AMINO ACID PRODRUGS

SCOPE OF THE INVENTION

The invention relates to amino acid derivatives of pharmaceutical compounds, methods of treating particular ailments, which are ameliorated by the administration of these drugs and pharmaceutical compositions containing these drugs.

The current invention involves improving many physicochemical, biopharmaceutical, and clinical efficacy of various drugs using amino acids as covalently bonded carriers for these drugs.

The development of chemical compounds for the treatment of disorders, maladies and diseases has become increasingly difficult and costly. The probability of success for such development is often discouragingly low. Further, the time for development can approach or exceed ten years, leaving large numbers of patients without remedy for an extended period of time.

Even in cases in which effective pharmaceutical compounds have been developed, there are often disadvantages associated with their administration. These disadvantages can include aesthetic, and pharmacokinetic bafflers affecting the effectiveness of some existing pharmaceutical compounds, For example, unpleasant taste or smell of a pharmaceutical compound or composition can be a significant barrier to patient compliance with respect to the administration regimen. The undesirable solubility characteristics of a pharmaceutical compound can lead to difficulty in formulation of a homogeneous composition. Other disadvantages associated with known pharmaceutical compounds include: poor absorption of orally administered formulations; poor bioavailability of the pharmaceutical compounds in oral formulations; lack of dose proportionality; low stability of pharmaceutical compounds *in vitro* and *in vivo;* poor penetration of the blood/brain barrier; excessive first-pass metabolism of pharmaceutical compounds as they pass through the liver; excessive enterohepatic recirculation; low absorption rates; ineffective compound release at the site of action; excessive irritation,

for example, gastro-intestinal irritability and/or ulceration; painful injection of parenterally administered pharmaceutical compounds and compositions; excessively high dosages required for some pharmaceutical compounds and compositions, and other undesirable characteristics. Some pharmaceutical compounds are processed by the body to produce toxic by-products with harmful effects.

The art is continually seeking new chemical compounds for the treatment of a wide variety of disorders, with improved properties to overcome the disadvantages of known pharmaceutical compounds mentioned above.

The present invention has overcome many problems associated with currently marketed drugs by making prodrugs. The concept of prodrugs is well known, and there are a number of examples of such prodrugs enumerated in the literature and there are a number of prodrugs available in the market, including such diverse groups as statin drugs, ACE inhibitors, antiviral drugs such as Acyclovir and the like.

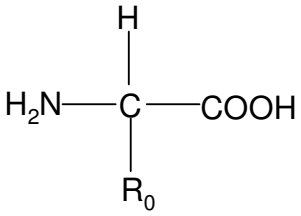
The present invention, however, uses amino acids as the moiety to make the prodrugs. The prodrugs of the present invention have a number of advantages. For example, when amino acid prodrugs are administered by a number of routes such as oral, IV, rectal or other such methods, these prodrugs are converted into active drug molecules. A significant advantage of the amino acid prodrug is that it is non-toxic, and hence either assimilated into the body or safely excreted. This is quite unlike the characteristics exhibited by a number of prodrugs available in the market, where the promoiety itself is toxic, as is the case with statin drugs, Enalapril, Benazapril and the like group of ace inhibitors, and a number of antibiotics such as pivoxil, isopropyl, Axetil, Cilexetil and the like groups, which are highly toxic, thereby reducing the therapeutic index of the active drug.

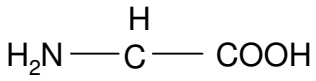
On the other hand, the amino acid prodrugs of the present invention also impart a number of advantages as shown herein below.

SUMMARY OF THE INVENTION

The present invention is directed to a pharmaceutically active prodrug, having amino acid covalently bonded to a pharmaceutical compound to form said acid prodrug, which is administered in this form to the subject, such as a mammal.

The amino acid is an ideal model to be used as a prodrug, because it is capable of forming various types of linkages between itself and the drug. By definition, an amino acid has at least two functionalities thereon, an amino group (NH_2) and a carboxy group (COOH). For example, the α- amino acids have the well known structure



wherever R_0 is the side group or chain of the amino acid. The  as defined herein, is the main chain of the amino acid. Thus, for example, beside the amino group and the carboxyl group on the main chain, the side chain may have functional groups thereon. It is the functional group on the amino acid moiety that permits the covalent linkage to occur between the amino acid and the drug.

The drug or medicament useful in the present invention contains functional groups thereon that permit the drug to react with and form a covalent bond with the amino acid. Examples of functional groups present on the drugs include NH_2, OH, COOH or acid derivatives thereof, such as esters, amides and the like.

The mode of attachment between the pharmaceutical compound and the amino acid can be via:

1) An ester bond (-CO-O-) arising from condensation of a carboxylic acid and an alcohol or phenolic hydroxyl group, or through transesterification, for example:

a) Where the pharmaceutical compound has an aliphatic or aromatic hydroxyl group an ester bond can be formed with the backbone carboxylic acid group of the amino group under esterification conditions; or

b) Where the pharmaceutical compound has an aliphatic or aromatic hydroxyl group and the amino acid has a side chain carboxylic acid group, an ester bond can be formed therebetween under esterification conditions; or

c) Where the pharmaceutical compound has a carboxylic acid group and the amino acid has a side chain aliphatic or aromatic hydroxyl group, an ester bond can be formed therebetween under esterification condition; or

d) Where the pharmaceutical compound has an ester group with a substituted or unsubstituted acyloxy (e.g., alkoxy- or arylalkoxy-, aryloxy carbonyl) substituent (compound-O-CO-substituent) and the amino acid has a backbone carboxylic acid group, an ester bond can be formed therebetween through transesterification; or

e) Where the pharmaceutical compound has an ester group with a substituted or unsubstituted acyloxy (e.g., alkoxy- or arylalkoxy-, aryloxy carbonyl) substituent (compound-O-CO-substituent) and the amino acid has a side chain carboxylic acid group, an ester bond can be formed therebetween through transesterification; or

f) Where the pharmaceutical compound has an ester group with a substituted or unsubstituted alkoxy- or arylalkoxy- or aryloxy carbonyl substituent (compound-CO-O-Substituent) and the amino acid has a side chain aliphatic or aromatic hydroxyl group, an ester bond can form therebetween though transesterification; or

g) The alcohol and carboxylic acid moieties may be on the same molecule such they can form an internal ester. For example, certain compounds like Gabapentin can form an internal ester under ester forming conditions, is also included with the scope of the present invention.

2) An amide bond (-CO-NH -) arising from condensation of a carboxylic acid and an amine, for example:

a) Where the pharmaceutical compound has an amino group and the amino acid has a backbone carboxylic acid group, an amide can be formed under amide forming conditions; or

b) Where the pharmaceutical compound has an amino group and the amino acid has a side chain carboxylic acid group, an amide bond can form therebetween under amide forming conditions; or

c) Where the pharmaceutical compound has a carboxylic acid group and the amino acid has a backbone amino group, an amide bond can form therebetween under amide forming conditions; or

d) Where the pharmaceutical compound has a carboxylic acid group and the amino acid has a side chain amino group, an amide bond can be formed therebetween under amide forming conditions.

Thus, the present invention is directed to the prodrugs thus formed. As shown hereinbelow the prodrug thus formed has advantages not realized relative to the drug without the amino acid attached thereto. For example, it can improve bioavailability, efficacy, be less toxic, exhibit greater solubility in water and/or improve the ability of the drug to pass into the cell membrane or through blood brain barrier, exhibit less side effects, such as gastro-intestinal irritability, enhanced therapeutic index and the like.

Thus, the present invention is directed to a method of improving the therapeutic quality of a drug wherein the improvement in the therapeutic quality is selected from the group consisting of improved efficacy, enhanced therapeutic index, increased solubility in the mammal's internal fluid, improved passage through the cell membrane, improved passage through the blood brain barrier, decreased side effects, such as significantly decreased irritation and/or ulcerations, less toxicity, enhanced absorption ratio and the like relative to the corresponding drug administered to the patient in the non-prodrug form, said method comprising reacting the drug with an amino acid to form a covalent bond therebetween and administering the product thereof (hereinafter "prodrug") to a patient. The prodrugs of the present invention have at least one improved quality. In

fact, they exhibit preferably at least two of the improved qualities cited hereinabove. Other advantages of the prodrug include the wide availability of the amino acids and the ease in which the reactions take place. The reaction to form the amide is generally efficient and yield are very high, presumably above about 70% and more preferably above about 80% and most preferably above about 90%.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 graphically compares the efficacy of L- serine ester of (±) Ibuprofen (F1), L-threonine ester (±) Ibuprofen (F2) and L-hydroxyproline ester of (±) Ibuprofen (F3), (±) Ibuprofen (i.e., the racemic mixture) and Ibuprofen (S)(+), after one hour dosing, based on the antagonizing property of Acetylcholine induced writhe in Albino mice.

Figure 2 graphically compares the efficacy of L- serine ester of (±) Ibuprofen, (F1), L-threonine ester of, (I) Ibuprofen (F2), L-hydroxyproline ester of (±) Ibuprofen (F3), ± Ibuprofen and S(+) Ibuprofen after 3 hour dosing, based on the antagonizing property of Acetylcholine induced writhes in albino mice.

Figure 3 depicts graphically the dose response relationship to mean clotting time (MCT) in minutes for the L-serine ester of acetylsalicylic acid (Formulation 1).

Figure 4 depicts graphically the dose response relationship to mean clotting time (MCT) minutes for the L-hydroxyproline ester of acetylsalicylic acid (Formulation 2).

Figure 5 depicts the dose response relationship to mean clotting time (MCT) in minutes for the L-threonine ester of acetylsalicylic acid (Formulation 3)

Figure 6 depicts the dose response relationship to mean clotting time (MCT) in minutes for control (acetylsalicylic acid).

Figure 7 graphically compares the relative efficacy of L-serine (ester of acetyl salicyclic acid (F.1), L-threonine ester of acetyl salicylic acid (F.2), L-hydroxyproline ester of acetylsalicylic acid (F.3), and acetylsalicylic acid (PC) as a function of mean clotting time in minutes.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

As used here, the term "drug", "medicament ", and "pharmaceutical" are being used interchangeably and refer to the active compound that is administered to the patient without attachment of the amino acid thereto. Moreover, as used herein, the drug contains a functional group thereon capable of reacting with the amino acid, such as NH_2, OH, COOH or acylating derivatives thereof (e.g., ester, anhydride, amide, and the like) and the like. When the drug is linked to an amino acid, the term "amino acid prodrug" or "prodrug of the present invention" or synonym thereto is utilized.

Among the amino acids useful as promoieties (i.e., reacting with the drugs) are those containing the free amino and/or carboxylic acid groups of all conventional amino acids. Of those, some preferred embodiments include those amino acids having relatively high solubility in aqueous media, for example, in deionized water at unbuffered aqueous solution at 25°C, of at least 100 g/L, and more preferably, at least 250 g/L, and even more preferably at least 500 g/L. For example, glycine and proline have solubilities in deionized water at 25°C of approximately 250 g/L and 1620 g/L, respectively.

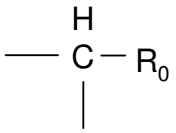
Other amino acids useful as promoieties are those containing basic amino side chains, such as lysine. For example, lysine has solubility in deionized water at 25°C of approximately 700 g/L.

Among other amino acids useful as promoieties are those containing hydroxyl side chains, such as hydroxyproline, serine, and threonine. For example, threonine, hydroxyproline and serine have solubilities in deionized water at 25°C of approximately 100 g/L, 369 g/L and 420 g/L, respectively.

Other preferred embodiments include those amino acids with relatively low solubility in aqueous media, for example, in deionized water at 25°C of at most 10 g/L, or for example, at most 2 g/L, or for example at most 0.6 g/L. For example, the solubility of tyrosine in deionized water at 25°C is approximately 0.5 g/L. Such prodrugs could be used to produce formulations with extended release characteristics, due to the limited solubility of the prodrugs.

Among other amino acids useful as promoieties are those containing carboxylic acid side chains, such as glutamic acid and aspartic acid. Other amino acids useful as promoieties are the non-essential amino acids, and the non-naturally occurring amino acids.

The following reaction schemes depict the reactions discussed hereinabove with respect to reaction of hydroxyl, carboxyl and amine containing drugs with various amino acids. In the schemes below, R is the drug less the functional OH, COOH or NH_2 group



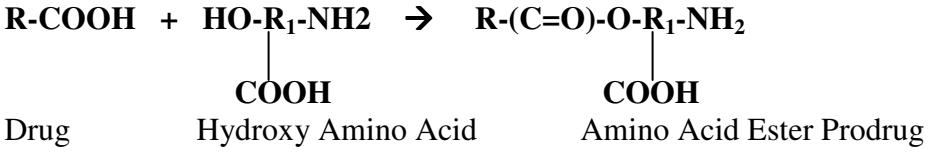
whichever is present, and R_1 is , wherein R_0 is the side chain of the amino acid listed hereinbelow:

Reaction Scheme A: Where the hydroxyl group of the drug is reacted with the carboxyl group of an amino acid to from the ester prodrug

R-OH + HOOC-R_1- NH_2 → R-O-(C=O)-R_1- NH_2

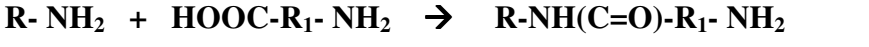
Drug Amino Acid Amino acid Ester Prodrug

Reaction Scheme B: Where the carboxyl group of the drug is reacted with the hydroxyl group of a hydroxy amino acid wherein the hydroxy group is on the side chain to form the ester prodrug.



Drug Hydroxy Amino Acid Amino Acid Ester Prodrug

Reaction Scheme C: Where the amine group of the drug is reacted with the carboxyl group of the amino acid to from the amide prodrug

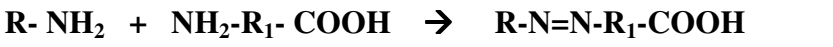


Drug Amino Acid Amino Acid Amide Prodrug

Reaction Scheme D: Where the carboxyl group of the drug is reacted with the carboxyl group of the amino acid to form the anhydride prodrug.

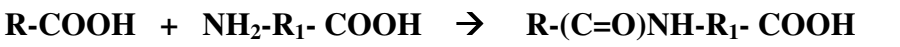
Drug Amino Acid Amino Acid Anhydride Prodrug

Reaction Scheme E: Where the amine group of the drug is reacted with the amine group of the amino acid to form the azo prodrug derivative.



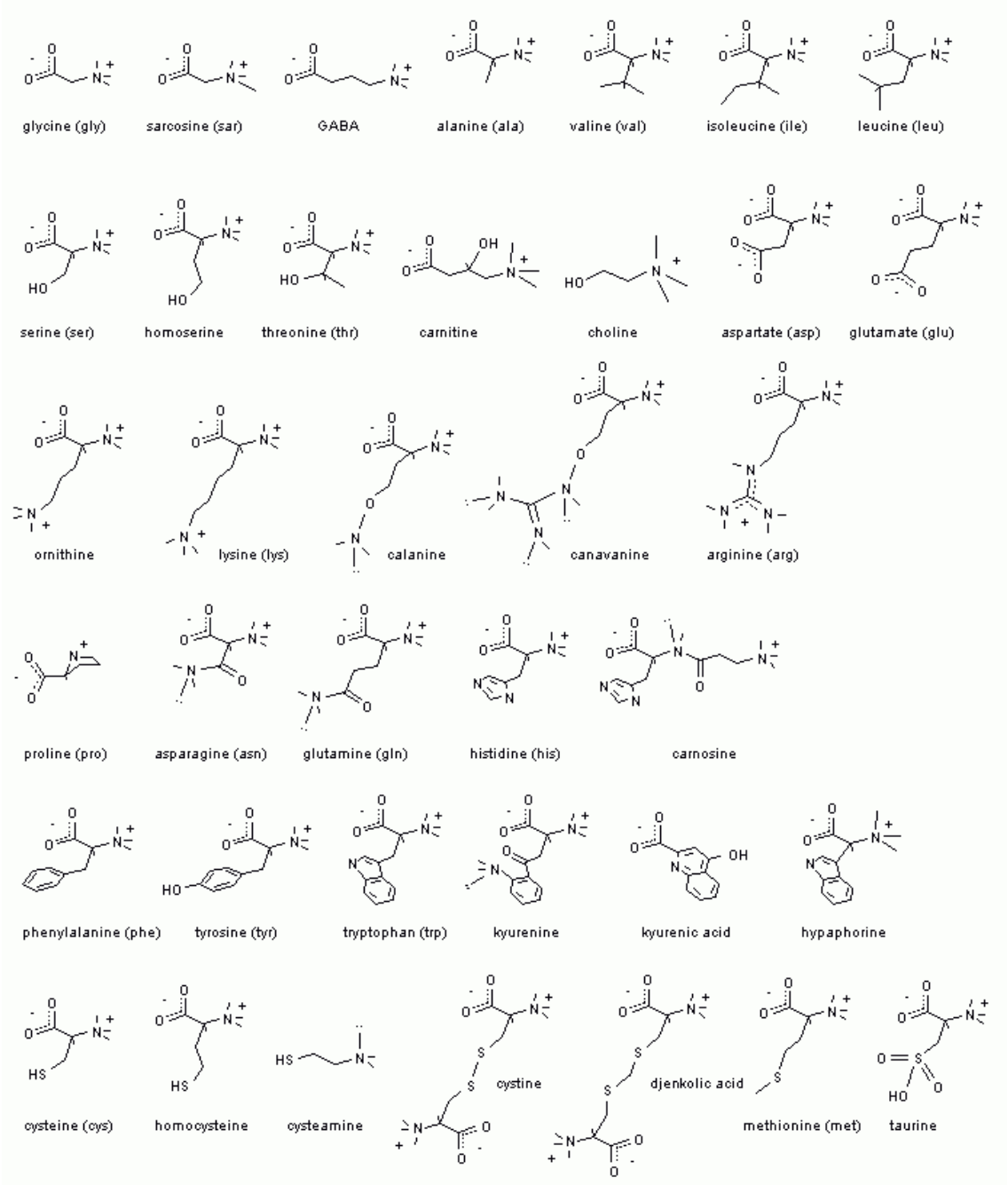
Drug Amino Acid Amino Acid Azo Prodrug

Reaction Scheme F: Where the carboxyl group of the drug is reacted with amine group of the amino acid to form the amide prodrug.



Drug Amino Acid Amino Acid Amide Prodrug

In the above schemes A-F, the preferred amino acids used are shown hereinbelow:



As used herein the term "amino acid" refers to an organic compound having therein a carboxyl group (COOH) and an amino group (NH_2) or salts thereof. In solution, these two terminal groups ionize to form a double ionized, through overall neutral entity identified as zwitterions. The amine donates an electron to the carboxyl group and the ionic ends are stabilized in aqueous solution by polar water molecules.

It is the side groups that distinguish the amino acids from each other. Some amino acids, such as lysine, have amino groups on the side chain; other amino acids have side chains containing hydroxy groups, such as threonine, serine, hydroxyproline, and tyrosine; some amino acids have carboxy groups on the side chain, such as glutamic acid or aspartic acid. These functional groups on the side chain also can form a covalent bond with the drug, such as esters, amides, and the like. When these side groups become involved in these linkages, such as hydroxy group, the bond may be depicted as OAA, wherein AA is an amino acid residue having a side chain with a hydroxy group, but without the hydroxy group. Thus, AA by this definition, refers to the amino acid without the hydroxy side group since it took part in the reaction in forming the ester. Moreover, when an ester is formed between the hydroxy group of the amino acid and the OH group of the drug, the hydroxy group on the carboxy group forms a byproduct with the hydrogen of the hydroxy group, thus, the resulting product does not have the OH group on the carboxy group, but just the acyl moiety. When the bond is depicted as C(=O)-NHAA, this means that the amino acid forms as an amide bond between the carboxy group on the drug and the amino group of the amino acid. However, as written, since the NH from the amide bond comes from the amino acid, AA is the amino acid without the amino group.

The preferred amino acids are the naturally occurring amino acids. It is more preferred that the amino acids are the ∀-amino acids. It is also preferred that the amino acids are in the L-configuration. The preferred amino acids include the twenty essential amino acids. The preferred amino acids are Lysine (Lys), Leucine (Leu), Isoleucine (Ile), Glycine (Gly), Aspartic Acid (Asp), Glutamic Acid (Glu), Methionine (Met), Alanine (Ala), Valine (Val), Proline (Pro), Histidine (His), Tyrosine (Tyr), Serine (Ser), Norleucine (Nor), Arginine (Arg), Phenylalanine (Phe), Tryptophan (Trp), Hydroxyproline (Hyp), Homoserine (Hsr), Carnitine (Car), Ornithine (Ort), Canavanine (Cav), Asparagine (Asn), Glutamine (Gln), Carnosine (Can), Taurine (Tau), djenkolic Acid (Djk), γ-aminobutyric Acid (GABA), Cysteine (Cys) Cystine (Dcy), Sarcosine

(Sar), Threonine (Thr) and the like. The even more preferred amino acids are the twenty essential amino acids, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser.

The prodrugs are prepared from a drug having a group thereon which can react with the amino acid.

The preferred drugs that are reacted with amino acids in accordance with various schemes are as follows:

Reaction Schemes

Drugs	A	B	C	D	E	F
Cyclosporins	YES					
Lopinavir	YES		YES		YES	
Ritonavir	YES		YES		YES	
Cefdinir		YES	YES	YES	YES	YES
Zileuton	YES		YES		YES	
Nelfinavir	YES		YES		YES	
Flavoxate		YES		YES		YES
Candesarten		YES	YES	YES	YES	YES
Propofol	YES					
Nisoldipine		YES	YES	YES	YES	YES
Amlodipine		YES	YES	YES	YES	YES
Ciprofloxacin		YES	YES	YES		YES
Ofloxacin		YES	YES	YES		YES
Fosinopril		YES		YES		YES
Enalapril		YES		YES		YES
Ramipril		YES		YES		YES
Benazepril		YES		YES		YES
Moexipril		YES		YES		YES
Trandolapril		YES		YES		YES

	Cromolyn	YES	YES		YES		YES
	Amoxicillin	YES	YES	YES	YES	YES	YES
	Cefuroxime	YES	YES	YES	YES	YES	YES
	Ceftazimide	YES	YES	YES	YES	YES	YES
	Cefpodoxime	YES	YES	YES	YES	YES	YES
	Atovaquone	YES					
	Gancyclovir	YES		YES		YES	
	Penciclovir	YES		YES		YES	
	Famciclovir	YES		YES		YES	
	Acyclovir	YES		YES		YES	
	Niacin		YES		YES		YES
	Bexarotene		YES		YES		YES
	Propoxyphene	YES					
	Salsalate	YES	YES		YES		YES
	Acetaminophen	YES					
	Ibuprofen		YES		YES		YES
	Lovastatin	YES	YES		YES		YES
	Simavastatin	YES	YES		YES		YES
	Atorvastatin	YES	YES		YES		YES
	Pravastatin	YES	YES		YES		YES
	Fluvastatin	YES	YES		YES		YES
	Nadolol	YES					
	Valsartan		YES		YES		YES
	Methylphenidate		YES	YES	YES		YES
	Sulfa Drugs			YES		YES	
	Sulfasalazine					YES	
	Methylprednisolone	YES					
	Medroxyprogesterone	YES					
	Estramustine	YES					
	Miglitol	YES					

	Mefloquine	YES		YES			
	Capacitabine			YES			
	Danazol	YES					
	Eprosartan		YES		YES		YES
5	Divalproex		YES		YES		YES
	Fenofibrate		YES		YES		YES
	Gabapentin*		YES	YES	YES	YES	YES
	Omeprazole			YES			
	Lansoprazole			YES			
10	Megestrol	YES					
	Metformin					YES	
	Tazorotene		YES		YES		YES
	Sumitriptan			YES			
	Naratriptan			YES			
15	Zolmitriptan			YES			
	Aspirin		YES		YES		YES
	Olmesartan		YES		YES		YES
	Sirolimus	YES					
	Tacrolimus	YES					
20	Clopidogrel		YES		YES		YES
	Amphotericin B	YES	YES		YES		YES
	Tenofovir	YES					
	Unoprostone		YES		YES		YES
	Fulvestrant	YES					
25	Cefditoren		YES		YES		YES
	Efavirenz			YES			
	Eplerenone		YES		YES		YES
	Treprostinil	YES	YES		YES		YES
	Adefovir	YES					
30							

The prodrug of the present invention contains amino groups and as such are basic in nature. They are capable of forming a wide variety of pharmaceutically acceptable salts with various inorganic and organic acids. These acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmaceutically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitride, sulfate, bisulfate, phosphate, formate, acetate, citrate, tartate, lactate, and the like.

As indicated herein, in one embodiment, the present invention is directed to a prodrug wherein the prodrug comprises a drug, e.g., cyclosporine and an amino acid esterified to the MeBmt (x-y=CH=CH) or dihydro MeBmt moiety, (x-y=CH_2CH_2). The amino acid is attached to the cyclosporine and to the other other drugs by a covalent bond.

The compounds of the present invention are prepared by art recognized techniques. For examples, if the drug contains an OH group, said as cyclosporin, then an amino acid or an acylating derivatives thereof, such as the acid halide, e.g., amino acid fluoride, amino acid chloride, or an amino acid alkyl ester wherein alkyl group contains 1-6 carbon atoms is reacted with the carboxy group of the drug, e.g., cyclosporine under esterification condition. Preferably, the reaction is conducted in the presence of an acid, such as hydrochloric acid, hydrobromic acid, p-toluenesulfonic acid and the like. Alternatively, as described hereinabove, if the drug has an amino group thereon, then the amino acid may be reacted with the drug under amide forming conditions to form an amide as the covalent bond. Or if the drug has a carboxy group or acylating derivative thereon, it may be reacted with the amino group of the amino acid to form an amide under amide forming conditions to form an amide bond between the amino acid and the drug. Additionally if the drug has a carboxy group therein, the hydroxy group of the side chain of the amino acid may be reacted with the carboxy group or acylating derivative, therein under esterification conditions to form the ester linkage between the amino acid and the drug, as described hereinabove.

If the amino acid has a group thereon which is reactive under the reaction conditions it is protected by a protecting group known in the art. After the completion of the reaction, the protecting group is removed. Examples of protecting groups that could be used are described in the book entitled, "Protective Group in Organic Synthesis" by Theodora W. Greene, John Wiley & Sons, 1981, the contents of which are incorporated by reference.

For example, if amino acids with carboxylic groups in their side chains, for example, aspartic acid and glutamic acid, are used in the aforementioned synthesis, these will generally require protection of the side chain carboxylic acid. Suitable protecting groups can be esters, such as cyclohexyl esters, t-butyl esters, benzyl esters, allyl esters, 9-fluorophenyl-methyl groups or adamantyl groups, such as 1-or 2-adamantyl which can be protected after the esterfication reaction is completed using techniques known to one of ordinary skill in the art.

If amino acids with hydroxyl groups in their side chains, for example, serine, threonine, hydroxyproline, and the like and amino acids with phenolic groups in their side chains, for example, tyrosine, and the like are used in the aforementioned esterification, reaction, they will desirably require protection of the chain hydroxyl or phenolic group. Suitable for protecting groups for the hydroxyl side chain groups can be ethers, such as benzyl ether or t-butyl ether. Removal of the benzyl ether can be effected by liquid hydrogen fluoride, while the t-butyl ether can be removed by treatment with trifluoroacetic acid. Suitable protecting groups for phenolic side chain groups can be ethers, as above, including benzyl or t-butyl ether or 2,6-dichlorobenzyl, 2-bromobenzyloxycarbonyl, 2,4-dintrophenyl and the like.

Moreover, the products can be purified to be made substantially pure by techniques known to one of ordinary skill in the art, such as by chromatography, e.g., HPLC, crystallization and the like. By substantially "pure" it is meant that the product contains no more than about 10% impurity therein.

The prodrugs can be made into pharmaceutical compositions including prodrugs of, or pharmaceutical acceptable salts, pharmaceutical acceptable solvates, esters, enantiomers, diastereomers, N-Oxides, polymorphs, thereof, as described herein, along with a pharmaceutical acceptable carrier, and optionally but desirably pharmaceutically acceptable excipients using techniques known to one of ordinary skill in the art.

The prodrugs utilized in the present method are used in therapeutically effective amounts.

The physician will determine the dosage of the prodrugs of the present invention which will be most suitable and it will vary with the form of administration and the particular compound chosen, and furthermore, it will vary depending upon various factors, including but not limited to the patient under treatment and the age of the patient, the severity of the condition being treated and the like and the identify of the prodrug administered. He will generally wish to initiate treatment with small dosages substantially less than the optimum dose of the compound and increase the dosage by small increments until the optimum effect under the circumstances is reached. It will generally be found that when the composition is administered orally, larger quantities of the active agent will be required to produce the same effect as a smaller quantity given parenterally. The compounds are useful in the same manner as the corresponding drug in the non-prolong form and the dosage level is of the same order of magnitude as is generally employed with these other therapeutic agents. When given parenterally, the compounds are administered generally in dosages of, for example, about 0.001 to about 10,000 mg/kg/day, also depending upon the host and the severity of the condition being treated and the compound utilized.

In a preferred embodiment, the compounds utilized are orally administered in amounts ranging from about 0.01 mg to about 1000 mg per kilogram of body weight per day, depending upon the particular mammalian host or the disease to be treated, more preferably from about 0.1 to about 500 mg/kg body weight per day. This dosage

regimen may be adjusted by the physician to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The prodrug may be administered in any convenient manner, such as by oral, intravenous, intramuscular or subcutaneous routes.

The prodrug may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly into the food of the diet. For oral therapeutic administration, the prodrug may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% of the prodrug. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of the prodrug used in such therapeutic compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention contain between about 200 mg and about 4000 mg of prodrug. The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier.

Various other materials may be present as coatings or otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such

as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations. For example, sustained release dosage forms are contemplated wherein the active ingredient is bound to an ion exchange resin which, optionally, can be coated with a diffusion barrier coating to modify the release properties of the resin or wherein the prodrug of the present invention is associated with a sustained release polymer known in the art, such as hydroxypropylmethylcellulose and the like.

The prodrug may also be administered parenterally or intraperitoneally. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, e.g., PEG 100, PEG 200, PEG 300, PEG 400, and the like, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form is usually sterile and must be fluid to the extent that syringability exists. It must be stable under the conditions of manufacture and storage and usually must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and one or more liquid polyethylene glycol, e.g. as disclosed herein and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to

include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the prodrug in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders, the above solutions are vacuum dried or freeze-dried, as necessary.

The prodrug can also be applied topically, as e.g., through a patch using techniques known to one of ordinary skill in the art. The prodrug can be administered buccally by preparing a suitable formulation of the prodrug of the present invention and utilizing procedures well known to those skilled in the art. These formulations are prepared with suitable non-toxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of buccal dosage forms. Some of these ingredients can be found in Remington's Pharmaceutical Sciences, 17th edition, 1985, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact nature of the buccal dosage form desired, e.g., tablets, lozenges, gels, patches and the like. All of these buccal dosage forms are contemplated to be within the scope of the present invention and they are formulated in a conventional manner.

The formulation of the pharmaceutical compositions may be prepared using conventional methods using one or more physiologically and/or pharmaceutically acceptable carriers or excipients. Thus, the compounds and their pharmaceutically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, or rectal administration. For oral administration, the pharmaceutical compositions may take the

form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinized maize starch, polyvinylpyrrolidone, or hydroxypropylmethyl cellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc, or silica); disintegrants (for example, potato starch, or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulfate). The tablets may be coated by methods well known in the art.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups, or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives, such as suspending agents (for example, sorbitol syrup, corn syrup, cellulose derivatives or hydrogenated edible oils and fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active prodrug.

The prodrug of the present invention may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspension, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the prodrug may be in the powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The prodrugs of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the prodrug of the present invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the prodrugs may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions containing the prodrugs of the present invention may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredients. The pack may for example comprise metal or plastic foil, such as blister pack. The pack or dispenser device may be accompanied by instructions for administration.

In tablet form, it is desirable to include a lubricant which facilitates the process of manufacturing the dosage units; lubricants may also optimize erosion rate and drug flux. If a lubricant is present, it will be present on the order of 0.01 wt. % to about 2 wt. %, preferably about 0.01 wt. % to 0.5 wt, %, of the dosage unit. Suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, sodium stearylfumarate, talc, hydrogenated vegetable oils and polyethylene glycol. As will be appreciated by those skilled in the art, however, modulating the particle size of the components in the dosage unit and/or the density of the unit can provide a similar effect-- i.e., improved manufacturability and optimization of erosion rate and drug flux--without addition of a lubricant.

Other components may also optionally be incorporated into the dosage unit. Such additional optional components include, for example, one or more disintegrants, diluents, binders, enhancers, or the like. Examples of disintegrants that may be used include, but are not limited to, crosslinked polyvinylpyrrolidones, such as crospovidone (e.g., Polyplasdone® XL, which may be obtained from GAF), cross-linked carboxylic methylcelluloses, such as croscanmelose (e.g., Ac-di-sol®, which may be obtained from FMC), alginic acid, and sodium carboxymethyl starches (e.g., Explotab®, which may be obtained from Edward Medell Co., Inc.), agar bentonite and alginic acid. Suitable diluents are those which are generally useful in pharmaceutical formulations prepared using compression techniques, e.g., dicalcium phosphate dihydrate (e.g., Di-Tab®, which may be obtained from Stauffer), sugars that have been processed by crystallization with dextrin (e.g., co-crystallized sucrose and dextrin such as Di-Pak®, which may be obtained from Amstar), calcium phosphate, cellulose, kaolin, mannitol, sodium chloride, dry starch, powdered sugar and the like. Binders, if used, are those that enhance adhesion. Examples of such binders include, but are not limited to, starch, gelatin and sugars such as sucrose, dextrose, molasses, and lactose. Permeation enhancers may also be present in the novel dosage units in order to increase the rate at which the active agents pass through the buccal mucosa. Examples of permeation enhancers include, but are not limited to, dimethylsulfoxide ("DMSO"), dimethyl formamide ("DMF"), N,N-dimethylacetamide ("DMA"), decylmethylsulfoxide ("C_{10}MSO"), polyethylene glycol monolaurate ("PEGML"), glycerol monolaurate, lecithin, the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecylcyclazacycloheptan-2-one (available under the trademark Azone.RTM. from Nelson Research & Development Co., Irvine, Calif.), lower alkanols (e.g., ethanol), SEPA® (available from Macrochem Co., Lexington, Mass.), cholic acid, taurocholic acid, bile salt type enhancers, and surfactants such as Tergitol®, Nonoxynol-9® and TWEEN-80®.

Flavorings may be optionally included in the various pharmaceutical formulations. Any suitable flavoring may be used, e.g., mannitol, lactose or artificial sweeteners such as

aspartame. Coloring agents may be added, although again, such agents are not required. Examples of coloring agents include any of the water soluble FD&C dyes, mixtures of the same, or their corresponding lakes.

In addition, if desired, the present dosage units may be formulated with one or more preservatives or bacteriostatic agents, e.g., methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chloride, or the like.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents for pharmaceutical active substances well known in the art. Except insofar as any conventional media or agent is incompatible with the prodrug, their use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of prodrug calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The prodrug is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore described. A unit dosage, for example, contains the principal active compound in amounts ranging from about 10 mg e.g. in humans, or as low as 1 mg (for small animals) to about 2000 mg. If placed in solution, the concentration of the prodrug preferably ranges from about 10 mg/mL to about 250 mg/mL. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients. In the case of buccal administration, the prodrugs are preferably in the buccal unit dosage form present in an amount ranging from about 10 to about 50 mg.

The prodrugs of the present invention are effective in treating disease or conditions in which the corresponding drug (without the amino acid prodrug of the present invention) normally are used.

As used herein the term "treating" refers to reversing, alleviating or inhibiting the progress of a disease, disorder or condition, or one or more symptoms of such disease, disorder or condition, to which such term applies. As used herein, "treating" may also refer to decreasing the probability or incidence of the occurrence of a disease, disorder or condition in a mammal as compared to an untreated control population, or as compared to the same mammal prior to treatment. For example, as used herein, "treating" may refer to preventing a disease, disorder or condition, and may include delaying or preventing the onset of a disease, disorder or condition, or delaying or preventing the symptoms associated with a disease, disorder or condition. As used herein, "treating" may also refer to reducing the severity of a disease, disorder or condition or symptoms associated with such disease, disorder or condition prior to a mammal's affliction with the disease, disorder or condition. Such prevention or reduction of the severity of a disease, disorder or condition prior to affliction relates to the administration of the composition of the present invention, as described herein, to a subject that is not at the time of administration afflicted with the disease, disorder or condition. As used herein "treating" may also refer to preventing the recurrence of a disease, disorder or condition or of one or more symptoms associated with such disease, disorder or condition. The terms "treatment" and "therapeutically," as used herein, refer to the act of treating, as "treating" is defined above.

As used herein the term "patient" or "subject" refers to a warm blooded animal, and preferably mammals, such as, for example, cats, dogs, horses, cows, pigs, mice, rats and primates, including humans. The preferred patient is humans.

The prodrugs of the present invention exhibit the same utility as the corresponding drug without the amino acid linkage. The prodrug exhibits an enhanced therapeutic quality. That is, they exhibit at least one and more preferably at least two enhanced therapeutic qualities relative to the drug which has not been transformed to the prodrug of the present invention prior to administration. These include, but are not limited to

a. Improved taste, smell
b. Desired octanol/water partition coefficient (i.e., solubility in water/fat)

The various amino acids have different solubility in aqueous solutions. By selecting a particular amino acid, the octanol water partition coefficient can be affected. For example, many drugs in the following list are highly hydrophobic. The amino acids are highly hydrophilic. For example, assume propofol is the drug and lysine is the amino acid. Propofol is completely insoluble in water, while lysine is soluble to the extent of 700 mg/ml. When these two diverse molecules are esterified via an ester bond, the resulting lysine ester of propfol has a solubility in water in excess of 250 mg/ml.

On the other hand, cromolyn sodium is highly water soluble. For all practical purpose, it is not absorbed when administered orally. By affecting its water solubility one could improve absorption. In this case, one would look for conditions opposite to that of propofol, i.e., the goal is to decrease water solubility. By choosing apporoprieate low water soluble amino acids, such as tyrosine, one can achieve proper hydrophilic/lipophilic balance.

c. Improved stability in-vitro and in-vivo
d. Enhanced penetration of blood-brain barrier
e. Elimination of first-pass effect in liver, i.e., the drug not metabolized in liver and therefore more drug in system circulation
f. Reduction of entero-hepatic recirculation (this improves bio-availability)
g. Painless injections with parenteral formulations
h. Improved bio-availability
i. Improved changes in the rate of absorption (increase vs lack thereof)

j. Reduced side effects
k. Dose proportionality

A dose proportionality claim requires that when the drug is administered in escalating doses, proportionally escalating amounts of active drug is delivered into the blood stream. This is measured by determining the area under the plasma concentration vs. time curve obtained after administering a drug via any route other than IV route and measuring the same in plasma/blood. A simple mathematical procedure is as follows: For example, a drug is administered at e.g., 3 different doses, 10, 100 and 1000 mg, orally to a patient, the area under the plasma concentration time curve (AUC) is measured. Then each total AUC is divided by the dose, and the result should be the same for all three doses. If it is the case, then there is dose proportionality. Lack of dose proportionality indicates any one or more of the pharmacokinetic/pharmacological mechanisms are saturable, including absorption, metabolism or the number of receptor sites available for pharmacological response.

For example in the above study, assume the AUC values of 100, 1000 and 10,000 are obtained, in this case the dose proportionality is inappropriate. When there is lack of dose proportionality, there is either more or less amount of drug in the plasma, depending upon which mechanism is saturable. The following are the possibilities: Saturable Absorption. If this is the case, as the dose is increased, proportionally less and less of the drug is absorbed, hence overall AUC will decrease as the dose is increased.

Saturable metabolism of elimination. If thus is the case, then more and more of the drug will be circulating in the blood, and the AUC will increase with increasing dose.

Saturable pharmacological receptor sites: In this case, since all the receptor sites will eventually be occupied by the drug, any additional drug will not increase the response. Thus, increasing dose will not result in increasing response.

Dose proportionality is an excellent response profile, since one can predict accurately the pharmacological response and curative power at all doses. Thus dose proportionality is a desirable quality for any drug. Furthermore, achievement of dose proportionality is also dependent upon the formulation, and fed/fasted differences.

l. Selective hydrolysis of the prodrug at site of action
m. Controlled release properties
n. Targeted drug delivery
o. Reduction in toxicity, hence, improved therapeutic ratio
p. Reduced dose
q. Alteration of metabolic pathway to deliver more drug at the site of action
r. Increased solubility in aqueous solution
s. Enhanced efficacy

Thus, various dosage forms available with amino acid pro-drugs and they are prepared by conventional methods:

i. Oral liquid dosage (Controlled release and immediate release liquids containing sugar and sugar free, dye and dye free, alcohol and alcohol free formulations, including chewable tablets)
ii. Oral solid dosage (Controlled release and immediate release tablets, capsules and caplets
iii. Intravenous (Injections, both ready to use and lyophilized powders)
iv. Intramuscular (Injections, both ready to use and lyophilized powders)
v. Subcutaneous (Injections, both ready to use and lyophilized powders)
vi. Transdermal (Mainly patches)
vii. Nasal (Sprays, formulations for nebulizer treatments)
viii. Topical (Creams, ointments)
ix. Rectal (Creams, ointments and suppositories)
x. Vaginal (Creams, ointments and pessaries)
xi. Ocular (Drops and ointments)

xii. Buccal (Chewable and now chewable tables)

Many drugs discussed herein, especially in the table hereinbelow are characteristically highly hydrophobic and readily precipitate in the presence of even very minor amounts of water, e.g., on contact with the body (e.g., stomach fluids). It is accordingly extremely difficult to provide e.g., oral formulations which are acceptable to the patient in terms of form and taste, which are stable on storage and which can be administered on a regular basis to provide suitable and controlling patient dosing.

Proposed liquid formulations, e.g., for oral administration of a number of drugs shown herein in the table have heretofore been based primarily on the use of ethanol and oils or similar excipient as carrier media. Thus, the commercially available drink-solutions of a number of drugs employ ethanol and olive oil or corn oil as carrier medium in conjunction with solvent systems comprising e.g., ethanol and LABRIFIL and equivalent excipient as carrier media. For example, the commercially available Cyclosporin drink solution employs ethanol and olive oil or corn oil as carrier medium in conjunctions with a Labroid as a surfactant. See e.g., U.S. Patent NO. 4,388,307. Use of the drink solution and similar composition as proposed in the art is however accompanied by a variety of difficulties.

Further, the palatability of the known oil based system has proved problematic. The taste of the known drink-solution of several drugs is, in particular, unpleasant. Admixture with an appropriate flavored drink, for example, chocolate drink preparation, at high dilution immediately prior to ingestion has generally been practiced in order to make regular therapy at all acceptable. Adoption of oil-based systems has also required the use of high ethanol concentrations which is itself inherently undesirable, in particular where administration to children is foreseen. In addition, evaporation of the ethanol, e.g., from capsules (adopted in large part, to meet problems of palatability, as discussed or other forms (e.g., when opened)) results in the development of a drug precipitate. Where such compositions are presented in, for example, soft gelatin encapsulated form,

this particular difficulty necessitates packaging of the encapsulated product in an air-tight component, for example, an air-tight blister or aluminum-foil blister package. This in turn renders the product both bulky and more expensive to produce. The storage characteristics of the aforesaid formulations are, in addition, far from ideal.

Bioavailability levels achieved using existing oral dosage system for a number of drugs described herein are also low and exhibit wide variation between individuals, individual patient types and even for single individuals at different times during the course of therapy. Reports in the literature indicates that currently available therapy employing the commercially available drug drink solution provides an average absolute bioavailability of approximately 10-30% only, with the marked variation between individual groups, e.g., between liver (relatively low bioavailability) and bone-marrow (relatively high bioavailability) transplant recipients. Reported variation in bioavailability between subjects has varied from one or a few percent for some patients, to as much as 90% or more for others. And as already noted, marked change in bioavailability for individuals with time is frequently observed. Thus, there is a need for a more uniform and high bioavailability of a number drugs shown herein in patients.

Use of such dosage forms is also characterized by extreme variation in required patient dosing. To achieve effective therapy, drug blood or blood serum levels have to be maintained within a specified range. This required range can in turn, vary, depending on the particular condition being treated, e.g., whether therapy is to prevent one or more pharmacological actions of a specific drug and when alternative therapy is employed concomitantly with principal therapy. Because of the wide variations in bioavailability levels achieved with conventional dosage forms, daily dosages needed to achieve required blood serum levels will also vary considerably from individual to individual and even for a single individual. For this reason it may be necessary to monitor blood/blood-serum levels of patients receiving drug therapy at regular and frequent intervals. Monitoring of blood/blood-serum levels has to be carried out on a regular

basis. This is inevitably time consuming and inconvenient and adds substantially to the overall cost of therapy.

It is also the case that blood/blood serum levels of a number of drugs described herein achieved using available dosage systems exhibit extreme variation between peak and trough levels. That is for each patient, effective drug levels in the blood vary widely between administrations of individual dosages.

There is also a need for providing a number of drugs described herein, especially the beta-lactum antibiotics, Cyclosporin, cephalosporins, steroids, quinolone antibiotics and Cyclosporin, in a water-soluble form for injection. It is well known that Cremaphore L® (CreL) used in current formulations of a number of drugs described hereinbelow is a polyoxyethylated derivative of castor oil and is a toxic vehicle. There have been a number of incidences of anaphylaxis due to the castor oil component. At present there is no formulation that would allow many of these drugs to be in aqueous solution at the concentrations needed due to poor water solubility of the drug.

Beyond all these very evident practical difficulties lies the occurrence of undesirable side reactions already alluded to, observed employing available oral dosage forms.

Several proposals to meet these various problems have been suggested in the art, including both solid and liquid oral dosage forms. An overriding difficulty which has however remained is the inherent insolubility of the several of the drugs shown in the table hereinbelow in aqueous media, hence preventing the use of a dosage form which can contain the drugs in sufficiently high concentration to permit convenient use and yet meet the required criteria in terms of bioavailability, e.g. enabling effective absorption from the stomach or gut lumen and achievement of consistent and appropriately high blood/blood-serum levels.

The particular difficulties encountered in relation to oral dosing with these drugs have inevitably led to restrictions in the use of specific drug therapy for the treatment of relatively less severe or endangering disease conditions. For example, taking Cyclosporin as a test drug, a particular area of difficulty in this respect has been the adoption of Cyclosporin therapy in the treatment of autoimmune diseases and other conditions affecting the skin, for example for the treatment of atopic dermatitis and psoriasis and, as also widely proposed in the art, for hair growth stimulation, e.g. in the treatment of alopecia due to ageing or disease.

Thus while oral Cyclosporin therapy has shown that the drug is of considerable potential benefit to patients suffering e.g. from psoriasis, the risk of side-reaction following oral therapy has prevented common use. Various proposals have been made in the art for application of Cyclosporins, e.g. Cyclosporin, in topical form and a number of topical delivery systems have been described. Attempts at topical application have however failed to provide any demonstrably effective therapy.

However, the present invention overcomes the problems described hereinabove. More specifically, the prodrug of the present invention significantly enhances its solubility in aqueous solutions relative to the non-prodrug form of the pharmaceutical, thereby avoiding the need to utilize a carrier, such as ethanol or castor oil when administered as a solution. Moreover, the prodrugs of these drugs, in accordance with the present invention, do not exhibit the side effects of the prior art formulations. Further, it has been found that when many of the drugs in the table hereinbelow is administered in its prodrug form in accordance with the present invention, there is enhanced oral absorption, thereby enhancing significantly its bioavailability and its efficacy.

The preferred drugs used in combination with the amino acids are forming prodrugs are listed hereinbelow in the following table and the benefits found are as listed in the penultimate column of the table. In the table, the key is as follows:

a) Improved taste smell

b) Desired Octanol/water partition coefficient (i.e. solubility in water)

c) Improved stability in vitro and in vivo

d) Penetration of blood-brain barrier

e) Elimination of first pass effect in liver

f) Reduction of enterohepatic recirculation

g) Painless injections with parenteral formulations

h) Improved bioavailability

i) Increased rate of absorption

j) Reduced side effects

k) Dose proportionability

l) Selective hydrolysis of the prodrug at site of actions

m) Controlled release properties

n) Targeted drug delivery

o) Reduction in toxicity, hence improved therapeutic ratio

p) Reduced dose

q) Alteration of metabolic pathway to deliver more drug at site of action.

Moreover, the table indicates the utility of the prodrug. The utility of the prodrug is the same as the corresponding drug (without the amino acid moiety attached). The utiluity is described in the literature such as the Physicians Desk Reference, 2004 edition, the contenets of which are incorporated by reference.

Amino Acid Prodrugs of	Applicable Dose Range	Preferred Dose Range	Most Preferred Dose Range	Amino Acids that can be reacted with the drug to form the ster/amide/azo/anhydride prodrugs	Preferred Amino Acids	Most Preferred Amino Acids	Improvements with prodrugs utility immuno	Utility
Cyclosporin Preferred Forms Oral Tab/Cap Oral Liquid IV Injections	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar or dipeptide of combination of any two amino acids especially AA-Gly, where Gly is a spacer attached to cyclosporin and AA is the above-cited amino acids.	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar or dipeptides of combination of any two amino acids especially AA-Gly, where Gly is a spacer attached to cyclosporin and AA is the above-cited amino acids.	Lys, Pro, & Gly and dipeptides of Lys-Gly, Pro-Gly, Gly-Gly	b,e, f, g, h, k, l, o, and p	prophylaxis of organ rejection, e.g., kidney, liver and heart allogenic transplants, treatment of rheumatoid arthritis and psoriosis
	5-1000 mg 1-25 mg/ml 10-250 mg per 5 ml	20-250 mg 5-15 mg/ml 25-100 mg per 5 ml	25-100 mg 10 mg/ml 50 mg/5ml					
Lopinavir Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Pro, Gly, & Ala	b, h, j, k, and o	treatment of HIV infections, e.g., AIDS
	0.1-1 g m 0.1-1 gm/5 ml	200-800 mg 0.2-0.8 g/5 ml	400-500 mg 400 mg/5ml					

Cefdinir Preferred Forms Oral Tab/Cap Oral Liquid IV Infusions	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Tyr, & Thr	a, b, e, f, h, i, o, and p	antibiotic treatment of diseases caused by Haemophilus influenzae, including B-lactamase producing strains, e.g., Haemophilus parainfluenzae (including ∃-lactosamase producing strains) and moraxella catarihalis (including ∃-lactamase producing strains), and streptococcus pyogenes; such as pneumonia, bronchitis and sinusitis, pharyngitis and tonsillitis
	0.1-1gm 0.1-1 gm/5ml 0.01-1 gm/100 ml	0.2-0.5 gm 0.2-0.5gm/5ml 20-500 mg/100 ml	200-400 mg 0.2-0.4gm/5ml 50-150 mg/100 ml					
Zileuton Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Sar, Ala, Pro	b, h, i, j, k, o, p	treatment of asthma
	200-1200 mg 200-1200 mg/5ml	200-800 mg 200-800 mg/5ml	300-400 mg 200-400 mg/5ml					
Nelfinavir Preferred forms Oral Tab/Cap Oral Powder IV Formulation	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Sar, Ala, Pro	b, h, i, j, k, o, p	treatment of HIV, infected patients, e.g., AIDS
	0.05-1 gm 10-250 mg/gm 10-250 mg/100 ml	0.1-0.5 gm 20-200 mg/gm 20-200 mg/100ml	0.2-0.4 gm 40-100 mg/gm 40-100 mg/100ml					

Flavoxate Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Tyr, & Thr	b, h, i, j, k, l, o,& p	treatment of urinary spasms
	10-1000 mg 10-1000 mg/5ml	20-500 mg 20-500 mg/5ml	50-250 mg 50-250 mg/5ml					
Candesarten Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Tyr, & Thr	b, c, e, f, h, i, j, k, l, o, p, q	treatment of hypertension
	1-100 mg 1-100 mg/5ml	2-75 mg 2-75 mg/5ml	4-50 4-50 mg/5ml					
Propofol Preferred Forms IV Infusions	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Sar, Pro, Ala, & Val	b, c, d, g, h, j, k, l, m, n, o, p, q	provides central nervous system anethesia
	1-25 mg/ml	2.0-20 mg/ml	5-15 mg/ml					
Nisoldipine Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Ser, & Hyp	b, e, h, i, j, o	calcium channel blocker, treatment of hypertension
	2-100 mg 2-100 mg/5ml	2.5-75 mg 2.5-75 mg/5ml	5-50 mg 5-50 mg/5ml					

Amlodipine Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base IV			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Ser, & Hyp	b, e, h, i, j, o	calcium channel blocker, treatment of hypertension
	0.1 – 20 mg 0.1-20 mg/5ml	1-10 mg 1-10 mg/5ml	2.5-5 mg 2.5-5 mg/5ml					
Ciprofloxacin Preferred Forms Oral Tab/Cap Oral Liquid IV Bulk (Sterile)	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	a, b, c, g, h, i, j, k, o, p	Antibiotic; inhibits various bacteria, e.g., pseudomonas aeruginosa, staphylococcus aureus or proteus mirabilis; treatment of corneal ulcers, conjunctivitis, acute otitis externa,
	0.1-1.5 gm 0.05-1gm/5ml 2-25 mg/ml	0.1-1.0 gm 0.08-1 gm/5ml 3-20 mg/ml	0.2-0.8 gm 0.12-1 g/5ml 5-15 mg/ml					
Ramipril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	j, o	treatment of hypertension
	0.1-20 mg 0.1-20 mg/5ml	0.5-12 mg 0.5-12 mg/5ml	1-10 mg 1-10 mg/5ml					
Trandolapril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly & Lys	j, o	treatment of hypertension
	0.1-10 mg 0.1-10 mg/5ml	0.5-7.5 mg 0.5-7.5 mg/5ml	1-4 mg 1-4 mg/5ml					

Fosinopril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	j, o	treatment of hypertension
	1-100 mg 1-100 mg/5ml	2-75 mg 2-75 mg/5ml	5-50 mg 5-50 mg/5ml					
Enalapril Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly & Lys	j, o	treatment of hypertension
	0.5-100 mg 0.5-100 mg/5ml	1-50 mg 1-50 mg/5ml	2-25 mg 2-25 mg/5ml					
Benazepril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	j, o	treatment of hypertension
	1-100 mg 1-100 mg/5ml	2-75 mg 2-75 mg/5ml	2.5-50 mg 2.5-50 mg/5ml					
Perindopril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	j, o	treatment of hypertension
	0.1-20 mg 0.1-20 mg/5ml	0.5-15 mg 0.5-15 mg/5ml	1-10 mg 1-10 mg/5ml					

Moexipril Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base 1-30 mg 1-30 mg/5ml	2-20 mg 2-20 mg/5ml	5-15 mg 5-15 mg/5ml	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly & Lys	j, o	treatment of hypertension
Cromolyn Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base 10-200 mg 10-200 mg/5ml	20-100 mg 20-100 mg/5ml	20-50 mg 20-50 mg/5ml	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, & Pro	b, c, h, i, j, k, l, n, o, p, q	inhibits release of histamine and leukotrienes from mast cell; treatment of mastocytosis, asthma
Amoxicillin Preferred forms Oral Tab/Cap* Oral Liquid Oral Powder (*also chewable)	All Doses expressed as drug base 0.1-1.5 gm 0.1-1.5 gm/5ml 0.1-0.75 gm	0.2-1.2 gm 0.2-1.2 gm/5ml 0.1-0.6 gm	0.25-1 gm 0.25-1 gm/5ml 0.125-0.5 gm	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly & Lys	a, b, c, h, i, j, k, l, o, p	antibiotic effective against ∃-lactamase negative strains causing infections of ear, nose, throat, e.g., streptococcus, staphylococcus or H influenzae;treatment of infections of genitourinary tract due to E. coli, P. mirabilis, E. faecalis, infections of skin due to streptococcus, staphylococcus or E. coli, infections of lower respiratory tract due to streptoccus, staphyloccus or H. influenzae, and gonorrhea
Cefuroxime Preferred Forms	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro,	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala,	Hyp, Ser, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, o,	antibiotic; treatment of pharyngitis/tonsillitis caused

Oral Tab/Cap Oral Liquid	10-1000 mg 10-1000 mg/5ml	50-750 mg 50-750 mg/5ml	100-600 mg 100-600 mg/5ml	His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar		p	by streptococcus, acute bacterialotitis media caused by streptococcus, H influenzae, moraxella catarihalis or streptococcus, urinary tract infections caused by E. coli or Klebsiella pneumonia, gonorrhea, skin infections cause by staphylococcus or straptococcus

Ceftazidime Preferred Forms Powder for IV Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	a, b, c, g, h, i, j, k, l, o, p, q	antibiotic, treatmtent of lower respiratory tract infections, including pneumonia caused by pseudomonas, H. influenzae, Klebsiella, Enterbacter, E. coli, proteus mirabilis, streptococcus, staphylococcus; skin and skin structure infections, caused by pseudomonas aeruginosa, Klebsiella, E. coli, Proteus enterbacter, staphylococcus, streptococcus, urinary tract infections casued by pseudomonas aeruginosa, enterbacter, proteus, Klebsiella, E. coli; bone and joint infections caused by pseudomonas, eruginosa, Klebsiella, Enterbacter, or staphylococcus; gynecologic infections including endometritis, pelvic cellulits and infections of the female genital tract caused by E. coli, intra-abdominal infections and central nervous system infections, including meningitis
	0.1-5 gm 0.1-1 gm 0.1-2.5 gm/5ml	0.25-4 gm 0.25-1 gm 0.25-2 gm/5ml	0.5-2 gm 0.5-1 gm 0.5-1 gm/5ml					

Cefpodoxime Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly & Lys	a, b, c, g, h, i, j, k, l, o, p, q	antibiotic especially against streptococcus, H. influenzae, moraxella catarrhalis; treatment of acute otis media, pharyngitis, tonsillitis, pneumonia, bronchitis, gonorrhea, and rectal infections in women
	10-500 mg 10-500 mg/5ml	25-350 mg 25-350 mg/5ml	50-250 mg 50-250 mg/5ml					
Atovaquone Preferred Forms Oral Tab/Cap Oral Liquid For Pediatric Use	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Ala, Pro & Ser	a, b, h, i, j, k, o, p	treatment of malaria caused by plasmodium parasite
	50-1000 mg above/5 ml 10-150 mg/5ml	100-500 mg above/5 ml 25-100 mg/5ml	200-300 mg above/5 ml 50-75 mg/5ml					
Acyclovir Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Sar, Hyp Pro & Ser	b, c, h, i, j, k, o, p	treatment of human cytomegalovirus (HCMV)
	50-1000 mg 50-1000 mg/5ml	100-750 ml 100-750 mg/5ml	150-500 mg 150-500 mg/5ml					

Gancyclovir Preferred Forms Oral Tab/Cap Oral Liquid IV Infusions	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, o, p	treatment of human cytomegalo virus (HCMV)
	0.1-1 gm 0.1-1gm/5ml 10-200 mg/ml	0.2-0.8 gm 0.2-0.8mg/5ml 25-100 mg/ml	0.2-0.6 gm 0.2-0.6mg/5 ml 30-60 mg/ml					
Penciclovir Preferred Forms Powder for IV Topical Cream Oral Cap/Tab	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, o, p	treatment of human cytomegalovirus (HCMV)
	10-1000mg/ml 0.1-5% 10-500 mg	25-750 mg/ml 0.25-3% 20-300 mg	50-500 mg/ml 0.5-2.5% 25-250 mg					
Niacin ER Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Tyr, Gly & Lys	a, b, h, i, j, l, m, n, o, p, q	lipid management, e.g. reducing lipid concentration
	0.2-2 gm 0.2-2 gm/5ml	0.25-1.5 gm 0.25-1 gm/5ml	0.5-1 gm 0.5-1 gm/5ml					
Bexarotene Preferred Forms Oral Tab/Cap Oral Liquid Topical Gel	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	b, c, h, i, j, k, l, o, p	treatment of skin conditions, especially those requiring activation of retinoid X receptors
	10-500 mg above/5ml 0.1-5%	25-250 mg above/5m 0.25-2.5%l	50-100 mg above/5 ml 0.5-1.5%					

Propoxyphene Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	a, b, c, h, i, j, k, l, o, p	treatment of pain
	20-400 mg 20-400 mg/5ml	25-250 mg 25-250 mg/5ml	30-150 mg 30-150 mg/5ml					
Salsalate Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Gly, & Lys	b, c, h, i, j, k, o, p	treatment of inflammatory conditions
	0.2-2 gm 0.2-2 gm/5ml	0.25-1.5 gm 0.25-1.5 gm/5ml	0.3-1 gm 0.3-1 gm/5ml					
Acetaminophen Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Sar, Gly, & Lys	a, b, c, e, h, i, j, k, o, p	treatment of pain or fever
	20-1000 mg 20-1000 mg/5ml	50-800 mg 50-800 mg/5ml	100-600 mg 100-600 mg/5ml					
Ibuprofen Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Tyr, Gly & Lys	a, b, h, i, j, l, m, n, o, p, q	treatment of pain, fever or inflammation
	20-1000 mg 20-1000 mg/5ml	50-800 mg 50-800 mg/5ml	100-600 mg 100-600 mg/5ml					

Lovastatin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, l, o, p	lowers choloesterol concentration; inhibits HMG-CoA reductase
	1-100 mg 1-100 mg/5ml	2-80 mg 2-80 mg/5ml	5-50 mg 5-50 mg/5ml					
Simavastatin Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, l, o, p	lowers choloesterol concentration; inhibits HMG-CoA reductase
	1-200 mg 1-200 mg/5ml	2-150 mg 2-150 mg/5ml	2.5-100 mg 2.5-100 mg/5ml					
Atorvastatin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, l, o, p	lowers choloesterol concentration; inhibits HMG-CoA reductase
	1-250 mg 1-250 mg/5ml	2-125 mg 2-125 mg/5ml	5-100 mg 5-100 mg/5ml					
Pravastatin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, & Lys	b, c, e, f, h, i, j, k, l, o, p	lowers choloesterol concentration; inhibits HMG-CoA reductase
	1-250 mg 1-250 mg/5ml	2-125 mg 2-125 mg/5ml	5-75 mg 5-75 mg/5ml					

Fluvastatin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Tyr, Gly & Lys	b, c, e, f, h, i, j, k, l, o, p	lowers choloesterol concentration; inhibits HMG-CoA reductase
	1-250 mg 1-250 mg/5ml	2-125 mg 2-125 mg/5ml	5-75 mg 5-75 mg/5ml					
Nadolol Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Ser, & Pro	b, h, i, j, k, l, o, p	treatment of angina pectoris and hypertension; ∃-adrenergic receptor antagonist
	1-250 mg 1-250 mg/5ml	5-225 mg 5-225 mg/5ml	10-200 mg 10-200 mg/5ml					
Valsartan Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Ser, Thr, Lys, Gly & Sar	b, f, i, j, k, l, o, p	treating hypertension, angiotension II antagonist
	10-500 mg 10-500 mg/5ml	25-250 mg 25-250 mg/5ml	50-200 mg 50-200 mg/5ml					
Methyl phenidate Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Hyp, Sar, & Ser	a, b, c, h, j, k, l, o, p	treatment of attention deficit disorders and narcolepsy
	1-50 mg 1-50 mg/5ml	2-40 mg 2-40 mg/5ml	2.5-25 mg 2.5-25 mg/5ml					

Trovafloxacin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Ser, Pro, Hyp & Thr	a, b, e, h, j, k, o, p	antibiotic; inhibits bacteria such as E. coli, pseudomonas, aeruginosa, H. influenzae, streptococous, Klebsiella, staphylococcus, mycoplasma pneumoniae, peptostreptococcus, prevotella; treatment of pneumonia, postsurgical infections; gynecolgic and pelvic infections, such as endomyometritis, parametritis, septic abortions,and post-partum infections; skin infections, e.g., diabetic foot infections
	10-500 mg 10-500 mg/5ml	50-300 mg 50-300 mg/5ml	80-250 mg 80-250 mg/5ml					
5-AS* Preferred Forms Oral Tab/Cap Oral Liquid (*5-Amino-Salicylic acid)	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Glu, Gly, Tyr & Lys	b, c, i, j, l, m, n, o, p, q	treatment of tuberculosis
	1-200 mg 1-200 mg/5ml	5-150 mg 5-150 mg/5ml	10-125 mg 10-125 mg/5ml					

Methyl prednisolone Preferred Forms IM Injection Topical Cream	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Pro, Sar & Ser	b, c, g, j, l, m, n, o, p, q	treatment of inflammation especially from infections, tissue damage, allergy and auto-immune disease
	2-200 mg/ml 0.001-5%	5-150 mg/ml 0.01-2.5%	10-100 mg/ml 0.1-2%					
Medroxy Progesterone Preferred forms IM Injections	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Pro, Sar & Ser	b, c, g, j, l, m, n, o, p, q	providing contraception
	1 mg – 4 gm/ml	10 mg – 2 gm/ml	40 mg – 1 gm/ml					
Estramustine Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Pro, Ala, Sar & Val	b, c, h, i, j, k, l, o, p	treatment of cancer especially treatment of metastatic or progressive carcinoma of prostate
	10-500 mg 10-500 mg/5ml	25-250 mg 25-250 mg/5ml	50-200 mg 50-200 mg/5ml					
Miglitol Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Pro, & Ser	b, c, i, j, n, q	treatment of type II diabetes
	1-250 mg 1-250 mg/5ml	2-150 mg 2-150 mg/5ml	10-125 mg 10-125 mg/5ml					

Mefloquine Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Pro, Val, Ala	a, b, c, h, i, j, k, l, o, p, q	treatment of malaria
	10-500 mg 10-500 mg/5ml	100-400 mg 25-400 mg/5ml	150-300 mg 150-300 mg/5ml					
Danazol Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Hyp, Pro, Ala, Val, Ser & Thr	a, b, c, e, f, g, h, i, j, k, l, n, o, p, q	treatment of endometriosis and fibrostatic breast disease
	2-500 mg 2-500 mg/5ml	10-350 mg 10-350 mg/5ml	25-250 mg 25-250 mg/5ml					
Eprosartan Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly & Val	b, c, h, i, j, k, l, o, p	ACE inhibitors, treatment of hypertension
	0.1-1 gm 0.1-1 gm/5ml	200-800 mg 200-800 mg/5ml	300-750 mg 300-750 mg/5ml					
Divalproex Na Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly, & Val	a, b, c, f, h, i, j, k, l, o, p	treatment of epilepsy
	50-800 mg 50-800 mg/5ml	75-750 mg 75-750 mg/5ml	100-600 mg 100-60 mg/5ml					

Fenofibrate Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly, & Ala	b, c, h, i, j, k, l, o, p, q	treatment of hypercholestemia
	10-800 mg 10-800 mg/5ml	20-750 mg 20-750 mg/5ml	100-600 mg 100-600 mg/5ml					
Gabapentin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Cyclic Deriv. & Tyr	b, c, d, e, f, h, i, j, k, l, n, o, p, p	treatment of convulsions
	10-800 mg 10-800 mg/5ml	25-750 mg 25-750 mg/5ml	50-500 mg 50-500 mg/5ml					
Lansoprazole Preferred forms Oral Tab/Cap Oral Liquid	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Pro, Sar, Ser & Val	b, e, f, h, i, j, k, l, o, p	suppression of gastric acid secretion by inhibition of (H+, K+) ATP-ase enzyme system at the secretory surface of the gastric parietal cell; treatment of gastric hyperacidity
	1-60 mg 1-6- mg/5ml	2-50 mg 2-50 mg/5ml	10-40 mg 10-40 mg/5ml					
Omeprazole Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Val, Pro & Sar	b, e, f, h, i, j, k, l, o, p	suppression of gastric acid secretion by inhibition of (H+, K+) ATP-ase enzyme system at the secretory surface of the gastric parietal cell, treatment of gastric hyperacidity
	1-200 mg 1-200 mg/5ml	2-100 mg 2-100 mg/5ml	5-60 mg 5-60 mg/5ml					

Megestrol Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Sar, Pro, Ser & Ala	b, c, h, i, j, k, l, n, o, p	treatment of anorexia; improving appetite in anorexic and patients suffering from AIDS
	2-100 mg 2-100 mg/5ml	4-80 mg 4-80 mg/5ml	20-60 mg 20-60 mg/5ml					
Metformin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Asp, Glu, Lys & Azo dimer	o, p	treatment of hyperglycemia; aids insulin to improve transport of glucose into cells
	0.2 – 3 gm 0.2-1 gm/5ml	0.25-1.5 gm 0,25-1.5 mg/5ml	0.5-1 gm 0.5-1 gm/5ml					
Tazarotene Preferred Forms Topical Gel	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, & Gly	B, c, h, I, j, k, l, o, p	treatment of psoriasis and acne especially those caused by pathogenic microorganisms, allergy and inflammation
	0.01-0.3%	0.02-0.25%	0.025-0.125%					
Sumatriptan Preferred Forms Oral Tab/Cap Oral Liquid IM Injections	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Ala, Pro, Sar & Val	b, c, d, g, h, i, j, k, l, n, o, p, q	5 HT subtype receptor agonist, treatment of migraine headaches
	5-250 mg 5-250 mg/5ml 1-36 mg/ml	10-200 mg 10-200 mg/5ml 2-24 mg/ml	20-125 mg 20-125 mg/5ml 4-20 mg/ml					

Naratriptan Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Val, Ala, & Pro	b, h, i, j, k, l, o, p	5 HT subtype receptor agonist; treatment of migraine headaches
	0.1-10 mg 0.1-10 mg/5ml	0.25-5 mg 0.25-5 mg/5ml	0.5-4 mg 0.5-4 mg/5ml					
Zolmitriptan Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Sar, Val, Ala, & Pro	b, h, i, j, k, l, o, p	5 HT subtype receptor agonist;treatment of migraine headaches
	0.1-12 mg 1-12 mg/5ml	0.5-10 mg 0.5-10 mg/5ml	1-7.5 mg 1-7.5 mg/5ml					
Aspirin Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly, & Ala	a, b, c, e, f, g, h, j, k, l, m, n, o, p, q	antipyretic, anti-inflammatory, analgesic, thrombolytic; treatment of hyperthermia, myocardial infarction and thrombolysis
	10-1000 mg 10-1000 mg/ml	20-800 mg 20-800 mg/ml	25-600 mg 25-600 mg/ml					
Olmesartan Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly, & Ala	b, h, i, j,k, l, o, p	ACE inhibitor, treatment of hypertension
	1-100 mg 1-100 mg/5ml	2-80 mg 2-80 mg/5ml	4-50 mg 4-50 mg/5ml					

Sirolimus Preferred Forms Oral Tab/Cap Oral Liquid IM Injections	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly, & Ala	b, h, i, j,k, l, o, p	immunosuppressant in surgical human patients with transplants; antibiotic; treating vitiligo psoriass, acne
	0.1-20 mg 0.1-20 mg/5ml	0.5-10 mg 0.5-10 mg/5ml	1-8 mg 1-8 mg/5ml					
Tacrolimus Preferred Forms Oral Tab/Cap Oral Liquid IV Infusions	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Ala, Thr, Sar & Pro	b, c, g, h, i, j, k, l, o, p	immunosuppressant in surgical human patients with transplants; antibiotic; treating vitiligo psoriass, acne
	0.1-20 mg above/5ml 1-20 mg/ml	0.2-15 mg above/5ml 2-15 mg/ml	0.25-10 mg above/5ml 2.5-8 mg/ml					
Pimecrolimus Preferred Forms Oral Tab/Cap Oral Liquid Ointment Cream	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Ala, Thr, Sar & Pro	b, c, g, h, i, j, k, l, o, p	immunosuppressant in surgical human patients with transplants; antibiotic treating vitiligo psoriass, acne
	0.1-20 mg above/5ml 0.01 – 10%	0.2-15 mg above/5ml 0.1-5%	0.25-10 mg above/5ml 0.5-2%					
Clopidogrel Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			20-125 mg 20-125 mg/5ml	25-100 mg 25-100 mg/5ml	Ser, Hyp, Thr, Lys, Ala, & Gly	b, c, h, i, j, k, l, m, o, p, q	treatment of myocardial infections
	10-250 mg 10-250 mg/5ml	20-125 mg 20-125 mg/5ml	25-100 mg 25-100 mg/5ml					

Amphotericin B Preferred Forms IV Infusion Topical Cream	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Ala, & Gly	b, c, g, i, j, l, m, n, o, p, q	treatment of fungus, expecially those acting on cell membrane changing its permeability
	0.5-20 mg/kg/day 0.01-10%	1-15 mg/kg/day 0.1-5%	2-10 mg/kg/day 0.5-2%					
Tenofovir Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Ala, Pro, Ser & Sar	b, c, h, i, j, k, l, o, p	inhibitor of HIV virus, treatment of AIDS infections
	10-900 mg 10-900 mg/5ml	50-750 mg 50-750 mg/5ml	100-500 mg 100-500 mg/5ml					
Unoprostone Preferred Forms Ocular Drops	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Tyr, Pro, & Lys	b, c, h, i, j, k, l, n, o, p, q	treatment of glaucoma, especially caused by age; lowers intraocular pressure
	0.01-1%	0.05-0.5%	0.01-0.25%					
Fulvestrant Preferred Forms IM Injection	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Pro, Ala, Val & Sar	B, c, g, j, l, o, p	treating cancer, especially breast cancer
	2-1250 mg/5ml	10-1000 mg/5ml	20-500 mg/5ml					

Cefditoren Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Gly, Lys & Ala	b, c, h, i, j, k, l, o, p	antibiotics, for treatment of infection by microorganisms, especially inhibits H. influenzae; Haemophilus para-influenzae, streptococcus, Maraxella catarrhalis; treatment of bronchitis, pharyngitis, tonsillitis, skin infections
	20-500 mg 20-500 mg/5ml	100-400 mg 100-400 mg/5ml	150-300 mg 150-300 mg/5ml					
Efavirenz Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Pro, Ala, Sar, & Val	b, c, h, i, j, k, l, o, p	inhibitor of HIV-1 specific, non-nucleoside, reverse transcirptase; treatment of AIDS infections
	0.2-1.2 gm 0.2-1.2 gm/5ml	300-800 mg 300-800 mg/5ml	400-750 mg 400-750 mg/5ml					
Eplerenone Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Ser, Hyp, Thr, Lys, Gly & Val	b, c, h, i, j, k, l, o, p	treatment of hypertension, blocks binding of aldosterone to mineralo-corticoid receptors
	10-250 mg 10-250 mg/5ml	15-200 mg 15-200 mg/5ml	20-150 mg 20-150 mg/5ml					

Treprostinil Preferred Forms SC infusion Oral Tab/Cap	All Doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Gly, Lys, Val, Hyp, Thr & Ser	b, c, g, h, i, j, k, l, o, p	Inhibits platelet aggregation and vasodilation of systemic and pulmonary vascular bed, treatment of cardiovascular related conditions
	0.1-100 mg/ml 10-1000 mg	0.2-50 mg/ml 20-800 mg	0.5-20 mg/ml 25-500 mg					
Adefovir Preferred Forms Oral Tab/Cap Oral Liquid	All doses expressed as drug base			Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Ser, Nor, Arg, Phe, Trp, Hyp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy, Thr, and Sar	Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Tyr, Thr, Arg, Phe, Trp, Gln, Asn, Cys and Ser, Hyp, Sar	Lys, Gly, Val, Ser, Hyp, & Pro	b, c, h, i, j, k, l, o, p	HIV reverse transcriptase inhibitors; treatment of HIV infections and AIDS
	1-100 mg 1-100 mg/5ml	2-50 mg 2-50 mg/5ml	5-20 mg 5-20 mg/5ml					

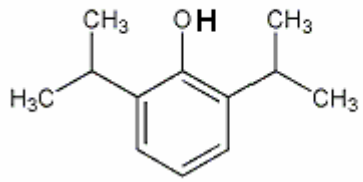
The following non-limiting examples further illustrate the invention:

Synthesis of Various Amino Acid Derivatives of Selected Drugs

I. Propofol Derivatives

Propofol (2,6-diisopropylphenol) is a low molecular weight phenol which widely used as a central nervous system anesthetic, and posses sedative and hypnotic activities. It is administered intravenously in the induction and maintenance of anesthesia and/or sedation in mammals. The major advantages of Propofol are that it can induce anesthesia rapidly, minimal side effects and upon withdrawal, the patient recovers quickly without prolonged sedation.

PROPOFOL



Propofol has been shown to have a large number of therapeutic applications, which are quite varying and somewhat surprising. For example, it has been shown to be an effective antioxidant, anti-emetic, anti-pruritic, anti-epileptic, anti-inflammatory, and even seems to possess anti-cancer properties.

Mechanism of Action:

The mechanism of action of Propofol has been extensively studied. Its central nervous system anesthetic activity has been shown to be related its high affinity for a specific subclass of GABA receptors (Collins G.G.S., 1988, Br. J. Pharmacology. 542, 225-232). However, there are a number of different receptors in the brain which are substrates for propofol, hence its varied activities.

Propofol also has significant biological effect as an antioxidant. Because of this generalized activity of propofol, it is theoretically useful in the treatment of a number of inflammatory processes where oxidation is an important factor. For example, cyclooxygenase mediated prostaglandin synthesis results in inflammation. By inhibiting oxidation in the respiratory tract, one could use propofol in the treatment of acid aspiration, adult/infant respiratory distress syndrome, airway obstructive diseases, asthma, cancer and a number of other similar pathological conditions.

Since oxidative tissue damage is a very common occurrence, it has been suggested that propofol could be useful in the treatment of Parkinson's disease, Alzheimer disease, Friedrich's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal chord injuries, and various other neurodegenerative diseases.

Propofol is currently available in the US market as an intravenous emulsion marketed by Astra Zenaca under the brand name Diprivan®. It is one the most widely used short acting central nervous system anesthetics in the market. The concentration of propofol is 10 mg/mL in non-pyrogenic sterile emulsion and the formula contains soybean oil, glycerol, egg lecithin, disodium edetate and sodium hydroxide.

A significant disadvantage of Propofol is that it is completely insoluble in water. Even at very low concentrations of 10 mg/mL, the drug precipitates out of an aqueous solution in room temperature. Therefore, manufacturers of this formulation use heroic methods to emulsify this product in water using extraordinarily complex and toxic emulsifying agents. For example, manufacturers of the IV formulations use egg lecithin, Cremaphor L®, castor oil, and other similar emulsifiers.

However, use of such emulsifiers is associated with number of problems. It is well now that various types of Cremaphor L® emulsifiers can precipitate allergic reactions. Egg lecithin and castor oil have been shown to produce anaphylactic shock in some patients. Furthermore, maintenance of stability of propofol in these emulsions is short lived and

more expensive. Moreover, the presence of egg lecithin and castor oil make the emulsion prone to microbial growth. It may be possible to dissolve propofol in water by complexing it with cyclodextrin, but cyclodextrin has not been approved by the FDA for use in intravenous therapy.

Heretofore, no one has made a safe prodrug of propofol. The British patents 1,102,011, and 1,160,468 and US Patent 3,389,138 describe the various phenol esters of amino acids, wherein the propofol is attached to a number of side chains which when released in the body produce toxic effects.

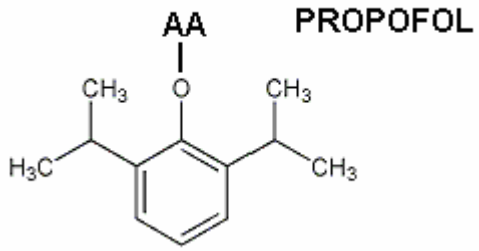
US Patent 6,451,854 describe a number of substituted alpha amino acetic acid esters of propofol, wherein propofol and the side chain were substituted with a number of different chemical groups. All the N,N-disubstituted glycine esters of propofol have not shown to be non-toxic and there many of the compounds described are derivative of propofol. Thus when released in the body after the cleavage of ester by the enzymes, many of the active drugs released are not propofol, and hence they do not possesses any toxicity data and are entirely new molecules with unknown therapeutic efficacy in man.

In yet another published paper on the water soluble salts of amino acid esters of anesthetic agent propofol, (Int. J. Pharmaceutics, 175[2]: 195-204, 1998) authors have synthesized a number of water soluble derivatives of propofol. However, when these prodrugs are cleaved by esterase enzymes, substituted non-natural amino acids with unknown toxicity profile are released in the body.

Until now there has been no pharmaceutical preparation has been available in the market that can deliver propofol without harmful side effects. The present invention however, has produced a number of water soluble, non-toxic derivatives of propofol which are suitable for delivering propofol in the body without any harmful side effects and without the needs for toxic and expensive additives, solubilizers and emulsifiers.

Accordingly, in one aspect, the present invention is directed to a class of prodrugs of Propofol. The prodrug consists of the carboxyl group of an amino acid esterified to the free hydroxyl group present on the propofol molecules.

More specifically, one aspect of the present invention is directed to, the compounds of the formulae



or pharmaceutically acceptable salts thereof; wherein AA is an amino acid, in which the carboxyl group of AA is reacted with the hydroxyl group of the Propofol.

In antoher aspect, the present invention is also directed to a pharmaceutical composition comprising a therapeutically effective amount of the various Propofol prodrugs above and a pharmaceutical carrier therefor.

In another embodiment, the present invention is directed to a method of treating a patient in need of propofol therapy, which method comprises administering to said patient an effective amount of the Propofol.

In a further embodiment, the present invention is directed to a method of enhancing the solubility of propofol in an aqueous solution comprising reacting the hydroxyl functionality of the Propofol and isolating the products thereof.

In a still further embodiment, the present invention is directed to a method of substantially and in a therapeutically efficacious manner, reducing or eliminating the potential toxic side effects of current formulations containing toxic exepients when

administered to a patient which comprises reacting the hydroxyl functionality of the propofol molecule with carboxyl function of selected amino acids to form an ester covalent bond respectively and isolating the product thereof and administering said product to the patient.

The current invention shows that when unsubstituted naturally occurring amino acids are esterified to propofol, the resulting prodrugs are highly water soluble, (>200 mg/L in water), release non-toxic amino acids upon cleavage in the body and require none of the toxic emulsifier, additives and other exepients.

Furthermore, it has been shown that the current invention also produced drugs, while they are prodrugs of propofol of the present invention are highly effective central nervous system anesthetics. Thus the current amino acid prodrugs are effective central nervous system anesthetics, with or without releasing the active parent drug.

The amino acid esters of the present invention are at least 10 times more soluble that propofol in water in room temperature. Especially the glycine, proline and lysine esters of propofol are soluble at the range of more than 100 mg/ml, and in case of lysine it is greater than 250 mg/mL.

While the prodrugs of the present invention are not expected to possess any antioxidant activity due to blockage of the phenolic group responsible for such; however the present inventor has found that the prodrugs of propofol are effective anesthetics with or without releasing propofol. The propofol prodrugs described release the propofol when administered in vivo and the resulting drug maintains its pharmacological and anti-oxidant properties.

The prodrug of propofol of the present invention clearly provides a number of advantages over propofol, for example, all of the side chains cleaved from these prodrugs are naturally occurring essential amino acids and hence are non-toxic. This

results in high therapeutic index. Secondly all the prodrugs are readily cleaved in the body to release propofol. Furthermore, due their high water solubility, they can be easily administered by either forming an in-situ solution just before IV administration using lyophilized sterile powder or providing the drug in solution in prefilled syringe or bottles for infusion. The aminoacid esters are more stable than propofol since OH group in propofol is blocked to oxidation. Thus the propofol prodrugs of the present invention are more effective then propofol itself without the toxicity and other pharmaceutical problems associated with current marketed formulations.

The prodrugs of propofol of the present invention possess anti-inflammatory, anti-oxidant, anti-cancer, anti-convulsive, anti-emetic and anti-pruritic properties.

These prodrugs of propofol of the present invention are effective in treating diseases or conditions in which Propofol normally are used. The prodrugs disclosed herein are transformed within the body to release the active compound and enhances the therapeutic benefits of the Propofol by reducing or eliminating biopharmaceutical and pharmacokenetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals. Since the prodrugs are more soluble in water then Propofol, it does not need to be associated with a carrier vehicle, such as alcohol or castor oil which may be toxic or produce unwanted side reactions. Moreover, oral formulations containing the prodrugs of Propofol are absorbed into the blood and are quite effective.

Thus, the prodrug of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs.

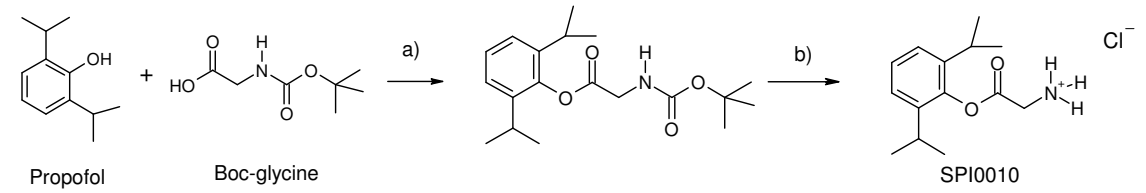
Furthermore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

Overview:

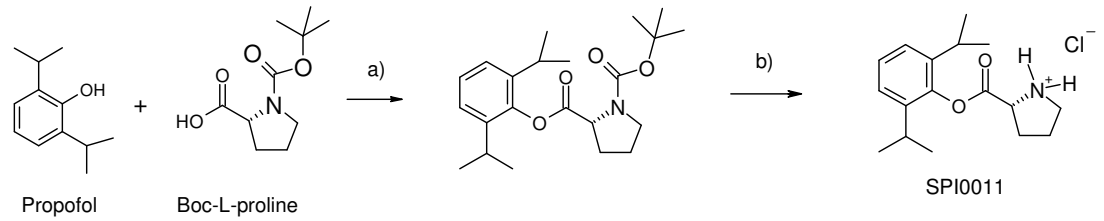
The procedure for the synthesis of the glycine, L-proline, and L-lysine esters of Propofol is depicted hereinbelow. However, these are exemplary and any amino acid produrugs thereof can be prepared using the following methodology. The complete procedure and analytical data is given in the **Experimental Section**. In general, as shown in the following scheme Propofol (10 g) was coupled with the N-Boc protected amino acid (1 equivalent) with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC) in the presence of a catalytic amount of 4-(N,N-dimethyamino)-pyridine (DMAP). The EDC was removed by extraction with water. After drying over sodium sulfate, filtration, and concentration the crude protected amino acid ester of Propofol was purified by flash chromatography to generate the protected esters in 50-60% yield. The protecting groups were then removed by stirring the protected esters in diethyl ether saturated with hydrochloric acid (gas) at room temperature. Yields for the deprotection step were generally 60-95%. After filtration and drying the hydrochloride salts of the glycine and L-proline esters of Propofol did not require additional purification. The hydrochloride salt of the L-lysine-Propofol ester was crystallized once from ethanol to remove a trace of mono-protected L-lysine-Propofol ester.

Synthetic Sequence:

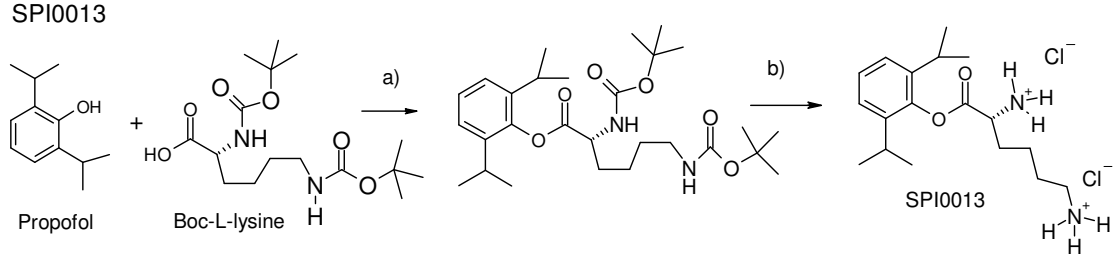
1. SPI0010



2. SPI0011



3. SPI0013



SCHEME

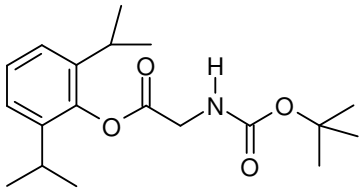
Synthesis of the glycine, L-proline, and L-lysine esters of Propofol: a) EDC, DMAP, CH_2Cl_2; b) HCl (g), Et_2O.

Experimental Section:

The synthesis of **SPI0010**, **SPI0011** and **SPI0013** were conducted in batches. Generally a small-scale experiment was performed first followed by a larger batch. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

1) SPI0010

Propofol (9.98 g, 55.97 mmole) was dissolved in dichloromethane (200 mL) at room temperature, under an argon atmosphere. N-t-Butyloxocarbonyl-glycine (11.2 g, 63.91 mmole) was added along with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 11.1g, 57.9 mmole) and 4-(N,N-dimethylamino)-pyridine (DMAP, 1.5 g, 12.27 mmole). After stirring for 21 hours under an argon atmosphere at room temperature, water (200 mL) was added and the layers were separated. The dichloromethane layer was washed again with water (200 mL) and dried for 1 hour over sodium sulfate (5 g). After filtration and concentration under reduced pressure, the remaining oil was purified by flash chromatography on silica gel (250 g), eluting with hexanes/ethyl acetate (10:1). The procedure generated the protected N-BOC protected glycine ester of Propofol as a white solid (11.34g, 60% yield).



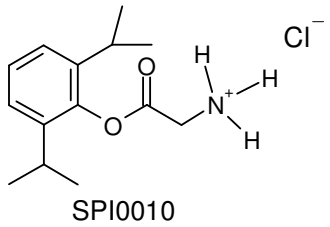
tert-Butoxycarbonylamino-acetic acid 2,6-diisopropylphenyl ester:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.25-7.13 (m, 3H), 5.18 (br s, 1H), 4.22 (d, 2H, J= 5.7 Hz), 2.89 (m, 2H), 1.46 (s, 9H), 1.18 (d, 12H, J= 6.9 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.35, 155.75, 145.22, 140.35, 126.90, 124.14, 80.32, 42.66, 28.54, 27.79, 23.57.

The Propofol-Boc-gycine ester (11.28 g, 33.6 mmole) was dissolved in anhydrous diethyl ether (200 mL) at room temperature. Hydrochloric acid (gas) was passed through the solution for 45 minutes while stirring. The mixture was allowed to stir at room temperature for 48 hours under an argon atmosphere. After 48 hours hexanes (200 mL) were added and the precipitate was filtered. The white solid was dried under high

vacuum for 5 hours at 88 °C. The experiment produced **SPI0010** (8.73 g, 95% yield, purity 99.9% by HPLC) as a white solid.



SPI0010

Amino-acetic acid 2,6-diisopropyl-phenyl ester, hydrochloride:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.77 (br s, 3H), 7.20-7.08 (m, 3H), 4.14 (m, 2H), 2.87 (m, 2H), 1.11 (d, 12H, J= 7 Hz).

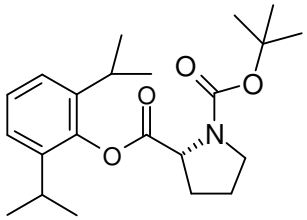
^{13}C NMR (75 MHz, $CDCl_3$): δ =166.42, 144.84, 140.42, 127.10, 124.06, 40.47, 27.61, 23.55.

CHN analysis:
calc.: C 61.87, H 8.16, N 5.15; found: C 61.14, H 8.20, N 5.14.

2) SPI0011

Propofol (10.03 g, 56.23 mmole) was dissolved in dichloromethane (100 mL) at room temperature, under an argon atmosphere. N-t-Butyloxocarbonyl-L-proline (14.04 g, 65.22 mmole) was added along with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 11.95 g, 62.33 mmole) and 4-(N,N-dimethylamino)-pyridine (DMAP, 1.1 g, 9.0 mmole). After stirring for 3 hours under an argon atmosphere at room temperature, water (100 mL) was added and the layers were separated. The dichloromethane layer was washed again with water (100 mL) and dried for 1 hour over sodium sulfate (5 g). After filtration and concentration under reduced pressure, the remaining oil was purified by flash chromatography on silica gel (250 g), eluting with hexanes/ethyl acetate (10:1). The procedure generated the protected N-BOC protected

L-proline ester of Propofol as a clear oil (11.34g, 66% yield) that solidified on standing in the freezer.

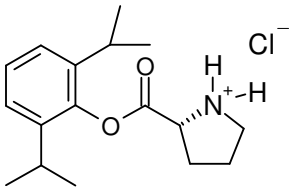


Pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2,6-diisopropyl-phenyl) ester:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31-7.20 (m, 3H), 4.73 (m, 1H), 3.70-3.50 (m, 2H), 3.20-2.94 (m, 2H), 2.46-2.20 (m, 2H), 2.20-2.0 (m, 2H), 1.55 (m, 9H), 1.25 (m, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.87, 171.01, 154.34, 153.93, 145.35 145.23, 140.06, 140.21, 126.69, 126.53, 123.95, 80.28, 79.89, 59.14, 46.67, 46.42, 31.10, 30.17, 28.61, 28.56, 28.56, 27.44, 27.18, 23.47.

The Propofol-Boc-L-proline ester (13.95 g, 37.14 mmole) was dissolved in anhydrous diethyl ether (100 mL) at room temperature. Hydrochloric acid (gas) was passed through the solution for 60 minutes while stirring. The mixture was allowed to stir at room temperature for 22 hours under an argon atmosphere. After 22 hours hexanes (50 mL) were added and the precipitate was filtered. The white solid was dried under high vacuum for 5 hours at 88 °C. The experiment produced **SPI0011** (9.1 g, 81% yield, purity 99.1% by HPLC) as a white solid.



SPI0011

Pyrrolidine-2(S)-carboxylic acid 2,6-diisopropyl-phenyl ester, hydrochloride:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.15 (br s, 2H), 7.27-7.14 (m, 3H), 4.78 (t, 1H, J= 7.8 Hz), 3.56 (m, 2H), 2.85 (m, 2H), 2.64 (m, 1H), 2.40 (m, 1H), 2.20 (m, 1H), 2.05 (m, 1H), 1.18 (m, 12H).

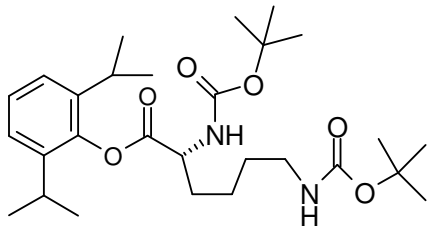
^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.30, 144.23, 139.74, 126.98, 123.96, 51.58, 38.21, 29.32, 26.64, 26.18, 23.71, 23.02, 21.67.

CHN analysis:
calc.: C 65.48, H 8.40, N 4.49; found: C 65.50, H 8.43, N 4.50.

3) SPI0013

The dicyclohexylamine salt of di-N-boc-L-lysine (23.62 g, 0.0447 mole) was added to diethyl ether (200 mL) and potassium hydrogen sulfate (9.14 g) in water (200 mL) that was cooled in an ice/water bath. After strirring for 20 minutes, the layers were separated. The ether layer was extracted three times with cold water (100 mL). The ether layer was then dried over sodium sulfate (15 g) for one hour, filtered, and concentrated under reduced pressure. The procedure generated the free acid of N,N'- di-boc-L-lysine (15.5 g, 100% recovery).

Propofol (8.0 g, 45 mmole) was dissolved in dichloromethane (100 mL) at room temperature, under an argon atmosphere. N, N'-di-t-Butyloxocarbonyl-L-lysine (15.5 g, 44.7 mmole) was added along with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 8.62 g, 45 mmole) and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.55 g, 4.5 mmole). After stirring for 3 hours under an argon atmosphere at room temperature, water (100 mL) was added and the layers were separated. The dichloromethane layer was washed again with water (100 mL) and dried for 1 hour over sodium sulfate (5 g). After filtration and concentration under reduced pressure, the remaining oil was purified by flash chromatography on silica gel (250 g), eluting with hexanes/ethyl acetate (9:1). The procedure generated the protected N-BOC protected L-lysine ester of Propofol as a white foam (12.42 g, 55% yield).

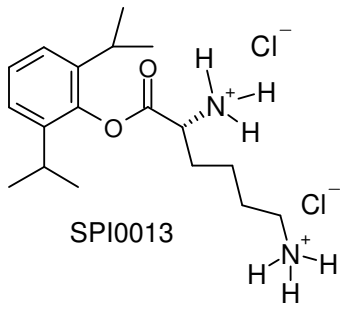


2(S),6-Bis-t-butoxycarbonylamino-hexanoic acid 2,6-diisopropyl-phenyl ester:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28-7.15 (m, 3H), 5.22 (d, 1H, J= 8.4 Hz), 4.70 (m, 1H), 4.59 (m, 1H), 3.17 (m, 2H), 2.93 (m, 2H), 2.09 (m, 1H), 1.86 (m, 1H), 1.67-1.54 (m, 4H), 1.48 (s, 9H), 1.46 (s, 9H), 1.20 (m, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.82, 156.10, 155.65, 145.25, 140.30, 126.80, 124.03, 80.14, 79.28, 53.76, 40.29, 32.09, 28.66, 28.54, 27.48, 23.91, 23.10.

The Propofol-di-Boc-L-lysine ester (12.34 g, 24.37 mmole) was dissolved in anhydrous diethyl ether (250 mL) at room temperature. Hydrochloric acid (gas) was passed through the solution for 60 minutes while stirring and cooling in an ice/water bath. The mixture was allowed to stir at room temperature for 48 hours under an argon atmosphere. After 48 hours the precipitate was filtered and crystallized from ethanol (100 mL). The white solid was dried under high vacuum for 4 hours at 90 °C. The experiment produced **SPI0013** (5.5 g, 60% yield, purity 98.6% by HPLC) as a white solid.



SPI0013

2(S),6-Diamino-hexanoic acid 2,6-diisopropyl-phenyl ester, dihydrochloride:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.05 (br s, 3H), 8.35 (br s, 3H), 7.26-7.13 (m, 3H), 4.43 (t, 1H, J= 6 Hz), 3.0-2.6 (m, 4H), 2.09 (m, 2H), 1.80-1.50 (m, 4H), 1.10 (d, 12H, J= 7 Hz).

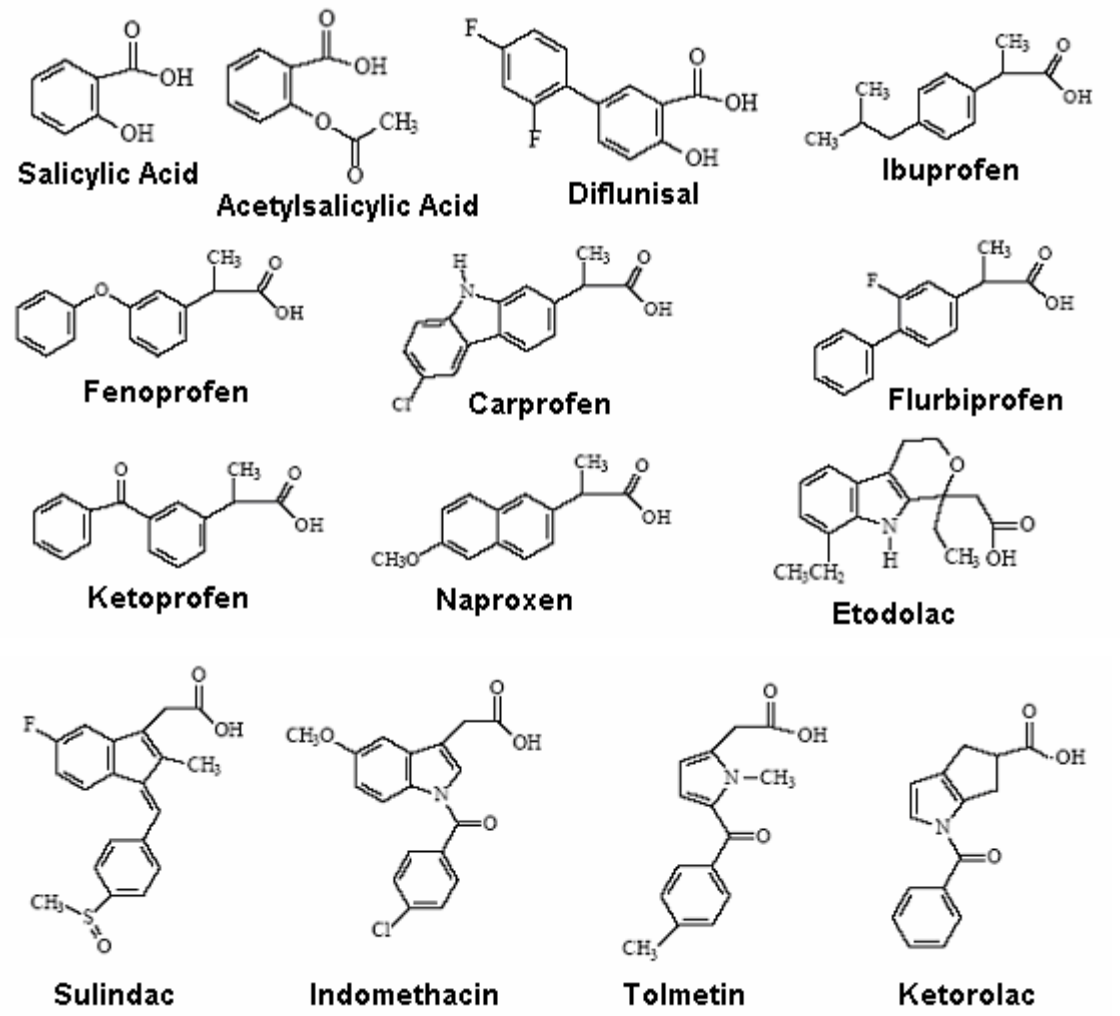
^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.30, 144.23, 139.74, 126.98, 123.96, 51.58, 38.21, 29.32, 26.64, 23.71, 23.02, 21.67.

CHN analysis:
calc.: C 56.99, H 8.50, N 7.38; found: C 56.48, H 8.56, N 7.30.

II. PRO DRUGS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

The NSAIDs comprise a class of structurally distinctive, carboxylic acid moiety attached to a planar aromatic functionality, Examples include: acetyl salicyclic acid, salicyclic acid, diflunisal, ibuprofen, fenoprofen, carprofen, flurbiprofen, ketoprofen, naproxen, sulindac, indomethacin, etodolac, tolmetin, ketorolac, diclofenac, and meclofenamate. The NSADIs posess anti-inflammatory, analgesic, antipyretic and anti-clotting activity.

Examples of the chemical structures of this uniques class of compounds showing wide variety of pharmacological activities are shown below.



NSAIDs are widely used for the treatment of acute and chronic pain, management of edema, tissue damage resulting from inflammatory joint diseases and also, effective anti-clotting agents in the treatment of myocardial infraction. A number of the agents also possess antipyretic activity in addition to analgesic and anti-inflammatory action, thus useful in reducing fever.

Some drugs in the above group have also been prescribed for Rheumatoid Arthritis, Osteoarthritis, acute gout, ankolysing spondylitis, and dysmenorrhea.

Mechanism of Action:

The major mechanism by which the NSAIDs produce their therapeutic effect is via inhibition of prostaglandin synthesis. Specifically NSAIDs inhibit cyclooxygenases, such as COX-1 and COX-2 enzymes, where these two enzymes are responsible for synthesis of prostaglandins. While COX-1 enzyme is important for the regulation of platelet aggregation, regulation of blood flow in kidney and stomach, and regulation of gastric acid secretion, COX-2 enzyme plays an important role in the pain and inflammatory processes. NSAIDs significantly increase clotting time and can be used for prophylaxis of thromboembolism and myocardial infarction.

All NSAIDs are relatively medium to strong organic acids with pKa's in the 3-6 range. Most of them are carboxylic acid derivatives. Acidic group is essential for COX inhibitory activity and in physiological pH, all the NSAIDs are ionized. All of them have quite varying hydrophilic lipophilic balance, and these are functions of their aryl, aromatic and aliphatic side chains and other heterocyclic variations in their structures. Most of the NSAIDs are highly bound to plasma proteins and often competitively replace other drugs which have similar affinity for plasma proteins. Hence concomitant administration of NSAIDs with other therapeutic class must be carefully evaluated to prevent drug interactions. Most of the drugs, due to acidic carboxyl group are metabolized by the mammals via conjugation. The major pathway of metabolic clearance of a number of NSAIDs is glucuronidation followed by renal elimination.

Use of acetylsalicylic acid (aspirin) in the prophylaxis of coronary heart diseases is now well known, and this drug has proved to be a lifesaver for a number of patients with myocardial infarction. Several additional uses have already been documents for aspirin, for example, it was recently reported in the medical journal Lancet (Vol 349, p 1641) that aspirin reduces the risk of stroke in patients with early warning signs of transient ischemic heart attacks. Pre-eclampsia and fetal growth retardation, both caused by blockages of the blood vessels of the placenta, are two of the commonest complications of pregnancy – there are millions of cases of pre-eclampsia in the world each year. In a

trial involving more than 9000 women in 16 countries, a daily dose of 60mg aspirin reduced the risk of pre-eclampsia by 13 per cent. (Aspirin Foundation website). Aspirin has also been shown to be effective in some studies to prevent colon cancer, lung cancer and pancreatic cancer in post-menopausal women. Since aspirin can improve blood flow, its usefulness in the treatment of diabetes certain forms of dementia such as Alzheimer's disease are becoming increasingly clear.

Because of their unique pharmaceutical potential, the NSAIDs have attracted considerable attention in the press. The primary area of clinical investigation for above drugs has been as non-steroidal anti-inflammatory agents, in particular in relation to their application to patients suffering from pain, arthritis, (Rheumatoid and Osteo) other inflammatory reactions, fever and for the prophylaxis of coronary heart diseases. These drugs are also used in the treatment of migraine headache, menstrual syndromes, back pain and gout.

Despite the very major contribution which NSAIDs have made, difficulties have been encountered in providing more effective and convenient means of administration (e.g., galenic formulations, for example, oral dosage form, which are both convenient and for the patient as well as providing appropriate bioavailability and allowing dosaging at an appropriate and controlled dosage rate) as well as the reported occurrence of undesirable side reactions; in particular severe gastric and duodenal ulcers, mucosal erythema, and edema, erosions, perforations, blood in stool, ulcerative colitis have been obvious serious impediments to their wider use or application. *The dual injury theory* involves NSAID-mediated direct damage, followed by a systemic effect in which prostaglandin synthesis is inhibited. Topical injury may also occur as a result of the biliary excretion of active hepatic metabolites and subsequent duodenogastric reflux. (Arthritis and Rheumatism 1995; 38(1):5-18) The effects are additive; either topical or systemic mechanisms alone are sufficient to produce gastro duodenal mucosal damage.

Moreover, the above mentioned NSAIDs are characteristically highly hydrophobic and readily precipitate in the presence of even very minor amounts of water, e.g., on contact with the body (e.g., stomach fluids). It is accordingly extremely difficult to provide e.g., oral formulations which are acceptable to the patient in terms of form and taste, which are stable on storage and which can be administered on a regular basis to provide suitable and controlling patient dosaging.

Proposed liquid formulations, e.g., for oral administration of NSAIDs, have heretofore been based primarily on the use of natural gums, like Xanthan, cellulose, citric acid, and lime flavor etc. See e.g., U.S. Patent NO. 5,780,046. Commercially available NSAIDs drink-solution employs incompatible orange color and berry flavor, citric acid, Xanthan Gum, polysorbate 80, pregelatinized starch, glycerin, sodium benzoate, and additional artificial colors and flavors. Use of the drink solution and similar composition as proposed in the art is however accompanied by a variety of difficulties.

Further, the palatability of the known oil based system has proved problematic. The taste of the known drink-solution is, in particular, unpleasant. Admixture with an appropriate flavored drink, for example, chocolate drink preparation, at high dilution immediately prior to ingestion has generally been practiced in order to make regular therapy at all acceptable. Adoption of oil based systems has also required the use of high ethanol concentrations to itself inherently undesirable, in particular where administration to children is forseen. In addition, evaporation of the ethanol, e.g., from capsules (adopted in large part, to meet problems of palatability, as discussed or other forms (e.g., when opened) results in the development of a NSAID precipitate. Where such compositions are presented in, for example, soft gelatin encapsulated form; this particular difficulty necessitates packaging of the encapsulated product in an air-tight component, for example, an air-tight blister or aluminum-foil blister package. This in turn renders the product both bulky and more expensive to produce. The storage characteristics of the aforesaid formulations are, in addition, far from ideal.

Gastric irritability of the NSAIDs has been a topic of great concern to the practicing physicians and as well as patients. Acute uses of aspirin, fenoprofen, flurbiprofen, indomethacin, ketorolac, meclofenamate, mefanamic acid, and piroxicam produce serious GI side effects. Even Ibuprofen is shown to cause severe gastric lesions upon long term use. Gastrointestinal toxicity is the most frequently encountered side effect associated with NSAIDs and presents considerable concern. Approximately one half of all hospital admissions for a bleeding ulcer are attributed to the use of NSAIDs, aspirin, or the two taken in combination during the week prior to admission. (Faulkner G, Prichard P, Somerville K, et al. Aspirin and bleeding peptic ulcers in the elderly. Br Med J. 1988; 297:1311-1313). A survey of Tennessee Medicaid patients who were hospitalized with GI complications showed that patients who used NSAIDs had approximately a fourfold greater risk for developing GI hemorrhage or peptic ulcer disease than patients not taking NSAIDs. (Griffin MR, Piper JM, Daugherty JR, et al. Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. Ann Intern Med. 1991; 114:257-263). Serious GI events, according to the FDA, occur in as many as 2% to 4% of patients per year who are taking continuous NSAID therapy for rheumatoid arthritis. The relative risk of gastric ulcer (4.725), duodenal ulcer (1.1 to 1.6), bleeding (3.8), perforation, and death are all increased by NSAID use when such patients are compared to those who do not take these products. In 1989, patients with rheumatoid arthritis had approximately 20,000 hospitalizations per year with an estimated cost of $10,000 per stay. (Fries JF, Miller SR, Spitz PW, et al. Toward an epidemiology of gastropathy associated with nonsteroidal anti-inflammatory drug use. J Gastroenterology. 1989; 96:647-655).

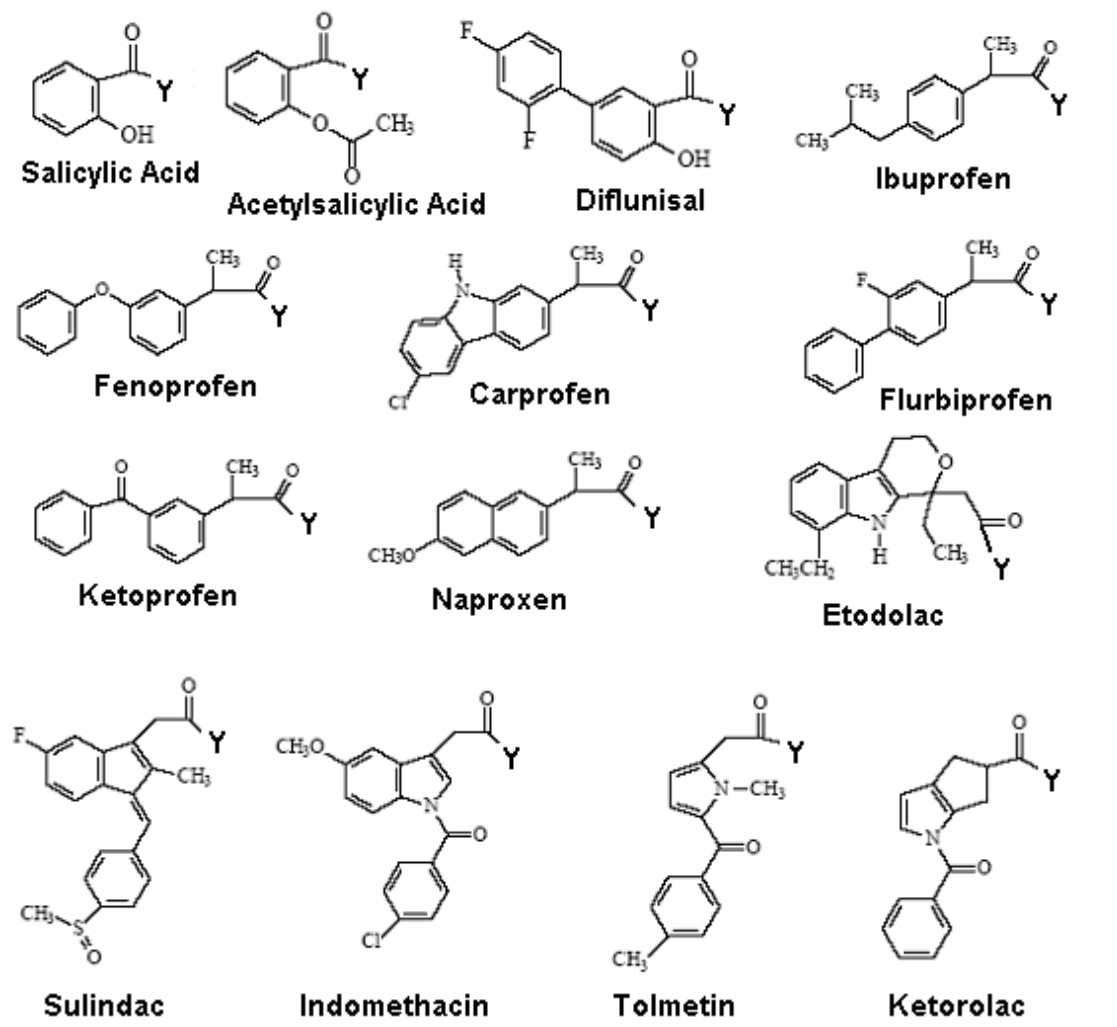
There is also a need for providing some of the NSAIDs in a water soluble form for injection. It is well known that high concentrations of alcohol and tromethamine used to form a salt in the current formulations of Ketorolac are toxic. At present there is no formulation that would allow the NSAIDs to be in aqueous solution at the concentrations needed due to poor water solubility of the drug.

Beyond all these very evident practical difficulties lies the occurrence of undesirable side reactions already alluded to, observed employing available oral dosage forms.

Several proposals to meet these various problems have been suggested in the art, including both solid and liquid oral dosage forms. An overriding difficulty which has however remained is the inherent insolubility of the NSAIDs in aqueous media, hence preventing the use of a dosage form which can contain NSAIDs in sufficiently high concentration to permit convenient use and yet meet the required criteria in terms of bioavailability, e.g. enabling effective resorption from the stomach or gut lumen and achievement of consistent and appropriately high blood/blood-serum levels.

The present prodrugs of NSAIDs overcome the problems described hereinabove. More specifically, an embodiment of the present invention is directed to a prodrug of NSAID which significantly enhances its solubility in aqueous solutions, thereby avoiding the need to utilize a carrier, such as ethanol or castor oil when administered as a solution. Moreover, the prodrugs of NSAID, in accordance with the present invention, do not exhibit the side effects of the prior art formulations. Further, the prodrugs of the present invention are almost completely devoid of gastric irritability upon oral administration, thereby enhancing significantly the therapeutic index of the prodrugs tested and their efficacy.

Accordingly, in one aspect, the present invention is directed to a prodrug of NSAIDs. The preferred prodrugs of the NSAIDs have the formula



or pharmaceutically acceptable salts thereof; wherein Y is either NH-AA or O-AA and AA is an amino acid, in which either an amine group or the hydroxyl group of AA is reacted with the carboxylic acid group of the NSAIDs.

The present invention is also directed to a pharmaceutical composition comprising a therapeutically effective amount of the various NSAIDs above and a pharmaceutical carrier therefor.

In another embodiment, the present invention is directed to a method of treating a patient in need of NSAID therapy, which method comprises administering to said patient an effective amount of the NSAIDs.

In a further embodiment, the present invention is directed to a method of enhancing the solubility of NSAID in an aqueous solution comprising reacting the carboxyl functionality of each of the NSAIDs and isolating the products thereof.

In a still further embodiment, the present invention is directed to a method of substantially and in a therapeutically efficacious manner, reducing or eliminating the gastric mucosal damage of NSAIDs when administered to a patient which comprises reacting the carboxyl functionality of each of the NSAID molecule with either amine or hydroxyl function of selected amino acids to form either an amide or ester covalent bond respectively and isolating the product thereof and administering said product to the patient.

A. Synthesis of Ibuprofen Amino Acid Derivaties

Overview:

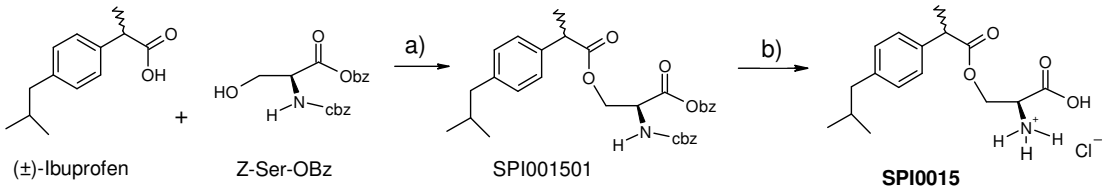
The procedure for the synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of Ibuprofen is outlined in **Synthetic Sequence** section. The complete procedure and analytical data is given in the **Experimental Section**. Again, these synthetic schemes are exemplary. The scheme is applicable for other amino acids in the preparation of the NSAID prodrugs of the present invention. In general, (±)-Ibuprofen (4-10 g, in batches) was coupled with the N-benzyloxy/benzyl ester protected amino acids (1 equivalent) with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 1 equivalent) in the presence of a catalytic amount of 4-(N,N-dimethyamino)-pyridine (DMAP). Once the reactions were complete, any excess EDC was removed by extraction with water, DMAP was removed by extraction with dilute acid, and Ibuprofen was removed by extraction with sodium bicarbonate. After drying over sodium sulfate, filtration, and concentration the crude protected amino acid esters

of (±)-Ibuprofen were either used directly or purified by flash chromatography on silica gel to generate the protected esters in good yield (85-95%). The column chromatography was generally not necessary if a slight excess of Ibuprofen and coupling agent were used, and a thorough extraction procedure was conducted. The protecting groups were removed by hydrogenation (25-35 psi H_2) in the presence of 10% palladium on carbon and hydrochloric acid. Yields for the deprotection step ranged from 70-90%. After filtration and drying the hydrochloride salts of the serine and threonine esters of (±)-Ibuprofen were purified by crystallization. The hydrochloride salt of the L-hydroxyproline-Ibuprofen ester was a gel that would not solidify/crystallize. In this case the hydrogenation was repeated without the use of acid and the neutral compound was purified.

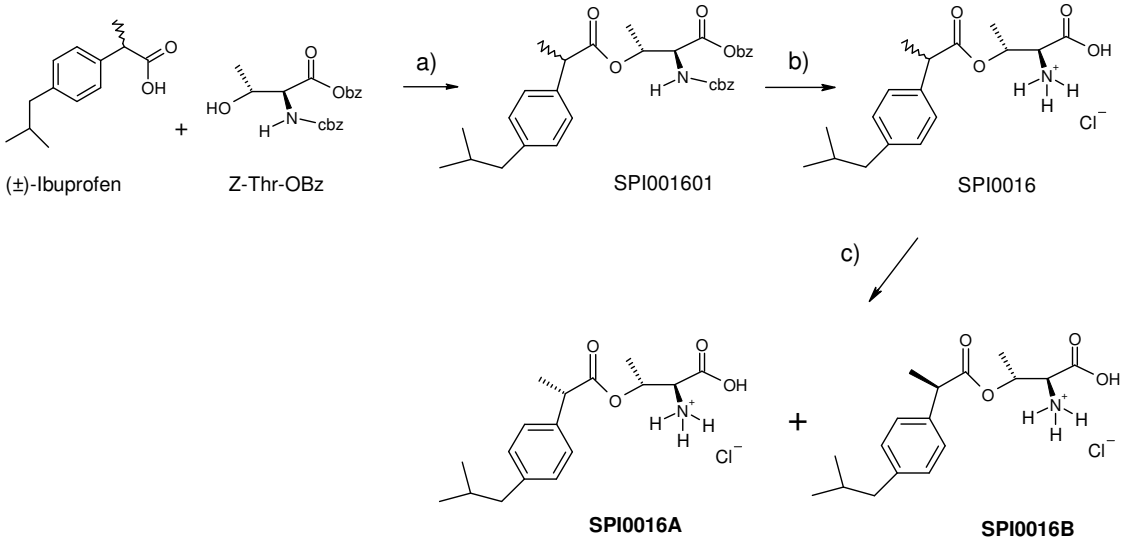
Because the Ibuprofen started as a mixture of enantiomers, the final products were delivered as a mixture of diastereomers except for the threonine ester. In the case of the threonine ester of Ibuprofen, washing with water, acetone or acetonitrile could readily separate the final diastereomeric salts. The insoluble isomer (SPI0016A) was determined to be the active isomer by comparison with an authentic standard prepared from S-(+)-Ibuprofen. The serine and hydroxyproline esters of (±)-Ibuprofen could not be readily separated in this fashion.

Synthetic Sequence:

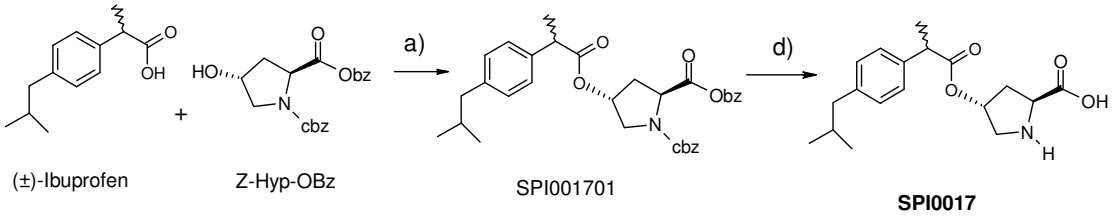
1. SPI0015



2. SPI0016A and SPI0016B



3. SPI0017



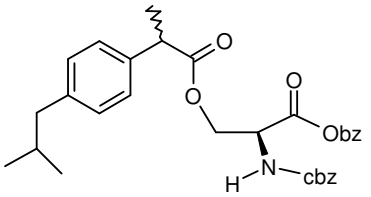
Synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen: a) EDC, DMAP, CH_2Cl_2; b) HCl, 10% Pd/C, EtOH c) acetone, d) 10% Pd/C, EtOH.

Experimental Section:

The synthesis of **SPI0015**, **SPI0016** and **SPI0017** were conducted in two or three batches. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Sigma-Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

1) Preparation of (±)-Ibuprofen-L-serine ester, hydrochloride (SPI0015).

(±)-Ibuprofen (5.04 g, 24.4 mmole), N-carbobenzyloxy-L-serine benzyl ester (8.11 g, 24.6 mmole), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 4.87g, 25.4 mmole), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.40 g, 3.27 mmole) were dissolved in dichloromethane (150 mL) at room temperature, under an argon atmosphere. After stirring for 22 hours under an argon atmosphere at room temperature, water (100 mL) was added and the layers were separated. The dichloromethane layer was washed again with water (100 mL) and dried for 1 hour over sodium sulfate (5 g). After filtration and concentration under reduced pressure, the remaining oil was purified by flash chromatography on silica gel (250 g), eluting with hexanes/ethyl acetate (3:1). The procedure generated the protected L-serine-(±)-Ibuprofen ester (**SPI001501**) as a colorless solid (11.4 g, 90% yield).



SPI001501

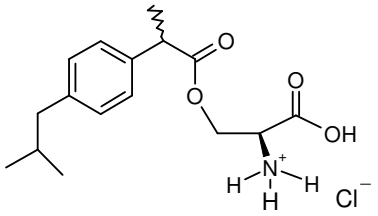
2(S)-Benzyloxycarbonylamino-3-[2(R,S)-(4-isobutyl-phenyl)-propionyloxy]-propionic acid benzyl ester:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40-7.20 (m, 10H), 7.14-7.01 (m, 4H), 5.50 (d, ½H, *J*= 8.4 Hz), 5.29 (d, ½H, *J*= 8.4 Hz), 5.11-5.02 (m, 2.5H), 4.90 (d, ½H, *J*= 12 Hz), 4.62

(m, 1H), 4.49-4.43 (m, 1H), 4.36-4.32 (m, 1H), 3.59 (m, 1H), 2.39-2.35 (m, 2H), 1.78 (m, 1H), 1.42-1.39 (m, 3H), 0.85 (d, 6H, *J*= 6.6 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.05, 169.19, 169.07, 155.68, 140.73, 137.20, 136.12, 135.05, 134.91, 129.44, 128.67, 128.65, 128.60, 128.41, 128.33, 128.30, 128.19, 127.19, 127.16, 67.75, 67.32, 64.51, 64.32, 53.71, 45.16, 45.02, 30.35, 22.60, 18.27.

The protected Ibuprofen-L-serine ester (22.50 g, 43.4 mmole) was dissolved in ethanol (200 mL) at room temperature and added to a Parr bottle that contained 10% palladium on carbon (3.86 g, 50% wet) under a nitrogen atmosphere. Hydrochloric acid (10 mL 37% HCl in 30 mL water) was added and the nitrogen atmosphere was replaced with hydrogen gas (25 psi). After 4 hours of shaking, the palladium catalyst was removed by filtration through celite. The ethanol/water was removed under reduced pressure. The remaining white solids were washed with water (25 mL), acetone (20 mL) and dried under high vacuum (4 hours at 88 °C). The experiment produced (±)-Ibuprofen-L-serine ester, hydrochloride **SPI0015** (11.3 g, 80% yield) as a colorless solid.



SPI0015

2(S)-Amino-3-[2(R,S)-(4-isobutylphenyl)-propionyloxy]-propionic acid, hydrochloride; ((R,S)-Ibuprofen-L-Serine ester, hydrochloride):

^{1}H NMR (300 MHz, DMSO): δ = 8.92 (br s, 3H), 7.22 (t, 2H, J= 7.5 Hz), 7.10 (d, 2H, *J*= 7.5 Hz), 4.56 (m, 1H), 4.37-4.20 (m, 2H), 3.83 (q, 1H, *J*= 6.9 Hz), 2.41 (d, 2H, *J*= 6.9 Hz), 1.80 (m, 1H), 1.41 (d, 3H, *J*= 6.9 Hz), 0.85 (d, 6H, *J*= 6.9 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 173.36, 173.32, 168.08, 168.04, 139.70, 128.96, 129.92, 127.20, 127.05, 62.47, 51.59, 51.49, 44.28, 44.00, 43.90, 29.68, 22.28, 18.70, 18.42.

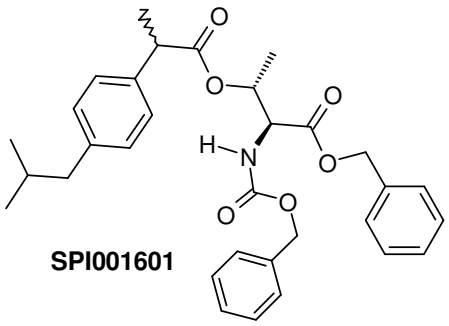
HPLC analysis:
99.13% purity; rt = 3.133 min; Luna C18 5u column (sn 167917-13); 4.6x250 mm; 254 nm; 50% ACN/50% TFA buffer (0.1%); 35 C; 20 ul inj.; 1ml/min; 1 mg/mL sample size; sample dissolved in mobile phase.

CHN analysis:
calc.: C 58.27, H 7.33, N 4.25; found: C 58.44, H 7.46, N 4.25.

Melting point: 169.5 - 170.5 °C

2a) Preparation and Separation of (±)-Ibuprofen-L-threonine ester, hydrochloride (SPI0016A and SPI0016B).

(±)-Ibuprofen (4.15 g, 20.11 mmole), N-carbobenzyloxy-L-threonine benzyl ester (6.90 g, 20.11 mmole), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 3.95 g, 20.6 mmole), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.25 g, 2.0 mmole) were dissolved in dichloromethane (50 mL) at room temperature, under an argon atmosphere. After stirring for 19 hours, the dichloromethane layer was washed with water (50 mL), 5% hydrochloric acid (2×25 mL), water (25 mL), saturated sodium bicarbonate (2×25 mL), and water (50 mL). After drying for one hour over sodium sulfate (5 g), filtration, and concentration under reduced pressure, the remaining oil was used without further purification. The procedure generated the protected L-threonine-(±)-Ibuprofen ester (**SPI001601**) as a light yellow oil (10.2 g, 95.3% yield), which solidified on standing.



SPI001601

2(S)-Benzyloxycarbonylamino-3-[2(R,S)-(4-isobutyl-phenyl)-propionyloxy]-butyric acid benzyl ester:

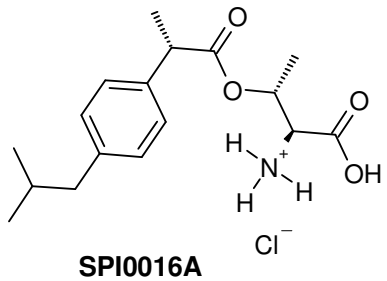
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40-7.15 (m, 10H), 7.14-7.01 (m, 4H), 5.48-5.25 (m, 2H), 5.11-5.01 (m, 3H), 4.90 (d, ½H, *J*= 12 Hz), 4.68 (d, ½H, *J*= 12 Hz), 4.48 (m, 1H), 3.60-3.48 (m, 1H), 2.39(m, 2H), 1.79 (m, 1H), 1.42-1.35 (m, 3H), 1.27 (d, 1.5 H, *J*= 6.6 Hz), 1.17 (d, 1.5 H, *J*= 6.6 Hz), 0.85 (m, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.32, 169.70, 169.30, 156.55, 140.75, 137.38, 137.22, 136.14, 135.07, 134.99, 129.45, 129.41, 128.65, 128.39, 128.22, 127.21, 127.14, 70.97, 70.70, 67.81, 67.66, 67.53, 57.83, 45.19, 30.39, 22.61, 18.57, 18.30, 17.18, 16.87.

The protected Ibuprofen-L-threonine ester (10.15 g, 19.0 mmole) was dissolved in warm ethanol (150 mL) and added to a Parr bottle that contained 10% palladium on carbon (3.4 g, 50% wet) under a nitrogen atmosphere. Hydrochloric acid (6 mL 37% HCl in 20 mL water) was added and the nitrogen atmosphere was replaced with hydrogen gas (30 psi). After 3 hours of shaking, the palladium catalyst was removed by filtration through celite (30 g). The ethanol/water was removed under reduced pressure. The experiment produced (±)-Ibuprofen-L-threonine ester, hydrochloride (**SPI0016A** and **SPI0016B**, 6.4 g, 97% crude yield) as a colorless solid. The crude mixture of diastereomers was stirred in acetone (200 mL) for 2 hours at room temperature under an argon atmosphere. After 2 hours the solids (2.84 g, **SPI0016A**) were filtered. The filtrate (**SPI0016B**, 3.0 g) was concentrated under reduced pressure.

1.) Purification of **SPI0016A** (active isomer):

After 3 batches of the S-Ibuprofen-L-threonine ester (**SPI0016A**) had been completed, the batches were combined (8.78 g total) and crystallized three times from DIUF water (100 mL). Each time a small amount of zwitterion was generated. In order to regenerate the salt, the solid generated (from each crystallization) was dissolved in 1% hydrochloric acid in ethanol (3 mL 37% hydrochloric acid in 100 mL ethanol). The ethanol solution was then concentrated under reduced pressure at room temperature. After the third crystallization and regeneration procedure, the salt (5.6 g) was stirred in acetonitrile (100 mL) for 44 hours at room temperature, under an argon atmosphere. The salt was then filtered and dried under high vacuum at 50-55 ° until the weight was constant (5.5 g).



SPI0016A

2(S)-Amino-3(R)-[2(S)-(4-isobutyl-phenyl)-propionyloxy]-butyric acid; (S-Ibuprofen-L-threonine ester, hydrochloride, active isomer):

^{1}H NMR (300 MHz, DMSO): δ = 8.76 (br s, 3H), 7.19 (d, 2H, J= 8.1 Hz), 7.11 (d, 2H, J= 8.1 Hz), 5.28 (dq, 1H, J= 6.3, 3.6 Hz), 4.14 (q, 1H, J= 3.6 Hz), 3.80 (q, 1H, J= 7.2 Hz), 2.41 (d, 2H, J= 7.2 Hz), 1.80 (m, 1H), 1.37 (d, 3H, J= 7.2 Hz), 1.21 (d, 3H, J= 6.3 Hz), 0.85 (d, 6H, J= 6.6 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 172.66, 168.24, 139.68, 137.24, 128.95, 126.97, 67.98, 55.35, 44.23, 43.83, 29.66, 22.24, 18.52, 16.47.

CHN analysis:

calc.: C 59.38, H 7.62, N 4.07; found: C 59.17, H 7.63, N 4.04.

HPLC analysis:

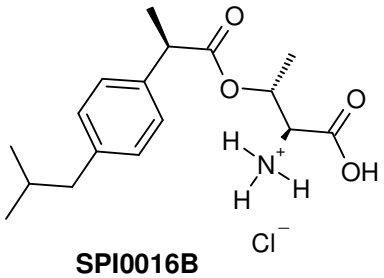
98.28% purity; r.t.= 6.951 min.; 60% TFA (0.1%)/40% acetonitrile; 1 mL/min; 37.5 C; Luna C18, 3u column (SN 167917-13), 4.6x250 mm; 22 ul injection.

Optical rotation: + 24.5 °

Melting Point: 189-190 °C

2) Purification of **SPI0016B** (inactive isomer):

After 3 batches of the R-Ibuprofen-L-threonine ester (**SPI0016B**) had been completed, the batches were combined (9.02 g total) and crystallized from DIUF water (50 mL). A small amount of zwitterion was generated during the crystallization. In order to regenerate the salt, the solid generated was dissolved in 1% hydrochloric acid in ethanol (3 mL 37% hydrochloric acid in 100 mL ethanol). The ethanol solution was then concentrated under reduced pressure at room temperature. The remaining salt (5.93 g) was crystallized three times from hot toluene (100 mL) with the addition of a small amount on acetone (1 mL). The salt was then filtered and dried under high vacuum at room temperature until the weight was constant (5.1 g).



SPI0016B

2(S)-Amino-3(R)-[2(R)-(4-isobutyl-phenyl)-propionyloxy]-butyric acid; (R-Ibuprofen-L-threonine ester, hydrochloride, inactive isomer):

^{1}H NMR (300 MHz, DMSO): δ = 8.82 (br s, 3H), 7.23 (d, 2H, J= 7.8 Hz), 7.10 (d, 2H, J= 7.8 Hz), 5.27 (m, 1H), 4.18 (m, 1H), 3.80 (q, 1H, J= 7.2 Hz), 2.41 (d, 2H, J= 7.2 Hz), 1.81 (m, 1H), 1.41 (d, 3H, J= 6.9 Hz), 1.34 (d, 3H, J= 6.3 Hz), 0.85 (d, 6H, J= 6.3 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 72.56, 168.08, 139.64, 136.98, 128.84, 127.14, 68.8, 55.29, 44.28, 29.69, 22.28, 18.24, 16.41.

CHN analysis:
calc.: C 59.38, H 7.62, N 4.07; found: C 59.30, H 7.60, N 4.05.

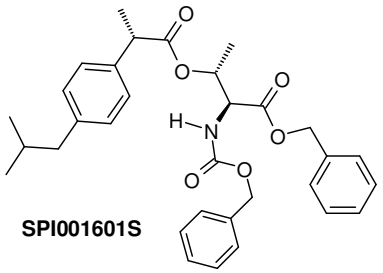
HPLC analysis:
98.43% purity; r.t.= 6.19 min.; 60% TFA (0.1%)/40% acetonitrile; 1 mL/min; 37.5 C; Luna C18, 3u column (SN 167917-13), 4.6x250 mm; 22 ul injection.

Optical Rotation: + 10.4 °

Melting Point: 176-177 °C

2b) Preparation of the S-(+)-Ibuprofen-L-threonine ester, hydrochloride standard (SPI0016S).

S-(+)-Ibuprofen (2.0 g, 9.69 mmole), N-carbobenzyloxy-L-threonine benzyl ester (3.25 g, 9.91 mmole), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 1.90 g, 9.91 mmole), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.12 g, 1.0 mmole) were dissolved in dichloromethane (25 mL) at room temperature, under an argon atmosphere. After stirring for 4 hours, the dichloromethane layer was washed with water (25 mL), 5% hydrochloric acid (25 mL), saturated sodium bicarbonate (2×25 mL), and water (25 mL). After drying for one hour over sodium sulfate (5 g), filtration, and concentration under reduced pressure, the remaining oil was used without further purification. The procedure generated the protected S-(+)-Ibuprofen-L-threonine ester (**SPI001601S**) as a light yellow oil (5.01 g, 98 % yield), which solidified on standing.



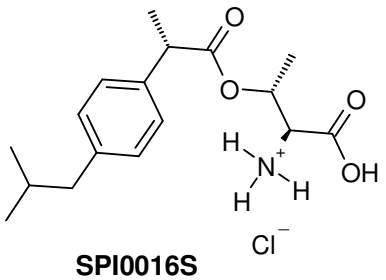
SPI001601S

2(S)-Benzyloxycarbonylamino-3-[2(R,S)-(4-isobutyl-phenyl)-propionyloxy]-butyric acid benzyl ester:

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35-7.23 (m, 10H), 7.10 (d, 2H, *J*= 7.8 Hz), 7.05 (d, 2H, *J*= 7.8 Hz), 5.48-5.25 (m, 2H), 5.17-5.01 (m, 4H), 4.50 (dd, 1H, *J*= 9.6, 1.8 Hz), 3.50 (q, 1H, *J*= 7.2 Hz), 2.40 (d, 2H, *J*= 7.2 Hz), 1.80 (m, 1H), 1.37 (d, 3H, *J*= 7.2 Hz), 1.17 (d, 3 H, *J*= 6.3 Hz), 0.86 (d, 6 H, *J*= 6.6 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.29, 169.69, 156.51, 140.68, 137.21, 136.08, 135.06, 129.40, 128.70, 128.66, 128.57, 128.38, 128.24, 127.14, 70.70, 67.80, 67.53, 57.87, 45.19, 45.11, 30.39, 22.61, 18.57, 16.87.

The protected S-(+)-Ibuprofen-L-threonine ester (5.0 g, 9.40 mmole) was dissolved in warm ethanol (100 mL) and added to a Parr bottle that contained 10% palladium on carbon (1.0 g, 50% wet) under a nitrogen atmosphere. Hydrochloric acid (1 mL 37% HCl in 10 mL water) was added and the nitrogen atmosphere was replaced with hydrogen gas (32 psi). After 2 hours of shaking, the palladium catalyst was removed by filtration through celite (30 g). The ethanol/water was removed under reduced pressure. The experiment produced S-(+)-Ibuprofen-L-threonine ester, hydrochloride (**SPI0016S**, 2.8 g, 85% crude yield) as a colorless solid. The salt was stirred in acetone (50 mL) for 3 hours at room temperature under an argon atmosphere. After 3 hours the solids (2.24 g, 69% yield) were filtered and dried under high vacuum at room temperature, until the weight was constant.



SPI0016S

2(S)-Amino-3(R)-[2(S)-(4-isobutyl-phenyl)-propionyloxy]-butyric acid;
(S-Ibuprofen-L-threonine ester, hydrochloride, active isomer):

^{1}H NMR (300 MHz, DMSO): δ = 8.76 (br s, 3H), 7.19 (d, 2H, J= 8.1 Hz), 7.11 (d, 2H, J= 8.1 Hz), 5.28 (dq, 1H, J= 6.3, 3.6 Hz), 4.14 (q, 1H, J= 3.6 Hz), 3.80 (q, 1H, J= 7.2 Hz), 2.41 (d, 2H, J= 7.2 Hz), 1.80 (m, 1H), 1.37 (d, 3H, J= 7.2 Hz), 1.21 (d, 3H, J= 6.3 Hz), 0.85 (d, 6H, J= 6.6 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 172.66, 168.24, 139.68, 137.24, 128.95, 126.97, 67.98, 55.35, 44.23, 43.83, 29.66, 22.24, 18.52, 16.47.

HPLC analysis:
98.28% purity; r.t.= 6.951 min.; 60% TFA (0.1%)/40% acetonitrile; 1 mL/min; 37.5 C; Luna C18, 3u column (SN 167917-13), 4.6x250 mm; 22 ul injection.

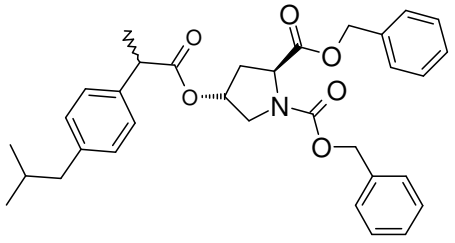
Optical rotation: + 26.5 °

Melting Point: 189-190 °C

3) Preparation of the (±)-Ibuprofen-L-hydroxyproline ester (SPI0017).

(±)-Ibuprofen (5.10 g, 24.7 mmole), N-carbobenzyloxy-L-hydroxyproline benzyl ester (8.80 g, 24.7 mmole), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 5.10g, 26.0 mmole), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.30 g, 2.40 mmole) were dissolved in dichloromethane (100 mL) at room temperature, under an

argon atmosphere. After stirring for 24 hours under an argon atmosphere at room temperature, water (100 mL) was added and the layers were separated. The dichloromethane layer was washed again with water (100 mL), 5% sodium bicarbonate (2×50 mL) and dried for 1 hour over sodium sulfate (5 g). After filtration and concentration under reduced pressure, the remaining oil was used without further purification. The procedure generated the protected (±)-Ibuprofen-L-hydroxyproline ester (**SPI001701**) as a light yellow oil (11.5 g, 85% yield).



SPI001701

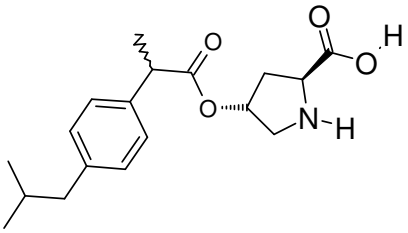
4(R)-[2-(4-Isobutyl-phenyl)-propionyloxy]-pyrrolidine-2(S)-carboxylic acid; ((R,S)-Ibuprofen-L-hydroxyproline ester):

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33-7.02 (m, 14H), 5.25-4.95 (m, 5H), 4.51-4.19 (m, 1H), 3.75-3.50 (m, 3H), 2.40 (d, 2H, *J*= 6.9 Hz), 2.15 (m, 1H), 1.81 (m, 1H), 1.44 (d, 3H, *J*= 7.0 Hz), 0.87 (d, 6H, *J*= 6.6 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.99, 171.93, 171.72, 154.68, 154.15, 140.70, 137.23, 137.04, 136.23, 135.44, 135.23, 129.41, 128.59, 128.47, 128.35, 128.19, 128.08, 127.89, 127.02, 72.86, 72.16, 67.40, 67.18, 67.09, 58.12, 57.83, 52.66, 52.49, 52.13, 45.15, 36.63, 35.67, 32.07, 30.33, 29.23, 22.90, 22.58, 18.36.

The protected Ibuprofen-L-hydroxyproline ester (11.40 g, 43.4 mmole) was dissolved in ethanol (150 mL) at room temperature and added to a Parr bottle that contained 10% palladium on carbon (2.73 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (34 psi). After 5 hours of shaking, the palladium catalyst was removed by filtration through celite. The ethanol was removed

under reduced pressure. The remaining white solids (6.60 g) were washed with DIUF water (50 mL), diethyl ether (50 mL) and dried under high vacuum until the weight was constant. The experiment produced (±)-Ibuprofen-L-hydroxyproline ester **SPI0017** (5.64 g, 84% yield) as a colorless solid.



SPI0017

4(R)-[2-(4-Isobutyl-phenyl)-propionyloxy]-pyrrolidine-2(S)-carboxylic acid; ((R,S)-Ibuprofen-L-hydroxyproline ester):

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 (d, 2H, J= 7.2 Hz), 7.09 (d, 2H, J= 7.2 Hz), 5.27 (m, 1H), 4.40 (t, 0.5H, J= 7 Hz), 4.24 (t, 0.5 H, J= 9 Hz), 3.75 (m, 1H), 3.61(m, 1H), 3.28 (d, 0.5H, J= 13 Hz), 3.15 (d, 0.5H, J= 13 Hz), 2.42-2.10 (m, 4H), 1.78 (m, 1H), 1.40 (br t, 3H, J= 6 Hz), 0.82 (d, 6H, J= 6 Hz). (mixture of diastereomers)

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.28, 173.23, 168.98, 139.88, 137.33, 137.23, 129.12, 127.26, 127.17, 72.58, 57.60, 57.50, 50.24, 50.12, 44.34, 44.15, 34.31, 34.16, 29.77, 22.34, 18.43, 18.23. (mixture of diastereomers)

HPLC analysis:

100% purity; r.t.= 5.35, 5.22 min.; 55% TFA (0.1%), 45% ACN; 1 mL/min; 32.3 C, Luna C18, serial # 188255-37; 20 ul inj..

CHN analysis:

calc.: C 67.69, H 7.89, N 4.39; found: C 67.47, H 7.87, N 4.30.

Melting Point: 198-199 °C

Efficacy (anti nociceptive potential) of Synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen by employing acetylcholine induced abdominal constriction method in male albino mice:

The present study was conducted to evaluate the efficacy of L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen taking into account the antagonizing property on acetylcholine induced writhe as an index in albino mice. Ibuprofen (racemic mixture) and ibuprofen (S)-(+) served as reference controls.

Different new formulations of ibuprofen and reference controls viz., ibuprofen (racemic mixture) and ibuprofen (S)-(+) were administered by gavage to male albino mice (Swiss strain), using 5% (v/v) Tween 80 in milli Q water as the vehicle. The study was conducted at two dose levels viz. 50mg and 100mg/kg body weight along with a vehicle control group. At each dose level 10 animals were used. All the doses were expressed as ibuprofen molar equivalents. The doses used as well as the molar equivalents are presented below.

Table 2: Formulation: Molar Equivalent:

Formulation	Molar equivalent
S-(+)-Ibuprofen-L-threonine ester	0.833 units are equivalent to 1 unit of Ibuprofen
(±)-Ibuprofen-L-serine ester	1.6 units are equivalent to 1 unit of Ibuprofen
(±)-Ibuprofen-L-hydroxyproline ester	1.55 units are equivalent to 1 unit of Ibuprofen

Table 3: Test Item: Group: Dose(mg/kg): Equivalent wt. Of the test item:

Test Item	Group	Dose (mg per kg) [in terms of Ibuprofen]	Equivalent weight of the Test item [mg/kg]
Vehicle	Vehicle control Group	0.0	--
S-(+)-Ibuprofen-L-threonine ester	Test Group 1	50.0	41.65
	Test Group 2	100.0	83.30
(±)-Ibuprofen-L-serine ester (Ibuprofen S)	Test Group 3	50.0	80.0
	Test Group 4	100.0	160.0
(±)-Ibuprofen-L-hydroxyproline ester	Test Group 5	50.0	77.5
	Test Group 6	100.0	155.0
Ibuprofen (racemic mixture)	Test Group 7	50.0	50.0
	Test Group 8	100.0	100.0
Ibuprofen S +	Test Group 9	50.0	25.0
	Test Group 10	100.0	50.0

5 The efficacy in terms of antagonizing effect on acetylcholine induced single writhe at two dose levels – 50.0 and 100.0 mg/kg for the three formulations and reference controls are presented below.

Table 4: Test Item: Group: Dose (mg/kg): Number of animals showing absence of single writhe (out of 10)

Test Item	**Group**	**Dose (mg per kg) [in terms of Ibuprofen]**	**Number of animals showing absence of single writhe (number of animals per dose = 10)**	
			One hour after dosing	**Three hours after dosing**
Vehicle	Vehicle control	0.0	0	0
S-(+)-Ibuprofen-L-threonine ester	Low dose	50.0	1	0
	High dose	100.0	3	0
(±)-Ibuprofen-L-serine ester	Low dose	50.0	4	2
	High dose	100.0	6	4
(±)-Ibuprofen-L-hydroxyproline ester	Low dose	50.0	5	4
	High dose	100.0	7	7
Ibuprofen (racemic mixture)	Low dose	50.0	4	2
	High dose	100.0	6	6
Ibuprofen S +	Low dose	50.0	5	1
	High dose	100.0	6	6

Statistical analysis employing Chi – square test procedure did not show any statistically significant difference among the formulations in comparison to reference control, while comparing the number of animals not showing writhe in each groups, as the respective "p" was found to be greater than 0.05, the level of significance.

From clinical observation based on the number of animals not showing writhes due to administration of acetylcholine, (±)-Ibuprofen-L-hydroxyproline ester was found to be

more effective in antagonizing the acetylcholine induced writhe when compared to other formulations and Ibuprofen (racemic) and Ibuprofen (S)-(+).

Table 5: Summary of Efficacy of L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen, Ibuprofen (racemic mixture) and Ibuprofen (S)-(+) - Based on Antagonizing Property of Acetylcholine Induced Writhe in Albino Mice

Dose (mg/kg) [in terms of Ibuprofen]	Test Item	Number of animals showing absence of single writhe (number of animals per dose = 10)	
		One hour after dosing	Three hours after dosing
50mg/kg	Vehicle control	0	0
	S-(+)-Ibuprofen-L-threonine ester	1	0
	(±)-Ibuprofen-L-serine ester	4	2
	(±)-Ibuprofen-L-hydroxyproline ester	5	4
	Ibuprofen (racemic mixture)	4	2
	Ibuprofen (S)-(+)	5	1

Table 6:

100mg/kg	Vehicle control	0	0
	S-(+)-Ibuprofen-L-threonine ester	3	0
	(±)-Ibuprofen-L-serine ester	6	4
	(±)-Ibuprofen-L-hydroxyproline ester	7	7
	Ibuprofen (racemic mixture)	6	6
	Ibuprofen (S)-(+)	6	6

The data were subjected to statistical analysis employing Chi – square test procedure for evaluating the efficacy of the new formulations in comparison to the reference controls. The test did not show any statistically significant difference among the formulations in comparison to reference control, while comparing the number of animals not showing writhe in each groups, as the respective "p" was found to be greater than 0.05, the level of significance.

The data is also summarized in FIGS. 1 and 2. From clinical observations and bar diagram for comparative efficacy (FIGS. 1 and 2) based on the number of animals not showing writhes due to administration of acetylcholine, (±)-Ibuprofen-L-hydroxyproline ester was found to be more effective in antagonizing the acetylcholine induced writhe when compared to other formulations and Ibuprofen (racemic) and Ibuprofen (S)-(+).

CONCLUSION

The present study was conducted to evaluate the relative efficacy of new formulations of ibuprofen. For this the antagonizing property of new formulations on acetylcholine writhes was taken as an index to determine the relative efficacy of the formulations. Ibuprofen (racemic mixture and ibuprofen (S)-(+) served as reference controls. The study was conducted at two dose levels (50.0 and 100.0 mg/kg) along with a vehicle control group.

The efficacy in terms of antagonizing effect of acetylcholine induced single writhe at two dose levels – 50.0 and 100.0 mg/kg for the three formulations and reference controls are presented below.

Table 7: Test Item: Group: Dose (mg/kg): No. of animals showing absence of single writhe (out of 10)

Test Item	**Group**	**Dose (mg per kg) [in terms of Ibuprofen]**	**Number of animals showing absence of single writhe (number of animals per dose = 10)**	
			One hour after dosing	**Three hours after dosing**
Vehicle	Vehicle control	0.0	0	0
S-(+)-Ibuprofen-L-threonine ester	Low dose	50.0	1	0
	High dose	100.0	3	0
(±)-Ibuprofen-L-serine ester	Low dose	50.0	4	2
	High dose	100.0	6	4
(±)-Ibuprofen-L-hydroxyproline ester	Low dose	50.0	5	4
	High dose	100.0	7	7
Ibuprofen (racemic mixture)	Low dose	50.0	4	2
	High dose	100.0	6	6
Ibuprofen (S)-(+)	Low dose	50.0	5	1
	High dose	100.0	6	6

Statistical analysis employing Chi – square test procedure did not show any statistically significant difference among the formulations in comparison to reference control, while comparing the number of animals not showing writhe in each groups, as the respective "p" was found to be greater than 0.05, the level of significance.

However from clinical observation based on the number of animals not showing writhes due to administration of acetylcholine (±)-Ibuprofen-L-hydroxyproline ester was found

to be more effective in antagonizing the acetylcholine induced writhe when compared to other formulations and Ibuprofen (racemic) and Ibuprofen (S)-(+).

Gastric mucosal irritation potential of L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen in fasted male albino rats

SUMMARY

The present study was conducted to determine the relative potential of new formulations of ibuprofen (L-serine, L-threonine, and L-hydroxyproline esters of (±)-Ibuprofen) to cause gastric mucosal irritation/lesions in fasted male albino rats. Ibuprofen (racemic mixture) and Ibuprofen(S)-(+) served as reference controls.

Different new formulations of ibuprofen and ibuprofen (racemic mixture) and ibuprofen(S)-(+) were administered by gavage to fasted male albino rats (Wistar strain), using 5% solution of Tween 80 in milli Q water as the vehicle. The study was conducted at two dose levels viz. 200mg and 300mg/kg body weight along with a vehicle control group. At each dose level 5 animals were used. All the doses were expressed as ibuprofen (racemic mixture) molar equivalents. The doses used as well as the molar equivalents were presented below.

Table 8: Formulation: Molar Equivalent

Formulation	Molar equivalent
S-(+)-Ibuprofen-L-threonine ester	0.833 units are equivalent to 1 unit of Ibuprofen
(±)-Ibuprofen-L-serine ester	1.60 units are equivalent to 1 unit of Ibuprofen
(±)-Ibuprofen-L-hydroxyproline ester	1.55 units are equivalent to 1 unit of Ibuprofen

The various groups used are tabulated hereinbelow:

Table 9: Test item: group: Dose (mg/kg) Equivalent wt.

Test item	**Group**	**Dose (mg per kg) [in terms of Ibuprofen]**	**Equivalent weight of the Test item [mg/kg]**
Vehicle	Vehicle control Group	0.0	--
S-(+)-Ibuprofen-L-threonine ester	Test Group 1	200.0	0.0
	Test Group 2	300.0	166.6
(±)-Ibuprofen-L-serine ester	Test Group 1	200.0	249.9
	Test Group 2	300.0	320.0
(±)-Ibuprofen-L-hydroxyproline ester	Test Group 1	200.0	480.0
	Test Group 2	300.0	310.0
Ibuprofen (racemic mixture)	Test Group 1	200.0	465.0
	Test Group 2	300.0	300.0
Ibuprofen (S)- (+)	Test Group 1	200.0	100.0
	Test Group 2	300.0	150.0

The rats were fasted for a period of 18 to 22 hours before dosing. The test item was administered as a single dose by gavage. Three hours after drug administration, the animals were killed humanely by CO_2 gas inhalation. The stomach was dissected out and observed for

- the quantity of mucous exudate,
- degree of hyperemia and thickening of stomach wall,
- hemorrhagic spots (focal or diffuse), nature of hemorrhages (petechial or ecchymotic) along with the size and
- perforations or any other lesions

The observations on gastric mucosal irritation of animals of various groups were summarized below

Table 10: Test item: Group: Dose (mg/kg): Observation

Test Item	**Group**	**Doe mg/kg (as per ibuprofen)**	**Observation**
Vehicle control	Vehicle control Group	0.0	None of the animals showed any evidence of gastric mucosal irritation
S-(+)-Ibuprofen-L-threonine ester	Test Group 1	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation
	Test Group 2	300.0	None of the dosed animals showed any evidence of gastric mucosal irritation.
(±)-Ibuprofen-L-serine ester	Test Group 1	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation.
	Test Group 2	300.0	None of the dosed animals showed any evidence of gastric mucosal irritation
(±)-Ibuprofen-L-hydroxyproline ester	Test Group 1	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation
	Test Group 2	300.0	None of the dosed animals showed any evidence of gastric mucosal irritation
Ibuprofen (racemic mixture)	Test Group 1	200.0	Gastric mucosal irritation was observed in one animal out of 5 animals dosed.

	Test Group 2	300.0	Gastric mucosal irritation was observed in two animals out of 5 animals dosed.
Ibuprofen (S) - (+)	Test Group 1	200.0	Gastric mucosal irritation was observed in all the 5 animals dosed.
	Test Group 2	300.0	Gastric mucosal irritation was observed in three animals out of 5 animals dosed.

The results of the present study showed that none of the formulations of ibuprofen had caused any evidence of irritation of gastric mucosa in fasted male albino rats of male sex at the two dose levels tested (200mg and 300mg/kg body weight). In contrast both ibuprofen (racemic mixture) and ibuprofen (S) – (+) had caused irritation of gastric mucosa at the two dose levels tested. Further ibuprofen(S) - (+) was found to be more gastric mucosal irritant than ibuprofen (racemic mixture).

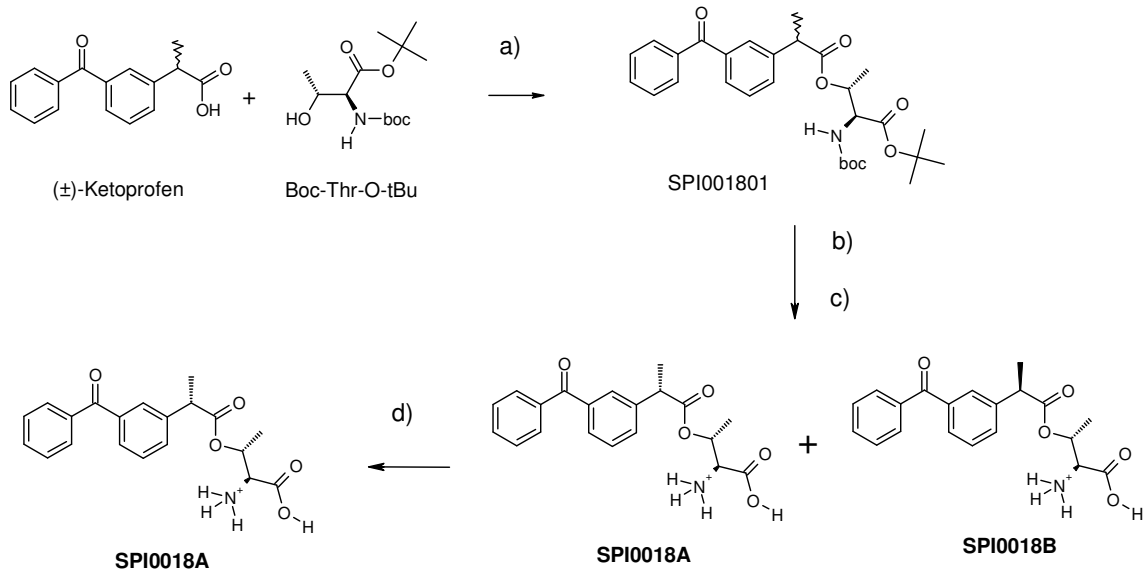
Overview Ketoprofen S(+) Threonine Ester Synthesis:

The procedure for the synthesis of the L-threonine esters of Ketoprofen is outlined in **Synthetic Sequence** section. This synthesis is exemplary and is equally applicable for the other amino acids. The complete procedure and analytical data is given in the **Experimental Section**. In general, (±)-Ketoprofen (5 g) was coupled with N-boc-L-threonine t-butyl ester[1] (1 equivalent) with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 1 equivalent) in the presence of a catalytic amount of 4-(N,N-dimethyamino)-pyridine (DMAP). Once the reaction was complete, any excess EDC was removed by extraction with water, DMAP was removed by extraction with dilute acid, and Ketoprofen was removed by extraction with sodium bicarbonate. After drying over sodium sulfate, filtration, and concentration the crude

protected L-threonine-(±)-Ketoprofen was purified by flash chromatography on silica gel to generate the protected L-threonine ester in good yield (98%). The protecting groups were removed by treatment with 2M hydrochloric acid in diethyl ether to cleave the boc group, followed by treatment with trifluoroacetic acid to remove the t-butyl ester. After drying, the mixture of L-threonine-R,S(±)-Ketoprofen esters was separated by crystallization from acetonitrile. The hydrochloride salt of the L-threonine-S(+)-Ketoprofen ester preferentially precipitated from acetonitrile. A sample of an optically pure standard was prepared starting with S(+)-ketoprofen for comparison. After drying and analysis, a sample of L-threonine-S(+)-Ketoprofen ester, hydrochloride (1.75 g) was separated from the mixture.

Synthetic Sequence:

SPI0018A



Synthesis of the L-threonine esters of (±)-Ketoprofen: a) EDC, DMAP, CH_2Cl_2; b) HCl (2M); c) TFA; d) ACN (crystallization).

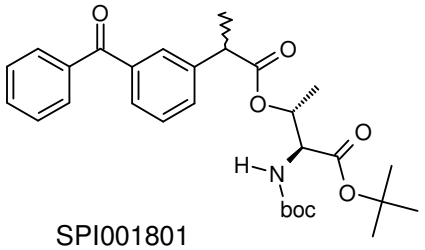
Experimental Section:

The synthesis of **SPI0018A** was conducted in a single batch. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Sigma-

Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

Preparation and Separation of S(+)-Ketoprofen-L-threonine ester, hydrochloride (SPI0018A).

(±)-Ketoprofen (5.32 g, 20.92 mmol), N-t-butylcarbonyl-L-threonine t-butyl ester (Boc-Thr-OtBu, 5.17 g, 18.72 mmol, (prepared in accordance with the literature), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 4.0 g, 20.9 mmol), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.22 g) were dissolved in dichloromethane (50 mL) at room temperature, under an argon atmosphere. After stirring for 5 hours, the dichloromethane layer was washed with water (50 mL), 5% hydrochloric acid (2×25 mL), water (25 mL), saturated sodium bicarbonate (2×25 mL), and water (50 mL). After drying for one hour over sodium sulfate (5 g), filtration, and concentration under reduced pressure, the remaining oil (10.3 g) was purified by column chromatography on silica gel (150 g), eluting with hexanes/ethyl acetate (2:1). After combining the product containing fractions, concentration and drying under high vacuum, the procedure generated the protected L-threonine-(±)-Ketoprofen ester (**SPI001801**) as a clear oil (9.42 g, 98% yield).



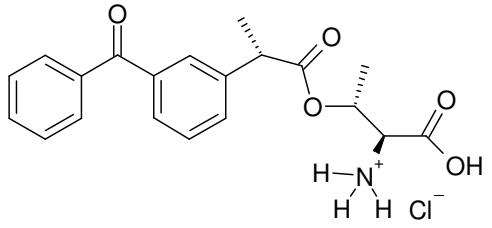
SPI001801

3-[2(R,S)-(3-Benzoyl-phenyl)-propionyloxy]-2(S)-tert-butoxycarbonylamino-butyric acid tert-butyl ester: (mix of diastereomers)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83-7.42 (m, 9H), 5.43 (dd, 1H, *J*= 13.2, 6.9 Hz), 5.10 (dd, 1H, *J*= 20.7, 9.3), 4.29 (t, 1H, *J*= 11.7 Hz), 3.75 (q, 1H, *J*= 7.2 Hz), 1.50-1.42 (m, 19.5H), 1.30-1.18 (m, 4.5H).

^{13}C NMR (75 MHz, $CDCl_3$): δ =. 196.18, 172.62, 172.55, 168.85, 168.58, 155.81, 140.33, 140.23, 137.86, 137.39, 132.46, 132.42, 131.54, 131.38, 130.00, 129.31, 129.13, 129.02, 128.54, 128.27, 82.50, 82.37, 80.05, 71.38, 71.22, 57.59, 57.52, 45.46, 45.31, 28.40, 27.98, 27.84, 18.54, 18.48, 17.19, 16.84.

The protected (R,S)-Ketoprofen-L-threonine ester (9.42 g, 18.41 mmol) was dissolved in dichloromethane (25 mL) under an argon atmosphere, at room temperature. Anhydrous hydrochloric acid in diethyl ether (2M, 25 mL) was added to the solution and the mixture was allowed to stir for 17 hours at room temperature. The mixture was concentrated under reduced pressure. The remaining foam (8.2 g) was dissolved in a mixture of dichloromethane (10 mL) and trifluoroacetic acid (20 mL). After stirring at room temperature for 6.5 hours the solution was concentrated under reduced pressure. Toluene (25 mL) was added to the remaining oil and the mixture was concentrated a second time. A mixture of ethanol (20 mL) and anhydrous hydrochloric acid in diethyl ether (2M, 20 mL) was added and the solution was concentrated a third time. After drying under high vacuum for 2 hours at room temperature, the experiment produced (±)-Ketoprofen-L-threonine ester, hydrochloride (mix of diastereomers, 7.11 g, 98% crude yield) as an off-white solid. The crude mixture of diastereomers (7.0 g) was crystallized 3 times from acetonitrile (200 mL). After the third crystallization, the remaining white solid was dried under high vacuum at 50 °C until the weight was constant (4 hours). The experiment produced L-threonine-S(+)-Ketoprofen ester, hydrochloride **SPI0018A** (2.2 g, 30% yield from SPI001801).



SPI0018A

2(S)-Amino-3(R)-[2(S)-(3-benzoyl-phenyl)-propionyloxy]-butyric acid, hydrochloride (L-threonine-S(+)-Ketoprofen ester, hydrochloride):

^{1}H NMR (300 MHz, DMSO): δ = 14.08 (br s, 1H), 8.72 (br s, 3H), 7.74-7.51 (m, 9H), 5.29 (t, 1H, J= 4.5 Hz), 4.16 (m, 1H), 3.97 (q, 1H, J= 6.3 Hz), 1.42 (d, 3H, J= 6.9 Hz), 1.23 (d, 3H, J= 6.3 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 195.34, 172.26, 168.21, 140.42, 137.05, 136.74, 132.66, 131.66, 129.48, 128.73, 128.49, 128.30, 68.23, 55.31, 44.00, 18.44, 16.45.

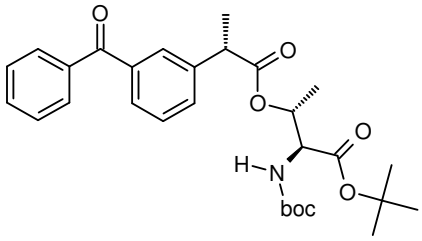
CHN analysis:
calc.: C 61.30, H 5.66, N 3.57; found: C 61.02, H 5.58, N 3.58.

HPLC analysis:
98.28% purity; r.t.= 25.14min.; 55% DIUF water (0.1% TFA)/45% methanol; 1 mL/min; 36.4 C; Luna C18, 5u column (serial # 211739-42), 4.6x250 mm; 20 ul injection.

Optical rotation: + 27.0 ° (20 C, 174.4 mg/10 mL ethanol, 589 nm); Melting Point: 166-167 °C

Preparation of the S-(+)-Ketoprofen-L-threonine ester, hydrochloride standard.

(+)-Ketoprofen (1.87 g, 7.74 mmol), N-t-butylcarbonyl-L-threonine t-butyl ester (Boc-Thr-OtBu, 2.25 g, 8.14 mmol, prepared in accordance with the literature method), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (EDC, 1.65 g, 8.60 mmol), and 4-(N,N-dimethylamino)-pyridine (DMAP, 0.1 g) were dissolved in dichloromethane (25 mL) at room temperature, under an argon atmosphere. After stirring for 4 hours, the dichloromethane layer was washed with water (25 mL). After drying for one hour over sodium sulfate (5 g), filtration, and concentration under reduced pressure, the remaining oil was used without purification. The procedure generated the protected L-threonine-(+)-Ketoprofen ester as a clear oil (4.01 g, ~100% yield).



^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81-7.42 (m, 9H), 5.43 (m, 1H), 5.10 (d, 1H, *J*= 9.3), 4.29 (d, 1H, *J*= 9.6 Hz), 3.75 (q, 1H, *J*= 7.2 Hz), 1.50-1.42 (m, 21H), 1.18 (d, 3H, J= 6.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.4, 172.79, 168.99, 155.94, 140.44, 137.99, 137.51, 132.59, 131.50, 130.13, 129.31, 129.25, 129.15, 128.66, 128.40, 82.68, 80.24, 71.37, 57.71, 45.43, 28.53, 28.10, 18.99, 16.96.

The protected (S)-Ketoprofen-L-threonine ester (3.92 g, 7.66 mmol) was dissolved in anhydrous hydrochloric acid in diethyl ether (2M, 50 mL) and stirred for 17 hours at room temperature. The mixture was concentrated under reduced pressure. The remaining foam (3.4 g) was dissolved in a mixture of dichloromethane (20 mL) and trifluoroacetic acid (20 mL). After stirring at room temperature for 6.5 hours the solution was concentrated under reduced pressure. Toluene (25 mL) was added to the remaining oil and the mixture was concentrated a second time. A mixture of ethanol (20 mL) and anhydrous hydrochloric acid in diethyl ether (2M, 20 mL) was added and the solution was concentrated a third time. After drying under high vacuum for 2 hours at room temperature, the experiment produced S(+)-Ketoprofen-L-threonine ester, hydrochloride (3.05g crude) as an off-white solid. The crude material was stirred with acetone (50 mL) for 2 hours at room temperature under an argon atmosphere. The remaining white solid was filtered and dried under high vacuum at 50 °C until the weight was constant (4 hours). The experiment produced L-threonine-S(+)-Ketoprofen ester, hydrochloride (2.04 g, 67 % yield).

^{1}H NMR (300 MHz, DMSO): δ = 14.08 (br s, 1H), 8.72 (br s, 3H), 7.74-7.51 (m, 9H), 5.29 (t, 1H, J= 4.5 Hz), 4.16 (m, 1H), 3.97 (q, 1H, J= 6.3 Hz), 1.42 (d, 3H, J= 6.9 Hz), 1.23 (d, 3H, J= 6.3 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 195.34, 172.26, 168.21, 140.42, 137.05, 136.74, 132.66, 131.66, 129.48, 128.73, 128.49, 128.30, 68.23, 55.31, 44.00, 18.44, 16.45.

HPLC analysis:
99.43% purity; r.t.= 25.14min.; 55% DIUF water (0.1% TFA)/45% methanol; 1 mL/min; 36.4 C; Luna C18, 5u column (serial # 211739-42), 4.6x250 mm; 20 ul injection.

Optical rotation: + 27.1 ° (20 C, 177.8 mg/10 mL ethanol, 589 nm); Melting Point: 166-167 °C

C. Amino Acid Derivaties of Aspirin

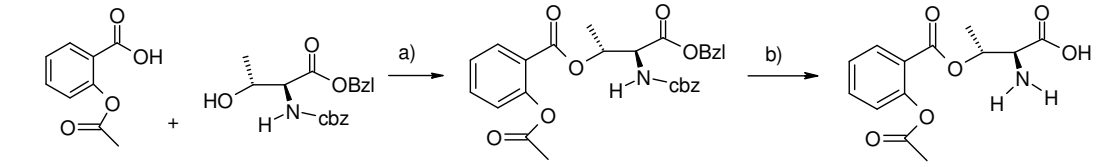
Overview:

The procedure for the synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of acetylsalicylic acid is outlined in **Synthetic Sequence** section and is exemplary for other amino acids. The complete procedure and analytical data is given in the **Experimental Section**. In general, acetylsalicyloyl chloride (10 g-25 g, in batches) was coupled with the N-benzyloxy/benzyl ester protected amino acids in the presence of pyridine. Once the reactions were complete (24 to 48 hours at room temperature), the mixture was poured into ice-cold 2N hydrochloric acid. The dichloromethane fraction was then washed with sodium bicarbonate, water and brine. After drying over sodium sulfate, filtration, and concentration the crude protected amino acid esters of acetylsalicylic acid were purified by flash chromatography on silica gel. The procedure generated the protected amino acid esters of acetylsalicylic acid in yields ranging from 68% to 95%. The protecting groups were removed by hydrogenation (20 psi H_2) in the presence of 10% palladium on carbon. The amino acid esters of acetylsalicylic acid were extracted away from the palladium catalyst with water, concentrated, and dried.

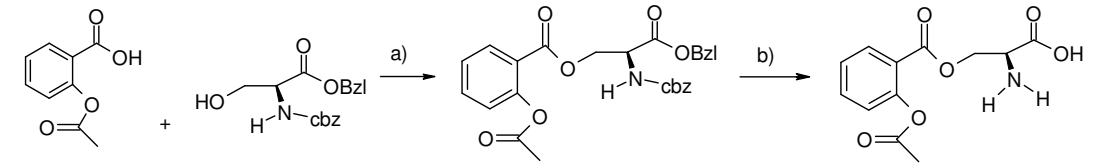
The final compounds were washed with solvent (water, dioxane, acetonitrile, and/or dichloromethane) until pure and dried under high vacuum until a constant weight was achieved.

Synthetic Sequence:

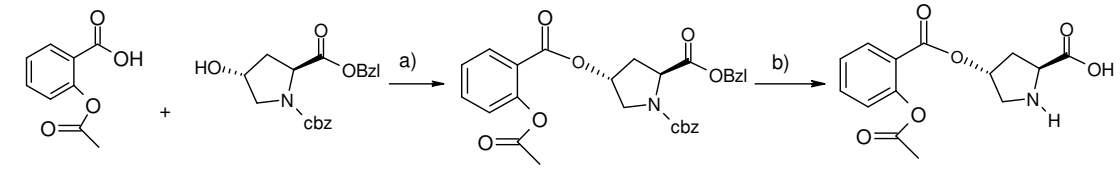
1. SPIB00102



2. SPIB00101



3. SPIB00103



Synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of acetylsalicylic acid: a) pyridine, CH_2Cl_2; b) 10% Pd/C, EtOH, EtOAc.

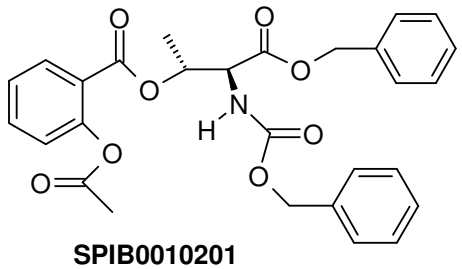
Experimental Section:

The synthesis of **SPIB00101**, **SPIB00102** and **SPIB00103** was conducted in one or two batches. Reagents mentioned in the experimental section were purchased at the highest

obtainable purity from Lancaster, Sigma-Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

1) SPIB00102: 2-O-Acetylsalicylic acid (2S, 3R)-(-)-threonine ester

A mixture of N-carbobenzyloxy-L-threonine benzyl ester (Z-Thr-OBzl, 21.77 g, 63.40 mmole) and pyridine (25 mL) in anhydrous dichloromethane (500 mL) was cooled in an ice bath while under a nitrogen atmosphere. Acetylsalicyloyl chloride (17.63 g, 88.76 mmole) was added and the mixture was allowed to warm to room temperature and stir overnight. After 24 hours, the mixture was poured into ice-cold 2N hydrochloric acid (400 mL). After mixing, the layers were separated and the dichloromethane fraction was washed with water (500 mL), saturated sodium bicarbonate solution (500 mL), water (500 mL), brine (500 mL) and dried over sodium sulfate (25 g). After filtration, concentration under reduced pressure, and drying under high vacuum, the remaining yellow oil (35. 43 g) was purified by flash chromatography on silica gel (300 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with hexanes/ethyl acetate (3:1). After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected acetylsalicylic-L-threonine ester **SPIB0010201** (28.1 g, 88% yield) as a colorless oil.

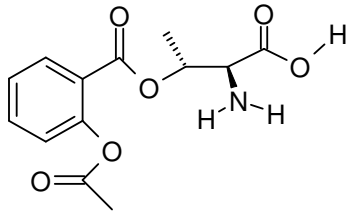


SPIB0010201

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (1H, d, *J*= 7.5 Hz), 7.51 (1H, dt, *J*= 7.5, 1.5 Hz), 7.34-7.17 (11H, m), 7.06 (1H, d, *J*= 7.2 Hz), 5.62 (2H, m), 5.13 (4H, m), 4.65 (1H, dd, *J*= 9.6, 2.4 Hz), 2.29 (3H, s), 1.38 (3H, d, *J*= 6.6 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.35, 169.22, 162.73, 156.26, 150.41, 135.79, 134.67, 133.77, 131.24, 128.35, 128.24, 128.08, 127.95, 125.78, 123.51, 122.61, 71.22, 67.72, 67.26, 57.64, 20.98, 16.88.

The protected acetylsalicylic-L-threonine ester **SPIB0010201** (14.50 g, 28.68 mmole) was dissolved in ethanol (100 mL) and ethyl acetate (100 mL) at room temperature and added to a Parr bottle that contained 10% palladium on carbon (3.0 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (20 psi). After 20 hours of shaking, the palladium catalyst was removed by filtration through celite. The remaining solids (palladium/celite and product) were washed with water (600×4 mL) until the product was removed. The ethanol and water fractions were concentrated under reduced pressure at room temperature. The remaining solids were washed with water (20 mL) and dioxane (20 mL) for 48 hours. After filtration, the remaining white solid was dried at room temperature under high vacuum until the product weight was constant (16 hours). The experiment produced acetylsalicylic-L-threonine ester, **SPIB00102** (4.40 g, 55% yield) as a white solid.



SPIB00102

^{1}H NMR (300 MHz, D_2O-DCl): δ = 8.00 (1H, dd, J= 7.8, 1.5 Hz), 7.74 (1H, dt, J= 7.8, 1.5 Hz), 7.47 (1H, dt, J= 7.8, 1.5 Hz), 7.27 (1H, dd, J= 7.8, 1.5 Hz), 5.76 (1H, dq, J= 6.9, 3.0 Hz), 4.49 (1H, d, J= 3.0 Hz), 2.39 (3H, s), 1.55 (3H, d, J= 6.9 Hz).

^{13}C NMR (75 MHz, D_2O-DCl): δ = 173.03, 168.84, 163.97, 149.56, 135.32, 131.26, 126.85, 123.48, 121.49, 69.16, 56.36, 20.45, 15.86.

HPLC analysis:

98.7% purity; rt= 6.233 min; Luna C18 5u column (sn 167917-13); 4.6x250 mm; 254 nm; 35% MeOH/65% TFA (0.1%) pH= 1.95; 35 C; 20 ul inj.; 1ml/min; sample dissolved in mobile phase with 1 drop phosphoric acid.

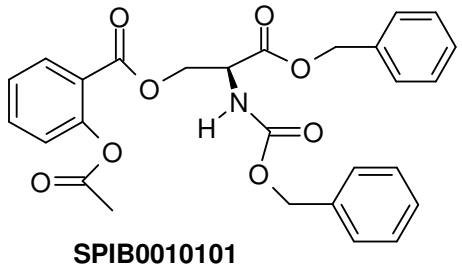
CHN analysis:

calc.: C 55.51, H 5.38, N 4.98; found: C 55.37, H 5.40, N 5.03.

Melting point: 153.5 °C (dec.)

2) SPIB00101: 2-O-Acetylsalicylic acid (2S)-(+)-serine ester

A mixture of N-carbobenzyloxy-L-serine benzyl ester (Z-Ser-OBzl, 23.17 g, 70.34 mmole) and pyridine (30 mL) in anhydrous dichloromethane (500 mL) was cooled in an ice bath while under a nitrogen atmosphere. Acetylsalicyloyl chloride (21.07 g, 106.1 mmole) was added and the mixture was allowed to warm to room temperature and stir over two days. After 48 hours, the mixture was poured into ice-cold 2N hydrochloric acid (400 mL). After mixing, the layers were separated and the dichloromethane fraction was washed water (500 mL), saturated sodium bicarbonate solution (500 mL), water (500 mL), brine (500 mL) and dried over sodium sulfate (25 g). After filtration, concentration under reduced pressure, and drying under high vacuum, the remaining brown solid (47.19 g) was purified by flash chromatography on silica gel (200 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with hexanes/ethyl acetate (3:1). After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected acetylsalicylic-L-serine ester **SPIB0010101** (32.97 g, 95% yield) as a white solid.



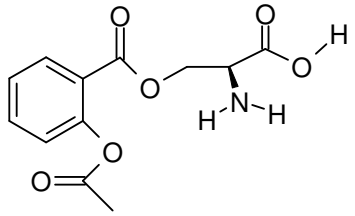
SPIB0010101

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (1H, d, *J*= 7.8 Hz), 7.55 (1H, dt, *J*= 7.8, 1.5 Hz), 7.33-7.21 (11H, m), 7.08 (1H, d, *J*= 7.5 Hz), 5.68 (1H, d, *J*= 8.4 Hz), 5.20 (2H, s), 5.12 (2H, s), 4.77 (1H, m), 4.66 (1H, dd, *J*= 11.4, 3.3 Hz), 4.57 (1H, dd, *J*= 11.4, 3.3 Hz), 2.30 (3H, s).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.45, 169.09, 163.68, 163.35, 155.57, 150.77, 135.87, 134.75, 134.07, 131.44, 128.50, 128.43, 128.27, 128.14, 128.04, 125.92, 123.71, 122.18, 67.83, 67.27, 64.63, 53.55, 21.03.

The protected acetylsalicylic-L-serine ester **SPIB0010101** (21.0 g, 42.7 mmole) was dissolved in ethanol (100 mL) and ethyl acetate (100 mL) at room temperature and added to a Parr bottle that contained 10% palladium on carbon (4.20 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (20 psi). After 5 hours additional 10% palladium catalyst (4.26 g) was added and the hydrogen atmospere was returned (20 psi). After an additional 20 hours of shaking at room temperature, the palladium catalyst was removed by filtration through celite. The remaining solids (palladium/celite and product) were washed with water (1500×2 mL) until the product was removed. The ethanol and water fractions were concentrated under reduced pressure at room temperature. The remaining solid (7.17 g) was dissolved in DIUF water (4.3 L), filtered through celite to remove insoluble material, and concentrated under high vacuum at room temperature. The white solid was then washed with 1,4-dioxane (100 mL) and DIUF water (50 mL) overnight. After 24 hours the solid was filtered and dried under high vacuum until the weight was constant (24 hours).

The experiment produced the acetylsalicylic-L-serine ester **SPIB00101** (6.17 g, 54% yield) as a white solid.



SPIB00101

^{1}H NMR (300 MHz, D_2O-DCl): δ = 8.05 (1H, dd, J= 7.8, 1.5 Hz), 7.75 (1H, dt, J= 7.8, 1.5 Hz), 7.47 (1H, dt, J= 7.8, 0.9 Hz), 7.27 (1H, dd, J= 7.8, 0.9 Hz), 4.87 (1H, dd, J= 12.6, 4.2 Hz), 4.79 (1H, dd, J= 12.6, 3.0 Hz), 4.62 (1H, dd, J= 4.2, 3.0 Hz), 2.39 (3H, s).

^{13}C NMR (75 MHz, D_2O-DCl): δ = 173.01, 168.58, 164.54, 149.72, 135.39, 131.59, 126.87, 123.62, 121.15, 62.38, 52.05, 20.44.

HPLC analysis:
98.1% purity; r.t.= 5.839 min.; 65% TFA (0.1%)/35% methanol; 1 mL/min; 35 C; Luna C18, 3u column (SN 184225-37), 4.6x250 mm; 22 ul injection; DAD1B, Sig= 240, 4 Ref= 550,100.

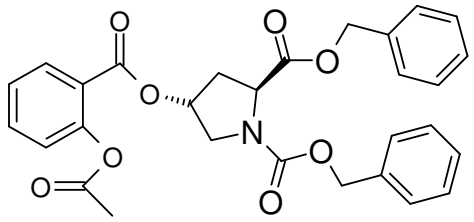
CHN analysis:
calc.: C 53.93, H 4.90, N 5.24; found: C 54.02, H 5.00, N 5.23.

Melting point: 147.0 °C (dec.)

3) SPIB00103: 2-O-Acetylsalicylic acid (2S, 4R)-4-hydroxyproline ester

A mixture of N-carbobenzyloxy-L-hydroxyproline benzyl ester (Z-Ser-OBzl, 21.5 g, 60.5 mmole)[1] and pyridine (25 mL) in anhydrous dichloromethane (500 mL) was cooled in an ice bath while under a nitrogen atmosphere. Acetylsalicyloyl chloride (13.2

g, 66.6 mmole) was added and the mixture was allowed to warm to room temperature and stir overnight. After 24 hours, additional acetylsalicyloyl chloride (5.0 g, 25.2 mmole) was added and the mixture was allowed to stir overnight. After 48 hours, the mixture was poured into ice-cold 1N hydrochloric acid (500 mL). After mixing, the layers were separated and the dichloromethane fraction was washed with water (500 mL), saturated sodium bicarbonate solution (500 mL), water (500 mL), brine (500 mL) and dried over sodium sulfate (25 g). After filtration, concentration under reduced pressure, and drying under high vacuum, the remaining yellow oil (40.7 g) was purified by flash chromatography on silica gel (460 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with heptane/ethyl acetate (3:1). After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected acetylsalicylic-L-hydroxyproline ester **SPIB0010301** (21.31 g, 68% yield) as a colorless oil.

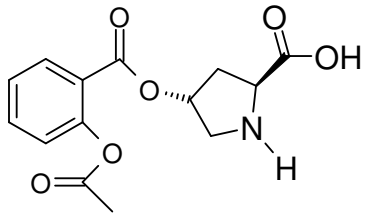


SPIB0010301

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.92 (1H, d, *J*= 7.8 Hz), 7.56 (1H, t, *J*= 7.8 Hz), 7.34-7.21 (10H, m), 7.09 (1H, d, *J*= 7.8 Hz), 5.48 (1H, s), 5.21 (2H, m), 5.03 (2H, d, J=15 Hz), 4.57 (1H, m), 3.85 (2H, m), 2.53 (1H, m), 2.28 (4H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.72, 171.49, 169.25, 163.47, 163.30, 154.52, 153.93, 150.54, 136.05, 135.94, 135.21, 135.00, 134.17, 134.12, 128.43, 128.32, 128.28, 128.20, 128.05, 127.98, 127.94, 127.79, 125.89, 123.70, 122.46, 122.38, 73.24, 72.59, 67.33, 67.11, 66.97, 58.02, 57.69, 52.47, 52.15, 36.74, 35.65, 20.90.

The protected acetylsalicylic-L-hydroxyproline ester **SPIB0010301** (10.6 g, 20.5 mmole) was dissolved in ethanol (75 mL) and ethyl acetate (75 mL) at room temperature and added to a Parr bottle that contained 10% palladium on carbon (3.0 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (20 psi). After 17 hours of shaking at room temperature, the reaction mixture was washed with water (500 mL) for two hours. The organic layer (top) was removed via pipette and the aqueous layer was filtered through celite. The water fraction was concentrated under reduced pressure at room temperature. The remaining solid (6.71 g) was then washed with anhydrous dichloromethane (35 mL) overnight. After 24 hours the solid was filtered and dried under high vacuum until the weight was constant (24 hours). The experiment produced acetylsalicylic-L-hydroxyproline ester, **SPIB00301** (2.87 g, 47.7 % yield) as a white solid.



SPIB00103

^{1}H NMR (300 MHz, D_2O-DCl): δ = 8.09 (1H, d, J= 7.5 Hz), 7.75 (1H, t, J= 7.5 Hz), 7.48 (1H, t, J= 7.5 Hz), 7.28 (1H, d, J= 7.5 Hz), 5.69 (1H, m), 4.76 (1H, t, J=7.5 Hz), 3.86 (1H, dd, J= 13.5, 3.9 Hz), 3.74 (1H, d, J= 13.5 Hz), 2.81 (1H, dd, J= 15.0, 7.5 Hz), 2.60 (1H, m), 2.40 (3H, s).

^{13}C NMR (75 MHz, D_2O-DCl): δ = 173.13, 170.25, 164.31, 149.65, 135.36, 131.54, 126.87, 123.54, 121.37, 73.86, 58.34, 50.95, 34.38, 20.48.

HPLC analysis:

98.3% purity; r.t.= 7.201 min.; 65% TFA (0.1%)/35% methanol; 1 mL/min; 35 C; Luna C18, 3u column (SN 184225-37), 4.6x250 mm; 22 ul injection; DAD1B, Sig= 240, 4 Ref= 550,100.

CHN analysis:

calc.: C 57.34, H 5.16, N 4.78; found: C 57.09, H 5.23, N 4.91.

Melting point: 162 °C (dec.)

Gastric Mucosa irritation potential of the L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid compared to acetylsalicylic acid:-

The present study was conducted to determine the relative potential of new formulations of aspirin (**L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid)** to cause gastric mucosal irritation/lesions in fasted male albino rats. Aspirin served as reference control.

Different new formulations of aspirin and aspirin were administered by gavage to fasted male albino rats (Wistar strain), using 0.5% (w/v) Carboxymethylcellulose (CMC) in Phosphate Buffer (pH 2.6) solution as the vehicle. The study was conducted at two dose levels viz. 100mg and 200mg/kg body weight along with a vehicle control group. At each dose level 5 animals were used. All the doses were expressed as aspirin molar equivalents. The doses used as well as the molar equivalents were presented below.

Table 11:Formulation: Molar equivalent

Formulation	Molar equivalent
L-serine ester of acetylsalicylic acid	1.483 units are equivalent to 1 unit of aspirin
L-Hydroxyproline ester of acetylsalicylic acid	1.628 units are equivalent to 1 unit of aspirin
L-threonine ester of acetylsalicylic acid	1.561 units are equivalent to 1 unit of aspirin.

Table 12: Test Item: Group: Dose (mg per kg) [in terms of acetylsalicylic acid] : Equivalent weight of the Test item [mg]

Test Item	**Group**	**Dose (mg per kg) [in terms of acetylsalicylic acid]**	**Equivalent weight of the Test item [mg]**
Vehicle control	Vehicle control Group	0.0	--
L-serine ester of acetylsalicylic acid	Test Group 1	100.0	148.3
	Test Group 2	200.0	296.6
L-Hydroxyproline ester of acetylsalicylic acid	Test Group 1	100.0	162.8
	Test Group 2	200.0	325.6
L-threonine, ester of acetylsalicylic acid	Test Group 1	100.0	156.1
	Test Group 2	200.0	312.2
Reference control acetylsalicylic acid	Test Group 1	100.0	100.0
	Test Group 2	200.0	200.0

The rats were fasted for a period of 18 to 22 hours before dosing. The test item was administered as a single dose by gavage. Three hours after drug administration, the animals were killed humanely by CO_2 gas inhalation. The stomach was dissected out and observed for

- the quantity of mucous exudate,
- degree of hyperemia and thickening of stomach wall,
- hemorrhagic spots (focal or diffuse), nature of hemorrhages (petechial or ecchymotic) along with the size and
- perforations

The observations on gastric mucosal irritation of animals of various groups were summarized below

Table 13: Test Item: Group: Dose mg/kg (as acetylsalicylic acid): Observation

Test Item	Group	Dose mg/kg (as acetylsalicylic acid	Observation
Vehicle control	Vehicle control Group	0.0	None of the animals showed any evidence of gastric mucosal irritation
L-serine ester of acetylsalicylic acid	Test Group 1	100.0	None of the dosed animals showed any evidence of gastric mucosal irritation
	Test Group 2	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation.
L-Hydroxyprolin ester of acetylsalicylic acid	Test Group 1	100.0	None of the dosed animals showed any evidence of gastric mucosal irritation.
	Test Group 2	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation
L-threonine, ester of acetylsalicylic acid	Test Group 1	100.0	None of the dosed animals showed any evidence of gastric mucosal irritation
	Test Group 2	200.0	None of the dosed animals showed any evidence of gastric mucosal irritation
Reference control (acetylsalicylic acid)	Test Group 1	100.0	None of the dosed animals showed any evidence of gastric mucosal irritation
	Test Group 2	200.0	All the 5 animals dosed, showed evidence of gastric mucosal irritation.

In conclusion it was observed that none of the L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid induced any evidence of irritation of gastric mucosa at the two doses tested *viz.,* 100 and 200mg/kg body weight In contrast, aspirin (acetylsalicylic acid) has caused irritation of the gastric mucosal in all the fasted male albino rats at the dose level of 200mg/kg. However at the dose level of 100mg/kg aspirin failed to cause any evidence of gastric mucosal irritation in the male rats.

Further none of the animals of different test groups showed any clinical symptoms of toxicity through out the observation period of three hours.

Efficacy of L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid compared to acetylsalicylic acid on Clotting Time in rats estimated one hour after dosing

Observations of blood clotting time

The data on the mean clotting time (MCT) of the animals of low, intermediate and high dose groups of different formulations, vehicle control and positive control groups estimated one hour after dosing were presented below (Table 14):

Table 14: Summary of Mean Clotting Time (± S.D.) in Minutes - L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid and Aspirin (Positive control): Low dose: Intermediate dose: High dose

	Low Dose	**Intermediate Dose**	**High Dose**
Vehicle control	4.9 ± 1.10		
L-serine ester of acetylsalicylic acid	5.7 ± 1.34	6.8 ± 1.48	6.9 ± 1.37
L-Hydroxyprolin ester of acetylsalicylic acid	6.1 ± 1.10	5.7 ± 0.82	7.5 ± 1.18
L-threonine, ester of acetylsalicylic acid	5.2 ± 1.14	5.6 ± 0.84	7.4 ± 0.97
Positive control (acetylsalicylic acid)	6.2 ± 1.40	8.1 ± 1.97	9.8 ± 1.32

FIG. 3-6 depict the group mean data of animals regarding the dose relationship + mean clotting time in minutes for the L-series ester of aspirin and for the control.

The statistical analysis showed a significant improvement at 5% significance level in the efficacy for the high dose and mid dose when compared to the vehicle control group (Figure 7).

FIG. 4 shows the group mean data of animals. It provides the dose response relationship to mean clotting time (MCT) in minutes with respect to L-hydroxyproline ester of asperin. The statistical analysis of FIG. 4 showed a significant improvement at 5% significance level in the efficacy for the high dose and low dose when compared to the vehicle control group (Figure 6)

FIG. 5 depicts the dose response relationship to mean clotting time (MCT) in minutes of L-threonine ester of acetylsalicylic acid. The statistical analysis showed a significant improvement at 5% significance level in the efficacy for the high dose when compared to the vehicle control.

FIG. 6 depicts the dose response relationship to mean clotting time for acetylsalicylic acid. The statistical analysis showed a significant improvement at 5% significance level in the efficacy for the intermediate and high dose when compared to the vehicle control. The dose response effect were statistically significant and clearly evident (Figure 7).

CONCLUSION

The present study was conducted to evaluate the efficacy of new formulations of aspirin using blood clotting time as an index in albino rats. Aspirin served as positive control. The study was conducted at three dose levels with the new formulations and positive control along with a vehicle control group.

Doses

The doses for the main study were selected based on the dose range finding experiments with acetylsalicylic acid. All the doses were expressed as aspirin molar equivalents. The doses used for the main experiment for different formulations and positive control were same and presented below.

Table 15: Test Item: Low Dose (mg/kg): Intermediate dose 9mg/kg): High dose (mg/kg)

Test Item	**Low Dose (mg/kg)**	**Intermediate Dose (mg/kg)**	**High Dose (mg/kg)**
L-serine ester of acetylsalicylic acid	1.0	4.0	10.0
L-Hydroxyprolin ester of acetylsalicylic acid	1.0	4.0	10.0
L-threonine, ester of acetylsalicylic acid	1.0	4.0	10.0
Aspirin (Positive control)	1.0	4.0	10.0

Efficacy (Blood clotting time)

The efficacy in terms of time required for the blood clotting time at different dose levels – low, intermediate and high dose for different formulations and acetylsalicylic acid are presented below.

Table 16: Low dose: Intermediate dose: High dose

	Low Dose	**Intermediate Dose**	**High Dose**
Vehicle control	4.9 ± 1.10		
L-serine ester of acetylsalicylic acid	5.7 ± 1.34	6.8 ± 1.48	6.9 ± 1.37
L-Hydroxyprolin ester of acetylsalicylic acid	6.1 ± 1.10	5.7 ± 0.82	7.5 ± 1.18
L-threonine, ester of acetylsalicylic acid	5.2 ± 1.14	5.6 ± 0.84	7.4 ± 0.97
Positive control	6.2 ± 1.40	8.1 ± 1.97	9.8 ± 1.32

L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid are as significant as acetylsalicylic acid with respect to clotting time observed after one hour but are far superior in terms of the absence of gastric irritation at all levels compared to acetylsalicylic acid.

Efficacy of L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid compared to acetylsalicylic acid on Clotting Time in rats estimated two hours after dosing

The present study was conducted to evaluate the efficacy of L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid compared to acetylsalicylic acid using blood clotting time, estimated 2 hours (± 10 minutes) after dosing, as an index in albino rats. Aspirin served as positive control. Male albino rats were exposed to aspirin and to 3 new formulations of aspirin at one dose level of 20mg/kg body weight. No vehicle control group was used. The doses were expressed as aspirin molar equivalents. The doses used for the main experiment for different formulations and positive control was presented below.

Table 17: Test Item: Dose in terms of Acetylsalicylic acid 9mg/kg)

Test Item	Dose in terms of Acetylsalicylic acid (mg/kg)
L-serine ester of acetylsalicylic acid	20.0
L-Hydroxyproline ester of acetylsalicylic acid	20.0
L-threonine ester of acetylsalicylic acid	20.0
Aspirin (Positive control)	20.0

Efficacy (Blood clotting time)

The efficacy in terms of time required for the blood clotting time at the dose level of 20 mg/kg body weight for different formulations and aspirin (positive control) are presented below.

Observations of Blood clotting time

The data on the mean clotting time (MCT) of the animals, estimated 2 hours (± 10 minutes) after dosing, at the dose level of 20mg/kg body weight for different formulations, vehicle control and positive control are presented below

Table 18: Summary of Mean Clotting Time (± S.D.) in Minutes of L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid compared to acetylsalicylic acid (Positive control)

	Dose (20mg/kg)
L-serine ester of acetylsalicylic acid	3.8 ± 0.92
L-Hydroxyproline ester of acetylsalicylic acid	4.2 ± 1.32
L-threonine ester of acetylsalicylic acid	5.3 ± 1.06
Positive control (acetylsalicylic acid)	5.4 ± 1.17

L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid were found to be effective on clotting time.

In conclusion**,** it was observed that based on the time required for the blood to clot (clotting time), when estimated 2 hours after dosing, the amino acid prodrugs were efficacous. However, the L-threonine ester of acetylsalicyclic acid was found to have relatively better efficacy than the other two formulations.

As shown by FIG. 7 the statistical analysis showed that L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid are as effective as acetylsalicylic acid there is no significant difference at 5% significance level for L-Hydroxyproline ester of acetylsalicylic acid and L-threonine ester of acetylsalicylic with respect to positive control for the mean blood clotting time observed after two hours. However, combined with the gastric irritation potential, the L-serine, L-threonine, and L-Hydroxyproline esters of acetylsalicylic acid are far superior.

There are a number of screening tests to determine the utility of the prodrugs created according to the disclosed methods. These include both in vitro and in vivo screening methods.

The in vitro methods include acid/base hydrolysis of the prodrugs, hydrolysis in pig pancreas hydrolysis in rat intestinal fluid, hydrolysis in human gastric fluid, hydrolysis in human intestinal fluid, and hydrolysis in human blood plasma. These assays are described in Simmons, DM, Chandran, VR and Portmann, GA, Danazol Amino Acid Prodrugs: In Vitro and In Situ Biopharmaceutical Evaluation, Drug Development and Industrial Pharmacy, Vol 21, Issue 6, Page 687, 1995, the contents of all of which are incorporated by reference.

The compounds of the present invention are effective in treating diseases or conditions in which NSAIDs normally are used. The prodrugs disclosed herein are transformed within the body to release the active compound and enhances the therapeutic benefits of the NSAIDs by reducing or eliminating biopharmaceutical and pharmacokenetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals. Since the prodrugs is more soluble in water then Ibuprofen or other NSAIDs, it does not need to be associated with a carrier vehicle, such as alcohol or castor oil which may be toxic or produce unwanted side reactions. Moreover, oral formulations containing the NSAID prodrugs are absorbed into the blood and are quite effective.

Thus, the prodrug of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs.

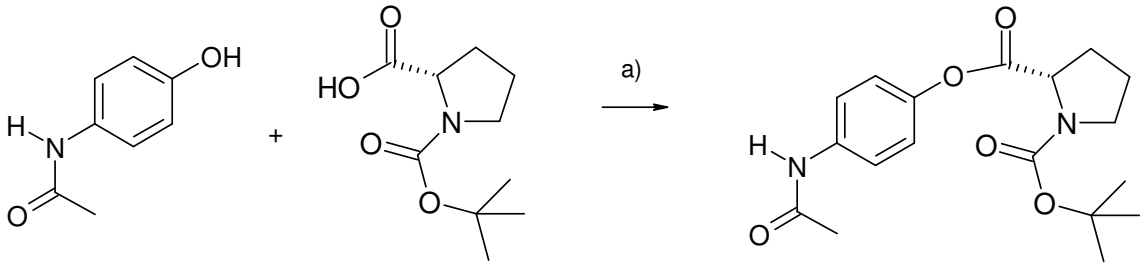
Furthermore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

IV. Proline Derivative of Acetaminophen

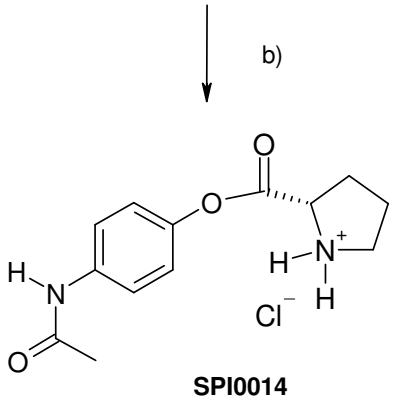
Overview:

The procedure for the synthesis of the L-proline ester of acetaminophen is outlined in **Synthetic Sequence** section. The synthesis is exemplary. The complete procedure and analytical data is given in the **Experimental Section**. Acetaminophen (10 g) was coupled with Boc-L-proline with EDC in the presence of a catalytic amount of DMAP. Once the reaction was complete (3 hours at room temperature), the solution was washed with water. After drying over sodium sulfate, filtration, and concentration the crude protected amino acid ester of acetaminophen was purified by flash chromatography on silica gel. The procedure generated the protected L-proline ester of acetaminophen in 72%. The protecting group was removed by dissolving the ester in dichloromethane and passing hydrogen chloride through the solution at room temperature. After filtration, the final salt was stirred in tetrahydrofuran until pure. The yield for the deprotection step was 91.4% after filtration and drying under high vacuum at 90 °C for 4 hours.

Synthetic Sequence:



Acetaminophen boc-L-proline SPI001401



SPI0014

Synthesis of the L-proline ester of acetaminophen: a) EDC, DMAP, CH_2Cl_2; b) HCl (g), CH_2Cl_2.

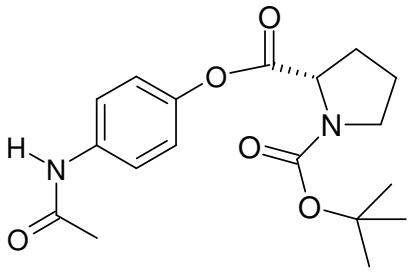
Experimental Section:

The synthesis of **SPI0014** was conducted in one batch. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Lancaster, Sigma-Aldrich, or Acros, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

SPI0014: Pyrrolidine-2(S)-carboxylic acid 4-acetylamino-phenyl ester, hydrochloride

A mixture of Boc-L-proline (14.39 g, 68.80 mmole), acetaminophen (10.02 g, 66.28 mmole), EDC (12.9 g, 67.29 mmole) and DMAP (1.10 g, 9.0 mmole) in anhydrous dichloromethane (100 mL) was stirred for 3 hours at room temperature under an argon

atmosphere. After 3 hours, water (120 mL) was added. After mixing for 5 minutes, the layers were separated and the dichloromethane fraction was washed with water (120 mL) and dried over sodium sulfate (5 g). After filtration, concentration under reduced pressure, and drying under high vacuum, the remaining oil (24.10 g) was purified by flash chromatography on silica gel (100 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with hexanes/ethyl acetate (1:2). After concentration of the product containing fractions under reduced pressure and drying at high vacuum until the weight was constant, the experiment produced the protected acetaminophen-L-proline ester **SPI001401** (16.71 g, 72.3% yield) as a white solid (foam).



SPI001401

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.83 (1/2 H, s), 8.70 (1/2 H, s), 7.58 (1/2 H, d, *J*= 7.5 Hz), 7.46 (1/2 H, d, *J*= 7.5 Hz), 6.96 (2 H, m), 4.47 (1 H, m), 3.59-3.45 (2H, m), 2.36 (1 H, m), 2.17-1.90 (6 H, m), 1.46 (9 H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.91, 171.75, 169.02, 154.44, 153.78, 146.36, 146.21, 121.44, 121.23, 120.82, 80.41, 80.17, 59.16, 46.78, 46.55, 31.06, 30.11, 28.50, 24.57, 24.28, 23.78.

The protected acetaminophen-L-proline ester **SPI001401** (16.60 g, 47.64 mmole) was dissolved in dichloromethane (400 mL) and hydrogen chloride gas was passed through the solution for 2 hours at room temperature. The remaining solids were allowed to settle (for 1 hour). The dichloromethane was carefully decanted away from the white precipitate. Tetrahydrofuran (200 mL) was added to the precipitate and the mixture stirred for 2 hours under an argon atmosphere. After filtration, the remaining white solid

was dried under high vacuum at 90 °C until the product weight was constant (4 hours). The experiment produced acetaminophen-L-proline ester, hydrochloride **SPI0014** (12.4 g, 91.4% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCL_3$-DMSO): δ = 10.41 (1H, br s), 10.26 (1H, s), 9.55 (1H, br s), 7.70 (2H, d, J= 9 Hz), 7.12 (2H, d, J= 9 Hz), 4.66 (t, 1H, J= 8.4 Hz), 3.33 (2H, m), 2.43 (1H, m), 2.28 (1H, m), 2.08 (s, 3H), 2.04 (2H, m).

^{13}C NMR (75 MHz, $CDCL_3$-DMSO): δ = 168.08, 167.25, 144.55, 137.40, 121.12, 119.64, 58.53, 45.33, 27.74, 23.86, 23.08.

HPLC analysis:
99.45% purity; rt= 5.733 min; Luna C18 5u column (sn 167917-13); 4.6x250 mm; 254 nm; 15% MeOH/85% hexane sulfonate buffer (110mMol, pH= 6); 35 C; 20 ul inj.; 1ml/min; 5 mg/mL sample size.

CHN analysis:
calc.: C 54.84, H 6.02, N 9.84; found: C 54.66, H 5.98, N 9.65.

Melting point: 221-222 °C

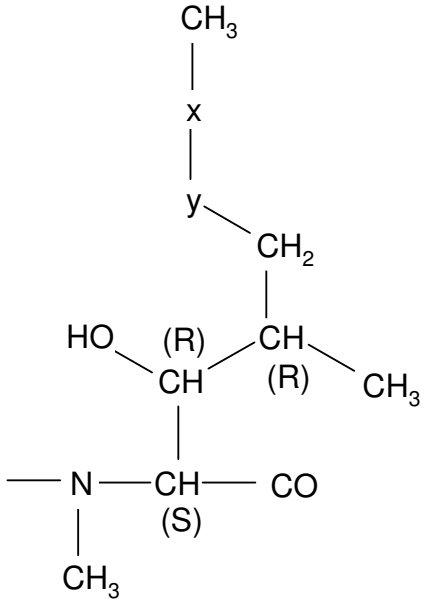
V. Amino Acid Derivative of Cyclosporine A

The macrocyclic immunosuppresants comprise a class of structurally distinctive, cyclic, poly, N-methylated undecaptides, and similar semi-synthetic macrolide structures commonly possessing pharmacological, in particular immunosuppressive, anti-inflammatory and/or anti-parasitic activity. The first of the cyclosporine to be isolated was the naturally occurring fungal metabolite Ciclosporin or Cyclosporine also known as cyclosporine A, which has the formula:

MeBmt-∀Abu-Sar-MeLeu-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal

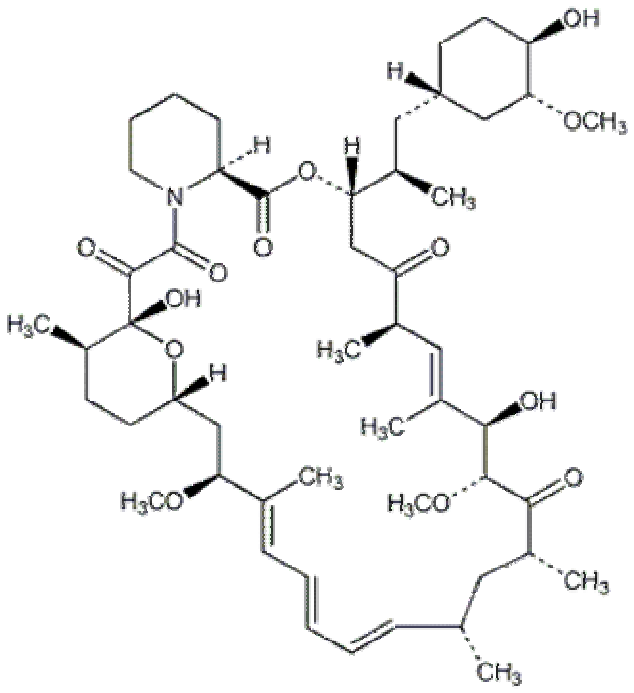
1 2 3 4 5 6 7 8 9 10 11

wherein MeBmt represents N-methyl-(4R)-4-but-2E-en-1-yl-4-methyl-(L) threonyl residue of the formula



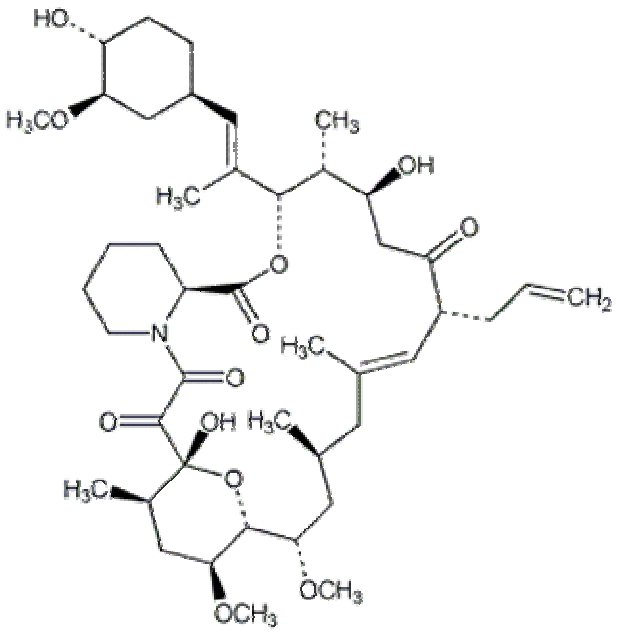
in which –x-y- is CH=CH –(trans). Other similar products include, sirolimus (b), tacrolimus (c), and pimecrolimus (d), having the following structures:

(b)



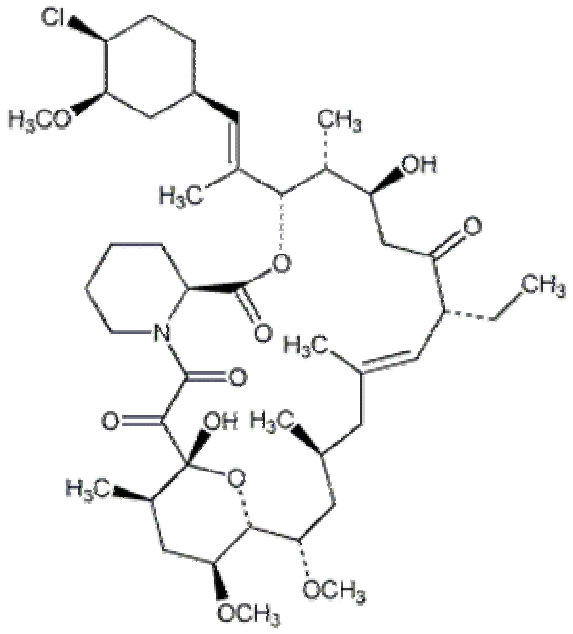
Sirolimus

(c)



Tacrolimus

(d)



Pimecrolimus

The class comprised by the cyclosporines is thus now very large indeed and includes, for example, $[Thr]^2$-, $[Val]^2$-, $[Nva]^2$- and $[Nva]^2$-$[Nva]^5$-Ciclosporin (also known as cyclosporines C, D, G and M respectively), $[Dihydrop\text{-}MeBmt]^1$-$[Val]^2$-ciclosporin (also known as dihydro-cyclosporine D), $[(D)Ser]^8$-Ciclosporin, $[MeIle]^{11}$-Ciclosporin, $[(D)MeVal]^{11}$-Ciclosporin (also known as cyclosporine H), $[MeAla]^6$-Ciclosporin, $[(D)Pro]^3$-Ciclosporin and so on.

In accordance with conventional nomenclature for cyclosporines, these are defined throughout the present specification and claims by reference to the structure of cyclosporine (i.e., Cyclosporine A). This is done by first indicating the amino acid residues present which differ from those present ion Ciclosporin (e.g., "$[(D)Pro]^3$" to indicate that the cyclosporine in question has a –(D)Pro- rather than –Sar- residue at the 3-position) and then applying the term Cyclosporine to characterize remaining residues which are identical to those present in Cyclosporine A.

As used herein, the term “cyclosporines” refers to the various types of cyclosporines, in which x-y in the MeBmt residue has a cis or trans CH=CH or in which x-y therein is also included in those derivatives in which one or more of those amino acids in positions 2-11 of Cyclosporine A is replaced by a different amino acid. It is preferred; however, that not more than two of the amino acids are replaced in the formula of cyclosporine A and more preferentially not more than one of the amino acids is replaced by an amino acid.

In addition, amino acid residues referred to by abbreviation, e.g., -Ala-, -MeVal- and -∀Abu-, are, in accordance with conventional practice, to be understood as having the (L)-configuration unless otherwise indicated, e.g. as in the case of “-(D)Ala-“. Residue abbreviations preceded by “Me” as in the case of “-MeLeu-”, represent ∀-N-methylated residues. Individual residues of the cyclosporine molecule are numbered, as in the art, clockwise and starting with the residue –MeBmt-, dihydro-MeBmt- etc…in position 1. The same numerical sequence is employed throughout the present specification and claims.

Because of their unique pharmaceutical potential, the macrocyclic immunosuppressants have attracted considerable attention in the press. The term “macrocyclic immuno-suppressants” includes various natural and semi-synthetic derivatives of cyclosporine, and other macrolides such as sirolimus, tacrolimus and pimecrolimus. The primary area of clinical investigation for above drugs has been as immunosuppressive agents, in particular in relation to its application to recipients of organ transplants, e.g., heart, lung, combined heart-lung, liver, kidney, pancreatic, bone-marrow, skin and corneal transplants, and in particular allogenic organ transplants. These drugs are also used in the treatment of psoriasis, atompic dermatitis, rheumatoid arthritis and nephritic syndrome.

Macrocyclic immunosuppressants are also useful for treating various autoimmune diseases and inflammatory conditions and especially inflammatory conditions with an

aetiology, including an autoimmune component, such as arthritis (for example, rheumatoid arthritis, arthritis chronica progredient and arthritis deformons) and rheumatic diseases. Specific autoimmune diseases for which cyclosporine therapy has been proposed or applied include, autoimmune hematological disorder (including, e.g., hemolytic anemia, aplastic anemia, pure red cell anemia, and idiopathic thrombocytopaenia), systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulamatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease, including, e.g., ulcerative colitis and Crohn's disease), endocrine opthalmopathy Graves disease, sarcoidosis, multiple sclerosis, primary billiary cirrhosis, juvenile diabetes (diabetes mellitus type I), uvetis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstial lung fibrosis, psoriatic arthritis, atopic dermatitis and glomerulonephritis (with and without nephrotic syndrome, e.g., including idiopathic nephritic syndrome or minimal change nephropathy).

Furthermore, macrocyclic immunosuppressants also have applicability as an anti-parasitic, in particular anti-protozoal agent, and are suggested to be useful for treating malaria, coccidiomycosis and schistomsomiasis. More recently, they have been taught to be useful as an agent for reversing or abrogating anti-neoplastic agent resistance contumors, and the like.

Despite the very major contribution which macrocyclic immunosuppressants have made, difficulties have been encountered in providing more effective and convenient means of administration (e.g., galenic formulations, for example, oral dosage form, which are both convenient and for the patient as well as providing appropriate bioavailability and allowing dosaging at an appropriate and controlled dosage rate) as well as the reported occurrence of undesirable side reactions; in particular nephrotoxic reactions have been obvious serious impediments to its wider use or application.

Moreover, the above mentioned macrocyclic immunosuppressants are characteristically highly hydrophobic and readily precipitate in the presence of even very minor amounts of water, e.g., on contact with the body (e.g., stomach fluids). It is accordingly extremely difficult to provide e.g., oral formulations which are acceptable to the patient in terms of form and taste, which are stable on storage and which can be administered on a regular basis to provide suitable and controlling patient dosaging.

Proposed liquid formulations, e.g., for oral administration of macrocyclic immunosuppressants, have heretofore been based primarily on the use of ethanol and oils or similar excipients as carrier media. Thus, the commercially available macrocyclic immunosupressant drink-solution employs ethanol and olive oil or corn-oil as carrier medium in conjunction with solvent systems comprising e.g., ethanol and LABRIFIL and equivalent excipients as carrier media. Thus, the commercially available macrocyclic immunosupressant drink solution employs ethanol and olive oil or corn-oil as carrier medium in conjunctions with a Labrifil as a surfactant. See e.g., U.S. Patent NO. 4,388,307. Use of the drink solution and similar composition as proposed in the art is however accompanied by a variety of difficulties.

Further, the palatability of the known oil based system has proved problematic. The taste of the known drink-solution is, in particular, unpleasant. Admixture with an appropriate flavored drink, for example, chocolate drink preparation, at high dilution immediately prior to ingestion has generally been practiced in order to make regular therapy at all acceptable. Adoption of oil based systems has also required the use of high ethanol concentrations to itself inherently undesirable, in particular where administration to children is forseen. In addition, evaporation of the ethanol, e.g., from capsules (adopted in large part, to meet problems of palatability, as discussed or other forms (e.g., when opened) results in the development of a macrocyclic immunosupressant precipitate. When such compositions are presented in, for example, soft gelatin encapsulated form an additional problem arises. This particular difficulty necessitates packaging of the encapsulated product in an air-tight component, for

example, an air-tight blister or aluminum-foil blister package. This in turn renders the product both bulky and more expensive to produce. The storage characteristics of the aforesaid formulations are, in addition, far from ideal.

Bioavailability levels achieved using existing oral macrocyclic immunosupressant dosage system are also low and exhibit wide variation between individuals, individual patient types and even for single individuals at different times during the course of therapy. Reports in the literature indicates that currently available therapy employing the commercially available macrocyclic immunosupressant drink solution provides an average absolute bioavailability of approximately 30% only, with the marked variation between individual groups, e.g., between liver (relatively low bioavailability) and bone-marrow (relatively high bioavailability) transplant recipients. Reported variation in bioavailability between subjects has varied from one or a few percent for some patients, to as much as 90% or more for others. And as already noted, marked change in bioavailability for individuals with time is frequently observed. Thus, there is a need for a more uniform and high bioavailability of macrocyclic immunosupressant in patients.

Use of such dosage forms is also characterized by extreme variation in required patient dosaging. To achieve effective immunosuppressive therapy, blood or blood serum levels compounds of the cyclosporin have to be maintained within a specified range. This required range can in turn, vary, depending on the particular condition being treated, e.g., whether therapy is to prevent transplant rejection or for the control of an autoimmune disease, or condition and on whether or not alternative immunosuppressive therapy is employed concomitantly with any of the immunosuppressants of the formula a-d therapy. Because of the wide variations in bioavailability levels achieved with conventional dosage forms, daily dosages needed to achieve required blood serum levels will also vary considerably from individual to individual and even for a single individual. For this reason it is necessary to monitor blood/blood-serum levels of patients receiving macrocyclic immunosuppressant therapy at regular and frequent intervals. Monitoring of blood/blood-serum levels, which is generally performed by

RIA or equivalent immunoassay technique, e.g. employing monoclonal antibody based technology, has to be carried out on a regular basis. This is inevitably time consuming and inconvenient and adds substantially to the overall cost of therapy.

It is also the case that blood/blood serum macrocyclic immunosuppressant levels achieved using available dosage systems exhibit extreme variation between peak and trough levels. That is for each patient, effective macrocyclic immunosuppressant levels in the blood vary widely between administrations of individual dosages.

There is also a need for providing macrocyclic immunosuppressant in a water soluble form for injection. It is well known that Cremephore L used in a current formulations of macrocyclic immunosuppressants is a polyoxyethylated derivative of castor oil and is a toxic vehicle. There have been a number of incidences of anaphylaxis due to the castor oil component. At present there is no formulation that would allow the macrocyclic immunosuppressants to be in aqueous solution at the concentrations needed due to poor water solubility of the drug.

Beyond all these very evident practical difficulties lies the occurrence of undesirable side reactions already alluded to, observed employing available oral dosage forms.

Several proposals to meet these various problems have been suggested in the art, including both solid and liquid oral dosage forms. An overriding difficulty which has however remained is the inherent insolubility of the macrocyclic immunosuppressants in aqueous media, hence preventing the use of a dosage form which can contain macrocyclic immunosuppressants in sufficiently high concentration to permit convenient use and yet meet the required criteria in terms of bioavailability, e.g. enabling effective resorption from the stomach or gut lumen and achievement of consistent and appropriately high blood/blood-serum levels.

The particular difficulties encountered in relation to oral dosaging with macrocyclic immunosuppressants have inevitably led to restrictions in the use of macrocyclic immunosuppressant therapy for the treatment of relatively less severe or endangering disease conditions. A particular area of difficulty in this respect has been the adoption of macrocyclic immunosuppressant therapy in the treatment of autoimmune diseases and other conditions affecting the skin, for example for the treatment of atopic dermatitis and psoriasis and, as also widely proposed in the art, for hair growth stimulation, e.g. in the treatment of alopecia due to ageing or disease.

Thus while oral macrocyclic immunosuppressant therapy has shown that the drug is of considerable potential benefit to patients suffering e.g. from psoriasis, the risk of side-reaction following oral therapy has prevented common use. Various proposals have been made in the art for application of macrocyclic immunosuppressants, e.g. cyclosporine, in topical form and a number of topical delivery systems have been described. Attempts at topical application have however failed to provide any demonstrably effective therapy.

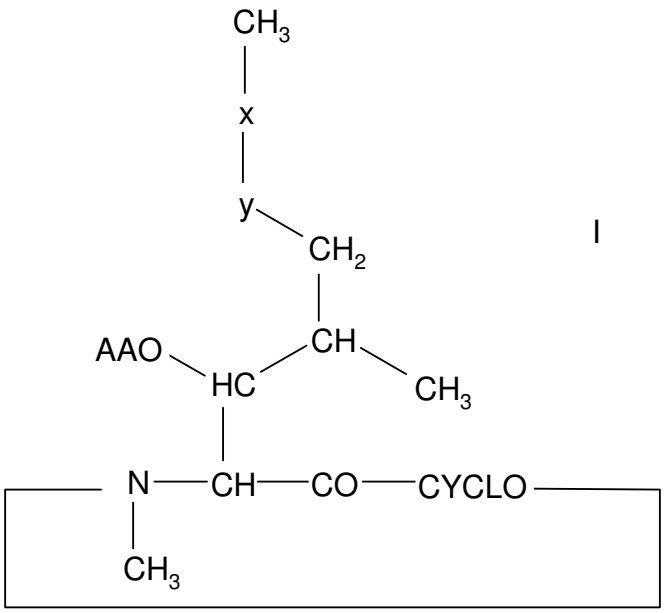
However, the present invention overcomes the problems described hereinabove. More specifically, an embodiment of the present invention is a prodrug of macrocyclic immunosuppressant which significantly enhances its solubility in aqueous solutions, thereby avoiding the need to utilize a carrier, such as ethanol or castor oil when administered as a solution. Moreover, the prodrugs of macrocyclic immunosuppressant, in accordance with the present invention, do not exhibit the side effects of the prior art formulations. Further, the inventor has found that the macrocyclic immunosuppressant prodrugs of the present invention enhance its absorption when administered in the prodrug form to a patient, thereby enhancing significantly its bioavailability and its efficacy.

Accordingly, in one aspect, the present invention is directed to a prodrug of macrocyclic immunosuppressants. The prodrug consists of an amino acid esterified to the free

hydroxy group present on the side chain of cyclosporine, sirolimus, tacrolimus and either one of the hydroxyl groups of the pimecrolimus molecule.

For example, an aspect of the present invention is directed to, the compounds of the formulas

(a)



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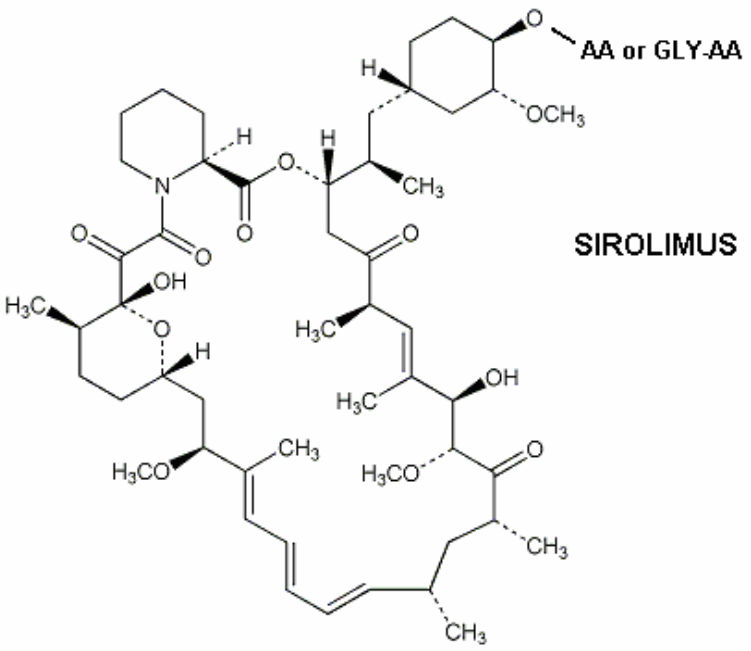
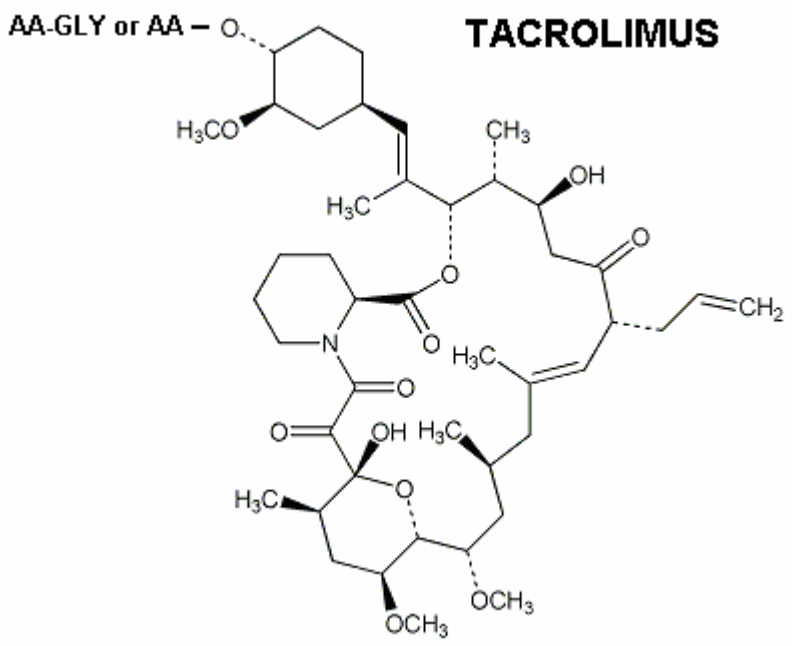
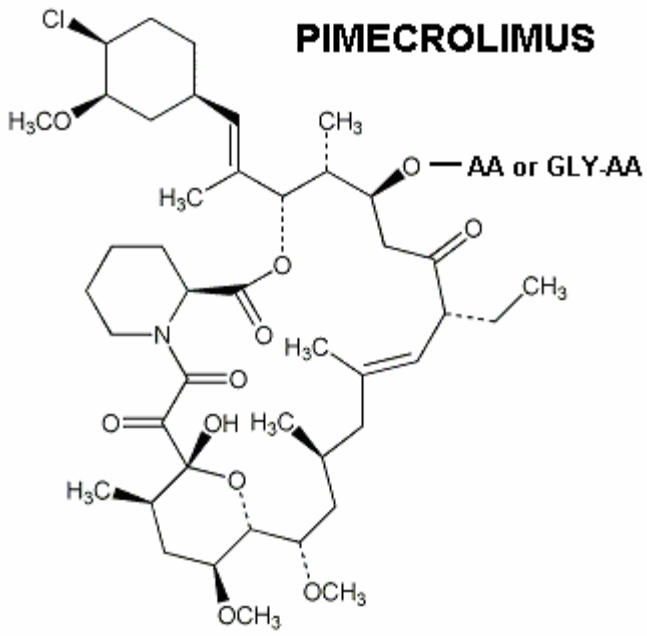
(b)



(c)



(d)



or pharmaceutically acceptable salts thereof;

wherein CYCLO represents the residues at positions 2-11 of the cyclosporine molecule; x-y is CH=CH or CH_2CH_2 and AA is an amino acid or a dipeptide of the formula GLY-AA. In the latter case, GLY is glycine and AA is any ∀-amino acid. In the dipeptide structure, an AA is attached to the drug via OH group using glycine as the spacer. Glycine is esterified to cyclosporine and then glycine is bonded to any AA via amide linkage using amino group of glycine and carboxylic acid group of AA.

The present invention is also directed to a pharmaceutical composition comprising a therapeutically effective amount of the compounds of the Formulas a-d above and a pharmaceutical carrier therefor.

In another embodiment, the present invention is directed to a method of treating a patient in need of macrocyclic immunosuppressant therapy (or cyclosporin therapy), which method comprises administering to said patient an effective amount of the compounds of Formulas a-d.

In a further embodiment, the present invention is directed to a method of enhancing the solubility of macrocyclic immunosuppressant in an aqueous solution comprising reacting the hydroxy functionality in the MeBmt moiety at position 1 of the cyclosporine molecule as well as the specified hydroxyl functions in formulas b-d with an amino acid or acylating derivative thereof under ester forming conditions or by using a simple amino acid or a dipeptide structure wherein the AA is attached to drug using glycine as the spacer and isolating and isolating the product thereof. Further, in another embodiment, the present invention is directed to a method of enhancing the dose proportionality of cyclosporin comprising reacting the hydroxy functionality in the MeBmt moiety at position 1 of the cyclosporin molecule with an amino acid or acylating derivative thereof under ester forming conditions and isolating the product thereof and administering the product to a patient.

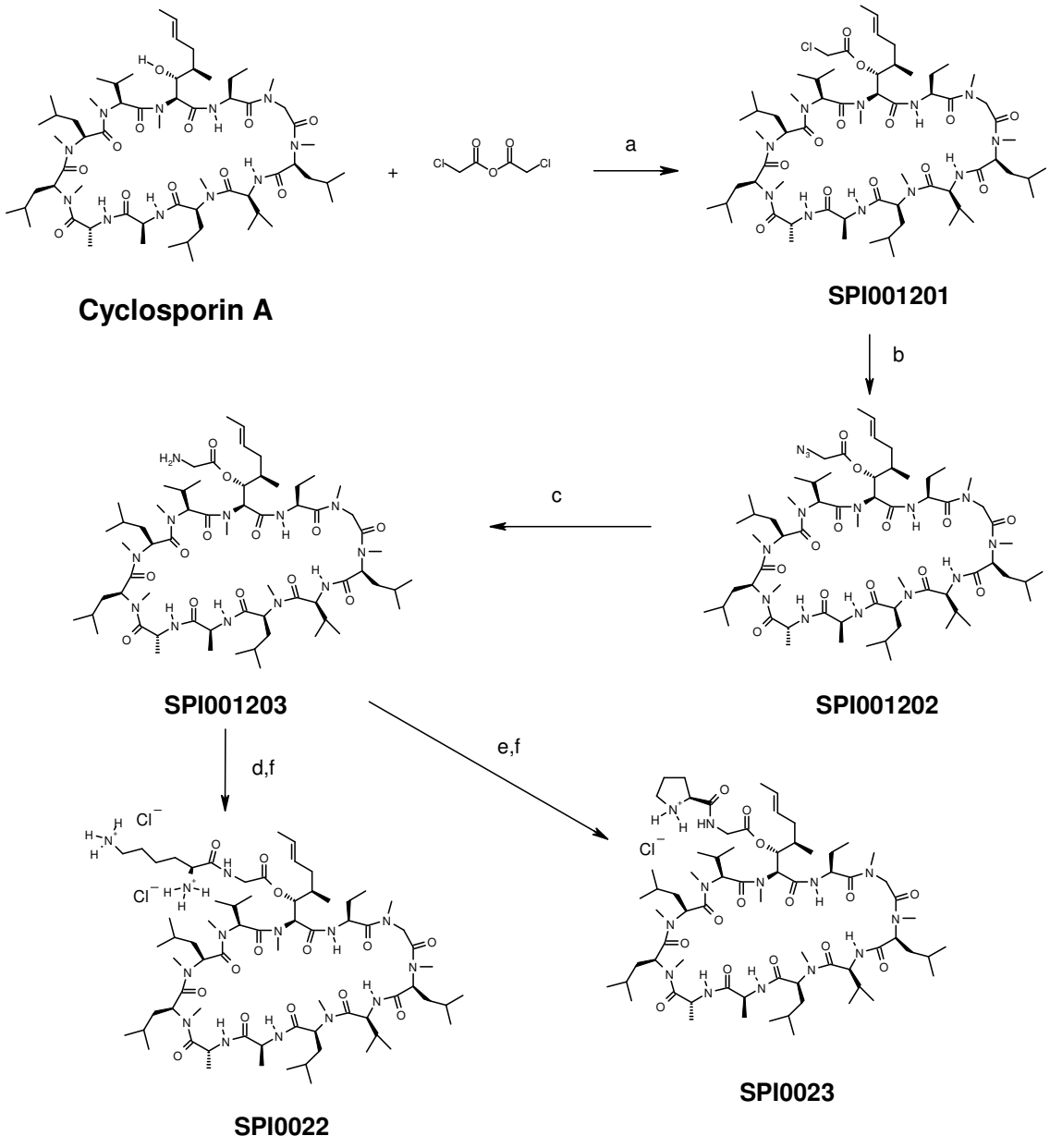
In a still further embodiment, the present invention is directed to a method of enhancing the bioavailability of macrocyclic immunosuppressants when administered to a patient which comprises reacting the hydroxy functionality in the MeBmt moiety in position of the cyclosporine molecule with an amino acid or acylating derivative under ester forming conditions and as well as the specified hydroxyl functions in formulas b-d with an amino acid or acylating derivative thereof under ester forming conditions or by using a simple amino acid or a dipeptide structure wherein the AA is attached to drug using glycine as the spacer and isolating the product thereof and administering said product to the patient.

Overview:

The procedure for the synthesis of the N-(L-proline)-glycine and N-(L-lysine)-glycine esters of Cyclosporine A is outlined in **Synthetic Sequence** section. These examples are exemplary of the synthetic scheme using amino acids. The complete procedure and analytical data is given in the **Experimental Section**. Cyclosporine A (15 g) was coupled with chloroacetic anhydride (4 equivalent) in anhydrous pyridine. The experiment produced the chloroacetate ester of Cyclosporine A (**SPI001201**, 14 g, 88%

yield) in good yield. The chloroacetate ester (10.1 g) was then treated with sodium azide in DMF to generate the azidoacetate ester of Cyclosporine A (**SPI001202**, 9.9 g, 97% yield). The azidoacetate (9.8 g) was then reduced with tin chloride (9 g) to prepare the glycine ester of Cyclosporine A (8.54 g, 89% yield). The glycine ester of Cyclosporine A (**SPI001203**) was then coupled with a two-fold excess of either boc-L-proline or Boc-L-lysine using EDC as the coupling agent. After purification by column chromatography, the boc protecting groups were removed from the dipeptide esters of Cyclosporine A at low temperature (5 °C) by treatment with 2M hydrochloric acid in diethyl ether. The L-lysine-glycine ester salt of Cyclosporine A did not require additional purification and was dried. The L-proline-glycine ester salt of Cyclosporine A required purification. The salt was converted to the free-base with sodium bicarbonate and purified by filtration through silica gel (eluting with acetone). The salt was then formed at low temperature with dilute anhydrous hydrochloric acid and dried.

Synthetic Sequence:

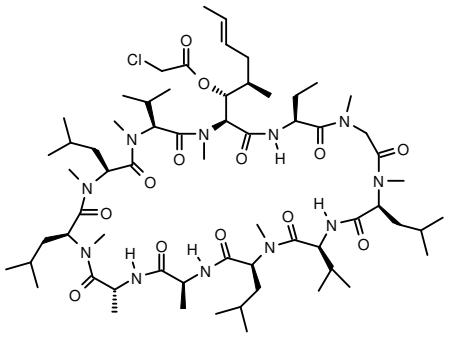


Synthesis of the N-(L-proline)-glycine and N-(L-Lysine)-glycine esters of Cyclosporine A: a) pyridine; b) NaN_3, DMF; c) $SnCl_2$, methanol; d) boc-L-lysine, EDC; e) boc-L-proline, EDC; f) HCl, Et_2O.

Experimental Section:

The synthesis of **SPI0022** and **SPI0023** was conducted in batches. Generally a small-scale experiment was performed first followed by a larger batch. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinckrodt. The Cyclosporine A (USP grade) used in these procedures was provided by Signature Pharmaceuticals, Inc.

1) SPI001201



Cyclosporine A (15.01 g, 0.0124 moles) was dissolved in anhydrous pyridine (35 mL) at room temperature, under an argon atmosphere. The solution was cooled to 5 °C in an ice/water batch and chloroacetic anhydride (9.10 g, 0.053 moles) was added. After stirring for 10 minutes, the ice bath was removed and the solution was allowed to stir under an argon atmosphere at room temperature for 17 hours. After 17 hours, diethyl ether (200 mL) was added. The ether was washed with water (2×100 mL) and dried for 1 hour over sodium sulfate (10 g). After filtration and concentration under reduced pressure, the remaining yellow foam was dried under high vacuum (1 hour at room temperature) and purified by flash chromatography on silica gel (200 g), eluting with heptane/acetone (2:1). After combining and concentrating the product containing fractions, the remaining light yellow foam (14.8 g) was purified a final time by crystallization from hot diethyl ether (140 mL). After cooling (-10 °C, 2 hours), filtration, and drying under high vacuum, the procedure generated the chloroacetate ester of Cyclosporine A **SPI001201** as a white solid (14.0 g, 88.3% yield).

Cyclosporine A chloroacetate ester:

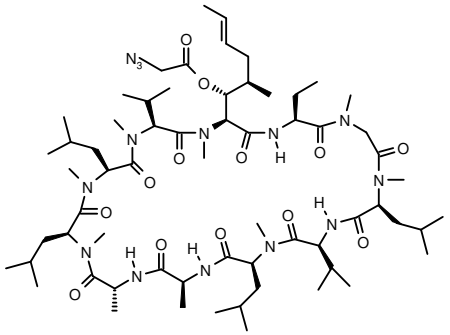
^{1}H NMR (300 MHz, $CDCl_3$):

δ = 8.50 (d, 1H, J= 9.6 Hz), 7.95 (d, 1H, J= 6.6 Hz), 7.46 (d, 1H, J= 9.0 Hz), 7.40 (d, 1H, J= 7.8 Hz), 5.35-4.52 (m, 15 H), 4.37 (t, 1H, J= 7.2 Hz), 4.12 (d, 1H, J= 14.7 Hz), 3.89 (d, 1H, J= 14.7 Hz), 3.45-3.0 (m, 15 H), 2.8-2.5 (m, 6H), 2.5-1.5 (m, 16H), 1.5-0.7 (m, 53 H).

^{13}C NMR (75 MHz, $CDCl_3$):

δ = 173.78, 173.37, 172.86, 172.61, 171.28, 171.18, 170.91, 170.79, 168.78, 167.64, 167.18, 128.77, 126.68, 75.46, 65.95, 58.89, 57.47, 55.80, 55.31, 54.86, 54.34, 50.19, 48.91, 48.35, 48.02, 44.80, 40.96, 39.44, 37.07, 35.93, 33.85, 33.25, 32.40, 31.74, 31.50, 30.38, 30.12, 29.82, 29.53, 25.13, 24.92, 24.78, 24.40, 23.99, 23.75, 22.85, 21.94, 21.41, 21.25, 20.84, 19.85, 18.79, 18.32, 17.89, 17.82, 15.46, 15.24, 10.08.

2) SPI001202



The chloroacetate ester of Cyclosporine A **SPI001201** (10.10 g, 7.89 mmole) was dissolved in anhydrous N,N-dimethlformamide (30 mL) at room temperature. Sodium azide (2.15 g, 33.0 mmole) was added. The mixture was allowed to stir at room temperature for 24 hours in the dark, under an argon atmosphere. After 24 hours, diethyl ether (150 mL) was added and the precipitate was filtered. The ether was washed with water (2×100 mL), dried over sodium sulfate (15 g) for 30 minutes, filtered, and

concentrated under reduced pressure. The remaining white solid was dried under high vacuum for 1 hour at room temperature. The experiment produced the azidoacetate ester of Cyclosporine A **SPI001202** (9.90 g, 97% yield) as a white solid, which was used without further purification.

Cyclosporine A azidoacetate ester:

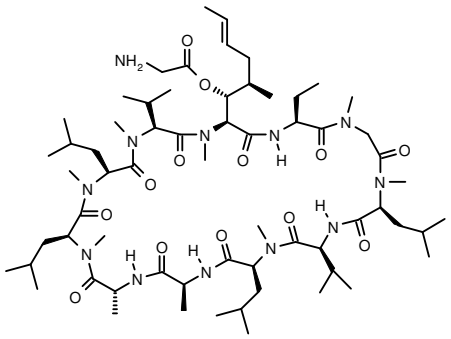
^{1}H NMR (300 MHz, $CDCl_3$):

δ = 8.48 (d, 1H, J= 9.3 Hz), 7.95 (d, 1H, J= 6.9 Hz), 7.45 (d, 1H, J= 9.0 Hz), 7.39 (d, 1H, J= 7.8 Hz), 5.5-4.5 (m, 15 H), 4.31 (t, 1H, J= 6.6 Hz), 4.04 (d, 1H, J= 17.3 Hz), 3.53 (d, 1H, J= 17.3 Hz), 3.45-3.0 (m, 15 H), 2.8-2.5 (m, 6H), 2.5-1.5 (m, 16H), 1.5-0.7 (m, 53 H).

^{13}C NMR (75 MHz, $CDCl_3$):

δ = 173.76, 173.32, 172.82, 172.53, 171.13, 170.89, 170.76, 170.69, 169.70, 168.20, 167.49, 128.63, 126.61, 74.96, 58.91, 57.39, 55.56, 55.21, 54.80, 54.23, 50.14, 48.99, 48.23, 48.24, 47.93, 44.71, 40.89, 39.33, 39.22, 37.02, 35.83, 33.81, 32.96, 32.31, 31.67, 31.42, 30.31, 30.09, 29.76, 29.47, 25.08, 24.92, 24.84, 24.67, 24.51, 24.40, 23.94, 23.82, 23.71, 21.85, 21.33, 21.25, 20.82, 19.79, 18.71, 18.25, 17.92, 17.81, 15.17, 10.03.

3) SPI001203



The azidoacetate ester of Cyclosporine A **SPI001202** (9.80 g, 7.62 mmole) was dissolved in methanol (250 mL) at room temperature. Water (40 mL) was added

followed by tin (II) chloride (5 g, 26.3 mmole). The solution was allowed to stir for 1 hour at room temperature when an additional quantity of tin (II) chloride (4 g, 21.0 mmole) was added. The solution was allowed to stir for an additional 2 hours at room temperature. Water (200 mL) containing ammonium hydroxide (40 mL, 29%) was added. After filtration, the solution was concentrated (to 200 mL) under reduced pressure. The remaining aqueous solution was extracted with ethyl acetate (2×200 mL). The ethyl acetate fractions were combined, dried over sodium sulfate (20 g), filtered and concentrated under reduced pressure. The remaining clear foam was purified by filtration through silica gel (150 g), eluting with dichloromethane/methanol (20:1). The procedure generated the glycine ester of Cyclosporine A as a clear, solid foam (8.54g, 89% yield).

Glycine ester of Cyclosporine A:

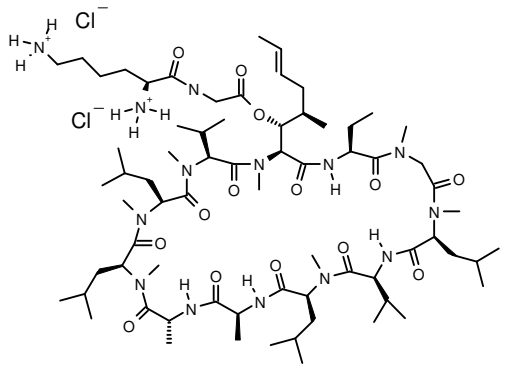
^{1}H NMR (300 MHz, $CDCl_3$):

δ = 8.60 (d, 1H, J= 9.6 Hz), 8.06 (d, 1H, J= 6.9 Hz), 7.53 (d, 1H, J= 8.4 Hz), 7.51 (d, 1H, J= 6.6 Hz), 5.7-4.52 (m, 15 H), 4.41 (t, 1H, J= 6.9 Hz), 3.5-3.0 (m, 17 H), 2.82-2.5 (m, 8H), 2.5-1.5 (m, 16H), 1.5-0.7 (m, 53 H).

^{13}C NMR (75 MHz, $CDCl_3$):

δ = 174.10, 173.67, 173.23, 172.72, 172.55, 171.18, 171.10, 170.73, 170.61, 169.68, 167.77, 128.82, 126.42, 73.83, 58.57, 57.32, 55.99, 55.20, 54.74, 54.31, 50.08, 48.82, 48.28, 47.90, 44.70, 43.81, 40.74, 39.33, 39.24, 37.02, 35.84, 33.72, 33.07, 32.39, 31.72, 31.41, 30.25, 29.98, 29.74, 29.51, 25.05, 24.81, 24.73, 24.54, 24.31, 23.91, 23.78, 23.68, 21.86, 21.33, 21.25, 20.68, 19.76, 18.74, 18.24, 17.94, 17.79, 15.18, 10.03.

4) SPI0022



The glycine ester of Cyclosporine A (SPI001203, 2.0 g, 1.59 mmole) was dissolved in anhydrous dichloromethane (25 mL) with boc-L-lysine (1.31 g, 3.78 mmole) and EDC (0.75 g, 3.9 mmole), under an argon atmosphere at room temperature. The boc-L-lysine was prepared from the dicyclohexylamine salt (2.0 g in 50 mL ether) by extraction with cold potassium hydrogen sulfate solution (1 g in 50 mL water) followed by cold water (2×50 mL). The ether containing the boc-L-lysine was dried over sodium sulfate (5 g), filtered, concentrated and dried under high vacuum for one hour at room temperature. A few crystals of DMAP were added to the mixture of EDC, boc-L-lysine, and the glycine ester of Cyclosporine A and the solution was allowed to stir for 4 hours at room temperature. The dichloromethane solution was extracted with DIUF water (50 mL), 5% sodium bicarbonate solution (50 mL), and with DIUF water (50 mL). After drying over sodium sulfate (10 g), the dichloromethane solution was filtered and concentrated under reduced pressure. The remaining white foam (3.01 g) was purified by flash column chromatography on silica gel (50 g), eluting with heptane/acetone (2:1). The product containing fractions were combined, concentrated under reduced pressure, and dried under high vacuum. The purified protected intermediate (2.34 g white solid, 92.8% yield) was placed in a flask under an argon atmosphere, which was cooled in an ice-water bath. Cold anhydrous 2 M hydrochloric acid in diethyl ether (20 mL) was added and the solution stirred for 8 hours (at 5 °C). The mixture was slowly allowed to warm to room temperature overnight. After stirring for a total of 20 hours, the flask was cooled again in an ice-water bath for 30 minutes. The product was filtered and dried under high vacuum for 1 hour at room temperature and then at 50 °C for 4 hours. The

experiment produced Cyclosporine A N-(L-lysine)-glycine ester, dihydrochloride trihydrate (**SPI0022**, 1.59 g, 73.9% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, NMR data is for the free base):

δ = 8.58 (d, 1H, J= 9.3 Hz), 8.04 (d, 1H, J= 6 Hz), 7.80 (d, 1H, J= 6 Hz), 7.49 (d, 2H, J= 8.4 Hz), 5.70-4.6 (m, 17 H), 4.41 (m, 1H), 4.28 (dd, 1H, J= 17, 7.2 Hz), 3.67 (d, 1H, J= 17 Hz), 3.46 (s 3H), 3.4-2.8 (m, 16 H), 2.8-2.5 (m, 8H), 2.5-1.35 (m, 24H), 1.5-0.7 (m, 50 H).

^{13}C NMR (75 MHz, $CDCl_3$, NMR data is for the free base):

δ = 175.23, 173.77, 173.34, 172.75, 172.63, 171.34, 171.22, 170.94, 170.84, 170.91, 169.89, 169.70, 128.74, 126.67, 74.41, 58.82, 57.43, 55.91, 55.21, 54.81, 54.42, 50.17, 48.89, 48.31, 47.98, 44.78, 41.92, 40.82, 40.69, 39.44, 39.32, 27.19, 35.91, 34.88, 33.71, 33.25, 33.12, 32.44, 31.83, 31.50, 30.38, 30.06, 29.81, 29.55, 25.14, 24.90, 24.52, 24.43, 24.00, 23.76, 21.93, 21.42, 21.29, 20.81, 19.84, 18.82, 18.32, 17.96, 17.86, 15.21, 10.10.

CHN analysis:

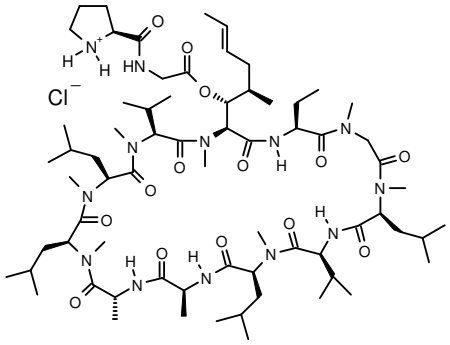
Calculated for $C_{70}H_{128}Cl_2N_{14}O_{15}$-$3H_2O$: C 55.50, H 8.92, and N 12.74; found: C 58.28, H 8.98, and N 13.16.

HPLC analysis:

99.60% purity; r.t.= 14.763 min.; 80% acetonitrile/20% Tris base in DIUF water; 1 mL/min; 60C; Synergi Hydro RP, 4u column (serial # 163383-7), 4.6x250 mm; 20 ul; UV= 210 nm.

Melting point: 196.0-198 °C (uncorrected)

5) SPI0023



The glycine ester of Cyclosporine A (SPI001203, 7.50 g, 5.95 mmole) was dissolved in anhydrous dichloromethane (50 mL) with boc-L-proline (2.56 g, 11.90 mmole) and EDC (2.28 g, 11.9 mmole), under an argon atmosphere at room temperature. A few crystals of DMAP were added to the mixture of EDC, boc-L-proline, and the glycine ester of Cyclosporine A and the solution was allowed to stir for 3 hours at room temperature. The dichloromethane solution was extracted with DIUF water (50 mL), 5% sodium bicarbonate solution (2×50 mL), and with DIUF water (50 mL). After drying over sodium sulfate (10 g), the dichloromethane was filtered and concentrated under reduced pressure. The remaining white foam (9.50 g) was purified by flash column chromatography on silica gel (150 g), eluting with heptane/acetone (2:1 followed by 1:1). The product containing fractions were combined, concentrated under reduced pressure, and dried under high vacuum (7.94 g white solid, 91.7% yield) for 10 minutes at room temperature.

The purified protected intermediate (6.46 g) was placed in a flask under an argon atmosphere, which was cooled in an ice-water bath. Cold anhydrous 2 M hydrochloric acid in diethyl ether (150 mL) was added and the solution stirred for 8 hours (at 5 °C). The mixture was slowly allowed to warm to room temperature overnight. After stirring for a total of 20 hours, the flask was cooled again in an ice-water bath for 30 minutes. The product was filtered and dried under high vacuum for 30 minutes at room temperature. The Cyclosporine A N-(L-proline)-glycine ester, hydrochloride (5.17 g, 84.6% yield, and 90% purity by HPLC) was converted to the free base by dissolving the

salt in DIUF water (25 mL) that contained sodium bicarbonate (1 g). The free base was extracted with dichloromethane (3×25 mL), which was dried over sodium sulfate (5 g), filtered and concentrated. The remaining off-white solid (5 g) was purified by filtration through silica gel (100 g), eluting with acetone. The product containing fractions were combined, concentrated under reduced pressure, and dried under high vacuum for 30 minutes at room temperature. The hydrochloride salt was regenerated by dissolving the free base (3.8 g) in diethyl ether (25 mL) and adding it to anhydrous 2M hydrochloric acid (5 mL) in heptane (50 mL), while cooling in an ice-water bath. After 20 minutes at 5 °C, the white solid was filtered and dried under high vacuum for 6 hours at room temperature. The experiment produced Cyclosporine A N-(L-proline)-glycine ester, hydrochloride (**SPI0023**, 3.8 g) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$):

δ = 14.20 (br s, 2H), 8.62 (d, 1H, J= 10 Hz), 8.06 (d, 1H, J= 6.9 Hz), 7.61 (d, 1H, J= 8.1 Hz), 7.48 (d, 1H, J= 9 Hz), 5.70-5.50 (m, 3H), 5.40-4.60 (m, 12H), 4.37 (m, 1H), 4.20 (d, 1H, J= 18 Hz), 3.97 (d, 1H, J= 18 Hz), 3.70 (m, 1H), 3.45 (s, 3H), 3.23-3.08 (m, 12H), 2.66 (s, 3H), 2.60 (s, 3H), 2.50-1.80 (m, 15H), 1.78-1.20 (m, 15H), 1.15-0.66 (m, 46H).

^{13}C NMR (75 MHz, $CDCl_3$):

δ = 174.15, 173.49, 172.67, 172.59, 171.86, 171.20, 171.13, 171.02, 170.83, 169.68, 168.77, 167.55, 128.30, 127.10, 80.09, 75.58, 62.65, 59.35, 57.36, 55.53, 55.30, 54.78, 54.35, 53.60, 50.25, 50.09, 48.92, 48.18, 48.12, 44.62, 40.59, 40.02, 39.43, 39.30, 37.13, 35.88, 33.74, 33.07, 32.19, 32.01, 31.86, 31.50, 31.43, 30.43, 29.93, 29.72, 29.30, 29.16, 27.56, 26.04, 25.00, 24.86, 24.74, 24.39, 20.96, 19.81, 18.71, 18.26, 18.09, 17.85, 17.79, 15.09, 14.30, 10.00.

CHN analysis:

Calculated for $C_{69}H_{122}ClN_{13}O_{14}$: C 59.48, H 8.83, and N 13.07; found: C 59.84, H 9.02, and N 12.65.

HPLC analysis:
99.59% purity; r.t.= 10.613 min.; 85% acetonitrile/15% Tris base in DIUF water; 1.2 mL/min; 60C; Synergi Hydro RP, 4u column (serial # 163383-7), 4.6x250 mm; 20 ul; UV= 210 nm.

Melting point: 197.0-199 °C (uncorrected)

These prodrugs of cyclosporin of the present invention are effective in treating diseases or conditions in which macrocyclic immunosuppressants normally are used. These prodrugs are transformed within the body to release the active compound and enhances the therapeutic benefits of the macrocyclic immunosuppressants by reducing or eliminating biopharmaceutical and pharmacokenetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals. Since the prodrugs are more soluble in water then cyclosporine or other macrocyclic immunosuppressants, it does not need to be associated with a carrier vehicle, such as alcohol or castor oil which may be toxic or produce unwanted side reactions. Moreover, oral formulations containing the prodrugs of the prodrugs are absorbed into the blood and are quite effective.

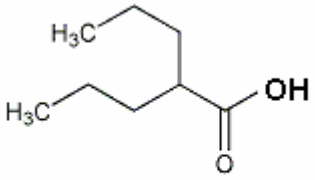
Thus, the prodrug of cyclosporin of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs.

Furthermore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

VI. Valproic Acid Esters

Valproic acid (2-Propylpentanoic acid) is low molecular weight carboxylic acid derivative which is widely used as an anti-convulsive agent, useful in the treatment of epilepsy and also possess vasodilatation activity in the brain to relieve migraine

headaches. It is administered orally to control epileptic episodes in humans and also alleviate severe pain associated with migraine headaches.



VALPROIC ACID

Valproic acid has been shown to have a large number of therapeutic applications, which are quite varying and somewhat surprising. For example, in addition to its efficacy in the treatment of epilepsy and migraine headaches, it has been shown to be effective in the treatment of certain psychiatric illnesses, such as bipolar disorder, mood stabilization, control of aggression, impulsivity in personality disorder, agitation in dementia, and has also been of use as adjunct therapy in the treatment of post traumatic stress disorder (PTSD).

Mechanism of Action:

In spite of being used in the treatment of epilepsy for a number of years, the exact mechanism of action of Valproic acid is still unknown. It has been postulated that it exerts its action by increasing concentration of gamma-amino butyric acid (GABA) in the brain. Gamma-amino butyric acid is a neurotransmitter, a chemical that nerves use to communicate with one another.

Valproate is the drug of choice in myoclonic epilepsy, with or without generalized tonic-clonic seizures, including juvenile myoclonic epilepsy of Janz that begins in adolescence or early adulthood. Photosensitive myoclonus is usually easily controlled. Valproate also is effective in the treatment of benign myoclonic epilepsy, postanoxic myoclonus, and, with clonazepam, in severe progressive myoclonic epilepsy that is characterized by tonic-clonic seizures as well. It also may be preferred in certain stimulus-sensitive (reflex, startle) epilepsies.

Although Valproate may be effective for infantile spasms; it is relatively contraindicated in children whose spasms are due to hyperglycinemia or other underlying metabolic (mitochondrial) abnormalities. In general, atonic and akinetic seizures in patients with Lennox-Gastaut syndrome are difficult to control, but Valproate is the drug of choice for treatment of mixed seizure types. Since this drug has been useful in some patients who are refractory to all other antiepileptic drugs, it may warrant a trial in nearly all nonresponsive patients regardless of seizure type.

In spite of it usefulness, hepatotoxicity may be fatal, but is idiosyncratic and not preventable by routinely monitoring liver enzymes. Hepatotoxicity occurs in very young children, most often those on multiple anticonvulsants. Valproate-induced cytopenias may be dose-related and warrant monitoring of complete blood counts during therapy. Encephalopathy with hyperammonemia without liver function test abnormalities may occur. Pregnant women in first month are at risk for neural tube defects.

Valproic acid is a low molecular weight liquid with characteristic odor. Taken orally it has unpleasant taste and can severely irritate mouth and throat. In order to convert Valproic acid into a solid dosage form convenient for oral administration, a number of derivatives with covalent and ionic bond with the carboxylic acid have been made. A simple sodium salt of Valproic acid, resulting in Valproate sodium is available as a solid. However a stable coordination complex, know as Divalproex sodium was formed by partial neutralization of two molecules of Valproic acid with one atom of sodium. This product is the most widely available Valproic acid hemi salt marketed by Abbott Laboratories in the USA under the brand name Depakote®. Depakote® is also available in extended release formulation for oral administration.

A significant disadvantage of Valproic acid is that it in liquid form it is difficult to administer. Furthermore, administration of Valproic acid in different forms does not uniformly produce desired bioavailability. For example, the overall bioavailability of Valproate from Valproic acid, its sodium salt, Divalproex®, and their extended release

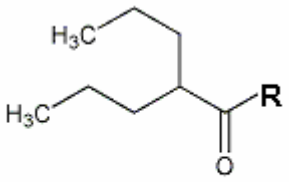
formulations are not quite interchangeable. Since continuous monitoring of plasma profile of Valproic acid is essential, any change in plasma concentration due to changes in the formulation adversely affect overall therapeutic outcome.

In order to improve the therapeutic effectiveness, uniform blood profile, develop pharmaceutically elegant formulation and reduce first pass metabolism, present invention discusses prodrugs of Valproic acid which overcome some of the difficulties stated above.

Until now there has been no pharmaceutical preparation has been available in the market that can deliver Valproic acid with out harmful side effects. The present invention however, has produced a number of water soluble, non-toxic derivatives of Valproic acid which are suitable for delivering Valproic acid consistently in the body without any harmful side effects and without the needs for expensive additives, and exepients.

Accordingly, in one aspect, the present invention is directed to a class of prodrugs of Valproic acid. The prodrug consists of the hydroxyl group of an amino acid esterified to the free carboxyl group present on the Valproic acid molecules. In another embodiment, the amine group of the amino acid is reacted with COOH group to form an amide linkage.

More specifically, an embodiment of the present invention is directed to, the compounds of the formula



VALPROIC ACID

or pharmaceutically acceptable salts thereof; wherein R is either NH-AA or O-AA and AA is an amino acid, in which either an amine group or the hydroxyl group is reacted with the carboxylic acid group of Valproic Acid.

The present invention is also directed to a pharmaceutical composition comprising a therapeutically effective amount of the various Valproic acid prodrugs above and a pharmaceutical carrier therefor.

In another embodiment, the present invention is directed to a method of treating a patient in need of Valproic acid therapy, which method comprises administering to said patient an effective amount of the Valproic acid.

In a further embodiment, the present invention is directed to a method of converting liquid Valproic acid into a solid powder by reacting the carboxyl functionality of the Valproic acid with either amine or hydroxyl functionality of an amino acid and isolating the products thereof.

In a still further embodiment, the present invention is directed to a method of substantially and in a therapeutically efficacious manner, reducing or eliminating the potential first pass metabolism thereby improving the consistent therapeutic effect by administering to a patient a prodrug which comprises reacting the COOH functionality of the Valproic acid molecule with either NH2 or OH functionality of selected amino acids to form an ester or amide covalent bond respectively and isolating the product thereof and administering said product to the patient.

It has been found that when unsubstituted naturally occurring amino acids are esterified to Valproic acid, the resulting prodrugs are pharmaceutically elegant free flowing powders, and are rapidly absorbed into the body and release non-toxic amino acids upon cleavage in the body and require none of the emulsifiers, additives and other exepients.

Furthermore, it has been shown that the current invention also produced drugs, while they are prodrugs of Valproic acid; they were highly effective anti-epileptics and were exhibiting such effect intact. Thus the current amino acid prodrugs are effective anti-epileptics and useful in the treatment of a number of psychiatric illnesses and exhibit such potential with or without releasing the active parent drug.

The Valproic acid prodrug bulk density is much higher than the corresponding sodium salts, and they are suitable for compacting large weight tablets and capsules. Furthermore, their do not exhibit bitter taste and unusual odor of the Valproic acid.

While the prodrugs my invention are not supposed to possess any acidic activity due to blockage of the carboxylic acid group responsible for such, however it has been shown that the prodrugs are effective anti-epileptics with or without releasing Valproic acid. However, all of the Valproic acid prodrugs described are released in vivo the active drug with all its pharmacological and psychoactive properties.

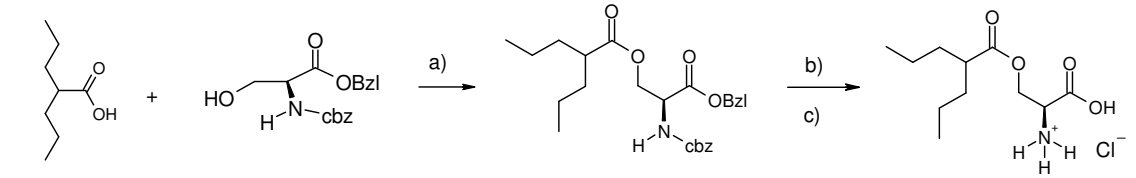
The prodrug of Valproic acid clearly provides a number of advantages over Valproic acid, for example, all of the side chains cleaved from these prodrugs are naturally occurring essential amino acids hence are non-toxic. This results in high therapeutic index. Secondly all the prodrugs are readily cleaved in the body to release Valproic acid. Furthermore, due their high water solubility, they can be easily administered by either forming an in-situ solution just before IV administration using lyophilized sterile powder or providing the drug in solution in prefilled syringe or bottles for infusion. The aminoacid esters are more stable than Valproic acid since COOH group in Valproic acid is blocked to reaction with bases. Thus the Valproic acid prodrugs invented here are more effective then Valproic acid itself without the toxicity and other pharmaceutical problems associated with current marketed formulations.

The procedure for the synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of valproic acid (2-propylpentanoic acid) is outlined in **Synthetic Sequence**

section and is exemplary for the preparation of the various prodrugs of the present invention. The complete procedure and analytical data is given in the **Experimental Section**. In general, valproic acid (2-8 g, in batches) was coupled with the N-benzyloxy/benzyl ester protected amino acids using EDC in the presence of a catalytic amount of DMAP. Once the reactions were complete (20 hours at room temperature), the mixture was extracted with DIUF water, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was either used directly for the deprotection step or purified by column chromatography. The procedure generated the protected amino acid esters of valproic acid in yields ranging from 72% to 92%. The protecting groups were removed by hydrogenation (30 psi H_2) in the presence of 10% palladium on carbon. The amino acid esters of valproic acid were extracted away from the palladium catalyst with ethanol, concentrated, and dried. The final salts were formed by acidification with hydrochloric acid. The crude salts (yields ranging from 57% to 92%) were then purified by the methods described in the Experimental Section.

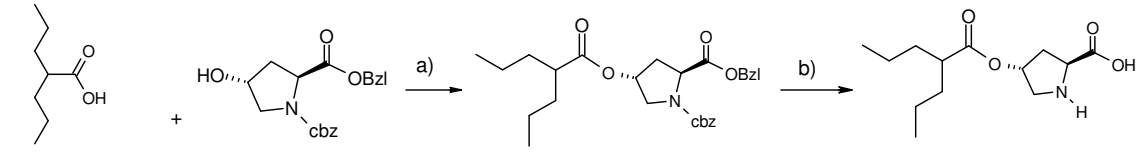
Synthetic Sequence:

1. SPIC001



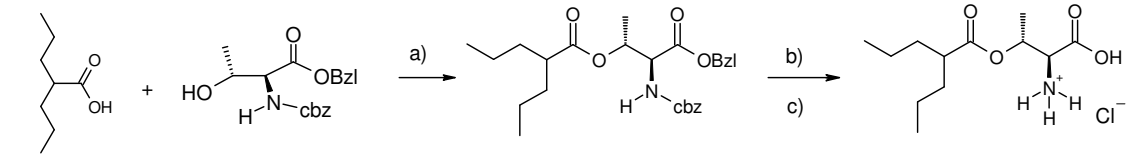
Valproic acid | Z-Ser-OBzl | SPIC00101 | **SPIC001**

2. SPIC002



Valproic acid | Z-Hyp-OBzl | SPIC00201 | **SPIC002**

3. SPIC003



Valproic acid | Z-Thr-OBzl | SPIC00301 | **SPIC003**

5

Synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of valproic acid: a) EDC, DMAP, CH_2Cl_2; b) H_2, 10% Pd/C, EtOH, EtOAc; c) HCl.

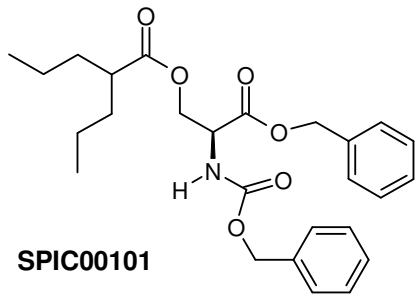
Experimental Section:

The synthesis of **SPIC001**, **SPIC002** and **SPIC003** was conducted in one or two batches. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Lancaster, Sigma-Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

1) SPIC001: 2-Propylpentanoic acid 2(S)-amino-2-carboxy-ethyl ester, hydrochloride

(L-Serine-valproic acid ester, hydrochloride)

A mixture of 2-propylpentanoic acid (valproic acid, 6.48 g, 44.93 mmole), N-carbobenzyloxy-L-serine benzyl ester (Z-Ser-OBzl, 14.80 g, 44.93 mmole), EDC (8.61 g, 44.91mmole), and DMAP (549 mg, 4.49 mmole) in anhydrous dichloromethane (50 mL) was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, the dichloromethane was washed with water (3×50 mL), dried over magnesium sulfate (5 g), filtered and concentrated under reduced pressure. The remaining colorless oil (20.87 g) was purified by column chromatography on silica gel (150 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with hexanes/ethyl acetate (3:1). After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected L-serine-valproate ester **SPIC00101** (18.9 g, 92% yield) as a colorless oil.

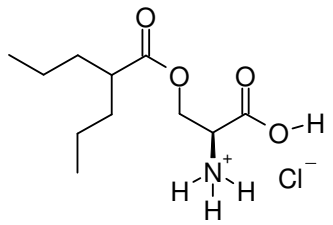


SPIC00101

^{1}H NMR (300 MHz, DMSO): δ = 7.96 (1H, d, *J*= 8.1 Hz), 7.35 (10H, m), 5.14 (2H, s), 5.05 (2H, s), 4.51 (1H, m), 4.29 (2H, m), 2.29 (1H, m), 1.50-1.25 (4H, m), 1.25-1.10 (4H, m), 0.80 (6H, t, J= 6.6 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 174.88, 169.15, 155.85, 136.58, 135.45, 128.26, 128.18, 127.47, 127.71, 127.57, 66.32, 65.66, 62.47, 53.09, 44.20, 33.86, 33.79, 19.95, 13.85.

The protected L-serine-valproate ester **SPIC00101** (18.9 g, 41.48 mmole) was dissolved in ethanol (60 mL) and ethyl acetate (60 mL) at room temperature and added to a Parr bottle (500 mL) that contained 10% palladium on carbon (3.0 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (30 psi). After 4 hours of shaking, additional palladium catalyst (1.0 g) in ethanol\ethyl acetate (1:1, 100 mL) was added and the reaction mixture shook overnight under hydrogen gas (30 psi) at room temperature. After 24 hours the catalyst was removed by filtration through a thin layer of activated carbon. The ethanol and ethyl acetate were concentrated under reduced pressure at room temperature. After drying under high vacuum, the remaining solids were acidified with hydrochloric acid in diethyl ether (2M, 24.6 mL). The mixture was stored in a refrigerator for two hours before filtration and washing with additional cold diethyl ether (10 mL). After filtration, the remaining white solid was dried at room temperature under high vacuum until the product weight was constant (24 hours). The experiment produced L-serine-valproic acid ester, hydrochloride **SPIC001** (6.34 g, 57% yield) as a white solid.



SPIC001

^{1}H NMR (300 MHz, DMSO): δ = 8.73 (br s, 3H), 4.47 (dd, 1H, J= 12.9, 4.5 Hz), 4.31 (dd, 2H, J= 12.9, 3.6 Hz), 2.36 (m, 1H), 1.50 (m, 2H), 1.39 (m, 2H), 1.20 (m, 4H), 0.84 (t, 6H, J= 7 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 174.67, 168.19, 61.84, 51.16, 44.12, 33.76, 33.58, 20.07, 19.92, 13.97, 13.89.

HPLC analysis:
98.49% purity; rt= 4.767 min; Luna C18 5u column (sn 167917-13); 4.6x250 mm; 254 nm; 33% ACN/66% DIUF water; 35 C; 20 ul inj.; 1ml/min; 20 mg/mL sample size; sample dissolved in mobile phase.

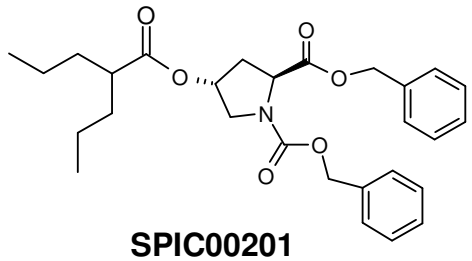
CHN analysis:
calc.: C 49.34, H 8.28, N 5.23; found: C 49.22, H 8.35, N 5.24.

Melting point: 159-160 °C

2) SPIC002: 4(R)-(2-Propyl-pentanoyloxy)-pyrrolidine-2(S)-carboxylic acid

(L-Hydroxyproline-valproic acid ester)

A mixture of 2-propylpentanoic acid (valproic acid, 4.32 g, 30 mmole), N-carbobenzyloxy-L-hydroxyproline benzyl ester (Z-Hyp-OBzl, 10.66 g, 30 mmole)[1], EDC (5.74 g, 30 mmole), and DMAP (366 mg, 3 mmole) in anhydrous dichloromethane (30 mL) was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, the dichloromethane was washed with water (3×30 mL), dried over magnesium sulfate (5 g), filtered and concentrated under reduced pressure. The remaining colorless oil **SPIC00201** (11.95 g, 24.7 mmole, 82.4% yield) was used without purification.



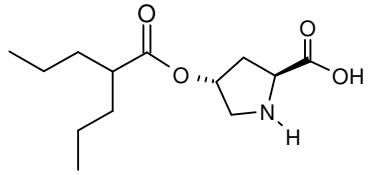
SPIC00201

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29 (10H, m), 5.28-5.00 (5H, m), 4.55 (1/2H, t, *J*= 8 Hz), 4.46 (1/2H, t, *J*= 8 Hz), 3.80-3.60 (2H, m), 2.43-2.16 (3H, m), 1.60-1.45 (2H, m), 1.40-1.32 (2H, m), 1.28-1.20 (4H, m), 0.86 (6H, m).

^{13}C NMR (75 MHz, DMSO): δ = 174.74, 171.40, 171.05, 153.79, 153.31, 136.34, 136.20, 135.57, 135.38, 128.24, 128.13, 127.95, 127.87, 127.67, 127.52, 127.28, 127.10, 72.29, 71.53, 66.34, 66.10, 57.66, 57.19, 52.27, 51.89, 44.13, 40.33, 35.78, 34.79, 34.04, 33.92, 33.35, 20.00, 19.91, 13.79, 13.73.

The protected L-hydroxyproline-valproate ester **SPIC00201** (17.24 g, 35.79 mmole) was dissolved in ethanol (50 mL) and ethyl acetate (100 mL) at room temperature and added to a Parr bottle (500 mL) that contained 10% palladium on carbon (3.5 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (30 psi). After 15 hours of shaking, the catalyst was removed by filtration through a thin layer of celite and activated carbon. The ethanol and ethyl acetate mixture was concentrated under reduced pressure at room temperature. After drying overnight under high vacuum at room temperature, the experiment produced L-hydroxyproline-valproic acid ester **SPIC002** (9.2 g, 99.8% yield) as a white solid. In order to remove trace impurities, the zwitterion was purified by reverse-phase column chromatography (50 g ODS silica gel) in two batches. The zwitterion was placed on the column in DIUF water and eluted with mixture of DIUF water/methanol (2:1, 1:1, 1:2, 100% methanol).

The product containing fractions were combined, concentrated under reduced pressure at 20 °C (or less), and dried under high vacuum at room temperature until the weight was constant (24 hours, 6.4 g white solid recovered).



SPIC002

^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.40 (br s, 1H), 8.32 (br s, 1H), 5.28 (m, 1H), 4.11 (t, 1H, J= 7.2 Hz), 3.59 (m, 1H), 3.34 (br d, 1H, J= 10.5 Hz), 2.50-2.22 (m, 3H), 1.62-1.50 (m, 2H), 1.50-1.32 (m, 2H), 1.32-1.19 (m, 4H), 0.88 (t, 6H, J= 7.2 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.99, 173.35, 71.83, 59.56, 49.77, 45.08, 36.19, 34.51, 20.87, 14.31.

HPLC analysis:
99.20% purity; r.t.= 7.228 min.; 70% DIUF water/30% acetonitrile; 1 mL/min; 36.8C; Luna C18, 5u column (serial # 167917-13), 4.6x250 mm; 22 ul injection; sample dissolved in mobile phase.

CHN analysis:
calc.: C 60.68, H 9.01, N 5.44; found: C 60.58, H 9.12, N 5.48.

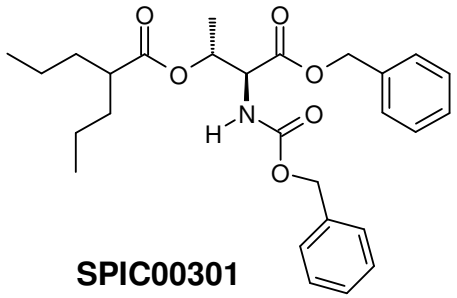
Melting point: 179.0-180.0 °C

3) SPIC003: 2-Propyl-pentanoic acid 2(S)-amino-2-carboxy-1(R)-methyl-ethyl ester, hydrochloride

(L-Threonine-valproic acid ester, hydrochloride)

A mixture of 2-propylpentanoic acid (valproic acid, 4.32 g, 30 mmole), N-carbobenzyloxy-L-threonine benzyl ester (Z-Thr-OBzl, 10.30 g, 30 mmole), EDC (5.74 g, 30 mmole), and DMAP (366 mg, 3.0 mmole) in anhydrous dichloromethane (30 mL) was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, the dichloromethane was washed with water (3×30 mL), dried over magnesium sulfate (5 g), filtered and concentrated under reduced pressure. The remaining colorless oil (13.44 g) was purified by column chromatography on silica gel (100 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with hexanes/ethyl acetate (4:1).

After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected L-threonine-valproate ester **SPIC00301** (12.65 g, 89.8% yield) as a colorless oil.



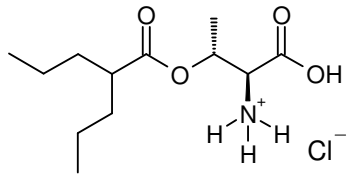
SPIC00301

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40-7.05 (11H, m), 5.45 (1H, m), 5.17-5.02 (4H, m), 4.53 (1H, d, J= 9.6 Hz), 2.24 (1H, m), 1.58-1.40 (2H, m), 1.40-1.15 (9H, m), 0.86 (6H, m).

^{13}C NMR (75 MHz, DMSO): δ = 174.24, 169.29, 156.48, 136.61, 135.34, 128.26, 128.20, 127.74, 127.67, 127.58, 69.04, 66.33, 65.78, 57.62, 44.50, 33.89, 33.80, 20.03, 19.91, 16.40, 13.87.

The protected L-threonine-valproate ester **SPIC00301** (12.65 g, 26.9 mmole) was dissolved in ethanol (50 mL) and ethyl acetate (50 mL) at room temperature and added to a Parr bottle (500 mL) that contained 10% palladium on carbon (2.53 g, 50% wet) under a nitrogen atmosphere. The nitrogen atmosphere was replaced with hydrogen gas (30 psi). After 20 hours the catalyst was removed by filtration through a thin layer of activated carbon, washing with ethanol (25 mL). The ethanol and ethyl acetate were concentrated under reduced pressure at room temperature. After drying under high vacuum, the remaining solids (6.13 g) were acidified with hydrochloric acid (3.1 mL conc.) in DIUF water (50 mL). The solution was filtered a second time through activated carbon and dried overnight in a freeze-dryer. The experiment produced L-threonine-valproic acid ester, hydrochloride **SPIC003** (6.52 g, 86.0 % yield) as a white solid.

The combined batches of the L-threonine-valproic acid ester, hydrochloride **SPIC003** (8.8 g) were purified by crystallization form acetonitrile. After the salt was dissolved in hot acetonitrile (225 mL), the material was treated activated acrbon, filtered, and placed in a 5 °C refrigerator overnight. The white solids were filtered after 18 hours, washed with cold acetonitrile (10 mL), and dried under high vacuum at room temperature until the product weight was constant (24 hours). The process recovered L-threonine-valproic acid ester, hydrochloride **SPIC003** (6.82 g, 77.5 % recovery) as a white solid.



SPIC003

^{1}H NMR (300 MHz, DMSO): δ = 8.71 (br s, 3H), 5.28 (m, 1H), 4.16 (d, 1H, J= 2.7Hz), 2.33 (m, 1H), 1.56-1.40 (m, 2 H), 1.37-1.27 (m, 5H), 1.21-1.13 (m, 4H), 0.84 (t, 6H, J= 6.6 Hz).

^{13}C NMR (75 MHz, DMSO): δ = 173.97, 168.19, 67.69, 55.42, 44.43, 33.95, 33.78, 20.07, 19.95, 16.54, 13.94.

HPLC analysis:
98.88% purity; r.t.= 4.864 min.; 70% DIUF water/30% acetonitrile; 1 mL/min; 40C; Luna C18, 5u column (serial # 211739-42), 4.6x250 mm; 20 ul injection; sample dissolved in mobile phase.

CHN analysis:
calc.: C 51.15, H 8.59, N 4.97; found: C 51.29, H 8.59, N 4.98.

Melting point: 144 °C

Solubility of the above esters wee determined in water at room temperature by dissolving excess of each of the drug and allowing them to settle for a few hours. The resulting solutions were centrifuged at 1500rpm for 3 min and the supernatant liquid was analyzed. It was shown that these esters possess solubility in water in excess of 50 mg/mL.

There are a number of screening tests to determine the utility of the prodrugs created according to the disclosed methods. These include both in vitro and in vivo screening methods.

The in vitro methods include acid/base hydrolysis of the prodrugs, hydrolysis in pig pancreas, hydrolysis in rat intesetinal fluid, hydrolysis in human gastric fluid, hydrolysis in human intestinal fluid, and hydrolysis in human blood plasma. These assays are described in Simmons, DM, Chandran, VR and Portmann, GA, Danazol, Amino Acid Prodrugs: In Vitro and In Situ Biopharmaceutical Evaluation, Drug Development and Industrial Pharmacy, Vol. 21, Issue 6, Page 687, 1995, the contents of all of which are incorporated by reference.

Prodrugs of Valproic acid of the present invention are effective in treating diseases or condidiotns in which Valproic acid normally are used. The prodrugs disclosed herein are transformed within the body to release the active compond and enhances the therapeutic benefits of the Valproic acid by reducing or eliminating biopharmaceutical and pharmacokinetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals.

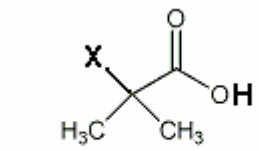
Thus, the prodrug of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs.

Furthermore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

VII WATER SOLUBLE PRODRUGS OF FIBRIC ACID DERIVATIVES

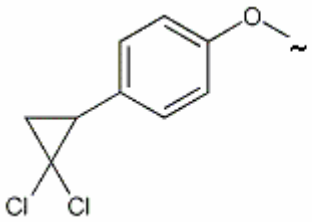
Fibric acid derivatives are useful anti-hyperlipidemic drugs useful in the treatment of hyperlipidemia in mammals where the symptoms are elevated triglycerides, low HDL (High density lipoproteins or “good” cholesterol, and elevated cholesterol. Fibric Acid derivatives are also useful in reducing LDL (Low density lipoproteins, or “bad”

cholesterol). The general structure of the fibric acid analogs is represented below, where X is various mixed aliphatic and aromatic functionalities. Specific derivatives included in this formula are clofibric acid, fenofibric acid, ciprfibrate and gemfibrozil and the like.

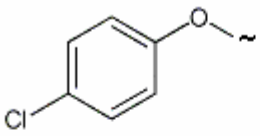


FIBRIC ACID ANALOGS

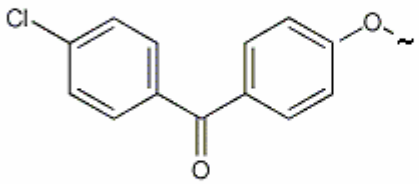
Typical examples of the chemical moiety X in the above structure are shown below.



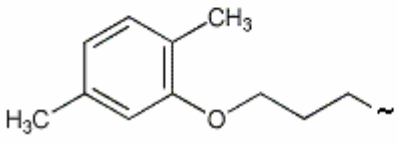
Ciprofibric Acid



Clofibric Acid



Fenofibric Acid



Gemfibrozil

Fibric acid analogs shown in the structure above have been shown to have a large number of therapeutic applications, which are quite varying and somewhat surprising. Broadly, these derivatives are useful in the treatment dyslipidemia and dyslipoproteinemia. Dyslipidemia and dyslipoproteinemia are herein defined to include the group selected from hypercholesterolemia, abnormal and elevated levels of cholesterol, abnormal and elevated levels of LDL cholesterol, abnormal and elevated levels of total cholesterol, abnormal and elevated levels of plasma cholesterol, abnormal and elevated levels of triglycerides, hypertrigylceridaemia, abnormal levels of lipoproteins, abnormal and elevated levels of low density lipoproteins (LDLs), abnormal and elevated levels of very low density lipoproteins, abnormal and elevated levels of very low intermediate density lipoproteins, abnormal levels of high density lipoproteins, hyperlipidemia, hyperchylomicronemia, abnormal levels of chylomicrons, related disorders, and combinations thereof such as those described in The ILIB Lipid Handbook for Clinical Practice, Blood Lipids and Coronary Heart Disease, Second Edition, A. M. Gotto et al, International Lipid Information Bureau, New York, N.Y., 2000, which is hereby incorporated by reference.

Mechanism of Action:

The mechanism of action of Fibric acid derivatives seen in clinical practice have been explained in-vivo in transgenic mice and in vitro in human hepatocyte cultures by the activation of peroxisome proliferator activated receptor alpha (PPAR-alpha). Through this mechanism, Fibric acid derivatives increase lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III (an inhibitor of lipoprotein lipase activity).

The resulting fall in triglycerides produces an alteration in the size and composition of LDL from small, dense particles (which are thought to be atherogenic due their susceptibility to oxidation), to large buoyant particles. These larger particles have greater affinity for cholesterol receptors and are catabolized rapidly. Activation of PPAR-alpha also induces an increase in the synthesis of apoproteins A-I, A-II, and HDL cholesterol.

Fibric Acid derivatives are also useful in the treatment of gout, as they reduce serum uric acid levels in hyperurecemic patients.

Hyperlipidemia types include type I, type IIa, type IIb, type III, type IV, and type V. These types can be characterized according to the levels relative to normal of lipids (cholesterol and triglycerides) and lipoproteins described above. Different classifications are derived from Drug Facts and Comparisons, 52nd Edition (1998) page 1066 which is hereby incorporated by reference.

Many of the fibric acid derivatives when administered orally do not have sufficient bioavilability and absorption are variable, erratic and depended upon food. In fact absolute bioavialbility of many of the fibric acid derivatives is not possible since the prodrugs of fibric acids currently marketed as insoluble in water, hence a parenteral formuation is difficult or not available. Furthermore, since these drugs usually administered as esters, they are in fact prodrugs. These prodrugs have to be metabolized in the body to release active drug, which are the fibric acids. However, due to the ester formation of these drugs, they are quite insoluble in water, hence are difficult to formulate, and are not easily broken down in the body to release active drugs.

Many of the Fibric acid derivatives are low to medium molecular weight solids with characteristic odor. Taken orally it has unpleasant taste and can severely irritate mouth and throat. Taken with food provides more blood concentration compared to fasting. This fed/fast difference in bioavailability is more pronounced when Fibric acid derivatives are compared against their corresponding prodrug derivatives. Overall bioavailability has been reported anywhere between 40-60 and quite variable among patients.

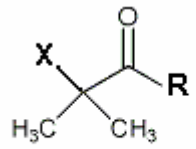
One of the significant problems associated with currently marketed fibric acid derivatives being that when these prodrugs are cleaved in the body, they release the prodrug moiety, which themselves are highly toxic. For example, in the case of

fenofibrate and gemfibrozil isopropyl alcohol is released as the esterase enzyme cleave the pro-moiety from the fenofibric acid. It is well know isopropanol is highly toxic when released into any of the mammalian tissues.

In order to improve the therapeutic effectiveness, uniform blood profile, develop pharmaceutically elegant formulation and improve the solubility of the drug in water, present invention discusses alternative prodrugs of Fibric acid derivatives which overcome many of the difficulties stated above.

Accordingly, in one aspect, the present invention is directed to alternate class of prodrugs of Fibric acid derivatives. The prodrug consists of the hydroxyl group of an amino acid esterified to the free carboxyl group present on the Fibric acid derivatives molecules. In another embodiment, the amine group of the amino acid is reacted with COOH of the fibric acids to form an amide linkage.

More specifically, in one aspect of the present invention is directed to, the compounds of the formulas



FIBRIC ACID ANALOGS

where x is as defined hereinabove

or pharmaceutically acceptable salts thereof; wherein R is either NH-AA or O-AA and AA is an amino acid, in which either an amine group or the hydroxyl group is reacted with the carboxylic acid group of Fibric acid derivatives.

The present invention is also directed in an embodiment to a pharmaceutical composition comprising a therapeutically effective amount of the various Fibric acid derivatives prodrugs above and a pharmaceutical carrier therefor.

In another embodiment, the present invention is directed to a method of treating a patient in need of Fibric acid derivatives therapy, which method comprises administering to said patient an effective amount of the Fibric acid derivatives.

In a further embodiment, the present invention is directed to a method of converting liquid Fibric acid derivatives into a solid powder by reacting the carboxyl functionality of the Fibric acid derivatives with either amine or hydroxyl functionality of an amino acid and isolating the products thereof.

In a still further embodiment, the present invention is directed to a method of substantially and in a therapeutically efficacious manner, make the derivatives absorbed easily upon oral administration thereby improving the consistent therapeutic effect by administering to a patient a prodrug which comprises reacting the COOH functionality of the Fibric acid derivatives molecule with either NH_2 or OH functionality of selected amino acids to form an ester or amide covalent bond respectively and isolating the product thereof and administering said product to the patient.

It was determined that when unsubstituted naturally occurring amino acids are esterified to Fibric acid derivatives, the resulting prodrugs are pharmaceutically elegant free flowing powders, and are rapidly absorbed into the body and release non-toxic amino acids upon cleavage in the body and require none of the emulsifiers, additives and other exepients.

Furthermore, it has been found that the current invention also produced drugs, while they are prodrugs of Fibric acid derivatives; they were highly effective anti-hyperlipidemics and were exhibiting such effect intact. Thus the current amino acid prodrugs are

effective anti-hyperlipidemics and useful in the treatment of a number of high cholesterol related illnesses and exhibit such potential with or without releasing the active parent drug.

While the prodrugs of fibric acidn of the present invention are not expected to possess any acidic activity due to blockage of the carboxylic acid group responsible for such, however it has been shown that the prodrugs of fibric acid are effective anti-hyperlipidemics with or without releasing Fibric acid derivatives. However, all of the Fibric acid derivatives prodrugs described are released in vivo the active drug with all its pharmacological and cholesterol lowering properties.

The present invention clearly provides a number of advantages over Fibric acid derivatives, for example, all of the side chains cleaved from these prodrugs are naturally occurring essential amino acids hence are non-toxic. This results in high therapeutic index. Secondly all the prodrugs are readily cleaved in the body to release Fibric acid derivatives. Furthermore, due their high water solubility, they can be easily administered by either forming an in-situ solution just before IV administration using lyophilized sterile powder or providing the drug in solution in prefilled syringe or bottles for infusion. The aminoacid esters are more stable than Fibric acid derivatives since COOH group in Fibric acid derivatives is blocked to reaction with bases. Thus the Fibric acid derivatives prodrugs described here are more effective then Fibric acid derivatives itself without the toxicity and other pharmaceutical problems associated with current marketed formulations.

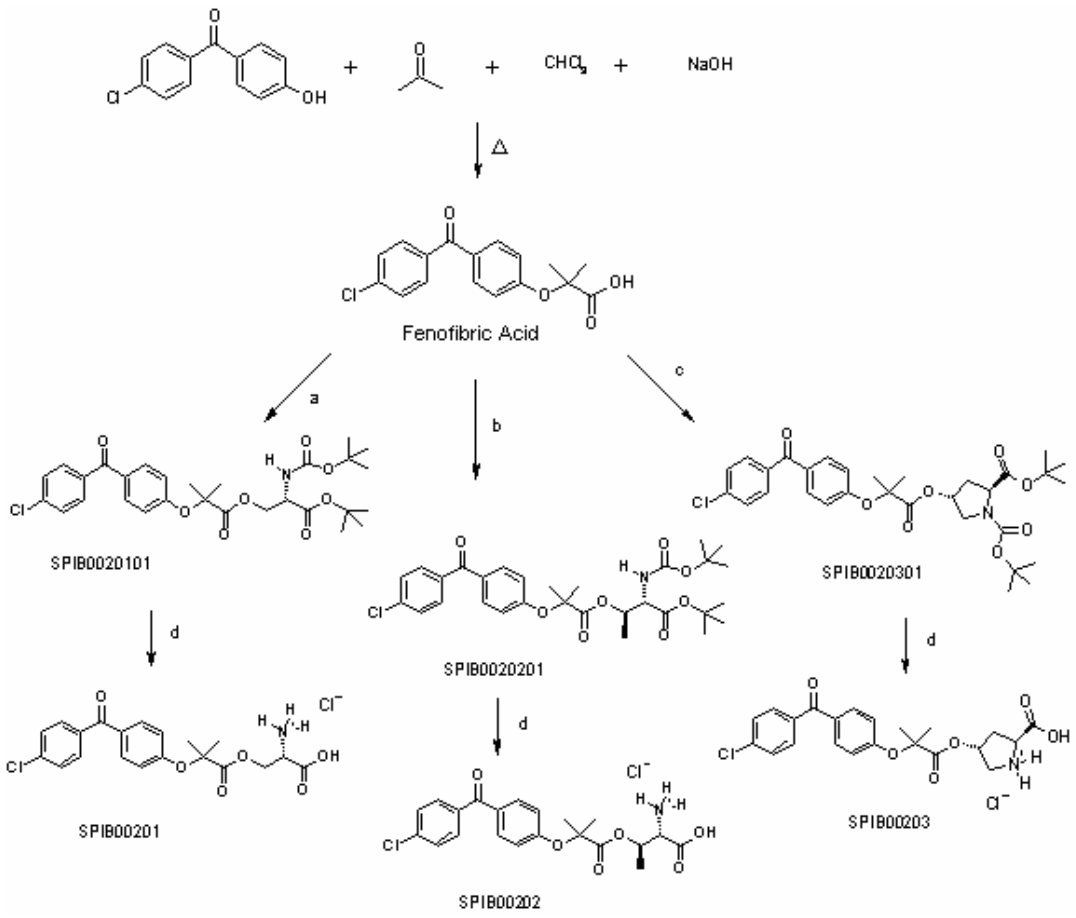
The prodrugs of this invention are anti-hyperlipidemic drugs useful in the treatment of hyperlipidemia in mammals where the symptoms are elevated triglycerides, low HDL (High density lipoproteins or “good” cholesterol, and elevated cholesterol. Fibric Acid derivatives are also useful in reducing LDL (Low density lipoproteins, or “bad” cholesterol).

Typical examples of synthesis of L-threonine, L-hydroxyproline and L-serine esters of Fibric acid derivatives are shown in the synthetic processes outlined below. These procedures are applicable to all other compounds of the Fibric acid derivatives class as well.

Synthesis of Fibric acid derivatives Prodrugs

The procedure for the synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of fenofibric acid is outlined in **Synthetic Sequence** section and is exemplary. The complete procedure and analytical data is given in the **Experimental Section**. In general, fenofibric acid (100 g batches) was prepared from 4-chloro-4'-hydroxybezophenone in accordane with the procedures in the literature. Fenofibric acid was coupled with the t-butyl esters of N-Boc protected amino acid (L-serine, L-threonine, and L-hydroxyproline) using EDC as the coupling agents and a catalytic amount of DMAP. The protecting groups were removed at low temperature (5 °C, 3-6 days) with a mixture of hydrochloric acid in acetic acid (1M) with dichloromethane. The amino acid ester salts of fenofibric acid were purified by crystallization from ethyl acetate, and dried under high vacuum.

Synthetic Sequence:

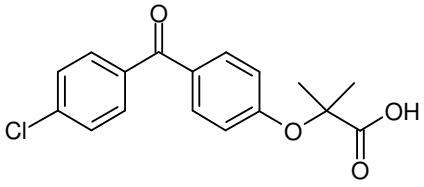


Synthesis of the L-serine, L-threonine, and L-hydroxyproline esters of fenofibric acid: a) Boc-Ser-OtBu, EDC, DMAP, CH_2Cl_2; b) Boc-Thr-OtBu, EDC, DMAP, CH_2Cl_2; c) Boc-Hyp-OtBu, EDC, DMAP, CH_2Cl_2; d) HCl, AcOH, CH_2Cl_2.

Experimental Section:

The synthesis of **SPIB00201**, **SPIB00202** and **SPIB00203** was conducted in one or two batches. Reagents mentioned in the experimental section were purchased at the highest obtainable purity from Lancaster, Sigma-Aldrich, Acros, or Bachem, except for solvents, which were purchased from either Fisher Scientific or Mallinkrodt.

1) Synthesis of fenofibric acid:



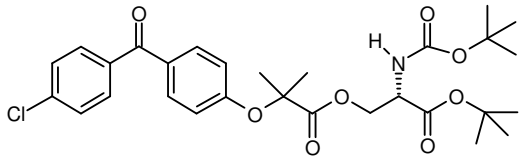
A mixture of 4-chloro-4'-hydroxybezophenone (116 g, 0.500 mole) and sodium hydroxide (120 g, 3.00 mole) in acetone (1 L) was heated to reflux for 2 hours. The heating was stopped and the heating source was removed. A mixture of chloroform (179 g, 1.50 mole) in acetone (300 mL) was added drop-wise. The reaction mixture was stirred overnight without heating. The mixture was heated to reflux for 8 hours and then allowed to cool to room temperature. The precipitate was removed by filtration and washed with acetone (100 mL). The filtrate was concentrated under reduced pressure to give a brown oil. Water (200 mL) was added to the brown oil and was acidified (to pH=1) with 1N hydrochloric acid. The precipitate, which formed was filtered and dried under high vacuum. The remaining yellow solid (268 g) was recrystallized from toluene in 4 batches (400 mL toluene each). After filtration and drying under high vacuum, the experiment produced **fenofibric acid** (116 g, 73% yield) as a light yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.22 (1H, s, br), 7.72 (4H, d, J= 8.4 Hz), 7.61 (2H, d, *J*= 7.8 Hz), 6.93 (2H, d, *J*= 7.8 Hz), 1.60 (6H, s).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 192.96, 174.18, 159.35, 136.84, 136.12, 131.67, 131.02, 129.12, 128.43, 116.91, 78.87, 25.13.

2) SPIB00201: L-serine-fenofibric acid ester

To a mixture of fenofibric acid (11.6 g, 36.3 mmol), N-carbobenzyloxy-L-serine t-butyl ester (Boc-Ser-OtBu, 8.62 g, 33.0 mmol), EDC (7.59 g, 39.6 mmol), and DMAP (484 mg, 3.96 mmol) cooled in an ice-water bath was added anhydrous dichloromethane (150 mL) dropwise. After the addition was complete, the ice bath was removed and the reaction mixture was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, the additional dichloromethane (200 mL) was added and the solution was washed with water (2×200 mL) and brine (200 mL). After drying over sodium sulfate and filtration, the solution was concentrated under reduced pressure. The remaining yellow oil (21.2 g) was purified by column chromatography on silica gel (400 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with heptane/ethyl acetate (3:1). After concentration of the product-containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected L-serine-fenofibric acid ester **SPIB0020101** (16.2 g, 87% yield) as a light yellow oil.

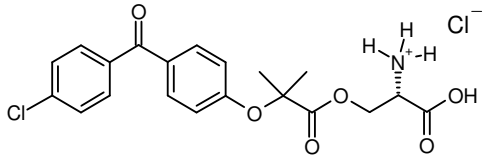


^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (2H, d, *J*= 9.0 Hz), 7.72 (2H, d, *J*= 9.0 Hz), 7.45 (2H, d, *J*= 8.7 Hz), 6.86 (2H, d, *J*= 8.7 Hz), 5.04 (1H, d, *J*= 6.9 Hz), 4.55-4.42 (3H, m), 1.66 (3H, s), 1.65 (3H, s), 1.43 (9H, s), 1.39 (9H, s).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 193.92, 172.99, 168.07, 159.24, 154.87, 138.24, 136.19, 131.94, 131.06, 130.40, 128.41, 117.26, 82.88, 80.13, 79.24, 65.44, 53.44, 28.27, 27.92, 25.70, 25.30.

To a stirred solution of the protected L-serine-fenofibric acid ester **SPIB0020101** (16.2 g, 28.8 mmol) in anhydrous dichloromethane (100 mL) cooled to 5 °C, under an argon atmosphere was added a solution of hydrogen chloride in acetic acid (400 mL, 1M, 400

mmol) drop-wise. The reaction mixture stirred for 3 days at 5 °C. After three days the mixture was concentrated under reduced pressure and dried under high vacuum to remove acetic acid. To the remaining light yellow oil (24.7 g) was added ethyl acetate (100 mL). The solution was concentrated and dried a second time. To the remaining light yellow oil (17.0 g) was added ethyl acetate (65 mL). The mixture was heated to reflux for 5 minutes and cooled to room temperature. The precipitate was removed by filtration and dried under high vacuum overnight at room temperature, then at 43 °C for one hour. The experiment produced the L-serine-fenofibric acid ester, hydrochloride **SPIB00201** (7.66 g, 60% yield) as a white solid.



^{1}H NMR (300 MHz, DMSO-d_6): δ = 14.12 (1H, s, br), 8.77 (3H, s, br), 7.72 (4H, m), 7.62 (2H, d, *J*= 8.4 Hz), 6.92 (2H, d, *J*= 9.0 Hz), 4.62 (1H, dd, *J*= 12.0, 4.2 Hz), 4.50 (1H, dd, *J*= 12.0, 2.4 Hz), 4.41 (1H, m), 1.64 (3H, s), 1.63 (3H, s).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 193.06, 171.70, 168.06, 158.72, 136.93, 136.06, 131.73, 131.09, 129.62, 128.49, 117.64, 79.02, 62.99, 51.11, 25.04, 24.94.

HPLC analysis:

100% purity; r.t.= 4.361 min.; 55% TFA (0.1%), 45% ACN; 1 mL/min; 32.3 C, Luna C18, serial # 167917-13; 20 ul inj., NB275-49.

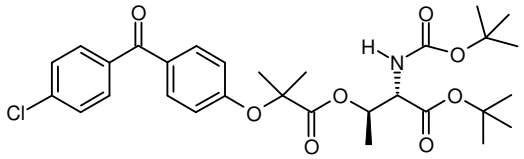
CHN analysis:

calc.: C 54.31, H 4.79, N 3.17; found: C 54.37, H 4.78, N 3.12.

Melting point: 151°C (dec.)

3) SPIB00202: **L-threonine-fenofibric acid ester**

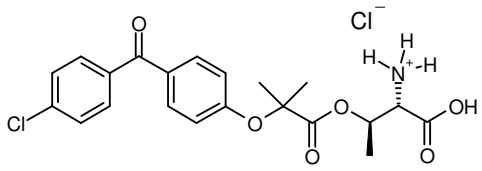
To a mixture of fenofibric acid (25.5 g, 79.9 mmol), N-carbobenzyloxy-L-threonine t-butyl ester (Boc-Thr-OtBu, 20.0 g, 72.6 mmol, prepared by the literature method), EDC (16.7 g, 87.1 mmol), and DMAP (1.06 g, 8.71 mmol) cooled in an ice-water bath was added anhydrous dichloromethane (200 mL), dropwise. After the addition was complete, the ice bath was removed and the reaction mixture was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, additional EDC (1.39 g, 7.26 mmol) was added and the experiment was allowed to stir over the weekend at room temperature under an argon atmosphere. After 4 days, additional dichloromethane (300 mL) was added and the solution was washed with water (300 mL) and brine (300 mL). After drying over sodium sulfate and filtration, the solution was concentrated under reduced pressure. The remaining yellow oil (53.5 g) was purified by column chromatography on silica gel (500 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with heptane/ethyl acetate (3:1). After concentration of the product-containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected L-threonine-fenofibric acid ester **SPIB0020201** (34.1 g, 82% yield) as a white foam.



^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (2H, d, *J*= 8.4 Hz), 7.72 (2H, d, *J*= 8.4 Hz), 7.45 (2H, d, *J*= 8.4 Hz), 6.87 (2H, d, *J*= 8.4 Hz), 5.47 (1H, m), 4.98 (1H, d, *J*= 9.9 Hz), 4,31 (1H, d, *J*= 9.9 Hz), 1.65 (3H, s), 1.64 (3H, s), 1.45 (9H, s), 1.42 (9H, s), 1.22 (3H, d, *J*= 6.3 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 193.94, 172.14, 168.70, 159.26, 155.62, 138.28, 136.18, 131.90, 131.08, 130.37, 128.43, 117.40, 82.70, 80.17, 79.38, 72.02, 57.46, 28.30, 27.99, 26.44, 24.79, 16.90.

To a stirred solution of the protected L-threonine-fenofibric acid ester **SPIB0020201** (34.1 g, 59.2 mmol) in anhydrous dichloromethane (100 mL) cooled to 5 °C, under an argon atmosphere was added a solution of hydrogen chloride in acetic acid (600 mL, 1M, 600 mmol) drop-wise. The reaction mixture was kept for 6 days at 5 °C. The mixture was concentrated under reduced pressure and dried under high vacuum to remove acetic acid. To the remaining white solid (45.8 g) was added ethyl acetate (500 mL). The mixture was heated to reflux for 10 minutes and cooled to room temperature. The precipitate was removed by filtration and dried under high vacuum overnight at room temperature. The experiment produced the L-threonine-fenofibric acid ester, hydrochloride **SPIB00202** (26.3 g, 97% yield) as a white solid.



^{1}H NMR (300 MHz, DMSO-d_6): δ = 14.10 (1H, s, br), 8.84 (3H, s, br), 7.73 (4H, m), 7.63 (2H, d, *J*= 8.1 Hz), 6.89 (2H, d, *J*= 8.7 Hz), 5.44 (1H, m), 4.31 (1H, s), 1.64 (3H, s), 1.62 (3H, s), 1.38 (3H, d, *J*= 6.3 Hz).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 193.04, 171.00, 168.13, 158.76, 136.90, 136.08, 131.70, 131.06, 129.49, 128.48, 117.41, 78.99, 69.40, 55.21, 25.59, 24.22, 16.06.

HPLC analysis:

98.59% purity; r.t.= 4.687 min.; 55% TFA (0.1%), 45% ACN; 1 mL/min; 32.3 C, Luna C18, serial # 167917-13; 20 ul inj., NB275-49, DAD1 B, Sig=210.4, Ref=550,100.

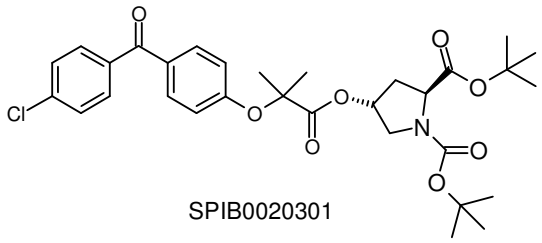
CHN analysis:

calc.: C 55.27, H 5.08, N 3.07; found: C 54.98, H 5.13, N 3.03.

Melting point: 160.5 °C (dec.)

4) SPIB00203: **L-hydroxyproline-fenofibric acid ester**

To a mixture of fenofibric acid (24.9 g, 78.1 mmol), N-carbobenzyloxy-L-hydroxyproline t-butyl ester (Boc-Hyp-OtBu, 20.4 g, 71.0 mmole, prepared in accordance with the procedure in the literature), EDC (16.3 g, 85.2 mmol), and DMAP (1.04 g, 8.52 mmol) cooled in an ice-water bath was added anhydrous dichloromethane (200 mL) dropwise. After the addition was complete, the ice bath was removed and the reaction mixture was stirred under an argon atmosphere at room temperature for 20 hours. After 20 hours, additional EDC (1.63 g, 8.52 mmol) was added and the experiment was allowed to stir over the weekend at room temperature under an argon atmosphere. After 4 days the solution was washed with water (200 mL) and brine (200 mL). After drying over sodium sulfate and filtration, the solution was concentrated under reduced pressure. The remaining yellow oil (49.4 g) was purified by column chromatography on silica gel (500 g, 0.035-0.070 mm, 6 nm pore diameter), eluting with heptane/ethyl acetate (2:1). After concentration of the product containing fractions under reduced pressure and drying under high vacuum until the weight was constant, the experiment produced the protected L-hydroxyproline-fenofibric acid ester **SPIB0020301** (26.4 g, 63% yield) as a colorless oil.

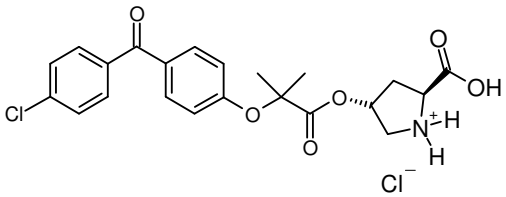


SPIB0020301

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.76 (2H, d, *J*= 8.1 Hz), 7.73 (2H, d, *J*= 8.1 Hz), 7.46 (2H, d, *J*= 8.1 Hz), 6.84 (2H, d, *J*= 8.1 Hz), 5.32 (1H, m), 4.13 (0.38H, t, *J*= 7.8 Hz), 4.00 (0.62H, t, *J*= 7.8 Hz), 3.67 (1.62H, m), 3.46 (0.38H, d, J=12.6 Hz), 2.29 (1H, m), 2.15 (1H, m), 1.68 (3H, s), 1.66 (3H, s), 1.44-1.38 (18H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 193.88, 172.98, 171.14, 159.25, 153.48, 138.23, 136.16, 131.99, 131.08, 130.36, 128.44, 117.03, 116.91, 81.48, 80.32, 80.20, 79.19, 74.03, 73.26, 58.23, 51.88, 51.58, 36.33, 35.31, 31.92, 28.29, 28.00, 25.89, 24.95.

To a stirred solution of the protected L-hydroxyproline-fenofibric acid ester **SPIB0020301** (26.0 g, 44.2 mmol) in anhydrous dichloromethane (100 mL) cooled to 5 °C, under an argon atmosphere was added a solution of hydrogen chloride in acetic acid (450 mL, 1M, 450 mmol) drop-wise. The reaction mixture stirred for 4 days at 5 °C. After four days the mixture was concentrated under reduced pressure and dried under high vacuum to remove acetic acid. To the remaining yellow oil (31.5 g) was added ethyl acetate (200 mL). The mixture was sonicated and then concentrated under reduced pressure and dried under high vacuum. To the remaining white solid (23.2 g) was added ethyl acetate (300 mL). The ethyl acetate mixture was heated to reflux for 10 minutes and cooled to room temperature. The precipitate was removed by filtration and dried under high vacuum overnight at room temperature. The experiment produced the L-hydroxyproline-fenofibric acid ester, hydrochloride **SPIB00203** (15.8 g, 76% yield) as a white solid.



^{1}H NMR (300 MHz, DMSO-d_6): δ = 14.07 (1H, s, br), 10.75 (1H, s, br), 9.40 (1H, s, br), 7.71 (4H, d, *J*= 8.1 Hz), 7.60 (2H, d, *J*= 8.1 Hz), 6.96 (2H, d, *J*= 8.1 Hz), 5.42 (1H, m), 4.24 (1H, t, *J*= 9.0 Hz), 3.61 (1H, dd, *J*= 13.2, 4.2 Hz), 3.28 (1H, d, *J*= 13.2 Hz), 2.35 (2H, m), 1.66 (3H, s), 1.64 (3H, s).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 193.00, 171.52, 169.14, 158.81, 136.87, 136.09, 131.81, 131.05, 129.48, 128.46, 117.28, 78.99, 73.79, 57.54, 50.23, 34.13, 25.69, 24.49.

HPLC analysis:

100% purity; r.t.= 8.369 min.; 60% DIUF water (0.1% TFA)/40% acetonitrile; 1 mL/min; 36.4 C; Luna C18, 5u column (serial # 191070-3), 4.6x250 mm; 20 ul injection; DAD1 A, Sig = 210.4, Ref = 550,100.

HPLC-MS (ESI): calculated: M^+ = 431; found M+H= 432.3

Melting point: 187.5 °C (dec.)

Solubility of the above esters were determined in water at room temperature by dissolving excess of each of the drug and let them settle for few hours. The resulting solutions were centrifuged at 1500rpm for 3 min and the supernatant liquid was analyzed. It was shown that these esters possess solubility in water in excess of 50 mg/mL.

EXPERIMENTAL

Rats were checked for time zero triglyceride level in blood. Then the rats were set on high sugar diet, such as 30% surcorse in water for 1 week. Then at the end of 1 week, rats were tested for triglycerides, and were put on normal diet. From day 7-14 the rats were administered either test or control drug. Triglycerides were again tested on the 14th day in rat blood.

In the Fenofibrate (control) vs L-Serine Ester of Fenofibric acid (test drug), 3 rats each for each of the drug and control at equivalent doses of 50, 100 and 200 mg/kg were tested.

The results are shown below.

SUMMARY – DOSE RANGE FINDING STUDY – HYPOLIPIDEMIC PROPERTY – FENOFIBRATE AND ITS FORMULATION

Test Substance: L-Serine Ester of Fenofibric Acid

Vehicle: 1% Tween 80 in milli Q -water

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Test Item	Dose. Mg/kg)	Animal No.	Triglycerides (mg/dl)		
			Day zero	Day 7	Day 14
Vehicle	0	1	81	168	121
		2	88	171	222
		3	114	133	162
Reference control Fenofibrate	50	4	95	157	101
		5	92	228	76
		6	80	150	73
	100	7	110	204	62
		8	115	195	69
		9	96	167	93
	200	10	144	90	48
		11	56	106	51
		12	58	125	38
L-Serine Ester of Fenofibric Acid	50	13	88	148	86
		14	94	145	86
		15	100	127	73
	100	16	109	-	46
		17	129	100	69
		18	71	183	47
	200	19	74	240	83
		20	81	158	61
		21	42	77	46

From the above results, it can be concluded the highly water soluble serine ester was effectively performed.

10 There are a number of screening tests to determine the utility of the prodrugs created according tio the disclosed methods. These include both <u>in vitro</u> and <u>in vivo</u> screening methods.

The in vitro methods include acid/base hydrolysis of the prodrugs, hydrolysis in pig pancreas hydrolysis in rat intestinal fluid, hydrolysis in human gastric fluid, hydrolysis in human intestinal fluid, and hydrolysis in human blood plasma. These assays are dscribed in Simmons, DM, Chandran, VR and Portmann, GA, Danazol Amino Acid Prodrugs: In Vitro and In Situ Biopharmaaceutical Evaluation, Drug Development and Industrial Pharmacy, Vol 21, Issue 6, Page 687, 1995, the contents of allof which are incorporated by reference.

The prodrugs of Fibric Acid of the present invention are effective in treating diseases or conditions in which Fibric acid derivatives normally are used. The prodrugs disclosed herein are transformed within the body to release the active compound and enhances the therapeutic benefits of the Fibric acid derivatices by reducing or eliminating biopharmaceutical and pharmacokinetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals.

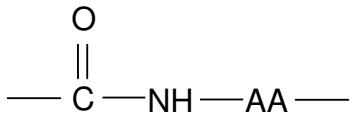
Thus, the prodrug of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs.

Furtheremore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

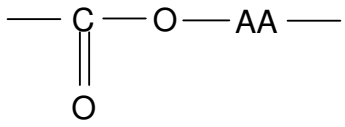
The prodrugs of Fibric acid of the present invention are effective in treating diseases or conditions in which Fibric acid derivatives normally are used. The prodrugs disclosed herein are transformed within the body to release the active compound and enhances the therapeutic benefits of the Fibric acid derivatives by reducing or eliminating biopharmaceutical and pharmacokenetic barriers associated with each of them. However it should be noted that these prodrugs themselves will have sufficient activity without releasing any active drug in the mammals.

Thus, the prodrug of fibric acid of the present invention enhances the therapeutic benefits by removing biopharmaceutical and pharmacokenetic barriers of existing drugs. Furthermore, the prodrugs are easily synthesized in high yields using reagents which are readily and commercially available.

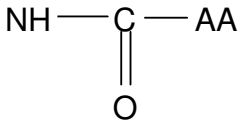
In the formula hereinabove and in the claims it is to be understood that the AA has the following definition in the following contents

1) 

AA in this definition refers to the amino acid residue without an amino group either on the main chain or the side chain.

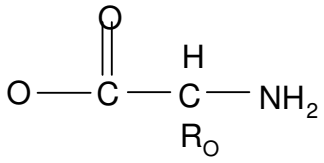
2) 

AA in this definition is an amino acid residue less the hydroxy group on the side chain.

3) 

AA refers to an amino acid group without the carboxy group, either on the main chain or side group.

4) OAA- This is a ester bond between the hydroxy group of the drug and the carboxy group of the amino acid either on the main chain or side chain. Thus, as written OAA is



wherein R_O is the side chain amino acid as defined hereinabove.

Alternatively, it may refer to an ester bond between the carboxy group of the drug and the hydroxy group on the side chain of those amino acids which have a hydroxy group thereon such as threonine, serine, hydroxyproline, tyrosine and the like. The hydroxy group forms part of the ester linkage which is depicted hereinabove with O. Thus, as written, the AA refers to an amino acid with a hydroxy group on the side chain, but as depicted OAA, the AA is without the hydroxy group since the oxygen atom is depicted in the formula.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

IN THE CLAIMS:

1. A method for enhancing at least two of the therapeutic qualities of a drug having a functionality group selected from the group consisting of hydroxy, amino, carboxy or acylating derivatives of said carboxy group, said improved therapeutic quality being selected from the group consisting of:

(a) improved taste or smell

(b) desired octanol/water partition coefficient

(c) improved stability

(d) enhanced penetration of blood-brain barrier

(e) elimination of first pass effect in the liver

(f) reduction of intero-hepatic recirculation

(g) painless injection with parental formulation

(h) improved bioavailability

(i) improved changes in the rate of absorption

(j) reduced side effects

(k) dose proportionality

(l) selective hydrolysis of the prodrug at site of action

(m) controlled release properties

(n) targeted drug delivery

(o) reduction in toxicity

(p) reduced dose

(q) alteration of metabolic pathway to deliver more drug at site of action

(r) increased solutility in aqueous solution and

(s) enhanced efficacy,

the method comprising (a) reacting the drug with an amino acid under conditions effective to form a covalent bond between the drug and the amino acid and (b) administering the product of (a) to a patient in need thereof, wherein said amino acid is a naturally occurring α-L-amino acid.

2. The method according to Claim 1 wherein the amino acid is Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Nor, Arg, Phe, Trp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Dcy and Sar.

3. The method according to Claim 1 or 2 wherein the amino acid is Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Arg, Phe, Trp, Gln, Asn, or Cys.

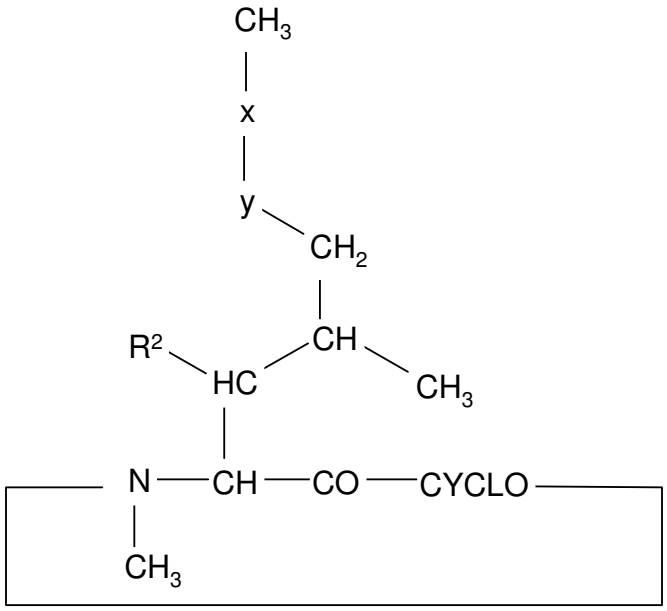
4. The method according to any one of Claims 1 – 3 wherein the the drug is Cyclosporin, Lopinavir, Ritonavir, Cefdinir, Zileuton, Nelfinavir, Flavoxate, Candesarten, Propofol, Nisoldipine, Amlodipine, Ciprofloxacin, Ofloxacin, Fosinopril, Enalapril, Ramipril, Benazepril, Perinodopril, Moexipril, Trandolapril, Cromolyn, Amoxicillin, Cefuroxime, Ceftazidime, Cefpodoxime, Atovaquone, Gancyclovir, Penciclovir, Famciclovir, Acyclovir, Niacin, Bexarotene, Propoxyphene, Salsalate, Acetaminophen, Ibuprofen, Lovastatin, Simavastatin, Atorvastatin, Pravastatin, Fluvastatin, Nadolol, Valsartan, Methylphenidate, Sulfa Drugs, Trovafloxacin, 5-amino-Salicyclic acid, Sulfasalazine, Methylprednisolone, Medroxyprogesterone, Estramustine, Miglitol, Mefloquine, Capacitabine, Danazol, Eprosartan, Valproic Acid, Gabapentin, Omeprazole, Lansoprazole, Megestrol, Metformin, Tazarotene, Sumitriptan, Naratriptan, Zolmitriptan, Aspirin, Olmesartan, Sirolimus, Tacrolimus, Pimecrolimus, Clopidogrel, Amphotericin, Tenofovir, Unoprostone, Fulvestrant, Cefditoren, Efavirenz, Eplerenone, Treprostinil, Adefovir, Salicyclic Acid, Diflunisal, Fenoprofen, Carprofen, Flurbiprofen, Ketoprofen, Naproxen, Etodolac, Sulindac, Indomethacin, Tolmetin, Ketorolac, Ciprofric Acid, Clofibric Acid, Fenofibric Acid, Salazine, or Gembifrozil or a pharmaceutically acceptable salt of any of said drug.

5. The method according to any one of Claims 1-4 wherein AA is Threonine, hydroxyproline or Serine.

6. The method according toany one of Claims 1-5 wherein the amino acid is Threonine.

7. The product from a reaction between a drug having a functionality selected from the group consisting of hydroxy, amino, carboxy or acylating derivative of said carboxy group and a L-α-amino acid under conditions sufficient to form a covalent bond from the reaction of said functional group and said amino acid, or the pharmaceutically acceptable salt thereof, wherein the drug is selected from the group consisting of cyclosporin, lopinavir, Cefdinir, Zileuton, Nelfinavir, Flavoxate, Candesartan, Propolfol, nisolidipine, amlodipine, Ciprofloxacicin, Ofloxacin, Enlarpril, Ramipril, Fosinopril, Benazepril, Perinlopril, Moexipril, Trandolapril, Cromolyn, Amoxicillin, Cepholosporin, Cefuroxime, Ceflazidime, Cefpodoxime, Atovaquone, Penciclovir, Famciclovir, Niacin, Bexarotene, Propoxyphine, Salsalate, Acetaminophen, Ibuprofen, Lovastatin, Simavastatin, Atorvastatin, Pravastatin, Fluvastatin, Nadolol, Valsartan, methylphenidate, Sulfa drug, Sulfasalazine, Trivafloxacin, methyl prednisolone, Medroxyprogesterone, Estramustine, Miglitol, Mefloquine, Capacitabine, Danazol, Eprosartan, Valproic acid, Gabapentin, Omeprazole, Lansoprazole, Megestrol, Metformin, Tazarotene, Sumitriptan, Naratriptan, Zolmitriptan, Olmesartan, Aspirin Sirolimus, Tacrolimus, Pimecrolimus, Clopidogrel, Amphotericin, Tenofovir, Unoprostone, Fulvestrant, Cefditoren, Efavirenz, Eplerenone, salicyclic acid, 5-amino salicyclic acid, diflunisol, fenoprofen, carprofen, Flurbiprofen, Ketoprofen, naproxen, etodolac, sulindac, indomethacin, tolmetin, ketorolac, ciprofibric acid, clofibric acid, gemfibrozil, Fenofibric acid, or Salazine or a pharmaceutically acceptable salt of any of said drug and wherein the amino acid is Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Nor, Arg, Phe, Trp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Sar or Dcy.

8. The product according to Claim 7 wherein the product is:


CH3
x
y
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CH3
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CH3

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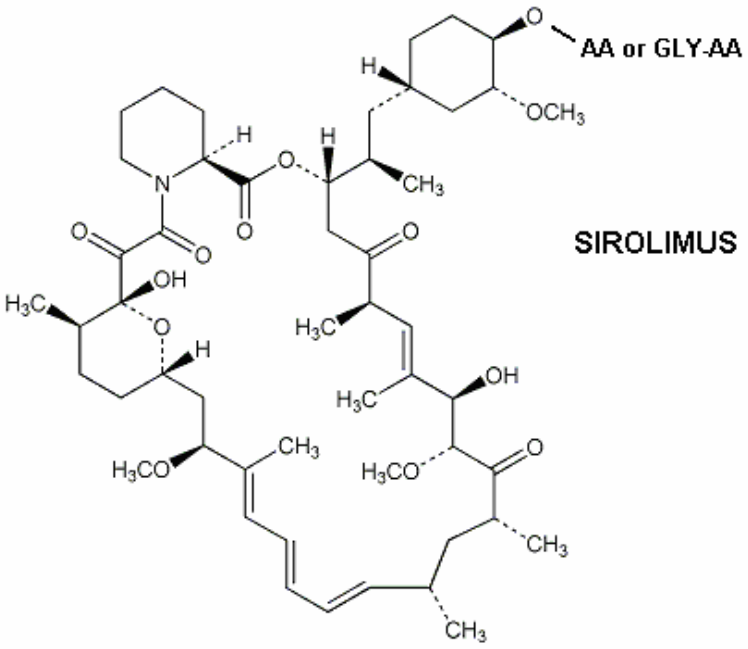
AA or GLY-AA
SIROLIMUS

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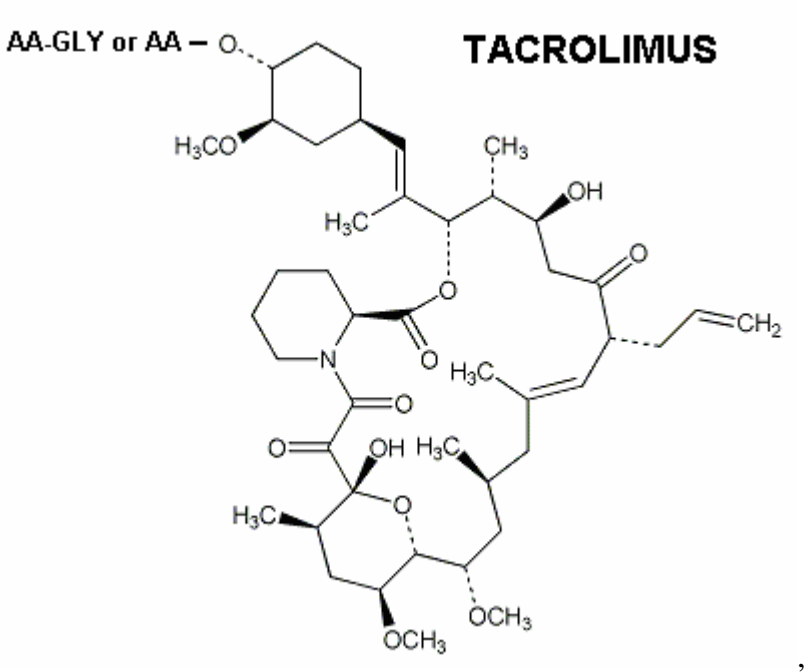
AA-GLY or AA —
TACROLIMUS

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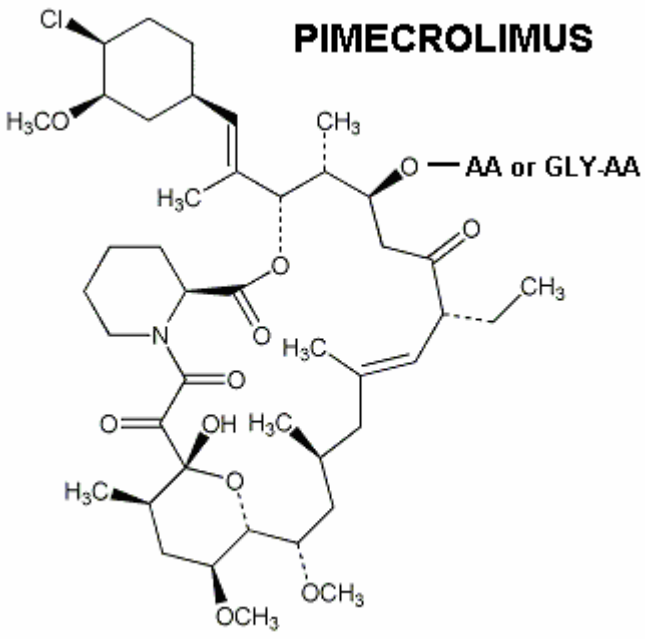
PIMECROLIMUS
AA or GLY-AA

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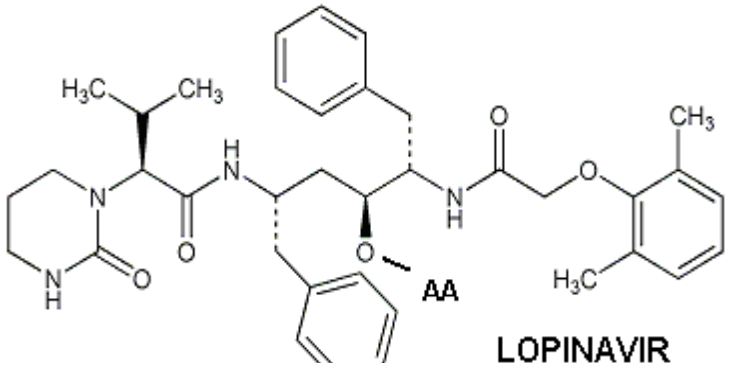

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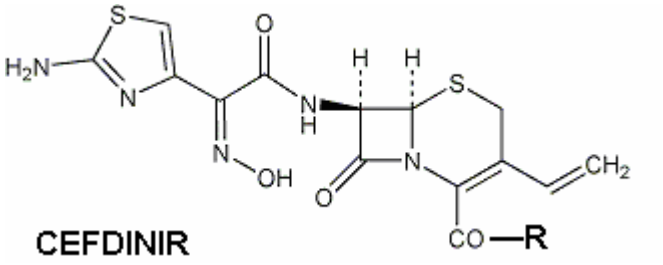

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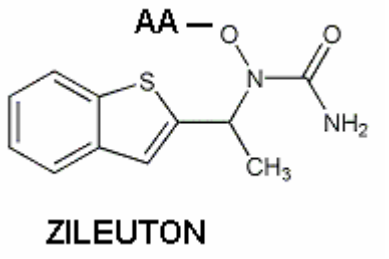

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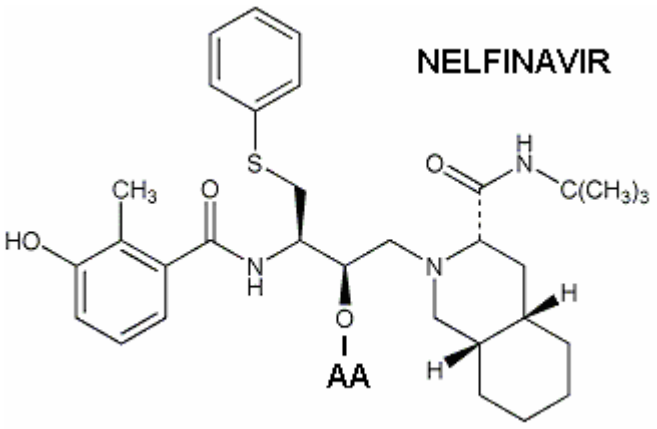

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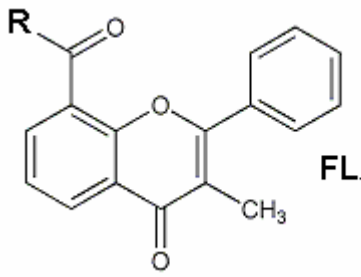

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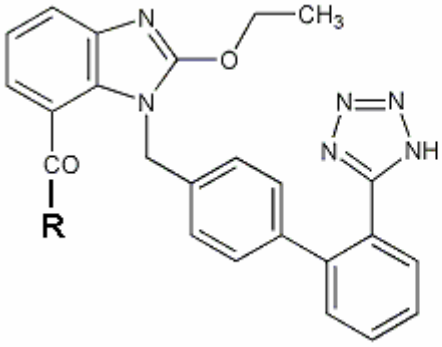

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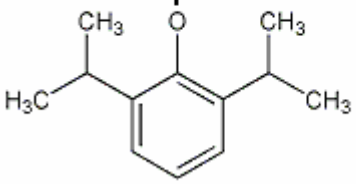

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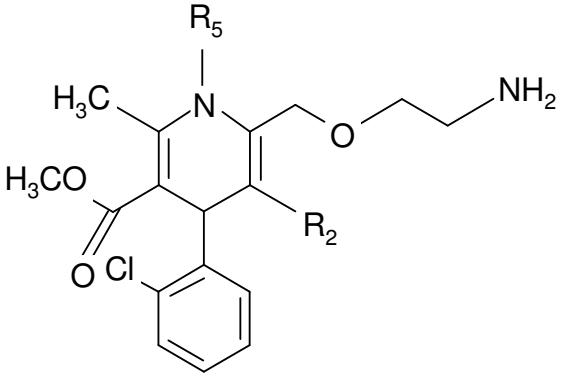

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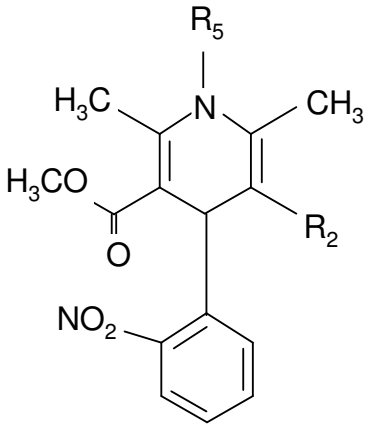

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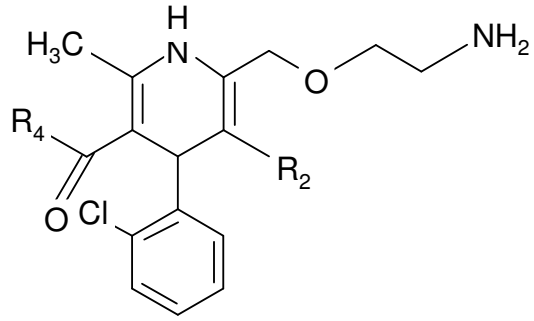
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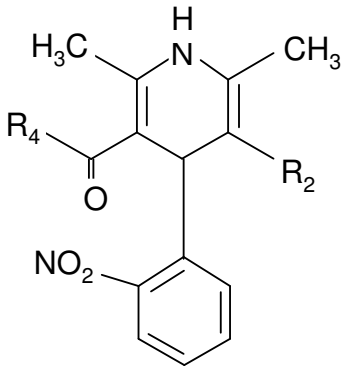
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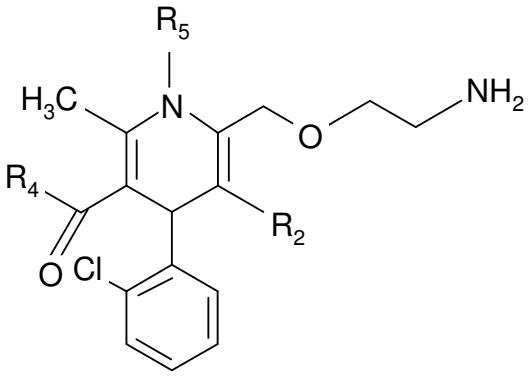
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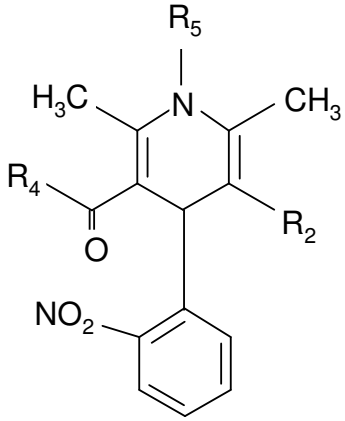
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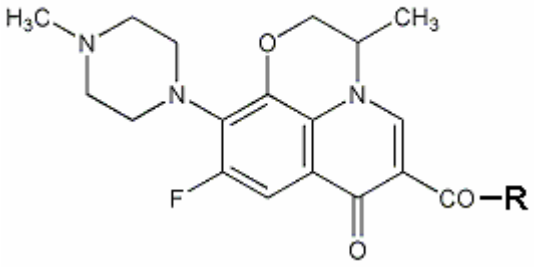
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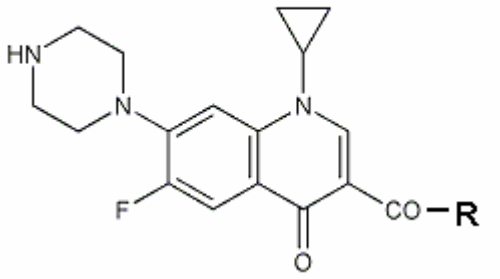

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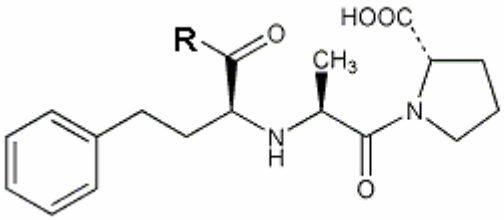

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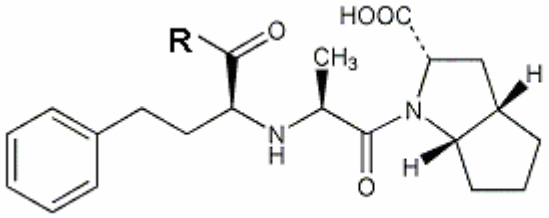

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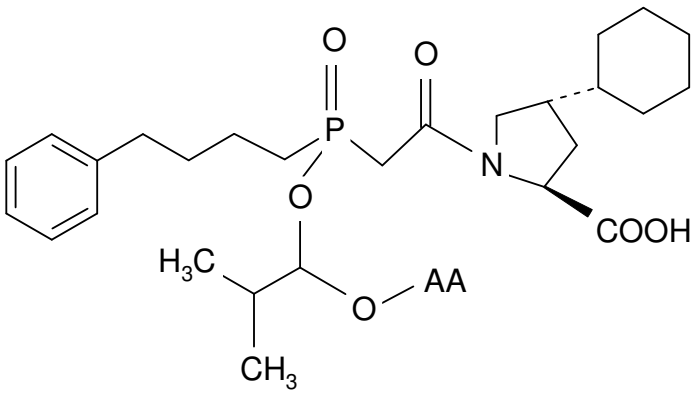

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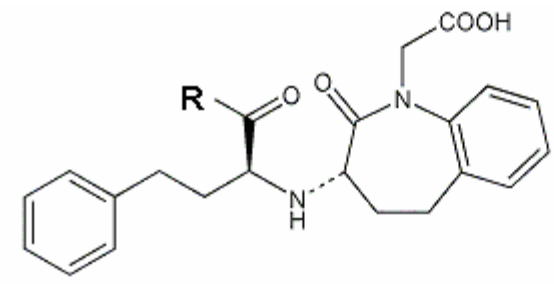

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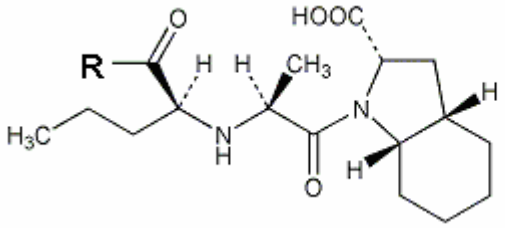

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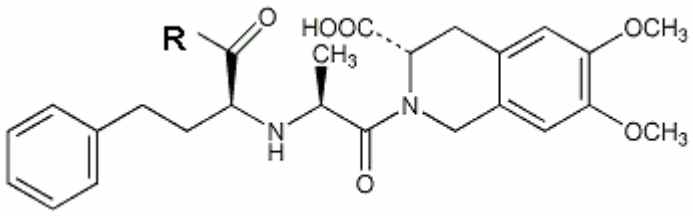

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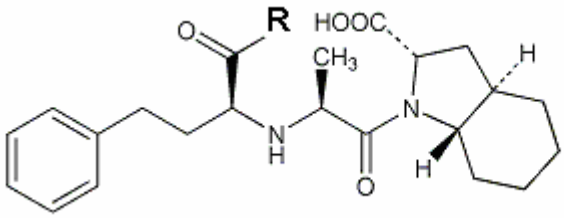

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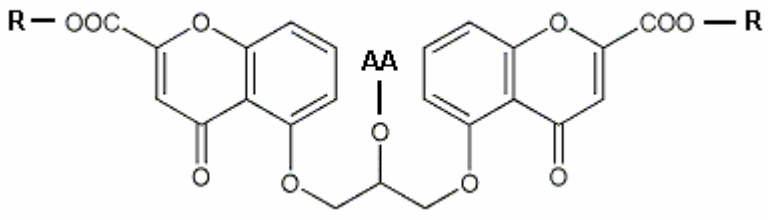

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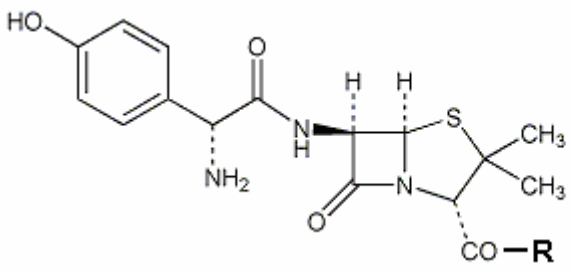

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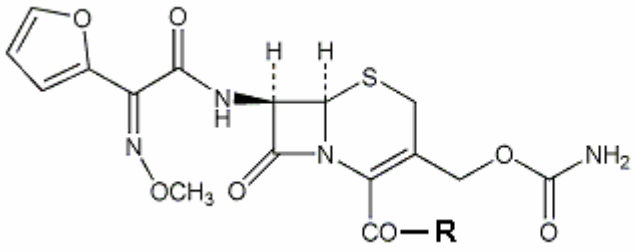

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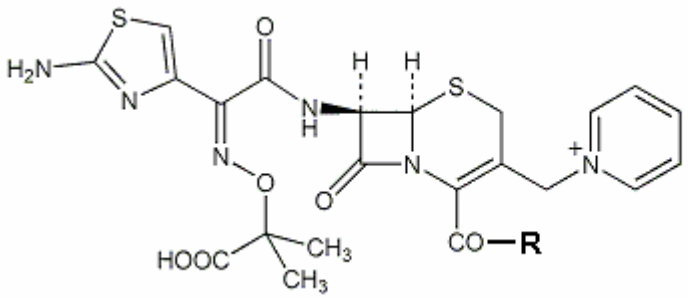

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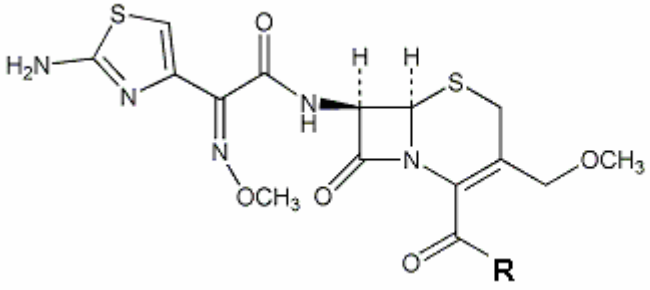

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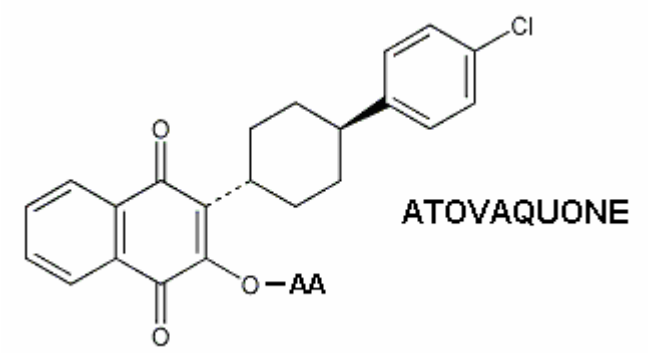
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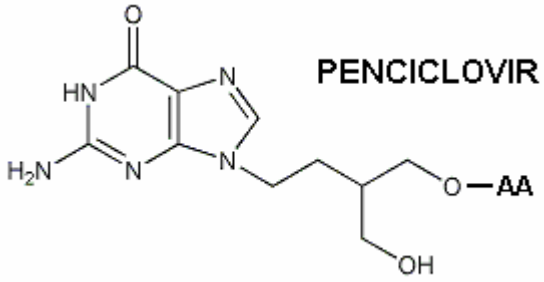
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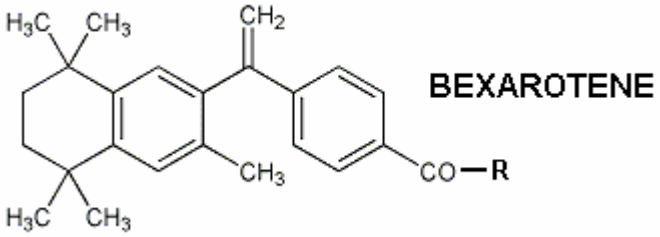
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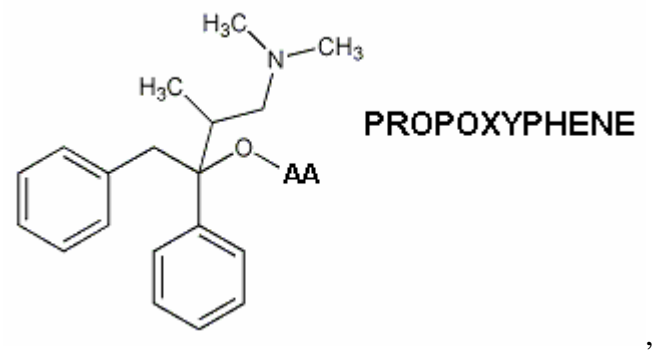
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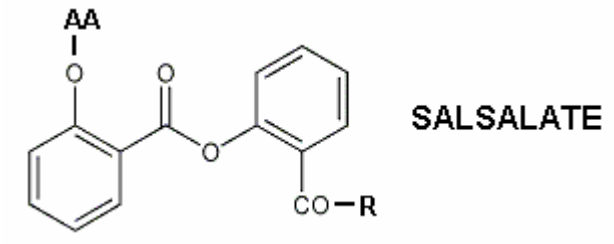
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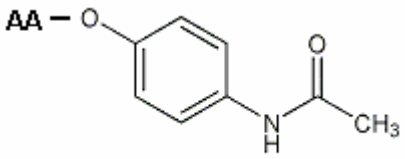
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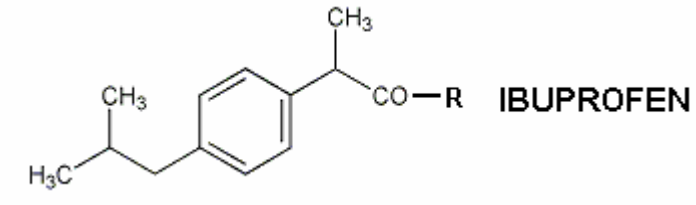
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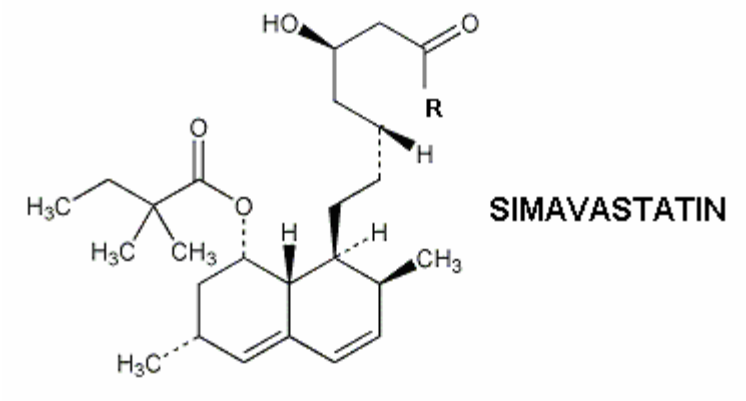

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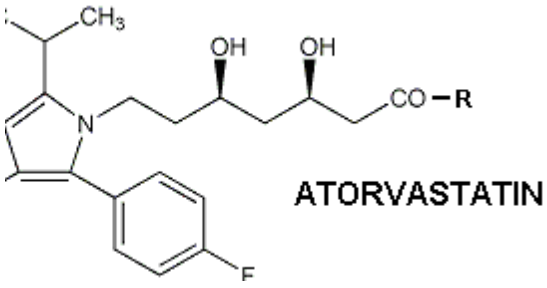

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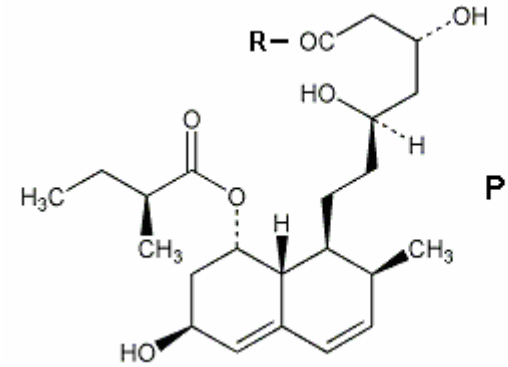

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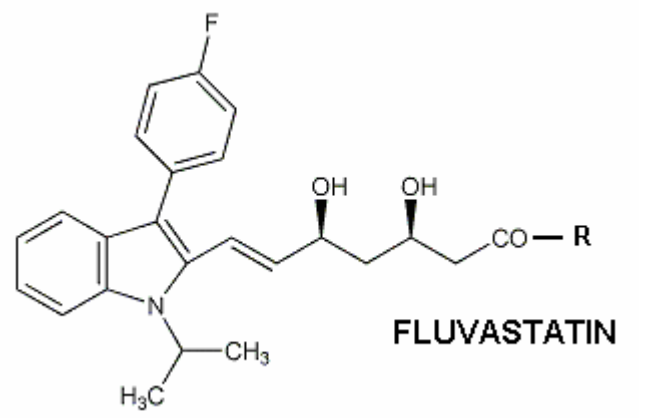
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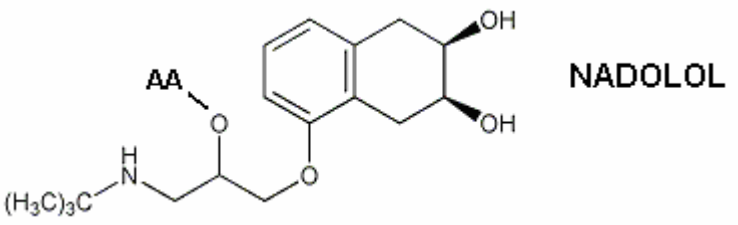
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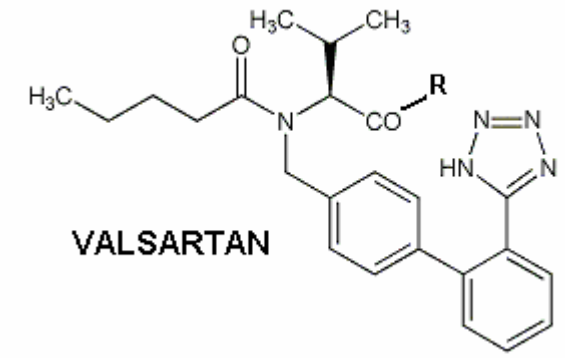
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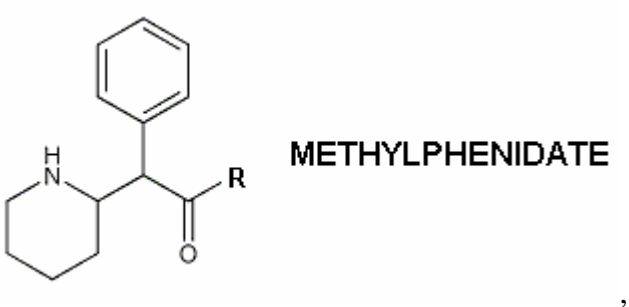

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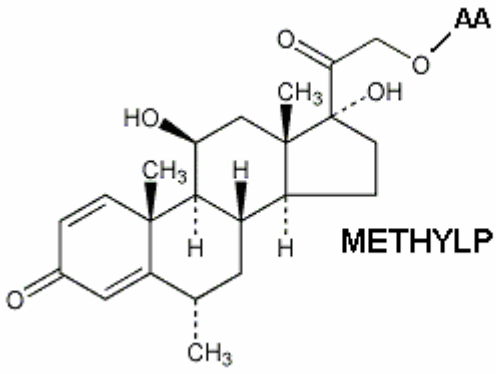

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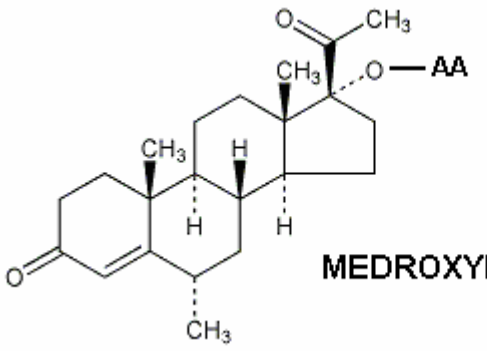

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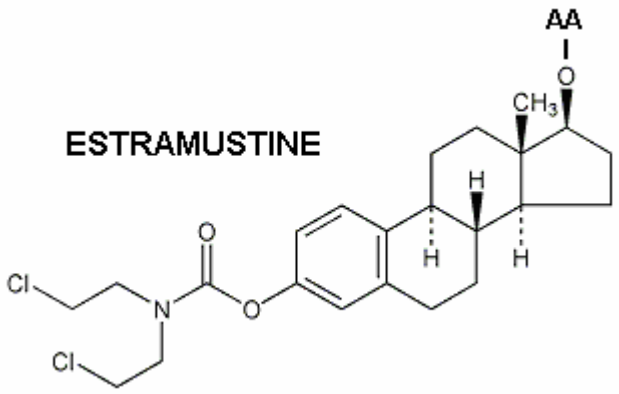
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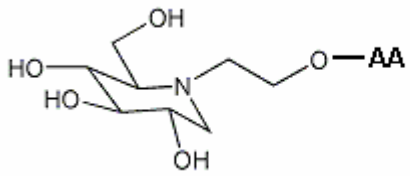
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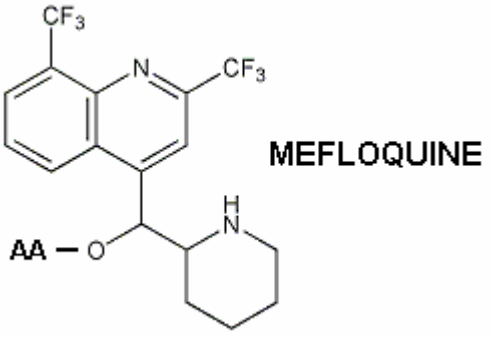
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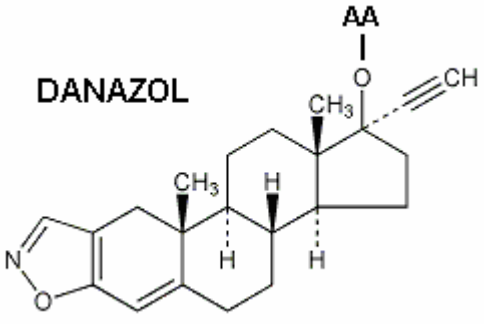
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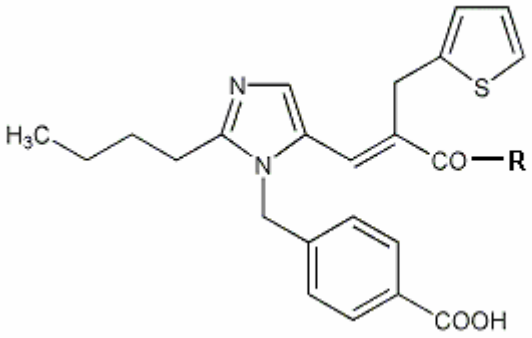

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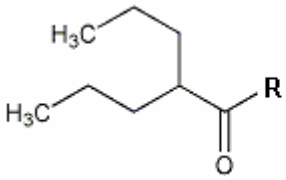

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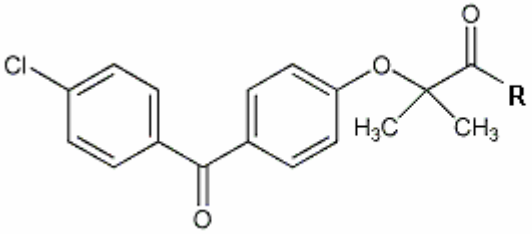

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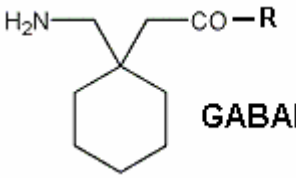

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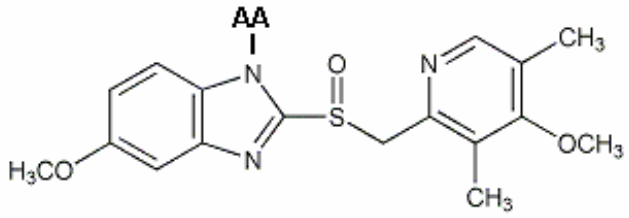

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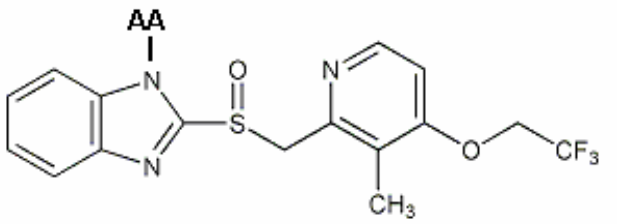

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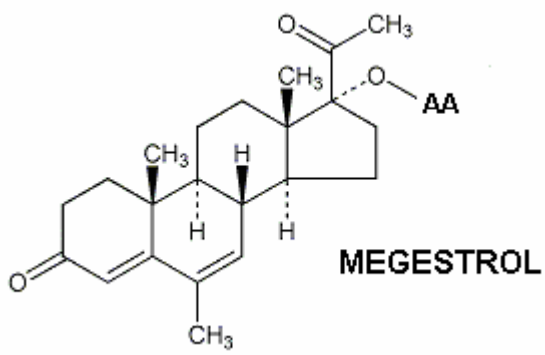

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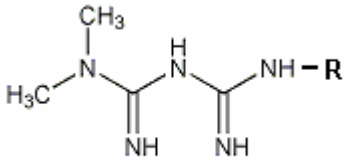

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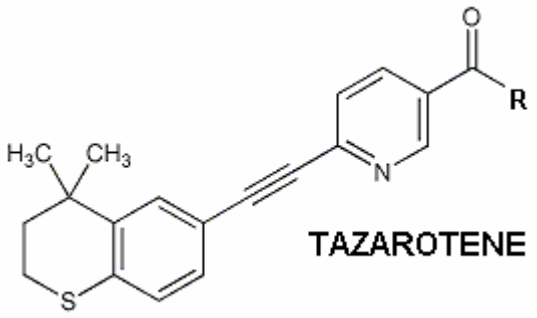

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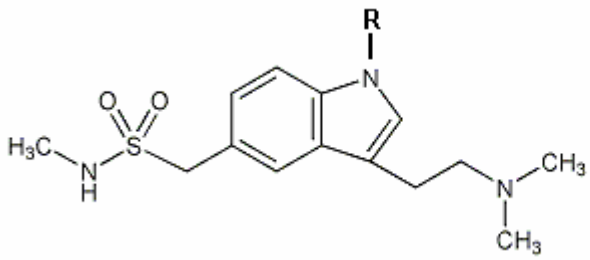

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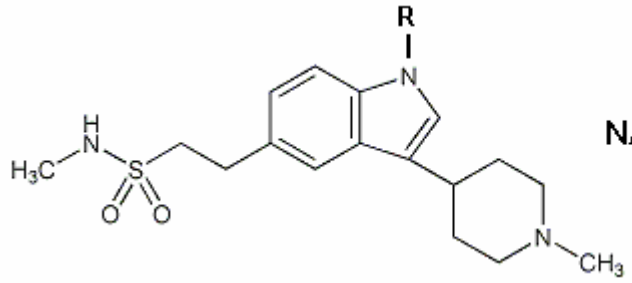

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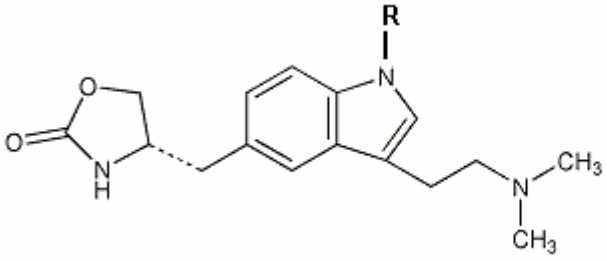

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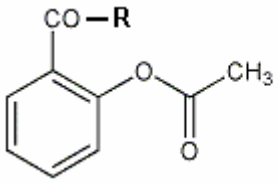

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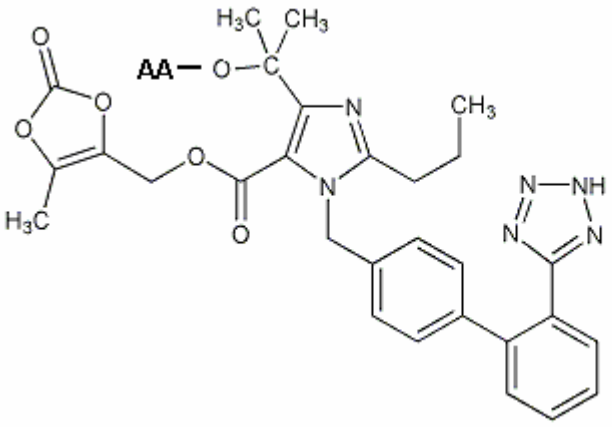

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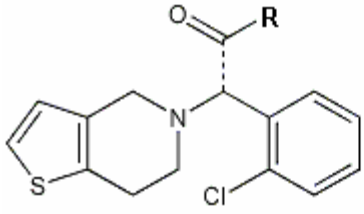

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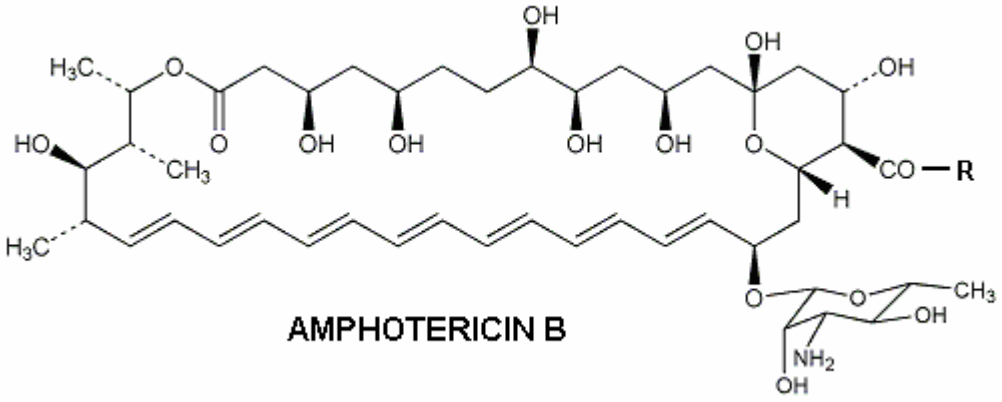

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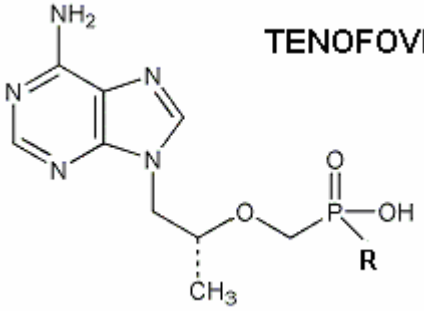

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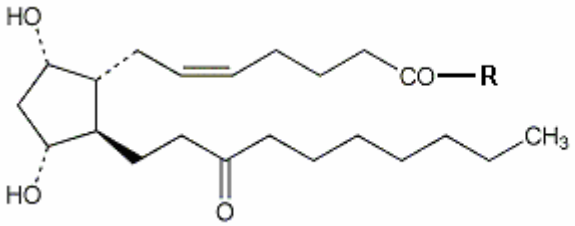

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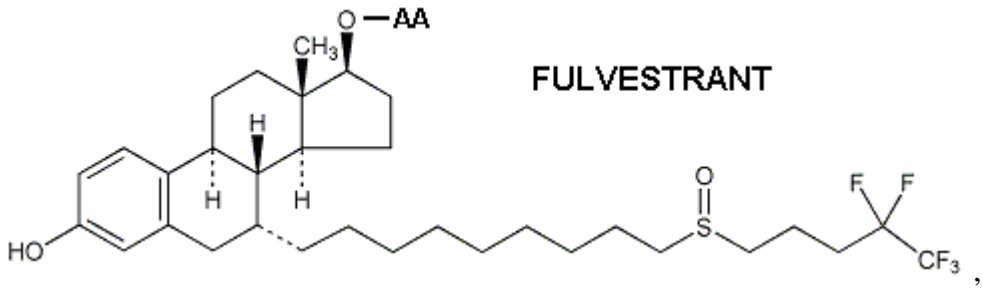

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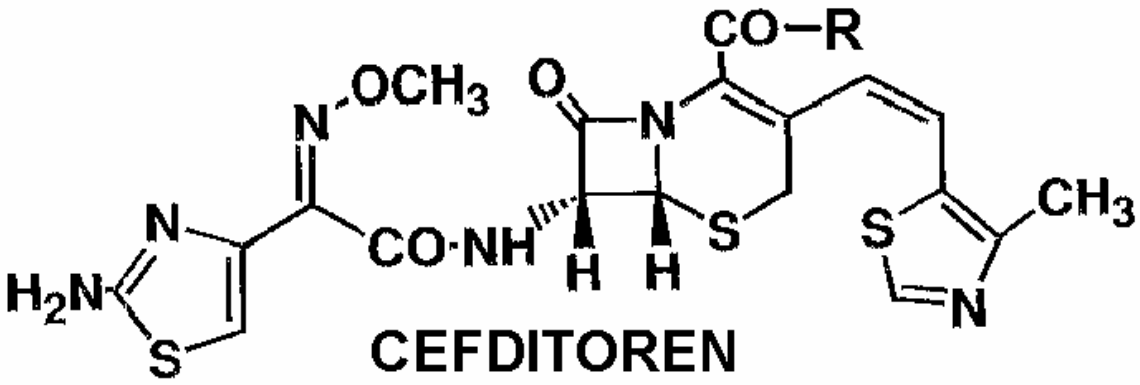

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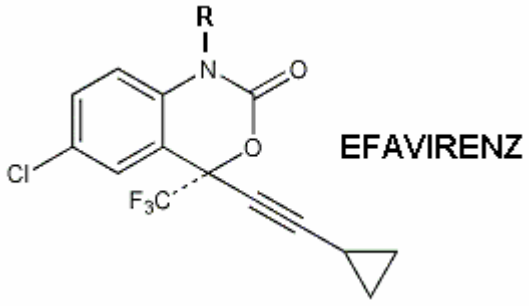

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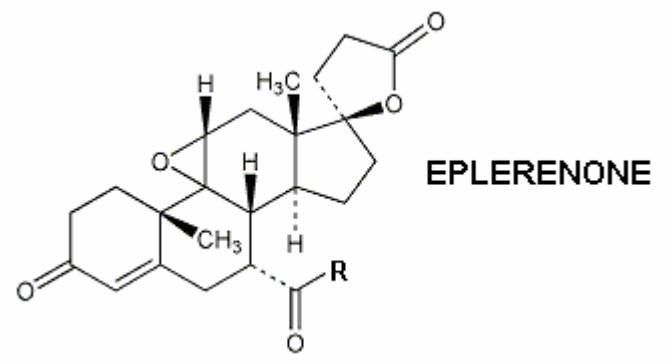

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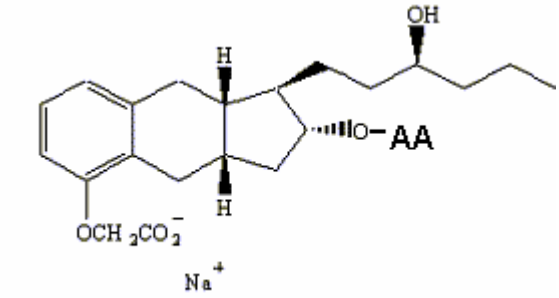

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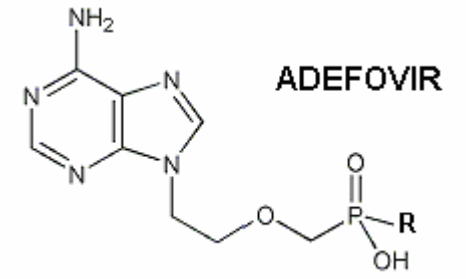

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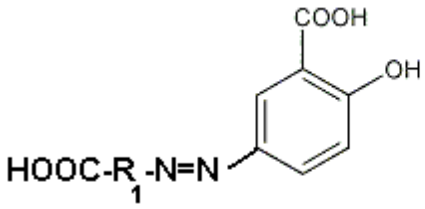
R_8N=N-AA_5-COOH,

SULFO DRUG AZO DERIVATIVE ,

R_8NH CO-AA_5NH_2

SULFA DRUG AMIDE DERIVATIVE

or



GLUTAMIC ACID AZO
SALICYLIC ACID DERIVATIVE

,

or a pharmaceutically acceptable salt thereof,

wherein

AA is an amino acid without the hydroxyl group on the carboxy group, and is attached via the acyl group

R is AA_3 or OAA_6,

CYCLO represents the residues at positions 2 – 11 of a cyclosporin molecule;

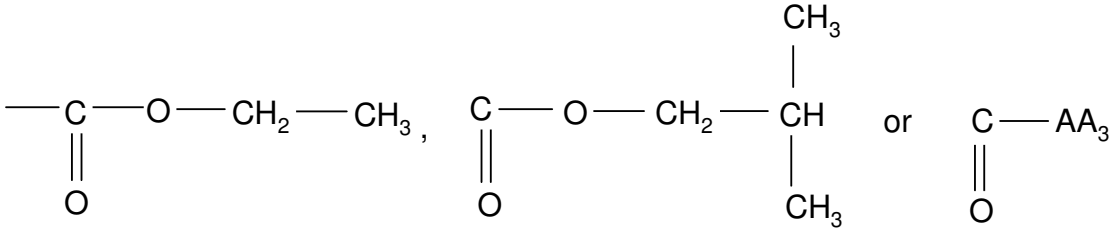
x-y is CH=CH or CH_2=CH_2;

R^2 is OAA or OGlyAA;

AA_1 is an amino acid without the hydroxy group on the carboxy group and is attached via the acyl group;

AA_6 is an amino acid residue having a hydroxy group on the side chain less said hydroxyl group, and wherein AA_6 is attached via an ester bond through its side chain hydroxy group;

R_2 is



R_4 is AA_3 or OAA_6;

R_5 is AA_2;

AA_2 is an amino acid residue less the hydroxy group on the carboxy group, and is attached via the acyl group;

AA_3 is an amino acid less the hydrogen atom on the amino group, and it is attached via the amino group;

R_3 is ethyl;

R_1 is an amino acid without the carboxy and amino groups;

R_6 is AA_1;

R_7 is O-AA_1;

AA_5 is an amino acid without the amino and carboxy groups;

R_8 is a sulfanilamide moiety of the general class of sulfa drugs; and

R_9 is AA_3 or OAA_6, and

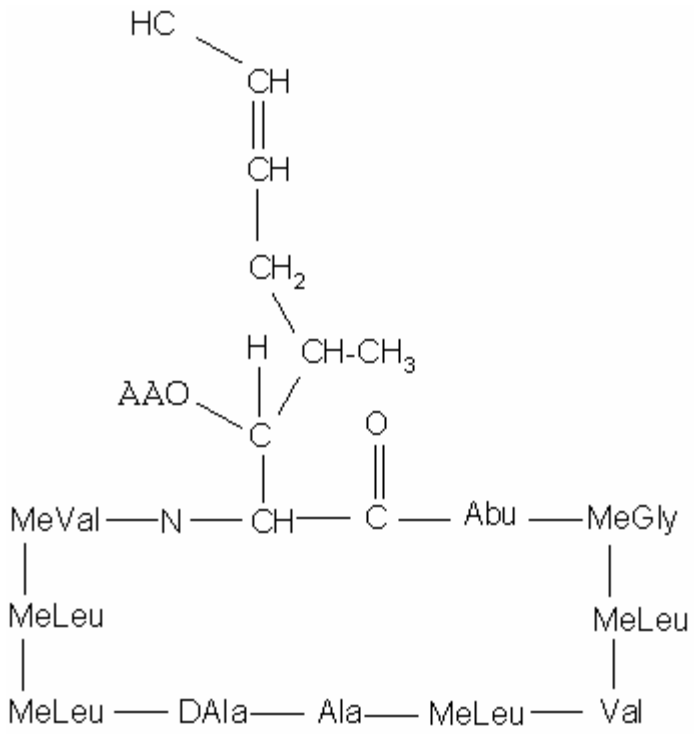
AA, AA_1, AA_2, AA_3, and AA_5 are independently L-amino acids and are Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Val, Pro, His, Nor, Arg, Phe, Trp, Hsr, Car, Ort, Cys, Cav, Asn, Gln, Can, Tau, Djk, GABA, Dcy or Sar, and AA_6 is an L-amino acid selected from Thr, Hyp, Ser or Tyr.

9. The product according to Claim 8 wherein AA_1, AA_2, AA_3, AA_4, and AA_5 are independently Thr, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Arg, Phe, Trp, Gln, Asn, Cys or Ser.

10. The method according to Claim 9 wherein AA_1, AA_2, AA_3, AA_4, AA_5 and AA_6 are independently Threonine, hydroxyproline or serine.

11. The compound according to Claim 10 wherein AA_1, AA_2, AA_3, AA_4, AA_5 and AA_6 are Thr.

12. The compound according to Claim 8 wherein the compound has the formula



13. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to any one of Claims 8 – 12 and a pharmaceutically acceptable salt thereof.

14. A method of enhancing the bioavailability of a drug having a functionally selected from the group consisting of hydroxy, amino, carboxy or acylating derivative of said carboxy group, said drug being selected from the group consisting of Cyclosporin, Lopinavir, Cefdinir, Zileuton, Nelfinavir, Flavoxate, Candesarten, Propofol, Nisoldipine, Amlodipine, Ciprofloxacin, Ofloxacin, Fosinopril, Enalapril, Ramipril, Benazepril, Moexipril, Trandolapril, Cromolyn, Amoxicillin, Cefuroxime, Ceftazidime, Cefpodoxime, Atovaquone, Gancyclovir, Penciclovir, Famciclovir, Acyclovir, Niacin, Bexarotene, Propoxyphene, Salsalate, Acetaminophen, Ibuprofen, Lovastatin, Simavastatin, Atorvastatin, Pravastatin, Fluvastatin, Nadolol, Valsartan,

Methylphenidate, Methyl Prednisolone, Sulfa Drugs, Sulfasalazine, Medroxyprogesterone, Estramustine, 5-aminosalicyclic acid, Miglitol, Mefloquine, Danazol, Eprosartan, Divalproex, Fenofibrate, Gabapentin, Omeprazole, Lansoprazole, Megestrol, Metformin,, Tazarotene, Sumitriptan, Naratriptan, Zolmitriptan, Aspirin, Olmesartan, Sirolimus, Tacrolimus, Pimecrolimus, Clopidogrel, Amphotericin, Tenofovir, Unoprostone, Fulvestrant, Cefditoren, Efavirenz, Eplerenone, Treprostinil, and Adefovir, , said method comprising reacting the drug with an amino acid under conditions effective to form a covalent bond between the drug and the amino acid and administering the product thereof to a patient in need thereof, wherein said amino acid is a L-amino acid selected from the groups consisting of Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Nor, Ara, Phe, Trp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Sar or Dcy.

15. A method of enhancing the solubility in an aqueous solution of a drug having a functionality selected from the group consisting of hydroxy, amino, carboxy or acylating derivative of said carboxy group, said drug being selected from the group consisting of lopinavir, cefdinir, amlodipine, nisoldipine, zileuton, nelfinavir, flavoxate, candesartan, cromolyn (sodium), atovaquone, niacin, bexarotene, propoxyphene, salsalate, acetaminophen, ibuprofen, valsartan, methyl phenidate, methyl prednisolone, medroxy progesterone, cephalosporin antibiotic, estramustine, miglitol, mefloquine, danazol, eprosartan, divalproex, fenofibrate, gabapentin, megestrol, metformin, tazarotene, aspirin, olmesarten, clopidogrel, amphotericin, tenofovir, unoprostone, fulvestrant, cefditoren, efavirenez, eplerenone, treprostinil adefovir and salazine, said method comprising reacting the covalent bond with an amino acid under conditions effective to form a drug between the drug and the amino acid and administering the product thereof to a patient in need thereof, wherein said amino acid is a L-amino acid selected from the group consisting of Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Nor, Ara, Phe, Trp, Hsr, Car, Ort, Cav, Asn, Gln, Can, Tau, Djk, GABA, Cys, Sar or Dcy.

16. The method according to Claim 14 or 15 wherein the amino acid is Thr, Hyp, Ser, Tyr, Lys, Leu, Ile, Gly, Asp, Glu, Met, Ala, Val, Pro, His, Arg, Phe, Trp, Gln, Asn, or Cys.

17. The method according to Claim 14 or 15 wherein the amino AA is threonine, hydroxyproline or serine.

18. A method of enhancing the safety profile with longer anesthetic effect of propofol in an aqueous solution comprising reacting the hydroxy functionality of the propofol molecule with an amino acid or acylating derivative thereof under ester forming conditions and isolating the product thereof.

19. A method of enhancing the solubility of a quinolone antibiotic having a carboxy group or acylating group in an aqueous solution comprising reacting the carboxylic acid functionality of the quinolone antibiotic with an amino acid or acylating derivative thereof under either ester or amide forming conditions and isolating the product thereof.

20. A method of enhancing the bioavailability of a quinolone antibiotic having a carboxyl group or acylating group when administered to a patient which comprises reacting the carboxylic acid functionality of the quinolone antibiotic with an amino acid or acylating derivative under ester or amide forming conditions, isolating the product thereof and administering said product to the patient.

21. A method of enhancing the solubility of ace inhibitors having a carboxy or acylating group in an aqueous solution comprising reacting the carboxylic acid functionality of the ace inhibtor molecule with an amino acid or acylating derivative thereof under either ester or amide forming conditions and isolating the product thereof.

22. A method of enhancing the bioavailability of ace inhibitors having a carboxy or acylating group when administered to a patient which comprises reacting the carboxylic acid functionality of the ace inhibitors molecule with an amino acid or acylating derivative under ester or amide forming conditions, isolating the product thereof and administering said product to the patient.

23. A method of reducing the gastric irritation of ibuprofen comprising reacting the carboxylic acid functionality of the ibuprofen molecule with an amino acid or acylating derivative under either ester or amide forming conditions and administering the product to a patient in need thereof, said product resulting less gastric irritability then ibuprofen.

24. A method of enhancing the solubility of a proton pump inhibitor in an aqueous solution comprising reacting the amine functionality of the proton pump inhibitor with the carboxylic acid moiety of the amino acid or acylating derivative thereof under amide forming conditions and isolating the product thereof.

25. A method of enhancing the bioavailability of a proton pump inhibitor when administered to a patient which comprises reacting the amine functionality of the proton pump inhibitor with the carboxylic acid moiety of an amino acid or acylating derivative thereof under amide forming conditions, isolating the product thereof and administering said product to the patient.

26. A method of enhancing the solubility of Selective 5-HT receptor agonists in an aqueous solution comprising reacting the amine functionality of the Selective 5-HT receptor agonists molecule with the carboxylic acid moiety of an amino acid or acylating derivative thereof under amide forming conditions and isolating the product thereof.

27. A method of enhancing the bioavailability of Selective 5-HT receptor agonists when administered to a patient which comprises reacting the amine functionality of the

Selective 5-HT receptor agonists with the carboxylic acid moiety of an amino acid under amide forming conditions, isolating the product thereof and administering said product to the patient.

28. A method of reducing the gastric irritability in the stomach resulting from aspirin administration to a mammal which method comprises reacting the carboxylic acid functionality of the aspirin molecule with an amino acid or acylating derivative thereof under either amide or ester forming conditions, and administering the formed product to said mammal, wherein the gastric irritability is reduced relative to aspirin.

29. A method of enhancing the solubility of immunosuppresants in an aqueous solution comprising reacting the hydroxy functionality of the immunosuppresants with an amino acid or acylating derivative thereof under ester forming conditions and isolating the product thereof.

30. A method of enhancing the bioavailability of immunosuppresants when administered to a patient which comprises reacting the hydroxy functionality of the immunosuppresants with an amino acid or acylating derivative under ester forming conditions, isolating the product thereof and administering said product to the patient.

31. A method of enhancing the solubility of a sulfa drug in an aqueous solution comprising reacting the amine of the sulfa drug with an amino acid or acylating derivative thereof under either azo or amide forming conditions and isolating product thereof.

32. A method of enhancing the bioavailabilty of a sulfa drug when administered to a patient which comprises reacting the amine functionality of the sulfa drug with an amino acid or acylating derivative under azo or amide forming conditions, isolating the product thereof and administering said product to the patient.

33. A method of providing a clear aqueous intravenous formulation comprising a Sulfa drug when administered to a patient which comprises reacting the amine of the Sulfa drug with an amino acid or acylating derivative under azo or amide forming conditions, isolating the product thereof and administering said product to the patient parenterally.

34. A method of enhancing the solubility in an aqueous solution of a nucleoside analog having an OH functionality comprising reacting the hydroxyl functionality of the nucleoside analog with an amino acid or acylating derivative thereof under ester forming conditions or isolating the product thereof.

35. A method for enhancing the bioavailability of a nucleoside analog having an OH group thereon which comprise reating the hydroxyl functionality of the nucleoside analog with an amino acid or acylating derivative thereof under under ester forming conditions and isolating the product thereof and administering the product to a patient.