US006066653A

United States Patent [19]

Gregg et al.

[11] Patent Number: 6,066,653

[45] Date of Patent: May 23, 2000

[54] **METHOD OF TREATING ACID LIPASE DEFICIENCY DISEASES WITH AN MTP INHIBITOR AND CHOLESTEROL LOWERING DRUGS**

[75] Inventors: **Richard E. Gregg**, Pennington, N.J.; **John R. Wetterau, II**, Langhorne, Pa.

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

[21] Appl. No.: **09/005,437**

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

Related U.S. Application Data

[60] Provisional application No. 60/036,183, 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

4,346,227	8/1982	Terahara et al.	560/119
5,712,279	1/1998	Biller et al.	514/252

FOREIGN PATENT DOCUMENTS

643057A1	3/1995	European Pat. Off. .
WO96/26205	8/1996	WIPO .

OTHER PUBLICATIONS

Scriver et al "The Metabolic and Molecular Bases of Inherited Disease", Seventh Edition, vol. II, Chapter 82, "Acid Lipase Deficiency: Wolman Disease and Cholesteryl Ester Storage Disease", pp. 2563–2587, (1995).

Scriver et al "The Metabolic and Molecular Bases of Inherited Disease", Seventh Edition, vol. II, Chapter 85, "Niemann–Pick Disease Type C: A Cellular Cholesterol Lipidosis", pp. 2625–2639, (1995).

Primary Examiner—Kimberly Jordan

Attorney, Agent, or Firm—Burton Rodney; Ronald S. Hermenau

[57] **ABSTRACT**

A method is provided for inhibiting or treating diseases associated with acid lipase deficiency by administering to a patient an MTP inhibitor, alone or optionally, in combination with another cholesterol lowering drug, such as pravastatin.

27 Claims, No Drawings

METHOD OF TREATING ACID LIPASE DEFICIENCY DISEASES WITH AN MTP INHIBITOR AND CHOLESTEROL LOWERING DRUGS

This application claims the benefit of U.S. Provisional Application Ser. No. 60/036,183, filed Jan. 17, 1997.

FIELD OF THE INVENTION

The present invention related to a method for inhibiting or treating diseases associated with acid lipase deficiency, including Wolman disease and/or cholesteryl ester storage disease, by administering an MTP inhibitor alone or in combination with another cholesterol lowering drug, such as pravastatin.

BACKGROUND OF THE INVENTION

Wolman disease and cholesteryl ester storage disease are characterized by a deficiency in activity of lysomal acid lipase which results in massive accumulation of cholesteryl esters and triglycerides in most tissues of the body. Cholesteryl esters and triglycerides are derived from plasma lipoproteins taken up by the cells and are substrates for acid lipase. Acid lipase is responsible for the hydrolysis of cholesteryl esters and triglycerides in the lysosomes.

If plasma cholesterol levels are lowered sufficiently, then cholesteryl ester and triglyceride accumulation in the lysosomes and the consequences of the accumulation could be minimized.

Wolman disease is the more severe of the two diseases and is almost always fatal before the age of 1 year. In contrast, cholesteryl ester storage disease may go undetected until adulthood by which time lipid deposition is widespread. Hyperbetalipoproteinemia is common in cholesteryl ester storage disease, and premature atherosclerosis may be severe.

To date, there has been no specific therapy for acid lipase deficiency other than attempts at suppression of cholesterol synthesis and apolipoprotein B production by 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in combination with cholestyramine treatment and a diet excluding foods rich in cholesterol and triglycerides. The above apparently provided improvement in only one or two cases of cholesteryl ester storage disease. Thus, for the most part, Wolman disease and cholesteryl ester storage disease have been untreatable.

See Scriver, C. R. et al "The Metabolic and Molecular Bases of Inherited Disease", Vol. II (1995), Chap. 82, "Acid Lipase Deficiency: Wolman Disease and Cholesteryl Ester Storage Disease", pp. 2563–2587.

Until now, there have not been any therapeutic agents available which could lower plasma cholesterol levels sufficiently to minimize cholesteryl ester and triglyceride accumulation in the lysosomes.

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 or treating a disease associated with acid lipase deficiency, including Wolman disease and/or cholesteryl ester storage disease (CESD), 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.

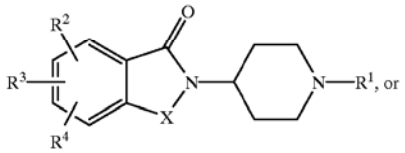
In accordance with the present invention, the MTP inhibitor lowers plasma cholesterol (LDL-cholesterol) to at least about 50% of normal LDL blood level, preferably down to less than about 25% of normal, and most preferably down to less than about 15% of normal, and lowers triglycerides to at least about 50% of normal triglyceride blood level, and preferably down to about 25% or less of normal, and thereby minimizes cholesteryl ester and triglyceride accumulation in the lysosomes.

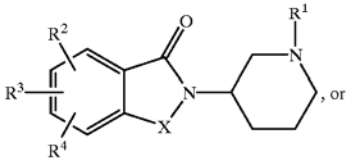
The terms "another cholesterol lowering drug or agent" or "another delipidating drug" will be employed interchangeably herein.

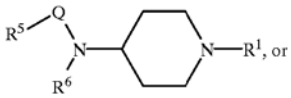
MTP inhibitors to be employed in the methods of the invention include MTP inhibitors disclosed in Canadian Patent Application Ser. No. 2,091,102 described hereinbefore (corresponding to U.S. application Ser. No. 117,362), WO 92/26205 published Aug. 29, 1996, U.S. application Ser. No. 472,067, filed Jun. 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.

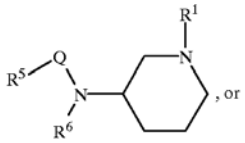
All of the above U.S. applications are incorporated herein by reference.

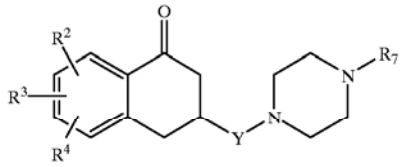
The MTP inhibitors disclosed in U.S. Application Ser. No. 472,067, filed June 6, 1995 (file DC21e) are piperidine compounds of the structure

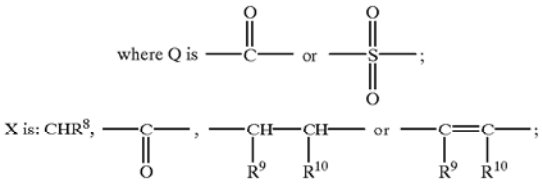




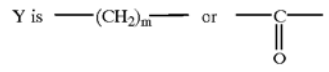








R^8, R^9 and R^{10} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, or cycloalkylalkyl;



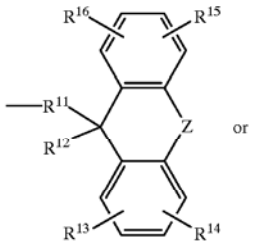
wherein m is 2 or 3;

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;

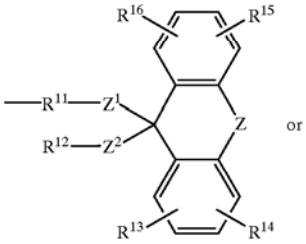
or R^1 is a fluorenyl-type group of the structure

A



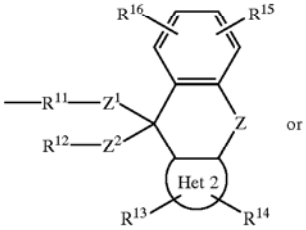
or

B



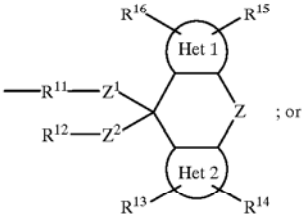
or

C



or

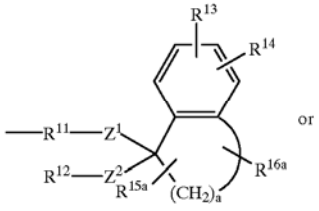
D



; or

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

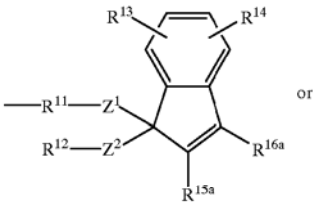
E



or

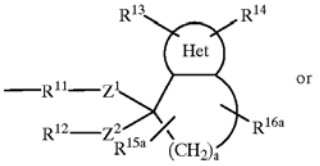
-continued

F



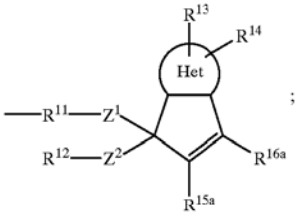
or

G



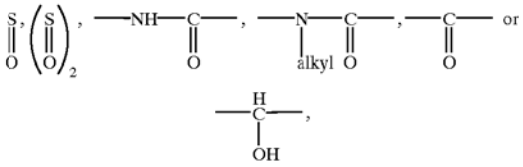
or

H



;

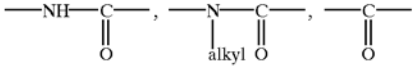
Z^1 and Z^2 are the same or different and are independently a bond, O, S,



or

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

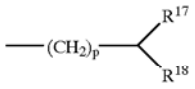
(2) when Z^2 is a bond, R^{12} cannot be heteroaryl or heteroarylalkyl;

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,

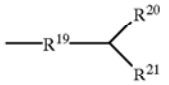
alkynyl, alkoxy, alkylsulfonyl, arylsulfonyl, alkylthio, arylthio, aminocarbonyl, alkylcarbonyloxy, arylcarbonylamino, alkylcarbonylamino, arylalkyl, heteroaryl, heteroarylalkyl, or aryloxy;

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;

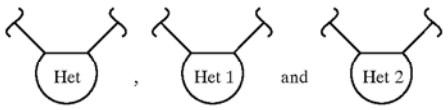
R^{21} is II, alkyl, aryl, alkylaryl, arylalkyl, aryloxy, arylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, cycloalkyl, cycloalkylalkyl or cycloalkylalkoxy;

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;

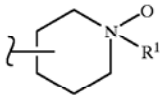
R^6 is hydrogen or C_1–C_4 alkyl or C_1–C_4 alkenyl; all optionally substituted with 1, 2, 3 or 4 groups which may independently be any of the substituents listed in the definition of R^5 set out above;

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

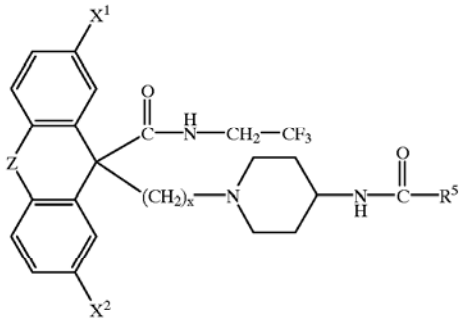
N-oxides



R 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;

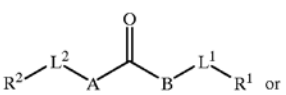
X^1 and X^2 are independently selected from H or halo;

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 Ser. No. 60/017,224, filed May 9, 1996 (file HX79a*) have the structure

I



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.