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Biller et al.

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[45] **Date of Patent: Apr. 14, 1998**

[54] **INHIBITORS OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN AND METHOD**

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[21] Appl. No.: **472,067**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 391,901, Feb. 21, 1995, abandoned, which is a continuation-in-part of Ser. No. 284,808, Aug. 5, 1994, abandoned, which is a continuation-in-part of Ser. No. 117,362, Sep. 3, 1993, Pat. No. 5,595,872.

[51] **Int. Cl.**[6] **C07D 211/98**; C07D 409/06; C07D 405/06; C07D 211/56; C07D 211/58; A61K 31/445

[52] **U.S. Cl.** **514/252**; 546/203; 546/204; 546/205; 546/206; 546/194; 546/196; 546/198; 546/199; 546/200; 546/201; 546/208; 546/202; 546/193; 546/189; 546/187; 546/244; 546/212; 546/214; 546/224; 546/213; 544/235; 544/238; 544/277; 544/406; 544/407; 544/287; 544/130; 544/360; 544/88; 544/405; 544/364; 544/391; 544/399; 514/266; 514/319; 514/318; 514/320; 514/321; 514/322; 514/325; 514/324; 514/316; 514/259; 514/235.5; 514/255

[58] **Field of Search** 546/203, 204, 546/205, 197, 194, 206, 196, 198, 199, 200, 201; 514/325, 319, 321, 318, 329, 320, 322, 252, 266; 544/406, 407, 235, 238, 277

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(List continued on next page.)

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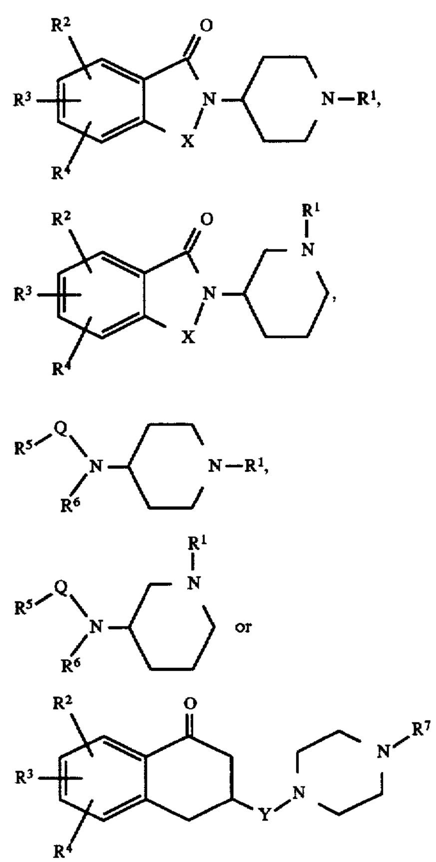
[57] **ABSTRACT**

Compounds are provided which inhibit microsomal triglyceride transfer protein and thus are useful for lowering serum lipids and treating atherosclerosis and related diseases. The compounds have the structure



wherein R^1 to R^7, Q, X and Y are as defined herein.

38 Claims, No Drawings

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INHIBITORS OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN AND METHOD

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of application Ser. No. 391,901 filed Feb. 21, 1995, now abandoned, which is a continuation-in-part of application Ser. No. 284,808 filed Aug. 5, 1994, now abandoned, which is a continuation-in-part of application Ser. No. 117,362 filed Sep. 3, 1993, now U.S. Pat. No. 5,595,872, each of which is hereby incorporated by reference.

FIELD OF THE INVENTION

This invention relates to novel compounds which inhibit microsomal triglyceride transfer protein, and to methods for decreasing serum lipids and treating atherosclerosis employing such compounds.

BACKGROUND OF THE INVENTION

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 protein from bovine liver has been isolated and characterized. Wetterau & Zilversmit, *Chem. Phys. Lipids* 38, 205–22 (1985). Polyacrylamide gel electrophoresis (PAGE) analysis of the purified protein suggests that the transfer protein is a complex of two subunits of apparent molecular weights 58,000 and 88,000, since a single band was present when purified MTP was electrophoresed under nondenaturing condition, while two bands of apparent molecular weights 58,000 and 88,000 were identified when electrophoresis was performed in the presence of sodium dodecyl sulfate (SDS). These two polypeptides are hereinafter referred to as 58 kDa and 88 kDa, respectively, or the 58 kDa and the 88 kDa component of MTP, respectively, or the low molecular weight subunit and the high molecular weight subunit of MTP, respectively.

Characterization of the 58,000 molecular weight component of bovine MTP indicates that it is the previously characterized multifunctional protein, protein disulfide isomerase (PDI). Wetterau et al., *J. Biol. Chem.* 265, 9800–7 (1990). The presence of PDI in the transfer protein is supported by evidence showing that (1) the amino terminal 25 amino acids of the bovine 58,000 kDa component of MTP is identical to that of bovine PDI, and (2) disulfide isomerase activity was expressed by bovine MTP following the dissociation of the 58 kDa–88 kDa protein complex. In addition, antibodies raised against bovine PDI, a protein which by itself has no TG transfer activity, were able to immunoprecipitate bovine TG transfer activity from a solution containing purified bovine MTP.

PDI normally plays a role in the folding and assembly of newly synthesized disulfide bonded proteins within the lumen of the endoplasmic reticulum. Bulleid & Freedman, *Nature* 335, 649–51 (1988). It catalyzes the proper pairing of cysteine residues into disulfide bonds, thus catalyzing the proper folding of disulfide bonded proteins. In addition, PDI has been reported to be identical to the beta subunit of

human prolyl 4-hydroxylase. Koivu et al., *J. Biol. Chem.* 262, 6447–9 (1987). The role of PDI in the bovine transfer protein is not clear. It does appear to be an essential component of the transfer protein as dissociation of PDI from the 88 kDa component of bovine MTP by either low concentrations of a denaturant (guanidine HCl), a chaotropic agent (sodium perchlorate), or a nondenaturing detergent (octyl glucoside) results in a loss of transfer activity. Wetterau et al., *Biochemistry* 30, 9728–35 (1991). Isolated bovine PDI has no apparent lipid transfer activity, suggesting that either the 88 kDa polypeptide is the transfer protein or that it confers transfer activity to the protein complex.

The tissue and subcellular distribution of MTP activity in rats has been investigated. Wetterau & Zilversmit, *Biochem. Biophys. Acta* 875, 610–7 (1986). Lipid transfer activity was found in liver and intestine. Little or no transfer activity was found in plasma, brain, heart, or kidney. Within the liver, MTP was a soluble protein located within the lumen of the microsomal fraction. Approximately equal concentrations were found in the smooth and rough microsomes.

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 has 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).

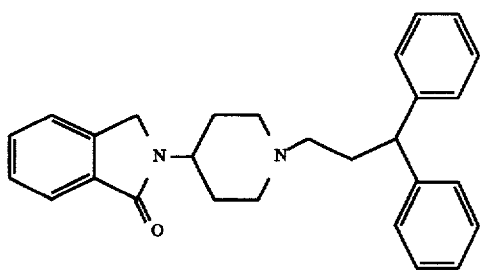
Subjects with abetalipoproteinemia are afflicted with numerous maladies. Kane & Havel, supra. Subjects have fat malabsorption and TG accumulation in their enterocytes and hepatocytes. Due to the absence of TG-rich plasma lipoproteins, there is a defect in the transport of fat-soluble vitamins such as vitamin E. This results in acanthocytosis of erythrocytes, spinocerebellar ataxia with degeneration of the fasciculus cuneatus and gracilis, peripheral neuropathy, degenerative pigmentary retinopathy, and ceroid myopathy. Treatment of abetalipoproteinemic subjects includes dietary restriction of fat intake and dietary supplementation with vitamins A, E and K.

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.

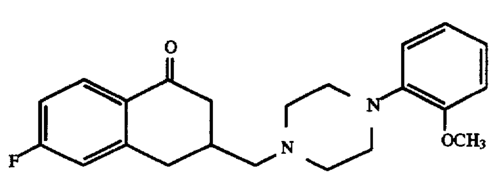
Olofsson and colleagues have studied lipoprotein assembly in HepG2 cells. Bostrom et al., *J. Biol. Chem.* 263, 4434–42 (1988). Their results suggest small precursor lipoproteins become larger with time. This would be consistent with the addition or transfer of lipid molecules to nascent lipoproteins as they are assembled. MTP may play a role in this process. In support of this hypothesis, Howell and Palade, *J. Cell Biol.* 92, 833–45 (1982), isolated nascent lipoproteins from the hepatic Golgi fraction of rat liver. There was a spectrum of sizes of particles present with varying lipid and protein compositions. Particles of high density lipoprotein (HDL) density, yet containing apoB, were found. Higgins and Hutson, *J. Lipid Res.* 25, 1295–1305 (1984), reported lipoproteins isolated from Golgi were consistently larger than those from the endoplasmic reticulum, again suggesting the assembly of lipoproteins is a progressive event.

Recent reports (Science, Vol. 258, page 999, 1992; D. Sharp et. al., Nature, Vol. 365, page 65, 1993) demonstrate that the defect causing abetalipoproteinemia is in the MTP gene, and as a result, the MTP protein. 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.

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)) reports MTP inhibitors which also block the production of apoB containing lipoproteins 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



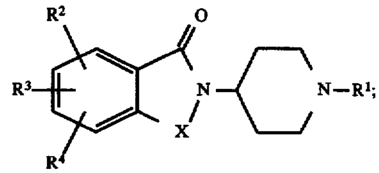
which has the name 2-[1-(3,3-diphenylpropyl)-4-piperidinyl]-2,3-dihydro-3-oxo-1H-isoindole hydrochloride and



which has the name 1-[3-(6-fluoro-1-tetralanyl)methyl]-4-O-methoxyphenyl piperazine

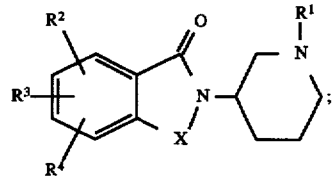
SUMMARY OF THE INVENTION

In accordance with the present invention, novel compounds are provided which are inhibitors of MTP and have the structure



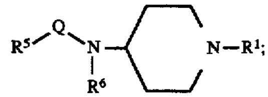
I

or



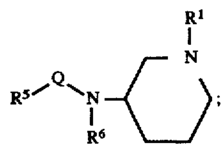
Ii

or



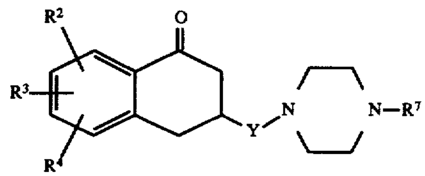
II

or



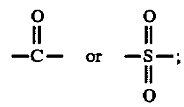
IIi

or

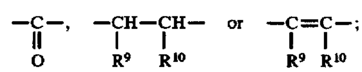


III

where Q is

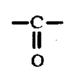


X is: CHR^8,



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

Y is —$(CH_2)_m$— or

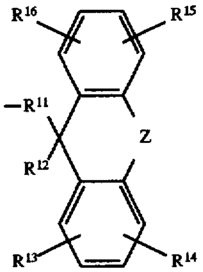


where m is 2 or 3;

R^1 is alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl (wherein alkyl has at least 2 carbons, preferably at least 3 carbons), diarylalkyl, arylalkenyl, diarylalkenyl, arylalkynyl, diarylalkynyl, diarylalkylaryl, heteroarylalkyl (wherein alkyl has at least 2 carbons, preferably at least 3 carbons), cycloalkyl, or cycloalkylalkyl (wherein alkyl has at least 2 carbons, preferably at least 3 carbons); all of the aforementioned R^1 groups being 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, cycloalkyl-alkyl, heteroaryl, fluorenyl, heteroarylalkyl, hydroxy or oxo; or

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

A



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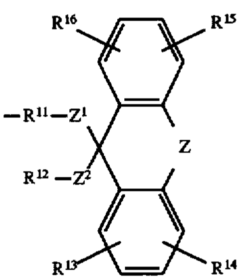
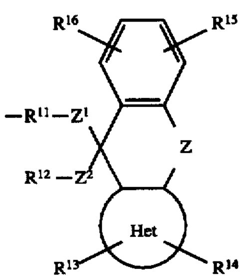
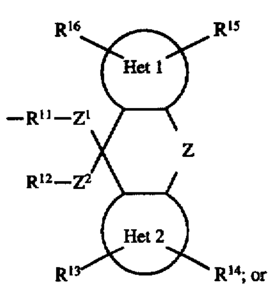


C



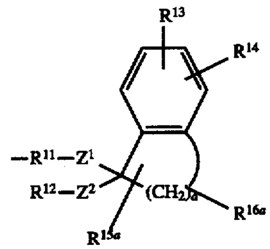
or

D



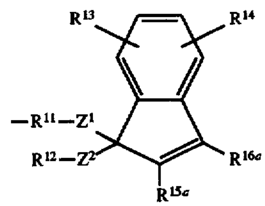
R^1 is an indenyl-type group of the structure

E



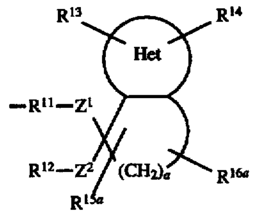
(a = 2,3 or 4)

E



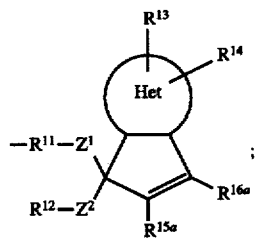
or

G

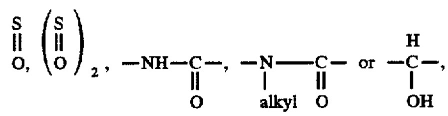


or

H

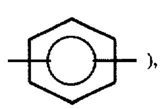


Z^1 and Z^2 are the same or different and are independently a bond, O, S,



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 (for example



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