. The plate was removed from the incubator with the drug-containing media aspirated. After adding 100 µL of media containing 10% MTT reagent to each well to reach a final concentration of 0.5 mg/mL, the plate was incubated at 37°C and 5% CO_2 for 3 h. The developed crystals were completely and undisturbed withdrawn from the growth medium. Following the production of formazan, 100 µL of dimethyl sulfoxide, a solubilizing solution, was added, and the plate was gently agitated in a rotary shaker. The absorbance was measured using the microplate reader at wavelengths of 570 nm and 630 nm. The percentage of growth inhibition was calculated after removing the background and the blank, and the dose-response curve for the cell line was utilized to produce the concentration of the test drug needed to inhibit cell growth by 50% (IC_{50}).[20]



Scheme 1: Synthetic route for the preparation of title compounds

RESULTS AND DISCUSSION

As shown in Table 1, it presents the physical data of N-substituted Schiff bases of 7-amino-4-methyl-coumarin (Sb1–Sb5). All synthesized compounds were obtained in good yields ranging from 65.6% to 87.3%. The melting points were sharp and lay between 153 and 163°C, indicating good purity of the compounds. The molecular formulae were consistent with the proposed structures. Thin-layer chromatography showed single spots with Rf values between 0.55 and 0.72, confirming the formation of pure Schiff bases. The nitro-substituted compound Sb3 showed the highest yield, while halogen-substituted derivatives also exhibited satisfactory yields and stability.

Figure 2 represents the general structure of the synthesized N-substituted Schiff bases of 7-amino-4-methyl-coumarin.

Figure 3 shows the general structure of N-substituted Schiff bases of 7-amino-4-methyl-coumarin, confirming the formation of the azomethine (–C=N–) linkage between the amino group of coumarin and substituted aromatic aldehydes. The presence of different substituents (r) on the aromatic ring represents structural variation among the synthesized Schiff bases, which is expected to influence their physicochemical and biological properties.

The IR spectrum of 7-[4-nitrobenzylideneamino]-4-methyl-2H-chromen-2-one confirms the successful formation of the Schiff base. A characteristic absorption band corresponding to the azomethine (C=N) stretching vibration was observed, indicating condensation between the amino group of

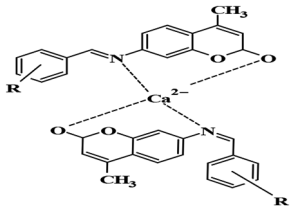


Figure 2: Proposed structure of calcium metal complex

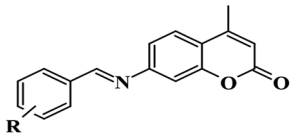


Figure 3: Structure of N-substituted Schiff bases of 7-amino-4-methyl-coumarin

coumarin and the aldehyde. The presence of a strong band due to the lactone carbonyl (C=O) group of the coumarin ring was also evident. In addition, characteristic bands corresponding to the nitro ($–NO_2$) group were observed, supporting the presence of the nitro-substituted aromatic ring. The disappearance of the primary amine ($–NH_2$) stretching band further confirms Schiff base formation, as shown in Figure 4.

Table 2 and Figures 5-7 showed the effect of calcium metal complexes (MC1–MC5) on the viability of A549 lung cancer cells as determined by the MTT assay. All tested complexes exhibited a concentration-dependent decrease in cell viability in the range of 20–100 µg/mL. Among the complexes, MC4 (2,6-dichloro substituted) demonstrated the highest

Table 1: Physical data of N-substituted Schiff bases of 7-amino-4-methyl-coumarin

S. No.	Compound code	R	% Yield	Melting point (℃)	Molecular formula	Rf value
1	Sb1	2-Cl	72.5	160–163	$C_{17}H_{12}ClNO_2$	0.60
2	Sb2	3-Br	65.6	155–157	$C_{17}H_{12}BrNO_2$	0.62
3	Sb3	4-NO_2	87.3	153–155	$C_{17}H_{13}N_2O_4$	0.58
4	Sb4	2,6-Cl_2	84.6	159–162	$C_{17}H_{11}Cl_2NO_2$	0.72
5	Sb5	2,5-$(OCH_3)_2$	77.8	154–156	$C_{19}H_{17}NO_4$	0.55

Table 2: Cell viability (A549) of calcium metal complexes of N-substituted Schiff bases of 7-amino-4-methyl-coumarin by MTT assay

Compound code	R	Concentration (µg/mL)						
		0	15	20	40	60	80	100
MC1	2-Cl	–	–	0.90	0.86	0.75	0.63	0.55
MC2	3-Br	–	–	0.86	0.71	0.62	0.51	0.43
MC3	4-NO_2	–	–	0.89	0.69	0.54	0.42	0.39
MC4	2,6-Cl_2	–	–	0.81	0.69	0.57	0.46	0.34
MC5	2,5-OCH_3	–	–	0.79	0.71	0.62	0.58	0.37
Standard drug (Cisplatin)		–	0.137	–	–	–	–	–
Control			100	–	–	–	–	–

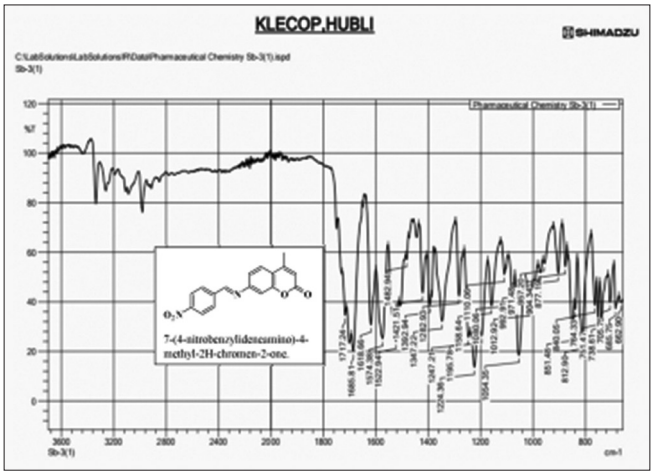


Figure 4: Infrared of 7-[4-nitrobenzylideneamino]-4-methyl-2H-chromen-2-one

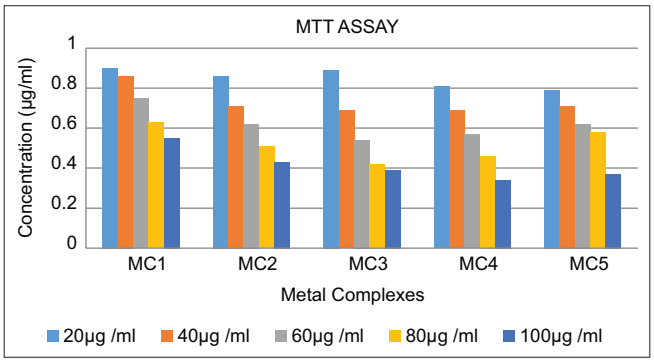


Figure 5: % cell viability results of calcium metal complexes

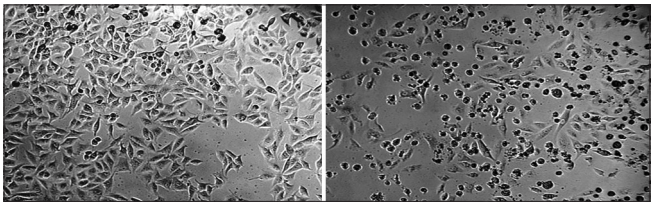


Figure 6: Microphotography of A549 untreated and cisplatin treated, respectively

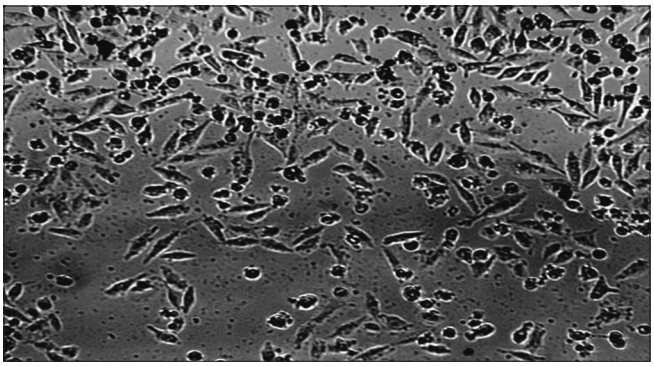


Figure 7: Microphotography of compound MC4 treated A549

cytotoxic activity, reducing cell viability to 34% at 100 µg/mL, followed by MC3 (4-NO_2) and MC5 (2,5-OCH_3). The bromine-substituted complex MC2 showed moderate activity, while MC1 exhibited comparatively lower cytotoxicity. The standard drug cisplatin showed strong cytotoxic activity, whereas the control group maintained 100% cell viability. These results indicate that calcium complexation and the

Table 3: Physical data of calcium metal complexes of N-substituted Schiff bases of 7-amino-4-methyl-coumarin derivatives

S. No.	Compound code	R	% Yield	Melting point (℃)	Molecular formula	Rf value
1	MC1	2-Cl	74.87	209–211	$C_{34}H_{24}O_4N_2Cl_2Ca$	0.56
2	MC2	3-Br	72.65	219–222	$C_{34}H_{24}O_4N_2Br_2Ca$	0.51
3	MC3	4-NO_2	68.77	299–302	$C_{34}H_{24}O_8N_4Ca$	0.53
4	MC4	2,6-Cl_2	88.47	209–212	$C_{34}H_{24}O_4N_2Cl_2Ca$	0.65
5	MC5	2,5-$(OCH_3)_2$	72.65	372–375	$C_{38}H_{36}O_8N_2Ca$	0.48

nature of substituents significantly influence anticancer activity.

DISCUSSION

The present study demonstrates the successful synthesis and characterization of N-substituted Schiff bases of 7-amino-4-methyl-coumarin and their corresponding calcium metal complexes, followed by evaluation of their *in vitro* anticancer activity against the A549 lung cancer cell line.

The physical data of the synthesized Schiff bases [Table 1] indicated good yields, sharp melting points, and consistent Rf values, confirming the formation and purity of the ligands. Substituent variation on the aromatic ring influenced the yield and physicochemical properties, with the nitro-substituted ligand (Sb3) exhibiting the highest yield. These observations suggest that electron-withdrawing groups favor Schiff base formation.

On complexation with calcium ions, notable changes were observed in the physicochemical properties [Table 3]. The calcium complexes (MC1–MC5) exhibited significantly higher melting points compared to their parent ligands, indicating increased thermal stability due to metal–ligand coordination. Changes in Rf values further supported the formation of new chemical entities. The proposed coordination through azomethine nitrogen and carbonyl oxygen is consistent with the observed spectral data.[21]

IR spectral analysis provided strong evidence for Schiff base formation and metal coordination. The appearance of the characteristic azomethine (–C=N–) stretching band and the disappearance of the primary amine (–NH_2) band confirmed successful condensation. In calcium complexes, the presence of additional bands attributed to metal–oxygen or metal–nitrogen interactions indicated effective chelation, which is known to enhance biological activity by increasing lipophilicity and membrane permeability.[21]

The *in vitro* anticancer evaluation using the MTT assay revealed that all calcium complexes exhibited a concentration-dependent reduction in A549 cell viability [Table 2 and Figure 5]. Among the synthesized complexes, MC4 (2,6-dichloro substituted) showed the highest cytotoxic activity, with cell viability reduced to approximately 34% at 100 µg/mL and an IC_{50} value of 73.14 µg/mL. The enhanced activity of MC4 may be attributed to the presence of electron-withdrawing chloro substituents, which can improve cellular uptake and strengthen metal–ligand interactions. Complexes MC3 and MC5 also showed appreciable activity, whereas MC1 and MC2 exhibited comparatively moderate effects.[22]

Morphological changes observed in microphotographic images [Figures 6 and 7] further supported the MTT assay results. Cells treated with MC4 showed significant shrinkage, reduced cell density, and loss of normal cellular architecture compared to untreated cells, indicating effective induction of cytotoxicity. These changes were comparable to those observed with the standard drug cisplatin.[23]

Overall, the results suggest that calcium complexation enhances the anticancer potential of coumarin-based Schiff bases and that substituent nature plays a crucial role in modulating biological activity. The promising cytotoxic profile of MC4 highlights its potential as a lead compound for further *in vivo* studies and mechanistic investigations in lung cancer therapy.

CONCLUSION

In the present work, a series of N-substituted Schiff bases of 7-amino-4-methyl-coumarin and their corresponding calcium metal complexes were successfully synthesized and characterized using physicochemical and spectral techniques such as FT-IR and ^{1}H NMR. The physical data, including yield, melting point, and Rf values, confirmed the formation and purity of the synthesized ligands and their calcium complexes. Spectral studies validated Schiff base formation through azomethine (–C=N–) linkage and confirmed metal coordination in the complexes.

The *in vitro* anticancer activity of the synthesized calcium complexes was evaluated against the A549 lung cancer cell line using the MTT assay. All complexes exhibited concentration-dependent cytotoxicity. Among them, the dichloro-substituted calcium complex MC4 showed the most potent activity, with a significant reduction in cell viability and an IC_{50} value of 73.14 µg/mL. Morphological changes observed in treated cells further supported the cytotoxic potential of MC4.

Overall, the study demonstrates that calcium metal complexation enhances the anticancer activity of coumarin-based Schiff bases and that the nature of substituents plays a key role in modulating biological activity. The promising results obtained for MC4 suggest that it may serve as a potential lead compound for further *in vivo* evaluation and development of safer and effective anticancer agents for lung cancer.

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AUTHORS' CONTRIBUTIONS

All authors have accepted responsibility for the manuscript's content and consented to its submission. They have thoroughly reviewed all results and unanimously approved the final version of the manuscript.

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