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Research article

Gemifloxacin-transition metal complexes as therapeutic candidates: antimicrobial, antifungal, anti-enzymatic, and docking studies of newly synthesized complexes

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ABSTRACT

In the era of acquired microbial resistance (AMR), resulting in the ineffectiveness of antibiotics is of keen interest for researchers in current scenarios. Ten novel metal complexes of gemifloxacin have been synthesized by reacting it with essential and trace elements in a 2:1 ratio predetermined conducto-metrically. As these metals are either present in the body or co-administered as metallic supplements can alter the level of antibiotics. Therefore, Metal complexes of Gemifloxacin, an important member of the fluoroquinolone family, were synthesized. The possible coordination of gemifloxacin with these metals has been proposed by the electronic and elemental data obtained through molar conductance, elemental analysis, and spectroscopic techniques like ultraviolet-visible (UV-Vis), infrared (IR), and proton-nuclear magnetic resonance (^{1}H NMR) studies.

In the light of these studies, the monoanionic bidentate ligand behavior of gemifloxacin in complexation with metals has been revealed. For *in-vitro* microbial studies, these newly synthesized complexes were tested against eleven different bacteria including Gram + ve and Gram -ve organisms, and one fungal strain. The results were compared with the parent drug by applying ANOVA through SPSS software version 22. Therefore, it has been found that among all synthesized metal complexes, the *G-M01* complex exhibits increased activity against *B. subtilis, P. mirabilis, E. coli, K. pneumonia,* and *C. freundii*. Complex *G-M02, G-M03, G-M04,* and *G-M10* show more pronounced activity than Gemifloxacin against *S. aureus* and *M. luteus*. Moreover, the binding orientations of the synthesized metal complexes into the binding site of the urease enzyme revealed that all the docked metal complexes oriented away from the Ni *bi*-center, and the inactivation of urease is due to their interaction with entrance flap residues.

1. Introduction

In the field of related bioprocesses and medicinal chemistry involved in human physiology, metal chelates/complexes of antibiotics played a pi-vital role. In various reported studies, the pharmacological behavior of antibiotics is very much dependent upon the coordination/complexation with the essential and trace elements (Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}) present inside the body [1]. As these are required for the effective transportation of drugs intracellularly, inhibition of bacterial cell wall synthesis or protein, and regulation of bacterial resistance via efflux mechanisms [2] adopted by various micro-organisms for the development of Acquired Microbial Resistance (AMR). The antibacterial response of quinolones is due to coordination with metal ions like Fe^{2+}, Cu^{2+}, and Al^{3+} [3] responsible for their reduced bioavailability.

Synthesis of metal complexes with diverse antibiotics has always been a keen center for research studies, due to the important role of these

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essential and trace elements in various physiological responses of the body alone/in synergism with antibiotics [4, 5]. Previously synthesized quinolone-metal complexes prove that these complexes exert change/variation in the biological response of these antibiotics against microorganisms and disease conditions [6].

Fluoroquinolones are a group of synthetic broad-spectrum antibiotics having various active members like Ciprofloxacin, Levofloxacin, Sparfloxacin, Gatifloxacin, Moxifloxacin, Gemifloxacin, etc. These members of the quinolones family show a significant effect against infection including the majority of Gram + ve and Gram -ve strains [7].

Our research group previously has reported the synthesis and biological profiling of essential and traces metal complexes of several fluoroquinolones (including 3rd and 4th generation agents) complexes, revealing changes in the physicochemical, spectroscopic and biological responses [8, 9, 10]. As evident from the research data, quinolone's absorption is well altered when given concomitantly with multivitamins, or, antacids, and other bivalent cations containing supplements [11, 12, 13]. In our previous study, we have already reported sparfloxacin metal complexes' response as antifungal agents where Fe^{2+}–SPFX and Cd^{2+}–SPFX complexes proved to be more potent than the parent molecule [8].

To study the interface of Gemifloxacin (Figure 1) with metals, the complexation of Gemifloxacin was done with some essential trace metals. Synthesized metal complexes were examined with physical parameters like color, solubility, % yield, and melting point. Some of the quinolones may act as bidentate, showing the involvement of 4-oxo, the carbonyl group found adjacent along with metal cations in Gemifloxacin.

Complexes were then characterized spectroscopically through UV-Vis, FT-IR, and ^{1}H NMR, while the presence of metal in the complex is further confirmed by elemental analysis (CHN). In addition, microbiological evaluations were achieved against selected fungal specie and different Gram + ve and Gram -ve bacterial species. Enzyme inhibition studies have been performed to check the anti-enzymatic activity of prepared metal complexes against urease and α-chymotrypsin enzyme. While docking simulations of the synthesized metal complexes were carried out in the binding site of the urease enzyme.

2. Materials and methods

Gemifloxacin mesylate (Figure 1) was gifted for this work by Pharm Evo (Pvt Ltd.) Pakistan, while all the chemicals and solvents were of analytical grade. For spectral studies, Shimadzu Model FT-IR Prestige-21 spectrophotometer using Shimadzu IR solution 1.2software, Bruker AMX 500 MHz spectrometer for 1H-NMR, and Carlo Erba 1106 were used for CHN analysis. Conductometric analysis was carried out on Vernier Lab Pro TM having Logger Pro 3.2 software, while the Argentometric method was used to determine the chloride levels. Autodock version 4.2 was used for docking studies.

2.1. Stoichiometric study

Job's method of continuous variations is the most applied technique for determining the stoichiometric ratio of coordination compounds through UV-Vis spectrophotometer [14, 15, 16] along with conductometric titration. Both methods were adopted from our previous studies with slight modifications [8, 9].

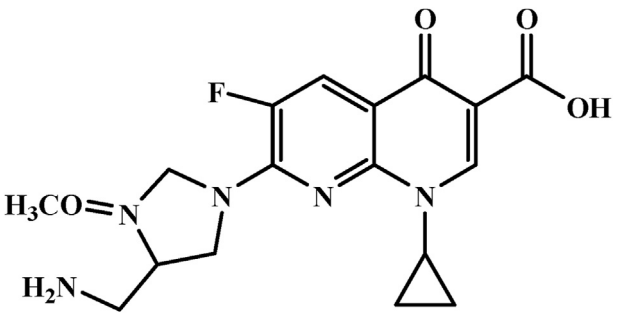

Figure 1. Gemifloxacin

2.2. Synthesis of GMFX-solid complexes

Ca, Cr, Mg, Mn, Co, Fe, Ni, Zn, Cu, and Cd complexes with GMFX were prepared by adding 20 ml of GMFX (L) 0.2 mmol/dm^3 to 20 ml of metallic chloride (M) 0.1 mmol/dm^3 in hot methanol, followed by refluxing (60 °C, 3 h) with intermittent stirring on a water bath. Solutions were then filtered and left to dry under vacuum over silica gel, 3 ml of diethyl ether was added to obtain fine crystals/dried complexes, earlier [8].

Further, their physicochemical characteristics including color, percent yields, melting points, and solubility were noted. These complexes were then characterized by spectroscopic techniques such as UV, IR, ^{1}H-NMR, and elemental analysis.

2.3. Microbiological evaluation

Microbiological screening of newly formed metal complexes has been performed against some bacteria (including Gram + ve and Gram-ve) and one fungal specie by disc diffusion method [17, 18] with some minor modifications. The chosen strains were *Micrococcus luteus* ATCC 10786, *Bacillus subtilus* ATCC 6051-U, *S. features* ATCC 24843, *Staphylococcus aureus* ATCC 29213, *Proteus mirabilis* ATCC 29906, *Salmonella typhi* ATCC 2881, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klesbellia pneumonia* ATCC 43816, *Shigella flexneri ATCC* 29903, *Citrobacter freundii* ATCC 13316 and *Candida albicans ATCC* 3147 gifted by Dr. Essa Laboratory and Diagnostics (Pvt.) Ltd.

Sterile Distill water with 0.8% soft agar having 110 CFU ml^{-1} (optical density OD ≈ 0.3 nm) was used to prepare each strain of bacterial suspension. This mixture has been poured into each Petri dish (90 mm) [15]. Dried antibacterial discs (6mm)-OXOID (Milan, Italy) were prepared by soaking each metal complex solution and reference drug at 5, 10, and 20 μg/mL concentrations, using water as a solvent, applied over each culture of the organism. For negative control DMSO discs were used, followed by incubation (18–24 h) at 37 °C for antibacterial activity and 48 h for antifungal activity. Inhibition zones (in mm) were determined by measuring through a digital Vernier caliper and then compared with GMFX and negative control. The results were obtained in triplicates against each organism.

2.4. Statistical analysis

One-way ANOVA was applied to the microbial study using statistical software SPPSS version 22, USA.

2.5. Enzyme inhibition studies

For the assay of urease inhibition, the method was adopted from our previously reported work with some modifications having thiourea as standard [13]. Chymotrypsin inhibitory activity was studied at 410nm by adopting Cannel Method using Chymostatin as standard. Calculations were performed on SoftMax Pro software (Molecular Devices) [19, 20].

2.6. Docking studies

Docking simulations of the synthesized metal complexes were carried out in the binding site of the urease enzyme. The accession code for the downloaded enzyme was 4UBP. Autodock version 4.2 was used for docking studies. The docking studies were carried out by using our previously reported procedures [21, 22]. Metals van der Waals and other parameters were obtained from the Autodock website. Diovery studio visualizer was used to analyze the 3-D interaction plots of the ligand-enzyme complexes.

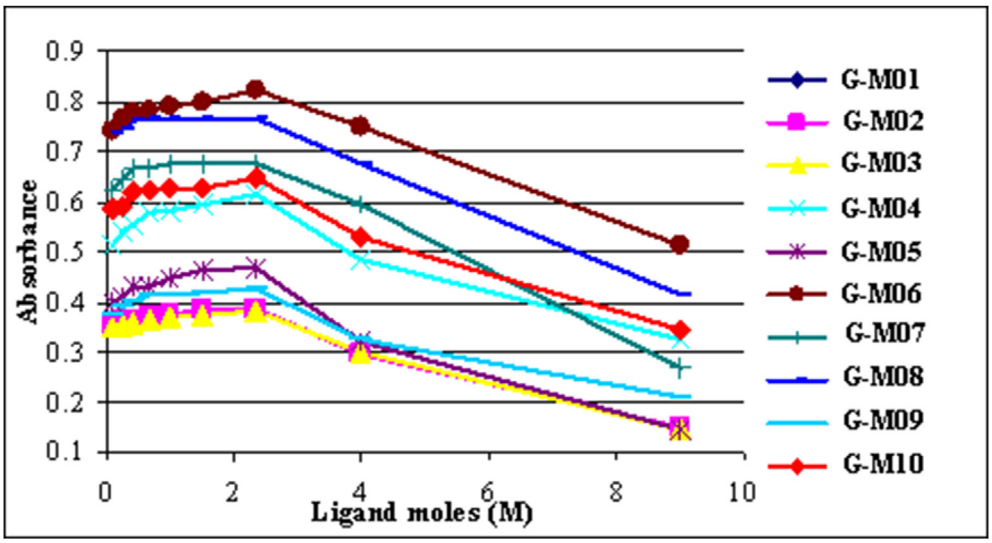

Figure 2. Showing complexation of gemifloxacin with transition metals.

3. Result and discussion

For the synthesis of all the ten GMFX-metal complexes, their stoichiometric ratio was predetermined via conductometric analysis and found 1:2 (M: L) and was then synthesized Figures 2 and 3) having good percentage yields. Proposed Structural formulas (Calculated) of the metal complexes were in good agreement with the elemental analysis results (Table 1). GMFX-metal complexes were insoluble in dichloromethane, benzene, and chloroform while completely soluble in DMSO, methanol, and water.

3.1. Spectral studies

Infrared spectroscopic data is used to characterize the metal complexes of quinolones, focusing on typical vibrations and carboxylate coordination, as a diagnostic tool. IR spectra of GMFX are quite comprehensive where OH stretching vibrations of COOH and N–H of piperazinyl ring exhibit a broad spilled band between 3500-3100 cm^{-1} [13,17]. Determination of metal binding mode with the carboxylate group of gemifloxacin revealed its bidentate bridging complexation in all Gemifloxacin-metal complexes and was determined by the difference ($\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$) (Table 2) [13].

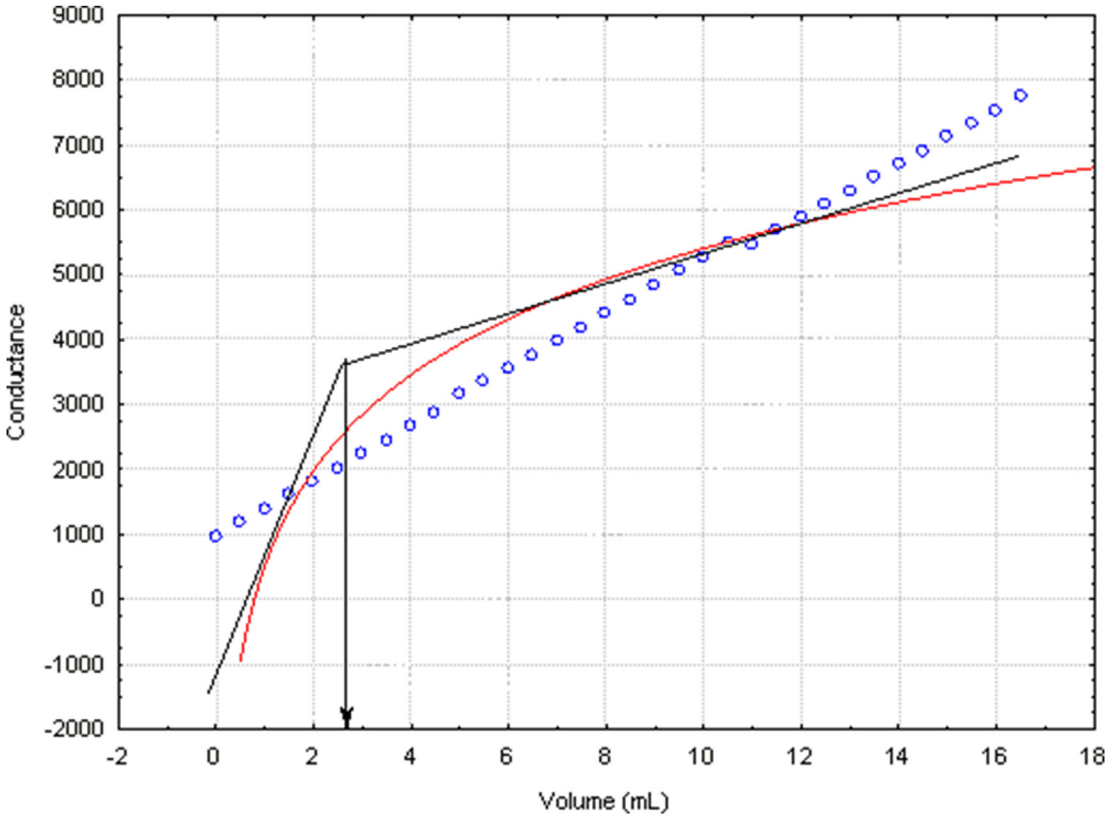

Figure 3. Representation of gemifloxacin-metal complexes ratio via conductance.

Table 1. Physicochemical data and *Elemental Analysis* of gemifloxacin and newly developed transition metal complexes.

Metal-Complexes	% Composition Found (Calculated)				M.P^{0}C	Color	Yield (%)
	C	H	N	M*			
GMFX	47.01 (47.16)	4.98 (5.07)	14.43 (14.10)	-	235	Light green	-
$[Mg\ (GMFX)_2(H_2O)_2]\cdot 2Cl_2$	45.95 (45.86)	5.10 (4.86)	14.51 (14.07)	9.71 (10.05)	100	Green	69
$[Ca\ (GMFX)_2H_2O]\cdot 2Cl_2$	45.05 (44.35)	5.41 (4.9)	14.01 (13.61)	8.99 (9.55)	178	Maroon	74
$[Cr\ (GMFX)_2(H_2O)]\cdot 2Cl_2\cdot H_2O$	42.98 (42.38)	5.62 (5.05)	13.62 (13.01)	9.33 (9.02)	90	Brown	75
$[Mn\ (GMFX)_2(H_2O)]\cdot 2Cl_2$	43. 54 (42.98)	5.78 (4.94)	13.56 (13.19)	8.97 (9.32)	80	Green	74
$[Fe\ (GMFX)_2(H_2O)_2]\cdot 2Cl_3$	43.43 (42.94)	5.89 (4.93)	13.43 (13.18)	9.54 (9.89)	79	White	80
$[Co(GMFX)_2H_2O]\cdot 3Cl_2$	36.94 (36.29)	4.92 (4.00)	12.04 (11.11)	9.43 (10.15)	150	Maroon	82
$[Ni(GMFX)_2H_2O]\cdot 2Cl_2$	36.85 (36.21)	4.85 (4.00)	11.92 (11.11)	9.05 (9.63)	120	Brown	71
$[Cu(GMFX)_2H_2O]\cdot 2Cl_2\cdot H_2O$	43.95 (943.36)	5.25 (4.79)	13.93 (13.31)	8.74 (9.02)	140	Green	76
$[Zn\ (GMFX)_2H_2O]\cdot 2Cl_2$	44.12 (43.29)	5.29 (4.78)	13.85 (13.28)	8.92 (9.33)	150	Brown	70
$[Cd\ (GMFX)_2H_2O]\cdot 2Cl_2$	42.15 (41.44)	5.12 (4.58)	13.01 (12.72)	9.62 (10.03)	130	Maroon	73

G-M01 (Mg $(GMFX)_2$), G-M02 (Ca $(GMFX)_2$), G-M03 (Cr $(GMFX)_2$), G-M04 (Mn $(GMFX)_2$), G-M05 (Fe $(GMFX)_2$), G-M06 (Co $(GMFX)_2$), G-M07 (Ni $(GMFX)_2$), G-M08 (Cu $(GMFX)_2$), G-M09 (Zn $(GMFX)_2$), G-M10 (Cd $(GMFX)_2$). *Metal

In GMFX, the carboxylic group peak appeared at 1718 cm^{-1} [23,24] which is shifted in all synthesized metal complexes ranging between 1627-1685 cm^{-1} and 1399- 1412 cm^{-1} and were assigned as asymmetric, υ (CO2) asym, and symmetric, υ (CO_2) sym, respectively. A broad band at 549-786 $cm^{-1}$is confirms the presence of OH molecule bonded with metal and with oxygen [8, 25, 26, 27].

The proton NMR spectra of synthesized complexes have been recorded in $CdCl_3$, and the spectra of complexes were compared with Gemifloxacin. HNMR spectra of the complexes revealed the presence of all the signals of the parent molecule. Aliphatic and piperazine protons practically remained unchanged as they lay far from the binding ligand site [8, 9]. The peaks at δ: 8.66 (s) and δ: 7.24(d) ppm indicated aromatic H-2 and H-5 protons of GMFX respectively which are close to the coordination site of GMFX with metals. In developed complexes H-2 proton gives a new signal at δ: 8.56–8.81 ppm while the H-5 proton appeared at δ: 7.42–7.91 ppm [28]. The overall changes of the 1H-NMR spectra of the complexes were indicative of the coordination of Gemifloxacin to the metal via the pyridine and one carboxylate oxygen atom (Table 2) as shown in (Figure 4).

3.2. Atomic absorption spectroscopy

Atomic absorption spectroscopy is used widely to determine the metals via direct analysis in solution form at variable concentrations. The Graph was plotted against absorbance and concentration at a specified wavelength of each metallic solution, revealing a linear relationship. Results obtained from the elemental analysis and atomic absorption assure the proposed structural formula (Figure 4) confirming their formation in the proposed ratio as given in Table 1.

3.3. Microbiological screening for gemifloxacin-metal complex

Formed complexes were further evaluated for their antimicrobial activity against a broad spectrum of Gram + ve and Gram-ve bacteria along with fungal specie (01) by disc diffusion method at different concentrations of 5, 10, and 20 μgmL^{-1}, selected based on having antimicrobial response. Some of these organisms have their role in the acquired microbial resistance (AMR) against antibiotics by adopting morphological and biochemical modifications. *C. albicans* is a fungal organism that is involved in minor to major nosocomial urinary tract infections across the world especially in females [29] with a death rate of 1.5 million per year [30]. It has been observed in our earlier research that sparfloxacin metal complexes showed some pronounced activity against fungal species [19]. Therefore, in the current microbial study of GMFX-metal complexes, *C. albicans* was also tested. S.D, F-value, Dunnet test, and one-way ANOVA, $P < 0.001$of all the complexes was carried out by using SPSS as mentioned in Table 3 a,b.

Statistical parameters confirm that G-M04 showed the highest antibacterial activity against *M. Luteus*, *B. Subtitis*, *P. mirabilis* (5 μgmL^{-1}), and *S. aureus* (20 μgmL^{-1}). G-M03 attributes great activity against *S. aureus* and *C.ferundii* (5 μgmL^{-1}) at their lowest concentration. Among all, G-M10 showed maximum activity against *S.typhi*, *E. coli*at lowest concentration 5 μgmL^{-1} while showed activity against *S. aureus* at 20 μgmL^{-1}. G-M05 exhibits activities that are equivalent to the parent molecule (GMFX) against *S.flexneri* at 20 μgmL^{-1}. It was observed that the G-M01 metal complex showed maximum antibacterial activity at a concentration of 20 μgmL^{-1} against *B. subtilis*, *P. mirabilis, E. coli, K. pneumonia,* and *C. ferundii* while against *M. luteus* and *B. subtilus,* all

Table 2. Spectroscopic data of gemifloxacin-metal complexes (FT-IR and ^{1}H-NMR).

Complexes	O–H stretching	υ (C=O)	υ $(CO_2)_{as}$	υ $(CO_2)_s$	[a] Δ	υ (M = O)	H2	H5
GMFX	3473	1627	1718[b]	-	-	898	8.66	7.24
G-M01	3412	1674	1531	1409	122	549	8.60	7.91
G-M02	3438	1685	1540	1406	134	561	8.61	7.91
G-M03	3392	1657	1532	1411	121	553	8.59	7.59
G-M04	3421	1680	1541	1405	136	628	8.56	7.90
G-M05	3429	1654	1533	1399	134	640	8.81	7.42
G-M06	3486	1627	1536	1407	129	786	8.60	7.46
G-M07	3438	1655	1531	1410	121	746	8.58	7.95
G-M08	3395	1653	1535	1408	127	690	8.65	7.65
G-M09	3433	1647	1545	1412	133	673	8.60	7.81
G-M10	3455	1651	1531	1402	129	732	8.65	7.53

Table 3 (a). Antibacterial activity of GMFX-metal complexes against studied organisms (% zone of inhibitions in mm).

Organism	*M. luteus*	*B. subtilis*	*S. features*	*S. aureus*	*P. mirabilis*	*S. typhi*
GMFX	18.41 ± 0.05	22.21 ± 0.21	16.33 ± 0.06	16.2 ± 0.12	20.38 ± 0.05	17.27 ± 0.22
G-M01	12.13 ± 0.07* 33.39	17.28 ± 0.16* 23.03	18.36 ± 0.16* -12.57	16.44 ± 0.05* -0.24	21.31 ± 0.06* -4.87	12.08 ± 0.09* 30.33
G-M02	15.31 ± 0.12* 15.93	7.33 ± 0.17* 67.35	16.26 ± 0.07 0.31	14.26 ± 0.12* 13.05	10.31 ± 0.02* 49.26	12.27 ± 0.02* 29.24
G-M03	11.25 ± 0.18* 38.22	14.16 ± 0.07* 36.93	16.17 ± 0.1 0.86	16.22 ± 0.1* 1.1	18.14 ± 0.18* 10.73	13.26 ± 0.15* 23.53
G-M04	14.28 ± 0.12* 21.58	16.22 ± 0.11* 27.75	18.32 ± 0.22* -12.32	16.23 ± 0.1* 1.04	17.24 ± 0.16* 15.16	14.25 ± 0.2* 17.82
G-M05	12.33 ± 0.14* 32.29	10.27 ± 0.1* 54.25	14.18 ± 0.17* 13.06	12.16 ± 0.15* 25.85	15.14 ± 0.08* 25.49	11.3 ± 0.08* 34.83
G-M06	12.26 ± 0.19* 32.67	10.22 ± 0.18* 54.48	14.35 ± 0.1* 12.02	14.14 ± 0.22* 13.78	16.23 ± 0.19* 20.13	11.19 ± 0.15* 35.47
G-M07	14.3 ± 0.21* 21.47	7.22 ± 0.043* 67.84	14.31 ± 0.09* 12.26	14.25 ± 0.12* 13.11	11.34 ± 0.09* 44.19	13.23 ± 0.02* 23.7
G-M08	10.18 ± 0.22* 44.1	9.3 ± 0.08* 58.57	15.32 ± 0.09* 6.07	11.18 ± 0.13* 31.83	12.2 ± 0.06* 9.96	11.23 ± 0.03* 35.24
G-M09	13.27 ± 0.2* 27.13	9.29 ± 0.14* 58.62	15.23 ± 0.2* 6.62	15.19 ± 0.1* 7.38	18.22 ± 0.11* 10.33	12.27 ± 0.12* 29.24
G-M10	14.26 ± 0.13* 21.69	10.19 ± 0.19* 54.61	18.26 ± 0.15* -11.96	18.05 ± 0.03* -10.06	16.25 ± 0.16* 20.03	15.28 ± 0.18* 11.88
ANOVA ($P < 0.001$), df = 10, 32						
F-value	529.86	4089.23	357.35	739.32	2229.46	784.722

n = 3, mean ± S.D, % ZI * indicates significance, and the -ve sign shows an increase in activity at a concentration of 20 μgmL^{-1}.

Table 3(b). Antibacterial activity of GMFX-metal complexes against studied organisms (% zone of inhibitions in mm).

Organism	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*S. flexneri*	*C. freundii*	*C. albicans*
GMFX	20.42 ± 0.06	25.19 ± 0.17	16.11 ± 0.12	17.08 ± 0.13	14.18 ± 0.13	15.25 ± 0.14
G-M01	15.3 ± 0.1* 24.48	15.13 ± 0.15* 39.94	16.42 ± 0.13–0.43	14.35 ± 0.15* 16.76	16.35 ± 0.03* -15.06	12.09 ± 0.04* 20.62
G-M02	12.14 ± 0.11* 40.08	12.29 ± 0.11* 51.21	16.4 ± 0.02–0.30	14.21 ± 0.13* 17.58	1.4 ± 0.07* 90.15	14.19 ± 0.1* 6.83
G-M03	16.26 ± 0.12* 19.74	17.18 ± 0.11* 31.8	12.26 ± 0.23* 25.01	14.46 ± 0.05* 16.13	16.18 ± 0.05* -13.86	13.35 ± 0.14* 12.34
G-M04	14.12 ± 0.08* 30.31	20.36 ± 0.11* 19.17	12.16 ± 0.18* 25.63	15.24 ± 0.06* 11.6	13.13 ± 0.07* 7.6	11.2 ± 0.23* 26.46
G-M05	13.3 ± 0.18* 34.35	20.05 ± 0.03* 20.4	14.2 ± 0.08* 13.15	16.27 ± 0.1* 5.63	12.2 ± 0.19* 14.14	0 ± 0* 100
G-M06	13.34 ± 0.13* 34.16	12.15 ± 0.19* 51.77	9.27 ± 0.04* 43.3	11.09 ± 0.01* 35.67	13.18 ± 0.1* 7.25	11.16 ± 0.09* 26.72
G-M07	12.49 ± 0.01* 38.35	14.17 ± 0.2* 43.75	12.21 ± 0.15* 25.32	14.22 ± 0.06* 17.52	14.3 ± 0.17–0.63	16.21 ± 0.25* -6.43
G-M08	12.16 ± 0.09* 39.98	14.26 ± 0.2* 43.39	14.19 ± 0.1* 13.21	9.39 ± 0.05* 45.53	14.23 ± 0.16–0.14	10.26 ± 0.16* 32.59
G-M09	14.17 ± 0.19* 30.06	16.26 ± 0.04* 35.45	13.37 ± 0.09* 18.23	13.2 ± 0.03* 23.43	9.13 ± 0.09* 35.75	11.31 ± 0.27* 25.74
G-M10	17.29 ± 0.06* 14.66	20.11 ± 0.12* 20.17	12.22 ± 0.14* 25.26	16.25 ± 0.2* 5.74	14.22 ± 0.15–0.07	14.39 ± 0.06* 5.25
ANOVA ($P < 0.001$), df = 10, 32						
F-value	1232.13	2449.111	928.252	1280.24	705.60	2153.10

n = 3, mean ± S.D, % ZI * indicates significance, and the -ve sign shows an increase in activity at a concentration of 20 μgmL^{-1}.

complexes exhibited activity less than Gemifloxacin. To our expected surprise, two metal complexes G-M08 and G-M10 exhibit response against the fungal strain. G-M08 showed the highest antifungal activity against *C. albican* at 10 μgmL^{-1} whereas G-M10 showed activity at 20 μgmL^{-1}.

These altered intrinsic activity responses of the complexes can be explained based on their lipid membrane involved in lipid-soluble materials penetrating ability and cell permeability responsible for controlling the antimicrobial resistance. Reportedly, overlapped ligand orbital involved in partial sharing of the +ve charge of the metal ion with Ligand

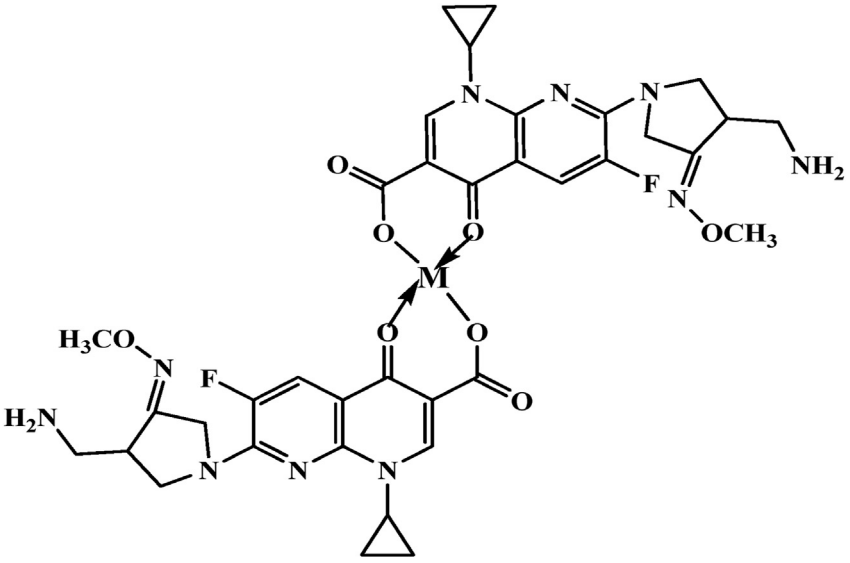

Figure 4. Proposed structure of gemifloxacin-transition metal complex.

Table 4. Enzymatic profiling of GMFX-transition metal complexes.

Enzymes	urease	α-chymotripsin
Complexes	IC50 ± SEM (μm)	IC50 ± SEM (μm)
GMFX	-	-
G-M01	155 ± 0.60	0
G-M02	169.23 ± 0.35	0
G-M03	145.56 ± 0.07	0
G-M04	154.48 ± 0.60	0
G-M05	139.21 ± 0.97	0
G-M06	166.35 ± 0.33	0
G-M07	142.61 ± 0.09	0
G-M08	168.21 ± 0.43	0
G-M09	148.98 ± 0.59	0
G-M10	158.41 ± 0.63	0
Standard	21.00 ± 0.12	5.7 ± 0.13

(Drug) attributes to the reduced polarity [31]. This change in hydrophilic and lipophilic character contributes to the blocking of the enzymatic metal binding site of microorganisms due to transportation through the bacterial cell wall [32].

3.4. Enzyme inhibition studies

Enzyme inhibitory activity revealed that GMFX metal complexes showed good inhibitory activity against urease enzyme (G-M02–78.8%, G-M08–77.6%) as compared to α-chymotrypsin which shows overall below 30% inhibition in all tested complexes (Table 4 and Figure 5).

3.5. Docking studies

We investigated the possible binding orientations of the synthesized metal complexes into the binding site of the urease enzyme. Urease enzyme in complex with acetohydroxamic acid (HAE) was obtained from Protein Data Bank. The accession code for the downloaded enzyme was 4UBP. AutoDock4.2 with Lamarckian Genetic Algorithm (LGA) was used for docking. After validation of the docking protocol by using a re-dock method, all the synthesized metal complexes were docked into the binding site of 4UBP.

The analysis of interaction plots of the docked ligand-enzyme complexes revealed that these bulky metal complexes were not able to coordinate with the Ni *bi*-center present in the active site. However, the complexes interacted with the important histidine and flap residues present at the entrance of the active site of the enzyme (Figure 6). Among flap residues, Cys322 is an important flap residue and is involved in the positioning of other active site residues. Other residues of the flap region are His323, Arg339, Asp363, Ala366, and Met367.

Three-dimensional interaction plots of chromium, iron, and manganese metal complexes are shown in Figure 6. It can be seen from the interaction plots that the studied complexes interact with the flap residues via hydrogen bond interactions. Chromium complex forms four hydrogen bond interactions with Ala279, His323, Arg339, and Cys322. Met367 forms π-sulfur interactions. Lys169 forms hydrogen bonds as well as halogen interactions (Figure 7a). Manganese complex forms hydrogen bonds interaction with Lys169, His324, Arg339, and Ala366. His324 also interacts with compounds *via* bifurcated π-π stacking interactions (Figure 7b). The Iron complex also forms hydrogen bond interactions with Lys169, His324, Arg339, and Ala366. Cys322 and Asp324 form a halogen bond with the fluorine atom (Figure 7c). The computed binding energy values for the chromium, Manganese, and Iron complexes are -5.63 kcal/mol, -5.04 kcal/mol, and -6.49 kcal/mol respectively.

Our results revealed the monoanionic bidentate behavior of gemifloxacin in complexation with metals. The characterization was carried out of newly synthesized GMFX-metal complexes that include elemental analysis along with Spectroscopic techniques (UV, IR, NMR). In IR spectra, the shifting of a carboxylic peak in metal complexes confirms the involvement of the carboxylic group in complexation. The Shifting of the carboxylic group peak (1718 cm^{-1}) in metal complexes between 1627-1685 cm^{-1} and 1399- 1412 cm^{-1} indicates the formation of GMFX-

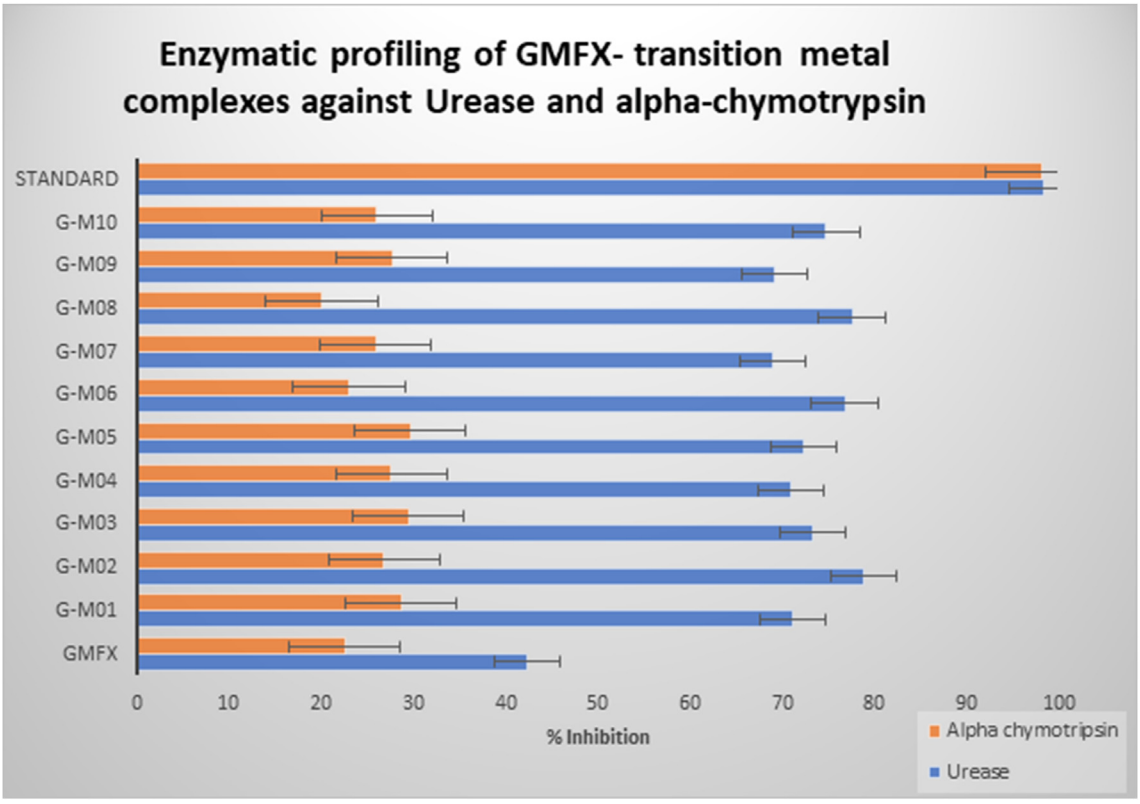

Figure 5. Enzymatic profiling of GMFX-transition metal complexes against Urease and alpha chymotrypsin.

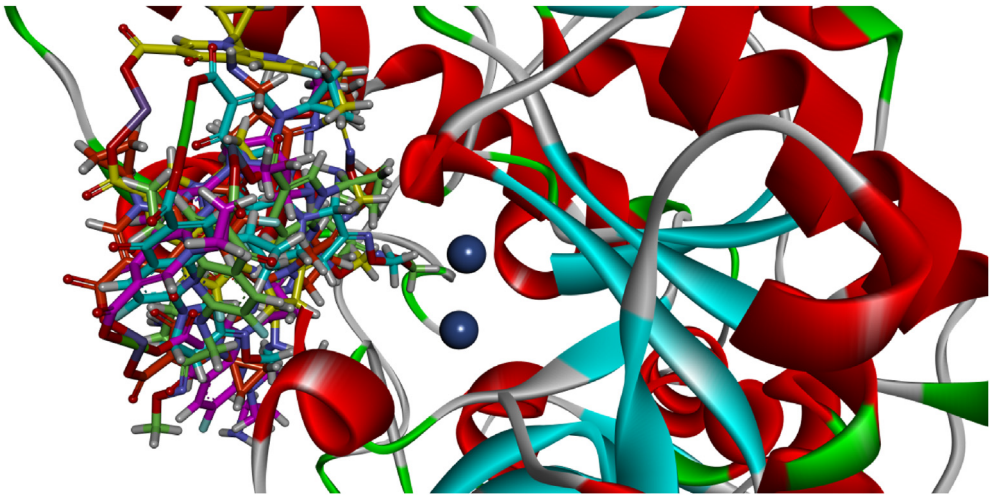

Figure 6. Ribbon diagram of overlaid binding poses of some of the metal complexes in the binding site of urease (PDB ID = 4UBP). All the docked complexes are shown in stick while Ni atoms are represented by blue spheres.

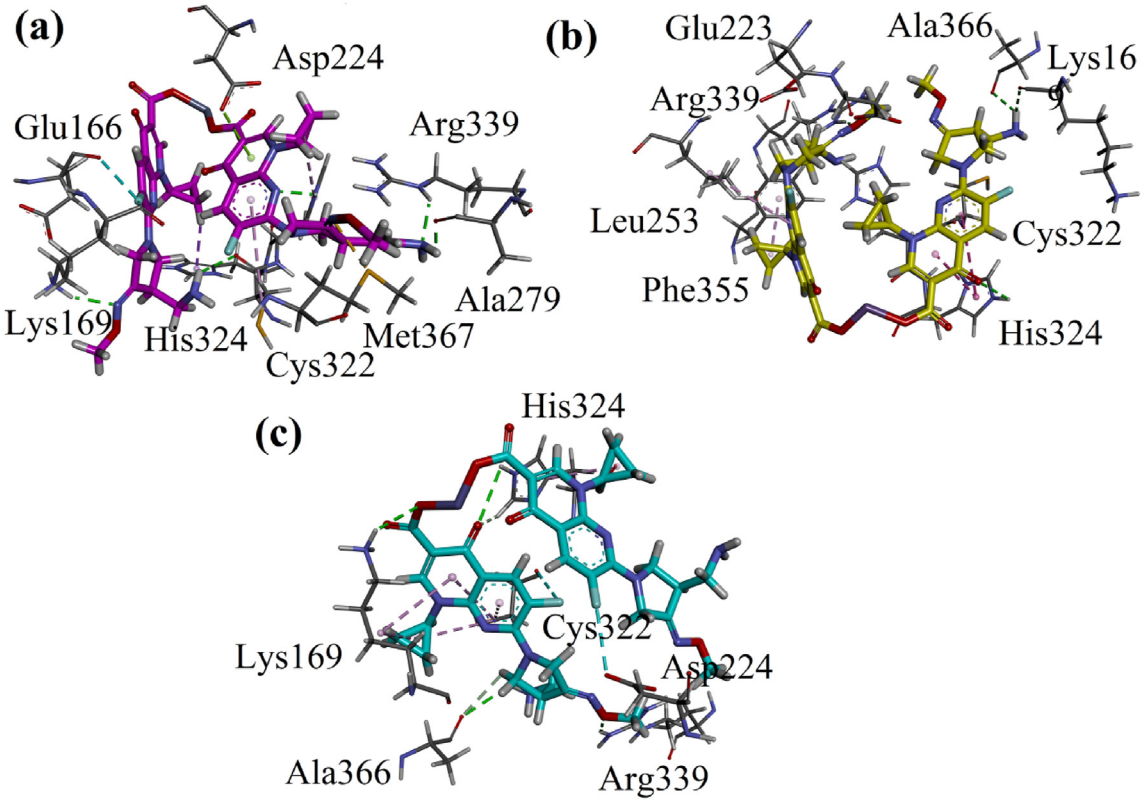

Figure 7. a–c 3-D interaction plots of represented metal complexes in the binding site of 4UBP. (a) Chromium complex (b) Manganese complex and (c) iron complex.

metal complexes in bidentate mode. ^{1}H-NMR spectra of metal complexes also confirm the complexation with metals thru pyridine.

Furthermore, the study also discovered that all synthesized complexes hold good antibacterial activities and remarkable antifungal activity against tested fungus i.e., *C. albicans*. Against *P. mirabilis* G-M01 and *K. pneumonia* G-M01 and G-M02 complexes exhibits enhanced activity in comparison to GMFX. G-M01 and G-M03 complexes showed increased activity against *C. freunii*, while the G-M07 complex against *C. albicans* and G-M10 complex against *S. aureus* showed increased activity in comparison to Gemifloxacin. Testing against S. features, G-M01, G-M04, and G-M10 complexes showed increased activity while other complexes are equivalent or less active than Gemifloxacin. Against *M. luteus* and *B. subtilus*, all complexes exhibited less activity than the parent drug.

4. Conclusion

Here, Gemifloxacin-transition metal complexes were synthesized successfully, which was not reported earlier. Different physicochemical and spectral (UV-Vis, FT-IR, and ^{1}H-NMR) characterization of the synthesized has been done, followed by elemental analysis and atomic absorption studies for structural confirmation. These studies confirmed the bidentate chelation via carboxylic and carbonyl groups of ketone with metal. Among all the synthesized metal complexes *G-M04* complex exhibits increased activity against most microbial organisms. Furthermore, complexes *G-M02, G-M03,* and *G-M10* are more active antimicrobial in nature than Gemifloxacin against *C. albicans, S. features,* and *C. freundii.* As formed complexes show remarkable antifungal activity against

C. albicans in tested concentrations either by killing them or inhibiting their replication by blocking active sites. So they can be screened against more fungal species and can work as potential antifungal agents. Binding orientations of the synthesized metal complexes were investigated in the binding site of the urease enzyme. The binding orientation pattern revealed that all the docked metal complexes oriented away from the Ni *bi*-center and the inactivation of urease is due to their interaction with entrance flap residues.

These complexes can be further studied and used clinically as antimicrobial and antifungal agents; however, further mechanism-based studies are required for a better understanding of their anti-urease effects.

Declarations

Author contribution statement

Sana Shamim: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Somia Gul: Performed the experiments; Wrote the paper.

Abdur Rauf, Umer Rashid: Analyzed and interpreted the data.

Ajmal Khan: Performed the experiments.

Rafat Amin: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Faiza Akhtar: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] K. Pratishtha, P. Rama, K.B. Satinder, Antibiotic-metal complexes in wastewaters: fate and treatment trajectory, Environ. Int. 157 (2021) s106863.

[2] L. Lambs, M. Venturini, B. Decock, H. Kozlowski, G. Berthon, Metal ion-tetracycline interactions in biological fluids. Part 8. Potentiometric and spectroscopic studies on the formation of Ca(II) and Mg(II) complexes with 4-dedimethylamino-tetracycline and 6-desoxy-6-demethyl-tetracycline, J. Inorg. Biochem. (33) (1988) 193–210.

[3] M. Kara, B.B. Hasinoff, D.W. McKay, N.R. Campbell, Clinical and chemical interactions between iron preparations and ciprofloxacin, Br. J. Clin. Pharmacol. (31) (1991) 257–261.

[4] N. Raman, S. Esthar, Thangaraja, A new Mannich base and its transition metal (II) complexes—synthesis, structural characterization and electrochemical study, Journal of Chemical Sciences 116, 2004, pp. 209–213 (4).

[5] A. Frei, J. Zuegg, A.G. Elliott, M. Baker, S. Braese, C. Brown, et al., Metal complexes as a promising source for new antibiotics, Chem. Sci. 11 (10) (2020) 2627–2639.

[6] R. Singh, A. Debnath, D. Masram, D. Rathore, Synthesis and Biological Activities of Selected Quinolone-Metal Complexes 2231, 2013, p. 606X.

[7] I. Turel, The interactions of metal ions with quinolone antibacterial agents, Coord. Chem. Rev. 232 (1-2) (2002) 27–47.

[8] S. Gul, N. Sultana, M.S. Arayne, S. Shamim, M. Akhtar, Khan, Sparfloxacin-metal Complexes as Urease Inhibitors: Their Synthesis, Characterization, Antimicrobial, and Antienzymatic Evaluation 2013, 2013.

[9] N. Sultana, M.S. Arayne, S. Gul, S. Shamim, Sparfloxacin–metal complexes as antifungal agents–Their synthesis, characterization and antimicrobial activities, J. Mol. Struct. 975 (1-3) (2010) 285–291.

[10] S. Arayne, N. Sultana, S. Shamim, A. Naz, Synthesis Characterization and Antimicrobial Activities of Azithromycin Metal Complexes, 2014.

[11] A. Mohamed, A. Abdel, A. Salah, S. Montaser, A. Gamal, R. Abuo, Towards anticancer fluoroquinolones, Rev. Article 352 (7) (2019).

[12] C. Agnieszka, P. Rama, K. Satinder, Brara P, M. Droguia, Vermab R, Y. Surampallic, Review: fluoroquinolones metal complexation and its environmental impacts, Coord. Chem. Rev. 376 (1) (2018) 46–61.

[13] S. Shamim SG, A. Khan, A. Ahmed, and Afeefa Gul antimicrobial, antifungal and enzymatic profiling of newly synthesized heavy metal complexes of gemifloxacin, Pharmaceut. Chem. J. 55 (10) (2022).

[14] E.K. Efthimiadou, M.E. Katsarou, A. Karaliota, G. Psomas, Copper (II) complexes with sparfloxacin and nitrogen-donor heterocyclic ligands: structure–activity relationship, J. Inorg. Biochem. 102 (4) (2008) 910–920.

[15] M. Mabrouk, S.F. Hammad, M.A. Abdelaziz, F.R. Mansour, Ligand exchange method for determination of mole ratios of relatively weak metal complexes: a comparative study, Chem. Cent. J. 12 (1) (2018) 1–7.

[16] F.R. Mansour, N.D. Danielson, Ligand exchange spectrophotometric method for the determination of mole ratio in metal complexes, Microchem. J. 103 (2012) 74–78.

[17] D.A. Martins, L.R. Gouvea, G.S.V. Muniz, S.R. Louro, DdGJ. Batista, MdNC. Soeiro, et al., Norfloxacin and N-Donor Mixed-Ligand Copper (II) Complexes: Synthesis, Albumin Interaction, and Anti-trypanosoma Cruzi Activity 2016, 2016.

[18] S. Shamim, I. Begum, W. Gul, T. Quds, M. Imran, E. Shah, et al., Antibacterial, antifungal and enzymatic activities of azithromycin heavy metal complexes: newly synthesized and characterized, Pak. J. Pharm. Sci. 34 (2021).

[19] S. Sagdinc, S. Bayarı, Spectroscopic studies on the interaction of ofloxacin with metals, J. Mol. Struct. 691 (1-3) (2004) 107–113.

[20] M.S. Arayne, N. Sultana, S. Gul, A. Khan, Novel derivatives of 5-amino-1-cyclopropyl-7-[(3 R, 5 S) 3, 5-dimethylpiperazine-1-yl]-6, 8-difluoro-4-oxo-quinoline-3-carboxylic acid: their synthesis, antimicrobial, antifungal, and urease inhibitory studies, Med. Chem. Res. 23 (3) (2014) 1248–1256.

[21] S.A. Sadeek, W.H. El-Shwiniy, Preparation, structure and microbial evaluation of metal complexes of the second generation quinolone antibacterial drug lomefloxacin, J. Mol. Struct. 981 (1-3) (2010) 130–138.

[22] S.A. Sadeek, S.M. Abd El-Hamid, El-Aasser MM, Synthesis, characterization, antimicrobial and cytotoxicity studies of some transition metal complexes with gemifloxacin, Monatshefte für Chemie-Chemical Monthly 146 (12) (2015) 1967–1982.

[23] W. Rehman, R. Yasmeen, F. Rahim, M. Waseem, C.-Y. Guo, Z. Hassan, et al., Synthesis biological screening and molecular docking studies of some tin (IV) Schiff base adducts, J. Photochem. Photobiol. B Biol. 164 (2016) 65–72.

[24] U. Rashid, F. Rahim, M. Taha, M. Arshad, H. Ullah, T. Mahmood, et al., Synthesis of 2-acylated and sulfonated 4-hydroxycoumarins: in vitro urease inhibition and molecular docking studies, Bioorg. Chem. 66 (2016) 111–116.

[25] V.W.-W. Yam, V.K.-M. Au, S.Y.-L. Leung, Light-emitting self-assembled materials based on d8 and d10 transition metal complexes, Chem. Rev. 115 (15) (2015) 7589–7728.

[26] A. Rusu, I.A. Lungu, O.L. Moldovan, C. Tanase, G. Hancu, Structural characterization of the millennial antibacterial (Fluoro)Quinolones-Shaping the fifth generation, Pharmaceutics 8 (8) (2021) 1289, 13.

[27] Suaifan Gary, A.A.M. Mohammed, Fluoroquinolones structural and medicinal developments (2013-2018): where are we now? Bioorg. Med. Chem. 27 (14) (2019) 3005–3060.

[28] H.M. Walaa, F.M. Nessma, G.M. Gehad, Spectroscopic characterization and molecular docking studies of acetyl ferrocene-derived Schiff base ligand with phenanthroline and some transition metal ions, J. Therm. Anal. Calorim. 131 (3) (2017).

[29] P. Behzadi, E. Behzadi, R. Ranjbar, Urinary tract infections and Candida albicans, Central Eur. J. Urol. 68 (1) (2015) 96.

[30] I. Onianwah, The Incidence and Prevalence of Candida albicans infection of the urogenital tract of females between the ages of 18 and 45 years old: a Case study of Patients receiving treatment in Ashford and Patrice clinic in Port Harcourt, Int. Res. J. Environ. Sci. 3 (4) (2014) 101–104. http://www.isca.in/.

[31] S. Wijnants, J. Vreys, P. Van Dijck, Interesting antifungal drug targets in the central metabolism of Candida albicans, Trends Pharmacol. Sci. (2021).

[32] A.R. Najlaa, M.Z. Ehab, G.M. Gehad, A.E. Hayam, Synthesis, spectroscopic characterization, molecular docking, and evaluation of antibacterial potential of transition metal complexes obtained using triazole chelating ligand, J. Chem. (1) (2020) 1–12.