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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF NOVEL OXADIAZOLE DERIVATIVES

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1, 3, 4-oxadiazole, Secondary amines, Synthesis, Anti-inflammatory activity, antibacterial activity

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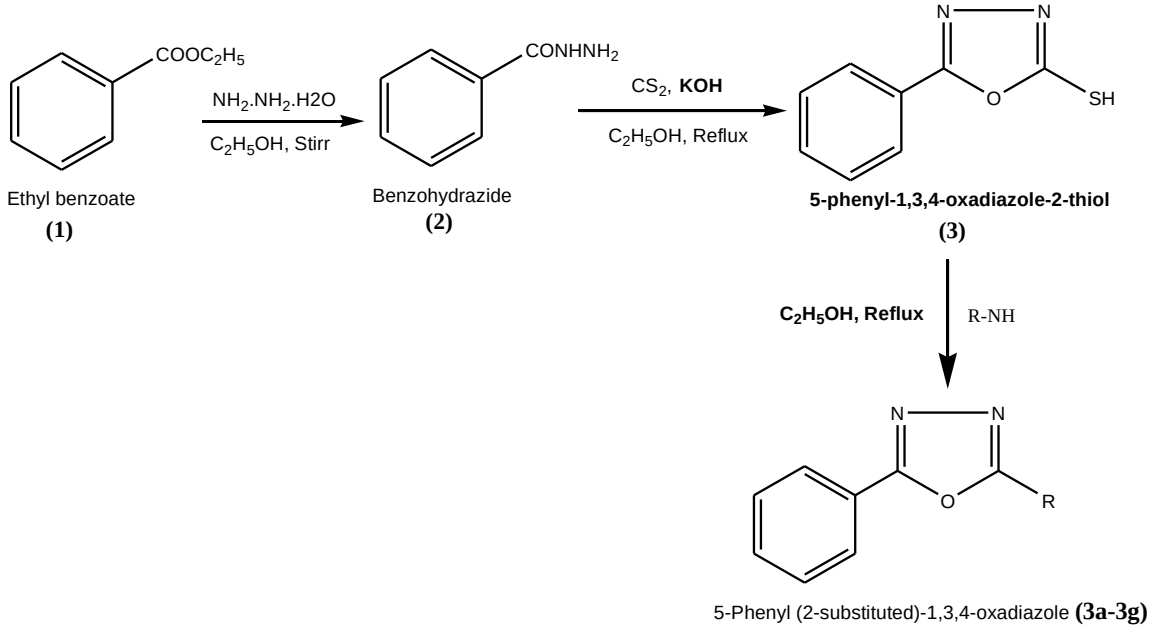
ABSTRACT

New 5- phenyl- 1, 3, 4- oxadiazole- 2- thiol derivative was synthesized by the ring closure reactions of benzohydrazides with carbon disulphide in presence of ethanolic KOH followed by substitution with secondary amines at 2^{nd} position. All the newly synthesized compounds were characterized by IR, NMR and LC-MASS spectral data. Most of them were tested for their anti-inflammatory and antibacterial activity.

INTRODUCTION: Oxadiazoles are used as scaffolds on which pharmacophores are arranged to provide potent and selective drugs. The synthesis of 1, 3, 4-oxadiazoles is of considerable interest due to their various biological activities such as anti-bacterial [1], anti-fungal [2], antitubercular activity [3], analgesic [4], anti-inflammatory [5] and anti-convulsant activity [6]. The novel oxadiazole derivatives are synthesized by cyclic condensation of hydrazine hydrate with CS_2 in the presence of alcoholic KOH to give corresponding mercapto derivatives of oxadiazole and can be further substituted with secondary amines [7].

The selected compounds are evaluated for their anti-inflammatory and antibacterial activity [8]. The completions of above reactions are confirmed by performing TLC. The structures of above synthesized compounds are confirmed by IR, ^{1}H NMR and LC-MASS spectroscopic methods. The IR spectra of benzohydrazides shows characteristic absorption band in the region of 3300 cm^{-1}, 1604cm^{-1}, 1658cm^{-1} due to -NH, -NH_2 and C=O group respectively. IR spectra of 5-Phenyl-1,3,4-oxadiazole-2-thiol shows absorption band in the region of 2567cm^{-1}(SH), 1610cm^{-1} (C=N), 1082cm^{-1}(C-O-C). The structure of 5-Phenyl-1,3,4-oxadiazole-2-thiol is well supported by ^{1}H NMR spectra which shows 7.50-7.59 (m,3H, Ar-H), 7.89-7.91 (m, 2H, Ar-H), 2.58 (s, 1H, SH). The sequence of reactions is as shown in following Scheme.

EXPERIMENTAL: All the melting points were determined in open capillaries, expressed in °C and are uncorrected. The purity of the synthesized compounds and completion of reactions were ascertained by performing thin layer chromatography on silica gel G in various solvent systems and visualized by exposure to iodine vapors or UV- radiation. The IR spectra of the compounds were recorded on SHIMADZU IR AFFINITY FTIR using KBr discs and the values are expressed in cm^{-1}. The ^{1}H NMR spectra of the selected compounds were recorded on FT ^{1}H NMR spectrophotometer in DMSO-d_6 using TMS as an internal standard and the values are expressed in ppm. The MASS Spectra of synthesized compounds were measured by Micromass Q-TOF MICRO.



SCHEME 1

STEP 1: Synthesis of Benzohydrazide (2): Ethyl benzoate (2.78 ml, 0.04 moles) was dissolved in ethanol (10ml) and hydrazine hydrate (3.88ml, 0.08 moles) was added drop wise and stirred for 5 hour. In between, the completion of the reaction was monitored by TLC. After completion of reaction, the solvent was distilled out to get the solid product of Benzohydrazide **(2)**. The product was dried and recrystallized from ethanol.

Yield: 68%, **M.P.**: 98-100^{o}C, **R_f** :0.67

STEP 2: Synthesis of 5-Phenyl-1, 3, 4-oxadiazole-2-thiol (3): Benzohydrazide **(2)** (1.36 gm, 0.01 mole) was dissolved in ethanol. To this potassium hydroxide pellets (0.4gm, 0.01mole) was added followed by drop wise addition of Carbon disulphide (3ml, 0.11mole). The reaction mixture was stirred for 15 minutes. Then it was refluxed until the evolution of hydrogen sulphide gas ceased. In between, the completion of the reaction was monitored by TLC. The Potassium salt of the compound obtained was poured cold water and then neutralized by using glacial acetic acid). The separated product was filtered, washed with water, dried and recrystallized from ethanol to yield compound **(3).**

Yield: 63%, **M.P.**:160-162^{o}C, **R_f :** 0.72

General procedure for Synthesis of 5-Phenyl (2-substituted)-1, 3, 4-oxadiazole (3a-3g): 5-phenyl-1, 3, 4-oxadiazole-2-thiol **(3)** (0.84g, 0.005 mole) was refluxed with various secondary amines (0.005 mole) in ethanol (10ml) for 8 hr. In between, the completion of the reaction was monitored by TLC. Then reaction mixture was poured into crushed ice and neutralized with glacial acetic acid. The separated product was filtered, washed with cold water, dried and recrystallized from ethanol to yield compound **(3a-3g).**

Spectral characterization data:

3: **IR (cm^{-1}) (KBr):** 3089cm^{-1}(Ar-CH), 2567cm^{-1}(SH), 1610cm^{-1} (C=N), 1571 cm^{-1} (Ar C=C), 1184cm^{-1}(C-O-C).

^{1}H NMR (δ, ppm) DMSO-d_6: 7.50-7.59(m,3H, Ar-H), 7.89-7.91 (m, 2H, Ar-H), 2.58 (s, 1H, SH).

3a: **IR (cm^{-1}) (KBr):** 3099 (C-H, Ar), 2954 (C –H Str in CH_3), 1610(C=N), 1571(Ar C=C), 1446(C-N), 1190(C-O-C), 769 (C_6H_5).

^{1}H NMR (δ, ppm) DMSO-d_6: 7.50-7.91(m, 5H, Ar-H).

3b: **IR (cm^{-1}) (KBr):** 3145 (Ar –CH), 2953(CH-Str in CH_3), 2856(CH-Str in CH_2), 1610(C=N), 1571(ArC=C), 1446(C-N), 1190(C-O-C), 769(C_6H_5).

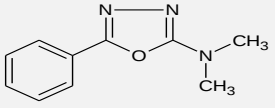
^{1}H NMR (δ, ppm) DMSO-d_6: 7.44-7.59(m, 3H, ArH), 7.89-7.91(m, 2H, (Ar-H), b 7.97-7.99(q, 2H, -CH_2), 7.79-7.82(t, 3H, -CH_3).

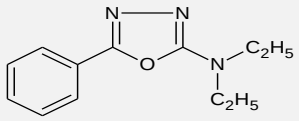
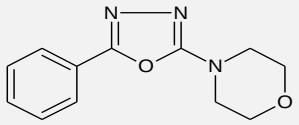
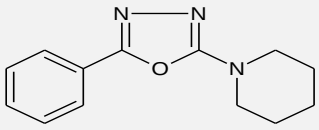
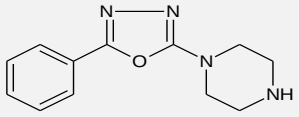
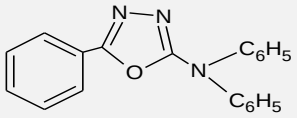
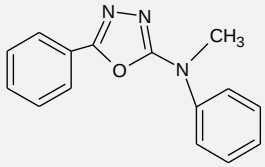
Mass (m/z) : (M^{+1}) 218.5.

3e: **IR (cm^{-1}) (KBr):** 3350(N-H Stretching), 3099(Ar-CH), 1610(C=N), 1571(ArC=C), 1190(C-O-C), 1446(C-N), 769(C_6H_5).

^{1}H NMR (δ, ppm) DMSO-d_6: 7.50-7.91(m, 13H, Ar-H), 14.41(s, 1H, NH).

TABLE 1: PHYSICAL DATA OF SYNTHESIZED COMPOUNDS (3A-3G)

Comp. Code	Structure	% of Yield	M.P. (^{0}C)	R_f Value
3a		70	190-192	0.63

3b		75	185-188	0.76
3C		73	188-190	0.65
3d		72	205-207	0.62
3e		74	185-188	0.66
3f		86	195-198	0.74
3g		80	200-203	0.72

Biological Evaluation [9]:

Antibacterial activity: Seven compounds from the series were screened for their antibacterial activity. Antibacterial activity was carried out against *P. vulgaris, P. aeuroginosa, E. coli* by the cup plate method at a conc. of 50µg/ml and 100µg/ml (**Table 2**). The standard drug used was Tetracycline and DMF was kept as control. The Percent of inhibition of all the test compounds were compared with the standard.

$$\% \text{ Antibacterial Activity Relative to Tetracycline} = \frac{ZI_t}{ZI_s} \times 100 \quad \ldots\ldots\ldots (1)$$

Where, ZI_t = Mean Zone of Inhibition of Test compounds; ZI_s = Mean Zone of Inhibition of Standard drug (Tetracycline)

Anti-inflammatory activity [10]: The Anti-inflammatory activity of the synthesized compounds **3a-3g** were evaluated in vivo by the carrageenan induced paw oedema method in rat. The compounds were tested at an oral dose of 100 mg/kg of body weight, and were compared with the standard drug (Indomethacin) at 1st, 2nd, 3rd and 4th hour of inflammation induction by carrageenan treatment. The percentage of inhibition for each group was calculated and the data was expressed. All the readings are reported in **Table 3** and represented graphically in **Fig. 3 & 4.** All the test compounds showed significant anti-inflammatory activity statistically. In comparison to control, all the test compounds and standard drug shows promising anti-inflammatory activity.

$$\text{Percentage of inhibition} = \frac{C_{mv} + T_v}{C_{mv}} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

Where, C_{mv} = Mean Edema volume of Control Group at any time "t"; T_v = Edema volume of individual mice treated with Test/ Standard Compound at any time "t"

RESULTS AND DISCUSSIONS:

TABLE 2: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Comp.	*E. coli*				*P. vulgaris*				*P. aeruginosa*			
	Zone of Inhibition (mm)		% inhibition		Zone of Inhibition (mm)		% inhibition		Zone of Inhibition (mm)		% inhibition	
	50µg/ ml	100µg/ ml	50µg/ ml	100µg/ ml	50µg/ ml	100µg/ ml	50µg/ ml	100µg/ ml	50µg/ ml	100µg/ ml	50µg/ ml	100µg/ ml
3a	8	12	33.28	46.08	6	12	28.56	52.08	7	11	36.82	52.36
3b	7	10	29.12	38.4	8	10	38.08	43.4	6	10	31.56	47.6
3c	17	21	70.72	80.64	14	19	66.64	82.46	12	17	63.12	80.92
3d	13	18	54.08	69.12	10	13	47.6	56.42	11	14	57.86	66.64
3e	15	19	62.4	72.96	11	15	52.36	65.1	10	18	52.6	85.68
3f	10	16	41.6	61.44	8	13	38.08	56.42	9	13	47.34	61.88
3g	9	13	37.44	49.92	7	11	33.32	47.74	6	11	31.56	52.36
DMSO (Control)	-	-	-	-	-	-	-	-	-	-	-	-
Tetracycline (Std.)	24	26	100	100	21	23	100	100	19	21	100	100

* (- no inhibition)

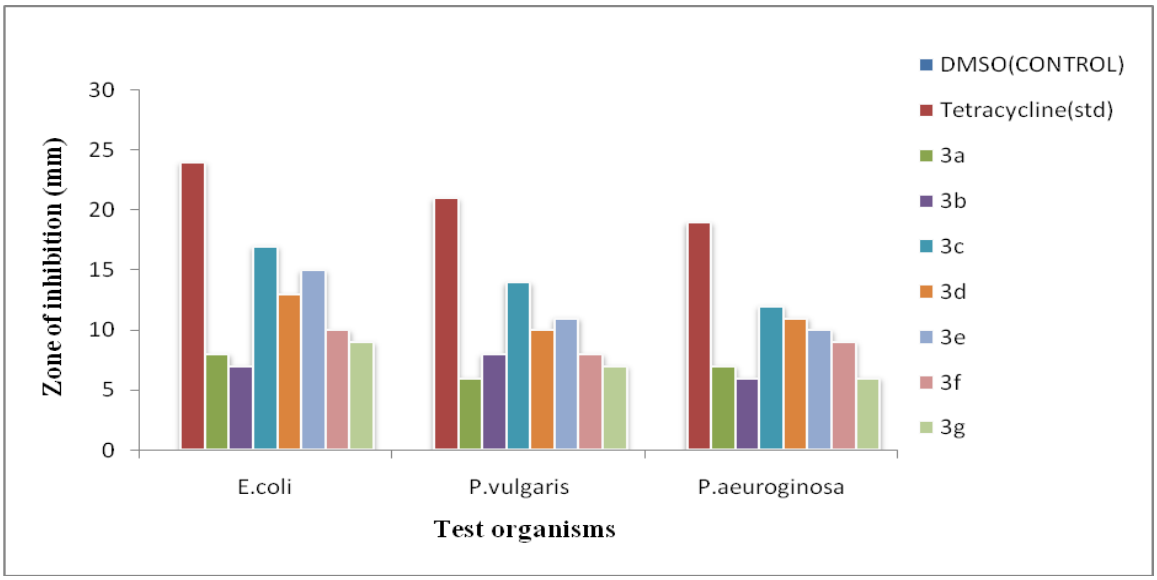


FIG. 1: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (50µG/ML)

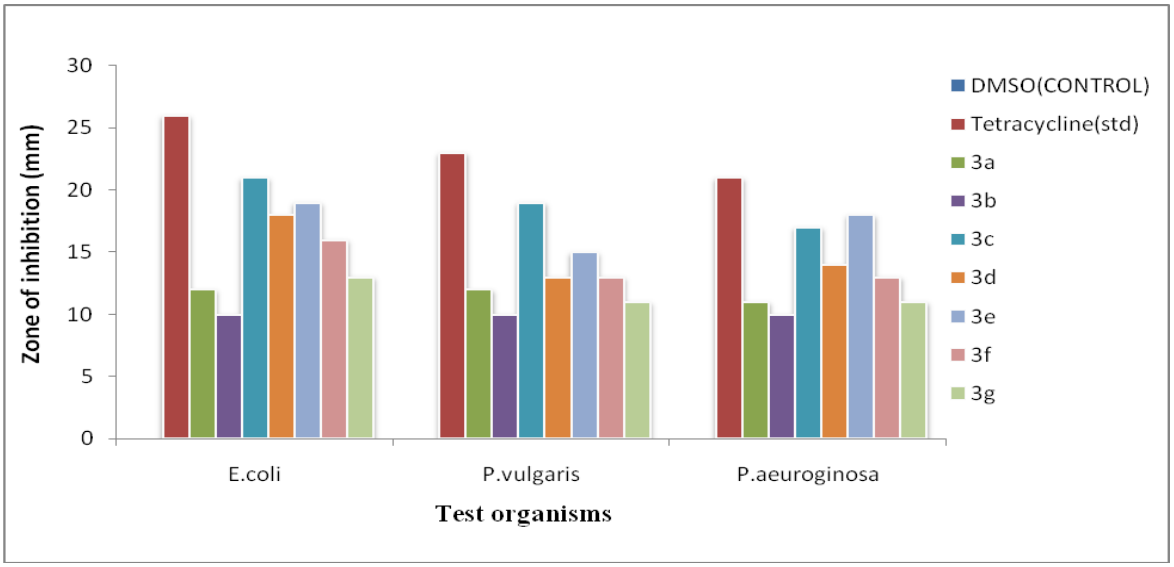


FIG. 2: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (100µG/ML)

TABLE 3: ANTI-INFLAMMATORY ACTIVITIES OF SYNTHESIZED COMPOUNDS

Groups	Paw Volume (1hr)	% inhibition (1hr)	Paw volume (2hr)	% inhibition (2hr)	Paw volume (3hr)	% inhibition (3hr)	Paw volume (4hr)	% inhibition (4hr)
Control	0.67	0 ± 0.025	0.72	0 ± 0.035	0.79	0 ± 0.04	0.80	0± 0.035
Standard	0.57	42.34 ± 0.015	0.60	54.63 ± 0.025	0.61	62.76 ± 0.03	0.61	70.86± 0,03
3a	0.48	9.64 ± 0.005	0.55	26.32 ± 0.015	0.58	32.54 ± 0.015	0.62	40.34±0.005
3b	0.42	22.72 ± 0.015	0.57	39.54 ± 0.01	0.61	44.56 ± 0.015	0.64	51.89± 0.015
3c	0.53	27.33 ± 0.015	0.65	44.34 ± 0.01	0.68	49.23 ± 0.02	0.71	55.23± 0.01
3d	0.46	19.67 ± 0.005	0.65	33.56 ± 0.005	0.64	39.65 ± 0.015	0.66	43.89± 0.025
3e	0.42	16.36 ± 0.01	0.66	32.45 ± 0.01	0.69	38.76 ± 0.01	0.72	41.87± 0.01
3f	0.51	10.56 ± 0.01	0.67	26.58 ± 0.015	0.70	35.43 ± 0.015	0.74	38.67± 0.01
3g	0.37	13.56 ± 0.01	0.55	28.76 ± 0.01	0.58	34.56 ± 0.005	0.61	37.23± 0.005

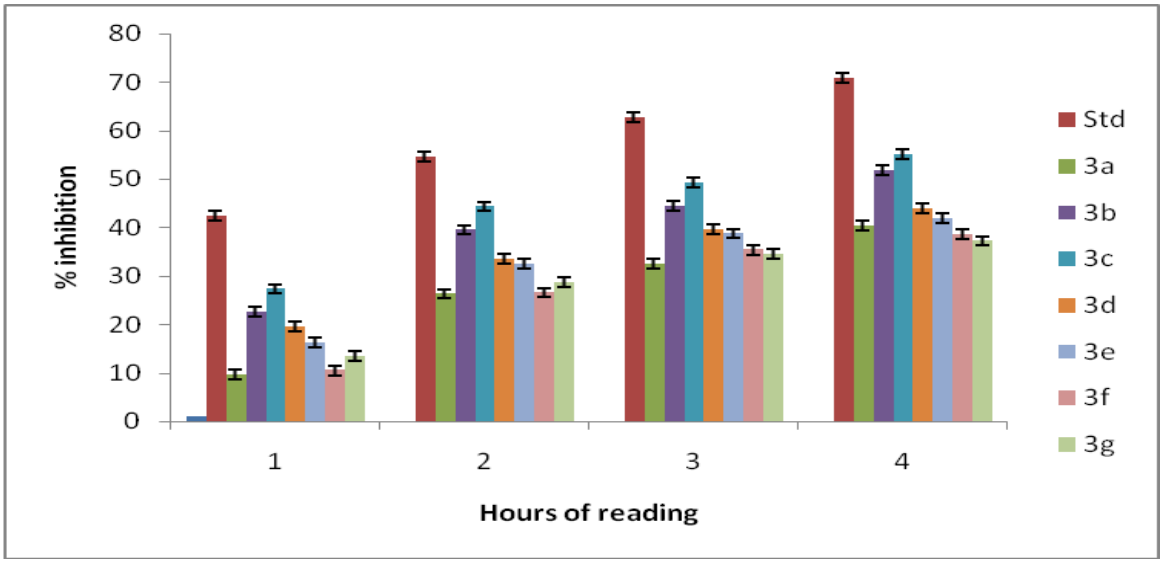


FIG. 3: ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUNDS

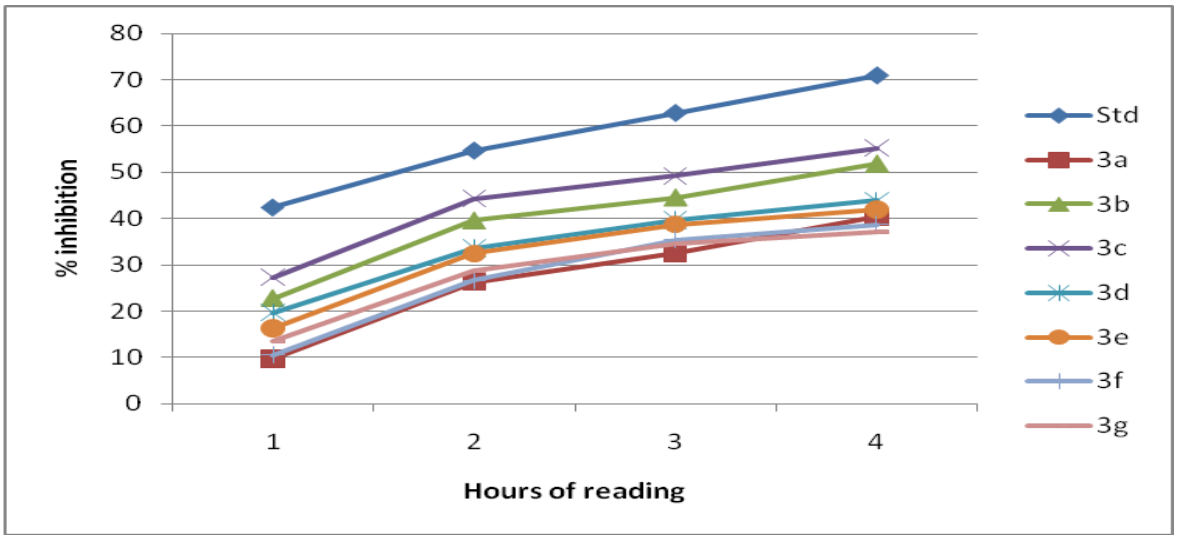


FIG. 4: ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUNDS

CONCLUSION: A new class of oxadiazoles were synthesized by the ring closure reactions of benzohydrazides with carbon disulphide in presence of ethanolic KOH followed by substitution with secondary amines at 2nd position. It may be assumed that further modifications may produce compounds of better activity with less toxic effects. The results of biological tests make novel oxadiazoles interesting lead molecules which certainly hold great promise for discovering safer biologically active agents.

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