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Synthesis, Antibacterial and Antifungal Activities of New *Bis*-5(6)-nitrobenzimidazoles

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Seventeen *bis*-5(6)-nitrobenzimidazole compounds and their salt derivatives were synthesized from the reaction of 5(6)-nitrobenzimidazole and appropriate alkyl dihalides and alkyl halides. The synthesized compounds were identified by ^{1}H, ^{13}C NMR, FT-IR spectroscopic techniques and elemental analysis. All compounds studied in this work were screened for their *in vitro* antimicrobial activitiy against the standard strains such as *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* and *Candida tropicalis*. Ten of the compounds were found effective to inhibit the growth of gram positive (*E. faecalis* and *S. aureus*) at MIC values between 50-400 µg/mL. Seven of the compounds exhibit antimicrobial activity against gram negative bactreria (*E. coli* and *P. aeruginosa*) at the concentrations studied (50-400 µg/mL). Nearly all compounds tested in this work showed antifungal activity with a range of the MICs between 50 and 800 µg/mL.

Key Words: 5(6)-Nitrobenzimidazole, *Bis*-benzimidazoles, Antibacterial activitiy, Antifungal activitiy, *in vitro* studies, Synthesis.

INTRODUCTION

Properties of benzimidazole and its derivatives have been studied for over hundred years. Special interest of researchers triggered by the fact that 5,6-dimethylbenzimidazole is a component[1] of naturally occurring vitamin B-12. The benzimidazole ring is an important pharmacophore in modern drug discovery[2]. Indeed, benzimidazole derivatives are interesting heterocycles because they are present in many naturally occurring products and various drugs. A large number of benzimidazole derivatives were shown to exhibit important biological properties, such as antibacterial, antifungal, antihelminthic, antiallergic, antineoplastic, local analgesic, antihistaminic, antileishmanial, vasodilator, hypotensive, spasmoliytic and antiulcer activities[3-14]. In recent years, considerable attention has been made for the synthesis of *bis*-benzimidazole compounds because of their properties in cancer therapy. Various studies with *bis*-benzimidazole derivatives have shown that binding affinity to calf thymus DNA

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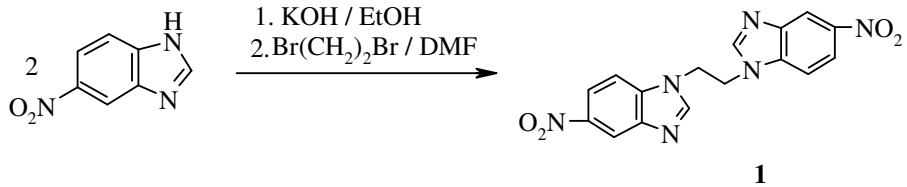
correlates positively *in vitro* topoisomerase inhibitory potency cytotoxicity[15-17]. *Bis*-benzimidazoles have also potent activity against a number of microorganisms including those that lead to AID-related infections[18]. *Bis*-benzimidazole compounds also showed versatile pharmacological activities such as antifungal, antihelmintic, antiviral, anticoagulant antiinflamatory activities[19-21]. We have also reported the synthesis of some *bis*-benzimidazole compounds and their selective *in vitro* antibacterial, antifungal activity against standart strains, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* and *Candida tropicalis*[8,9].

Therefore, it seemed of interest to study derivatives of new *bis*-5(6)-nitrobenzimidazoles bearing various alkyl or substituted alkyl groups in position 1.1' and 3.3' and explore their antibacterial and antifungal activities against standard strains.

EXPERIMENTAL

Bis-5(6)-nitrobenzimidazoles used in this work were prepared from 5(6)-nitrobenzimidazole and appropriate alkyl dihalides in good to high yields according to the procedure indicated in **Scheme-I** and as described in the experimental section. 5(6)-Nitrobenzimidazole was synthesized from the reaction of 1,2-diamino-4-nitrobenzimidazole and formic acid similar to the benzimidazole synthesis[22].

General: All chemicals using in this study [except 5(6)-nitrobenzimidazole] were purchased from Aldrich Chemical Co. (Dorest, UK) or Merck (Darmstadt, Germany). ^{1}H and ^{13}C NMR spectra (chemical shift in δ, ppm) were recorded using Bruker DPX-300 high performance digital FT-NMR (Bruker WM360, Bruker Instruments, Inc., Billercia, USA) spectrometers. Infrared spectra were recorded as KBr pellets in the range 4000-400 cm^{-1} on an ATI Unicam, Mattson 1000 spectrophotometer (Unicam Ltd., Cambridge, UK). Elemantal analysis were performed by the elemental analysis laboratory of The scientific and techonological research centre of Inonu University at Malatya (Turkey). Melting points were recorded using an electrothermal melting point apparatus, electrothermal 9200 (electrothermal Engineering Ltd., Essex , UK) and uncorrected.



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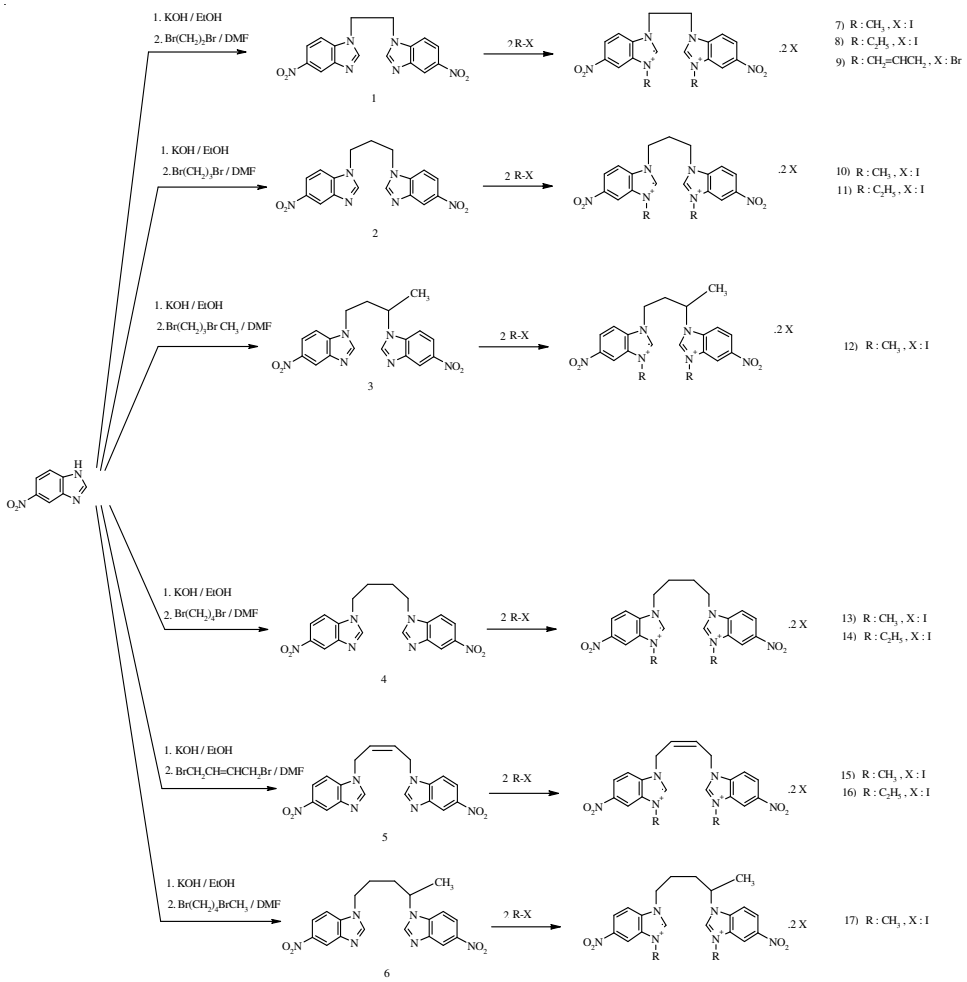
Preparation of 1,1'-ethylene-*bis*-5(6)-nitrobenzimidazole (**1**)

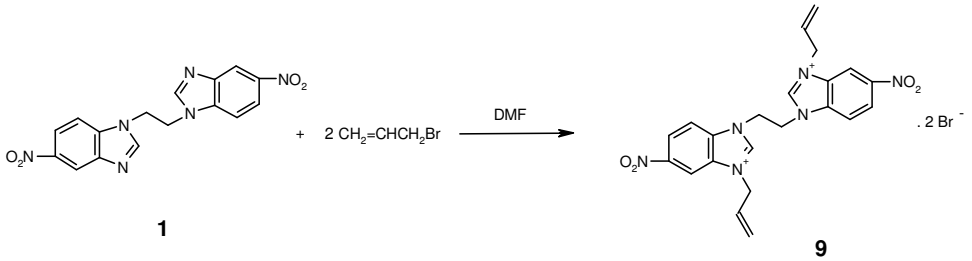
A solution of 5(6)-nitrobenzimidazole (4.76 g; 29.17 mmol) and KOH (1.64 g, 29.23 mmol) in EtOH (30 mL) was heated under reflux for 48 h. The mixture was cooled, after which the solvent was removed from the filtrate *in vacuo*. The formed

5(6)-nitrobenzimidazole potassium salt and 1,2-ethylene dibromide (2.90g, 15.43 mmol) was heated in dimethylformamide (10 mL) under reflux for 3 h. The mixture was then cooled and all volatiles were removed *in vacuo* and water was added to precipitate 1,1'-ethylene-*bis*-5-nitrobenzimidazole. The precipitate was filtered and dried *in vacuo*. The *bis*-5-nitrobenzimidazole was crystallized from a DMF-EtOH (2:1) mixture (yield 3.19 g, 62 %; m.p. 247-248 ºC). Analysis calculated for $C_{16}H_{12}N_6O_4$: C 54.54, H 3.40, N 23.86 %; found: C 54.32, H 3.18, N 23.18 %.



Scheme-I. Synthesis pathways of the new 5(6)-nitrobisbenzimidazole derivatives

Similarly, compounds **2**, **3**, **4**, **5** and **6** were synthesized from appropriate benzimidazole and alkyl dihalides. The related data of the synthesised compounds are given in Tables 1-3.



1 **9**

Preparation of 3,3'-diallyl-1,1'-ethylene-*bis*-5(6)-nitrobenzimidazolium dibromide (**9**)

TABLE-1
COLOUR, MELTING POINTS, YIELDS AND ANALYTICAL DATA OF NITROBENZIMIDAZOLE DERIVATIVES

Compound No.	Colour	m.p. (ºC)	Yields (%)	Elemental analysis (%): Calcd. (Found)		
				C	H	N
1	Yellow	248	62	54.54 (54.32)	3.40 (3.18)	23.86 (23.18)
2	Yellow	223	82	55.73 (55.18)	3.82 (3.62)	22.95 (22.12)
3	Yellow	176	55	56.84 (56.05)	4.21 (4.11)	22.10 (21.80)
4	Yellow	247	85	56.84 (56.72)	4.21 (4.18)	22.10 (22.02)
5	Yellow	> 250	78	57.14 (57.04)	3.70 (3.53)	22.22 (22.08)
6	Yellow	182	67	57.86 (57.35)	4.56 (4.16)	21.31 (21.05)
7	Yellow	245	78	33.96 (33.18)	2.83 (2.53)	13.20 (13.02)
8	Yellow	238	82	36.15 (36.02)	3.31 (3.24)	12.65 (12.32)
9	Brown	222	76	44.44 (43.91)	3.70 (3.43)	14.14 (13.37)
10	Yellow	225	71	35.08 (35.02)	3.07 (3.02)	12.92 (12.28)
11	Yellow	146	74	37.17 (37.00)	3.54 (3.41)	12.39 (12.26)
12	Yellow	192	72	36.15 (36.04)	3.31 (3.12)	12.65 (12.07)
13	Yellow	227	73	36.15 (36.10)	3.31 (3.15)	12.65 (12.42)
14	Red	143	69	38.16 (38.01)	3.75 (3.61)	12.14 (12.08)
15	Yellow	218	82	36.26 (36.12)	3.02 (3.01)	12.69 (12.29)
16	Red	175	71	38.27 (38.03)	3.47 (3.28)	12.17 (12.05)
17	Yellow	191	68	37.17 (37.06)	3.54 (3.44)	12.39 (12.03)

A mixture of 1,1'-ethylene-*bis*-5(6)-nitrobenzimidazole (0.85 g, 2.41 mmol) and allyl bromide (0.70 g, 5.78 mmol) in dimethylformamide (DMF, 5 mL) was heated under reflux for 5 h. The mixture was then cooled and the volatiles were removed *in vacuo*. The residue was crystallized from a DMF/EtOH (2:1) mixture (yield: 1.09 g, 76 %; m.p. 221-222 ºC). Analysis, calculated for $C_{22}H_{22}N_6OBr_2$: C 44.44, H 3.70, N 14.14 %; found: C 43.91, H 3.43, N 13.37 %.

Similarly, compounds **7**, **8**, **10**, **11**, **12**, **13**, **14**, **15** and **16** were synthesised from appropriate benzimidazole and alkyl dihalides. The related data of the synthesized compounds are given in Tables 1-3.

Biological activity

Methods of antimicrobial testing: Antimicrobial activities of the compounds were determined by using agar dilution procedure outlined by the National Committee

for Clinical Laboratory standards[23,24]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains such as *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* and *Candida tropicalis* obtained from the Department of Microbiology, Faculty of Medicine, Ege University, Turkey. The stock solutions of the compounds were prepared in dimethyl sulfoxide which had no effect on the microorganisms in the concentrations studied. All of the dilutions were done with distilled water. The concentrations of the tested compounds were 800, 400, 200, 100, 50 and 25 μg/mL. Ampicilin and fluconazole from FAKO (Istanbul, Turkey) were used as a reference compound for the experimental conditions. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFU s/mL) was spread over the surface of agar plates. All the inoculated plates were incubated at 35 °C and results were evaluated after 16-20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC).

TABLE-2
^{1}H NMR SPECTROSCOPIC DATA, WITH ASSIGNMENTS
OF NITROBENZIMIDAZOLE DERIVATIVES

Com. No.	2-CH	Bridge (-CH_2CH_2-)	R	Aromatics
1	8.94(2H,s)	4.90(4H,t)	–	8.25(6H,m)
2	8.68(2H,s)	2.50(2H,m), 4.50(4H, t)	–	8.28(6H,m)
3	8.61(2H,s)	1.70(3H,d), 2.40(2H, m), 4.45(2H, t), 4.90(1H, m)	–	8.11(6H,m)
4	8.80(2H,s)	1.80(4H,s), 4.40(4H, d)	–	8.10(6H,m)
5	8.60(2H,s)	5.05(4H,d), 5.99(2H, m)	–	8.13(6H,m)
6	8.70(2H,s)	1.60(3H,d), 1.90(4H, m), 4.40(2H, t), 4.85(1H, m)	–	8.30(6H,m)
7	9.81(2H,s)	5.21(6H,t)	4.14(6H,d)	8.55(6H,m)
8	9.92(2H,s)	5.14(6H,t)	1.44(6H,t), 4.59(4H, q)	8.54(6H,m)
9	10.12(2H,s)	5.22(4H,t)	5.42(4H,d), 5.48(4H, q), 6.03(2H,m)	8.52(6H,m)
10	10.10(2H,s)	2.56(2H,m), 4.80(4H, t)	4.18(6H,d)	8.53(6H,m)
11	10.17(2H,s)	2.70(2H,m), 4.80(4H, t)	1.57(6H,t), 4.60(4H, q)	8.54(6H,m)
12	10.04(2H,s)	1.72(3H,d), 2.80(2H, m), 4.15(2H, t), 4.70(1H, m)	4.19(6H,d)	8.40(6H,m)
13	10.03(2H,s)	2.10(4H,s), 4.70(4H, d)	4.20(6H,d)	8.51(6H,m)
14	10.12(2H,s)	2.09(4H,s), 4.60(4H, d)	1.55(6H,t), 4.58(4H, q)	8.50(6H,m)
15	9.98(2H,s)	5.36(4H,d), 6.27(2H, m)	4.19(6H,d)	8.52(6H,m)
16	10.13(2H,s)	5.37(4H,d), 6.32(2H, m)	1.55(6H,t), 4.62(4H, q)	8.40(6H,m)
17	9.98(2H,s)	1.63(3H,d), 2.06(4H, m), 4.61(2H, t), 5.10(1H, m)	4.13(6H,d)	8.50(6H,m)

Chemical shifts (δ) relative to TMS. Abbreviations, s = singlet, d = doublet, t = triplet, q = quarted, m = multiplet, spectra for compounds **1**-**17** recorded in DMSO-d_6.

TABLE-3
^{13}C NMR SPECTROSCOPIC DATA WITH ASSIGNMENTS
OF NITROBENZIMIDAZOLE DERIVATIVES

Com. No.	^{13}C NMR spectroscopic data
1	44.89, 107.88, 111.11, 116.15, 117.72, 118.45, 120.21, 133.42, 138.53, 142.78, 143.27, 148.00, 148.46, 149.60
2	29.95, 42.87, 45.42, 108.33, 111.65, 115.42, 117.83, 119.96, 133.43, 138.54, 142.56, 143.22, 147.41, 148.41, 149.35
3	17.81, 46.78, 107.95, 111.38, 116.03, 117.45, 118.40, 120.08, 125.91, 130.43, 133.42, 138.02, 143.10, 148.12, 149.34
4	27.08, 44.54, 108.23, 111.58, 116.14, 117.64, 118.38, 120.21, 133.51, 142.92, 143.13, 145.12, 148.08, 149.22
5	46.13, 108.26, 111.76, 116.18, 117.68, 118.21, 120.30, 128.91, 129.41, 133.38, 138.47, 143.09, 148.38, 149.61
6	20.88, 26.68, 33.08, 44.38, 52.93, 108.09, 111.44, 116.20, 117.65, 118.20, 120.26, 133.13, 138.12, 143.02, 148.26, 149.70
7	31.25, 34.28, 34.69, 36.28, 111.52, 115.28, 121.97, 122.33, 131.11, 132.23, 135.03, 136.05, 146.27, 147.98, 162.79
8	14.44, 14.62, 34.89, 43.37, 46.57, 111.43, 115.25, 115.77, 122.36, 131.43, 135.24, 146.27, 147.16
9	34.71, 46.52, 49.92, 111.58, 115.52, 121.41, 122.42, 130.77, 131.35, 135.22, 146.31, 147.77
10	28.44, 31.27, 34.66, 44.72, 111.23, 111.55, 115.39, 122.09, 131.30, 132.29, 135.20, 136.10, 146.14, 147.53
11	14.45, 28.36, 31.26, 34.89, 36.30, 43.40, 44.82, 111.31, 115.45, 122.15, 131.44, 135.29, 146.22, 146.69, 162.77
12	24.20, 34.33, 44.29, 111.35, 115.33, 121.96, 131.97, 135.86, 146.03, 146.72, 147.39, 147.91
13	25.92, 31.26, 34.57, 36.29, 47.06, 54.83, 111.28, 115.40, 122.12, 131.23, 132.22, 135.15, 136.05, 146.10, 147.47, 162.77
14	14.46, 25.90, 31.25, 36.28, 43.35, 47.17, 111.37, 115.46, 122.11, 131.37, 135.24, 146.18, 146.64, 146.92
15	34.33, 44.70, 48.41, 54.83, 111.20, 115.33, 121.84, 128.17, 130.95, 132.34, 134.81, 135.84, 145.98, 147.58
16	14.38, 34.91, 43.28, 48.59, 111.37, 115.44, 121.98, 128.56, 129.44, 131.18, 135.03, 146.09, 146.40, 146.78
17	20.58, 25.34, 32.35, 34.47, 47.06, 54.94, 111.24, 115.30, 121.99, 130.92, 132.19, 135.03, 136.00, 146.09, 147.43

Chemical Shifts (δ, ppm) relative to $Si(CH_3)_4$= 0; spectra for compounds **1**-**17** recorded in DMSO-d_6 .

RESULTS AND DISCUSSION

The structure of all the synthesized compounds were identified by ^{1}H, ^{13}C NMR, FT-IR and micro analysis. Colour, yields, melting points and analytical data of the newly synthesized compounds are given in Table-1. ^{1}H NMR spectral data are given in Table-2 and ^{13}C NMR spectral data in Table-3. Benzimidazole itself and the 5- or 6-substituted derivatives can show a tautomerism of the imidazole ring[25,26]. Hence, the starting compounds and the products names are given as included tautomerism situation.

The antimicrobial and antifungal activity results (MIC) are given in Tables-4 and 5, respectively.

TABLE-4
MINIMUM ANTIBACTERIAL INHIBITORY CONCENTRATIONS
(μg/mL) OF NITROBENZIMIDAZOLE DERIVATIVES

Compound No.	Tested microorganisms[a]			
	A	B	C	D
Ampicillin	3.12	0.39	0.78	> 75
1	> 800	> 800	> 800	> 800
2	200	100	400	400
3	> 800	> 800	> 800	> 800
4	200	200	400	400
5	200	200	> 800	> 800
6	50	50	100	200
7	> 800	> 800	> 800	> 800
8	> 800	> 800	> 800	> 800
9	> 800	800	800	> 800
10	200	200	400	400
11	200	100	400	400
12	> 800	> 800	> 800	> 800
13	800	800	800	800
14	400	200	800	800
15	100	50	400	800
16	100	100	> 800	> 800
17	400	400	400	400

[a]: A = *E. faecalis* B = *S. Aureus*, C = *E. Coli*, D = *P. aeruginosa.*

TABLE-5
MINIMUM ANTIFUNGAL INHIBITORY CONCENTRATIONS
(μg/mL) OF NITROBENZIMIDAZOLE DERIVATIVES

Compound No.	Tested organism	
	C. albicans	*C. tropicals*
Fluconazole	1.25	1.25
1	800	800
2	200	100
3	800	800
4	200	200
5	400	200
6	50	50
7	800	800
8	800	800
9	800	800
10	400	200
11	200	100
12	> 800	> 800
13	800	800
14	400	200
15	100	50
16	200	100
17	200	200

In this work, 17 new *bis*-5(6)-nitrobenzimidazole derivatives were synthesized and tested against standard strains of gram-positive (*E. faecalis* and *S. aureus*) and gram-negative (*E. Coli* and *P. Aeruginosa*) bacteria and yeasts (*Candida albicans* and *Candida tropicalis*). As can be seen in Table-4, the compounds **2**, **4**, **5**, **6**, **10**, **11**, **14**, **15**, **16** and **17** were found effective in inhibiting the growth of gram-positive bacteria with MICs values between 50-400 µg/mL. The compounds **2**, **4**, **6**, **10**, **11**, **15** and **17** exhibit considerable antimicrobial activity against gram-negative bacteria with MICs values between 100-400 µg/mL. On the other hand, the compounds **2**, **4**, **5**, **6**, **10**, **11**, **14**, **15**, **16** and **17** were found effective against *C. tropicalis* with MIC values of 50-200 µg/mL. These compounds also showed antifungal activity against *C. albicans* with a range of MICs between 50-400 µg/mL. Among the tested compounds, **6** and **15** were the most effective compounds with MICs 50 µg/mL against *C. tropicalis*. The compound **6** also found the most effective against *S. aureus* and *E. faecalis* with MIC's 50 µg/mL. From the data, it is suggested that the double bond in the *bis*-benzimidazole bridge may play a crucial role in the antimicrobial activity against gram-positive bacteria and yeasts. On the other hand, it can be concluded that the antibacterial activity of the compounds is related to cell wall structure of the bacteria.

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