Synthesis, Characterization and Biological Evaluation of N-stabilized Metal Complexes of Schiff Bases

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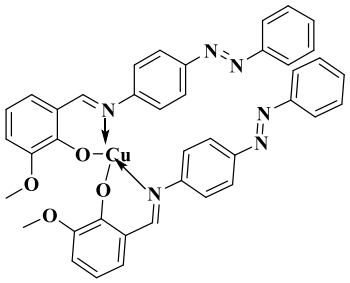
Abstract: Despite harmonious circumstances, a simple and effective approach for the synthesis of Schiff base metal complexes has been described. The necessity for creating innovative chemotherapeutic drugs with a wide range of biological activities and functions has been highlighted by the synthesis and characterization of physiologically active Schiff base metal complexes. The absence of solvents, catalysts, and complicated work-up conditions make this synthetic procedure appealing for obtaining unique physiologically active complex derivatives. The interaction between Schiff base metal complexes with different oxidation states has been comprehensively examined in this study, with a yield ranging from 69% to 80%. The produced compounds have been examined using FT-IR, 1HNMR, and 13CNMR spectroscopy methods. Using thrombolysis and hemolysis, the pharmacological properties of these compounds have been assessed. The potential of all the target compounds (10a-e) as thrombolytic and hemolytic agents is modest to medium. Compound 10c had the best thrombolytic capability and the least amount of cytotoxicity among the synthesised derivatives.

Keywords: Biomedical applications, cytotoxicity, hemolysis, metal complex, schiff base ligand (L), thrombolysis.

I INTRODUCTION

Cancer is a treacherous, sometimes lethal disease that develops aberrant cells with the potential to spread uncontrolled and kill healthy portions of the body[1]. A tumour is a mass of tissues made up of these cells [2]. It may be benign or cancerous. Benign tumours are typically not dangerous, are reversible with surgery, and do not grow back [3]. Moreover, unlike malignant tumours, which are cancerous, this tumor's cells do not spread to other areas of the body and seldom pose a danger to life [4]. These tumours' aberrant cells proliferate and divide again in an unpredictable way. These cells have the ability to penetrate and harm organs and tissues nearby [5]. These tumours have the ability to release tumour cells that may travel from their initial location to develop new tumours in other organs or break out and enter the lymphatic or circulation[6, 7]. A malignant cell divides by using nutrition and oxygen, and it may result in tumours, weakened immune systems, and other alterations that stop the body from working properly [8]. As of January 1, 2016, 15.5 million Americans were expected to have a history of cancer, according to a 2018 survey done by the American Cancer Society [9]. As to a study carried out by the Indian Council of Medical Research (ICMR) National Cancer Registry Programmed [10],over 1300 Indians pass away from cancer every day.

The death rate from cancer rose by almost 6% between 2012 and 2014 [11]. This deadly illness is treated with immunotherapy [12], hormone therapy [13], chemotherapy [14], and other treatments. These treatments do, however, also come with serious adverse effects. A more recent and innovative method of curing this deadly and dangerous illness without any negative side effects is called "precision medicine" or "personalized medicine" [15].



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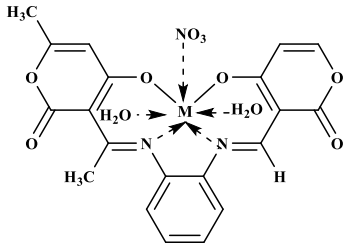

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Fig.1: Anticancer Schiff base ligands

Recent years have seen the discovery of several Schiff bases and their metal complexes with striking and potential anticancer properties as shown in figure 1 [16]. The term "schiff bases" (RCH=NR', where R and R' are substituted alkyl, aryl, cycloalkyl, or heterocyclic groups) is sometimes used to describe carbonyl compounds are formed when primary amines condense.

Hugo Schiff published the first study on Schiff bases in 1864 [16]. These substances are sometimes referred to as azomethines, imines, or anils. It has been proposed that Schiff bases' C=N connection plays a significant part in demonstrating the biological activity [17]. The chemical and biological

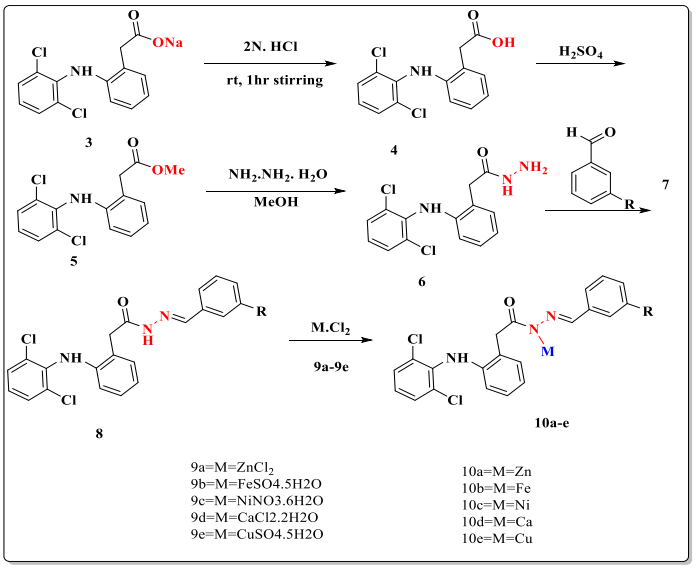
ramifications of a single pair of electrons in the nitrogen atom's sp^2 hybridised orbital in the azomethine group have also been studied [18]. In metal coordination chemistry, schiff bases are regarded as "privileged ligands" [19] because of their unique C=N group feature and broad and varied synthesis. Schiff bases and their complexes have shown to be very physiologically active drugs with anti-malaria properties in the field of medicine [20-24], antibacterial[25], antifungal [26,27], antiviral, anticancer, antitumor, antiinflammatory and anti-HIV activities properties[16, 28-31]. Additionally, schiff bases are used in heterogeneous and homogeneous catalysis [32].

For these reasons, chain & physical properties as well an incomparable variety of their possible applications in a great number of fields and t h e i r complexes are important for advances in the coordination chemistry [33]. Here, we successfully synthesise a new bidentate SBL using a single-pot method, together with the associated Zinc(II), iron(II), Nickle(II), Copper(I), and Calcium complex.Molecule characterized by UV-visible, FT-IR and fuorescence spectroscopy method was trustworthy. Furthermore, their anticancer activity was also evidenced by the in vitro demonstration of cytotoxicity on Vero, HeLa, and MCF7 cancer cell lines.

II EXPERIMENTAL

A. General information

This synthesised sequence used only analytical-grade reagents and solvents. Through a simple one-pot chemical synthesis, this work effectively synthesised a bidentate SBL and its unique Zinc(II), Iron(II), Nickle(II), Copper(I), and Calcium (II). The molecule was characterised using analytical methods such as UV-vis, FT-IR[34], and fuorescence spectroscopy, all of which were considered credible. Furthermore, a thorough in vitro demonstration of their cytotoxic effects on cancer cell lines Vero, HeLa, and MCF7 was provided, and they underwent thin-layer chromatography (TLC). However, spots started to form due to the UV (ultra violet) radiation.



Scheme 1: Target molecule synthesis (10a–e)

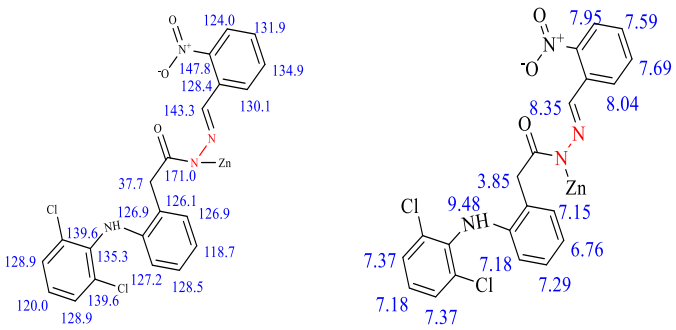


Fig.2: NMR Analysis of 10a

B. Synthesis

Metal salts were reacted with (E)-2-(2,6-dichlorophenyl) amino) phenyl)-N-methyl-N-(2-nitrobenzylidene) acetohydrazide (0.05g, 0.0001mol) for three hours while stirring continuously under reflux conditions. This resulted in the formation of the metal complex (L). Next, the development of the reaction as seen by Thin-Layer Chromatography (TLC) through to the conclusion. Yields of 69% to 80% are achieved as the reaction mixture cools.

C. Hemolysis

Five millilitres of blood were drawn from albino mice, and the sample was centrifuged at 1000 rpm for about five minutes. Following its separation, the pellet containing RBCs washed four times in cold, phosphate-buffered saline with a pH of 7.4. Subsequently, 180 µL of a chemical combination (20 µL) was introduced to the red blood cells. For thirty minutes, the sample was incubated at 37°C. After that, it was removed from the incubator and given five minutes to cool. It was then centrifuged at 13,000 rpm for five minutes. Then to each tube of residual material again at 100µL of cold phosphate-buffered saline. Both a positive control and a negative control, dimethyl sulfoxide2,20-azino-bis (3-etilbenzothiazolin -6-sulphonic acid), were used [35-38]

$$\text{RCBs Lysis (\%)} = \frac{sanmpleabsorbance - negativecontrolabsorbance}{positivecontrolabsorbance} \times 100$$

D. Thrombolytic Potential

Blood was collected by venipuncture from albino mice and transfer directly into sterile, preweighed Eppendorf tubes. Eppendorf tubes with specimens were incubated at 37°C for one hour to appropriate clot formation[21, 22]. After that the sample was taken out, and weights of tubes were subjected to determine starting mass in weight for initial clot[39, 40]. They were then incubated at 37°C for three hours, filled, one by one with 100 µL of DMSO- dissolved solutions into Eppendorf tubes. ABTS was the positive reference and water is negative control. Eppendorf tubes were re-weighed after aspirating the 2nd serum to determine extent of clot lysis [35].

$$\text{Clot outlay (\%)} = \frac{weightoforiginalclot - weightfinalclot}{weightoffirdtclot} \text{ X } 100$$

III RESULTS

The 2-(2,6-dichlorophenyl) amino) phenyl)-N-methyl-N-(2-nitrobenzylidene) acetohydrazide metal complex 53%-67%, Kumari Nagaraj et al., were synthesized by treating adequate hydrazine and 4-choloro-3 nitobenzaldehyde under reflux for three hours than proceeded Thin-Layer Chromatography (TLC).

A.Hemolysis Analysis

In contrast to the higher levels of toxicity shown by **10a** and **10d** , It's noteworthy to observe that Compound 184d caused the greatest amount of lysis of red blood cells (RBCs), 5.7% more than the reference control ABTS. Compounds **10b** and **10c** also significantly increased hemolysis, as seen by Table 1 (rates of 1.45% and 3.80%, respectively).

Additionally, Table 1 illustrates that Compound **10e** had the lowest cytotoxic effect of all the derivatives studied, at only 0.9%. The information that is now available suggests that the kind and orientation of the compounds' functional groups have a major impact on their hemolytic activity. The molecules that are generated show varying degrees of hemolytic activity, from mild to severe, indicating possible applications in the treatment of cancer

B. Thrombolysis Analysis

We evaluated the potential for thrombolysis of each synthesised molecule. These compounds generally exhibited very little thrombolytic action. Compounds **10d** and **10b** had more thrombolytic capability in comparison to the industry standard control, ABTS, whereas other derivatives shown intermediate capacity. Compound **10b** had the highest lysis rate (65.1%) when compared to ABTS. Compound **10c** had the lowest lysis rate, 40.2%, according to Table 1. Research is now being done to determine if functional groups have a beneficial inductive impact on thrombolysis.

III DISCUSSION

Table1: NMR analysis and MP of all synthesized compound

Compounds	Melting points	NMR Values
4	151ºC	These are the off-white amorphous samples: 37.299 (Ar-H dd, 1H), 7.37 (Ar-H, d, 2H), 9.48 (Amide, s, 1H), 7.15 (Ar-H, d, 1H), 7.16 (Ar-H, d, 1H), 7.18 (Ar-H, dd, 1H), 6.67 (Ar-H, dd, 1H), and 12.39 (OH, s, 1H).
5	96ºC	The following compounds are off-white and amorphous: 3.66 (CH3, s, 3H), 3.69 (CH2, s, 2H), 6.67 (Ar-H, dd, 1H), 7.15 (Ar-H, d, 1H), 7.16 (Ar-H, d, 1H), 7.18 (Ar-H, dd, 1H), 7.299 (Ar-H dd, 1H), 7.37 (Ar-H, d, 2H), 9.48 (amide, s, 1H).
7	171ºC	off-white amorphous materials: 47.299 (Ar-H dd, 1H), 7.37 (Ar-H, d, 2H), 9.08 (NH, t, 1H), 7.15 (Ar-H, d, 1H), 7.16 (Ar-H, d, 1H), 7.18 (Ar-H, dd, 1H), 4.42 (amide, d, 2H), 6.67 (Ar-H, dd, 1H), and 9.48 (amide, s, 1H)
8	143.5ºC	Amorphous, off-white: 3.85 (CH2, s, 2H), 6.76 (Ar-H, dd, 1H), 7.15 (Ar-H, d, 1H), 7.16 (Ar-H, d, 1H), 7.18 (Ar-H, dd, 1H), 7.299 (Ar-H, dd, 1H), 7.37 (Ar-H, d, 2H), 7.59(Ar-H, dd, 1H), 7.69 (Ar-H, d, 1H), 8.08 (Ar-H, d, 1H), 8.57(C=N, s, 1H), 9.48(NH, s, 1H), 11.07(amide, s, 1H).
10a-e	251ºC	Amorphous, off-white: 3.66 (CH2, s, 2H), 6.76 (Ar-H, dd, 1H), 7.15 (Ar-H, d, 1H), 7.16 (Ar-H, d, 1H), 7.18 (Ar-H, dd, 1H), 7.299 (Ar-H, dd, 1H), 7.37 (Ar-H, d, 2H), 7.68 (Ar-H, d, 1H), 8.06 (Ar-H, d, 1H), 9.48 (amide, s, 1H), 9.97 (C=N, s, 1H).

Table2: Target molecule hemolysis and thrombolysis potential mean ± standard deviation (10a-e)

Sr. #	Derivatives	% of Thrombolysis ± SD	% of Hemolysis ± SD
1	184a	47.2 ± 0.08	2.0 ± 0.121
2	184b	65.1 ± 0.08	1.4 ± 0.004
3	184c	40.2 ± 0.08	3.8 ± 0.005
4	184d	54.2 ± 0.08	5.7 ± 0.041

5	184e	30.5 ± 0.08	0.9 ± 0.009
S	ABTS	86	95.9

[a]Experiments were performed in triplicates and expressed as mean ± SD* $p<0.05$, was considered significant

IV CONCLUSION

Because of its low cost and environmentally friendly settings, this synthetic approach offers a simple method for producing Schiff base metal derivatives in high yields (69-80%). Hemolysis and thrombolysis were used to assess the metabolic profile of the target molecules (10a–e). Schiff base ligands (L), which provide a range of structural motifs and bioactive capabilities, were successfully synthesised and characterised in Zinc (II), iron (II), Nickle (II), Copper (II), and Cobalt (II) metal complexes.

Complex Fe(II) showed the least amount of cytotoxicity among the compounds studied; in contrast, Zinc (II), iron (II), Nickle (II), Copper (II), and Calcium (II) induced the greatest lysis of red blood cells (RBCs) at a rate of 5.7%. With a lysis rate of 65.1%, complex Fe(II) had the highest. On the other hand, complex Ni (II) had the lowest lysis rate, at 30.5%. Numerous Schiff base complexes involving metals may be interesting candidates for novel therapeutics that are appropriate for treating a variety of cancer types, some of which have high incidence rates.

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