ANTIMICROBIAL AND SOD-LIKE ACTIVITIES OF *N,N′*-BIS(FERROCENYLMETHYLENE)ETHYLENEDIAMINE SCHIFF BASE AND IT'S METAL COMPLEXES

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ABSTRACT

The synthesized *N,N′*-bis(ferrocenylmethylene)ethylenediamine Schiff base and it's metal complexes were screened *in vitro* for antimicrobial activity against two Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 7064), four Gram-negative (*Escherichia coli* ATCC 25922, *Citrobacter koseri* DBCC 01, *Entorebacter aeruginosa* ATCC 13048, *Salmonella typhimurium* DBCC 02) and antifungal activity against a *Candida albicans*. MIC values of the compounds ranged from 27 to 533 µg/mL. Compound **(5)** showed very strong activity against *S. aureus* with the best MIC (27 µg/mL). The lowest MIC for *C. albicans was* obtained 107 µg/mL. The superoxide dismutase activity of Schiff base and it's metal complexes has been measured and discussed. SOD-like activities of bis ferrocenyl Schiff base ligand metal complexes were investigated using NBT. While only the Cu (II) complex showed SOD-like (40 µm IC_{50}) activity, the SOD-like activity value was not determined in the other complex and ligand.

Keywords: Antimicrobial activity, Ferrocene, Schiff base, Schiff base metal complex, SOD-like activity.

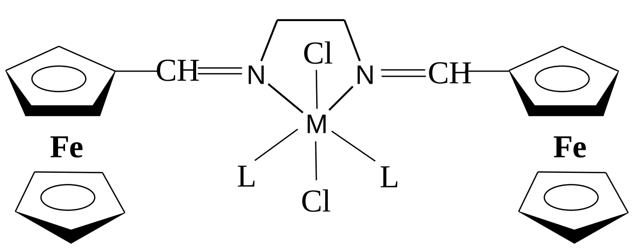
1. INTRODUCTION

The syntheses of the ferrocenyl derivatives and exploration of their properties have become an attractive research area since ferocene was found in 1951.

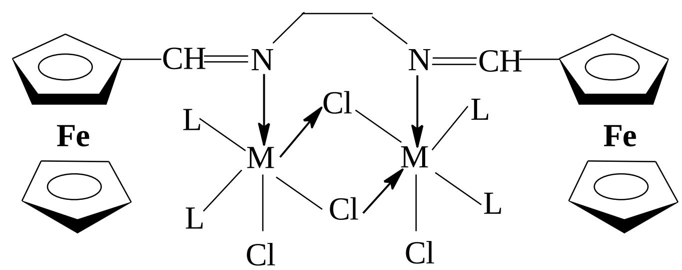
Due to the fascinating properties of the ferrocene moiety as well as the diversity ferrocenyl derivatives have attracted the attention of many researchers in the field of organometallic chemistry for using in various applications such as materials science [1], homogeneous catalysts [2], molecular sensors [3-6], non-linear optics [7-10], liquid crystals [11], and electrochemistry [12-15]. Many ferrocenyl compounds show intresting biological activities such as antibacterial, antimalarial, antifungal, antiviral, cytotoxic, antioxidant and anticancer effects [15-40]. Superoxide anion, H_2O_2, organic peroxide, and hydroxyl radical known as reactive oxygen species (ROS) are produced in metabolism as a response to various stimuli and their formation and destructions are in balance under physiological conditions. Superoxide dismutase enzymes (SODs) serve a vital role in defending aerobic living-organisms from oxidative damage by catalyzing the dismutation of superoxide radical anions into molecular oxygen and hydrogen peroxide so the function of SODs are involved in anticancer and antiaging mechanisms [32, 40].

Especially complexes of ferrocenyl Schiff bases have been studied by several groups [12, 14, 41-52]. The ferrocenyl moiety represents a quie bulky group with unique spatial requirements due to its sandwich shape, may cause electronic effects on the coordination behaviour of the donor centers of these ligands that may influence the in vitro antibacterial, antifungal and cytotoxic properties of organometallic based compounds, thus making them attractive pharmacophore for drug design.

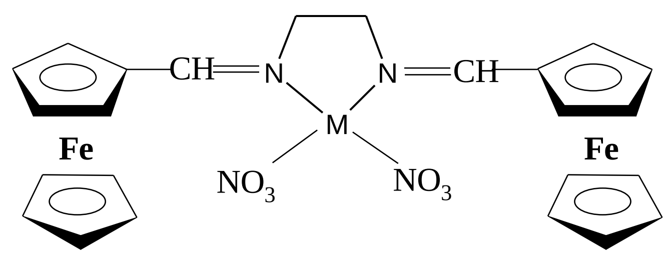
In recent years, we have synthesized and characterized a number of bis ferrocenyl Schiff base (*N,N′*-bis(ferrocenylmethylene)ethylenediamine (FcNN)) and its metal complexes (Figure 1) ($SnCl_4FcNN$ (**1**), $SnCl_2FcNN(H_2O)_2$ (**2**), $Sn_2(CH_3)_4Cl_4FcNN$ (**3**), Ti_2Cl_8FcNN(**4**), $CdFcNN(NO_3)_2$ (**5**), and $CuFcNN(NO_3)_2$ (**6**)) [14]. Following our previous studies, we focused on the investigation on the antimicrobial and SOD-like activities of these complexes.



a) L:(Cl) M= Sn(IV) **(1)**, L:(H_2O) M=Sn(II) **(2).**



b) L: (CH_3), M= Sn(IV) **(3)**, L:(Cl) M=Ti(IV) **(4).**



c) M= Cd(II) **(5)**, Cu(II) **(6).**

Figure 1. Structural formula of the metal complexes.

2. EXPERIMENTAL

2.1. Microbiological methods

2.1.1 Target microorganisms

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Citrobacter koseri* DBCC 01, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium*, DBCC 02, *Bacillus cereus* ATCC 7064 and *Candida albicans* ATCC 10239 were used for the antimicrobial activity as a reference cultures obtained from the Department of Biology, Manisa Celal Bayar University.

2.1.2 Diffusion assay

First *In vitro* antimicrobial studies were carried out by the agar well diffusion method [55] with slight modifications against test microorganisms. All conditions in the implementation of this experiment were made according to Avcı Özbek et al. [22]. In addition, some commercial antibiotics were used as to determine the sensitivity of the strains (Table 1). Experiments were repeated three times, and the results were expressed as average values.

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2.1.3 Determination of minimum inhibition concentration (MIC)

The microtiter broth dilution technique was performed using the CLSI standards [56-58]. Serial dilutions of the compounds and antibiotics were prepared as 2560 – 20 µg/mL and 150 – 0.293 µg/mL, respectively. All other application conditions of the experiment were made according to Avcı Özbek et al. [22]. The average of three values was calculated and that was the MIC for the test compounds and microorganisms.

2.2 Superoxidedismutase (SOD) like activity

The method used to study the SOD-like activity is similar to that described by Beauchampand Fridovich [59], using nitrobluetetrazoliumchloride (NBT) as indicator, and the xanthine–xanthineoxidase system as the superoxide radical generator (in a 10 mM pH 7.8 phosphatebuffer solution). SOD-like mimic activity was determined by using the nitrobluetetrazolium reduction method spectrophotometrically at 560 nm.

The IC_{50} values were determined by regression analysis and interpolation of the % inhibition versus assay concentration curve for at least five experimental points foreach system, with inhibition value sranging from 10 to 70%. In all cases a linearity greater than 0.960 was achieved. Each experiment was performed in triplicate.

3. RESULTS AND DİSCUSSİON

3.1 Antimicrobial activity

The antimicrobial activities of the bis ferrocenyl Schiff base ligand (N,N'-bis(ferrocenylmethylene)ethylenediamine (FcNN)) and its metal complexes ($SnCl_4$FcNN (**1**), $SnCl_2$FcNN$(H_2O)_2$ (**2**), $Sn_2(CH_3)_4Cl_4$FcNN (**3**), Ti_2Cl_8FcNN(**4**), CdFcNN$(NO_3)_2$ (**5**), and CuFcNN$(NO_3)_2$ (**6**)) were determined against 4 Gr(-) and 2 Gr(+) bacteria, as well as 1 fungus. The results of antimicrobial activities are presented in Table 1. The ferrocenyl Schiff base ligand (FcNN) showed activity against *Escherichia coli* and *Bacillus cereus* but no activity against other microorganisms. According to our results, only four compounds showed antimicrobial activity and their effectiveness is in the range of 12-34 mm inhibition zone. Two metal complexes ($Sn_2(CH_3)_4Cl_4$FcNN and (**3**), Ti_2Cl_8FcNN(**4**)) were found to be microbially inactive. The CdFcNN$(NO_3)_2$ (**5**), and CuFcNN$(NO_3)_2$ (**6**) complexes showed the best bacterial inhibition among all the complexes with inhibition zone diameter ranged from 12.0 to 34.0 mm indicating that the coordination of the bis ferrocenyl Schiff base ligand (FcNN) to these metal complexes has enhanced its antimicrobial activity.

Compound CdFcNN$(NO_3)_2$ (**5**) showed high activity against *S. aureus* and *E. aerogenes w*ith 34 mm. However, the compounds differ significantly in their activity against test microorganisms (Table 1, Figure 2). Comparison of antimicrobial activity results of **FcNN, 4, 5** and **6** are given in Figure 3.

Table 1. Antimicrobial activity of synthesized compounds and some antibiotics against test microorganisms.

		Compounds						Antibiotics		
Microorganisms[a]	**FcNN**	**1**	**2**	**3**	**4**	**5**	**6**	**Ampicillin**	**Gentamicin**	**Nystatin**
Escherichia coli	10	12[b]	-	-	10	22	14	6[R]	16	NA
Staphylococcus aureus	-	12	-	-	10	34	26	16	20	NA
Citrobacter koseri	-	-	-	-	10	32	10	26	24	NA
Enterobacter aerogenes	-	16	-	-	14	34	20	6[R]	22	NA
Salmonella typhimurium	-	14	-	-	12	30	26	6[R]	26	NA
Bacillus cereus	8	-	-	-	8	22	12	6[R]	18	NA
Candida albicans	-	-	-	-	-	28	24	NA	NA	24

[a] Bacteria tested in MHA medium, yeast in YPDA.

[b] Inhibition zone diameter in millimeters, not including well and disc diameter (6 mm). Mean values, n = 3; NA - not applicable; R – resistant.

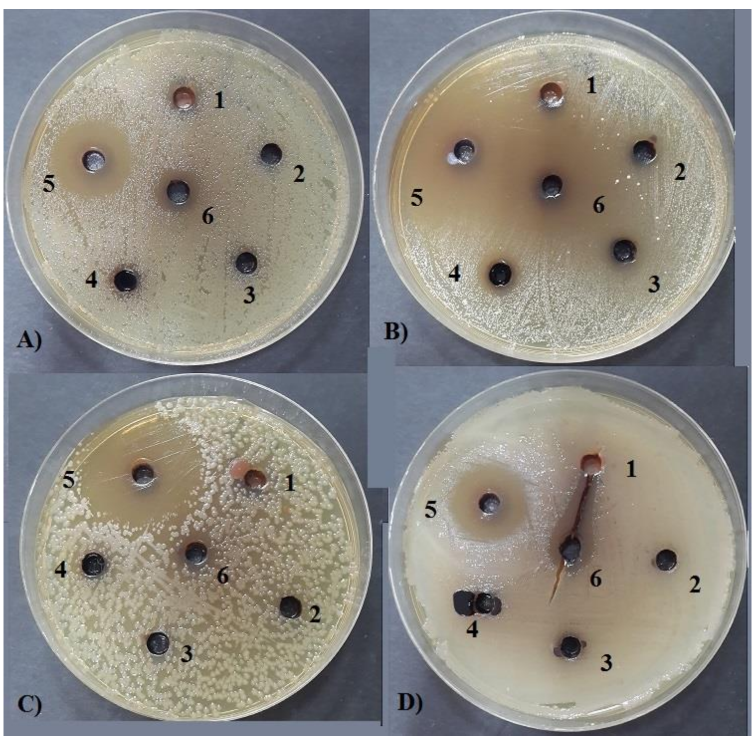

Figure 2. Antibacterial activity of compounds against some microorganisms; A) *E. coli*, B) *S. aureus*, C) *C. koseri* and D) *B. cereus*.

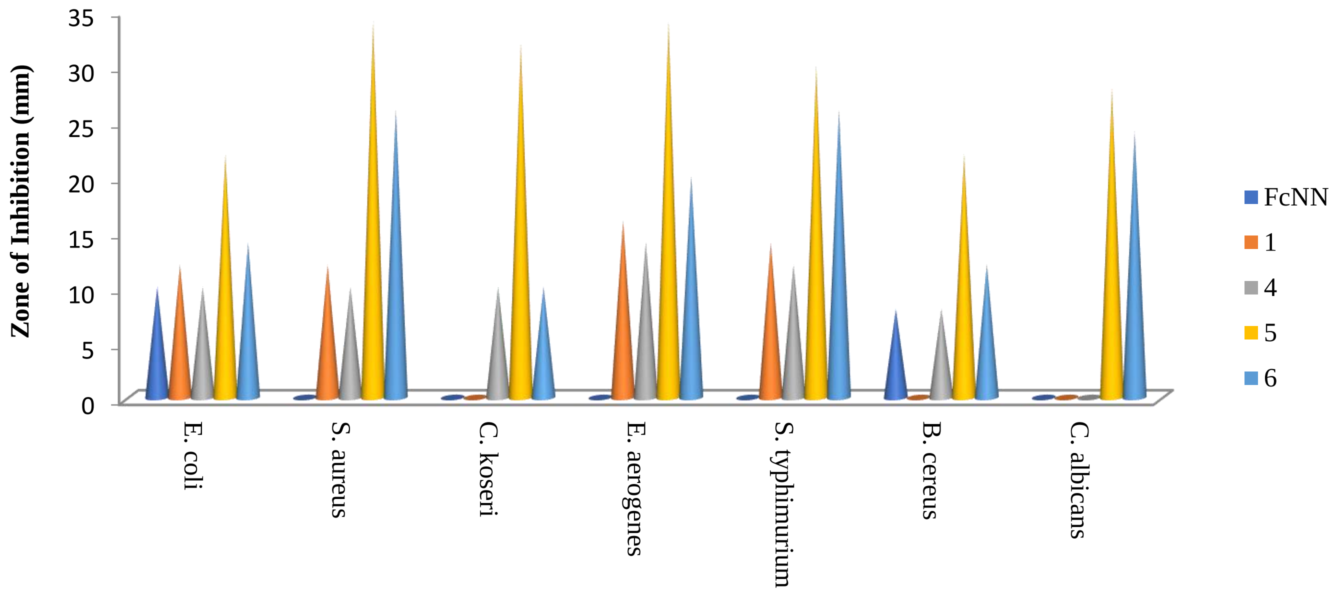

Figure 3. Comparison of antimicrobial activity of **FcNN, 1, 4, 5, 6** against microorganisms.

MIC assays revealed that the tested compounds exhibited variable MICs and selective antimicrobial activity, depending on the microbial strains. Significant antimicrobial effects, expressed as MIC of tested compounds against selected microorganisms are presented in Table 2. MIC values of the compounds ranged from 27 to 533 µg/mL. Compound **(5)** showed very strong activity against *S. aureus* with the best MIC (27 µg/mL). The lowest MIC for *C. albicans*was obtained was 107 µg/mL. The results of the present investigation clearly indicate that the efficiacy of compounds are comparable to positive control antibiotics.

Table 2. MIC values of the compounds and standard antibiotics (µg/mL) against selected microorganisms

Compounds & standard antibiotics	*Microorganisms*[a]						
	E. coli	*S. aureus*	*C. koseri*	*E. aerogenes*	*S. typhimurium*	*B. cereus*	*C. albicans*
(4)	ND	ND	ND	427±185	533±185	ND	NA
(5)	267±92[b]	27±12	107±46	133±46	213±92	160±0	107±46
(6)	320±0	67±23	ND	160±0	213±92	ND	133±46
Ampicillin	20±1.4	9±0	9±1.4	10±1.4	6±1.4	8±1.4	ND
Gentamicin	5±1.4	4±0.7	3±0.7	4±0	2±0.7	2±0.7	ND
Nystatin	NA	NA	NA	NA	NA	NA	0.7±0.2

[a]Bacteria tested in MHB medium, yeast in YPDB.
[b]Data presented as the mean value of three determinations ± standard deviation. ND - not determined, NA – not applicable.

Therefore, it was interesting to note that antibiotic-resistant bacteria showed an increased sensitivity to the compounds **5** and **6**. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of the tested compounds and these might have different modes of action on test organisms.

The literature contains a number of reports on the antimicrobial properties of ferrocenyl Schiff base metal complexes [18, 20, 53, 54]. Abd-Elzaher [18] have investigated the antibacterial activities of ferocenyl Schiff bases (from condensation of acetylferrocene with 2-aminophenol,(LH1) 2-amino-5-picoline(LH2) and 2-amino-5-chlorophenol (LH3) respectively) and its metal complexes. From the results of antimicrobial evaluation, the prepared complexes of $Cu(L1)_2$, $Zn(L1)_2{\cdot}2H_2O$, $Co(L2)_2{\cdot}2H_2O$, $Ni(L2)_2{\cdot}2H_2O$, $Cu(L2)_2$ and $Zn(L2)_2{\cdot}2H_2O$ showed significant antimicrobial activity against *C. Albicans.*

In other study, Liu *et al.*, have synthesized the metal complexes of *S*-benzyl-*N*-(1-ferrocenyl-3-(4-methylbenzene)acrylketone) dithiocarbazate (**HL1**) and *S*-benzyl-*N*-(1-ferrocenyl-3-(4- chlorobenzene)acrylketone)dithiocarbazate (**HL2**) and tested for their antibacterial and antifungal properties. The results revealed that Zn(II) complexes of both the ligands and Cu(II) complex of the **HL2** were shown to have significant activity against all bacterial strains [20]. Mahmoud *et al*., synthesized metal complexes of $[M(L)Cl(H_2O)_3]$ {M = Ni(II), Cu(II)} and $[M(L)Cl(H_2O)_3].nH_2O$ {M = Co(II), n = 1 and M = Zn(II), n = 2} of (*Z*)-(4-(1-((2-carboxycyclohexa-2,4-dien-1-yl)imino)ethyl) [*bis*(η^5cyclopenta-1,3-dien-1yl)] iron for their evaluation as antimicrobial agents against some bacteria and fungi. From the results of the antimicrobial evaluation, the prepared ferrocenyl complexes exhibited the highest antibacterial and antifungal activities [53]. Ferrocenyl Schiff base ligand (1-(1-[2-hydroxyphenyl-2-imino]methyl)-ferrocene) and its metal (Co(II), Ni(II), Cu(II), and Pd(II)) complexes synthesized by Ahmed Mourtada Elseman *et al*. [54] The synthesized compounds were investigated for their antimicrobial activities against three gram-negative bacteria. The Pd(II) complex exhibited the highest potential against *Aspergillus fumigatus* and *Geotrichum candidum*. In present study, compound $CdFcNN(NO_3)_2$ **(5)** showed very strong activity against *S. aureus* with the best MIC (27 µg/mL).

3.2 Superoxidedismutase (SOD)- like activity

Metal complexes have received attention in the development of SOD-like mimic activities for scavenging of the superoxide free radical [60,61]. SOD-like activities of the bis ferrocenyl Schiff base ligand (N,N'-bis(ferrocenylmethylene)ethylenediamine (FcNN)) and its metal complexes ($SnCl_4FcNN$ (**1**), $SnCl_2FcNN(H_2O)_2$ (**2**), $Sn_2(CH_3)_4Cl_4FcNN$ (**3**),

Ti_2Cl_8FcNN(**4**), CdFcNN$(NO_3)_2$ (**5**), and CuFcNN$(NO_3)_2$ (**6**)) were investigated using NBT. These data are shown in Table 3. Except compound $Cu(NO_3)_2$FcNN (**6**), the ferrocenyl Schiff base ligand and its complexes did not show effective catalytic activity against the dismutation of the superoxide anion.

The Cu (II) complex showed an IC_{50} value of 41 mm, while the IC_{50}value was not determined in other synthesized complexes and ligand. The IC_{50} value of $Cu(NO_3)_2$FcNN (**6**) complex has been compared with the IC_{50} values of some Cu complexes that do not contain ferrocenyl-substitued in litareture [61-66]. As listed in Table 3 and Figure 4, the IC_{50} value of $Cu(NO_3)_2$FcNN (**6**) complex is higher than compared with the earlier reported Cu(II) complexes ([Cu(bipy)$_2$(NO$_3$)]ClO$_4$, CuZnSOD and Cu$_2$Zn$_2$SOD) [61-62, 64-66]. Also, the IC_{50} value of complex (**6**) is lower than compared the earlier reported [Cu(L) (HL)]ClO$_4$ and [CuL$_1$L$_2$Cl]Cl complexes [61-62].

Table 3. IC_{50} values of metal complex and references.

S.N.	Metal complexes	IC_{50}value (µM.)	References
1	FcNN	ND	Thiswork
2	$SnCl_4$FcNN (**1**)	ND	Thiswork
3	$SnCl_2$FcNN$(H_2O)_2$ (**2**)	ND	Thiswork
4	$Sn_2(CH_3)_4Cl_4$FcNN (**3**)	ND	Thiswork
5	Ti_2Cl_8FcNN (**4**)	ND	Thiswork
6	CdFcNN$(NO_3)_2$ (**5**)	ND	Thiswork
7	CuFcNN$(NO_3)_2$ (**6**)	41 ± 5.2	Thiswork
8	[Cu(L) (HL)]ClO$_4$	49	Singh *et al.*
9	[Cu(bipy)$_2$(NO$_3$)]ClO$_4$	33	Zhou *et al.*
10	[CuL$_1$L$_2$Cl]Cl	98	Joseph and Rani
11	CuZnSOD	0.0043	Klug *et al.*
12	CuZnSOD	0.04	Siddiqi *et al.*
13	Cu$_2$Zn$_2$SOD	0.01	Guinot *et al.*

ND - not determined.

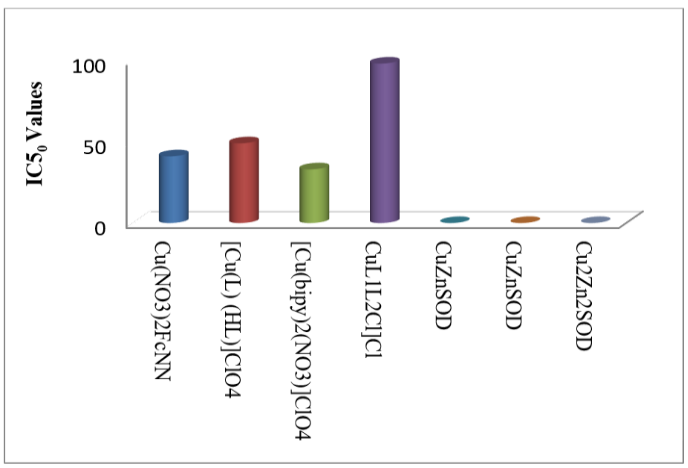

Figure 4. Comprasion of IC_{50} values of (**6**) and references.

CONCLUSION

In this study, antimicrobial activity of the Schiff base ligand (N,N'-bis(ferrocenylmethylene)ethylenediamine (FcNN)) and its metal complexes was investigated by the agar well diffusion method, againts different bacterial species, such as; two Gram-positive, *Staphylococcus aureus* and *Bacillus cereus* and four Gram-negative, *Escherichia coli*, *Citrobacter koseri*, *Entorebacter aeruginosa* and *Salmonella typhimurium* and against fungi *Candida albicans*. The antimicrobial results show that the compound CdFcNN$(NO_3)_2$ **(5)** showed very strong activity against *S. aureus* with the best MIC (27 µg/mL). The compound could be of potential for a novel class of metal-based antibactericidal agents.

Also the SOD-like activity of Schiff bases and its complexes were investigated by NBT and IC_{50} values were evaluated. Except compound $Cu(NO_3)_2$FcNN (**6**), Schiff base ligand and its complexes did not exhibit the SOD activity. SOD-like activity (IC_{50}) of (**6**) was measured to be 41 µM. The IC_{50} value obtained for compound (**6**) may indicate that this copper complex could be a SOD-like mimetic.

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