

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 May 2009 (14.05.2009)

PCT

(10) International Publication Number
WO 2009/062200 A1

(51) International Patent Classification:
A01N 37/10 (2006.01)

Tennessee 38139 (US). THOMPSON, Karin [US/US];
909 Lackey Road, Marion, Arkansas 72364 (US).

(21) International Application Number:
PCT/US2008/083068

(74) Agent: RUSSELL, Donna; 1492 Anthony Way, Mount
Juliet, Tennessee 37122 (US).

(22) International Filing Date:
10 November 2008 (10.11.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/002,611 9 November 2007 (09.11.2007) US

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA,
CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE,
EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,
ZW.

(71) Applicant (for all designated States except US): UNIVER-
SITY OF TENNESSEE RESEARCH FOUNDATION
[US/US]; Suite 403, 1534 White Avenue, Knoxville, Ten-
nessee 37996 (US).

(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, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL,
NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG,
CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

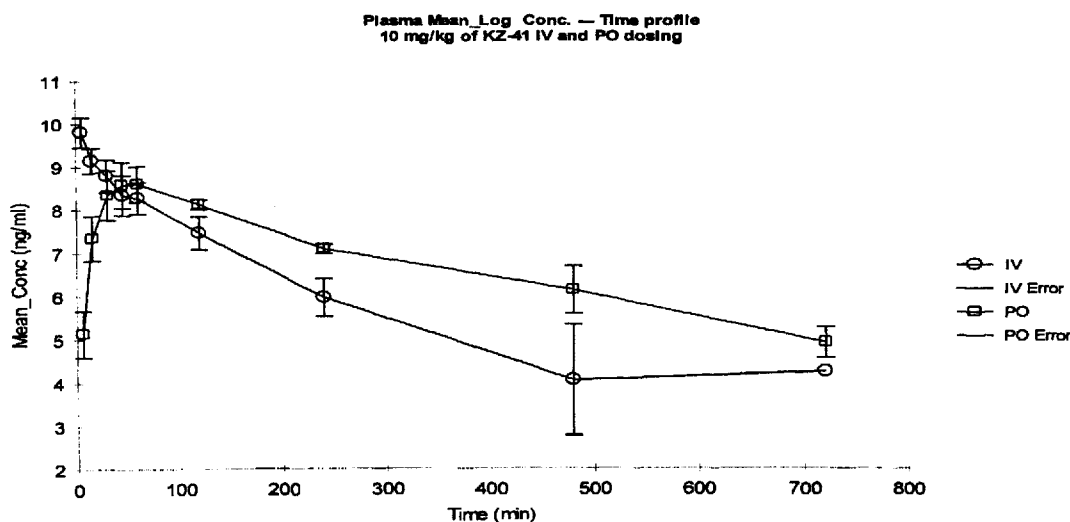
(72) Inventors; and

(75) Inventors/Applicants (for US only): YATES, Charles
[US/US]; 1596 Quinn Road, Collierville, Tennessee
38017 (US). ZENG, Kui [CN/US]; 8375 Westfair Circle
North, Germantown, Tennessee 38139 (US). MILLER,
Duane [US/US]; 8706 Maple Creek Cove, Germantown,

Published:
— with international search report

(54) Title: ANTI-INFLAMMATORY QUINIC ACID DERIVATIVES FOR ORAL ADMINISTRATION

Fig. 12



(57) Abstract: Disclosed are compounds comprising analogs of quinic acids or shikimic acids having anti-inflammatory properties. The compounds are suitable for oral administration, stable, and demonstrate significant efficacy in inhibiting NF- κ B, inhibiting leukocyte adhesion, and inhibiting other factors and cytokines known to be involved in inflammatory disease.

Anti-Inflammatory Quinic Acid Derivatives for Oral AdministrationCross-Reference to Related Applications

5 [0001] This application claims the benefit of priority of United States provisional patent application number 61/002,611, filed on November 9, 2007, the contents of which are incorporated herein by reference.

Field of the Invention

10 [0002] The invention relates to quinic and shikimic acid derivatives. More specifically, the invention relates to amide derivatives of quinic acids and shikimic acids and to methods for their use as anti-inflammatory agents.

Background of the Invention

15 [0003] Non-steroidal anti-inflammatory agents are prescribed to patients at a rate of approximately 70 to 100 million prescriptions a year. Approximately 30+ billion dollars are spent each year for the purchase of anti-inflammatory agents, and researchers are continually searching for new and better anti-inflammatory compositions. A significant percentage of human disease is considered to be "inflammatory disease" or is considered to
20 have a significant inflammatory component as part of the causation or progression of the disease. Inflammatory diseases may affect any organ system, and although they may be more prevalent in older individuals, particular races, or may be more common in one gender, they can affect anyone. These diseases include neurodegenerative disorders, asthma, hepatitis, acute respiratory disorder (ARD), chronic obstructive pulmonary disease
25 (COPD), hepatitis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, nephritis, glomerulonephritis, ischemia/reperfusion injury, septic shock, and sarcoidosis,

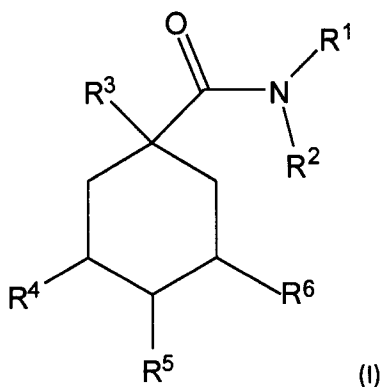
just to name a few. The numbers of individuals who are affected by these diseases are significant. For example, approximately 15% of the population of the United States suffers from rheumatic disorders.

[0004] As with other pharmaceutical approaches to disease, medicinal side effects are always a concern. Some agents have shown great promise as anti-inflammatory agents, but their side effects have made them either unacceptable for human or animal administration or the side effects have resulted in their use in only the most necessary of circumstances, where those effects are outweighed by the significant need to improve the comfort of the patient.

[0005] New anti-inflammatory compositions are needed to meet the needs of millions of patients.

Summary of the Invention

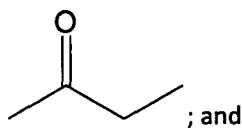
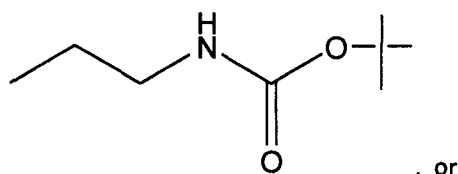
[0006] The invention relates to compositions which may be described as analogs of quinic acid, those analogs having a structure as in Formula I



where:

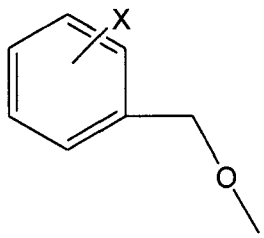
R^1 and R^2 are each independently hydrogen, straight or branched alkyl, cycloalkyl,

aryl, benzyl, arylalkyl, heterocyclic amine, aminoalkyl,



R^3 , R^4 , R^5 , and R^6 are each independently hydrogen or hydroxyl.

5 [0007] Compounds of the invention may also comprise compounds of Formula I



where R^3 is substituted with

, as well as compounds of Formula I where R^3 is absent and a double bond links carbons 1 and 6 of the ring.

[0008] Another aspect of the invention is a method for inducing an anti-inflammatory effect in a human or animal, the method comprising administering to the human or animal a therapeutically effective amount of a compound of Formula I. In one aspect, the method for inducing an anti-inflammatory effect in a human or animal may comprise administering to the human or animal a compound of Formula I to decrease cellular NF- κ B activity. In another aspect, the method for inducing an anti-inflammatory effect in a human or animal may comprise administering to the human or animal a compound of Formula I to decrease leukocyte adhesion.

Brief Description of the Drawings

[0009] Fig. 1 is a scheme (Scheme 1) describing the synthesis of amides of the present invention. Amide groups for individual compounds are indicated by -NH-R^1 , and R^1 is shown for each corresponding compound synthesized.

5 [0010] Fig. 2 is an alternate scheme (Scheme 2) for the synthesis of compound KZ-41.

[0011] Fig. 3 is a scheme (Scheme 3) for the synthesis of compound KZ-09 and the heterocyclic amine-substituted KZ-45.

10 [0012] Fig. 4 is a scheme (Scheme 4) for the synthesis of compounds of the present invention.

[0013] Fig. 5 is a scheme (Scheme 5) for synthesis of additional compounds, including amides compounds having a substitution at R^3 .

[0014] Fig. 6 is a scheme (Scheme 6) describing conditions for the synthesis of the tertiary alcohol analog of quinic acid.

15 [0015] Fig. 7 is a scheme (Scheme 7) describing conditions for the synthesis of analogs wherein the hydroxyl at position R^4 has been removed

[0016] Fig. 8 is a scheme (Scheme 8) describing conditions for the synthesis of additional analogs KZ-93 and KZ-91.

20 [0017] Fig. 9 is a graph illustrating the results of an SEAP assay performed using the indicated quinic acid analogs. The effects of the synthesized quinic acid analogs on NF- κ B activity were assessed using a cell-based high-throughput screening system comprising A549 cells stably transfected with a plasmid containing a secreted alkaline phosphatase (SEAP) reporter gene driven by an NF- κ B response element. Upon TNF- α stimulation, NF- κ B translocates to the nucleus and binds the kappaB response element, causing transcriptional

activation. This activation drives production of the SEAP reporter, which can be measured in the cell culture supernatant as a surrogate marker for NF-kB.

[0018] Cells were plated on 24 well plates at 3×10^4 cells/well and allowed to grow overnight. A549 NF-kB SEAP reporter cells were treated with 10 ng/ml TNF-alpha and the
5 quinic acid analog (1 micromolar) indicated on the x-axis. After 18 hours, SEAP activity (indicated on the y-axis) was measured.

[0019] Fig. 10 is a graph illustrating the effect of quinic acid analog KZ-41 on leukocyte activity determined by the mouse dorsal flap model.

[0020] Fig. 11 is a graph illustrating the results of an assay to compare stability of
10 analog KZ-41 with that of quinic acid (QA) to degradation by bacterial enzymes. Percentage of drug remaining is shown on the Y-axis and the time exposed to the enzymes is shown on the X-axis. Results for quinic acid are indicated by the diamonds (◆) and results for KZ-41 are indicated by the squares (■).

[0021] Fig. 12 is a graph illustrating the levels of analog KZ-41 when provided orally
15 (PO) or by intravenous administration (IV). Unlike native or synthetic quinic acid, which is degraded by enzymes produced by bacteria in the intestines, KZ-41 is not degraded and is available for absorption into the circulation.

Detailed Description

20 [0022] The inventors have synthesized derivatives of quinic acids and shikimic acids to provide compounds having significant anti-inflammatory activity. These compounds are designed for oral delivery, and studies using the MTS assay, a cell-proliferation assay that measures cytotoxicity, indicate that the compounds exhibit no cytotoxicity at 100

micromolar levels. The compounds are stable at room temperature, as well as water-soluble.

[0023] Quinic acid may be synthesized by methods previously described in the literature, or may be isolated from a variety of plant extracts, such as Cat's Claw extract.

5 These extracts have previously been shown to have anti-inflammatory properties. Subsequent research demonstrated that a significant portion of this activity is attributable to the quinic acid portion of the plant extract. Extracts have been used therapeutically for a variety of diseases including allergy, arthritis, chemotherapy side effects, cancer, bacterial/fungal infections, gastrointestinal inflammation and gastric ulcers. Oral
10 administration of a hot water Cat's Claw extract, C-Med-100[®] enhanced all leukocyte populations in a doxorubicin-induced leucopenia rat model, whereas Neupogen[®], a granulocyte colony stimulator commonly used to treat chemotherapy-induced leucopenia, increased only the neutrophil fraction.

[0024] Quinic acids are, however, catabolized by enzymes produced by gut bacteria.

15 In rats, less than 10% of an orally-administered dose of quinic acid is recovered. The inventors therefore set out to produce quinic acid derivatives that retain or improve the beneficial effects of quinic acid, while being effective for oral administration. They discovered that amide substitutions such as, for example, carboxamide substitutions, produced compounds that retain the beneficial effects associated with quinic and shikimic
20 acids, and in some cases provide a significantly improve effect, these same compounds being resistant to gut bacteria enzyme degradation. The compounds they have synthesized are not catabolized by bacterial enzymes, but are water-soluble, stable at room temperature, and effective for producing anti-inflammatory effects. As demonstrated by

the graph in Fig. 12, the compounds may be administered orally to provide levels of compound in the tissue similar to, or better than, that produced by intravenous (IV) administration. These properties make the compounds attractive options for therapeutic use as anti-inflammatory agents.

5 [0025] The compounds are relatively easy to synthesize, and the inventors have found more than one approach for their synthesis, using combinations of steps for molecular rearrangements or the addition of functional groups generally known to those of skill in the art, as shown in the accompanying figures. In one example, synthesis of quinic acid analogs of the invention was performed by first forming the lactone. To the quinic acid
10 starting material was added PTSA in refluxing benzene and DMF. To form the various individual analogs, the lactone was allowed to react with a variety of amines (see Fig. 1, R groups) in acetic acid at 85°C, as indicated in Fig. 1.

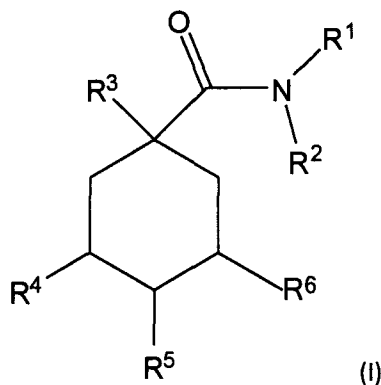
 [0026] Briefly, as shown in Fig. 4 (Scheme 4), compounds of the invention may be formed by protecting the alcohols of shikimic acid by converting them to acetates, followed
15 by activation of the carboxylic acid moiety and treatment with propyl amine (R=nPr), followed by deacetylation with LiOH to give the desired amide.

 [0027] Schemes for synthesis of additional compounds are described in Fig. 5 (Scheme 5). Briefly, compounds may be synthesized as shown, using chemistry previously described by Kaila, *et al* (Kaila, N., *et al.*, (2005) J. Med. Chem. 48: 4346-4357), but with the
20 addition of the indicated amines to improve the effects and the resistance to bacterial enzyme degradation.

 [0028] The scheme for synthesis of the tertiary alcohol is shown in Scheme 6 (Fig. 6). It utilizes the formation of 22 from quinic acid, as outlined by Hanessian (Hanessian, S. *et al.* (1997) J. Org. Chem. 62: 465-473). The two alcohols are then protected by silyl protecting

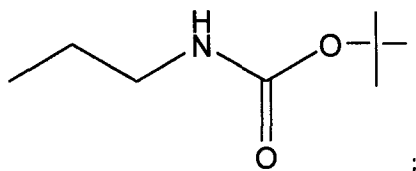
groups and the benzyl protecting group is removed to give the intermediate alcohol that is oxidized by pyridinium dichromate to give ketone 24. The epoxide may be opened with propyl amine ($R=nPr$) followed by silyl protection of the newly generated alcohol to give 25.

[0029] Compounds of the invention may be described by Formula I



wherein:

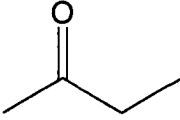
R^1 and R^2 are each independently hydrogen, straight or branched alkyl, cycloalkyl, aryl, benzyl, arylalkyl, heterocyclic amine, aminoalkyl, ketone, or



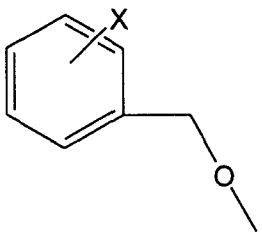
and

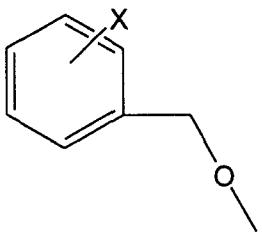
R^3 , R^4 , R^5 , and R^6 are each independently hydrogen or hydroxyl.

[0030] In one aspect, R^1 and R^2 form a piperidine ring with N, and in another aspect of the invention is a compound such as compound KZ-41 wherein when R^1 or R^2 is hydrogen, the other of R^1 or R^2 is alkyl. In one aspect, the alkyl is $-C_3H_7$ and each of R^3 - R^6 is hydroxyl.

[0031] In another aspect of the invention, the ketone may be  and each of R³-R⁶ may be hydroxyl.

[0032] Compounds of the invention may also comprise compounds of Formula I



where R³ is substituted with , as well as compounds of Formula I

5 where R³ is absent and a double bond exists between carbons 1 and 6 of the ring.

[0033] Compounds of the present invention may conveniently be administered in a pharmaceutical composition containing the compound in combination with a suitable excipient. Such pharmaceutical compositions can be prepared by methods and contain excipients which are well known in the art. Such methods and ingredients may be found in

10 Remington's Pharmaceutical Sciences (Alfonso Gennaro *et al.*, eds., Lippincott, Williams & Wilkins, Baltimore, Maryland, 20th ed., 2000). The compounds and compositions of the present invention can be administered parenterally (for example, by intravenous, intraperitoneal or intramuscular injection), topically, orally, or rectally.

[0034] For oral therapeutic administration, the active compound may be combined

15 with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, and wafers, for example. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 100% of the weight of a given unit dosage form. The amount of active

compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[0035] The tablets, troches, pills, capsules, and the like may also contain the following: binders 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, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. The active compound may also be incorporated into sustained-release preparations and devices.

[0036] Pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the preparation of sterile injectable or infusible solutions or dispersions. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (*e.g.*, glycerol, propylene glycol, liquid polyethylene glycols), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the

formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants.

[0037] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in the appropriate solvent with various other ingredients
5 described above, followed by filter sterilization.

[0038] For topical administration, the present compounds may be applied in pure form in liquid compositions or administered as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

[0039] Useful dosages of the compounds of formula I can be determined by
10 comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art (for example, see U.S. Pat. No. 4,938,949).

[0040] Another aspect of the invention is a method for administering to a human or animal patient a compound of the invention to provide an anti-inflammatory effect.
15 Compounds of the invention have demonstrated anti-inflammatory effects both *in vivo* and *in vitro*. Of the compounds tested, several demonstrated efficacy for inhibiting NF-kB activity, with the N-propyl amide substitution (designated KZ-41) being especially effective. Compounds of the invention also demonstrated efficacy in decreasing leukocyte adhesion, as well as decreasing the levels or activity of a variety of cytokines known to contribute to
20 inflammatory disease or diseases having a significant inflammatory component.

[0041] Therapeutically effective dosages may therefore be administered orally, or by IV or other appropriate means to achieve an anti-inflammatory effect. In one aspect, compositions of the invention may be administered to inhibit NF-kB activity for diseases such as, for example, rheumatoid arthritis. Compositions of the invention may also be

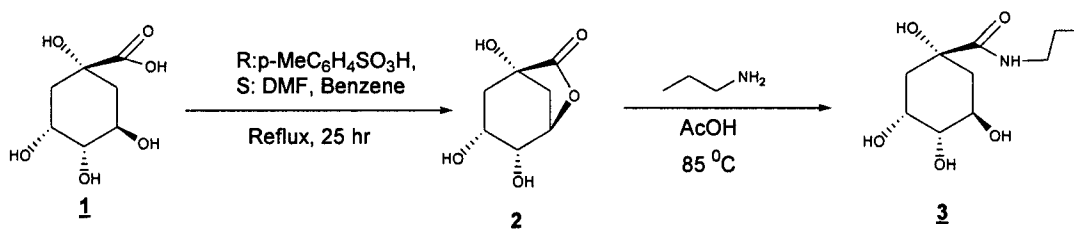
effective for the treatment of viral illness, acute respiratory distress syndrome (ARDS), and other disease states associated with increased TNF-alpha and NF-kB activity.

[0042] The invention may be further described by means of the following non-limiting examples:

5

Examples

Synthesis of Specific QA analogs



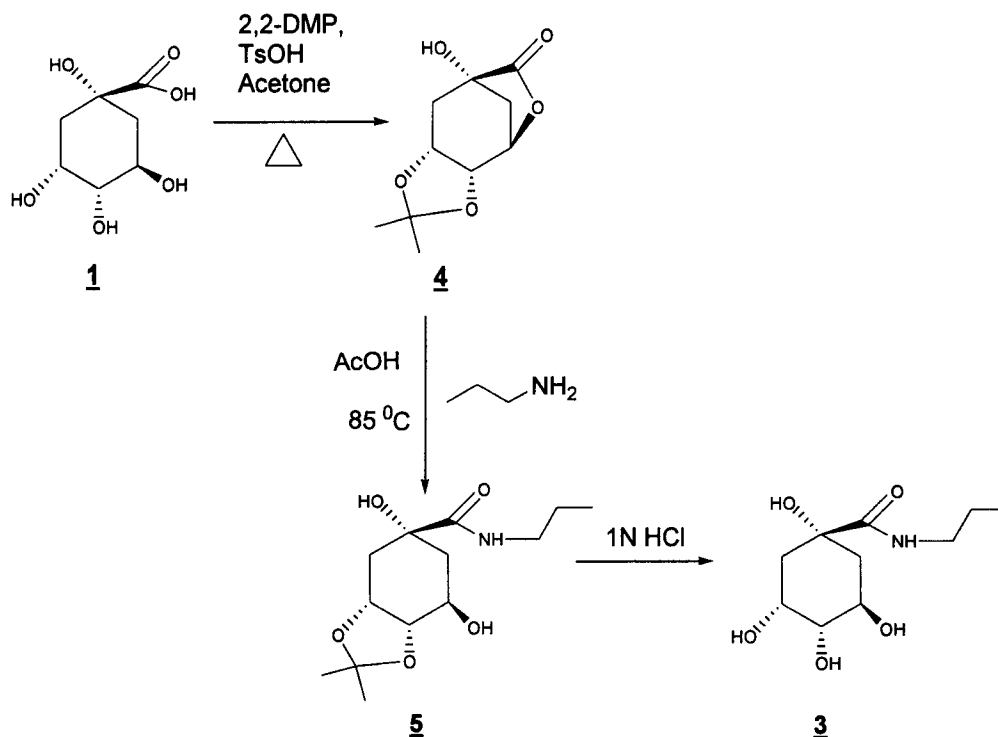
10

Scheme 9

[0043] **1,3,4-Trihydroxy-6-oxa-bicyclo[3.2.1]octan-7-one (2)**. In a 200 mL round-bottom flask fitted with a stirring bar, reflux condenser, Dean-Stark trap, and argon inlet, 5 g of quinic acid (**1**, 26 mmol) was placed and 10 mL of dry DMF was added via syringe and the
 15 slurry stirred at room temperature. Next, benzene 60 mL and p-toluenesulfonic acid 0.5 g were added, and the slurry was heated to reflux for 26 h. TLC was used confirm the completion of reaction. A 1:1 mixture of EtOAc and 12eptanes (100 mL) was added to the cooled reaction mixture. The mixture was stirred for 1 h at room temperature and filtered. The collected solid was again stirred with a 1:1 mixture of EtOAc and 12eptanes (100 mL) for
 20 1 hr at room temperature and filtered. Titration was repeated one more time with a 1:1 mixture of EtOAc and 12eptanes (100 mL) and the precipitate collected to give 3.5 g of Lactone **2** (78% yield). Mp: 192-193 °C, R_f 0.25 (EtOAc); MS: [M-H]⁻: 171; ¹H NMR (300 MHz, DMSO) δ 1.72 (t, J = 1.6 Hz, 1H), 1.82-1.88 (m, 1H), 2.07-2.13 (m, 1H), 2.25 (d, J = 1.1 Hz,

1H), 3.49 (ddd, J = 11.4, 6.3, 5.7 Hz, 1H), 3.82 (t, J = 4.5 Hz, 1H), 4.61 (t, J = 5.1 Hz, 1H), 4.816 (d, J = 6.0 Hz, 1H), 5.23 (d, J = 4.5 Hz, 1H), 5.89 (s, 1H).

[0044] **1,3,4,5-tetrahydroxy-cyclohexane-carboxylic acid propylamide(3)** In a 50 mL round-bottom flask fitted with a stirring bar, reflux condenser, 0.196 g Lactone **2** (1.125 mmol), and propylamine (0.83 mL, 0.6g, 10.13 mmol) were combined, then glacial acetic acid (0.19 mL, 0.20 g, 3.36 mmol) was added. The solution was warmed to 85 °C in an oil bath for 30 min, at which time TLC (CHCl₃ :MeOH:NH₃OH =100:10:1) indicated complete consumption of the starting lactone. Reaction mixture was purified by flash column with the same solvent as TLC. Product **3** was collected, yielding 206mg (80% yield). Mp: 132-133 °C ,
R_f 0.03 (CHCl₃ :MeOH:NH₃OH =100:10:1); MS: [M-H]⁻ = 232; ¹H NMR (300 MHz, DMSO) δ 0.81 (t, J = 6.0 Hz, 3H), 1.41 (ddd, J = 13.8, 7.5, 6.9 Hz, 2H) 1.66-1.83 (m, 4H), 3.02 (dd, J =6.9, 6.6 Hz, 2H), 3.22 (t, J = 6.0 Hz, 1H), , 3.75 (ddd, J = 13.8, 9.0, 5.7 Hz, 1H), 3.94 (s, 1H), 4.69 (d, J = 4.5 Hz, 1H), 4.90 (d, J =5.4 Hz, 1H), 5.19 (d, J =3.6 Hz, 1H), 5. 47 (s, 1H), 7.76 (t, J = 5.4 Hz, 1H).



Scheme 10

[0045] **3,4-O-Isopropylidene-1,5-quinic Lactone (4)** A mixture of quinic acid (1) (20g, 104.1 mMol), p-toluenesulfonic acid monohydrate (0.2g), 2,2-dimethoxypropane (38g, 364 mMol), and acetone (100 mL) was heated to reflux for 2 hr. The reaction mixture was cooled down, and solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), washed with 5% aqueous sodium bicarbonate (100 mL). The aqueous phase was back-extracted with ethyl acetate (50x 2 mL). The combined organic extracts were dried (Na₂SO₄), filtered, and the solvents were removed in vacuo. The residue was crystallized with hot ethyl acetate, got white solid 16g (80% yield). mp: 147-149 °C R_f = 0.3 (EtoAC: Hex = 3:7). MS: [M+Na]⁺ = 237. ¹H NMR (CDCl₃) δ 1.37 (s, 3H), 1.55 (s, 3H), 2.21 (dd, J = 14.7, 3.0 Hz, 1H), 2.30-2.44 (m, 2H), 2.68 (d, J = 11.7 Hz, 1H), 2.83 (s, 1H), 4.32-4.36 (m, 1H), 4.51-4.58 (m, 1H), 4.79 (dd, J = 2.6, 6.3 Hz, 1H).

[0046] **5,7-Dihydroxy-2,2-dimethyl-hexahydro-benzo[1,3]dioxole-5-carboxylic acid propylamide (5)**. Lactone (**4**), (10g, 46.7 mMol), propylamine (30 mL), and glacial acetic acid (10 mL) was refluxed under argon with oil bath (85 °C) for 45 min. Solvents were removed under reduced pressure. The residue was washed with saturated sodium bicarbonate solution (100 mL), extracted with ethyl acetate (100 x 3 mL). The combined organic extracts were dried (Na₂SO₄), filtered, and the solvents were removed in vacuo. The residue was crystallized with hot ethyl acetate, got white solid 9.7g (76 % yield). mp: 85-86 °C, R_f = 0.15 (EtoAC: Hex = 2:1). MS: [M+Na]⁺ = 296. ¹H NMR (DMSO) δ 0.83 (t, J = 6.0 Hz, 3H), 1.24 (s, 3H), 1.38 (s, 3H), 1.40-1.45 (m, 2H), 1.61-1.64 (m, 2H), 1.72 (dd, J =14.7, 5.7 Hz, 1H), 2.04 (dd, J =14.7, 5.7 Hz, 1H), 3.01 (dd, J =6.6, 7.2 Hz, 2H), 3.80 (ddd, J = 9.9, 6.3, 3.6 Hz, 2H), 4.31 (d, J =5.4 Hz, 1H), 5.01 (d, J = 5.1 Hz, 1H), 5.28 (s, 1H), 7.73 (t, J = 5.7 Hz, 1H).

[0047] **1,3,4,5-tetrahydroxy-cyclohexane-carboxylic acid propylamide(3)** The amide (**5**) (5.0g) was dissolved in water (10mL), then added aq. 1M HCl (40 mL) at room temperature. The reaction mixture was stirred at room temperature for 1hr. Solvent was removed under reduced pressure. Residue HCl was removed by adding EtOEt (20 x 3 mL) under vacuo. The residue was further dried under oil pump. Product (**3**) was collected, yielding product 4.26g (100% yield). mp: 132-133 °C, R_f 0.03 (CHCl₃ :MeOH:NH₃OH =100:10:1); MS: [M-H]⁻ = 232; ¹H NMR (300 MHz, DMSO) δ 0.81 (t, J = 6.0 Hz, 3H), 1.41 (ddd, J = 13.8, 7.5, 6.9 Hz, 2H) 1.66-1.83 (m, 4H), 3.02 (dd, J =6.9, 6.6 Hz, 2H), 3.22 (t, J = 6.0 Hz, 1H), , 3.75 (ddd, J = 13.8, 9.0, 5.7 Hz, 1H), 3.94 (s, 1H), 4.69 (d, J = 4.5 Hz, 1H), 4.90 (d, J =5.4 Hz, 1H), 5.19 (d, J =3.6 Hz, 1H), 5.47 (s, 1H), 7.76 (t, J = 5.4 Hz, 1H).

Effects of Quinic Acid Amides on NF- κ B Activity

[0048] The effects of the synthesized quinic acid analogs on NF- κ B activity were assessed using a cell-based high-throughput screening system comprising A549 cells stably transfected with a plasmid containing a secreted alkaline phosphatase (SEAP) reporter gene
5 driven by an NF- κ B response element. Upon TNF- α stimulation, NF- κ B translocates to the nucleus and binds the kappaB response element, causing transcriptional activation. This activation drives production of the SEAP reporter, which can be measured in the cell culture supernatant as a surrogate marker for NF- κ B.

[0049] Cells were plated on 24 well plates at 3×10^4 cells/well and allowed to grow
10 overnight. A549 NF- κ B SEAP reporter cells were treated with 10 ng/ml TNF- α and the quinic acid analog (1 micromolar) indicated on the x-axis of Fig. 4. After 18 hours, SEAP activity (indicated on the y-axis of Fig. 9 was measured.

Effect of Quinic Acid Amides on Leukocyte Activity

15 [0050] The effect of quinic acid analogs on leukocyte-endothelial cell interactions in response to LPS was also tested in a murine dorsal window imaging model. Briefly, animals were outfitted with imaging windows in the dorsal skin flap. Animals received systemic LPS and leukocyte rolling and adhesion were quantified four hours later. Rolling and adhesion were significantly increased in LPS-treated animals compared to vehicle alone (Fig. 10).
20 Quinic acid analogs of the invention significantly reduced leukocyte adhesion, while having little effect on leukocyte rolling.

[0051] Male nude mice age 8-10 weeks, weighing 25-30 grams were used for the study. All surgical procedures were carried out aseptically. After the animal was anesthetized, a suitable area of skin on the dorsum was marked for hair removal with a

clipper, followed by use of hair removal lotion. The surgical area was disinfected with alcohol and betadine. A thin dorsal skin fold was lifted from the back and sandwiched between two titanium plates. The titanium plates were firmly fixed with three screws and nuts through the holes made in the fold. The skin was sutured to the top of the plates for
5 added stability. A circular section of the skin on one side of the skin fold, along with its cutaneous tissue and fascia was incised through the window of the assembly to expose the blood vessels and the striated muscle of the opposing skin of the dorsal skin fold. Antibiotic ointment containing bacitracin zinc, polymyxin B sulfate and neomycin sulfate was applied to the edges of the incised wound and a glass window fixed with a snap ring on top of the
10 exposed area inside the dorsal skin fold assembly. Animals received buprenorphine (s.c. 0.1 mg/kg) immediately following surgery and every 12 hours for 48 hours for pain.

Quinic Acid Amides Are Not Degraded by Bacterial Enzymes

[0052] Experiments involving *Gluconobacter oxydans* were performed as described
15 by Adachi et al. (Adachi, O., et al., (2003) Biosci. Biotechnol. Biochem. 67: 2124-2131). Briefly, *G. oxydans* (ATCC, Manassas, VA) was cultured in basal media consisting of glycerol, 3 grams of yeast extract, and 1 gram of polypepton in 1 liter of tap water. In order to induce quinate dehydrogenase (QDH), quinate (2 g) was added and the pH adjusted to 7.0. Microorganisms were grown in medium (100 mL) at 30°C. For cell-mass production, a seed
20 culture in 100 mL of medium was done overnight and transferred to 5 liters of fresh medium in a table top shaker incubator and cultivated for 12 hr under aeration. Harvested cells were suspended in medium (5 mM potassium phosphate and pH 6.5) and centrifuged to pellet cells. Cells were stored without freezing.

[0053] Cell suspensions were prepared by homogenizing harvested cells at a ratio of 10 g of wet cells per 10 mL of 5 mM potassium phosphate, pH 6.5. Cell suspensions were passed twice through a French pressure cell press. Intact cells were removed by low speed centrifugation and the cell-free extract was further centrifuged (68,000 x g for 90 min) to
5 separate the membrane and cytoplasmic soluble fraction. The membrane fraction was washed by homogenizing the precipitate in buffer and centrifugation repeated.

[0054] The membrane fraction (5 grams dried) was incubated with QA analogs (1 mM) under different pH conditions (pH 3-10) at 30°C. Culture media was sampled every 30 minutes for up to 3 hours. Three hours was chosen, as previous results have shown
10 complete depletion of QA in this time frame (Adachi, *et al.* (2003) Biosci. Biotechnol. Biochem. 67: 2124-2131). Samples will be stored at -80°C until analysis.

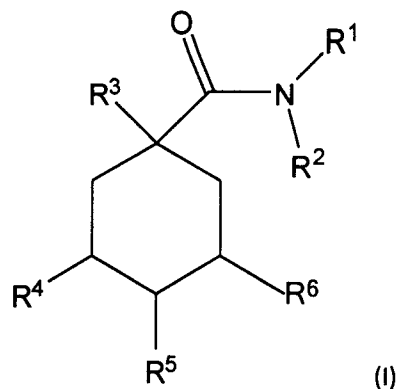
[0055] Samples were evaporated to dryness at 30°C and reconstituted with 150 mL of 1:1 (v/v) mixture of 0.5% ammonia in water and acetonitrile. Internal standards were added to compensate for the variability of the mass spectrometric response. Prepared
15 samples were injected onto the LC-MS/MS for analysis. The Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD) consisted of a SCA-10A vp system controller, two LC-10AD VP liquid chromatogram pumps, and a CTC PAL (CTC Analytics AG, Zwingen, Switzerland) autosampler. A Waters Nova-Pak® C₁₈ 3 mm (2.1 x 50 mm) column protected by a 2-cm C₁₈ guard column maintained at ambient temperature was used in the
20 analysis. The mobile phase A consisted of 0.5% ammonia in water and mobile phase B consisted of 0.5% ammonia in acetonitrile. The flow rate was set at 0.3 mL/min with a gradient from 60% to 40% A in 4 min, isocratic for 3 min, then returned to 40% in 2 min. MS/MS analysis was performed using a Sciex ®API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The mass spectrometry system was

operated with turbo ionspray ionization source under negative ion mode. The spray needle voltage was set at 5,000 V, with the turbo gas temperature set at 400 °C, and the declustering potential set at 60 V. For this study, samples were analyzed using a Q1 scan for product ion identification, a product ion scan for characterization of metabolites or enzymatic products and by using multiple-reaction monitoring (MRM) for quantitation of QA. Quantitation was performed using one set of MRM transitions: parent QA ions (MH^- /191)[®] selective daughter ions m/z 127. A switch valve was used before the mass spectrometer to remove buffer matrix in the sample. The peak areas, peak ratios, linear regression, assayed concentrations, and other quantitative analysis calculations were conducted Sciex Analyst 1.4 software. A calibration curve, which is a weighted linear regression curve relating to peak area ratios of the analyte/internal standard to the concentration of analyte in the calibration standard, was established for each QA analog. The analyte concentrations in the samples was determined by comparison to the calibration curve using the Analyst software.

[0056] Results are shown in Fig.11, where results for quinic acid are indicated by the diamonds (◆) and results for KZ-41 are indicated by the squares (■).

What is claimed is:

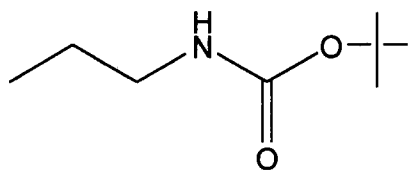
1. A compound of Formula (I)



5

wherein:

R^1 and R^2 are each independently hydrogen, straight or branched alkyl, cycloalkyl, aryl, benzyl, arylalkyl, heterocyclic amine, aminoalkyl, ketone, or



- 10 and R^3 , R^4 , R^5 , and R^6 are each independently hydrogen or hydroxyl.

2. The compound of claim 1 wherein R^1 is hydrogen, R^2 is alkyl and R^3 , R^4 , R^5 , and R^6 are each hydroxyl.

3. The compound of claim 2 wherein R^2 is $-C_3H_7$.

4. The compound of claim 1 wherein R^1 is cyclohexyl.

- 15 5. The compound of claim 1 wherein R^1 is aminoalkyl.

6. A method comprising administering to a human or animal subject a therapeutically effective amount of a compound of Formula (I) to produce an anti-inflammatory effect in the human or animal subject.
7. The method of claim 6 wherein the compound of Formula (I) further comprises a compound wherein R^1 is hydrogen, R^2 is alkyl and R^3 , R^4 , R^5 , and R^6 are each hydroxyl.
8. The method of claim 6 wherein the anti-inflammatory effect is inhibition of NF- κ B activity.
9. The method of claim 6 wherein the anti-inflammatory effect is inhibition of leukocyte adhesion.

Fig. 1

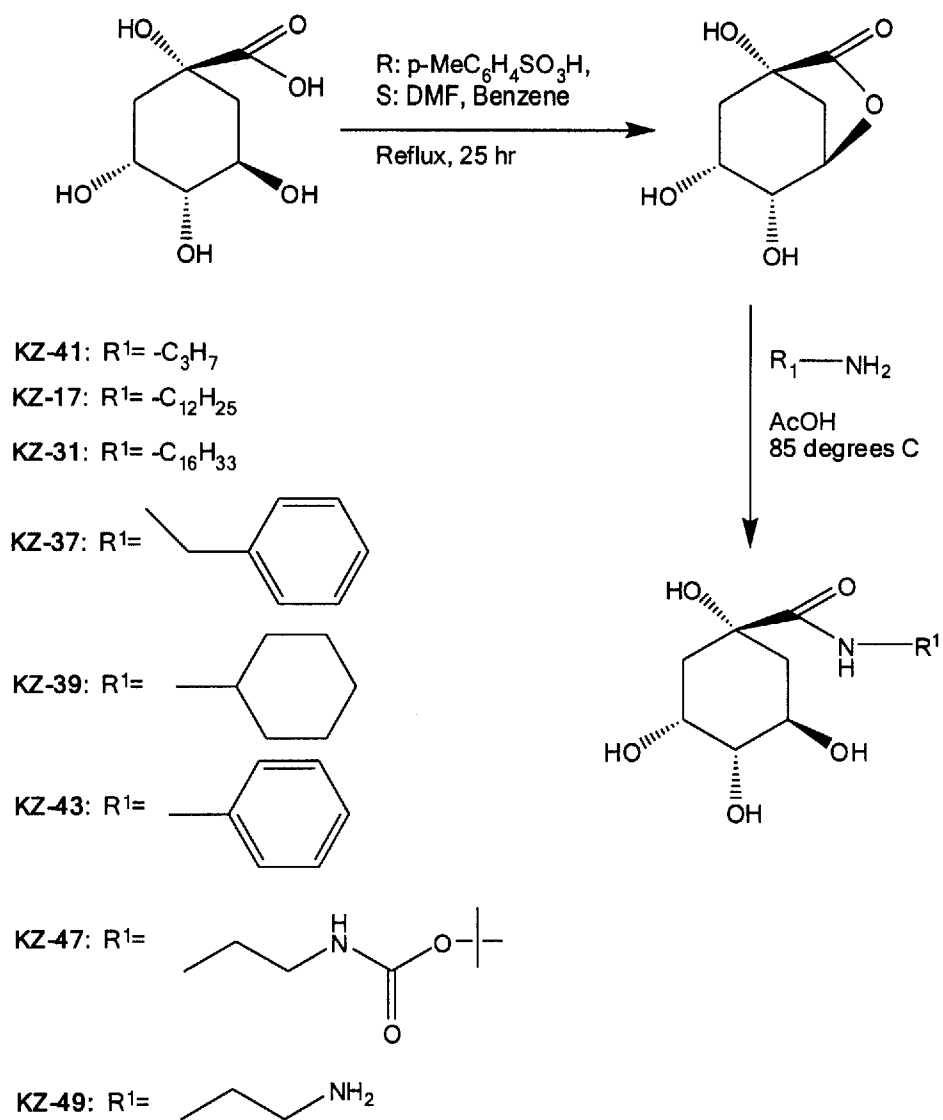


Fig. 2

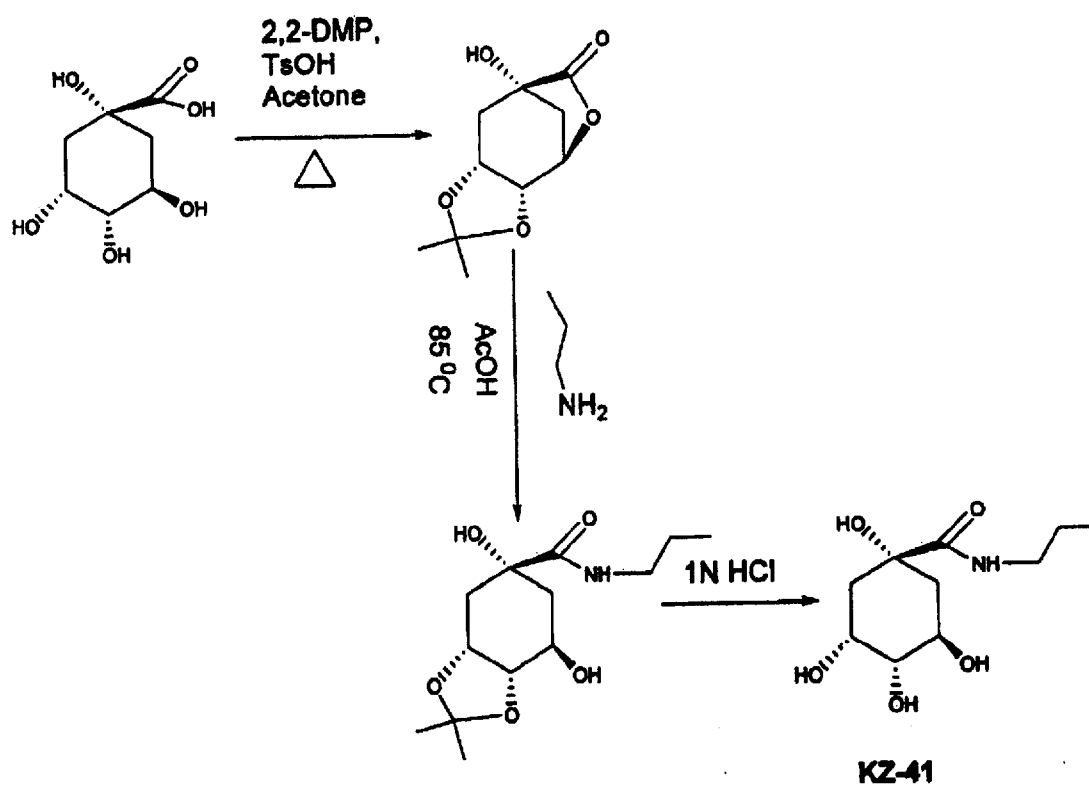


Fig. 3

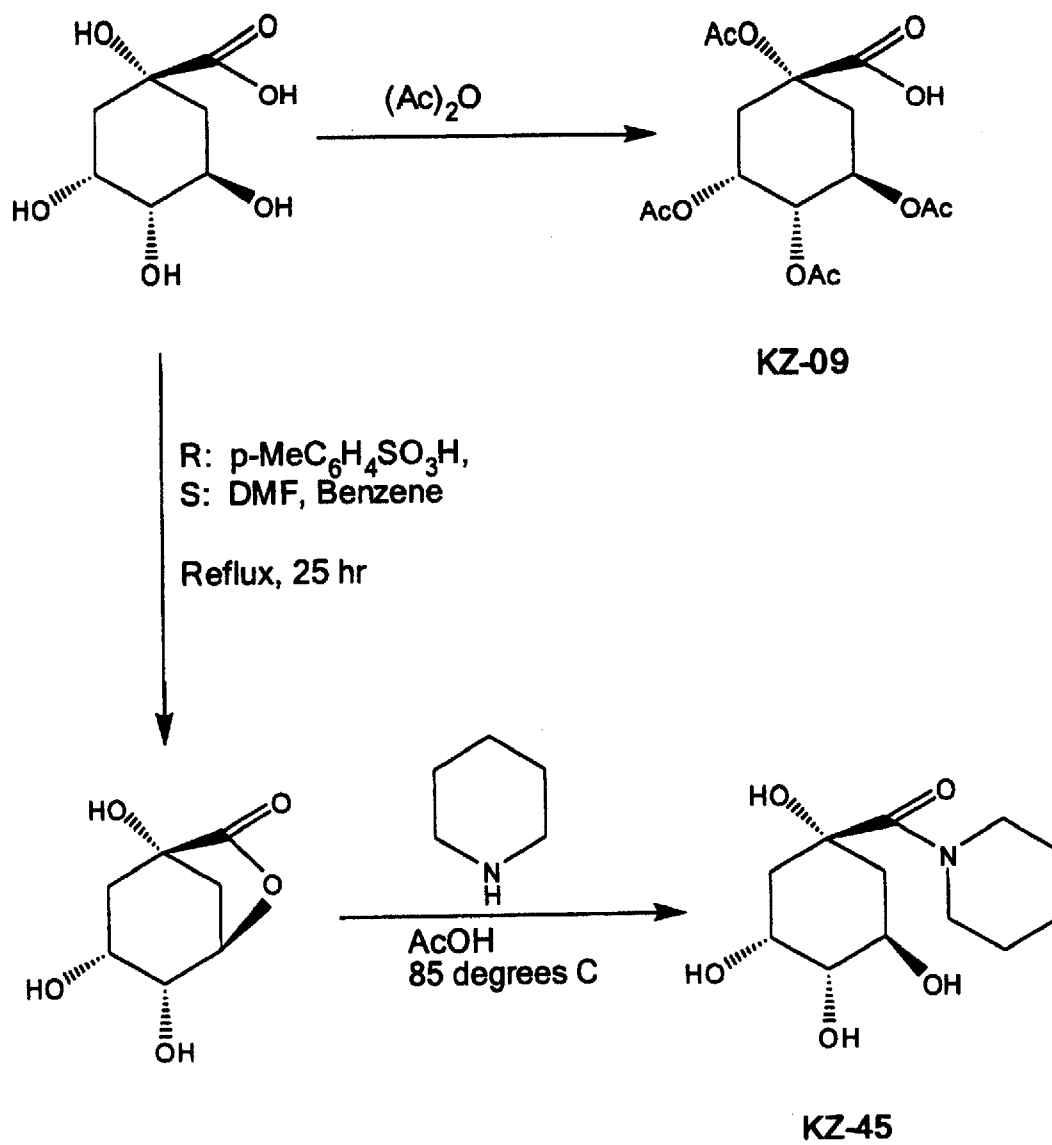
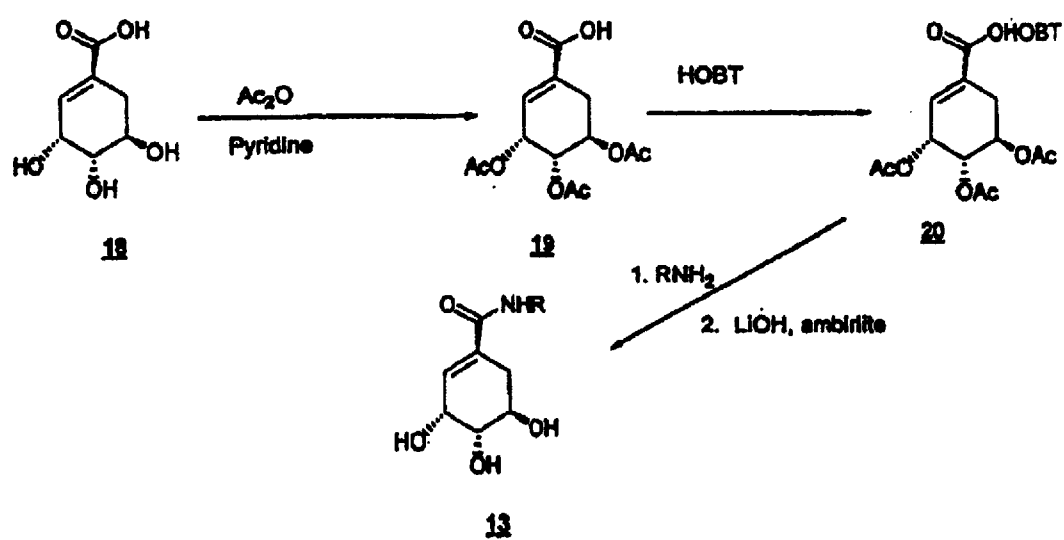


Fig. 4



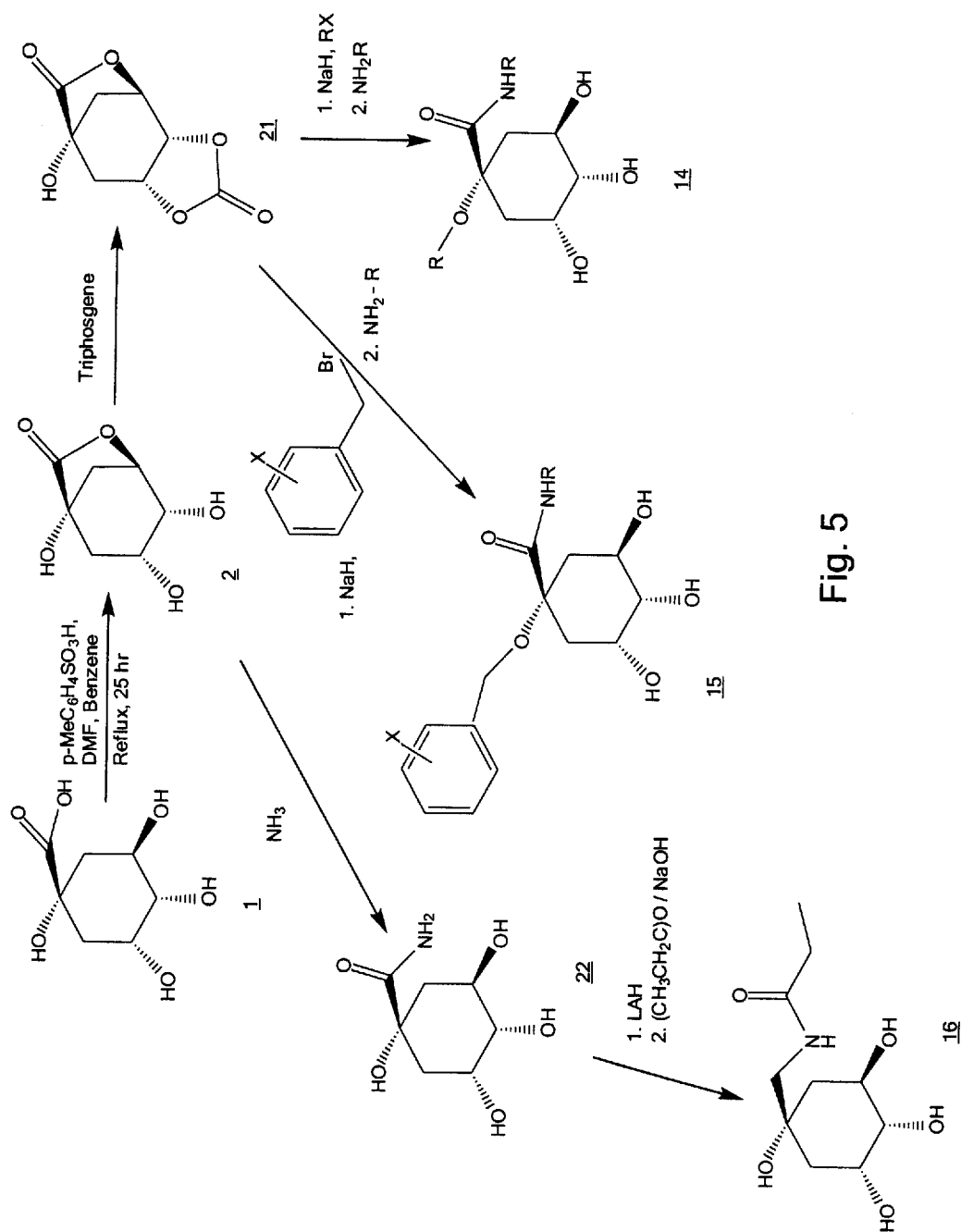


Fig. 5

Fig. 6

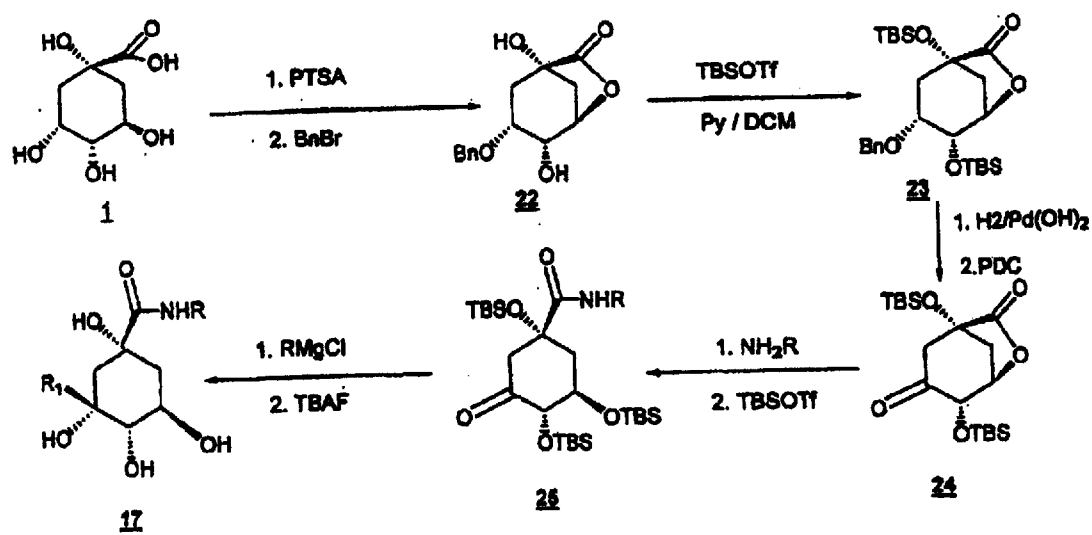


Fig. 7

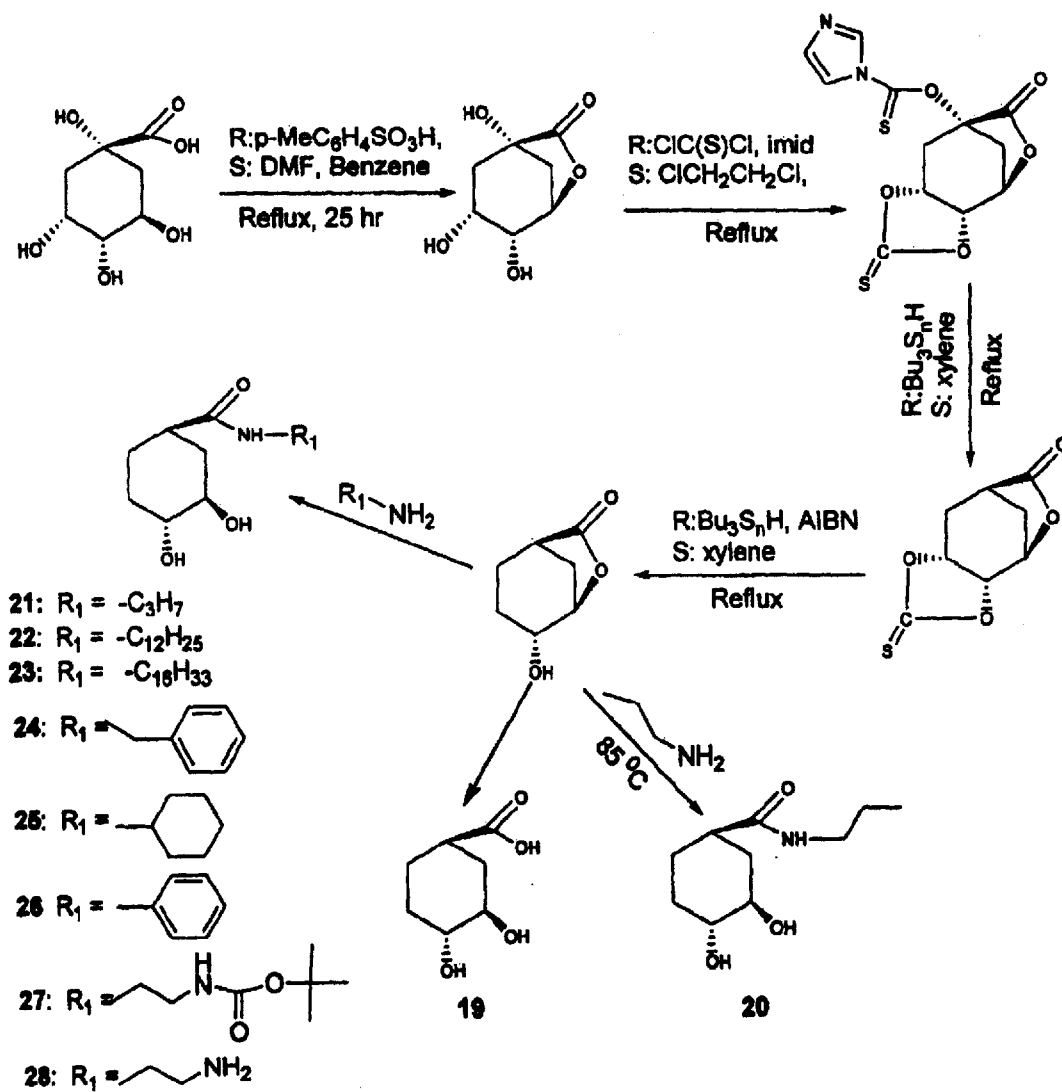


Fig. 8

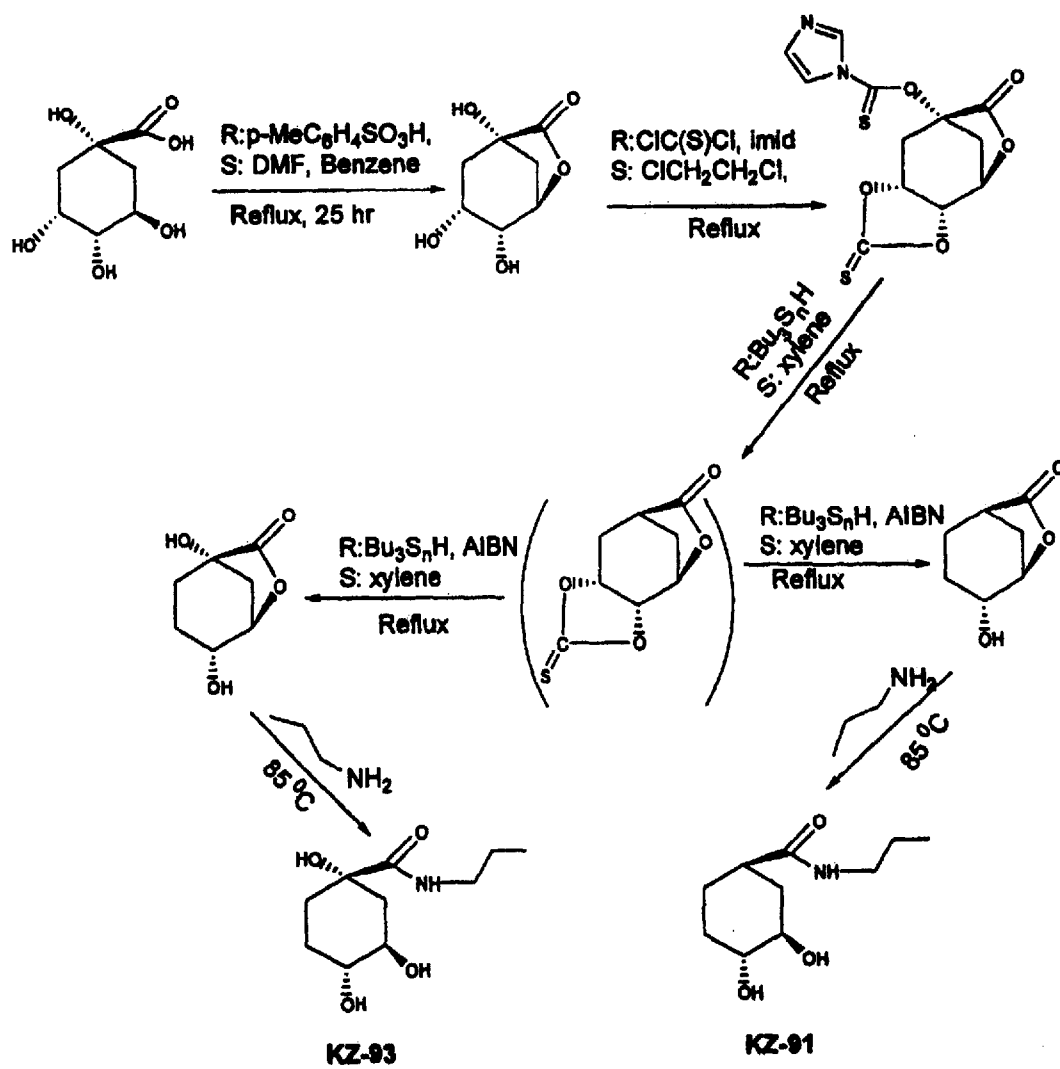


Fig. 9

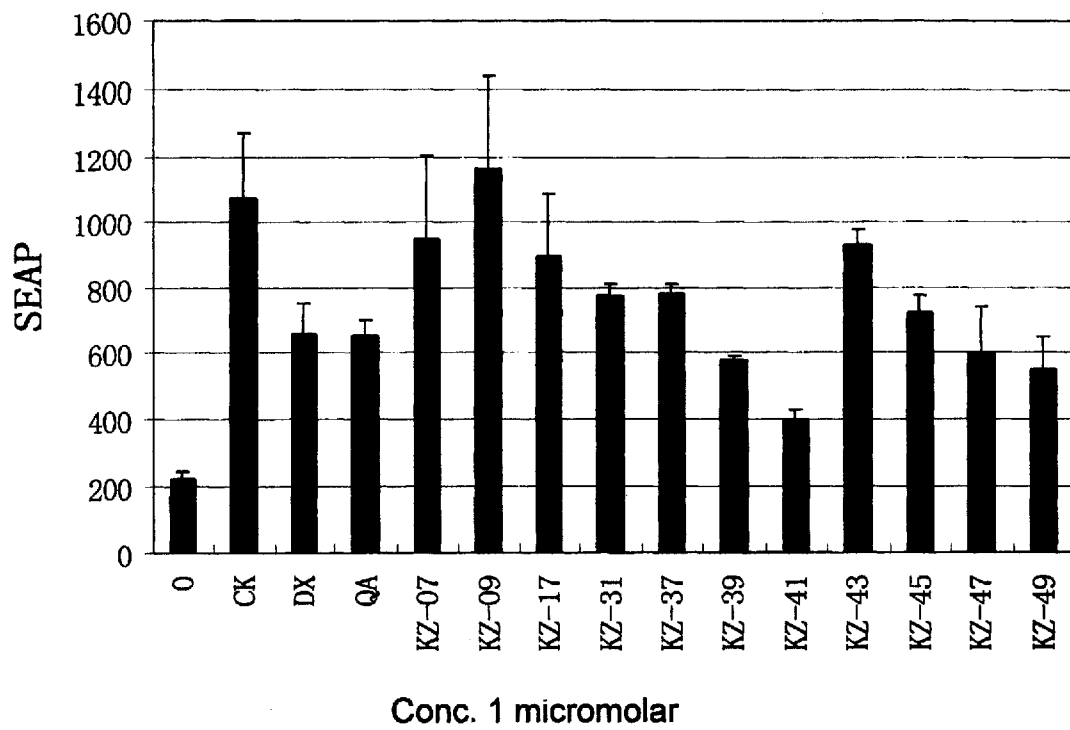
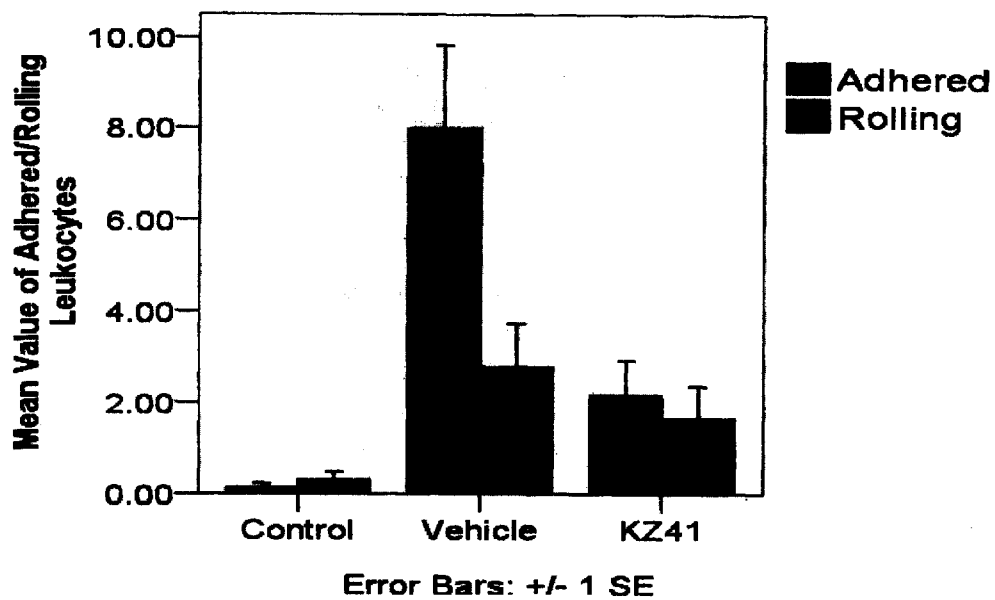
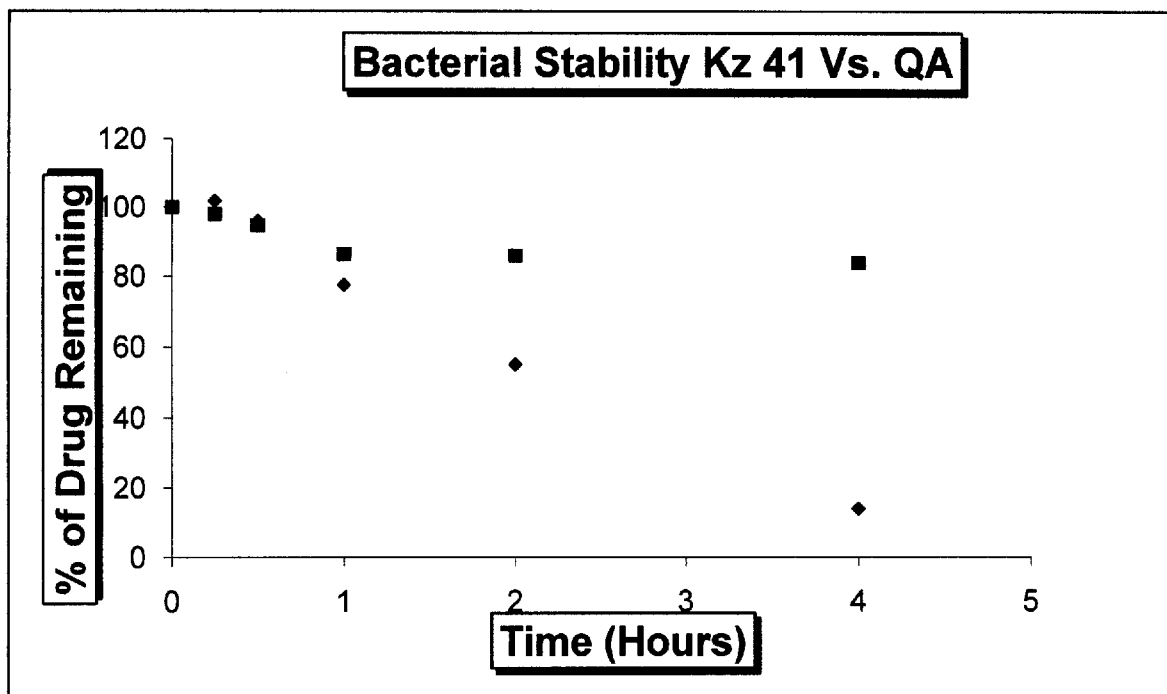


Fig. 10



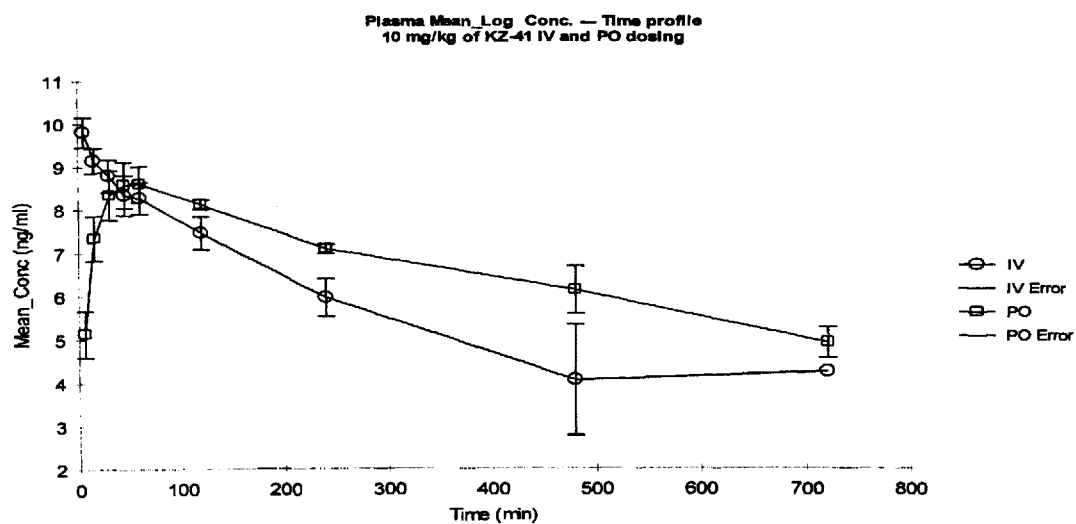
11/12

Fig. 11



■ - KZ-41
◆ - Quinic Acid

Fig. 12



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/83068

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 37/10 (2009.01)

USPC - 514/570,829

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)

USPC: 514/570

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

USPC: 514/570,829

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

Pub West: (PGPB,USPT,EPAB,JPAB), Google patent, USPTO

Search Terms: Quinic acid, derivatives, analogs, amides, anti-inflammatory, hydroxyl, shikimic acid, inhibition, leucocyte, NF-kB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2,918,477 A (Alberti et al.) 22 December 1959 (22.12.1959) (col1 ln 15) (col 2 ln 10,40-55)	1 ----- 2-9
Y	US 5,656,721 A (Albert et al.) 12 August 1997 (12.08.1997) (col 6 ln 29-30) (col 8 ln22) (col10 ln 58-59) (col 11 ln 10) (col 12 ln 40)	2-9
Y	US 2005/0176825 A1 (Pero) 11 August 2005 (11.08.2005) para [0017] [0031]	8-9

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

18 January 2009 (18.01.2009)

Date of mailing of the international search report

29 JAN 2009

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. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

JB