Brief Articles

Novel Adrenoceptor Antagonists with a Tricyclic Pyrrolodipyridazine Skeleton[||]

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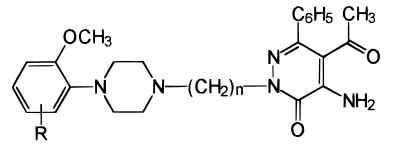
A still unknown tricyclic heterocyclic system (**5**) was synthesized from 6-hydroxy-2-methylpyridazin-3-one and its structure identified as 2,8-dichloro-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one by spectroscopic investigations. Selective condensation of **5** with 2-[4-(2-substituted-phenyl)piperazin-1-yl]ethylamine gave the 2-arylpiperazinylethylamino-8-chloro derivatives **6a**−**c**, which were investigated in binding studies toward the three α_1-adrenergic and 5-HT_{1A}-serotonergic receptor subtypes. They displayed high potency on all the assays and some selectivity for α_{1a} and α_{1d} subtypes.

Introduction

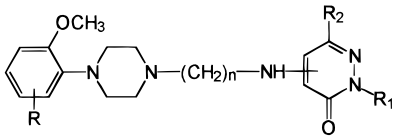
α_1-Adrenoceptors (α_1-AR) are members of the superfamily of G protein-coupled receptors (GPCR) that transduce signals across the cell membrane, thus initiating a variety of intracellular biochemical events. Like all G protein-coupled receptors, the AR family shares the presence of seven hydrophobic regions that are believed to form a bundle of α-helical transmembrane domains (TM), connected by alternating intracellular and extracellular hydrophilic loops.[1] They are at present classified into three subtypes, designated as α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}), with lowercase subscripts being used to indicate the recombinant receptors and uppercase subscripts to denote the native receptors.[2−4] The presence of these different α_1-AR subtypes in blood vessels and other smooth muscles points to the importance of developing selective drugs for receptor classification and characterization as well as for therapeutic effectiveness.

In the course of our studies in the field of new and potentially selective adrenoceptor antagonists, we synthesized a class of 4,5-disubstituted-6-phenylpyridazinones (**1**), having an arylpiperazinylalkyl chain at position 2. The members of this class carrying an ethylenic spacer between the protonated arylpiperazine and the heterocycle (e.g., **1a**) showed slight α_{1d}/α_{1a}, high α_{1d}/α_{1b}, and very high α_{1a}/5-HT_{1A} and α_{1d}/5-HT_{1A} selectivities.[5] Later on, to better evaluate the importance of the substituents on the pyridazinonic ring, we planned the synthesis of a series of derivatives (**2**) having the arylpiperazinylalkyl chain at different positions of the ring and with a less complex substitution pattern (Chart 1).

Chart 1



1 (**1a**: R=H, n=2)



2

One of the key intermediates for the synthesis of such compounds was devised into the dichloro derivative **4**, used to prepare 4-alkylamino-substituted compounds. We designed its synthesis from the known 6-hydroxy-2-methylpyridazin-3-one (**3**)[6] which, in analogy to that reported in the literature by Druey et al. for the 2-phenyl analogue,[7] could be transformed into **4** by treatment with $POCl_3$ and PCl_5. However, no evidence of **4** was detected in the reaction mixture, but a different compound (**5**) resulted as the sole reaction product. Extensive spectroscopic investigation allowed us to assign to **5** the structure of a still unknown tricyclic system, namely, 2,8-dichloro-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one. After regioselective introduction of the appropriate arylpiperazinylethylamino chain on **5**, the so-obtained **6a**−**c** were tested in binding screening to adrenergic and serotonergic receptors (Scheme 1).

We report here on the synthesis of **5** and **6a**−**c**, on the spectroscopic analyses performed for their structural determination, and on the affinity and selectivity of **6a**−**c** toward α_1-adrenergic and 5-HT_{1A}-serotonergic receptors.

[||] Dedicated to Professor Gottfried Heinisch on the occasion of his 60th birthday.

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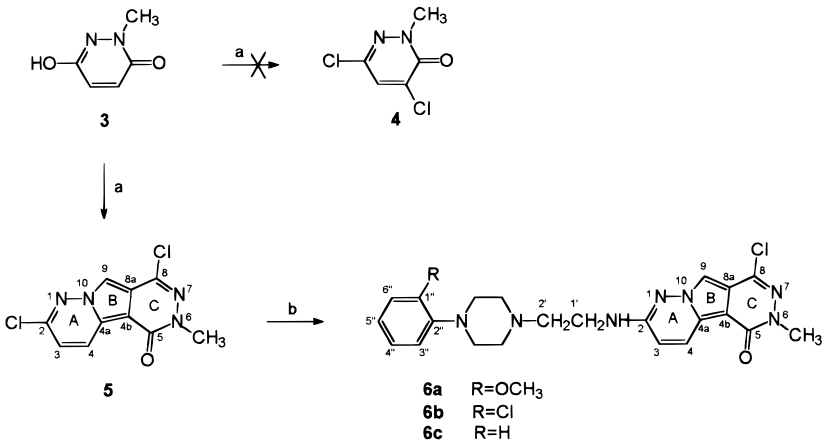
Scheme 1[a]



[a] (a) 1. $POCl_3$, Δ, 2. PCl_5, Δ; (b) 2-[4-(2-substituted-phenyl)piperazin-1-yl]ethylamine, EtOH, Δ.

Table 1. ^1H and ^{13}C NMR Data of Compounds **5** and **6a**−**c**[a]

	5	6a	6b	6c
		^1H NMR		
H-3	7.06 (d, $J = 10$)	6.55 (d, $J = 9.5$)	6.46 (d, $J = 9.5$)	6.46 (d, $J = 9.5$)
H-4	8.62 (d, $J = 10$)	8.28 (d, $J = 9.5$)	8.28 (d, $J = 9.5$)	8.28 (d, $J = 9.5$)
H-9	8.00 (s)	7.69 (s)	7.70 (s)	7.70 (s)
N-CH_3	3.84 (s)	3.81 (s)	3.81 (s)	3.81 (s)
1′-CH_2		3.61 (m)	3.50 (m)	3.51 (m)
2′-CH_2		2.94 (m)	2.73 (m)	2.71 (m)
piperazine H		3.25 (m), 2.94 (m)	3.11 (m), 2.73 (m)	3.24 (m), 2.71 (m)
phenyl H		6.85−7.07 (m)	6.90−7.40 (m)	6.65−6.98 (m), 7.24−7.32 (m)
O-CH_3		3.88 (s)		
		^{13}C NMR		
C-2	150.3	154.7	154.8	154.8
C-3	115.8	109.4	109.1	109.1
C-4	129.6	127.5	127.5	127.4
C-4a	123.9	122.1	122.1	122.1
C-4b	106.4	105.9	105.9	105.9
C-5	157.0	157.5	157.5	157.5
C-8	131.5	132.0	132.0	132.0
C-8a	121.6	119.2	119.2	119.2
C-9	111.7	110.5	110.5	110.6
N-CH_3	38.4	38.1	38.1	38.1
1′-CH_2		37.0	37.7	37.6
2′-CH_2		56.1	55.9	55.9
piperazine C		49.6, 53.0	51.1, 52.9	49.1, 52.8
phenyl C		111.2, 118.3, 121.0, 123.5, 140.3, 152.1	120.2, 123.7, 127.4, 128.7, 130.6, 149.0	116.0, 119.8, 129.1, 151.0
O-CH_3		55.3		

[a] Chemical shifts in ppm, coupling constants in Hz.

Chemistry

Reaction of compound **3** with PCl_5 in $POCl_3$ at reflux gave a single crystalline product (**5**) in good yield, whose structure was determined through spectroscopic investigations. Compound **5** was condensed with 2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine[5] to give **6a,** whose structure was determined similarly to that of **5**. Treatment with two differently substituted piperazinylalkylamines gave **6b,c**, which were easily identified on the basis of the assignment previously done for **6a**.

Structural Identification

The ^{13}C NMR spectrum of **5** in $CDCl_3$ clearly indicates the presence of 10 carbon atoms (Table 1), thus suggesting the possibility of a condensation between two pyridazinonic moieties. However, the ^1H NMR spectrum showed a very small number of signals relative to a dimeric structure; in fact, only one methyl group at 3.84 ppm and only two doublets ($J = 10$ Hz) at 7.06 and 8.62 ppm are present together with only one other signal, a singlet (1H) at 8.00 ppm. The mass spectrum ($M + 1 = 268$ *m/z* and two chlorine atoms present) suggests a molecular formula of $C_{10}H_6Cl_2N_4O$, also confirmed by elemental analysis. These data are compatible with a condensation−oxidation process which involves the carbonyl and the methyl group of a molecule and the positions 4 and 5 of the other. In fact, the number of unsaturations calculated from the molecular formula suggests that besides two hexa-atomic rings deriving from the two molecules of the precursor **3**, a new ring is present and structure **5** can be hypothesized. To confirm the correctness of the structure, a series of NMR experiments have been performed. The ^1H NMR NOE experiments performed are compatible with those of **5**; in fact, no significative NOE effects on irradiation of the methyl group at 3.84 ppm and on irradiation of the hydrogen atom at 8.00 ppm were observed. Due to the scarcity of hydrogen atoms, NMR experiments based on

Table 2. Affinity Constants of **1a**, **6a**−**c**, Prazosin, BMY7378, and 8-OH-DPAT toward Native α_1 and 5-HT_{1A} Receptors and Cloned α_1-Adrenoceptor Subtypes[a]

	K_i (nM), native receptors (rat brain)			K_i (nM), cloned receptors (human brain)		
compd	α_1	5-HT_{1A}	ratio 5-HT_{1A}/α_1	α_{1a}	α_{1b}	α_{1d}
1a[b]	24.6	123	5.01	4.75	22.1	0.93
6a	73.3	16.3	0.22	1.10	15.2	3.04
6b	54.6	56.5	1.03	5.93	13.6	11.9
6c	129.2	21.2	0.16	1.93	15.6	6.56
prazosin	0.74	2360	3190	0.58	0.28	0.29
BMY7378	282	0.37	0.0013	378	70.9	1.28
8-OH-DPAT	18000	2.33	0.00013			

[a] Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation.[15] The affinities estimated were derived from displacement of [^{3}H]prazosin binding for α_1-adrenoceptors and [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin for 5-HT_{1A} receptors. Each experiment was performed in triplicate. K_i values were from 2 to 3 experiments which agreed within 20%. [b] See ref 5.

^{13}C were indispensable for a sure structural assignment. Thus, a gated decoupled ^{13}C NMR spectrum and several heteronuclear 2D COLOC experiments were performed and allowed to confirm the structure **5** as compatible with the observed carbon−hydrogen couplings. The same experiments were then performed on **6a**. Its mass spectrum and its elemental analysis were compatible with the substitution of a chlorine atom of **5** by the amine. In the determination of its structure, special attention was given to the position of the substitution as, in principle, either the chlorine atom at C-2 or the atom at C-8 of **5** could be substituted. The ^{1}H NMR spectrum presents all the expected signals for the arylpiperazinylethylamino group besides the same signals already present in **5**. The position of the substitution was easily determined by NOE effects; in fact, on irradiation of NH, the signal of H-3 was enhanced by 7.5% while H-4 and H-9 remained unaffected; vice versa, on irradiation of H-3 a 6.6% enhancement of NH was observed (together with a 4% enhancement for H-4). A ^{13}C NMR gated decoupled experiment and several heteronuclear 2D COLOC experiments were performed in order to confirm the structure of **6a** and to assign all the carbon atoms. Similarly to **5**, in addition to all the expected large $^1J_{CH}$, several $^2J_{CH}$, $^3J_{CH}$, and $^4J_{CH}$ could be determined; in particular, all the vicinal trans couplings (6−8 Hz) were found: H-9 with C-4a and C-4b, H-3 with C-4a, H-4 with C-2; moreover, the geminal coupling between H-9 and C-8a showed a similar value, thus confirming the presence of a pentaatomic ring in the structure, as in the unsaturated ring compounds the five-membered rings are exceptional in showing large positive couplings.[8] Several smaller couplings are also in agreement with the structure: for example, the *N*-methyl group with C-5 and C-8, H-9 with C-8, etc. A comparison of the ^{13}C NMR resonances of the tricyclic moiety of compounds **5** and **6a** shows significant shifts of the carbon atoms of ring A leaving quite unaffected the signals of ring C, thus confirming the substitution of the chlorine atom at C-2. It should be noted that the NMR resonances of the carbon atoms of ring C observed for compounds **5** and **6a** appear in full agreement with the data reported in the literature[9] for 6-chloro-2-methylpyridazin-3(2*H*)-one and its adduct with diazapropane.

The structures of **6b,c** were confirmed on the basis of their ^{1}H and ^{13}C NMR spectra, also reported in Table 1.

Biological Results

Compound **6a** was tested for its affinity toward native α_1-receptor cells ([^{3}H]prazosin as specific ligand on rat cerebral cortex), 5-HT_{1A} receptor ([^{3}H]-8-OH-DPAT, rat hippocampus), and the human cloned α_1-receptor subtypes expressed on CHO cells ([^{3}H]prazosin). Prazosin, 8-OH-DPAT, and BMY7378 were used as reference standards. The results, reported in Table 2, show that it is almost equipotent toward both native receptors, its K_i values being in the nanomolar range. However, when tested toward α_1-receptor subtypes, it displays a slight preference toward α_{1a} and α_{1d} (K_i = 1.10 and 3.04 nM, respectively), with respect to α_{1b} (K_i = 15.2 nM). Its antagonism for the adrenergic receptor was also verified on the epididymal vas deferens and aorta from Sprague−Dawley rats (see Experimental Section): pK_b = 7.94 and 8.47, respectively, were found.

The interesting results obtained with **6a** intrigued us to verify if the presence of different substituents on the phenyl ring of the side chain could affect either its potency or its selectivity. Compounds **6b** (R = Cl) and **6c** (R = H) were therefore synthesized and tested in binding studies. As can be seen from the data reported in Table 2, their behavior was quite similar to that of **6a**. In fact, both compounds displayed higher affinity for the α_1-adrenergic subtypes than for the 5-HT_{1A} receptor. However, while the selectivity of **6c** was fully comparable to that of **6a**, the 2-chlorophenyl derivative **6b** did not distinguish at all between α_{1b} and α_{1d}, being only slightly more potent toward α_{1a}.

Conclusions

Treatment of compound **3** with PCl_5 in $POCl_3$ gave an unexpected compound whose structure, elucidated by spectroscopic investigations, presents the still unknown pyrrolo[1,2-*b*:3,4-*d*′]dipyridazine skeleton. Introduction of an arylpiperazinylethylamino group at position 2 of the heterocyclic system gave compounds **6a**−**c** which were investigated in binding studies toward adrenergic and serotonergic receptors. The relevant affinity of **6a**−**c** toward α_1-adrenoceptor subtypes provides a basis for us to propose them as a model for new, possibly more selective, agents. The recent hypothesis[10] that the three α_1-AR subtypes have different structural/dynamics behavior and that, in particular, the α_{1a}-AR subtype could be more flexible and sensitive to the environmental conditions with respect to the other two subtypes suggests that the new tricyclic system **5** appended to a properly substituted phenylpiperazinyl

moiety could lead to agents with improved selectivity with respect to **6a**−**c**.

Moreover, due to the novelty of their tricyclic moiety, compounds **6** could be a potential substrate to be developed in different areas, since the pyridazine derivatives have been reported in the past decade to display a wide range of pharmacological activities.[11]

Experimental Section

Chemistry. Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. Elemental analyses for the test compounds were within ±0.4 of the theoretical values. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 230−400 mesh) was used for flash chromatography. The structures of all compounds were consistent with their analytical and spectroscopic data.

2,8-Dichloro-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one, 5. Compound **3**[6] (1.8 g, 0.014 mol) was refluxed with excess $POCl_3$ (7 mL) for 2 h and cooled to 50 °C, PCl_5 (6.4 g, 0.031 mol) added in small portions, and the mixture refluxed overnight. After cooling, the mixture was poured onto iced water (30 mL), the pH adjusted to about 6 by 6 N NaOH, and then the mixture thoroughly extracted by dichloromethane (3 × 20 mL). After evaporation of the solvent, the residue was purified by flash chromatography, eluting with cyclohexane/ethyl acetate (7/3), and finally crystallized from acetic acid: mp = 244.5 °C; yield 1.83 g (95.3%). Anal. ($C_{10}H_6Cl_2N_4O$) C, H, Cl, N.

8-Chloro-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamino}-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one, 6a. A solution of **5** (100 mg, 0.37 mmol) and 2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine[12] (330 mg, 1.40 mmol) in EtOH (5 mL) was refluxed for 72 h. After evaporation of the solvent, the residue was purified by flash chromatography, eluting with dichloromethane/methanol (98/2), to give 50 mg of **6a**: yield 28.8%; mp = 98−99 °C. Anal. ($C_{23}H_{26}ClN_7O_2$) C, H, Cl, N.

8-Chloro-2-{2-[4-(2-chlorophenyl)piperazin-1-yl]ethylamino}-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one, 6b. Compound **6b** was prepared following the same procedure above-reported for **6a**, starting from **5** and 2-[4-(2-chlorophenyl)piperazin-1-yl]ethylamine:[12] yield 32.2%; mp = 104−105 °C. Anal. ($C_{22}H_{23}Cl_2N_7O$) C, H, Cl, N.

8-Chloro-2-[2-(4-phenylpiperazin-1-yl)ethylamino]-6-methylpyrrolo[1,2-*b*:3,4-*d*′]dipyridazin-5(6*H*)-one, 6c. Compound **6c** was prepared following the same procedure above-reported for **6a**, starting from **5** and 2-(4-phenylpiperazin-1-yl)ethylamine: yield 19.4%; mp = 164−165 °C. Anal. ($C_{22}H_{24}ClN_7O$) C, H, Cl, N.

NMR Experiments. The NMR spectra of **5** and **6a**−**c** were recorded with a Bruker AC-E 300 spectrometer, equipped with an Aspect 3000 computer, a process controller, and an array processor, in deuteriochloroform for 0.2 M solutions at 298 K. For the COLOC spectra 256 experiments of 24 scans each were run with a spectral width of 20 000 Hz in F_2 (size 4K) and 2 000 Hz in F_1 (size 1K). The data were then zero-filled to give a final 4K × 1K matrix. The delay times Δ_1 and Δ_2 were fixed to values suitable to enphasize long-range couplings, for example, 42 and 21 ms, respectively, in the experiment to detect 12-Hz couplings or 167 and 83 ms in the experiment to detect 3-Hz couplings.

The gated decoupled experiments were performed using a standard pulse sequence (with operating Bruker software). The spectra were collected using 32K data sets over a spectral width of 20 000 Hz with a relaxation delay (D_1) of 5 s.

The NOE difference spectra were obtained using standard steady-state difference experiments. The signal was irradiated with sufficient power (45 L) to saturate only this signal repeating the process until 16 transients had been accumulated. The process was repeated with the decoupler set far off resonance, and the total procedure was repeated 200 times. The resulting FIDs were subtracted and Fourier transformed using an exponential multiplicator with 3-Hz line broadening.

Radioligand Binding Assays at Native Receptors. Binding studies on native α_1-adrenergic and 5-HT_{1A}-serotonergic receptors were carried out in membranes of rat cerebral cortex (α_1) and hippocampus (5-HT_{1A}). Male Sprague−Dawley rats (200−300 g; Charles River, Italy) were killed by cervical dislocation, and different tissues were excised, immediately frozen, and stored at −70 °C until use. Tissues were homogenized (2 × 20 s) in 50 volumes of cold Tris-HCl buffer (pH 7.4), using a Politron homogenizer (speed 7). Homogenates were centrifuged at 49000*g* for 10 min, resuspended in 50 volumes of the same buffer, incubated at 37 °C for 15 min, and then centrifuged and resuspended twice more. The final pellets were suspended in 100 volumes of Tris-HCl buffer (pH 7.4), containing 10 μM pargiline and 0.1% ascorbic acid. Membranes were incubated in a final volume of 1 mL for 30 min at 25 °C with 0.1−0.5 nM [^{3}H]prazosin (α_1) or 0.5−1.5 nM [^{3}H]-8-OH-DPAT (5-HT_{1A}), in the absence or presence of competing drugs; nonspecific binding was determined in the presence of 10 μM phentolamine (α_1) or 10 μM 5-HT (5-HT_{1A}).

The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters. The filters were then washed with ice-cold buffer, and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

Radioligand Binding Assay at Cloned α_1-Adrenoceptors. Binding to cloned human α_1-adrenoceptor subtypes was performed in membranes from CHO cells (Chinese hamster ovary cells) transfected by electroporation with DNA expressing the gene encoding each α_1-adrenoceptor subtype. Cloning and stable expression of the human α_1-adrenoceptor gene was performed as previously described.[13] CHO cell membranes (30 μg of protein) were incubated in 50 mM Tris-HCl, pH 7.4, with 0.1−0.4 nM [^{3}H]prazosin, in a final volume of 1 mL for 30 min at 25 °C, in the absence or presence of competing drugs (1 pM−10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% poly(ethylenimine)-pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

Data Analysis. The inhibition of specific binding of the radioligands by the tested drugs was analyzed to estimate the IC_{50} value by using the nonlinear curve-fitting program Allfit.[14] The IC_{50} value was converted to an affinity constant (K_i) by the equation of Cheng and Prusoff.[15]

Functional Studies. Evaluation of compound **6a** for α_1-antagonism was performed on epididymal vas deferens and aorta, obtained from Sprague−Dawley rats (350−450 body weight) sacrificed by cervical dislocation. The epididymal portion of each vas deference and thoracic aorta was isolated, freed of adhering connective tissue, and placed in Krebs solution containing NaCl (112 mM), glucose (11.1 mM), $NaHCO_3$ (25 mM), KCl (4.7 mM), $CaCl_2$ (2.5 mM), $KHPO_4$ (1.2 mM), and $MgSO_4$ (1.2 mM). Desmethylimipramine (0.1 μM) and corticosterone (1 μM) to block neuronal and extraneuronal uptake of noradrenaline, (±)-propanolol (1 μM) to block β-receptors, and yohimbine (0.1 μM) to block α_2-receptors were added to the Krebs solution. Vas deference segments (20 mm long) and aortic strips (2 × 30 mm long) were mounted for isotonic tension recording in 20-mL organ bath, containing Krebs buffer aerated constantly with 95% O_2−5% CO_2 and maintained at 37 °C, and loaded with a resting tension of 0.5 and 1.5 g, respectively. The tissues were allowed to equilibrate for 60 min with washing every 20 min. After the equilibration period, tissues were primed by addition of 10 μM noradrenaline. After another washing and equilibration period of 60 min, a noradrenaline concentration−response curve was constructed (basal curve). Following washout of noradrenaline, single concentrations of the compounds were incubated for 30 min before repeating the noradrenaline concentration−response curve. Responses were expressed as percentage of the maximal contraction observed in the basal noradrenaline

concentration−response curve. Schild plot parameters were evaluated by linear regression analysis according to Tallarida and Murray.[16]

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