6.1 Introduction

Cancer has been a cause of concern for human throughout. With the second half of the 20th century molecular and cellular basis of cancer was understood. Based on these findings effective treatment have been developed. Regardless of all the developments made so far cancer is still a major cause of concern due to a great number of limitations [1]. Cisplatin synthesised by Peyrone in 1844 has been used as effective anticancer drug owing to its ability to bind to DNA via a covalent mode [2]. However the need for new metal based complexes has risen due to the limitation of cisplatin in terms of its resistance to tumour cells. The mechanism of cisplatin has been extensively monitored and was found to bind to DNA preferentially through covalent intrastrand binding. Lerman [3] suggested non-covalent mode such as intercalation for binding of small molecules with nucleic acid. This mode of binding was found to induce structural and property modification of DNA [4], which is the primary step to mutagenesis [5]. Various
interactive forces like hydrogen bonding, hydrophobic and electrostatic interaction stabilizes the binding of small molecules to DNA. The antibiotic actinomycin D or daunomycin [6] have shown intercalative mode of binding. Two additional non-covalent mode of binding has been reported: groove binding and external electrostatic effect [7,8].

Metal complexes are suited to study the mode of binding to DNA as they offer the possibility of evaluating the effect of sterric and electronic factor through systematic changes of the metal and coordination sphere. The non-covalent interactions between the ancillary ligands of the metal complexes and DNA are employed to induce selectivity of binding [9,10] towards particular DNA sequence depending on the steric complementarity between the bound complex and the DNA back bone. Among the other factors that affects binding affinity [11,12] are size, shape and hydrophobicity of the intercalating ligand.

Curcumin a spice used to give flavour and yellow colour to curry has found to exhibit various biological applications including anti-tumour property that include apoptosis induction and growth inhibition in vitro, also the ability to inhibit tumorigenesis in vivo [13-16]. Large quantity of curcumin can be consumed without toxicity; as a result it remains as a valuable compound for drug development. The advantage of bringing about changes in curcumin scaffold is the lack of toxicity. One such analogue of curcumin is curcumin pyrazole which has been evaluated for anti-malarial activity [17], enhancement of memory [18] and restoration of membrane homeostasis disrupted after brain trauma [19]. Pyrazole, characterized by a 5-membered ring structure consist of three carbon atoms and two nitrogen atoms in adjacent position. Pyrazole are aromatic compound of the heterocyclic diazole. They belong to a class of alkaloids due to its pharmacological effect on human. Pyrazole derivatives are subjected to many research studies as they have widespread biological activity such as anti-inflammatory [20], antiviral [21], antihistaminic [22], fungicides [23] and anti-cancer [24]. The pyrazole
derivate of curcumin and its complexes has been synthesised with the aim evaluating its antioxidant and DNA binding property.

6.2 Experimentals

6.2.1 Materials

Curcumin     E.MERCK
Hydrazine hydrate    E.MERCK
Deoxyribonucleic Acid    SRL
Tris (Hydroxymethyl) Aminomethane    SRL
Acetic acid glacial    S. D. FINE CHEM LTd. Mumbai

6.2.2 Methods

6.2.2.1. Preparation of curcumin pyrazole

Curcumin-I (1.2 mmol) was dissolved in glacial acetic acid (5 mL) and Hydrazine hydrate (1.5 mL) was added to the solution as described in the Scheme 1. The solution was refluxed for 8 h and solvent was removed by vacuum. The obtained solid residue was dissolved in ethyl acetate and washed with water. Organic portion was collected, dried over sodium sulphate and concentrated in vacuum. Crude product was purified by column chromatography using silica gel with hexane/ ethyl acetate 60:40 as eluent [17].

![Scheme-1 Preparation of curcumin pyrazole](image)
6.2.2.2 Preparation of metal complexes of curcumin pyrazole

Curcumin pyrazole (0.27 mmol) and the metal salts (0.27 mmol) solution were prepared separately in methanol. To the curcumin pyrazole solution containing catalytic amount of piperidine, the metal salt solution was added with continuous stirring, Scheme 2. The resultant solution stirred for 4 h and the product separated was filtered, washed with cold methanol several times to remove the residual reactant and dried in vacuum.

![Scheme 2: Preparation of metal complexes of curcumin pyrazole](image)

**6.2.2.3 Cyclic voltammetric studies**

The cyclic voltammogram of curcumin pyrazole complexes were done with complexes of \(1 \times 10^{-5} \text{ M}\) in DMSO with Tetrabutylammonium perchlorate as the supporting electrolyte.

**6.2.2.4 Preparation of stock solution of DNA**

The stock solution of Herring sperm DNA was prepared in Tris-HCl buffer and the concentration was determined by measuring the absorption intensity at 260 nm with a known \(\varepsilon\) value of 6600 M\(^{-1}\) cm\(^{-1}\)[25]. The stock solutions were stored at 4\(^{\circ}\) C and used within 4 days.
6.3 Result and Discussion

6.3.1 Characterisation of curcumin Pyrazole

6.3.1.1 Electronic absorption spectra

Maximum absorption band of Curcumin pyrazole was obtained at 328 nm indicating the π-π* transition in DMSO.

6.3.1.2 Infrared spectra

Infrared spectroscopy was used to determine the chemical bonds in a molecule. The broad absorption band centred at 3487 cm⁻¹ can be interpreted as v(O-H) stretching. A peak at 3148 cm⁻¹ corresponds to ν(N-H) of pyrazole. The sharp band at 1623 cm⁻¹ which is characteristic to ν(-C=O) of curcumin, disappeared and a new band around 1549-1600 cm⁻¹ appeared was assigned to pyrazole ring ν(-C=N). The intense band at 1513 cm⁻¹ can be attributed to ν(C=C). The sharp peak at 1271 cm⁻¹ corresponds to ν(–C-O) of phenol. A medium intense band at 1029 cm⁻¹ can be ascribed to ν(-OCH₃). The obtained IR spectra was as in Fig 6.1.

![Fig 6.1 IR spectra of curcumin pyrazole](image)
6.3.1.3 NMR spectra

$^1$H NMR (300MHz, DMSO-$D_6$) $\delta$ 3.9 (s,6H,-OCH$_3$), 6.73 (d,2H,$J=16$Hz), 6.81-7.32 (6H,aromatic), 7.5 (d,2H,$J=16$Hz), 9.66 (s,2H,-OH) is represented in Fig 6.2.

![Figure 6.2 NMR of curcumin pyrazole](image)

6.3.2 Characterisation of metal complexes curcumin pyrazole (1-5)

6.3.2.1 Elemental analysis

Analytical data obtained are as shown in the Table 6.1.
Table 6.1 Elementary data of metal complexes of curcumin pyrazole

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Compound</th>
<th>C (%) obtain (Cal)</th>
<th>H (%) obtain (Cal)</th>
<th>N % Obtain (Cal)</th>
<th>Metal obtain (Cal)</th>
<th>Cl%</th>
<th>Molar conductance (Ohm⁻¹ cm² mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mn-CurPy</td>
<td>53.68 (53.46)</td>
<td>4.90 (4.49)</td>
<td>5.67 (11.64)</td>
<td>7.67 (7.51)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Mg-CurPy</td>
<td>57.65 (57.17)</td>
<td>4.98 (4.80)</td>
<td>6.77 (6.35)</td>
<td>5.78 (5.51)</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Cu-CurPy</td>
<td>52.87 (52.50)</td>
<td>4.55 (4.41)</td>
<td>5.77 (13.23)</td>
<td>7.67 (7.38)</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Ni-CurPy</td>
<td>53.24 (53.04)</td>
<td>4.88 (4.45)</td>
<td>5.97 (12.34)</td>
<td>7.67 (7.46)</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>Co-CurPy</td>
<td>53.76 (53.92)</td>
<td>4.99 (4.52)</td>
<td>5.57 (12.60)</td>
<td>7.79 (7.58)</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

Fig 6.3 MALDI of Cu-curcumin pyrazole
The detection of mass using MALDI (Shimadsu Biotech Axima in +mode) suggests the formation of 2:2 complexes for curcumin pyrazole. Fig 6.3 represents the mass spectrum of Cu-curcumin pyrazole where the highest peak at 960 corresponds to the 2:2 complex formed, other being the molecular cation of various fragments of the complex [26]. The molar conductance measurement indicates non-electrolytic nature of all the complexes.

Based on the analytical, molar conductance and mass data complexes have been assigned the molecular formula \([M_2(CurPy)_2(H_2O)_2.Cl_2]\).

6.3.2.2 Electronic spectra

Electronic spectra of the complexes in DMSO are given in the Table-6.2.

**Table-6.2 UV-visible spectral data of curcumin pyrazole metal complexes in DMSO 10^{-5} \text{mol L}^{-1}\**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Compound</th>
<th>(\lambda_{\text{max}}) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mn-CurPy</td>
<td>342</td>
</tr>
<tr>
<td>2</td>
<td>Mg-CurPy</td>
<td>337</td>
</tr>
<tr>
<td>3</td>
<td>Cu-CurPy</td>
<td>339</td>
</tr>
<tr>
<td>4</td>
<td>Ni-CurPy</td>
<td>340</td>
</tr>
<tr>
<td>5</td>
<td>Co-CurPy</td>
<td>338</td>
</tr>
</tbody>
</table>

6.3.2.3 Infrared spectra

The various IR bands and their assignments of curcumin pyrazole and its metal complexes are given in the Table 6.3. The strong C=N stretching frequency of curcumin pyrazole at 1549 cm\(^{-1}\) is being shifted to higher wave length region in its metal complexes. The C=N stretching frequencies of curcumin pyrazole in its complex with Mn(II), Mg(II), Cu(II), Ni(II) and Co(II) were 1589 cm\(^{-1}\), 1588 cm\(^{-1}\), 1587 cm\(^{-1}\), 1586 cm\(^{-1}\), and 1585 cm\(^{-1}\), respectively.
1592 cm\(^{-1}\), 1597 cm\(^{-1}\) and 1591 cm\(^{-1}\) respectively. The high frequency shift of the ring \(\nu(-C=\text{N})\) suggest pyrazole ring nitrogen are involved in ligation. In the IR spectra of curcumin pyrazole complexes Fig 6.4-6.8, the -OH band do not show considerable shift from 3447 cm\(^{-1}\). Hence indicating that the phenolic -OH groups are not involved in the complexation.

### Table-6.3 IR bands and their assignments for curcumin pyrazole and its metal complexes

<table>
<thead>
<tr>
<th>Compund</th>
<th>(\nu(\text{O-H})) (cm(^{-1}))</th>
<th>(\nu(\text{N-H})) pyrazole (cm(^{-1}))</th>
<th>(\nu(-\text{C=\text{N}})) pyrazole (cm(^{-1}))</th>
<th>(\nu(\text{C=C})) phenol (cm(^{-1}))</th>
<th>(\nu(\text{O-CH}_3)) (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin pyrazole</td>
<td>3447</td>
<td>3148</td>
<td>1549</td>
<td>1513</td>
<td>1271</td>
</tr>
<tr>
<td>Mn-CurPy (1)</td>
<td>3443</td>
<td>-</td>
<td>1589</td>
<td>1512</td>
<td>1272</td>
</tr>
<tr>
<td>Mg-CurPy (2)</td>
<td>3428</td>
<td>-</td>
<td>1594</td>
<td>1510</td>
<td>1275</td>
</tr>
<tr>
<td>Cu-CurPy (3)</td>
<td>3428</td>
<td>-</td>
<td>1592</td>
<td>1512</td>
<td>1270</td>
</tr>
<tr>
<td>Ni-CurPy (4)</td>
<td>3426</td>
<td>-</td>
<td>1597</td>
<td>1514</td>
<td>1265</td>
</tr>
<tr>
<td>Co-CurPy (5)</td>
<td>3440</td>
<td>-</td>
<td>1591</td>
<td>1512</td>
<td>1269</td>
</tr>
</tbody>
</table>

Figure 6.4 IR spectra of Mn-curcumin pyrazole complex
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Figure 6.5 IR spectra of Mg-curcumin pyrazole complex

Figure 6.6 IR spectra of Cu-curcumin pyrazole complex

Figure 6.7 IR spectra of Ni-curcumin pyrazole complex
6.3.2.4 Thermal Analysis

TGA was used to determine degradation temperatures and absorbed moisture content. TG curves Fig 6.9, suggest that there is coordinated water in the complexes. Decomposition of the complexes occurs at a higher temperature when compared to the ligand curcumin pyrazole.
Figure 6.9 TG-DTG curves  (i) Curcumin pyrazole  (ii) Mn-curcumin pyrazole  (iii) Mg-curcumin pyrazole, (iv) Cu-curcumin pyrazole (v) Ni-curcumin pyrazole (vi) Co-curcumin pyrazole

Curcumin pyrazole was stable up to 200°C and after 400°C it underwent complete decomposition. All complexes showed the presence of coordinated water.
6.3.2.5 Cyclic voltammetric studies

The cyclic voltammogram of curcumin pyrazole complexes in DMSO are given in the Fig 6.10-6.14 and Table 6.4. Study were done with complexes of concentration \( (1 \times 10^{-5} \text{ M}) \) in DMSO with Tetrabutylammonium perchlorate as the supporting electrolyte. The \( I_{pc}/I_{pa} \) shows a one electron transport take place in all the complexes.

Table 6.4 Cyclic voltammetric data of curcumin pyrazole complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( E_{pc}(\text{mV}) )</th>
<th>( E_{pa}(\text{mV}) )</th>
<th>( E_{1/2}(\text{mV}) )</th>
<th>( \Delta E_p(\text{mV}) )</th>
<th>( I_{pc}/I_{pa} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn-CurPy</td>
<td>-491</td>
<td>-245</td>
<td>-368</td>
<td>246</td>
<td>.895</td>
</tr>
<tr>
<td>Mg-CurPy</td>
<td>-861</td>
<td>-707</td>
<td>-784</td>
<td>154</td>
<td>.913</td>
</tr>
<tr>
<td>Cu-CurPy</td>
<td>-884</td>
<td>-690</td>
<td>-787</td>
<td>194</td>
<td>1.1</td>
</tr>
<tr>
<td>Ni-CurPy</td>
<td>-992</td>
<td>-747</td>
<td>-670</td>
<td>245</td>
<td>1.1</td>
</tr>
<tr>
<td>Co-CurPy</td>
<td>-901</td>
<td>-712</td>
<td>-807</td>
<td>189</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fig 6.10 Cyclic voltammogram of Mn-curcumin pyrazole
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Fig. 6.11 Cyclic voltammogram of Mg-curcumin pyrazole

Fig. 6.12 Cyclic voltammogram of Cu-curcumin pyrazole
Synthesis of curcumin pyrazole and its metal complexes: Antioxidant activity and DNA binding study of curcumin pyrazole complexes

Fig. 6.13 Cyclic voltammogram of Ni-curcumin pyrazole

Fig. 6.14 Cyclic voltammogram of Ni-curcumin pyrazole
6.3.3 Antioxidant activity study

The details of the DPPH solution prepared are discussed in Chapter 2. The concentration of curcumin pyrazole used for the DPPH assay varied from 13-30 mmol. The solvents used for the study were methanol, acetonitrile, acetone, ethyl acetate and 1,4-dioxane.

6.3.3.1 Solvent effect on antioxidant property of curcumin pyrazole

The result of antioxidant activity of curcumin pyrazole using DPPH assay method is as shown in Fig 6.15. The solvents used for the study were methanol, acetonitrile, acetone, ethyl acetate and 1,4-dioxane. The antioxidant activity was found to be far high in methanol and least in 1,4-dioxane. The magnitude of antioxidant activity in the five solvents follow the order 1,4-dioxane < ethyl acetate < acetone < acetonitrile < methanol. IC_{50} values of curcumin pyrazole are as given in Table 6.5 from which it were evident that the antioxidant activity is dependent on medium in which study has been done.

![Fig 6.15 Kinetic solvent effect on antioxidant activity of curcumin pyrazole](image)
Table 6.5 IC₅₀ values of curcumin- pyrazole and curcumin-I in various solvents

<table>
<thead>
<tr>
<th>SOLVENTS</th>
<th>Curcumin-I IC₅₀(µM)</th>
<th>Curcumin-pyrazole IC₅₀(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Acetone</td>
<td>110</td>
<td>89</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>180</td>
<td>137</td>
</tr>
<tr>
<td>1,4 Dioxane</td>
<td>341</td>
<td>271</td>
</tr>
</tbody>
</table>

The antioxidant activity of curcumin pyrazole was found to be more in comparison to curcumin-I. This might be due to resonance stabilization of the free radical centre of pyrazole ring similarly as of phenols and are as depicted in Fig 6.16.

![Resonance stabilisation of phenoxide and pyrrole radical](image)

**Fig 6.16 Resonance stabilisation of phenoxide and pyrrole radical**

6.3.3.1.1 Antioxidant activity in polar protic solvents

Among the solvents used to study the antioxidant property of curcumin pyrazole by DPPH assay, the maximum activity was shown in methanol mediated reactions. This anomalous behaviour was due to protic nature of methanol forming intermolecular hydrogen bonding among solvent thereby preventing ArOH…S.
interaction as in Scheme 3. Hence, ArOH (substrate) to S (solvent) equilibrium do not exist in methanol, facilitating the ionisation of ArOH depending on the bulk property of the solvent, its relative permittivity (dielectric constant, \( \varepsilon_r \)), molecular property, its relative ability to solvate stabilising anions (ArO\(^-\)), as quantified by Swain et al’s A value \[27\]. Methanol having high \( \varepsilon_r \) and A values \( (33, 0.75) \) supports ionisation and hence ArOH looses proton to form stable phenoxy ion at very fast rate. Consequently quenching of DPPH occurs at low concentration of ArOH, giving a very low IC\(_{50}\) value \[28\]. All these fact support the existence of SPLET in methanol.

![Scheme 3 Anomalous behaviour of ArOH in protic solvent- methanol](image)

6.3.3.1.2 Antioxidant activity in polar aprotic solvents

In polar aprotic solvents the order of antioxidant activity was acetonitrile > acetone > ethyl acetate. The IC\(_{50}\) values were as in Table 6.5. The polar aprotic solvent with high dielectric constant \( (37, 21) \) was expected to give high activity in terms of dielectric constant \[29\], which was true for acetonitrile but not for acetone. The high activity in acetonitrile as when compared to the very low activity in acetone can be interpreted in terms of the solvent interaction of these medium as represented in Fig 3.9 in Chapter 3. The hydrogen bonding interactions between highly electronegative oxygen of acetone and hydrogen of phenol give rise to strong ArOH….S (solvent) interactions \[30\], the primary factor contributing to the low rate of H-abstraction from a phenol to a free radical. Hence phenol deprotonation is difficult and only those molecules which are free for the deprotonation as in Scheme 4 \[29\] will be available to interact with DPPH to form DPPHH via HAT. HAT being slow will subsequently lead to higher IC\(_{50}\) value for all the substrates in acetone as when compared to very low value in acetonitrile. As
acetone supports ionization, the measured rate would be the sum of the rates for the HAT (black) and SPLET (red) Scheme 5 [31]. Whether the SPLET mechanism will be significant compared with the HAT mechanism will depend on the phenol’s acidity, the radical’s electron affinity, the ability of the solvent to support phenol ionization. In acetone, contribution from HAT will be more when compared to SPLET as evident from higher IC$_{50}$ value. Acetonitrile in which solvent interaction is very less and the major reaction is expected to proceed via SPLET, as evident from its low IC$_{50}$ value and antioxidant activity comparable to that in methanol.

Scheme-4 [11] Origin of kinetic solvent effects in a HAT reaction in polar aprotic solvents –that do not support ionisation

Scheme-5 [26] Origin of kinetic solvent effects in a mixed HAT and SPLET reaction in polar aprotic solvents –that support ionisation
6.3.3.1.3 Antioxidant activity in non-polar solvents

In the non-polar solvent 1,4-dioxane used to study the reaction kinetics the activity was very low when compared to methanol due to the low ε and A values (2.2, 0.19) [28]. Dioxane being a non-polar solvent that may not support ionisation, it is assumed that reaction will take a path of slower HAT as shown in Scheme 6 [32]. HAT being very slow will require higher concentration of the antioxidant to bring 50% inhibition of DPPH. This in turn is expected to give higher IC$_{50}$ value in solvent like 1,4-dioxane.

\[
\text{ArOH} + \text{DPPH} \rightarrow \text{ArO} + \text{DPPHH}
\]

Scheme 6 [31] HAT mechanism in non-polar, non-ionising solvent

6.3.3.2 Antioxidant property of metal complexes of curcumin pyrazole

Curcumin pyrazole (0.5 mg mL$^{-1}$)/metal complex (0.5 mg mL$^{-1}$) solutions in DMSO were prepared separately and added to a methanol solution of DPPH (0.01 mmol) within the range of 10-120 µL and made up to a final volume of 3 mL using methanol as solvent. The scavenging ability of curcumin pyrazole and its metal complexes were monitored spectrophotometrically in terms of decrease in absorbance at 517 nm after 20 min.

The DPPH scavenging activity of metal complexes were less than that of curcumin pyrazole and are depicted in Fig. 6.17. The IC$_{50}$ value of the complexes decreases in the order of Mn(II) > Mg(II) > Cu(II) > Ni(II) > Co(II) as shown in Table 6.6.
Fig 6.17 Antioxidant activity of metal complexes of curcumin pyrazole

Table-6.6 IC<sub>50</sub> value in μM of Curcumin pyrazole and its metal complexes

<table>
<thead>
<tr>
<th>Materials</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin-I</td>
<td>13</td>
</tr>
<tr>
<td>Mn-CurPy</td>
<td>14</td>
</tr>
<tr>
<td>Mg-CurPy</td>
<td>16</td>
</tr>
<tr>
<td>Cu-CurPy</td>
<td>29</td>
</tr>
<tr>
<td>Ni-CurPy</td>
<td>31</td>
</tr>
<tr>
<td>Co-CurPy</td>
<td>44</td>
</tr>
</tbody>
</table>

6.3.4 DNA- binding studies

Interaction of metal complexes with DNA has lead to investigation for new chemotherapeutic agent in biotechnology and medicine [33]. Small molecule have found to react with specific site along a DNA strand, which has been used as a model for protein nucleic acid interaction and has provided a new route to drug design and development. Interaction of metal complexes with DNA is used as probe for DNA structure in solution and as chemotherapeutic agents [33,34]. In our
work, interaction of metal complexes of curcumin pyrazole with DNA has been studied using spectral, electrochemical and CD techniques.

### 6.3.4.1 Electronic absorption titration

Electronic absorption spectroscopy is one of the most common technique used in DNA-binding studies of metal complexes. The magnitude of spectral perturbation is an evidence for extent of binding [35]. The two effect characteristics of interaction of metal complexes with DNA are hypochromism and hyperchromism. Hypochromic effect is attributed to interaction between electronic states of complex with DNA bases [36]. Hyperchromism can be ascribed to external contact or partial uncoiling of helical structure exposing more bases of the DNA [37].

The electronic absorption titrations were carried out using fixed amount of each metal complex of curcumin pyrazole (30 µM) with increasing concentration of DNA as in Fig 6.18(a)-6.21(a). The Mn(II), Mg(II), Cu(II) and Co(II) complexes (1,2,3 & 5) of curcumin pyrazole gave absorption band in the region of 320-328 nm in DMSO- buffer mixture were assigned due to $\pi-\pi^*$ transition. All the complexes exhibited hypochromism in their absorption maximum with no change in band position.

To further illustrate the binding strength of the curcumin pyrazole complexes with DNA, the intrinsic binding constant $K_b$ was determined from the spectral titration data. By monitoring the changes in absorption at corresponding $\lambda_{\text{max}}$ with increasing HS-DNA concentration and $K_b$ can be calculated using the Eq (1) [38].

\[
\frac{[\text{DNA}]}{(c_a-c_{\text{f}})} = \frac{[\text{DNA}]}{(c_b-c_{\text{f}})} + \frac{1}{K_b (e_b-e_{\text{f}})}
\tag{1}
\]

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Where [DNA] is the concentration of DNA in base pair, $\varepsilon_a$ is the apparent extinction coefficient for each addition of DNA to the complex ($A_{\text{abs}}/[\text{Complex}]$), $\varepsilon_f$, extinction coefficient for free metal complex and $\varepsilon_b$, extinction coefficient for metal complex in the fully bound form.

A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ gives binding constant $K_b$ as the ratio of slope by intercept. The magnitude of $K_b$ value gives the extent of binding. The absorption spectra of pyrazole complexes of Mn(II), Mg(II), Cu(II) and Co(II) of $(3 \times 10^{-5}$M) in Tris-HCl buffer pH 7.1 in the absence (R=0) and presence of increasing amount of DNA are represented in Fig 6.18(a)-6.21(a). A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ for the titration of DNA with complex for binding constant are represented in Fig 6.18(b)-6.21(b). The binding constant values obtained for the present curcumin pyrazole complexes can be represented as in Table 6.7. From the binding constant values the obtained order was Mn(II) < Co(II) < Mg(II) < Cu(II). These values and order obtained where comparable to that of complexes of macrocyclic tetraaza diacetyl curcumin ligand [39] with Cu(II), Co(II) and Mn(II) as metals. The comparison of binding constant values can be represented as in Table 6.6.

Table 6.7 The binding constant, $K_b$ (M$^{-1}$) for curcumin pyrazole complexes and tetraaza diacetyl curcumin complexes

<table>
<thead>
<tr>
<th>SL No</th>
<th>Complexed metal</th>
<th>Binding Constant $K_b$ (M$^{-1}$)</th>
<th>Curcumin-pyrazole</th>
<th>Tetraaza diacetyl curcumin [39]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mn (II)</td>
<td>2.9×10$^4$</td>
<td>3.5×10$^4$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Co (II)</td>
<td>5.4×10$^4$</td>
<td>5.2×10$^4$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mg (II)</td>
<td>1.6×10$^5$</td>
<td>6.3×10$^5$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cu (II)</td>
<td>2.4×10$^5$</td>
<td>1.4×10$^5$</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 6

Curcumin and its derivatives as Antioxidants and DNA Intercalators

Fig 6.18 (a) Absorption spectra of Mn-CurPy complex (3×10^{-5}) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R=0.3, 0.4, 0.5, 0.6, 0.7 & 0.9) of increasing amount of DNA. R=[DNA]/[Complex]

Fig 6.18 (b) A plot of [DNA]/(ε_a-ε_b) versus [DNA] for the titration of DNA with complex

Fig 6.19 (a) Absorption spectra of Co-CurPy complex (3×10^{-5}) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R= 0.2, 0.4, 0.6, & 0.8) of increasing amount of DNA. R=[DNA]/[Complex]

Fig 6.19 (b) A plot of [DNA]/(ε_a-ε_b) versus [DNA] for the titration of DNA with complex
Synthesis of curcumin pyrazole and its metal complexes: Antioxidant activity and DNA binding study of curcumin pyrazole complexes

Fig 6.20 (a) Absorption spectra of Mg-CurPy complex ($3 \times 10^{-5}$) in Tris-HCl buffer of pH 7.1 in the absence ($R=0$) and presence ($R=0.2, 0.4, 0.6, 0.8 & 1$) of increasing amount of DNA. $R=[\text{DNA}]/[\text{Complex}]

Fig 6.20 (b) A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_0)$ versus $[\text{DNA}]$ for the titration of DNA with complex

Fig 6.21 (a) Absorption spectra of Cu-CurPy complex ($3 \times 10^{-5}$) in Tris-HCl buffer of pH 7.1 in the absence ($R=0$) and presence ($R=0.2, 0.4, 0.6 & 1.2$) of increasing amount of DNA. $R=[\text{DNA}]/[\text{Complex}]

Fig 6.21 (b) A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_0)$ versus $[\text{DNA}]$ for the titration of DNA with complex
6.3.4.2 Cyclic Voltammetric studies

Electrochemical method is complementary to UV method of investigating metallointercalation and coordination of metal ions to DNA. Voltammetric techniques can be used as a tool to study interactions of small molecules with either weak absorption bands or those having electronic transitions overlapping with the DNA molecule. The advantage of this technique is that multiple oxidation states of the same species as well as mixtures of several interacting species can be observed simultaneously [40]. The Cu(II) and Mn(II) complex of curcumin pyrazole were selected for voltammetric studies Fig 6.22 & 6.23 and their redox behaviour were studied in the absence and presence of DNA at room temperature at the scan rate of 100 mVs\(^{-1}\). On increasing the concentrations of DNA, the peak currents and \(E_{pc}\) decreased, Table 6.8.

Electrochemical studies can be used to quantify the binding of the metal complex to DNA, in terms of change in current and formal potential upon addition of DNA, due to the difference in diffusion rate of an equilibrium mixture of free and DNA-bound metal complex to the electrode surface [40]. The effect of varying concentration of DNA with fixed concentration of complex, was a decrease in anodic and cathodic peak currents. The decrease in current could be attributed to slow diffusion of the metal complex bound to the large, slowly diffusing DNA [40]. The variation in current and formal potential suggests an association of the complex with DNA. According to Brads report [40], the decrease in potential (\(E_{pc}\)) and current (\(I_{pc}\)), imply that the interaction mode is electrostatic or groove binding. The DPV of copper complex of curcuminpyrazole ascertain the changes found in CV.
Synthesis of curcumin pyrazole and its metal complexes: Antioxidant activity and DNA binding study of curcumin pyrazole complexes

Fig 6.22 (a) Cyclic voltammogram and (b) Differential pulse voltammogram of Cu-pyrazole complex (3x10^-5) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R= 0.4, 0.6 & 1) of increasing amount of DNA

Fig 6.23 Cyclic voltammogram of Mn-pyrazole complex (3x10^-5) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R= 0.4, 0.6 & 1) of increasing amount of DNA

Table 6.8 Voltammetric behaviour of pyrazole complexes in Tris HCl buffer pH 7.1 in the absence and presence of DNA

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Epc(mV)</th>
<th>Epa(mV)</th>
<th>ΔEpc(mV)</th>
<th>E1/2(mV)</th>
<th>ΔE1/2 (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu-CurPy</td>
<td>0</td>
<td>276</td>
<td>310</td>
<td>34</td>
<td>294</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>240</td>
<td>317</td>
<td>77</td>
<td>279</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>180</td>
<td>230</td>
<td>50</td>
<td>205</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>96</td>
<td>211</td>
<td>115</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>Mn-CurPy</td>
<td>0</td>
<td>224</td>
<td>-19</td>
<td>-243</td>
<td>102</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>216</td>
<td>-27</td>
<td>-243</td>
<td>94</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>202</td>
<td>-28</td>
<td>-230</td>
<td>87</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>198</td>
<td>-32</td>
<td>-230</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>
6.3.4.3 Circular dichroism studies

Circular Dichroism (CD) is a useful technique to probe non-covalent drug-DNA interactions, which affect the electronic structure of the molecules and also alter their electronic spectroscopic behaviour [41,42]. CD spectroscopy allows for quick characterization of the drug-DNA interaction using a small amount of sample, as low as (20 µg mL\(^{-1}\)) [43]. When electromagnetic radiation reaches DNA, the macromolecules present a certain degree of alignment in the direction of the electric field vector and this molecular alignment is measured by the light polarized absorbance. Depending on the binding modes, spectrum of DNA will be modified with increasing concentration of small drugs or metal complexes and characteristic conformation changes would be observed in DNA.

The theoretical (i.e., quantum chemical) description of CD spectra of molecules as large as DNA is very complex and hence do not provide structural information on molecules at atomic level. CD spectroscopy is therefore used primarily for empirical studies of DNA and it has many advantages over other methods of conformational analysis. It is extremely sensitive, permitting work with very low amount of DNA (20 µg mL\(^{-1}\)), the samples can easily be titrated with various agents (like salts, alcohols or acids) that induce conformational isomerizations in DNA and CD measurements are fast and relatively inexpensive that allows comparative studies of related molecules of DNA under many conditions [43].

A solution of HS-DNA shows a positive band (275 nm) from base stacking interaction and a negative band at (245 nm) from right-handed helicity of DNA characteristic of DNA in the right-handed B form [44]. When HS-DNA was titrated with increasing concentration of curcumin pyrazole complex of Mn(II) and
Cu(II) the bands were modified Fig.6.24 (a) and (b). The change in intensity of DNA band Table 6.9, suggests a conformational change in DNA which is relative to the mode of binding of complex to DNA. The CD spectra shows slight perturbation of the bands with decrease in intensity in both positive and negative bands and zero cross over at 258 nm suggesting the stacking mode and disturbance in the orientation of base pairs of DNA. With increasing concentration of curcumin pyrazole complex of Mn(II) and Cu(II), the peaks at 275 and 245 nm of DNA shift to 1–3 nm without any change in the zero-cross over at 258 nm is indicative of groove binding mode [45]. This suggests that binding of the complexes to DNA induces certain conformational changes in DNA of B-like to a more A-like structure [46]. The simple groove binding and electrostatic interaction of small molecules show less or no perturbation on the base-stacking and helicity bands, while for the classical intercalator like methylene blue enhances the intensities of both the bands stabilizing the right-handed B conformation of DNA [47].

![CD spectra of HS-DNA](image)

**Fig 6.24 (a)** CD spectra of HS-DNA (3×10^{-5}) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R=0.4 & 1) of Cu-CurPyrazole

**Fig 6.24 (b)** CD spectra of HS-DNA (3×10^{-5}) in Tris-HCl buffer of pH 7.1 in the absence (R=0) and presence (R=0.4 & 0.8) of Mn-CurPyrazole
Table 6.9 CD parameter for DNA-complex interaction of pyrazole complex of Copper and Manganese

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Positive band</th>
<th>Negative band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>CD (mdeg)</td>
</tr>
<tr>
<td>Cu-CurPy</td>
<td>0.00</td>
<td>275</td>
<td>9.9892</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>275</td>
<td>9.5114</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>278</td>
<td>8.5809</td>
</tr>
<tr>
<td>Mn-CurPy</td>
<td>0.00</td>
<td>275</td>
<td>10.6248</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>275</td>
<td>9.2534</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
<td>278</td>
<td>8.9438</td>
</tr>
</tbody>
</table>

6.4 Conclusions

Curcumin pyrazole has been synthesised and are structurally characterised. The antioxidant property of the compound has been studied using DPPH. Pyrazole derivative of curcumin was found to be more active than curcumin. Solvent effect on antioxidant property of pyrazole was checked in methanol, acetonitrile, acetone, ethyl acetate and 1,4-dioxane. The observed antioxidant activity was found to be high in methanol and least in 1,4-dioxane. The magnitude of antioxidant activity in the five solvents follow the order 1,4-dioxane < ethyl acetate < acetone < acetonitrile < methanol. The variation in antioxidant activity with solvent could be explained in terms of type of solvent used depending on its dielectric constant and interaction of the pyrazole with solvent medium.

Pyrazole derivative were used as ligand for the synthesis of metal complexes which has been subjected to DNA binding studies using various techniques like UV-Vis, CV and CD. The observed results showed that the curcumin-pyrazole complexes interact with DNA with increasing concentration of DNA. From the plot of $[\text{DNA}] / ([e_{\text{a}} - e_{\text{i}}])$ versus $[\text{DNA}]$ of the titration of DNA with...
Synthesis of curcumin pyrazole and its metal complexes: Antioxidant activity and DNA binding study of curcumin pyrazole complexes

Complex, the binding constant ($K_b$) value was calculated and the order obtained is Mn(II) < Co(II) < Mg(II) < Cu(II).

Cyclic voltammetric measurement studies were done with curcumin pyrazole complex of Mn(II) and Cu(II). On increasing the concentrations of DNA, the peak currents and Epc decreased. The variation in current and formal potential suggested an association of the complex with DNA. From the electrochemical data the mode of binding was suggested as electrostatic or groove binding.

Circular dichroism studies were also carried out to ascertain the mode of binding of complex to DNA. In CD fixed concentration of DNA was titrated against increasing concentration of curcumin pyrazole complex of Cu(II) and Mn(II). The spectra showed slight perturbation on the bands with decrease in intensity in both positive and negative bands with increasing concentration of curcumin pyrazole complex of Mn(II) and Cu(II). The peaks at 275 and 245 nm of Mn(II) and Cu(II) complex shift to 1–3 nm without any change in the zero-cross over at 258 nm which was indicative of groove binding mode.

6.5 References


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Chapter 6


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Conclusions

Over the past few decades there has increasing demands on plant derived compounds as antioxidant. The unanswered question of the present scenario, whether medicines discovered today are safer, more effective and more affordable than the traditional age-old medicines that are in use for centuries for the welfare of mankind. Since the above thought advises us to revisit and revive the use of traditional remedies which far more superior than the modern medicine in terms of efficiency and having fewer side effects. Curcumin is one such medicine that has been used by the people of the Indian subcontinent for centuries with no known side effects, not only as a component of food, but also to treat a wide variety of ailments. Its history goes back over 5000 years, to the heyday of Ayurveda (which means the science of long life). Although its capability to preserve food is been utilised, the ability to give colour to food and to add taste to the food is also well acknowledged, its health-promoting effects are less well documented or appreciated. It was once considered a cure for jaundice, an appetite suppressant and digestive. In Indian and Chinese medicines, turmeric was used as anti-inflammatory agents to treat gas, colic, toothaches, chest pains and menstrual difficulties. This spice was also used to help with stomach and liver problems, heal wounds and scars and as a cosmetic.

In the thesis we represent our findings related to curcumin and its derivatives in terms of structure activity relationship. Efforts have been furnished to synthesis few derivatives modifying the 1,3-diketomoeity and keeping the phenolic part intact. This mode of study was selected keeping in mind the controversy regarding the active part of curcumin in deciding its antioxidant
activity. From the study result we were able to furnish conclusions regarding the relation between structure and activity.

Thesis is divided into six chapters. The first chapter provides an insight into the different biological activity of curcumin. The studies that have attempted to explain the activity in terms of its structure is also discussed in the chapter; subsequently the relevance and scope of the present work.

**Chapter 2:** The metal [Mn(II), Mg(II), Cu(II), Ni(II) and Co(II)] complexes of curcumin with 1:1 ligand to metal ratio has been synthesized and characterized. The DPPH scavenging activity of metal complexes were less than that of curcumin-I. The antioxidant activity of the complexes decreases in the order of Mn(II) > Mg(II) > Cu(II) > Ni(II) > Co(II). The difference in activity of curcumin-I and its complexes can be inferred in terms of involvement of different reaction centre of curcumin in free radical quenching. Intracellular ROS content was determined by oxidative conversion of cell-permeable DCFH-DA to fluorescent 2′,7′ dichlorofluorescein (DCF) in H9c2 cells. The fluorescence imaging data was used to quantify the presence of ROS in H9c2 cells. The intensity of fluorescence was decreased in the H2O2 stress induced groups that were subjected to pre-treatment with curcumin/its metal complexes for 24 h. This hold up the fact that even in the cell lines the metal complexes in which the enol centre was blocked shows activity comparable to curcumin, further emphasizing the findings of the DPPH study that phenolic centre is the major centre for antioxidant activity. The complexes have comparable antioxidant activity to parent curcumin-I, establishing the minimal involvement of keto-enol moiety of curcumin as the antioxidant centre and hold up the phenolic -OH as the prime centre for the antioxidant activity.
**Chapter 3:** To establish mechanistic paths for the electron transfer from the phenolic group of curcumin to free radical (DPPH) solvent effect study has been done. The study of antioxidant property of curcumin-I, II and metal complexes of curcumin-I using DPPH in five solvent showed an activity in the order of methanol > acetonitrile > acetone > ethyl acetate > 1,4-dioxane. The complex in which enol form was blocked also showed the same trend as curcumin-I and II but slightly less active when compared to the parent compound. This point’s to the fact that even though the enol form is not available for the radical formation, sufficient scavenging of DPPH occurs and can be concluded as the consequence of phenoxide ion formation. Curcumin-I is more reactive than curcumin-II in all the solvent selected for the study, curcumin-I has two methoxy groups which is electron donating compared to one methoxy in curcumin-II. In the complexes formed it was the enolate ion that chelate to metals. Metals being electropositive decrease the electron density at -OH group thereby declining the possible electron donation to DPPH and hence the antioxidant activity. This is consistent with the observation that metal complexes are slightly less active than the parent compound in all the solvent. As curcumin-I, II and metal complexes show the same trend in antioxidant activity in solvents selected for the study, it is suggestive that mechanistic path way followed by the substrates are the same.

**Chapter 4:** Two derivatives of curcumin, salicylidenecurcumin (CD1) and benzalidenecurcumin (CD2) were prepared by the Knoevenagel condensation of curcumin-I with the aldehydes-salicylaldehyde and benzaldehyde along the active methylene group and their efficacy evaluated against selenite cataract model. The study was designed to compare the biological activity of curcumin and two of its Knoevenagel
condensates, salicylidene-curcumin (CD1) and bezaledene-curcumin (CD2) on *in vitro* selenite induced cataract models. The study establishes the contribution of diketo group (in the absence of keto/enol tautomerism) in deciding its activity. The derivatives of curcumin, CD1 and CD2 were found to be effective in overcoming biochemical changes occurring in cataractous lenses. In comparison, the derivatives of curcumin, CD1 was superior to CD2 in the attenuation of selenite induced cataract. The presence of an additional -OH group in the salicyledene is proposed as the cause of an appreciable increase in antioxidant property. Our findings suggest that the curcumin derivative, salicyledene-curcumin (CD1) is endowed with enhanced antioxidant property and keto-enol structure is not a necessary requirement for deciding antioxidant activity. The order of increase in antioxidant property was curcumin < CD2 < CD1. The higher activity of bezaledene-curcumin (CD2) when compared to curcumin with the same number of phenolic OH may be due to the extended conjugated system.

*Chapter 5:* Curcumin complexes have been subjected to DNA binding studies using various techniques like UV-Vis, CV and CD. The results showed that with increasing concentration of DNA, the complexes interact with DNA in groove binding mode. Morphological alterations of H9c2 cells after 48h of treatment with curcumin and its metal complexes of [Cu(II), Mn(II), Ni(II) and Co(II)] showed that cells had normal spindle shaped morphology at all (1, 5, 10, 15 and 20 µM) concentrations of various samples. MTT assay showed that curcumin and its metal complexes showed a reduction in cell viability in a dose-dependent manner when compared to untreated cells.
Chapter 6: Curcumin pyrazole has been synthesised and structurally characterised. The antioxidant property of the compound has been studied using DPPH. Pyrazole derivative of curcumin was found to be more active than curcumin. The magnitude of antioxidant activity in the five solvents follow the order 1,4-dioxane < ethyl acetate < acetone < acetonitrile < methanol. Pyrazole derivative were used as ligand for the synthesis and characterisation of metal complexes which has been subjected to DNA binding studies using various techniques like UV-Vis, CV and CD. The binding constant ($K_b$) value was calculated and the order obtained was Mn(II) < Co(II) < Mg(II) < Cu(II) and the mode of binding was suggested as electrostatic or groove binding.

Scope: Since curcumin pyrazole was found to be more active than parent curcumin, the in vitro study in terms of cytotoxicity and cell availability could be checked to establish its advantage over the parent material. ROS quenching ability of curcumin pyrazole and its metal complexes in cell lines could also be monitored so as to confirm its activity.