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Toxicological effects, biological aspects and spectral characterization of organoboron(III) complexes of sulfonamide-imines

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The toxicological effects, biological aspects and spectral characterization of organoboron(III) complexes of sulfonamide-imines derived by the condensation of salicylaldehyde with different sulfa-drugs are described. The benzene-soluble, high-molecular-weight complexes have been characterized using a wide range of analytical and spectroscopic techniques, viz. UV, IR, ^{1}H and ^{11}B NMR. On the basis of these studies, it is inferred that the imines derived from sulfa drugs and salicylaldehyde behave as dibasic tridentate ligands and thus provide a tetrahedral environment around the boron atom. Finally, all these complexes have been screened for their antimicrobial activity against a variety of fungal and bacterial strains and their toxicological effects on male albino rats examined at the dosages employed. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: boron(III) complexes; antifertility; antibacterial; antifungal; toxicity; sulfonamide-imines

INTRODUCTION

Sulfonamides were the first drugs found to act selectively and could be used systematically as preventive and therapeutic agents against various diseases.[1–4] Various sulfonamide-imines have been synthesized and tested for their potential pharmacological applications as antacids,[5] and for their antidiarrhoeal and antiulcer activities.[6] Twelve new[7] *N*-arylsulfonamido-2-chloro-8-methyl-quinoline-3-yl-azomethines were recently prepared and screened for their *in vitro* antibacterial activity towards Gram positive and Gram negative bacterial strains and antifungal activity towards *Aspergillus niger* at a concentration of 40 mg/ml. Silver(I) and zinc(II) complexes of aminobenzolamide (5-sulfanilyalmido-1,3,4-thiadiazole-2-sulfonamide) derivatives[8] proved to be effective antifungal agents against several *Aspergillus* and *Candida* spp., with minimum inhibitory concentrations (MICs) in the range 1.8–5.0 μg ml^{-1}. The probable mechanism of antifungal action of these complexes seems to be due to inhibition of phosphomannose isomerase, a key enzyme in the biosynthesis of yeast cell walls. The antifertility effects of some sulfonamides and related compounds and their accumulation in the epididymis of male rats have also been reported recently.[9]

In this paper, we have considered the chelating behaviour of sulfonamide-imines and the characterization of their boron(III) complexes. Ligands, along with their chelates, have been tested *in vitro* against a number of fungal and bacterial strains and *in vivo* on male albino rats by performing serum analyses, blood analyses and fertility tests. Only those compounds showing good antimicrobial activities have been selected for oral administration.

EXPERIMENTAL

All the chemicals used were dried and distilled before use.

Preparation of the ligands

For the preparation of sulfonamide-imines, sulfa-drugs, viz. sulfathiazole, sulfaphenazole, sulfadiazine, sulfamerazine and sulfisoxazole were condensed with an equimolar amount of salicylaldehyde in ethanol. The reaction mixture was refluxed for about 4 h on a water bath and then allowed to cool to room temperature. The solid product obtained was filtered, recrystallized from the same solvent and dried *in vacuo*. The imines so obtained were analysed before use:

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1. Salicylaldimine sulfathiazole (L_1H_2), $C_{16}H_{13}N_3S_2O_3$, yellow solid, m.p. 222 °C.
2. Salicylaldimine sulfaphenazole (L_2H_2), $C_{22}H_{18}N_4SO_3$, orange solid, m.p. 183 °C.
3. Salicylaldimine sulfadiazine (L_3H_2), $C_{17}H_{14}N_4SO_3$, yellow solid, m.p. 249 °C.
4. Salicylaldimine sulfamerazine (L_4H_2), $C_{18}H_{16}N_4SO_3$, yellow solid, m.p. 219 °C.
5. Salicylaldimine sulfisoxazole (L_5H_2), $C_{16}H_{17}N_4SO_4$, yellow solid m.p. 160 °C.

Synthesis of the complexes

A calculated amount of phenyldihydroxyborane was taken in dry benzene in a 100 ml round-bottomed flask and an equimolar amount of the imine was added to it. The contents were refluxed for several hours and the progress of the reaction was ascertained by noting the liberated water azeotropically. The excess of the solvent was first distilled off and then removed using a vacuum pump. The resulting product was repeatedly washed with dry cyclohexane and finally dried *in vacuo* for 3–4 h. The details of these reactions and the analyses of the resulting boron derivatives are given in Table 1.

Analytical methods and physical measurements

Carbon and hydrogen analyses were performed at the RSIC Chennai. Nitrogen and sulfur were estimated by Kjeldahl's and Messenger's methods respectively. Boron was estimated as boric acid formed by refluxing a weighed amount of the complex in methanol containing a few drops of sulfuric acid and mannitol and then collecting the distillate in distilled water. The boric acid so formed is titrated against sodium hydroxide using phenolphthalein as an indicator. Molecular weights were determined by the Rast camphor method. IR spectra were recorded as KBr discs on a Perkin–Elmer 577 grating spectrophotometer in the range 4000–200 cm^{-1}. ^1H NMR and ^{11}B NMR spectra were obtained using a JEOL FX90Q spectrometer. Tetramethylsilane (TMS) was used as the internal reference for ^1H NMR spectra and $BF_3 \cdot Et_2O$ was used as the external reference for ^{11}B NMR spectra.

Antimicrobial activity

All the organic moieties and their corresponding boron complexes were tested for *in vitro* growth inhibition activity against Gram negative and Gram positive bacteria, viz. *Escherichia coli* and *Staphylococcus aureus*, and against phytopathogenic fungi, viz. *Macrophomina phaseolina* and *Fusarium oxysporum.* Muller Hinton broth (beef extract, peptone, starch and agar–agar) and potato–dextrose agar broth (infusion of boiled potatoes, dextrose, agar–agar) were employed as culture media for the bacteria and fungi respectively. The stock solutions of the compounds (1 mg l^{-1}) were prepared by dissolving in MeOH and testing was carried out using a twofold serial dilution technique.[10] In this technique, 1.8 ml of inoculated broth (test organisms maintained on 100 ml broths by incubating at 28 ± 1 °C for 24 h) was taken in a test tube and 0.2 ml of the stock solution of the test compound was added to it from the first dilution. The process was repeated to give a set of eight dilutions containing test compound concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg l^{-1}. A set of tubes containing the inoculated broths with an equivalent quantity of MeOH was kept as a control. Each test was replicated twice. The tubes were then placed for incubation. The incubation period for the bacteria and fungi was 48 h at 28 °C and 72 h at 37 °C respectively. The lowest concentration of the compound that resulted in the complete inhibition of the visible mycelium growth after incubation was taken as the MIC value. The conventional bactericide *Streptomycin* and the fungicide 2-(methoxy carbomyl) benzimidazole (*Bavistin*) were used as standards for comparing the activity of the compounds.

Toxicological effects

Fertile Sprague Dawley male albino rats (*Rattus norvegicus*) that were 6–8 weeks old and had an average body weight between 150 and 200 g were divided into three groups of five animals each to test the different compounds. They

Table 1. Physical properties and analytical data of organoboron(III) complexes of ONN donor ligands derived from sulfa-drugs

Reactant (g [mmol])		Product		Elemental analysis, found (calc.) (%)					
$PhB(OH)_2$	Ligand	(Colour and state)	M.p. (°C)	C	H	N	S	B	Mol. wt.
0.75 [6.15]	L_1H_2	$PhB(L_1)$	164	59.25	3.48	9.32	14.28	2.29	421
	2.21 [6.15]	Mustard yellow solid		(59.34)	(3.62)	(9.44)	(14.40)	(2.43)	(445.33)
0.79 [6.48]	L_2H_2	$PhB(L_2)$	138	62.58	3.85	10.32	5.85	2.94	509
	2.92 [6.48]	Orangish yellow solid		(62.69)	(3.95)	(10.44)	(5.98)	(2.02)	(536.44)
0.82 [6.73]	L_3H_2	$PhB(L_3)$	182	62.59	3.80	12.65	7.39	2.33	417
	2.38 [6.73]	Reddish yellow solid		(62.74)	(3.89)	(12.72)	(7.28)	(2.46)	(440.29)
0.86 [7.05]	L_4H_2	$PhB(L_4)$	110	63.58	4.13	12.20	6.97	2.27	426
	2.60 [7.05]	Mustard yellow solid		(63.45)	(4.22)	(12.33)	(7.06)	(2.38)	(454.31)
0.88 [7.22]	L_5H_2	$PhB(L_5)$	130	60.85	4.54	9.86	7.28	2.39	402
	2.51 [7.22]	Light yellow solid		(60.99)	(4.65)	(9.70)	(7.40)	(2.50)	(433.29)

were housed in plastic cages at room temperature (21 ± 1 °C) with a photoperiod of 12 h light/12 h dark and provided with standard laboratory chow (Ashirwad Food Industries Ltd, Chandigarh, India) and tap water *ad libitum*. The rats were weighed weekly to check for changes in the body weight. Group I was used as a control group and received 0.2 ml olive oil/day/animal. Groups II and III received 100 mg and 125 mg respectively (both per kilogram body weight per day) of ligand (L_1H_2) and compound PhB (L_1) mixed in olive oil (see Table 4). These were administered orally by pearl point needle. The animals were weighed and autopsied on day 31 under light ether anaesthesia and the blood from the heart was collected in pre-heparinized tubes. Serum was then obtained by centrifugation at 3000 rpm and stored at −20 °C. Biochemical estimations of serum were done colorimetrically at wavelengths of 540 and 620 nm. Haematological parameters were estimated in the collected blood; the antifertility was checked by counting the motile and immotile sperm in the cauda epididymis and the sperm density was determined in the cauda epididymis and testes using a haemocytometer.

RESULTS AND DISCUSSION

Synthesis and spectral aspects

Reactions of phenyldihydroxyborane with dibasic tridentate imines derived from sulfa-drugs proceed with the liberation of water, which is removed azeotropically in the form of the binary azeotrope of benzene and water. All the newly synthesized compounds are coloured solids and are found to be soluble in common organic solvents like methanol and chloroform. The complexes are monomeric in nature, as indicated by their molecular weight determinations. The low molar conductance values (7–15 Ω^{-1} cm^2 mol^{-1}) reveal the non-electrolytic nature of all the complexes.

The IR spectra of the ligands exhibit two frequencies of medium intensity at 3140 and 3160 cm^{-1} and a band of varying intensity at 3090 ± 10 cm^{-1} that may be assigned to the hydrogen-bonded OH group ν(NH) vibrations of the SO_2–NH group. The possible hydrogen bonding will give resonance structures I and II to the ligand (Fig. 1).

However, these bands disappear from the spectra of the boron complexes, indicating the possible loss of the proton on complexation and subsequent formation of B–O and B–N bonds.

A strong absorption band at 1100 cm^{-1} in the ligand is assigned to C–O stretching vibration of the hydrogen-bonded ring system which shifts to a lower frequency range 1095–1088 cm^{-1} in the boron complexes. This shift is probably due to the increased mass of the boron linked to oxygen, as well as to the possible weakening of the C–O linkage. The characteristic azomethine (>C═N) stretching vibration bands appear in the region 1620–1600 cm^{-1}, and these shifted towards a higher frequency in the spectra of the boron complexes, suggesting the coordination of the azomethine nitrogen.

Besides these, there are absorption bands[11] near 1605, 1575, 1480 and 1430 cm^{-1} for six-membered heterocyclic aromatic rings and three bands near 1590, 1490 and 1400 cm^{-1} for five-membered heterocyclic aromatic groups, which remain unaffected in the complexes. There are also strong, broad

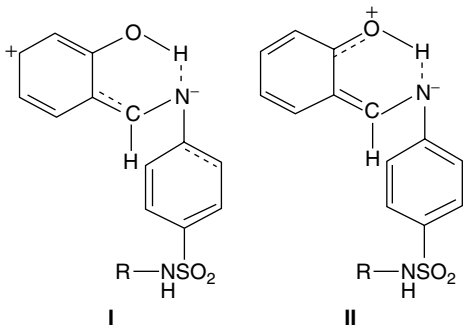

Figure 1. Resonating structures of ligands.

Table 2. ^{1}H NMR spectral data (δ, ppm) of ligands and their organoboron(III) complexes[a]

Compound	OH (s)	SO_2NH (s)	Ar–H (m)	H–C═N (s)	^{11}B NMR (s)
L_1H_2	13.08	10.20	7.55–6.76	8.40	—
PhB(L_1)	—	—	7.63–6.88	8.56	3.22
L_2H_2	12.48	10.15	7.48–6.69	8.32	—
PhB(L_2)	—	—	7.60–6.75	8.48	9.36
L_3H_2	12.50	10.26	7.90–6.50	8.80	—
PhB(L_3)	—	—	8.38–6.84	8.86	5.64
L_4H_2	12.86	10.23	7.45–6.28	8.76	—
PhB(L_4)	—	—	7.62–6.53	8.92	9.44
L_5H_2	12.62	10.12	7.75–7.08	8.44	—
PhB(L_5)	—	—	7.88–7.14	8.51	6.18

[a] s = singlet, m = multiplet.

bands[12] between 1380 and 1300 cm^{-1} and between 1150 and 1050 cm^{-1} due to symmetric and asymmetric vibrations of the $-SO_2-$ group, which either remain unaffected or observed at slightly higher frequencies, signifying the absence of coordination through the $-SO_2-$ group.

The appearance of new bands of medium to strong intensity in the spectra of boron complexes in the regions 1550–1525 cm^{-1} and 1360–1320 cm^{-1} may be assigned to ν(B←N) and ν(B–O) modes respectively. A strong absorption band observed in the spectra of boron complexes in the region 1285–1250 cm^{-1} can be assigned to the ν(Ph–B) grouping.

In the ^{1}H NMR spectra of the ligands, a broad singlet due to the OH proton is observed at *ca* 12.48–13.08 ppm. Another broad singlet observed at *ca* 10.12–10.26 ppm can be assigned to the SO_2NH proton. Neither of these signals appear in the spectra of the boron complexes, which suggests the deprotonation of the –OH and –NH groups on complexation and subsequent coordination of oxygen, as well as nitrogen, to the boron atom.

The aromatic proton signals, which appear in the form of a complex multiplet in the spectra of imines in the region δ 7.90–6.28 ppm, undergo deshielding on chelation.

A signal due to the H–C═N proton appears in the region δ 8.32–8.80 ppm in the spectra of all the ligands. It suffers a downfield shift, indicating deshielding, possibly due to the donation of the lone pair of electrons by the azomethine nitrogen to the boron atom and formation of an N → B dative bond.

The ^{11}B NMR spectral data of all the complexes have also been recorded using $BF_3 \cdot Et_2O$ as the external reference. The chemical shift values obtained for all the complexes are in the range δ 3.22–8.44 ppm, and these correspond to the reported values for tetracoordinated boron signals. The ^{1}H and ^{11}B NMR spectral data of the ligands and their complexes are given in Table 2.

On the basis of the foregoing studies, it can be inferred that the imines derived from sulfa-drugs and salicylaldehyde behave as dibasic tridentate ligands and thus provide a tetrahedral environment around the boron atom, as depicted in Fig. 2.

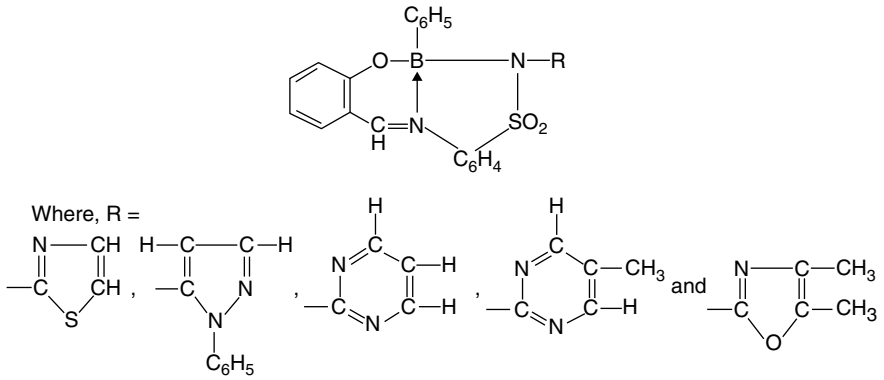

Figure 2.

Table 3. Antimicrobial data of ONN donor ligands derived from sulfa-drugs and their boron(III) derivatives

Compound	Minimum inhibitory concentration (mg L^{-1})			
	E. coli (−)	S. aureus (+)	M. phaseolina	F. oxysporum
L_1H_2	50	6.25	50	12.5
$PhB(L_1)$	25	1.56	12.5	12.5
L_2H_2	100	50	>100	50
$PhB(L_2)$	50	25	100	25
L_3H_2	25	25	100	50
$PhB(L_3)$	6.25	12.25	50	25
L_4H_2	50	50	>100	100
$PhB(L_4)$	12.5	25	50	50
L_5H_2	>100	100	>100	>100
$PhB(L_5)$	100	100	100	100
Standard[a]	1.00	0.01	0.78	3.13

[a] Streptomycin for bactera; Bavistin for fungi.

Biochemical aspects

The hypothesis proposed by Woods and Fildes[13] regarding the action of sulfonamides has provided a group of compounds that can be tolerated in the human body with minimal toxicity and which are injurious to many bacteria. Sulfonamides are structural analogues of *p*-aminobenzoic acid, which is required by many bacteria to synthesize their own folic acid for a number of metabolic reactions. Thus, sulfonamides can fit the same enzyme and become incorporated to form an altered folate that is metabolically injurious to the bacteria. In contrast, humans cannot synthesize folate and must obtain it in the diet; therefore, sulfonamides will not affect the host. Many sulfonamide-imines have recently been[7,14] prepared and screened for their *in vitro* antibacterial and antifungal activity. Also, the peculiar behaviour of organoboron compounds in chemical and biological processes (such as in plastic stabilizers, polymerization accelerators, lubricants, bactericides[15,16] and in cancer treatment[17,18]) has led us to synthesize some biologically active organoboron complexes of sulfonamide-imines so as to contribute in the field of bioinorganic chemistry. Consequently, the compounds synthesized have been evaluated for their antibacterial and antifungal actions, as well as for their effects on fertile male albino rats by doing serum biochemistry, haematology and sperm dynamics.

It can be seen from Table 3 that the bacteriotoxicity and fungitoxicity of sulfonamide-imines may be significantly enhanced on chelation with phenyldihydroxyborane. The complexes showed good antibacterial and moderate antifungal activity. Among the complexes, PhB(L_1) exhibited growth inhibition started from 1.56 mg l^{-1} against *S. aureus*. The bacterial activity of the ligands $H_a - L_4H_a$ and their complexes against Gram negative *E. coli* varied between 6.25 and 100 mg l^{-1}, but *E. coli* was least affected by the L_5H_2 ligand (MIC > 100 mg l^{-1}). However, the activity increased on complexation. Not much variation was observed in the activity of the ligands L_2H_2, L_3H_2, L_4H_2 and L_5H_2 and their organoboron complexes against *M. phaseolina*, but the ligand L_1H_2 and its complex exhibited better growth inhibition which varied between 12.5 and 50 mg l^{-1} against *F. oxysporum* and *M. phaseolina*.

Although no ligand or complex showed activity comparable to that of the standard, the salicylaldimine sulfathiazole (L_1H_2) ligand and its boron complex nevertheless gave the most promising results with the bacteria and fungi. Thus, these were selected for testing on male albino rats to check for toxicological effects.

The results regarding the toxicological effects of salicylaldimine sulfathiazole (L_1H_2) and its boron complex PhB(L_1) on the serum of male albino rats (Table 4) reveal: (1) a significant lowering ($P \leq 0.01$) in glucose level, which may be associated with starvation, hyperinsulinism and decreased activity of endocrine glands; and (2) a slight increase ($P \leq 0.05$) in alanine aminotransferase and aspartate aminotransferase, which may be due to pathological changes such as necrosis of

Table 4. Effects of ligand (L_1H_2) and its boron complex PhB(L_1) on male albino rats[a]

	Serum analysis			Blood analysis				Sperm dynamics			
								Density (10^6/ml)			
Treatment	Glucose (mg/dl)	Alanine amino-transferase (units/ml)	Aspartate amino-transferase (units/ml)	Total erythrocyte count ($10^6/mm^3$)	Total leucocyte count (mm^3)	Haemoglobin (g)	Haematocrit (%)	Motility (%)	Testes	Cauda epididymis	Fertility (%)
Group I											
Control	102.17 ± 3.73	132.20 ± 7.00	76.18 ± 2.31	6.56 ± 0.21	5300 ± 253.34	15.25 ± 0.32	50.44 ± 1.59	69.61 ± 4.34	4.15 ± 1.86	21.70 ± 2.06	100 (+)
Group II (100 mg kg^{-1} body wt/day/animal)											
L_1H_2	71.35 ± 1.76**	150.41 ± 1.54*	86.06 ± 1.20*	5.42 ± 0.73	6998.23 ± 13.37**	12.64 ± 0.22	45.91 ± 0.48*	38.19 ± 1.22	1.04 ± 0.76	10.63 ± 1.18	45 (−)
PhB(L_1)	89.13 ± 1.96*	143.42 ± 1.13	76.17 ± 0.34	6.04 ± 0.18**	6194.29 ± 112.43	14.73 ± 1.10	47.38 ± 1.93	31.67 ± 1.22	1.89 ± 0.24**	8.82 ± 0.55	50 (−)
Group III (125 mg kg^{-1} body wt/day/animal)											
L_1H_2	77.15 ± 2.59**	144.16 ± 1.67	78.43 ± 1.55	5.40 ± 0.68	6582.15 ± 12.53	12.52 ± 12.53*	45.86 ± 0.37	36.07 ± 0.92***	1.51 ± 0.33	9.13 ± 0.69	50 (−)
PhB(L_1)	93.74 ± 1.07	134.07 ± 1.83	75.95 ± 1.61	6.15 ± 0.46	6213.45 ± 59.28*	14.35 ± 0.98	47.12 ± 2.69	29.84 ± 1.15***	1.06 ± 0.47	6.75 ± 1.19*	55 (−)

[a] Mean ± SEM of five animals. * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

hepatocytes causing increase in the permeability of cell membranes, resulting in the release of transferases into the blood stream. These effects occurred in a dose-dependent manner compared with control rats. The present study finds support from the work of other toxicologists.[19–21] No significant changes in other parameters are observed. Lowering of glucose level was more pronounced in rats exposed to ligand than to its boron complex.

The haematological results (Table 4) reveal that the ligands showed significant ($P \leq 0.01$, $P \leq 0.05$) decreases in total erythrocyte count, haemoglobin (Hb) and haematocrit percent value and a significant ($P \leq 0.05$) decrease in leucocyte count compared with metal complexes in a dose-dependent manner in male albino rats. Deficiency of vitamin B_{12} in the liver may be the reason for impaired synthesis of nucleic acid, resulting in defective maturation of erythrocytes and their nuclei.[22] The decrease in the Hb content can be related either to a poor intake of the iron-rich diet by intoxicated albino rats or to the interference of the compound with enzymes of the red cells that synthesize the Hb. Thus, the destruction of erythrocytes by compounds leads to a fall in Hb. Similar results were also reported by Reeves *et al*.,[23] Siddiqui *et al*.[24] and Quadri *et al*.[25] The observed increase in leucocyte production (leucocytosis) justified the assumption that these compounds act as chemical stressors. The stress causes a slight increase in adrenaline level and, in consequence, to neutrophil and lymphotic leucocytosis.[26,27]

Regarding sperm motility and sperm density, a significant reduction ($P \leq 0.05$) was observed in rats exposed to ligand (L_1H_2) and its boron complex [PhB(L_1)], with the complex showing a more pronounced reduction in these parameters than the ligand (Table 4). The decrease in sperm motility may be due either to an alteration in the enzymatic activities of the oxidative phosphorolytic process required for adenosine triphosphate production, which in turn is necessary for the forward movement of spermatozoa, i.e. their motility, which may cause infertility[28] or to an androgen deprivation effect of the compounds. Biologically active gonadotrophins (luteinizing hormone and follicle-stimulating hormone) are necessary for normal sperm production, development and maturation in testes.[29] The suppression of gonadotrophins may decrease sperm density in testes.[30] The decline in sperm density in the cauda epididymis may be due to an alteration in androgen metabolism. The physiological and biochemical integrity of the epididymis are dependent on androgens.[31]

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