Synthesis and Antitumor Activity of 1,5,6-Substituted *E*-3-(2-Chloro-3-indolylmethylene)-1,3-dihydroindol-2-ones[1]

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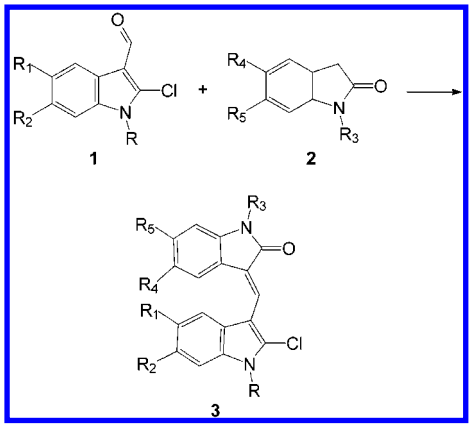
Synthesis and antitumor activity of new *E*-3-(2-chloro-3-indolylmethylene)-1,3-dihydroindol-2-ones are described. All compounds prepared were active in the primary test (three human cell lines) and entered the second level (60 human cell lines). The most active antitumor derivatives bear the same substituents in the chloroindole ring and are not CDK1 inhibitors. A COMPARE analysis showed that they could act as tubulin binders. In most cell lines, *E*-3-(2-chloro-5-methoxy-6-methyl-3-indolylmethylene)-1,3-dihydroindol-2-one was a growth inhibitor more potent than vincristine.

Introduction

We describe in this paper the synthesis and antitumor activity of a new series of *E*-3-(2-chloro-3-indolylmethylene)-1,3-dihydroindol-2-ones (**3**; see Scheme 1). In our first paper on this topic,[2] analogous compounds were reported, taking into account only the N substitution (R, R_3) with a methyl group. In the introduction of this paper, we pointed out that the parent compound 3-(3-indolylmethylene)-1,3-dihydroindol-2-one, first synthesized in 1969 by a German team, was described as an antitumor agent in 1977 by a Japanese team.

In our subsequent paper,[3] the substitution at the 5 position (R_1, R_4) was also considered. This previous work disclosed the interesting activity of several compounds and demonstrated that at least one of the two NH groups should be unsubstituted in order to maintain the antitumor activity. According to this finding, we prepared new compounds **3** including also a substitution at position 6 (R_2, R_5) and a different substituent at position 5 of the 2-chloroindole (R_1). The growth inhibition of all the new compounds (**3a**−**o**) was tested on three human tumor cell lines, and according to the protocols developed at the National Cancer Institute (NCI, Bethesda, MD), the active compounds were tested on 60 tumor cell lines. The derivatives that were active in this test were also evaluated as positive inotropic agents in search for potential coanthracyclinic activity. We suggested this term to indicate the pharmacological behavior of a molecule endowed with both antitumor activity (to reduce the anthracycline toxicity by reducing its dosage) and positive inotropic activity (to counteract the heart depression induced by anthracyclines).[1,3−5] The same derivatives active on the 60 lines were also tested as potential inhibitors of cyclin-dependent kinase 1 (CDK1) in order to verify whether the antitumor activity was related to this mechanism of action. A paper reporting some 2-chloro-3-indolylmethylene-1,3-dihydroindol-2-ones as CDK1 inhibitors has been published separately.[6]

Scheme 1[a]



[a] For R, see Table 1.

Chemistry

The reaction between a 2-chloroindolaldehyde **1** and the equivalent of an oxindole **2** was performed in methanol in the presence of piperidine. We recently described[6] two of the compounds reported in Table 1 (**3b**, **3k**). For one of these (**3b**), triethylamine was employed because piperidine gave nucleophilic displacement of chlorine at the 2 position with the formation of 3-(2-piperidinyl-3-indolylmethylene)-1-phenyl-1,3-dihydroindol-2-one. This behavior was previously noticed for an analogous compound.[2]

Aldehyde **1c** (Scheme 2), a starting material for the preparation of **3n**, could not be obtained by treatment of its corresponding methoxy derivative with $AlCl_3$ as described in the synthesis of **1d** from **1a**,[7] but it was possible to prepare it by means of the Vilsmeier reaction on **2c**, prepared in turn from **2a**. With an analogous procedure, compound **1d** was synthesized from **2d**, prepared in turn from **2b**.

All compounds **3** were obtained as pure geometrical isomers. From our previous experience in this field,[2,3] this means that they are in the *E* configuration because

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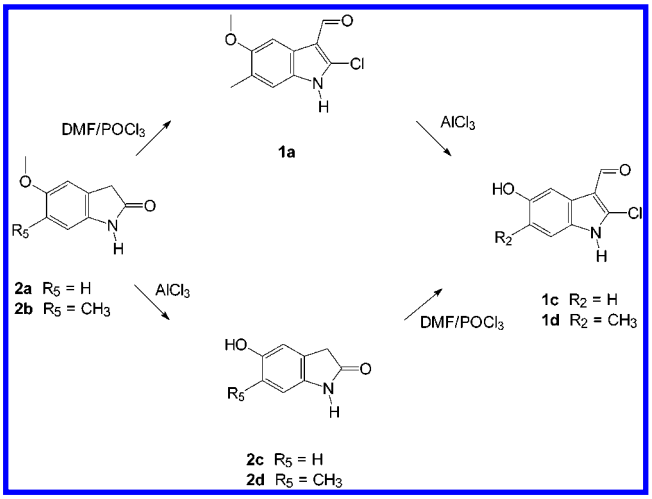
Table 1. Compounds **3**

compd	R	R_1	R_2	R_3	R_4	R_5	formula	MW	mp, °C	solvent
3a	H	H	H	H	OCH_3	CH_3	$C_{19}H_{15}ClN_2O_2$	338.8	235−240 dec	MeOH
3b	H	H	H	C_6H_5	H	H	$C_{23}H_{15}ClN_2O$	370.8	(6)	
3c	H	OCH_3	H	H	OCH_3	CH_3	$C_{20}H_{17}ClN_2O_3$	368.8	295−300 dec	MeOH
3d	H	OCH_3	H	C_6H_5	H	H	$C_{24}H_{17}ClN_2O_2$	400.9	190−194 dec	EtOH
3e	H	OCH_3	CH_3	H	H	H	$C_{19}H_{15}ClN_2O_2$	338.8	200−205 dec	pet. ether
3f	H	OCH_3	CH_3	CH_3	H	H	$C_{20}H_{17}ClN_2O_2$	352.8	290−295 dec	MeOH
3g	H	OCH_3	CH_3	H	OCH_3	H	$C_{20}H_{17}ClN_2O_3$	368.8	205−210 dec	EtOH
3h	H	OCH_3	CH_3	H	OCH_3	CH_3	$C_{21}H_{19}ClN_2O_3$	382.8	245−250 dec	EtOH
3i	H	OCH_3	CH_3	C_6H_5	H	H	$C_{25}H_{19}ClN_2O_2$	414.9	205−208 dec	EtOH
3j	CH_3	H	H	H	OCH_3	CH_3	$C_{20}H_{17}ClN_2O_2$	352.8	290−295 dec	MeOH
3k	C_6H_5	H	H	H	H	H	$C_{23}H_{15}ClN_2O$	370.8	(6)	
3l	C_6H_5	H	H	H	OCH_3	H	$C_{24}H_{17}ClN_2O_2$	400.9	188−192 dec	EtOH
3m	C_6H_5	H	H	H	OCH_3	CH_3	$C_{25}H_{19}ClN_2O_2$	414.9	180−185 dec	MeOH
3n	H	OH	H	H	H	H	$C_{17}H_{11}ClN_2O_2$	310.7	210−215 dec	EtOH
3o	H	OH	OCH_3	H	H	H	$C_{18}H_{13}ClN_2O_2$	324.8	215−217 dec	EtOH

Scheme 2



we never isolated a pure *Z* isomer directly from the reaction mixture. In the few cases where it was possible to isolate a pure *Z* isomer after several crystallizations, its solution was unstable, giving within a few hours a mixture of *E* and Z. The solutions of compounds **3** were stable, and the signals (Table S1) are in agreement with those previously published for analogous derivatives.[2,3] Moreover, it is possible to observe that the chloroindole NH is more deshielded than the oxindole NH and the substituent on this group has a deshielding effect on the methine bridge. Nevertheless, one compound (**3c**) was subjected to a series of NOE experiments (see Supporting Information) that confirmed the *E* configuration.

Pharmacological Results

(a) In Vitro Growth Inhibition and Cytotoxicity.[8] As a primary screening, compounds **3a**−**o** were evaluated for their cytotoxic potency on three human cell lines such as NCI-H460 lung cancer, MCF7 breast cancer, and SF-268 glioma. A compound is considered active when it reduces the growth of any of the cell lines to 32% or less (negative numbers indicate cell kill), and it is passed on for evaluation in the full panel of 60 cell lines. As shown in Table 2, all the compounds reported in Table 1 were active.

The panel of 60 human tumor cell lines is organized into subpanels representing leukemia, melanoma, and cancers of the lung, colon, kidney, ovary, breast, prostate, and central nervous system.

Table 2. Three-Cell Panel: Growth Percentages after Inoculation with Compounds **3a**−**o**

NCI code (NSC)	compd (10^{-4} M)	NCI-H460 (lung)	MCF7 (breast)	SF-268 (CNS)
711612	**3a**	−35	−35	−48
711614	**3b**	−1	−3	6
711613	**3c**	−23	−23	−54
711615	**3d**	2	3	15
711616	**3e**	−39	−54	−30
711619	**3f**	−18	4	−1
711617	**3g**	−48	−54	−44
711618	**3h**	−63	−51	−67
711620	**3i**	−10	1	1
711608	**3j**	−50	3	−14
711609	**3k**	−16	8	−17
711610	**3l**	−28	−6	12
711611	**3m**	5	8	51
717202	**3n**	−82	−69	−34
717201	**3o**	−80	−83	−98

The test compounds (**3a**−**o**) were dissolved in DMSO and evaluated using five concentrations at 10-fold dilutions, the highest being 10^{-4} M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. Compounds **3e**−**i** were the most active, and it is interesting to point out that all these derivatives bear the same substituents in the chloroindole ring. Table 3 reports the results obtained (only from these compounds) expressed as the log of the molar concentration, taking into consideration the 50% growth inhibitory power (GI_{50}), the cytostatic effect (TGI = total growth inhibition), and the cytotoxic effect (LC_{50}).

(b) CDK1 Inhibitory Activity.[9] As a first attempt to investigate the mechanism of the antitumor activity of compounds **3a**−**o**, they were tested in a CDK1/cyclin B kinase inhibition assay. This kinase and other members of the CDK family play a central role in the control of the cellular cycle, and it has been demonstrated that the deregulation of their activity may be involved in various human tumors.[10] Compounds **3b** and **3d**−**m**,**o** did not result to be CDK1 inhibitors, whereas compounds **3a** (IC_{50} = 20 μM) and **3c**,**n** (IC_{50} = 30 μM) showed a borderline inhibitory activity but without any correlation with the antitumor activity on the 60 cell lines.

(c) Positive Inotropic Activity. The positive inotropic activity of the most potent antitumor agents (**3e**−**i**) was evaluated according to the procedure described[5] (see Supporting Information). At 10^{-6}−10^{-4} M, they did not increase the contraction force of the guinea-pig papillary muscle.

Table 3. Growth Inhibition, Cytostatic and Cytotoxic Activity of Compounds **3e**−**i** on the 60-Cell Panel[a]

	3e[b]			**3f**[b]			**3g**[b]			**3h**[b]			**3i**[b]			vincristine sulfate[c]		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
leukemia	−7.2	−4.7	−4.1	−6.6	−4.6	−4.0	−6.3	−5.1	−4.2	−6.3	−4.8	−4.2	−5.6	−4.4	−4.0	−7.0	−4.8	−3.2
NSCLC	−6.9	−4.7	−4.3	−6.1	−5.0	−4.1	−5.6	−4.8	−4.2	−5.5	−4.8	−4.2	−5.5	−4.5	−4.1	−6.6	−4.8	−3.6
colon	−7.1	−5.1	−4.6	−6.5	−5.0	−4.2	−6.0	−5.0	−4.4	−5.4	−4.7	−4.2	−5.6	−4.7	−4.2	−7.0	−5.4	−4.1
CNS	−7.1	−5.6	−4.4	−6.7	−5.6	−4.5	−6.1	−5.2	−4.7	−6.2	−5.3	−4.6	−5.5	−4.7	−4.2	−6.9	−5.2	−3.7
melanoma	−6.9	−5.0	−4.6	−6.2	−4.6	−4.0	−6.0	−5.0	−4.5	−5.5	−4.8	−4.3	−5.5	−4.9	−4.3	−6.8	−5.1	−3.6
ovarian	−7.1	−5.2	−4.4	−6.5	−5.2	−4.3	−6.1	−5.3	−4.3	−5.7	−4.9	−4.4	−5.6	−4.8	−4.3	−6.5	−4.7	−3.5
renal	−6.7	−4.7	−4.3	−5.7	−4.2	−4.0	−5.7	−4.8	−4.3	−5.6	−4.8	−4.3	−5.4	−4.4	−4.1	−6.5	−4.7	−3.6
prostate	−6.9	−4.8	−4.4	−6.4	−4.7	−4.0	−5.9	−5.1	−4.4	−5.9	−4.9	−4.4	−5.5	−4.9	−4.0	−6.9	−5.2	−3.5
breast	−7.0	−5.3	−4.2	−6.8	−5.1	−4.0	−6.0	−5.0	−4.2	−5.6	−4.7	−4.1	−5.6	−5.0	−4.1	−6.5	−5.1	−3.5
MG_MID[d]	−7.0	−5.0	−4.3	−6.4	−4.9	−4.1	−6.0	−5.0	−4.3	−5.7	−4.8	−4.3	−5.5	−4.7	−4.1	−6.7	−5.0	−3.6

[a] All values are log of the molar concentration. [b] Highest concentration tested is 10^{-4} M. [c] Highest concentration tested is 10^{-3} M. [d] MG_MID = calculated mean panel.

Conclusions

Compounds **3e**−**i** showed good growth inhibition activity with GI_{50} values ranging from −5.4 to −7.2. In particular, *E*-3-(2-chloro-5-methoxy-6-methyl-3-indolylmethylene)-1,3-dihydroindol-2-one **3e** was as active as vincristine in prostate cancer and more potent in all the other lines. The substituents at the 5 and 6 positions of the 2-chloroindole moiety are critical because all the disubstituted compounds (**3e**−**i**) were active on the 60 lines; the lack of even one of these substituents or their presence in the 2-indolinone moiety results in loss of activity. Also the N substitution in the 2-indolinone moiety is important because the most active compounds are unsubstituted (**3e**) or bear a small group (**3f**) whereas the introduction of a bulky group (**3i**) results in a 10- to 15-fold reduction of activity.

As a second attempt to investigate the mechanism of the antitumor activity, compounds **3e**−**g** (i.e., the most active considering the calculated mean panel of Table 3) have been analyzed by means of COMPARE.[11] This program compared the patterns evinced by the test compounds with the patterns produced by the standard anticancer agents in the NCI database[12] and determined that the test compounds most likely were tubulin binders. Among these drugs, we chose vincristine because it bears two indole rings and was present in all the compounds tested (Table S2 in Supporting Information). The available antitumor activity data of vincristine are reported in Table 3 for comparison purposes (the highest dose tested was 10^{-3} instead of 10^{-4}).

Experimental Section

(a) Chemistry. The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values. Bakerflex plates (silica gel IB2−F) were used for TLC; the eluent was petroleum ether/acetone in various proportions. Kieselgel 60 (Merck) was used for column chromatography. The IR spectra were recorded in Nujol on a Nicolet Avatar 320 E.S.P.; ν_{max} is expressed in cm^{-1}. The ^1H NMR spectra were recorded in $(CD_3)_2SO$ on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in δ (ppm) and J in Hz. (Table S1 in Supporting Information). The synthesis of **2d** and **1c** is reported below; all other starting compounds are commercially available or previously described.

5-Hydroxy-6-methyl-2-indolinone 2d. 5-Methoxy-6-methyl-2-indolinone **2b** (6 mM) was dissolved in 60 mL of xylene and treated with 2 g of anhydrous $AlCl_3$. The mixture was refluxed for 2 h, the solvent was evaporated under reduced pressure, and the residue was treated with ice−water. The resulting precipitate was collected by filtration and crystallized from ethanol with a yield of 90%. $C_9H_9NO_2$ (163.2): mp 269−270 °C (dec). IR, ν_{max} (cm^{-1}): 3165, 1671, 1194, 861, 683. ^1H NMR, δ (ppm): 2.07 (3H, s, CH_3), 3.33 (2H, s, CH_2), 6.51 (1H, s, ind), 6.66 (1H, s, ind), 8.79 (1H, s, OH), 10.02 (1H, s, NH).

2-Chloro-5-hydroxyindol-3-carboxaldehyde 1c. The Vilsmeier reagent was prepared at 0−5 °C by dropping $POCl_3$ (100 mM) into a stirred solution of DMF (50 mM) in $CHCl_3$ (10 mL). 5-Hydroxindol-2-one **2c** (20 mM), dissolved in a mixture of $CHCl_3$ (70 mL) and DMF (10 mL), was added dropwise to the Vilsmeier reagent while maintaining stirring and cooling. The reaction mixture was kept for 24 h at room temperature, chloroform was removed under reduced pressure, and the resulting residue was treated with ice−water. The mixture was neutralized with $NaHCO_3$, and the crude aldehyde thus obtained was collected by filtration and crystallized from ethanol with a yield of 65%. $C_9H_6ClNO_2$ (195.6): mp 230−231 °C (dec). IR, ν_{max} (cm^{-1}): 3180, 1640, 1250, 1195, 855. ^1H NMR, δ (ppm): 6.75 (1H, dd, ind-6, J = 2.3, 8.7), 7.23 (1H, d, ind-7, J = 8.7), 7.47 (1H, d, ind-4, J = 2.3), 9.23 (1H, s, OH), 9.91 (1H, s, CH), 12.84 (1H, s, NH)

General Procedure for the Synthesis of Compounds 3. The appropriate 2-chloroaldehyde **1** (10 mM) was dissolved in methanol (100 mL) and treated with the equivalent of the appropriate indolinone **2** and piperidine (1 mL). The reaction mixture was refluxed for 3−5 h (according to a TLC test), and the precipitate formed on cooling was collected by filtration and crystallized. The yield was 60−70% for compounds **3a**,**f**,**h**−**l** and 40−50% for compounds **3b**−**e**,**g**,**m**−**o**. In some cases the precipitation was favored by partial evaporation of the solvent and/or acidification.

The spectroscopic data for the 2-chloroderivatives are reported in Table S1. In an attempt to synthesize compound **3b** in the presence of piperidine, the following derivative was isolated: *E*-3-(2-piperidinyl-3-indolylmethylene)-1-phenyl-1,3-dihydroindol-2-one. $C_{28}H_{25}N_3O$ (419.5): mp 175−179 °C (dec, MeOH). IR, ν_{max} (cm^{-1}): 3250, 1660, 1570, 1210, 1160. ^1H NMR, δ (ppm): 1.56 (6H, s, piperidine), 3.38 (4H, s, piperidine), 6.81 (2H, m, ar), 6.89 (1H, m, ar), 7.00 (3H, m, ar), 7.10 (1H, t, ar, J = 7.2), 7.29 (1H, d, ar, J = 7.2), 7.42 (1H, m, ar), 7.52 (4H, m, ar), 7.92 (1H, s, CH), 11.46 (1H, s, NH).

For experimental information on (b) antitumor activity,[8] (c) positive inotropic activity,[5] and (d) CDK1 inhibitory activity,[9] see their references and also the Supporting Information.

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Supporting Information Available: Experimental section (NOE and biology), Table S1 (IR and NMR data), and Table S2 (PCC values from COMPARE). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Potential Antitumor Agents 32. For part 31, see: Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Giorgi, G.; Salvini, L.; Garaliene V. Synthesis and Antitumor Activity of Substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones. *Anti-Cancer Drug Des.* **2001**, *16*, 167−174.

(2) Andreani, A.; Locatelli, A.; Rambaldi, M.; Leoni, A.; Bossa, R.; Fraccari, A.; Galatulas, I. Potential Antitumor Agents. 25. Synthesis and Cytotoxic Activity of 3-(2-Chloro-3-indolylmethylene)1,3-dihydroindol-2-ones. *Anticancer Res.* **1996**, *16*, 3585−3588.

(3) Andreani, A.; Locatelli, A.; Leoni, A.; Morigi, R.; Chiericozzi, M.; Fraccari, A.; Galatulas, I.; Salvatore, G. Synthesis and Potential Coanthracyclinic Activity of Pyridylmethylene and Indolylmethylene lactams. *Eur. J. Med. Chem.* **1998**, *33*, 905−909.

(4) Andreani, A.; Locatelli, A.; Leoni, A.; Rambaldi, M.; Morigi, R.; Bossa, R.; Chiericozzi, M.; Fraccari, A.; Galatulas, I. Synthesis and Potential *Coanthracyclinic* Activity of Substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones. *Eur. J. Med. Chem.* **1997**, *32*, 919−924.

(5) Andreani, A.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Recanatini, M.; Garaliene, V. Potential Antitumor Agents. 29. Synthesis and Potential Coanthracyclinic Activity of Imidazo-[2,1-*b*]thiazole Guanylhydrazones. *Bioorg. Med. Chem.* **2000**, *8*, 2359−2366.

(6) Andreani, A.; Cavalli, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Recanatini, M.; Garnier, M.; Meijer, L. Imidazo[2,1-*b*]thiazolylmethylene- and Indolylmethylene-2-indolinones: A New Class of Cyclin-Dependent Kinase Inhibitors. Design, Synthesis, and CDK1/Cyclin B Inhibition. *Anti-Cancer Drug Des.* **2000**, *15*, 447−452.

(7) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Greci, L.; Andreani, F. Study on Compounds with Potential Antitumor Activity. III. Hydrazone Derivatives of 5-Substituted 2-Chloro-3-formyl-6-methylindole. *Farmaco, Ed. Sci.* **1979**, *34*, 132−138.

(8) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.

(9) Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. Biochemical and Cellular Effects of Roscovitine, a Potent and Selective Inhibitor of the Cyclin-Dependent Kinases cdc2, cdk2 and cdk5. *Eur. J. Biochem.* **1997**, *243*, 527−536.

(10) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. Cyclin-Dependent Kinase Inhibitors: Useful Targets in Cell Cycle Regulation. *J. Med. Chem.* **2000**, *43*, 1−18.

(11) http://dtp.nci.nih.gov/docs/compare/compare_methodology.html.

(12) http://dtp.nci.nih.gov/docs/cancer/searches/standard_agent_table.html.

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