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Synthesis, spectroscopic characterization and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff bases derived from 5-bromo-salicylaldehyde

Ahmed Nuri Kursunlu^{a,*}, Ersin Guler^a, Fatih Sevgi^b, Birol Ozkalp^b

^a Department of Chemistry, Selcuk University, Campus, 42075 Konya, Turkey
^b Vocational School of Health Services, Selcuk University, Campus, 42075 Konya, Turkey

HIGHLIGHTS

• The Schiff bases derived from salicylaldehyde containing bromine have an important situation for antimicrobial studies.

• The antibacterial properties of the Schiff bases and their metal complexes are illuminated.

• The results of this study can be useful to researchers attempting for future work to study the antifungal activities.

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1. Introduction

ABSTRACT

In this study, the new Schiff base ligands derived from condensation of amine and 5-bromo-salicylaldehyde were characterized. All compounds, the Schiff bases and the metal complexes, were characterized by elemental analyzes, FT-IR, ¹H NMR, ¹³C NMR and magnetic susceptibility measurements. The synthesized ligands, along with their metal (II) complexes, were screened for their in vitro antibacterial activity against four Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella enteri-tidis*) and four Gram-positive (*Streptococcus pyogones, Bacillus cereus, Staphylococcus aureus and* Methicillin-resistant *S. aureus*) bacterial strains by using disc diffusion and broth microdilution techniques.

The minimum inhibitory concentration (MIC) values were calculated by microplate reader at 620 nm to optimum result. It was found that all compounds showed mild to moderate antibacterial activity and the Schiff base ligands had a better antibacterial activity than their complexes.

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In the past decades, the problem of multi-drug resistant microorganisms has reached on alarming level around the world. Therefore, the synthesis of new compounds that are used for the effective treatment of microbial infections without side effects is a biomedical problem [1]. The synthesis of new Schiff base complexes becomes widespread due to their potential application in chemistry, biochemistry, medicine and technology [2]. The synthesis, characterizations and structure-activity relations of Schiff bases were studied previously by some researchers [3]. It is well known that several Schiff base complexes are used as anti-inflammatory, anti-pyretic, chemosensor, anti-diabetic, anti-bacterial, anti cancer and anti-HIV [1,4]. Schiff bases containing halogen groups and their metal complexes have a special interest due to their antimicrobial properties [5]. Schiff bases derived from substituted salicylaldehydes and various amines and their metal complexes have been widely investigated because of their wide

applicability [6–9]. The chelating ligands containing O and N donor atoms show broad biological activity and have special interest on bonding to metal ions [10,11]. It is also reported that salicylaldehyde derivatives, with one or more halo-atoms in the aromatic ring, shows biological activities like antitumor, antibacterial and antifungal activities [12,13].

These investigations led to the conception that the Schiff bases of 5-bromo-salicylaldehyde would possess potential antimicrobial properties. In this study, the synthesis-spectroscopic characterization of the new Schiff bases derived from 5-bromo-salicylaldehyde and their metal complexes and their antibacterial properties were reported. The results of this study can be useful to researchers attempting to gain more understanding of the antimicrobial activity of Schiff base ligands and their metal complexes.

2. Experimental

2.1. Instruments

The melting points of the ligands and the metal complexes were measured by a Buchi-B-540 melting point apparatus. The pH val-







^{*} Corrosponding author. Tel.: +90 332 223 3876. E-mail address: ankursunlu@gmail.com (A.N. Kursunlu).

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ues were measured with a WTW pH, 537 pH meter. The IR spectra were recorded by using KBr pellet technique in a Mattson-1000 FT-IR spectrophotometer. The percentages of carbon, hydrogen, nitrogen and metal in all compounds were determined using a *TruSpec* elemental analyzer. ¹H NMR and ¹³C NMR measurements of Schiff bases were obtained on a *Varian*, 400 MHz spectrometer at room temperature. The mass spectra for ligands were obtained with a Varian MAT 711 spectrometer. The magnetic measurements of the metal complexes were carried out by *Sherwood Scientific Magnetic Susceptibility Balance* in solid state at room temperature. The MIC values were measured by Bio-Tek μ Quant microplate reader.

2.2. Materials

All chemicals used were of the analytical reagent grade and of highest purity available. 2-Chloroethylamine hydrochloride (Fluka), 2-bromooethylamine hydrobromide (Fluka)5-bromo-2hydroxybenzaldehyde (Merck) and triethylamine (Merck) were used for the synthesis of the ligands and copper(II)acetate monohydrate (Fluka); cobalt(II)acetate dihydrate (Fluka) and nickel(II)acetate tetrahydrate (Sigma); zinc(II)acetate dihydrate (AcrosOrganics) were used to make their complexes. The absolute methanol, ethylacetate, ethanol (Riedel-de Haen) and diethylether (Merck) were used as organic solvents.

2.3. The preparation of (E)-4-bromo-2-((2chloroethylimino)methyl)phenol (L_1) and (E)-4-bromo-2-((2bromoethylimino)methyl)phenol (L_2)

A 1:1 equimolar methanolic solution of 2-chloroethylamine hydrochloride (30 mmol) and 5-bromo-2-hydroxybenzaldehyde (30 mmol) were mixed and gently heated for 30 min with constant stirring, 4.2 cm³ of triethylamine (30 mmol) was gradually added to this mixture to remove the hydrochloride salt of 2-chloro ethylamine. Stirring was continued for an additional 30 min. The yellow precipitate was washed with ethylacetate and dried under vacuo. The obtained yellowish residue was recrystallized from a mixture of hot ethanol and diethylether (1:2) and was dried overnight in a vacuum desiccator [14]. It was named as (E)-4-bromo-2-((2-chloroethylimino)methyl)phenol (L₁). Anal. Calc. for (%) C₉H₉NOBrCl (L₁) (262.53 g/mol): C, 41.17; H, 3.46; N, 5.34; Found: C, 41.07; H, 3.37; N, 5.41. ¹H NMR [400 MHz, CDCl₃] δ 3.80 (2H, t, CH₂), 3.92 (2H, t, CH₂), 6.88(1H, d, ArH), 7.39 (2H, m, ArH), 8.31(1H, s, CH=N), 13.02 (1H, s, ArOH).¹³C NMR [400 MHz, CDCl₃] δ 44.0, 60.9, 110.4, 119.3, 120.1 134.0, 135.5, 160.3, 166.1. Anal. Calc. MS (ESI) *m*/*z* 262.95 (M+); Found: 262.63.

The (E)-4-bromo-2-((2-bromoethylimino)methyl)phenol (L₂) was prepared according to same procedure. Anal. Calc. for (%) C_9H_{9-} NOBr₂ (L₂) (306.98 g/mol): C, 35.21; H, 2.96; N, 4.56; Found: C,

35.01; H, 3.03; N, 4.33. ¹H NMR [400 MHz, CDCl₃] δ 3.68 (2H, t, CH₂), 3.82 (2H, t, CH₂), 6.92(1H, d, ArH), 7.41 (2H, m, ArH), 8.44(1H, s, CH=N), 13.00 (1H, s, ArOH). ¹³C NMR [400 MHz, CDCl₃] δ 34.0, 68.9, 112.5, 119.5, 122.8 136.0, 137.5, 159.2, 166.0. Anal. Calc. MS (ESI) *m/z* 306.90 (M+); Found: 307.58.

2.4. Synthesis of complexes

The metal complexes were prepared by the addition of a hot methanol solution of ligand to an equimolar amount (5 mmol) of the metal salts [$(Cu(CH_3COO)_2 \cdot H_2O, Co(CH_3COO)_2 \cdot 4H_2O, Ni(CH_3COO)_2 \cdot 4H_2O, Zn(CH_3COO)_2 \cdot 2H_2O]$]. The appropriate reaction mixtures were stirred and refluxed at 80 °C for 3 h under nitrogen. The separated complexes were filtered off, washed several times with ethanol, and dried under vacuum in the presence of anhydrous CaCl₂ (Tables 1 and 2).

The general reaction for the preparation of the complexes of Schiff base ligands are: MX₂. $nH_2O + L \rightarrow ML_2$. n_1H_2O where M = Cu(II), Co(II), Ni(II), Zn(II) (X = CH₃COO⁻), n = 0-4 and $n_1 = 0-2$ (see Fig. 1).

2.5. Biological activity

The test microorganisms; *Escherichia coli* RSKK 340, *Klebsiella pneumoniae* RSKK 06017, *Pseudomonas aeruginosa* RSKK 06021, *Salmonella enteritidis* RSKK 96046, *Streptococcus Pyogones* RSKK 413/214, *Bacillus cereus* RSKK 1122, *Staphylococcus aureus* RSKK 96090 were obtained from the Refik Saydam National Public Health Agency. Methicillin-resistant *S. aureus* (MRSA) is clinically isolated. The organisms were maintained on agar slope at 4 °C and sub-cultured for 24 h before use.

Ceftriaxone (CRO) 30 mcg (Bio Disc) was used as a positive control for disc diffusion tests. Ceftriaxone (Eczacıbaşı), was used as a positive control for broth microdilution test. DMSO (Merck) was used as a negative control. 96-well microtiter plates (TPP 92096) were used for microdilution tests. Nutrient broth (Difco) and Mueller Hinton Agar (Difco) were used.

2.5.1. Antibacterial testing by disc diffusion

The antibacterial activities of ligands and their metal complexes in dimethylsulfoxide (DMSO) were performed in vitro by the disc diffusion method [15]. The bacterial inoculums were prepared from overnight grown cultures (24 h) in Nutrient broth (Difco) and turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 cfu/ml). Aliquots (100 µL) of inoculums were spread over the (Difco) plates with a sterile glass spreader. The sterilized paper discs (Oxoid, 6 mm diameter) were wetted with 10 µL of a solution of each compound to be tested, in the concentration of 0.02 g/ml (200 µg per disc) in DMSO. Ceftriaxone and

 Table 1

 Analytical and physical data of the Schiff base ligands and their complexes.

Compound		Color	Yield (%)	m.p (°C)	Elemental analys	sis found (calcd.) (%	%)		μ eff (B.M.)
					С	Н	Ν	М	
L ₁		Bright yellow	85	93	41.07 (41.17)	3.46 (3.37)	5.34 (5.41)	-	-
L ₂		Bright yellow	83	102	35.01 (35.21)	3.03 (2.96)	4.33 (4.56)	-	-
L ₁	Co(II)	Dark green	55	183	34.75 (34.98)	3.33 (3.26)	4.42 (4.53)	9.74 (9.54)	1.67
	Ni(II)	Bright green	57	220	37.16 (37.19)	2.77 (3.01)	4.82 (4.74)	10.09 (10.46)	Dia.
	Cu (II)	Med. green	62	228	36.86 (36.72)	2.75 (2.95)	4.78 (4.46)	10.83 (10.95)	1.80
	Zn(II)	Yellow-white	63	203	36.74 (37.08)	2.74 (2.87)	4.76 (4.71)	11.12 (11.56)	Dia.
L ₂	Co(II)	Brown	59	192	30.58 (30.76)	2.85 (2.64)	3.96 (3.78)	8.34 (8.46)	1.72
	Ni(II)	Bright green	62	229	32.24 (32.44)	2.40 (2.27)	4.18 (4.07)	8.75 (9.04)	Dia.
	Cu(II)	Med. green	57	242	32.01 (32.19)	2.39 (2.44)	4.15 (4.12)	9.41 (9.66)	1.76
	Zn(II)	Yellow-white	61	225	31.92 (32.32)	2.38 (2.34)	4.14 (4.09)	9.66 (9.97)	Dia.

Table 2

Characteristic IR (cn	n ⁻¹) bands of liga	nds and their complexe	s.
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Compound	ls	ν(C=N)	ν(OH)	$\nu(CH_2)$	ν(C—Cl)	<i>v</i> (C—Br)	ν(M—N)
L ₁		1635	3453	2852-2894	823	654	-
L ₂		1641	3338	2892-2964	887	677	-
L ₁	Co(II) Ni(II) Cu(II) Zn(II)	1612 1612 1620 1620	3480 - - -	2887-2929 2941-2964 2894-2925 2925-2960	831 819 823 827	684 692 688 684	554 550 550 569
L ₂	Co(II) Ni(II) Cu(II) Zn(II)	1612 1612 1620 1620	3480 - - -	2887-2929 2941-2964 2894-2925 2925-2960	831 819 823 827	684, 671 692, 677 688, 670 684, 666	554 550 550 569



Fig. 1. The general scheme of the complexes.

DMSO were used as controls. The plates were incubated at $37 \,^{\circ}$ C for 24 h and the zone diameters of exact growth inhibition were measured. The values are reported in Table 3 as a mean of three replicates.

2.5.2. Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentrations (MIC) were determined by broth microdilution methods [16]. For MIC determination, the inoculums were prepared like the following procedure: the broth culture of each bacterial strains was adjusted to a turbidity equivalent to a 0.5 McFarland standard for 4–6 h and were diluted in Nutrient broth media to give concentration of $\approx 10^6$ cfu/mL for bacteria. Twofold serial dilutions of compounds and Ceftrioxane were prepared in Nutrient broth in 96-well plates starting from a stock solution of compounds (2.048 mg/ml DMSO). An equal volume of bacterial inoculum was added to each well on the microtiter plate. In this manner final concentration of compounds range from 512, 256, 128...to 0.5 µg/mL and 5×10^5 cfu/ mL bacteria in each well (last wells are broth only control well). Then, the inoculated microtiter plates were incubated at 37 °C for 24 h, and the growths were spectrophotometrically recorded at 620 nm by using a microplate reader (µQuant, BioTek). The MIC values were defined as the lowest concentration of compounds whose absorbances were comparable to the negative control wells (broth only, without inoculum). Ceftrioxane was used for comparative purposes and quality control of the method. The MIC values are reported as the mean of three experiments.

3. Results and discussion

3.1. Infrared and elemental analysis

In the IR spectra of the Schiff base ligands, a sharp C=N band was observed in the range of $1635-1641 \text{ cm}^{-1}$, respectively (Table 2) [17]. The other series of weak bands between 2852 and 2894 cm⁻¹ are related to *v*(C-H) modes of vibrations. The ligands also display bands at 3453 and 1366 cm⁻¹ which are assigned to *v*(OH) and *v*(C-O) stretching vibrations of the phenolic-OH, respectively. Additionally, the peaks were observed between 877 and 654 cm⁻¹ that attributed to chlorine and bromine.

After complexation, the vibration bands of the Schiff bases were decreased in intensity and shifted towards higher area about 15–30 cm⁻¹ in the metal complexes [18]. This shift to lower wavenumbers or disappearance of peaks support complex formations [19]. Moreover, the ν (M–N) band for the complexes are appeared in the wave number range 550–569 cm⁻¹ [20].

Table 3

Inhibitory zone (diameter) mm of compounds against tested bacterial strains by the disc diffusion Gram-negative Gram-positive method.

Compounds	E. coli	K. pneumoniae	P. aeruginosa	S. enteritidis	S. pyogones	B. cereus	S. aureus	MRSA
L ₁	16	15	16	13	20	17	19	12
$Co(L_1)_2 (H_2O)_2$	10	9	8	8	12	8	9	-
$Ni(L_1)_2$	10	11	10	10	14	10	11	9
$Cu(L_1)_2$	9	8	8	8	8	8	9	-
$Zn(L_1)_2$	10	10	10	11	15	9	10	9
L ₂	16	13	17	18	14	21	11	
Co (L ₂) ₂ (H ₂ O) ₂	9	10	7	9	11	8	9	-
$Ni(L_2)_2$	9	12	9	10	13	8	12	8
$Cu(L_2)_2$	8	8	9	8	9	10	10	-
$Zn(L_2)_2$	10	10	11	12	16	14	12	9
CRO	28	29	17	28	25	26	33	-
DMSO	-	_	_	_	_	-	-	-



Fig. 2. The NMR spectra of L_1 -Ni(II), L_1 -Zn(II) complexes and L_2 , respectively.



Fig. 3. UV–Vis spectra of L_1 -Co(II), L_1 -Ni(II), L_1 -Cu(II) and L_1 -Zn(II) complexes.

Tab	ole 4										
Mir	nimal inhibitory	concentration	(MIC) in $\mu g/$	mL of compo	ounds against	t tested	bacterial s	strains by	y microdilu	tion metho	od.
				-	_	-	_			_	

Compounds	E. coli	K. pneumoniae	P. aeruginosa	S. enteritidis	S. pyogones	B. cereus	S. aureus	MRSA
L ₁	64	64	128	64	64	32	32	16
$Co(L_1)_2$	NT	NT	NT	NT	NT	NT	NT	NT
$(H_2O)_2$								
$Ni(L_1)_2$	128	128	256	64	64	64	64	128
$Cu(L_1)_2$	NT	NT	NT	NT	NT	NT	NT	NT
$Zn(L_1)_2$	64	64	256	128	64	32	32	64
L ₂	64	128	128	128	32	32	32	16
Со	NT	NT	NT	NT	NT	NT	NT	NT
$(L_2)_2(H_2O)_2$								
$(H_2O)_2$								
$Ni(L_2)_2$	128	128	256	128	128	64	64	256
$Cu(L_2)_2$	NT	NT	NT	NT	NT	NT	NT	NT
$Zn(L_2)_2$	64	64	128	256	128	32	32	64
	<0.5	<0.5 Ceftriaxone	128	32	<0.5	<0.5	<0.5	512

NT: not tested because of insolubility in DMSO/media.

3.2. NMR and mass spectral analysis

The NMR spectra confirm the identity of the newly prepared ligands. In order to identify the solution structures of the Schiff base ligands, ¹H and ¹³C NMR spectra were recorded in chloroform(CDCl₃) as a solvent. The ¹H NMR and ¹³C NMR spectra of the ligands show no evidence of unreacted carbonyl or amine compound. On the basis of NMR spectra, the new Schiff base ligands were judged to be greater than 98% pure and were used without further purification.

The triplets at 3.82–3.92 and 3.68–3.82 ppm in the proton NMR spectra of the L_1 and L_2 can be attributed to the protons of the $-CH_2$ groups, respectively. The singlet peaks at 7.41 and the two doublets at 6.89–6.92, 7.39–7.41 ppm range may be assigned to the protons of the benzene ring. The protons of azomethine group (HC=N) are observed at 8.31–8.44 ppm as singlet. In addition, the chemical shifts of the proton on phenolic hydroxy groups are around 13.00 ppm.

The ¹³C NMR spectra of the L₁ and L₂ have similar properties. In ¹³C NMR, the signal due to the azomethine carbon atom was observed at 160.3–166.3 ppm, respectively. The ligands give also signals at 44.0–34.0 and 60.9–68.9 ppm that are due to the carbon atoms of $-CH_2$ groups. In the spectra of the ligands, the signals were observed in the 110.4–112.5, 119.3–119.5, 120.1–122.8, 134.0–136.0, 135.5–137.5 and 166.1–166.0 ppm range may be attributed to the carbon atoms of the benzene ring and the hydroxyl groups. Similarly, The LC–MS (ESI+) spectra of the Schiff base ligands were investigated and the molecular ions (m/z) were found: 262.63–307.58, respectively. So, these results show that the goal ligands were synthesized.

Fig. 2 shows that the NMR spectra of L_1 -Ni(II), L_1 -Zn(II) complexes and L_2 , respectively. The shift of the peaks indicated that the complexation reactions were successful for L_1 and similarly for L_2 . So, it is well known that Schiff bases derived from salicylal-dehyde are a good ligand for complex formation [6–9].

3.3. Magnetic susceptibility measurements

The structures of the complexes are supported by magnetic moment data. The magnetic moment of the Cu(II) complexes were observed in the range of 1.80–1.76 B.M. which corresponds to a single unpaired electron with a very slight orbital contribution. The geometry of the Cu(II) complexes is a square-planar or a distorted tetrahedral [21]. The Co(II) complexes reported herein are high spin at room temperature magnetic moment values of 1.67– 1.72 B.M, respectively. These results indicate that, the complexes of Co(II) have a six coordinate and probably octahedral geometry [22]. The Zn(II) and Ni(II) complexes are diamagnetic as expected and their geometries are probably tetrahedral [23].

3.4. UV-Vis studies

The UV-Vis spectra of all complexes were recorded in DMSO from 250 to 650 nm (Fig. 3). The low intense bands between 350 and 800 nm are due to d-d transitions and the intensity bands in the 250–300 nm range are assigned to the intraligand π – π * and $n-\pi^*$ transitions. The cobalt complexes of L₁ and L₂ have two bands in the visible region at 560-573 nm. The cobalt(II) octahedral complexes generally show three transitions in the visible region ${}^{4}T_{1g} \rightarrow {}^{4}T_{2g}$ and ${}^{4}A_{2g} \rightarrow {}^{4}T_{1g}$ [16]. [Ni(L₁)₂] and [Ni(L₂)₂] exhibit three spectral bands at 335-367, 410-425 and 625-653 nm due to ${}^3T_{1f} \rightarrow {}^3T_{1p}$. The spectra of the copper complexes show two absorption at 370-382 and 650-665 nm, which can be assigned to $^2B_{1g} \rightarrow ^2B_{2g}$ and $^2B_{1g} \rightarrow ^2E_g$ transition corresponding to a square planar structure. Two of these transitions are indicated by weaker shoulders in the absorption spectrum [23]. For copper complexes. the absorption bands in the range of 370-382 and 410-425 nm are assigned to $M \rightarrow L$ charge-transfer (MLCT) or $L \rightarrow M$ chargetransfer (LMCT) [24]. The absorption bands observed at lower energy and in lower intensities between 625-653 nm may be attributed to the d-d transitions. The d-d transitions were not observed in the visible region for zinc(II) complexes of L_1 and L_2 consistent with the d^{10} configuration of the Zn(II) ion [25].

3.5. Biological activity

The in vitro antibacterial activity was tested by disc diffusion method [15] and broth microdilution technique [16] using pathogenic strains of *E. coli* RSKK 340, *K. pneumoniae* RSKK 06017, *P. aeruginosa* RSKK 06021, *S. enteritidis* RSKK 96046, *S. pyogones* RSKK 413/214, *B. cereus* RSKK 1122, *S. aureus* RSKK 96090 and Methicillin-resistant *S. aureus* (MRSA). Ceftriaxone was used as positive control and quality control of assays. The zone of inhibition and MIC's values are listed in Tables 3 and 4, respectively. Since Cu(II) and Co(II) complexes of ligands are not soluble enough in DMSO media, their antimicrobial activity have not been determined by the microdilution method; these compounds screened only on disc diffusion method (see Fig. 4).

The screening data indicate that ligands are more active than their metal complexes. While MIC value of control antibiotic has $512 \mu g/mL$ against MRSA; surprisingly the ligands, (*E*)-4-bromo-2-((2-chloroethylimino)methyl)phenol and (*E*)-4-bromo-2-((2bromoethylimino)methyl)phenol, showed potent inhibitory activity with 16 μ g/mL MIC value. The ligands showed moderate activity



Fig. 4. The antimicrobial active terminals of the complexes.

all of the Gram-positive and Gram-negative strains with MIC ranges (16–128 μ g/mL). The Co(II) and Cu(II) complexes of the ligands had no valuable inhibitory activity with low inhibition zones. The Ni(II) and Zn(II) complexes generally have a strong antimicrobial effect against studies bacteria's (Tables 3 and 4).

4. Conclusion

We synthesized the new Schiff base ligands containing bromine and chlorine derived from salicylaldehyde. Then, Cu(II), Co(II), Ni(II) and Zn(II) complexes of these Schiff base ligands were obtained in appropriate conditions. Among the synthesized compounds, the Schiff base ligands (L_1 and L_2) with chlorine, bromine and —OH substituents showed moderate inhibition activity against some Gram-positive and Gram-negative bacteria including MRSA. The Zn(II) and Ni(II) complexes of the prepared ligands have a strong antimicrobial effect upon the studies bacterias. The results of this study can be useful to researchers attempting for future work to study the antifungal activities of the Schiff base complexes. Based on the results obtained, slight modifications of the L_1 and L_2 might produce potent and potential compounds which could be used as antimicrobial agents such as Ceftriaxone.

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