

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
3 February 2005 (03.02.2005)

PCT

(10) International Publication Number
WO 2005/009429 A1

- (51) International Patent Classification⁷: **A61K 31/14**, 31/045
- (21) International Application Number: PCT/US2004/021260
- (22) International Filing Date: 29 June 2004 (29.06.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 10/623,114 18 July 2003 (18.07.2003) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: COMPOSITIONS CONTAINING PRODRUGS OF FLORFENICOL AND METHODS OF USE

(57) Abstract: The present invention provides compositions and methods for administering florfenicol to mammals. The compositions contain a prodrug of florfenicol in a pharmaceutically acceptable carrier. In one embodiment the prodrug is an esterized form of florfenicol. Examples of suitable prodrugs include one or a combination of the following: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate. In another embodiment the prodrug is converted into the florfenicol in vivo by the action of one or more endogenous esterases. The invention also provides new compounds, pharmaceutical compositions containing the compounds, and methods for their administration.

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COMPOSITIONS CONTAINING PRODRUGS OF FLORFENICOL AND METHODS OF USE

Field of the Invention

[0001] The present invention pertains to prodrugs for use in mammals.

Background of the Invention

[0002] The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

[0003] Florfenicol is a structural analog of chloramphenicol and thiamphenicol, having a fluorine atom instead of the hydroxyl group located at C-3 in the structure of chloramphenicol and thiamphenicol. Bacterial resistance to chloramphenicol and thiamphenicol is usually attributed to acetylation of the 3' hydroxyl group by the action of chloramphenicol acetyl transferase. Since florfenicol does not possess the 3' hydroxyl group such a mechanism of inactivation is not possible with florfenicol, thus making florfenicol a highly useful antibiotic.

[0004] Florfenicol is active against a variety of microorganisms including *Citrobacter*, *Proteus mirabilis*, *Proteus sp.*, *Shigella*, *Salmonella*, *Providencia*, *Bacteroides*, *Staphylococcus aureus*, *Enterococci*, *Pasteurlla haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, and *Haemophilus influenza*. Conversely, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Acinetobacter*, have been found to be resistant to florfenicol. Literature suggests that typical MICs (minimum inhibitory concentrations) of florfenicol for various bacteria range from 0.3- 1 µg/ml.

[0005] Dosage schedules of antibiotics are designed to maintain serum or tissue levels above the MIC for the target organisms for a period of time sufficient to clear the infection. Drugs that are cleared rapidly must be administered more often to maintain effective levels. In the case of some highly hydrophilic drugs, the drugs are cleared from the system so rapidly

that their use requires multiple daily doses. Therefore, there is a need for increasing the effectiveness of known hydrophilic antibacterial compounds, such as florfenicol.

Summary of the Invention

[0006] In one aspect, the present invention provides compositions containing a prodrug of florfenicol and a pharmaceutically acceptable carrier in an injectable composition. In one embodiment the prodrug of florfenicol is present in the composition at a concentration of at least 200 mg/ml, while in another embodiment the prodrug of florfenicol is present in the composition at a concentration of about 300 mg/ml. In still another embodiment the prodrug is an esterized form of florfenicol. In various embodiments the prodrug is one or a combination of the following: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nonanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate. In one embodiment the prodrug is converted into the florfenicol in vivo by the action of an esterase.

[0007] By "prodrug" is meant a chemical derivative compound that is transformed in vitro to yield florfenicol through the action of one or more mammalian enzymes. In one embodiment the mammalian enzyme is an esterase. In various embodiments prodrugs are derivatized through an ester linkage between a hydroxyl group of the drug molecule and the carboxyl group of an external acid, or vice versa, to form an esterized form of florfenicol. The term "prodrug ester group" refers to any of many ester-forming groups that are hydrolyzed in the mammalian body. Examples of prodrug ester groups and external acids include acetyl, propionyl, butyryl, pentanyl, hexanyl, heptanyl, octanyl, nonyl, decanyl, undecanyl, dodecanyl, and phthalyl. Other examples of suitable prodrug ester groups and external acids can be found in the book "Pro-drugs as Novel Delivery Systems," by Higuchi and Stella, Vol. 14 of the American Chemical Society Symposium Series, American Chemical Society (1975).

[0008] By a "pharmaceutically acceptable carrier" is meant a non-toxic, inert, solid, semi-solid, or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type. In one embodiment the pharmaceutically acceptable carrier is propylene glycol. But many other

carriers are available. Examples of pharmaceutically acceptable carriers include, but are not limited to, pyrrolidone, N-methyl pyrrolidone, polyethylene glycol, propylene glycol, glycerol formal, isosorbide dimethyl ether, ethanol, dimethyl sulfoxide, tetrahydrofurfuryl alcohol, triacetin, or any combination of these, or another solvent found to have similar acceptable properties such as being non-toxic and soluble in water. In one embodiment the carrier is propylene glycol in glycerol formal (e.g., in 10% glycerol formal). Other pharmaceutically acceptable carriers include sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, arachis oil, sesame oil, olive oil, corn oil, soybean oil, and saw flower oil, ; glycols, such as propylene glycol; polyols such as glycerin, sorbitol, mannitol and polyethylene glycol; esters such as ethyl oleate, isopropyl myristate, and ethyl laurate; agar; ethyl alcohol; as well as other non-toxic compatible substances used in pharmaceutical formulations. Any of these or mixtures thereof can be prepared in a liquid form or dissolved in a suitable liquid to form the carrier. Still other pharmaceutically acceptable carriers include water insoluble or water immiscible solvents. Examples of these include, but are not limited to, isopropyl myristate, and ethyl lactate.

[0009] By “injectable” is meant a composition or formulation that is suitable for placing into a syringe and injecting into the mammalian body. The composition and vehicle are tissue-compatible and should not rely on constituents that are likely to elicit an allergic response. Injectable compositions can be injected into the mammalian body without causing adverse effects due to the presence of solid materials in the composition. Solid materials include, but are not limited to, particles, crystals, a gummy mass, and a gel. The injectable compositions can be injected intra-muscularly, intra-venously, sub-cutaneously, intra-dermally, intra-peritoneally, or parenterally, or other suitable modes of injection into the mammalian body.

[0010] One injectable composition of the invention contains one or more compounds of the present invention dissolved in glycerol formal with 10% propylene glycol. In one embodiment the composition contains a suspension or solution of one or more compounds of

the present invention dissolved in an oil, for example arachis oil or sesame oil. The compositions can also contain one or more compounds of the present invention dissolved in a solvent such as pyrrolidone, N-methyl pyrrolidone, polyethylene glycol, propylene glycol, glycerol formal, isosorbid dimethyl ether, ethanol, dimethyl sulfoxide, tetrahydrofurfuryl alcohol, triacetin, or any combination of these, or another solvent found to have similar acceptable properties such as being non-toxic and soluble in water. The compositions can optionally contain a chelating agent, for example ethylenediamine tetracetic acid and an anti-oxidant, for example sodium metabisulphite.

[0011] In another aspect the present invention provides novel compounds. The compounds are one or more compounds having a structure selected from the following: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate.

[0012] In another aspect the present invention provides pharmaceutical compositions for administration to a mammal. The pharmaceutical compositions contain one or more compounds described herein and a pharmaceutically acceptable carrier, which can be supplied in an injectable form.

[0013] In another aspect the present invention provides methods of administering florfenicol to a mammal. The methods involve administering a composition containing a prodrug of florfenicol to the mammal. In one embodiment the composition is administered to the mammal by injection. The composition can form a drug depot in the mammal. The prodrug is converted in vivo by one or more endogenous enzymes into florfenicol. In one embodiment the prodrug is present in the composition at a concentration of at least 250 mg/ml. The prodrug is any of the compound prodrugs described herein, or a combination thereof. In one embodiment the endogenous enzymes are esterases. In various embodiments the mammal is a bovine, an equine, a porcine, an ovine, a canine, or a feline. The compositions can be administered orally, or injected sub-cutaneously, intra-peritoneally, intra-dermally, mucosally, intra-muscularly, or by other means.

[0014] A “drug depot” is a reservoir of prodrug situated in the body of the mammal and established by the injection of prodrug through the skin. The drug depot may have a solid, semi-solid, or liquid form, and represents a concentration of prodrug in the treated mammal. In one embodiment the drug depot is a precipitate of the prodrug. The depot then becomes a source of prodrug, and therefore active drug, for the treated mammal. The drug depot can be created and situated in a muscle of the mammal by intra-muscular injection, but can also be situated in other tissue types such as in the dermal layer or in connective tissue. In one embodiment the depot emits prodrug and the prodrug is converted into active drug in the body. In another embodiment the prodrug is converted into active drug at or near the drug depot. In another embodiment the composition in its entirety acts as a drug depot.

[0015] By “endogenous enzymes” is meant those enzymes produced or synthesized within the organism by the organism’s own protein synthesis apparatus. Endogenous enzymes are those normally found within the organism. They may be produced by the mammalian body continuously or produced in response to a particular stimulus. “Esterase” is a generic term for those enzymes that catalyze the hydrolysis of ester bonds. By “intra-muscular” is meant within a muscle. Thus, administration by intra-muscular injection means injected into a muscle.

[0016] In another aspect the present invention provides methods of administering florfenicol to a mammal. The methods involve injecting into the mammal a composition comprising one or more compounds of the present invention, wherein the one or more compounds is/are converted into florfenicol in vivo by the action of one or more endogenous esterases. In various embodiments the one or more compounds is/are present in the composition at a concentration of at least 250 mg/ml.

[0017] The summary of the invention described above is not limiting and other features and advantages of the invention will be apparent from the following detailed description, as well as from the claims.

Brief Description of the Drawings

[0018] Figure 1 provides a schematic illustration of the prodrug conversion process.

[0019] Figure 2 provides a schematic illustration of the synthesis of florfenicol ester prodrugs.

[0020] Figure 3 provides a graphical illustration of the in-vitro release kinetics of florfenicol and florfenicol ester prodrugs.

[0021] Figure 4 provides a graphical illustration of the pharmacokinetics of florfenicol and florfenicol ester prodrugs in cattle.

[0022] Figure 5 provides a graphical illustration of the pharmacokinetics of florfenicol butyrate in cats.

[0023] Figure 6 provides a graphical illustration of the pharmacokinetics of florfenicol hexanoate in cats.

[0024] Figure 7 provides a graphical illustration of the pharmacokinetics of florfenicol butyrate and hexanoate in cats.

[0025] Figure 8 provides a graphical illustration of serum concentrations of florfenicol butyrate v. NUFLOR[®] (Schering-Plough Corp., Kenilworth, NJ) in cats.

[0026] Figures 9a-n provide illustrations of examples of compounds of the present invention.

Detailed Description of the Invention

[0027] The compositions and methods of the present invention have distinct and surprising advantages over previously available compositions and methods. While the solubilization of florfenicol usually requires relatively strong and harsh solvents, the prodrugs described herein are highly soluble in mild pharmacologically acceptable solvents, such as glycerol formal.

[0028] The prodrug compositions of the present invention are efficiently converted to active florfenicol by esterases endogenous in the mammalian system. The butyrate and hexanoate prodrug derivatives are less soluble in water than free florfenicol and display slower release kinetics, while the acetate prodrug derivative is more soluble in water and displays a shorter release time. These chemical characteristics are used to advantage in the present invention. In general the release rate is related to the solubility of the drug in water or biological fluids. When solubility is low, a more viscous depot is formed that releases prodrug more slowly. When solubility is high, the prodrug is released more quickly from the depot resulting in a faster release rate. Florfenicol can be made more hydrophobic by derivatization into various esters. In general, the more hydrophobic the derivative the lower the solubility. Thus, in general the hexanoate derivative is less soluble than the butyrate derivative, which is less soluble than native florfenicol. Thus, when a "fast kill" or "initial burst" of drug is desired, a more soluble prodrug is advantageous to obtain a shorter release time. Longer release times offer the benefit of preventing residual organisms from growing.

[0029] As further described below, a single intra-muscular injection of a florfenicol butyrate prodrug in cattle provided active florfenicol in the serum at higher levels and for longer periods of time than intra-muscular injections of NUFLOR[®] (Schering-Plough Corp., Kenilworth, NJ), which is a commercially available form of florfenicol. Furthermore, a single subcutaneous injection of the butyrate and hexanoate ester prodrug formulations in cats was found to provide serum florfenicol concentrations above the minimum inhibitory concentration (MIC) for a period of 4-6 days, while serum concentrations of NUFLOR[®] drop off significantly after 2 days (Figure 4).

[0030] Without being bound by any particular theory, it is believed that after injection the water-soluble pharmaceutically acceptable carrier diffuses away from the injection site, thereby resulting in the formation of a drug depot containing the prodrug. In one embodiment the drug depot is a precipitate of the prodrug. As the pro-drug slowly dissolves and is released from the depot into the serum over time, it is hydrolyzed by endogenous esterases into active florfenicol, thereby providing a continuous source of florfenicol at effective levels. Thus, the difficulties associated with florfenicol are overcome in the present

invention by converting the relatively hydrophilic florfenicol molecule into a highly hydrophobic molecule that forms in vivo drug depots providing long release times.

[0031] Further information regarding the compositions and methods of the present invention is provided with reference to the following non-limiting examples.

Example 1 - Ester Formation

[0032] Esters can be formed by the reaction of acids with alcohols. The formation of esters can be viewed as the substitution of the OH group on the acid by the alcohol group, and the proton on the alcohol combined with the acid OH to form water.

[0033] In one embodiment of the invention, the ester is formed by the reaction of an acid anhydride with an alcohol (florfenicol). Thus, an acid anhydride of the derivative(s) being formed is obtained and reacted under catalytic conditions with florfenicol to form the ester derivative. The catalytic conditions are provided by the presence of a strong acid, such as perchloric or concentrated sulfuric acid. The reaction is rapid, irreversible, and lies far to the right. Yields of nearly 100% are obtainable using little or no excess acid anhydride. The reaction can be depicted as:



[0034] In another embodiment florfenicol can be reacted with a carboxylic acid in the presence of a catalyst. This reaction principle is known as "Fischer esterification." Appropriate catalysts include strong acids such as concentrated sulfuric acid, hydrogen chloride, or p-toluenesulfonic acid. This type of reaction is an equilibrium reaction, which must be refluxed for several hours. Higher yields can be obtained by using excessive amounts of acid or alcohol, as the reaction does not lie far to the right.

[0035] In a third method, esters can be formed by reacting an acid chloride with florfenicol. The reaction is quantitative and rapid. The acid chloride is the corresponding

derivative to be synthesized. This reaction produces hydrogen chloride and pyridine can be added to neutralize this reaction product.

[0036] The above three methods of forming esters are provided only as examples. Other methods are known to those of ordinary skill in the art, and more methods may yet be discovered, that can be applied in the present invention. Such other methods are also contemplated for use herein. Since esters can be formed from a wide variety of acids, a very large number of esters is possible. In the methods, combinations of several acids can be combined with florfenicol to form a mixture of several different ester derivatives.

Example 2 – Preparation of Prodrugs

[0037] Florfenicol acetate, florfenicol butyrate, and florfenicol hexanoate ester pro-drugs were synthesized as shown in Figure 2, using a simple one step synthetic procedure. The procedure utilized was the acid anhydride procedure.

[0038] Thus, florfenicol was suspended in the corresponding acid anhydride followed by the addition of a catalytic amount of perchloric acid under conditions suitable for esterization to occur such as, for example, stirring at room temperature for about 3 hours. Generally, this embodiment involves adding solid florfenicol to liquid anhydride. The florfenicol is not soluble in anhydride and stays as a suspension. Once perchloric acid is added, product begins to form and dissolve in the solution. After sufficient time a completely clear solution is obtained and product begins to crystallize out of solution. The following examples are suitable procedures for preparing prodrugs.

[0039] Florfenicol butyrate: 225 g of florfenicol was suspended in 330 ml of butyric anhydride in one liter round bottom flask equipped with a over head stirrer and a drying tube. To this suspension was added one ml of perchloric acid, while stirring vigorously, with a pipette. A clear solution was obtained in a few minutes. Stirring was continued for another 3 hours during which the florfenicol butyrate pro-drug was crystallized. The solid product was filtered using a Buckner funnel under vacuum, and washed thoroughly with hexane. The product was finally crystallized in ethanol and the crystallized product was washed with hexane and dried to obtain around 230 g of solid florfenicol butyrate.

[0040] Florfenicol acetate: 250 g of florfenicol was suspended in 330 ml of acetic anhydride in one liter round bottom flask equipped with a over-head stirrer and a drying tube. To this suspension was added one ml of perchloric acid, while stirring vigorously with a pipette. A clear yellow solution was obtained in a few minutes. Stirring was continued for another 3 hours. After 3 hrs, the reaction mixture was poured into 1000 g of ice and stirring continued to arrive at a suspension of product. Solid present was filtered and resuspended in 500 ml of water, and then stirred for 10 min to remove residual acetic acid. The solid product was filtered using a Buckner funnel under vacuum and then recrystallized in ethanol. The recrystallized product was washed with hexane and dried to obtain around 260 g of solid florfenicol acetate.

[0041] Florfenicol hexanoate: 100 g of florfenicol was suspended in 500 g of hexanoic anhydride in one liter round bottom flask equipped with an overhead stirrer and a drying tube. To this suspension was added 3 ml of perchloric acid, while stirring vigorously, with a pipette. A clear solution was obtained in a few minutes. Stirring was continued for another 3 hours during which the product, florfenicol hexanoate pro-drug was recrystallized. The solid product was filtered using Buckner funnel under vacuum, washed thoroughly with hexane. The product was finally recrystallized in ethanol and the recrystallized product was washed with hexane and dried to obtain around 117 g of solid florfenicol hexanoate.

[0042] Florfenicol phthalate: 3.9 g of florfenicol, 3.6 g of phthalic anhydride and 7.2 ml of triethyl amine in 36 ml of dioxane were taken in a 100 ml round bottom flask equipped with a magnetic stir bar and a drying tube. The reaction mixture was stirred at room temperature for 6 hours. After 6 hours, the contents were poured into 300 ml of cold water. The resulting gummy solid was dissolved in dichloromethane and washed thoroughly with saturated bicarbonate several times followed by water. The organic layer was dried over anhydrous sodium sulfate and concentrated over roto-evaporator. The semisolid obtained was recrystallized in a mixture of ethylacetate and hexane to obtain a solid product of 4.2 grams.

Example 3 – In vitro Release Kinetics

[0043] The following example illustrates the preparation of formulations and analysis of the release kinetics for florfenicol acetate, florfenicol butyrate, and florfenicol hexanoate.

Other prodrug formulations can be prepared and analyzed using the same principles such as, for example, florfenicol propionate, florfenicol pentanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate. Each prodrug was prepared as an injectable formulation at 300 mg/ml in non-aqueous carriers including propylene glycol, glycerol formal, N-methyl-pyrrolidone (NMP), and polyethylene glycol (PEG).

[0044] 7.5 grams of florfenicol butyrate, florfenicol hexanoate, and florfenicol acetate were transferred to individual 25 ml volumetric flasks. 2.5 ml of propylene glycol was added to each solid, followed by the addition of glycerol formal to a final volume of 25 ml. The flask was placed on a shaker for about 2 hours to obtain a clear homogeneous solution.

[0045] *In vitro* release kinetics of the prodrug formulations and a solution of florfenicol in NMP were studied using a dialysis technique. A 0.5 ml aliquot of each formulation was injected into a SLIDE-A-LYZER[®] dialysis cassette (Pierce Biotechnology, Inc., Rockford, IL), and each cassette was suspended in a flask containing 150 ml of phosphate-buffered saline, 10 mM phosphate, 120 mM NaCl, pH 7.4.

[0046] The ester prodrug was observed to precipitate in the bag within about an hour. The acetate prodrug was found to be more soluble in aqueous carriers, while the butyrate and hexanoate derivatives formed precipitates more readily and were less soluble in aqueous carriers such as the phosphate buffer. Aliquots were removed from the flasks at various intervals and florfenicol esters were determined by HPLC. The *in vitro* release samples were directly injected after filtering through a 0.2 um filter in phosphate-buffered saline.

[0047] Samples were analyzed on a reversed phase C-18 column with detection at 223 nm, using acetonitrile and water as the solvent system. The gradient started at 25% acetonitrile rising to 60% acetonitrile over 10 minutes. From 20-42 minutes the acetonitrile was lowered to 25%. The flow rate was 1.2 ml/min.

[0048] Figure 3 illustrates the release kinetics for each florfenicol prodrug. The acetate prodrug was found to display faster release kinetics compared to florfenicol, while the butyrate and hexanoate derivatives displayed slower release kinetics compared to native florfenicol.

Example 4 – Minimum Inhibitory Concentrations

[0049] The Minimum Inhibitory Concentrations (MICs) were determined for each ester derivative in the presence and absence of pig liver esterase (PLE) with florfenicol (NUFLOR[®], Schering-Plough, Kenilworth, NJ) as a control. The results are summarized in Table 1. The results illustrate that the prodrugs have comparable MICs to the native florfenicol in the presence of PLE, while no activity was observed in the absence of the enzyme. Higher MICs were observed with the acetate prodrug than with the butyrate and hexanoate prodrugs, presumably because acetate is a poor substrate for PLE.

Table 1: Determination of MICs for Florfenicol Derivatives

	MIC (ug/ml)							
	Nuflor [®]	FFAc	FFBut	FFHex.	Nuflor [®] w/est.	FFAc w/est.	FFBut w/est.	FFHex w/est.
<i>E. coli</i> 25922	6.25	>200	>200	>200	6.25	200	25	12.5
<i>Klebsiella pneumoniae</i> 31488	1.56	200	>200	>200	1.56	100	12.5	6.25
<i>Pseud. aeruginosa</i> 27853	200	>200	>200	>200	>200	>200	>200	>200
<i>Serratia marcescens</i> 14757	25	>200	>200	>200	25	200	100	25
<i>Proteus mirabilis</i> 29906	6.25	200	>200	>200	6.25	200	25	6.25

Dilution series: 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56

Key

FFAc = Florfenicol acetate

FFBut = Florfenicol butyrate

FFHex = Florfenicol hexanoate

w/est. = with esterase

Example 5 – Hydrolysis of Prodrug by Esterases

[0050] The conversion of florfenicol esters to florfenicol by the esterases present in dog and cat serum and whole blood was studied. Each of the prodrugs to be analyzed was prepared at a concentration of 300 mg/ml solution of the prodrug in 10% propylene glycol in glycerol formal. 0.1 ml aliquots of these preparations were spiked into 1.933 ml of blood and serum and the conversion to active florfenicol at 37 °C was studied. 100 ul aliquots were loaded onto a solid phase extraction (SPE) cartridge and the drug/pro-drug was extracted. The results are summarized in Table 2 and illustrate that all three esters are substrates to both canine and feline esterases.

Table 2: Percent Hydrolysis of Florfenicol Esters in Canine Plasma and Whole Blood After 24 Hrs

	Plasma	Whole blood
Florfenicol	100	100
Florfenicol acetate	51	96
Florfenicol butyrate	80	96
Florfenicol hexanoate	94	88

Example 6 – Animal to Animal Variations

[0051] Animal to animal variations in hydrolysis rates were also examined. Florfenicol butyrate was spiked into whole blood from five different dogs and the percent conversion to florfenicol after 30 minutes was determined by HPLC analysis. The results are shown in Table 3 and illustrate that no significant change in the hydrolysis rate was observed within the group of five animals.

**Table: 3 Percent Hydrolysis of Florfenicol Butyrate in
Canine Whole Blood After 30 Minutes**

Dog#	% Hydrolysis
34410	83
35752	94
35803	98
35870	90
35872	100

Example 7 – Intra-Muscular Administration to Cattle

[0052] This example describes the administration of various florfenicol ester prodrug formulations by injection into cattle with the serum being analyzed for the presence of florfenicol and prodrug.

[0053] HPLC analysis of the serum from the injected animals showed a florfenicol peak and no ester derivative peaks. These results illustrate the conversion of prodrug to active florfenicol in vivo.

[0054] Florfenicol butyrate was formulated at 350 mg/ml in 10% propylene glycol in glycerol formal as described above and injected intramuscularly into four calves at a dosage of 40 mg/kg calf. Commercially available NUFLO[®] was used as a control and was injected as a single dose of 20 mg/kg as per label. Injections were administered to the muscles of the neck or the buttocks. Serum samples from the injected animals were analyzed for florfenicol and the results are summarized in Figure 4. With NUFLO[®], the serum concentrations built up rapidly to a high of about 4.5 mg/ml, and then declined rapidly. With the florfenicol butyrate the serum concentrations also built up rapidly but decreased more slowly, thus extending the area under curve and retaining active florfenicol in the serum at effective levels for a longer period of time. Thus, during days 2-7 the serum concentrations obtained using florfenicol butyrate were higher compared to those where NUFLO[®] was used. These data

illustrate that the pro-drug formulation provided for the controlled release of florfenicol, thus keeping therapeutic levels of florfenicol in the serum for a longer period time, while the NUFLOR[®] formulation resulted in a rapid build up and a rapid decrease.

Example 8 – Sub-Cutaneous Florfenicol Butyrate in Cats

[0055] Florfenicol butyrate was formulated at 300 mg/ml in 10% propylene glycol in glycerol formal as described above and injected sub cutaneously into two cats at a dose of 40 mg/kg of cat. Both animals showed serum concentrations of florfenicol of greater than 1.0 ug/ml (the MIC) for 5-6 days, with serum concentrations peaking at 4-5 ug/ml. An illustration of the data is provided in Figure 5.

[0056] In a separate experiment florfenicol butyrate was formulated at 350 mg/ml in 10% propylene glycol in glycerol formal and injected subcutaneously into five cats at a dosage of 40 mg/kg. Commercially available NUFLOR[®] was used as a control and was also injected at a dosage of 40 mg/kg. Serum samples from the injected animals were analyzed for the presence of florfenicol, and the results are graphically depicted in Figure 8. Concentrations of NUFLOR[®] built up rapidly in the serum and then declined rapidly over two days. Conversely, the concentration of florfenicol butyrate built up less rapidly and declined more slowly than that of NUFLOR[®]. Thus, the florfenicol butyrate was released more slowly than NUFLOR[®]. Figure 8 demonstrates that serum concentrations of florfenicol butyrate are higher compared to NUFLOR[®] for days 2-6. Therefore, the florfenicol pro-drug formulation provided the controlled release of the active at therapeutic levels for a longer period of time than NUFLOR[®].

Example 9 – Sub-Cutaneous Florfenicol Hexanoate in Cats

[0057] In this example florfenicol hexanoate was formulated at 300 mg/ml in 10% propylene glycol in glycerol formal as described above and injected sub-cutaneously into two cats at a dose of 40 mg/kg of cat. Both animals showed serum florfenicol concentrations above 1.0 ug/ml for 6 days with high concentrations at 2.5-4 ug/ml. The results are illustrated in Figure 6.

Example 10 – Mixture of Florfenicol Derivatives Sub-Cutaneous in Cats

[0058] In this example a 1:1 ratio mixture of florfenicol butyrate and florfenicol hexanoate was formulated at 300 mg/ml in 10% propylene glycol in glycerol formal as described above and injected sub-cutaneously into two cats at a dose of 40 mg/kg of cat. Both animals showed serum concentrations of florfenicol of greater than 1.0 ug/ml for 4-6 days, with high concentrations of 2.5-10 ug/ml. The results are graphically illustrated in Figure 7.

Example 11 – Florfenicol Acetate Sub-Cutaneous in Cats

[0059] In this example florfenicol acetate was formulated at 300 mg/ml in 10% propylene glycol in glycerol formal as described above and injected sub-cutaneously into a cat at a dose of 40 mg/kg of cat. Serum samples were analyzed periodically at 0, 1, 6, and 24 hours after the injection. The results are summarized below in Table 4, which indicates the serum concentrations of florfenicol and acetate at the indicated time intervals after injection. A considerably higher concentration of drug was found at 24 hours after injection than found when using the butyrate and hexanoate derivatives. Un-hydrolyzed acetate ester was also noted at a serum concentration of about 5 ug/ml.

[0060] Without being bound by any particular theory, it is believed by the inventors that the acetate esters are released more rapidly into the bloodstream than the butyrate and hexanoate esters. However, the hydrolysis rate is less with the acetate ester, which may be caused by it being a poorer substrate for the endogenous esterases.

	Time (hrs)			
	<u>0 hours</u>	<u>1 hour</u>	<u>6 hours</u>	<u>24 hours</u>
[Florfenicol] (ug/ml)	0	4.18	15.49	3.93
[Acetate] (ug/ml)	0	5.70	5.4	0.5

[0061] The invention illustratively described herein may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of

excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0062] The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents.

CLAIMS

What is claimed is:

1. A composition comprising:

a prodrug of florfenicol and a pharmaceutically acceptable carrier, provided in an injectable composition.
2. The composition of claim 1 wherein the prodrug of florfenicol is present in the composition at a concentration of at least 200 mg/ml.
3. The composition of claim 2 wherein the prodrug of florfenicol is present in the composition at a concentration of about 300 mg/ml.
4. The composition of claim 1 wherein the prodrug comprises an esterified form of florfenicol.
5. The composition of claim 4 wherein the prodrug is selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate.
6. The composition of claim 5 wherein the composition is selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol hexanoate, florfenicol phthalate.
7. The composition of claim 5 wherein the prodrug is converted into florfenicol in vivo by the action of an esterase.
8. A compound selected from the group consisting of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate.

9. A pharmaceutical composition for administration to a mammal comprising a compound of claim 8 and a pharmaceutically acceptable carrier.

10. The pharmaceutical composition of claim 9 wherein the compound is selected from the group consisting of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol hexanoate, florfenicol octanoate, florfenicol decanoate, florfenicol dodecanoate, and florfenicol phthalate.

11. A method of administering florfenicol to a mammal comprising:
administering a composition containing a prodrug of florfenicol to the mammal, wherein the prodrug is converted in vivo by endogenous enzymes into florfenicol.

12. The method of claim 11 wherein the composition is administered by injection.

13. The method of claim 11 wherein the composition forms a drug depot in the mammal when injected.

14. The method of claim 11 wherein the prodrug is present in the composition at a concentration of at least 250 mg/ml.

15. The method of claim 14 wherein the prodrug is selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate.

16. The composition of claim 15 wherein the prodrug is selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol hexanoate, and florfenicol phthalate.

17. The composition of claim 15 wherein the prodrug is converted into the florfenicol in vivo by the action of one or more esterases.

18. The method of claim 11 wherein the mammal is selected from the group consisting of: a bovine, an equine, an ovine, a porcine, a canine, and a feline.

19. The method of claim 12 wherein the prodrug is injected into the mammal intra-muscularly.

20. A method of administering florfenicol to a mammal comprising:

injecting into the mammal a composition comprising a compound selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol propionate, florfenicol butyrate, florfenicol pentanoate, florfenicol hexanoate, florfenicol heptanoate, florfenicol octanoate, florfenicol nonanoate, florfenicol decanoate, florfenicol undecanoate, florfenicol dodecanoate, and florfenicol phthalate;

wherein the one or more compounds is/are converted into florfenicol in vivo by the action of one or more endogenous esterases.

21. The method of claim 20 wherein the one or more compounds are selected from the group consisting of one or a combination of: florfenicol acetate, florfenicol butyrate, florfenicol hexanoate, florfenicol propionate, and florfenicol phthalate.

22. The method of claim 20 wherein the one or more compounds is/are present in the composition at a concentration of at least 250 mg/ml.

23. The method of claim 20 wherein the mammal is selected from the group consisting of: a bovine, an equine, an ovine, a porcine, a canine, and a feline.

24. The method of claim 23 wherein the formulation is injected into the mammal intra-muscularly.

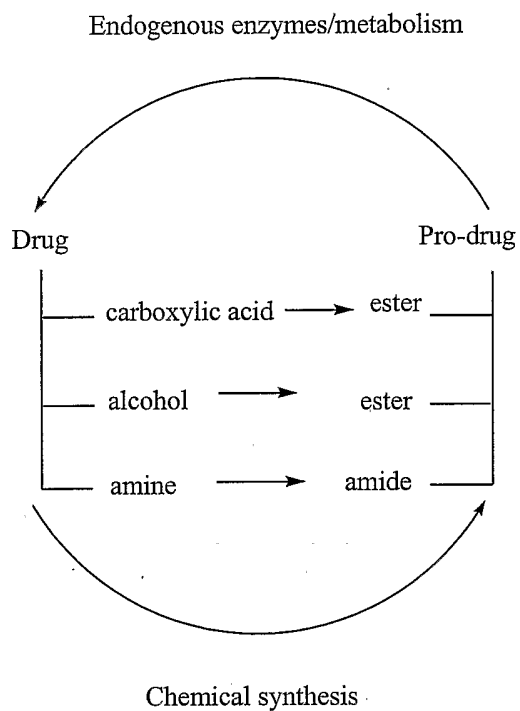


FIG. 1

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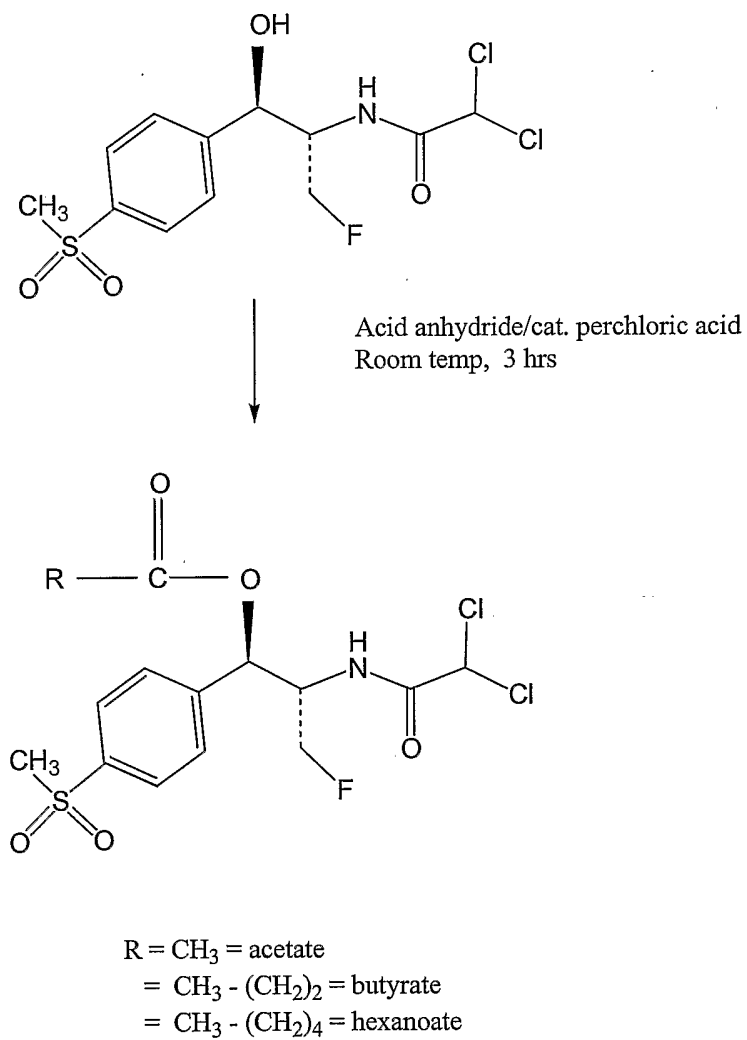


FIG. 2

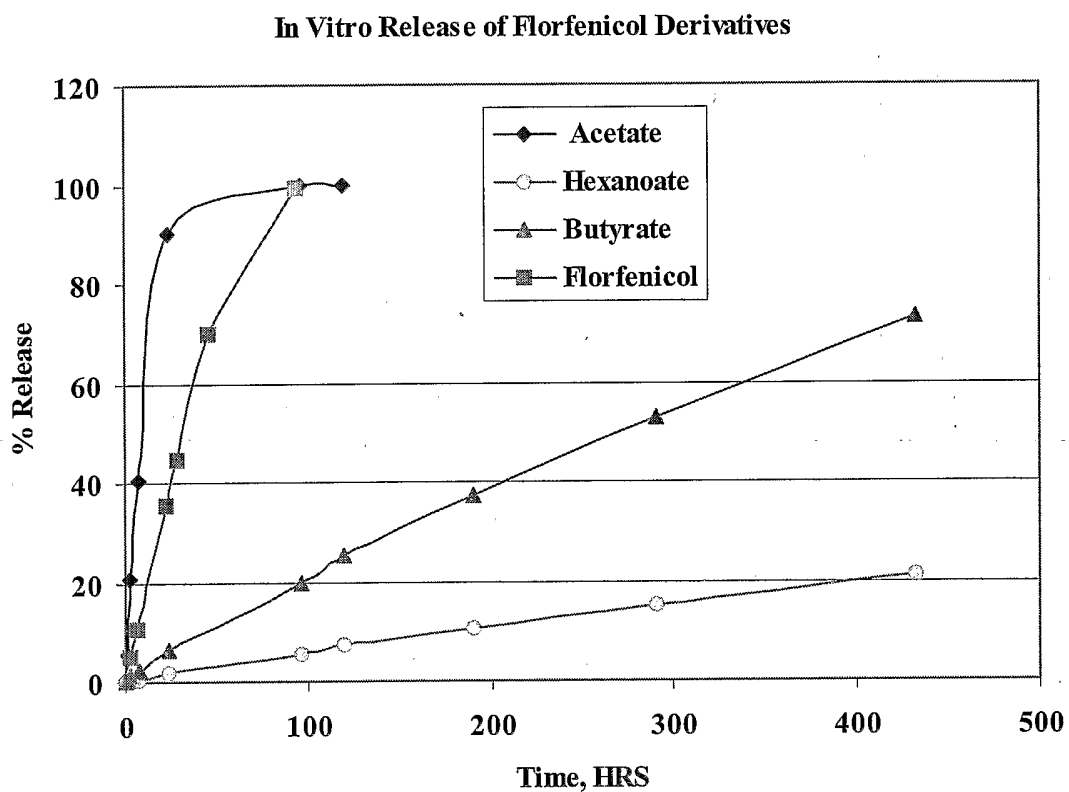


FIG. 3

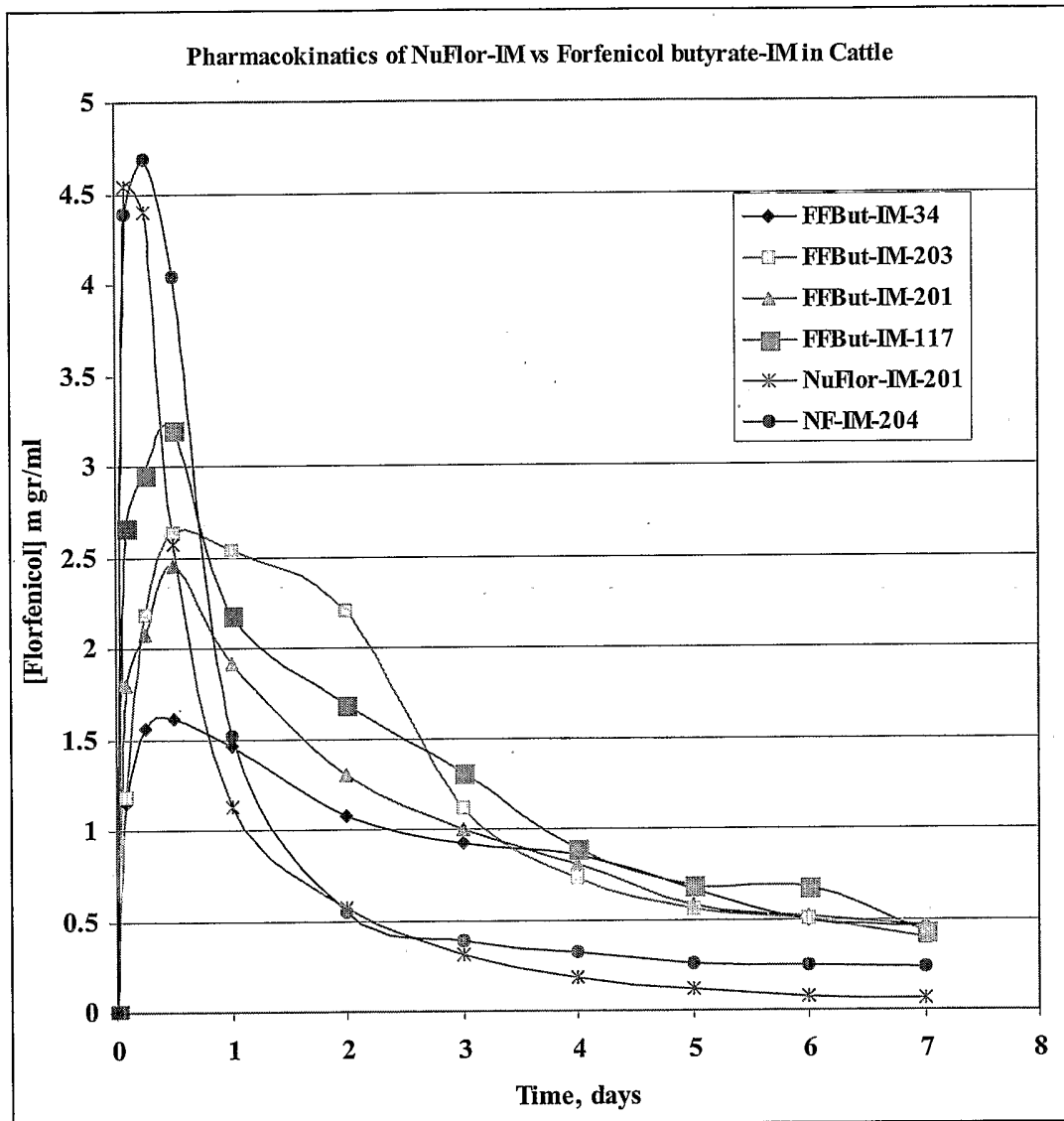


FIG. 4

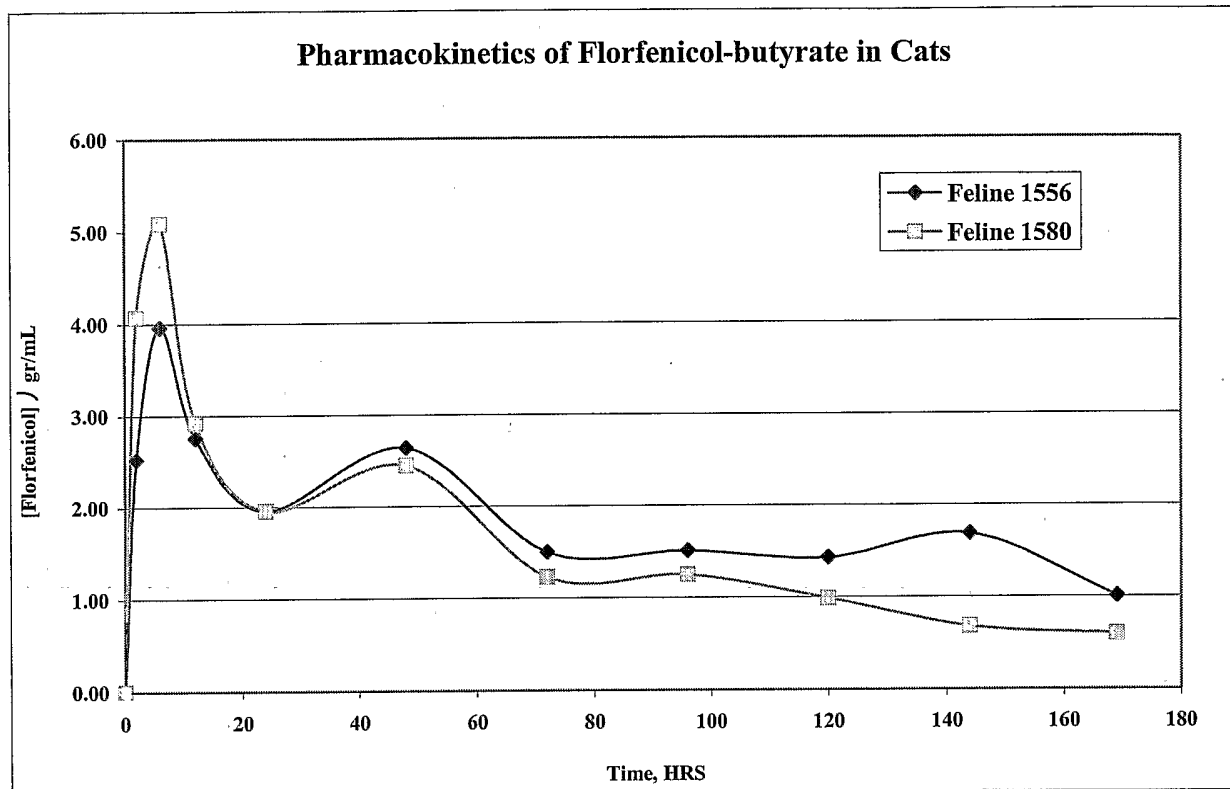


FIG. 5

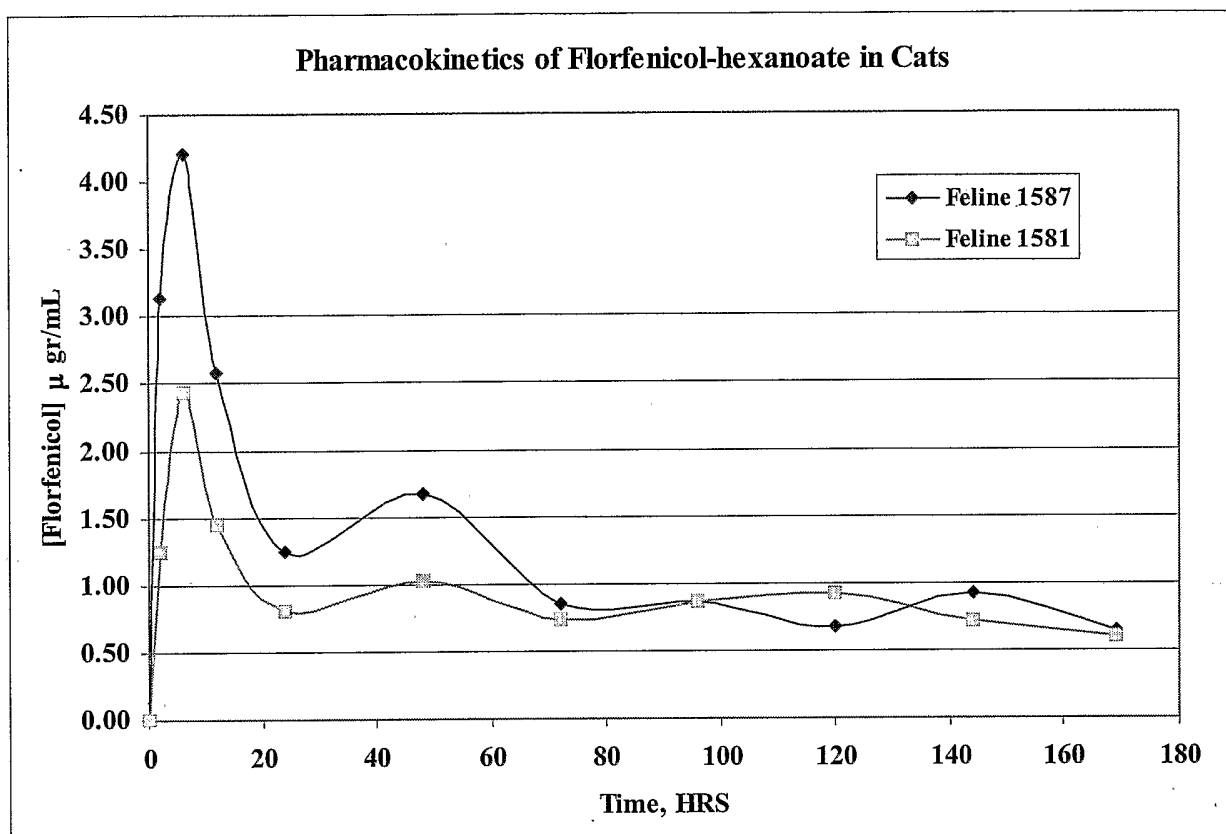


FIG. 6

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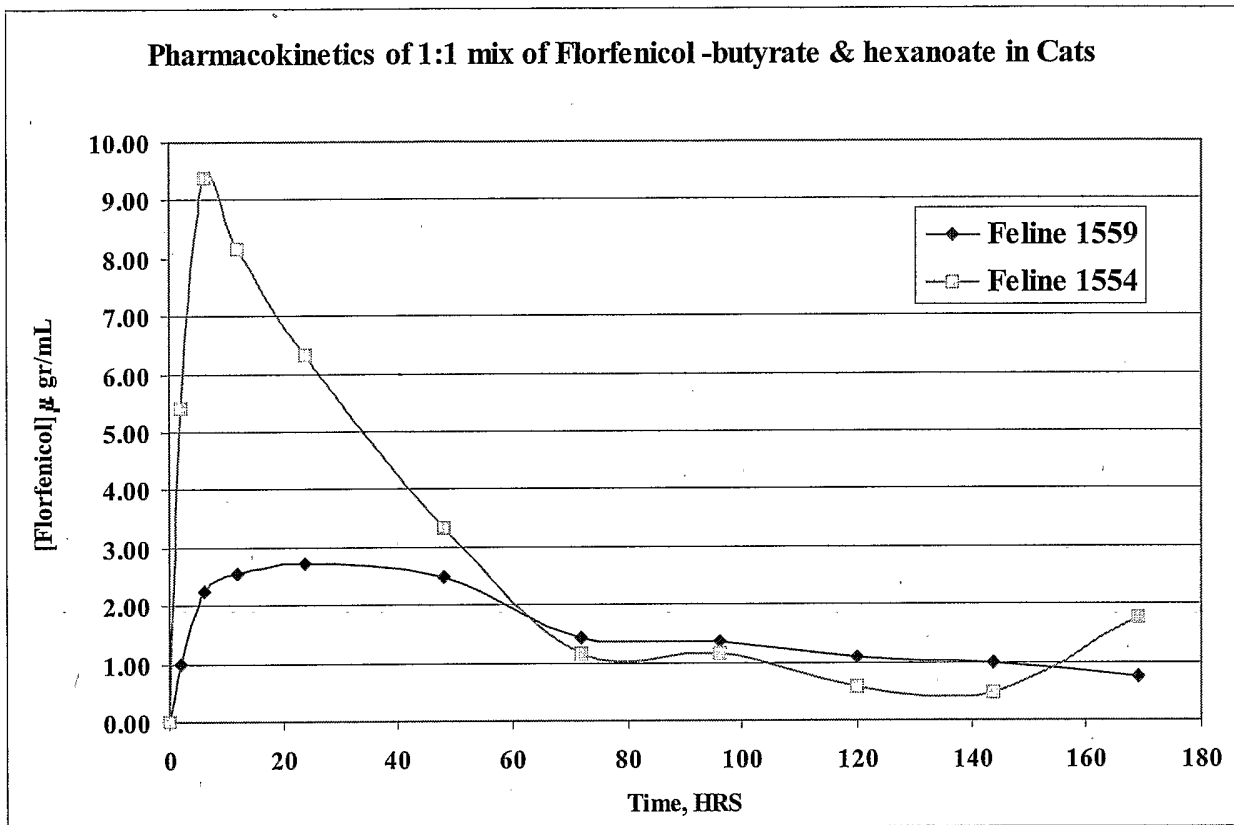


FIG. 7

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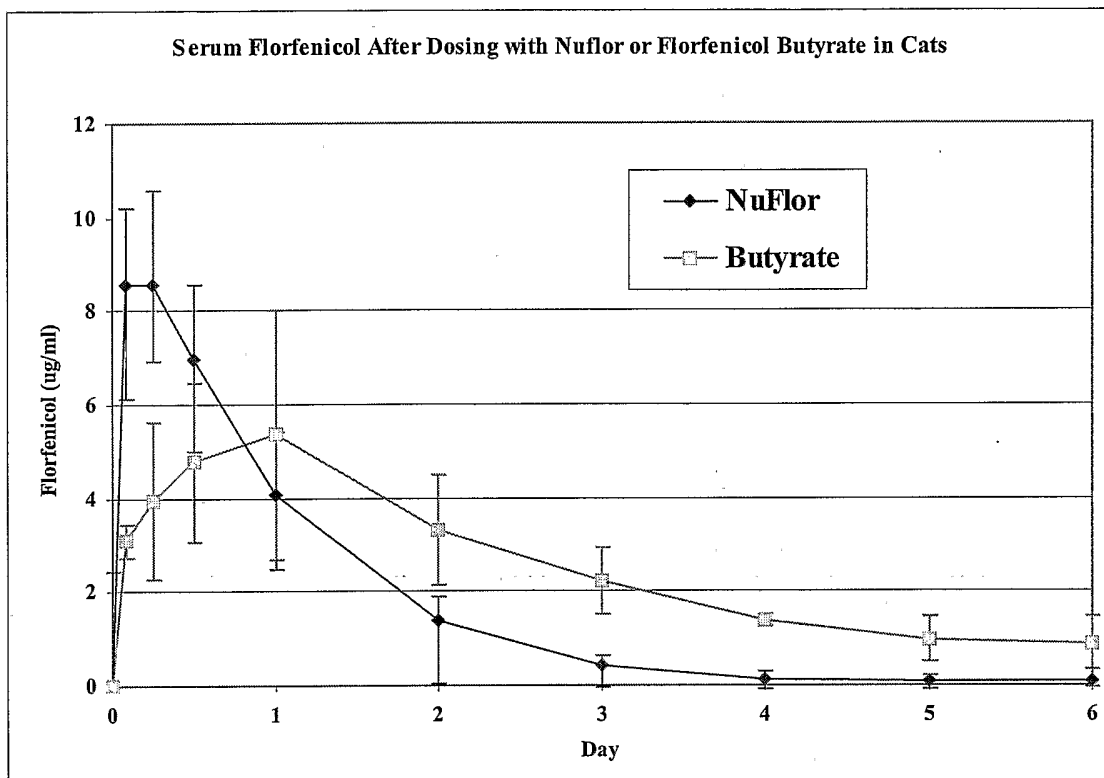


FIG. 8

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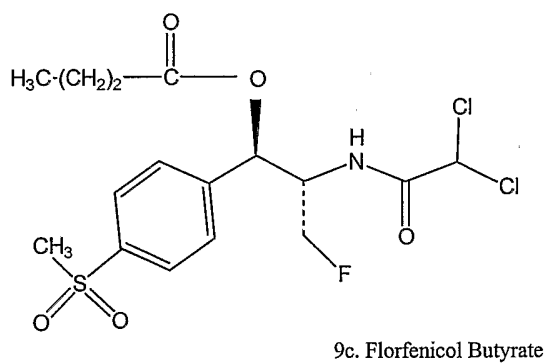
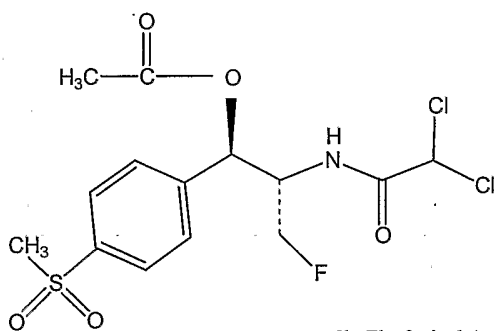
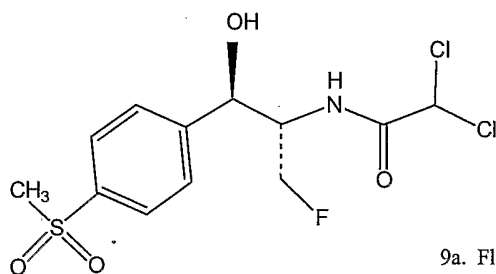


FIG. 9a-c

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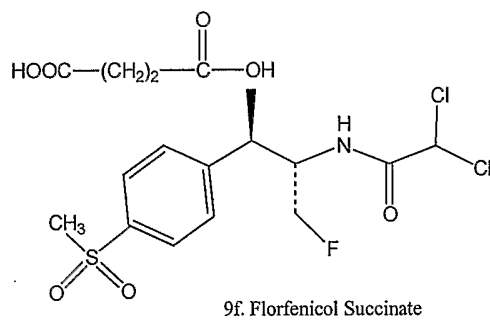
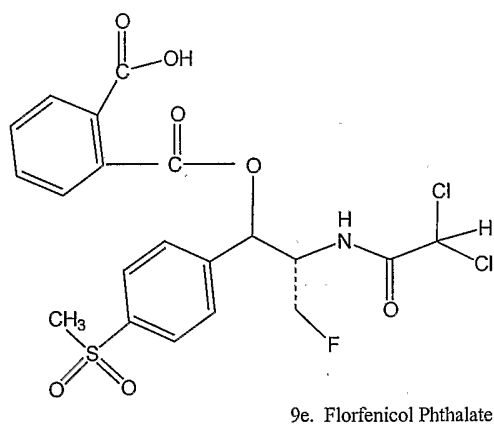
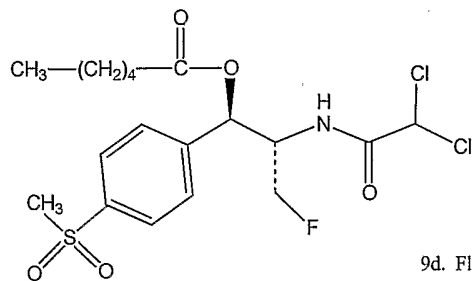
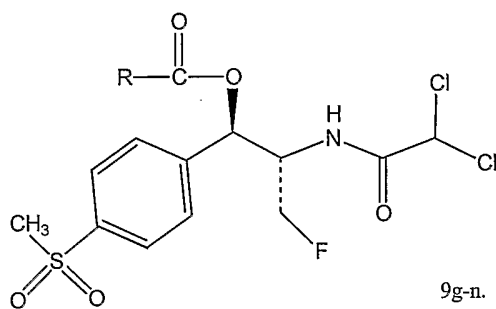


FIG. 9d-f

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9g-n. Florfenicol Derivatives

- Florfenicol Propionate: $\text{R} = \text{CH}_3\text{CH}_2-$
Florfenicol Pentanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_3-$
Florfenicol Heptanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_5-$
Florfenicol Octanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_6-$
Florfenicol Nanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_7-$
Florfenicol Decanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_8-$
Florfenicol Undecanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_9-$
Florfenicol Dodecanoate: $\text{R} = \text{CH}_3(\text{CH}_2)_{10}-$

FIG. 9g-n

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/21260

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/14, 045
 US CL : 514/643, 724

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 514/643, 724

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 02/41899 A1 (PHOENIX SCIENTIFIC, INC.) 30 May 2002 (30.05.2002), abstract, page 2, lines 1-20m claims.	1 ----- 2-24

Further documents are listed in the continuation of Box C. See patent family annex.

<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 21 October 2004 (21.10.2004)	Date of mailing of the international search report 06 DEC 2004
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Sreenivasan Padmanabhan Telephone No. 703-308-1235 <div style="text-align: right;"> DEBORAH A. THOMAS PARALEGAL SPECIALIST GROUP 1000 pat </div>
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