The proton transfer complex has been synthesized by mixing a 1:1 ratio of 8-aminoquinoline (donor) and chloranilic acid (acceptor) in methanol. FTIR, $^{13}$C NMR, $^1$H NMR, Powder XRD and UV-visible studies confirmed the formation of the newly synthesized compound. These methods ascertain that cations and anions combine to form weak hydrogen bonds as $\text{N}^+\text{H}\cdots\text{O}$. The physical properties such as energy of interaction (ECT), resonating energy (RN), ionization potential ($\text{IP}$), and oscillator strength ($f$), transition dipole strength ($D$) and free energy ($\Delta G$) were estimated through UV-visible spectrosopy. The thermal stability of this complex and extensive erosion was analyzed by TGA/DTA study. Benesi-Hildebrand equation was used to determine 1:1 stoichiometry of this complex and to calculate the molar extinction coefficient ($\varepsilon_{\text{CT}}$), the formation constant ($K_{\text{CT}}$) and other physical parameters. The nature of transfer of charge relations plays a vital role in chemistry and in biological systems. The synthesized proton transfer complex has been screened for antibacterial activities against different bacteria and antifungal activities against different fungi. The proton transfer complex also displays outstanding interaction with the human protein (globulin) protein. The DFT calculations by B3LYP/6-311G* basis set gave theoretical establishment and HOMO ($-5.468$ eV) to LUMO ($-3.328$ eV) electronic energy gap ($\Delta E$) as $2.140$ eV. Theoretical analysis proves the biological characteristics as well. Molecular docking displays that CT complex is fully bound to the protein and determines the free binding energy value of $-290.18$ kcal/mol (FEB).

A new organic charge transfer complex has been prepared, characterized and explored for antibacterial, antifungal and protein binding properties. The experimental results are supported by theoretical analysis.
1. Introduction

The sharing of electronic charge between two appropriate molecular moieties, one of which has the ability to donate, is called a donor and the other can accept the charges. The charge transfer (CT) interaction follows with the transfer of charge from the donor moiety to the acceptor moiety, which was first introduced by Mulliken (1950; Mulliken & Pearson, 1969) and later studied by Foster (1969). Nowadays, it is very challenging to optimize and synthesize the CT-complex due to contact between donor and acceptor organic moieties (Khan et al., 2020a) with intermolecular hydrogen bond (N−H⋯O) by exchanging of charges between donor and acceptor (Khan & Ahmad, 2010; Shakya & Khan, 2021). The development of color in this complex structure varies on the low energy (LUMO) of acceptor and the interaction of the donor’s highly occupied molecular orbit, which absorbs radiation in the visible region (Hamed et al., 1998). Initially, Matsunaga and co-workers (Kasha, 1991; Saito & Matsunaga, 1974) offered the photonic charge transfer complex. As implied by Pauling, this complex is a remarkable example of hydrogen bonding (Pauling 1960). Atkins substantiated the dipole-dipole force in this complex (Atkins, 1990).

Charge transfer complex has a wide range of uses in human life due to its acquisition of various biological and physicochemical assets. The CT-complex has been used in a number of optical materials (Di Bella et al., 1993), photo-catalysts (Dabestani et al., 1998), electrical conductor (Rodina et al., 1995; Sakurai et al., 1997), semiconductor (Eychmüller & Rogach, 2000; Grossel & Weston, 1996; Nour, 2000) and chemosensors (Khan & Shakya, 2019; Shakya et al., 2020; Zhang et al., 2018). Such kind of complexes are also used in medicine (Büyükşan et al., 1999; Takahashi et al., 1993) and many biological schemes (Dozal et al., 2000; Khan et al., 2011, 2013, 2018; Refat et al., 2006). They are also used as drugs for enzyme catalysis, insecticide, antibacterial iron transfer, DNA-binding and antifungal over lipophilic membranes (Dozal et al., 2000; Gutmann et al., 1992; Khan et al., 2020b; Papadakis, 2019; Papadakis et al., 2016; Sondhi et al., 2001).

In this article, we have reported the CT-complex of 8-aminoquinoline (8-AQ) as a donor with chloranilic acid (ChA) as acceptor (Figure 1). Several groups have reported in which chloranilic acid (ChA) acts as an acceptor in the formation of Charge transfer complex with pyrazole (Khan et al., 2020c), p-nitroaniline (Zulkarnain et al., 2017), 2, 3-diaminopyridine (AI-Ahmary et al., 2018), 4-aminoquinoline (AI-Ahmary et al., 2016), 2-amino-4 methylthiazole (Miyay et al., 2018), 5,6-dimethyl benzimidazole (Singh et et al., 2016), and many with other donor’s molecule.

The proton transfer or CT-complex based on the donor and acceptor molecules have been identified and established by the highly sensitive UV–visible spectroscopic technique (Khan et al., 2020d), FTIR, NMR (1H & 13C), TGA/DTA, etc. UV-Vis spectrophotometric studies were accomplished in various solvents, which highlights the effect of polarity in the formation of this complex. Important parameters like ionization potential (I0), formation constant (KCT), oscillator strength (f) extinction coefficient (εCT), resonance energy (R0) energy (ECT), free energy (∆G) and transition dipole strength (μEN) can be successfully explored using this method. TGA and DTA are known to conclude thermodynamic properties of this CT-complex at different temperatures. The proton transfer complex of 8AQ and ChA has been supplementarily investigated by the Powder XRD. Furthermore, molecular docking studies are used to discuss the antimicrobial properties of such newly obtained CT-complex and DFT calculations were also investigated to achieve various theoretical data.

2. Experimental study

2.1. Preparation of CT complex and reagents used

For the synthesis of CT-complex, chloranilic acid (ChA) was used as acceptor and 8-aminoquinoline (8-AQ) was used as a donor. The solvents such as double distilled water, chloroform, acetonitrile, methanol and ethanol obtained from Merck were extracted before use.

The saturated solution of ChA (0.1446 g, 1 mmol) and 8-AQ (0.2089 g, 1 mmol) were mixed together in methanol to obtain CT-complex. The mixture was stirred vigorously for one hour and stood for a few hours. The CT-complex was identified by preliminary analysis and obtained molecular formula as C15H10N2O4Cl2, molecular weight =353.16, and element percentages as C = 50.96%, H = 2.83%, N = 7.93%, O = 18.12% and Cal = 20.10%. The reaction scheme is shown in Scheme 1.

2.2. Instrumental measurements

The 2020 FTIR spectrometer has been used to trace the FTIR spectra of 8-AQ, ChA and the synthesized complex using the KBr disc technique. Electronic absorption spectra of 8-AQ, ChA and synthesized complex have been recorded by using Perkin Elmer Lambda-45 UV–visible spectrophotometer in UV–visible region (200–470 nm) in methanol, DMF and DMSO solvent at room temperature. Both 8-AQ and ChA have been visualized separately by spectrophotometric titration for maximum absorption wavelength. The reaction mixture of donor and acceptor was permitted to stand overnight at room
temperature for establishing CT-complex structure. Using pure solvent as a reference solution, the wavelength at maximum absorption was determined for the resultant solution. Fluorescence study was conducted using Spectrofluorophotometer (Shimadzu, Japan) Model RF-5301PC equipped with Xenon lamp upon excitation of 300 nm. The $^1$H-NMR spectrum of CT-complex was recorded by JEOL-JNMLA-400 FT (400 MHz) NMR spectrometer in DMSO. The composition of proton transfer complex was confirmed by using WATERS-Q-TOF PREMIER-HAB213 mass spectrometer with an electron spray ionization (ESI) procedure. The TGA and DTA analysis of 8-AQ and ChA and CT-complex were verified using instrument EXSTAR TGA/DTA 6300 model in nitrogen atmosphere by the 20°C/min heating rate.

2.3. DFT calculations

DFT calculations have been obtained by Gaussian 09 package software (Frisch & Trucks, 2004). Energy minimized structures of free 8AQ, ChA and synthesized charge transfer complex has been explored. Complete geometric optimizations work with DFT and Beck’s three-parameter hybrid exchange activities for the CT-complex, which was a basic set of Popel—B3 LYP/6-311G**. The calculated IR frequencies through this method are found to be positive, which shows that the optimized geometry is at a minimum on the potential energy surface. In the FTIR spectrum, bands that appeared are assigned with full accuracy, using animated modes of vibrations. Relative structure visualization was done using Chemcraft 1.5 software (Zhurko & Zhurko, 2016).

2.4. Antimicrobial and protein binding studies

The antibacterial activity of synthesized complex has been verified in vitro against Escherichia coli, Bacillus subtilis and Staphylococcus aureus. This CT-complex has also been demonstrated for its antifungal property in DMSO by standard agar disk diffusion method against Aspergillus niger, Candida albicans and Fusarium oxysporum.

In the protein binding, CT-complex interaction with globulin protein was studied by fluorescence spectroscopy. Spectrofluorophotometer RF-5301PC equipped with Xenon lamp has been employed upon excitation of 280 nm. The globulin protein (CT-globulin) was liquefied in phosphate buffer (pH 7.4). CT-complex binding with globulin protein was studied by observing the change in initial fluorescence of CT-complex on adding varied protein concentrations.
2.5. Molecular docking

To apply the molecular docking technique, HEX 8.0 software was used (Ritchie & Venkatraman, 2010). Structure of 8-AQ and ChA and synthesized CT-complex has been mapped through CHEMSKETCH software and pdb format is obtained. The structure of globulin protein with PDB ID as 1D2S has been downloaded from an online protein data bank. CHIMERA software was used to get the structure outlook of the image.

3. Results and discussion

3.1. Electronic spectra

Electronic absorption spectrum of chloranilic acid (ChA), 8-aminoquinoline (8-AQ) and CT-complex in methanol, DMF and DMSO have been noted in UV-visible region from 200 to 700 nm as exposed in Figure 2. Complex absorption spectra are explored by the Gaussian function

\[ y = y_0 + \left( \frac{A}{w \sqrt{\pi}} \right) \exp \left[ -2\left( \frac{x-x_c}{w} \right)^2 \right] \]

The concentration of the 8AQ has been kept much more than ChA in the reaction mixture (Hasani & Rezaei, 2006). The concentration of the 8AQ is altered over an extensive range of 1.5 × 10^{-4} M, 2.0 × 10^{-4} M, 2.5 × 10^{-4} M, 3.0 × 10^{-4} M to 3.5 × 10^{-4} M, while the concentration of ChA is kept fixed at 1 × 10^{-4} M in each of the reaction combinations. The new absorption maxima bands confirm the formation of CT-complex at 242 nm, 275 nm and 302 nm in methanol, DMF and DMSO, respectively, which were not found in UV-visible spectra of free donor and acceptor. The absorption of CT-complex is marginally moved to longer wavelength and shorter energy absorption perceived in the integrated solution by donor and acceptor is labeled by Mullikan (Mulliken & Pearson, 2006). The CT-complex occurrence was further evidenced by foster (Foster, 1969). Using the Benesi-Hildebrand equation (Table 1), the formation constant, the alteration in enthalpy and the alteration in the free energy of this complex at room temperatures were assessed.

\[ \frac{[A]_0}{A} = 1/(K_{CT} \varepsilon_{CT}) \times 1/[D]_0 + 1/\varepsilon_{CT} \]

where \([A]_0\) is the original concentration of acceptor and \([D]_0\) is the original concentration of donor. \(\varepsilon_{CT}\) and \(K_{CT}\) are molar absorptivity and formation constant of this complex, respectively. When \(\frac{[A]_0}{A}\) versus \(1/[D]_0\) was mapped, the straight line was achieved, which supports the 1:1 establishment of the present complex, as mentioned in Figure 3. The slope and intercept of the plot are equivalent to \(1/(K_{CT} \varepsilon_{CT})\) and \(1/\varepsilon_{CT}\), respectively. It has been endorsed that molar absorptivity growths with rising in temperature show that CT-complex stability decreases with an increase in temperature and \(\Delta G^\circ\) negative value intimates that the complexation process is spontaneous.

3.1.1. Determination of transition energy, ionization potentials, resonance energy, free energy, oscillator strength and transition dipole moment

The CT-complex energy has been analyzed operating the following relation (Benesi & Hildebrand, 1949).

\[ \varepsilon_{CT} = \frac{1243.667}{\lambda_{CT}} \]

where \(\lambda_{CT}\) CT-complex wavelength. The complex band is described in Table 1.

By Aloisi and Pignataro equation, the Ionization potential of the donor in charge transfer complex has been estimated (Aloisi & Pignataro, 1973).

\[ I_D(ev) = 5.76 + 1.53 + 10 - 4\lambda_{CT} \]
Figure 2. Electronic absorption of acceptor \((1 \times 10^{-4} \text{ M})\), CT-complex \((1 \times 10^{-4} \text{ M} + 1 \times 10^{-4} \text{ M})\) and donor \((1 \times 10^{-4} \text{ M})\) in (a) DMF, (b) Me-OH and (c) DMSO at room temperature.

Table 1. Absorption data for spectrophotometric determination of stoichiometry and formation constant \((K_{CT})\) and molar extinction coefficient \((\varepsilon_{CT})\) of the 8-AQ: ChA CT-complex at room temperature.

| Concentration of donor (M) | Concentration of acceptor (M) | Absorbance at \(\lambda_{CT}\) (nm) | Formation constant \((K_{CT})\) l mol\(^{-1}\) | Molar extinction coefficient \((\varepsilon_{CT})\) l cm\(^{-1}\) mol\(^{-1}\) |
|---------------------------|-------------------------------|-----------------------------------|----------------------------------|----------------------------------|
| Methanol                  |                               |                                   |                                  |                                  |
| 1.5 \times 10^{-4}        | 1.0 \times 10^{-4}           | At 242 nm                         | 3.008 \times 10^{3}             | 15.10 \times 10^{4}             |
| 2.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 2.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 3.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 3.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
| Dmf                       |                               |                                   |                                  |                                  |
| 1.5 \times 10^{-4}        | 1.0 \times 10^{-4}           | At 275 nm                         | 1.885 \times 10^{3}             | 11.01 \times 10^{4}             |
| 2.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 2.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 3.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 1.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
| DMSO                      |                               |                                   |                                  |                                  |
| 1.5 \times 10^{-4}        | 1.0 \times 10^{-4}           | At 303 nm                         | 1.728 \times 10^{2}             | 5.330 \times 10^{4}             |
| 2.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 2.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 3.0 \times 10^{-4}        |                              |                                   |                                  |                                  |
| 3.5 \times 10^{-4}        |                              |                                   |                                  |                                  |
where \( I_D \) is donor ionization potential, \( \lambda_{CT} \) is the wavenumbers of the complex in cm\(^{-1}\).

Briegleb and Czekalla (1960) equation is used to determine the resonance energy

\[
\varepsilon_{CT} = \left(7.7 \times 10^{-4}\right)/[h\nu_{CT}/|\Delta k|] - 3.5
\]

The CT-complex standard free energy (\( \Delta G^\circ \)) was determined (Briegleb & Czekalla, 1960) by exercising the following equation.

\[
\Delta G^\circ = -2.303RT\log K_{CT}
\]

where \( \Delta G^\circ \) is the CT-complex free energy at KJ mol\(^{-1}\), \( K_{CT} \) is the formation constant. The values are presented in Table 2.

The oscillator strength (\( f \)) is expended to the precise transition probability of charge transfer band (Martin et al., 1969). We determine oscillator strength (\( f \)) from CT absorption spectrum by exercising the following formulation.

\[
f = 4.32 \times 10^{-9}\varepsilon_{CT}\Delta\nu/\nu_{1/2}
\]

where \( \Delta\nu_{1/2} \) and \( \varepsilon_{CT}\Delta\nu_{1/2} \) is the half-width and the maximum extinction coefficient of the band. The experimental CT-complex oscillator strengths are shown in Table 2. The Gaussian curve result for the dissimilar system is listed in Table 3.

### 3.2. FT-IR spectra

The infrared spectra of 8-AQ (donor), ChA (acceptor) and synthesized complex are presented in Figure 4(a). FTIR spectrum was also investigated by the DFT, B3LYP/6-311++G (d, p) level of theory to support the experimental results. The comparison of experimental FTIR spectra of the CT-complex and their constituents with the simulated spectra is shown in Figure 4(b). Few significant vibrational signals appeared in the FTIR spectrum of CT-Complex have been found comparable with simulated IR spectrum and animated modes listed in Table 4. The comparison between reactant moieties and the formed complex has been done in terms of intensity and wavelength and found a small shift, which endorsed the formation of CT-complex. This shift in bands is accredited to expected symmetry and charge in the electronic structure after CT-complex formation. ChA is a strong electron acceptor as well as a strong acid (Elsayed & Agarwal, 1982), so proton transfer from 8-AQ to the ChA is projected to form a stable CT-complex. The broad frequency assigns the O-H stretching at 3237 cm\(^{-1}\) in 8-AQ, which was shifted to 3208 cm\(^{-1}\) in the CT-complex. The asymmetric stretching frequencies of the amino group \( \nu_{as(NH_2)} \) show a negligible change in frequency from 3450 cm\(^{-1}\) (for 8-AQ) to 3449 cm\(^{-1}\) in the spectrum of CT-complex and \( \nu_{as(NH_2)} \) is shifted from 3350 cm\(^{-1}\) (for 8-AQ) to 3361 cm\(^{-1}\) for the CT-complex. The stretching frequency of aromatic C-H of CT-complex is shifted to 3059 cm\(^{-1}\) from 3154 cm\(^{-1}\) for individual 8-AQ. In addition, a new vibrational band attributing to stretching frequency has appeared at 3094 cm\(^{-1}\), which is a characteristic trait of FT-IR spectrum of the CT-complex, as this frequency is absent in the spectra of free 8-AQ and ChA. This band appears due to stretching vibration of a proton involved in donor donation site (Bellamy & Bellamy, 1975). This results in the protonation of -NH group of the 8-AQ over proton transfer from the acidic center –OH on the ChA to the basic center on the donor -NH group (Adam & Refat, 2015, 2016; Islam et al., 2019). Furthermore, the out of plane bending vibrational band \( \gamma(C-H) \) is shifted to 724 for the complex compared with 760 cm\(^{-1}\) for 8-AQ itself. These observations confirm the charge transfer process from 8-AQ towards ChA. The presence of proton transfer interaction in the formed complex is explained based on the appearance of a new vibrational band at 3094 cm\(^{-1}\) assigned to \( \nu(NH^+)/(O-H-N^-) \) due to the presence of O-H-N hydrogen bonding between 8-AQ and ChA. Hence, the infrared spectral studies revealed the interaction between 8-AQ and ChA through charge transfer and hydrogen bonding interactions.

### 3.3. NMR spectroscopy

#### 3.3.1. \(^1\)H-NMR spectra

The proton NMR spectrum of the CT-complex was established in DMSO-d\(_6\) as shown in Figure 5 and also in CDCl\(_3\) (Figure S1, ESI) for better clarity. The central goal of the \(^1\)H-NMR spectra is to show the presence of the charge transfer interactions in the designed hydrogen bond charge transfer (HBTC) complex, leading to proton transfer interactions in this complex. The proton \(^1\)H NMR of the produced complex shows six different peaks as, (8.919, s, 2H), (8.427, t, 2H), (8.637, t, 1H), (7.532, d, 2H), (7.326, m, 1H) and broad signal of -NH2 group at (5.64, s, 2H). The proton at 8.919 ppm belongs to chloranilic acid, which shows a downfield shift after CT-complex formation. ChA is a strong electron acceptor as well as a strong acid (Elsayed & Agarwal, 1982), so proton transfer from 8-AQ to the ChA is projected to form a stable CT-complex. The broad frequency assigns the O-H stretching at 3237 cm\(^{-1}\) in 8-AQ, which was shifted to 3208 cm\(^{-1}\) in the CT-complex. The asymmetric stretching frequencies of the amino group \( \nu_{as(NH_2)} \) show a negligible change in frequency from 3450 cm\(^{-1}\) (for 8-AQ) to 3449 cm\(^{-1}\) in the spectrum of CT-complex and \( \nu_{as(NH_2)} \) is shifted from 3350 cm\(^{-1}\) (for 8-AQ) to 3361 cm\(^{-1}\) for the CT-complex. The stretching frequency of aromatic C-H of CT-complex is shifted to 3059 cm\(^{-1}\) from 3154 cm\(^{-1}\) for individual 8-AQ. In addition, a new vibrational band attributing to stretching frequency has appeared at 3094 cm\(^{-1}\), which is a characteristic trait of FT-IR spectrum of the CT-complex, as this frequency is absent in the spectra of free 8-AQ and ChA. This band appears due to stretching vibration of a proton involved in donor donation site (Bellamy & Bellamy, 1975). This results in the protonation of -NH group of the 8-AQ over proton transfer from the acidic center –OH on the ChA to the basic center on the donor -NH group (Adam & Refat, 2015, 2016; Islam et al., 2019). Furthermore, the out of plane bending vibrational band \( \gamma(C-H) \) is shifted to 724 for the complex compared with 760 cm\(^{-1}\) for 8-AQ itself. These observations confirm the charge transfer process from 8-AQ towards ChA. The presence of proton transfer interaction in the formed complex is explained based on the appearance of a new vibrational band at 3094 cm\(^{-1}\) assigned to \( \nu(NH^+)/(O-H-N^-) \) due to the presence of O-H-N hydrogen bonding between 8-AQ and ChA. Hence, the infrared spectral studies revealed the interaction between 8-AQ and ChA through charge transfer and hydrogen bonding interactions.

#### 3.3.2. \(^{13}\)C NMR spectra

To additionally corroborate the formation of CT-complex and the hydrogen bonding interactions in 8-AQ and ChA
complex, the CT-complex carbon $^{13}$C NMR spectrum has been obtained in DMSO-d$_6$ solvent. The recorded spectrum is presented in Figure 6. The spectrum clearly displays the peaks for the carbons of both 8-AQ as well as ChA verifying the establishment of CT-complex. Because of an increase in the electron density in ChA by charge transfer from 8-AQ, the carbon atoms of ChA showed high field shift, i.e. towards low chemical shift value. Consequently, the carbon $^{13}$C NMR spectrum also confirms the CT-complex and hydrogen bond formation between 8-AQ and ChA.

Table 2. Wavelength ($\lambda_{\text{CT}}$), ionization potential ($I_D$), energy of interaction ($E_{\text{CT}}$) resonance energy ($R_N$), oscillator strength ($f$), dipole moment ($\mu_{\text{EN}}$), free energy ($\Delta G$) and correlation coefficient ($r$) of the 8-AQ:ChA CT-complex.

| Solvent | $\lambda_{\text{CT}}$ (nm) | $I_D$ (eV) | $E_{\text{CT}}$ (eV) | $R_N$ (eV) | $\Delta G$ (kJ mol$^{-1}$) | $f$ ($10^{-3}$) | $\mu_{\text{EN}}$ (D) | $r$    |
|---------|-----------------|----------|-----------------|----------|------------------|-------------|-----------------|------|
| Methanol| 242             | 11.42    | 5.139           | 0.643    | 22.46            | 1.06        | 60.17           | 0.99314|
| Acetonitrile | 275             | 10.95    | 4.572           | 0.403    | 19.84            | 1.73        | 75.16           | 0.99908|
| DMSO    | 303             | 10.62    | 4.104           | 0.228    | 18.68            | 1.17        | 20.8            | 0.98990|

Table 3. Gaussian curve analysis for the CT-spectrum of 8-AQ with ChA in different solvents.

| Solvent | Area of the curve ($A$) | Width of the curve ($w$) | Centre of the curve ($x_c$) |
|---------|-------------------------|-------------------------|----------------------------|
| Ethanol | 25.16 ± 1.045           | 24.79 ± 0.876           | 240.58 ± 0.071             |
| Methanol| 68.03 ± 1.118           | 46.93 ± 0.428           | 240.58 ± 0.048             |
| Acetonitrile | 32.17 ± 0.139           | 63.05 ± 0.175           | 276.69 ± 0.099             |
| DMSO    | 97.13 ± 5.109           | 129.7 ± 2.609           | 304.12 ± 0.031             |

Figure 4. (a) FT-IR spectra of (A) 1:1 CT-complex; (B) ChA; and (C) 8-AQ in the range of 4000–500. (b) Computational FTIR (black) of the CT-complex through DFT using B3LYP/6-311++G (d, p) level of theory compared with Experimental FTIR (green).
3.4. Powder X-ray diffraction studies

The characteristic powder X-ray spectra recorded in the P-Analytical XPERT-PRO diffractometer with anode components Cu, Kα1, Kα2 and Kβ, radiations of wavelength 1.54 Å, 1.54 Å and 1.39 Å, respectively, from 50°–70° angle at generator settings 35 mA, 40 kV. Kα1 has a barely smaller wavelength and double intensity as Kα2. PXRD pattern of this complex is shown in Figure 7. In the synthesized CT-complex, the most solid Bragg’s peak has been found at diffraction angles 2θ of 27.01°, which authenticates that the resulting CT-complex has crystalline properties due to the presence of the sharp packs. The data in P-XRD pattern is a good deal for the establishment of this complex. The proton transfer mechanism between the hydroxyl group of ChA and 8-AQ also performs a significant function in the process of crystallization and self-assembly in molecules.

3.5. Thermal stability

In order to ensure CT interaction amongst 8-AQ and ChA, and thermal stability of synthesized CT-complex, thermo-gravimetric (TGA) and differentiated (differentiated) for 8-AQ, ChA and prepared complex were performed. TGA and DTA studies of donor, acceptor and complex have been represented in Figure 8 and data listed in Table 5. Thermogram of CT-complex exhibits that 18.50% and 36.28% of complex decomposes at 204.34 and 298.21 °C, in two steps, respectively, shown in Figure 8. The CT-complex exothermic peaks in the DTA thermogram spectra have been noticed at ΔH = −82.38 at 204.52 °C. The 8-AQ shows one decomposition step at 202.73 °C with about 100.54% weight loss and the ChA also decomposes at 270.16 °C, in one step with about 99.50% weight loss. The DTA thermogram spectra of 8-AQ in Figure 8 illustrations that two exothermic peaks are located about 99.50% weight loss. The DTA and TGA outcomes provide evidence of CT-complex establishment.

3.6. Theoretical studies

DFT is a quantum mechanical method that provides a much reliable structure as compared to classical methods. DFT provides the electronic structure for an ensemble formed by an
acceptor (8-AQ) and a donor (ChA). DFT studies were performed by orca 4.1.1 and visualization was done using Avogadros Orca 4.1. The geometry optimization was done using a hybrid basis set B3LYP (6-31 G) as it is widely accepted and provides accurate results for small molecular systems. Several significant molecular parameters were assessed at optimized geometry of the complex and its ingredients. The charge transfer was seen during complex formation from atomic charge calculations. Optimized structure showed that the two intermolecular hydrogen bonds of high strength formed during CT-complex formation. Jeffrey and Jeffrey, 1997; categorizes H bonds with donor-acceptor distances of 2.2–2.5 Å as strong (mostly covalent), 2.5–3.2 Å as moderate (mostly electrostatic) and 3.2–4.0 Å as weak (electrostatic). The hydrogen bond, bond strength also depends on the bond angle X–H–Y (X and Y are N, O, F). More is the bond angle closer towards 180° stronger is the hydrogen bond. The two hydrogen bonds N8–H10–O26 and N6–H10–O26 and
N14–H31–O26 were seen to form with bond lengths 1.925 Å and 1.631 Å (Figure 9), respectively. The bond angle values for both the hydrogen bonds were 161.60° and 159.60°, respectively. These strong hydrogen bonds are considered responsible for holding the acceptor and donor molecule intact and making CT-complex formation process spontaneous. 3D-plot of frontier molecular orbitals with HOMO-LUMO gap is shown in Figure 10. The charge delocalization, the kinetic stability and the chemical reactivity of CT-complex can be explained from these orbitals, i.e., HOMO, LUMO and their energies. HOMO-LUMO analysis of CT-complex showed that HOMO is localized on 8-AQ whereas LUMO is localized on ChA. The high dipole moment value for CT-complex was calculated from its electronic and nuclear contribution and was found to be 3.905 D. The high value of the dipole moment can also be considered as a deriving force for CT-complex formation.

3.7. Pharmacology

3.7.1. Antibacterial activities

The antibacterial activity of the prepared complex of 8-AQ and ChA has been tested in vitro using agar well plate diffusion method against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Cruickshank et al., 1995). 100 μL of bacterial test pathogen strains were spread on Mueller-Hinton agar (MHA) plates, and different concentrations of newly synthesized 8-AQ and ChA complex (10, 20, 40, 80 μg/mL) were introduced on the agar plate. These plates were finally incubated at 37°C for 24 h and the zone of inhibition has been restrained. Gentamicin at a concentration of 80 μg/mL served as control (Collins, 1976).

The significant inhibitory activity of newly synthesized 8-AQ and ChA complex was noticed against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains by dose-dependent manner shown in Figure 11 and Table 6. The complex at a concentration of 80 μg/mL (D4) showed the inhibition more than gentamicin (80 μg/mL) against bacterial strain of *Bacillus subtilis*. The highest zone inhibition was observed in D4 (80 μg/mL) against bacterial strain of *Bacillus subtilis*, followed by *Escherichia coli* > *Staphylococcus aureus*. The antibacterial activity of the complex may be due to the disruption of cell membrane resulting in increases in ROS generation, which leads to oxidative stress.

3.7.2. Antifungal activity

The newly synthesized complex has been examined for its antifungal assets versus *Aspergillus niger*, *Fusarium oxysporum* and *Candida albicans*. For this activity, the complex was engaged at different concentrations (10, 20, 40, 80 μg/mL). The antifungal activity of the complex has competed with the standard antifungal drug Nystatin (80 μg/mL). The antifungal activity was determined by measuring zone of inhibition (mm). For fungi, growth inhibition was identified *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* when considering it with altered concentration (10, 20, 40, and 80 μg/mL) of newly synthesized donor and acceptor complex as exposed in Figure 12 and Table 7. The visible growth inhibition was noticed at a small concentration 10 μg/mL.
inhibition was followed the dose-dependent manner, with the increase in the concentration of the newly synthesized 8-AQ: ChA complex.

3.8. Protein binding studies

The fluorescence quenching system was taken to censor molecular contacts due to its high sensitivity (Hu et al., 2005; Tan et al., 2009). To achieve the protein binding nature of the CT-complex, we examined the relations of the CT-complex with the protein by the technique of fluorescence spectroscopy. Firstly, the synthesized CT-complex of $10^{-4}$ M concentration solution was prepared in different polar solvents (acetone, acetonitrile, chloroform, DMF, DMSO, ethanol and methanol), keeping slit width of 20.0 nm for source and 10.0 nm for detector to observe the fluorescence response of the CT-complex (Figure 13). It was observed that the synthesized CT-complex has good luminescent property. Further, to study the binding nature of protein with CT-complex, 1 mM solution of globulin and CT-complex was prepared in phosphate buffer ($\text{pH} = 7.4$). The fluorescence quenching was recorded upon excitation at 280 nm, keeping slit width of 10.0 nm and 5.0 nm for the detector. It was observed that on the increment addition of globulin solution to CT-complex solution, there is a decrease in initial intensity (Figure 14). The decrease in intensity or quenching on adding globulin suggests the interaction between protein and CT-complex. Stern–Volmer plot of quenching is an upward curvature (Figure 14) that produces the characteristic fluorescence emission spectra of complex. To determine the protein binding capability of the CT-complex, the modified Stern–Volmer equation (MSV) was used to calculate the binding constant, which was found to be $8.368 \text{M}^{-1}$ (Shukla et al., 2012),

$$\frac{F_0}{F_0 - F} = \frac{1}{F_0} + \frac{1}{K_a} \frac{1}{Q}$$

Here, $F_0$ and $F$ are fluorescence intensity before and after the addition of quencher, $[Q]$ (protein) denotes the quencher concentration and $f_a$ is fraction of fluorophore (CT-complex) accessible to the quencher (protein). $K_a$ is binding constant, which was obtained with the help of the intercept and slope (Figure 14). The data was plotted against emission intensity and quencher concentration shown in Figure 13. Our work concluded that the charge transfer complexes significantly bound to protein, which has potential molecular goals in designing of anti-neoplastic drugs.

3.9. Molecular docking

The acceptable docked pose was obtained from the computational molecular docking of CT-complex with human protein (globulin). The possible binding site between protein (globulin) and CT-complex was experimented to strengthen our experimental results and envisaging different types of interactions between protein & CT-complex systems. Molecular docking interactions between protein (globulin) and CT-complex was exhibited in Figure 15. The approximate binding distance of globulin protein and CT-complex was found to be Tyr $84 = 2.3 \text{Å}$, Asn $82 = 1.9 \text{Å}$, Phe $67 = 2.6 \text{Å}$ and Thr $60 = 2.8 \text{Å}$. Figure 16 shows the hydrogen surface, aromatic surface and hydrophobic surface of interacting protein and CT-complex (Akram et al., 2020; Khan et al., 2021; Shakya et al., 2019). The FEB value (free energy of binding) as $-290.18 \text{kJ mol}^{-1}$ was found for the globulin & CT-complex system shown in Figure 16. The higher value of binding energy shows stronger interaction between globulin and CT-complex.

4. Conclusion

The designed CT-complex of 8-aminoquinoline (8-AQ) and chloranilic acid (ChA) has been confirmed by infrared spectra, $^1$H & $^{13}$C NMR and PXRD. Studies have shown that the formation of 1:1 CT-complex is only because of the exchange of charge between ChA and 8-AQ, creating the extensive
hydrogen bonding in the synthesized complex. IR spectroscopy determines various bonding modes in the complex. The NMR studies further verified the structure of the synthesized complex. Spectrophotometric studies provide formation constant and various physical parameters of the complex in DMSO, methanol and DMF. There hydrogen bonding interaction, $N^+–H–O^-$ among reactant moieties, evidencing the establishment of the complex between 8-AQ and ChA. The 1:1 stoichiometry could be ascertained with the help of the Benesi-Hildebrand equation. TGA/DTA analysis also suggested that the new CT-complex is more thermodynamically stable than its constituents. The charge transfer complex exhibits wonderful biological activity in terms of antimicrobial activity and interaction with protein. Thus, the complex

| Bacterial Strain     | D1 (10 μg/mL) | D2 (20 μg/mL) | D3 (40 μg/mL) | D4 (80 μg/mL) | Gentamicin (80 μg/mL) |
|----------------------|--------------|---------------|--------------|--------------|-----------------------|
| Escherichia coli     | 6.2 ± 0.19   | 12.4 ± 0.58   | 19.0 ± 0.14  | 22.2 ± 0.62  | 35.0 ± 0.62           |
| Bacillus subtilis    | 15.0 ± 1.44  | 19.8 ± 0.13   | 26.7 ± 0.24  | 29.1 ± 0.24  | 32.0 ± 1.04           |
| Staphylococcus aureus| 8.0 ± 0.55   | 12.4 ± 1.01   | 15.18 ± 1.0  | 17.2 ± 0.74  | 20.01 ± 0.92          |

Antibacterial activity of CT-complex at various concentrations.

| Fungal Strain        | D1 (10 μg/mL) | D2 (20 μg/mL) | D3 (40 μg/mL) | D4 (80 μg/mL) | Nystatin (80 μg/mL) |
|----------------------|--------------|---------------|--------------|--------------|---------------------|
| Aspergillus niger    | 8.2 ± 0.82   | 15 ± 0.44     | 22.8 ± 1.0   | 22.2 ± 1.01  | 24.2 ± 1.483        |
| Candida albicans     | 7.4 ± 0.46   | 16.1 ± 0.54   | 17.2 ± 0.31  | 19.4 ± 0.21  | 22.6 ± 1.140        |
| Fusarium oxysporum   | 10.1 ± 0.73  | 12 ± 0.27     | 14.1 ± 0.12  | 17.6 ± 0.20  | 24.6 ± 1.140        |

Antifungal activity of CT-complex at various concentrations.
is significant in supplying of a wide diversity of anti-neoplastic drugs and complex can be more surveyed for its bioorganic applications. The DFT studies also substantiate the establishment of stable complex formation theoretically. Molecular docking offers binding sight CT-complex with 1D2S (globulin) and determines FEB value (free energy of binding) as $-290.18 \text{ kcal mol}^{-1}$.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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