CHAPTER 4

Transition metal chemistry of 'tritopic' 4-aminoantipyrine scaffold based heterocycles: Efficient anticonvulsants in Wistar rats.
Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than sixty million people worldwide according to epidemiological studies [1]. In epilepsy, the normal pattern of neuronal activity becomes disturbed causing strange sensations, emotions and behaviors or sometimes convulsions, muscle spasms and loss of consciousness. This is a kind of brain disorder in which clusters of nerve cells or neurons in the brain sometimes signal abnormally which leads to the said disorders. Mainly two kinds of epilepsy have been identified [2] one with grandmal and other with petitmal. The majority of antiepileptic drugs have been in use since 1985. They do not provide satisfactory seizure control in all patients and typically cause notable adverse side effects such as ataxia, nausea, mental dulling and hepatotoxicity [3,4] which may limit their maximal usefulness. For these reasons there is growing interest to find more effective and safer antiepileptic drugs, is, therefore, imperative and challenging in medicinal chemistry. Antiepileptic drugs (AEDs) are important part of the treatment program for epilepsy and the main aim of AEDs is to suppress seizures without inducing adverse side effects. A significant rising interest in the design of metal compounds as drugs and diagnostic agents is currently observed in the area of scientific study, appropriately termed medicinal inorganic chemistry [5]. Investigations in this area focus mainly on the speciation of metal species in biological media, based on possible interactions of these metal ions with diverse biomolecules, in an
effort to contribute to future development of new therapeutics or diagnostic agents [6]. A wide range of metal complexes are already in clinical use and encourage further studies for new metallodrugs, such as metal-mediated antibiotics, antibacterial, antiviral, antiparasitic, radiosensitizing agents and anticancer compounds. In literature we can find that metal complexes which are derived from nitrogen containing heterocyclic molecules have prominent importance in the biochemical study, due to their wide range of pharmacological activity. Among which 4-aminoantipyrine based metal complexes have been known to possess a variety of applications in biological, clinical, analytical and pharmacological areas [7-8].

Literature survey reveals that the compounds of nitrogen heterocyclic system which contains at least one carbonyl group and phenyl or alkyl substituents attached to the heterocyclic system [9-10] will lead to increase the lipophilicity. This common template is present in the structure of older generation of AEDS such as phenobarbital, pirimidone, phenytoin which all known to be active against MES. Such structural requirements are observed in the 4-aminoantipyrine molecule. In this regard we have selected 4-aminoantipyrine as the core molecule for the synthesis of the Schiff base metal complexes.

The synthesized compounds were evaluated as anticonvulsant agents in experimental epilepsy models, i.e., maximal electroshock test (MES).
Experimental

Synthesis of precursor

4-Aminoantipyrine, isatin were purchased from Sd-fine chemicals. Preparation of 2-hydroxy-3-formylquinoline is done as per the procedure given in chapter 3.

Preparation of the ligands

The ligand \( L^2 \) was prepared by the method reported earlier [11]. To hot ethanolic solution of 4-aminoantipyrine (2.03 g, 0.01 mole) 2-hydroxy-3-formylquinoline/ isatin (0.01 mole) was added for the preparation of ligand \( L^1H \) and \( L^2 \) respectively. The reaction mixture was refluxed for 3-4 hours on water bath and the separated solid was filtered and dried (The schematic presentation shown in Scheme-1).

\{(L^1H, M. P.: >250 °C, Yield: 78 %), (L^2, M. P.: 159 °C, Yield: 76 %)\}.

![Scheme-1](image-url)
Preparation of complexes

Ethanolic solution of metal(II) chloride \{CoCl_2·6H_2O (0.237 g, 0.001 mole), NiCl_2·6H_2O (0.237 g, 0.001 mole), CuCl_2·2H_2O (0.170 g, 0.001 mole) and ZnCl_2 (0.136 g, 0.001 mole)\} was added with stirring to an ethanolic solution of the ligand \{(L^1H (0.358 g, 0.001 mole), L^2 (0.332 g, 0.001 mole)\} and refluxed at water bath temperature for 3-4 h. So obtained complexes were filtered off, washed with ethanol and dried under vacuum over P_2O_5.

Pharmacology

Animals for the investigation

Wistar rats of either sex weighing between 180-200 g were used in the present investigation with prior permission from institutional animal ethics committee (IAEC). Animal studies were performed as per the rules and regulations of CPCSEA. The animals were acclimatized to the experimental room having temperature 23 ± 2 °C, controlled humidity conditions, and 12:12 hour light and dark cycle. The Wistar rats were housed in sterile Plexiglas transparent cages containing sterile paddy husk as bedding material with maximum of 4 animals in each cage. The rats were fed on autoclaved standard rat food pellets and water *ad libitum*.

Acute toxicity study [12]

For screening any synthesized compound for any of its pharmacological properties, it is customary to carryout acute toxicity study to determine the safe effective dose of the novel compound. Wistar rats of either sex weighing
between 180-200 g were starved for 18 h prior to the experiment. The animals were divided into the group of eight each, after recording their body weight. The test sample solutions of suitable concentration in 1% gum acacia were administered orally in different groups. Initially all test samples were administered with 12.5 mg/kg body weight, if all the animals survived with this dose, then the samples were tested at higher dose range viz., 25, 50, 100, 200, 400 mg/kg so on, if the test samples caused 100% death at this dose the lower dose range was treated as LD$_{50}$ dose. Finally the lethal dose fixed for the reporting compounds is 700 mg/kg for ligands $L^{1}H$, $L^{2}$ and their copper and zinc complexes. The administered dose is the one tenth of the threshold dose.

**Testing of compounds for anticonvulsant activity against maximal electroshock generic seizures in Wistar rats [12]**

Previously weighed and numbered Wistar rats were categorized into eight groups each consisting of 4 rats. One group was used as control and the other for the phenytoin drug treatment and remaining six for the novel sample treatment. The drug phenytoin, control and test samples in gum acacia were administered orally to the respective group of animals. Corneal ear clip electrodes were placed on the cornea of the rats and 150 mA of electric current was applied for 0.2 s by means of electroconvulsiometer to all groups. Each animal was placed into individual plexiglas transparent case and was observed for 30 min. Time (in seconds) in various phases of convulsion viz., tonic flexion, tonic extensor, clonic convulsions and stupor was noted. All the experimental groups were compared with the respective control treated with vehicle.
Statistical analysis

Values are expressed as mean ± SEM, statistical difference between means were determined by performing one-way ANOVA followed by Dunnett's test. P<0.05 was considered as significant difference in the present study.

Result and discussion

Complexes obtained in the present study were non-hygroscopic and in the form of amorphous solids. They are insoluble in water, EtOH and MeOH but soluble in DMF and DMSO. Ligand to metal ratio in few complexes is found to be 2:1 and 1:1 for other complexes. The C H N data of the synthesized ligands and their complexes are complied in Table 1. Melting points of all the complexes are found to be above 300 °C and yield of the complexes are about 60%. Compounds were evaluated for pharmacological properties and have exhibited promising anticonvulsant activity towards the electroshock induced seizures in Wistar rats.

IR spectral studies

The IR spectra provide valuable information regarding the nature of the functional group coordinated to the metal atom are presented in Table-2. The ligands (L¹H, L²) shown broad bands around 1715, 1703 cm⁻¹ and are attributed to the v(C=O) of antipyrine molecule. A sharp band at 1680 cm⁻¹ is assigned to the v(C=O) of isatin system in case of ligand L². Upon complexation the v(C=O) of the antipyrine molecule has shifted to lower frequency region suggesting the coordination of carbonyl group to the metal ion. The carbonyl
stretches frequency of the isatin moiety at 1680 cm$^{-1}$ has been lowered by 60-70 cm$^{-1}$ in the spectra of complexes (C5-C8). This clearly indicates the coordination of the carbonyl group to the metal ion. The spectra of both the ligands show the characteristic $\nu(>C=\text{N})$ bands in the region 1570–1500 cm$^{-1}$. In the spectra of the complexes this band appears at lower frequency region indicating the coordination of the azomethine nitrogen atom to the metal ion [13]. The ligand acts as tridentate chelating agent coordinated to the metal ion via the one nitrogen $\nu(>\text{C}=\text{N})$ and two oxygen atoms. Moreover, ligand L$^1$H and L$^2$ have shown sharp bands at 3456 and 3140 cm$^{-1}$ which are the characteristic features of the $-\text{OH}$ and ring $-\text{NH}$ of quinoline and isatin molecule respectively. Due to the hydrated nature of the complexes it makes difficult to observe the absence and presence of the ($-\text{OH}$) and ring ($-\text{NH}$) of quinoline and isatin molecule in all the complexes. Presence of broad band around 3400 cm$^{-1}$ in all the complexes is the indication of coordinated/lattice celled water molecule. The low frequency non-ligand band in the 470 cm$^{-1}$ region is assigned to $\nu$(M-N) (spectra 1-4).

$^1$H NMR spectral studies

$^1$H NMR analysis of the ligands (L$^1$H and L$^2$) and their zinc complexes (C4 and C8) were carried out in DMSO-$d_6$ solvent. Spectrum of the ligand (L$^1$H) shows the peaks around 10.1 and 8.8 ppm which are ascribed to the $-\text{OH}$ and azomethine proton respectively. The signal due to azomethine proton exhibits a downfield shift in its zinc (C4) complex suggesting the coordination of azomethine nitrogen atom to the metal ion [14]. Similarly, complex (C4)
Chapter-4 Anticonvulsant activity

displays the absence of quinoline –OH proton, which indicates the coordination of –OH group to the metal atom via deprotonation. In case of ligand \( \text{L}^2 \) peak at 11.1 ppm is due to the presence of the ring (-NH) of isatin moiety. But, the ring (-NH) of isatin molecule retained in the spectrum of its complex \( \text{C8} \) is evidence for its non-involvement in the complexation. The peak resonating around 6-8 ppm and 2-3 ppm in both the ligands and their zinc complexes are attributed to the aromatic proton and methyl proton respectively (spectra 5-6).

**Molar conductivity measurements**

Molar conductivities of the complexes were measured in DMSO solution with \( 10^{-3} \) M concentration. All the complexes show molar conductance values in the range 7.4-16.5 mho cm\(^2\) mol\(^{-1}\) Table-1. These low conductance values suggest that the complexes are non-electrolytic in nature [15].

**Electronic spectral studies**

The electronic spectra of the ligands \( \text{L}^1\text{H} \) and \( \text{L}^2 \) display the bands around 260 and 370 nm, which are assigned to the \( \pi-\pi^* \) and \( n-\pi^* \) transitions respectively. The \( n-\pi^* \) transition are accompanied with the \((>\text{C}=\text{N}-)\) azomethine group of the ligands. In complexes low shift of this band is the indication of the coordination of azomethine nitrogen to the metal ion [16]. The cobalt complexes \( \text{C1 and C5} \) have shown the band around 425 nm which is attributed to the LMCT. In addition to this, complex \( \text{C1} \) displays the band at 593 nm assigned to d-d transition. In the same way the nickel complexes \( \text{C2 and C6} \) exhibit the bands around 565 and 573 nm which support the octahedral geometry of the complexes. For the copper \( \text{C3 and C7} \) and zinc \( \text{C4 and C8} \)
complexes band observed around 430-460 nm are due to the LMCT transition (spectra 7-10).

EPR spectral studies

EPR studies of paramagnetic transition metal(II) complexes yield information about the distribution of the unpaired electrons and hence about the nature of the bonding between the metal ion and ligands. The EPR spectra of Cu(II) complexes (C3 and C7) were recorded at room temperature. Complexes exhibit isotropic signal with the g value at 2.06 and 2.08 respectively (spectrum 11).

FAB Mass studies

FAB Mass spectral data of the complexes C3 and C7 also supported by the elemental and analytical results. Although for all the complexes the peaks at the highest m/z value cannot always be assigned with certainty the isotopic pattern consistent with a 1:2 and 1:1 (M:L) stoichiometry for the complexes C3 and C7 with the corresponding molecular ion peaks 815 and 506 respectively. The spectra also contain peaks due to molecular cations and show some prominent peaks corresponding to the various fragments of the complexes (spectra 12-13).

Magnetic studies

The experimentally determined room temperature magnetic susceptibilities of the complexes are presented in Table-1. For the cobalt complexes C1 and C5 magnetic moment values are found to be 5.19 and 5.13 BM respectively, which correspond to the presence of three unpaired electrons with octahedral
geometry. [17]. The magnetic moment of the nickel complexes C2 and C6 are observed to be 3.13 and 3.09 BM respectively, which are attributed for the high-spin configuration and show the octahedral environment around Ni$^{2+}$ ion in both the complexes [17]. Copper complexes C3 and C7 have shown magnetic moment values 1.96 and 1.91 BM. respectively, which corresponds to the presence of one unpaired electron. Thus the magnetic moment values along with analytical data support octahedral geometry for the complexes.

**Thermal analysis**

Thermal behavior of complexes having composition [Cu(C$_{21}$H$_{16}$N$_4$O$_2$)$_2$]2H$_2$O and [Cu(C$_{19}$H$_{16}$N$_4$O$_2$)Cl$_2$ H$_2$O]H$_2$O, have been studied in the temperature range 40-900 °C with heating rate of 10 °C min$^{-1}$ in a nitrogen atmosphere. Complex [Cu(C$_{21}$H$_{16}$N$_4$O$_2$)$_2$]2H$_2$O decomposes in two steps. In the first step of decomposition weight loss observed in the temperature range around 97-99 °C which corresponds to the loss of the lattice celled water molecules. The dehydrated complex continues to loose its weight in the temperature range up to 600 °C. Beyond 600 °C plateau is observed which is the indication of the formation of corresponding metal oxide. Similarly in the complex [Cu(C$_{19}$H$_{16}$N$_4$O$_2$)Cl$_2$ H$_2$O]H$_2$O three steps of decomposition were observed. The first step is due to the loss of lattice celled water molecule which is observed around 90-98 °C. Weight loss due to coordinated water molecules was observed at 180 °C. The third step is due to loss of ligand molecule which was observed around 400-500 °C. Beyond 500 °C decomposition was not observed due to the formation of stable metal oxide. Weight losses from the TG, agree well with the theoretical calculations (Graph 1).
Cyclic Voltammetry study

Cyclic voltammetry is a highly versatile electro-analytical technique. In recent years it has become most popular technique for studying electrochemical reactions. The electrochemical behavior of ligands and their complexes has been investigated in DMSO solution. Only copper(II) complex (C7) is found to be redox active in the potential range -1.0 to 1.0 and was studied with the scan rates of 0.5, 1.0 and 1.5 V. Copper complex [Cu(C19H16N4O2)Cl2H2O].H2O exhibit well defined quasireversible redox peak corresponding to the oxidation of metal species Cu^{II} \rightarrow Cu^{III}, with anodic peak at 0.320 V (Epa), followed by the respective cathodic waves in the reverse scan at 0.625 V (Epc) due to Cu^{III} \rightarrow Cu^{II} reduction. The value of Epa and Epc of the quasireversible Cu^{II}/ Cu^{III} couple were slightly affected in the scan rate variation studies, suggesting no change in the quasireversibility. The peak separation between Epa and Epc (\AEp= 305 mV) is found to be greater than 59 mV supports the quasireversibility of the redox couple, corresponding to the one electron process [18] (spectra 14).

Pharmacology study

Maximal electroshock method (MES) is used as the preliminary method for anticonvulsant studies of the synthesized compounds viz., L'H, L2, C3, C4, C7 and C8 at 70 mg/kg body weight of the Wistar rats. The pharmacological data of these compounds are presented in Table-3. Information presented in Table-3 represents a thorough and detailed presentation of test results obtained at specific times of seizure induction in all the phases I, II, III and IV of
convulsion. The satisfactory anticonvulsant action of above said compounds is quite long and comparable to phenytoin, which is used as the standard anticonvulsant drug, at the dose of 20 mg/kg body weight. Flexion is the first phase of convulsion at 150 mA alternating current was delivered for 0.2 s via corneal electrodes to the Wistar rats. At this phase, the recurrent seizures were occurred in the rats by the appearance of neck jerking. The time taken by the group of rats administered with phenytoin to get recover is 3.10 ± 0.5, which is almost same in case of all the tested samples. Abolition of the hind limb tonic extensor component indicates the test compound’s ability to inhibit MES-induced seizure is observed in the extensor phase. The samples C3, C4, C7 and C8 have taken the short time period at this phase compared to the ligands (L$^1$H and L$^2$). Finally, the animals were almost inactive in clonus and stupor phase. But the animals were recovered in case of standard phenytoin drug.

![Figure 1](image_url)

**Figure 1** The tentative structure of the complexes (C1-C8)

**Conclusion**

In this part of the work, we have presented the synthesis and structural investigations of 4-aminoantipyrine based Schiff base ligands and their later
first row transition metal(II) complexes. The structure of the ligands and their metal complexes were confirmed by various spectral and elemental techniques. The ligand to metal (L:M) stoichiometry is found to be 2:1 and 1:1 in case of complexes of \( L^1H \) and \( L^2 \) respectively. The present study suggest the coordination of the ligands to the metal ion in N, O, O fashion. All the complexes are found to be non-electrolytic in nature with octahedral geometry. The prepared compounds were evaluated for pharmacological properties and have exhibited promising anticonvulsant activity towards the electroshock induced seizures in Wistar rats. The complexes have shown better activity as compared to their ligands.
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<th>C</th>
<th>H</th>
<th>N</th>
<th>M</th>
<th>Cl</th>
<th>Molar conductance in $\lambda_m$ mho cm² mol⁻¹</th>
<th>Magnetic moment in $\mu_{eff}$ BM</th>
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<td>L¹H</td>
<td>$(C_2H_16N_4O_2)$</td>
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<td>4.97/5.02</td>
<td>15.13/15.64</td>
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<td>--</td>
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<td>C1</td>
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<td>62.01/62.15</td>
<td>4.14/4.43</td>
<td>13.16/13.81</td>
<td>7.02/7.26</td>
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<td>7.8</td>
<td>5.19</td>
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<td>61.98/62.17</td>
<td>4.15/4.44</td>
<td>13.19/13.81</td>
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<td>7.4</td>
<td>3.13</td>
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<td>13.63/13.73</td>
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<td>--</td>
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<td>$[Zn(C_2H_16N_4O_2)_2]_2H_2O$</td>
<td>61.18/61.72</td>
<td>3.96/4.40</td>
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<td>16.55/16.86</td>
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<td>C5</td>
<td>$[Co(C_3H_13N_4O_2)_2Cl_2H_2O]$</td>
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<td>3.91/4.02</td>
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<td>$[Ni(C_3H_13N_4O_2)_2Cl_2H_2O]$</td>
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<td>3.94/4.02</td>
<td>11.09/11.27</td>
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Table 2 IR spectral data of the ligands and their complexes in cm\(^{-1}\)

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<th>Compound</th>
<th>(\tilde{v}(C=\text{N})) (azomethine)</th>
<th>(\tilde{v}(C=\text{O})) (antipyrine)</th>
<th>(\tilde{v}(C=\text{O})) isatin</th>
<th>(\tilde{v}(O-H))</th>
<th>(\tilde{v}(M-N))</th>
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<td>3431</td>
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Table 3 Anticonvulsant activity (MES test) data of ligands and their copper and zinc complexes

<table>
<thead>
<tr>
<th>Treatment/ Dose mg/kg</th>
<th>Flexion</th>
<th>Extensor</th>
<th>Clonus</th>
<th>Stupor</th>
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<tr>
<td>Control</td>
<td>3.16 ± 0.28</td>
<td>12 ± 0.80</td>
<td>19.0 ± 3.0</td>
<td>220 ± 5.0</td>
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<td>Gum acacia 1 % W/W</td>
<td>3.10 ± 0.5</td>
<td>1 ± 0.01</td>
<td>5.1 ± 4.6</td>
<td>170 ± 15.5</td>
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<td>Phenytoin 20 mg/kg</td>
<td>3.19 ± 0.4</td>
<td>11 ± 0.8</td>
<td>20.0 ± 0.6</td>
<td>215 ± 7.0</td>
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<td>Ligand L'H 70 mg/kg</td>
<td>3.03 ± 0.4</td>
<td>5.0 ± 0.7</td>
<td>4.8 ± 6.2</td>
<td>156 ± 15.0</td>
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<tr>
<td>Copper (C3) complex 70 mg/kg</td>
<td>3.01 ± 0.6</td>
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<td>3.9 ± 7.3</td>
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<td>Zinc(C4) complex 70 mg/kg</td>
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<td>11 ± 0.5</td>
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<td>Ligand L 2 70 mg/kg</td>
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<td>Copper (C7) complex 70 mg/kg</td>
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<td>6.5 ± 0.7</td>
<td>4.2 ± 5.0</td>
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</table>
Chapter 4

Spectra

Spectrum 1 IR spectrum of L'H

Spectrum 2 IR spectrum of C3 complex
Spectrum 3 IR spectrum of ligand $L^2$

Spectrum 4 IR spectrum of C7 complex
Chapter 4

Spectra

Spectrum 5 $^1$H NMR spectrum of L$^1$H

Spectrum 6 $^1$H NMR spectrum of L$^2$
Chapter-4  
Spectra

Spectrum 7 UV-Visible spectrum of $L^1H$  
Spectrum 8 UV-Visible spectrum of complex C1

Spectrum 9 UV-Visible spectrum of $L^2$  
Spectrum 10 UV-Visible spectrum of complex C7

Spectrum 11 EPR spectrum of complex C3
Spectrum 12 FAB Mass spectrum of complex C3

Spectrum 13 FAB Mass spectrum of complex C7
Graph-1. TG-DTA thermogram of complex C7

Spectrum 14 Cyclic Voltammogram spectrum of complex C7
References


