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Synthesis, Characterization and Antimicrobial studies of novel 2-Pyrazoline derivatives

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Abstract: A simple,efficient method for the synthesis of some novel 2-pyrazoline derivatives is reported. Chalcones were prepared by conventional Claisen-schmidt condensation of piperonal and substituted acetophenones,Pyrazoline were prepared by condensing chalcones with hydrazine hydrate/isoniazid/tolyl sulfonylhydrazide. The structures of the synthesized compounds were confirmed by FTIR, ^{1}H NMR, mass and elemental spectral data. The synthesized compounds have been screened for their antimicrobial activity against different micro-organisms. A significant level of activity was observed.
Keywords: Chalcones, hydrazine hydrate,isoniazid,benzene sulfonyl chloride, pyrazolines, Antimicrobial Activity.

Introduction:

The chemistry of chalcones has generated intensive scientific studies throughout the world. Especially interest has been focused on the synthesis and biodynamic activities of chalcones. The most convenient methods are the Claisen-Schimdt condensation of equi molar quantities of an arylmethylketones with aryl aldehyde in the presence of alcoholic alkali. Chalcones are used to synthesize several derivatives like cyanopyridines, pyrazolines isoxazoles, pyrimidines, having different heterocyclic ring systems. In our present study,pyrazolines are synthesized by condensing chalcone with hydrazides.

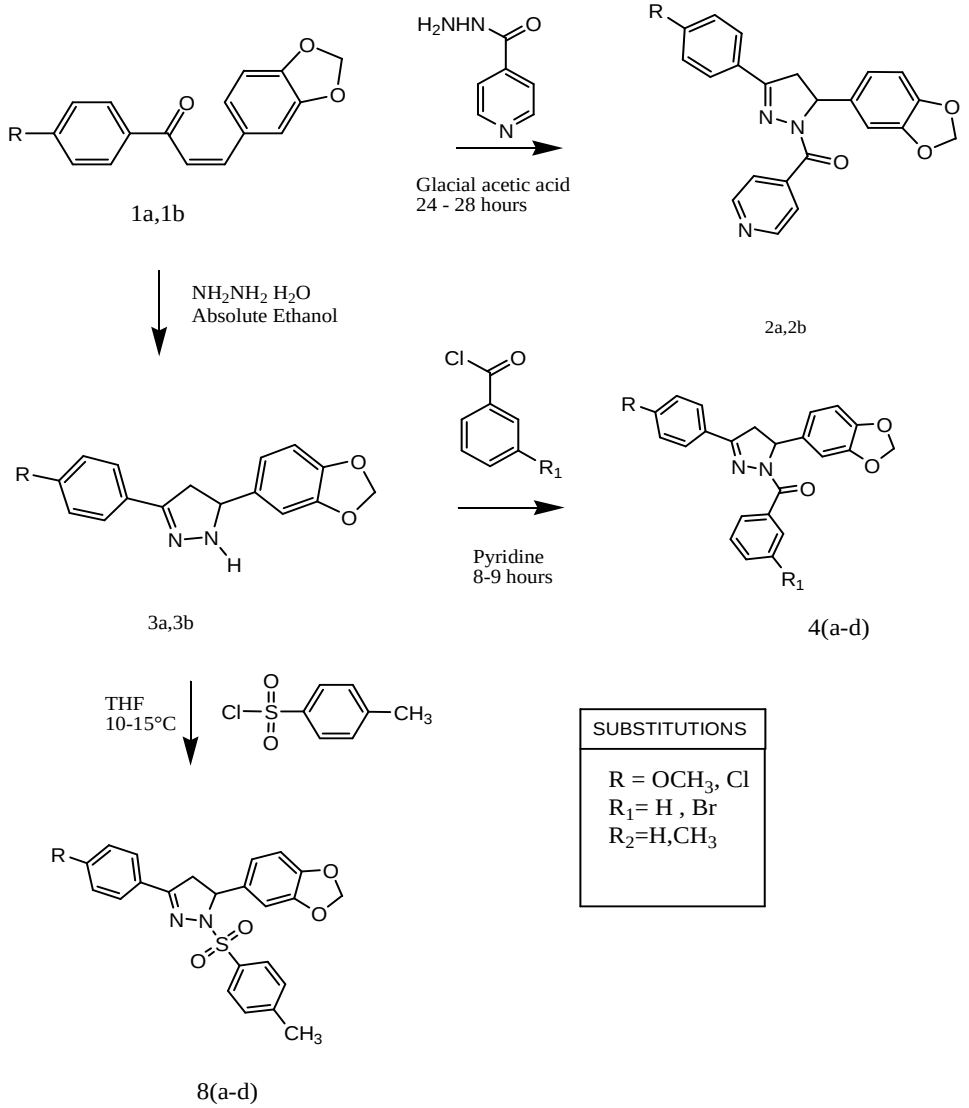
Piperonal,a naturally occurring derivative of piperine compound(the pyrrolidine amide of piperic acid) is an aromatic aldehyde.In our present study, piperonal is the aldehyde moiety in the chalcone preparation. Methylenedioxy group in piperonal have some biological activity [1,2]. 2-Pyrazoline derivatives have also been reported in the literature to exhibit various pharmacological activities such as antimicrobial [3-8], anti-inflammatory [9] and antihypertensive [10]. Its derivatives, possess a wide range of biological and physiological activities such as antitumor, antiarthritic, analgesic, anti diabetic, fungicidal, bactericidal, immunosuppressive activities.[6,8]. On the other hand antitubercular activity of isoniazid is well documented[11,12]. On the other hand, sulfur containing heterocycles possess pharmacological activities widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. In view of these observations and in continuation of our earlier work [13],we are now reporting some novel 2-pyrazoline derivative containing benzodioxole , isoniazid / benzenesulfonyl moiety.

Materials and Methods

All the reagents were purchased from Aldrich and used as received. Glacial acetic acid and dry solvents were supplied by Spectrochem, India. The ^{1}H NMR was performed by ^{1}H NMR chemical shift values were reported on the scale in ppm relative to TMS, The ^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker AMX

400.spectrometer (400MHz). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR model. Column chromatography was performed with silica gel 60-120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for their reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposing them to iodine vapor or KMnO4 reagents. Melting points were determined by Buchi B-545 apparatus. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer.Yield reported is the isolated yield after purification of the compounds.

SCHEME



General procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(4-substituted phenyl)-4,5-dihydropyrazol-1-yl)(pyridine-4-yl)methanone(2a).

Equimolar amount of chalcone(0.01M) and Isoniazid(0.01M) in glacial acid(25 ml) was refluxed for 24-28 hours. Reaction was monitored by TLC, after the completion of reaction, it was diluted with water (10 mL) and extracted with ethyl acetate (50 mL) and dried over Na2SO4. The organic layer was concentrated under reduced pressure to give crude product. The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 70% ethyl acetate.

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl)(pyridine-4-yl)methanone(2a):Off White powder,Yield:68 %,M.P:125°C ,

IR(KBr):1660cm^{-1}(C=O),1565cm^{-1}(C=N), 1490 cm^{-1}(C=C),1134 cm^{-1} (C-N str)

^{1}H NMR:400MHz($CDCl_3$) : 3.22 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2), 3.68(dd, *J* = 4.8Hz, 5.6Hz, 1H, -CH_2), 5.2(q, *J* = 24Hz, 1H, -CH), 5.93(q, *J* = 20Hz, 2H, -O-CH_2-O), 7.2-7.3(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar),7.9-8.8(d,pyridyl,2H $_a$,2H_b,J=8Hz)

LC-MS: m/z 402 (M+1).

Elemental analysis: calculated C;68.82;H,4.77;N 10.47;O,15.97 Found: C;68.81;H,4.78;N 10.45;O,15.99

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-Chlorophenyl)-4,5-dihydropyrazol-1-yl)(pyridine-4-yl)methanone(2b): Off White powder,Yield:75%,M.P:196°C ,

IR(KBr):1664cm^{-1}(C=O),1567cm^{-1}(C=N), 1491 cm^{-1}(C=C),1134 cm^{-1} (C-N str)

^{1}H NMR:400MHz($CDCl_3$) : 3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2), 3.7(dd, *J* = 4.8Hz, 5.6Hz, 1H, -CH_2), 5.2(q, *J* = 24Hz, 1H, -CH), 6.2(q, *J* = 20Hz, 2H, -O-CH_2-O), 7.08-7.22(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar),7.9-8.1(d,pyridyl,2H_a ,2H_b,J=8Hz)

LC-MS: m/z 406.5 (M+1).

Elementalanalysis:
calculated:C;65.11;H,3.97;N 10.35;O,11.83,found: C;65.12;H,3.96;N 10.37;O,11.81

Procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(4-substitutedphenyl)-4,5-dihydro-1H-pyrazole: 3(a-b) A mixture of chalcone(0.01M) and hydrazine hydrate 99% (0.01M) was refluxed in absolute ethanol for 8 hours. Reaction was monitored by TLC, with eluent 8:2 petether:ethylacetate. Reaction was completed. Then the resulting solid obtained was dried and washed with water. The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 70% ethyl acetate. The solid was dried and recrystallized from abs.ethanol .

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole:3(a)

Brown powder, Yield: 78%, M.P:118°C

IR(KBr):1568 cm^{-1}(C=N), 1493 cm^{-1}(C=C),1130 cm^{-1} (C-N str)

^{1}H NMR:400MHz($CDCl_3$) : 3.06(dd, *J* = 8Hz, 8Hz, 1H, -CH_2), 3.56(dd, *J* = 5Hz, 5.6Hz, 1H, -CH_2), 5.11(q, *J* = 20Hz, 1H, -CH), 5.8(q, *J* = 20Hz, 2H, -O-CH_2-O), 7.8(m,J=2Hz,4H), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar), 8.6(s,1H, NH). LC-MS: m/z 297(M+1).

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole3(b) :

Brown powder,Yield: 78%,M.P:133°C

IR(KBr):1564cm^{-1}(C=N), 1495 cm^{-1}(C=C),1130 cm^{-1} (C-N str)

^{1}H NMR:400MHz($CDCl_3$) : 3.09(dd, *J* = 8Hz, 8Hz, 1H, -CH_2), 3.74(dd, *J* = 5Hz, 5.6Hz, 1H, -CH_2), 5.11(q, *J* = 20Hz, 1H, -CH), 5.8(q, *J* = 20Hz, 2H, -O-CH_2-O), 7.4-.7.5(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar), 8.6(s,1H, NH).LC-MS: m/z 301.5(M+1).

General procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(4-substituted phenyl)-4,5-dihydro pyrazol-1-yl)phenyl methanone(4a-d).

To the intermediate(3)(1mmol),substituted benzoyl chloride(1 mmol) was refluxed in pyridine(8-10hours). Reaction was monitored by TLC, with eluent 7:3 petether:ethylacetate. . The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in petroleum ether and slowly increased to 60% ethyl acetate.The solid was dried and recrystallised from ethanol.

5-**(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl)phenyl methanone(4a):**

Brown powder,Yield:74%,M.P:136-138°C

IR(KBr):1668(C=O),1562cm^{-1}(s,C=N), 1496 cm^{-1}(m,C=C),1135cm^{-1} (s,C-N str)

^{1}H NMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.9-8.8(m,5H,) 3.1(dd, *J* = 11Hz, 11Hz, 1H, -CH_2), 3.72(dd, *J* = 4.2Hz, 5.6Hz, 1H, -CH_2), 5.1(q, *J* = 24Hz, 1H, -CH), 5.93(q, *J* = 20Hz, 2H, -O-CH_2-O), 7.2-7.4(m, 3H, Ar), 7.50(d, *J* = 1.2Hz, 1H, Ar), 7.06(t, *J* = 16Hz, 2H, Ar) 7.06 (m, *J* = 16Hz, 2H, Ar)) LC-MS: m/z 401(M+1). Calculated:C,71.99;H,5.03;N,7.00;O,15.98.Found: C,71.98;H,5.04;N,7.02;O,15.96

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl)(3-bromophenyl)methanone (4b)

Brown powder,Yield:66%,M.P:159-160°C

IR(KBr):1667(C=O),1566cm^{-1}(s,C=N),1498cm^{-1}(m,C=C),1139cm^{-1}(s,C-Nstr)

^{1}H NMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC-MS: m/z 480 (M+1).Calculated:C,60.14;H,4.00;Br,16.67; N,5.84;O,13.35.

Found:C,60.12;H,4.02;Br,16.69;N,5.86;O,13.35

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl) phenyl methanone(4c):

Brown powder, Yield:72%,M.P:143-144°C

IR(KBr):1665(C=O),1565cm^{-1}(s,C=N),1492cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr)^{1}HMR:400MHz($CDCl_3$) : 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), (m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J*= 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC-MS: m/z 405.5 (M+1).Calculated:C,68.23;H, 4.23;Cl,8.76N,6.92;O,11.86.Found: C,68.20;H, 4.26;Cl,8.74N,6.94;O,11.86

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)(3-bromophenyl)methanone (4d):

Brown powder,Yield:72%,M.P:188-189°C

IR(KBr):1670(C=O),1568cm^{-1}(s,C=N),1486cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr)^{1}HMR:400MHz($CDCl_3$): 400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC-MS: m/z 484.5(M+1).

Elemental analysis Calculated:C,57.11;H,3.33;Br;16.52;Cl;7.33;N,5.79;O,9.92.

Found: C,57.13;H,3.31;Br;16.50;Cl; 7.35;N,5.78;O, 9.93

General procedure for synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-(4-substitutedphenyl)-4,5-dihydro-1-tosyl-1H-pyrazole6(a-b) :

P-Toluene sulfonyl chloride (0.002M) was dissolved in tetrahydrofuran(2ml) with stirring.The stirred mixture was cooled in an ice bath to 5-10°C;followed by gradual addition of a solution of compound[3] in tetrahydrofuran so that the temperature was maintained between 10-15°C.Stirring was continued for half an hour after the addition was complete. Reaction mixture was directly concentrated to remove organic volatiles. The residue was dissolved in water (20 mL), and extracted with ethyl acetate (30 mL) and the combined organic layer was washed with water (30mL), brine solution (20 mL), dried over Na_2SO_4 and evaporated to dryness to get the crude product. The crude product was recrytallized with diethyl ether and dried under vacuum.The solid separated was filtered,dried and crystallised from ethanol.

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1-tosyl-1H-pyrazole6(a):

Brown powder,Yield:73%,M.P:106-107°C IR(KBr)1565cm^{-1}(s,C=N),1492cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr) 1172,1384(s,SO_2) ^{1}HMR:400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H,-CH_2) LC-MS: m/z451(M+1). Calculated:C,63.98;H,4.92;N,6.22;O,17.79;S,7.12 Found: C,63.96; H,4.94; N,6.20;O,17.81;S,7.13.

5-(benzo[d][1,3]dioxol-5-yl)-3-(4-chlorophenyl)-4,5-dihydro-1-tosyl-1H-pyrazole6(b):

Brown powder,Yield:76%,M.P:112-114°C IR(KBr)1565cm^{-1}(s,C=N),1492cm^{-1}(m,C=C),1134cm^{-1}(s,C-Nstr) 1172,1384(s,SO_2) ^{1}HMR:400MHz($CDCl_3$) : 7.23-7.00 (m, 5H, Ar),), 6.80-6.74(m, 3H, Ar), 5.1(q, *J* = 24Hz, 1H, -CH),6.02 (t, *J* = 20Hz, 3H, -CH, -O-CH_2-O)3.98 (dd, *J* = 12Hz, 12Hz, 1H, -CH_2),3.3 (dd, *J* = 8Hz, 8Hz, 1H, -CH_2) LC-MS: m/z 455.5(M+1).Elemental analysis

Calculated:C,61.45;H,4.42;F,5.02;N,6.83;O,16.49;S,7.81 Found:C,57.38;H,3.46;Br,18.21;N,6.36;O,14.59.

Antimicrobial Activity

We have investigated newly synthesised pyrazolines for their antibacterial activity against *Escherichia coli, Staphylococcus aureus , Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterial strains by the disc diffusion method. Solvent and growth controls were kept, the zones of inhibition and minimum inhibitory concentrations (MIC) noted. Results of these studies are given in Table-1 and compared with the standard ciprofloxin. We have investigated newly synthesised pyrazolines were screened for their antifungal activity against *Aspergillus niger, Candida albicans* obtained. Antifungal activity was determined by measuring the inhibition zone and (MIC) was noted. The results of these studies were given in Table-2 and compared with the standard Keta conazole.

Table-1 : Antibacterial activities of the newly synthesised compounds (Zone of Inhibition in mm,#MIC in µg/mL given in parenthesis)

Compounds (10 µg/ml)	*S. aureus*	*E. coli*	*P. aeruginosa*	*Klebsilla pneumoniae*
2a	23(6.25)	21.5 (6.25)	23.5(6.25)	20(6.25)
2b	23(6.25)	21.5 (6.25)	23.5(6.25)	20(6.25)
4a	<10(50)	17.5(12.5)	<10(50)	<10(50)
4b	<10(50)	17.5(12.5)	<10(50)	<10(50)
4c	<10(50)	17.5(12.5)	<10(50)	<10(50)
4d	<10(50)	17.5(12.5)	<10(50)	<10(50)
6a	23(6.25)	21.5 (6.25)	23.5(6.25)	17(6.25)
6b	23(6.25)	21.5 (6.25)	23.5(6.25)	19(12.5)
ciprofloxacin	24.5(6.25)	25(6.25)	24(6.25)	25.5(6.25)

MIC: Minimum inhibitory concentration.

Table-2 : Antifungal activities of the newly synthesised compounds (Zone of Inhibition in mm,#MIC in µg/mL given in parenthesis)

Compounds (10 µg/ml)	*Aspergillus niger*	*Candida albicans*
2a	32(6.25)	35.5(6.25)
2b	30(6.25)	32(6.25)
4a	<10(50)	<10(50)
4b	<10(50)	<10(50)
4c	<10(50)	<10(50)
4d	<10(50)	<10(50)
6a	30(6.25)	33(6.25)
6b	35(6.25)	36(6.25)
ketaconazole	38.5(6.25)	34.5(6.25)

MIC: Minimum inhibitory concentration.

Results and Discussion

A series of novel 2-pyrazoline derivatives were synthesised and evaluated for their antibacterial and antifungal activity. All the derivatives were efficiently synthesised by two or three step process.The structure of the newly synthesised compounds was elucidated by their ^{1}H NMR,LC-MS/MS, IR spectral data and melting point analysis. We have investigated newly synthesised pyrazoline bearing piperonal for their antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacterial strains by the disc diffusion method. Solvent and growth controls were kept, the zones of inhibition and minimum inhibitory concentrations (MIC) noted. Results of these studies are given in Table-2 and compared with the standard ciprofloxin. Most of them showed the moderate to low antibacterial activity.Among the compound 2a,2b was showed good inhibition towards all the four bacteria tested. Compounds 6a,6b were showed good activity in *Staphylococcus aureus,Escherichia coli* and Pseudomonas aeruginosa. Compounds 4a,4b and 5c shows moderate to low active against all the strains tested. Synthesised pyrazolines were screened for their antifungal activity against *Aspergillus niger, Candida albicans*. Antifungal activity was determined by

measuring the inhibition zone. The results of these studies were given in Table-2 and compared with the standard Keta conazole. Most of the compounds synthesised showed the moderate to low activity against all the fungi tested. Particularly compounds 2a,2b,6a,6b were active against all the above fungi tested.

Conclusion

We have successfully synthesised a new series of 2-pyrazoline derivatives and moreover, some of compounds contains bioactive heterocyclic moiety. The antimicrobial screening suggests that all the newly synthesised compounds showed moderate to good activity against the tested organisms.

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