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New Bioactive Co(II) and Ni(II) Complexes with Ofloxacin Mixed-Ligand

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Abstract: *Four mixed-ligand metal(II) complexes with the molecular formulae [M(ofl)(bipy)]$NO_3 \cdot H_2O$ and [M(ofl)(phen)]$NO_3 \cdot H_2O$ were synthesized by the reaction of Co(II) and Ni(II) with ofloxacin (Hofl) in the presence of 1,10-phenanthroline (phen) / 2,2'-bipyridine (bipy) (1:1:1 molar ratio). The data obtained from elemental analysis, molar conductance, spectral infrared, reflectance spectra and magnetic studies gave information about the coordination of the ligands and the geometry of the metallic ion. The results suggest that Hofl is deprotonated and bidentately bound to the metal ion through the pyridone oxygen and the carboxylato oxygen while phen and bipy act as neutral bidentate ligands coordinated through the nitrogen atoms. Tetrahedral geometry is proposed for all complexes. Thermal analysis data led to useful information concerning the composition, dehydration and thermal behaviour of the complexes. Ofloxacin and the metal complexes were tested for antibacterial activity and also for antioxidant activity. The variations of physicochemical parameters were monitored, which explain the antibacterial behaviour of these compounds. The interactions between metal complexes and bacterial receptors have lower energy values compared to the ofloxacin ligand. The molecular docking studies allow the identification of the biological target and the predictions of the bonds between the studied compounds and the receptors of E. coli (3t88), S. aureus (3q8u) and P. aeruginosa (4lkd).*

Keywords: *ofloxacin, mixed-ligand, metal complexes, DFT calculations, molecular docking*

1.Introduction

In recent years, researchers have been trying to discover new chemical compounds in order to prevent microbial resistance to antibiotics which represents one of the major problems facing the medical world.

Ofloxacin (Hofl), 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3. 1.05,13]trideca-5(13), 6,8,11-tetraene-11- carboxylic acid, is a second-generation fluoroquinolone drug which is active against both Gram positive and Gram-negative bacteria. Ofloxacin functions by inhibiting DNA gyrase - an enzyme necessary to separate replicated DNA- thereby inhibiting bacterial cell division [1].

One of the main objectives when obtaining ofloxacin metal complexes was to ascertain the nature of the functional groups responsible for metal-ligand binding [2,3]. The synthesis of two monocrystalline complexes $[Cu(oflo)_2(H_2O)] \cdot 2H_2O$ and $[Co(oflo)_2(MeOH)_2] \cdot 4MeOH$ was successful and their structural analysis was performed by X-ray diffraction method. There are reports in the literature about the synthesis, structural characterization and antibacterial activity of ofloxacin metal complexes [4-8]. But, it was found that when a nitrogen-donating coligand (1,10-phenanthroline or 2,2'-bipyridine) is added in the coordination sphere, in addition to ofloxacin which is the primary ligand, the obtained complexes show a much improved antibacterial activity. It is known that 1,10-phenanthroline and 2,2'-bipyridine present considerable interest in coordination chemistry as chelating bidentate ligands for transition metal ions.

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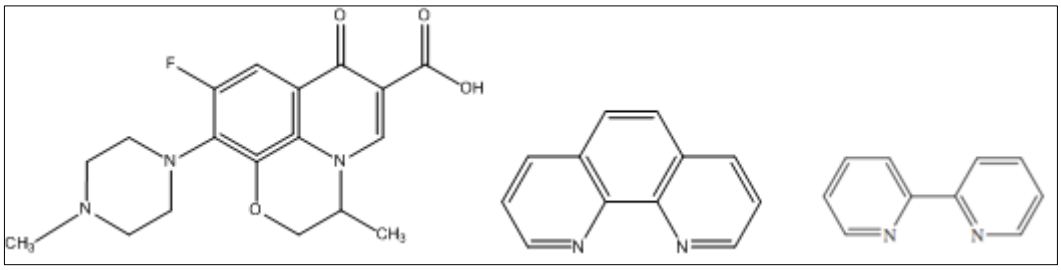
Their coordination ability is determined by their structural features, namely a planar heteroaromatic system with two nitrogen atoms which permit the formation of a chelate with the metal ion [9, 10]. The literature comprises papers in which phen and bipy are used as coligand in the obtaining of metal complexes which have applications in chemistry, biology and medicine [11-21].

New complexes of ofloxacin with the metal ions Zn(II), Zr(IV), Ce(III), Th(IV) and U(VI) in the presence of 2,2'-bipyridine were prepared and the biological behaviour of the metal complexes was better than the one of the parent drug [22]. The metal complexes $[ZrO(Ofl)(Phen)(H_2O)]NO_3 \cdot 2H_2O$, $[Zn(Ofl)(Phen)(H_2O)_2](CH_3COO) \cdot 2H_2O$ and $[UO_2(Ofl)(Phen)(H_2O)](CH_3COO) \cdot H_2O$ of ofloxacin and 1,10-phenanthroline were prepared [23]. Antimicrobial activity of the compounds was evaluated against some bacteria and fungi species. The activity data show that most metal complexes have better antibacterial activity than that of the ofloxacin drug.

The present paper deals with the synthesis, structural characterization and antibacterial evaluation of new Co(II) and Ni(II) complexes involving ofloxacin as primary ligand, 2,2'-bipyridine or 1,10-phenanthroline as coligands (Figure 1). The detailed investigation of the interaction of the studied complexes with bacterial receptors, *Escherichia coli* (EA, code 3t88), *Staphylococcus aureus* (SA, code 3q8u) and *Pseudomonas aeruginosa* (PA, code 4lkd) [24], was conducted through molecular docking studies. The molecular docking is a method of studying the interaction between the ligand and the receptor, which allows finding the most stable conformations between the two interaction participants [25].



(A) **(B)** **(C)**

Figure 1. Structural formula of ofloxacin (Hofl) (A); 1,10-phenanthroline (phen) (B); 2,2'-bipyridine (bipy) (C)

2. Materials and methods

2.1. Materials and instruments

The used chemicals and solvents were of analytical grade. Ofloxacin, $Co(NO_3)_2 \cdot 6H_2O$ and $Ni(NO_3)_2 \cdot 6H_2O$ were from Sigma-Aldrich and 1,10-phenanthroline and 2,2'-bipyridine were from Merck. M.L.W. microelementary CHN analyser was used for the chemical analysis of carbon, hydrogen and nitrogen. Varian-AA775 spectrophotomer was used to determine the metal contents of the complexes using atomic absorption technique. A Perkin Elmer 157 instrument in anhydrous KBr pellets recorded IR spectra in the range 4000-400 cm^{-1}. The UV-Vis diffuse reflectance spectra were recorded on a Jasco V670 spectrometer, in the range of 200-900 nm, using MgO as standard. Sanyo Gallenkamp apparatus was used for the melting points and OK-102 conductivity meter was used for determining the molar conductivities. Faraday balance that works at room temperature was used for magnetic susceptibility measurement. Thermogravimetric analyses (TGA) coupled with differential thermal analysis (DTA) were recorded using a Mettler Toledo TGA/SDTA851e instrument under 80 mL/min synthetic air flow, at a heating rate of 10°C/min. The TG analyses of the Co complexes were performed from 25 - 1000°C, while the Ni samples were recorded in the 25 - 1200°C temperature range.

2.2. Methods and techniques

Biological activity studies

The synthesized metal complexes and the free ofloxacin, were screened for antibacterial activity against pathogenic Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) clinically isolated bacterial strains and comparisons were made. The paper disk diffusion method was adopted for the determination of antibacterial activity [26]. The preparation of agar plates for the inoculation with bacterial strains: Mueller-Hinton agar with 2% glucose was suspended for 15 min in 1L of distilled water and then boiled until the agar was completely dissolved. The mixture was sterilized in an autoclave for 15 min at 120°C, poured into sterile Petri dishes and then stored at 40°C for being inoculated with bacterial strains. 30 μg of compound dissolved in 0.01 mL DMSO was placed using a micropipette on paper discs having a diameter of 3 mm. Each paper disc was placed on an agar Petri dish, which had previously been inoculated with a bacterial strain, and then incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the inhibition zone diameter (mm).

The activities of the ofloxacin ligand and its metal complexes were confirmed by calculating the activity index (AI) according to the following relation [27]:

$$AI = \frac{\text{Inhibition zone of complex compound (mm)}}{\text{Inhibition zone of ofloxacin ligand (mm)}} \cdot 100$$

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The capacity of synthesized metal complexes at various concentrations (0-3000μg/mL) to scavenge the radical 2,2-diphenyl-1-picrylhydrazyl were assessed colorimetrically [15]. 1.90 mL of 0.075 mM DPPH methanolic solution was mixed with 100 μL sample solution in DMSO and vortexed thoroughly. The absorbance of the mixtures was measured at 517 nm after 30 min. A blank reagent was used to study the stability of DPPH over the test time and the percentage of DPPH radical scavenging activity of samples was evaluated according to the formula:

$$\text{DPPH \% scavenging} = [(A_0\text{-}A_S).100] / A_0$$

where A_0 is the absorbance of DPPH alone, A_S is the absorbance of (DPPH + sample)

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation scavenging activity was measured colorimetrically at 734 nm [28]. ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark, at room temperature, for 16 h before use. The ABTS radical cation solution was diluted with 80% methanol to an absorbance of 0.70 at 734 nm. 100μL of the sample solution in DMSO was mixed with 2.9 mL of diluted ABTS radical cation solution. After reaction for 6 min, the absorbance at 734 nm was measured. ABTS radical cation scavenging activity of samples was evaluated according to the formula:

$$\text{ABTS \% scavenging} = [(A_0\text{-}A_S).100] / A_0$$

where A_0 is the absorbance of ABTS alone, A_S is the absorbance of (ABTS + sample)

Software used in analysis

First, the molecules were modelled and optimized using the Hyperchem 8.0 program [29] using a MM + force field. Optimization was then completed using the DFT method in Gaussian 09 packet software [30], with Becke-3-Lee-Yang-Parr (B3LYP) supplemented with the standard 6-311G(d) basis set. To study the interaction of the complexes with different bacteria (proteins), in this paper we used the Autodock 4.2 program [31]. The target proteins were taken from the Protein Data Bank [24] and

optimized with Modrefiner software [32]. The used ligands were optimized with Gaussian Software, and the interaction products resulting from the docking analysis were visualized in the Pymol software [33].

2.3. Synthesis, analytical and spectral data for metal complexes

An ethanolic solution of 1.2 mM ofloxacin deprotonated with 1.2 mM KOH and an ethanolic solution of 0.6 mM phen or 0.6 mM bipy were added simultaneously and slowly to an ethanolic solution of 0.6 mM $Ni(NO_3)_2 \cdot 6H_2O$ or 0.6 mM $Co(NO_3)_2 \cdot 6H_2O$. The *p*H of the solution was adjusted to 7 with 1 M HCl. The mixture solution was stirred for 2 h, reduced in volume on a rotavapor and was allowed to stand at room temperature over night. The precipitate was filtered, washed with ethanol and diethyl ether and dried over anhydrous $CaCl_2$.

[Co(ofl)(bipy)] $NO_3 \cdot H_2O$ (1): $CoC_{28}H_{29}FN_6O_8$: yield: 82 %, color: orange; found (calcd.) %: C, 51.08 (51.26);H, 4.75 (4.42); N, 12.94 (12.81);Co, 8.79 (8.99). λ_M=117$\Omega^{-1}cm^{-2}mol^{-1}$;FT-IR (KBr,$cm^{-1}$): 1624ν(COO)asym, 1384ν(COO)sym, 1570ν(C=O)pyridone, 510ν(M-O), 424ν(M-N).

[Co(ofl)(phen)] $NO_3 \cdot H_2O$ (2): $CoC_{30}H_{29}FN_6O_8$: yield: 85 %, color: dark orange; found (calcd.) %: C, 52.68(52.98);H, 4.75(4.26);N, 12.12(12.36);Co, 8.88(8.67). λ_M=113$\Omega^{-1}cm^{-2}mol^{-1}$;FT-IR (KBr,$cm^{-1}$): 1620ν(COO)asym, 1384ν(COO)sym, 1574ν(C=O)pyridone, 504ν(M-O), 428ν(M-N).

[Ni(ofl)(bipy)] $NO_3 \cdot H_2O$ (3): $NiC_{28}H_{29}FN_6O_8$: yield: 90 %, color: green; found (calcd.) %: C, 51.57 (51.26); H, 4.87 (4.42); N, 12.12 (12.81); Ni, 8.67 (8.97). λ_M=128$\Omega^{-1}cm^{-2}mol^{-1}$; FT-IR (KBr,$cm^{-1}$): 1624ν(COO)asym, 1384ν(COO)sym, 1574ν(C=O)pyridone, 509ν(M-O), 424ν(M-N).

[Ni(ofl)(phen)]$NO_3 \cdot H_2O$ (4): $NiC_{30}H_{29}FN_6O_8$: yield: 87 %, color: light green; found (calcd.) %: C, 52.78 (52.98);H, 4.87 (4.26); N, 11.89 (12.36);Ni, 8.18 (8.65). λ_M=128$\Omega^{-1}cm^{-2}mol^{-1}$;FT-IR (KBr, cm^{-1}): 1620ν(COO)asym, 1380ν(COO)sym, 1579ν(C=O)pyridone, 510ν(M-O), 434ν(M-N).

3. Results and discussions

The complexes were synthesized with a 82-90% yield through the reaction of KOH deprotonated ofloxacin with $Ni(NO_3)_2 \cdot 6H_2O$ or $Co(NO_3)_2 \cdot 6H_2O$ in the presence of one co-ligand 1,10-phenantroline or 2,2´- bipyridine according the following equation:

$$M(NO_3)_2 \cdot 6H_2O + Hofl + KOH + phen \rightarrow [M(ofl)(phen)]\ NO_3 \cdot H_2O + KNO_3 + 6H_2O$$

where M = Ni(II) or Co(II) and ofl = deprotonated ofloxacin.

The analytical data of the metal complexes show the formation of 1: 1: 1 [M: Hofl: phen] ratio. The microcrystalline complexes are stable in air and coloured, having the melting point up to 230°C. They are insoluble in water and common organic solvents but are soluble in DMF and DMSO. The molar conductivities of metal complexes measured in 10^{-3} $molL^{-1}$ DMF have high values (113-128 $\Omega^{-1}cm^2\ mol^{-1}$), which indicate their electrolyte nature [34]. These results are in accordance with the elemental analysis data.

3.1. Structural investigations of metal complexes

Infrared spectra of the metal complexes were analyzed in comparison with free ligands spectra in order to obtain information about the donor atoms from each ligand. The assignments of the IR spectra were made on the basis of literature and Nakamoto [35]. The important IR spectral data of the ofloxacin ligand (Hofl) and metal complexes are summarized in Table 1.

Table 1. IR spectral data for free ligand ofloxacin (Hofl) and complexes (cm^{-1})

Compound	ν(OH); ν(H_2O)	ν(C=O) / ν(C-O) carboxyl	ν_{asym} COO^- $\nu_{sym}COO^-$	ν(C=O) pyridone	ν(M-O) ν(M-N)	Δ
Hofl	3460	1713 1257	-	1622	-	-
$[Co(ofl)(bipy)]NO_3 \cdot H_2O$	3524	-	1624 1384	1570	510 424	240
$[Co(ofl)(phen)]NO_3 \cdot H_2O$	3545	-	1620 1384	1574	504 428	236
$[Ni(ofl)(bipy)]NO_3 \cdot H_2O$	3524	-	1624 1384	1574	509 424	240
$[Ni(ofl)(phen)]NO_3 \cdot H_2O$	3540	-	1620 1380	1579	510 434	240

The IR spectrum of free Hofl exhibits bands at 3460, 1713 and 1257 cm^{-1}, respectively, which are attributed to ν(O-H), $\nu(C=O)_{carboxyl}$ and $\nu(C-O)_{carboxyl}$ stretching vibrations of the carboxyl group. Another important band is at 1622 cm^{-1} which is attributed to $\nu(C=O)_{pyridone}$ stretching vibration. In the IR spectra of the complexes, the ν(O-H) disappears because of the deprotonation of the carboxyl group when ofloxacin is coordinated to the metal ion; $\nu(C=O)_{carboxyl}$ and $\nu(C-O)_{carboxyl}$ are shifted in the range 1624-1620 cm^{-1} and 1384-1380 cm^{-1} and are attributed as asymmetric $\nu(COO)_{asym}$ and symmetric $\nu(COO)_{sym}$ stretching vibration of the carboxylato group. The difference $\Delta = \nu(COO)_{asym} - \nu(COO)_{sym}$ is useful to indicate the coordination mode of the ligand. Values of the Δ parameter > 200 cm^{-1} suggest the monodentate coordination of the carboxilato group [36, 37]. The $\nu(C=O)_{pyridone}$ vibration is shifted from 1622 cm^{-1} to 1579 - 1570 cm^{-1} upon bonding to the metal ion. The changes of the IR spectra suggest that ofloxacin is deprotonated and is bidentately coordinated to the metallic ion by the pyridone oxigen and a carboxylato oxigen.

The IR spectrum of phen or bipy contains bands corresponding to the ring frequencies ν(C=C) and ν(C=N) at 1503, 1421 cm^{-1} of free phen and at 1527, 1443 cm^{-1} of free bipy. These bands are shifted to higher frequencies with 10-15 cm^{-1} upon coordination to the metallic ion. Also, the IR spectrum of phen or bipy contains characteristic bands due to ρ(C-H) vibrations at 853 and 738 cm^{-1} for free phen which appear at 722, 726 cm^{-1} in **2** and **4** while the bands at 851 and 691 cm^{-1} of free bipy appear at 771, 767 cm^{-1} in **1** and **3**. The shifts of the ring vibrations confirm the presence and coordination of phen and bipy in the complexes [35, 38, 39]. All spectra of the metal complexes contain bands at 504-506 cm^{-1} and 424-428 cm^{-1} which are assigned to ν(M-O) and ν(M-N), respectively [35,40]. The bands at 3524 - 3545 cm^{-1} are due to ν(O-H) or ν(H_2O). As reported in literature, coordinated water molecules also exhibit *ρr(*H_2O*)* rocking at 834, 863 cm^{-1} and *ρw(*H_2O*)* wagging at 567, 541 cm^{-1} [35]. The absence of these spectral bands in the spectra of complexes indicates that the water molecules are not coordinated, so they are crystallization water which was further confirmed by thermal studies. Finally, the band at 1351cm^{-1} confirms the presence of uncoordinated NO_3^- anion [35].

The results of IR data show that the deprotonated ofloxacin is bidentately bound to the metal ion through the pyridone oxygen and the carboxylato oxygen; 1,10-phenanthroline and 2,2'-bipyridine act as neutral bidentate ligands coordinated through two nitrogen donor atoms.

Ultraviolet-visible spectra and magnetic measurements

The electronic spectra of ofloxacin ligand and metal complexes were recorded in solid state. The absorption bands observed in the electronic spectrum of the ofloxacin at 44444 cm^{-1} (225nm) and 33784 cm^{-1} (296nm) and 28985 cm^{-1} (345nm), are assigned to intraligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. These bands also appeared in the electronic spectra of the complexes, but they were shifted to lower or higher values, indicating the coordination of the ligand to the central metallic ions. The electronic spectrum of $[Co(ofl)(bipy)]NO_3 \cdot H_2O$ exhibits two bands at 17450 cm^{-1} (573 nm) and 11430 cm^{-1} (874 nm), which are assigned to $^4A_2(F) \rightarrow {}^4T_1(F)$ and $^4A_2(F) \rightarrow {}^4T_1(P)$, respectively, typical of a tetrahedral geometry [41]. The complex has magnetic moment of 5.12 BM which is in agreement with

the values for tetrahedral Co(II) complexes [42,43]. The electronic spectrum of [Co(ofl)(phen)]$NO_3 \cdot H_2O$ displays two bands at 17384 cm^{-1} (575 nm) and 11330 cm^{-1} (882 nm), which are assigned to $^4A_2(F) \rightarrow {}^4T_1(F)$ and $^4A_2(F) \rightarrow {}^4T_1(P)$, respectively [41]. The proposed tetrahedral geometry of the complex is confirmed from the value of 5.12 BM [42, 43]. The electronic spectrum of [Ni(ofl)(bipy)]$NO_3 \cdot H_2O$ presents three absorption bands at 22870 cm^{-1} (437 nm), 15770 cm^{-1} (634 nm) and 14220 cm^{-1} (703 nm) assignable to $^1A_{1g} \rightarrow {}^1B_{1g}$, $^3T_1(F) \rightarrow {}^3T_1(P)$ and $^1A_{1g} \rightarrow {}^1A_{2g}$ transitions, respectively, characteristic of tetrahedral Ni(II) complexes [41, 44]. These data are in accordance with the magnetic moment of 3.51 B.M. whose value is characteristic of tetrahedral geometry of Ni(II) ion. The electronic spectrum of [Ni(ofl)(phen)]$NO_3 \cdot H_2O$ exhibits three bands in tetrahedral environment corresponding to 22880 cm^{-1} (437 nm) [$^1A_{1g} \rightarrow {}^1B_{1g}$,], 15870 cm^{-1} (630 nm) [$^3T_1(F) \rightarrow {}^3T_1(P)$] and 14320 cm^{-1} (698 nm) [$^1A_{1g} \rightarrow {}^1A_{2g}$] and the value of 3.42 B.M. also indicates tetrahedral geometry for Ni(II) [45, 46].

3.2.Thermal analysis

Thermogravimetric analyses were performed to confirm the structure and thermal behaviour of the complexes. All samples show the loss of residual solvent from 25 to 125°C, corresponding to 1.8 - 2.6 water molecules per mol of complex (Table 2). [Co(ofl)(bipy)]$NO_3 \cdot H_2O$, also exhibits a definite solvent mass loss event between 65 and 125°C, corresponding to ~1.3 water molecules (Figure 2 inset). Thus, the amount of adsorbed water was computed from the 25 - 65°C mass loss, while the crystallization water content was computed from the 65 - 125°C temperature range for all samples [47]. The mass loss corresponding to crystallization water is close to the theoretical values for a stoichiometric 1:1 complex : water ratio. This fact indicates that all complexes contain 1 molecule of crystallization water (Table 2). The combustion of the organic substituents takes place in two distinct temperature ranges, 200-600°C and 800-1050°C. The first mass loss event corresponds to the partial combustion of the organic groups to carbon and gases and it consists of several superimposed thermal loss events. The derivative of the TG curve shows a sharp mass loss around 400°C for all samples, which likely corresponds to the combustion of ofloxacin. The mass loss between 800-1000°C for Co complexes and between 850-1050°C for the Ni complexes is attributed to the loss of unreacted carbon, as well as the transition between (II, III) mixed oxides to NiO or CoO. Experiments up to 800°C show the formation of a black precipitate, likely containing unreacted carbon. This is supported by the fact that the residual mass at 800 is higher than the theoretical mass of the mixed oxides or carbonates. The total mass loss for the complexes between 160 – 1050°C shows good agreement with the value computed on the basis of their molar mass (Table 2) indicating the successful formation of the complexes. The thermal stability of the complexes was computed as the onset temperature of the organic combustion from the dTG curve (Table 2). Interestingly, the sample 4-[Ni(ofl)(phen)]$NO_3 \cdot H_2O$ has increased thermal stability with respect to the other materials (261 °C *versus* 202 - 204°C).

Table 2. Decomposition temperature (T_d), theoretical (th.) *versus* experimental (exp.) mass loss during thermogravimetric analyses

Sample	T_d (°C)	Temperature (°C)	Mass Loss (%)		Residue
			Exp.	Th.	
1- [Co(ofl)(bipy)]$NO_3 \cdot H_2O$	204	25-65	3.4	-	[Co(ofl)(bipy)]$NO_3 \cdot H_2O$
		65 - 125	3.9	2.7	[Co(ofl)(bipy)]NO_3
		160-1000	87.4	87.9	CoO
2-[Co(ofl)(phen)]$NO_3 \cdot H_2O$	203	25-65	4.0	-	[Co(ofl)(phen)]$NO_3 \cdot H_2O$
		65 - 125	2.5	2.6	[Co(ofl)(phen)]NO_3
		160-1000	88.6	88.7	CoO
3-[Ni(ofl)(bipy)]$NO_3 \cdot H_2O$	202	25-65	3.3	-	[Ni(ofl)(bipy)]$NO_3 \cdot H_2O$
		65 - 125	1.6	2.7	[Ni(ofl)(bipy)]NO_3
		160-1050	87.2	87.2	NiO
4-[Ni(ofl)(phen)]$NO_3 \cdot H_2O$	261	25-65	3.7	-	[Ni(ofl)(phen)]$NO_3 \cdot H_2O$
		65 - 125	2.3	2.7	[Ni(ofl)(phen)]NO_3
		160-1050	87.4	87.5	NiO

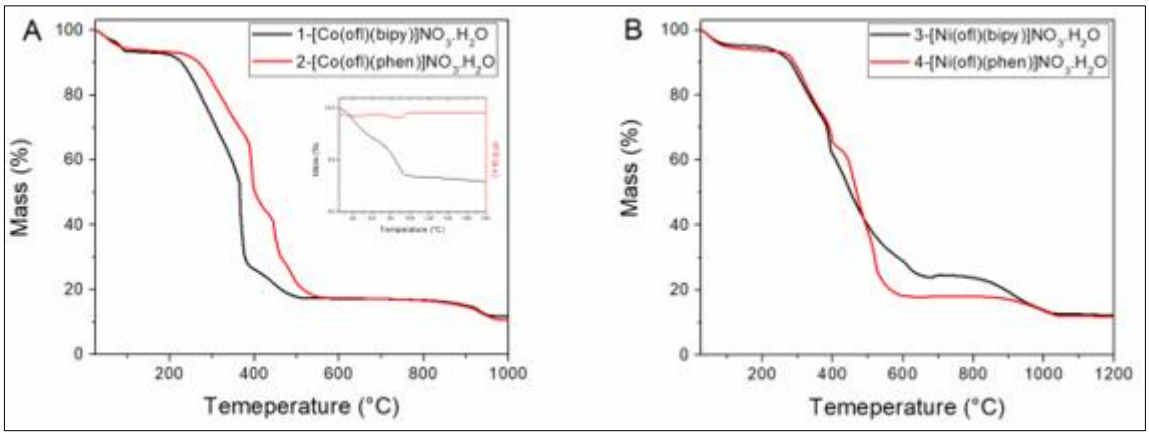


Figure 2. Thermogravimetric analyses of the Co (A) and Ni (B) complexes. Inset shows the 25-180 °C region of 1-[Co(ofl)(bipy)]$NO_3 \cdot H_2O$

3.3. Antioxidant activity

Recent research has shown that free radicals play a causal role in the development of many diseases (inflammation, cardiovascular disease, cancer, diabetes, neurodegenerative disease, atherosclerosis) and aging processes. Free radicals are formed in living cells in the normal course of aerobic metabolism as a consequence of the many redox processes involved. A balance is maintained between their formation and scavenging. The excessive formation of free radicals can damage biomolecules, such as DNA, proteins, lipids and carbohydrates, and lead to numerous diseases. Living cells develop protective mechanisms to maintain low levels of free radicals, including enzymatic and non-enzymatic antioxidant systems. In this context, exogenous antioxidant with free radical scavenging capacity may be important in preventing and treating diseases involving free radicals. Antioxidant activity can be determined by various methods that differ in terms of their assay principles and experimental conditions.

In this work, the antioxidant activity of the synthesized complexes was evaluated by two methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS (2,2'-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging assay, which are the most commonly used.

The antioxidant activity of the complexes evaluated by both methods are presented in Figure 3(a, b). The obtained results indicate the following: (i) all metal complexes have antioxidant activity in both cases which varies in the same way: (3) > (1) > (2) > (4); (ii) the values of the antioxidant activity obtained for ABTS radical cation scavenging effect were consistently higher than those obtained through DPPH radical scavenging assay. This behaviour can be explained by the different reaction kinetics between the synthesized complexes and the two free radicals used in similar concentration ranges.

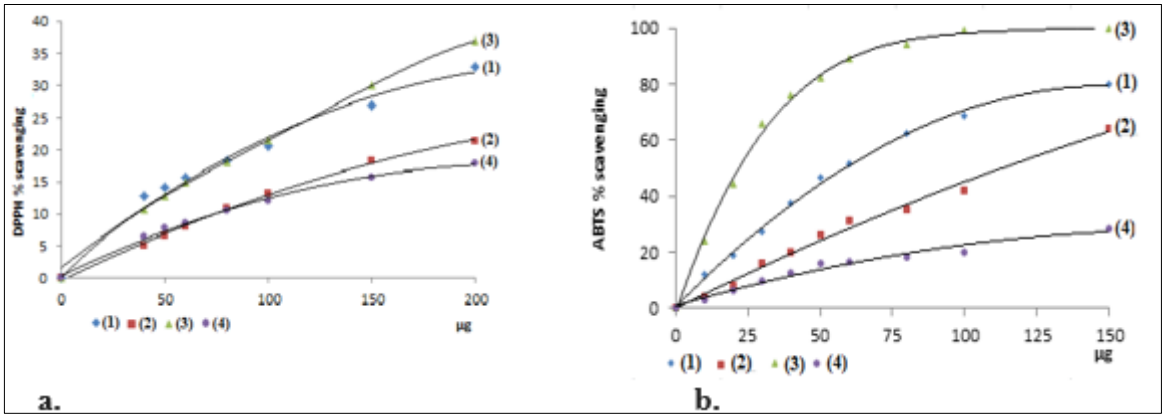


Figure 3. DPPH radical scavenging activities of the metal complexes (a); ABTS radical scavenging activities of the metal complexes (b)

The obtained results using both DPPH and ABTS methods show that the synthesized complexes have the property of neutralizing the free radicals and their use as exogenous antioxidants is recommended in the treatment of diseases involving free radicals and oxidative stress.

3.4. Bacteriological studies. Ofloxacin and the metal complexes were tested for *in vitro* antibacterial activity using the diffusion method against, *S. aureus, E. coli and P.aeruginosa*. The antibacterial activity was estimated on the basis of the size of the inhibition zone (mm) and the results are presented in Table 3. The solvent DMSO alone does not show any antibacterial effect so it is used in bacteriological studies.

Table 3. The inhibition zone diameter (mm) of ofloxacin and the complexes

Compound	Gram-positive bacteria	Gram-negative bacteria	
	S. aureus	***E. coli***	*P. aeruginosa*
(1) **[**Co(ofl)(bipy)]$NO_3 \cdot H_2O$	**32**	**32**	**34**
(2) [Co(ofl)(phen)] $NO_3 \cdot H_2O$	**34**	**34**	**38**
(3) **[**Ni(ofl)(bipy)] $NO_3 \cdot H_2O$	30	30	30
(4) **[**Ni(ofl)(phen)] $NO_3 \cdot H_2O$	**32**	**32.5**	**32**
Ofloxacin	30	30	30
DMSO	-	-	-

Ofloxacin is moderately active against the bacterial species while its antibacterial activity becomes better in some cases when it is coordinated to the metal ions as follows: (2) > (4) > (1) > (3) for *E. coli*; (2) > (1) = (4) > (3) for *S. aureus*; (2) > (1) > (4) > (3) for *P. aeruginosa.* Referring to complexes, we note that the Co(II) complexes are more active as compared with Ni(II) complexes. There are two theories that can explain this increased activity of complexes: (i) the Overtone concept [48] which considers that the fat solubility of the cell membrane has an important role in the antibacterial activity because it allows the passage of only soluble materials in lipids; (ii) Tweedy's chelation theory [49] which explains that the polarity of the metal ion is highly reduced because of the ligand orbital overlap and of the positive charge division of the central metallic ion with the donor atoms of the ligand.

The activity index values of the complex compounds are presented in Figure 4 and from these data, we can conclude that [Co(ofl)(phen)]$NO_3 \cdot H_2O$ complex has the highest activity index.

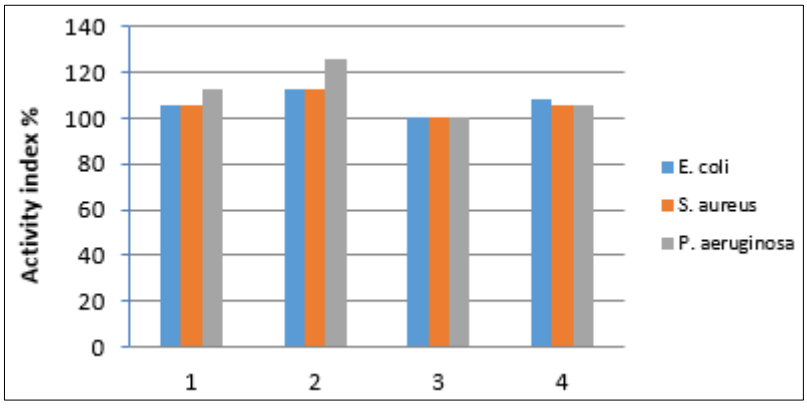


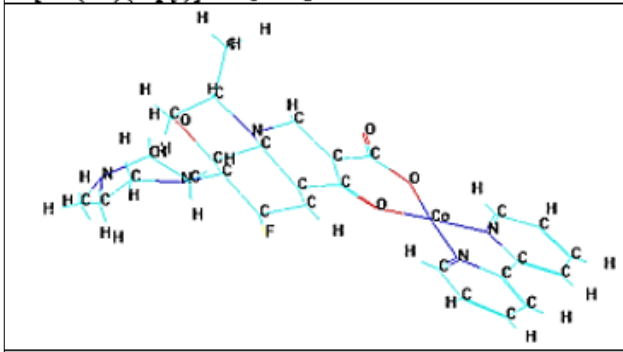
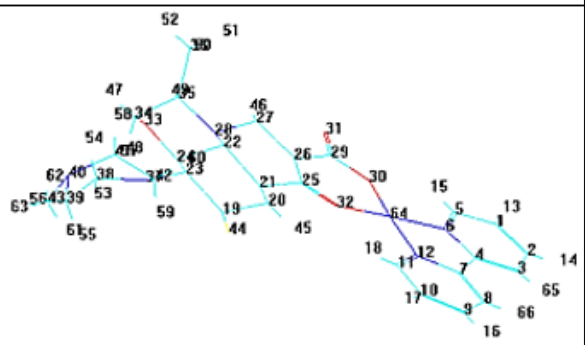
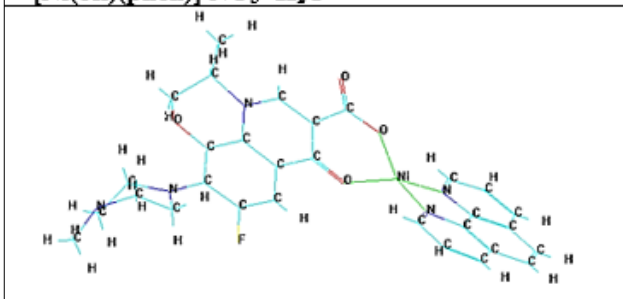
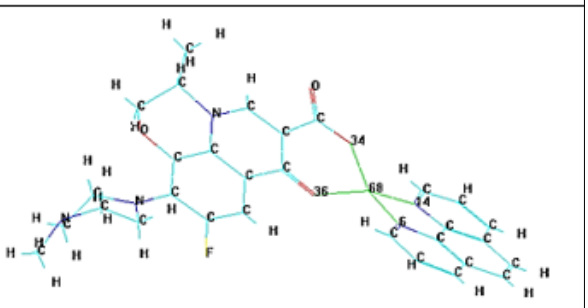
Figure 4. The activity index values of the complex compounds

3.5. Conformational analysis

In the studied metal complexes, the central metallic ion has tetrahedral coordination. The optimization of molecular geometries, by DFT calculations, led to tetrahedral structures for the metal complexes (Table 4). The angular values around the metallic ions which generate complexes are deviated

from 180°, indicating that the metallic ion and donor atoms of the ligands are not in the same plane. The tetrahedral conformation occurs due to the π-conjugate ring system. The theoretical data presented in this table regarding bond lengths and angles, along with the experimental ones, is an appropriate support for determining the geometry of the studied metal complexes.

Table 4. Angles and bond lengths for two of the studied metal complexes

[Co(ofl)(bipy)] $NO_3 \cdot H_2O$	
	
O_{32}-Co_{64}-N_6 = 177.248 ^{0} O_{30}-Co_{64}-N_{12} = 174.525 ^{0}	O_{30}-Co_{64} = 1.8195 Å; Co_{64}-N_{12} = 1.9245 Å O_{32}-Co_{64} = 1.8474 Å; Co_{64}-N_6 = 1.9052 Å
[Ni(ofl)(phen)] $NO_3 \cdot H_2O$	
	
O_{34}-Ni_{68}-N_6 = 160.295 ^{0} O_{36}-Ni_{68}-N_{14} = 160.596 ^{0}	O_{34}-Ni_{68} = 1.9135 Å; Ni_{68}-N_6 = 1.9209 Å O_{36}-Ni_{68} = 1.9963 Å; Ni_{68}-N_{14} = 1.9943 Å

3.6. Variations in physicochemical parameters and their correlation with antibacterial activity

The calculation method based on the Density Functional Theory (DFT) B3LYP / 6-311G of the Gaussian program allows the evaluation of the different structural parameters of these compounds when considering all the interactions between the electrons. The values of these descriptors are indicated in Table 5, where Cpd.- compound; ofl.- ofloxacin; μ-Dipole moment; α - Polarizability; EE - Electronic Energy; Cv - Heat capacity; S-Entropy; CP - Partition Coefficient (octanol/water); E_t - Total energy.

Table 5. Values of parameters obtained with DFT

Cpd.	μ (D)	α (a.u.)	EE (Ha)	Cv (cal/mol.K)	S (cal/mol.K)	CP	E_t (kcal/mol)
(1)	7.4342655	502.55533	-3139.9605	129.639	211.001	4.7385	700.59
(2)	7.1939755	531.78833	-3216.172	133.684	212.411	5.2249	714.96
(3)	8.3973444	455.42433	-3265.4826	130.988	217.049	3.5051	550.39
(4)	7.724512	485.898	-3341.6951	134.977	219.158	3.9911	631.86
Hofl	7.4688162	235.81367	-1262.5741	87.832	158.269	0.1788	103.65

As can be seen in this table, the dipole moments of both Co complexes are lower than that of the ofloxacin ligand. As it is well known, the dipole moment is a measure of the polarization of a molecular system. Thus, for Co complexes, the coordination decreases the polarity of the central metallic ion, on the one hand, due to the partial sharing of its positive charge with the electron-donating groups of the ligands and, on the other hand, due to the delocalisation of the π electrons in the entire system formed following the coordination. This decrease in the polarity of cobalt ion can cause the lipophilic

(hydrophobic) character of the central metallic ion to increase, and therefore favours the passage of Co complexes through the lipid membrane of the bacteria. This explains the pronounced antibacterial activity of these compounds over the ofloxacin ligand and, in particular, of the **[**Co(ofl)(phen)]$NO_3 \cdot H_2O$ complex.

Also, for the four complexes, the dipole moment (μ) increases in the following order (2) < (1) < (4) < (3), and the partition coefficient octanol / water(CP) decreases in the order: (2) > (1) > (4) > (3). In the same order, the substances are also placed in terms of inhibitory activity on Gram-negative bacteria *P. aeruginosa* ((2) > (1) > (4) > (3)).

On the other hand, the second parameter presented in Table 6 is polarizability α, which is, as it is well known, the deformability of a molecule in an electric field. As can be seen, the most deformable combination is the (2) complex, and the polarizability decreases following the order: (2) > (1) > (4) > (3), having the same variation as the inhibitory activity presented by these complexes concerning the Gram-negative bacteria *P. aeruginosa.*

The other parameters vary approximately proportional to the molecular weight of the studied complexes. A comparative study of total energies of metal complexes and ofloxacin ligand reveals that the highest total energy value corresponds to the (2) complex (Table 5), so it is the least stable and most reactive of all the studied metal complexes. Higher total energy values for complexes compared to ligand energy indicate the low stability of metal complexes relative to that of the ofloxacin ligand (103.65 kcal / mol), which explains the pronounced antibacterial activity of the complexes compared to the ligands.

For the substances studied, other descriptors of molecular type were determined (molecular surface, molecular surface area SA and molecular volume, V), as well as frontier orbital descriptors HOMO-LUMO (Table 6). These molecular orbitals determine how the molecule interacts with other species. Global descriptors of reactivity were calculated (χ – absolute electronegativity, η - chemical hardness, μ - chemical potential, σ - global softness and ω – electrophilicity index), by means of the following relations [50,51]:

$$\chi = -\frac{|E_{HOMO} + E_{LUMO}|}{2} \quad ; \quad \eta = -\frac{|E_{LUMO} - E_{HOMO}|}{2}$$

$$\mu = -\chi \qquad \sigma = \frac{1}{2\eta} \qquad \omega = \frac{\mu^2}{2\eta}$$

The values calculated for these descriptors are shown in Table 7.

Table 6. Structural parameter values for complexes and ofloxacin ligand

Cpd.	SA ($Å^2$)	V ($Å^3$)	E_{HOMO}, eV	E_{LUMO}, eV	ΔE	χ, eV	η, eV	μ, eV	σ, $(eV)^{-1}$	ω, eV
(1)	771.922	435.228	-7.8054	-1.9652	5.8402	-4.8853	-2.9201	4.8853	-0.1712	-4.0859
(2)	788.188	440.739	-7.7360	-1.2545	5.4815	-4.4952	-2.7408	4.4952	-0.1824	-3.6857
(3)	784.047	429.292	-8.2751	-1.4322	6.8429	-4.8537	-3.4215	4.8537	-0.1461	-3.4419
(4)	799.971	443.827	-8.0954	-2.0552	6.0402	-5.0753	-3.0201	5.0753	-0.1655	-4.2631
Hofl	562.356	290.953	-8.9674	-0.9427	8.0247	-4.9550	-4.0123	4.9550	-0.1246	-3.0592

The difference $\Delta E = E_{LUMO} - E_{HOMO}$ is a very important molecular parameter from a chemical point of view because a small value indicates that the molecule is very reactive and thus less stable and vice versa. According to the values presented in Table 6, the complexes are more reactive than the ofloxacin ligand. The most reactive is the (2) complex, with the lowest difference ΔE, while the least reactive is the (3) complex, i.e. the most stable, with the greatest difference ΔE. This result is consistent with the variation in the antibacterial activity of these complexes.

The energy E_{HOMO} is considered a measure of the ionizing potential of a molecule and allows the estimation of its electron donating capacity. Therefore, the higher the values of this energy, the better the electron donor molecule. From the data of Table 6, it is clear that the best electron donor is the (2) complex (E_{HOMO} = -7.7360 eV). Instead, the energy E_{LUMO} is considered a measure of the affinity of a

molecular system for the electron and represents the ability of a molecule to receive an electron. Regarding the other calculated parameters, a variation of the η descriptor is observed similar to polarizability (2) > (1) > (4) > (3) and an inverse variation for the descriptor σ. The greatest electronegativity belongs to the (2) complex, which means that this molecular system has the most pronounced donor capacity - electron acceptors capacity of all the studied complexes.

Statistical correlation of inhibitory activity on Gram - negative bacteria *P. aeruginosa* with different descriptors led to the following results, which are presented in Table 7.

Table 7. Correlation coefficients $AA = a_0 + a_iX_i$, X_i – descriptors

Descriptor	SA	V	CP	E_t	SA, E_t	V, E_t
R^2 (%)	20.9	23.5	46.7	43.1	76.2	80.7

The best correlations are the last two, indicating the dependence of the inhibitory activity on a molecular form descriptor (SA or V) and on an energy descriptor (E_t) (Figure 5 a, b). As can be seen, the largest contribution to the formation of antibacterial activity has the SA and V molecular form descriptors, but they have negative contributions of 66.05 and 76.08%, respectively. In both cases, the energy descriptor has a positive contribution to the biological response, namely 33.95% in the first case and 23.92% in the second case. This means that in the design of new compounds with optimized antibacterial activity, the descriptors that have positive contributions to the formation of activity should be used, and those descriptors that negatively contribute to the formation of the biological response should be avoided [52].

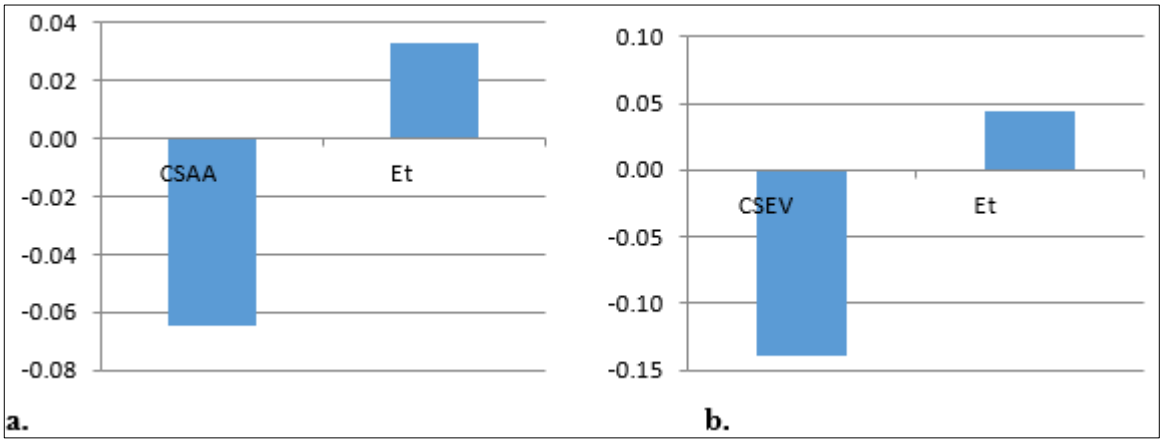


Figure 5. Contribution plot of descriptors X_i for correlation $AA = f(SA, E_t)$ (a); Contribution plot of descriptors X_i for correlation $AA = f(V, E_t)$ (b)

3.7. Molecular docking

It is a study method that allows: (i) simulation of the molecular recognition process by obtaining optimized conformations for both participants in the interaction and (ii) an appropriate orientation between them so that the energy of the formed system is minimal.

In this context, the interactions between the metal complexes and *E. coli* (EA, code 3t88), *S. aureus* (SA, code 3q8u) and *P. aeruginosa* (PA, code 4lkd) bacteria were assessed. Docking results led to conformational matches between these compounds and receptors (3t88, 3q8u and 4lkd). The docking of the first complex $[Co(ofl)(bipy)]NO_3 \cdot H_2O$ in active sites of biological receptors is shown in Figure 6.

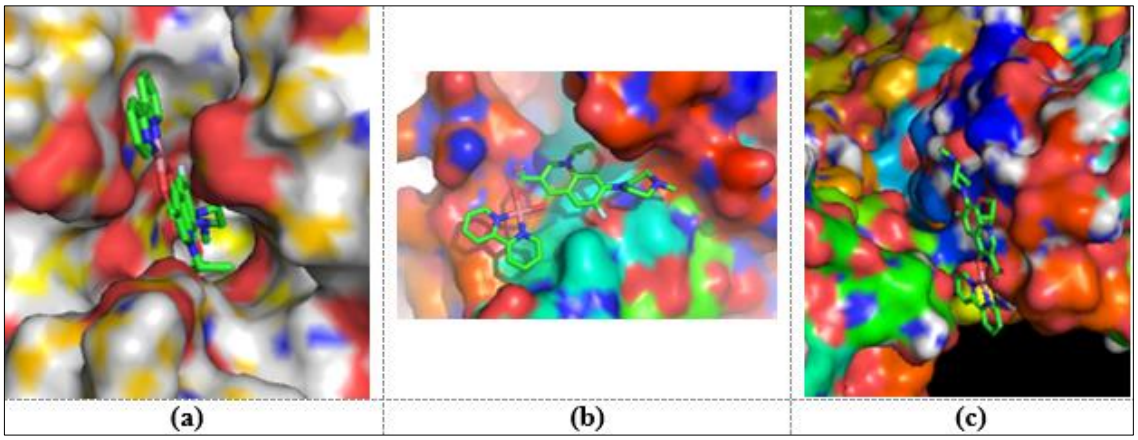


Figure 6. [Co(ofl)(bipy)]$NO_3 \cdot H_2O$ in interaction with *E. Coli* (a), *S. aureus* (b) And *P. aeruginosa* (c) receptors

The energies calculated in these docking studies are presented in Table 8, where EA-*E. coli*, SA-*S. aureus*, PA-*P. aeruginosa*.

Table 8. Energy values obtained in docking calculations

Cpd.	Receptor	Est. free energy of binding (kcal/mol)	Est. inhibition constant (K_i) (nM)	vdW + bond + desolv energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total internal energy (kcal/mol)
(1)	EC	-11.30	5.21	-9.51	-2.08	-0.74
	SA	-9.91	54.32	-8.61	-1.60	-0.71
	PA	-10.70	14.30	-7.17	-3.83	-0.58
(2)	EC	-11.77	2.35	-9.27	-2.80	-0.74
	SA	-10.65	15.50	-9.36	-1.59	-0.73
	PA	-10.88	10.62	-7.36	-3.81	-0.48
(3)	EC	-10.77	12.68	-9.13	-2.84	-0.57
	SA	-9.54	101.41	-9.15	-1.58	-0.65
	PA	-11.92	1.82	-9.84	-3.28	-0.55
(4)	EC	-12.14	1.25	-10.97	-2.37	-0.74
	SA	-10.12	38.42	-9.87	-1.44	-0.73
	PA	-10.91	10.06	-9.66	-2.44	-0.74
Hofl	EC	-6.52	16.67	-6.20	-1.21	-0.59
	SA	-5.94	43.97	-5.80	-1.04	-0.35
	PA	-7.81	1.90	-7.75	-0.95	-0.47

For a better understanding of the activity of the studied metal complexes, we investigated more closely the molecular docking of the most active complex in the series, the (2) complex, with the most pronounced inhibitory activity on bacteria used for antibacterial analysis. Binding modes are represented in Figure 7.

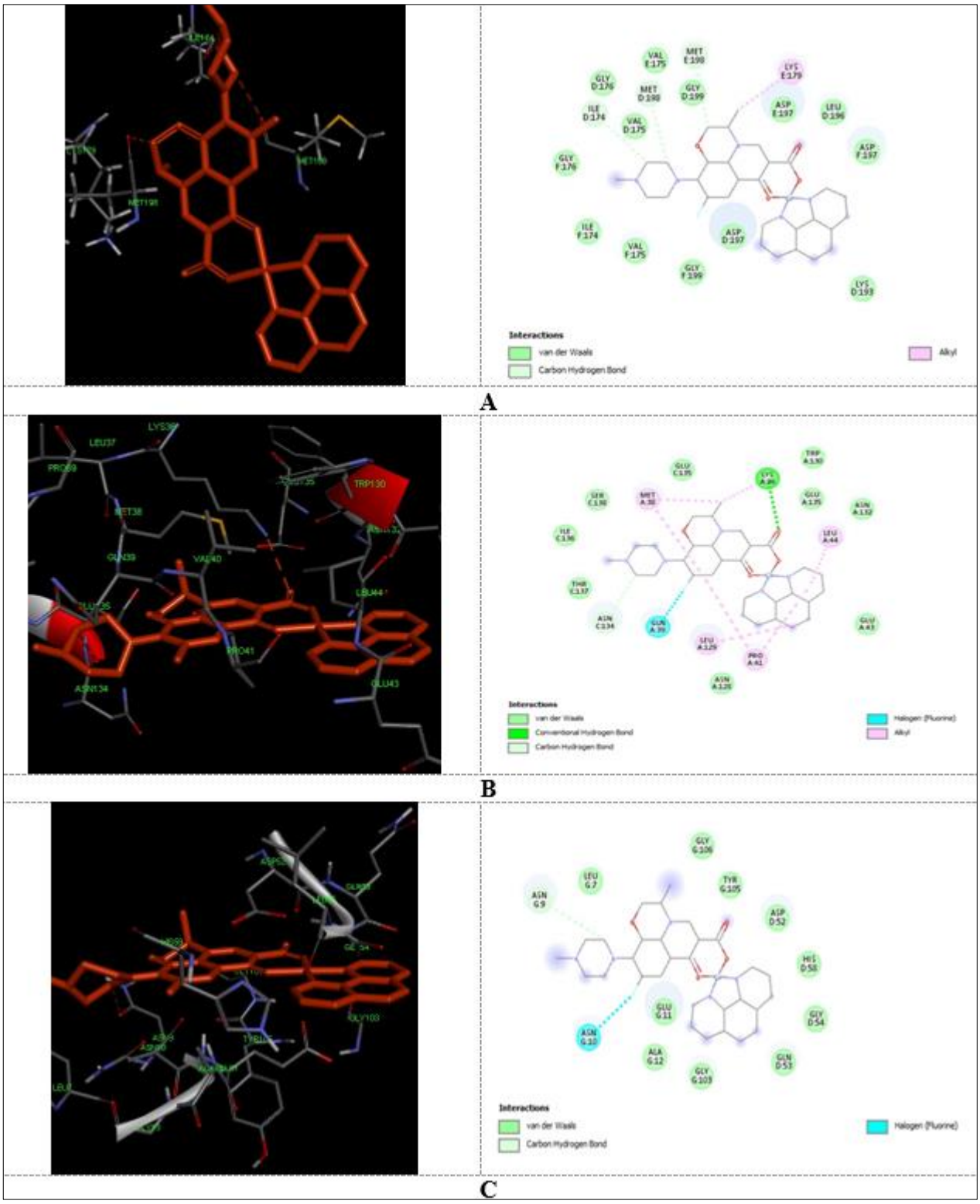


Figure 7. Three-dimensional interactions generated by Discovery Studio 4.1 [23] between the (2) complex and *E. coli* **(A)**, *S. aureus* **(B)** and *P. aeruginosa* **(C)** receptors and the corresponding 2D diagram. The amino acid residues in the receptors are represented using lines, and the structure of the complex is represented using the stick model. The interactions are represented as lines interrupted in different colours as in the legend (right)

As can be seen, the interactions between the metal complex and the bacterial protein residues are van der Waals and carbon-hydrogen bond interactions in all three analyzed interactions. In the case B of interaction, the complex [Co(ofl)(phen)]$NO_3 \cdot H_2O$ bonds to the residue Lys36 of the *S. aureus* receptor by hydrogen bonds, in this case the oxygen from the carboxyl group of the ofloxacin is involved. Also, in the first two cases, hydrophobic interactions occur between participants (π-alkyl).

4. Conclusions

Four complexes of cobalt(II) and nickel(II) with ofloxacin and 1,10-phenanthroline or 2,2'-bipyridine were synthetized and characterized using spectroscopic methods. The ligand ofloxacin acts as monoanionic bidentate (OO) ligand and 1,10-phenanthroline or 2,2'-bipyridine acts as neutral bidentate (NN) ligand. From the data of the elemental analysis and molar conductivities, the complexes have the general formulae [M(ofl)(bipy)]$NO_3 \cdot H_2O$ and [M(ofl)(phen)]$NO_3 \cdot H_2O$ which are also confirmed by the thermal analysis. All complexes have tetrahedral geometry. The obtained results using both DPPH and ABTS methods show that the synthesized complexes present a good antioxidant activity. Ofloxacin and its metal complexes were tested for their antibacterial activity and the results showed that Co(II) complexes are more active as compared with Ni(II) complexes. The performed studies suggested the existence of an inverse correlation between the dipole moment and the antibacterial activity of the complexes. Also, there is a direct link between the octanol / water partition coefficient and the biological activity. Such studies can serve as models of comparison and may be useful in designing stronger antibacterial agents.

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