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Synthesis, Structure, and Pharmacological Evaluation of Co(III) Complex Containing Tridentate Schiff Base Ligand[1]

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Abstract—A Co(III) Schiff base complex with the formula $[Co(L)_2]Cl \cdot H_2O$ (**I**) (L = monoanionic tridentate Schiff base ligand derived from salicylaldehyde and ethylene diamine) has been synthesized and characterized by elemental analysis and spectroscopic techniques. The structure of **I** was confirmed by single crystal X-ray diffraction studies (CIF file CCDC no. 1009967), which showed the presence of two Schiff base molecules in an octahedral fashion. The complex has been screened for its in vitro antibacterial and anticancer activities. Complex **I** was found to be more active in anti-microbial and anti-cancer activity than the ligand.

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INTRODUCTION

Schiff bases and their complexes have a variety of applications including biological, clinical and analytical, and have been extensively studied over the past few decades [1–3]. Schiff base metal complexes are very popular due to their diverse chelating ability [4]. They play an important role in both synthetic and structural research, because of their preparative accessibility and structural diversity [5]. Due to variable magnetic property and catalytic activity, they can also serve as efficient models for the metalloproteins and enzymes [6, 7]. In view of the interest involved in the Schiff base complexes, many cobalt Schiff base complexes have been synthesized and characterized [8, 9]. We report herein the synthesis and biological evaluation of Schiff base complexes of cobalt.

EXPERIMENTAL

Materials, methods and measurements. All the chemicals were purchased from Sigma Aldrich. Elemental analyses of the compounds were carried out using the ElementarVario EL III CHN analyzer. The FT-IR spectra were recorded on a Shimadzu FT-IR spectrophotometer in the 4000–400 cm^{-1} region. The UV-Vis spectra were recorded on an Elico SL 159 UV-Vis spectrophotometer. The molar conductance of the complex was measured using a Systronics conductivity bridge at room temperature in DMSO solution. The thermal behavior of Co(III) complex was studied using PerkinElmer STA 6000 thermo analyzer. The antimicrobial activity of the ligand and its Co complex was carried out by disc diffusion method. The Schiff base ligand was prepared from salicylaldehyde and ethylene diamine according to the reported procedure [10, 11]. The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity.

Synthesis of Co(III) complex (I). The Schiff base ligand (0.0328 g, 0.2 mmol) in ethanol (10 mL) was added to a solution of $CoCl_2 \cdot 6H_2O$ (0.0237 g, 0.1 mmol) in ethanol (10 mL), and the mixture was heated under reflux for 5 h. The product obtained was filtered, washed and dried. The yield was 67%, m.p. = 218°C, orange solid.

For $C_{18}H_{24}N_4O_3ClCo$			
anal. calcd., %:	C, 49.22;	H, 5.46;	N, 12.76,
Found, %:	C, 49.22;	H, 5.47;	N, 12.76.

IR (KBr; ν, cm^{-1}) 3380 s, 3420 s, 1634 s, 1448 w, 1386 w, 1298 w, 1154 w, 1123 w, 673 s, 598 m, 553 m, 548 m.

X-ray determination of structure I. A Bruker APEX 2 X-ray (three-circle) diffractometer was employed for crystal screening, unit cell determination, and data collection. The X-ray radiation employed was generated from a Mo sealed X-ray tube (λ = 0.70173 Å with a potential of 40 kV and a current of 40 mA) fitted with a graphite monochromator in the

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Table 1. Crystal data and structural refinement parameters for **I**

Parameter	Value
Formula weight	438.79
Temperature, K	110.15
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions:	
a, Å	10.036(3)
b, Å	11.842(3)
c, Å	32.425(8)
Volume, Å^3	3853.6(16)
Z	8
ρ_{calcd}, mg/m^3	1.513
Absorption coefficient, mm^{-1}	1.056
F(000)	1824
Crystal size, mm	0.32 × 0.24 × 0.18
θ Range for data collection, deg	2.387–27.478
Index ranges	$-13 \le h \le 13$, $-15 \le k \le 15$, $0 \le l \le 40$
Reflections collected	14280
Independent reflections (R_{int})	4282 (0.0290)
Data/restraints/parameters	4282/0/247
Goodness-of-fit on F^2	1.052
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0293$, $wR_2 = 0.0730$
R indices (all data)	$R_1 = 0.0352$, $wR_2 = 0.0751$
Largest diff. peak and hole, e Å^{-3}	0.348 and −0.399

parallel mode (175 mm collimator with 0.5 mm pinholes). Systematic reflection conditions and statistical tests of the data suggested the space group *Pbca*. A solution was obtained readily using SHELXTL (XS) [12]. Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters. The structure was refined (weighted least squares refinement on F^2) to convergence [13]. Olex2 was employed for the final data presentation and structure plots. The crystal data and structural refinement parameters are summarized in Table 1. Bond lengths and bond angles for the complex have been incorporated in Table 2. A few selected H-bonding parameters are given in Table 3.

Supplementary material for the crystal structure has been deposited with the Cambridge Crystallographic Data (no. 1009967; deposit@ccdc.cam.ac.uk or http://ccdc.cam.ac.uk).

Antimicrobial activity. The antibacterial activity of the ligand and the Co(III) complex was tested against the bacteria *S. aureus, B. subtilis, S. paratyphi* and *K. pneumoniae* by the disc diffusion method using agar nutrient medium. The antifungal activity was screened for the organisms *A. niger* and *C. albicans* cultured on potato dextrose agar medium, by the disc diffusion method. The plates were incubated for 24 and 72 h for bacteria and fungi respectively. Then, the test solutions were diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration of the samples was noted [14].

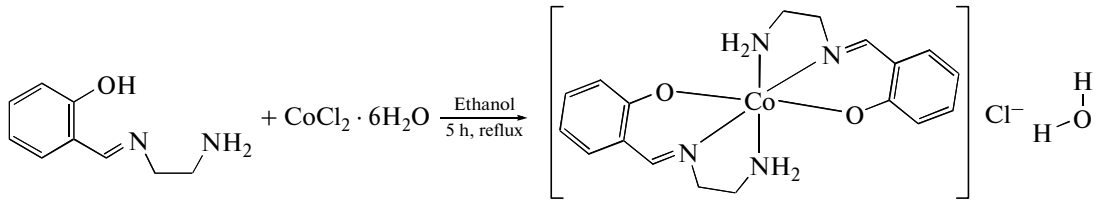
Anticancer activity-cell treatment procedure and MTT assay. The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/mL. Cell suspensions were seeded into 96-well plates at plating density of 10000 cells/well and incubated to allow for cell attachment at 37°C. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in DMSO and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 μL of these different sample dilutions were added to the appropriate wells already containing 100 μL of medium, resulted the required final sample concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37°C. The medium containing without samples was served as control and triplicate was maintained for all concentrations.

3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring, converting

the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 μL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μL of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the formula, % cell Inhibition = 100 − Abs (sample)/Abs (control) × 100. Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ (concentration) and IC_{50} was determined using graph pad prism software.

RESULTS AND DISCUSSION

An octahedral Co(III) complex was prepared from the reaction between $CoCl_2 \cdot 6H_2O$ and Schiff base (derived from the condensation of salicylaldehyde with ethylene diamine):



The analytical and spectral data of the complex revealed monoanionic tridentate coordination of the ligand with Co^{3+} ion. The complex is soluble in common polar solvents but readily soluble in DMF and DMSO to give stable solutions at room temperature. The molar conductance of Co(III) complex is 78 S mol^{-1} cm^{-1} showing their 1 : 1 electrolyte nature [15].

In the FT-IR spectrum of the ligand, a strong band is observed at 1666 cm^{-1} for the free azomethine group. In the Co(III) complex, this band is shifted to lower wave number (1634 cm^{-1}) indicating the coordination of the Schiff base through azomethine nitrogen [3]. A strong band observed at 1274 cm^{-1} in the free ligand has been assigned to phenolic C–O stretching. On complexation, this band is shifted to a higher frequency region (1298 cm^{-1}) proving that the other coordination site is phenolic oxygen atom [16]. Strong bands around 3433–3450 cm^{-1} are due to primary amine N–H stretching. In the complex, the absorptions due to $-NH_2$ have been observed at a lower frequency region 3380–3420 cm^{-1} indicating that the amine nitrogen atom is coordinated to the Co^{3+} ion [10].

The electronic absorption spectra of the ligand and its Co(III) complex were recorded in DMF at 25°C. The electronic spectrum of the ligand showed two bands at 250 and 330 nm, which correspond to charge transfer transitions. The electronic spectrum of Co(III) complex displayed bands at 285, 350 and 400 nm. The absorptions around 280–350 nm are ascribed to metal to ligand charge transfer transitions. The absorption at 400 nm may be assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ transition. The observed spectra are similar to those reported for other six coordinated Co(III) complexes [17–20].

The thermogravimetric analysis gives information about the thermal stability of **I** and suggests a general scheme for thermal decomposition of the chelate. In the present investigation, heating rates were suitably controlled at 10°C/min under nitrogen atmosphere and the weight loss was measured from the ambient temperature up to 800°C. The thermogram of **I** showed two decomposition steps within the temperature range 35–500°C correspond to the loss of water molecule of hydration, chlorine and other gases with a mass loss of 34% (calcd. 32.02%) accompanied by two exothermic peaks with T_{max} = 129.02°C and 371.89°C on the DTA curve, attributed to the removal of the non-coordinated part of the ligand. The subsequent steps (>500°C) correspond to the removal of the organic part of the complex, leaving metal oxide as a residue. The overall weight loss amounts to 65% (calcd. 62.6%). This mass loss corresponds to the pyrolysis of Co(III) complex leaving Co_2O_3 as residue. An exothermic peak with T_{max} = 551°C on the DTA was observed for this step [21]. The thermogram of complex **I** is depicted in Fig. 1.

The surface morphological study of the pure CoO nanoparticle obtained by the thermal decomposition of complex **I** at 500°C was carried out and the images are shown in Fig. 2.

The structure of **I** consists of one uncoordinated Cl^- and one hydrated water molecule (Fig. 3). The Co^{3+} ion is coordinated by four nitrogen (two imine and two amine) and two oxygen atoms from two ligands forming a slightly distorted octahedral geometry with Co–N bond distances range from 1.8960(14) to 1.9547(14) Å and Co–O bond distances range from 1.8894(12) to 1.8979(11) Å. The Co–N (amine) bond lengths (Co(1)–N(2) 1.9547(14); Co(1)–N(4) 1.9600(14) Å) are longer than Co–N (imine) bond lengths (Co(1)–N(1) 1.8960(14); Co(1)–N(3) 1.8978(14) Å). The *cis* angles in the octahedron are only slightly devi-

Table 2. Selected bond lengths (Å) and bond angles (deg) for **I**

Bond	*d*, Å
Co(1)–O(2)	1.8979(11)
Co(1)–O(1)	1.8894(12)
Co(1)–N(2)	1.9547(14)
Co(1)–N(3)	1.8978(14)
Co(1)–N(1)	1.8960(14)
Co(1)–N(4)	1.9600(14)
Angle	**ω, deg**
O(2)Co(1)N(2)	89.23(6)
O(2)Co(1)N(4)	177.90(5)
O(1)Co(1)O(2)	90.77(5)
O(1)Co(1)N(2)	179.99(7)
O(1)Co(1)N(3)	88.74(5)
O(1)Co(1)N(1)	94.76(5)
O(1)Co(1)N(4)	87.17(6)
N(2)Co(1)N(4)	92.83(6)
N(3)Co(1)O(2)	94.22(6)
N(3)Co(1)N(2)	91.26(6)
N(3)Co(1)N(4)	85.36(6)
N(1)Co(1)O(2)	87.58(6)
N(1)Co(1)N(2)	85.24(6)
N(1)Co(1)N(3)	176.05(6)
N(1)Co(1)N(4)	92.97(6)
C(15)O(2)Co(1)	124.20(10)
C(6)O(1)Co(1)	124.20(11)

ated from the value of 90°, O(1)Co(1)O(2) has a value of 90.77(5)° and O(2)Co(1)N(2) has a value of 89.23(6)°, and the *trans* angles have an average value of 178°. The equatorial plane consists of three nitrogen (two imine and one amine) atoms and one oxygen atom, and the axial positions are occupied by amine nitrogen and oxygen. Hydrogen bonding is observed between the amine hydrogen atoms and the oxygen atom of the hydrated water molecule and chloride ion (Fig. 4).

Complex **I** was evaluated for their pharmacological activity against four different gram positive and gram negative bacteria, *S. aureus, B. subtilis, S. paratyphi* and *K. pneumoniae*, and two fungi *A. niger* and *C. albicans*. The results of antibacterial and antifungal screening have been compared with the standard Ciproflosacin and Clotrimazole in each case. It is evident from Fig. 5 that the activities of Schiff base are increased upon complexation with Co(III), which can be explained on the basis of chelation theory. Complex **I** showed pronounced activity against *S. aureus, S. paratyphi* and *A. niger* and considerable activity against *B. subtilis*, *K. pneumoniae* and *C. albicans*. It is evident from Table 4 that the MIC values of the complex against *S. aureus, S. paratyphi* and *A. niger* are 250, 125 and 500 μg/mL, respectively. The increasing antimicrobial activity of Co(III) chelate with increase in concentration is due to the effect of Co^{3+} ion on the normal cell process. Furthermore, the mode of action of the compounds may involve formation of hydrogen bond through azomethine group with the

Table 3. Geometric parameters of hydrogen bonds of **I***

D–H···A	Distance, Å			Angle DHA, deg
	D–H	H···A	D···A	
O(1*w*)–H(1*wA*)···Cl(1)$^{\#1}$	0.85	2.30	3.1489(16)	174
N(2)–H(2*A*)···Cl(1)$^{\#2}$	0.99	2.31	3.2237(15)	154
N(2)–H(2*B*)···Cl(1)	0.99	2.29	3.2455(15)	160
N(4)–H(4*A*)···Cl(1)$^{\#2}$	0.99	2.35	3.3308(17)	173
N(4)–H(4*B*)···O(1*w*)	0.99	1.95	2.927(2)	169

* Symmetry transformations used to generate equivalent atoms: $^{\#1}$ $x-1, y, z$; $^{\#2}$ $x-1/2, y, -z+3/2$.

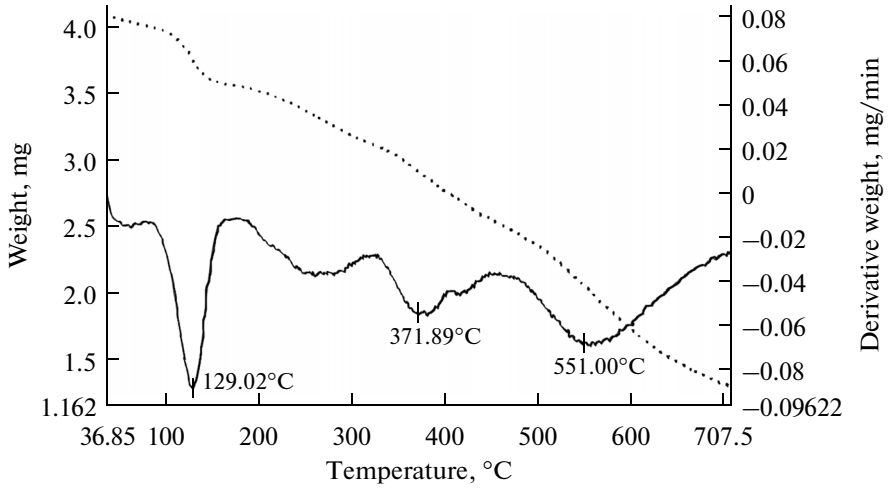


Fig. 1. Thermogram of complex **I**.

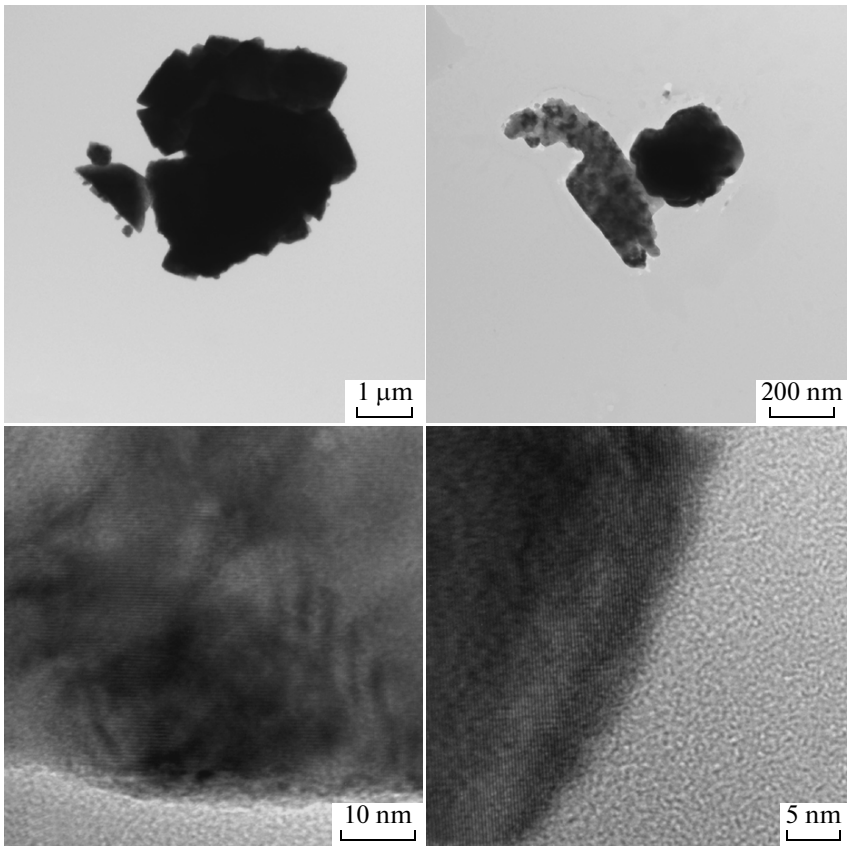


Fig. 2. TEM images showing the surface morphology of CoO at different nm range.

active centers of cell constituents, resulting in interference with the normal cell process [22–27].

In vitro cytotoxicity study was carried out for complex **I** against the human breast cancer cell line (MCF 7) by means of a colorimetric micro culture MTT assay which measures mitochondrial dehydrogenase activity as an indication of cell viability. It is evident that the number of cells decreased with an increase in the concentration of **I**. Complex **I** showed moderate activity which is evidenced from low IC_{50}

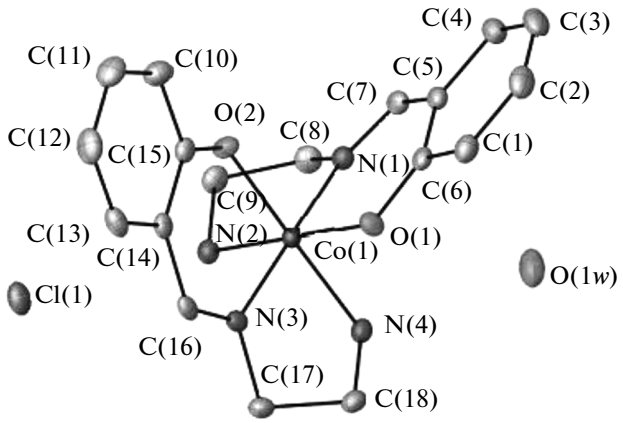


Fig. 3. Molecular structure of complex **I**.

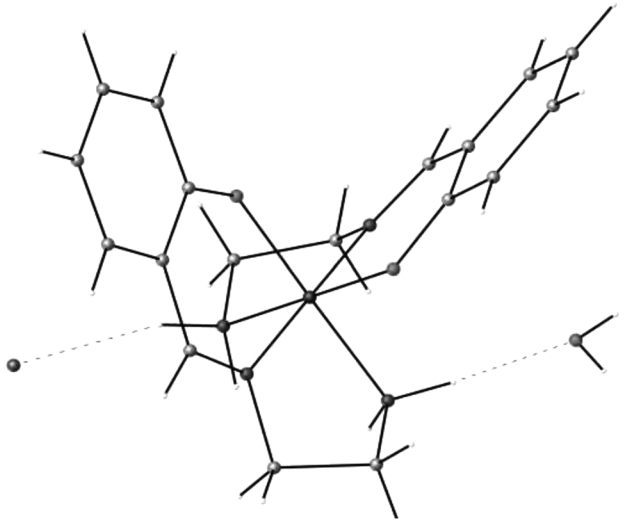


Fig. 4. Hydrogen bonding interactions in structure **I**.

Table 4. MIC values for **I** against *S. aureus, S. paratyphi* and *A. niger*

Organisms	μg/mL						
	1000	500	250	125	62.5	31.25	15.625
S. aureus				+	+	+	+
S. paratyphi					+	+	+
A. niger			+	+	+	+	+

values (50% inhibitory concentration after exposure for 48 h in MTT assay) of <100 μg/mL [28, 29].

Thus, Co(III) Schiff base complex has been synthesized and characterized by various spectral techniques and the structure was confirmed by single crystal XRD study. The Co(III) is six coordinated with four nitrogen and two oxygen atoms from two ligands. Complex **I** has been screened for the in vitro antimicrobial activity against various test organisms and their MIC values are also reported. It showed moderate to good activity against various bacterial and fungal strains. The in vitro cytotoxicity study shows that complex **I** exhibits anti tumor activity against the human breast cancer cells with $IC_{50} < 100$ μM.

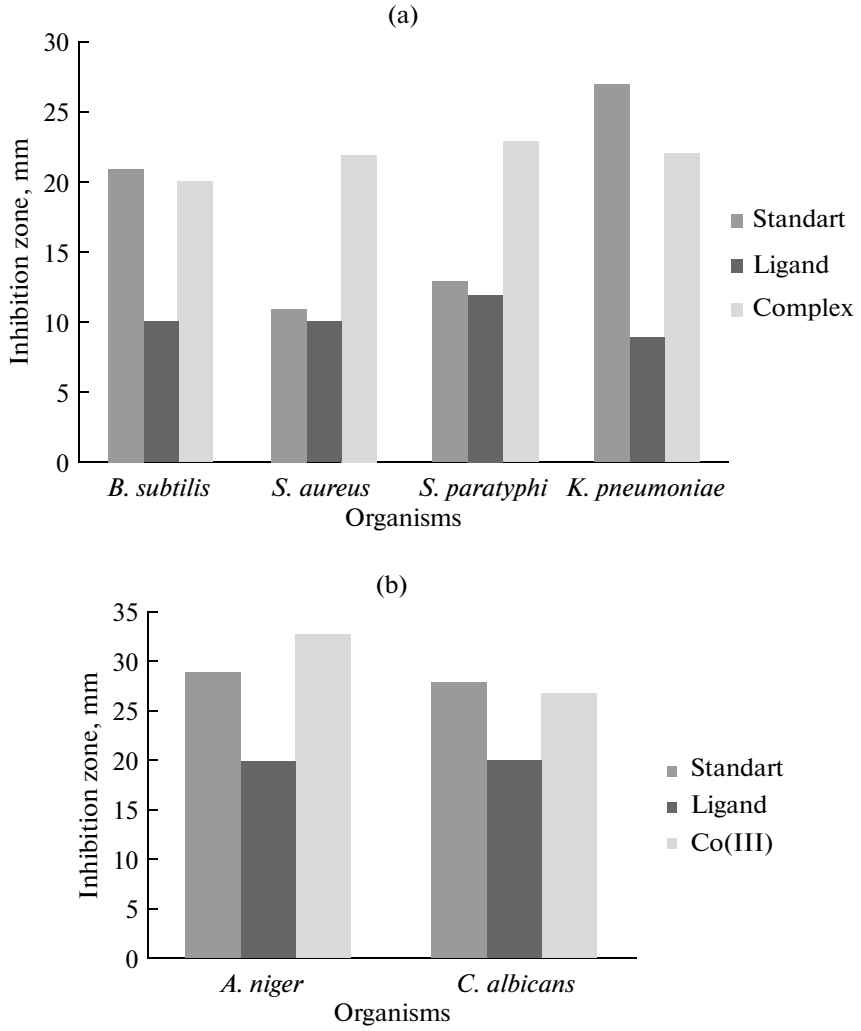


Fig. 5. Antibacterial (a) and antifungal (b) activities of complex **I**.

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