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Gregg

[11] **Patent Number: 6,057,339**

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[54] **METHOD OF INHIBITING OR TREATING PHYTOSTEROLEMIA WITH AN MTP INHIBITOR**

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[73] Assignee: **Bristol-Myers Squibb Company**, Princeton, N.J.

[21] Appl. No.: **09/005,430**

[22] Filed: **Jan. 10, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/035,591, Jan. 17, 1997.

[51] **Int. Cl.**[7] **A61K 31/445**; A61K 31/21

[52] **U.S. Cl.** **514/325**; 514/510; 514/824

[58] **Field of Search** 514/325, 510, 514/824

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,502,045	3/1996	Miettinen et al.	514/182
5,712,279	1/1998	Biller et al.	514/252

FOREIGN PATENT DOCUMENTS

WO96/26205	8/1996	WIPO .	

OTHER PUBLICATIONS

Scriver et al, "The Metabolic and Molecular Bases of Inherited Disease", Seventh Edition, vol. II, Bjorkhem et al, "Inborn Errors in Bile Acid Biosynthesis and Storage of Sterols Othern Than Cholesterol", pp. 2073–2099, (1995).

Cobb et al, "Sitosterolemia–Opposing Effects of Cholestytamine and Lovastatin on Plasma Sterol Levels in a Homozygous Girl and Her Heterozygous Father", (Rockefeller Univ., Biochem. Genet & Betab Lab, New York, NY), Abstract No. 96:434984, Metabolism–Clinical and Experimental, vol. 45, No. 6, pp. 673–679, Jun. 1996.

CA: 124:174453, Parsons et al., 1995.

CA 125:132403, Hidaka et al., 1995.

Primary Examiner—Kimberly Jordan
Attorney, Agent, or Firm—Burton Rodney; Ronald S. Hermenau

[57] **ABSTRACT**

A method is provided for inhibiting onset of or treating phytosterolemia by administering to a patient an MTP inhibitor, alone or optionally, in combination with another cholesterol lowering drug, such as pravastatin.

25 Claims, No Drawings

METHOD OF INHIBITING OR TREATING PHYTOSTEROLEMIA WITH AN MTP INHIBITOR

This application claims benefit to U.S. Provisional Application 60/035,591, filed Jan. 17, 1997.

FIELD OF THE INVENTION

The present invention related to a method for inhibiting onset of or treating phytosterolemia, by administering an MTP inhibitor alone or in combination with another cholesterol lowering drug, such as pravastatin.

BACKGROUND OF THE INVENTION

As indicated in Scriver, C. R. et al "Metabolic Basis of Inherited Diseases" Vol. II (1995), Chap. 65, Inborn Errors in Bile Acid Biosynthesis and Storage of Sterols Other than Cholesterol, Bjorkhem, I. and Boberg, K. M., pp. 2073–2099, phytosterolemia (also referred to as sitosterolemia) is a rare inherited sterol storage disease involving increased intestinal absorption of phytosterol or shellfish sterols and decreased fecal secretion. It is characterized by "tendon and tuberous xanthomas and by a strong predisposition to premature coronary atherosclerosis . . . . Increased amounts of phytosterols (plant sterols), such as sitosterol and campesterol and their 5α-stanols, are found in blood, plasma, erythrocytes, and different tissues, especially in the xanthomas and arteries of affected subjects. Increased serum cholesterol and cholesterol have also been found in many patients." (p. 2073)

Patients afflicted with phytosterolemia have been found to have an increased incidence of coronary heart disease at an early age most likely due to early development of atherosclerosis at an early age. Bjorkhem et al, supra, indicate at page 2090 that "the mechanism behind the atherosclerosis is unexplained, but a high content of plant sterols in the circulating lipoproteins might promote their deposition in the arterial wall."

The microsomal triglyceride transfer protein (MTP) catalyzes the transport of triglyceride (TG), cholesteryl ester (CE), and phosphatidylcholine (PC) between small unilamellar vesicles (SUV). Wetterau & Zilversmit, *Chem. Phys. Lipids* 38, 205–22 (1985). When transfer rates are expressed as the percent of the donor lipid transferred per time, MTP expresses a distinct preference for neutral lipid transport (TG and CE), relative to phospholipid transport. The microsomal triglyceride transfer protein from bovine liver has been isolated and extensively characterized (1). This has led to the cloning of cDNA expressing the protein from several species, including humans (2). MTP is composed of two subunits. The small subunit is the previously characterized multifunctional protein, protein disulfide isomerase. This is supported by biochemical analysis of the protein (3) as well as co-expression studies performed in insect Sf9 cells using the baculovirus expression system. Expression of soluble active MTP requires the co-expression of PDI and the unique large subunit of MTP (4).

1: Wetterau, J. R. and Zilversmit, D. B. (1985) Chem. Phys. Lipids 38, 205–222.
Wetterau, J. R., et al, (1990) J. Biol. Chem. 265, 9800–9807.
Wetterau, J. R., et al, (1991) Biochemistry 30, 4406–4412.
Atzel, A., and Wetterau, J. R. (1993) Biochemistry 32, 10444–10450.
Atzel, A., and Wetterau, J. R. (1994) Biochemistry 33, 15382–15388.
Jamil, H., et al, (1995) J. Biol. Chem. 270, 6549–6554.
2. Sharp, D. et al, (1993) Nature 365, 65–69.
Lin, M. C. M., et al, J. Biol. Chem. 269, 29138–29145.
Nakamuta, M., et al, (1996) Genomics 33, 313–316.
3. Wetterau, J. R., et al, (1990) J. Biol. Chem. 265, 9800–9807.
Wetterau, J. R., et al, (1991) Biochemistry 30, 9728–9735.
4. Ricci, B., et al, (1995) J. Biol. Chem. 270, 14281–14285.

In vitro, MTP catalyzes the transport of lipid molecules between phospholipid membranes. Presumably, it plays a similar role in vivo, and thus plays some role in lipid metabolism. The subcellular (lumen of the microsomal fraction) and tissue distribution (liver and intestine) of MTP have led to speculation that it plays a role in the assembly of plasma lipoproteins, as these are the sites of plasma lipoprotein assembly. Wetterau & Zilversmit, *Biochem. Biophys. Acta* 875, 610–7 (1986). The ability of MTP to catalyze the transport of TG between membranes is consistent with this hypothesis, and suggests that MTP may catalyze the transport of TG from its site of synthesis in the endoplasmic reticulum (ER) membrane to nascent lipoprotein particles within the lumen of the ER.

Abetalipoproteinemia is an autosomal recessive disease characterized by a virtual absence of plasma lipoproteins which contain apolipoprotein B (apoB). Kane & Havel in *The Metabolic Basis of Inherited Disease,* Sixth edition, 1139–64 (1989). Plasma TG levels may be as low as a few mg/dL, and they fail to rise after fat ingestion. Plasma cholesterol levels are often only 20–45 mg/dL. These abnormalities are the result of a genetic defect in the assembly and/or secretion of very low density lipoproteins (VLDL) in the liver and chylomicrons in the intestine. The molecular basis for this defect had not been previously determined. In subjects examined, triglyceride, phospholipid, and cholesterol synthesis appear normal. At autopsy, subjects are free of atherosclerosis. Schaefer et al., *Clin. Chem.* 34, B9–12 (1988). A link between the apoB gene and abetalipoproteinemia has been excluded in several families. Talmud et al., *J. Clin. Invest.* 82, 1803–6 (1988) and Huang et al., *Am. J. Hum. Genet.* 46, 1141–8 (1990).

Recent reports (5) demonstrate that the defect causing abetalipoproteinemia is in the MTP gene, and as a result, the MTP protein. When examined, individuals with abetalipoproteinemia have no MTP activity, as a result of mutations in the MTP gene, some of which have been characterized. These results indicate that MTP is required for the synthesis of apoB containing lipoproteins, such as VLDL, the precursor to LDL. It therefore follows that inhibitors of MTP would inhibit the synthesis of VLDL and LDL, thereby lowering VLDL levels, LDL levels, cholesterol levels, and triglyceride levels in animals and man.

5. Wetterau, J. R., et al, (1992) Science 258, 999–1001.
Sharp, D., et al, (1993) Nature 365, 65–69.
Ricci, B., et al, (1995) J. Biol. Chem. 270, 14281–14285.
Shoulders, C. C., et al, (1993) Hum. Mol. Genetics 2, 2109–2116.
Narcisi, T. M. E., et al, (1995) Am. J. Hum. Genet. 57, 1298–1310.

Rehberg, E. F., et al, J. Biol. Chem (in press).

Canadian Patent Application No. 2,091,102 published Mar. 2, 1994 (corresponding to U.S. application Ser. No. 117,362, filed Sep. 3, 1993 (file DC21b)) which is incorporated herein by reference), reports MTP inhibitors which also block apoB containing lipoprotein secretion in a human hepatic cell line (HepG2 cells). This provides further support for the proposal that an MTP inhibitor would lower apoB containing lipoprotein and lipid levels in vivo. This Canadian patent application discloses a method for identifying the MTP inhibitors.

The use of microsomal triglyceride transfer protein (MTP) inhibitors for decreasing serum lipids including cholesterol and triglycerides and their use in treating atherosclerosis, obesity, hyperglycemia, and pancreatitis is disclosed in WO 96/26205, published Aug. 29, 1996, U.S. application Ser. No. 472,067, filed Jun. 6, 1995 (file DC21e), U.S. application Ser. No. 548,811, filed Jan. 11, 1996 (file DC21h), U.S. provisional application Ser. No. 60/017,224, filed May 9, 1996 (file HX79a*), U.S. provisional application Ser. No. 60/017,253, filed May 10, 1996 (file HX82*), U.S. provisional application Ser. No. 60/017,254, May 10, 1996 (file HX84*) and U.S. provisional application Ser. No. 60/028,216, filed Oct. 1, 1996 (file HX86*).

All of the above U.S. applications are incorporated herein by reference.

DESCRIPTION OF THE INVENTION

In accordance with the present invention, a method is provided for inhibiting onset of or treating phytosterolemia, in mammalian species, wherein a therapeutically effective amount of a microsomal triglyceride transfer protein (MTP) inhibitor is administered to a patient in need of treatment.

The MTP inhibitor may optionally be administered in combination with another cholesterol lowering drug or delipidating agent.

The MTP inhibitor alone or optionally in combination with another cholesterol lowering drug is administered systemically, such as orally or parenterally or transdermally, to patients in need of treatment.

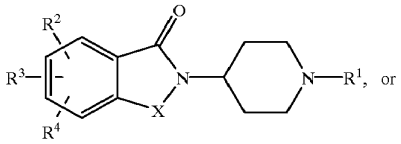
In accordance with the present invention, the MTP inhibitor lowers plasma cholesterol (LDL-cholesterol) to at least about 50% of normal LDL blood level, preferably down to less than about 25% of normal, and lowers triglycerides to at least about 50% of normal triglyceride blood level, and preferably down to about 25% or less of normal, and thereby reduces plasma cholesterol and resulting atherosclerosis.

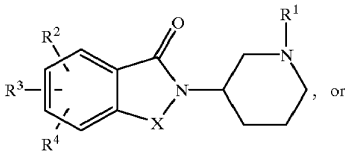
The terms "another cholesterol lowering drug or agent" or "another delipidating drug" will be employed interchangeably herein.

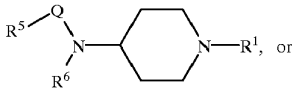
MTP inhibitors to be employed in the methods of the invention include MTP inhibitors disclosed in Canadian Patent Application No. 2,091,102 described hereinbefore (corresponding to U.S. application Ser. No. 117,362), WO 92/26205 published Aug. 29, 1996, U.S. application Ser. No. 472,067, filed Jun. 6, 1995 (file DC21e), U.S. application Ser. No. 548,811, filed Jan. 11, 1996 (file DC21h), U.S. provisional application Ser. No. 60/017,224, filed May 9, 1996 (file HX79a*), U.S. provisional application Ser. No. 60/017,253, filed May 10, 1996 (file HX82*), U.S. provisional application Ser. No. 60/017,254, filed May 10, 1996 (file HX84*), and U.S. provisional application Ser. No. 60/028,216, filed Oct. 1, 1996 (file HX86*). Preferred are each of the preferred MTP inhibitors disclosed in each of the above applications.

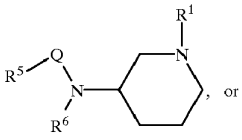
All of the above U.S. applications are incorporated herein by reference.

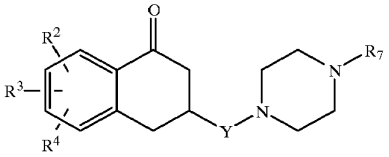
The MTP inhibitors disclosed in U.S. application Ser. No. 472,067, filed Jun. 6, 1995 (file DC21e) are piperidine compounds of the structure

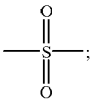






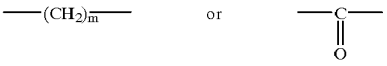




where Q is  or  ;

X is: CHR^8, 

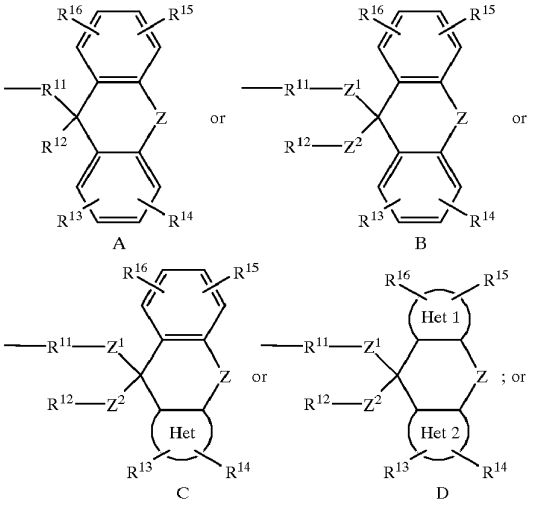
R^8, R^9 and R^{10} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, or cycloalkylalkyl;

Y is 

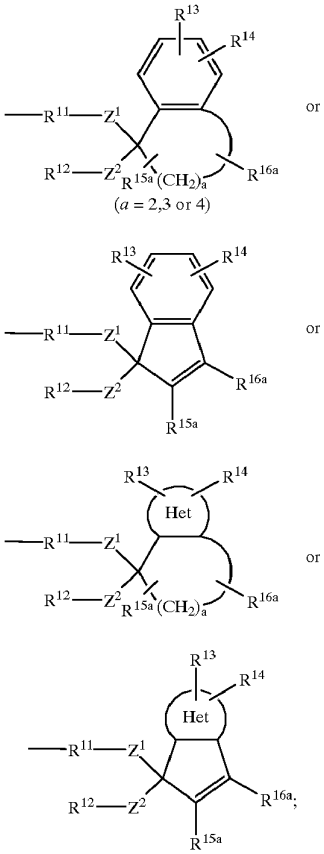
wherein m is 2 or 3;

R^1 is alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl wherein alkyl has at least 2 carbons, diarylalkyl, arylalkenyl, diarylalkenyl, arylalkynyl, diarylalkynyl, diarylalkylaryl, heteroarylalkyl wherein alkyl has at least 2 carbons, cycloalkyl, or cycloalkylalkyl wherein alkyl has at least 2 carbons, all optionally substituted through available carbon atoms with 1, 2, 3 or 4 groups selected from halo, haloalkyl, alkyl, alkenyl, alkoxy, aryloxy, aryl, arylalkyl, alkylmercapto, arylmercapto, cycloalkyl, cycloalkylalkyl, heteroaryl, fluorenyl, heteroarylalkyl, hydroxy or oxo;

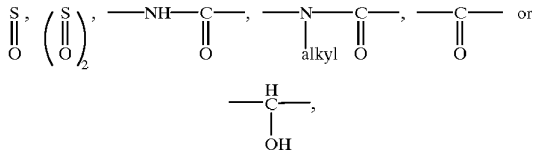
or R^1 is a fluorenyl-type group of the structure



R^1 is an indenyl-type group of the structure

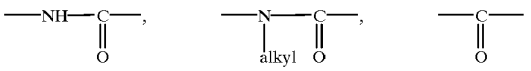


Z^1 and Z^2 are the same or different and are independently a bond, O, S,



with the proviso that with respect to B, at least one of Z^1 and Z^2 will be other than a bond; R^{11} is a bond, alkylene, alkenylene or alkynylene of up to 10 carbon atoms; arylene or mixed arylene-alkylene; R^{12} is hydrogen, alkyl, alkenyl, aryl, haloalkyl, trihaloalkyl, trihaloalkylalkyl, heteroaryl, heteroarylalkyl, arylalkyl, arylalkenyl, cycloalkyl, aryloxy, alkoxy, arylalkoxy or cycloalkylalkyl, with the provisos that preferably

(1) when R^{12} is H, aryloxy, alkoxy or arylalkoxy, then Z^2 is



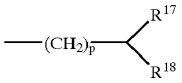
or a bond and

(2) when Z^2 is a bond, R^{12} cannot be heteroaryl or heteroarylalkyl;

Z is a bond, O, S, N-alkyl, N-aryl, or alkylene or alkenylene from 1 to 5 carbon atoms; R^{13}, R^{14}, R^{15}, and R^{16} are independently hydrogen, alkyl, halo, haloalkyl, aryl, cycloalkyl, cycloheteroalkyl, alkenyl, alkynyl, hydroxy, alkoxy, nitro, amino, thio, alkylsulfonyl, arylsulfonyl, alkylthio, arylthio, aminocarbonyl, alkylcarbonyloxy, arylcarbonylamino, alkylcarbonylamino, arylalkyl, heteroaryl, heteroarylalkyl or aryloxy;

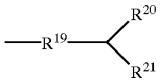
R^{15a} and R^{16a} are independently hydrogen, alkyl, halo, haloalkyl, aryl, cycloalkyl, cycloheteroalkyl, alkenyl, alkynyl, alkoxy, alkylsulfonyl, arylsulfonyl, alkylthio, arylthio, aminocarbonyl, alkylcarbonyloxy, arylcarbonylamino, alkylcarbonylamino, arylalkyl, heteroaryl, heteroarylalkyl, or aryloxy;

or R^1 is a group of the structure



wherein p is 1 to 8 and R^{17} and R^{18} are each independently H, alkyl, alkenyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl or cycloalkylalkyl at least one of R^{17} and R^{18} being other than H;

or R^1 is a group of the structure



wherein R^{19} is aryl or heteroaryl;

R^{20} is aryl or heteroaryl;

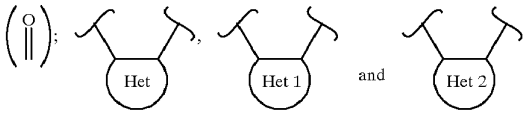
R^{21} is H, alkyl, aryl, alkylaryl, arylalkyl, aryloxy, arylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, cycloalkyl, cycloalkylalkyl or cycloalkylalkoxy;

R^2, R^3, R^4 are independently hydrogen, halo, alkyl, alkenyl, alkoxy, aryloxy, aryl, arylalkyl, alkylmercapto, arylmercapto, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, hydroxy or haloalkyl;

R^5 is independently alkyl, alkenyl, alkynyl, aryl, alkoxy, aryloxy, arylalkoxy, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkyl, cycloalkylalkyl, polycycloalkyl, polycycloalkylalkyl, cycloalkenyl, cycloheteroalkyl, heteroaryloxy, cycloalkenylalkyl, polycycloalkenyl, polycycloalkenylalkyl, heteroarylcarbonyl, amino, alkylamino, arylamino, heteroarylamino, cycloalkyloxy, cycloalkylamino, all optionally substituted through available carbon atoms with 1, 2, 3 or 4 groups selected from hydrogen, halo, alkyl, haloalkyl, alkoxy, haloalkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloheteroalkyl, cycloheteroalkylalkyl, aryl, heteroaryl, arylalkyl, arylcycloalkyl, arylalkenyl, arylalkynyl, aryloxy, aryloxyalkyl, arylalkoxy, arylazo, heteroaryloxo, heteroarylalkyl, heteroarylalkenyl, heteroaryloxy, hydroxy, nitro, cyano, amino, substituted amino, thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkylcarbonyl, arylcarbonyl, arylaminocarbonyl, alkoxycarbonyl, aminocarbonyl, alkynylaminocarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonylamino, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonyl, alkylsulfonyl, arylsulfonylamino, heteroarylcarbonylamino, heteroarylsulfinyl, heteroarylthio, heteroarylsulfonyl, alkylsulfinyl;

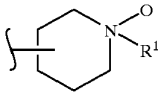
R^6 is hydrogen or C_1–C_4 alkyl or C_1–C_4 alkenyl; all optionally substituted with 1, 2, 3 or 4 groups which may independently be any of the substituents listed in the definition of R^5 set out above;

R^7 is alkyl, aryl or arylalkyl wherein alkyl by itself or as part of arylalkyl is optionally substituted with oxo



are the same or different and are independently selected from heteroaryl containing 5- or 6-ring members; and

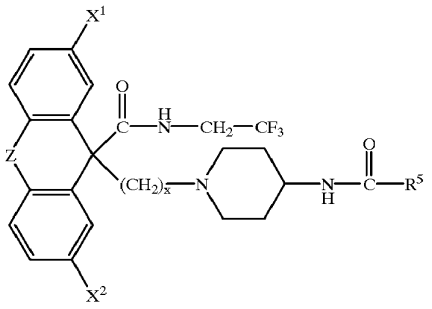
N-oxides



thereof; and

pharmaceutically acceptable salts thereof; with the provisos that preferably where in the first formula X is CH_2, and R^2, R^3 and R^4 are each H, then R^1 will be other than 3,3-diphenylpropyl, and preferably in the fifth formula, where one of R^2, R^3 and R^4 is 6-fluoro, and the others are H, R^7 will be other than 4-(2-methoxyphenyl).

The MTP inhibitors disclosed in U.S. application Ser. No. 548,811 filed Jan. 11, 1996 (file DC21h), have the structure



including the piperidine N-oxide thereof or a pharmaceutically acceptable salt thereof, wherein Z is a bond, O or S;

X^1 and X^2 are independently selected from H or halo;

x is an integer from 2 to 6;

R^5 is heteroaryl, aryl, heterocycloalkyl or cycloalkyl, each R^5 group being optionally substituted with 1, 2, 3 or 4 substituents which may be the same or different.

The MTP inhibitors disclosed in U.S. provisional application No. 60/017,224, filed May 9, 1996 (file HX79a*) have the structure

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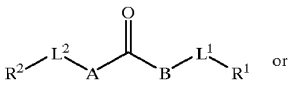

or

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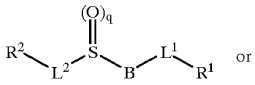

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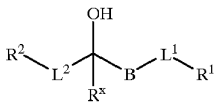


including pharmaceutically acceptable salts thereof, wherein q is 0, 1 or 2;

A is

(1) a bond;

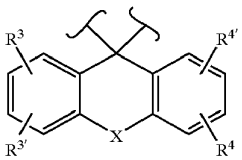
(2) —O—; or

(3)

;

where R^5 is H or lower alkyl or R^5 together with R^2 forms a carbocyclic or heterocyclic ring system containing 4 to 8 members in the ring.

B is a fluorenyl-type group of the structure:



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