J. Bangladesh Acad. Sci. 46(2); 203-211: December 2022 DOI: 10.3329/jbas.v46i2.63622

Journal of Bangladesh Academy of Sciences

Journal homepage: http://www.bas.org.bd/publications/jbas.html

Research Article

Iron (II) and Zinc (II) complexes of gemifloxacin mesylate: synthesis, characterization, serum binding profiling, and evaluation of antimicrobial activity

Fahima Aktar, Md. Jamal Hossain[1], Md. Zakir Sultan[2] and Mohammad A. Rashid[*]

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

ARTICLE INFO

Article History

Received: 01 December 2022
Revised: 20 December 2022
Accepted: 26 December 2022

Keywords: Gemifloxacin mesylate, TGA, FT-IR, Antimicrobial activity, Drug-protein interaction.

ABSTRACT

Gemifloxacin mesylate is a synthetic fluoroquinolone derived antimicrobial agent. Two metal complexes of gemifloxacin mesylate were synthesized *viz.* gemifloxacin-Fe and gemifloxacin-Zn. The formed complexes were characterized by using TLC, TGA and FT-IR spectra analyses. The formed complexes were then evaluated for antimicrobial activity. The newly formed two complexes were assessed against nine bacterial and one fungal strain, where gemifloxacin (30 µg/disc) was used as a reference standard. The new complexes showed better activity than the standard reference drug against *Klebsiella pneumoniae* among nine bacterial strains. But two bacterial species, *Acinetobacter lwoffii* and *Enterococcus faecium*, and the fungal strain *Candida albicans* showed complete resistance to the newly synthesized complexes and the reference standard. The drug protein interaction with the Bovine Serum Albumin (BSA) was also studied. The interaction mechanism was explored, suggesting that gemifloxacin and its Zn (II) complex interact with BSA via a static process while the Fe (II) complex interacts via a dynamic process and the lower Ksv value also indicated that drug - BSA complexes were formed in ground-state.

Introduction

Gemifloxacin mesylate (GeFo, Figure 1), chemically [(R,S)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylicacid methane-sulfonate] is a broad-spectrum oral antibiotic of fluoroquinolone group (Piam et al., 2012).

GeFo has *in vitro* potent antibacterial activity against respiratory tract infection pathogens because it has good penetration into respiratory secretions, for example, into alveolar macrophages and the epithelial lining fluid, having enough concentrations at the site of the infection (Bolon, 2009; Turel, 2002; Andriole, 2000). GeFo gives activity by inhibiting host DNA synthesis as it inhibits both DNA gyrase and topoisomerase IV enzyme, essential for the growth of Bacteria. Fluoroquinolone antibiotics have also been found to have anti-inflammatory and anticancer effects, as revealed by some recently carried out experiments (Kuhlmann et al., 1998; Uddin et al., 2021). The drug-metal interaction of diverse deprotonated fluoroquinolones has been extensively studied. In many cases. the metal complexes of drugs are found to be more active than the parent compound (Johnson et al., 1999; Grossman et al., 2005; Kan et al., 2013). Considering the fact, two metal complexes (Iron (II) and Zinc (II)) with GeFo have been synthesized as an attempt to assess the physicochemical properties, protein profiling and to investigate their antibacterial properties.

[*]Corresponding author: <arpharm64@du.ac.bd>

[1]*Department of Pharmacy, State University of Bangladesh, Dhanmondi, Dhaka, Bangladesh*

[2]*Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, Bangladesh*

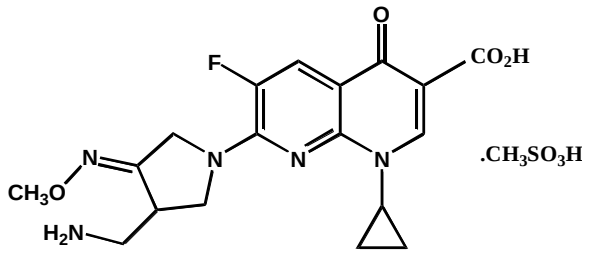


Fig. 1. Gemifloxacin mesylate.

Materials and methods

Chemicals and equipment

Analytical grade solvents and chemicals were used for all experimental purposes. The API of antibiotic gemifloxacin mesylate (purity 100.9%) was received as gift samples from Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh. BSA solution of pH 7.4 was prepared using phosphate buffer. The experimentally used TLC plates (HSF-254) were purchased from Merck, Germany.

Preparation of pH 7.4 buffer solutions

Phosphate buffer was made ready by dissolving 235 ml of 0.01M K_2HPO_4 with 65 ml of 0.01M KH_2PO_4 and the mixture was raised to a volume of 1000 ml with DM water (Hossain et al., 2020a; Saha et al., 2012).

Synthesis of Gemifloxacin mesylate-Fe and Gemifloxacin mesylate-Zn complexes

The solid metal complexes, gemifloxacin mesylate-Fe *(*GeFo-Fe) and gemifloxacin mesylate-Zn (GeFo-Zn), were prepared by mixing a hot methanolic solution of the ligand (1 mM) with that containing the required amount of the appropriate metal chloride (0.5 mM). The reaction mixture was continuously refluxed on a water bath for 6.0-7.5 h at 60 ℃. The solutions were then filtered and left for crystallization at room temperature. In each case, a fine solid product was obtained, which was washed with the solvent. Gemifloxacin mesylate-metal complexes were synthesized with a 1:2 M ratio. Following the formation of the complexes, several chromatographic and spectroscopic analyses were done to characterize the complexes.

Thin layer chromatography (TLC)

To evaluate the formation of metal complexes, TLC was performed using methanol-chloroform-toluene (20:40:40) as the mobile phase.

Thermogravimetric analysis (TGA)

The thermogravimetric analyses (TGA) of the metal complexes were carried out at temperatures up to 600 ^oC by the thermogravimetric analyzer (TGA-50, Shimadzu, Japan). An aliquot of each complex (~3 mg) was heated in an aluminum pan with temperature rising rate at 10 °C/min under a nitrogen gas flow rate of 20 mL/min.

Fourier transform infrared spectrophotometry (FTIR)

The FTIR analyses of metal drug complexes were carried out at the wavelength from 400 cm^{-1} to 4000 cm^{-1}. About 100 mg of pure and dried KBr was added to 1 mg of each dried sample, then homogenously mixed with a mortar-pestle and pressed mechanically to make a pellet under the pressure of 8-10 tons. The prepared disc was placed in the IR beam path to acquire the spectrum.

Fluorescence quenching for profiling ligand-protein binding

The fluorescence studies were conducted at different concentrations of drug at 5 µM, 10 µM, 20 µM and 50 µM while BSA concentration was fixed at 10 µM. Most of the 295-400 nm range of fluorescence emission spectra were reported at 280 nm excitation wavelength. The test tubes containing the solution of BSA and the drug or its complexes were heated at least 10 min before the measurements (in fluorescence spectrophotometer F-7000) (Tanwir et al., 2012; Hossain et al., 2020b; Hossain et al., 2020c).

Antimicrobial screening

To assay the antimicrobial activity, the disc diffusion method (Biemer, 1973; Aktar et al., 2009) was used to evaluate the metal-drug complexes against nine bacteria (five gram positive bacteria viz.

Enterococcus faecium, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis* with four gram-negative bacteria viz. *Acinetobacter lwoffii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and 1 fungal species (*Candida albicans*) collected as pure cultures from the Department of Genetic Engineering, University of Dhaka, Dhaka, Bangladesh. The antimicrobial activity of the drug metal complexes was evaluated by mapping the diameter of the zone of inhibition expressed in mm. Mean values were taken by repeating the experiment thrice times (Khatun et al., 2021; Salve et al., 2022).

Results and Discussion

Characterizations

Both crystalline and amorphous drug complexes were obtained. To prove the complexation, TLC was carried in methanol-chloroform-toluene (20:40:40). Single spot from the two complexes differed from the parent drug were found (Table 1). Each spot indicated the presence of a new complex.

Table 1. R_f values of pure GeFo and two metal complexes GeFo-Zn and GeFo-Fe.

Drug and complex	R_f value
GeFo	0.2
GeFo-Zn	0.4
GeFo-Fe	0.5

TGA of standard GeFo in Figure 2(a) revealed that 13.02% decomposition occurred at 237.80 °C and 46.98% at 490.84 °C. While metal complex GeFo-Zn exhibited 17.36% decomposition at 241.47 °C and 54.15% decomposition at 566.30 °C in Fig. 2(b). Another, the metal complex GeFo-Fe showed 22.24% decomposition at 284.37 °C and 79.49% decomposition at 566.65 °C Fig. 2(c).

FT-IR by providing information about the presence or absence of specific functional groups helps to compare two similar molecular structures. Moreover, when two pure samples display the same IR spectra, they can be described as the same compounds (Aktar et al., 2019). In contrast, any shifts or disappearance of peaks are directed toward the presence of a different compound. The IR spectra obtained after drug-metal interaction demonstrated a new pattern of peaks compared to pure drug powder.

The FTIR spectrum of gemifloxacin mesylate showed a peak at 1043.49 cm^{-1} corresponding to (C-F) bending and at 1463.97cm^{-1} for O-CH_3 bending (Fig. 3), at 1199.72 cm^{-1} for R-COOH Stretching, at 1631.78 cm^{1} for N-H scissoring, at 1716.65cm^{-1} for aromatic -C=O stretching and at 898.83 cm^{-1}due to C-H rocking (Nagasree et al., 2016). In both GeFo-Zn and GeFo-Fe these characteristic peaks of the parent compound were shifted (Serafin and Stańczak, 2009). The complexation may occur as in Fig. 4.

TGA thermograms were obtained from pure drugs and metal complexes. The TGA thermograms are shown in Fig. 2.

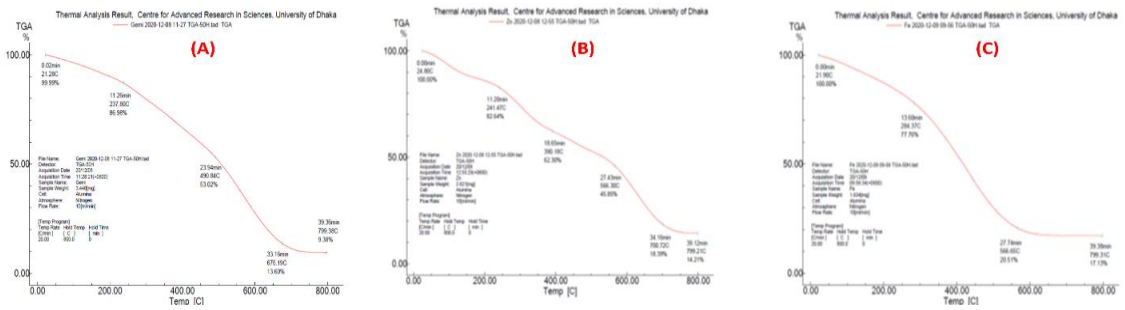


Fig. 2. TGA thermograms of A) pure GeFo, B) metal complex GeFo-Zn, C) metal complex GeFo-Fe.

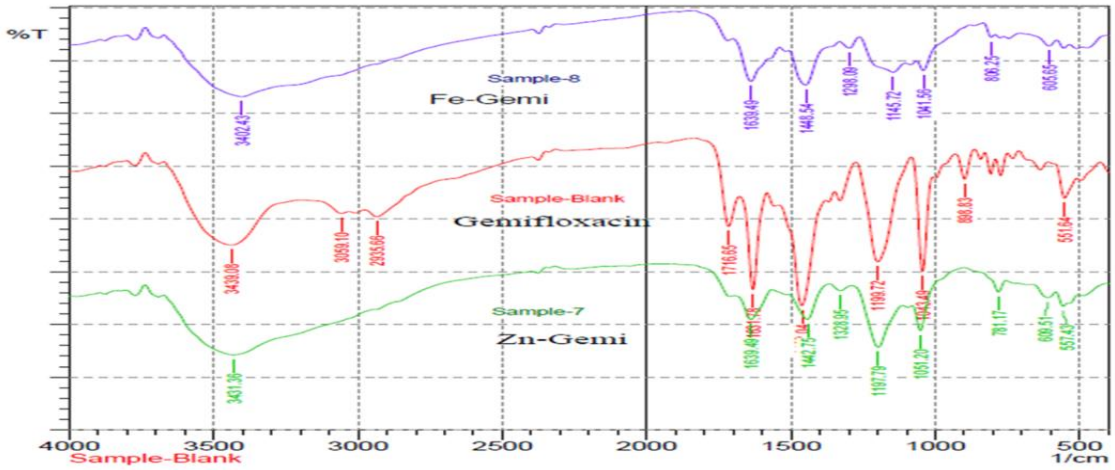


Fig 3. Overlaid FT-IR Overlaid FT-IR spectra of standard drug GeFo, metal complexes GeFo-Zn and GeFo-Fe.

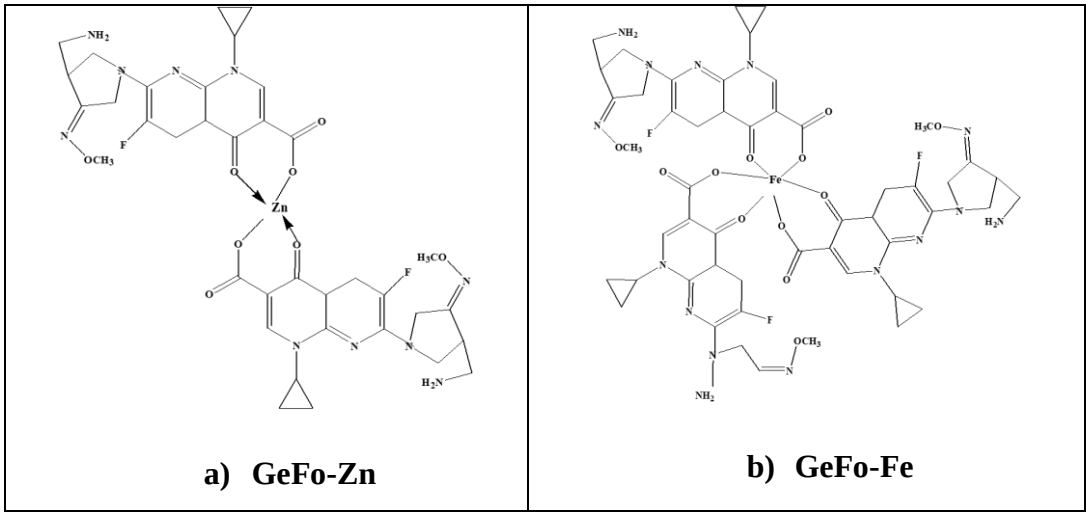


Fig. 4. Postulated structures of a) GeFo-Zn, and b) GeFo-Fe.

GeFo and GeFo-Zn & GeFo-Fe complexes-induced fluorescence quenching of BSA

Due to the fluorescence emission of its phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) residues, BSA has the inherent fluorescence property. Tryptophan is the dominant innate fluorophore as it is sensitive to local environmental changes due to conformational transition, subunit association, complex formation, and denaturation (Ghisaidoobe and Chung, 2014). Fluorescence quenching study provides precious information about the interaction mechanism between protein and ligand (Hossain et al., 2021a). Herein, fluorescence spectra of BSA (10 µM) upon exposure to various concentrations of GeFo and its metal complexes (0-50 µM) were measured and shown in Fig. 5. In the absence of the drug and drug complexes, BSA showed a typical emission spectrum with a maximum peak at 340 nm at 280 nm excitation wavelength. The fluorescence intensity of BSA gradually declined with the increased concentrations of drug and metal complexes.

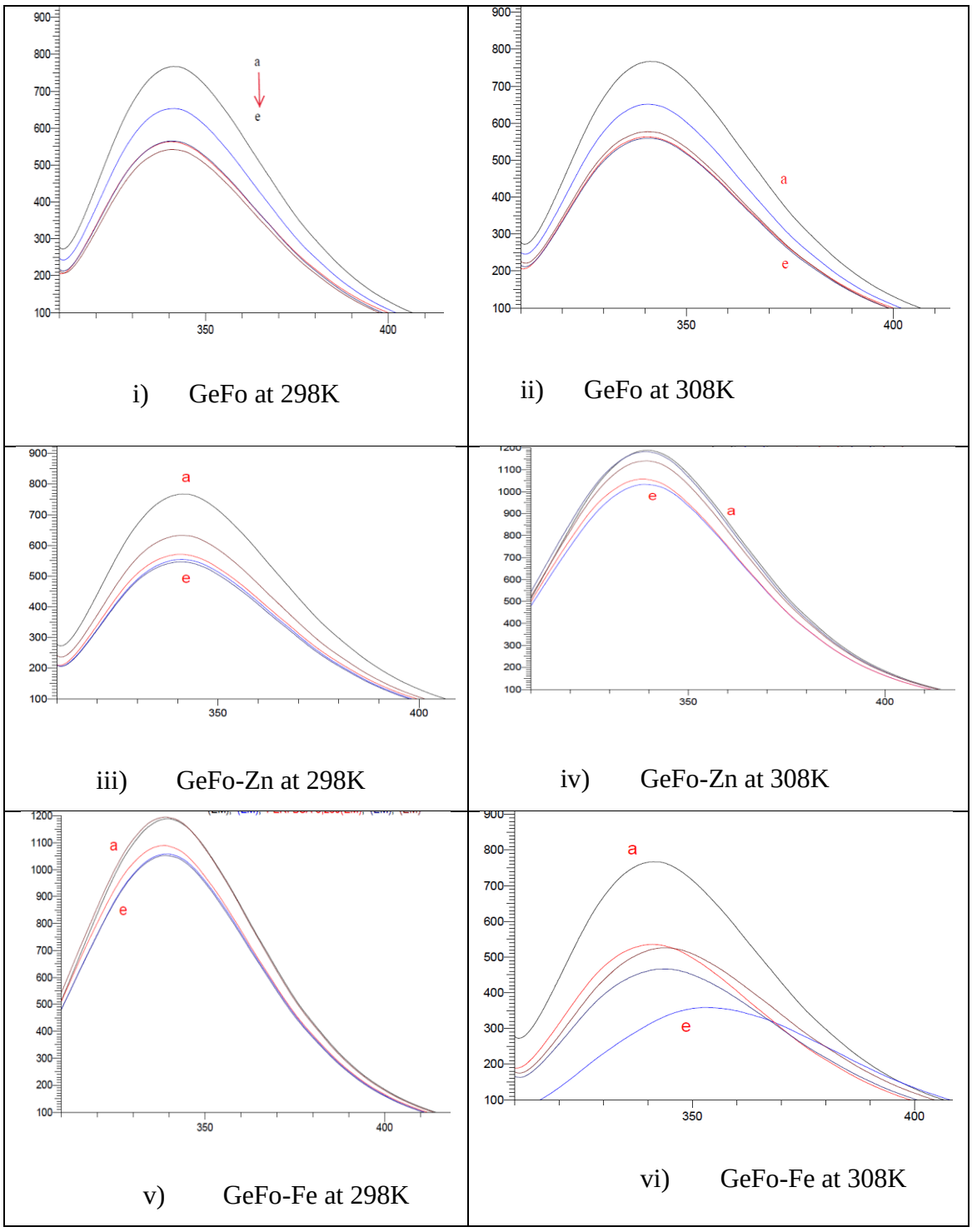
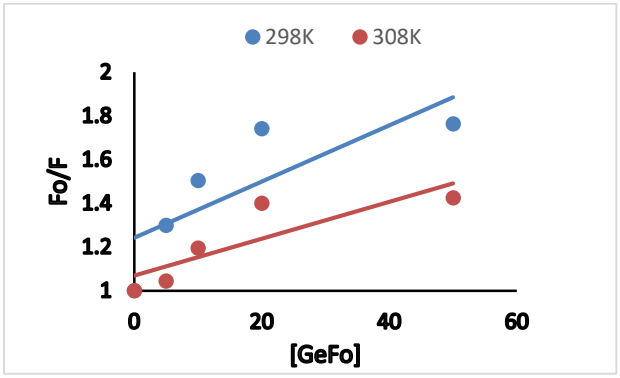


Figure 5. Fluorescence spectra of BSA (10 µM) in the presence of drugs and its metal complexes (a-e: 0, 5, 10, 20, and 50 µM) upon excitation with 280 nm wavelength at 298K and 308 K.

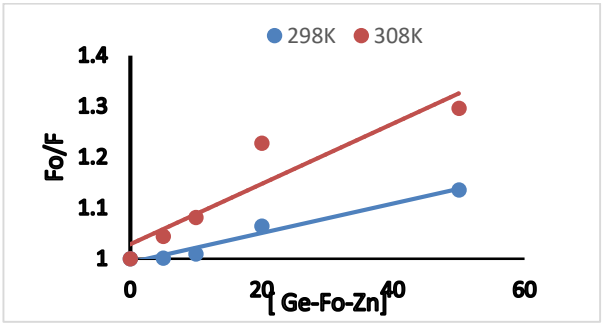
The Stern-Volmer equation (Equation (1)) was applied at the excitation wavelength of 280 nm to the elucidation of the interaction mechanism between BSA and the drugs. The plots of F_0/F versus ligand concentration ([Q]) fitted with the Stern-Volmer equation also help to distinguish between dynamic and static interactions between protein and its ligand. The static mechanism refers to the ground-state complex formation, while the dynamic mechanism means to the collisional encountering process (Hossain et al., 2021b). In the Stern-Volmer plot, a linear trend proposes that the interaction is guided by a single mechanism, either a static or dynamic process. If the Stern-Volmer constant (Ksv) increases with increasing temperature, then the dynamic process is mainly involved in the interaction system. As the temperature rising enhances diffusion and collision of protein and its ligand, that ultimately promotes the dynamic process as indicated by the increased slope of the Stern-Volmer plot. On the other hand, the rising temperature destabilizes the protein-ligand complex and substantially reduces the Ksv in the static quenching process (Hossain et al., 2021b).

$$F_0/F = 1 + Ksv[Q] = 1 + kq\,\tau_o[Q] \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

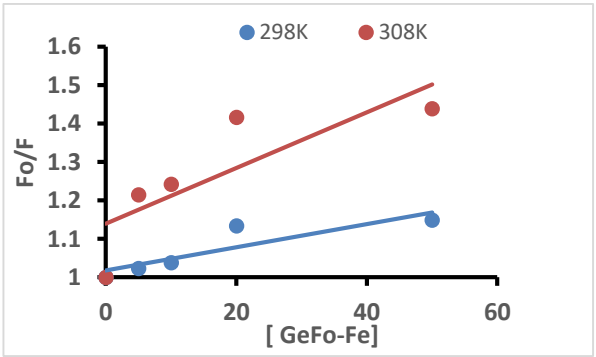
F_0 and F are the fluorescence intensities in the absence and the presence of quencher (drug and metal complexes), respectively. [Q] stands for the concentration of quencher. Ksv and kq are the Stern–Volmer constant and the biomolecular quenching rate constant, respectively. $\tau_o$is the average lifetime of the fluorophore (BSA) in the absence of quencher [$\tau_o = 1 \times 10^{-8}$s] (Hossain et al., 2021a; Hossain et al., 2021b). In this study, the fluorescence quenching was studied by measuring the fluorescence at an excitation wavelength of 280 nm at two different temperatures (298 K and 308 K). The fluorescence of BSA was dramatically reduced when exposed to the drug and drug complex in a range of 0-50 µM (Fig. 5). The quenching was inversely proportional to the temperature, where attenuation of fluorescence quenching was observed with the rising temperature. The F0/F versus drug and drug metal concentrations plots were perfectly fitted with a linear model of the Stern-Volmer equation (Fig. 6a, 6b and 6c), recommending a single mechanism involved in the BSA-drug interaction.



(a) [GeFo]×10^{-6} M



(b) [GeFo-Zn]×10^{-6}M



(c) [GeFo-Fe]×10^{-6}M

Fig. 6. (a) Gemifloxacin, GeFo, (b) Gemifloxacin-Zn, GeFo-Zn, (c) Gemifloxacin-Fe, GeFo-Fe the Stern-Volmer plots of bovine serum albumin (BSA) in the presence of drugs at 298 and 308 K. [GeFo], [GeFo-Zn] and [GeFo-Fe]=0–50 µM; [BSA]=10 µM; λex =280 nm.

As the temperatures increased, the Ksv values decreased for GeFo and GeFo-Zn, while for GeFo-Fe complex Ksv value was increased (Table 2), suggesting the primary involvement of static and dynamic quenching processes, respectively. Moreover, the biomolecular quenching rate constants (kq) were found at a level of 10^{12} $M^{-1}s^{-1}$, which was 100 times higher than the maximum scattering collisional quenching constant of various quenchers (2×10^{10} $M^{-1}s^{-1}$) (Liu et al., 2018). Hence, the findings suggested that GeFo and GeFo-Zn interact with BSA via a static process by the ground-state complex formation, while GeFo-Fe interaction with BSA is a dynamic process.

Antimicrobial activities

In antimicrobial activity screening, the metal complexes showed less inhibitory activity than the standard gemifloxacin mesylate (30 µg/disc) except against the *K. pneumoniae.* Against *K. pneumoniae* both the Fe and Zn complexes showed higher inhibitory activity than the standard, which is 17±0.54 mm at dose GeFo-Zn (30 µg/disc), 18±0.32 mm at dose GeFo-Zn (60 µg/disc), 14 ±0.58 mm at dose GeFo-Fe (30µg/disc) and 18±0.15 mm at dose GeFo-Zn (60 µg/disc) while for GeFo it was 12±0.16 mm. Both the standard drug and the Fe and Zn complexes showed resistance against *A. lwoffii*, *E. faecium* and fungal species *C. albicans.* Detailed results are shown in Table 3.

Table 2. Stern-Volmer constants for BSA-GeFo, BSA-GeFo-Zn, and BSA-GeFo-Fe interaction at different temperatures.

Sample	T (K)	1/T	Ksv $\times 10^4$ (M^{-1})	kq $\times 10^{12}$ ($M^{-1}S^{-1}$)
GeFo	298	0.0033	1.29±0.38	1.29±0.38
	308	0.0032	0.84±1.23	0.84±1.23
GeFo-Zn	298	0.0033	0.2± 0.05	0.2± 0.05
	308	0.0032	0.05±0.87	0.05±0.87
GeFo-Fe	298	0.0033	0.3±0.11	0.3±0.11
	308	0.0032	0.7±0.05	0.7±0.05

Table 3. Antimicrobial activity of standard drug gemifloxacin (30 µg/disc), Zn complexes (30 µg/disc, 60 µg/disc), and Fe complexes (30 µg/disc, 60 µg/disc) against ten different micro-organisms.

List of microorganisms	Zone of Inhibition (mm)				
Bacteria	Zn-gemi 30 µg/disc	Zn-gemi 60 µg/disc	Fe-gemi 30 µg/disc	Fe-gemi 60 µg/disc	Gemifloxacin mesylate 30 µg/disc
Acinetobacter lwoffii	R	R	R	R	R
Enterococcus faecium	R	R	R	R	R
Pseudomonas aeruginosa	12±0.23	17±0.54	9±0.54	15±0.56	24±0.34
Streptococcus pneumoniae	13±0.17	20±0.32	12±0.24	18±0.34	28±0.16
Staphylococcus aureus	9±0.32	22±0.32	14±0.21	20±0.23	25±0.25
Streptococcus pyogenes	8±0.21	14±0.65	7±0.43	9±0.65	16±0.24
Escherichia coli	22±0.43	25±0.73	11±0.37	15±0.26	30±0.15
Bacillus subtilis	17±0.57	23±0.12	7±0.43	12±0.12	24±0.14
Klebsiella pneumoniae	17±0.54	18±0.32	14±0.58	18±0.15	12±0.16
Fungi					
Candida albicans	R	R	R	R	R

Note: R indicates the resistances against the corresponding microorganisms.

Conclusion

The newly synthesized two metal complexes of gemifloxacin mesylate *viz.* GeFo-Fe and GeFo-Zn were characterized using TLC, TGA, and FT-IR spectra analyses. The formed complexes were then evaluated for antimicrobial activity. Nine bacteria species and one fungal strain were assessed against the newly formed two complexes using gemifloxacin (30 µg/disc) as standard. Among nine bacteria strains, two species and the fungal strain showed complete resistance to the newly synthesized complexes and the standard. Against *Klebsiella* spp. the new complexes showed better activity than the standard drug. The drug-protein interaction was also studied using BSA. The interaction mechanism among the drug and metal complexes with BSA suggested that gemifloxacin and its Zn complex interacted with BSA via a static process while the Fe complex interacted via a dynamic process and the lower Ksv value also indicated that Drug -BSA complexes were formed in ground-state. Research suggests that the zinc and iron complexes of the antibiotic gemifloxacin mesylate may change the drug's efficacy. The findings open possibility of promoting the production and utilization of metal complexes of antibiotics for treating resistant bacteria.

Acknowledgment

The authors are grateful to Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh, for providing APIs for the research; the Department of Genetic engineering, University of Dhaka, for providing fresh cultures of microorganisms and for providing laboratory facilities for antimicrobial study; Centre for Advanced Research in Sciences (CARS) for instrumental support; Mr. Ali (Lab Attendant, Department of Genetic engineering) and Mr. Konik Kumar Sarkar (Lab attendant, Drug Analysis and Research Laboratory, CARS, DU) for helping us during the research work.

Financial support:

The authors have received no financial support for conducting the research.

Data Availability:

All the data associated with this research are available in the manuscript.

Author contributions:

Fahima Aktar: Conceptualization, study design, methodology, data analysis and interpretation, drafting the original version.

Md. Jamal Hossain: Methodology, data curation, data analysis and interpretation, writing-reviewing and editing.

Md. Zakir Sultan: Methodology, data analysis and interpretation, writing-reviewing, and editing

Mohammad A. Rashid: Conceptualization, super-vision, writing-reviewing, and editing.

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