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Synthesis and pharmacological activities of some new 2-[1-(6-methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidene thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one derivatives

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In this study, thirteen new compounds having a 2-[1-(6-methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one structure were synthesised using *N*-[2-(6-methoxy-2-naphthyl)propanoyloxysuccinimide, *N*-[2-(6-methoxy-2-naphthyl)propanoyl]thiosemicarbazide and 3-[1-(6-methoxy-2-naphthyl)ethyl]-5-mercapto-1,2,4-triazole. The structures and physical properties of the compounds were elucidated by IR, ^{1}H NMR, mass spectroscopy and elemental analysis. The antiinflammatory activity and gastric ulceration potential of the compounds were tested using naproxen as a reference compound.

1. Introduction

It is well known that the therapeutic use of nonsteroidal antiinflammatory drugs (NSAIDs) is often limited by common side effects such as dyspepsia, gastric ulceration and nephrotoxicity due to the inhibitory effect on prostaglandin which has a cytoprotective role in the human body [1, 2]. Furthermore, it has been shown that the otocoid leukotrienes that are produced by lipoxygenase may cause gastric ulceration [3]. To overcome these effects the compounds described were studied in comparison with naproxen, the only pure stereospecific arylpropionic acid derivative to be used clinically as a NSAID in the (*S*) configuration [4, 5]. Probably the inhibition of both cyclooxygenase and 5-lipoxygenase enzymes not only increases antiinflammatory effects but also decrease side effects. Furthermore, previous studies by Kothari [6], Mullican [7] and Tozkoparan [8–10] suggested that structures having 1,2,4-triazole and thiazolo [3,2-*b*]-1,2,4-triazole-5(6*H*)-one rings possessed antiinflammatory activity. Combining a clinically used antiinflammatory drug like naproxen with these structures may be useful both to enhance effectiveness and decrease gastric side effects. This prompted us to synthesise 1-[(6-methoxy-2-naphthyl)ethyl]-5-mercapto-1,2,4-triazole and 2-[1-(6-methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one derivatives.

2. Investigations, results and discussion

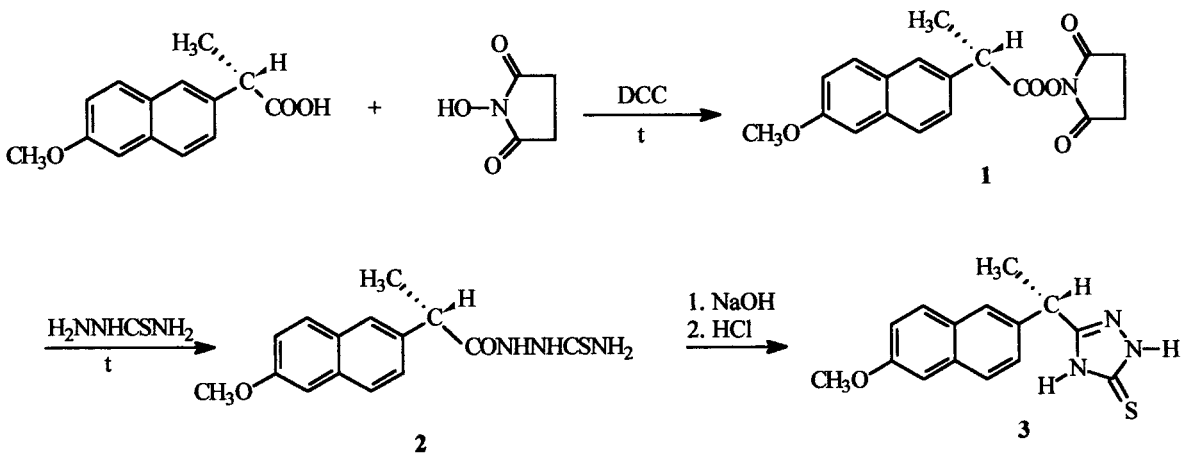
2.1. Chemistry

The synthesis of the compounds is outlined in Schemes 1 and 2. Naproxen was reacted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide to obtain *N*-[2-(6-methoxy-2-naphthyl)propanoyloxy]succinimide **(1)** as the starting compound. This compound was then reacted with thiosemicarbazide to yield *N*-[2-(6-methoxy-2-naphthyl)-propanoyl]thiosemicarbazide **(2)**.

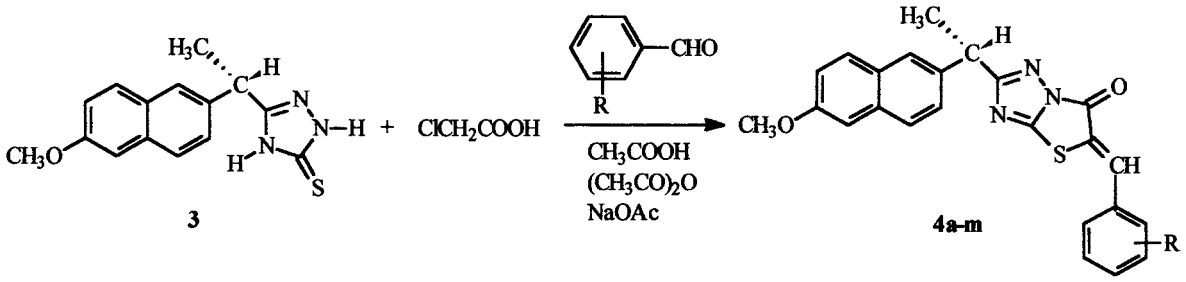
The cyclization of the 1,2,4-triazole-3-thione ring was achieved in an alkaline medium and the product was then obtained by adjusting the pH to 3.0. Since the reaction has different side products such as thiadiazole derivatives, the reaction was monitored by TLC to obtain 3-[1-(6-methoxy-2-naphthyl)ethyl]-5-mercapto-1,2,4-triazole **(3)** in high yield (Scheme 1).

There are several methods, mainly using 5-mercapto-1,2,4-triazole, to obtain thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones for example reacting 5-mercapto-1,2,4-triazole and α-halogenated acids in the presence of alkalihydroxide followed

Scheme 1

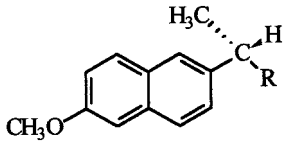


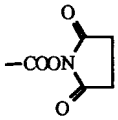
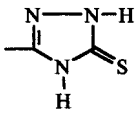
Scheme 2

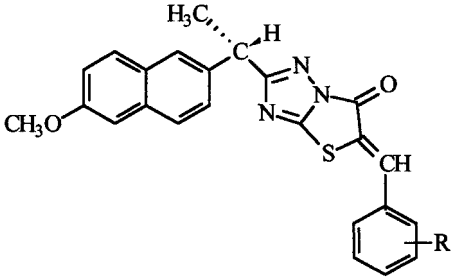


by cyclization to thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one using polyphosphoric acid or phosphorus oxychloride [11, 12]. In fact, we used another method combining the different steps in one. This method simply involves the reaction of 3-[1-(6-methoxy-2-naphthyl)ethyl]-5-mercapto-1,2,4-triazole with chloroacetic acid and benzaldehyde derivatives in the presence of dry sodium acetate in a mixture of acetic acid and acetic anhydride to obtain the 2-[1-(6-methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones (Scheme 2). Melting points, reaction yields, and $[\alpha]_D^{20}$ values of the compounds synthesized are shown in Table 1.

Table 1: Melting points, reaction yields and optical rotation of the compounds synthesized



Compd.	R	M.p. (°C)	Yield (%)	$[\alpha]_D^{20}$
1		118–120	88	+ 8.08
2	–CONHNHCSNH$_2$	178–9	72	+ 30.0
3		214–216	80	+ 32.5



4a	–H	199–202	88	+35.0
4b	4-CH$_3$	154–155	61	+35.2
4c	4-OCH$_3$	172–174	58	+34.6
4d	4-Br	183–185	61	+34.9
4e	4-Cl	163–165	62	+35.7
4f	4-F	183–184	68	+35.3
4g	4-NO$_2$	163–164	63	+34.8
4h	3-CH$_3$	175–176	57	+35.3
4i	3-OCH$_3$	191–192	54	+34.9
4j	3-Br	181–182	76	+35.2
4k	3-Cl	169–170	79	+35.5
4l	3-F	187–189	82	+34.9
4m	3-NO$_2$	197–198	73	+35.1

The structural properties of the compounds were elucidated by IR, ^{1}H NMR and mass spectrometric analyses.

IR spectral data of all compounds exactly fitted predictions based on the positions of functional groups.

In ^{1}H NMR spectra for all compounds the protons appeared for the aromatic ring at δ 7.00–7.80, at δ 1.50–2.00 (d) for the methyl, at δ 4.00–4.60 (q) for the methine group of the side chain from naproxen and at δ 3.75–3.95 (s) for the ring methoxy group. For **3**, the N–H of triazole ring was found at δ 13.00. In **4b** and **4h** the methyl protons substituted in the phenyl group were situated at δ 2.40–2.50 (s). In **4c** and **4I** the methoxy protons substituted in the phenyl group were situated at δ 3.85 (s). In previous studies [8, 10] the benzylidene derivatives produced were shown to exist in the *cis (Z)* configuration by ^{1}H NMR and X-ray [13] studies. For the 2-[1-(6-methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones **4a–m** the benzylidene proton was seen at δ 8.15 (s) and the products showed single spots in TLC. This prompted us to assume that these derivatives are also in the *cis (Z)* configuration. Mass spectra were obtained for compounds **4a–m** using the electron impact techniques as compounds **1–3** are expected to be the derivatized skeleton. Molecular ion, base peaks and cleavages from the parent molecule were almost the same for all of the derivatives except halogen substituted ones. As an example, mass data for **4d** except isotopic peaks are given in part 3.1.4 and further data can be supplied by the author.

2.2. Pharmacological activity

For preliminary activity screening, all test drugs were administered to mice at doses of 100 mg/kg (body weight) using naproxen at a dose of 400 mg/kg as a reference substance; test compounds which posessed more than 20% inhibitory effect in any of the measurement ranges (**4a–f**, **4i–k**, **4m**) and **2** (under derivatized skeleton) were selected for further evaluation of the activity-dose relationship using 50 mg/kg and 25 mg/k dose levels with naproxen as a reference substance in 200 and 100 mg/kg doses (Table 2).

Among the compounds examined in this study, **4d** and **4f** possessed the most clear and consistent activity. For these two compounds ED_{50} values were calculated using the Litchfield-Wilcoxon method which gave 229.0 mg for **4d** and **5.7** mg for **4f** (for the 180 min values). Although an exact dose-response relationship could not be established, compounds **4c** and, to a lesser extent, **4i** deserve attention and may be considered for further evaluation.

Table 2: Antiinflammatory activity and gastric ulceration effects of the selected compounds at 25 and 50 mg/kg doses, in a carrageen-induced hind paw edema model in mice

Compd.	Dose, mgr/kg (per os)	Ulcer score	Swelling in thickness (× 10–2 mm) Percent inhibitory activity			
			90 min	180 min	270 min	360 min
Control		0/6	56.0 ± 5.3	84.9 ± 4.9	59.3 ± 10.5	52.7 ± 8.2
2	25	1/6	56.0 ± 7.8	60.9 ± 3.2* (28.3)	48.5 ± 2.7* (18.2)	34.3 ± 4.1* (34.9)
	50	2/6	36.5 ± 6.0 (34.9)	61.3 ± 12.3 (29.6)	45.8 ± 5.9 (22.8)	52.4 ± 7.6 (0.3)
4a	25	0/6	59.5 ± 3.0	78.2 ± 6.9 (7.9)	57.5 ± 3.2 (3.0)	40.0 ± 2.4* (24.1)
	50	0/6	22.3 ± 3.8 ** (60.2)	55.5 ± 14.4 (34.6)	61.3 ± 10.4	49.2 ± 11.8
4b	25	0/6	52.7 ± 9.5 (5.9)	80.4 ± 8.1 (5.3)	60.2 ± 8.5	42.5 ± 7.3 (19.4)
	50	0/6	43.6 ± 7.7 (22.0)	79.5 ± 8.8 (8.7)	61.6 ± 10.6	43.8 ± 7.9 (16.9)
4c	25	0/6	60.1 ± 16.3	93.7 ± 11.0	63.5 ± 5.8	53.9 ± 7.3
	50	0/6	27.0 ± 3.5* (51.7)	62.0 ± 2.9 (26.9)	41.7 ± 9.6 (29.7)	40.7 ± 3.5 (22.7)
4d	25	0/6	54.3 ± 9.8 (3.1)	43.5 ± 7.5* (48.8)	41.0 ± 6.8* (30.9)	30.4 ± 1.2** (42.3)
	50	0/6	16.7 ± 5.4** (70.2)	53.3 ± 5.5 (37.2)	44.8 ± 10.9 (24.5)	36.11 ± 7.7 (31.5)
4e	25	0/6	72.5 ± 6.7	111.8 ± 16.1	70.4 ± 6.1	42.3 ± 7.9 (19.7)
	50	0/6	37.4 ± 7.5 (33.1)	75.3 ± 3.3 (11.3)	54.2 ± 4.0 (8.5)	36.1 ± 4.5 (31.5)
4f	25	1/6	52.9 ± 11.7 (5.5)	66.6 ± 7.0 (21.6)	40.0 ± 2.2*** (32.5)	32.6 ± 1.5** (38.1)
	50	1/6	34.4 ± 3.8* (38.5)	51.1 ± 6.2* (39.7)	36.0 ± 4.1* (39.2)	40.5 ± 7.5 (23.0)
4i	25	0/6	60.1 ± 8.0	117.7 ± 11.7	79.9 ± 10.6	63.3 ± 7.4
	50	0/6	30.7 ± 7.2** (45.2)	60.6 ± 13.9 (28.6)	50.3 ± 10.6 (15.2)	39.4 ± 9.0 (25.2)
4j	25	0/6	55.1 ± 4.8 (1.6)	79.5 ± 4.9 (6.4)	44.2 ± 1.3** (25.5)	32.4 ± 2.0** (38.5)
	50	3/6	48.3 ± 13.3 (13.8)	72.8 ± 12.3 (16.4)	59.9 ± 10.7	56.8 ± 15.0
4k	25	0/6	69.2 ± 12.8	109.7 ± 15.8	67.4 ± 4.0	52.3 ± 6.8
	50	1/6	33.5 ± 7.3 (40.2)	56.7 ± 8.9 (33.2)	51.2 ± 12.5 (13.7)	37.7 ± 9.1 (28.5)
4m	25	0/6	38.4 ± 6.5 (31.4)	79.4 ± 9.9 (6.5)	47.7 ± 4.6 (19.6)	44.4 ± 5.9 (15.7)
	50	0/6	33.8 ± 8.8 (39.6)	64.3 ± 11.1 (24.3)	64.7 ± 8.1	49.0 ± 7.1 (70)
Naproxen	100	2/6	33.2 ± 7.8* (40.7)	59.1 ± 7.7 (30.4)	41.5 ± 2.1 (30.0)	36.4 ± 3.7 (30.9)
	200	3/6	38.2 ± 11.3 (31.8)	43.1 ± 8.3* (49.2)	28.1 ± 8.1** (52.6)	27.6 ± 5.5** (47.6)
	400	5/6	39.3 ± 9.3* (29.8)	57.8 ± 7.6* (31.9)	45.8 ± 2.4** (22.8)	39.7 ± 4.0 (24.7)

*: $p < 0.05$; ***: $p < 0.001$ significant from the control

The stomachs of the animals were also examined for gastric ulceration and in spite of the high gastric ulcer incidence with the reference compound naproxen the synthesised active compounds were generally found safe from the point of view of ulcer induction. In particular **4d** was found safe at 25 and 50 mg/kg doses, but **4f** also showed a low risk of gastric lesions.

In the literature, using the same method, the antiinflammatory activity of naproxen has been tested at a dose of 200 mg/kg [14]. In our study, this reference substance was studied at the 400–100 mg/kg dose range and it appeared that the antiinflammatory activity of naproxen was not dependent on changes in dose but that gastric ulceration increased with the dose.

The synthesised compounds **4a–m** have significant anti-inflammatory activity, and furthermore the ulcerogenic activity of these compounds was lower than that of the reference compound, with compounds **4a**, **4b**, **4f**, **4g**, **4i** and **4m** showing almost no ulcerogenic activity. Given these results, these compounds may show a specific activity to enzymes or receptors. COX I and COX II studies will be planned for these compounds.

3. Experimental

All chemicals used in this study were supplied by Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. The IR spectra were recorded on a Perkin Elmer FT-IR Spectrometer 1720 X (Beaconsfield, UK) as KBr disc (υ, cm^{-1}). ^1H NMR spectra in deuterium chloroform were obtained on a Bruker AC 200 MHz FT NMR (Bruker, Karlsruhe, Germany) using TMS as an internal standard (chemical shift in δ, ppm). MS of compounds **4a–m** were obtained on a Finnigan MAT GCQ Mass spectrometer (EI, 70 eV). The $[\alpha]_D^{20}$ values were obtained and calculated using a Bellingham-Stanley P20 Polarimeter. Elemental analyses were performed with a Leco CHNS-932 Element Analyser (Philadelphia, USA) at the Scientific and Technical Research Council of Turkey. All compounds gave satisfactory elemental analysis. Silica gel HF 254 + 366 (E. Merck, Darmstadt, Germany) was used for TLC analysis.

3.1. Synthesis of the compounds

3.1.1. (+) N-[2-(6-Methoxy-2-naphthyl)propanoyloxy] succinimide **(1)**

22 mmol of naproxen and 28 mmol of *N*-hydroxysuccinimide were dissolved in dry tetrahydrofuran and cooled to 0 °C. 28 mmol of dicyclohexylcarbodiimide (DCC) was added, stirred for 2 h and kept at 4 °C for 24 h. The precipitate was filtered; THF was evaporated under reduced pressure and the oily residue was crystallised from diethyl ether.
IR (cm^{-1}): 1785 (C=O, ester), 1628 (C=O, amide), 1269 (C=O, ester), 1205 (C−O, ether).
^1H NMR (δ, ppm), ($CDCl_3$): 1.80 (3 H; d, J = 7,3 Hz; $C\underline{H_3}$−CH), 3.90 (3 H; s; O−$C\underline{H_3}$), 4.50 (1 H; q J = 7,1 Hz; C$\underline{H}$−CH_3), 7.15–7.75 (6 H; m; ar).

3.1.2. (+) N-[2-(6-Methoxy-2-naphthyl)propanoyl] thiosemicarbazide **(2)**

10 mmol of 1 was dissolved in 25 ml of dry tetrahydrofuran and 10 mmol of thiosemicarbazide was dissolved in 2 ml dimethylsulphoxide. The reaction mixture was refluxed until the reaction was complete by monitoring with TLC. The compound produced **2** was poured into water.
IR (cm^{-1}): 3187 (N−H), 1667 (C=O, amide), 1607 (C=S), 1214 (C−O, ether).
^1H NMR (δ, ppm), ($CDCl_3$): 1.5 (2 H; s; CS−$N\underline{H_2}$), 1.80 (3 H; d, J = 7,3 Hz; $C\underline{H_3}$−CH), 2.00 (1 H; s; N$\underline{H}$−CS), 2.50 (1 H; s; CO−N$\underline{H}$), 3.90 (3 H; s; O−$C\underline{H_3}$), 4.50 (1 H; q J = 7,1 Hz; C$\underline{H}$−CH_3), 7.15–7.75 (6 H; m; ar).

3.1.3. (+) 3-[1-(6-Methoxy-2-naphthyl)ethyl]-5-mercapto-1,2,4-triazole **(3)**

6 mmol of **2** was refluxed in 50 ml 10% NaOH for 6 h, and cooled to room temperature and the pH of the mixture was adjusted to 3.0 using conc. HCl. The precipitate was filtered and crystallised from $EtOH : H_2O$ (1 : 1).
IR (cm^{-1}): 1635 (C=S), 1207 (C−O, ether), 1587; 1456; 1396 (triazole ring).
^1H NMR (δ, ppm), ($CDCl_3$): 1.60 (3 H; d, J = 7,3 Hz; $C\underline{H_3}$−CH), 3.75 (3 H; s; O−$C\underline{H_3}$), 4.50 (1 H; q J = 7,1 Hz; C$\underline{H}$−CH_3), 7.00–7.75 (6 H; m; ar) 13.00 (1 H; s; N−$\underline{H}$).

3.1.4. (+)2-[1-(6-Methoxy-2-naphthyl)ethyl]-6-(substituted)benzylidenethiazolo[3,2-b]-1,2,4-triazole-5(6H)-ones **4a–m**

Equimolar amounts (4 mmol) of **3**, aromatic aldehydes, chloroacetic acid, sodium acetate (anh.), 6 ml acetic anhydride and 8 ml acetic acid were refluxed for 2 h. The reaction mixture was then poured into ice water. The precipitate was filtered, washed with water and 10% sodium bicarbonate and then crystallised with suitable solvents.
IR (cm^{-1}): 1735 (C=O, lactam), 1606; 1402 (C=N and C=N−N), 1316 (C−N), 1214 (C−O, ether).
^1H NMR (δ, ppm), ($CDCl_3$): 1.80 (3 H; d, J = 7,3 Hz; $C\underline{H_3}$−CH), 3.95 (3 H; s; O−$C\underline{H_3}$), 4.50 (1 H; q J = 7,1 Hz; C$\underline{H}$−CH_3), 7.00–7.80 (10 H; m; ar), 8.15(1 H; s; Ar−C$\underline{H}$)
MS (for compound **4d**): 492 (M+) (26.61%), 491, 478, 450, 405, 369, 354, 326, 296, 283, 251, 236 (100%), 210, 185, 153, 129, 55.

3.2. Pharmacology

Local breed albino mice of both sexes weighing approximately 20–25 g were used. All the animals were left for two days under laboratory conditions for acclimatization and maintained on a standard pellet diet and water ad libidum before the day of the experiment. On the last day food was withdrawn and they were given water only. Test samples and reference compounds were suspended in 0.5% carboxymethyl cellulose and administered to each mouse by using a gastric gavage needle. The control group animals, however, received the same volume of dosing vehicle. A minimum of six animals was used in each group.

3.2.1. Antiinflammatory activity

The carrageen-induced hind paw edema model according to the method reported by Kasahara et al. [15] was used with some modifications for antiinflammatory activity testing.
Naproxen (200 mg/kg) was used as the reference compound. Test samples were administered orally 60 min before the injection of 25 µl of freshly prepared solution of carrageen (0.5 mg/ml) in physiological saline (154 mM NaCl) into supplanter tissue of the right hind paw of each mouse. The same volume of saline solution was injected into that of the left hind paw as an internal control. The difference in foot pad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo) at intervals differed from those described by Kasahar et al. The foot thickness of each mouse was measured four times at 90 min intervals up to 360 min. Statistical differences between the treatment and the control group of animals were evaluated by a two-tailed Student's t-test. Percent inhibitory effects were estimated according to the following equation, where n was the average difference in thickness between the left and right hind paw of the control group and n' was that of the test group of animals.

$$\text{Inhibition } (\%) = [(n - n')/n] \times 100$$

3.2.2. Gastric ulceration studies

All the animals were subjected to this experimental process. They were sacrificed immediately after the last measurement, ie. 7 h after the application of each test drug, under ether anaesthesia and stomachs were removed, opened through the greater curvature, washed and fixed in 5% formalin solution. The stomachs were then examined for lesions under a dissecting microscope.

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