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# SYNTHESIS OF 1, 3, 5-TRIARYL-4, 6-DIOXO-PYRROLO [3, 4-*d*]-7, 8-DIHYDROPYRZOLES AND THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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**ABSTRACT**

1,3,5-triaryl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrzoles (**3**) were synthesized by 1,3-dipolar cycloaddition reaction of nitrile imines generated *in situ* by the catalytic dehydrogenation of aromatic aldehyde hydrazones (**2**) with *N*-aryl maleimides (**1**). The new molecules were characterized by spectral analysis and were evaluated in vitro for their antibacterial, antifungal and antioxidant activities.

**Keywords**: Chloramine-T, maleimides, pyrazoles, antibacterial, antifungal, antioxidant.



## INTRODUCTION

Maleimide compounds are an important class of substrates for biological and chemical application[1]. In organic chemistry the maleimide functionality can be used as a synthetic platform in total synthesis due to its Michael-accepting ability and dienophillic nature[2-4]. In maleimide one of the carbonyl groups acts as an amide carbonyl and the other acts as a ketonic group, thus the double bond in maleimide portion is activated due to α,β-unsaturated system for the nucleophillic attack. The chemistry of five membered heterocycles has flourished with considerable intensity because of their synthetic and biological applications. A number of pyrazole derivatives have been reported to act as herbicides, fungicides, hypnotics, sedatives, antibacterial, analgesics, anti-inflammatory, anticonvulsant, diuretic and anti-mitotic activities[5]. Pyrazole derivatives were known to exhibit antimicrobial, antioxidant activities and reducing power abilities[6-7].

Nitrile imines undergo 1,3-dipolar cycloaddition reactions with various dipolarophiles leading to variety of pyrazoles, pyrazolines, pyrazolidines and other heterocyclic compounds. Numerous pyrazole derivatives by the reaction of *in situ* generated nitrile imines obtained from hydrazidoyl halides with sodium salt of active methylene compounds[8]. The oxidation of aldehyde hydrazones with CAT provides nitrile imines[9]. 1-Aryl-3-[nitro-2-thienyl]-4-aroyl pyrazoles synthesized by the 1,3-dipolar cycloaddition of 3-arylsydnones with 1-aryl-3-[5-nitro-2-thienyl]-2-propyn-1-ones have shown antibacterial and antifungal activity[10]. The versatile synthons 4-[2-bromoacetyl]-5-methyl-1-phenyl-3-phenylcarbomoyl-*1H*-pyrazole and 4-[(E)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbomoyl-*1H*-pyrazole were used as precursors for the synthesis of series of phenylpyrazoles with different aromatic ring systems[11]. Pyrazole derivatives are potent TNAP inhibitors exhibiting $IC_{50}$ values as low as 5nM[12].

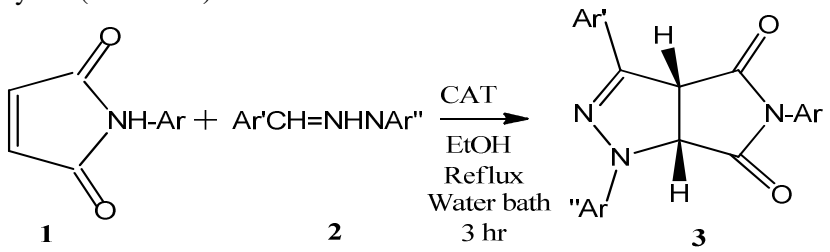
In view of the enormous amount of biological applications of associated with the pyrazolines and maleimide compounds, the molecules with both the moieties in them were synthesized with hope to get the molecules of diverse biological activity. This paper describes the synthesis of title compounds and in vitro evaluation of their antimicrobial and antioxidant activities.

## EXPERIMENTAL

The melting points were measured with micro melting point apparatus and are uncorrected. The chemicals/reagents used were purchased from sigma-aldrich chemicals (India) and Merck Chemicals

(India). IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (chemical ionization) and the important fragments are given with the relative intensities in the bracket. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Thin layer chromatography (TLC) was performed on a pre-coated Silica Gel sheets (HF 254, sd-fine) using benzene:ethyl acetate (7:2) eluent and visualization of the spots was done in iodine vapour and UV light. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane:ethyl acetate (8:1) as eluent.

In a typical reaction a mixture of *N*-aryl maleimide (**1**), aldehyde hydrazone (**2**) and chloramine-T in ethyl alcohol were refluxed on a water bath for three hours. After the completion of reaction followed by usual work up; the reaction afforded a 1,3,5-triaryl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazoles (**3**) in moderate to good yield (Scheme-1).




**1** **2** **3**

**3** (a) Ar = $C_6H_5$, Ar' = $C_6H_5$, Ar'' = $C_6H_5$
(b) Ar = $C_6H_5$, Ar' = 4-$OCH_3C_6H_4$, Ar'' = $C_6H_5$
(c) Ar = $C_6H_5$, Ar' = 3,4-$(OCH_3)_2C_6H_3$, Ar'' = $C_6H_5$
(d) Ar = $C_6H_5$, Ar' = 3,4,5-$(OCH_3)_3C_5H_2$, Ar'' = $C_6H_5$
(e) Ar = $C_6H_5$, Ar' = 4-$ClC_6H_4$, Ar'' = $C_6H_5$
(f) Ar = 4-$ClC_6H_4$, Ar' = $C_6H_5$, Ar'' = $C_6H_5$

Scheme-1

**General method for the synthesis of 1,3,5-triaryl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrzoles (3)**

In a typical reaction, a mixture of *N*-aryl maleimide **1** (0.01mole), phenyl hydrazone **2a** (0.01mole) and chloramine-T (0.02mole) in 20ml of 95% ethyl alcohol was refluxed on a water bath for 3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was extracted with ether (30ml), washed thoroughly with 10% sodium hydroxide (2 X 20mL) solution, water (3 X 20mL), finally with saturated brine solution (1 X 15mL) and then dried over anhydrous sodium sulphate. The solvent was evaporated to dryness on hot water bath. The product **3a** was obtained as a reddish brown gummy mass in 68% yield. The same procedure was used in all cases.

**Antimicrobial activity**

Antimicrobial activity of the synthesized compounds (**3a-f**) was done by paper disc diffusion method[13]. The compounds in methanol solution were tested against Gram-negative bacteria species *Escherichia coli, Salmonella typhimurium,* Gram-positive bacteria species *Bacillus substilis*, *Staphylococcus aureus* and against fungi species *Aspergillus niger, Aspergillus flavus, C. albicans, Fusarium oxysporium.* Streptomycin and Griseofulvin were used as standard drugs against bacteria and fungi species respectively. Minimum inhibitory concentrations (MICs) of the test samples (**3a-f**) against the different bacterium and fungi were determined by broth dilution technique. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound and controls were inoculated with approximately 5 x $10^5$ c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 hrs at $37^0$C and the growth was monitored visually and spectrophotometrically. The lowest concentration

required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). All the experiments were carried out in triplicate and the results were taken as a mean of three determinations.

**Antioxidant activity**

The antioxidant activity of the compounds (**3a-f**) on DPPH radical was estimated according to the method of Lai and co-workers[14]. Butylated hydroxyl toluene (BHT) was used as standard oxidant. The tests were performed in triplicate at different concentrations (10-50 µg/mL) and the results were taken as a mean of three determinations.

**1,3,5-Triphenyl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrzoles (3a)**

$^1$H NMR ($CDCl_3$): δ 3.26 (d, 1H, *J=6.22Hz*, CH $C_8$-H), 4.33 (d, 1H, *J=6.90Hz*, NCH $C_7$-H), 6.76-7.50 (m, 15H, Ar, Ar', Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 41.0 ($C_8$), 50.2 ($C_7$), 112.2 (2C, Ar"-C), 117.0 (1C, Ar"-C), 120.1 (2C, Ar-C), 124.0 (1C, Ar-C), 128.5 (4C, Ar', Ar-C), 129.0 (2C, Ar'-C), 129.4 (2C, Ar"-C), 130.2 (1C, Ar'-C), 131.4 (1C, Ar'-C), 140.6 (1C, Ar-C), 144.0 (1C, Ar"-C), 156.2 ($C_3$), 170.4 ($C_4$,$C_6$, CO). MS (relative intensity) m/z: 367 ($M^+$, 24), 276 (32), 264 (46), 248 (18), 236 (26), 220 (66), 192 (52), 171 (38), 143 (16), 91 (100). Anal. Calcd. for $C_{23}H_{17}N_3O_2$; C, 75.19%; H, 4.66%; N, 11.44%. Found; C, 75.11%; H, 4.52%; N, 11.33%.

**3-(4-Methoxy phenyl)-1,5-diphenyl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazole (3b)**

Obtained as brown oil in 62% yield. $^1$H NMR ($CDCl_3$): δ 3.38 (d, 1H, *J=6.45Hz*, CH $C_8$-H), 3.80 (s, 3H, $OCH_3$), 4.50 (d, 1H, *J=6.65Hz*, NCH $C_7$-H), 6.55 (s, 2H, Ar"-$C_2$-H, $C_6$-H ), 6.65 (s, 1H, Ar"-$C_4$-H), 6.80 (s, 2H, Ar'-$C_3$-H, $C_5$-H), 7.10-7.52 (m, 9H, Ar, Ar', Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 41.8 ($C_8$), 50.2 ($C_7$), 56.2 ($OCH_3$), 112.5 (2C, Ar"-C), 114.2 (2C, Ar'-C), 116.6 (1C, Ar"-C), 120.3 (2C, Ar-C), 123.3 (2C, Ar'-C), 124.1 (1C, Ar-C), 128.7 (2C, Ar-C), 129.0 (2C, Ar'-C), 129.2 (2C, Ar"-C), 130.2 (2C, Ar'-C), 140.8 (1C, Ar-C), 143.6 (1C, Ar"-C), 156.8 ($C_3$), 164.4 (1C, Ar'-C), 170.0 (2C, CO). MS (relative intensity) m/z: 397 ($M^+$, 28), 367 (20), 276 (34), 264 (28), 248 (46), 236 (22), 220 (58), 192 (40), 171 (18), 143 (26), 91 (100). Anal. Calcd. for $C_{24}H_{19}N_3O_3$; C, 72.53%; H, 4.82%; N, 10.57%. Found. C, 72.40%; H, 4.76%; N, 10.46%.

**3-(3,4-Dimethoxy phenyl)-1,5-diphenyl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazole (3c)**

Obtained as light brown oil in 48% yield. $^1$H NMR ($CDCl_3$): δ 3.50 (d, 1H, *J=5.6Hz*, CH $C_8$-H), 3.85 (s, 6H, $OCH_3$), 4.60 (d, 1H, *J=6.25Hz*, NCH $C_7$-H), 6.50 (s, 2H, Ar"-$C_2$-H, $C_6$-H ), 6.70 (s, 1H, Ar"-$C_4$-H), 6.85 (s, 1H, Ar'-$C_5$-H, $C_5$-H), 7.05-7.65 (m, 9H, Ar, Ar', Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 42.9 ($C_8$), 52.8 ($C_7$), 56.8 (2C, $OCH_3$), 112.1 (2C, Ar"-C), 115.3 (2C, Ar'-C), 116.9 (1C, Ar"-C), 120.8 (2C, Ar-C), 122.3 (1C, Ar'-C), 124.4 (1C, Ar-C), 124.8 (1C, Ar'-C), 128.4 (2C, Ar-C), 129.8 (2C, Ar"-C), 143.2 (1C, Ar"-C), 147.5 (1C, Ar'-C), 149.8 (1C, Ar'-C), 157.6 ($C_3$), 171.0 (2C, CO). MS (relative intensity) m/z: 427 ($M^+$, 22), 397 (28), 367 (20), 276 (38), 264 (24), 248 (52), 236 (24), 220 (60), 192 (44), 171 (16), 143 (28), 91 (100). Anal. Calcd. for $C_{25}H_{21}N_3O_4$; C, 70.25%; H, 4.95%; N, 9.83%. Found. C, 70.12%; H, 4.85%; N, 9.71%.

**1,5-Diphenyl-3-(3,4,5-trimethoxy phenyl)-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazole (3d)**

Obtained as light brown oil in 66% yield. $^1$H NMR ($CDCl_3$): δ 3.40 (d, 1H, *J=6.25Hz*, CH $C_8$-H), 3.96 (s, 9H, $OCH_3$), 4.38 (d, 1H, *J=6.30Hz*, NCH $C_7$-H), 6.50-6.80 (m, 5H, Ar', Ar"-H), 7.10-7.60 (m, 7H, Ar, Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 41.7 ($C_8$), 50.2 ($C_7$), 56.4 (3C, $OCH_3$), 108.0 (2C, Ar'-C), 112.6 (2C, Ar"-C), 116.6 (1C, Ar"-C), 120.6 (2C, Ar-C), 124.6 (1C, Ar-C), 125.8 (1C, Ar'-C), 128.8 (2C, Ar-C), 129.6 (2C, Ar"-C), 135.2 (1C, Ar'-C), 140.2 (1C, Ar-C), 143.6 (1C, Ar"-C), 148.3 (2C, Ar'-C), 155.8 ($C_3$), 171.8 (2C, CO). MS (relative intensity) m/z: 457 ($M^+$, 20), 427 (16), 397 (28), 367 (36), 276 (40), 264 (24), 248 (56), 236 (22), 220 (62), 192 (48), 171 (20), 143 (32), 91 (100). Anal. Calcd. for $C_{26}H_{23}N_3O_5$; C, 68.26%; H, 5.07%; N, 9.19%. Found. C, 68.14%; H, 4.99%; N, 9.10%.

**3-(4-Chlorophenyl)-1,5-diphenyl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazole (3e)**
Obtained as light gummy mass in 43% yield. $^1$H NMR ($CDCl_3$): δ 3.25 (d, 1H, *J=6.40Hz*, CH $C_8$-H), 4.44 (d, 1H, *J=6.80Hz*, NCH $C_7$-H), 6.50 (s, 2H, Ar"-$C_2$-H,$C_6$-H), 6.60 (s, 1H, Ar"-$C_4$-H, 7.00-7.65 (m, 11H, Ar, Ar', Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 41.9 ($C_8$), 50.4 ($C_7$), 112.3 (2C, Ar"-C), 116.3 (1C, Ar"-C), 120.2 (2C, Ar-C), 124.2 (1C, Ar-C), 125.8 (1C, Ar'-C), 128.5 (2C, Ar-C), 129.1 (4C, Ar', Ar"-C), 129,4 (1C, Ar'-C), 130.3 (2C, Ar'-C), 136.5 (1C, Ar-C), 143.2 (1C, Ar"-C), 155.1 ($C_3$), 172.6 (2C, CO). MS (relative intensity) m/z: 403 ($M^+$, $^{37}$Cl, 12), 401 ($M^+$, $^{35}$Cl, 28), 310 (42), 282 (32), 264 (16), 254 (64), 236 (26), 227 (20), 171 (52), 143 (36), 91 (100). Anal. Calcd. for $C_{23}H_{16}N_3O_2Cl$; C, 68.74%; H, 4.01%; N, 10.46%, Cl, 8.82%. Found. C, 68.61%; H, 3.94%; N, 10.32%, Cl, 8.73%.

**5-(4-Chlorophenyl)-1,3-diphenyl-4,6-dioxo-pyrrolo[3,4-*d*]-7,8-dihydropyrazole (37f)**
Obtained as light brown gummy mass in 38% yield. $^1$H NMR ($CDCl_3$): δ 3.25 (d, 1H, *J=6.40Hz*, CH $C_8$-H), 4.44 (d, 1H, *J=6.80Hz*, NCH $C_7$-H), 6.50 (s, 2H, Ar"-$C_2$-H,$C_6$-H), 6.60 (s, 1H, Ar"-$C_4$-H, 7.00-7.65 (m, 11H, Ar, Ar', Ar"-H). $^{13}$C NMR ($CDCl_3$): δ 41.8 ($C_8$), 50.8 ($C_7$), 112.6 (2C, Ar"-C), 117.2 (1C, Ar"-C), 121.7 (2C, Ar-C), 128.6 (2C, Ar'-C), 129.0 (2C, Ar'-C), 129.5 (4C, Ar, Ar"-C), 129.9 (1C, Ar-C), 130.2 (1C, Ar'-C), 131.3 (1C, Ar'-C), 138.9 (1C, Ar-C), 143.6 (1C, Ar"-C), 155.4 ($C_3$), 170.2 (2C, CO). m/z: 401 ($M^+$, $^{35}$Cl, 26), 298 (40), 276 (22), 270 (28), 248 (64), 220 (36), 205 (20), 177 (52), 127 ($M^+$, $^{37}$Cl, 12), 125 (100), 91 (86). Anal. Calcd. for $C_{23}H_{16}N_3O_2Cl$; C, 68.74%; H, 4.01%; N, 10.46%, Cl, 8.82%. Found. C, 68.61%; H, 3.94%; N, 10.32%, Cl, 8.73%.

## RESULTS AND DISCUSSION

The IR, $^1$H NMR, $^{13}$C NMR, Mass spectra and elemental analysis provided the structure of the products. For instance, all the products gave the medium absorption bands due to C=N stretching in the wavelength region 1685-1645cm$^{-1}$ in the IR spectrum. The absorption bands expected due to C=C stretching of the precursors *N*-aryl maleimides in the region 680cm$^{-1}$ was found absent. The absorption bands expected due to the secondary N-H (=NH-) stretching of the other starting material phenyl hydrazones in the region 3310-3160cm$^{-1}$ were also found absent. These observations in the IR spectra favor the formation of the cycloadducts.

The $^1$H NMR spectra of the products displayed the signals due to CH ($C_8$-H) protons appeared as doublet in the region δ 3.2-3.5 ppm. The signals due to NCH ($C_7$-H) appeared in the down field as doublet in the region δ 4.2-4.6 ppm due to the fact that, this proton carrying carbon atom is bonded to the electronegative nitrogen atom. The coupling constant (*J*) values calculated for $C_7$-H and $C_8$-H protons were in the range *J= 5.6-6.9 Hz* which suggests that cycloaddition leading to the formation of *cis* adducts. The expected signals due to the olefinic C-H (=CH) protons of starting material *N*-aryl maleimides (**2**) in the region δ 6.90 - 7.0 ppm were found absent. The signals expected due to CH protons in the region δ 8.2-8.4 ppm and NH protons in the region δ 3.5-4.50 ppm of the other starting materials phenyl hydrazones (**2**) were also found absent.

The $^{13}$C NMR spectra gave the consistent pattern signals due to $C_3$- (CH=) carbon atoms in the region δ 155.2–157.6 ppm due to $C_7$- carbon atoms in the region δ 50.7-52.8 ppm and due to $C_8$- carbon atoms in the region δ 41.0-42.9 ppm. The expected signals due to C=C carbon atoms of the starting materials *N*-aryl maleimides in the region δ 135.0-137.0 ppm were found absent.

All cycloadducts gave significantly stable molecular ion peaks with relative abundance ranging from 16-66%. The common possible fragmentation patterns involve some rearrangement with the removal of smaller molecules viz. CO, $N_2$, $C_2H_2$, HCHO, $C_6H_5CN$, etc. All gave satisfactory elemental analysis with deviation of 0.1-0.3% from the theoretical values.

The experimental results indicated that the test compounds (**3a-f**) exhibited significant antibacterial activity against the bacterium *Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli* and moderately active against *Bacillus substilis*. The compounds (**3a-f**) were found highly resistant against the fungi *Aspergillus niger* and *Fusarium oxysporium,* moderate resistant *Aspergillus flavus* and very weak resistant against *C. albicans*. The results showed that the presence of electron donating substituents like –$OCH_3$ on the aromatic ring enhanced the inhibition against the all organisms. The compound **3e-f**

containing chloro-substituents have shown greater resistant against all the organisms in compared to the other test compounds. From the MIC's determination results, it was found that the compound **3e-f** containing chloro-substituents have the least MIC values against all the bacterium and fungi species tested.

All the synthesized compounds showed promising free radical scavenging ability, but of lesser activity when compared with the standard antioxidant. At the initial concentrations of (10-20 µg/mL), no much significant variations in the free radical scavenging ability of samples **3a-f** was observed. However, when the concentration was increased (30-50 µg/mL) all showed a promising radical scavenging ability. The compounds **3a-d** showed radical scavenging ability up to 55%, whereas compounds **3e-f** showed radical scavenging ability up to 40% with reference to the standard antioxidant. These experimental results indicate the potential electron donating ability of synthesized compounds.

## REFERENCES

1. Michael A Walker, *J. Org. Chem*., **60**, 5352 (1995).
2. S.W. Baldwin, P. Greenspan, C. Alaimo and A.T. Mcphail, *Tetrahedron Letters*, **32**, 5877 (1991).
3. Yoshitsugu Arai, Makoto Matsui, Akihito Fujii, Tohru Kontani, Toshiyuki Ohno, Toru Koizumi and Motoo Shiro, *J. Chem. Soc. Perkin Trans.,* **1**, 25 (1994).
4. Ronald Grigg, Sivagnanasundram Surendra kumar, Sunit Thianpatanagul and David Vipond, *J. Chem. Soc., Perkin Trans.,* **1**, 2693 (1988).
5. Anjani Solankee and Yogesh Prajapati, *Rasayan J. Chem*., **2**, 23 (2009).
6. K.B. Umesha, K.M.L. Rai and M.A. Harish Nayaka, *Int. J. Biomed. Sci.,* **5,** 359 (2009).
7. K.B. Umesha, K.M.L. Rai and K. Ajay Kumar, *Indian J. Chem*., **41B,** 1450 (2001).
8. A.S. Shawali, *Chem. Rev.*, **93**, 2731 (1993).
9. A. Hassner and K.M.L. Rai, *Synthesis*, 57 (1989).
10. N. Satheesha Rai, Balakrishna Kalluraya, B. Lingappa, Shaliny Shenoy and Vedavati G Puranic, *Eur. J. of Med. Chem*., **43**,1715(2008).
11. Ahmad M. Farag, Abdelrahman S. Mayhoub, Saber E. Barakat and Ashraf H. Bayomi, *Bioorg. and Med. Chem*., **16**, 4569 (2008).
12. S. Sidiqui, R. Ardecky, Y. Su, S. Narisawa, B. Brown, J.L. Millan, E. Sergienko and N.D. Cosford, *Bioorg. and Med. Chem. Lett*., **19**, 222 (2009).
13. S. Lemriss, B. Marquet, H. Ginestet, A. Fassouane and P. Bairon, *J. Mycol. Med.,* **13,** 189 (2003).
14. L.S. Lai, S.T. Chou and W.W Chao*, J. Agri. Food Chem*. **49,** 963 (2001).

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