SYNTHESIS, THERMAL STUDY AND BIOLOGICAL ACTIVITIES OF SCHIFF BASE LIGAND

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Abstract

Organic substances known as "schiff bases" have a wide range of biological activity, which can be enhanced by coordinating with various metal ions. Their compounds can even function as heterogeneous catalysts in chemical processes or as corrosion inhibitors. This work concentrated on the Schiff base generated from 2-amino-4-hydroxy-6-methyl pyrimidine due to the versatility of the Schiff base synthesis and its numerous possible applications. With the help of acetoacetic ester and guanidine, 2-amino-4-hydroxy-6-methylpyrimidine was successfully synthesized. Treatment of Phenylisothiocyanate with 2-amino-4-hydroxy-6-methylpyrimidine followed to yield 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide. The structure of the Schiff base ligand has been synthesized and characterized UV–visible, FTIR, ^{1}H NMR spectra, ^{13}C NMR spectra and thermal analysis and screened for antidiabetic and antimicrobial activity. The biological activity result and the spectroscopic data verified Schiff base production, demonstrating the antidiabetic, and antibacterial properties of 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide.

Key words: Schiff base legend, ^{13}C NMR, ^{1}H NMR, FTIR, Thermal study, Biological activities.

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Introduction

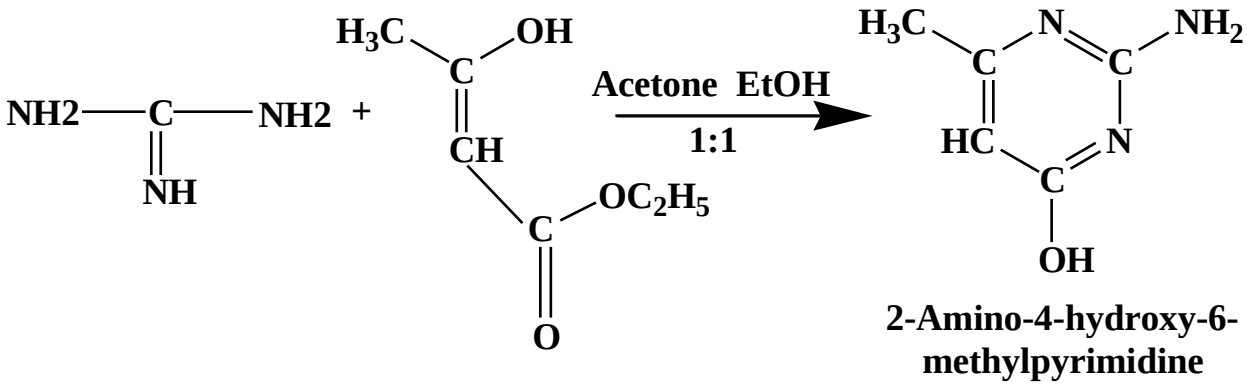
It is clear that many organic chemicals employed in medicine do not have a completely organic mode of action; rather, some are activated or biotransformed by metal ion metabolism. Inorganic elements play a vital role in biological and biological medical processes. Numerous medications have altered pharmacological and toxicological characteristics in the form of metal complexes and perhaps The inclusion of metals in the form of complexes demonstrated some degree of antibacterial, antifungal, anticancer, and anti-inflammatory activity in schiff bases, which are adaptable C=N (Imine) containing compounds with a broad spectrum of biological activity [1]. The substance that contains the azomethine group (-HC=N-) is called a Schiff base. Hugo Schiff initially described them in 1864 [2]. They are condensation products of ketones (or) aldehydes (aldehyde and ketones) with primary amines. In most cases, heat, base catalysis, or acids are used to form Schiff bases. Crystalline solids are a frequent type of Schiff base; they are weakly basic, but at least some of them combine with strong acids to generate insoluble salts. Schiff bases are employed as ligands in the formation of metal complexes with a variety of distinct structures or as intermediates in the synthesis of amino acids. A Schiff base functions as a Flexi-dentate ligand and often coordinates via the N atom of the azomethine group and the O atom of the depronated phenolic group [3]. The co-ordination chemistry of Schiff base azomethane depends heavily on nitrogen as well as other donor atoms like oxygen. Therefore, an effort is undertaken to examine the coordination chemistry of these interactions as well as the interaction of reduced Schiff base with transition metals of biological significance. The synthesis and characterisation of reduced Schiff base and its metal complexes were covered in the current work [4]. Additionally, the decreased Schiff base metal complexes' analgesic and antibacterial activity is assessed and contrasted with the benchmarks [5]. While aliphatic aldehydes are unstable and easily polymerize, aromatic aldehydes, particularly those with an efficient conjugation mechanism, generate stable Schiff bases [6]. Aldehydes create Schiff base ligands more easily than ketone (carbonyl carbon) ones.Schiff bases come in a variety of shapes and sizes. Because Schiff base compounds have such a diverse and flexible structure, a large range of Schiff base compounds and their behavior have been explored [7]. Schiff bases are typically composed of very stable complexes with metal ions and are bi, tri, or tetra-dentate chelate ligands. Numerous researchers have examined their chemical and physical characteristics in a variety of fields, including preparation applications, the identification, protection, and determination of aldehydes or ketones, the purification of carbonyl and amino compounds, and the synthesis of these compounds in intricate or delicate reactions [8,9]. Schiff base ligands are very important in chemistry, especially in the development of Schiff base complexes, since many of these complexes exhibit excellent catalytic activity in a variety of reactions at high temperatures and in the presence of moisture. Schiff base complexes have the potential to form stable complexes with metal ions. A review article showcasing the catalytic activity of Schiff base complexes is necessary because several reports on their applications in homogeneous and heterogeneous catalysis have been published in recent years. Schiff base metal complexes, specifically the ions Co(II), Cu(II), and Zn(II), were crucial in the advancement of coordination chemistry [10]. Because of their physiological DNA binding and cleavage capabilities, transition metal complexes have piqued interest. Current research focuses on the use of metal complexes as chemical nucleases. It has been shown that the present focus of study is on in-organic complexes as chemical nucleases. Inorganic complexes have proven to be useful as sequence-specific DNA binding agents in foot printing experiments, as well as as diagnostic agents in medical applications and genetic research.

Materials and Methods

Alkalar grade (Indian-made) chemicals were used to make alkyl/arylisothiocyanates in compliance with the literature, and the melting points of all synthesized compounds were determined in an open capillary without correction. KBr pellet IR spectra were recorded using a Perkin-Elmer spectrophotometer in the 4000-400 cm-1 range. TMS was used as the internal standard to obtain NMR spectra using CDCl3 and DMSO. On silica gel-G plates, TLC was utilized to confirm the purity of the compounds.

Synthesis of 2-amino-4-hydroxy-6-methyl pyrimidine

Using an acetone-ethanol (1:1) medium, guanidine and acetoacetic ester interacted for eight hours in a water bath. There was precipitate filtering. By way of precipitation, it formed crystals. (By employing acetic acid as a precipitant to extract it from its alkaline solution) [11,12].

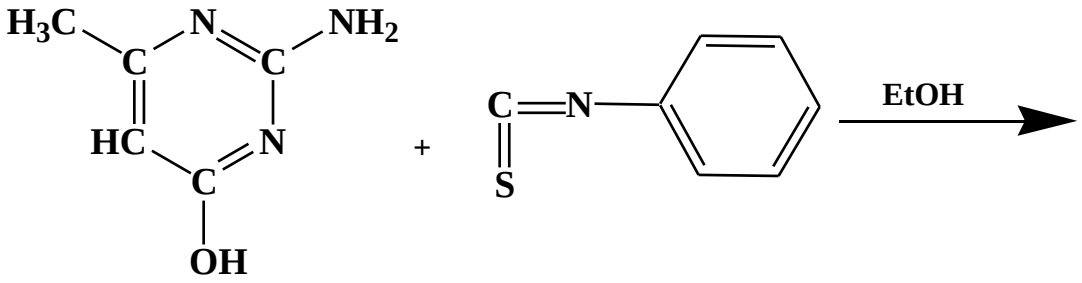


2-Amino-4-hydroxy-6-methylpyrimidine

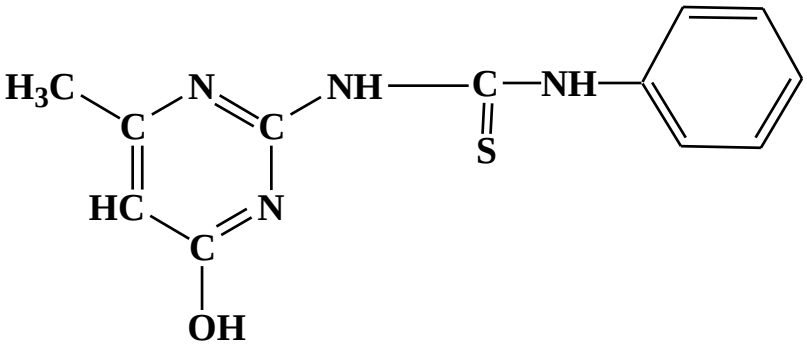
Scheme A- Synthesis of 2-amino-4-hydroxy-6-methyl pyrimidine

Synthesis of 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide

In an acetone-ethanol medium in a 1:1 molar ratio, the reaction between 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide and 2-amino-4-hydroxy-6-methylpyrimidine was conducted in a boiling water bath for four hours. Filtration is then used to extract 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide from the reaction mixture [13, 14].



2-Amino-4-hydroxy-6-methylpyrimidine **Phenylisothiocynate**



1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide

Scheme -2 Synthesis of 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide

Characterization: The spectral data for the IR, 1H-NMR, 13C NMR and UV-Vis analysis was done to illustrate Schiff base lignad.

Thermal analysis

The simultaneous TG/DT analysis of Schiff base ligand was studied from ambient temperature to 1000 ◦C in nitrogen atmosphere using a-Al2O3 as reference [15].

Biological activities

Antidiabetic activity

Schiff base ligand was allowed to screen for antidiabetic activity by α-Amylase and α-Glucosidase assay.

Inhibition assay of α-amylase

Shukla et al.'s approach, with a few modifications, was used for the enzymes inhibition procedure for -amylase [16]. For α-amylase, acarbose (AC) was the usual medication. The half maximum inhibitory concentration (IC50) was also used to express the percentage (%) of inhibition for all enzymes except for α-amylase.

$$\%\ \text{OF INHIBITION} = \frac{Absorbance\ of\ control - (absorbance\ of\ extract)}{Absorbance\ of\ control} \times 100$$

Inhibition assay of α-glucosidase

The Shukla et al. approach was followed with a minor modification for the -glucosidase enzyme inhibition procedure [17]. The reference medication for the α-glucosidase inhibition experiment was acarbose (AC). At 410 nm, the absorbance of the liberated p-nitrophenol was measured. Each experiment was run in triplicate, and a parallel setup without test drug served as the control. With the exception of α-glucosidase, the percentage (%) of inhibition for each enzyme was also represented as the half maximum inhibitory concentration (IC50), which was determined using the formula above.

Antimicrobial activity

Utilizing the disc-diffusion method, antibacterial activity was assessed. A variety of microorganisms were kept at -80 ºC after being taken from the Microbial Type Culture Collection (MTCC). Microorganisms were inoculated on nutrient agar (Microxpress Ltd.) in petri plates for purity assessments prior to use in the bioassay. Dimethylsulfoxide (DMSO) stock solutions of the Schiff base ligand were made. Each inoculation plate (70 mm) was covered with sterile discs that had been saturated with a 20 ml sample. The agar plates were prepared by spreading the individual bacterial strains across nutrient agar plates, impregnated with various test samples (20 ml/disc), and then seeded the agar plate's surface. After that, the plates were incubated for a day at 37 ◦C in a bacteriological incubator. The zones of inhibition were measured in millimeters (mm) in order to evaluate antibacterial capability. Less than 12 mm inhibition zones were thought to have little antibacterial action, whereas those between 12 and 18 mm were thought to have quite a bit of activity. Singh and associates (2015) [18].

Statistical analysis

Every experiment was conducted in triplicate, and the Mean± SD findings were given. One-way ANOVA was used for statistical analysis of the data, and Duncan's test was then performed. When $p>0.05$, mean values were deemed statistically significant.

Result and Discussion

Spectral data for Compound synthesized by Scheme-B

UV, λ_{max}(DMSO) nm: 358.4 and 425.5; (Fig- 3.5) **IR**(KBr) ν_{max} cm^{-1}:32.13.5, 16611, 1569.6, 1225.8, 1176.2; (Fig- 3.2) **^{13}C-NMR** (DMSO, D_2O, 400.140 MHz) δ ppm:176.38, 147.28, 136.13, 129.97, 122.41, 119.91, 103.57, 39.92; (Fig- 3.4) **^{1}H-NMR** (DMSO, 400 MHz)δ ppm { multiplicity: Singlet(s), doublet(d), triplet(t), multiplet(m), doublet- doublet(d-d)} : 2.508, (1H, d), 7.31 (1H, d), 6.92 (1H, d-d), , 9.410, 10.113, 10.789 (1H, s); (Fig- 3.3).

Interpretation of data

UV-vis absorption spectra of the Schiff base legend recorded at ambient temperature in DMSO (1 105 M). The electronic absorption spectra of Schiff base show two bands at 350.4 and 425.5 nm. The n-* transition of the azomethine (-CH-NH-) group is responsible for the lower energy band at 425.5 nm, whereas the -* transitions of the phenyl ring [19] are linked to the higher energy band at 350.4 nm. The infrared spectra of the isolated molecule show the existence of an OH group with a 3427.1 cm-1 intermolecular hydrogen bonding stretching frequency. The band at 3213.5 cm-1 shows an asymmetric stretching of the (NH)-moiety, whereas the band at 2746.4 cm-1 was formed by the C-H stretching of an alkene. The absorption peaks at 1661.1 cm-1 [20–23] show stretching of C=N. N-C=N stretching produced the band at 1569.6 cm-1. Phenolic OH was observed to be bending at 1382.4 cm-1. In order to simulate the vibrations of aromatic hydrocarbons brought on by the =C-O bending, the absorption bands at 1225.8 cm-1 were employed. Stretching and bending vibrations of the sort =(C=S) are visible in the absorption band at 1176.2 cm-1 [24–26]. The 13C-NMR spectra showed the presence of twelve carbon atoms. The 13C-NMR spectra show the solvent peak at 39.92. A signal is detected for the carbonyl (C-4) group at 176.38 ppm, and for aromatic carbon, the values are 147.28, 136.13, 129.97, 122.14, 119.91, and 103.57 ppm. The 1H-NMR spectra show a solvent signal at around 2.508. Isolated compound peaks at 10.783 and 9.410 [27–31] indicated the presence of phenolic proton at C3, C5, C7, and C4'. There were peaks with chemical shifts of 7.91 and 6.92 at C3', 5', C2', 6', C8, and C6 due to the presence of aromatic protons. It was comparable to the pure 1-(4-hydroxy-6-methylpyrimidino)-3-

phenylthiocarbamide, as shown by the findings of the UV, FTIR, ^{1}H-NMR, and ^{13}C-NMR spectra (Fig. 1).

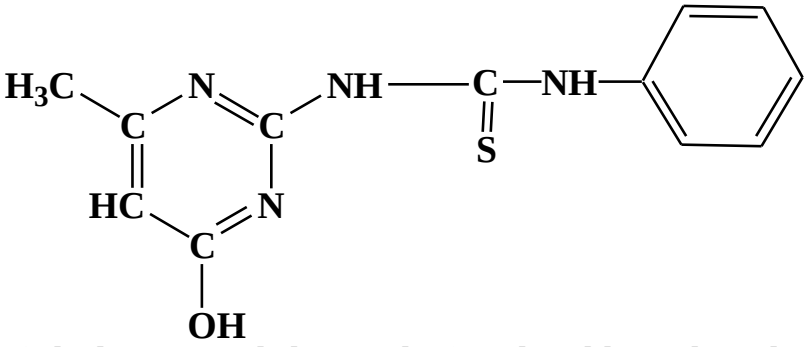


Fig 1. 1-(4-hydroxy-6-methylpyrimidino)-3-phenylthiocarbamide

Ligand's thermal stability is revealed by thermal analysis. The elimination of coordinated water molecules is shown by the first step of the ligand's TG curve, which has a sharp slope between 150 and 200 ◦C and a mass loss of 5.0% (calculated at 5.2%). The dehydration stage is represented by an endothermic peak on the DTA curve that ranges from 140 to 210 ◦C (DTmax = 170 ◦C). The anhydrous ligand first exhibits a slow disintegration from 250 to 500 ◦C, with 15.53% (calculated to be 17.25%) mass loss. The elimination of the ligand's non-coordinate component may be the cause of the broad exotherm DTmax = 410 ◦C in DTA. The second phase decomposes between 600 and 630 ◦C, and for this step, a steep endotherm in DTA is seen at 615 ◦C.

Table 1 shows the in vitro α-amylase inhibitory activity of Schiff basse ligand in comparison to acarbose. The alpha amylase inhibition on varying sample concentration is graphically shown in Figure 3, which also aids in the estimate of the ligand's IC_{50} value and standard acarbose. The concentration of ligand or standard medication needed to block 50% of the enzyme in reaction is known as the IC_{50} value. Each ligand represents an inhibitory activity against α-amylase that is dependent on concentration. Acarbose had an IC_{50} value of 40.14±0.08 µg/ml and a percentage alpha amylase inhibition of 52.64–97.51% at concentrations ranging from 50–1000 µg/ml. Better therapeutic efficacy and potency are correlated with lower IC_{50} values.

Table 1 compares the inhibitory action of Schiff base ligand against acarbose against in vitro - glucosidase. The inhibition of α-glucosidase when the ligand concentration is changed is illustrated visually in Figure 3, which also aids in determining the ligand's IC_{50} value and standard acarbose. Compared to synthetic medicines that have a lot of adverse effects, including abdominal discomfort, bloating, flatulence, and diarrhea, this active ligand may be employed as an antidiabetic medication [26].

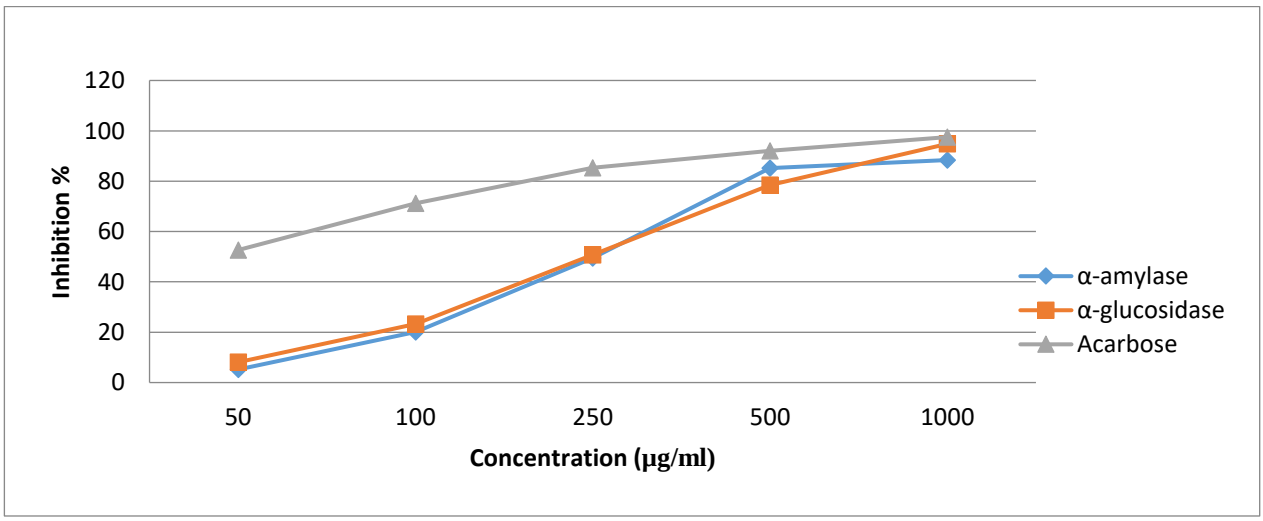


Fig. 3 Inhibition for antidiabetic assay of Schiff base ligand.

Antidiabetic activity IC_{50} (µg/ml) of Schiff base ligand	
α-amylase method	**α-glucosidase method**
260.3±0.39	290.36±0.28

Table 1 IC_{50} of antidiabetic activity of Schiff base ligand.

Using the disc diffusion method, Schiff base ligands were investigated to assess their antibacterial activity against food poisoning bacteria, including two strains of Gram positive bacteria (Listeria monocytogenes & Staphylococcus aureus) and two strains of Gram negative bacteria (Escherichia coli & Pseudomonas aeruginosa). The assessment of the ligand's antibacterial activity was noted in Table 2. With varying potencies of all tested harmful bacteria at a concentration of 25 mg/ml, the results showed that Schiff base ligand was possibly useful for inhibiting the microbiological growth of food poisoning bacteria.

Antimicrobial screening test of *Schiff base ligand* (25 mg/ml) against some bacterial strains.		
Bacterial species	**Schiff base lignad**	**Erythromycin**
L. monocytogenes	7.0±0.01	22.0±0.00
S. aureus	8.0±0.40	20.5±0.05
E. coli	8.5±0.3	15.5±0.01
P. aeruginosa	8.0±0.02	20.0±0.00

Table 2 Zone of inhibition (in mm) of bacterial species in *Schiff base ligand* and Erythromycin .

Conclusion

Schiff bases are often chelate ligands, tri-, or tetra-dentate, that have the ability to bind metal ions and form stable complexes. Thus, Schiff bases have had a major impact on both the development of inorganic biochemistry and coordination chemistry [32]. The carbon-nitrogen double bond of Schiff bases is readily reduced by metal complexes, just like the carbon-oxygen double bond. Schiff bases are hence flexible ligands that bind to metal ions via azomethine's nitrogen atoms. Schiff bases are chelate ligands that can bind metal ions to form stable complexes; these ligands are commonly bi-, tri-, or tetra-dentate [33–35]. Thus, Schiff bases have played a major role in the development of both inorganic biology and coordination chemistry.

Schiff bases possess a wide range of pharmacological effects and biological activities, such as antibacterial, antimalarial, anti-inflammatory, antiviral, and antipyretic properties, because of their imine (-C=N-) link [36]. Antibacterial and antifungal activity are just two of the many biological and pharmacological properties of transition metal-Schiff base complexes that have been found. Many Schiff-bases that are obtained from various amines have been shown to have interesting applications in industrial catalysis, materials chemistry, and catalytic processes [37]. Salicylaldehyde and the primary diamine condense to form the salen type ligands, which are characterized as flexible ligands for coordination chemistry due to the amine/aldehyde basic's ability to change the ligands' steric and electrical properties. The structural features of this important class of ligands include donor centers, which enable metal ions to project different geometries with other ligands. Consequently, a wide range of complexes were created by changing the metal ions in the Salen-type ligand [38–40]. These molecules have been thoroughly researched in many branches of chemistry. Researchers are interested in the tetradentate ligand, which comprises nitrogen-oxygen donor atoms, as a chelating agent because of its kinetic and thermodynamic stability [41]. Nitrogen is one of the imine groups (C=N) of Schiff-bases and their metallic complexes, which helps explain many of their unique biological properties as well as their capacity to chelate [42]. Metal-salens compounds are currently essential in the fields of inorganic biochemistry, catalysis, magnetism, medical imaging, sensors, nonlinear optical devices, solar cells, and building themes/building blocks [43, 44]. These materials can combine with practically any metal ion and are easy to produce. This molecule facilitates the interaction of metal ions with azomethine nitrogen. These kinds of metal complexes have been studied in great detail numerous times, and some of them provide as good illustrations of how inorganic biochemistry and catalysis have developed [45]. Their oxidation states are variable and they have different coordination geometries. Many compounds need copper to function because it is an element that is required by biology. Many scientists are interested in copper's coordination chemistry because of its intriguing biological

features. A wide variety of copper complexes based on Schiff bases have been successfully used as models in biological and supramolecular systems. Over the past few decades, a great deal of scientific research has been done on the antibacterial, redox, catalytic, and antioxidant activities, with a primary emphasis on biological applications [46-50]. In supramolecular chemistry, materials science, catalysis, coordination, and separation processes, as well as in applications in the biomedical fields and the synthesis of novel compounds with exceptional structures and properties, the significance of Schiff-base complexes has been extensively studied and reviewed.

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