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Synthesis, Characterization, *invitro* Antibacterial, Anti-inflammatory Evaluations of Novel 4-Quinolone containing Pyrazolidinedione derivatives

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Abstract: There has been a biggest problem of bacterial resistance ever since the development of anti-bacterial agents. The present research work focuses on the microwave assisted solvent less synthesis combined with conventional stirring and refluxation methods to form some novel substituted 4-quinolone pyrazolidinedione derivatives. The characterisation of n=9 derivatives was carried out using I.R, ^{1}H NMR and mass spectral analysis. The percentage yield of final compounds was found to be 22.15 to 69.68. Purity of the compounds was checked by using TLC and elemental analysis. These compounds showed a considerable anti-bacterial activity against *S. aureus, B. subtilis, Klebesiella pneumoniae and Proteus vulgaris* and anti-inflammatory activity using *Invitro* testing methods compared to Ciprofloxacin, Amoxicillin and Ibuprofen respectively.
Keywords: Quinolone, Fluoroquinolone, antibacterial agents, pyrazolidine-3,5-dione, anti-inflammatory agents.

Introduction and Experimental

Although a large number of anti-bacterial agents are available in the market for the treatment of deadly bacterial infections. But because of the growing resistance problem to these agents, the need for new antibacterial agents is also growing proportionately. One of the most used anti-bacterial classes of drugs is fluoroquinolones. They have shown considerable inhibition of topoisomerase II in bacteria[1]. These agents are drugs of choice for the treatment of urinary tract infections caused mainly by bacteria of enterobacter family. Serendipitous discovery 7-chloro derivative of 4-quinolone-3-carboxylic acid during the discovery of chloroquine pawed way for the further inventions of this category. These agents are not only explored for their antibacterial activity but also for their anticancer effect as well. These derivatives have been reported to have a good anti-mitotic action also[2].
Second aspect of a bacterial infection is inflammatory conditions. Inflammation is one of the first defence mechanisms which is activated once an antigen enters the human body and accompanied by fever, oedema and other symptoms. Various non steroidal anti-inflammatory drugs are available in the market for the treatment of these inflammatory conditions. The recent

development of pyrazolidine-3,5-diones has opened a new avenue for the search of better derivatives. These agents are also reported to have better anti-bacterial activity and are also reported to be angiotensin II receptor antagonist[3,4]. Recently one of the authors has synthesized 6-pyrazolidinedione substituted quinolone esters and carried out antibacterial activity. Since the qunioline-3-carboxylic esters were established to be very effective antimicrobial agents[5]. The present work was designed to carry out microwave assisted solvent less synthesis of 4-quinolone pyrazolindine-3,5-dione pharmacophores clubbed in the same molecule with an intention to get compounds showing broad spectrum antibacterial as well as anti-inflammatory activities. The study focuses on the characterization of these n=9 synthesized derivatives using spectral (I.R, ^{1}H NMR and Mass spectra) data and *invitro* biological evaluation of these n=9 agents for antibacterial and anti-inflammatory activities. The purity checks for the compounds were done using elemental analysis and TLC studies.

Materials and Methods:

The chemicals and reagents used in this were of AR and LR grade. They were procured from SpectroChem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy.

Synthesis of substituted ethyl esters of 4-oxo-1,4-dihydroquinoline-3-carboxylic acids [1A-1I][6,7,8]

Equimolar quantities of substituted anilines **(**0.01 mol) and diethyl (ethoxymethylene) malonate (EMME) (0.01mol), (almost colourless liquid, b.p. 279-281 °C) were taken in a beaker under solvent-free condition. When a clear solution was obtained by shaking, it was irradiated under microwave for min at high power (900 watts) while shaking the mixture at intervals of 30 sec. The resulting solution was eventually converted to pasty mass upon cooling with ice. This was followed by washing it with acetone and the residue was recrystallised using N, N-Dimethyl formamide (DMF) as solvent. Melting points, percentage yields and time of micro wave irradiation of all 1A-1I compounds are given in **table 1(a).**

Synthesis of substituted 4-oxo-1,4-dihydroquinoline-3-carboxylic ethyl ester hydrazide [2A-2H]

Equimolar quantities of ethyl esters of 4-oxo-1,4-dihydroquinoline-3-carboxylic acids and hydrazine hydrate was taken in presence of DMF as a solvent in an iodine flask. The reaction mixture was kept for stirring using magnetic stirrer for 16 to 18 hours. The clear solution obtained was poured in ice cold water with constant stirring. The solution was kept at room temperature for 20 to 24 hours to get precipitates of hydrazide. The solid precipitates were filtered, dried and recrystallised using DMF-ethanol (1:1) combination. Percentage yield and melting points of all the (2A-2I) derivatives are given in **table 1(b).**

Synthesis of 7-chloro-6-fluoro-4-oxo-*N*'-phenyl-1,4-dihydroquinoline-3-carbohydrazide [2I]

Equimolar quantities of ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate and phenyl hydrazine was taken in a 50ml of beaker. Now the suspension obtained is subjected to microwave irradiation at high power (900 watts) for 7-8min with stirring at an interval of 30 sec. The red coloured solution was cooled in ice. The mixture was washed with ethanol to give precipitates after keeping aside the clear solution for 17 hours. The solid precipitate obtained was filtered and recrystallised using ethanol. Percentage yield and melting point for the derivative is given it **table 1(b).**

Synthesis of substituted 4-quinolone containing pyrazolidine-3,5-dione derivatives [3A-3I]

An equimolar quantities of substituted 4-oxo-1,4-dihydroquinoline-3-carboxylic ethyl ester hydrazide (2A-2I) and diethyl malonate was taken in DMF-ethanol (1:1) solution and acetic acid (1ml) was added to it in a round bottom flask. The mixture was refluxed for 6-7 hrs. The reaction mixture was left in open dish for 2-3 hrs. The solid precipitate formed was filtered, dried and recrystallized using ethanol. Percentage yield and melting points for all the synthesized derivatives are presented in **table 1(c).**

Table 1(a): Substituted ethyl esters of 4-oxo-1,4-dihydroquinoline-3-carboxylic acids

Parameters	Codes								
	1A	**1B**	**1C**	**1D**	**1E**	**1F**	**1G**	**1H**	**1I**
R_1	Chloro	H	H	H	H	H	H	H	Chloro
R_2	Fluoro	H	F	Cl	Br	I	CH_3	NO_2	H
R_3	H	H	H	H	H	H	H	H	Ph
% Yield	50.39	46.96	52.07	40.15	40.05	47.23	45.86	46.30	50.39
Melting Point (°C)	290-295	270-275	296-298	310-315	318-320	306-308	295-301	260-261	290-292
Time of Irradiation (min)	1-1.5	1.5-2	4.5-5	2-3	2-3	2-3	1-1.3	0.4-0.5	1-1.5

General Scheme:

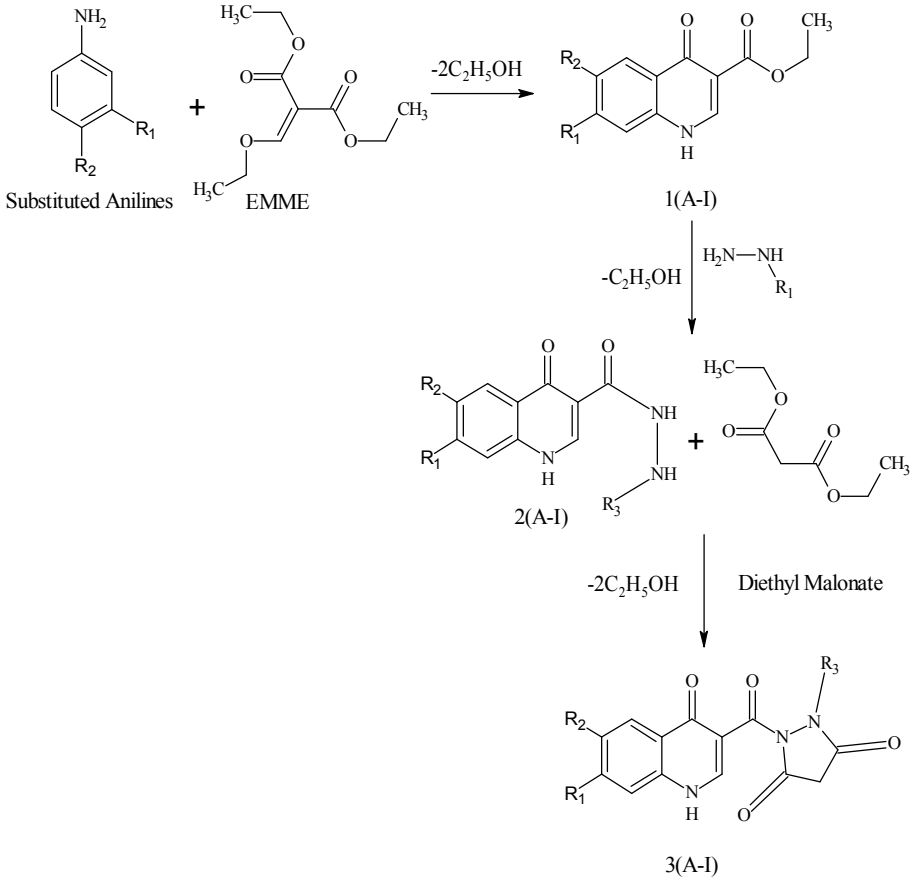

Table 1(b): Substituted 4-oxo-1,4-dihydroquinoline-3-carboxylic ethyl ester hydrazides

Parameters	Codes								
	2A	**2B**	**2C**	**2D**	**2E**	**2F**	**2G**	**2H**	**2I**
R_1	Chloro	H	H	H	H	H	H	H	Chloro
R_2	Fluoro	H	F	Cl	Br	I	CH_3	NO_2	H
R_3	H	H	H	H	H	H	H	H	Ph
% Yield	56.96	48.25	54.47	47.97	47.51	58.39	55.73	48.43	13.59
Melting Point (°C)	>350	>350	>350	>350	>350	>350	>350	>350	>350

Table 1(c): Substituted 4-quinolone containing pyrazolidine-3,5-dione derivatives

Parameters	Codes								
	3A	**3B**	**3C**	**3D**	**3E**	**3F**	**3G**	**3H**	**3I**
R1	Chloro	H	H	H	H	H	H	H	Chloro
R2	Fluoro	H	F	Cl	Br	I	CH_3	NO_2	H
R3	H	H	H	H	H	H	H	H	Ph
%Yield	22.11	48.48	35.5	40.41	69.68	49.63	50.12	60.75	45.09
Melting Point (°C)	>350	>350	>350	>350	>350	>350	>350	>350	>350

Biological Activity

1) **Anti-bacterial activity[8]:** Agar plates were prepared by pouring nutrient agar media into the petridishes and each of them were inoculated with a particular micro-organism, like gram-positive (*S. aureus & B. subtilis*) & gram-negative (*Klebsiella pneumonieae & Proteus vulgaris*). After agar was solidified, cups were made in the nutrient agar. The Anti-microbial test drugs 3A-3I is placed in the cups (1000µg). The drug diffuses through the agar around the cup. The plates are incubated at a temp of 37ºC for 24hrs for bacterial culture. The standard reference drugs used in the antibacterial screening were Ciprofloxacin and Amoxicillin (100 µg/ml).

2) Anti-inflammatory activity (*in vitro* Inhibition of albumin denaturation)[9]: The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) by carrying out sonication for 10-15mins and diluted with phosphate buffer (0.2M, pH 7.4). The final concentration of DMF in all solutions was less than 2.5%. Test solution (1ml) containing different concentration of synthesized compounds **[3A-3I]** was mixed with 1ml of 1mg/ml albumin solution in phosphate buffer and incubated at 27º±1ºC for 15 min. Denaturation was induced by keeping the reaction mixture at 60º±1ºC in water bath for 10-20 min. after cooling, the turbidity was measured at 660nm in U.V spectrophotometer. The percentage inhibition of denaturation was calculated from control where no synthesized compounds were added and compared against standard (Ibuprofen).

Results and Discussion

Synthesis of 1A-1I was carried out using microwave irradiation under solvent free condition, physical data of which is provided in **table 1(a)**. The yields were found to be par with that of the conventional methods which uses organic solvents. Thus this technique is a time saving and cost saving method. The technique was also found to be useful for synthesis of phenyl hydrazide under solvent less condition. Spectral results for all the intermediates and final compounds are summarized in **table 2(a)**. Elemental analysis of all the final 3A-3I compounds showed that they were highly pure; purity was also checked using TLC. The data for elemental analysis- calculated and found are reported in **table 2(b)**.

A considerable antibacterial activity was shown by all the synthesized derivatives compared to standard drugs. The biological activity data are provided in **table 3(a)** and 3**(b).** Compound 3D was found to be more effective against *S. aureus* while 3B and 3G have shown good activity against *B.subtilis*. 3G has shown some activity against *Klebsiella pneumoniae*. Compound 3C has shown good activity against *Proteus vulgaris*. From the *invitro* anti-inflammatory activity studies it was revealed that 3B and 3D gave comparatively better inhibition to albumin denaturation with increase in concentration from 0.2mg/ml; to 1.0 mg/ml.

Table 2(a): Spectral (IR, ^{1}NMR, Mass) results of synthesized compounds

S. No.	Code	Spectral Results
1.	**1A.**	3096(Ar, C-H str), 3151(Ar, N-H str), 1697(Ester, >C=O str), 2968(alkanes, C-H str), 1037(C-F str), 800(C-Cl str), 1475(C-N stretch of quinoline having C=C-N).
2.	**2A.**	3045(Ar, C-H str), 3170(Ar, N-H str), 1678(Amide, >C=O str), 989(C-F str), 796(C-Cl str), 3312(N-H str), 1471(C-N stretch of quinoline having C=C-N).
3.	**3A.**	2947(Ar, C-H str), 3046(Ar, N-H str), 1674(Amide, >C=O str), 991(C-F str), 795(C-Cl str), 1477(C-N stretch of quinoline having C=C-N). 7.9-8.3 (3H, Ar), 12.7 (1H, Ar N-H), 10.5 (1H, aliphatic amide N-H), 2.1 (2H, CH_2).324 (M^+) and other important peaks are 229, 97.
4.	**1B.**	3097 (Ar, C-H str), 3150 (Ar, N-H str), 1696 (Ester, >C=O str), 2961 (alkanes, C-H str), 1487(C-N stretch of quinoline having C=C-N).
5.	**2B.**	2984 (Ar, C-H str), 3097 (Ar, N-H str), 1674 (Amide, >C=O str), 1473 (C-N stretch of quinoline having C=C-N), 3289 (Amide, N-H str).
6.	**3B.**	2983 (Ar, C-H str), 3014 (Ar ,N-H str), 1674 (Amide, >C=O str), 1564 (pyrazolidinedione, >C=O str), 1475 (C-N stretch of quinoline having C=C-N), 3096 (N-H str).6.9-8.2 (5H, Ar), 12.2 (1H, Ar N-H), 11.3 (1H, aliphatic amide N-H), 2.1 (2H, CH_2). 272(M+1) and other important peaks are 185, 97.
7.	**1C.**	3015 (Ar, C-H str), 3101 (Ar, N-H str), 2979 (alkanes, C-H str), 1701 (Ester, >C=O str), 1481(C-N stretch of quinoline having C=C-N), 1031 (C-F str).
8.	**2C.**	3016 (Ar, C-H str), 3180 (Ar, N-H str), 1676 (Amide, >C=O str), 1481(C-N stretch of quinoline having C=C-N), 1001(C-F str), 3289 (N-H str).
9.	**3C.**	3017 (Ar, C-H str), 3084 (Ar, N-H str), 1676 (Amide, >C=O str), 996(C-F str), 1571

		(pyrazolidinedione, >C=O str), 1487(C-N stretch of quinoline having C=C-N), 3180 (N-H, str). 7.5-8.5 (4H, Ar), 12.2 (1H, Ar N-H), 11.3 (1H aliphatic amide N-H), 2.1 (2H, CH_2). 290(M+1) and other important peaks are 196, 94.
10.	**1D.**	3069 (Ar, C-H str), 3150 (Ar, N-H str), 2964 (alkanes, C-H str), 1692 (Ester, >C=O str), 800 (C-Cl str), 1458 (C-N stretch of quinoline having C=C-N).
11.	**2D.**	3045 (Ar, C-H str), 3076 (Ar, N-H str), 1670 (Amide, >C=O str), 815 (C-Cl str), 1474 (C-N stretch of quinoline having C=C-N), 3252 (N-H, str).
12.	**3D.**	3045 (Ar, C-H str), 3076 (Ar, N-H str), 1671 (Amide, >C=O str), 815 (C-Cl str), 1473(C-N stretch of quinoline having C=C-N), 1568 (pyrazolidinedione, >C=O str), 3168 (N-H, str). 6.9-7.5(4H, Ar), 12.7 (1H, Ar N-H), 11.3 (1H aliphatic amide N-H), 2.3 (2H, CH_2). 307(M+2) and other important peaks are 208, 97.
13.	**1E.**	3090 (Ar, C-H str), 3147 (Ar, N-H str), 2961 (alkanes, C-H str), 1693 (Ester, >C=O str), 597 (C-Br str), 1466 (C-N stretch of quinoline having C=C-N).
14.	**2E.**	3024 (Ar, C-H str), 3159 (Ar, N-H str), 1670 (Amide, >C=O str), 621 (C-Br str), 1470 (C-N stretch of quinoline having C=C-N), 3315 (N-H str).
15.	**3E.**	2981 (Ar, C-H str), 3160 (Ar, N-H str), 1720 (Amide, >C=O str), 621 (C-Br str), 1469 (C-N stretch of quinoline having C=C-N), 3247 (N-H str), 1531 (pyrazolidinedione, >C=O str). 7.6-7.8 (4H, Ar), 12.6 (1H, Ar N-H), 10.6 (1H aliphatic amide N-H), 2.1 (2H, CH_2). 350(M^+) and other important peaks are 253, 97.
16.	**1F.**	3069 (Ar, C-H str), 3147 (Ar, N-H str), 2962 (alkanes, C-H str), 1693 (Ester, >C=O str), 598 (C-I str), 1465 (C-N stretch of quinoline having C=C-N).
17.	**2F.**	3047 (Ar, C-H str), 3174 (Ar, N-H str), 1674 (Amide, >C=O str), 605 (C-I str), 1475 (C-N stretch of quinoline having C=C-N), 3312 (N-H str).
18.	**3F.**	3048 (Ar, C-H str), 1674 (Amide, >C=O str), 606 (C-I str), 1476 (C-N stretch of quinoline having C=C-N), 3251 (N-H str), 1562 (pyrazolidinedione, >C=O str). 7.8-8.3 (4H, Ar), 11.3 (1H, Ar N-H), 10.9 (1H aliphatic amide N-H), 2.0 (2H, CH_2). 398 (M+1) and other important peaks are 299, 99
19.	**1G.**	3098 (Ar, C-H str), 3150 (Ar, N-H str), 2961 (alkanes, C-H str), 1696 (Ester, >C=O str), 1377(C-H bending), 1487 (C-N stretch of quinoline having C=C-N).
20.	**2G**	3045 (Ar, C-H str), 3170 (Ar, N-H str), 2802 (alkanes, C-H str), 1670 (Amide, >C=O str), 1490 (C-N stretch of quinoline having C=C-N), 3314 (N-H str).
21.	**3G.**	3046 (Ar, C-H str), 1670 (Amide, >C=O str), 1451 (C-N stretch of quinoline having C=C-N), 3247 (N-H str), 1564 (pyrazolidinedione, >C=O str). 7.6-8.1 (4H, Ar), 12.8 (1H, Ar N-H), 12.2 (1H aliphatic amide N-H),2.1 (2H, CH_2), 1.3 (3H, CH_3). 285(M^+) and other important peaks are 271, 185, 97.
22.	**1H**	2997 (Ar, C-H str), 3107 (Ar, N-H str), 2909 (alkanes, C-H str), 1685 (Ester, >C=O str), 1508 (C-N stretch of quinoline having C=C-N), 1555 (Ar, N-O str).
23.	**2H.**	2995 (Ar, C-H str), 3151 (Ar, N-H str), 1684 (Amide, >C=O str), 1508 (C-N stretch of quinoline having C=C-N), 1557 (Ar, N-O str), 3340 (N-H stretching).
24.	**3H.**	2996 (Ar, C-H str), 3152 (Ar, N-H str), 1683 (Amide, >C=O str), 1508 (C-N stretch of quinoline having C=C-N), 1556 (Ar, N-O str), 3186 (N-H str), 1571 (pyrazolidinedione, >C=O str). 7.8-8.3 (4H, Ar), 11.3 (1H, Ar N-H), 10.9 (1H aliphatic amide N-H), 2.1 (2H CH_2). 317(M^+) and other important peaks are 218, 99.
25.	**1I.**	3096(Ar, C-H str), 3151(Ar, N-H str), 1697(Ester, >C=O str), 2968(alkanes, C-H str), 1037(C-F str), 800(C-Cl str), 1475(C-N stretch of quinoline having C=C-N).
26.	**2I.**	3012(Ar, C-H str), 3096 (Ar, N-H str), 1643(Amide, >C=O str), 3274 (Amide, N-H str), 1027 (C-F), 752 (C-Cl str), 1475 (C-N stretch of quinoline having C=C-N).
27.	**3I.**	2996 (Ar, C-H str), 3096 (Ar, N-H str), 1643(Amide, >C=O str), 1596 (pyrazolidinedione, >C=O str), 1027(C-F), 752(C-Cl str), 1476(C-N stretch of quinoline having C=C-N). 7.9-8.8 (8H, Ar), 12.7 (1H, Ar N-H), 2.1 (2H, CH_2). 400 and other important peaks are 229, 176, 99 and 77.

Table 2(b): Elemental analysis results of 3A-3I compounds.

Sr. No.	Code	Elements		
		C(Calculated)	H(Calculated)	N(Calculated)
1.	3A	48.25(48.24)	2.20(2.18)	12.99(12.98)
2.	3B	56.85(57.09)	3.81(2.77)	10.65(10.51)
3.	3C	57.65(57.57)	3.29(3.34)	15.38(15.49)
4.	3D	53.88(53.99)	2.83(2.79)	14.65(14.53)
5.	3E	51.25(51.08)	2.89(2.64)	13.78(13.75)
6.	3F	44.68(44.60)	2.35(2.30)	12.14(12.00)
7.	3G	39.45(39.32)	2.11(2.03)	10.65(10.58)
8.	3H	59.12(58.59)	3.85(3.38)	14.85(14.73)
9.	3I	49.32(48.38)	2.61(2.55)	17.65(17.72)

Table 3(a): Zone of Inhibition of 3A-3I compounds in mm

S. No.	Compound	Antibacterial activity zone of inhibition in (mm)			
		S.aureus **(gram-positive)**	***B. subtilis*** **(gram-positive)**	***Klebsiella pneumonieae*** **(gram-negative)**	***Proteus vulgaris*** **(gram-negative)**
1.	3A	17	18	21	29
2.	3B	17	20	24	26
3.	3C	19	17	20	30
4.	3D	22	18	22	28
5.	3E	20	18	22	29
6.	3F	19	19	23	27
7.	3G	18	20	25	28
8.	3H	20	17	21	25
9.	3I	19	18	20	23
10.	Ciprofloxacin	37	40	35	31
11.	Amoxicillin	43	39	35	41
14.	Control (DMF)	NI	NI	NI	NI

Note: All the values are mean of triplicates

NI: no inhibition

Table 3(b): Invitro anti-inflammatory activity table for 3A-3I compounds

S. No	Compound		Blank	Concentration (mg/ml)				
				0.2	**0.4**	**0.6**	**0.8**	**1.0**
1.	3A	**Inhibition of denaturation (%)**	0	22.1	33.2	45.7	49.3	51.9
2.	3B		0	20.0	28.7	33.7	49.1	59.8
3.	3C		0	21.2	19.3	23.9	41.2	54.3
4.	3D		0	22.3	36.2	45.0	50.0	66.9
5.	3E		0	10.2	31.6	42.0	46.2	52.4
6.	3F		0	10.8	22.8	29.8	39.6	43.1
7.	3G		0	25.0	31.3	34.8	41.2	50.6
8.	3H		0	27.9	33.5	41.7	52.1	52.2
9.	3I		0	20.1	24.3	32.3	41.6	51.6
10.	Ibuprofen (std)		0	28.0	41.0	54.5	65.1	82.4

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