US005827875A

United States Patent [19]

Dickson, Jr. et al.

[11] **Patent Number: 5,827,875**

[45] **Date of Patent: Oct. 27, 1998**

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

[75] Inventors: **John K. Dickson, Jr.**, Eastampton, N.J.; **Jeffrey A. Robl**, Newtown, Pa.; **Scott A. Biller**, Hopewell, N.J.

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

[21] Appl. No.: **842,132**

[22] Filed: **Apr. 23, 1997**

[51] **Int. Cl.**[6] **A61K 31/40**; C07D 207/26

[52] **U.S. Cl.** **514/424**; 548/528; 548/518; 548/550; 548/557; 514/422; 514/426

[58] **Field of Search** 548/528, 550, 548/557, 518, 525, 527; 514/422, 424, 426

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,910,931	10/1975	Cavalla et al.	260/293.62
3,963,745	6/1976	Cale, Jr. et al.	260/326.55
4,289,781	9/1981	Bengtsson et al.	424/267
4,367,232	1/1983	Boix-Igleasias et al.	424/267
4,576,940	3/1986	Tahara et al.	514/212
4,581,355	4/1986	Tahara et al.	514/212
4,607,042	8/1986	Pierce	514/323
4,826,975	5/1989	Picciola et al.	544/391
5,026,858	6/1991	Vega-Noverola et al.	546/224
5,028,616	7/1991	Desai et al.	514/321
5,032,598	7/1991	Baldwin et al.	514/318
5,098,915	3/1992	Desai et al.	514/324
5,130,333	7/1992	Pan et al.	514/460
5,189,045	2/1993	Peglion et al.	514/319
5,212,182	5/1993	Musser et al.	514/314
5,215,989	6/1993	Baldwin et al.	514/252
5,292,883	3/1994	Martin et al.	546/201
5,527,801	6/1996	Masuda et al.	514/255

FOREIGN PATENT DOCUMENTS

0584446A2	3/1994	European Pat. Off. .
0643057A1	3/1995	European Pat. Off. .
WO93/05778	4/1993	WIPO .
WO96/40640	12/1996	WIPO .

OTHER PUBLICATIONS

Bulleid & Freedman, Nature 335, 649–651 (1988). "Defective co–translational formation of disulphide bonds in protein disulphideisomerase–deficient microsomes".

Koivu et al., J. Biol. Chem. 262, 6447–6449 (1987). "A Single Polypeptide Acts both as the β Subunit of Prolyl 4–Hydroxylase and as a Protein Disulfide–Isomerase".

Kane & Havel in the Metabolic Basis of Inherited Disease, Sixth Edition, 1139–1164 (1989). "Disorders of the Biogenesis and Secretion of Lipoproteins Containing the B Apolipoproteins".

Schaefer et al., Clin. Chem. 34, B9–B12 (1988). "Genetics and Abnormalities in Metabolism of Lipoproteins".

Drayna et al., Nature 327, 632–634 (1987). "Cloning and sequencing of human cholesteryl ester transfer protein cDNA".

Pihlajaniemi et al., EMBO J. 6, 643–649 (1987). "Molecular cloning of the β–subunit of human prolyl–4–hydroxylase. This subunit and protein disulphide isomerase are products of the same gene".

Yamaguchi et al., Biochem. Biophys. Res. Comm. 146, 1485–1492 (1987). "Sequence of Membrane–Associated Thyroid Hormone Binding Protein From Bovine Liver: Its Identity with protein Disulphide Isomerase".

Edman et al., Nature 317, 267–270 (1985). Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin.

Kao et al., Connective Tissue Research 18, 157–174 (1988). "Isolation of cDNA Clones and Genomic DNA Clones of β–Subunit of Chicken Prolyl 4–Hydroxylase".

Wetterau, J. et al., Biochem 30, 9728–9735 (1991). "Protein Disulfide Isomerase Appears Necessary To Maintain the Catalytically Active Structure of the Microsomal Triglyceride Transfer Protein".

Morton, R.E. et al., J. Biol. Chem. 256, 1992–1995 (1981). "A Plasma Inhibitor of Triglyceride and Chloesteryl Ester Transfer Activities".

Wetterau, J. et al., Biochem. 30, 4406–4412 (1991). "Structural Properties of the Microsomal Triglyceride–Transfer Protein Complex".

Wetterau, J. et al., J. Biol. Chem. 265, 9800–9807 (1990). "Protein Disulfide Isomerase Is a Component of the Microsomal Triglyceride Transfer Protein Complex".

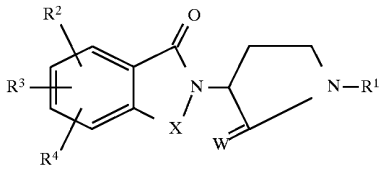
Wetterau, J. and Zilversmit, D.B., Chem. and Phys. of Lipids 38, 205–22 (1985). "Purification and Characterization of Microsomal Triglyceride and Cholesteryl Ester Transfer Protein From Bovine Liver Microsomes".

(List continued on next page.)

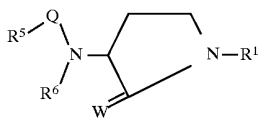
Primary Examiner—John Kight
Assistant Examiner—Charanjit S. Aulakh
Attorney, Agent, or Firm—Burton Rodney

[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



or



wherein R^1 to R^6, Q, W and X are as defined herein.

16 Claims, No Drawings

OTHER PUBLICATIONS

Wetterau, J. and Zilversmit, D.B., Biochimica et Biophysica Acta 875, 610–617 (1986). "Localization of intracellular triacylglycerol and cholesteryl ester transfer activity in rat tissues".

Wetterau, J. and Zilversmit, D.B., J. Biol. Chem. 259, 10863–10866 (1984). "A Triglyceride and Cholesteryl Ester Transfer Protein Associated with Liver Microsomes".

Wetterau, J., Grant Application entitled: "Intracellular Tryglyceride Transport and Metabolism".

Presentation Materials, Aspen Bile Acid/Cholesterol Conference, Aug. 15, 1992.

Wetterau, J. R., et al., Science, vol. 258, 999–1001, Nov. 6, 1992, "Absence of Microsomal Triglyceride Transfer Protein in Individuals with Abetalipoproteinemia".

Archibald, J. L., et al., Journal of Medicinal Chemistry, vol. 14, No. 11, pp. 1054–1059 (1991).

Cortizo, L. et al., J. Med. Chem., 34, pp. 2242–2247, 1991.

Hall, I. H. et al., Pharmaceutical Research, vol. 9, No. 10, pp. 1324–1329, 1992.

Hall, I. H., et al., Pharmacological Research Communications, vol. 19, No. 12, pp. 839–858, 1987.

Murthy et al., Eur. J. Med. Chem.–Chim. Ther., vol. 20, No. 6, pp. 547–550, 1985.

INHIBITORS OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN AND METHOD

FIELD OF THE INVENTION

This application is based on provisional application No. 60/017,253 filed May 10, 1996.

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.

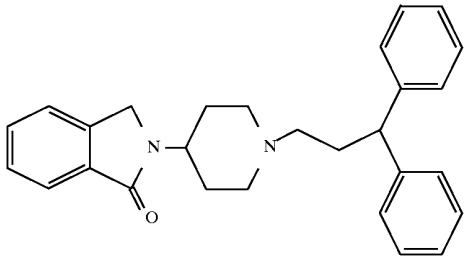
In vitro, MTP catalyzes the transport of lipid molecules between phospholipid membranes. Presumably, it plays a similar role in vivo, and thus plays some role in lipid metabolism. The subcellular (lumen of the microsomal fraction) and tissue distribution (liver and intestine) of MTP have led to speculation 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

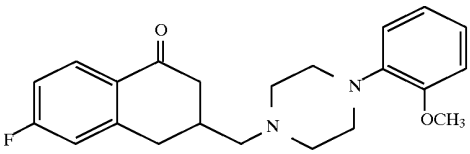
density lipoprotein (HDL) density, yet containing apoB, were found. Higgins and Hutson, *J. Lipid Res.* 25, 1295–1305 (1984), reported lipoproteins isolated from Golgi were consistently larger than those from the endoplasmic reticulum, again suggesting the assembly of lipoproteins is a progressive event.

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

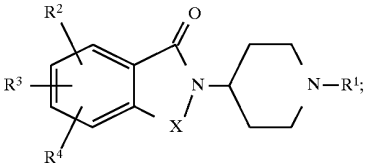


which has the name 2-[1-(3,3-diphenylpropyl)-4-piperidinyl]-2,3-dihydro-3-oxo-1H-isoindole hydrochloride and



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

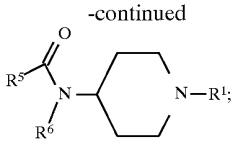
EP 0643057A1 published Mar. 15, 1995, discloses MTP inhibitors of the structure



I

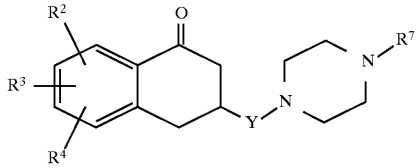
or

-continued



II

or



III

where X is: CHR^8, $-\underset{R^9}{\underset{|}{CH}}-\underset{R^{10}}{\underset{|}{CH}}-$ or $-\underset{R^9}{\underset{|}{C}}=\underset{R^{10}}{\underset{|}{C}}-$;

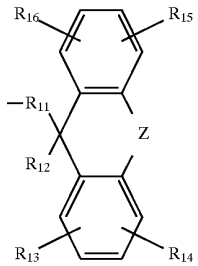
R^8, R^9 and R^{10} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, or cycloalkylalkyl;

Y is $-(CH_2)_m-$ or $-\underset{\underset{O}{\|}}{C}-$

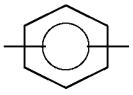
where m is 2 or 3;

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 of the aforementioned R^1 groups being optionally substituted through available carbon atoms with 1, 2, or 3 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 group of the structure

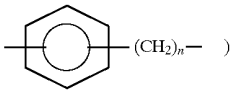


R^{11} is a bond, alkylene, alkenylene or alkynylene of up to 6 carbon atoms, arylene (for example



),

or mixed arylene-alkylene (for example



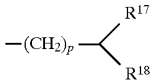
where n is 1 to 6;

R^{12} is hydrogen, alkyl, alkenyl, aryl, heteroaryl, haloalkyl, arylalkyl, arylalkenyl, cycloalkyl, aryloxy, alkoxy, arylalkoxy, heteroarylalkyl or cycloalkylalkyl;

Z is a bond, O, S, N-alkyl, N-aryl, or alkylene or alkenylene of from 1 to 5 carbon atoms;

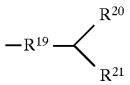
R^{13}, R^{14}, R^{15}, and R^{16} are independently hydrogen, alkyl, halo, haloalkyl, aryl, cycloalkyl, cycloheteroalkyl, alkenyl, alkynyl, hydroxy, alkoxy, nitro, amino, thio, alkylsulfonyl, arylsulfonyl, alkylthio, arylthio, carboxy, aminocarbonyl, alkylcarbonyloxy, alkylcarbonylamino, arylalkyl, heteroaryl, heteroarylalkyl, or aryloxy;

or R^1 is



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



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, haloalkyl, alkenyl, alkoxy, aryloxy, aryl, arylalkyl, alkylmercapto, arylmercapto, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, hydroxy or haloalkyl;

R^5 is alkyl of at least 2 carbons, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkyl, cycloalkylalkyl, polycycloalkyl, polycycloalkylalkyl, cycloalkenyl, cycloalkenylalkyl, polycycloalkenyl, polycycloalkenylalkyl, heteroarylcarbonyl, all of the R^5 and R^6 substituents being optionally substituted through available carbon atoms with 1, 2, or 3 groups selected from hydrogen, halo, alkyl, haloalkyl, alkoxy, haloalkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloheteroalkyl, cycloheteroalkylalkyl, aryl, heteroaryl, arylalkyl, arylcycloalkyl, arylalkynyl, aryloxy, aryloxyalkyl, arylalkoxy, arylazo, heteroaryloxo, heteroarylalkyl, heteroarylalkenyl, heteroaryloxy, hydroxy, nitro, cyano, amino, substituted amino (wherein the amino includes 1 or 2 substituents which are alkyl, or aryl or any of the other aryl compounds mentioned in the definitions), thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkylcarbonyl, arylcarbonyl, arylaminocarbonyl, alkoxycarbonyl, aminocarbonyl, alkynylaminocarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonylamino, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonyl, alkylsulfonyl, arylsulfonylamino; with the proviso that when R^5 is CH_3, R^6 is not H; and where R^5 is phenyl, the phenyl preferably includes an ortho hydrophobic substituent such as alkyl, haloalkyl, aryl, aryloxy or arylalkyl;

R^6 is hydrogen or C_1–C_4 alkyl or C_1–C_4 alkenyl;

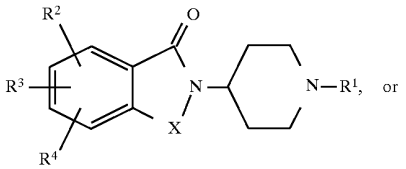
R^7 is alkyl, aryl or arylalkyl wherein alkyl or the alkyl portion is optionally substituted with oxo; and

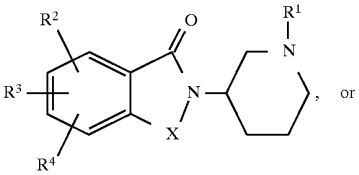
including pharmaceutically acceptable salts and anions thereof.

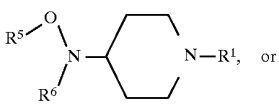
In the formula I compounds, where X is CH_2 and R^2, R^3 and R^4 are each H, R^1 will be other than 3,3-diphenylpropyl.

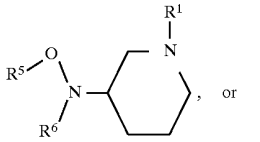
In the formula III compounds, where one of R^2, R^3 and R^4 is 6-fluoro, and the others are H, R^7 will be other than 4-O-methoxyphenyl.

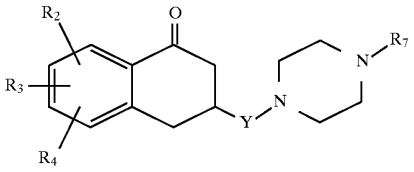
U.S. application Ser. No. 472,067, filed Jun. 6, 1995 (file DC21e) discloses compounds of the structure

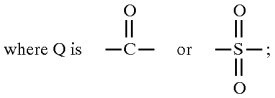


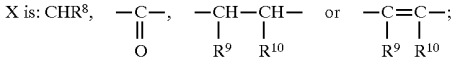




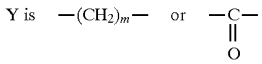




where Q is 

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 

wherein m is 2 or 3;

R^1 is alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl wherein alkyl has at least 2 carbons, diarylalkyl, arylalkenyl,

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.