Synthesis of *N*,*N*-dialkyl-1-(2-alkylthiopyrimidin-4-yl)piperidin-4-amines as potential heat shock protein inhibitors

V. N. Aldobaev,[a] M. A. Prezent,[b] and I. V. Zavarzin[b]★

[a]*Research Center for Toxicology and Hygienic Regulation of Biologics — Branch of the State Scientific Center "Institute of Immunology" of the Federal Medical and Biological Agency of Russia, 102A ul. Lenina, 142253 Bolshevik, Moscow Region, Russian Federation*
[b]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. E-mail: zavi@ioc.ac.ru*

A new efficient method for synthesizing promising heat shock protein inhibitors, *N*,*N*-dialkyl-1-(2-alkylthiopyrimidin-4-yl)piperidin-4-amines, by the reaction of 2-alkyl-4-chlorothiouracils with 4-(*N*-alkyl-*N*-methylamino)piperidines was developed. 2-Alkyl-4-chlorothiouracils were synthesized by alkylation of 2-thiouracil with alkyl iodides and subsequent treatment of the intermediates with $POCl_3$. 4-(*N*-Alkyl-*N*-methylamino)piperidines were prepared by reductive amination of 1-(*tert*-butoxycarbonyl)-4-piperidinone with methylamine followed by treatment of the intermediate with the appropriate aldehydes.

Key words: thiouracil, 1-(*tert*-butoxycarbonyl)-4-piperidinone, piperidines, reductive amination, nucleophilic substitution, pyrimidines, heat shock protein inhibitors, antitumor activity.

A growing number of evidences of drug-resistant human tumor cells require development of new drugs with new target disease-specific mechanisms of cytostatic activity that can increase the efficiency of cancer therapy. One of the promising therapeutic approaches in modern cancer therapy is targeting of heat shock proteins (HSPs) playing an important role in maintaining cellular homeostasis. Selective inhibition of at least one of the representatives of a large family of heat shock proteins promotes cancer cell apoptosis.[1—5]

Several recent publications[6—8] are focused on the synthesis of different small molecule inhibitors of HSP70. Structures of these inhibitors were identified by molecular docking. Thus, Zeng *et. al*.[8] have described synthesis of 67 derivatives of 4-aminopiperidine designed as promising HSP70 inhibitors. The authors also evaluate *in vitro* biological activity of the synthesized compounds, determined their dissociation constants using surface plasmon resonance studies, and examined the ability of the test compounds to inhibit HSP70 ATPase activity. It should be emphasized that the results obtained in some steps during the work[8] cannot be reproduced. The main aim of the present work is the development of new simple and efficient synthetic approach towards small molecule inhibitors of HSPs based on the analysis of both new structures constantly emerging in scientific literature and the results of SAR modelling.

We intended to synthesize derivatives of 4-aminopiperidine that exhibited high HSP70 inhibiting activity and to evaluate cytotoxicity of the synthesized compounds against tumor cells and fibroblasts (MSF7, HEP-2, and L-929) for searching for potential cytostatics and their further trials.

Synthetic approach described by Zeng *et. al*.[8] involves the successive substitution of the chlorine atoms of 2,4-dichloropyrimidine first with *tert*-butyl *N*-methyl-*N*-(piperidin-4-yl)carbamate and then with sodium methanethiolate, removal of the protecting group, and finally alkylation with the appropriate benzylic halides. However, we failed to reproduce substitution of the chlorine atom at the pyrimidine ring with sodium thiomethoxide. In our hands, the substitution failed to proceed under conditions described by Zeng *et. al*.[8] and under some other conditions. Therefore, it was required to develop new approaches to the most promising piperidines synthesized in work[8] and their closest homologs.

We developed a procedure that allows one to widen significantly the scope of combinatorial synthesis of the target compounds for screening using cell cultures to identify optimal drug candidates for further development as cytostatics.

Our approach (Scheme 1) takes a simple synthesis of 2-alkylthio-4-chloropyrimidines **3a**,**b** and 4-(*N*-alkyl-*N*-methyl)aminopiperidines **7a**—**e**. The target products **8a**—**g** were obtained in high yields by substitution of the chlorine atom of compounds **3a,b** with piperidines **7a**—**e** (see Scheme 1). It is possible to vary the alkyl substituent at

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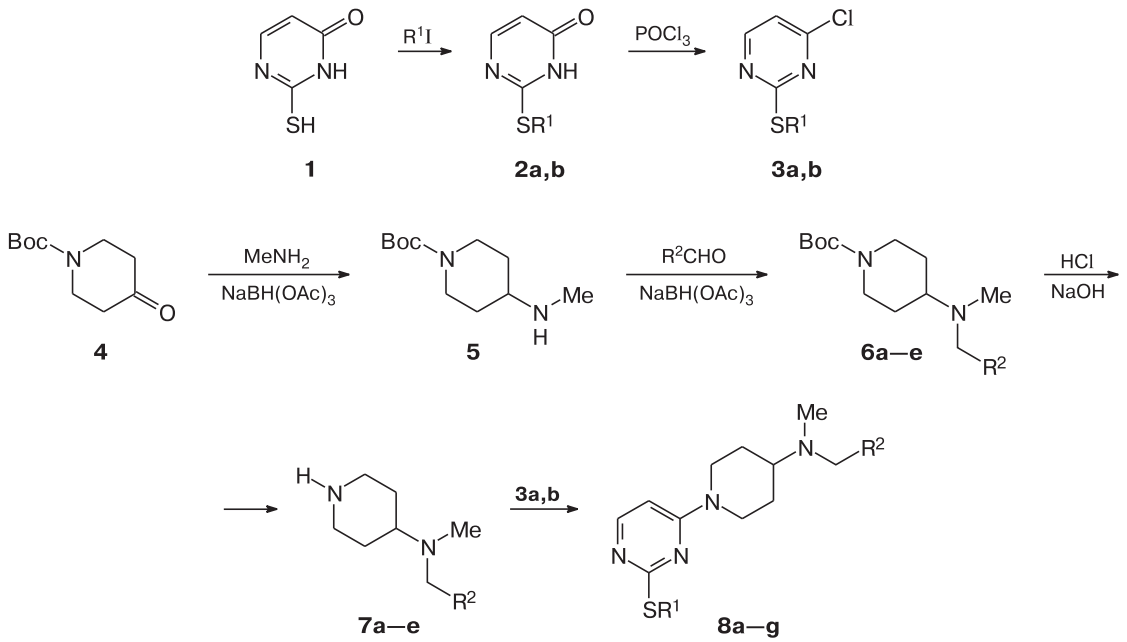
Scheme 1



2, **3:** R^1 = Me (**a**), Et (**b**)
6, **7:** R^2 = 2,6-$Cl_2C_6H_3$ (**a**), 4-NCC_6H_4 (**b**), 2-ClC_6H_4 (**c**), 2-chloro-3-pyridyl (**d**), 1,5-dimethylpyrazol-4-yl (**e**)

8	R^1	R^2	**8**	R^1	R^2
a	Me	2,6-$Cl_2C_6H_3$	**e**	Me	1,5-Dimethylpyrazol-4-yl
b	Me	4-NCC_6H_4	**f**	Et	2,6-$Cl_2C_6H_3$
c	Me	2-ClC_6H_4	**g**	Et	4-NCC_6H_4
d	Me	2-Chloro-3-pyridyl			

the sulfur atom, amine used for the modification of the carbonyl group, and aldehyde.

The synthesis started from the commercially available thiouracil (**1**) and 1-(*tert*-butoxycarbonyl)piperidin-4-one (**4**). Thiouracil was readily alkylated with alkyl iodides[9] and subsequent treatment of compounds **2a**,**b** with phosphorous oxychloride gives 4-chloropyrimidines **3a**,**b**.[10] Amination of substituted piperidinone **4** with methylamine[11] in the presence of $NaBH(OAc)_3$ gives compound **5**. 4-(*N*-Alkyl-*N*-methylamino)piperidines **7a**—**e** were synthesized by the reaction of compound **5** with aldehydes in the presence of $NaBH(OAc)_3$ followed by successive treatment first with acid and then with base.

Substitution of the chorine atom of 4-chloropyrimidines **3a**,**b** with amines **7a**—**e** was carried out in refluxing dioxane in the presence of diisopropylethylamine (DIPEA) to give the target compounds **8a**—**g**. Products **8a**—**g** are white solids well soluble in chloroform, benzene, and THF but sparingly soluble in hexane and water. ^{1}H NMR spectra of compounds **8a**—**g** exhibit characteristic signals of the NCH_2Ar(Het) group protons at δ 4.00 and the doublets of the CH= pyrimidine ring protons at δ 6.25 and 8.05. IR spectra of compounds **8a**—**g** show vibration bands of the C=N group at 1637—1655 cm^{-1}. High resolution mass spectra of these compounds reveal the $[M + H]^+$ and $[M + Na]^+$ peaks.

The developed synthetic approach allows varying the substituents both at the sulfur atom of the pyrimidine fragment and at the nitrogen atom of the piperidine moiety, which is advantageous over method described by Zeng *et. al.*[8]

It is of note that the replacement of methylamine (on the synthesis of piperidine **5**) with other amines serves for synthesizing new 4-dialkylaminopiperidine derivatives that can be involved in further transformations.

Currently, the synthesized compounds are under *in vitro* screening against selected cell lines in the Research Center for Toxicology and Hygienic Regulation of Biologics — Branch of the State Scientific Center "Institute of Immunology" of the Federal Medical and Biological Agency of Russia.

Experimental

^{1}H NMR spectra were run on a Bruker WM-300 spectrometer (a working frequency of 300 MHz) at 303 K. ^{13}C NMR spectra were acquired with a Bruker WM-300 instrument (a working frequency of 75 MHz). The chemical shifts are given in the δ scale

relative to the residual proton signals and the carbon signals of the deuterated solvents (δ 7.27 for $CDCl_3$ (^1H) and δ 39.50 for DMSO-d_6 (^{13}C)).

Melting points were measured with a Boetius apparatus at a heating rate of 4 °C min^{-1} and are given uncorrected. High resolution electrospray ionization (ESI) mass spectrometry was performed with a Bruker Daltonics MicrOTOF II instrument. The mass spectra were acquired over the *m/z* range of 50—3000 Da operating in either positive (capillary voltage of 4500 V) or negative (capillary voltage of 3200 V) ion modes; the probes were injected as the solutions in MeCN and MeOH *via* a syringe pump at a flow rate of 3 μL min^{-1}; interface temperature was 180 °C; nebulizer gas was nitrogen (flow rate of 4.0 L min^{-1}). Elemental analysis was carried out with a Perkin Elmer 2400 Series II CHNS/O analyzer. Column chromatography was performed with Acros Organics silica gel 60A (0.060—0.200 mm). Thiouracil, 1-(*tert*-butoxycarbonyl)piperidin-4-one, 2-chloropyridine-3-carbaldehyde, 1,5-dimethylpyrazole-4-carbaldehyde, 2-chlorobenzaldehyde, 2,6-dichlorobenzaldehyde, 4-cyanobenzaldehyde, $NaBH(OAc)_3$, and DIPEA were purchased from Aldrich.

The intermediates (2-alkyl-4-chloropyrimidines **3a**,**b**, 1-(*tert*-butoxycarbonyl)-4-(methylamino)piperidine (**5**), and compounds **7a—e**) were used in the next step without characterization and purification.

***N*-Alkyl-*N*-methyl-1-(2-alkylthiopyrimidin-4-yl)piperidin-4-amines 8a—g (general procedure).** To a solution of piperidine **5** (10 mmol) and the appropriate aldehyde (11 mmol) in CH_2Cl_2 (100 mL), $NaBH(OAc)_3$ (20 mmol) was added. After 20 h stirring, 10% aqueous NaOH (50 mL) was added and stirring was continued for 1 h. The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The oily residue was treated with 20% HCl (10 mL), the mixture was stirred for 6 h, basified with concentrated aqueous NaOH to pH 10, and extracted with ethyl acetate (2×30 mL). After drying, the combined organic extracts were transferred into a weighted flask and concentrated *in vacuo*. To the obtained oily amine **7a—e**, dioxane (20 mL), DIPEA (2 equiv.), and 4-chloropyrimidine **3a**,**b** (1.2 equiv.) were added, the mixture was refluxed for 6 h, cooled down, and concentrated *in vacuo*. The residue was extracted with dichloromethane, the extracts were washed with water, dried, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (elution first with petroleum ether—ethyl acetate (1 : 5) then with ethanol—dichloromethane (1 : 20), removal of the solvents *in vacuo*, and trituration of the residue with hexane gives the title products. Compounds **8a**,**b**,**e**,**f** were obtained as bases; compounds **8c**,**d**,**g** were converted into hydrochlorides by dissolving in MeOH saturated with HCl (10 mL) followed by removal of the solvent.

***N*-(2,6-Dichlorobenzyl)-*N*-methyl-1-(2-methylthiopyrimidin-4-yl)piperidin-4-amine (8a).** Yield 2.46 g (62%), m.p. 146—147 °C. IR (KBr), ν/cm^{-1}: 1637 (C=N). ^1H NMR ($CDCl_3$), δ: 1.70, 2.10 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.32 (s, 3 H, NMe); 2.50 (s, 3 H, SMe); 2.95 (s, 3 H, C(2)H_2, NCH); 4.00 (s, 2 H, CH_2 (benzyl)); 4.55 (s, 2 H, C(1)H_2); 6.25 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.15—7.40 (m, 3 H, C_6H_3); 8.05 (d, 1 H, =C(6)H, *J* = 2.0 Hz). ^{13}C NMR (DMSO-d_6), δ: 13.4 (SMe), 26.0 (C(3), C(5)), 38.9 (NMe), 43.1 (C(2), C(6)), 52.2 (NCH_2), 60.9 (C(4)), 99.0 (C(5′)), 128.6 (C(4″), C(6″)), 129.7 (C(5″)), 134.70 (C(2″)), 136.1 (C(1″), C(3″)), 155.5 (C(6′)), 160.2 (C(4′)), 170.0 (C(2′)). HRMS, found: *m/z* 397.1023 $[M + H]^+$, 419.0840 $[M + Na]^+$. Calculated for $C_{18}H_{22}Cl_2N_4S$: M + H = 397.1015, M + Na = 419.0834.

***N*-(4-Cyanobenzyl)-*N*-methyl-1-(2-methylthiopyrimidin-4-yl)piperidin-4-amine (8b).** Yield 2.44 g (69%), m.p. 181—183 °C. IR (KBr), ν/cm^{-1}: 2222 (CN), 1638 (C=N). ^1H NMR ($CDCl_3$), δ: 1.70, 2.10 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.32 (s, 3 H, NMe); 2.50 (s, 3 H, SMe); 2.95 (s, 3 H, C(2)H_2, NCH); 4.00 (s, 2 H, CH_2 (benzyl)); 4.55 (s, 2 H, C(1)H_2); 6.25 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.52, 7.63 (both d, 2 H each, C_6H_4, *J* = 7.0 Hz); 8.05 (d, 1 H, =C(6)H, *J* = 2.0 Hz). ^{13}C NMR (DMSO-d_6), δ: 13.3 (SMe), 27.0 (C(3), C(5)), 37.4 (NMe), 42.9 (C(2), C(6)), 56.2 (NCH_2), 60.3 (C(4)), 99.0 (C(5′)), 109.4 (C(1″)), 119.0 (CN), 129.1 (C(3″), C(5″)), 132.1 (C(2″), C(6″)), 146.5 (C(4″)), 155.5 (C(6′)), 160.2 (C(4′)), 170.0 (C(2′)). HRMS, found: *m/z* 354.1810 $[M + H]^+$, 376.1570 $[M + Na]^+$. Calculated for $C_{19}H_{23}N_5S$: M + H = 354.1746, M + Na = 376.1566.

***N*-(2-Chlorobenzyl)-*N*-methyl-1-(2-methylthiopyrimidin-4-yl)piperidin-4-amine hydrochloride (8c · HCl).** Yield 2.15 g (54%), m.p. 247—249 °C. IR (KBr), ν/cm^{-1}: 1637 (C=N). ^1H NMR (DMSO-d_6), δ: 1.95, 2.40 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.42 (s, 3 H, NMe); 2.60 (s, 3 H, SMe); 3.25 (s, 2 H, C(2)H_2); 3.80 (s, 1 H, NCH); 4.35, 4.60 (both d, 2 H each (benzyl), *J* = 5.0 Hz); 4.80 (s, 2 H, C(1)H_2); 7.05 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.40—7.60 (m, 3 H, C_6H_4, =C(6)H); 8.05, 8.20 (both d, 1 H each, C_6H_4, *J* = 7.0 Hz); 11.70 (br.s, 1 H, NH^+). ^{13}C NMR (DMSO-d_6), δ: 13.3 (SMe), 25.5 (C(3), C(5)), 38.9 (NMe), 43.1 (C(2), C(6)), 52.2 (NCH_2), 61.7 (C(4)), 99.40 (C(5′)), 127.7, 128.1, 129.9, 131.6, 134.1, 134.7 (C_6H_4), 145.2 (C(6′)), 159.2 (C(4′)), 164.90 (C(2′)). HRMS, found: *m/z* 363.1411 $[M + H]^+$, 385.1230 $[M + Na]^+$. Calculated for $C_{18}H_{23}ClN_4S$: M + H = 363.1405, M + Na = 385.1224.

***N*-[(2-Chloro-3-pyridyl)methyl]-*N*-methyl-1-(2-methylthiopyrimidin-4-yl)piperidin-4-amine hydrochloride (8d · HCl).** Yield 2.04 g (51%), m.p. 268—270 °C. IR (KBr), ν/cm^{-1}: 1655 (C=N). 2.00, 2.42 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.42 (s, 3 H, NMe); 2.55 (s, 3 H, SMe); 3.25 (s, 2 H, C(2)H_2); 3.80 (s, 1 H, NCH); 4.35, 4.60 (both d, 2 H, CH_2 (benzyl), *J* = 5.0 Hz); 4.80 (s, 2 H, C(1)H_2); 7.00 (d, 1 H, =C(5)H (pyrimidine), *J* = 2.0 Hz); 7.50 (t, 1 H, =CH (pyridine), *J* = 2.0 Hz); 8.10 (d, 1 H, =C(6)H (pyrimidine), *J* = 2.0 Hz); 8.50 (d, 1 H, =CH (pyridine), *J* = 2.0 Hz); 8.75 (d, 1 H, =CH (pyridine), *J* = 2.0 Hz); 12.50 (br.s, 1 H, NH^+). ^{13}C NMR (DMSO-d_6), δ: 13.3 (SMe), 25.2, 25.6 (C(3), C(5)), 37.9 (NMe), 43.1 (C(2), C(6)), 52.2 (NCH_2), 61.6 (C(4)), 99.4 (C(5′)), 123.60 (C(4″)), 129.7 (C(5″)), 143.2 (C(3″)), 145.7 (C(6′)), 156.9 (C(6″)), 151.5 (C(2″)), 159.0 (C(4′)), 165.0 (C(2′)). HRMS, found: *m/z* 364.1364 $[M + H]^+$, 387.1185 $[M + Na]^+$. Calculated for $C_{17}H_{22}ClN_5S$: M + H = = 364.1357, M + Na = 387.1177.

***N*-[(1,5-Dimethylpyrazol-4-yl)methyl]-*N*-methyl-1-(2-methylthiopyrimidin-4-yl)piperidin-4-amine (8e).** Yield 1.95 g (51%), m.p. 121—123 °C. IR (KBr), ν/cm^{-1}: 1637 (C=N). ^1H NMR (DMSO-d_6), δ: 1.70, 1.90 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.15 (s, 3 H, Me); 2.32 (s, 3 H, NMe); 2.40 (s, 3 H, NMe); 2.55 (s, 3 H, SMe); 2.95 (s, 3 H, C(2)H_2, NCH); 3.80 (s, 2 H, CH_2 (benzyl)); 4.50 (s, 2 H, C(1)H_2); 6.30 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.10 (s, 1 H, CH (pyrazole)); 7.95 (d, 1 H, =C(6)H, *J* = 2.0 Hz). ^{13}C NMR (DMSO-d_6), δ: 9.4 (Me), 13.4 (SMe), 25.0, 26.0 (C(3), C(5)), 36.7, 38.9 (2 NMe), 43.1, 44.3 (C(2), C(6)), 46.2 (NCH_2), 59.9 (C(4)), 99.00 (C(5′)), 128.6 (C(4″), C(6″)), 129.7 (C(5″)), 134.7 (C(2″)), 136.1 (C(1″), C(3″)), 155.5 (C(6′), 160.2 (C(4′)), 170.0 (C(2′)). HRMS, found:

m/z 347.2020 $[M + H]^+$, 369.1838 $[M + Na]^+$. Calculated for $C_{17}H_{26}Cl_2N_6S$: M + H = 347.2012, M + Na = 369.1831.

***N*-(2,6-Dichlorobenzyl)-1-(2-ethylthiopyrimidin-4-yl)-*N*-methylpiperidin-4-amine (8f).** Yield 2.54 g (62%), m.p. 123—124 °C. IR (KBr), ν/cm^{-1}: 1637 (C=N). ^{1}H NMR (DMSO-d_6), δ: 1.40 (t, 3 H, Me, *J* = 5.0 Hz); 1.70, 2.10 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.22 (s, 3 H, NMe); 2.70—2.95 (m, 3 H, C(2)H_2, NCH); 3.00 (q, 2 H, CH_2N, *J* = 5.0 Hz); 3.80 (s, 2 H, CH_2 (benzyl)); 4.55 (m, 2 H, C(1)H_2); 6.25 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.15—7.40 (m, 3 H, C_6H_3); 8.05 (d, 1 H, =C(6)H, *J* = 2.0 Hz). ^{13}C NMR (DMSO-d_6), δ: 14.8 (Me), 24.2 (SCH_2), 26.9 (C(3), C(5)), 36.0 (NMe), 43.1 (C(2), C(6)), 52.2 (NCH_2), 60.9 (C(4)), 99.0 (C(5′)), 128.6 (C(4′′), C(6′′)), 129.7 (C(5′′)), 134.7 (C(2′′)), 136.1 (C(1′′), C(3′′)), 155.5 (C(6′)), 160.2 (C(4′)), 170.0 (C(2′)). HRMS, found: *m/z* 411.1182 $[M + H]^+$, 433.0998 $[M + Na]^+$. Calculated for $C_{19}H_{24}Cl_2N_4S$: M + H = 411.1171, M + Na = 433.0991.

***N*-(4-Cyanobenzyl)-1-(2-ethylthiopyrimidin-4-yl)-*N*-methylpiperidin-4-amine hydrochloride (8g · HCl).** Yield 2.78 g (69%), m.p. 154—156 °C. IR (KBr), ν/cm^{-1}: 2245 (CN), 1637 (C=N). ^{1}H NMR (DMSO-d_6), δ: 1.40 (t, 3 H, Me, *J* = 5.0 Hz); 2.00, 2.40 (both s, 2 H each, C(3)H_2, C(4)H_2); 2.52 (s, 3 H, NMe); 3.30 (s, 4 H, CH_2N, C(2)H_2); 3.80 (s, 1 H, NCH); 4.35, 4.60 (both d, 2 H, CH_2 (benzyl), *J* = 5.0 Hz); 4.80 (s, 2 H, C(1)H_2); 7.10 (d, 1 H, =C(5)H, *J* = 2.0 Hz); 7.52 (s, 3 H, C_6H_4, =C(6)H); 8.20 (s, 2 H, C_6H_4); 12.20 (br.s, 1 H, NH^+). HRMS, found: *m/z* 368.1909 $[M + H]^+$, 390.1730 $[M + Na]^+$. Calculated for $C_{20}H_{25}N_5S$: M + H = 368.1903, M + Na = 390.1722.

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