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# Synthesis and evaluation of novel phthalimide derivatives as analgesic and antiinflammatory agents

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## Abstract

A series of phthalimide derivatives were synthesized and evaluated for their analgesic and antiinflammatory activity. The synthesized compounds **3a-j** were characterized by IR and NMR spectral data. All compounds were screened for their analgesic and antiinflammatory activity. Compounds **3a-j** were screened for antiinflammatory activity against carrageenin induced rat paw edema. Except compound **3b** all exhibited good antiinflammatory activity, while compounds **3d**, **3h** and **3j** exhibited good analgesic activity when screened against acetic acid induced writhings in mice.

**Key Words**: phthalimide, pyrazoline, antiinflammatory, analgesic.

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## Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli.The process of inflammation is not undesirable; it is a protective mechanism essential for survival [1]. On the other hand, in certain situations, the inflammation process may cause a considerable harm. Many diverse stimuli like thermal (heat or cold); chemical (foreign substances, foreign organisms, drugs); or mechanical (trauma) may initiate the inflammatory process. The most important activator of the inflammatory response is the mast cell, which initiates inflammation by releasing biochemical mediators (e.g. Histamine, chemotactic factors) from preformed cytoplasmic granules and synthesizing other mediators (e.g. prostaglandins, leukotrienes) in response to stimulus.

Enhanced tumor necrosis factor-α (TNF-α) synthesis is associated with the development of rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease [2]. Tumor necrosis factors (or the TNF family) refer to a group of cytokines family that can cause apoptosis.

The functioning of the immune system is finely balanced by the activities of pro-inflammatory, particularly, tumor necrosis factor-α (TNF-α) and anti-inflammatory mediators or cytokines. Unregulated activities of these mediators can lead to the development of serious inflammatory diseases. Though a number of popular NSAID are available to treat inflammation and pain, they all suffer from numerous mild to serious side effects [3]. Thus there is need for newer and safer analgesic and antiinflammatory agents. Thalidomide is one of the small-molecule TNF-α inhibitors. It has been approved to treat moderate to severe leprosy and clinical trials are ongoing for its use in cancer (multiple myeloma) and RA [4]. Discovery of thalidomide as TNF-α inhibitor has led to an impetus on development of phthalimide derivatives as potential analgesic-antiinflammatory agents [5-8]. In the present work we report synthesis of novel phthalimide derivatives and their screening for analgesic and anti-inflammatory activity in widely used animal models.

**Results and Discussion**

The intermediate chalcones **1a-j** and the corresponding pyrazolines **2a-j** were synthesized using reported procedures [9-11]. Pyrazolines **2a-j** when treated with phthalic anhydride in presence of glacial acetic acid furnished phthalimide derivatives **3a-j** in 60-80 % yields. The synthesized phthalimide derivatives were freely soluble in acetone and chloroform. All the compounds obtained are solids melting at around the range of 210-265 $^0$C (Table 1). The solid state IR (KBr,cm$^{-1}$) spectra of these compounds reveal a characteristic aromatic stretch between 3000-3100 cm$^{-1}$ and sharp carbonyl stretching vibration for phthalimide at 1708.99 cm$^{-1}$, 1734.06 cm$^{-1}$ and 1766.85 cm$^{-1}$ . No peak was observed for free amino group at around 3342.75 cm$^{-1}$ indicating incorporation of this group into the phthalimide ring. The $^1$H NMR spectra were recorded in $CDCl_3$. The pyrazoline 5-H revealed *dd* at around 5.25 ppm, and pyrazoline 4-$CH_2$ revealed *dd* at around 3.87 & 3.15 ppm. The aromatic protons appeared at 6.80-7.98 ppm as multiplet. Absence of singlet by amino protons confirmed incorporation of this group into the phthalimide ring.

The compounds **3a-j** were tested *in vivo* for antiinflammatory and analgesic activity using popularly used animal models. The data for anti-inflammatory activity is given in Table 2. The results indicate that except compound **3b** all the synthesized compounds significantly inhibit edema. Compound **3a** containing a *p*-chloro substitution on the phenyl ring was found to be most active. Carrageenin induced paw edema was taken as a prototype of exudative phase of inflammation. Inflammation is a biphasic event where, initial phase is due to release of histamine, serotonin and kinins in the first hour after carrageenin injection and more pronounced second phase is related to release of prostaglandin like substances in 2-3 h [ 12-14]. Hence significant inhibition of edema may be due to an inhibitory effect exerted on inflammatory mediators released in response to phlogogenic stimuli.

Results of analgesic activity presented in Table 3 indicate that compound **3j** containing *m*-bromo group on the phenyl ring is most active. Also compounds **3d** and **3h** show good analgesic

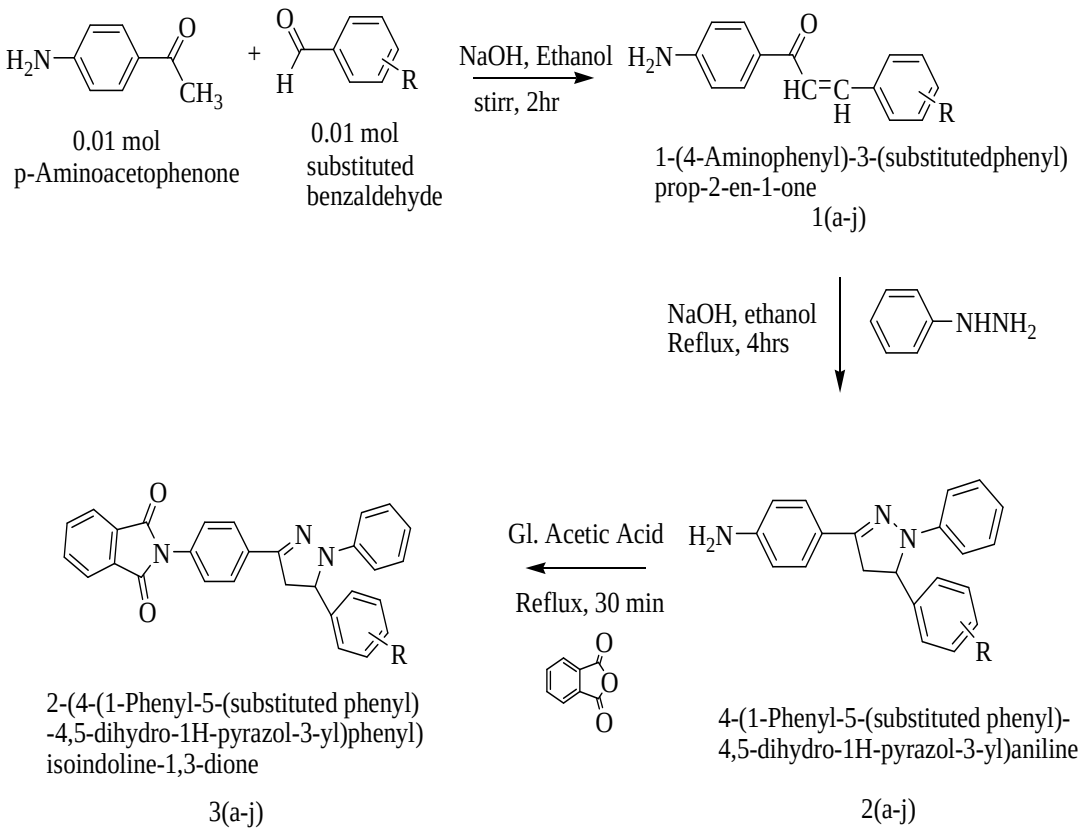
activity while compounds **3e** and **3i** are moderately active in acetic acid induced writhing in mice model.

## Materials and Methods

The melting points are uncorrected and were determined on Veego (model:-VMP-D) electronic apparatus. The IR spectra were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide. The $^1$H NMR spectra were recorded on Varian-Mercury 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard (chemical shift in δ ppm). All chemicals used in the synthesis were of laboratory grade.

**General procedure for synthesis of *2-(4-(4, 5-dihydro-1-phenyl-5-(substituted phenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3a-j).**

A mixture of 4-(4,5-dihydro-1-phenyl-5-(substituted phenyl)-1H-pyrazol-3-yl)-benzenamine **2a-j** (0.5 g) and phthalic anhydride (0.5 g) was taken in 100 ml RBF containing 5 ml of glacial acetic acid. The reaction mixture was heated under reflux for 30 min. and was then allowed to cool to room temperature. The solid thus obtained was filtered and recrystalized from ethanol. The entire synthetic route has been shown in scheme 1. The physical data is given in Table 1.




**Scheme 1. Synthesis of target compounds**

**Table1 : physical data of phthalimide derivatives (3a-j)**

| Code No | R- Group | M.P. ($^oC$) | $R_f$ Value | Yield(%) |
|---|---|---|---|---|
| 3a | *p*-Cl | 251-253 | 0.50 | 79 |
| 3b | *o*-Cl | 225-227 | 0.42 | 60 |
| 3c | *m*-Cl | 240-241 | 0.61 | 76 |
| 3d | *o*-$NO_2$ | 225-228 | 0.45 | 64 |
| 3e | *p*-$NO_2$ | 231-233 | 0.52 | 62 |
| 3f | *p*-$OCH_3$ | 208-210 | 0.60 | 77 |
| 3g | 3,4,5$(OCH_3)_3$ | 243-244 | 0.72 | 71 |
| 3h | H | 261-262 | 0.58 | 75 |
| 3i | *p*-Br | 215-216 | 0.65 | 69 |
| 3j | *m*-Br | 244-245 | 0.59 | 74 |

***2-(4-(4,5-Dihydro-1-phenyl-5-(4-chlorophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3a).**
IR (KBr, $cm^{-1}$): 3065.96 (Ar CH), 1734.06, 1708.99(C=O), 1516.10, 1498.74(C=N), 1491. 02(C=C), 831.35(C-Cl); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd,Ha), 3.83(1H,dd,Hb), 5.15(1H,dd, Hc), 6.8-8.0(m, Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(2-chlorophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3b).**
IR (KBr, $cm^{-1}$): 3065.96(Ar CH), 1734.06, 1712.85 (C=O), 1519.96, 1498.74(C=N), 1471.74(C=C), 831.36(C-Cl); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H, dd, Ha), 3.83(1H,dd, Hb), 5.75(1H,dd, Hc), 6.8-8.4(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(3-chlorophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3c).**
IR (KBr, $cm^{-1}$): 3057.27(ArCH), 1735.99, 1712.85(C=O), 1518.03, 1498.74(C=N), 1478.01(C=C), 833.28(C-Cl); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(2-nitrophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3d).**
IR (KBr, $cm^{-1}$): 3073.26(Ar CH), 1724.42, 1691.63( C=O), 1514.51(C=N), 1492.61(C=C), 1583.87, 1381.08($NO_2$); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(4-nitrophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3e).**
IR (KBr, $cm^{-1}$): 3073.26(Ar CH), 1724.42, 1691.63( C=O), 1514.51(C=N), 1492.61(C=C), 1555.87, 1381.08($NO_2$); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(4-methoxyphenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3f).**
IR (KBr, $cm^{-1}$): 3065.96(Ar CH), 3007.55(aliphatic CH), 1708.99, 1674.27(C=O), 1514.17, 1498.34(C=N), 1489.10(C=C); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc),3.78(3H, $OCH_3$) 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(3,4,5trimethoxyphenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3g).**
IR (KBr, $cm^{-1}$): 3053.42(Ar CH), 2935.76, 2837.38(aliphatic CH), 1737.92, 1712.85(C=O), 1496.81(C=N), 1462.09 (C=C); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc),3.78(9H, $OCH_3$) 6.8-8.0(m, Ar-H).

***2-(4-(4,5-Dihydro-1,5diphenyl)-1H-pyrazol-3-yl)phenyl) isoindoline-1,3-dione* (3h).**
IR (KBr, $cm^{-1}$): 3029.45(Ar CH), 1735.99, 1714.37(C=O), 1519.96, 1498.74(C=N); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(4-bromophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3i).**
IR (KBr, $cm^{-1}$): 3045.26(Ar CH), 1735.99, 1708.99(C=O), 1498.74(C=N), 1487.17(C=C), 786.25(C-Br); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***2-(4-(4,5-Dihydro-1-phenyl-5-(3-bromophenyl)-1H-pyrazol-3-yl)phenyl)-isoindoline-1,3-dione* (3j).**
IR (KBr, $cm^{-1}$): 3073.26(Ar CH), 1735.99, 1716.70(C=O), 1519.96, 1498.74(C=N), 1478.01(C=C), 789.35(C-Br); $^1H$ NMR ($CDCl_3$, δ ppm): 3.18(1H,dd, Ha), 3.83(1H,dd, Hb), 5.15(1H,dd, Hc), 6.8-8.0(m,Ar-H).

***Anti inflammatory activity against carrageenin induced rat paw edema***

Antiinflammatory activity was determined by carrageenin induced rat paw edema model of Winter et al [15]. Sprague Dawley rats of either sex weighing 150-250 g were used. Animals were housed in a group of six per cage. A 12:12 light: dark cycle was followed during the experiment. Animals had free access to food and water, however, food was withdrawn six hours before and during experiment. The standard groups received orally aspirin 250 mg/kg body weight suspended in 1% acacia in distilled water. The test group orally received synthesized compounds **3a-j** 250 mg/kg body weight suspended in 1% acacia in distilled water. The control animals received 1% acacia suspension. The test compounds and standard were administered one hour prior to the carrageenin injection. Before performing these experiments, ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Academy guidelines for the use and care of experimental animals (CPCSEA Reg. No.311).Acute inflammation was induced by injecting freshly prepared 0.1% w/v aqueous solution of carrageenin in the subplanter region of right hind paw. After carrageenin injection the paw volume was measured after 1, 2 and 3 h by plathysmometer (UGO-Basile, Italy). Any

significant reduction in the volume of the paw compared to the control group was considered as antiinflammatory response. The results are presented in Table 2 and graphically in Figure 1. Percent inhibition of inflammation after 3 h was calculated by using following formula.

$$\% \text{ inhibition} = (1- Vt_3-Vt_0/ Vc_3-Vc_0) \text{ X } 100.$$

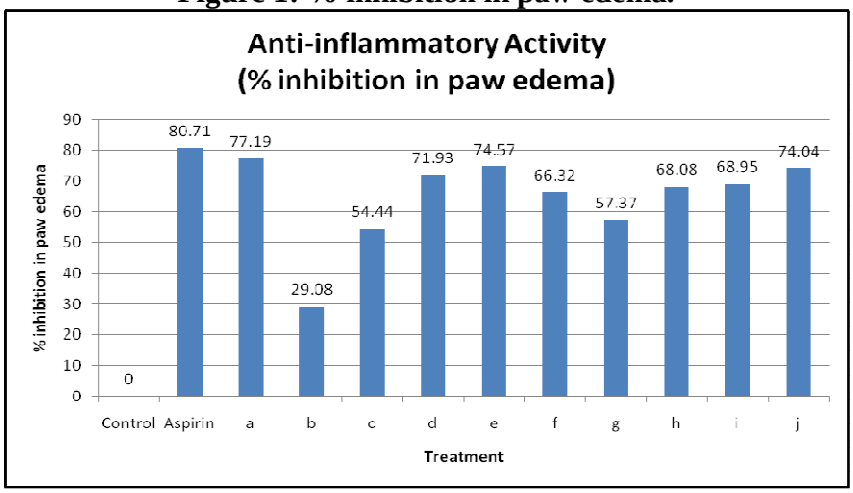
Where, Vt = mean relative change in paw volume in test group, Vc = mean relative change in paw volume in control group.

**Table 2 : Effect of phthalimide derivatives (3a-j) on carrageenin induced paw edema in rat**

| Group | R | Mean paw volume Mean±SEM | % Edema Inhibition In Rats(180 min) |
|---|---|---|---|
| Control | | 1.56 ± 0.0114 | 0.0 |
| Aspirin | | 1.10 ± 0.0151** | 80.71 |
| 3a | *p*-Cl | 1.21 ± 0.0274** | 77.19 |
| 3b | *o*-Cl | 1.51 ± 0.0157$^{ns}$ | 29.08 |
| 3c | *m*-Cl | 1.33 ± 0.0270** | 54.44 |
| 3d | *o*-$NO_2$ | 1.18 ± 0.0335** | 71.93 |
| 3e | *p*-$NO_2$ | 1.19 ± 0.0304** | 74.57 |
| 3f | *p*-$OCH_3$ | 1.29 ± 0.0298** | 66.32 |
| 3g | 3,4,5$(OCH_3)_3$ | 1.27 ± 0.0204** | 57.37 |
| 3h | H | 1.31 ± 0.0272** | 68.08 |
| 3i | *p*-Br | 1.25 ± 0.0168** | 68.95 |
| 3j | *m*-Br | 1.23 ± 0.0197** | 74.04 |

Dose: 250 mg/ kg body wt. for standard and test compounds. n=6, ** - P < 0.01, $^{ns}$- non significant, compared with control (saline) group. Data expressed as Mean ± SEM. Data was analyzed by one-way ANNOVA followed by Dunnett's test.

**Figure 1: % inhibition in paw edema.**



***Analgesic activity against acetic acid induced writhing in mice***

Analgesic activity was determined by acetic acid induced writhing in mice model of Koster et al [16]. Albino mice of either sex weighing 25-30 g were used. The test compounds **3a-j**, standard (diclofenac sodium 20 mg/kg body weight) and control were administered orally to mice and 0.6% acetic acid solution (10 ml/kg) was injected intraperitonialy after the administration of synthesized compounds and standard. The number of writhings in each mouse was observed for 20 min period starting 10 min after injection of acetic acid. Analgesic activity was expressed as percentage of inhibition of number of writhings, when compared with the control group. Percentage analgesic activity of compounds was calculated using following formula,

% analgesic activity =

$$\left( \frac{\text{No. of writhing in test - No. of writhing in control}}{\text{No. of writhing in control}} \right) \text{X } 100$$

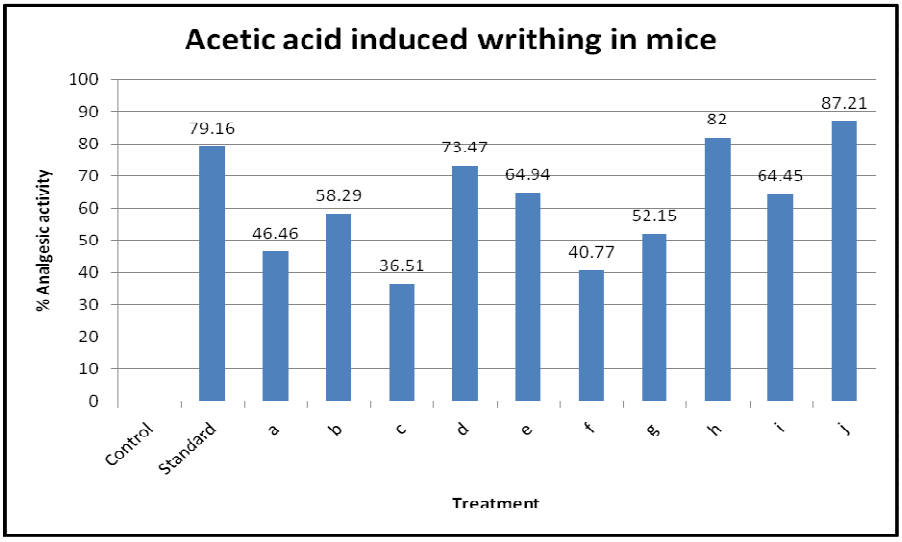
The results are presented in Table 3 and graphically in Figure 2.

**Table 3: Analgesic activity of phthalimide derivative (3a-j) in acetic acid induced writhing in mice.**

| Group | R | No of writhings in 10 min(mean±SEM) | % Analgesic activity |
|---|---|---|---|
| Control (saline) | - | 35.17 ± 1.167 | -- |
| Aspirin(Standard) | - | 4.5 ± 0.4282** | 79.16 |
| 3a | *p*-Cl | 7.33 ± 07601** | 46.46 |
| 3b | *o*-Cl | 18.83 ± 1.138** | 58.29 |
| 3c | *m*-Cl | 14.67 ± 1.838** | 36.51 |
| 3d | *o*-$NO_2$ | 22.33 ± 0.8819** | 73.47 |
| 3e | *p*-$NO_2$ | 9.33 ± 0.8819** | 64.94 |
| 3f | *p*-$OCH_3$ | 12.33 ± 1.453** | 40.77 |
| 3g | 3,4,5$(OCH_3)_3$ | 20.83 ± 0.7923** | 52.15 |
| 3h | H | 16.83 ± 1.167** | 82.00 |
| 3i | *p*-Br | 6.33 ± 0.9545** | 64.45 |
| 3j | *m*-Br | 12.5 ± 0.6191** | 87.21 |

Dose: 250 mg/ kg body wt. for test compounds and 20 mg/kg body wt for standard drug. n=6, ** - $P < 0.01$, $^{ns}$- non significant, compared with control (saline) group. Data expressed as Mean ± SEM. Data was analyzed by one-way ANNOVA followed by Dunnett's test.

**Figure 2: % inhibition of acetic acid induced writhing in mice**




## Conclusion

A novel series of phthalimide derivatives incorporating a pyrazoline ring have been successfully synthesized and tested for anti-inflammatory action. Compound **3a** was found to be most active in the carrageenin induced paw edema model while compound **3j** displayed best activity in the acetic acid induced writhing model.

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