

Spectroscopic characterization of novel D-amino acid-Schiff bases and their Cr(III) and Ni(II) complexes as antimicrobial agents

Nurşen Sarı · Nihat Pişkin · Hatice Öğütçü ·
Nurdan Kurnaz

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Abstract The aim of this work was to investigate the antibacterial and antifungal activities of novel D-amino acid-Schiff bases including fluorine atom and their Cr(III) and Ni(II) complexes. All these substances have been examined for antibacterial activity against pathogenic strains *Listeria monocytogenes 4b* ATCC19115, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC1280, *Salmonella typhi* H NCTC 901.8394, *Brucella abortus* (A.99, UK-1995) RSKK03026, *Staphylococcus epidermis* sp., *Micrococcus luteus* ATCC9341, and *Shigella dysenteriae* typ 10 NCTC 9351, and antifungal activity against *Candida albicans* Y-1200-NIH, Tokyo. The antimicrobial test results of these amino acid-Schiff base complexes exhibited better activity than some known antibiotics. In particular, diamagnetic Ni(II) complexes were more potent bactericides than all of the substances synthesized.

Keywords Schiff bases · Antimicrobial activity · Fluorine atom · Ni(II) complex · Cr(III) complex · Diamagnetic

Introduction

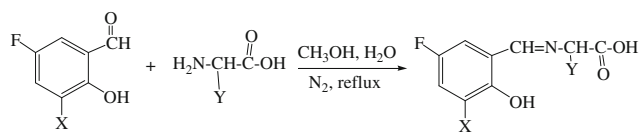
Schiff bases appear to be important intermediates in a number of enzymatic reactions involving enzyme interaction with an amino or a carbonyl group of a substrate. In bioinorganic chemistry, the interest in the Schiff base complexes derives from their ability to provide synthetic models for metal-containing sites in metalloproteins and to contribute to developments in medicinal chemistry. Thus, Schiff bases and their complexes have a variety of applications in biological, clinical, and analytical fields (Egloff *et al.*, 2009; Ellis *et al.*, 1997). Recently, there has been considerable interest in the chemistry of amino acid-Schiff base compounds due to their potential in nuclear medicine applications (Abram and Alberto, 2006; Gharagozlou and Boghaei, 2008).

However, the drug resistances against antibacterial agents may pose a problem in their use with medical purpose (Singh *et al.*, 2006). The problem could be overcome by the preparation of metal complexes, by a process of chelation with the coordination of transition metal ions. It is well known that N and O atoms play a key role in the coordination of metals. Amino acid-Schiff bases have N and O atoms as their basic elements. Schiff base derivatives containing donor atom can act as good chelating agents for the transition of metal ions (Avaji *et al.*, 2008; Takeuchi *et al.*, 1999; Sari *et al.*, 2008). Amino acid-Schiff base metal complexes have found wide applications in corrosion inhibitors (Negm and Zaki, 2009), tumor radio-imaging agents (Wang *et al.*, 2005), and pharmacologically active compounds (Wang *et al.*, 2007). However, no studies have been carried out on Schiff bases and their metal complexes derived from amino acid-Schiff bases including fluorine. This study aimed to fill in this gap. Novel amino acid-Schiff bases derivatives were investigated to find out the antibacterial properties of Schiff bases and

N. Sarı (✉) · N. Pişkin · N. Kurnaz
Department of Chemistry, Faculty of Science,
Gazi University, 06500 Tenikoklullar, Ankara, Turkey
e-mail: nursens@gazi.edu.tr

H. Öğütçü
Department of Biology, Faculty of Arts and Science,
Ahi Evran University, Kırşehir, Turkey

N. Kurnaz
Department of Chemistry, Faculty of Science,
Kırklareli University, Kırklareli, Turkey



Y — X

- H, -Cl 5-fluoro-3-chloro-2-hydroxybenzaldehyde-D-glycine
- CH₃, -Cl 5-fluoro-3-chloro-2-hydroxybenzaldehyde-D-alanine
- H, -H 5-fluoro-2-hydroxybenzaldehyde-D-glycine
- CH₃, -H 5-fluoro-2-hydroxybenzaldehyde-D-alanine

Scheme 1 Structure of amino acid-Schiff bases

their complexes (Scheme 1). Amino acid-Schiff base derivatives were synthesized by condensation methods. And then, Cr(III) and Ni(II) complexes were synthesis by template methods.

Chemicals and methods

5-Fluoro-2-hydroxy benzaldehyde, 3-chloro-5-fluoro-2-hydroxybenzaldehyde, D-glycine, D-alanine, Ni(II) chloride, and Cr(III) chloride were provided by Sigma-Aldrich Company. ¹H and ¹³C NMR spectra of the Schiff bases were recorded using a Bruker DPX-300 MHz and 100 MHz using TMS as an internal standard and CDCl₃ as solvent. FTIR spectra in the 4000–400 cm⁻¹ range were measured using KBr disks on a Mattson 1000 FTIR spectrophotometer. Carbon, hydrogen, and nitrogen content were obtained using LECO-9320 analyzer. Conductivity measurements were carried out at 20 °C in 10⁻³ M DMSO using a Siemens WPA CM 35 apparatus. The room temperature magnetic moments were measured using MK-1 model Gouy Balance of Christison Scientific Equipment Ltd. Metal contents were determined using a Philips PU 9285 atomic absorption instrument. Mass spectra were recorded on a Micro Mass-UK Platform II mass spectrometer.

5-Fluoro-2-hydroxybenzaldehyde-D-glycine

A solution of 5-fluoro-2-hydroxy benzaldehyde 0.35 g (2.5 × 10⁻³ mol) in MeOH (10 ml) was added drop wise to the 4 ml hot aqueous solution of the D-glycine 0.19 g (2.5 × 10⁻³ mol) and heated under reflux for 4 h in nitrogen atmosphere. After cooling, the mixture was filtered and allowed to stand. On standing for a further 4 days in nitrogen atmosphere, the solid crude formed was collected by filtration, dried in desiccators over CaCl₂. Yields: 52.

5-Fluoro-3-chloro-2-hydroxybenzaldehyde-D-glycine

A solution of 3-chloro-5-fluoro-2-hydroxybenzaldehyde 0.44 g (2.5 × 10⁻³ mol) in MeOH (10 ml) was added drop wise to the 4 ml hot aqueous solution of the D-glycine 0.19 g (2.5 × 10⁻³ mol) and heated under reflux for 4 h in nitrogen atmosphere. After cooling, the mixture was filtered and allowed to stand. On standing for a further 6 days in nitrogen atmosphere, the solid crude formed was collected by filtration, dried in desiccators over CaCl₂. Yields: 56.

5-Fluoro-2-hydroxybenzaldehyde-D-alanine

A solution of 5-fluoro-2-hydroxy benzaldehyde 0.35 g (2.5 × 10⁻³ mol) in MeOH (10 ml) was added drop wise to the 4 ml hot aqueous solution of the D-glycine 0.19 g (2.5 × 10⁻³ mol) and heated under reflux for 4 h in nitrogen atmosphere. After cooling, the mixture was filtered and allowed to stand. On standing for a further 4 days in nitrogen atmosphere, the solid crude formed was collected by filtration, dried in desiccators over CaCl₂. Yields: 68.

5-Fluoro-3-chloro-2-hydroxybenzaldehyde-D-alanine

A solution of 3-chloro-5-fluoro-2-hydroxybenzaldehyde 0.44 g (2.5 × 10⁻³ mol) in MeOH (10 ml) was added drop wise to the 4 ml hot aqueous solution of the D-alanine 0.22 g (2.5 × 10⁻³ mol) and heated under reflux for 4 h in nitrogen atmosphere. After cooling, the mixture was filtered and allowed to stand. On standing for a further 4 days in nitrogen atmosphere, the solid crude formed was collected by filtration, washed with a small volume of ethanol and dioxan, and then, finally crystallized from methanol, dried in desiccators over CaCl₂. Yields: 58.

General synthesis of complexes

Cr(III) and Ni(II) complexes of Schiff bases resulting from the condensation of 1 mol of aldehyde with 1 mol of D-amino acid has been isolated by metal template reaction. A sample of CrCl₃·6H₂O and NiCl₂·6H₂O (for Cr(III) and Ni(II); 0.67 and 0.59 g, respectively, 2.5 × 10⁻³ mol) was dissolved in methanol (25 ml). To the solution, amino acid (D-glycine 0.19 g, D-alanine 0.22 g; 2.5 × 10⁻³ mol) and aldehyde (5-fluoro-2-hydroxy benzaldehyde 0.35 g, 3-chloro-5-fluoro-2-hydroxybenzaldehyde 0.44 g; 2.5 × 10⁻³ mol) in methanol (25 ml) was added, immediately giving a colored solution. The resulting solution was stirred for ca. 5 h in nitrogen atmosphere, filtered, and allowed to stand. On standing for a further 10–20 days, the solid complexes formed was collected by filtration, washed with a

small volume of ethanol and acetone, and then, dried in a desiccator over CaCl_2 . Yields: 61–73.

Detection of antimicrobial activity

The bacterial subcultures chosen were *Listeria monocytogenes* 4b ATCC19115, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC1280, *Salmonella typhi* H NCTC-901.8394, *Brucella abortus* (A.99, UK-1995) RSKK03026, *Staphylococcus epidermis* sp., *Micrococcus luteus* ATCC 9341, and *Shigella dysenteria* type 10 NCTC 9351. An antifungal susceptibility test was used by *Candida albicans* Y-1200-NIH, Tokyo.

The ligands and the complexes were tested for their antimicrobial activity by the well-diffusion method. Each ligand and complex was kept dry at room temperature and dissolved (0.25 $\mu\text{g}/\text{ml}$) in DMF. DMF was used as solvent and also for control. It was found to have no antimicrobial activity against any of the tested organisms. 1% (v/v) of 24-h broth culture containing 10^6 CFU/ml was placed in sterile Petri dishes. Mueller-Hinton Agar (MHA) (15 ml) kept at 45 °C was then poured into the Petri dishes and allowed to solidify. Then 6-mm diameter wells were punched carefully using a sterile cork borer and were entirely filled with the test solutions. The plates were incubated for 24 h at 37 °C. On completion of the incubation period, the mean value obtained for the three holes was used to calculate the zone of growth inhibition of each sample. Bacterial and fungal subcultures were tested for resistance to six antibiotics (produced by Oxoid Lt., Basingstoke, UK):

ampicillin (preventing the growth of gram-negative bacteria), nystatin (binding to sterols in the fungal cellular membrane, altering the permeability, and allowing leakage of the cellular contents), kanamycin (used in molecular biology as agent to isolate bacteria), sulfamethoxazol (bacteriostatic antibacterial agent that interferes with folic acid synthesis in susceptible bacteria), amoxycillin (b-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms), Sulbactam.

Results and dissociations

Analytical data and some of the physical properties of the Schiff bases and their complexes are summarized in Table 1. The complexes are only soluble in DMF and DMSO, but insoluble in organic solvents like CCl_4 and benzene. Molar conductance values of the Cr(III) and Ni(II) complexes were found to be ca. 7 $\mu\text{S}/\text{cm}$ in 10^{-3} M DMF solutions, indicating the 1:1 electrolytic nature of the compounds. This state indicated the nonelectrolytic behavior for Cr(III) and Ni(II) complexes (Altundas *et al.*, 2010). The results obtained showed that the structure of complexes is the ML complexes according to the LC-mass spectroscopy of $[\text{M}-3\text{H}_2\text{O}]^+$ 253 (m/z : %11.2), $[\text{M}-3\text{H}_2\text{O}]^+$ 267 (m/z : %9.8), $[\text{M}-\text{H}_2\text{O}]^+$ 287.6 (m/z : %18.2), $[\text{M}-\text{H}_2\text{O}]^+$ 301.6 (m/z : %11.2), $[\text{M}-2\text{H}_2\text{O}]^+$ 281.7 (m/z : %11.2), $[\text{M}-2\text{H}_2\text{O}]^+$ 295.7 (m/z : %11.2), $[\text{M}-2\text{H}_2\text{O}]^+$ 317.1 (m/z : %11.2), and $[\text{M}-2\text{H}_2\text{O}]^+$ 330.1 (m/z : %11.2); for $[\text{Ni}(5\text{F-Gly})(\text{H}_2\text{O})_3]$, $[\text{Ni}(5\text{F-Ala})(\text{H}_2\text{O})_3]$, $[\text{Ni}(5\text{F-3Cl-Gly})(\text{H}_2\text{O})]$, $[\text{Ni}(5\text{F-3Cl-Ala})(\text{H}_2\text{O})]$, $[\text{Cr}(5\text{F-Gly})\text{Cl}(\text{H}_2\text{O})_2]$, $[\text{Cr}(5\text{F-Ala})\text{Cl}(\text{H}_2\text{O})_2]$, $[\text{Cr}(5\text{F-3Cl-Gly})\text{Cl}(\text{H}_2\text{O})_2]$, and $[\text{Cr}(5\text{F-3Cl-Ala})\text{Cl}(\text{H}_2\text{O})_2]$, respectively.

Table 1 Analytical and physical data for amino acid-Schiff bases and their Ni (II) and Cr(III) complexes

Compound	Empirical formula (formula weight, g)	m.p. (°C), μB	Elemental analysis, found (calcd) %				Electrolyte	
			C	H	N	M	$\mu\text{S}/\text{cm}$	Type
[5F-Gly]	$\text{C}_9\text{H}_8\text{NO}_3\text{F}$ (196.6)	82–83, –	53.61 (54.93)	4.98 (4.06)	8.28 (7.12)	–	KCl 14.38	1:1
[5F-Ala]	$\text{C}_{10}\text{H}_{10}\text{NO}_3\text{F}$ (210.6)	85–87, –	53.79 (56.98)	4.73 (4.74)	7.21 (6.64)	–	H_2O 2.21	ne
[5F-3Cl-Gly]	$\text{C}_9\text{H}_7\text{NO}_3\text{FCl}$ (231)	92–94, –	47.13 (46.75)	3.31 (3.03)	6.41 (6.06)	–	DMF 0.27	ne
[5F-3Cl-Ala]	$\text{C}_{10}\text{H}_9\text{NO}_3\text{FCl}$ (245)	96–97, –	49.37 (48.97)	3.19 (3.67)	6.12 (5.71)	–		
$[\text{Ni}(5\text{F-Gly})(\text{H}_2\text{O})_3]$	$\text{C}_9\text{H}_{12}\text{NO}_6\text{FNi}$ (307.7)	229, 2.88	34.75 (35.09)	3.77 (3.89)	4.33 (4.54)	18.52 (19.07)	6.23	ne
$[\text{Ni}(5\text{F-Ala})(\text{H}_2\text{O})_3]$	$\text{C}_{10}\text{H}_{14}\text{NO}_6\text{FNi}$ (321.7)	250>, 2.84	36.61 (37.30)	4.82 (4.35)	4.78 (4.36)	17.30 (18.24)	6.67	ne
$[\text{Ni}(5\text{F-3Cl-Gly})(\text{H}_2\text{O})]$	$\text{C}_9\text{H}_7\text{NO}_4\text{FCNi}$ (305.6)	115 ^a , D	35.11 (35.34)	4.55 (4.35)	5.01 (4.58)	19.37 (19.20)	7.03	ne
$[\text{Ni}(5\text{F-3Cl-Ala})(\text{H}_2\text{O})]$	$\text{C}_{10}\text{H}_9\text{NO}_4\text{FCNi}$ (319.6)	122, D	38.03 (37.54)	2.49 (2.81)	4.71 (4.38)	17.78 (18.36)	7.22	ne
$[\text{Cr}(5\text{F-Gly})\text{Cl}(\text{H}_2\text{O})_2]$	$\text{C}_9\text{H}_{10}\text{NO}_5\text{FCr}$ (317.7)	103, 4.08	33.47 (33.99)	3.56 (3.14)	4.61 (4.40)	15.22 (16.33)	7.28	ne
$[\text{Cr}(5\text{F-Ala})\text{Cl}(\text{H}_2\text{O})_2]$	$\text{C}_{10}\text{H}_{12}\text{NO}_5\text{FCr}$ (331.7)	112, 3.97	35.63 (36.17)	3.21 (3.61)	4.31 (4.22)	15.21 (15.64)	7.32	ne
$[\text{Cr}(5\text{F-3Cl-Gly})\text{Cl}(\text{H}_2\text{O})_2]$	$\text{C}_9\text{H}_{10}\text{NO}_5\text{FCl}_2\text{Cr}$ (353.1)	114, 4.10	30.11 (30.58)	2.27 (2.83)	3.51 (3.96)	14.72 (14.69)	7.47	ne
$[\text{Cr}(5\text{F-3Cl-Ala})\text{Cl}(\text{H}_2\text{O})_2]$	$\text{C}_{10}\text{H}_{11}\text{NO}_5\text{FCl}_2\text{Cr}$ (366.1)	119, 3.81	32.46 (32.77)	3.42 (3.00)	3.37 (3.82)	15.87 (14.17)	7.52	ne

D diamagnetic, ne nonelectrolytic

^a Decomposition temperature

Table 2 summarizes the main IR and UV–visible bands of the Schiff bases and their complexes. IR bands of Schiff bases in the 1663–1667 cm^{-1} , 3110–3125 cm^{-1} , 2798–2864/2446–2467 cm^{-1} regions are characteristic of $\nu(\text{CH}=\text{N})$, $\nu(\text{OH})$, and $\nu(\text{CH})_{\text{arom/alif}}$, respectively (Sarı and Gürkan, 2004). The bands in the 1621–1638 cm^{-1} and 1579–1590 cm^{-1} regions may, respectively, ascribe to $\nu_{\text{COO-(asym)}}$ and $\nu_{\text{COO-(sym)}}$ vibrations (Sarı and Gürkan, 2004). The electronic spectra of the Schiff bases in DMF show bands ca. 340 nm are attributed to the azomethine chromospheres $n \rightarrow \pi^*$ transition (Sarı *et al.*, 2003). The bands at higher energies (272–343 nm) are associated with the benzene $\pi \rightarrow \pi^*$ and $\sigma \rightarrow \sigma^*$ transition (Sarı *et al.*, 2003; Silverstein *et al.*, 1981). The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of the Schiff bases are presented in Table 3. In general, the duplets, quartet, or triplets observed at 7.10–7.85 ppm are assigned to aldehyde ring protons. The singlet at 8.27–8.41 ppm and 9.95–9.99 ppm are assigned to imine and aromatic hydroxyl protons, respectively. The protons of $-\text{CH}_3$, $-\text{CH}_2$, and $-\text{CH}$ of amino acid group in the Schiff bases are also observed as expected. The $^{13}\text{C-NMR}$ spectra data of the Schiff bases (Table 3) are also in accordance with the proposed structures.

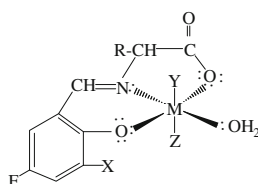
The azomethine and carboxylate bands in the IR spectra of the complexes appear in the range 1683–1691, 1651–1658, and 1549–1561 cm^{-1} , somewhat different than observed for the free ligands. These indicate that the azomethine nitrogen and the oxygen of the carboxylate group are coordinated to metal ion. The IR spectra of all complexes exhibit characteristic bands of coordination water at ca. 3450, 885, and 774 cm^{-1} assigned to $\nu(\text{OH})$, $\rho_r(\text{OH})$, and $\rho_w(\text{OH}_2)$ vibrations, respectively (Sarı and Gürkan, 2004). These observations clearly suggest that the

water molecules are coordinated to the metal ion (Scheme 2). The appearance of new bands in the 503–560 and 405–411 cm^{-1} regions are due to $\nu(\text{M-O})$ and $\nu(\text{M-N})$, respectively (Altundas *et al.*, 2010).

Since all Cr(III) complexes and octahedral Ni(II) complexes are paramagnetic, the $^1\text{H-NMR}$ spectra could not be obtained. $^1\text{H-NMR}$ spectra of $[\text{Ni}(5\text{F-3Cl-Gly})(\text{H}_2\text{O})]$ and $[\text{Ni}(5\text{F-3Cl-Gly})(\text{H}_2\text{O})]$ complexes were obtained due to the diamagnetic properties. The diamagnetic Ni(II) complexes exhibit signals in the range of 8.42–8.43 ppm due to $-\text{CH}=\text{N}$ protons. These signals are observed in the lower field than ligands. The signals ($^1\text{H-NMR}$ and IR spectra) of Ni(II) complexes are different from those of the corresponding ligands, suggesting the coordination through oxygen atoms in a phenol ring and azomethine groups. The ligands showed signals at 9.95–9.99 ppm, but the complexes do not contain OH signals as the ligands can coordinate Ni(II) ions with deprotonated phenolic oxygen (Table 3). More detailed information about the structure of the ligands and their Ni(II) complexes were provided by the $^{13}\text{C-NMR}$ spectra data. $^{13}\text{C-NMR}$ spectra of the ligands were assigned by comparison with those of their Ni(II) complexes. The signals of carbon atoms, which neighbor to an $-\text{OH}$ group, were different. This can be attributed to the coordination of the phenolic oxygen atom (Altundas *et al.*, 2010) (Table 3). C_9 and C_4 carbon atoms in the free Schiff bases showed a significant shift after complexation. Furthermore, doublets observed at 192.70–192.64 ppm and 192.71–192.68 ppm due to complexation, are assigned to carbon of $-\text{COOH}$. This case may be due to the coordination of the ligand to the metal atom by the azomethine nitrogen and phenolic oxygen. Paramagnetic $[\text{Ni}(5\text{F-Gly})(\text{H}_2\text{O})_3]$ and $[\text{Ni}(5\text{F-Ala})(\text{H}_2\text{O})_3]$ complexes show three d–d bands in

Table 2 Specific FTIR and UV-GB spectra data of amino acid-Schiff bases and their Ni (II) and Cr(III) complexes

Compound	ν_{OH} , $\nu_{\text{CH}=\text{N}}$	$\nu_{\text{CH(alif)}/\nu_{\text{CH(arom)}}$	$\nu_{\text{COOH (asim)}}$ / $\nu_{\text{COOH (sim)}}$	$\nu_{\text{Ni-O/Ni-N}}$	$\lambda_{\text{max}} (\epsilon)$
[5F-Gly]	3125, 1664	2466, 2305/2798	1628/1587	–	294 (1.267); 343 (0.967); 383 (0.55)
[5F-Ala]	3110, 1667	2467, 2310/2861	1621/1590	–	294 (1.981); 318 (3.898); 333 (3.91)
[5F-3Cl-Gly]	3114, 1665	2468, 2308/2855	1632/1584	–	272 (3.97); 314 (4.00); 337 (3.92); 361 (3.91)
[5F-3Cl-Ala]	3120, 1663	2467, 2310/2864	1638/1579	–	277 (3.913); 308 (3.93); 348 (3.95); 373 (3.59)
[Ni(5F-Gly)(H ₂ O) ₃]	3425, 1689	2767, 2606/3104	1651/1559	528/411	288 (2.961); 337 (2.377); 400 (0.281); 641(0.034); 865 (0.031)
[Ni(5F-Ala)(H ₂ O) ₃]	3425, 1684	2767, 2606/3107	1654/1556	534/410	284 (2.582); 389 (0.411); 636(0.036); 893 (0.84)
[Ni(5F-3Cl-Gly)H ₂ O]	3489, 1691	2765, 2680/3110	1658/1550	503/405	271 (2.449); 313 (3.994); 331 (3.96); 354 (3.938); 448 (0.441)
[Ni(5F-3Cl-Ala)(H ₂ O)]	3498, 1687	2667, 2597/3116	1658/1552	560/413	270 (3.486); 318 (3.872); 335 (3.931); 355 (3.581); 452 (0.514)
[Cr(5F-Gly)Cl(H ₂ O) ₂]	3465, 1691	2765, 2621/3115	1654/1551	516/408	286 (2.874); 325 (2.078); 358 (0.687); 627(0.566)
[Cr(5F-Ala)Cl(H ₂ O) ₂]	3473, 1687	2764, 2629/3112	1651/1549	521/411	283 (2.882); 369 (2.814); 537 (0.703)
[Cr(5F-3Cl-Gly)Cl(H ₂ O) ₂]	3485, 1683	2769, 2601/3113	1657/1554	519/412	271 (3.049); 306 (2.971); 329 (1.621); 571 (0.497)
[Cr(5F-3Cl-Ala)Cl(H ₂ O) ₂]	3475, 1685	2767, 2607/3117	1658/15561	517/409	284 (2.885); 321 (2.870); 335 (1.531); 623 (0.365)

Scheme 2 Structure of suggested for studied complexes

M: Cr(III) or Ni(II)

X: -H or -Cl; R: -H or -CH₃Y, Z: H₂O, for octahedral Ni(II) / -, for diamagnetic Ni(II)Y: H₂O; Z: -Cl for octahedral Cr(III)**Table 4** Antimicrobial activity of studied compounds (0.25 µg/µl) and standard reagents (diameter of zone inhibition (mm))

Microorganisms	Compound				Control	
	[5F-Gly], [Ni(5F-Gly)(H ₂ O) ₃], [Cr(5F-Gly)Cl(H ₂ O) ₂]	[5F-Ala], [Ni(5F-Ala)(H ₂ O) ₃], [Cr(5F-Ala)Cl(H ₂ O) ₂]	[5F-3Cl-Gly], [Ni(5F-3Cl-Gly)H ₂ O], [Cr(5F-3Cl-Gly)Cl(H ₂ O) ₂]	[5F-3Cl-Ala], [Ni(5F-3Cl-Ala)H ₂ O], [Cr(5F-3Cl-Ala)Cl(H ₂ O) ₂]		
Gram (+ve)						
<i>Sh.dys. typ 10</i>	11, -, 8	-, 10.5, -	18, 20, 10	11.5, 12.5, -	-	
<i>L. monocytogenes</i>	10, 10, 8.5	6.5, 9, 9	10, 8, 10	12.5, 10, 7	-	
<i>S. typhi H</i>	15, 13.5, 12	-, 10.5, 13.5	18, 6.5, 11.5	11.5, 16, 11.5	-	
<i>Br. abortus</i>	11.5, 8.5, 10	10.5, 13, 11.5	15, 12.5, 11	17.5, 15, 15	-	
Gram (-ve)						
<i>S. aureus</i>	7, 11.5, 7	6, 11.5, 10	18.5, 12.5, 10	16, 17.5, -	-	
<i>S. epidermis</i>	6, 8.5, 8.5	5, 13, 8.5	12.5, 14, -	12, 16.5, 7	-	
<i>M. luteus</i>	15, 12, 21	14, 11.5, 8.5	26.5, 24, 17.5	23.5, 31.5, 11	-	
<i>E. coli</i>	13, 11.5, 12.5	10, 10, -	16, 16.5, 8.5	15.5, 19.5, -	-	
Yeast						
<i>C. albicans</i>	10, 13, 13.5,	12.5, 10.5, 10,	15.5, 20.5, 10.5	15, 20, 10.5	-	
Pozitif control	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>S. typhi H</i>	<i>Br. abortus</i>	<i>C. albicans</i>
K30	25	15	25	20	-	-
SXT25	24	11	18	17	-	-
AMP10	30	16	10	11	-	-
AMC30	30	22	14	19	-	-
NYS100	-	-	-	-	-	20
SCF	-	-	-	-	12	-

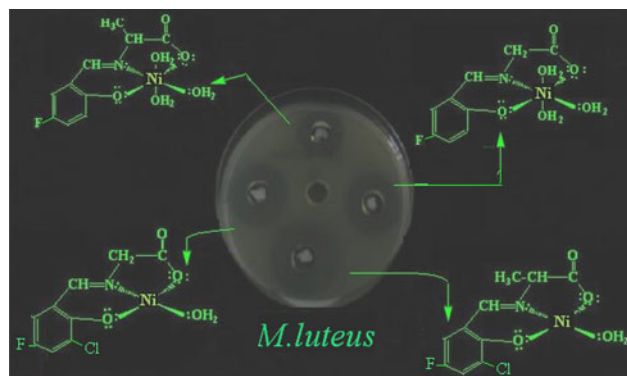
K30 Kanamycin 30 µg, SXT25 Sulphamethoxazol 25 µg, AMP10 Ampicillin 10 µg, AMC30 Amoxycillin 30 µg, NYS100 Nystatin 100 µg, SCF Sulbactam (30 µg)

the region 865–893 nm, 636–641 nm, and 389–400 nm. First band is assigned for ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, the second band for ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$, and third band ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)(\nu_3)$ transition. The UV-spectrum of Chromium (III) complexes shows three bands in the regions 537–627 nm, 329–369 nm, and 283–306 nm. The peak ca. 550, 350, and 290 nm corresponds to the ${}^4A_{2g} \rightarrow {}^4T_{2g}$, ${}^4A_{2g} \rightarrow {}^4T_{1g}(F)$, and ${}^4A_{2g} \rightarrow {}^4T_{1g}(P)$ electronic transition, respectively. The magnetic measurements for Cr(III) and two Ni(II) (for [Ni(5F-Gly)(H₂O)₃] and [Ni(5F-Ala)(H₂O)₃]) complexes showed three and two unpaired electrons, and the magnetic moment values 4.10–3.81 BM and 2.84–2.88 BM, respectively, for Cr(III) and paramagnetic Ni(II) ion suggesting consistency with their octahedral environment.

Biological activity of Schiff bases and their complexes

The ligands and their complexes were screened for antimicrobial activity in DMF solvent as a control substance. The compounds were tested with the same concentrations in DMF solution (0.25 µg/µl). All the synthesized compounds and antibiotic exhibited varying degree of inhibitory effects on the growth of different tested strains (Table 4). All the compounds were active against *L. monocytogenes*, *Br. abortus*, *M. luteus*, and *C. albicans*. *Br. abortus* is a gram-negative bacterium that causes premature abortion of a cattle fetus. What makes this bacterium so dangerous is that it can be transferred from an animal to a human host (Sari *et al.*, 2006). In humans, this

disease causes both acute and chronic symptoms, but can be treated with antibiotics. This research indicates that amino acid-Schiff bases and their Ni(II) and Cr(III) complexes are active against *Br. abortus* (Table 4). All the synthesized compounds showed moderate activity against *L.monocytogenes* except [5F-3Cl-Ala]. [5F-Ala] and its Cr(III) complexes were inactive in *S.typhi H*, whereas [5F-Gly] and their complexes were active gram (–ve), [5F-3Cl-Gly] and their complexes were active gram (+ve). In general, the metal complexes are more potent bactericides than the ligand. This enhancement in activity may be explained on the basis of chelation theory (Altundas *et al.*, 2010). Chelation reduces the polarity of the metal ion. Hence, a complex has lipophilic character, and increases the interaction between metal ion and the lipid is favored. This lead to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cellular processes (Murukan and Mohanan, 2007). As seen in Table 4, diamagnetic Ni(II) complexes showed a significant activity against gram (+ve) and gram (–ve) (Scheme 3). The investigated compound's antimicrobial activity values in this research were higher than that reported for other amino acid-Schiff base derivatives (Sakiyan *et al.*, 2004; Sari *et al.*, 2003). The main difference in the amino acid-Schiff base derivatives reported in this paper is the presence of the fluorine atom. The other amino acid-Schiff base derivatives included substituted benzyl, pyridine, or naphtha groups. Coordination structure of complexes plays an important role in their biological activity mechanisms. The results show that the diamagnetic Ni(II) complexes are more active against the tested yeast and as compared to the paramagnetic Ni(II) complexes. Diamagnetic Ni(II) complexes have square planer structure (D_{4h}). Square planer structure may be reducing the polarity of the metal ion due to the strong overlap in between the $d_{x^2-y^2}$ orbital of metal ion and donor orbital of the ligand. Thus, the lipophilic character of the central metal atom is enhanced, which results in a higher capability to penetrate



Scheme 3 Imaging of antimicrobial affectivities of Ni(II) complexes against *M. luteus*

the microorganisms through the lipid layer of the cell membrane.

Furthermore, the antibacterial activity of these compounds was also compared with seven commercial antibiotics, namely Kanamycin, Sulfamethoxazol, Ampicillin, Ciprofloxacin, Amoxycillin, Sulbactam, and Nystatin. It was seen that the synthesized compounds were effective as the antibiotics mentioned.

Conclusions

In this paper, new amino acid-Schiff base derivatives were synthesized by the condensation of 5-fluoro-2-hydroxy benzaldehyde, 3-chloro-5-fluoro-2-hydroxybenzaldehyde with D-glycine, D-alanine. And then, Ni(II) and Cr(III) complexes were prepared by the template method. The structures of the prepared compound were confirmed by elemental analysis, IR 1H and ^{13}C NMR spectral analysis. Diamagnetic Ni(II) complexes showed a significant activity against gram (+ve) and gram (–ve).

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