Combinations of Ileal Bile Acid Transport Inhibitors and Nicotinic Acid Derivatives for Cardiovascular Indications

This application claims priority of U.S. provisional application Ser. No. 60/142,550 filed Jul. 7, 1999 and of U.S. provisional application Ser. No. 60/113,955 filed Dec. 23, 1998.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to methods of treating cardiovascular diseases, and specifically relates to combinations of compounds, compositions, and methods for their use in medicine, particularly in the prophylaxis and treatment of hyperlipidemic conditions such as are associated with atherosclerosis, hypercholesterolemia, and other coronary artery disease in mammals. More particularly, the invention relates to ileal bile acid transporter (IBAT) inhibiting compounds. The invention also relates to nicotinic acid derivative compounds.

Description of Related Art

It is well-settled that hyperlipidemic conditions associated with elevated concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol are major risk factors for coronary heart disease and particularly atherosclerosis. Since high levels of LDL cholesterol increase the risk of atherosclerosis, methods for lowering plasma LDL cholesterol would be therapeutically beneficial for the treatment of atherosclerosis and other diseases associated with accumulation of lipid in the blood vessels. These diseases include, but are not limited

to, coronary heart disease, peripheral vascular disease, and stroke.

Atherosclerosis underlies most coronary artery disease (CAD), a major cause of morbidity and mortality in modern society. High LDL cholesterol (above about 180 mg/dl) and low HDL cholesterol (below 35 mg/dl) have been shown to be important contributors to the development of atherosclerosis. Other diseases or risk factors, such as peripheral vascular disease, stroke, and hypercholesterolaemia are negatively affected by adverse HDL/LDL ratios.

Interfering with the recirculation of bile acids from the lumen of the intestinal tract is found to reduce the levels of serum cholesterol in a causal relationship. Epidemiological data has accumulated which indicates such reduction leads to an improvement in the disease state of atherosclerosis. Stedronsky, in "Interaction of bile acids and cholesterol with nonsystemic agents having hypocholesterolemic properties," Biochimica et Biophysica Acta, 1210, 255-287 (1994) discusses the biochemistry, physiology and known active agents surrounding bile acids and cholesterol.

Transient pathophysiologic alterations are shown to be consistent with interruption of the enterohepatic circulation of bile acids in humans with an inherited lack of IBAT activity, as reported by Heubi, J.E., et al. See "Primary Bile Acid Malabsorption: Defective in Vitro Ileal Active Bile Acid Transport", Gastroenterology, 83, 804-11 (1982).

In another approach to the reduction of recirculation of bile acids, the ileal bile acid transport system is a putative pharmaceutical target for the treatment of hypercholesterolemia based on an interruption of the enterohepatic circulation with specific transport

inhibitors (Kramer, et al., "Intestinal Bile Acid Absorption" The Journal of Biological Chemistry, 268 (24), 18035-46 (1993).

In several individual patent applications, Hoechst Aktiengesellschaft discloses polymers of various naturally occurring constituents of the enterohepatic circulation system and their derivatives, including bile acid, which inhibit the physiological bile acid transport with the goal of reducing the LDL cholesterol level sufficiently to be effective as pharmaceuticals and, in particular for use as hypocholesterolemic agents. The individual Hoechst patent applications which disclose such bile acid transport inhibiting compounds are each separately listed below.

R1. Canadian Patent Application No. 2,025,294.
R2. Canadian Patent Application No. 2,078,588.
R3. Canadian Patent Application No. 2,085,782.
R4. Canadian Patent Application No. 2,085,830.
R5. EP Application No. 0 379 161.
R6. EP Application No. 0 549 967.
R7. EP Application No. 0 559 064.
R8. EP Application No. 0 563 731.

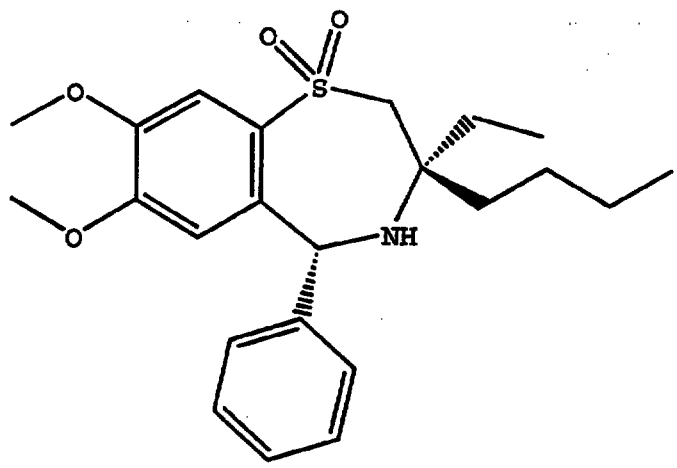
Selected benzothiepines are disclosed in world patent application number WO 93/321146 for numerous uses including fatty acid metabolism and coronary vascular diseases.

Other selected benzothiepines are known for use as hypolipaemic and hypocholesterolaemic agents, especially for the treatment or prevention of atherosclerosis as disclosed in application No. EP 508425. A French patent application, FR 2661676 discloses additional benzothiepines for use as hypolipaemic and

hypocholesterolaemic agents. Furthermore, patent application no. WO 92/18462 lists other benzothiepines for use as hypolipaemic and hypocholesterolaemic agents. U.S. Patent No. 5,994,391 (Lee et al.) Each of the benzothiepine hypolipaemic and hypocholesterolaemic agents described in these individual patent applications is limited by an amide bonded to the carbon adjacent the phenyl ring of the fused bicyclobenzothiepine ring.

Further benzothiepines useful for the treatment of hypercholesterolemia and hyperlipidemia are disclosed in patent application no. PCT/US95/10863. More benzothiepines useful for the prophylaxis and treatment of hypercholesterolemia and hyperlipidemia as well as pharmaceutical compositions of such benzothiepines are described in PCT/US97/04076. Still further benzothiepines and compositions thereof useful for the prophylaxis and treatment of hypercholesterolemia and hyperlipidemia are described in U.S. Application Serial No. 08/816,065.

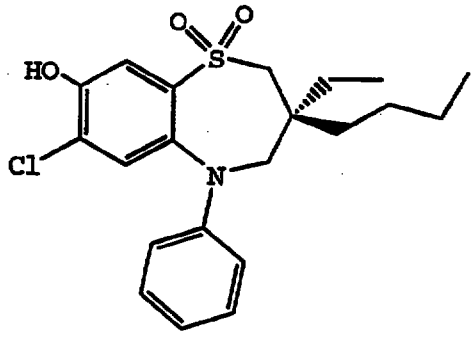
In vitro bile acid transport inhibition is disclosed to correlate with hypolipidemic activity in The Wellcome Foundation Limited disclosure of the Patent Application No. WO 93/16055 for "Hypolipidemic Benzothiazepine Compounds." That publication describes a number of hypolipidemic benzothiazepine compounds. Additional hypolipidemic benzothiazepine compounds (particularly 2,3,4,5-tetrahydrobenzo-1-thi-4-azepine compounds) are disclosed in Patent Application No. WO 96/05188. A particularly useful benzothiazepine disclosed in WO 96/05188 is the compound of formula B-2. Further hypolipidemic benzothiazepine compounds are described in Patent Application No. WO 96/16051.



B-2

(3R,5R)-3-butyl-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1-4-benzothiazepine 1,1-dioxide

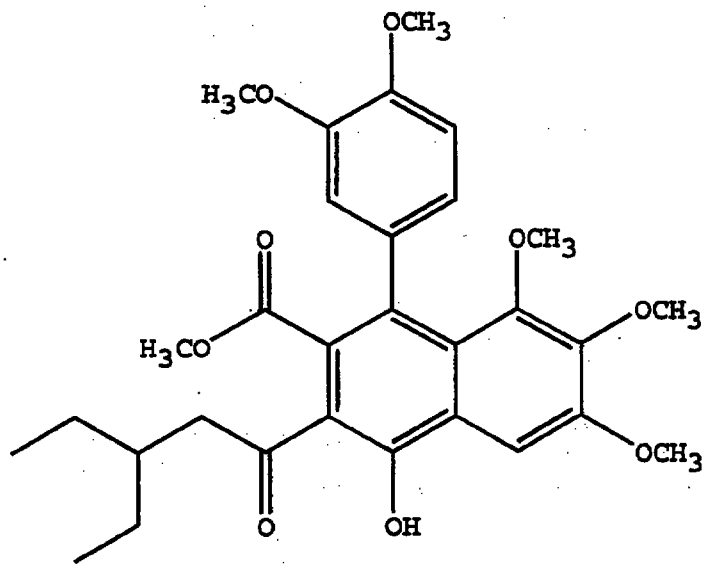
Other benzothiazepine compounds useful for control of cholesterol are described in PCT Patent Application No. WO 99/35135. Included in that description is the compound of formula B-7.



B-7

Further IBAT inhibitor compounds include a class of naphthalene compounds, described by T. Ichihashi et al. in J. Pharmacol. Exp. Ther., 284(1), 43-50 (1998). In this class, S-8921 (methyl 1-(3,4-dimethoxyphenyl)-3-(3-ethylvaleryl)-4-hydroxy-6,7,8-trimethoxy-2-naphthoate) is particularly useful. The structure of S-8921 is shown in formula B-20. Further naphthalene compounds or lignin derivatives useful for the treatment or prophylaxis of

hyperlipidemia or atherosclerosis are described in PCT Patent Application No. WO 94/24087.



B-20

Nicotinic acid (niacin) is a B-complex vitamin reported as early as 1955 to act as a hypolipidemic agent (R. Altschl, et al., Arch. Biochem. Biophys., 54, 558-9 (1955)). It is sometimes used to raise low HDL levels and lower VLDL and LDL levels. Useful commercial formulations of nicotinic acid include Niacor, Niaspan, Nicobid, Nicolar, Slo-Niacin. Nicotinic acid is contraindicated for patients having hepatic dysfunction, active peptic ulcer, or arterial bleeding. Another compound in this class useful for cardiovascular indications is niceritrol (T. Kazumi et al., Curr. Ther. Res., 55, 546-51). J. Sasaki et al. (Int. J. Clin. Pharm. Ther., 33 (7), 420-26 (1995)) describes a reduction in cholesterol ester transfer activity by niceritrol monotherapy. Acipimox (5-methyl pyrazine-2-carboxylic acid 4-oxide, U.S. Patent No. 4,002,750) is structurally similar to nicotinic acid and has antihyperlipidemic activity.

Some combination therapies for the treatment of cardiovascular disease have been described in the

literature. Combinations of IBAT inhibitors with HMG CoA reductase inhibitors useful for the treatment of cardiovascular disease are disclosed in U.S. Patent Application No. 09/037,308.

A combination therapy of fluvastatin and niceritrol is described by J. Sasaki et al. (Id.). Those researchers conclude that the combination of fluvastatin with niceritrol "at a dose of 750 mg/day dose does not appear to augment or attenuate beneficial effects of fluvastatin."

L. Cashin-Hemphill et al. (J. Am. Med. Assoc., 264 (23), 3013-17 (1990)) describe beneficial effects of a combination therapy of colestipol and niacin on coronary atherosclerosis. The described effects include nonprogression and regression in native coronary artery lesions.

A combination therapy of acipimox and simvastatin shows beneficial HDL effects in patients having high triglyceride levels (N. Hoogerbrugge et al., J. Internal Med., 241, 151-55 (1997)).

Sitostanol ester margarine and pravastatin combination therapy is described by H. Gylling et al. (J. Lipid Res., 37, 1776-85 (1996)). That therapy is reported to simultaneously inhibit cholesterol absorption and lower LDL cholesterol significantly in non-insulin-dependent diabetic men.

Brown et al. (New Eng. J. Med., 323 (19), 1289-1339 (1990)) describe a combination therapy of lovastatin and colestipol which reduces atherosclerotic lesion progression and increase lesion regression relative to lovastatin alone.

Buch et al. (PCT Patent Application No. WO 9911263) describe a combination therapy comprising amlodipine and a statin compound for treating subjects suffering from

angina pectoris, atherosclerosis, combined hypertension and hyperlipidemia, and to treat symptoms of cardiac arrest. Buch et al. describe in PCT Patent Application No. WO 9911259 a combination therapy comprising amlodipine and atorvastatin.

Scott et al. (PCT Patent Application No. WO 9911260) describe a combination therapy comprising atorvastatin and an antihypertensive agent.

Dettmar and Gibson (UK Patent Application No. GB 2329334 A) claim a therapeutic composition useful for reducing plasma low density lipoprotein and cholesterol levels, wherein the composition comprises an HMG CoA reductase inhibitor and a bile complexing agent.

The above references show continuing need to find safe, effective agents for the prophylaxis or treatment of cardiovascular diseases.

Summary of the Invention

To address the continuing need to find safe and effective agents for the prophylaxis and treatment of cardiovascular diseases, combination therapies of cardiovascular drugs are now reported.

Among its several embodiments, the present invention provides a combination therapy comprising the use of a first amount of an IBAT inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia, atherosclerosis, or hypercholesterolemia, wherein said first and second amounts together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of the compounds. For example one of the many embodiments of the present invention is a combination therapy comprising

therapeutic dosages of an IBAT inhibiting compound and a nicotinic acid derivative compound. A preferred embodiment of the present invention is a combination therapy comprising therapeutic dosages of a benzothiepine IBAT inhibiting compound and a nicotinic acid derivative compound.

A further embodiment of the instant invention comprises the use of any of the cardiovascular combination therapies described herein for the prophylaxis or treatment of hypercholesterolemia, atherosclerosis, or hyperlipidemia. Therefore, in one embodiment the present invention provides a method for the prophylaxis or treatment of a hyperlipidemic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount of the compounds.

In another embodiment, the present invention provides a method for the prophylaxis or treatment of an atherosclerotic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-atherosclerotic condition effective amount of the compounds.

In still another embodiment, the present invention provides method for the prophylaxis or treatment of hypercholesterolemia comprising administering to a patient in need thereof a combination in unit dosage form wherein

the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-hypercholesterolemic condition effective amount of the compounds.

Further scope of the applicability of the present invention will become apparent from the detailed description provided below. However, it should be understood that the following detailed description and examples, while indicating preferred embodiments of the invention, are given by way of illustration only since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following detailed description is provided to aid those skilled in the art in practicing the present invention. Even so, this detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

The contents of each of the references cited herein, including the contents of the references cited within these primary references, are herein incorporated by reference in their entirety.

a. Definitions

The following definitions are provided in order to aid the reader in understanding the detailed description of the present invention:

"Ileal bile acid transporter" or "IBAT" is synonymous with apical sodium co-dependent bile acid transporter, or ASBT.

"Benzothiepine IBAT inhibitor" means an ileal bile acid transport inhibitor which comprises a therapeutic compound comprising a 2,3,4,5-tetrahydro-1-benzothiepine 1,1-dioxide structure.

"Nicotinic acid derivative" means a therapeutic compound comprising a pyridine-3-carboxylate structure or a pyrazine-2-carboxylate structure, including acid forms, salts, esters, zwitterions, and tautomers. Nicotinic acid derivatives include, for example, nicotinic acid (niacin), niceritrol, and acipimox.

"Combination therapy" means the administration of two or more therapeutic agents to treat a hyperlipidemic condition, for example atherosclerosis and hypercholesterolemia. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single dosage form having a fixed ratio of active ingredients or in multiple, separate dosage forms for each inhibitor agent. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the hyperlipidemic condition.

The phrase "therapeutically effective" is intended to qualify the combined amount of inhibitors in the combination therapy. This combined amount will achieve the goal of reducing or eliminating the hyperlipidemic condition.

"Therapeutic compound" means a compound useful in the prophylaxis or treatment of a hyperlipidemic condition, including atherosclerosis and hypercholesterolemia.

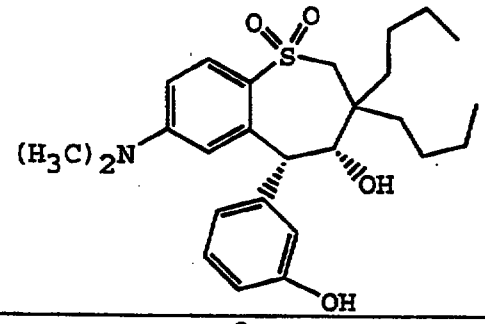
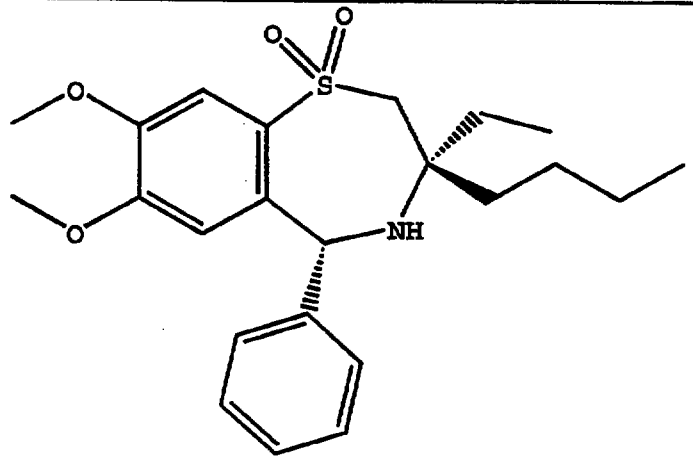
b. Combinations

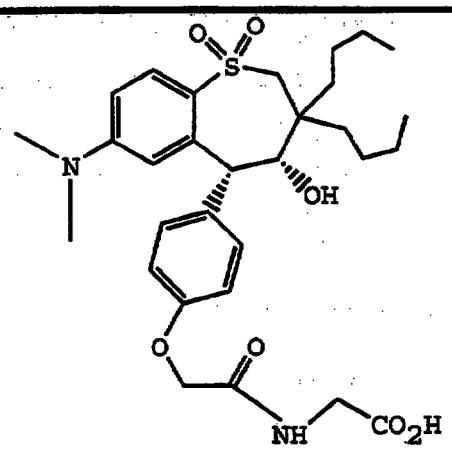
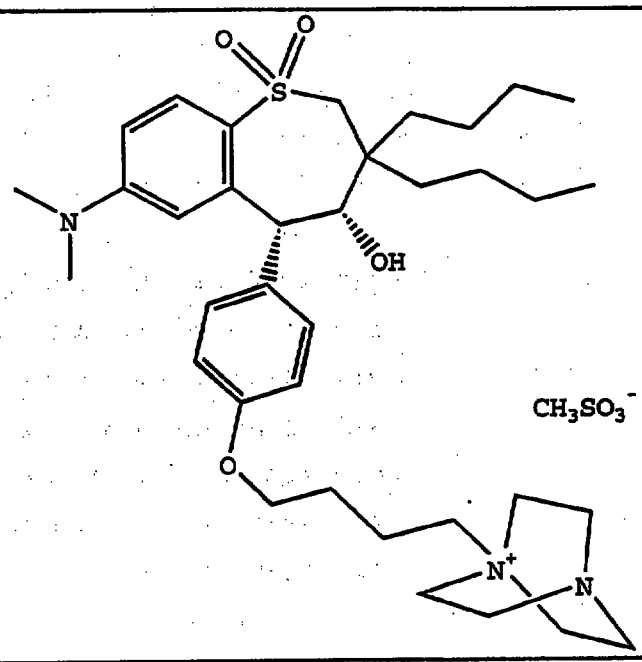
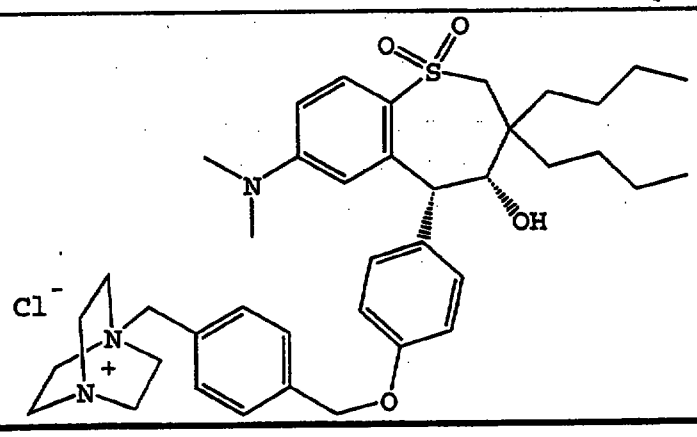
The combinations of the present invention will have a number of uses. For example, through dosage adjustment and medical monitoring, the individual dosages of the therapeutic compounds used in the combinations of the present invention will be lower than are typical for dosages of the therapeutic compounds when used in monotherapy. The dosage lowering will provide advantages including reduction of side effects of the individual therapeutic compounds when compared to the monotherapy. In addition, fewer side effects of the combination therapy compared with the monotherapies will lead to greater patient compliance with therapy regimens.

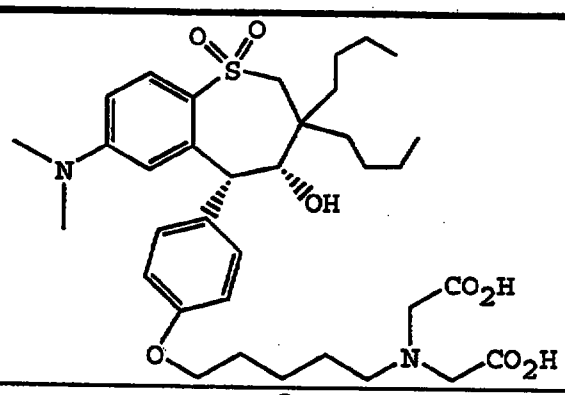
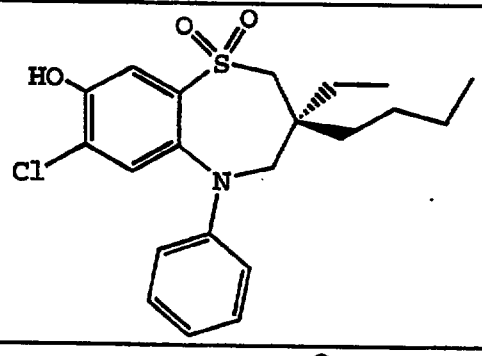
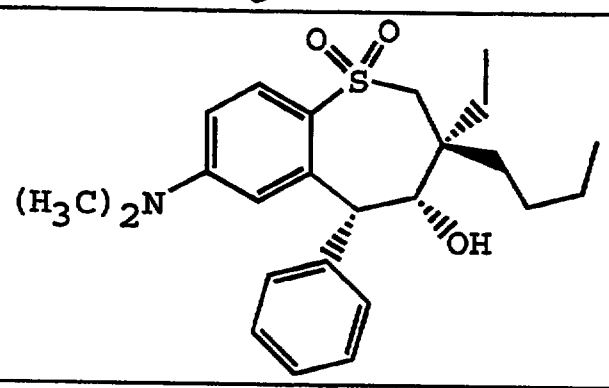
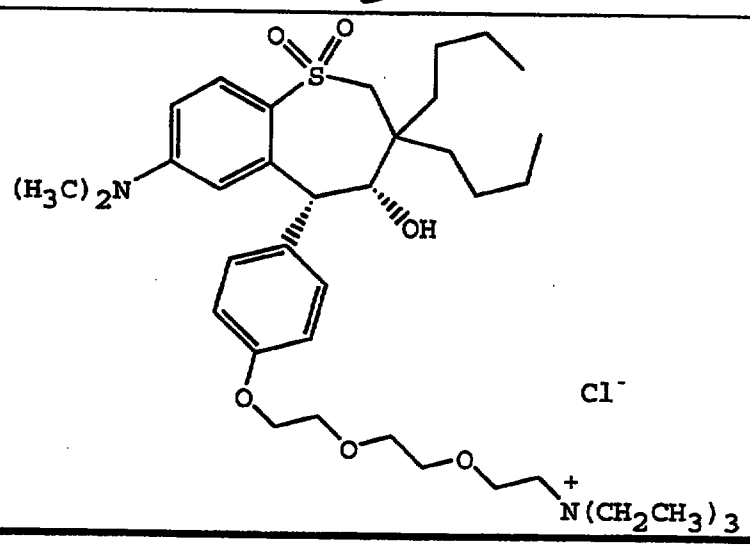
Compounds useful in the present invention encompass a wide range of therapeutic compounds. Some IBAT inhibitors useful in the present invention are disclosed in patent application no. PCT/US95/10863, herein incorporated by reference. More IBAT inhibitors are described in PCT/US97/04076, herein incorporated by reference. Still further IBAT inhibitors useful in the present invention are described in U.S. Application Serial No. 08/816,065, herein incorporated by reference. More IBAT inhibitor compounds useful in the present invention are described in WO 98/40375, herein incorporated by reference. Additional IBAT inhibitor compounds useful in the present invention are described in U.S. Application Serial No. 08/816,065, herein incorporated by reference. Further IBAT inhibiting compounds useful in the present invention are disclosed in U.S. Patent No. 5,994,391, herein incorporated by

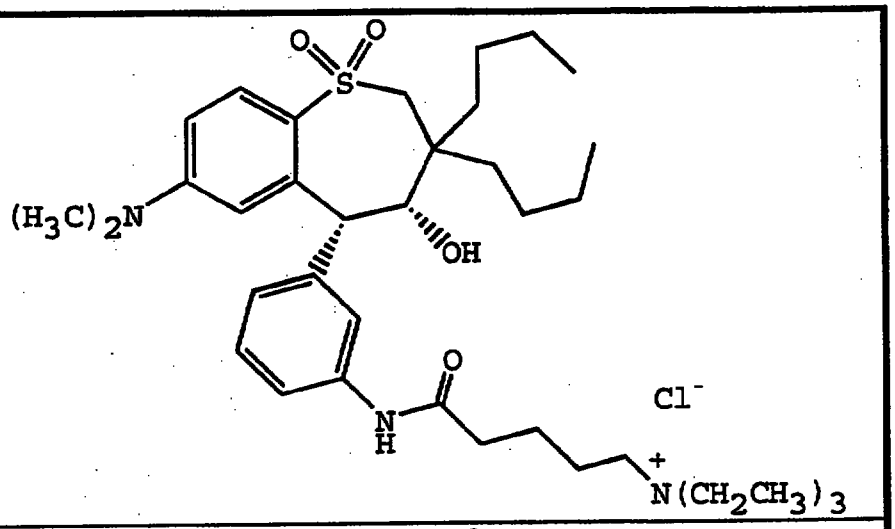
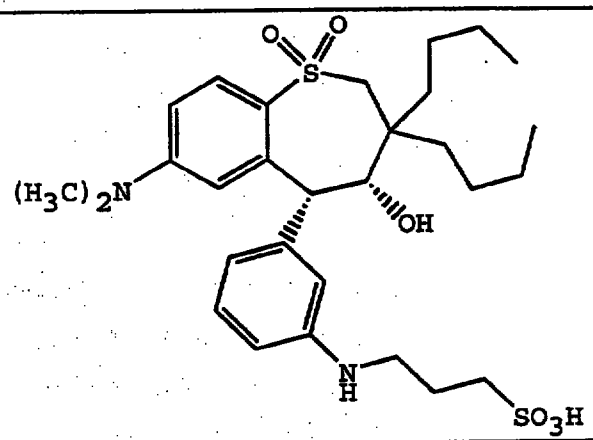
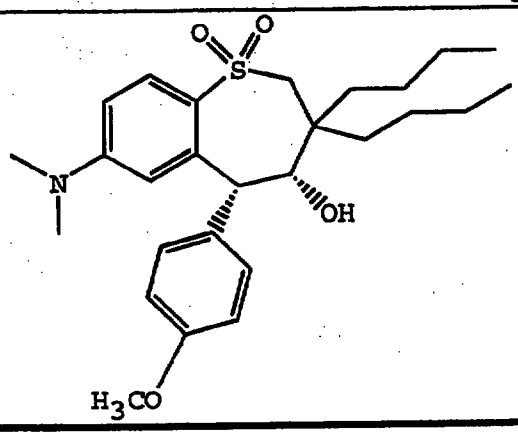
reference. IBAT inhibitors of particular interest in the present invention include those shown in Table 1, as well as the diastereomers, enantiomers, racemates, salts, and tautomers of the IBAT inhibitors of Table 1.

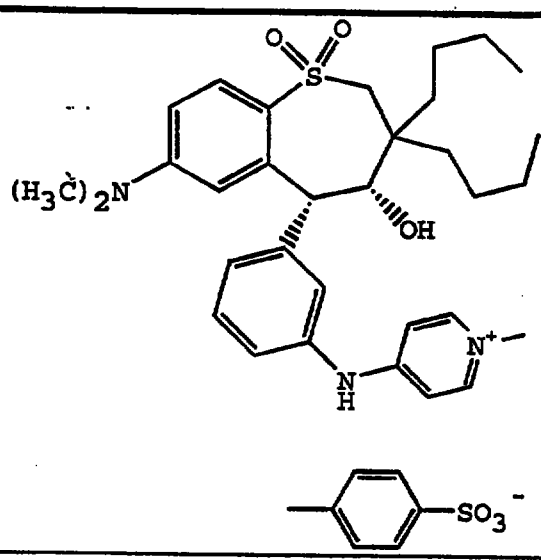
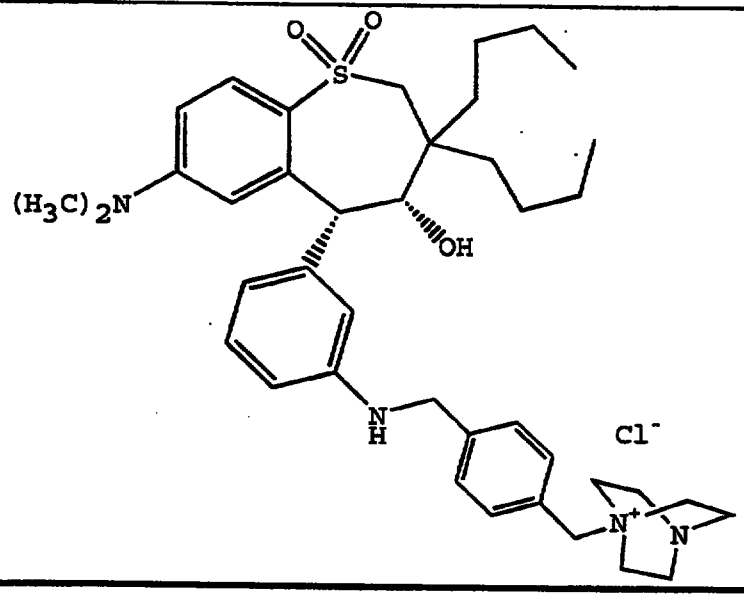
Table 1.

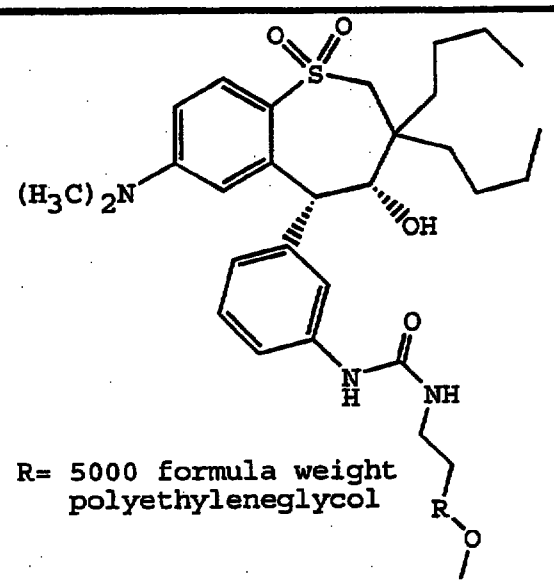
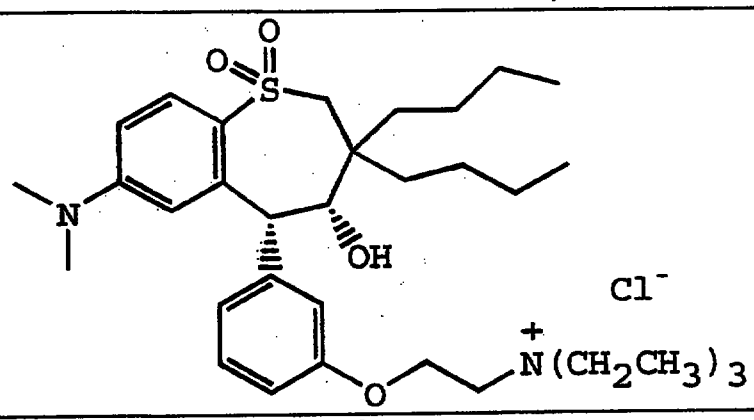
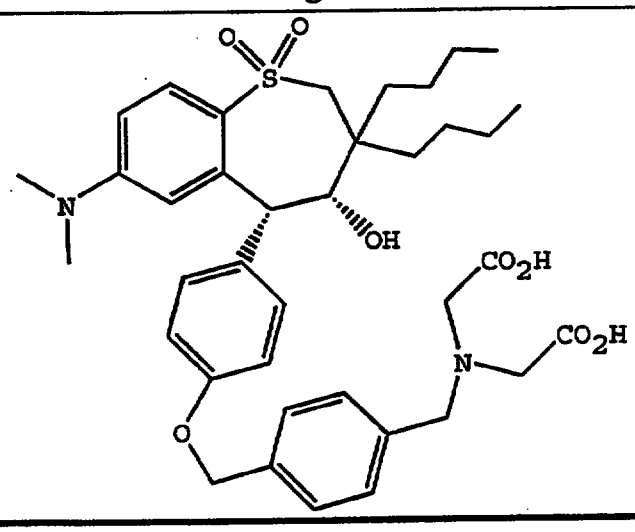
Compound Number	Structure
B-1	
B-2	 (3R,5R)-3-butyl-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1-4-benzothiazepine 1,1-dioxide

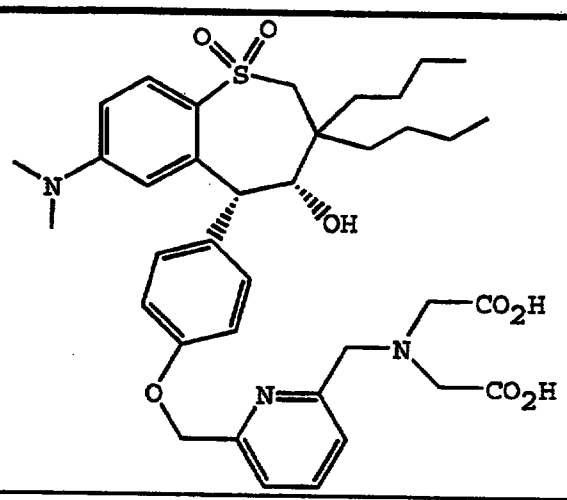
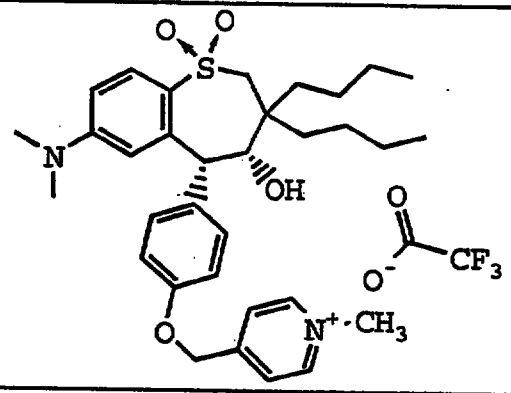
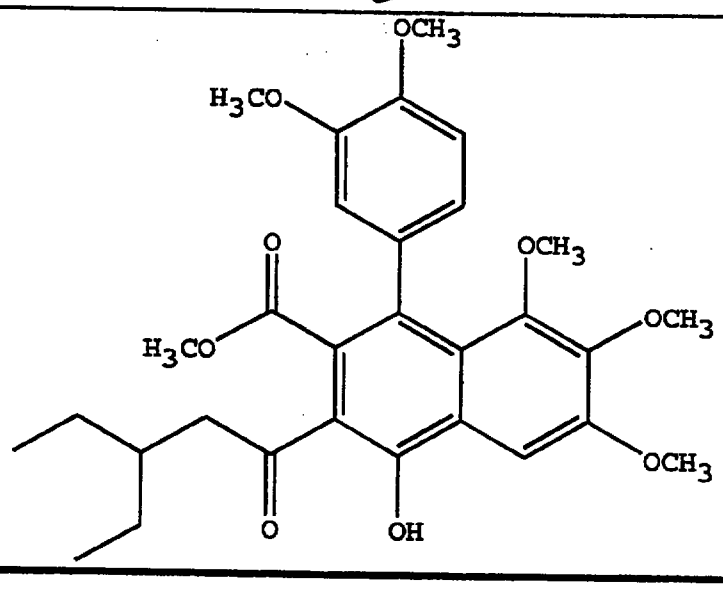
B-3	
B-4	
B-5	

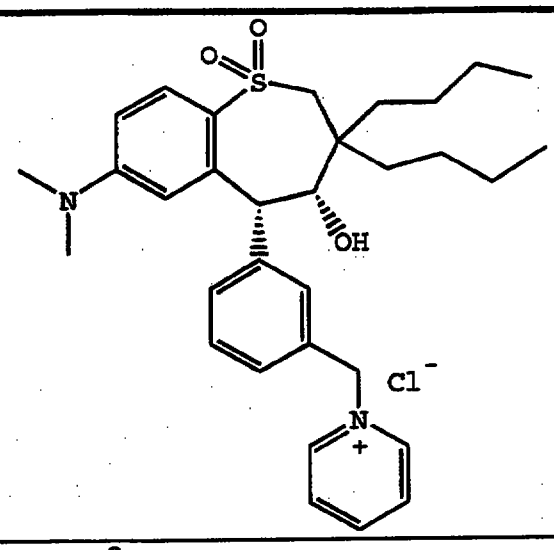
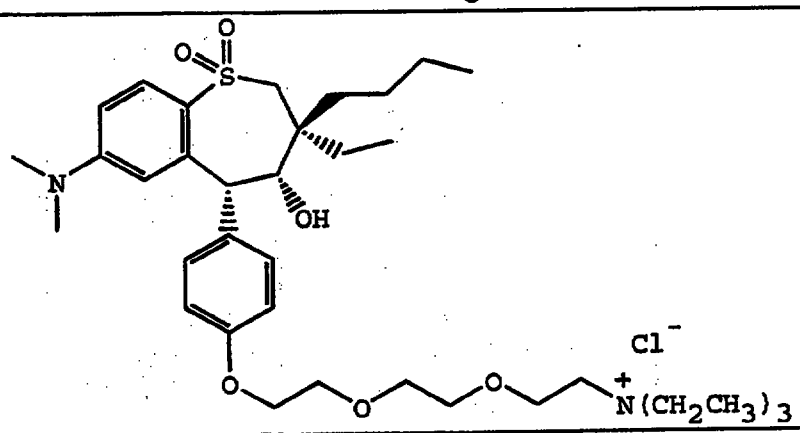
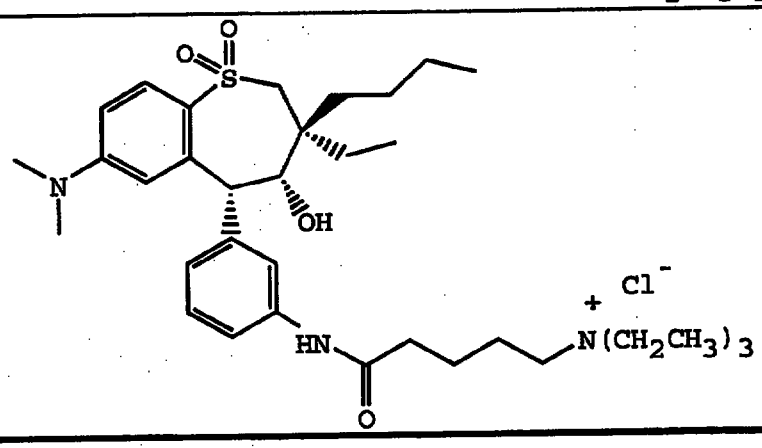
B-6	
B-7	
B-8	
B-9	

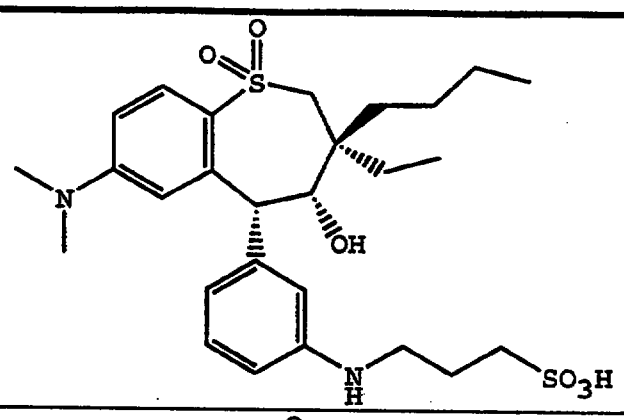
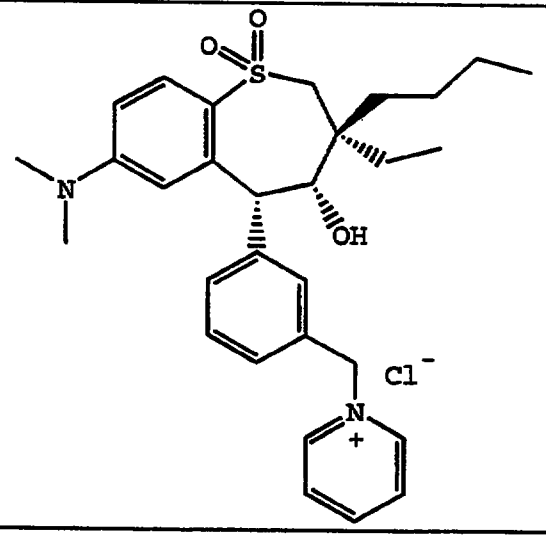
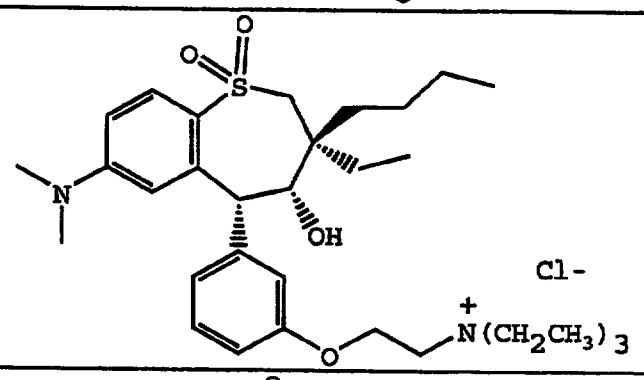
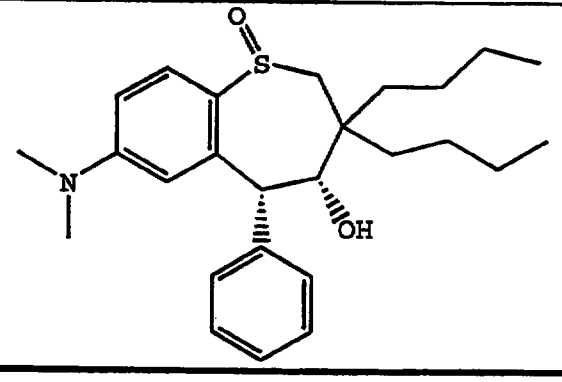
B-10	
B-11	
B-12	

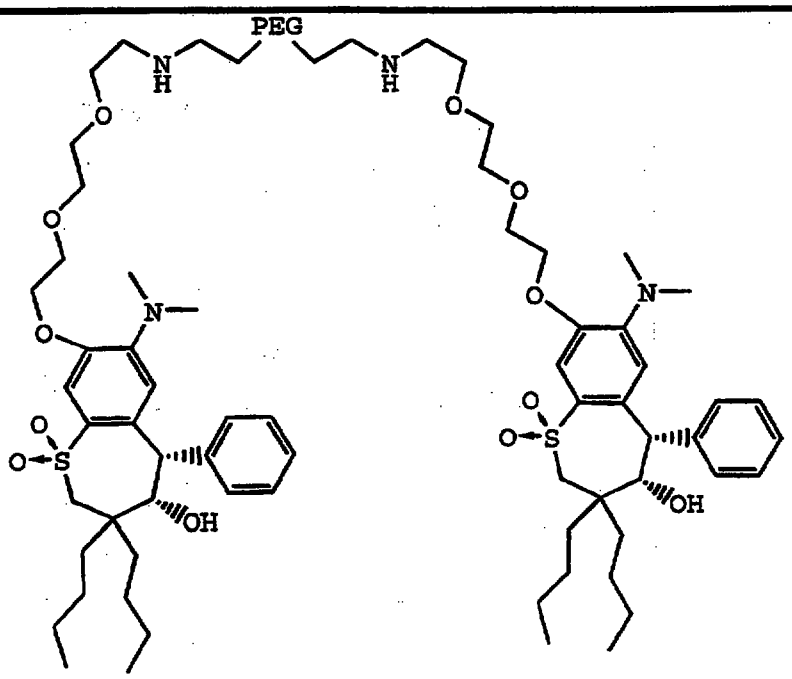
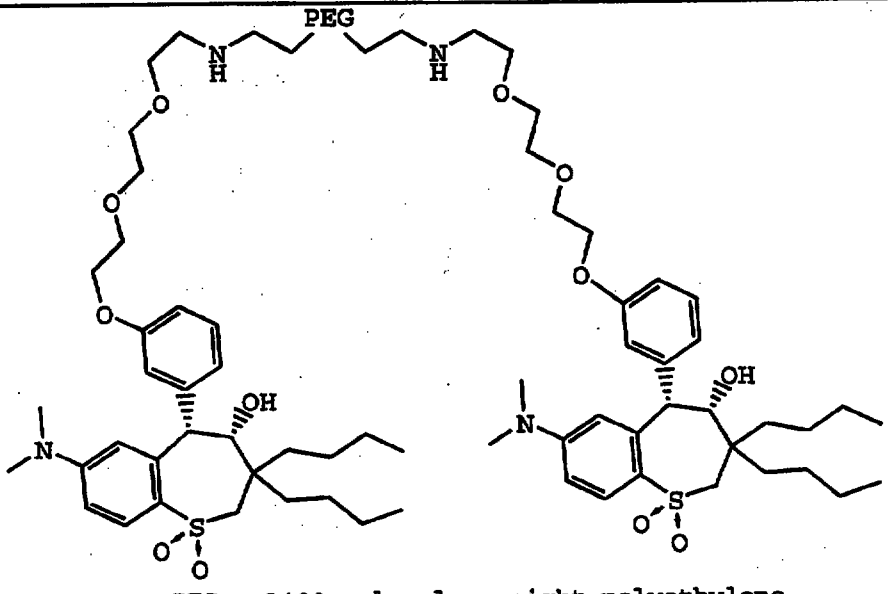
B-13	
B-14	

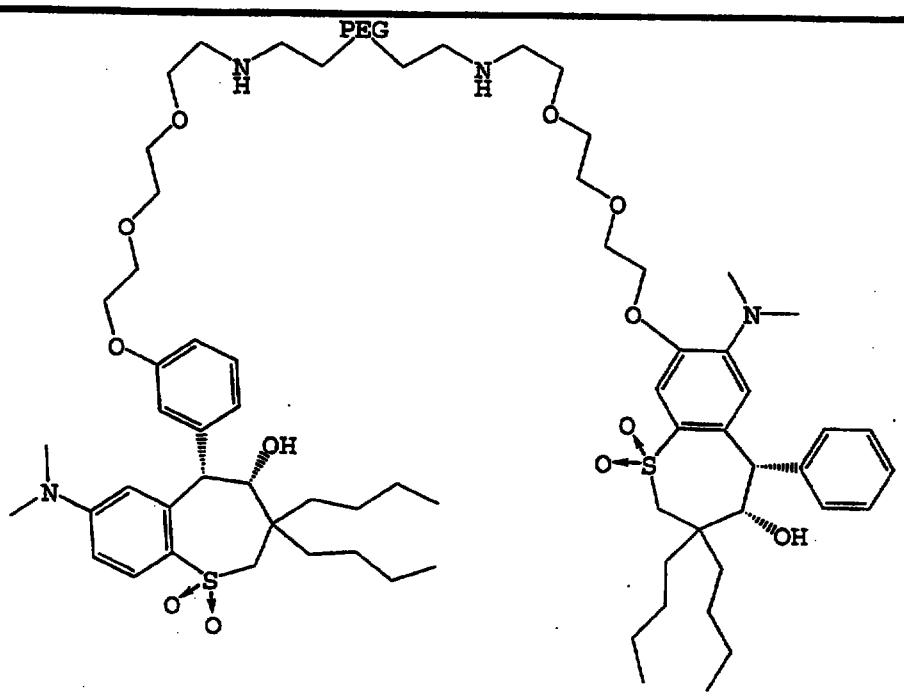
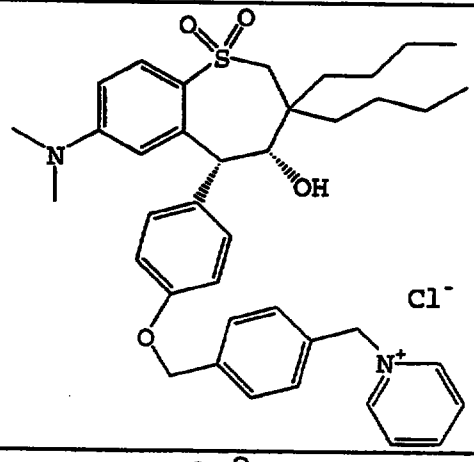
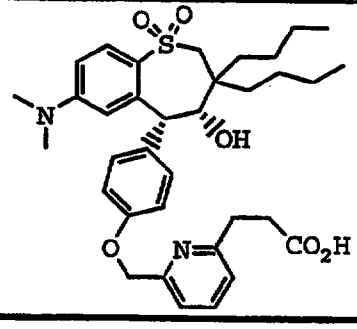
B-15	
B-16	
B-17	

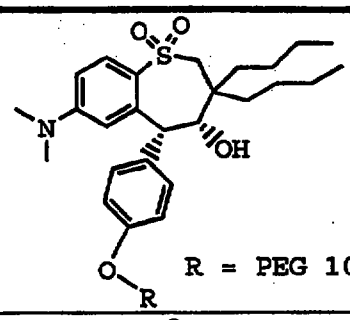
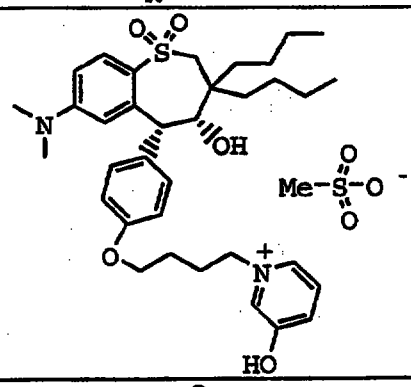
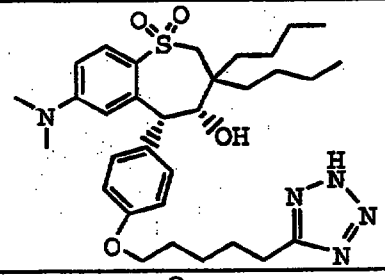
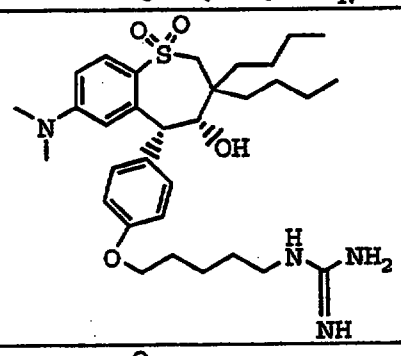
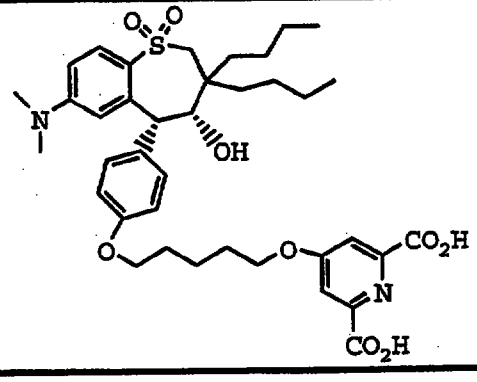
B-18	
B-19	
B-20	

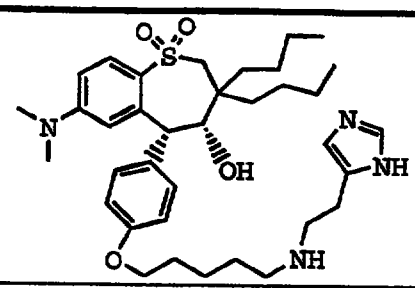
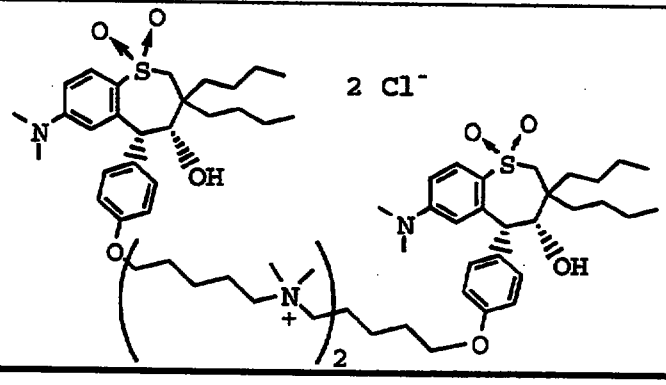
B-21	
B-22	
B-23	

B-24	
B-25	
B-26	
B-27	

B-28	 PEG = 3400 molecular weight polyethylene glycol polymer chain
B-29	 PEG = 3400 molecular weight polyethylene glycol polymer chain

B-30	 PEG = 3400 molecular weight polyethylene glycol polymer chain
B-31	
B-32	

B-33	
B-34	
B-35	
B-36	
B-37	

B-38	
B-39	

Nicotinic acid derivatives useful in the combinations and methods of the present invention comprise a wide variety of structures and functionalities. Preferred nicotinic acid derivatives for the present invention are described in Table 2. The therapeutic compounds of Table 2 can be used in the present invention in a variety of forms, including acid form, salt form, racemates, enantiomers, zwitterions, and tautomers. The individual patent documents referenced in Table 2 are each herein incorporated by reference.

Table 2.

Compound Number	Common Name	CAS Registry Number	Patent Document Reference
G-118	Nicotinic Acid	59-67-6	
G-117	Niceritrol	5868-05-3	GB 1022880
G-3	Acipimox	51037-30-0	GB 1351967

The compounds (for example, ileal bile acid transport inhibiting compounds or nicotinic acid derivative compounds) useful in the present invention can have no asymmetric carbon atoms, or, alternatively, the useful compounds can have one or more asymmetric carbon atoms. When the useful compounds have one or more asymmetric carbon atoms, they therefore include racemates and stereoisomers, such as diastereomers and enantiomers, in both pure form and in admixture. Such stereoisomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention.

Isomers may include geometric isomers, for example *cis*-isomers or *trans*-isomers across a double bond. All such isomers are contemplated among the compounds useful in the present invention.

The compounds useful in the present invention also include tautomers.

The compounds useful in the present invention as discussed below include their salts, solvates and prodrugs.

Dosages, Formulations, and Routes of Administration

The compositions of the present invention can be administered for the prophylaxis and treatment of hyperlipidemic diseases or conditions by any means, preferably oral, that produce contact of these compounds with their site of action in the body, for example in the ileum, plasma, or liver of a mammal, e.g., a human.

For the prophylaxis or treatment of the conditions referred to above, the compounds useful in the compositions and methods of the present invention can be used as the compound *per se*. Pharmaceutically acceptable

salts are particularly suitable for medical applications because of their greater aqueous solubility relative to the parent compound. Such salts must clearly have a pharmaceutically acceptable anion or cation. Suitable pharmaceutically acceptable acid addition salts of the compounds of the present invention when possible include those derived from inorganic acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, sulfonic, and sulfuric acids, and organic acids such as acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glycolic, isothionic, lactic, lactobionic, maleic, malic, methanesulfonic, succinic, toluenesulfonic, tartaric, and trifluoroacetic acids. The chloride salt is particularly preferred for medical purposes. Suitable pharmaceutically acceptable base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, and alkaline earth salts such as magnesium and calcium salts.

The anions useful in the present invention are, of course, also required to be pharmaceutically acceptable and are also selected from the above list.

The compounds useful in the present invention can be presented with an acceptable carrier in the form of a pharmaceutical composition. The carrier must, of course, be acceptable in the sense of being compatible with the other ingredients of the composition and must not be deleterious to the recipient. The carrier can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose composition, for example, a tablet, which can contain from 0.05% to 95% by weight of the active compound. Other pharmacologically active substances can also be present, including other compounds of the present invention. The pharmaceutical compositions of the invention can be prepared by any of the well known

techniques of pharmacy, consisting essentially of admixing the components.

Optionally, the combination of the present invention can comprise a composition comprising an ileal bile acid transport inhibiting compound and a nicotinic acid derivative compound. In such a composition, the ileal bile acid transport inhibitor and the nicotinic acid derivative can be present in a single dosage form, for example a pill, a capsule, or a liquid which contains both of the compounds.

These compounds can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic compounds or as a combination of therapeutic compounds.

The amount of compound which is required to achieve the desired biological effect will, of course, depend on a number of factors such as the specific compound chosen, the use for which it is intended, the mode of administration, and the clinical condition of the recipient.

In general, a total daily dose of an IBAT inhibitor can be in the range of from about 0.01 to about 1000 mg/day, preferably from about 0.1 mg to about 50 mg/day, more preferably from about 1 to about 10 mg/day.

Generally a total daily dose of a nicotinic acid derivative can be in the range of from about 500 to about 10,000 mg/day, preferably about 1000 to about 8000 mg/day, and more preferably still about 3000 to about 6000 mg/day in single or divided doses.

The daily doses described in the preceding paragraphs for the various therapeutic compounds can be administered to the patient in a single dose, or in proportionate multiple subdoses. Subdoses can be administered 2 to 6

times per day. Doses can be in sustained release form effective to obtain desired results.

In the case of pharmaceutically acceptable salts, the weights indicated above refer to the weight of the acid equivalent or the base equivalent of the therapeutic compound derived from the salt.

Oral delivery of the combinations of the present invention can include formulations, as are well known in the art, to provide prolonged or sustained delivery of the drug to the gastrointestinal tract by any number of mechanisms. These include, but are not limited to, pH sensitive release from the dosage form based on the changing pH of the small intestine, slow erosion of a tablet or capsule, retention in the stomach based on the physical properties of the formulation, bioadhesion of the dosage form to the mucosal lining of the intestinal tract, or enzymatic release of the active drug from the dosage form. For some of the therapeutic compounds useful in the present invention (e.g., an IBAT inhibitor or a nicotinic acid derivative), the intended effect is to extend the time period over which the active drug molecule is delivered to the site of action (e.g., the ileum) by manipulation of the dosage form. Thus, enteric-coated and enteric-coated controlled release formulations are within the scope of the present invention. Suitable enteric coatings include cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethylcellulose phthalate and anionic polymers of methacrylic acid and methacrylic acid methyl ester.

The combinations of the present invention can be delivered orally either in a solid, in a semi-solid, or in a liquid form. When in a liquid or in a semi-solid form, the combinations of the present invention can, for example, be in the form of a liquid, syrup, or contained

in a gel capsule (e.g., a gel cap). In one embodiment, when an IBAT inhibitor is used in a combination of the present invention, the IBAT inhibitor can be provided in the form of a liquid, syrup, or contained in a gel capsule. In another embodiment, when a nicotinic acid derivative is used in a combination of the present invention, the nicotinic acid derivative can be provided in the form of a liquid, syrup, or contained in a gel capsule.

When administered intravenously, the dose for a nicotinic acid derivative can, for example, be in the range of from about 150 mg/kg body weight to about 3000 mg/kg body weight, preferably from about 300 mg/kg body weight to about 2000 mg/kg body weight, more preferably from about 500 mg/kg body weight to about 1000 mg/kg body weight.

The dose of any of these therapeutic compounds can be conveniently administered as an infusion of from about 10 ng/kg body weight to about 100 ng/kg body weight per minute. Infusion fluids suitable for this purpose can contain, for example, from about 0.1 ng to about 10 mg, preferably from about 1 ng to about 10 mg per milliliter. Unit doses can contain, for example, from about 1 mg to about 10 g of the compound of the present invention. Thus, ampoules for injection can contain, for example, from about 1 mg to about 100 mg.

Pharmaceutical compositions according to the present invention include those suitable for oral, rectal, topical, buccal (e.g., sublingual), and parenteral (e.g., subcutaneous, intramuscular, intradermal, or intravenous) administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the

particular compound which is being used. In most cases, the preferred route of administration is oral.

Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of at least one therapeutic compound useful in the present invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. As indicated, such compositions can be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound(s) and the carrier (which can constitute one or more accessory ingredients). In general, the compositions are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the product. For example, a tablet can be prepared by compressing or molding a powder or granules of the compound, optionally with one or more assessory ingredients. Compressed tablets can be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). Molded tablets can be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid diluent.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising a compound of the present invention in a flavored base, usually sucrose, and acacia or tragacanth, and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions suitable for parenteral administration conveniently comprise sterile aqueous

preparations of a compound of the present invention. These preparations are preferably administered intravenously, although administration can also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations can conveniently be prepared by admixing the compound with water and rendering the resulting solution sterile and isotonic with the blood. Injectable compositions according to the invention will generally contain from 0.1 to 5% w/w of a compound disclosed herein.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit-dose suppositories. These can be prepared by admixing a compound of the present invention with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly (e.g., Vaseline), lanolin, polyethylene glycols, alcohols, and combinations of two or more thereof. The active compound is generally present at a concentration of from 0.1 to 50% w/w of the composition, for example, from 0.5 to 2%.

Transdermal administration is also possible. Pharmaceutical compositions suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain a compound of the present invention in an optionally buffered, aqueous solution, dissolved and/or dispersed in an adhesive, or dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%.

As one particular possibility, the compound can be delivered from the patch by electrotransport or iontophoresis, for example, as described in Pharmaceutical Research, 3(6), 318 (1986).

In any case, the amount of active ingredient that can be combined with carrier materials to produce a single dosage form to be administered will vary depending upon the host treated and the particular mode of administration.

The solid dosage forms for oral administration including capsules, tablets, pills, powders, gel caps, and granules noted above comprise one or more compounds useful in the present invention admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate or solubilizing agents such as cyclodextrins. In the case of capsules, tablets, powders, granules, gel caps, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or setting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic

parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Pharmaceutically acceptable carriers encompass all the foregoing and the like.

In combination therapy, administration of two or more of the therapeutic agents useful in the present invention may take place sequentially in separate formulations, or may be accomplished by simultaneous administration in a single formulation or separate formulations. Administration may be accomplished by oral route, or by intravenous, intramuscular, or subcutaneous injections. The formulation may be in the form of a bolus, or in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or diluents, or a binder such as gelatin or hydroxypropylmethyl cellulose, together with one or more of a lubricant, preservative, surface active or dispersing agent.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension, or liquid. Capsules, tablets, etc., can be prepared by conventional methods well known in the art. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount

of the active ingredient or ingredients. Examples of dosage units are tablets or capsules. These may with advantage contain one or more therapeutic compound in an amount described above. For example, in the case of an IBAT inhibitor, the dose range may be from about 0.01 mg/day to about 500 mg/day or any other dose, dependent upon the specific inhibitor, as is known in the art. Also, in the case of a nicotinic acid derivative, the dose range may be from about 0.01 mg to about 500 mg or any other dose, dependent upon the specific inhibitor, as is known in the art.

The active ingredients may also be administered by injection as a composition wherein, for example, saline, dextrose, or water may be used as a suitable carrier. A suitable daily dose of each active therapeutic compound is one that achieves the same blood serum level as produced by oral administration as described above.

The therapeutic compounds may further be administered by any combination of oral/oral, oral/parenteral, or parenteral/parenteral route.

Pharmaceutical compositions for use in the treatment methods of the present invention may be administered in oral form or by intravenous administration. Oral administration of the combination therapy is preferred. Dosing for oral administration may be with a regimen calling for single daily dose, or for a single dose every other day, or for multiple, spaced doses throughout the day. The therapeutic compounds which make up the combination therapy may be administered simultaneously, either in a combined dosage form or in separate dosage forms intended for substantially simultaneous oral administration. The therapeutic compounds which make up the combination therapy may also be administered sequentially, with either therapeutic compound being

administered by a regimen calling for two-step ingestion. Thus, a regimen may call for sequential administration of the therapeutic compounds with spaced-apart ingestion of the separate, active agents. The time period between the multiple ingestion steps may range from a few minutes to several hours, depending upon the properties of each therapeutic compound such as potency, solubility, bioavailability, plasma half-life and kinetic profile of the therapeutic compound, as well as depending upon the effect of food ingestion and the age and condition of the patient. Circadian variation of the target molecule concentration may also determine the optimal dose interval. The therapeutic compounds of the combined therapy whether administered simultaneously, substantially simultaneously, or sequentially, may involve a regimen calling for administration of one therapeutic compound by oral route and another therapeutic compound by intravenous route. Whether the therapeutic compounds of the combined therapy are administered by oral or intravenous route, separately or together, each such therapeutic compound will be contained in a suitable pharmaceutical formulation of pharmaceutically-acceptable excipients, diluents or other formulations components. Examples of suitable pharmaceutically-acceptable formulations containing the therapeutic compounds for oral administration are given above.

Treatment Regimen

The dosage regimen to prevent, give relief from, or ameliorate a disease condition having hyperlipemia as an element of the disease, e.g., atherosclerosis, or to protect against or treat further high cholesterol plasma or blood levels with the compounds and/or compositions of the present invention is selected in accordance with a

variety of factors. These include the type, age, weight, sex, diet, and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetics and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized, and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore deviate from the preferred dosage regimen set forth above.

Initial treatment of a patient suffering from a hyperlipidemic condition can begin with the dosages indicated above. Treatment should generally be continued as necessary over a period of several weeks to several months or years until the hyperlipidemic disease condition has been controlled or eliminated. Patients undergoing treatment with the compounds or compositions disclosed herein can be routinely monitored by, for example, measuring serum LDL and total cholesterol levels by any of the methods well known in the art, to determine the effectiveness of the combination therapy. Continuous analysis of such data permits modification of the treatment regimen during therapy so that optimal effective amounts of each type of therapeutic compound are administered at any point in time, and so that the duration of treatment can be determined as well. In this way, the treatment regimen/dosing schedule can be rationally modified over the course of therapy so that the lowest amount of the therapeutic compounds which together exhibit satisfactory effectiveness is administered, and so that administration is continued only so long as is necessary to successfully treat the hyperlipidemic condition.

A potential advantage of the combination therapy disclosed herein may be reduced dosage amount of any individual therapeutic compound, or all therapeutic compounds, effective in treating hyperlipidemic conditions such as atherosclerosis and hypercholesterolemia. The dosage lowering will provide advantages including reduction of side effects of the individual therapeutic compounds when compared to the monotherapy.

One of the several embodiments of the present invention comprises a combination therapy comprising the use of a first amount of an IBAT inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia or atherosclerosis, wherein said first and second amounts together comprise an anti-hyperlipidemic condition effective amount or an anti-atherosclerotic condition effective amount of said compounds. For example one of the many embodiments of the present invention is a combination therapy comprising therapeutic dosages of an IBAT inhibitor and a nicotinic acid derivative. A preferred embodiment of the present invention is a combination therapy comprising therapeutic dosages of a benzothiepine IBAT inhibitor and a nicotinic acid derivative.

The following non-limiting examples serve to illustrate various aspects of the present invention.

c. Examples

Table 3 illustrates examples of some combinations of the present invention wherein the combination comprises a first amount of an IBAT inhibitor and a second amount of a nicotinic acid derivative, wherein said first and second

amounts together comprise an anti-hyperlipidemic condition effective amount or an anti-atherosclerotic condition effective amount of said compounds.

Table 3.

Example Number	Component 1	Component 2
1	B-1	nicotinic acid (niacin)
2	B-2	nicotinic acid (niacin)
3	B-3	nicotinic acid (niacin)
4	B-4	nicotinic acid (niacin)
5	B-5	nicotinic acid (niacin)
6	B-6	nicotinic acid (niacin)
7	B-7	nicotinic acid (niacin)
8	B-8	nicotinic acid (niacin)
9	B-9	nicotinic acid (niacin)
10	B-10	nicotinic acid (niacin)
11	B-11	nicotinic acid (niacin)
12	B-12	nicotinic acid (niacin)
13	B-13	nicotinic acid (niacin)
14	B-14	nicotinic acid (niacin)
15	B-15	nicotinic acid (niacin)
16	B-16	nicotinic acid (niacin)
17	B-17	nicotinic acid (niacin)
18	B-18	nicotinic acid (niacin)
19	B-19	nicotinic acid (niacin)
20	B-20	nicotinic acid (niacin)
21	B-21	nicotinic acid (niacin)
22	B-22	nicotinic acid (niacin)
23	B-23	nicotinic acid (niacin)
24	B-24	nicotinic acid (niacin)
25	B-25	nicotinic acid (niacin)
26	B-26	nicotinic acid (niacin)
27	B-27	nicotinic acid (niacin)
28	B-28	nicotinic acid (niacin)
29	B-29	nicotinic acid (niacin)
30	B-30	nicotinic acid (niacin)
31	B-31	nicotinic acid (niacin)
32	B-32	nicotinic acid (niacin)
33	B-33	nicotinic acid (niacin)
34	B-34	nicotinic acid (niacin)
35	B-35	nicotinic acid (niacin)
36	B-36	nicotinic acid (niacin)
37	B-37	nicotinic acid (niacin)
38	B-38	nicotinic acid (niacin)

39	B-39	nicotinic acid (niacin)
40	B-1	niceritrol
41	B-2	niceritrol
42	B-3	niceritrol
43	B-4	niceritrol
44	B-5	niceritrol
45	B-6	niceritrol
46	B-7	niceritrol
47	B-8	niceritrol
48	B-9	niceritrol
49	B-10	niceritrol
50	B-11	niceritrol
51	B-12	niceritrol
52	B-13	niceritrol
53	B-14	niceritrol
54	B-15	niceritrol
55	B-16	niceritrol
56	B-17	niceritrol
57	B-18	niceritrol
58	B-19	niceritrol
59	B-20	niceritrol
60	B-21	niceritrol
61	B-22	niceritrol
62	B-23	niceritrol
63	B-24	niceritrol
64	B-25	niceritrol
65	B-26	niceritrol
66	B-27	niceritrol
67	B-28	niceritrol
68	B-29	niceritrol
69	B-30	niceritrol
70	B-31	niceritrol
71	B-32	niceritrol
72	B-33	niceritrol
73	B-34	niceritrol
74	B-35	niceritrol
75	B-36	niceritrol
76	B-37	niceritrol
77	B-38	niceritrol
78	B-39	niceritrol
79	B-1	acipimox

80	B-2	acipimox
81	B-3	acipimox
82	B-4	acipimox
83	B-5	acipimox
84	B-6	acipimox
85	B-7	acipimox
86	B-8	acipimox
87	B-9	acipimox
88	B-10	acipimox
89	B-11	acipimox
90	B-12	acipimox
91	B-13	acipimox
92	B-14	acipimox
93	B-15	acipimox
94	B-16	acipimox
95	B-17	acipimox
96	B-18	acipimox
97	B-19	acipimox
98	B-20	acipimox
99	B-21	acipimox
100	B-22	acipimox
101	B-23	acipimox
102	B-24	acipimox
103	B-25	acipimox
104	B-26	acipimox
105	B-27	acipimox
106	B-28	acipimox
107	B-29	acipimox
108	B-30	acipimox
109	B-31	acipimox
110	B-32	acipimox
111	B-33	acipimox
112	B-34	acipimox
113	B-35	acipimox
114	B-36	acipimox
115	B-37	acipimox
116	B-38	acipimox
117	B-39	acipimox

BIOLOGICAL ASSAYS

The utility of the combinations of the present invention can be shown by the following assays. These assays are performed *in vitro* and in animal models essentially using procedures recognized to show the utility of the present invention.

In Vitro Assay of compounds that inhibit uptake of [^{14}C]-Alanine

The alanine uptake assay can be performed in an identical fashion to the taurocholate assay, with the exception that labeled alanine is to be substituted for the labeled taurocholate.

Measurement of Rat Fecal Bile Acid Concentration (FBA)

Total fecal output from individually housed rats is to be collected for 24 or 48 hours, dried under a stream of nitrogen, pulverized, mixed, and weighed. Approximately 0.1 gram is weighed out and extracted into an organic solvent (butanol/water). Following separation and drying, the residue is dissolved in methanol and the amount of bile acid present will be measured enzymatically using the 3α-hydroxysteroid steroid dehydrogenase reaction with bile acids to reduce NAD. (*see* Mashige, F. et al. Clin. Chem., 27, 1352 (1981), herein incorporated by reference).

Rat Gavage Assay

Male Wister rats (275-300g) are to be administered IBAT inhibitors using an oral gavage procedure. Drug or vehicle (0.2% TWEEN 80 in water) is administered once a day (9:00-10:0 a.m.) for 4 days at varying dosages in a final volume of 2 mL per kilogram of body weight. (TWEEN 80 is a 20 molar polyethyleneoxide sorbitan monooleate surfactant manufactured by ICI Specialty Chemicals,

Wilmington, Delaware, U.S.A.) Total fecal samples are collected during the final 48 hours of the treatment period and analyzed for bile acid content using an enzymatic assay as described below. Compound efficacy will be determined by comparison of the increase in fecal bile acid (FBA) concentration in treated rats to the mean FBA concentration of rats in the vehicle group.

[^{3}H]taurocholate Uptake in Rabbit Brush Border Membrane Vesicles (BBMV)

Rabbit Ileal brush border membranes are to be prepared from frozen ileal mucosa by the calcium precipitation method describe by Malathi *et al.* (Biochimica Biophysica Acta, 554, 259 (1979), herein incorporated by reference). The method for measuring taurocholate is essentially as described by Kramer *et al.* (Biochimica Biophysica Acta, 1111, 93 (1992), herein incorporated by reference) except the assay volume will be 200 µl instead of 100 µl. Briefly, at room temperature a 190 µl solution containing 2µM [^{3}H]-taurocholate(0.75 µCi), 20 mM tris, 100 mM NaCl, 100 mM mannitol pH 7.4 is incubated for 5 sec with 10 µl of brush border membrane vesicles (60-120 µg protein). The incubation is initiated by the addition of the BBMV while vortexing and the reaction is to be stopped by the addition of 5 ml of ice cold buffer (20 mM Hepes-tris, 150 mM KCl) followed immediately by filtration through a nylon filter (0.2 µm pore) and an additional 5 ml wash with stop buffer.

Acyl-CoA; Cholesterol Acyl Transferase (ACAT)

Hamster liver and rat intestinal microsomes are to be prepared from tissue as described previously (J. Biol. Chem., 255, 9098 (1980), herein incorporated by reference) and used as a source of ACAT enzyme. The assay will

consist of a 2.0 ml incubation containing 24 μM Oleoyl-CoA (0.05 μCi) in a 50 mM sodium phosphate, 2 mM DTT pH 7.4 buffer containing 0.25 % BSA and 200 μg of microsomal protein. The assay will be initiated by the addition of oleoyl-CoA. The reaction proceeds for 5 min at 37° C and will be terminated by the addition of 8.0 ml of chloroform/ methanol (2:1). To the extraction is added 125 μg of cholesterol oleate in chloroform methanol to act as a carrier and the organic and aqueous phases of the extraction are separated by centrifugation after thorough vortexing. The chloroform phase is to be taken to dryness and then spotted on a silica gel 60 TLC plate and developed in hexane/ethyl ether (9:1). The amount of cholesterol ester formed will be determined by measuring the amount of radioactivity incorporated into the cholesterol oleate spot on the TLC plate with a Packard Instaimager.

Measurement of Hepatic Cholesterol Concentration (HEPATIC CHOL)

Liver tissue is to be weighed and homogenized in chloroform:methanol (2:1). After homogenization and centrifugation the supernatant is separated and dried under nitrogen. The residue is to be dissolved in isopropanol and the cholesterol content will be measured enzymatically, using a combination of cholesterol oxidase and peroxidase, as described by Allain, C. A. et al., Clin. Chem., 20, 470 (1974) (herein incorporated by reference).

Measurement of Hepatic HMG CoA-Reductase Activity (HMG COA)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by

centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for HMG CoA reductase activity by incubating for 60 minutes at 37° C in the presence of ^{14}C-HMG-CoA (Dupont-NEN). The reaction is stopped by adding 6N HCl followed by centrifugation. An aliquot of the supernatant is separated, by thin-layer chromatography, and the spot corresponding to the enzyme product is scraped off the plate, extracted and radioactivity is determined by scintillation counting. (Reference: Akerlund, J. and Bjorkhem, I. (1990) *J. Lipid Res.* 31, 2159).

Measurement of Hepatic Cholesterol 7-α-Hydroxylase Activity (7a-OHase)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for cholesterol 7-α-hydroxylase activity by incubating for 5 minutes at 37° C in the presence of NADPH. Following extraction into petroleum ether, the organic solvent is evaporated and the residue is dissolved in acetonitrile/ methanol. The enzymatic product will be separated by injecting an aliquot of the extract onto a C_{18} reversed phase HPLC column and quantitating the eluted material using UV detection at 240nm. (Reference: Horton, J. D., *et al.* (1994) *J. Clin. Invest.* 93, 2084).

Determination of Serum Cholesterol (SER.CHOL, HDL-CHOL, TGI and VLDL + LDL)

Total serum cholesterol (SER.CHOL) are to be measured enzymatically using a commercial kit from Wako Fine Chemicals (Richmond, VA); Cholesterol C11, Catalog No.

276-64909. HDL cholesterol (HDL-CHOL) will be assayed using this same kit after precipitation of VLDL and LDL with Sigma Chemical Co. HDL Cholesterol reagent, Catalog No. 352-3 (dextran sulfate method). Total serum triglycerides (blanked) (TGI) will be assayed enzymatically with Sigma Chemical Co. GPO-Trinder, Catalog No. 337-B. VLDL and LDL (VLDL + LDL) cholesterol concentrations will be calculated as the difference between total and HDL cholesterol.

Measurement of Hamster Fecal Bile Acid Concentration (FBA)

Total fecal output from individually housed hamsters is to be collected for 24 or 48 hours, dried under a stream of nitrogen, pulverized and weighed. Approximately 0.1 gram is weighed out and extracted into an organic solvent (butanol/water). Following separation and drying, the residue is dissolved in methanol and the amount of bile acid present is measured enzymatically using the 3α-hydroxysteroid steroid dehydrogenase reaction with bile acids to reduce NAD. (Mashige, F. et al. Clin. Chem., 27, 1352 (1981), herein incorporated by reference).

Dog Model for Evaluating Lipid Lowering Drugs

Male beagle dogs, obtained from a vendor such as Marshall farms and weighing 6-12 kg are fed once a day for two hours and given water ad libitum. Dogs may be randomly assigned to a dosing groups consisting of 6 to 12 dogs each, such as: vehicle, i.g.; 1mg/kg, i.g.; 2mg/kg, i.g.; 4 mg/kg, i.g.; 2 mg/kg, p.o. (powder in capsule). Intra-gastric dosing of a therapeutic material dissolved in aqueous solution (for example, 0.2% Tween 80 solution [polyoxyethylene mono-oleate, Sigma Chemical Co., St. Louis, MO]) may be done using a gavage tube. Prior to initiating dosing, blood samples may be drawn from the

cephalic vein in the morning before feeding in order to evaluate serum cholesterol (total and HDL) and triglycerides. For several consecutive days animals are dosed in the morning, prior to feeding. Animals are to be allowed 2 hours to eat before any remaining food is removed. Feces are to be collected over a 2 day period at the end of the study and may be analyzed for bile acid or lipid content. Blood samples are also to be taken, at the end of the treatment period, for comparison with pre-study serum lipid levels. Statistical significance will be determined using the standard student's T-test with $p<.05$.

Dog Serum Lipid Measurement

Blood is to be collected from the cephalic vein of fasted dogs in serum separator tubes (Vacutainer SST, Becton Dickinson and Co., Franklin Lakes, NJ). The blood is centrifuged at 2000 rpm for 20 minutes and the serum decanted.

Total cholesterol may be measured in a 96 well format using a Wako enzymatic diagnostic kit (Cholesterol CII) (Wako Chemicals, Richmond, VA), utilizing the cholesterol oxidase reaction to produce hydrogen peroxide which is measured colorimetrically. A standard curve from 0.5 to 10 µg cholesterol is to be prepared in the first 2 columns of the plate. The serum samples (20-40 µl, depending on the expected lipid concentration) or known serum control samples are added to separate wells in duplicate. Water is added to bring the volume to 100 µl in each well. A 100 µl aliquot of color reagent is added to each well and the plates will be read at 500 nm after a 15 minute incubation at 37 degrees centigrade.

HDL cholesterol may be assayed using Sigma kit No. 352-3 (Sigma Chemical Co., St. Louis, MO) which utilizes

dextran sulfate and Mg ions to selectively precipitate LDL and VLDL. A volume of 150 µl of each serum sample is to be added to individual microfuge tubes, followed by 15 µl of HDL cholesterol reagent (Sigma 352-3). Samples are to be mixed and centrifuged at 5000 rpm for 5 minutes. A 50 µl aliquot of the supernatant is to be then mixed with 200 µl of saline and assayed using the same procedure as for total cholesterol measurement.

Triglycerides are to be measured using Sigma kit No. 337 in a 96 well plate format. This procedure will measure glycerol, following its release by reaction of triglycerides with lipoprotein lipase. Standard solutions of glycerol (Sigma 339-11) ranging from 1 to 24 µg are to be used to generate the standard curve. Serum samples (20-40 µl, depending on the expected lipid concentration) are added to wells in duplicate. Water is added to bring the volume to 100 µl in each well and 100 µl of color reagent was also added to each well. After mixing and a 15 minute incubation, the plates will be read at 540 nm and the triglyceride values calculated from the standard curve. A replicate plate is also to be run using a blank enzyme reagent to correct for any endogenous glycerol in the serum samples.

Dog Fecal Bile Acid Measurement

Fecal samples may be collected to determine the fecal bile acid (FBA) concentration for each animal. Fecal collections may be made during the final 48 hours of the study, for two consecutive 24 hour periods between 9:00 am and 10:00 am each day, prior to dosing and feeding. The separate two day collections from each animal are to be weighed, combined and homogenized with distilled water in a processor (Cuisinart) to generate a homogeneous slurry.

About 1.4 g of the homogenate is to be extracted in a final concentration of 50% tertiary butanol/distilled water (2:0.6) for 45 minutes in a 37°C water bath and centrifuged for 13 minutes at 2000 x g. The concentration of bile acids (mmoles/day) may be determined using a 96-well enzymatic assay system (1,2). A 20 µl aliquot of the fecal extract is to be added to two sets each of triplicate wells in a 96-well assay plate. A standardized sodium taurocholate solution and a standardized fecal extract solution (previously made from pooled samples and characterized for its bile acid concentration) will also analyzed for assay quality control. Twenty-microliter aliquots of sodium taurocholate, serially diluted to generate a standard curve are similarly to be added to two sets of triplicate wells. A 230 µl reaction mixture containing 1M hydrazine hydrate, 0.1 M pyrophosphate and 0.46 mg/ml NAD is to be added to each well. A 50 µl aliquot of 3a-hydroxysteroid dehydrogenase enzyme (HSD; 0.8 units/ml) or assay buffer (0.1 M sodium pyrophosphate) are then added to one of the two sets of triplicates. All reagents may be obtained from Sigma Chemical Co., St. Louis, MO. Following 60 minutes of incubation at room temperature, the optical density at 340nm will be measured and the mean of each set of triplicate samples will be calculated. The difference in optical density ± HSD enzyme is to be used to determine the bile acid concentration (mM) of each sample based on the sodium taurocholate standard curve. The bile acid concentration of the extract, the weight of the fecal homogenate (grams) and the body weight of the animal are to be used to calculate the corresponding FBA concentration in mmoles/kg/day for each animal. The mean FBA concentration (mmoles/kg/day) of the vehicle group is to be subtracted

from the FBA concentration of each treatment group to determine the increase (delta value) in FBA concentration as a result of the treatment.

Intestinal Cholesterol Absorption Assay

A variety of compounds are shown to inhibit cholesterol absorption from the intestinal tract. These compounds lower serum cholesterol levels by reducing intestinal absorption of cholesterol from both exogenous sources (dietary cholesterol) and endogenous cholesterol (secreted by the gall bladder into the intestinal tract).

In hamsters the use of a dual-isotope plasma ratio method to measure intestinal cholesterol absorption has been refined and evaluated as described by Turley et al. (J. Lipid Res. 35, 329-339 (1994), herein incorporated by reference).

Male hamsters weighing 80-100 g are to be given food and water ad libitum in a room with 12 hour alternating periods of light and dark. Four hours into the light period, each hamster is administered first an intravenous dose of 2.5 μCi of [1,2-^{3}H]cholesterol suspended in Intralipid (20%) and then an oral dose of [4-^{14}C]cholesterol in an oil of medium chain triglycerides (MCT). The i.v. dose is given by injecting a 0.4 ml volume of the Intralipid mixture into the distal femoral vein. The oral dose is given by gavaging a 0.6 ml volume of the MCT oil mixture introduced intragastrically via a polyethylene tube. After 72 hours the hamsters are bled and the amount of ^{3}H and ^{14}C in the plasma and in the original amount of label administered are determined by liquid scintillation spectrometry. The cholesterol absorption will be calculated based on the following equation:

Percent cholesterol absorbed =

$$\frac{\text{\% of oral dose per ml of 72 hour plasma sample}}{\text{\% of i.v. dose per ml of 72 hour plasma sample}} \times 100$$

Microsomal triglyceride transfer protein (MTP) assay:

MTP can be purified from liver tissue or cultured cells (e.g. HepG2 cells) using standard methods as described by Ohringer et al. (Acta Crystallogr. D52, 224-225 (1996), herein incorporated by reference).

Subsequent analysis of MTP activity can be performed as described by Jamil et al. (Proc. Natl. Acad. Sci. 93, 11991-11995 (1996), herein incorporated by reference).

The basis of this assay is to measure the transfer of labeled triglycerides from a population of donor vesicles to a population of acceptor vesicles in the presence of MTP. Inhibitors of MTP can be evaluated by adding them to the mixture prior to the introduction of MTP. Donor vesicles are to be prepared by sonication of an aqueous mixture of egg phospholipids, cardiolipin, ^{3}H-labeled phospholipid and ^{14}C-labeled triglycerides. Acceptor vesicles are to be prepared by sonication of an aqueous mixture of egg phospholipids. The vesicle solutions are mixed together, with or without added MTP inhibitors, and MTP is to be added to initiate the transfer reaction. The assay will be terminated after 60 minutes by addition of 0.5 ml of DE-52 cellulose followed by centrifugation to pellet the donor molecules. The amount of ^{3}H and ^{14}C in the pellet and in the original amount of label in the mixture will be determined by liquid scintillation

spectrometry. The lipid transfer rate will be calculated based on first order kinetics using the expression:

$$[S] = [S]_0 \, e^{-kt}$$

where $[S]_0$ and $[S]$ are the fractions of ^{14}C label in the donor membrane pellet at times 0 and t, respectively, and the term k is the fraction of label transferred per unit time.

Plasma Lipids Assay in Rabbits

Plasma lipids can be assayed using standard methods as reported by J.R. Schuh et al., J. Clin. Invest., 91, 1453-1458 (1993), herein incorporated by reference. Groups of male, New Zealand white rabbits are placed on a standard diet (100g/day) supplemented with 0.3% cholesterol and 2% corn oil (Zeigler Bothers, Inc., Gardners, PA). Water is available ad lib. Groups of control and treated animals are killed after 1 and 3 months of treatment. Tissues are removed for characterization of atherosclerotic lesions. Blood samples are to be taken for determination of plasma lipid concentrations.

Plasma Lipids

Plasma for lipid analysis is to be obtained by withdrawing blood from the ear vein into EDTA-containing tubes (Vacutainer; Becton Dickenson & Co., Rutherford, NJ), followed by centrifugal separation of the cells. Total cholesterol was determined enzymatically, using the cholesterol oxidase reaction (C.A. Allain et al., Clin. Chem., 20, 470-475 (1974), herein incorporated by reference). HDL cholesterol was also measured

enzymatically, after selective precipitation of LDL and VLDL by dextran sulfate with magnesium (G.R. Warnick et al., Clin. Chem., 28, 1379-1388 (1982), herein incorporated by reference). Plasma triglyceride levels will be determined by measuring the amount of glycerol released by lipoprotein lipase through an enzyme-linked assay (G. Bucolo et al., Clin. Chem., 19, 476-482 (1973), herein incorporated by reference).

Atherosclerosis

Animals are to be killed by pentobarbital injection. Thoracic aortas are rapidly removed, immersion fixed in 10% neutral buffered formalin, and stained with oil red O (0.3%). After a single longitudinal incision along the wall opposite the arterial ostia, the vessels are pinned open for evaluation of the plaque area. The percent plaque coverage is determined from the values for the total area examined and the stained area, by threshold analysis using a true color image analyzer (Videometric 150; American Innovision, Inc., San Diego, CA) interfaced to a color camera (Toshiba 3CCD) mounted on a dissecting microscope. Tissue cholesterol will be measured enzymatically as described, after extraction with a chloroform/methanol mixture (2:1) according to the method of Folch et al. (J. Biol. Chem., 226, 497-509 (1957), herein incorporated by reference).

In Vitro Vascular Response

The abdominal aortas are rapidly excised, after injection of sodium pentobarbital, and placed in oxygenated Krebs-bicarbonate buffer. After removal of perivascular tissue, 3-mm ring segments are cut, placed in a 37°C muscle bath containing Krebs-bicarbonate solution, and suspended between two stainless steel wires, one of

which is attached to a force transducer (Grass Instrument Co., Quincy, MA). Force changes in response to angiotensin II added to the bath will be recorded on a chart recorder.

The examples herein can be performed by substituting the generically or specifically described therapeutic compounds or inert ingredients for those used in the preceding examples.

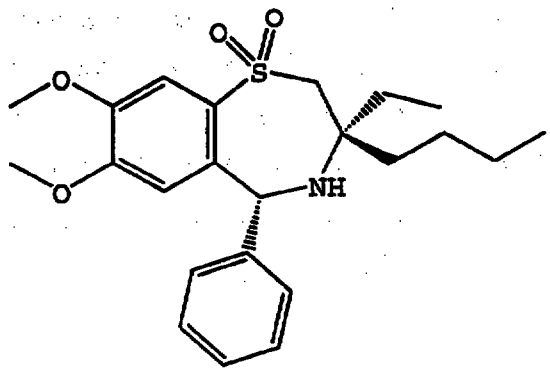
The invention being thus described, it is apparent that the same can be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications and equivalents as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

CLAIMS

What is claimed is:

1. A therapeutic combination comprising a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of the compounds.

2. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibiting compound has the structure of formula B-2:

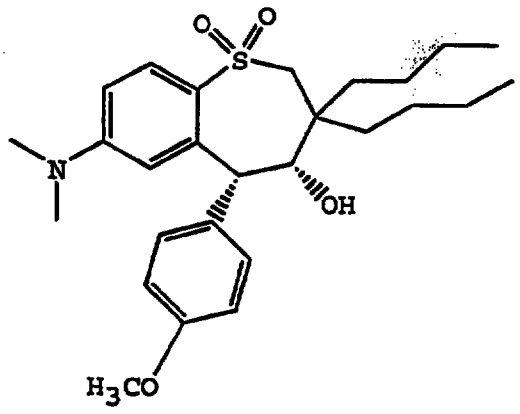


B-2

or an enantiomer or racemate thereof.

3. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibiting compound has the structure of formula B-12:

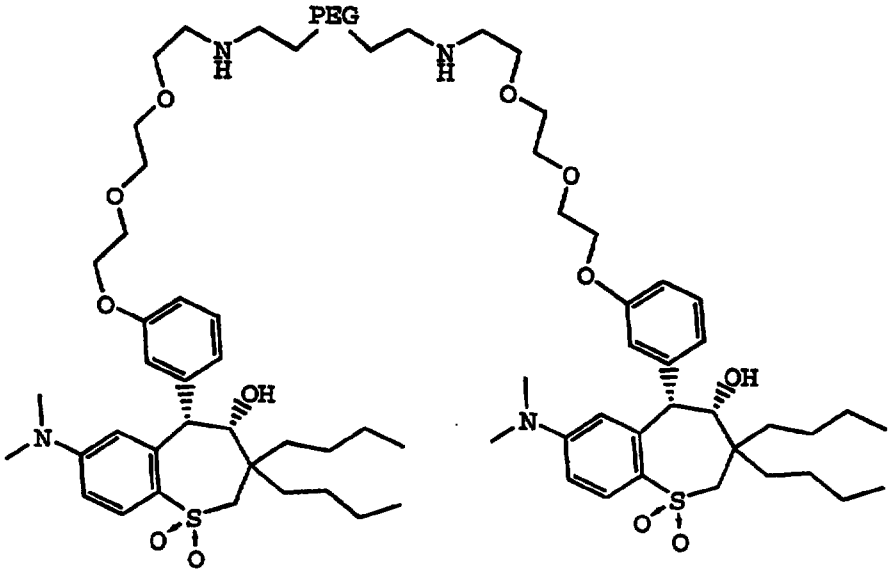




B-12

or an enantiomer or racemate thereof.

4. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibiting compound has the structure of formula B-29:

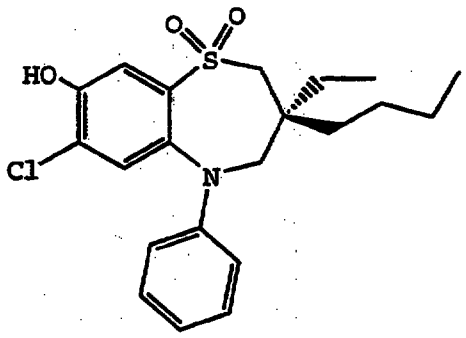


B-29

or an enantiomer or racemate thereof, wherein PEG is an about 3000 to about 4000 molecular weight polyethylene glycol polymer chain.

5. The therapeutic combination of claim 1 wherein the ileal bile acid transport inhibiting compound has the structure of formula B-7:

This Sheet Has Been Replaced



B-7

or an enantiomer or racemate thereof.

6. The therapeutic combination of claim 1 wherein the nicotinic acid derivative is nicotinic acid or a salt thereof.

7. The therapeutic combination of claim 1 wherein the nicotinic acid derivative is niceritrol.

8. The therapeutic combination of claim 1 wherein the nicotinic acid derivative is acipimox.

9. The therapeutic combination of claim 1 wherein the combination comprises a composition comprising the ileal bile acid transport inhibiting compound and the nicotinic acid derivative compound.

10. A method for the prophylaxis or treatment of a hyperlipidemic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount of the compounds.

11. A method for the prophylaxis or treatment of an atherosclerotic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-atherosclerotic condition effective amount of the compounds.

12. A method for the prophylaxis or treatment of hypercholesterolemia comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an ileal bile acid transport inhibiting compound and a second amount of a nicotinic acid derivative compound wherein the first amount and the second amount together comprise an anti-hypercholesterolemic condition effective amount of the compounds.

This Sheet Has Been Replaced