

## RESEARCH ARTICLE

# Non-template Tailored Macrocyclic Complexes: Synthesis, Characterization, Biological and Computational Studies

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## ABSTRACT

Some novel biologically active tetraaza-macrocyclic complexes of Fe (III) and Cr (III) were synthesized by non-template method. In this methodology, first macrocyclic ligand was synthesized by condensation reaction between triethylenetetramine and dimesone which was further reacted with trivalent Fe and Cr ions to synthesize the macrocyclic complexes having general formula  $[M(C_{14}H_{26}N_4)(X)](X)_2$  where X represents  $Cl^-$ ,  $NO_3^-$ ,  $CH_3COO^-$ . Various physiochemical studies suggested the square pyramidal geometry around the metal ion of all complexes. With the aid of PXRD technique, it was found that some of the complexes possessed monoclinic crystal system while other complexes showed to have orthorhombic crystal system. The DFT study was also carried out to examine the various quantum descriptors of ligand and its complexes. Moreover, newly synthesized macrocyclic ligand and its complexes with Fe and Cr ions were tested against fungal strain as well various bacterial strains. The results of antibacterial study showed that all complexes exhibited remarkable ability to inhibit the growth of microbes as compared to ligand. All complexes were found to have drug-likeness behavior as indicated by *in-silico* studies. Furthermore, these complexes were also tested for their antioxidant activity and it was observed that complexes showed better antioxidant activity as compared to ligand and standard solution of ascorbic acid.

## 1 | Introduction

Macrocyclic complexes have a productive potential in the biological realm. Consequently, scientists are constantly working toward the goal of synthesizing an increasing number of unique macrocyclic compounds. The macrocyclic complexes possess antimicrobial and antioxidant activities due to their variable oxidation state and structural conformations. For instance, some macrocyclic complexes have been reported to show various biological activities including antibacterial [1], antitumor [2] and antifungal [3], antioxidant [4] and pesticidal activities [5]. Moreover, macrocyclic complexes also act as MRI contrast

agents [6] and DNA binding agents [7]. Therefore, considering the aforementioned biological importance of macrocyclic complexes we wish to report the synthesis of novel macrocyclic complexes. In the present work, a ligand having macrocyclic frame was synthesized by condensation reaction between triethylenetetramine and dimesone followed by synthesis of Fe (III) and Cr (III) complexes of this ligand. Furthermore, the antioxidant and antibacterial activity of these complexes were investigated. Additionally, some computational studies were carried out like *In-silico* molecular docking (using Autodock4.2), screening of toxicity (using Pre ADMET Server), which indicates the biological significance of these complexes. Further, DFT studies

(with Gaussian09W) were also carried out to explore the stability of complexes as compared to ligand and the square pyramidal geometry around the metal ion in complexes.

## 2 | Materials and Methods

The analytical reagent grade triethylenetetramine, dimedone, acetone, acetic acid and DMSO were bought from Sigma-Aldrich. All trivalent salts of Cr and Fe were purchased from CDH. The solvents were utilized as received, without any further purification. For comparative antimicrobial activities, standard drugs Amphotericin-B (an antifungal drug) and Ciprofloxacin (an antibacterial drug) were used. The bacterial culture of *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* was collected from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. IR spectra of all complexes were recorded on MB-3000 FTIR spectrometer. The XEVO G2-XS QT mass spectrometer was used for obtaining the mass spectra of all complexes. Elemental analysis data was obtained from EuroEA elemental analyzer whereas electronic spectra were recorded using Perkin Elmer Lambda U.V. Spectrophotometer. The CM-183 conductivity meter was used to measure the conductance of all complexes. The magnetic susceptibilities of all complexes were determined using “PAR 155 magnetometer”. Perkin Elmer thermal analyzer was used to examine the thermal stability of all complexes. The powder X-ray diffraction studies were conducted on X’pret Pro, X-ray Diffractometer. The gravimetric approach was adopted to determine the metal contents present in chromium complexes. The back titration method was used to determine the metal content of the iron complexes. The electrical melting point machine was employed to determine the melting point of complexes by using fine capillaries.

### 2.1 | Synthesis of Macrocyclic Ligand

The ligand possessing macrocyclic frame was obtained from the condensation reaction between triethylenetetramine (20 mmol) and dimedone (20 mmol) in the methanolic solution (Scheme 1). In the reaction mixture few drops of conc. HCl was added. This mixture was refluxed for 24 h with continuous stirring. The precipitates so obtained were filtered, washed with methanol and dried in vacuum desiccators.

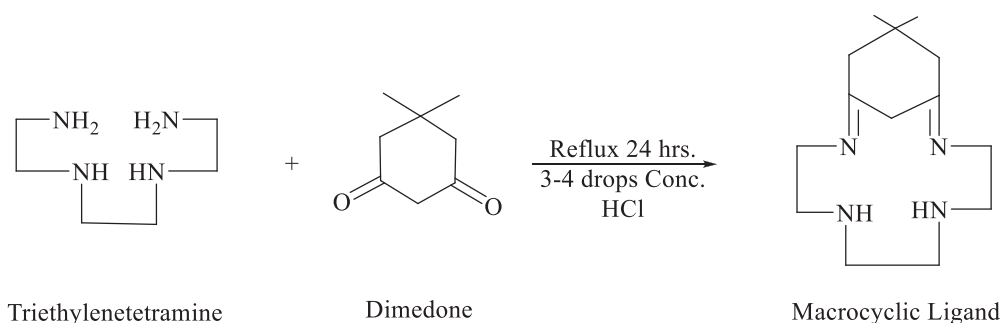
### 2.2 | Synthesis of Macrocyclic Complexes

The macrocyclic ligand and trivalent metal salt of Fe or Cr (1:1 M ratio) in methanol were refluxed with continuous stirring for 6–7 h (Scheme 2). Excess of the solvent was removed and the concentrated solution was cooled for 10–12 h. The colored precipitates of the complexes were obtained which were filtered and washed with ethanol and acetone. These complexes were then dried in vacuum desiccators.

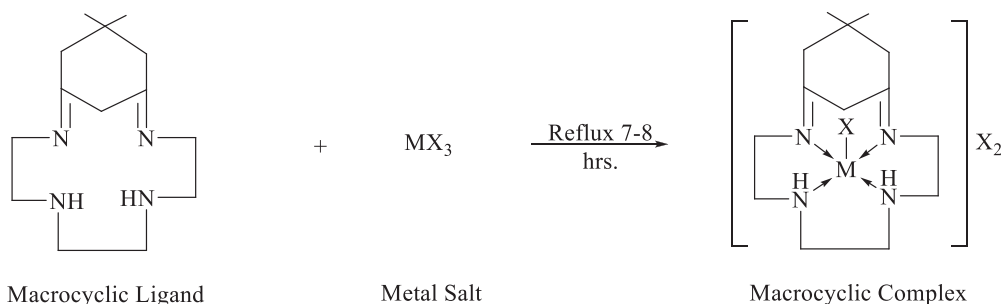
### 2.3 | Biological Activities

#### 2.3.1 | Antioxidant Activity

The free radical scavenging technique was used to investigate the antioxidant activity of ligand and its complexes. In this procedure, a 1 mM standard solution of DPPH was prepared in methanol. DMSO was used to make the standard solution of ascorbic acid, ligand and the solution of complexes having different concentrations ranging from 0 to 45 mg/mL. One milliliter of sample solution was added to a 3 mL methanolic solution



**SCHEME 1** | Synthesis of macrocyclic ligand.



**SCHEME 2** | Synthesis of trivalent macrocyclic complexes; where M = Cr and Fe and X = Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>.

of DPPH. The above prepared mixture solution was incubated for 30 min at room temperature. At 517 nm, the absorbance of the solutions was measured. The measured absorbance value of the control sample and the samples of different concentration were used to determine the dropped DPPH concentration. The percentage of radical scavenging activity of all complexes was determined using the following equation:

$$\% \text{ of Radical scavenging activity} = \frac{A_o - A_c}{A_o} \times 100 \quad (1)$$

Where  $A_o$  = absorbance of the control sample.  $A_c$  = absorbance of the sample concentration C.

### 2.3.2 | Antimicrobial Activity

The ligand and all complexes were evaluated against gram-positive (*B. subtilis*), gram-negative (*E. coli*), and yeast microorganisms (*C. albicans*). The agar well diffusion technique [8] was used to estimate the zone of inhibition (Table 1).

The obtained findings were compared with the commercially available antibacterial and antifungal drugs, such as Ciprofloxacin and Amphotericin-B, respectively. The following equation was used to calculate the activity index of all synthesized complexes:

$$\% \text{ activity index} = \frac{\text{ZOI of sample}}{\text{ZOI of standard drug}} \times 100 \quad (2)$$

## 2.4 | In-Silico Studies

### 2.4.1 | DFT Calculation

The global and chemical reactivity descriptors of newly synthesized ligand and all complexes were calculated by DFT study at B3LYP/6-31G(d,p) in Gaussian 09 W [9, 10] and visualized in Gaussian view 05 software [10].

### 2.4.2 | ADMET Studies

The ADMET tests were utilized to predict the drug-like features of all synthesized complexes [11]. The ADMET characteristics of all synthesized complexes were evaluated with the use of an online Swiss ADMET predictor.

### 2.4.3 | Molecular Docking

Simulation of docking was conducted using the AutoDock 4.2 software [12]. This helped to examine the interactions between proteins and complexes. The target site was established with the addition of Kollaman charges in PDB: 2Y1B. The complexes were treated as ligands after adding gasteiger charges to them [13]. The Lamarckian Genetic Algorithm (LGA) was used for analyzing the best conformers. For each complex, a maximum of 10 conformers were examined. The Discovery Studio 2017 was used to make a picture of the best conformer out of 10 conformers. These findings reveal the binding energy or the interactions between the protein and complex.

## 3 | Result and Discussion

Analytical data of synthesized macrocyclic ligand and its complexes with Cr (III) and Fe (III) ions gave satisfactory results that were in good agreement with the proposed general formula  $[M(L)(X)](X)_2$ , where  $M = \text{Cr (III) and Fe (III)}$ ;  $L = \text{C}_{14}\text{H}_{26}\text{N}_4$  and  $X = \text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{CH}_3\text{COO}^-$ . Moreover, it was determined that the molar conductance value of all synthesized complexes was found within the range of 149 to 162  $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$  (Table 2), which indicated that all complexes were of 1:2 electrolytic nature [14]. Further, it was noticed that all of these complexes were soluble in DMSO. The melting point of complexes was found in the range of 200°C–240°C indicating their thermal stability. By using different analytical and spectroscopic techniques, it was revealed that each of these complexes had a square pyramidal geometry around the central metal ion.

**TABLE 1** | The diameter of inhibition zone of all synthesized complexes and standard drugs.

| Code name   | Complex name                                                                    | Diameter of inhibition zone (mm) |                         |                         |
|-------------|---------------------------------------------------------------------------------|----------------------------------|-------------------------|-------------------------|
|             |                                                                                 | Gram +ve bacteria                | Gram –ve bacteria       | Fungi                   |
|             |                                                                                 | <i>Bacillus subtilis</i>         | <i>Escherichia coli</i> | <i>Candida albicans</i> |
| Ligand      | $\text{C}_{14}\text{H}_{26}\text{N}_4$                                          | 12                               | 13                      | NT                      |
| <b>C-1</b>  | $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{Cl})](\text{Cl})_2$     | 33                               | 38                      | NT                      |
| <b>C-2</b>  | $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{NO}_3)(\text{NO}_3)_2]$ | <b>38</b>                        | 37                      | 21                      |
| <b>C-3</b>  | $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{OAc})(\text{OAc})_2]$   | 27                               | 30                      | 19                      |
| <b>C-4</b>  | $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{Cl})](\text{Cl})_2$     | 32                               | 34                      | NT                      |
| <b>C-5</b>  | $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{NO}_3)(\text{NO}_3)_2]$ | 36                               | <b>42</b>               | <b>23</b>               |
| <b>C-6</b>  | $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{OAc})(\text{OAc})_2]$   | 25                               | 29                      | NT                      |
| <b>SD-1</b> | <b>Amphotericin-B</b>                                                           | —                                | —                       | 40                      |
| <b>SD-2</b> | <b>Ciprofloxacin</b>                                                            | 46                               | 46                      | —                       |

Note: The bold entries indicate that the zone of inhibition (mm) shown by Complex Nos. C-2 and C-5 against *B. subtilis* and *E. coli*, respectively is highest among all other complexes. Similarly, Complex C-5 shows the best antifungal activity as compared to other complexes.

**TABLE 2** | Analytical data of template impacted macrocyclic complexes.

| C. No.     | Color         | Mol. weight | Elemental analysis |                  |                  | Metal %          |                                                                          |
|------------|---------------|-------------|--------------------|------------------|------------------|------------------|--------------------------------------------------------------------------|
|            |               |             | Cald. (Found)      |                  |                  | Cal. (Found)     | Molar conductance (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> ) |
| C          | H             | N           |                    |                  |                  |                  |                                                                          |
| <b>L</b>   | Yellow        | 250.39      | 67.16<br>(66.92)   | 10.47<br>(10.11) | 22.38<br>(22.01) | —                | —                                                                        |
| <b>C-1</b> | Black         | 408.74      | 41.14<br>(41.03)   | 6.41<br>(6.33)   | 13.71<br>(13.59) | 12.72<br>(12.63) | 159.41                                                                   |
| <b>C-2</b> | Black         | 488.39      | 34.43<br>(34.26)   | 5.37<br>(5.28)   | 20.08<br>(19.93) | 10.65<br>(10.53) | 153.74                                                                   |
| <b>C-3</b> | Black         | 479.51      | 50.10<br>(49.98)   | 7.36<br>(7.30)   | 11.68<br>(11.59) | 10.84<br>(10.78) | 150.56                                                                   |
| <b>C-4</b> | Red           | 412.59      | 40.75<br>(40.53)   | 6.35<br>(6.22)   | 13.58<br>(13.45) | 13.54<br>(13.39) | 161.12                                                                   |
| <b>C-5</b> | Reddish Brown | 492.24      | 34.16<br>(33.97)   | 5.32<br>(5.21)   | 19.92<br>(19.80) | 11.35<br>(11.23) | 152.22                                                                   |
| <b>C-6</b> | Reddish Brown | 483.36      | 49.70<br>(49.58)   | 7.30<br>(7.16)   | 11.59<br>(11.47) | 11.55<br>(11.38) | 149.43                                                                   |

### 3.1 | Infrared Spectra

The peaks corresponding to  $\text{—NH}_2$  groups of triethylenetetramine and peaks corresponding to  $\text{>C=O}$  groups of dimedone were not observed in the IR spectrum of macrocyclic ligand synthesized by condensation between triethylenetetramine and dimedone. However, a new peak appeared in the IR spectrum of above said macrocyclic ligand at  $1680\text{cm}^{-1}$  which was attributed to  $\nu(\text{C=N})$  stretching vibrations [15]. This peak confirmed the condensation between  $\text{—NH}_2$  groups of triethylenetetramine and  $\text{>C=O}$  groups dimedone (Figure S1). Interestingly, in the IR spectra of all complexes (Figures S2–S6), this peak was shifted to lower wave number i.e. between  $1637$  and  $1586\text{cm}^{-1}$  (Figure 1). Which can be explained on the basis of drifting of electrons from the azomethine nitrogen to central metal atom to form coordinate bond between metal and nitrogen [16]. Furthermore, all complexes exhibited a single broad medium intensity band at about  $3200\text{--}3400\text{cm}^{-1}$ , which may be attributed to  $\nu(\text{N—H})$  stretching vibration [17]. The stretching vibration of  $\nu(\text{C—H})$  bonds in all complexes resulted in moderate intensity bands in the range of  $2800\text{--}3000\text{cm}^{-1}$ . Moreover, the presence of peaks in the spectra of nitrate complexes at  $1460\text{--}1420$ ,  $1325\text{--}1280$ , and  $1040\text{--}1010\text{cm}^{-1}$  revealed that the coordination mode of nitrate groups with metal ions is unidentate, whereas, in the IR spectra of acetate complexes, the asymmetric stretching vibration of the acetate ion group resulted into appearance of peak between  $1676$  and  $1669\text{cm}^{-1}$ , while the peaks between  $1305$  and  $1304\text{cm}^{-1}$  appeared due to the symmetric stretching vibrations. The difference between asymmetric and symmetric stretching is about  $375\text{--}365\text{cm}^{-1}$ , which is greater than  $144\text{cm}^{-1}$ , indicating the unidentate nature of coordination between acetate ion and the central metal ion.

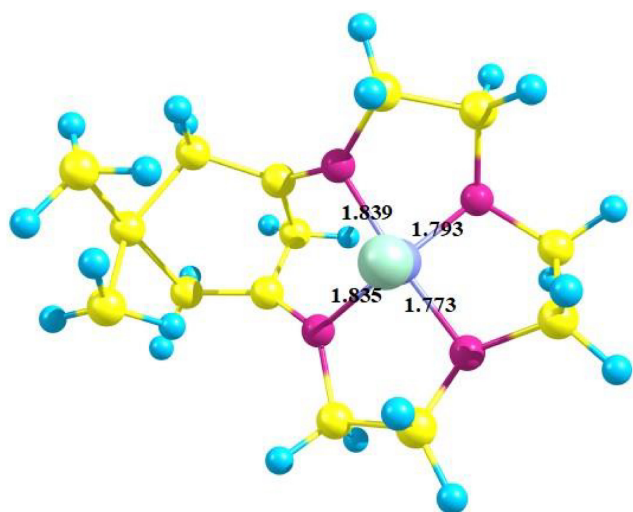
### 3.2 | Electronic Spectra

#### 3.2.1 | Cr (III) Complexes

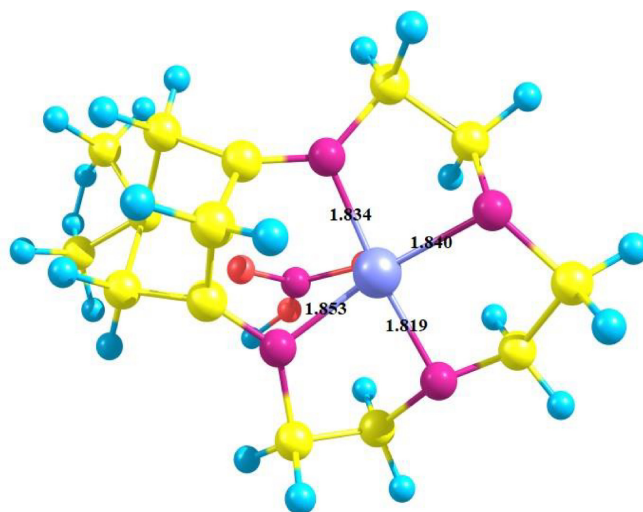
There few bands were observed in the electronic spectra of Cr (III) macrocyclic complexes, and they were located at  $786\text{--}634$ ,  $620\text{--}462$ ,  $407\text{--}290$ , and  $276\text{--}219\text{nm}$  (Figure S7). These bands might be attributed to  $d_z^2 \rightarrow d_{x^2-y^2}$ ,  $d_{xy} \rightarrow d_{x^2-y^2}$  and  $d_{xz}/d_{yz} \rightarrow d_{x^2-y^2}$  electronic transitions, respectively, whereas, lower wavelength band ( $276\text{--}219\text{nm}$ ) may be assigned to  $\pi \rightarrow \pi^*$  transitions. These bands are consistent with reported bands of square pyramidal geometry around the metal ion [18]. Furthermore, the magnetic moment of Cr (III) complexes was found to be approximately  $4.02\text{--}4.50$  B.M. at room temperature [19]. These values suggested that the Cr (III) ion has three unpaired electrons and satisfied the findings of its electronic spectra.

#### 3.2.2 | Fe (III) Complexes

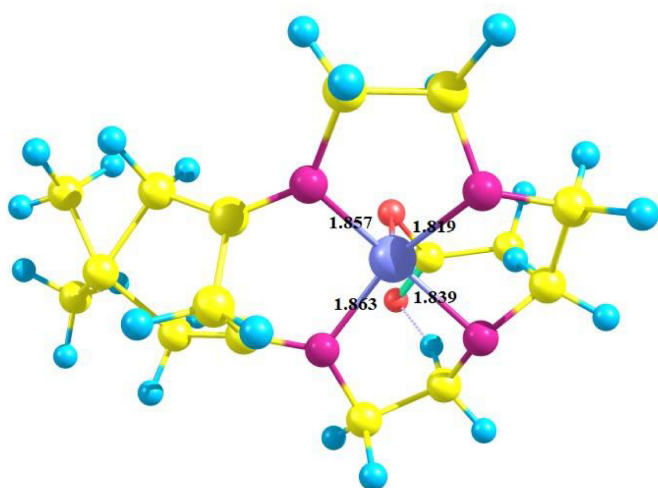
Different bands were observed in the electronic spectra of Fe (III) containing macrocyclic complexes at  $660\text{--}486\text{nm}$  (corresponds to  $d_z^2 \rightarrow d_{x^2-y^2}$  transition),  $462\text{--}361\text{nm}$  (corresponds to  $d_{xy} \rightarrow d_{x^2-y^2}$  transition), and  $257\text{--}205\text{nm}$  (assigned to  $d_{xz}/d_{yz} \rightarrow d_{x^2-y^2}$  transition) (Figure S8). All of these transitions are in close agreement with the transitions already reported for the square pyramidal geometry surrounding the Fe (III) metal ion [18]. In addition to this, the existence of five unpaired electrons was confirmed by the magnetic moment, i.e.,  $4.90\text{--}5.30$  B.M. at room temperature, which further validated the results of the electronic spectra analysis [19].



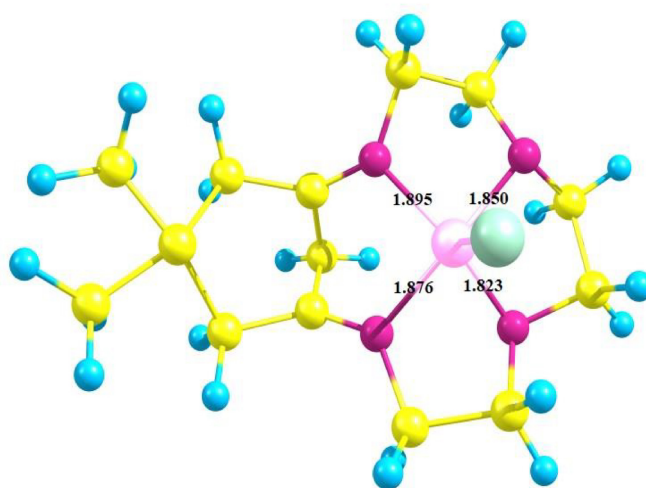
**C-1**



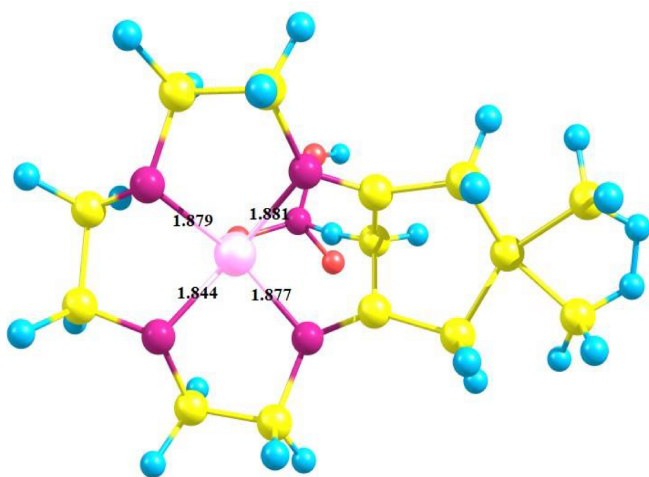
**C-2**



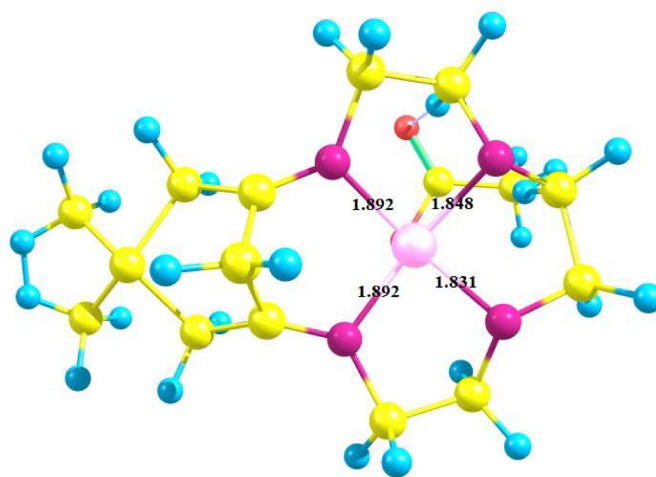
**C-3**



**C-4**



**C-5**



**C-6**

**FIGURE 1** | IR spectrum of C-3 complex.

### 3.3 | Mass Spectra

The mass spectra of ligand and complexes gave idea about their molecular mass. The molecular ion peaks of ligand and its complexes are given below:

**Ligand**— $[\text{C}_{14}\text{H}_{26}\text{N}_4]$ ;  $m/z = 249.93$ , (Cald. M. W. = 250.39);

**C-1**— $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{Cl})](\text{Cl})_2$ ;  $m/z = 409.16$ , (Cald. M. W. = 408.74);

**C-2**— $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{NO}_3)](\text{NO}_3)_2$ ;  $m/z = 487.35$ , (cald. M. W. = 488.39);

**C-3**— $[\text{Cr}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{OAc})](\text{OAc})_2$ ;  $m/z = 479.21$ , (Cald. M. W. = 479.51);

**C-4**— $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{Cl})](\text{Cl})_2$ ;  $m/z = 413.26$ , (Cald. M. W. = 412.59);

**C-5**— $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{NO}_3)](\text{NO}_3)_2$ ;  $m/z = 492.29$ , (Cald. M. W. = 492.24);

**C-6**— $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{OAc})](\text{OAc})_2$ ;  $m/z = 483.01$ , (Cald. M. W. = 483.36);

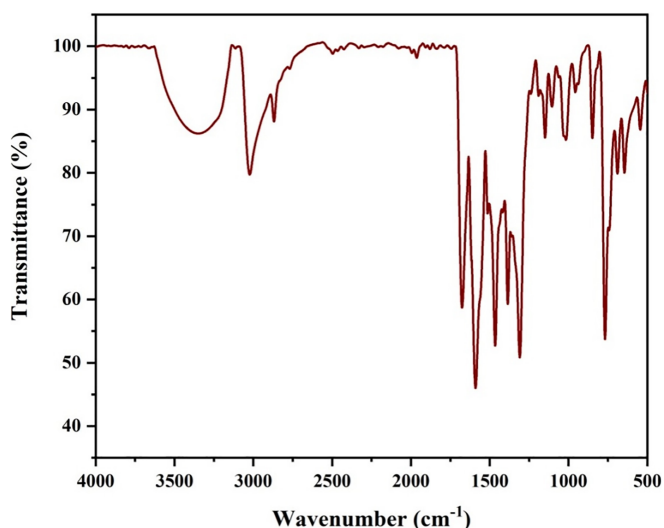


FIGURE 2 | Mass spectrum of C-6 complex.

The results obtained from mass spectra (Figures S9-S14) were in good agreement with the mass calculated from proposed molecular formula. The molecular formula was proposed on the basis of the results obtained from various physicochemical studies. For instance, the complex C-6  $[\text{Fe}(\text{C}_{14}\text{H}_{26}\text{N}_4)(\text{OAc})](\text{OAc})_2$  showed  $m/z$  at 483.01 which is very close to 483.36 i.e. the calculated molecular weight of this complex (Figure 2).

### 3.4 | PXRD Studies

The Powder X-Ray diffractograph technique was used to investigate the crystal systems of newly synthesized macrocyclic complexes at wavelength of 1.54 Å and a 4°–85° angle range. PXRD data of complexes were run into the FullProf software to estimate the lattice dimensions of each complex [20]. Based on these studies, it was found that the C-1, C-5, and C-6 complexes had orthorhombic crystal system, while the C-2, C-3, and C-4 complexes possessed monoclinic crystal system (Table 3) because these complexes fulfill  $a \neq b \neq c$ ;  $\alpha = \gamma = 90^\circ \neq \beta$  and  $a \neq b \neq c$ ;  $\alpha = \gamma = \beta = 90^\circ$  conditions of monoclinic and orthorhombic crystal systems, respectively.

### 3.5 | Thermal Studies

Thermogravimetric analysis was carried out to examine the thermal stability of all complexes in the temperature range of 20°C–800°C. In the thermogram curve of all complexes no significant mass loss was observed upto ~200°C which indicated the absence of water molecules inside or outside their coordination sphere. From the thermogram curve of all complexes two steps of decomposition were observed after ~200°C indicating the decomposition of the organic moiety and moiety coordinated to metal ion respectively. After that, the respective metal oxide was formed [21]. For instance, the C-2 complex (Figure 3) exhibited two stages of the decomposition at 252°C–264°C and 314°C–364°C indicating the decomposition of the organic moiety and metal coordinated moiety. Above 410°C, final decomposition led to the formation metal oxide.

### 3.6 | Molecular Modeling

The stability of all newly synthesized complexes (C-1 to C-6) was validated by optimized energy ranges between –2490 and –2038 eV while the optimized energy of ligand was –767.903 eV. This difference in the energies of ligand and its complexes explained the

TABLE 3 | The lattice parameters of synthesized macrocyclic complexes.

| Complex no. | ( $\alpha$ ) | ( $\beta$ ) | ( $\gamma$ ) | [a]   | [b]   | [c]  | System       |
|-------------|--------------|-------------|--------------|-------|-------|------|--------------|
| C-1         | 90°          | 90°         | 90°          | 23.93 | 14.56 | 6.70 | Orthorhombic |
| C-2         | 90°          | 92.93°      | 90°          | 5.48  | 7.92  | 4.47 | Monoclinic   |
| C-3         | 90°          | 99.61°      | 90°          | 7.99  | 6.49  | 4.27 | Monoclinic   |
| C-4         | 90°          | 115.02°     | 90°          | 8.16  | 3.98  | 3.68 | Monoclinic   |
| C-5         | 90°          | 90°         | 90°          | 8.71  | 5.47  | 2.63 | Orthorhombic |
| C-6         | 90°          | 90°         | 90°          | 8.09  | 6.81  | 3.58 | Orthorhombic |

more stability of all complexes as compared to ligand. The energy gap between the HOMO and the LUMO of all complexes was found to be  $-0.16$  to  $-0.05$  eV (Table 4). Using Koopmans' theorem (Table S1) accounts for all global parameters obtained from molecular parameters [22, 23]. Donor-acceptor behaviour of the metal–ligand system can be easily analyzed by ( $\eta$ ) hardness and ( $\sigma$ ) softness [24]. The small energy gap indicated the molecule to be soft whereas a large energy gap indicated that molecule to be hard. In additionally, hard molecules showed more stability as compared to soft molecules [25]. From the results of theoretical calculations, it was concluded that the complexes showed the following order of hardness ( $\eta$ ) and consequently stability also as **C-5** > **C-6** > **C-1** > **C-3** > **C-4** > **C-2** due to large energy gap between HOMO and LUMO, However, the above order will be reversed, if softness ( $\sigma$ ) or instability of the complexes taken into account [26]. For instance, theoretical calculations showed that the complex **C-2** has the lowest hardness value ( $\eta=0.0184$  eV) or the highest softness ( $\sigma=54.35$  eV), which means it is the least stable molecule due to the small energy gap between HOMO and LUMO (Figure 4).

Moreover, the bond lengths of all complexes were also analyzed by using the output file of Gaussian. The pictorial representations of all complexes with their bond lengths were explored in Figure 5. This values supported the presence of square pyramidal geometry around the metal ion in all synthesized macrocyclic complexes.

### 3.7 | ADMET Studies

For the development of a drug, a rule must be followed by all complexes known as Lipinski's rule, or Pfizer rule which is given by Christopher A. Lipinski in 1997. According to that rule molecular weight should not be greater than 500, an H-bond donor  $\leq 5$ , a logP  $\leq 5$  and an H-bond acceptor  $\leq 10$ , TPSA  $\leq 140$  Å. With this rule, it was found that all synthesized complexes were following Lipinski's rule or Pfizer's rule without any violation as shown in Table 5. Therefore, it may be concluded from ADMET studies that all synthesized complexes **C-1** to **C-6** possessed drug-likeness behavior.

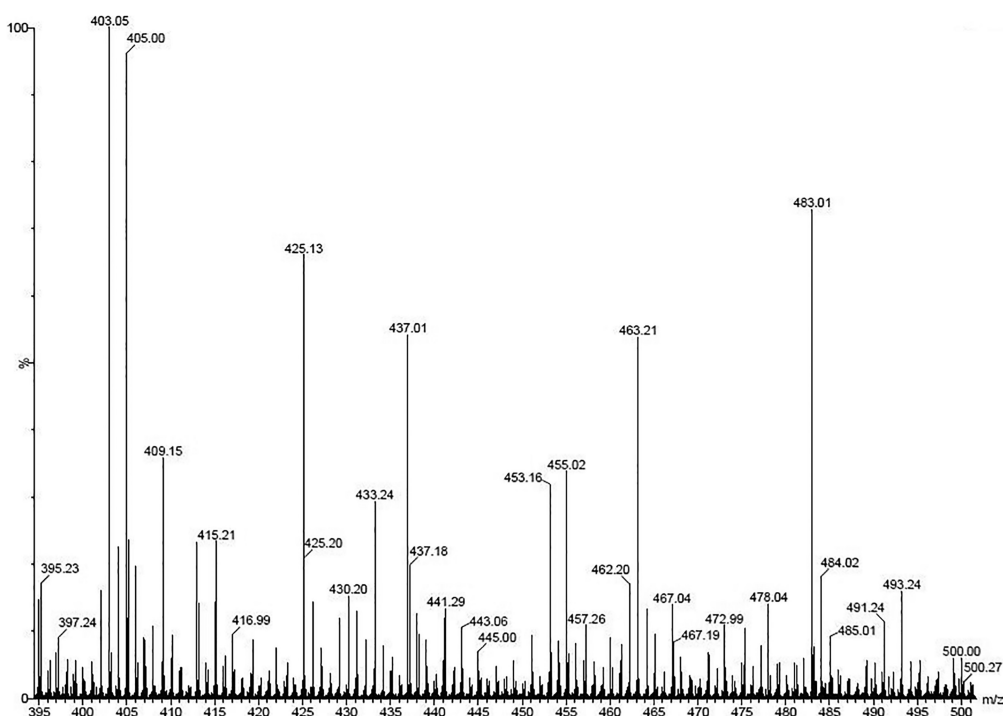


FIGURE 3 | TGA curve of C-2 complex.

TABLE 4 | Electronic parameters of energy optimized complexes.

| Code no.   | $E_{Total}$ (eV) | $E_{HOMO}$ (eV) | $E_{LUMO}$ (eV) | $\Delta E$ (eV) |
|------------|------------------|-----------------|-----------------|-----------------|
| Ligand     | −767.903         | −0.2074         | −0.0154         | 0.1920          |
| <b>C-1</b> | −2270.797        | −0.1436         | −0.0713         | 0.0723          |
| <b>C-2</b> | −2091.486        | −0.1098         | −0.0728         | 0.0369          |
| <b>C-3</b> | −2038.965        | −0.1389         | −0.0681         | 0.0708          |
| <b>C-4</b> | −2490.205        | −0.1495         | −0.0853         | 0.0642          |
| <b>C-5</b> | −2290.710        | −0.2209         | −0.0609         | 0.1600          |
| <b>C-6</b> | −2258.186        | −0.1578         | −0.0810         | 0.0768          |

### 3.8 | Antimicrobial Studies

The antimicrobial activities of ligand and its complexes were tested against gram-positive (*Bacillus subtilis*), gram-negative (*Escherichia coli*) and fungal strains (*Candida albicans*). From their results, it was observed that the all complexes showed higher activity index of antimicrobial activity as compared to macrocyclic ligand. From this result, it may be concluded that the transition metal ions enhanced the antimicrobial activity of the complexes. The activity index was calculated using Equation 2 as mentioned earlier. Among all complexes, **C-5**

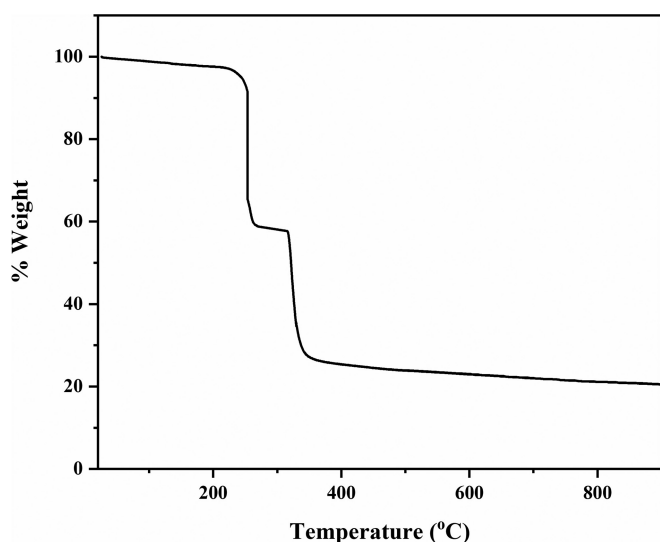
complex showed the best inhibition activity index i.e. 91.30 and 57.50 against *E. coli* and *C. albicans*, respectively (Figure 6). Whereas, the complex **C-2** showed high activity index i.e. 82.60 against *B. subtilis*.

### 3.9 | Docking Studies

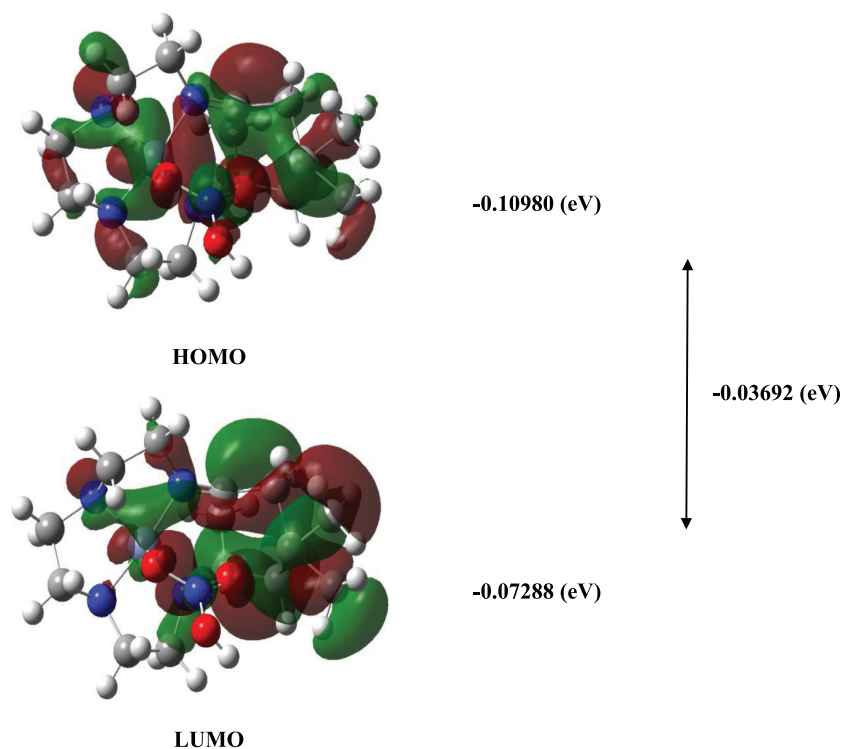
Results obtained from antimicrobial studies suggested that few complexes showed significant antimicrobial activity. Complex **C-5** exhibited the best antimicrobial activity against bacterial as well fungal strains. Therefore, the docking study of **C-5** with membranous protein of *Escherichia coli* (PDB: 2Y1B) was investigated. The docking results revealed that complex **C-5** showed various interactions with amino acid (Thr:47; Lys:90; Arg:89) of PDB: 2Y1B in 4<sup>th</sup> run out best 10 runs by  $-5.89$  kcal/mol (Table S2) binding energy of docking (Figure 7). These all interactions were shorted and visualized using Discovery studio 2020.

### 3.10 | Antioxidant Complexes

From the results of antioxidant activity it is clear that the variation in the concentration of all the complexes affect their antioxidant activity. In other words, the antioxidant activity is directly proportional to the concentration of complexes. All complexes exhibited remarkable antioxidant activity as compared to ligand and it was found that complex **C-2** showed the best antioxidant activity. Moreover, it was observed that all complexes of different concentration showed better antioxidant activity as compared to the standard solution ascorbic acid (Figure 8). The remarkable antioxidant activity of all complexes could be



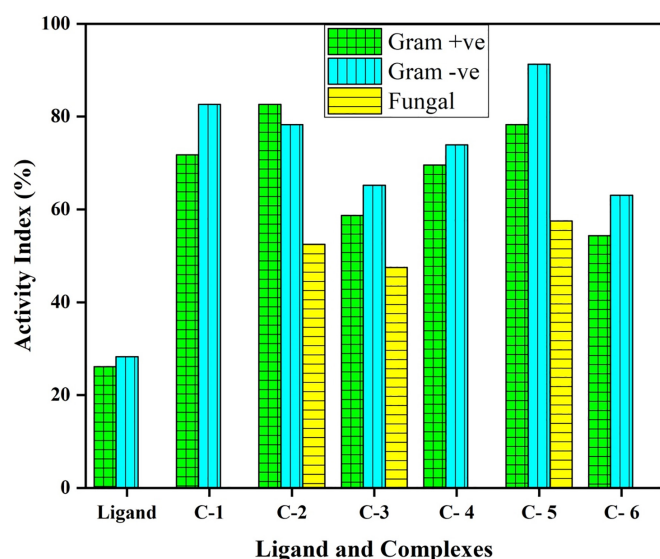
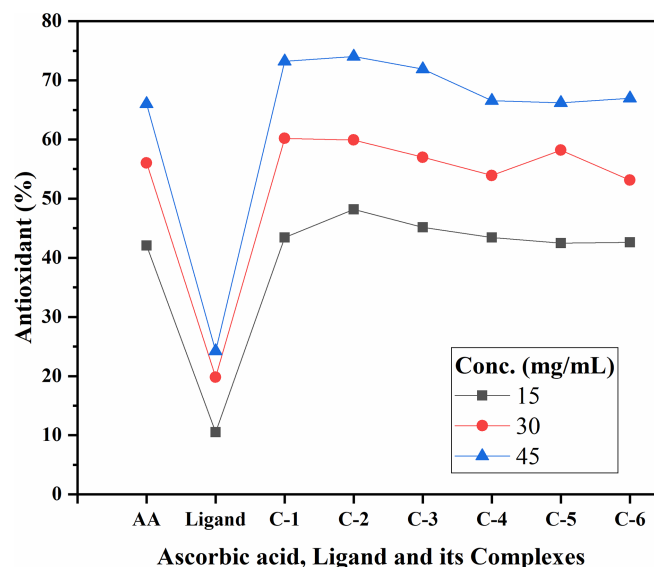
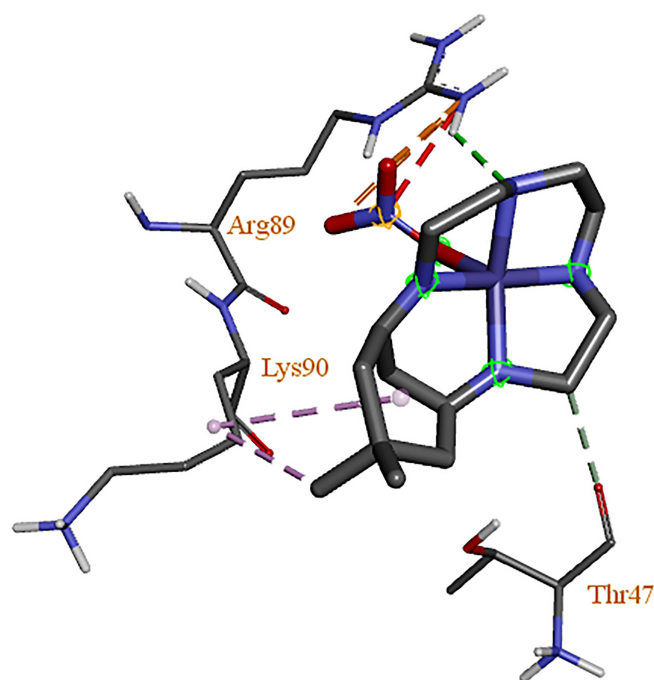
**FIGURE 4** | HOMO and LUMO representation with energy gap of C-2 complex.



**FIGURE 5** | Pictorial representation of all complexes with their bond lengths around the metal ion.

**TABLE 5** | Descriptors of ADMET properties.

| C. no. | Mol. weight | Physicochemical parameters |          |                        |                     | Drug likeness behavior |
|--------|-------------|----------------------------|----------|------------------------|---------------------|------------------------|
|        |             | Log P                      | TPSA (Å) | No. of H-bond acceptor | No. of H-bond Donor |                        |
| C-1    | 408.74      | 0.0                        | 48.78    | 4                      | 2                   | Yes (0;violation)      |
| C-2    | 488.39      | 0.0                        | 103.83   | 7                      | 2                   | Yes (0;violation)      |
| C-3    | 479.51      | 0.0                        | 75.08    | 6                      | 2                   | Yes (0;violation)      |
| C-4    | 412.59      | 0.0                        | 48.78    | 4                      | 2                   | Yes (0;violation)      |
| C-5    | 492.24      | 0.0                        | 103.83   | 7                      | 2                   | Yes (0;violation)      |
| C-6    | 483.36      | 0.0                        | 75.08    | 6                      | 2                   | Yes (0;violation)      |


**FIGURE 6** | Antimicrobial activity index of complexes.

**FIGURE 8** | Antioxidant activity in percentage of ligand and its complexes compared with standard ascorbic acid solution.

**FIGURE 7** | Best docking image of PDB: 2Y1B with complex C-5.

justified by the presence of hydrogen atom bonded with nitrogen in all complexes. This hydrogen is acidic in nature and its donation to the DPPH results into the formation of stable DPPH free radical [27].

## 4 | Conclusion

A macrocyclic ligand and its macrocyclic complexes of Cr (III) and Fe (III) ion were synthesized by non-template methodology. These complexes were characterized with the aid of various physiochemical and spectral techniques. The results obtained physiochemical and spectral studies of all complexes suggested them to have square pyramidal geometry around the metal ion. Further, PXRD technique revealed that complexes **C-1**, **C-5** and **C-6** possessed the orthorhombic crystal system whereas the complexes **C-2**, **C-3** and **C-4** were found to have monoclinic crystal system. Furthermore, the DFT study was also performed to calculate the various quantum chemical parameters of all complexes and on the basis of  $\Delta E$  (eV) values and it was concluded that all complexes were more stable as compared to ligand. From the ADMET studies, it was found that all complexes and ligand were found to have drug-likeness behavior without any violation of Lipinski's rule. So, all complexes and ligand were tested

against gram-positive bacterial strain i.e. *Bacillus subtilis*, gram-negative bacterial strain i.e. *Escherichia coli* and fungal strain i.e. *Candida albicans*. The results of antimicrobial activity indicated that the all complexes exhibited high inhibition potential as compared to ligand. Among all the complexes and ligand, the complex **C-5** demonstrated the best antimicrobial activity. The docking study of complex **C-5** with membranous protein of *E. coli* (PDB: 2Y1B) showed a number of significant interactions between them which further supported the results of antimicrobial activity of complex **C-5**. Moreover, all complexes were found to have high antioxidant activity as compare to ligand and standard ascorbic solution also. Among all complexes, the complex **C-2** showed best antioxidant activity. Therefore, it concluded that the presence of metal ions in the complexes enhanced the biological activities of these complexes.

## Author Contributions

**Jai Kumar:** conceptualization, methodology, software, writing – original draft, funding acquisition, investigation, writing – review and editing, validation, visualization, data curation. **Ramesh Kumar:** investigation, funding acquisition, writing – review and editing, visualization, software, formal analysis, supervision, resources, methodology, conceptualization, data curation. **Devender Singh:** conceptualization, visualization, formal analysis, supervision. **Harish Kumar:** software, supervision, visualization, conceptualization. **Arti Jangra:** funding acquisition, writing – review and editing, methodology, visualization, formal analysis, resources, conceptualization. **Praveen Kumar Gupta:** investigation, software, supervision, formal analysis, visualization.

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## Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.