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(54) Title: N-ACYLETHANOLAMIDE DERIVATIVES AND USES THEREOF

(57) Abstract: The present disclosure provides certain N-Acylethanolamide derivatives, and uses relating thereto.

N-ACYLETHANOLAMIDE DERIVATIVES AND USES THEREOF

Cross Reference to Related Applications

[1] This application claims priority to U.S. provisional application number 62/517,344, filed June 9, 2017, and U.S. provisional application number 62/407,796, filed October 13, 2016, the entirety of each of which is hereby incorporated by reference.

Background

[2] N-acylethanolamides are uniquely useful and valuable compounds. Studies have shown that N-acylethanolamides can be effective in the treatment of a variety of diseases, disorders, and conditions.

Summary

[3] N-acylethanolamides are widely recognized as potentially useful therapeutic compounds, and have been extensively studied in particular for their analgesic and/or anti-inflammatory effects.

[4] However, the present disclosure appreciates that N-acylethanolamide compounds often suffer from one or more poor pharmacological properties, for example resulting in limited bioavailability when administered by a particular route (e.g., oral) and/or low exposure to a particular target site of interest (e.g., bowel or, more specifically, lower bowel). In many cases, these poor properties can limit dosage, limit exposure or delivery to a particular site of interest, limit susceptibility to effective delivery by a particular route, etc and/or may therefore or otherwise necessitate alternative modes of administration.

[5] The present disclosure further appreciates that some or all of the poor pharmacological properties encountered with many N-acylethanolamide compounds may be relieved or obviated by provision of an appropriate prodrug. As is known in the art, a prodrug is in most cases a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation within the body in order to release the active drug. The present disclosure encompasses the insight that one source of pharmacological problems with certain N-acylethanolamide compounds may be failure of the compound to reach a relevant target site in sufficient level to achieve its desired biological effect. Alternatively or additionally, an N-acylethanolamide compound may interact with non-target sites, and/or may display undesirable side effects. The present disclosure provides certain derivative (e.g., prodrug) forms of N-acylethanolamide compounds that may relieve or obviate such problem(s) and/or source(s).

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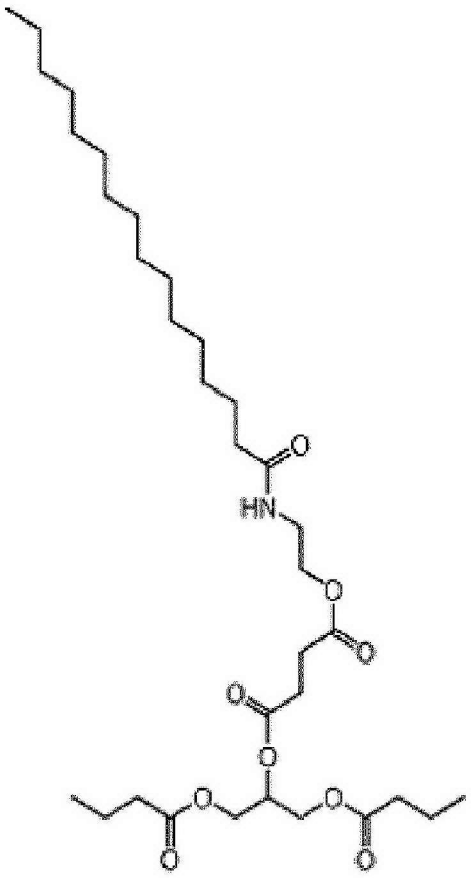
[5a] In a first aspect of the invention, there is provided a compound which is:



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[5b] In a second aspect of the invention, there is provided a pharmaceutical composition comprising the compound according to a first aspect of the invention and a pharmaceutically acceptable excipient

[5c] In a third aspect of the invention, there is provided a method of treatment of pain, the method comprising administering to a subject in need thereof the compound according to the first aspect of the invention or the pharmaceutical composition according to the second aspect of the invention.

[5d] In a fourth aspect of the invention, there is provided a method of treatment of inflammatory pain, the method comprising administering to a subject in need thereof the compound according to the first aspect of the invention or the pharmaceutical composition according to the second aspect of the invention.

[5e] In a fifth aspect of the invention, there is provided a method of treatment of neuropathic pain, the method comprising administering to a subject in need thereof the compound according to the first

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aspect of the invention or the pharmaceutical composition according to the second aspect of the invention

[5f] In a sixth aspect of the invention, there is provided use of the compound according to the first aspect of the invention in the manufacture of a medicament for the treatment of pain.

[5g] In a seventh aspect of the invention, there is provided use of the compound according to the first aspect of the invention in the manufacture of a medicament for the treatment of inflammatory pain.

[5h] In an eighth aspect of the invention, there is provided use of the compound according to the first aspect of the invention in the manufacture of a medicament for the treatment of neuropathic pain.

[6] In some embodiments, the present disclosure provides derivatives of N-acylethanolamides that exhibit improved pharmacological properties and/or display biological activity that is reasonably comparable to (or, in some cases may be better than) that of its parent N-acylethanolamide (or another appropriate reference N-acylethanolamide). In some embodiments, a provided N-acylethanolamide derivative compound may exhibit one or more properties such as, for example, increased oral bioavailability, increased cell permeability, increased water solubility, reduced first-pass effect, increased stability, active transport by intestinal transporters, avoidance of efflux transporters, and/or combinations thereof when compared to a reference N-acylethanolamide such as, for example, its parent N-acylethanolamide.

[7] In some embodiments, a compound for use in accordance with the present disclosure is one wherein an N-acylethanolamide is conjugated to a moiety selected from the group consisting of phosphate, butyric acid, glycerol, succinate, caprylic acid, gluconoic acid, eicosapentaeonoic acid, linoleic acid, succinate, and sucrose moieties, and combinations thereof. In some embodiments, an N-acylethanolamide is conjugated to one or more such moieties through use of a linker moiety. In some embodiments, an N-acylethanolamide is conjugated to two or more such moieties. In some embodiments, an N-acylethanolamide is conjugated to one, two, or three such moieties.

[8] In some embodiments, a provided compound has a chemical structure represented by formula **I-a**:

$$X_1\text{-}X_2$$

or a pharmaceutically acceptable salt thereof; wherein

X_1 is an N-acylethanolamide; and

X_2 is a moiety conjugated to the N-acylethanolamide.

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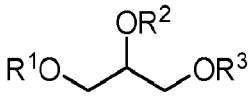
[9] In some embodiments, X_1 is selected from the group consisting of N-palmitoylethanolamide, N-oleoylethanolamide, and N-arachidonoy lethanolamide; in some particular embodiments, X_1 is N-palmitoylethanolamide. In some embodiments, X_2 comprises a moiety selected from the group consisting of phosphate, butyric acid, glycerol, succinate, caprylic acid, gluconoic acid, eicosapentaeonoic acid, linoleic acid, succinate, and sucrose moieties.

[10] In some embodiments, a provided compound has a chemical structure represented by formula **I**:



I

or a pharmaceutically acceptable salt thereof, wherein:

each R^1, R^2, or R^3 is independently hydrogen or $-T-R^4$, wherein at least one of R^1, R^2, or R^3 is $-T-R^4$;

$-T-$ represents a bivalent moiety; and

R^4 is an optionally substituted group selected from the group consisting of C_{1-40} aliphatic, -C(O)R, and X_1; wherein

R is selected from the group consisting of hydrogen and optionally substituted C_{1-20} aliphatic; and

X_1 is as defined above.

[11] In some embodiments, a provided compound has a chemical structure represented by formula **I-b**:

$$X_1\text{-}X_3$$

I-b

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is as defined above;

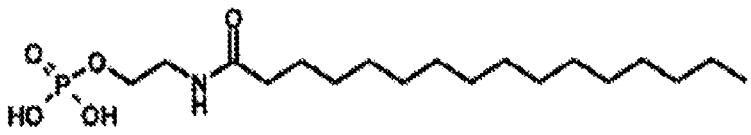
X_3 is an optionally substituted group selected from the group consisting of $-(CH_2)_m-P(O)(OR)_2$, C_{1-40} aliphatic, $-T-X_4$; further wherein

m is an integer select from the group consisting of 0-10;

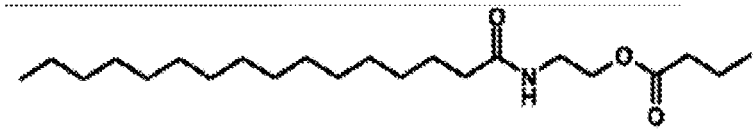
-T- is as defined above;

X_4 is a saccharide moiety, in some particular embodiments, X_4 is a disaccharide, for example, sucrose.

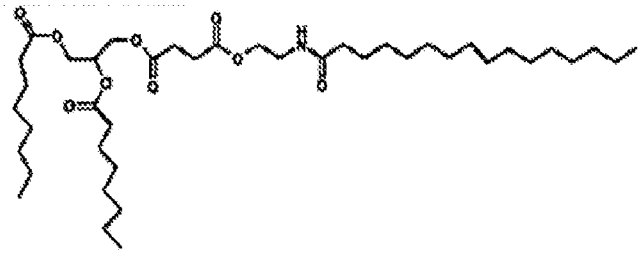
[12] In some embodiments, the present disclosure provides compounds such as, for example:



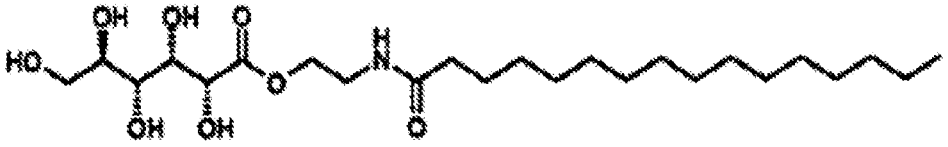
I-1



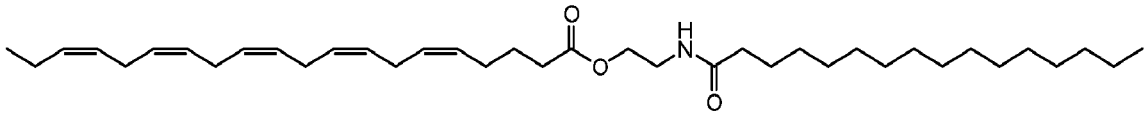
I-2



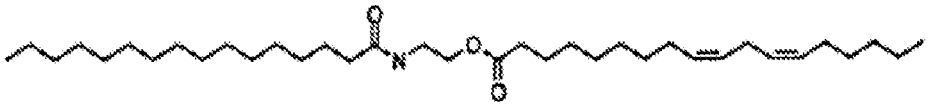
I-3



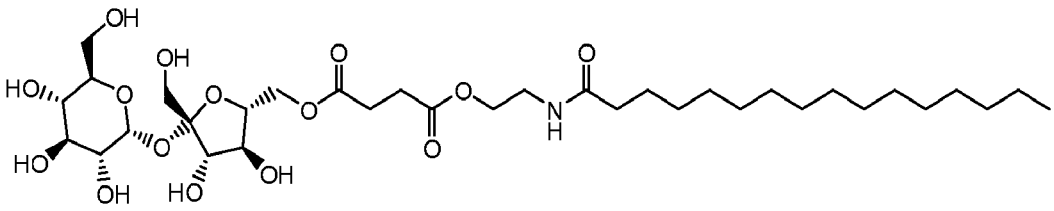
I-4



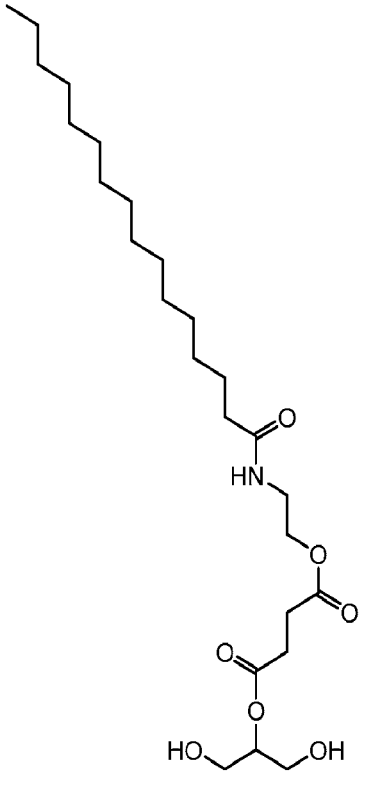
I-5



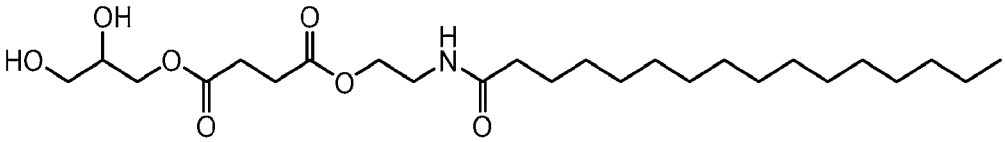
I-6



I-7



I-8 and



I-9

[13] In some embodiments, the present disclosure provides N-acylethanolamide prodrugs. In some embodiments, a provided prodrug may be characterized by one or more desirable physical properties, which may, for example, be assessed relative to an appropriate reference N-acylethanolamide (e.g., to the parent N-acylethanolamide of the provided prodrug); in some embodiments, such desirable physical properties may be or include, for example, enhanced aqueous solubility (which may facilitate, for example, formulation into a pharmaceutical composition, particularly for oral or parenteral administration), enhanced absorption from the digestive tract, enhanced stability under relevant storage conditions, etc.

[14] In some embodiments, a parent N-acylethanolamide compound is one that is characterized by limited aqueous solubility and/or limited oral bioavailability. For example, in some embodiments, a parent N-acylethanolamide compound is characterized by aqueous solubility below a relevant threshold and/or oral bioavailability below a relevant threshold.

[15] In some embodiments, a parent N-acylethanolamide compound is characterized by one or more pharmacological properties that impact its amenability to pharmaceutical formulation, for

example, so that challenges are encountered preparing pharmaceutical compositions containing a desirable unit dose amount and/or a desirable concentration of the compound. In some embodiments, the present disclosure provides derivatives of such parent N-acylethanolamide compounds; in some embodiments, derivatives provided by the present disclosure act as prodrugs of the relevant parent compounds in that, when administered to a subject (e.g., in the context of a pharmaceutical composition), the provided derivatives deliver the parent compound and/or an active metabolite thereof. In some embodiments, as described herein, provided N-acylethanolamide derivative compounds comprise one or more moieties modifying or otherwise linked to a parent N-acylethanolamide compound.

[16] In some embodiments, provided N-acylethanolamide derivative compounds are amenable to pharmaceutical formulations at unit doses and/or concentrations that are higher than those achieved with the relevant parent N-acylethanolamide compounds under comparable conditions.

[17] Among other things, the present disclosure provides compounds of formula **I** and pharmaceutically acceptable salts thereof, the preparation of the above-mentioned compounds, medicaments containing them and their manufacture, as well as technologies for identifying and/or characterizing useful such compounds, and use of provided compounds, for example in the treatment of one or more diseases, disorders, or conditions, for example as described herein.

Brief Description of the Drawings

[18] FIG. 1 is a flow chart indicating pain classification and representative indications. Distinguishing between different types of pain is critical for proper treatment. Pain can be classified by its duration into acute and chronic pain. Chronic pain is further classified by the source of pain production: nociceptive pain, which is transmitted by nociceptors from the site of injury or tissue damage (for example, inflamed joints in arthritis); neuropathic pain, which is initiated or caused by a primary lesion or dysfunction in the nervous system (further subdivided into central and peripheral, involving the central and peripheral nervous systems, respectively); visceral pain, which involves the internal organs; mixed pain, which is of mixed origin. Prevalence for selected chronic pain conditions in the United States is indicates. Sources: Centers for Disease Control and Prevention, National Center for Health Statistics, Arthritis Foundation, National Institutes of Diabetes and Digestive Kidney Diseases, American Pain Society, The American Pain Foundation.

[19] FIG. 2A is a bar graph illustrating visual analog 10-point scale measuring use of PEA to manage pain.

[20] FIG, 2B is a bar graph illustrating quality of life over a 24-point scale for paitients administered PEA to manage pain.

[21] FIG. 2C is a scatter plot measuring changes in pain intensity in patients treated with PEA and control groups at different observation times. Values are expressed as mean ± SEM.

[22] FIG. 2D is a bar graph measuring a visual analog of pelvic pain among Groups A, B, and C.

[23] FIG. 2E is a bar graph measuring a visual analog of dyspareunia among Groups A, B, and C.

[24] FIG. 2F is a bar graph measuring a visual analog of dysmenorrhea among Groups A, B, and C.

[25] FIG. 2G is a bar graph illustrating percentage regularization of status following treatment with butyric acid and insulin. In particular, 15 IBS-DP patients vs. 4 IBS-CP patients: 68% vs 14% and 71% vs 16% respectively in the intent to treat (ITT) and per-protocol (PP) groups ($p < 0.005$). *Statistically significant ($p < 0.005$).

[26] FIG. 2H is a scatter plot illustrating the endoscopic score (n=10) in patients with distal UC before and after treatment with sodium butyrate (black dot) or sodium chloride (white dot; control) enemas. Vertical bars indicate 1 SEM; *significant differences (endoscopic score, $P < 0.01$; histological grading, $P < 0.02$; upper-crypt labeling, $P < 0.03$; Wilcoxon test).

[27] FIG. 2I is a scatter plot illustrating the histological grading (n=10) in patients with distal UC before and after treatment with sodium butyrate (black dot) or sodium chloride (white dot; control) enemas. Vertical bars indicate 1 SEM; *significant differences (endoscopic score, $P < 0.01$; histological grading, $P < 0.02$; upper-crypt labeling, $P < 0.03$; Wilcoxon test).

[28] FIG. 2J is a scatter plot illustrating the upper-crypt labeling frequency (n=6) in patients with distal UC before and after treatment with sodium butyrate (black dot) or sodium chloride (white dot; control) enemas. Vertical bars indicate 1 SEM; *significant differences (endoscopic score, $P < 0.01$; histological grading, $P < 0.02$; upper-crypt labeling, $P < 0.03$; Wilcoxon test).

[29] FIG. 2K is a bar graph illustrating inhibitory effect of PEA (1–10 $mg \cdot kg^{-1}$, i.p.) on upper gastrointestinal transit in control mice. Transit was measured 28 days after OM or vehicle (30% ethanol) administration. Results (the means ± SEM of 9–10 mice for each experimental group) are expressed as a percentage of upper gastrointestinal transit. *$P < 0.05$, **$P < 0.01$,

significantly different from vehicle. The term "vehicle" refers to the vehicle used to dissolve PEA.

[30] FIG. 2L is a bar graph illustrating inhibitory effect of PEA (1–10 $mg \cdot kg^{-1}$, i.p.) on upper gastrointestinal transit in mice treated with OM (oil of mustard). Transit was measured 28 days after OM or vehicle (30% ethanol) administration. Results (the means ± SEM of 9–10 mice for each experimental group) are expressed as a percentage of upper gastrointestinal transit. $*P < 0.05$, $**P < 0.01$, significantly different from vehicle. Note that in (B) the term "vehicle" refers to the vehicle used to dissolve OM. the % transit of a vehicle or PEA in OM-treated mice.

[31] FIG. 2M is a bar graph illustrating 2,4,6-dinitrobenzenesulfonic acid-induced ("DNBS-induced") colitis in mice. Changes in body weight from control and DNBS-treated mice in the presence or absence of intraperitoneal (i.p.) PEA. Mice were weighed before DNBS (or vehicle) administration and immediately before killing. Tissues were analysed 3 days after vehicle or DNBS administration. PEA (0.1–10 $mg \cdot kg^{-1}$) was administered once a day for three consecutive days starting 24 h after the inflammatory insult (therapeutic protocol). Bars are mean ± SEM of 12–15 mice for each experimental group. $\#P < 0.001$ versus control (i.e. mice without intestinal inflammation). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ versus DNBS alone.

[32] FIG. 2N is a bar graph illustrating DNBS-induced colitis in mice. Changes in body weight from control and DNBS-treated mice in the presence or absence of orally administered (p.o.) PEA. Mice were weighed before DNBS (or vehicle) administration and immediately before killing. Tissues were analysed 3 days after vehicle or DNBS administration. PEA (0.1–10 $mg \cdot kg^{-1}$) was administered once a day for three consecutive days starting 24 h after the inflammatory insult (therapeutic protocol). Bars are mean ± SEM of 12–15 mice for each experimental group. $\#P < 0.001$ versus control (i.e. mice without intestinal inflammation). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ versus DNBS alone.

[33] FIG. 2O is a bar graph illustrating DNBS-induced colitis in mice. Changes in colon weight/colon length ratio from control and DNBS-treated mice in the presence or absence of i.p. PEA. Mice were weighed before DNBS (or vehicle) administration and immediately before killing. Tissues were analysed 3 days after vehicle or DNBS administration. PEA (0.1–10 $mg \cdot kg^{-1}$) was administered once a day for three consecutive days starting 24 h after the inflammatory insult (therapeutic protocol). Bars are mean ± SEM of 12–15 mice for each experimental group. $\#P < 0.001$ versus control (i.e. mice without intestinal inflammation). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ versus DNBS alone.

[34] FIG. 2P is a bar graph illustrating DNBS-induced colitis in mice. Changes in colon weight/colon length ratio from control and DNBS-treated mice in the presence or absence of p.o. PEA. Mice were weighed before DNBS (or vehicle) administration and immediately before killing. Tissues were analysed 3 days after vehicle or DNBS administration. PEA (0.1–10 mg·kg $^{-1}$) was administered once a day for three consecutive days starting 24 h after the inflammatory insult (therapeutic protocol). Bars are mean ± SEM of 12–15 mice for each experimental group. #$P < 0.001$ versus control (i.e. mice without intestinal inflammation). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ versus DNBS alone.

[35] FIG. 2Q is a scatter plot measuring the % inhibition (as measured by colon weight: length ratio) versus the amount of PEA administered for two populations (one via oral administration, the other intraperitoneal).

[36] FIG. 3A is a blank plasma chromatogram of PEA. The Y-axis measures intensity (cps) on a scale from 0 to 365; the X-axs measured time (min) on a scale from 0 to 1.0.

[37] FIG. 3B is a representative chromatogram of LLOQ for PEA, 2.5 ng/mL. The Y-axis measures intensity (cps) on a scale from 0 to 870; the X-axs measures time (min) on a scale from 0 to 1.0.

[38] FIG. 3C is a representative chromatogram of the ULOQ for PEA, 1000 ng/mL. The Y-axis measures intensity (cps) on a scale from 0 to $1.8x10^5$; the X-axs measures time (min) on a scale from 0 to 1.0.

[39] FIG. 3D is a representative calibration curve for PEA.

[40] FIG. 3E is a blank plasma chromatogram of PEA-prodrug **I-9**. The Y-axis measures intensity (cps) on a scale from 0 to 1000; the X-axs measures time (min) on a scale from 0 to 1.0.

[41] FIG. 3F is a representative chromatogram of LLOQ for PEA-prodrug **I-9**, 0.5 ng/mL. The Y-axis measures intensity (cps) on a scale from 0 to 1120; the X-axs measures time (min) on a scale from 0 to 1.0.

[42] FIG. 3G is a representative chromatogram for ULOQ for PEA prodrug **I-9**, 1000 ng/mL. The Y-axis measures intensity (cps) on a scale from 0 to $9x10^6$; the X-axs measures time (min) on a scale from 0 to 1.0.

[43] FIG. 3H is a representative calibration curve for PEA-prodrug **I-9**.

[44] FIG. 4A is a scatter plot of individual plasma concentrations of PEA after intravenous administration of PEA in male Sprague-Dawley rats at 1 mg/kg (Group 1).

[45] FIG. 4B is a scatter plot of average plasma concentrations of PEA after intravenous administration of PEA in male Sprague-Dawley rats at 1 mg/kg (Group 1).

[46] FIG. 5A is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-9** at 16 mg/kg in male Sprague Dawley rats.

[47] FIG. 5B is a scatter plot of average plasma concentrations of PEA after oral administration of **I-9** at 16 mg/kg in male Sprague-Dawley rats.

[48] FIG. 5C is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-6** at 19 mg/kg in male Sprague-Dawley rats (Group 2).

[49] FIG. 5D is a scatter plot of average plasma concentrations of PEA after oral administration of **I-6.**

[50] FIG. 5E is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-5** at 19.7 mg/kg in male Sprague-Dawley rats (Group 3).

[51] FIG. 5F is a scatter plot of average plasma concentrations of PEA after oral administration of **I-5** at 19.7 mg/kg in male Sprague-Dawley rats (Group 3).

[52] FIG. 5G is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-3** (PEA-Succinate-Glycerol-Di-Caprylic) at 24.5 mg/kg in male Sprague-Dawley rats (Group 4).

[53] FIG. 5H is a scatter plot of average plasma concentrations of PEA after oral administration of **I-3** (PEA-Succinate-Glycerol-Di-Caprylic) at 24.5 mg/kg in male Sprague-Dawley rats (Group 4).

[54] FIG. 5I is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-2** (PEA-BA) at 12/5 mg/kg in male Sprague-Dawley rats (Group 5).

[55] FIG. 5J is a scatter plot of average plasma concentrations of PEA after oral administration of **I-2** (PEA-BA) at 12.5 mg/kg in male Sprague-Dawley rats (Group 5).

[56] FIG. 5K is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-11** at 20.7 mg/kg in Male Sprague-Dawley Rats (Group 6).

[57] FIG. 5L is a scatter plot of average plasma concentrations of PEA after oral administration of **I-11** at 20.7 mg/kg in male Sprague-Dawley rats (Group 6).

[58] FIG. 5M is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-7** at 24.5 mg/kg in male Sprague-Dawley rats (Group 7).

[59] FIG. 5N is a scatter plot of average plasma concentrations of PEA after oral administration of **I-7** at 24.5 mg/kg in male Sprague-Dawley rats (Group 7).

[60] FIG. 6 is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-13** (in 20% (Solutol HS15:NMP 1:1) 10% PEG400, 70% H_2O) at 24.3 mg/kg in male Sprague-Dawley rats.

[61] FIG. 7A is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-12** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in male Sprague-Dawley rats (Group 1).

[62] FIG. 7B is a scatter plot of average plasma concentrations of PEA after oral administration of **I-12** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in male Sprague-Dawley rats (Group 1).

[63] FIG. 7C is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-12** (in 0.5% Methyl Cellulose in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in male Sprague-Dawley rats (Group 2).

[64] FIG. 7D is a scatter plot of average plasma concentrations of PEA after oral administration of **I-12** (in 0.5% Methyl Cellulose in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in male Sprague-Dawley rats (Group 2).

[65] FIG. 8A is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-15** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats (Group 1).

[66] FIG. 8B is a scatter plot of average plasma concentrations of PEA after oral administration of **I-15** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats (Group 1).

[67] FIG. 8C is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-14** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats (Group 1).

[68] FIG. 8D is a scatter plot of average plasma concentrations of PEA after oral administration of **I-14** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats (Group 1).

[69] FIG. 9A is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-8**.

[70] FIG. 9B is a scatter plot of average plasma concentrations of PEA after oral administration of **I-8**.

[71] FIG. 9C is a scatter plot of individual plasma concentrations of PEA after oral administration of **I-16**.

[72] FIG. 9D is a scatter plot of average plasma concentrations of PEA after oral administration of **I-16**.

[73] FIG. 10A is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 4 mg/kg in male Sprague-Dawley rats.

[74] FIG. 10B is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 4 mg/kg in male Sprague-Dawley rats.

[75] FIG. 10C is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 8 mg/kg in male Sprague-Dawley rats.

[76] FIG. 10D is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 8 mg/kg in male Sprague-Dawley rats.

[77] FIG. 10E is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 16 mg/kg in male Sprague-Dawley rats.

[78] FIG. 10F is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 16 mg/kg in male Sprague-Dawley rats.

[79] FIG. 10G is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 5.2 mg/kg in male Sprague-Dawley rats.

[80] FIG. 10H is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 5.2 mg/kg in male Sprague-Dawley rats.

[81] FIG. 10I is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 10.35 mg/kg in male Sprague-Dawley rats.

[82] FIG. 10J is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 10.35 mg/kg in male Sprague-Dawley rats.

[83] FIG. 10K is a scatter plot of individual plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats.

[84] FIG. 10L is a scatter plot of average plasma concentrations (ng/mL) and pharmacokinetic parameters for PEA after oral administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in male Sprague-Dawley rats.

[85] FIG. 11 is a bar graph illustrating the paw withdrawal latency (in seconds) as a function of amount of I-16 provided to an animal subject. The animal received either no I-16 (i.e., only the vehicle); 5 mg/kg equivalents of PEA (equivalent to 10.25 mg/kg of **I-16**); or 10 mg/kg equivalents of PEA (equivalent to 20.50 mg/kg of **I-16**).

[86] FIG. 12A is a timeline illustrating the dosing schedule of rats administered I-16 to evaluate analgesic effects in rat Chronic Constriction Injury (CCI) model.

[87] FIG. 12B is a bar graph illustrating mechanical allodynia in rats as a function of analgesic administered (vehicle; gabapentin; or **I-16**).

Definitions

A. *Chemical Definitions*

[88] As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic

Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[89] *Aliphatic*: As used herein, "aliphatic" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon, bicyclic hydrocarbon, or polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-100 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-20 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1, 2, 3, or 4 aliphatic carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof.

[90] *Alkyl*: As used herein, the term "alkyl" is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In some embodiments, alkyl has 1-100 carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 carbon atoms in its backbone (e.g., C_1-C_{20} for straight chain, C_2-C_{20} for branched chain), and alternatively, about 1-10. In some embodiments, a cycloalkyl ring has from about 3-10 carbon atoms in their ring structure where such rings are monocyclic or bicyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1-4 carbon atoms (e.g., C_1-C_4 for straight chain lower alkyls).

[91] *Alkenyl*: As used herein, the term "alkenyl" refers to an alkyl group, as defined herein, having one or more double bonds.

[92] *Alkynyl*: As used herein, the term "alkynyl" refers to an alkyl group, as defined herein, having one or more triple bonds.

[93] *Protecting Group*: The phrase "protecting group," as used herein, refers to temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl

ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. A "Si protecting group" is a protecting group comprising a Si atom, such as Si-trialkyl (e.g., trimethylsilyl, tributylsilyl, t-butyldimethylsilyl), Si-triaryl, Si-alkyl-diphenyl (e.g., t-butyldiphenylsilyl), or Si-aryl-dialkyl (e.g., Si-phenyldialkyl). Generally, a Si protecting group is attached to an oxygen atom. The field of protecting group chemistry has been reviewed (Greene, T. W.; Wuts, P. G. M. Protective Groups in Organic Synthesis, 2nd ed.; Wiley: New York, 1991). Such protecting groups (and associated protected moieties) are described in detail below.

[94] Protected hydroxyl groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Examples of suitably protected hydroxyl groups further include, but are not limited to, esters, carbonates, sulfonates, allyl ethers, ethers, silyl ethers, alkyl ethers, arylalkyl ethers, and alkoxyalkyl ethers. Examples of suitable esters include formates, acetates, proprionates, pentanoates, crotonates, and benzoates. Specific examples of suitable esters include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate (trimethylacetate), crotonate, 4-methoxy-crotonate, benzoate, p-benzylbenzoate, 2,4,6-trimethylbenzoate. Examples of suitable carbonates include 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl carbonate. Examples of suitable silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl ether, and other trialkylsilyl ethers. Examples of suitable alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, and allyl ether, or derivatives thereof. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyran-2-yl ether. Examples of suitable arylalkyl ethers include benzyl, p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl ethers.

[95] Protected amines are well known in the art and include those described in detail in Greene (1999). Suitable mono-protected amines further include, but are not limited to, aralkylamines, carbamates, allyl amines, amides, and the like. Examples of suitable mono-protected amino moieties include t-butyloxycarbonylamino (–NHBOC), ethyloxycarbonylamino, methyloxycarbonylamino, trichloroethyloxycarbonylamino, allyloxycarbonylamino (–NHAlloc),

benzyloxocarbonylamino (–NHCBZ), allylamino, benzylamino (–NHBn), fluorenylmethylcarbonyl (–NHFmoc), formamido, acetamido, chloroacetamido, dichloroacetamido, trichloroacetamido, phenylacetamido, trifluoroacetamido, benzamido, t-butyldiphenylsilyl, and the like. Suitable di-protected amines include amines that are substituted with two substituents independently selected from those described above as mono-protected amines, and further include cyclic imides, such as phthalimide, maleimide, succinimide, and the like. Suitable di-protected amines also include pyrroles and the like, 2,2,5,5-tetramethyl-[1,2,5]azadisilolidine and the like, and azide.

[96] Protected aldehydes are well known in the art and include those described in detail in Greene (1999). Suitable protected aldehydes further include, but are not limited to, acyclic acetals, cyclic acetals, hydrazones, imines, and the like. Examples of such groups include dimethyl acetal, diethyl acetal, diisopropyl acetal, dibenzyl acetal, bis(2-nitrobenzyl) acetal, 1,3-dioxanes, 1,3-dioxolanes, semicarbazones, and derivatives thereof.

[97] Protected carboxylic acids are well known in the art and include those described in detail in Greene (1999). Suitable protected carboxylic acids further include, but are not limited to, optionally substituted C_{1-6} aliphatic esters, optionally substituted aryl esters, silyl esters, activated esters, amides, hydrazides, and the like. Examples of such ester groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, and phenyl ester, wherein each group is optionally substituted. Additional suitable protected carboxylic acids include oxazolines and ortho esters.

[98] Protected thiols are well known in the art and include those described in detail in Greene (1999). Suitable protected thiols further include, but are not limited to, disulfides, thioethers, silyl thioethers, thioesters, thiocarbonates, and thiocarbamates, and the like. Examples of such groups include, but are not limited to, alkyl thioethers, benzyl and substituted benzyl thioethers, triphenylmethyl thioethers, and trichloroethoxycarbonyl thioester, to name but a few.

[99] *Substitution*: As described herein, compounds of the disclosure may contain optionally substituted and/or substituted moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this disclosure are preferably those that

result in the formation of stable or chemically feasible compounds. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[100] Suitable monovalent substituents include halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^o$, $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ-$, $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$, $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; $-SiR^\circ_3$; $-OSiR^\circ_3$; $-(C_{1-4}$ straight or branched alkylene$)O-N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene$)C(O)O-N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$-(5-6 membered heteroaryl ring), or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[101] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^\bullet$, $-(haloR^\bullet)$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^\bullet$, $-(CH_2)_{0-2}CH(OR^\bullet)_2$; $-O(haloR^\bullet)$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^\bullet$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^\bullet$, $-(CH_2)_{0-2}SR^\bullet$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^\bullet$, $-(CH_2)_{0-2}NR^\bullet_2$, $-NO_2$, $-SiR^\bullet_3$, $-OSiR^\bullet_3$, $-C(O)SR^\bullet$, $-(C_{1-4}$ straight or branched alkylene$)C(O)OR^\bullet$, or $-SSR^\bullet$ wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-}

$_4$ aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[102] Suitable divalent substituents include the following: =O, =S, $=NNR^*_2$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[103] Suitable substituents on the aliphatic group of R^* include halogen, $-R^\bullet$, -(halo$R^\bullet$), –OH, $-OR^\bullet$, –O(halo$R^\bullet$), –CN, –C(O)OH, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[104] In some embodiments, suitable substituents on a substitutable nitrogen include $-R^\dagger$, $-NR^\dagger_2$, $-C(O)R^\dagger$, $-C(O)OR^\dagger$, $-C(O)C(O)R^\dagger$, $-C(O)CH_2C(O)R^\dagger$, $-S(O)_2R^\dagger$, $-S(O)_2NR^\dagger_2$, $-C(S)NR^\dagger_2$, $-C(NH)NR^\dagger_2$, or $-N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[105] Suitable substituents on the aliphatic group of $R^\dagger$ are independently halogen, $-R^\bullet$, -(halo$R^\bullet$), –OH, $-OR^\bullet$, –O(halo$R^\bullet$), –CN, –C(O)OH, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–

membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[106] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention.

B. *Other Definitions*

[107] *Administration:* As used herein, the term "administration" typically refers to the administration of a composition to a subject or system. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc.. In some particular embodiments, administration may be bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[108] *Agent:* In general, the term "agent", as used herein, may be used to refer to a compound or entity of any chemical class including, for example, a polypeptide, nucleic acid, saccharide, lipid, small molecule, metal, or combination or complex thereof. In appropriate circumstances, as will be clear from context to those skilled in the art, the term may be utilized to refer to an entity that is or comprises a cell or organism, or a fraction, extract, or component thereof. Alternatively or additionally, as context will make clear, the term may be used to refer to a natural product in that it is found in and/or is obtained from nature. In some instances, again as will be clear from context, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form; in some embodiments, an agent may be utilized in crude form. In some embodiments, potential agents may be provided as collections or libraries, for example that may be screened to identify or characterize active agents within them. In some cases, the term "agent" may refer to a compound or entity that is or comprises a polymer; in some cases, the term may refer to a compound or entity that comprises one or more polymeric moieties. In some embodiments, the term "agent" may refer to a compound or entity that is not a polymer and/or is substantially free of any polymer and/or of one or more particular polymeric moieties. In some embodiments, the term may refer to a compound or entity that lacks or is substantially free of any polymeric moiety

[109] *Agonist*: Those skilled in the art will appreciate that the term "agonist" may be used to refer to an agent condition, or event whose presence, level, degree, type, or form correlates with increased level or activity of another agent (i.e., the agonized agent). In general, an agonist may be or include an agent of any chemical class including, for example, small molecules, polypeptides, nucleic acids, carbohydrates, lipids, metals, and/or any other entity that shows the relevant activating activity. In some embodiments, an agonist may be direct (in which case it exerts its influence directly upon its target); in some embodiments, an agonist may be indirect (in which case it exerts its influence by other than binding to its target; e.g., by interacting with a regulator of the target, so that level or activity of the target is altered).

[110] *Animal:* As used herein refers to any member of the animal kingdom. In some embodiments, "*animal*" refers to humans, of either sex and at any stage of development. In some embodiments, "*animal*" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms.

In some embodiments, an animal may be a transgenic animal, genetically engineered animal, and/or a clone.

[111] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[112] *Associated with:* Two events or entities are "associated" with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide, genetic signature, metabolite, microbe, etc) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[113] *Carrier:* as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. In some exemplary embodiments, carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like. In some embodiments, carriers are or include one or more solid components.

[114] *Comparable:* As used herein, the term "comparable" refers to two or more agents, entities, situations, sets of conditions, etc., that may not be identical to one another but that are sufficiently similar to permit comparison there between so that one skilled in the art will appreciate that conclusions may reasonably be drawn based on differences or similarities observed. In some embodiments, comparable sets of conditions, circumstances, individuals, or populations are characterized by a plurality of substantially identical features and one or a small

number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc to be considered comparable. For example, those of ordinary skill in the art will appreciate that sets of circumstances, individuals, or populations are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under or with different sets of circumstances, individuals, or populations are caused by or indicative of the variation in those features that are varied.

[115] *Composition:* Those skilled in the art will appreciate that the term "composition" may be used to refer to a discrete physical entity that comprises one or more specified components. In general, unless otherwise specified, a composition may be of any form – e.g., gas, gel, liquid, solid, etc.

[116] *Comprising:* A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as "comprising" or "consisting essentially of" one or more named elements or steps also describes the corresponding, more limited, and closed-ended composition or method "consisting of" (or "consists of") the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.

[117] *Dosage form* or *unit dosage form:* Those skilled in the art will appreciate that the term "dosage form" may be used to refer to a physically discrete unit of an active agent (*e.g.*, a therapeutic or diagnostic agent) for administration to a subject. Typically, each such unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when

administered to a relevant population (*i.e.*, with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

[118] *Dosing regimen:* Those skilled in the art will appreciate that the term "dosing regimen" may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (*i.e.*, is a therapeutic dosing regimen).

[119] *Encapsulated:* The term "encapsulated" is used herein to refer to substances that are completely surrounded by another material.

[120] *Excipient:* as used herein, refers to a non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[121] *Gel*: As used herein, the term "gel" refers to viscoelastic materials whose rheological properties distinguish them from solutions, solids, etc. In some embodiments, a composition is considered to be a gel if its storage modulus (G′) is larger than its modulus (G″). In some embodiments, a composition is considered to be a gel if there are chemical or physical cross-linked networks in solution, which is distinguished from entangled molecules in viscous solution.

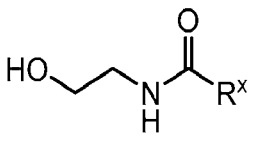
[122] *"Improved," "increased"* or *"reduced":* As used herein, these terms, or grammatically comparable comparative terms, indicate values that are relative to a comparable reference measurement. For example, in some embodiments, an assessed value achieved with an agent of interest may be "improved" relative to that obtained with a comparable reference agent. Alternatively or additionally, in some embodiments, an assessed value achieved in a subject or system of interest may be "improved" relative to that obtained in the same subject or system under different conditions (e.g., prior to or after an event such as administration of an agent of interest), or in a different, comparable subject (e.g., in a comparable subject or system that differs from the subject or system of interest in presence of one or more indicators of a particular disease, disorder or condition of interest, or in prior exposure to a condition or agent, etc). In some embodiments, comparative terms refer to statistically relevant differences (e.g., that are of a prevalence and/or magnitude sufficient to achieve statistical relevance). Those skilled in the art will be aware, or will readily be able to determine, in a given context, a degree and/or prevalence of difference that is required or sufficient to achieve such statistical significance.

[123] *Intraperitoneal:* The phrases "intraperitoneal administration" and "administered intraperitoneally" as used herein have their art-understood meaning referring to administration of a compound or composition into the peritoneum of a subject.

[124] *Moiety*: Those skilled in the art will appreciate that a "moiety" is a defined chemical group or entity with a particular structure and/or or activity, as described herein.

[125] *Oral:* The phrases "oral administration" and "administered orally" as used herein have their art-understood meaning referring to administration by mouth of a compound or composition.

[126] *Parent N-acylethanolamide compound*: A "parent" N-acylethanolamide compound, for purposes of the present disclosure, is a compound relative to which the present disclosure provides derivatives (e.g., to provide a compound of described herein). Typically, a parent N-acylethanolamide compound has a structure as set forth below:



wherein R^x is C_{1-40} aliphatic.

[127] In some embodiments, R^x is C_{1-40} aliphatic. In some embodiments, R^x is C_{1-35} aliphatic. In some embodiments, R^x is C_{1-30} aliphatic. In some embodiments, R^x is C_{1-25} aliphatic. In some embodiments, R^x is C_{1-20} aliphatic. In some embodiments, R^x is C_{1-15} aliphatic. In some embodiments, R^x is C_{1-10} aliphatic. In some embodiments, R^x is C_{1-5} aliphatic. In some

embodiments, R^X is C_{5-30} aliphatic. In some embodiments, R^X is C_{10-25} aliphatic. In some embodiments, R^X is C_{10-20} aliphatic. In some embodiments, R^X is C_{5-15} aliphatic. In some embodiments, R^X is C_{15-25} aliphatic. In some embodiments, a parent N-acylethanolamide compound is derived from a fatty acid selected from the group consisting of myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, linoleic acid, linoelaidic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, and cerotic acid. In some embodiments, a parent N-acylethanolamide compound is selected from the group consisting of N-palmitoylethanolamide, N-oleoylethanolamide, and N-arachidonoylethanolamide. In some embodiments, a parent N-acylethanolamide compound is N-palmitoylethanolamide. In some embodiments, a parent N-acylethanolamide compound is N-oleoylethanolamide. In some embodiments, a parent N-acylethanolamide compound is N-arachidonoylethanolamide.

[128] *Parenteral:* The phrases "parenteral administration" and "administered parenterally" as used herein have their art-understood meaning referring to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

[129] *Patient:* As used herein, the term "patient" refers to any organism to which a provided composition is or may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. In some embodiments, a patient is suffering from or susceptible to one or more disorders or conditions. In some embodiments, a patient displays one or more symptoms of a disorder or condition. In some embodiments, a patient has been diagnosed with one or more disorders or conditions. In some embodiments, the disorder or condition is or includes cancer, or presence of one or more tumors. In some embodiments, the patient is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition.

[130] *Pharmaceutical composition:* As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of

achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[131] *Pharmaceutically acceptable:* As used herein, the phrase "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[132] *Pharmaceutically acceptable carrier:* As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as 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, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[133] *Pharmaceutically acceptable salt:* The term "pharmaceutically acceptable salt", as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, *i.e.*, salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

[134] *Predetermined:* By predetermined is meant deliberately selected, for example as opposed to randomly occurring or achieved.

[135] *Prevent* or *prevention:* as used herein when used in connection with the occurrence of a disease, disorder, and/or condition, refers to reducing the risk of developing the disease, disorder and/or condition and/or to delaying onset of one or more characteristics or symptoms of the disease, disorder or condition. Prevention may be considered complete when onset of a disease, disorder or condition has been delayed for a predefined period of time.

[136] As used herein, the term "prodrug" refers to a compound that is a drug precursor which, following administration, released the drug in vivo via a chemical or physiological process (e.g., a prodrug released the drug upon reaching physiological pH or through enzyme action is converted to the desired drug form).

[137] *Reference:* As used herein describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[138] *Subject:* As used herein, the term "subject" or "test subject" refers to any organism to which a provided compound or composition is administered in accordance with the present disclosure *e.g.,* for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.,* mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*) and plants. In some embodiments, a subject may be suffering from, and/or susceptible to a disease, disorder, and/or condition.

[139] *Suffering from*: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[140] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, and/or condition is one who has a higher risk of developing the disease, disorder, and/or condition than does a member of the general public. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not have been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder,

and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[141] *Systemic:* The phrases "systemic administration," "administered systemically," "peripheral administration," and "administered peripherally" as used herein have their art-understood meaning referring to administration of a compound or composition such that it enters the recipient's system.

[142] *Tautomeric forms:* The phrase "tautomeric forms," as used herein, is used to describe different isomeric forms of organic compounds that are capable of facile interconversion. Tautomers may be characterized by the formal migration of a hydrogen atom or proton, accompanied by a switch of a single bond and adjacent double bond. In some embodiments, tautomers may result from prototropic tautomerism (*i.e.*, the relocation of a proton). In some embodiments, tautomers may result from valence tautomerism (*i.e.*, the rapid reorganization of bonding electrons). All such tautomeric forms are intended to be included within the scope of the present disclosure. In some embodiments, tautomeric forms of a compound exist in mobile equilibrium with each other, so that attempts to prepare the separate substances results in the formation of a mixture. In some embodiments, tautomeric forms of a compound are separable and isolatable compounds. In some embodiments of the disclosure, chemical compositions may be provided that are or include pure preparations of a single tautomeric form of a compound. In some embodiments, chemical compositions may be provided as mixtures of two or more tautomeric forms of a compound. In certain embodiments, such mixtures contain equal amounts of different tautomeric forms; in certain embodiments, such mixtures contain different amounts of at least two different tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain all tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain less than all tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain one or more tautomeric forms of a compound in amounts that vary over time as a result of interconversion. In some embodiments of the disclosure, the tautomerism is keto-enol tautomerism. One of skill in the chemical arts would recognize that a keto-enol tautomer can be "trapped" (*i.e.*, chemically modified such that it remains in the "enol" form) using any suitable reagent known in the chemical arts in to provide an enol derivative that may subsequently be isolated using one or more suitable techniques known in the art. Unless otherwise indicated, the present disclosure encompasses all tautomeric forms of relevant compounds, whether in pure form or in admixture with one another.

[143] *Therapeutic agent:* As used herein, the phrase "therapeutic agent" refers to an agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[144] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of a substance (*e.g.*, a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, *etc.* For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[145] *Therapeutic regimen*: A "therapeutic regimen", as that term is used herein, refers to a dosing regimen whose administration across a relevant population may be correlated with a desired or beneficial therapeutic outcome.

[146] *Treat:* As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits only early signs of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[147] *Unit dose*: The expression "unit dose" as used herein refers to an amount administered as a single dose and/or in a physically discrete unit of a pharmaceutical composition. In many

embodiments, a unit dose contains a predetermined quantity of an active agent. In some embodiments, a unit dose contains an entire single dose of the agent. In some embodiments, more than one unit dose is administered to achieve a total single dose. In some embodiments, administration of multiple unit doses is required, or expected to be required, in order to achieve an intended effect. A unit dose may be, for example, a volume of liquid (*e.g.*, an acceptable carrier) containing a predetermined quantity of one or more therapeutic agents, a predetermined amount of one or more therapeutic agents in solid form, a sustained release formulation or drug delivery device containing a predetermined amount of one or more therapeutic agents, etc. It will be appreciated that a unit dose may be present in a formulation that includes any of a variety of components in addition to the therapeutic agent(s). For example, acceptable carriers (e.g., pharmaceutically acceptable carriers), diluents, stabilizers, buffers, preservatives, etc., may be included as described infra. It will be appreciated by those skilled in the art, in many embodiments, a total appropriate daily dosage of a particular therapeutic agent may comprise a portion, or a plurality, of unit doses, and may be decided, for example, by the attending physician within the scope of sound medical judgment. In some embodiments, the specific effective dose level for any particular subject or organism may depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

Detailed Description of Certain Embodiments

[148] N-acylethanolamides have shown promise in treatment of various diseases, disorders, and conditions. In some embodiments, one or more compounds provided herein may be useful in treatment of such diseases, disorders and conditions. In some embodiments, one or more provided compounds may be useful, for example, in treatment of one or more neurologic diseases, disorders or conditions. In some embodiments, one or more compounds provided herein may be useful, for example, in treatment of pain, anxiety, depression, schizophrenia, amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease, neuropathic pain, cerebral ischemia, epilepsy, appetite loss, dental pain, osteoarthritis, reduced gastrointestinal motility, cancer, glaucoma, atopic dermatitis, respiratory infection, post-traumatic stress disorder, obesity, insomnia, sleepiness, and/or Irritable Bowel Syndrome with Diarrhea (IBS-D).

[149] In some embodiments, one or more compounds provided herein may be useful in reducing gastrointestinal motility in a subject. In some embodiments, one or more compounds provided herein may be useful in reducing cancer cell proliferation in a subject or in a biological sample. In some embodiments, one or more compounds provided herein may be useful in inducing lipolysis in a patient or in a biological sample.

[150] The success of N-acylethanolamides, as well as their sub-optimal pharmacological properties, has led to the development of derivatives, compositions, and prodrugs. Certain N-acylethanolamide derivatives display improved pharmacological properties. For example, polyethylene glycol derivatives of N-acylethanolamides result in improved physico-chemical properties for the treatment of inflammatory and itch- or pain-associated disorders. See, for example, US 2015/0157733 A1.

[151] However, such derivatives, compositions, and prodrugs have failed to produce N-acylethanolamides with improved oral bioavailability suitable for oral administration at high dosages. As a result, current administration of N-acylethanolamides must be parenteral, often intravenous. See, for example, Vacondio, F. et al. "Amino Acid Derivatives as Palmitoylethanolamide Prodrugs: Synthesis, In Vitro Metabolism, and In Vivo Plasma Profile in Rats" *PLoS One* **2015**, 10(6), e0128699.

[152] In some embodiments, the present invention provides derivatives of N-acylethanolamides with desirable pharmacological properties, for example, which may be or include one or more improved properties relative to appropriate N-acylethanolamide reference compounds (e.g., the parent compound of a particular derivative). In certain embodiments, the present disclosure provides derivatives of parent N-acylethanolamide compounds that are characterized by one or more suboptimal pharmacological properties.

[153] In certain embodiments, provided N-acylethanolamide derivative compounds may be characterized by one or more of the properties of increased oral bioavailability, increased cell permeability, increased water solubility, reduced first-pass effect, increased stability, active transport by intestinal transporters, or avoidance of efflux transporters, when compared to N-acylethanolamide reference compounds (e.g., the parent N-acylethanolamide compound of a particular derivative). In some embodiments, provided N-acylethanolamide derivative compounds may be characterized by increased oral bioavailability when compared to N-acylethanolamide reference compounds (e.g., the parent N-acylethanolamide compound of a particular derivative). In some embodiments, the present invention provides N-acylethanolamide derivative compounds that are administered orally. In some embodiments, the present invention

provides N-acylethanolamide derivative compounds that may be administered orally at high dosages.

[154] Furthermore, administration of provided N-acylethanolamide derivative compounds may lead to its ability to function in the treatment of diseases, disorders, or conditions.

Provided N-Acylethanolamide Derivatives

[155] In some embodiments, a compound for use in accordance with the present disclosure is one wherein an N-acylethanolamide is conjugated to a moiety selected from the group consisting of phosphate, butyric acid, glycerol, succinate, caprylic acid, gluconoic acid, eicosapentaeonoic acid, linoleic acid, succinate, and sucrose moieties, and combinations thereof. In some embodiments, an N-acylethanolamide is conjugated to one or more such moieties through use of a linker moiety. In some embodiments, an N-acylethanolamide is conjugated to two or more such moieties. In some embodiments, an N-acylethanolamide is conjugated to one, two, or three such moieties.

[156] In some embodiments, a provided compound has a chemical structure represented by formula **I-a**:

$$X_1\text{-}X_2$$

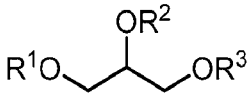
or a pharmaceutically acceptable salt thereof; wherein

X_1 is an N-acylethanolamide; and

X_2 is a moiety conjugated to the N-acylethanolamide.

[157] In some embodiments, X_1 is selected from the group consisting of N-palmitoylethanolamide, N-oleoylethanolamide, and N-arachidonoylethanolamide; in some particular embodiments, X_1 is N-palmitoylethanolamide. In some embodiments, X_2 comprises a moiety selected from the group consisting of phosphate, butyric acid, glycerol, succinate, caprylic acid, gluconoic acid, eicosapentaeonoic acid, linoleic acid, succinate, and sucrose moieties.

[158] In some embodiments, a provided compound has a chemical structure represented by formula **I**:



I

or a pharmaceutically acceptable salt thereof, wherein:

each R^1, R^2, or R^3 is independently hydrogen or $-T-R^4$, wherein at least one of R^1, R^2, or R^3 is $-T-R^4$;

–T– represents a bivalent moiety; and

R^4 is an optionally substituted group selected from the group consisting of C_{1-40} aliphatic, -C(O)R, and X_1; wherein

R is selected from the group consisting of hydrogen and optionally substituted C_{1-20} aliphatic; and

X_1 is as defined above.

[159] In some embodiments, a provided compound has a chemical structure represented by formula **I-b**:

$$X_1\text{-}X_3$$

I-b

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is as defined above;

X_3 is an optionally substituted group selected from the group consisting of $\text{-}(CH_2)_m\text{-}P(O)(OR)_2$, C_{1-40} aliphatic, $\text{-T-}X_4$; further wherein

m is an integer select from the group consisting of 0-10;

-T- is as defined above;

X_4 is a saccharide moiety, in some particular embodiments, X_4 is a disaccharide, for example, sucrose.

[160] In some embodiments, at least one of R^1, R^2, or R^3 is $-T-R^4$. In some embodiments, at least two of R^1, R^2, or R^3 is $-T-R^4$.

[161] In some embodiments, one of R^1, R^2, or R^3 is $-T-R^4$. In some embodiments, two of R^1, R^2, or R^3 are each independently $-T-R^4$. In some embodiments, R^1, R^2, and R^3 are each independently $-T-R^4$.

[162] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is $-T-R^4$. In some embodiments, R^2 is hydrogen. In some embodiments, R^2 is $-T-R^4$. In some embodiments, R^3 is hydrogen. In some embodiments, R^3 is $-T-R^4$.

[163] In some embodiments, R^1 and R^2 are hydrogen, and R^3 is $-T-R^4$. In some embodiments, R^2 and R^3 are hydrogen, and R^1 is $-T-R^4$. In some embodiments, R^1 and R^3 are hydrogen, and R^2 is $-T-R^4$. In some embodiments, R^1 is hydrogen, and R^2 and R^3 are each independently $-T-R^4$. In some embodiments, R^2 is hydrogen, and R^1 and R^3 are each independently $-T-R^4$. In

some embodiments, R^3 is hydrogen, and R^1 and R^2 are each independently $-T-R^4$. In some embodiments, each of R^1, R^2, and R^3 are independently $-T-R^4$.

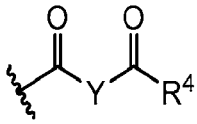
[164] In some embodiments, –T– represents a bivalent moiety.

[165] In some embodiments, –T– is a bivalent moiety derived from a dicarboxylic acid. In some embodiments, –T– is a bivalent moiety derived from an optionally substituted dicarboxylic acid selected from the group consisting of oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, maleic acid, fumaric acid, glutaconic acid, traumatic acid, muconic acid, glutinic acid, citraconic acid, mesaconic acid, malic acid, aspartic acid, glutamic acid, tartronic acid, tartaric acid, diaminopimelic acid, saccharic acid, mesoxalic acid, oxaloacetic acid, acetonedicarboxylic acid, arabinaric acid, phthalic acid, isophthalic acid, terephthalic acid, diphenic acid, and 2,6-napthalenedicarboxylic acid.

[166] In some embodiments, –T– is a bivalent moiety derived from optionally substituted oxalic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted malonic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted succinic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted glutaric acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted adipic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted pimelic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted suberic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted azelaic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted sebacic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted maleic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted fumaric acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted glutaconic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted traumatic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted muconic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted glutinic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted citraconic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted mesaconic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted malic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted

aspartic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted glutamic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted tartronic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted tartaric acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted diaminopimelic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted saccharic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted mesoxalic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted oxaloacetic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted acetonedicarboxylic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted arabinaric acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted phthalic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted isophthalic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted terephthalic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted diphenic acid. In some embodiments, –T– is a bivalent moiety derived from optionally substituted 2,6-naphthalenedicarboxylic acid.

[167] In some embodiments, $-T-R^4$ is:



wherein R^4 is as defined above; and

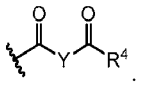
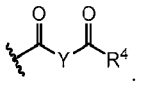
Y is a bivalent C_{1-20} straight or branched hydrocarbon chain.

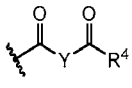
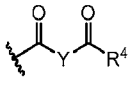
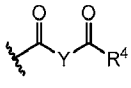
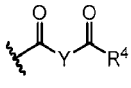
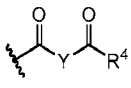
[168] In some embodiments Y is a bivalent C_{1-20} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-15} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-12} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-10} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-8} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-6} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-5} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-4} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-3} straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{1-2} straight or branched hydrocarbon chain.

[169] In some embodiments Y is a bivalent C_{1-20} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-15} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-12} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-10} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-8} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-6} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-5} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-4} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-3} straight hydrocarbon chain. In some embodiments Y is a bivalent C_{1-2} straight hydrocarbon chain.

[170] In some embodiments Y is a bivalent C_1 hydrocarbon chain. In some embodiments Y is a bivalent C_2 straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_3 straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_4 straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_5 straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_6 straight or branched hydrocarbon chain. In some embodiments Y is a bivalent C_{10} straight or branched hydrocarbon chain.

[171] In some embodiments, Y is propylene. In some embodiments, Y is ethylene. In some embodiments, Y is methylene.

[172] In some embodiments, at least one of R^1, R^2, or R^3 is  . In some embodiments, at least two of R^1, R^2, or R^3 are each independently  .

[173] In some embodiments, one of R^1, R^2, or R^3 is  . In some embodiments, R^1 is  . In some embodiments, R^2 is  . In some embodiments, two of R^1, R^2, or R^3 are each independently  . In some embodiments, R^1, R^2, and R^3 are each independently  .

[174] In some embodiments, R^4 is an optionally substituted group selected from the group consisting of C_{1-40} aliphatic, -C(O)R, and X_1. In some embodiments, R^4 is optionally substituted C_{1-40} aliphatic. In some embodiments, R^4 is optionally substituted C_{1-35} aliphatic. In some embodiments, R^4 is optionally substituted C_{1-30} aliphatic. In some embodiments, R^4 is optionally substituted C_{1-25} aliphatic. In some embodiments, R^4 is optionally substituted C_{1-20} aliphatic. In some embodiments, R^4 is optionally substituted C_{1-10} aliphatic. In some embodiments, R^4 is

optionally substituted C_{1-6} aliphatic. In some embodiments, R^4 is optionally substituted -C(O)R. In some embodiments, R^4 is X_1.

[175] In some embodiments, X_1 is selected from N-palmitoylethanolamide, N-oleoylethanolamide, or N-arachidonoylethanolamide. In some embodiments, X_1 is N-palmitoylethanolamide. In some embodiments, X_1 is N-oleoylethanolamide. In some embodiments, X_1 is N-arachidonoylethanolamide.

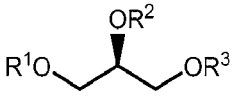
[176] In some embodiments, R is selected from the group consisting of hydrogen and optionally substituted C_{1-20} aliphatic. In some embodiments, R is hydrogen. In some embodiments, R is optionally substituted C_{1-20} aliphatic. In some embodiments, R is optionally substituted C_{1-10} aliphatic. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-3} aliphatic.

[177] In some embodiments, X_3 is an optionally substituted group selected from the group consisting of $-(CH_2)_m-P(O)(OR)_2$, C_{1-40} aliphatic, and -T-X_4. In some embodiments, X_3 is optionally substituted $-(CH_2)_m-P(O)(OR)_2$. In some embodiments, X_3 is optionally substituted C_{1-40} aliphatic. In some embodiments, X_3 is optionally substituted C_{1-30} aliphatic. In some embodiments, X_3 is optionally substituted C_{1-20} aliphatic. In some embodiments, X_3 is optionally substituted C_{1-10} aliphatic. In some embodiments, X_3 is optionally substituted C_{1-6} aliphatic. In some embodiments, X_3 is an optionally substituted -T-X_4.

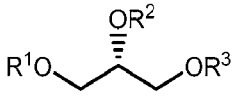
[178] In some embodiments, *m* is an integer select from the group consisting of 0-10. In some embodiments, *m* is an integer select from the group consisting of 0-5. In some embodiments, *m* is 0. In some embodiments, *m* is 1. In some embodiments, *m* is 2. In some embodiments, *m* is 3. In some embodiments, *m* is 4. In some embodiments, *m* is 5.

[179] In some embodiments, X_4 is a saccharide moiety. In some embodiments, X_4 is a disaccharide. In some embodiments, X_4 is sucrose.

[180] In some embodiments, a compound of formula **I** does not comprise a stereocenter within the glycerol backbone (e.g., when R^1 and R^3 are the same). In some embodiments, a compound of formula **I** comprises a stereocenter within the glycerol backbone (e.g., wherein R^1 and R^3 are different). In some embodiments, a compound of formula **I** is provided and/or utilized as a racemic mixture. In some embodiments, a compound of formula **I** is provided and/or utilized as a mixture of stereoforms that may or may not be a racemic mixture. In some embodiments, a compound of formula **I** is provided and/or utilized as a single enantiomer. In some embodiments, the present disclosure provides compounds of formula **I'** or **I''**:



I'



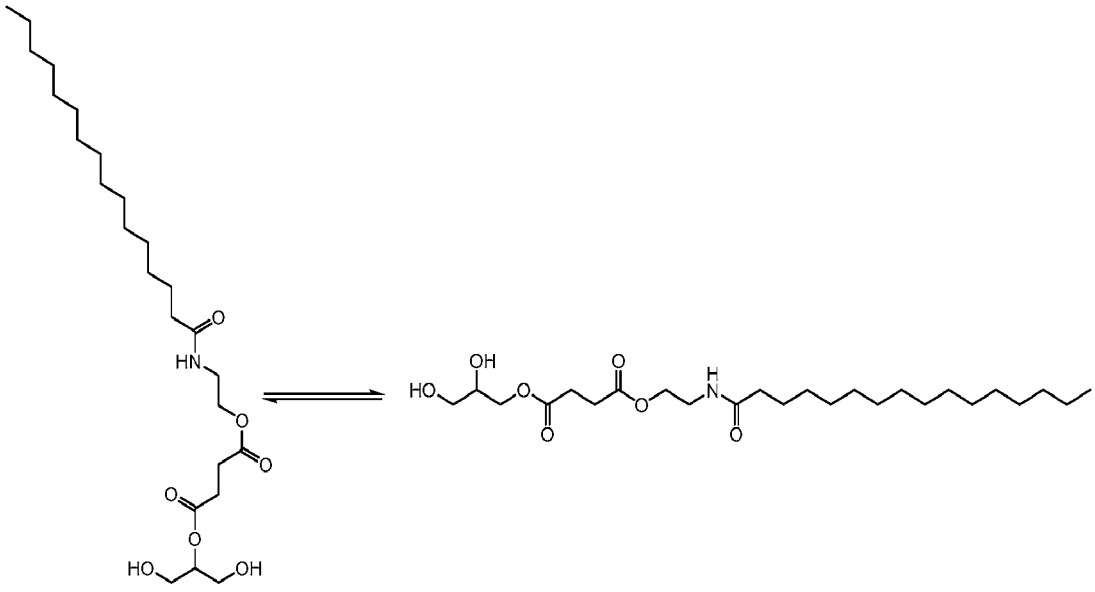
I''

or a pharmaceutically acceptable salt thereof;

wherein R^1, R^2, and R^3 are as defined above.

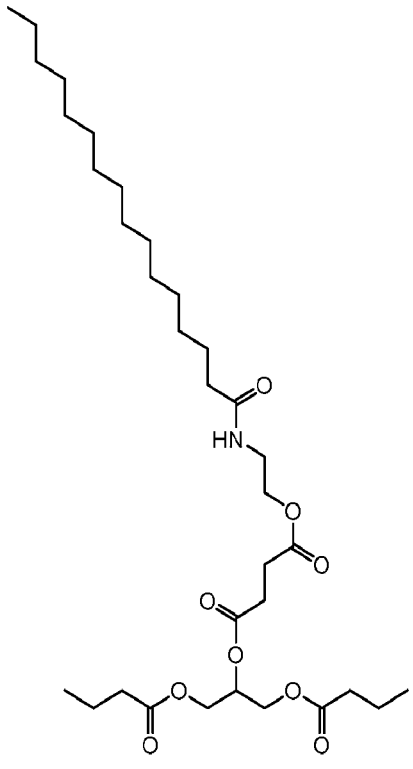
[181] The present disclosure also provides the insight that, in some embodiments, the position of the N-acylethanolamide, e.g., PEA, on the glycerol moiety may have an effect on its pharmacological properties. For example, a glycerol moiety with an N-acylethanolamide moiety conjugated to the 2 position (e.g., the position corresponding to *-OR^2 of formulae **I**, **I'**, or **I''**) may exhibit improved pharmacological properties over a glycerol moiety with an N-acylethanolamide moiety conjugated to the 1 or 3 position (e.g., the position corresponding to *-OR^1 or *-OR^3 of formulae **I**, **I'**, or **I''**). Without wishing to be bound to a particular theory, the present disclosure proposes that, in some embodiments, the 1 and 3 positions of the glycerol backbone may be more susceptible to cellular lipases than the 2 position.

[182] The present disclosure also provides the insight that, in some embodiments, a compound provided herein may isomerize, for example, undergoing positional isomerization. For example, the present disclosure proposes that, in some embodiments, when a glycerol moiety comprises a free alcohol (e.g., a free alcohol at a position corresponding to *-OR^1 or *-OR^3 of formulae **I**, **I'**, or **I''**), a moiety conjugated to glycerol (e.g., a moiety comprising an N-acylethanolamide at a position corresponding to *-OR^2 of formulae **I**, **I'**, or **I''**), may migrate to a free alcohol (e.g., migrate from a position corresponding to *-OR^2 of formulae **I**, **I'**, or **I''** to a position corresponding to *-OR^1 or *-OR^3 of formulae **I**, **I'**, or **I''**). For example, compounds **I-8** and **I-9** may interconvert among positional isomers.

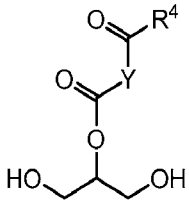


[183] In some embodiments, isomerization occurs prior to administration. In some embodiments, isomerization occurs after administration.

[184] In addition, the present disclosure provides the insight that, in some embodiments, a glycerol moiety that does not comprise a free alcohol will not isomerize. For example, in some embodiments, compound **I-16** does not undergo positional isomerization.



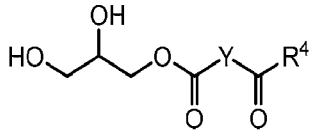
[185] In some embodiments, the present disclosure provides compounds of formula **II**:



or a pharmaceutically acceptable salt thereof;

wherein Y and R^4 are as defined above.

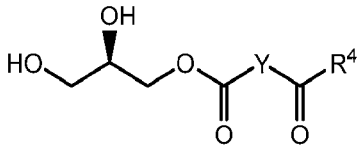
[186] In some embodiments, the present disclosure provides compounds of formula **III**:



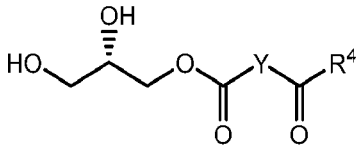
or a pharmaceutically acceptable salt thereof;

wherein Y and R^4 are as defined above.

[187] In some embodiments, the present disclosure provides compounds of formulae **III'** or **III''**:



III'

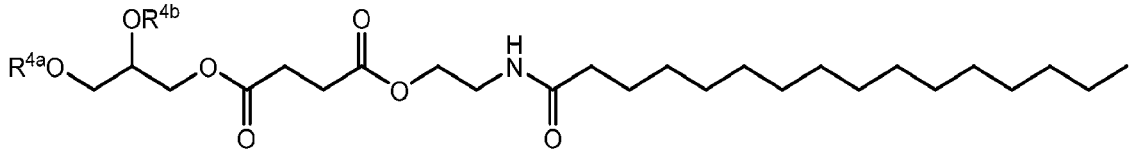


III''

or a pharmaceutically acceptable salt thereof;

wherein Y and R^4 are as defined above.

[188] In some embodiments, the present disclosure provides compounds of formula **IV**:



IV

or a pharmaceutically acceptable salt thereof;

wherein:

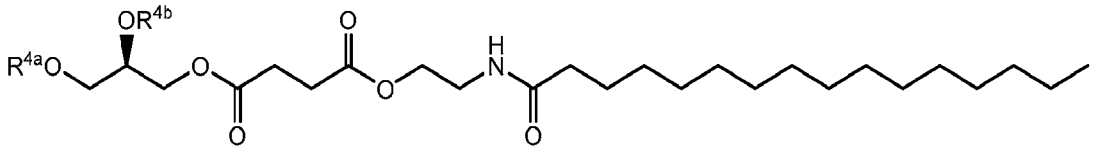
R^{4a} and R^{4b} are independently hydrogen, -C(O)R', or -C(O)-Y-C(O)OR';

wherein

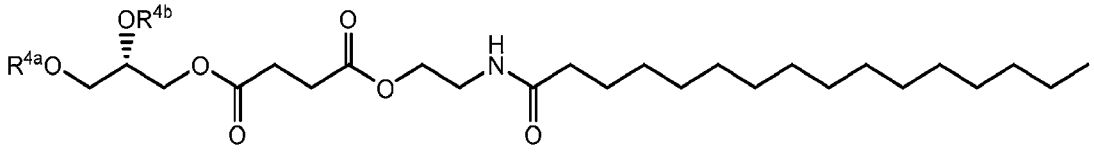
each R' is independently selected from the group consisting of hydrogen and an optionally substituted C_{1-20} aliphatic; and

each Y is independently as defined above and described herein.

[189] In some embodiments, the present disclosure provides compounds of formulae **IV'** or **IV''**:



IV'

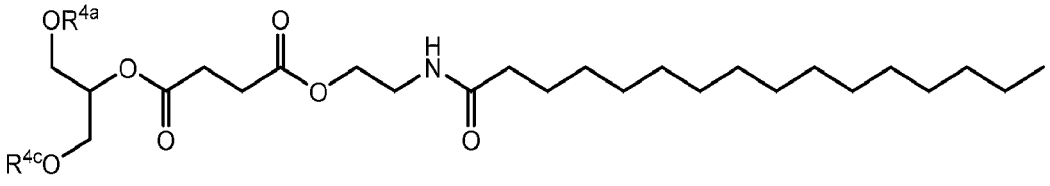


IV''

or a pharmaceutically acceptable salt thereof;

wherein R^{4a} and R^{4b} are as defined above and herein.

[190] In some embodiments, the present disclosure provides compounds of formula **V**:



V

wherein:

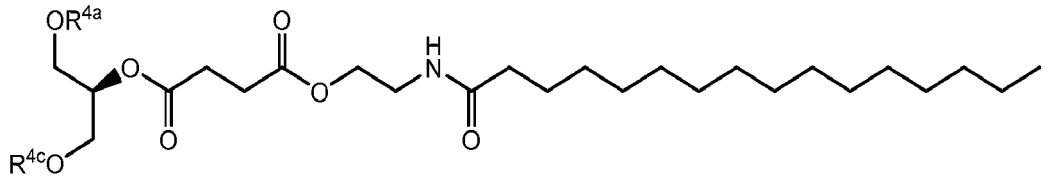
R^{4a} and R^{4c} are independently hydrogen, -C(O)R', or -C(O)-Y-C(O)OR';

wherein

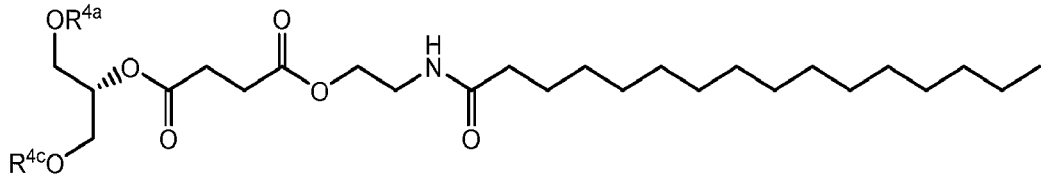
each R' is independently selected from the group consisting of hydrogen and an optionally substituted C_{1-20} aliphatic; and

each Y is independently as defined above and described herein.

[191] In some embodiments, the present disclosure provides compounds of formulae **V'** or **V''**:



V'



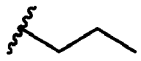
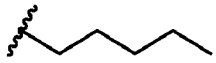
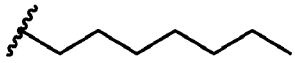
V''

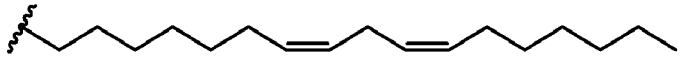
or a pharmaceutically acceptable salt thereof;

wherein R^{4a} and R^{4c} are as defined above and herein.

[192] In some embodiments, R^{4a} is hydrogen. In some embodiments, R^{4a} is -C(O)R'. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR'. In some embodiments, R^{4b} is hydrogen. In some embodiments, R^{4b} is -C(O)R'. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR'. In some embodiments, R^{4c} is hydrogen. In some embodiments, R^{4c} is -C(O)R'. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR'.

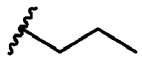
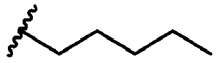
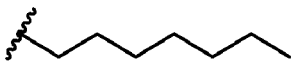
[193] In some embodiments, R' is hydrogen. In some embodiments, R' is optionally substituted C_{1-20} aliphatic. In some embodiments, R' is selected from the group consisting of:

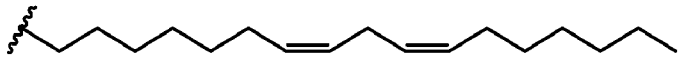
hydrogen,  ,  ,  ,

 , and

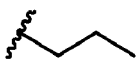
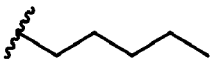
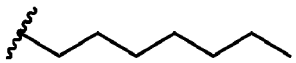
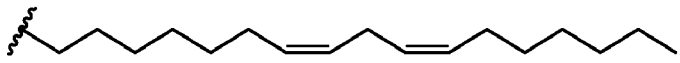
 .

[194] In some embodiments, R' is selected from the group consisting of:

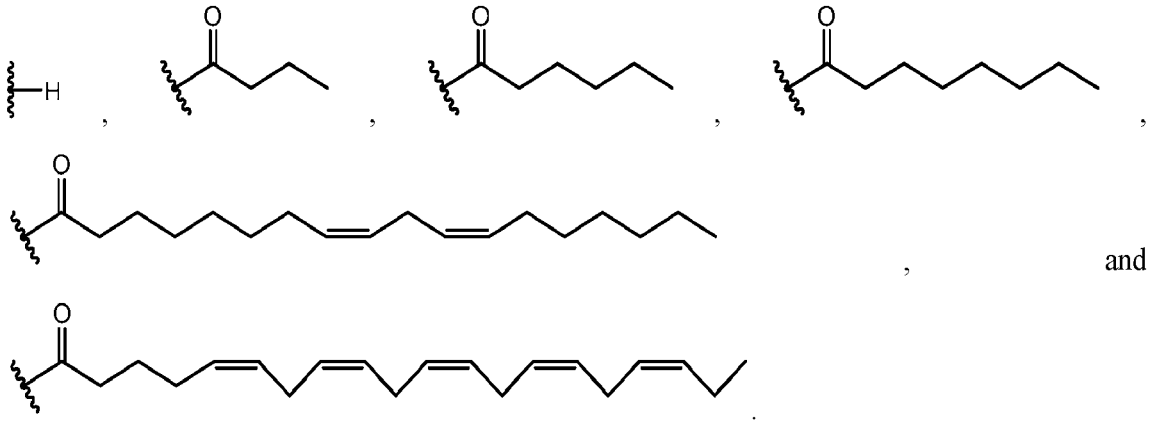
 ,  ,  ,

 , and

 .

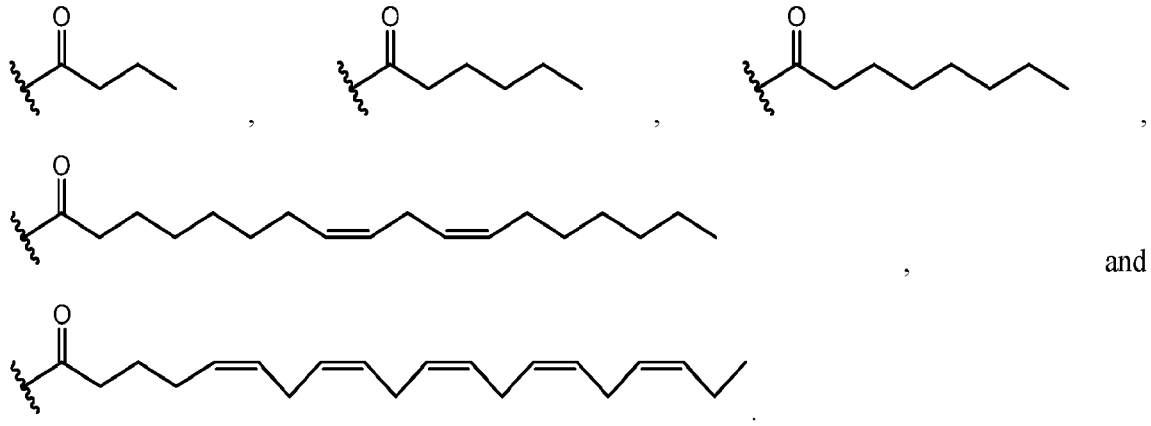
[195] In some embodiments, R' is  . In some embodiments, R' is  . In some embodiments, R' is  . In some embodiments, R' is  . In some embodiments, R' is  .

[196] In some embodiments, R^{4a} is -C(O)R'. In some embodiments, R^{4a} is -C(O)R', wherein R' is optionally substituted C_{1-20} aliphatic. In some embodiments, R^{4a} is selected from the group consisting of:

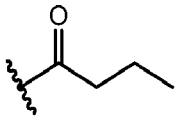
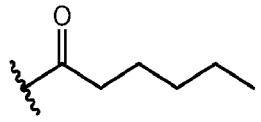
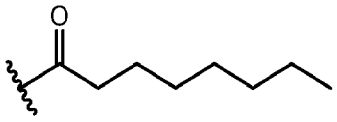
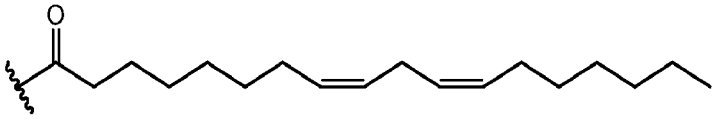


and

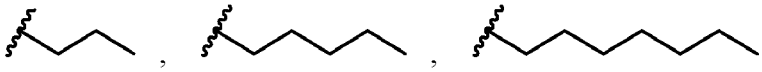
[197] In some embodiments, R^{4a} is selected from the group consisting of:

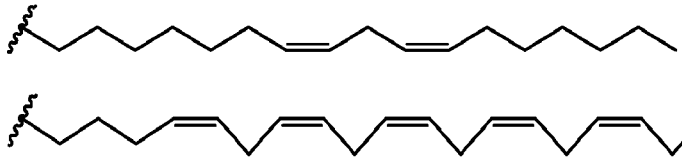


and

[198] In some embodiments, R^{4a} is  . In some embodiments, R^{4a} is  . In some embodiments, R^{4a} is  . In some embodiments, R^{4a} is  . In some embodiments, R^{4a} is  .

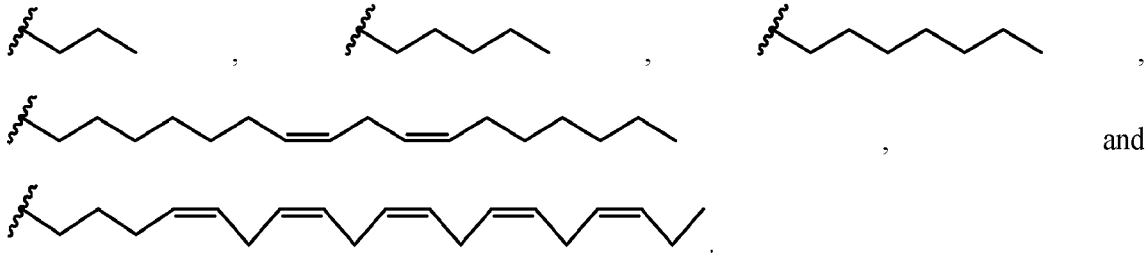
[199] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR". In some embodiments, R^{4a} is -C(O)-Y-C(O)OR", wherein R' is optionally substituted C_{1-20} aliphatic.

[200] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of hydrogen,  ,



and

[201] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein R' is hydrogen. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of



and

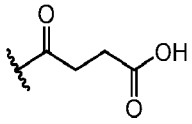
[202] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-6} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-4} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3} straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight or branched hydrocarbon chain.

[203] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-6} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-4} straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3}

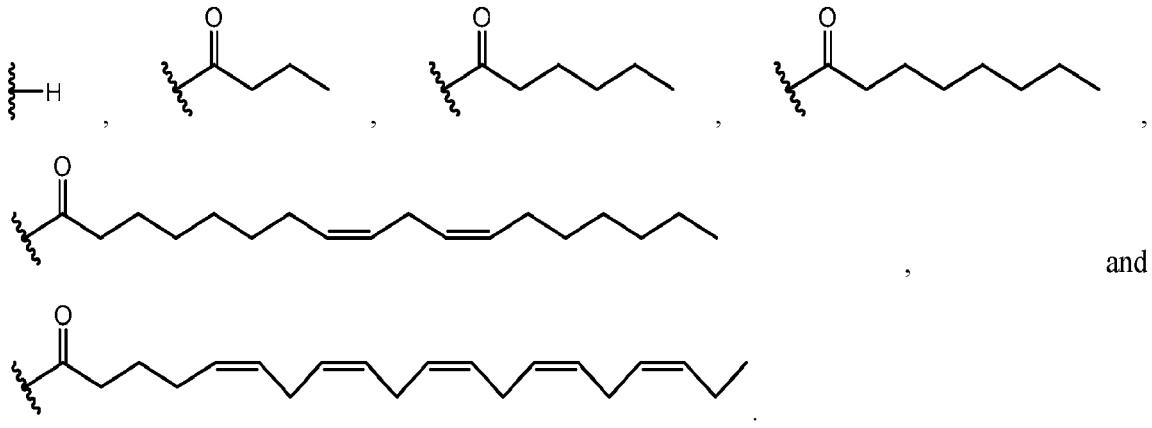
straight hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight hydrocarbon chain.

[204] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_1 hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_2 straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_3 straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_4 straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_5 straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_6 straight or branched hydrocarbon chain. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{10} straight or branched hydrocarbon chain.

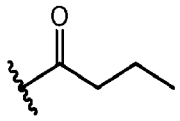
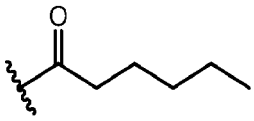
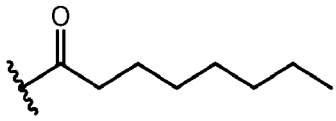
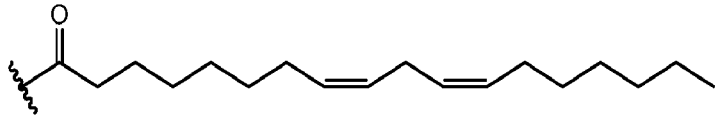
[205] In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein, Y is propylene. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is ethylene. In some embodiments, R^{4a} is -C(O)-Y-C(O)OR', wherein Y is methylene.

[206] In some embodiments, R^{4a} is  .

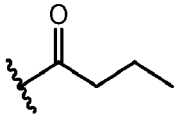
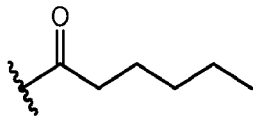
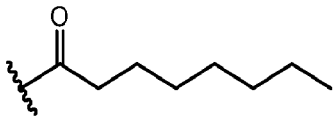
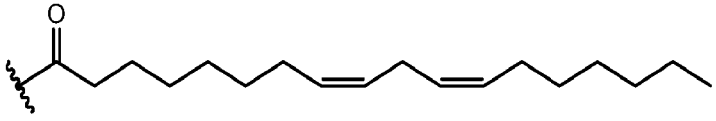
[207] In some embodiments, R^{4b} is -C(O)R'. In some embodiments, R^{4b} is -C(O)R', wherein R' is optionally substituted C_{1-20} aliphatic. In some embodiments, R^{4b} is selected from the group consisting of:



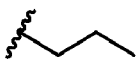
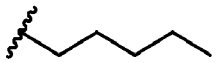
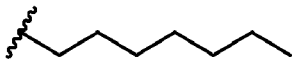
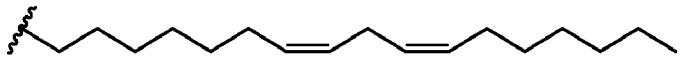
[208] In some embodiments, R^{4b} is selected from the group consisting of:

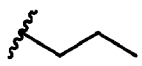
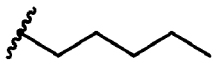
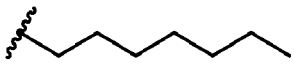

,

,

,

, and

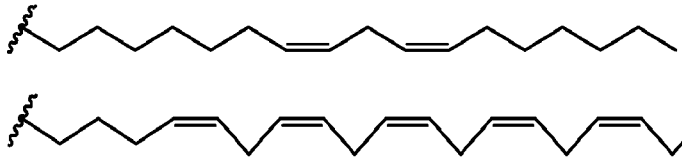
.

[209] In some embodiments, R^{4b} is  . In some embodiments, R^{4b} is  . In some embodiments, R^{4b} is  . In some embodiments, R^{4b} is  . In some embodiments, R^{4b} is  .

[210] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR'. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein R' is optionally substituted C_{1-20} aliphatic.

[211] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of hydrogen,  ,  ,  ,  , and  .

[212] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein R' is hydrogen. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of  ,  ,  ,



, and

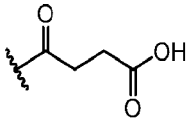
[213] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-6} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-4} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3} straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight or branched hydrocarbon chain.

[214] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-6} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-4} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3} straight hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight hydrocarbon chain.

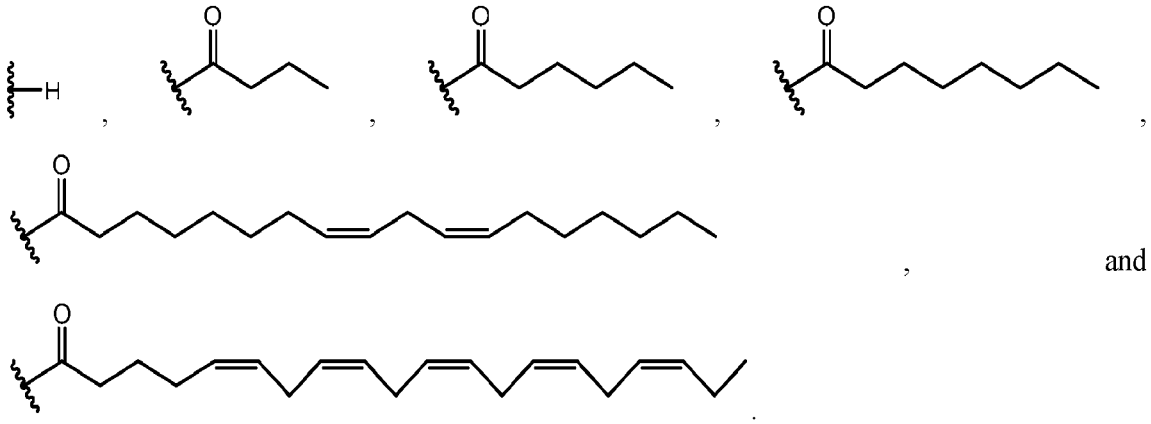
[215] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_1 hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_2 straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_3 straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_4 straight or branched hydrocarbon chain. In some

embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_5 straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_6 straight or branched hydrocarbon chain. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{10} straight or branched hydrocarbon chain.

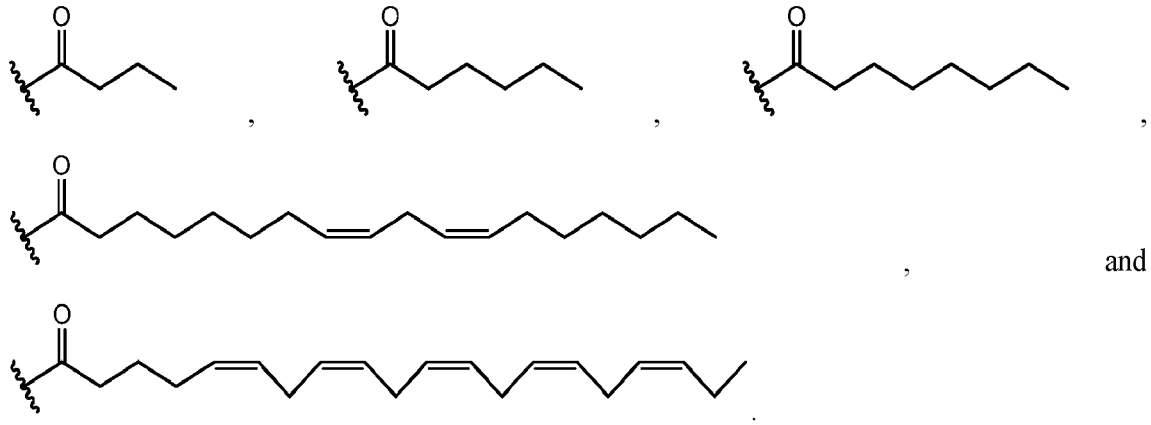
[216] In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein, Y is propylene. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is ethylene. In some embodiments, R^{4b} is -C(O)-Y-C(O)OR', wherein Y is methylene.

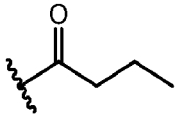
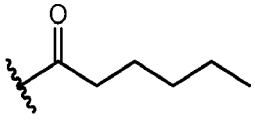
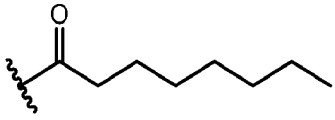
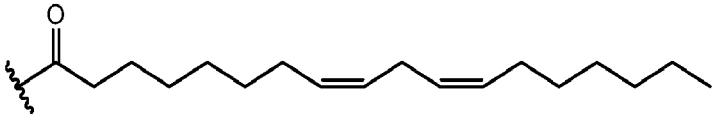
[217] In some embodiments, R^{4b} is  .

[218] In some embodiments, R^{4c} is -C(O)R'. In some embodiments, R^{4c} is -C(O)R', wherein R' is optionally substituted C_{1-20} aliphatic. In some embodiments, R^{4c} is selected from the group consisting of:

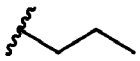
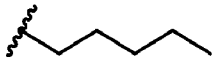
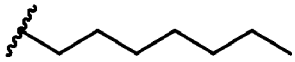
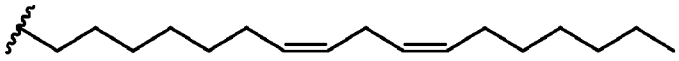

and

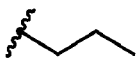
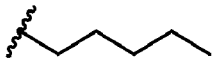
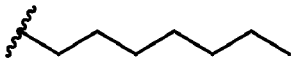
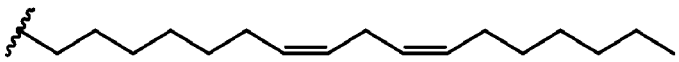
[219] In some embodiments, R^{4c} is selected from the group consisting of:


and

[220] In some embodiments, R^{4c} is  . In some embodiments, R^{4c} is  . In some embodiments, R^{4c} is  . In some embodiments, R^{4c} is  . In some embodiments, R^{4c} is  .

[221] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR'. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein R' is optionally substituted C_{1-20} aliphatic.

[222] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of hydrogen,  ,  ,  ,  , and  .

[223] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein R' is hydrogen. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein R' is selected from the group consisting of  ,  ,  ,  , and  .

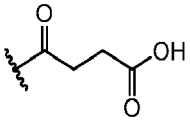
[224] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR',

wherein Y is a bivalent C_{1-6} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-4} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3} straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight or branched hydrocarbon chain.

[225] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-20} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-15} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-12} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-10} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-8} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-6} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-5} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)O-R', wherein Y is a bivalent C_{1-4} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-3} straight hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{1-2} straight hydrocarbon chain.

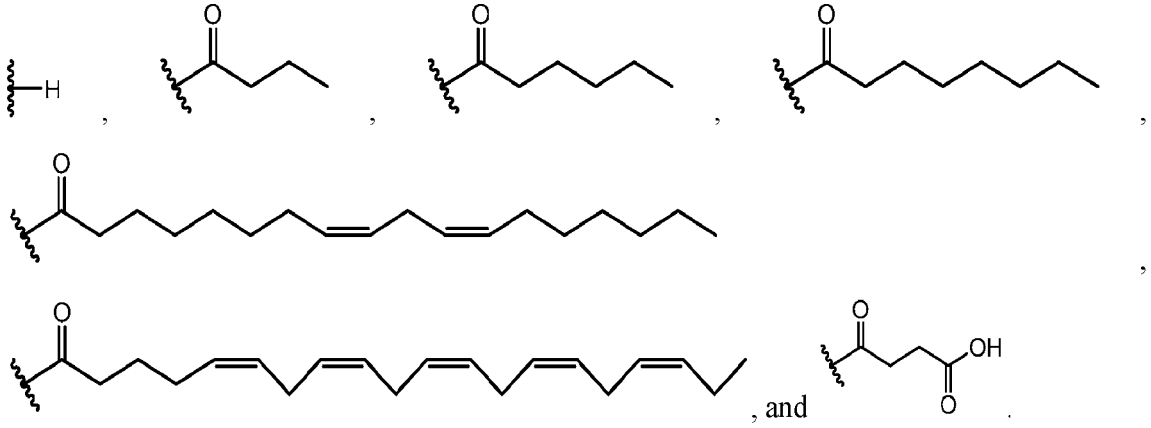
[226] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_1 hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_2 straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_3 straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_4 straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_5 straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_6 straight or branched hydrocarbon chain. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is a bivalent C_{10} straight or branched hydrocarbon chain.

[227] In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein, Y is propylene. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is ethylene. In some embodiments, R^{4c} is -C(O)-Y-C(O)OR', wherein Y is methylene.

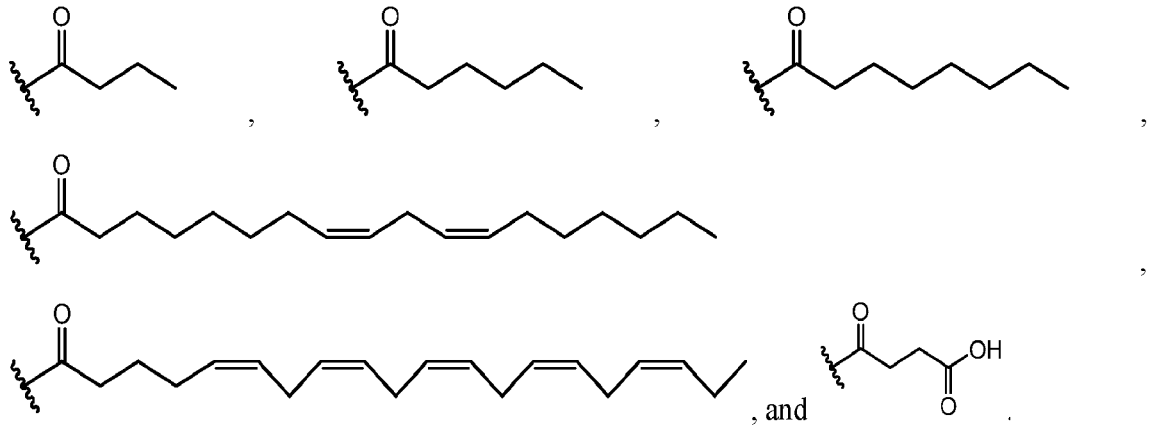
[228] In some embodiments, R^{4c} is 

.

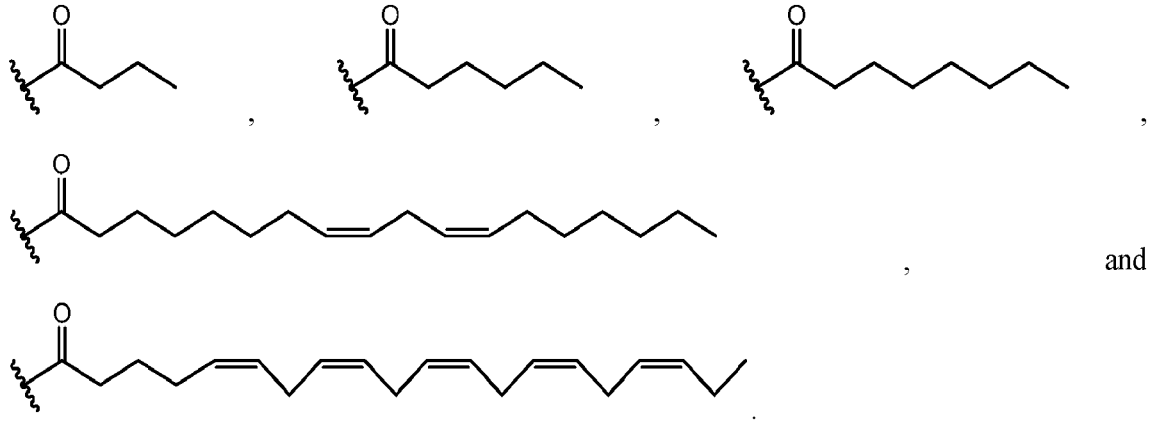
[229] In some embodiments, each of R^{4a}, R^{4b}, and R^{4c} is hydrogen. In some embodiments, each of R^{4a}, R^{4b}, and R^{4c} is independently selected from the group consisting of:



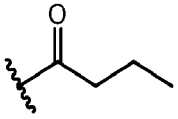
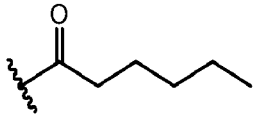
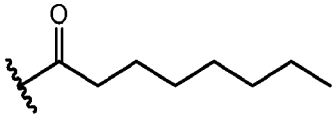
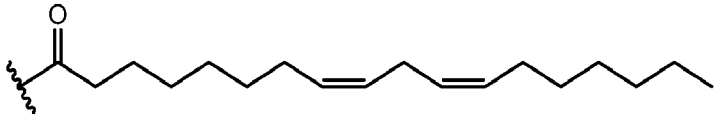
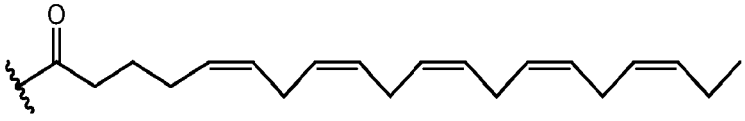
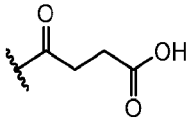
[230] In some embodiments, each of R^{4a}, R^{4b}, and R^{4c} is independently selected from the group consisting of:

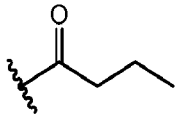
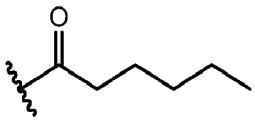
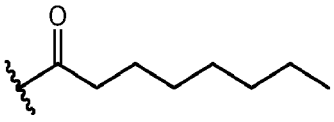
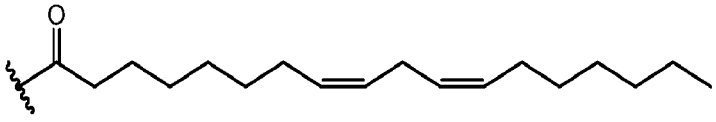
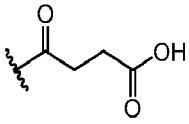


[231] In some embodiments, each of R^{4a}, R^{4b}, and R^{4c} is independently selected from the group consisting of:



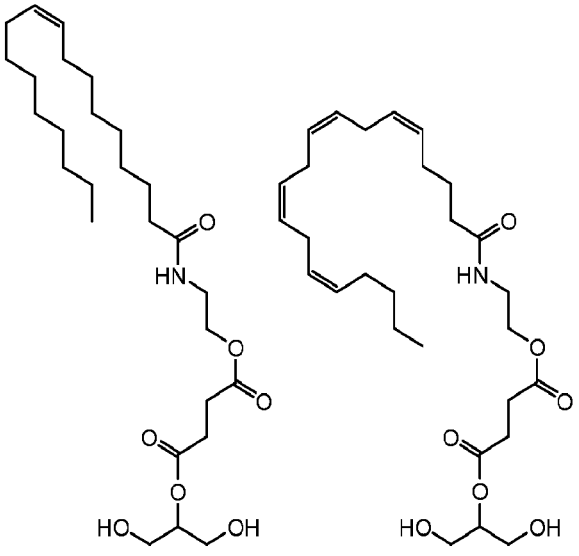
[232] In some embodiments, R^{4a} and R^{4b} or R^{4a} and R^{4c} are the same. In some embodiments, R^{4a} and R^{4b} are the same. In some embodiments, R^{4a} and R^{4b} are  . In some embodiments,

R^{4a} and R^{4b} are  . In some embodiments, R^{4a} and R^{4b} are  . In some embodiments, R^{4a} and R^{4b} are  . In some embodiments, R^{4a} and R^{4b} are  . In some embodiments, R^{4a} and R^{4b} are  . In some embodiments, R^{4a} and R^{4b} are  .

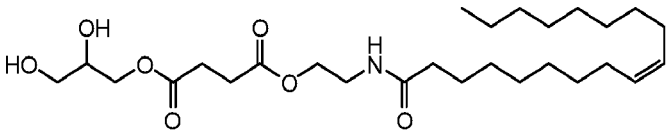
[233] In some embodiments, R^{4a} and R^{4c} are the same. In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  . In some embodiments, R^{4a} and R^{4c} are  .

[234] In some embodiments, the present disclosure provides N-acylethanolamide derivatives selected from those in Table 1.

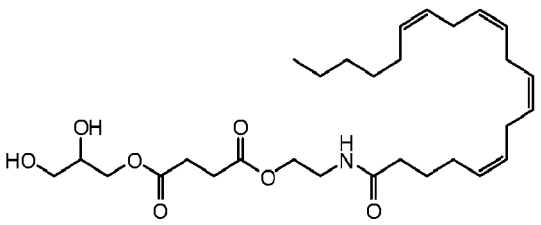
Table 1.



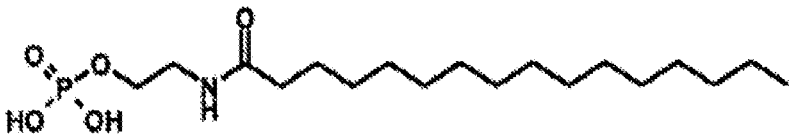
I-a-2 I-a-3



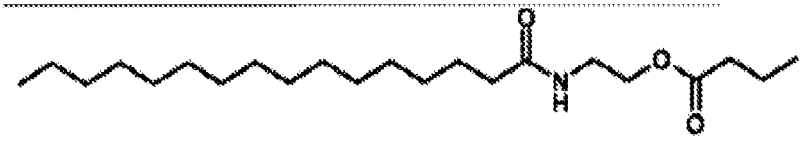
I-a-5



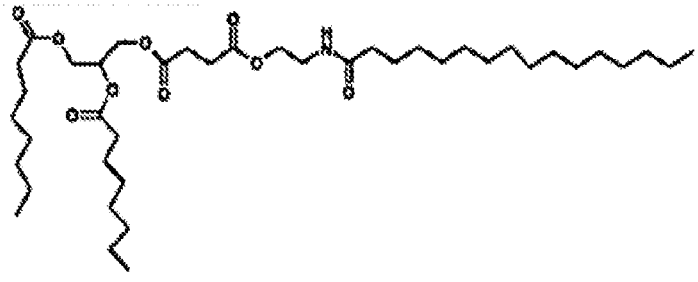
I-a-6.



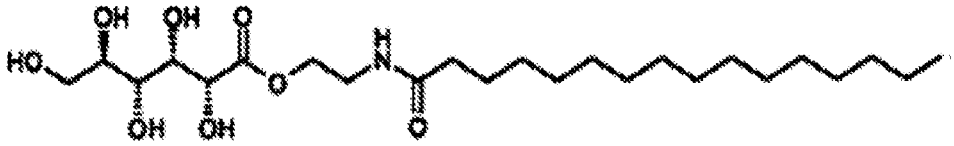
I-1



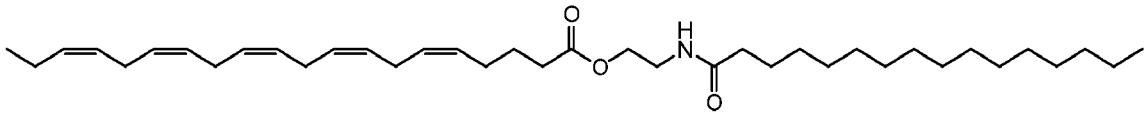
I-2



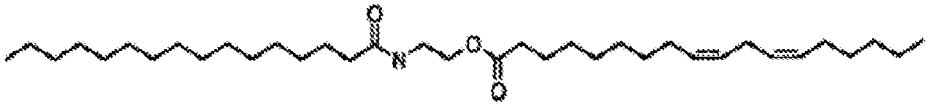
I-3



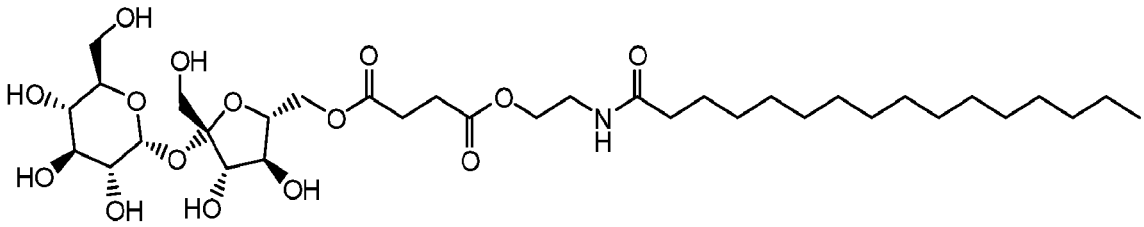
I-4



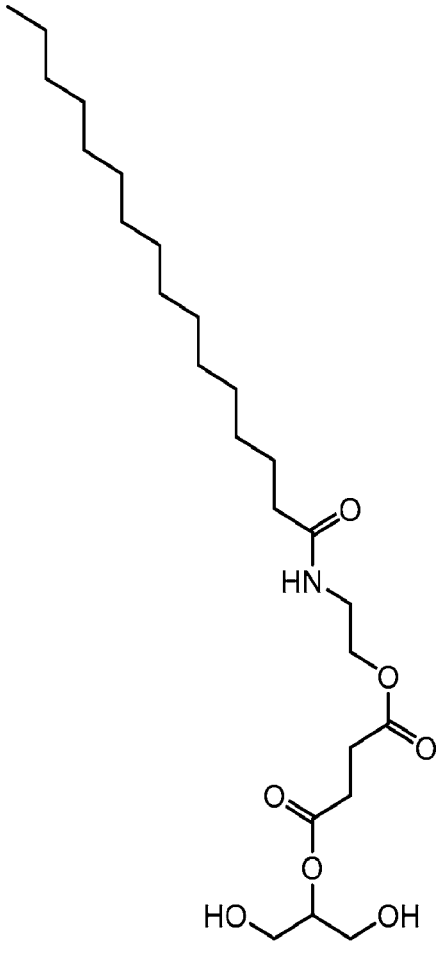
I-5



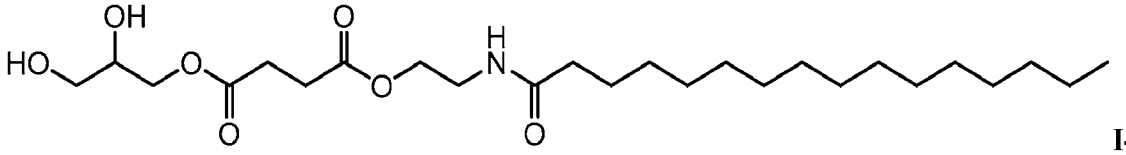
I-6



I-7



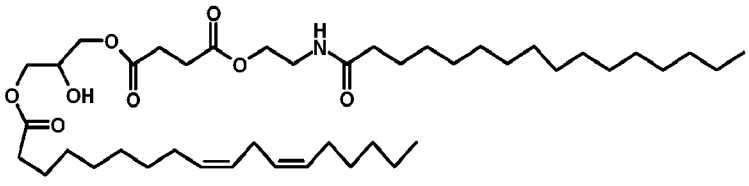
I-8



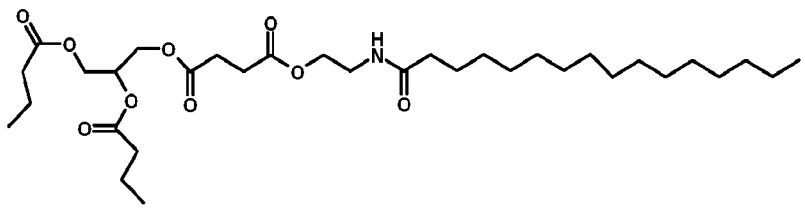
I-9

[235] In some embodiments, the present disclosure provides compounds selected from those in Table 1-a.

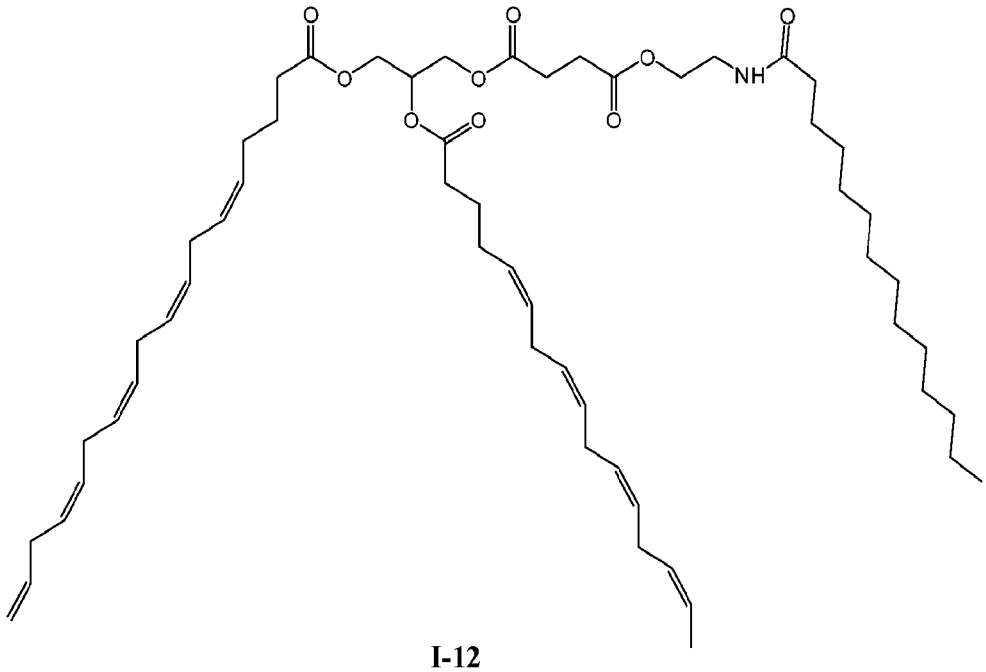
Table 1-a.



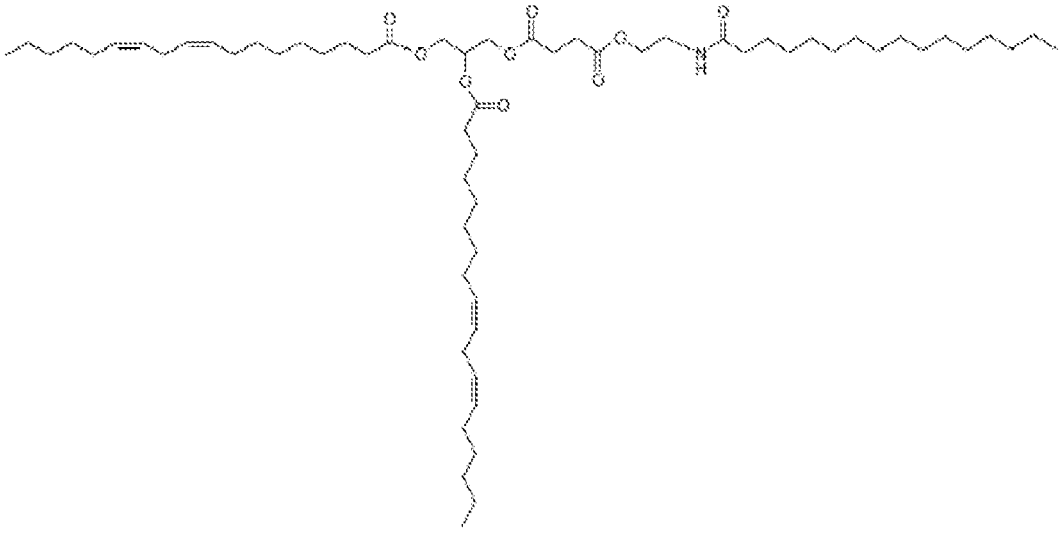
I-10



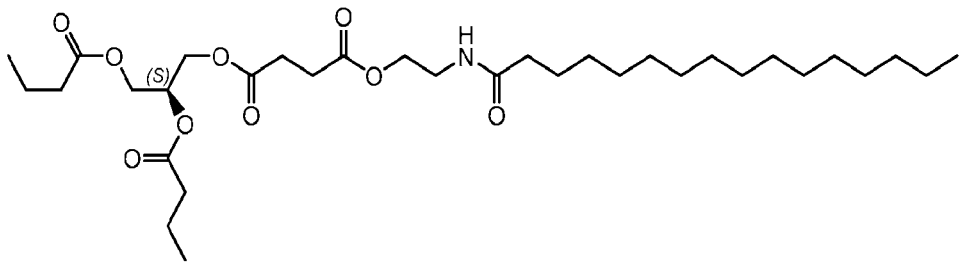
I-11



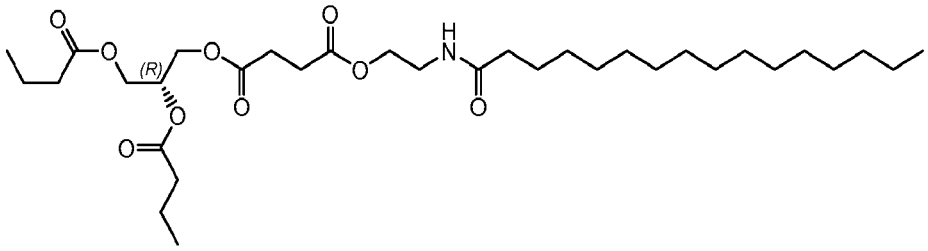
I-12



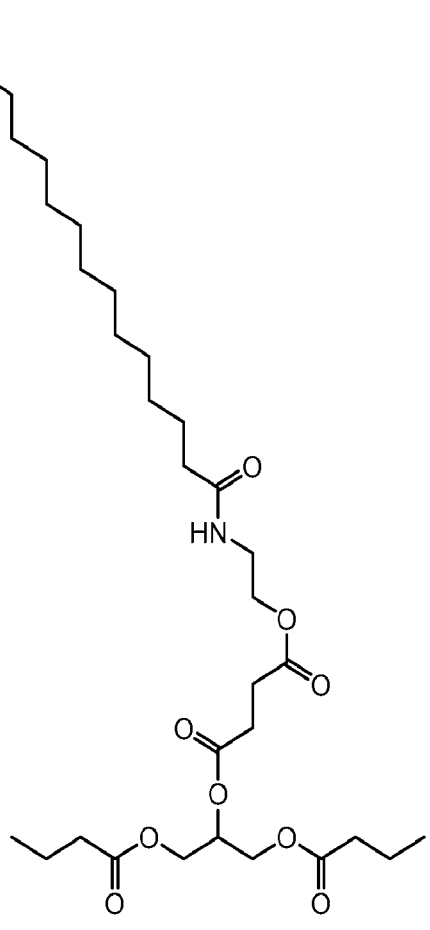
I-13



I-14



I-15



I-16

[236] In some embodiments, one or more hydrogen atoms are replaced with a deuterium atom(s). In some embodiments, one or more of R^1, R^2, or R^3 is or contains deuterium.

[237] In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are comparable to that of a reference compound (e.g., a parent N-acylethanolamide compound). In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are improved as compared to a reference compound (e.g., a parent N-acylethanolamide compound).

[238] In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound may show improved solubility in an aqueous system as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some particular embodiments, aqueous solubility may be assessed according to an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[239] In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound may show improved stability as compared to a reference compound (e.g., a parent N-acylethanolamide compound) In some particular embodiments, stability may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[240] In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound is metabolized differently as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound is metabolized at a different rate as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound is metabolized at a faster rate as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that the compound is metabolized at a slower rate as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some particular embodiments, metabolized rate may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[241] In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that when administered, the compound delivers a parent N-acylethanolamide compound or an active metabolite thereof.

[242] In some embodiments, provided N-acylethanolamide derivative compounds are characterized in that when administered, the compound exhibits an improved oral bioavailability

as compared to a reference compound (e.g., a parent N-acylethanolamide compound). In some particular embodiments, oral bioavailability may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[243] In some embodiments, a reference compound is or comprises a parent N-acylethanolamide compound. In some embodiments, a reference compound is or comprises palmitoylethanolamide.

[244] In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are comparable to that of palmitoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are improved as compared to palmitoylethanolamide.

[245] In some embodiments, provided N-acylethanolamide derivative compounds display increased solubility as compared to that of palmitoylethanolamide. In some particular embodiments, aqueous solubility may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[246] In some embodiments, provided N-acylethanolamide derivative compounds display increased stability that is comparable to that of palmitoylethanolamide.

[247] In some embodiments, provided N-acylethanolamide derivative compounds are metabolized differently as compared to that of palmitoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a faster rate as compared to that of palmitoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a slower rate as compared to that of palmitoylethanolamide.

[248] In some embodiments, provided N-acylethanolamide derivative compounds when administered deliver palmitoylethanolamide or an active metabolite thereof. In some embodiments, provided N-acylethanolamide derivative compounds when administered display improved oral bioavailability as compared to the administration of palmitoylethanolamide.

[249] In some embodiments, a reference compound is or comprises oleoylethanolamide.

[250] In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are comparable to that of oleoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are improved as compared to oleoylethanolamide.

[251] In some embodiments, provided N-acylethanolamide derivative compounds display increased solubility that is comparable to that of oleoylethanolamide. In some particular

embodiments, aqueous solubility may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[252] In some embodiments, provided N-acylethanolamide derivative compounds display increased stability that is comparable to that of oleoylethanolamide.

[253] In some embodiments, provided N-acylethanolamide derivative compounds are metabolized differently as compared to that of oleoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a faster rate as compared to that of oleoylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a slower rate as compared to that of oleoylethanolamide.

[254] In some embodiments, provided N-acylethanolamide derivative compounds when administered deliver oleoylethanolamide or an active metabolite thereof. In some embodiments, provided N-acylethanolamide derivative compounds when administered display improved oral bioavailability as compared to the administration of oleoylethanolamide.

[255] In some embodiments, a reference compound is or comprises arachidonylethanolamide.

[256] In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are comparable to that of arachidonylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds display one or more activities that is/are improved as compared to arachidonylethanolamide.

[257] In some embodiments, provided N-acylethanolamide derivative compounds display increased solubility that is comparable to that of arachidonylethanolamide. In some particular embodiments, aqueous solubility may be assessed, for example, using an appropriate assay. In some embodiments, an appropriate assay is known in the art and/or described herein.

[258] In some embodiments, provided N-acylethanolamide derivative compounds display increased stability that is comparable to that of arachidonylethanolamide.

[259] In some embodiments, provided N-acylethanolamide derivative compounds are metabolized differently as compared to that of arachidonylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a faster rate as compared to that of arachidonylethanolamide. In some embodiments, provided N-acylethanolamide derivative compounds are metabolized at a slower rate as compared to that of arachidonylethanolamide.

[260] In some embodiments, provided N-acylethanolamide derivative compounds when administered deliver arachidonylethanolamide or an active metabolite thereof. In some

embodiments, provided N-acylethanolamide derivative compounds when administered display improved oral bioavailability as compared to the administration of arachidonylethanolamide.

Uses

[261] In some embodiments, the present disclosure provides methods of identifying and/or characterizing derivatives of an N-acylethanolamide compound (e.g., a parent N-acylethanolamide compound), which method comprising the steps of:

providing a derivative compound comprising a moiety modifying or otherwise linked to an N-acylethanolamide; and

determining that the derivative compound has one or more improved pharmacologic properties relative to the N-acylethanolamide compound.

[262] In some embodiments, the present disclosure provides technologies for identifying, assessing, and/or characterizing one or more activities or attributes of one or more provided N-acylethanolamide derivative compounds.

[263] In some embodiments, the present disclosure provides methods of treating a subject suffering from or susceptible to a disease, disorder, or condition, which method comprises a step of:

administering an N-acylethanolamide derivative or composition disclosed herein to a subject in need thereof.

[264] In some embodiments, an N-acylethanolamide derivative or composition disclosed herein is administered in combination with one or more other agents that treat the relevant disease, disorders, or conditions (or one or more symptoms thereof) from which a relevant subject is suffering.

[265] Various diseases, disorders, and/or conditions may be affected by an N-acylethanolamide. In some embodiments, the present disclosure provides methods comprising administering to a subject suffering from or susceptible to a disease, disorder, or condition a pharmaceutically effective amount of a provided compound or composition.

[266] In some embodiments, a disease, disorder, or condition is or comprises pain. In some embodiments, pain may be chronic pain. In some embodiments, pain may be or include lower back pain. In some embodiments, a disease, disorder, or condition is or comprises chronic lower back pain. In some embodiments, a disease, disorder, or condition is or comprises sciatica. In some embodiments, a disease, disorder, or condition is or comprises radiculopathy. In some

embodiments, a disease, disorder, or condition is or comprises radiating pain. Certain pain classification and representative indications are depicted in FIG. 1.

[267] In some embodiments, a disease, disorder, or condition is or comprises anxiety. In some embodiments, a disease, disorder, or condition is or comprises depression. In some embodiments, a disease, disorder, or condition is characterized by one or more symptoms of schizophrenia.

[268] In some embodiments, a disease, disorder, or condition is or comprises a neurologic, disease, disorder, or condition. In some embodiments, a disease, disorder or condition is or comprises Huntington's disease. In some embodiments, a disease, disorder or condition is or comprises Parkinson's disease. In some embodiments, a disease, disorder or condition is or comprises Alzheimer's disease. In some embodiments, a disease, disorder, or condition is or comprises Amyotrophic Lateral Sclerosis (ALS, also known as Lou Gehrig's disease). In some embodiments, a disease, disorder, or condition is or comprises multiple sclerosis. In some embodiments, a disease, disorder, or condition is or comprises neuropathic pain. In some embodiments, a disease, disorder, or condition is or comprises cerebral ischemia. In some embodiments, a disease, disorder, or condition is or comprises epilepsy.

[269] In some embodiments, a disease, disorder, or condition is or comprises appetite loss. In some embodiments, a disease, disorder, or condition is or comprises dental pain. In some embodiments, a disease, disorder, or condition is or comprises osteoarthritis. In some embodiments, a disease, disorder, or condition is or comprises reduced gastrointestinal motility.

[270] In some embodiments, a disease, disorder, or condition is or comprises cancer.

[271] In some embodiments, a disease, disorder, or condition is or comprises an ophthalmic condition. In some embodiments, a disease, disorder, or condition is or comprises glaucoma.

[272] In some embodiments, a disease, disorder, or condition is or comprises atopic dermatitis. In some embodiments, a disease, disorder, or condition is or comprises respiratory infection. In some embodiments, a disease, disorder, or condition is or comprises post-traumatic stress disorder. In some embodiments, a disease, disorder, or condition is or comprises obesity. In some embodiments, a disease, disorder, or condition is or comprises insomnia. In some embodiments, a disease, disorder, or condition is or comprises sleepiness.

[273] In some embodiments, the present disclosure provides methods of reducing gastrointestinal motility in a patient, which method comprising the step of administering a compound or composition disclosed herein to a subject in need thereof.

[274] In some embodiments, the present disclosure provides methods of reducing cancer cell proliferation in a patient or in a biological sample, which method comprising the step of administering to said patient or contacting said biological sample with a compound or composition disclosed herein.

[275] In some embodiments, the present disclosure provides methods of inducing lipolysis in a patient or in a biological sample, which method comprising the step of administering to said patient or contacting said biological sample with a compound or composition disclosed herein.

[276] In some particular embodiments, provided compounds including a butyric acid moiety is useful in the treatment of IBS-D and/or for the treatment of pain. Butyric acid (BA) is a critical component of gut heath and has been shown to decrease pain, reduce frequency and increased consistency of bowel movements for IBS-D patients.

[277] In some embodiments, provided treatments that utilize one or more compounds as described herein (e.g., treatment of pain) may deliver PEA at a level corresponding to a dose greater than or equal to 1200 mg/day PEA. In some embodiments, provided treatments may involve administration once or twice daily.

[278] In some embodiments, provided treatments that utilize one or more compounds as described herein (e.g., treatment of IBS-D) may deliver PEA at a level corresponding to a dose of about 3 g/day PEA.

[279] In some embodiments, provided treatments with butyric-acid-moiety-containing compounds as described herein (e.g., treatment of pain and/or IBS-D) may deliver PEA at a level corresponding to a dose of about 3 g/day PEA and/or may deliver BA at a level corresponding to a dose of about 1 g/day BA.

[280] In some embodiments, one or more particular compounds provided herein may be useful in the treatment of a plurality of different diseases, disorders or conditions; om some such embodiments, the compound may be differently formulated when utilized for different diseases, disorders or conditions.

Compositions

[281] In some embodiments, compounds as provided herein are prepared and/or utilized in compositions, such as pharmaceutical compositions. In some embodiments, a provided pharmaceutical composition comprises a therapeutically effective amount of a provided compound, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and

pharmaceutically acceptable carriers. In some embodiments, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or optic administration. In some embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

[282] In some embodiments, the present disclosure provides a pharmaceutical composition comprising a provided compound or composition, in a mixture with a pharmaceutically acceptable excipient.

[283] In therapeutic and/or diagnostic applications, provide compounds can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington, The Science and Practice of Pharmacy, (20th ed. 2000).

[284] Provided compounds and compositions thereof are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.01 to about 10000 mg, from about 0.01 to about 1000 mg, from about 0.5 to about 100 mg, from about 1 to about 50 mg per day, and from about 5 to about 100 mg per day are examples of dosages that may be used. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician.

[285] Pharmaceutically acceptable salts are generally well known to those of ordinary skill in the art, and may include, by way of example but not limitation, acetate, benzenesulfonate, besylate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington, The Science and Practice of Pharmacy (20th ed. 2000). Preferred pharmaceutically acceptable salts include, for example, acetate, benzoate, bromide, carbonate, citrate, gluconate, hydrobromide, hydrochloride, maleate, mesylate, napsylate, pamoate (embonate), phosphate, salicylate, succinate, sulfate, or tartrate.

[286] Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained- low release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington, The Science and Practice of Pharmacy (20th ed. 2000). Suitable routes may include oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articullar, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

[287] For injection, provided agents may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[288] Use of pharmaceutically acceptable inert carriers to formulate provided compounds or compositions into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection.

[289] The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable provided compounds and compositions to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (*e.g.*, patient) to be treated.

[290] For nasal or inhalation delivery, provided compounds or compositions may also be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances such as, saline, preservatives, such as benzyl alcohol, absorption promoters, and fluorocarbons.

[291] In certain embodiments, provided compounds and compositions are delivered to the CNS. In certain embodiments, provided compounds and compositions are delivered to the cerebrospinal fluid. In certain embodiments, provided compounds and compositions are administered to the brain parenchyma. In certain embodiments, provided compounds and compositions are delivered to an animal/subject by intrathecal administration, or

intracerebroventricular administration. Broad distribution of provided compounds and compositions, described herein, within the central nervous system may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration.

[292] In certain embodiments, parenteral administration is by injection, by, *e.g.*, a syringe, a pump, *etc.* In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue, such as striatum, caudate, cortex, hippocampus and cerebellum.

[293] Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[294] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, gels, syrups, suspensions, powders, or solutions.

[295] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[296] In some embodiments, cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[297] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in a mixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[298] Depending upon the particular condition, or disease state, to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may be administered together with provided compounds or compositions. For example, chemotherapeutic agents or other anti-proliferative agents may be combined with provided compounds or compositions to treat proliferative diseases and cancer. Examples of known chemotherapeutic agents include, but are not limited to, adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, and platinum derivatives.

Methods of Making

[299] In some embodiments, the present disclosure provides methods of manufacturing a provided N-acylethanolamide derivative compound, which method comprising steps of:

conjugating, or otherwise linking, a N-acylethanolamide compound (e.g., a parent N-acylethanolamide compound) to a linker moiety;

conjugating, or otherwise linking, a moiety to the linker-N-acylethanolamide moiety.

[300] In some embodiments, the present disclosure provides methods of manufacturing a provided N-acylethanolamide derivative compound, which method comprising steps of:

conjugating, or otherwise linking, a moiety to a linker moiety;

conjugating, or otherwise linking, a N-acylethanolamide compound (e.g., a parent N-acylethanolamide compound) to the linker moiety.

[301] In some embodiments, a moiety is selected from the group consisting of phosphate, butyric acid, glycerol, succinate, caprylic acid, gluconoic acid, eicosapentaeonoic acid, linoleic acid, succinate, and sucrose moieties, and combinations thereof.

[302] In some embodiments, the present disclosure provides method of manufacturing a provided pharmaceutical composition, which method comprising a step of:

formulating a provided N-acylethanolamide derivative compound together with at least one pharmaceutically acceptable carrier.

Exemplification

Example 1: (N-Palmitoylethanolamide) PEA in pain

[303] This Example provides a meta-analysis of 12 PEA pain studies and shows, among other things, that 67% of treated patients versus 21% of placebo achieved a VAS scores <= 3. See FIGs. 2A-2F, and the table below, quoted from Guida et al., Dolor 2010; Paladini et al., Pain Physician Jounrla, Feb. 2016; and Cobellis et al., Eur. J. Ob. and Gyn, Jul 2010.

	Etiopathogenesis			
	Degenerative	Neuropathic	Mixed	Miscellaneous
Patient number	1174 (79.1%)	170 (11.5%)	82 (5.5%)	58 (3.9%)

Example 2: Treating IBS-D

[304] The present Example illustrates treatment of IBS-D according to some embodiments of the present disclosure.

[305] The present disclosure provides a strong rationale to combine butyric acid (BA)and PEA into a single dual active prodrug that will be metabolized in the gut to the two active constituents that will alleviate pain and improve fecal consistency

[306] In some embodiments, IBS-D treatment success comprises simultaneous improvement in (i) daily worst abdominal pain score by ≥30% as compared to baseline weekly average; and (ii) reduction in the Bristol Stool Scale (BSS) to <5 on at least 50% of the days within a 12-week time interval. Alternatively or additionally, treatment success may be or comprise improvement in daily worst abdominal pain in the absence of a concurrent bowel movement. See FIGs. 2G-2Q, and the tables below, quoted from Scarpellini et al., Digestive Liver Disease, 2007; Banasiewicz et al., Colorectal Disease, 2012; Scheppach et al., Gastroenterology, 1992; Capasso et al., Br. J. of Pharm, 2014; and Borrelli et al., Br. J. of Pharm., 2015. For example, short-chain fatty acid irrigation has been shown to ameliorate inflammation in diversion colitis. In the study by Scheppach, et al., the effect of butyrate enemas was tested in 10 patients with distal ulcerative colitis who had been unresponsive to or intolerant of standard therapy for 8 weeks. They were treated for 2 weeks with sodium butyrate (100 mmol/L) and 2 weeks with placebo in random order (single-blind trial). Before and after treatment, clinical symptoms were noted and the degree of inflammation was graded endoscopically and histologically. Rectal proliferation was assessed by autoradiography. After butyrate irrigation, stool frequency (n/day) decreased from 4.7 ± 0.5 to 2.1 ± 0.4 ($P < 0.01$) and discharge of blood ceased in 9 of 10 patients. The endoscopic score fell from 6.5 ± 0.4 to 3.8 ± 0.8 ($P < 0.01$). The histological degree of

inflammation decreased from 2.4 ± 0.3 to 1.5 ± 0.3 ($P < 0.02$). Overall crypt proliferation was unchanged, but the upper crypt-labeling index fell from 0.086 ± 0.019 to 0.032 ± 0.003 ($P < 0.03$). On placebo, all of these parameters were unchanged. These data support the view that butyrate deficiency may play a role in the pathogenesis of distal ulcerative colitis and that butyrate irrigation ameliorates this condition. Scheppach, et al., "Effect of Butyrate Enemas on the Colonic Mucosa in Distal Ulcerative Colitis," Gastroenterology, 103:51-56 (1992).

Demographic characteristics of patients included

	IBS-CP ($n = 28$)	IBS-CP ($n = 22$)	p
Gender (male/female)	8/20	8/14	ns
Age [mean (SD)]	32 ± 5	34 ± 5	ns
BMI (body mass index)	21 ± 8	22 ± 7	ns

Effects of therapy on severity of symptoms

Symptom	IBS-DP		IBS-CP	
	at inclusion	after treatment	at inclusion	after treatment
Abdominal pain	9.5 (1)[a]	6.1 (1)*	9.2 (1)	9.4 (1)
Meteorism	9.6 (1)	4.7 (1)*	9.3 (1)	9.0 (1)
Flatulence	5.5 (1)	4.0 (1)*	5.1 (1)	5.1 (1)

[a] figure in brackets = SD

* Statistically significant ($p < 0.005$).

	MSB (*N* = 34)		Placebo (*N* = 32)		
	Number of patients	Percentage of study group	Number of patients	Percentage of study group	*P*
After 4 weeks of study Patients reporting subjective relief in IBS symptoms YES	11	32	2	6.25	<0.01
After 12 weeks of study Patients reporting subjective relief in IBS symptoms YES	18	53	5	15.6	<0.01

	MSB (*N* = 34)			Placebo (*N* = 32)			
	Mean	SD	Median	Mean	SD	Median	*P*
Baseline							
Spontaneous abdominal pain	0.53	0.51	1	0.53	0.51	1	ns
Postprandial abdominal pain	0.44	0.50	0	0.44	0.50	0	ns
Abdominal pain during defaecation	0.35	0.49	0	0.56	0.50	1	ns
Urge sensation after the defaection	0.26	0.45	0	0.38	0.49	0	ns
Mucus in stool	0.15	0.36	0	0.13	0.34	0	ns
Changes in stool consistency	0.44	0.50	0	0.38	0.49	0	ns
Constipation	0.38	0.49	0	0.47	0.51	0	ns
After 4 weeks of study							
Spontaneous abdominal pain	0.382	0.493	0	0.50	0.51	0.5	ns
Postprandial abdominal pain	**0.324**	**0.475**	**0**	**0.56**	**0.50**	**1**	**0.0968**
Abdominal pain during defaecation	**0.176**	**0.387**	**0**	**0.59**	**0.50**	**1**	**0.0032**
Urge sensation after defaecation	0.235	0.431	0	0.41	0.50	0	ns
Mucus in stool	0.088	0.288	0	0.13	0.34	0	ns
Changes in stool consistency	0.382	0.493	0	0.41	0.50	0	ns
Constipation	0.353	0.485		0.47	0.51	0	ns
After 12 weeks of study							
Spontaneous abdominal pain	**0.21**	**0.41**	**0**	**0.50**	**0.51**	**0.5**	**0.0132**
Postprandial abdominal pain	**0.21**	**0.41**	**0**	**0.56**	**0.50**	**1**	**0.0031**
Abdominal pain during defaecation	**0.15**	**0.36**	**0**	**0.59**	**0.50**	**1**	**0.0002**
Urge sensation after defaecation	**0.15**	**0.36**	**0**	**0.44**	**0.50**	**0**	**0.0100**
Mucus in stool	0.12	0.33	0	0.22	0.42	0	ns
Changes in stool consistency	**0.18**	**0.39**	**0**	**0.41**	**0.50**	**0**	**0.0417**
Constipation	**0.24**	**0.43**	**0**	**0.47**	**0.51**	**0**	**0.0493**

ns, not significant

There were no adverse effects in either arm o the study. Values are shown on proportions.

Example 3: Method Development, Plasma Stability, and Method Qualification for PEA and Prodrugs

[307] The present example describes an LC-MS/MS method to determine PEA levels in Sprague-Dawley rat plasma and to determine the stability of compounds(s) provided here.

[308] An LC-MS/MS method for the determination of PEA and PEA-prodrug **I-9** in Sprague-Dawley rat plasma was developed. Each test article was infused onto an ABSciex API4000 mass spectrometer to determine optimized parameters. Next, liquid chromatography conditions were developed to obtain suitable specificity and to resolve the PEA and PEA-prodrug peaks.

[309] The stability of the PEA-prodrug **I-9** was assessed in Sprague-Dawley rat plasma containing sodium heparin as the anticoagulant, and in acidified rat plasma containing citric acid and formic acid. The acidified plasma was prepared by collecting Sprague-Dawley rat plasma over sodium heparin and adding 100 μL of 0.5 M citric acid per mL of blood. The blood was centrifuged to generate plasma, and then 100 μL of 10% formic acid was added per mL of plasma.

[310] PEA-prodrug was added to each matrix to a final concentration of 1 μg/mL. Triplicate aliquots (50 μL) were immediately collected and added to 150 μL of acetonitrile containing internal standard. The remaining plasma aliquots were split equally. One aliquot was allowed to stand at room temperature while the second was placed on ice. After ninety minutes, triplicate aliquots of each sample were collected and added to acetonitrile. The samples were centrifuged at 13000 rpm for ten minutes, and the resulting supernatant was analyzed using the developed LC-MS/MS method. The peak area response ratios (PARR) of the analyte and internal standard from the incubated samples were then compared to the initial sample to determine the percent PEA prodrug remaining.

[311] Following their initial analysis, the supernatant samples from the acidified plasma experiment were reinjected after storage on the autosampler (~8 °C) for two hours in order to assess the stability of the PEA-prodrug in the post-extract matrix.

[312] Results of the stability experiments are shown in Tables 3a, 3b, and 3c.

Table 3a: Plasma Stability for PEA-Prodrug **I-9** in Sprague Dawley Rat Plasma

	Peak Area Response Ratio	**Average Peak Area Response Ratio**	**% Remaining**
Intial (t=0)	2.78E-01	2.78E-01	ND
	2.75E-01		
	2.81E-01		
90 minutes, ice	2.02E-01	2.04E-01	73.4%
	2.03E-01		
	2.07E-01		
90 minutes, RT	8.38E-04	5.65E-04	0.203%
	4.97E-04		
	3.60E-04		

ND: not determined

Table 3b: Plasma Stability for PEA-Prodrug **I-9** in Acidified Sprague-Daley Rat Plasma

	Peak Area Response Ratio	**Average Peak Area Response Ratio**	**% Remaining**
Intial (t=0)	1.31E+00	1.34E+00	ND
	1.40E+00		
	1.31E+00		
90 minutes, ice	1.39E+00	1.43E+00	107%
	1.46E+00		
	1.44E+00		
90 minutes, RT	1.23E+00	1.29E+00	96.0%
	1.30E+00		
	1.33E+00		

ND: not determined

Table 3c: Post-Extract Stability for PEA-Prodrug **I-9** Extracted from Acidified Sprague-Dawley Rat Plasma

	Peak Area Response Ratio	Average Peak Area Response Ratio	% Remaining
Intial (t=0)	1.31E+00	1.34E+00	ND
	1.40E+00		
	1.31E+00		
2 Hour Storage on Autosampler (~8 °C)	1.38E+00	1.42E+00	106%
	1.46E+00		
	1.42E+00		

ND: not determined

[313] Results of the stability of in each matrix are presented in Tables 3a and 3b. The prodrug was found to be unstable when stored at room temperature (0.203% remaining) and on ice (73.4% remaining) for 90 minutes. When the PEA-prodrug **I-9** was fortified into rat plasma acidified with citric and formic acids, it was found to be stable at room temperature (107% remaining) and on ice (96.0% remaining) for 90 minutes. In addition, it was demonstrated that the PEA-prodrug **I-9** was stable in the post-extract matrix following storage on the autosampler (~8 °C) for two hours (Table 3c).

[314] The specificity, accuracy, and precision of the method for PEA and the PEA-prodrug **I-9** in acidified Sprague-Dawley rat plasma were evaluated via a single-day pre-study qualification. A single eight-point standard curve and quality control samples at three levels with six replicates each were extracted and analyzed for PEA or PEA-prodrug **I-9**. In addition, a dilution QC of a high concentration sample was prepared to demonstrate parallelism of the method. Standards and quality control samples were prepared from independently prepared stock solutions of each test compound. Results of the plasma qualification are presented in Tables 3d and 3e.

Table 3d: Method Qualification Results for PEA in Acidified Rat Plasma

Nominal Conc. (ng/mL)	12.5	100	500	10000
Measured Concentration (ng/mL)	10.3	105	509	10800
	12.6	107	477	11800
	11.6	110	451	10100
	12.2	96.8	496	13100
	11.0	101	441	10900
	14.0	109	448	10800
Average (ng/mL)	12.0	105	470	11250
Accuracy (%)	95.6	105	94.1	113
CV (%)	10.9	4.83	5.96	9.39
n	6	6	6	6

Table 3e: Method Qualification Results for PEA-Prodrug **I-9** in Acidified Rat Plasma

Nominal Conc. (ng/mL)	2.50	50.0	500	10000
Measured Concentration (ng/mL)	2.62	48.2	537	10600
	2.12	50.2	517	10700
	2.35	48.7	522	10300
	2.39	49.5	536	10600
	2.23	51.2	567	10900
	2.15	48.2	547	10900
Average (ng/mL)	2.31	49.3	538	10667
Accuracy (%)	92.4	98.7	108	107
CV (%)	8.03	2.44	3.35	2.11
n	6	6	6	6

[315] Plasma samples were extracted using the methods described below.

[316] Analytical stock solutions (1.00 mg/mL of the free drug) were prepared in DMSO.

[317] Sprague-Dawley rat blood was collected over sodium heparin and 0.5 M citric acid was added at a rate of 100 µL per mL of blood. Blood was centrifuged to collect plasma. A 100 µL aliquot of 10% formic acid was added to each mL of plasma.

[318] Standards were prepared in acidified rat plasma. Standards and quality control samples were prepared from independently prepared stock solutions of each analyte. Working solutions were prepared in 50:50 acetonitrile: water and then added to plasma to make calibration standards to final concentrations of 1000, 500, 100, 50, 10, 5, 1, and 0.5 ng/mL and quality control samples to final concentrations of 2.50, 50.0, and 500 ng/mL for PEA-prodrug **I-9**. For PEA, calibration standards were prepared to final concentrations of 1000, 500, 100, 50, 25, 10, 5, and 2.5 ng/mL and quality control samples to final concentrations of 12.50, 100, and 500 ng/mL.

A high concentration dilution QC was prepared at 10,000 ng/mL for each analyte. This sample was diluted 20-fold into the range of the assay prior to extraction.

[319] Plasma samples were extracted via acetonitrile precipitation. Standards and QCs: Add 10 µL of appropriate working solution to 50 µL of blank matrix in a 96-well plate. Blanks: Add 10 µL 50:50 acetonitrile: water to 50 µL of blank matrix in a 96-well plate. Samples: Add 10 µL 50:50 acetonitrile: water to 50 µL of study sample in a 96-well plate. Cap and mix. Add 150 µL of acetonitrile (containing 100 ng/mL ritonavir as an internal standard) to each well. Cap and mix at 1000 rpm for five minutes. Centrifuge the plate at 3000 rpm for ten minutes. Transfer a 150 µL aliquot of the resulting supernatant into a clean 96-well plate. Cap for analysis. HPLC and Mass spectrometry conditions are described in Table 3f. Chromatograms are exemplified in Figures 3A, 3B, 3C, 3D, 3E, 3F, 3G, and 3H.

Table 3f: HPLC and Mass Spectrometry Conditions

HPLC Conditions

Instrument: Waters Acquity UPLC
Column: Waters HSS C_{18}, 30 x 2.1 mm id, 1.8µm
Mobile Phase Buffer: 40 mM ammonium formate, pH 3.5
Aqueous Reservoir (A): 10% buffer, 90% water
Organic Reservoir (B): 10% buffer, 90% acetonitrile
Gradient Program

Time (min)	Grad. Curve	%A	%B
0.0	6	20	80
0.75	6	0	100
0.80	6	50	50
1.0	6	50	50

Flow Rate: 800 µL/min
Injection Volume: 5 µL
Run Time: 1.0 min
Column Temperature: 40°C
Sample Temperature: 8°C
Strong Autosampler Wash: 1:1:1 (v:v:v) water:methanol:isopropanol with 0.2% formic acid
Weak Autosampler Wash: 4mM ammonium formate

Mass Spectrometer Conditions

Instrument: PE Sciex API4000
Interface: Electrospray ("Turbo Ion Spray"
Mode: Multiple reactions monitoring (MRM)
Gases: CUR 30, CAD 10, GSI 50, GS2 50
Source Temperature: 500°C
Voltages and Ions Monitored*

Analyte	Polarity	Precursor Ion	Product Ion	IS	DP	EP	CE	CXP
PEA	Positive	300.2	62.1	5500	106	10	30	6
PEA-prodrug	Positive	474.5	282.4	5500	80	10	24	7
Ritonavir (Internal STD)	Positive	721.3	296.1	5500	65	10	25	18

IS: Ion Spray Voltage; DP: Declustering Potential; EP: Entrance Potential; CE: Collision Energy; CXP: Collision Cell Exit Potential; *All settings are in volts

Example 4: PEA Stability in Human and Rat Liver Microsomes, Human and Rat Intestinal S9 Fractions, and Simulated Gastric fluid

[320] The present Example describes PEA stability observed in Human and Rat Liver Microsomes, Human and Rat Intestinal S9 Fractions, and Simulated Gastric fluid.

Liver Microsomal Stability

[321] Mixed-gender human liver microsomes (Lot# 1210347) and male Sprague-Dawley rat liver microsomes (Lot# 1310030) were provided. The reaction mixture, minus cofactors, was prepared as described below. The test article was added into the reaction mixture at a final concentration of 1 µM. The control compound, testosterone, was run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37 °C for 3 minutes. The reaction was initiated by the addition of cofactor, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 µL) were withdrawn at 0, 10, 20, 30, and 60 minutes. All samples were immediately combined with 400 µL of ice-cold 50/50 acetonitrile/H_2O containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. All samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug **I-9** and the expected drug (PEA). Analytical conditions are outlined in Appendix 4-1. The test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 4a and 4b.

Reaction Composition

Liver Microsomes 0.5 mg/mL

NADPH (cofactor) 1 mM

UDPGA (cofactor) 1 mM

Potassium Phosphate, pH 7.4 100 mM

Magnesium Chloride 5 mM

Test Article 1 µM

Table 4a: PEA stability observed in Human and Rat Liver Microsomes

Test Article	Species	% Remaining of Initial (n=1)					Half-Life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-9	Human	100	1.2	<1.0	<1.0	<1.0	<10(1.6)	>0.139 (0.888)
	Rat	100	4.0	<1.0	<1.0	<1.0	<10(2.2)	>0.139 (0.642)

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Similarly, if the calculated half-life is less than the shortest time point, the half-life is expressed as < that time point and the calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	Cl_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	17	0.0792	≤41
	Rat	1.4	1.03	≤15

Table 4b: Measured concentrations of Prodrug and Drug

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-9	Human	I-9	0.18	0.0021	0	0	0
		PEA	0.83	0.60	0.51	0.41	0.20
	Rat	I-9	0.21	0.0085	0	0	0
		PEA	0.35	0.16	0.076	0.027	0.0030

Intestinal S9 Fraction Stability

[322] Mixed-gender human intestinal S9 fraction (Lot# 0710351) and male Sprague-Dawley rat intestinal S9 fraction (Lot# 0510116) were provided. The reaction mixture, minus cofactors, was prepared as described below. The test article was added into the reaction mixture at a final concentration of 1 μM. The control compounds, testosterone and 7-hydroxycoumarin, were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. The reaction was initiated by the addition of cofactor cocktail, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. All samples were immediately combined with 400 μL of icecold 50/50 acetonitrile/H_2O

containing 0.1% formic acid and internal standard to terminate the reaction. The samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. All samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug **I-9** and the expected drug (PEA). Analytical conditions are outlined in Appendix 4-1. The test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to single-phase exponential decay equation. Results are shown in Tables 4c and 4d.

Reaction Composition

Intestinal S9 Fraction 1.0 mg/mL

NADPH (cofactor) 1 mM

UDPGA (cofactor) 1 mM

PAPS (cofactor) 1 mM

GSH (cofactor) 1 mM

Potassium Phosphate, pH 7.4 100 mM

Magnesium Chloride 5 mM

Test Article 1 μM

Table 4c: PEA stability observed in Human and Rat Intestinal S9 Fraction

Test Article	Species	% Remaining of Initial (n=1)					Half-Life[a] (min)	CL_{int}[b] (mL/min/ mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-9	Human	100	59	16	47	<1.0	<10(9.2)	>0.0693 (0.0753)
	Rat	100	119	88	75	49	55	0.125

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Similarly, if the calculated half-life is less than the shortest time point, the half-life is expressed as < that time point and the calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	Cl_{int} (ml/min/mg protein)
Testosterone	Human	26	0.0269
	Rat	116	0.00597
7-HC	Human	7.3	0.943
	Rat	34	0.0201

Table 4d: Measured Concentrations of Prodrug and Drug

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-9	Human	I-9	0.61	0.36	0.099	0.029	0.0043
		PEA	0.21	0.50	0.69	0.65	0.66
	Rat	I-9	0.77	0.91	0.68	0.58	0.38
		PEA	0.031	0.088	0.13	0.16	0.24

Simulated Gastric Fluid Stability

[323] Studies were carried out in simulated gastric fluid (SGF). SGF was prepared by dissolving 2.0 g of NaCl and 3.2 g of purified pepsin (derived from porcine stomach mucosa) in 7 mL of 10 N HCl and sufficient water to make 1000 mL. The pH was adjusted to pH 1.2. Control experiments were also run without the addition of pepsin to the matrix. The test article was added into the SGF at 37 °C at a final concentration of 2 μM, and incubated in a shaking water bath at 37 °C. Individual tubes were dosed for each time point (0, 15, 30, 60, and 120 minutes). At the appropriate time, 500 μL of ice-cold acetonitrile containing 0.1% formic acid and internal standard was added to a single tube. The starting time of each tube was staggered so that all timepoints finished simultaneously. The samples were then mixed and centrifuged. Calibration standards were prepared in matched matrix. All samples and standards were assayed by LCMS/ MS using electrospray ionization for both the dosed prodrug **I-9** and the expected drug (PEA). Analytical conditions are outlined in Appendix 4-1. The test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 4e and 4f.

Table 4e: PEA stability observed in Simulated Gastric Fluid

Test Article	Species	% Remaining of Initial (n=1)					Half-Life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-9	SGF w/pepsin	100	95	80	92	86	>120
	SGF w/o pepsin	100	93	84	72	71	>120

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time.

Table 4f: Measured Concentrations of Prodrug and Drug

Dosed Test Article	Matrix	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-9	Human	I-9	1.52	1.45	1.21	1.40	1.31
		PEA	0.0046	0.0078	0.0083	0.011	0.017
	Rat	I-9	2.07	1.93	1.74	1.49	1.48
		PEA	0.0046	0.0066	0.0067	0.0079	0.017

Microsome/S9 Fraction Control

[324] As a control for the liver microsome and intestinal S9 fraction studies, additional experiments were run in reaction mixture, with the exclusion of microsomal proteins. The reaction mixture was prepared as described below. The test article was added into the reaction mixture at a final concentration of 2 μM, and then incubated in a shaking water bath at 37 °C. Individual tubes were dosed for each time point (0, 10, 20, 30, and 60 minutes). At the appropriate time, 500 μL of ice-cold acetonitrile containing 0.1% formic acid and internal standard was added to a single tube. The starting time of each tube was staggered so that all timepoints finished simultaneously. The samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. All samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug **I-9** and the expected drug (PEA). Analytical conditions are outlined in Appendix 4-1. The test article concentration at each timepoint was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Table 4g and 4h.

Reaction Composition

NADPH (cofactor) 1 mM

UDPGA (cofactor) 1 mM

PAPS (cofactor) 1 mM

GSH (cofactor) 1 mM

Potassium Phosphate, pH 7.4 100 mM

Magnesium Chloride 5 mM

Test Article 2 µM

Table 4g: Microsome/S9 Fraction Control

Test Article	Matrix	% Remaining of Initial (n=1)					Half-Life[a] (min)
		0 min	10 min	20 min	30 min	60 min	
I-9	Control	100	66	77	67	62	>60

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time.

Table 4h: Measured Concentration of Prodrug and Drug

Dosed Test Article	Species	Analyte	Concentration (µM)				
			0 min	10 min	20 min	30 min	60 min
I-9	Control	I-9	2.24	1.47	1.72	1.5	1.38
		PEA	0	0	0	0	0

Appendix 4-1

Liquid Chromatography

Column: Waters ACQUITY UPLC BEH C18 30 x 2.1 mm. 1.7 µm
M.P. Buffer: 25 mM ammonium formate buffer, pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.8 mL/minute
Gradient Program:

Time (min)	% A	% B
0.00	20	80
0.75	0	100
0.80	50	50
1.00	50	50

Total Run Time: 1.0 minutes
Autosampler: 10 µL Injection Volume
Wash 1: water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash 2: 0.1% formic acid in water

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 1.0 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-9	+474.5/282.4	80	10	24	7	5500	500	7	30	50	50
PEA	+300.2/62.1	106	10	30	6	5500	500	7	30	50	50
Warfarin(IS)	+309.2/251.1	80	10	30	5	5500	500	7	30	50	50

Example 5: Determination of the Oral Bioavailability of PEA Following Administration of PEA and PEA-Prodrug in Male Sprague-Dawley Rats

[325] The present Example describes PEA levels observed in Sprague-Dawley rats samples after oral administration of compound(s) provided herein.

[326] The oral bioavailability of palmitoylethanolamide (PEA) was evaluated following oral dosing of PEA, a marketed PEA product, Normast, or a PEA-prodrug **I-9**. PEA was also dosed intravenously at 1 mg/kg. Blood samples were collected up to 8 hours post-dose, and PEA and PEA-prodrug plasma concentrations were determined with a qualified LC-MS/MS method. Pharmacokinetic analysis was conducted by a non-compartmental model using Phoenix WinNonlin v.6.4 software.

Preparation of Dosing Formulations

[327] PEA for IV and PO dosing (St. Louis, MO). PEA-prodrug (lot 261-SB-85) and Normast (Epitech Group, lot D106C6) were provided. The IV dosing solution was prepared fresh on the day of dosing at 0.5 mg/mL in a vehicle comprised of 10% solutol HS15, 10% n-methylpyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400), and 70% water. For PO dosing, torpac capsules were loaded with an appropriate amount of PEA, PEA-prodrug, or normast powder. Doses in groups 2, 3, and 4 were prepared to deliver a similar amount of active drug per rat. The PEA-prodrug is 63.2% (w/w) active and Normast contains 72.7% active (w/w). The prodrug dose in group 5 was the maximum amount of powder that would fit into a single capsule.

[328] The pharmacokinetics of PEA and the PEA-prodrug were evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Rats intended for IV dosing were fitted with an additional JVC for dosing. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study.

Animal Dosing

[329] Blood samples (~ 300 µL) were collected from the rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 µL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 µL) was then transferred to a chilled, labeled

polypropylene tube containing 15 µL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 5a.

Table 5a: Study Design

Dose Group	Test Article	No. of Animals	Dosing Route	Dose (mg/kg)	Dosing Formulation Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sampling Time Points
1	PEA	3	IV	1	0.5	2	10% Solutol HS15, 10% NMP, 10% PEG400, 70% water	Pre-dose, 5, 15, 30 min, 1, 2, 4 and 8 hours
2	PEA	3	PO	~10	NA	1 capsule	Torpac capsule	Pre-dose, 15, 30 min, 1, 2, 4, and 8 hours
3	PEA-prodrug	3	PO	~16*	NA	1 capsule	Torpac capsule	
4	RLD (Normast)	3	PO	~16*	NA	1 capsule	Torpac capsule	
5	PEA-prodrug	3	PO	~126*	NA	1 capsule	Torpac capsule	

NMP: n-methyl pyrrolidone; *mg of actual pro-drug or drug product, not corrected for active content.

[330] An LC-MS/MS method for the determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[331] Pharmacokinetic parameters were calculated from the time course of the plasma concentration and are presented in Tables 3 through 9. Pharmacokinetic parameters were determined with Phoenix WinNonlin (v6.4) software using a non-compartmental model. Maximum plasma concentrations (C_0) after IV dosing were estimated by extrapolation of the first two time points back to t=0. Maximum plasma concentration (C_{max}) and the time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from the data. Area under the time concentration curve (AUC) was calculated using the linear trapezoidal rule with calculation to the last quantifiable data point, and with extrapolation to infinity if applicable. At least three quantifiable data points were required to determine the AUC. Plasma half-life ($t_{1/2}$) was calculated from 0.693/slope of the terminal elimination phase. Mean residence time, MRT, was calculated by dividing the area under the moment curve (AUMC) by the AUC. Clearance (CL) was calculated from dose/AUC. Steady-state volume of distribution (V_{ss}) was calculated from CL*MRT (mean residence time). Bioavailability was determined by dividing the

individual PO dose normalized AUC_{last} values by the average IV AUC_{last} value. Any samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

[332] The IV dosing solution was analyzed by LC-MS/MS. The measured dosing solution concentration is shown in Table 5b. The dosing solutions were diluted into rat plasma and analyzed in triplicate. All concentrations are expressed as mg/mL of the free base. Capsules were not analyzed. Nominal dosing concentrations were used in all calculations for these groups.

Table 5b: Measured Dosing Solution Concentrations (mg/mL).

Test Article	Route of Administration	Vehicle	Nominal Dosing Conc. (mg/mL)	Measured Dosing Solution Conc. (mg/mL)	% of Nominal
PEA	IV	10% Solutol HS15, 10% NMP, 10% PEG400, 70% water	0.5	0.382	76.3

NMP: n-methyl pyrrolidone

[333] Endogenous levels of PEA were found in all rats. Measured concentrations of PEA in plasma samples were corrected by subtracting the concentration of PEA measured in the pre-dose samples. These corrected values are reported in the tables below and were used to determine pharmacokinetic parameters. Any corrected values that were negative are reported as not determined (ND).

[334] Following IV dosing at 1 mg/kg, PEA had an average half-life of 0.596 ± 0.165 hours, an average clearance rate of 15.1 ± 3.15 L/hr/kg and an average volume of distribution of 9.12 ± 0.832 L/kg. Results are shown in Table 5c and FIGs. 4A and 4B.

Table 5c: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Intravenous Administration of PEA in Male Sprague-Dawley Rats at 1 mg/kg (Group 1).

Intravenous (1 mg/kg)					
Time (hr)	**Rat #**				
	907	**908**	**909**	**Mean**	**SD**
0 (pre-dose)	10.3	6.96	6.50	7.92	2.07
0.083	122	**137**	174	144	26.6
0.25	**47.2**	**49.6**	**47.7**	48.2	1.29
0.50	**27.5**	**30.7**	**35.5**	31.2	4.02
1.0	**9.60**	**7.94**	**16.1**	11.2	4.31
2.0	**5.20**	**5.64**	**9.90**	6.91	2.60
4.0	ND	ND	ND	ND	ND
8.0	ND	ND	ND	ND	ND
Animal Weight (kg)	0.275	0.265	0.262	0.267	0.007
Volume Dosed (mL)	0.55	0.53	0.52	0.53	0.02
C_0 (ng/mL)[1]	196	227	331	251	70.9
t_{max} (hr)[1]	0	0	0	0	0
$t_{1/2}$ (hr)	0.564	0.449	0.774	0.596	0.165
MRT_{last} (hr)	0.399	0.374	0.435	0.402	0.0306
CL (L/hr/kg)	17.4	16.4	11.5	15.1	3.15
V_{ss} (L/kg)	10.0	8.39	8.94	9.12	0.832
AUC_{last} (hr ng/mL)	53.3	57.2	75.8	62.1	012.0
AUC_{∞} (hr ng/mL)	57.6	60.8	86.8	68.4	16.0

C_0: maximum plasma concentration extrapolated to t=0; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; CL: clearance; V_{ss}: steady state volume of distribution; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; [1]Extrapolated to t=0.

[335] Following oral dosing of PEA in group 2 (average dose of 11.7 mg/kg), nearly all plasma samples were below the endogenous levels measured in the predose samples. The highest concentration measured in one animal was 5.30 ng/mL at 2 hours post dose. No AUCs or bioavailability values could be determined for this group. Results are shown in Table 5d.

Table 5d: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA After Oral Administration of PEA in Male Sprague-Dawley Rats at ~10 mg/kg (Group 2).

Oral (~10 mg/kg, PEA after PEA Dose)					
Time (hr)	**Rat #**				
	910	**911**	**912**	**Mean**	**SD**
0 (pre-dose)	8.04	8.30	6.00	7.45	1.26
0.25	ND	ND	ND	ND	ND
0.50	ND	ND	ND	ND	ND
1.0	ND	ND	2.34	ND	ND
2.0	ND	ND	5.30	ND	ND
4.0	ND	ND	ND	ND	ND
8.0	ND	ND	ND	ND	ND
Animal Weight (kg)	0.250	0.260	0.262	0.257	0.006
Volume Dosed (mg)	3.0	3.0	3.0	3.0	0.0
Dose (mg/kg)	12.0	11.5	11.5	11.7	0.295
C_{max} (ng/mL)	ND	ND	5.30	ND	ND
t_{max} (hr)	N	ND	2.0	ND	ND
$t_{1/2}$ (hr)	ND	ND	ND	ND	ND
MRT_{last} (hr)	ND	ND	ND	ND	ND
AUC_{last} (hr ng/mL)	ND	ND	ND	ND	ND
AUC_{∞} (hr ng/mL)	ND	ND	ND	ND	ND
Dose-normalized Values					
AUC_{last} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND
Bioavailability (%)	ND	ND	ND	ND	ND

C_0: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined.

[336] Following oral dosing of the PEA-prodrug in group 3 (average dose of 11.7 mg/kg active equivalents), nearly all plasma samples were below the endogenous levels measured in the predose samples. The highest concentration measured in one animal (rat 914) was 0.630 ng/ml at 0.25 hours post dose. The bioavailability in this animal was 0.108%. Results are shown in Table 5e and 5f.

Table 5e: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA-Prodrug After Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats at ~16 mg/kg (Group 3).

Oral (~16 mg/kg, PEA-prodrug after PEA-prodrug Dose)					
Time (hr)	**Rat #**				
	913	**914**	**915**	**Mean**	**SD**
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.25	BLOQ	BLOQ	BLOQ	ND	ND
0.50	BLOQ	BLOQ	BLOQ	ND	ND
1.0	BLOQ	BLOQ	BLOQ	ND	ND
2.0	BLOQ	BLOQ	BLOQ	ND	ND
4.0	BLOQ	BLOQ	BLOQ	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.251	0.266	0.259	0.259	0.008
Amount Dosed (mg)	4.8	4.8	4.8	4.8	0
Dose (mg/kg)	19.1	18.0	18.5	18.6	0.540
C_{max} (ng/mL)	ND	ND	ND	ND	ND
t_{max} (hr)	ND	ND	ND	ND	ND
$t_{1/2}$ (hr)	ND	ND	ND	ND	ND
MRT_{last} (hr)	ND	ND	ND	ND	ND
AUC_{last} (hr ng/mL)	ND	ND	ND	ND	ND
AUC_{∞} (hr ng/mL)	ND	ND	ND	ND	ND
Dose-normalized Values					
AUC_{last} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (0.5 ng/mL).

Table 5f: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA After Oral Administration of PEA Prodrug in Male Sprague-Dawley Rats at ~16 mg/kg (Group 3).

Oral (~16 mg/kg, PEA after PEA-prodrug Dose)					
Time (hr)	**Rat #**				
	913	**914**	**915**	**Mean**	**SD**
0 (pre-dose)	6.23	3.80	5.17	5.07	1.22
0.25	ND	0.630	ND	ND	ND
0.50	ND	ND	ND	ND	ND
1.0	ND	0.500	ND	ND	ND
2.0	ND	ND	ND	ND	ND
4.0	ND	0.230	ND	ND	ND
8.0	ND	ND	ND	ND	ND
C_{max} (ng/mL)	ND	0.630	ND	ND	ND
t_{max} (hr)	ND	0.25	ND	ND	ND
$t_{1/2}$ (hr)	ND	ND[3]	ND	ND	ND
MRT_{last} (hr)	ND	1.75	ND	ND	ND
AUC_{last} (hr ng/mL)	ND	0.763	ND	ND	ND
AUC_{∞} (hr ng/mL)	ND	ND[3]	ND	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	ND	0.0669	ND	ND	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND[3]	ND	ND	ND
Bioavailability (%)[2]	ND	0.108	ND	ND	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; [1]dose normalized values determined by dividing the parameter by the dose in mg/kg; [2]bioavailability determined by dividing the individual dose normalized AUC_{last} value by the average IV AUC_{last} value; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

[337] Following oral dosing of Normast in group 4 (average dose of 18.5 mg/kg), many plasma samples were below the endogenous levels measured in the predose samples. Average maximum plasma concentration (n=3) was 3.38 ± 2.17 ng/mL. Average bioavailability (n=2) was 0.561%. Results are shown in Table 5g.

Table 5g: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA After Oral Administration of Normast in Male Sprague-Dawley Rats at ~16 mg/kg (Group 4).

Oral (~16 mg/kg, PEA after Normast Dose)					
Time (hr)	**Rat #**				
	916	**917**	**918**	**Mean**	**SD**
0 (pre-dose)	4.43	2.36	4.05	3.61	1.10
0.25	ND	0.200	ND	ND	ND
0.50	ND	ND	2.19	ND	ND
1.0	1.11	ND	5.83	3.47	ND
2.0	ND	0.960	2.10	1.53	ND
4.0	2.62	1.69	ND	2.16	ND
8.0	ND	ND	ND	ND	ND
Animal Weight (kg)	0.264	0.25	0.263	0.259	0.008
Amount Dosed (mg)	4.8	4.8	4.8	4.8	0.0
Dose (mg/kg)	18.2	19.2	18.3	18.5	0.569
C_{max} (ng/mL)	2.62	1.69	5.83	3.38	2.17
t_{max} (hr)	4.0	4.0	1.0	3.0	1.7
$t_{1/2}$ (hr)	ND	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	ND	3.04	1.10	2.07	ND
AUC_{last} (hr ng/mL)	ND	3.18	6.24	4.71	ND
AUC_{∞} (hr ng/mL)	ND	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	ND	0.227	0.469	0.348	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	ND	0.366	0.756	0.561	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; [1]dose normalized values determined by dividing the parameter by the dose in mg/kg; [2]bioavailability determined by dividing the individual dose normalized AUC_{last} value by the average IV AUC_{last} value; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

[338] Following oral dosing of the PEA-prodrug in group 5 (average dose of 90.6 mg/kg active equivalents), many plasma samples were below the endogenous levels measured in the predose samples. Average maximum plasma concentration (n=3) was 2.52 ± 0.829 ng/mL. Bioavailability determined in one animal was 0.124%. Results are shown in Table 5h and 5j.

Table 5h: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA-Prodrug After Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats at ~126 mg/kg (Group 5)

Oral (~126 mg/kg, PEA-prodrug after PEA-prodrug Dose)					
Time (hr)	**Rat #**				
	919	**920**	**921**	**Mean**	**SD**
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.25	BLOQ	BLOQ	BLOQ	ND	ND
0.50	BLOQ	BLOQ	BLOQ	ND	ND
1.0	BLOQ	BLOQ	BLOQ	ND	ND
2.0	BLOQ	BLOQ	BLOQ	ND	ND
4.0	BLOQ	BLOQ	BLOQ	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.263	0.269	0.263	0.265	0.003
Amount Dosed (mg)	38	38	38	38	0
Dose (mg/kg)	144	141	144	143	1.86
C_{max} (ng/mL)	ND	ND	ND	ND	ND
t_{max} (hr)	ND	ND	ND	ND	ND
$t_{1/2}$ (hr)	ND	ND	ND	ND	ND
MRT_{last} (hr)	ND	ND	ND	ND	ND
AUC_{last} (hr ng/mL)	ND	ND	ND	ND	ND
AUC_{∞} (hr ng/mL)	ND	ND	ND	ND	ND
Dose-normalized Values					
AUC_{last} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (0.5 ng/mL).

Table 5j: Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA After Oral Administration of PEA Prodrug in Male Sprague-Dawley Rats at ~126 mg/kg (Group 5).

Oral (~126 mg/kg, PEA after PEA-prodrug Dose)					
Time (hr)	**Rat #**				
	919	**920**	**921**	**Mean**	**SD**
0 (pre-dose)	1.82	1.57	2.40	1.93	0.426
0.25	ND	ND	ND	ND	ND
0.50	ND	ND	ND	ND	ND
1.0	ND	3.47	ND	ND	ND
2.0	0.690	1.84	0.450	0.993	0.743
4.0	1.93	1.49	2.17	1.86	0.345
8.0	ND	ND	ND	ND	ND
C_{max} (ng/mL)	1.93	3.47	2.17	2.52	0.829
t_{max} (hr)	4.0	1.0	4.0	3.0	1.7
$t_{1/2}$ (hr)	ND	ND	ND	ND	ND
MRT_{last} (hr)	ND	2.06	ND	ND	ND
AUC_{last} (hr ng/mL)	ND	6.85	ND	ND	ND
AUC_{∞} (hr ng/mL)	ND	ND	ND	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	ND	0.0767	ND	ND	ND
AUC_{∞} (hr kg ng/mL/mg)	ND	ND	ND	ND	ND
Bioavailability (%)[2]	ND	0.124	ND	ND	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; [1]dose normalized values determined by dividing the parameter by the dose in mg/kg; [2]bioavailability determined by dividing the individual dose normalized AUC_{last} value by the average IV AUC_{last} value; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

[339] The PEA-prodrug was not detectable in any plasma samples. No pharmacokinetic parameters were determined for the PEA-prodrug.

Example 6: PEA Levels in Rabbit Plasma Samples

[340] The present Example describes PEA levels observed in rabbit plasma samples after oral administration of compound(s) provided herein. Blood samples were taken approximately five hours after the morning dosing.

[341] Instability of the PEA-prodrug **I-9** in untreated plasma samples has been demonstrated previously (see, e.g., Examples 3-5). Plasma samples from the current study were not treated to prolong the post-collection stability of the prodrug. Instead, all samples were allowed to stand, thawed and untreated, at room temperature for four hours prior to extraction and analysis. This strategy allowed for any remaining prodrug in the sample to convert to PEA. All reported concentrations are total PEA in the sample.

[342] Seventy-nine New Zealand White rabbit plasma samples were analyzed with a previously developed LC-MS/MS method for the detection of palmitoylethanolamide (PEA). A total of 79 plasma samples were received for analysis. All samples were received frozen in good condition. Samples were stored at -80 °C until analysis.

[343] An LC-MS/MS method for the determination of PEA in plasma was used to quantify samples in this study. See, e.g., Example 3. Study samples were extracted using the methods described in Examples 3-5.

[344] Results are shown in Tables 6a through 6d

Table 6a.: Individual and Average PEA Concentrations (ng/ML) in Group 1 (Vehicle treated) Plasma Samples.

Day	Animal ID					Average	SD
	R3145	R2038	R2769	R3147	R3154		
-5	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	BLOQ	2.77	BLOQ	BLOQ	ND	ND
7	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND

BLOQ: below the limit of quantitation (2.5 ng/mL); ND: not determined.

Table 6b: Individual and Average PEA Concentrations (ng/mL) in Group 2 (Normast treated at 32mg/kg/day) Plasma Samples

Day	Animal ID					Average	SD
	R2771	R3152	R3143	R3138	R3140		
-5	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	2.97	4.52	BLOQ	BLOQ	3.75	ND
7	BLOQ	BLOQ	BLOQ	5.29	BLOQ	ND	ND

BLOQ: below the limit of quantitation (2.5 ng/mL); ND: not determined.

Table 6c: Individual and Average PEA Concentrations (ng/mL) in Group 3 (PEA-prodrug treated at 32mg/kg/day) Plasma Samples

Day	Animal ID					Average	SD
	R3156	R2050	R2833	R2037	R2770		
-5	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
3	6.64	8.70	10.8	4.14	6.63	ND	ND
7	5.62	6.09	4.47	BLOQ	5.57	ND	ND

BLOQ: below the limit of quantitation (2.5 ng/mL); ND: not determined.

Table 6d: Individual and Average PEA Concentrations (ng/mL) in Group 4 (PEA-prodrug at 160 mg/kg/day) Plasma Samples

Day	Animal ID					Average	SD
	R3141	R3139	R3153	R3157	R3151		
-5	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
-3	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ND	ND
3	27.6	20.0	27.6	24.7	17.0	23.4	4.73
7	7.10	8.12	9.93	14.5	14.3	10.8	3.45

BLOQ: below the limit of quantitation (2.5 ng/mL); ND: not determined.

[345] One sample from the vehicle group (Group 1) is reported with a measureable PEA concentration (2.77 ng/mL, Day 3, Animal R2769). This positive result is likely due to the fact that PEA is an endogenous fatty acid. Several samples from the vehicle group and predose time points were near the LLOQ of the method (2.5 ng/mL), but this was the only such sample that exceeded the LLOQ.

Example 7: Determination of Bioavailability of PEA Following Oral (PO) Administration of PEA-Prodrugs in Male Sprague-Dawley Rats

[346] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs **I-2**, **I-3**, **I-5**, **I-6**, **I-7**, **I-9**, and **I-11** in male Sprague-Dawley rats.

[347] Oral bioavailability of palmitoylethanolamide (PEA) was evaluated following oral dosing of seven different PEA-prodrugs. Prodrugs were dosed orally to deliver a total PEA dose of 10 mg/kg. Following oral administration of the PEA-prodrugs, PEA plasma concentrations were determined with a qualified LC-MS/MS method.

Preparation of Dosing Formulations

[348] Pro-drugs were dosed so that a total dose of 10 mg/kg of PEA was administered. Each prodrug was formulated in a vehicle comprised of 10% Solutol, 10% n-methyl pyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400) and 70% water. Formulations were prepared fresh on the day of dosing.

Animal Dosing

[349] Pharmacokinetics of PEA were evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study.

[350] Blood samples (~ 300 μL) were collected from the rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 μL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 μL) was then transferred to a chilled, labeled polypropylene tube containing 15 μL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 7a.

Table 7a: Study Design

Dose Group	Test Article	Number of Animals	Dosing Route	Dose (mg/kg of prodrug)*	Dosing Solution Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sampling Time Points
1	**I-9**	3	PO	16	3	5.3	10% Solutol, 10% NMP, 10% PEG400, 70% water	Pre-dose, 5, 15, 30 min, 1, 2, 4, and 8 hours
2	**I-6**	3	PO	19	3	6.3		
3	**I-5**	3	PO	19.7	3	6.6		
4	**I-3**	3	PO	24.5	3	8.2		
5	**I-2**	3	PO	12.5	3	4.2		
6	**I-11**	3	PO	20.7	3	6.9		
7	**I-7**	3	PO	24.5	3	8.2		

NMP: n-methyl pyrrolidone; *dose of actual pro-drug, all deliver 10 mg/kg of PEA.

[351] An LC-MS/MS method for determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[352] Pharmacokinetic parameters were calculated from the time course of the plasma concentration and are presented in Tables 7b-7h and Figures 7A-7N. Maximum plasma concentration (C_{max}) and time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from the data. Area under the time concentration curve (AUC) was calculated using the linear trapezoidal rule with calculation to the last quantifiable data point, and with extrapolation to infinity if applicable. At least three quantifiable data points were required to determine AUC. Plasma half-life ($t_{1/2}$) was calculated from 0.693/slope of the terminal elimination phase. Mean residence time, MRT, was calculated by dividing area under the moment curve (AUMC) by AUC. Bioavailability was determined by dividing individual dose-normalized PO AUC_{last} values by the average IV AUC_{last} value (IV data Example 5). Samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

Results

[353] No adverse reactions were observed following the oral administration of PEA pro-drugs in male Sprague-Dawley rats in this study.

[354] The dosing solutions were not analyzed by LC-MS/MS. Concentrations are expressed as mg/ml of the free base. Nominal dosing level was used in all calculations.

[355] Individual and average plasma concentrations and pharmacokinetic parameters for PEA and are shown in Tables 7b-7h. Data are expressed as ng/mL of the free drug. Samples that were below the limit of quantitation were not used in the calculation of averages. Plasma concentration versus time data are plotted in Figures 7A-7N. Endogenous levels of PEA were found in all rats. Measured concentrations of PEA in plasma samples were corrected by subtracting the concentration of PEA measured in the pre-dose samples. These corrected values are reported in the tables below and were used to determine pharmacokinetic parameters. Any corrected values that were negative are reported as not determined (ND).

[356] Following PO dosing of **I-9** (Group 1), maximum plasma concentrations (average of 10.4 ± 2.29 ng/mL) were observed at 1 hour post dosing. Average half-life was not determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure based on the dose-normalized AUC_{last} was 1.61 ± 0.692 hr*kg*ng/mL/mg. Based on the IV data from study Example 5, the average oral bioavailability for **I-9** was 2.60 ± 1.11%. Results are shown in Table 7b and FIGs. 5A and 5B.

Table 7b. Individual and Average Plasma Concentrations (ng/ml) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-9** at 16/mg/kg in Male Sprague-Dawley Rats.

Oral (16 mg/kg **I-9** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	161	**162**	**163**	**Mean**	**SD**
0 (pre-dose)	2.74	3.83	3.30	3.29	0.545
0.083	1.83	ND	0.170	1.00	ND
0.25	7.56	2.41	5.50	5.16	2.59
0.50	7.36	7.07	11.4	8.61	2.42
1.0	9.36	8.77	13.0	10.4	2.29
2.0	3.93	1.84	5.33	3.70	1.76
4.0	ND	ND	0.640	ND	ND
8.0	ND	ND	ND	ND	ND
Animal Weight (g)	0.259	0.258	0.260	0.259	0.001
Amount Dosed (m:)	1.37	1.37	1.38	1.37	0.01
C_{max} (ng/mL)	9.36	8.77	13.0	10.4	2.29
t_{max} (hr)	1.00	1.00	1.00	1.00	0.00
$t_{1/2}$ (hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	0.933	0.913	1.28	1.04	0.208
AUC_{last} (hr ng/mL)	13.7	10.8	24.0	16.1	6.92
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	1.37	1.08	2.40	1.61	0.692
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	2.20	1.74	3.86	2.60	1.11

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (0.5 ng/mL); [1]Dose normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable date points trailing the C_{max}.

[357] Following PO dosing of **I-6** (Group 2), maximum plasma concentrations (average of 11.7 ± 5.39 ng/mL) were observed at fifteen minutes post dosing. Average halflife after PO dosing was 1.96 ± 2.04 hours. Average exposure based on the dose-normalized AUC_{last} was 1.40 ± 0.737 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-6** was 2.25 ± 1.19%. Results are shown in Tables 7c and FIGs. 5C and 5D.

Table 7c. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-6** at 19 mg/kg in Male Sprague-Dawley Rats (Group 2).

Oral (19 mg/kg **I-6** equals 10 mg/kg PEA)

Time (hr)	**Rat #**				
	164	**165**	**166**	**Mean**	**SD**
0 (pre-dose)	3.27	4.06	4.26	3.86	0.523
0.083	2.13	1.27	ND	1.70	ND
0.25	17.3	11.3	6.54	11.7	5.41
0.50	**16.5**	**7.24**	**5.73**	9.83	5.85
1.0	**5.17**	**3.40**	**5.04**	4.54	0.987
2.0	**5.43**	**2.31**	**4.46**	4.07	1.60
4.0	**0.220**	ND	ND	ND	ND
8.0	BLOQ	ND	ND	ND	ND
Animal Weight (g)	0.250	0.256	0.249	0.252	0.004
Volume Dosed (mL)	1.58	1.61	1.57	1.59	0.02
C_{max} (ng/mL)	17.3	11.3	6.54	11.7	5.39
t_{max} (hr)	0.250	0.250	0.250	0.250	0.000
$t_{1/2}$ (hr)	0.600	0.979	4.31	1.96	2.04
MRT_{last} (hr)	1.12	0.749	0.942	0.936	0.184
AUC_{last} (hr ng/mL)	22.4	9.10	10.3	14.0	7.37
AUC_{∞} (hr ng/mL)	22.6	12.4	ND[3]	17.5	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	2.24	0.910	1.03	1.40	0.737
AUC_{∞} (hr kg ng/mL/mg)	2.26	1.24	ND[3]	1.75	ND
Bioavailability (%)[2]	3.61	1.47	1.66	2.25	1.19

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-

normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined because the AUC_{∞} was a greater than 25% extrapolation above the AUC_{last}.

[358] Following PO dosing of **I-5** (Group 3), maximum plasma concentrations (average of 12.2 ± 5.52 ng/mL) were observed between fifteen and thirty minutes post dosing. Average half-life after PO dosing was 3.15 hours. Average exposure based on the dose normalized AUC_{last} was 2.38 ± 1.47 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-5** was 3.84 ± 2.37%. Results are shown in Table 7d and FIGs. 5E and 5F.

Table 7d. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-5** at 19.7 mg/kg in Male Sprague-Dawley Rats (Group 3).

Oral (19.7 mg/kg **I-5** equals 10 mg/kg PEA

Time (hr)	Rat #				
	167	**168**	**169**	**Mean**	**SD**
0 (pre-dose)	3.66	BLOQ	BLOQ	ND	ND
0.083	ND	3.92	6.53	5.23	ND
0.25	3.13	14.1	16.5	11.2	7.13
0.50	5.97	10.0	9.04	8.34	2.11
1.0	3.35	3.77	3.33	3.48	0.248
2.0	3.43	**4.84**	**8.43**	5.57	2.58
4.0	ND	**2.94**	**3.03**	2.99	ND
8.0	BLOQ	**1.46**	**1.69**	1.58	ND
Animal Weight (g)	0.255	0.256	0.251	0.254	0.003
Volume Dosed (mL)	1.68	1.69	1.66	1.68	0.02
C_{max} (ng/mL)	5.97	14.1	16.5	12.2	5.52
t_{max} (hr)	0.500	0.250	0.250	0.333	0.144
$t_{1/2}$ (hr)	ND[3]	3.53	2.77	3.15	ND
MRT_{last} (hr)	0.994	2.71	2.66	2.12	0.977
AUC_{last} (hr ng/mL)	7.27	29.0	35.3	23.8	14.7
AUC_{∞} (hr ng/mL)	ND[3]	36.4	42.0	39.2	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	0.727	2.90	3.53	2.38	1.47
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	3.64	4.20	3.92	ND
Bioavailability (%)[2]	1.17	4.67	5.68	3.84	2.37

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter

by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

[359] Following PO dosing of **I-3** (Group 4), maximum plasma concentrations (average of 9.83 ± 3.69 ng/mL) were observed between thirty minutes and 2 hours post dosing. Average half-life was not determined; however, the half-life of one rat was 0.779 hours. Average exposure based on the dose-normalized AUC_{last} was 1.99 ± 0.338 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-3** was 3.20 ± 0.544%. Results are shown in Table 7e and FIGs. 5G and 5H.

Table 7e. Individual and Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-3** at 24.5 mg/kg in Male Sprague-Dawley Rats (Group 4).

Oral (24.5 mg/kg **I-3** equals 10 mg/kg PEA)

Time (hr)	**Rat #**				
	170	**171**	**172**	**Mean**	**SD**
0 (pre-dose)	2.81	3.07	2.22	2.70	0.436
0.083	0.640	1.02	ND	0.830	ND
0.25	3.90	7.43	6.20	5.84	1.79
0.50	3.52	10.4	13.2	9.04	4.98
1.0	3.45	**8.03**	12.9	8.12	4.72
2.0	5.89	**6.61**	10.7	7.73	2.58
4.0	2.27	**0.640**	ND	1.46	ND
8.0	ND	BLOQ	ND	ND	ND
Animal Weight (g)	0.254	0.259	0.260	0.258	0.003
Volume Dosed (mL)	2.08	2.12	2.13	2.11	0.03
C_{max} (ng/mL)	5.89	10.4	13.2	9.83	3.69
t_{max} (hr)	2.00	0.500	0.500	1.00	0.866
$t_{1/2}$ (hr)	ND[3]	0.779	ND[3]	ND	ND
MRT_{last} (hr)	1.89	1.38	1.08	1.45	0.405
AUC_{last} (hr ng/mL)	16.0	22.3	21.4	19.9	3.38
AUC_{∞} (hr ng/mL)	ND[3]	23.0	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	1.60	2.23	2.14	1.99	0.338
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	2.30	ND[3]	ND	ND
Bioavailability (%)[2]	2.58	3.59	3.44	3.20	0.544

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined;

BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

[360] Following PO dosing of **I-2** (Group 5), maximum plasma concentrations (average of 8.83 ± 1.23 ng/mL) were observed between fifteen and thirty minutes post dosing. Average half-life was not determined; however, the half-life of one rat was 0.797 hours. Average exposure based on the dose-normalized AUC_{last} was 0.854 ± 0.164 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-2** was 1.38 ± 0.264%. Results are shown in Table 7f and FIGs. 5I and 5J.

Table 7f. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-2** at 12.5 mg/kg in Male Sprague-Dawley Rats (Group 5).

Oral (12.5 mg/kg **I-2** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	173	**174**	**175**	**Mean**	**SD**
0 (pre-dose)	2.67	1.41	1.73	1.94	0.655
0.083	0.220	3.07	2.02	1.77	1.44
0.25	7.73	10.2	8.47	8.80	1.26
0.50	7.83	**8.69**	8.37	8.30	0.435
1.0	3.80	**3.89**	1.22	2.97	1.52
2.0	3.11	**2.19**	1.11	2.14	1.00
4.0	ND	BLOQ	BLOQ	ND	ND
8.0	ND	ND	BLOQ	ND	ND
Animal Weight (g)	0.247	0.246	0.259	0.251	0.007
Volume Dosed (mL)	1.04	1.04	1.09	1.06	0.03
C_{max} (ng/mL)	7.83	10.2	8.47	8.83	1.23
t_{max} (hr)	0.500	0.250	0.250	0.333	0.144
$t_{1/2}$ (hr)	ND[3]	0.797	ND[5]	ND	ND
MRT_{last} (hr)	0.862	0.742	0.606	0.736	0.128
AUC_{last} (hr ng/mL)	9.09	9.84	6.70	8.54	1.64
AUC_{∞} (hr ng/mL)	ND[3]	ND[4]	ND[5]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	0.909	0.984	0.670	0.854	0.164
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[4]	ND[5]	ND	ND
Bioavailability (%)[2]	1.46	1.58	1.08	1.38	0.264

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time,

calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable data points trailing the C_{max}; [4]not determined because the AUC_{∞} was a greater than 25% extrapolation above the AUC_{last}; [5]not determined because the line defining the terminal elimination phase had an $r^2<0.85$.

[361] Following PO dosing of **I-11** (Group 6), maximum plasma concentrations (average of 15.5 ± 3.01 ng/mL) were observed between fifteen minutes and thirty minutes post dosing. Average half-life was not determined; however, the half-life of one rat was 0.685 hours. Average exposure based on the dose-normalized AUC_{last} was 2.79 ± 0.808 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-11** was 4.49 ± 1.30%. Results are shown in Table 7g and FIGs. 5K and 5L.

Table 7g. Individual Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-11** at 20.7 mg/kg in Male Sprague-Dawley Rats (Group 6).

Oral (20.7 mg/kg **I-11** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	176	**177**	**178**	**Mean**	**SD**
0 (pre-dose)	1.62	1.89	BLOQ	1.76	ND
0.083	3.26	1.41	4.33	3.00	1.48
0.25	12.7	11.1	15.2	13.0	2.06
0.50	**12.6**	18.7	14.9	15.4	3.10
1.0	**6.74**	10.6	13.1	10.2	3.20
2.0	**4.414**	5.73	5.80	5.22	0.939
4.0	**0.340**	0.510	0.828	0.559	0.248
8.0	BLOQ	0.520	2.62	1.57	ND
Animal Weight (g)	0.258	0.252	0.259	0.256	0.004
Volume Dosed (mL)	1.78	1.74	1.79	1.77	0.03
C_{max} (ng/mL)	12.7	18.7	15.2	15.5	3.01
t_{max} (hr)	0.250	0.500	0.250	0.333	0.144
$t_{1/2}$ (hr)	0.685	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.13	1.52	2.33	1.66	0.612
AUC_{last} (hr ng/mL)	19.5	28.7	35.5	27.9	8.08
AUC_{∞} (hr ng/mL)	19.8	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	1.95	2.87	3.55	2.79	0.808
AUC_{∞} (hr kg ng/mL/mg)	1.98	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	3.13	4.62	5.73	4.49	1.30

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined because the line defining the terminal elimination phase had an r^2 <0.85.

[362] Following PO dosing of **I-7** (Group 7), maximum plasma concentrations (average of 3.52 ng/mL) were observed between zero and fifteen minutes hour post dosing. Average half-life was not determined; however, the half-life of one rat was 0.736 hours. Average exposure based on the dose-normalized AUC_{last} was 1.23 hr*kg*ng/mL/mg. Based on the IV data from Example 5,

average oral bioavailability for **I-7** was 1.98%. Results are shown in Table 7h and FIGs. 5M and 5N.

Table 7h. Individual and Average Plasma Concentrations (mg/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-7** at 24.5 mg/kg in Male Sprague-Dawley Rats (Group 7).

Oral (24.5 mg/kg **I-7** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	179	**180**	**181**	**Mean**	**SD**
0 (pre-dose)	3.15	BLOQ	3.85	3.50	ND
0.083	ND	2.88	ND	ND	ND
0.25	1.65	3.89	ND	2.77	ND
0.50	**1.57**	3.28	ND	2.43	ND
1.0	**1.23**	2.36	ND	1.80	ND
2.0	**0.400**	3.43	ND	1.92	ND
4.0	ND	2.46	ND	ND	ND
8.0	ND	2.85	ND	ND	ND
Animal Weight (kg)	0.254	0.249	0.263	0.255	0.007
Volume Dosed (mL)	2.08	2.04	2.16	2.09	0.06
C_{max} (ng/mL)	3.15	3.89	ND	3.52	ND
t_{max} (hr)	0.000	0.250	ND	0.125	ND
$t_{1/2}$(hr)	0.736	ND[3]	ND	ND	ND
MRT_{last} (hr)	0.779	3.93	ND	2.35	ND
AUC_{last} (hr ng/mL)	2.19	22.4	ND	12.3	ND
AUC_{∞} (hr ng/mL)	2.61	ND[3]	ND	ND	ND
Dose-normalized Values[1]					ND
AUC_{last} (hr kg ng/mL/mg)	0.219	2.24	ND	1.23	ND
AUC_{∞} (hr kg ng/mL/mg)	0.261	ND[3]	ND	ND	ND
Bioavailability (%)[2]	0.352	3.61	ND	1.98	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined because the line defining the terminal elimination phase had an r^2 <0.85.

Example 8: PEA Stability in Human, Rat, Mouse and Dog Liver Microsomes, Human, Rat, Mouse and Dog Liver S9 Fraction, Human, Rat, Mouse and Dog Intestinal S9 Fraction, Human, Rat, Mouse and Dog Plasma, and Simulated Intestinal Fluid.

[363] The present Example describes PEA stability observed in 1) human, rat, mouse, and dog liver microsomes; 2) human, rat, mouse, dog liver S9 fraction; human, rat, mouse, and dog intestinal S9 fraction; 4) human, rat, mouse, and dog plasma; and 5) simulated intestinal fluid containing various enzymes.

Liver Microsomal Stability

[364] Mixed-gender human (Lot# 1210347), male Sprague-Dawley rat (Lot# 1310030), male CD-1 mouse (Lot# 1510043), and male Beagle dog (Lot# 0810143) liver microsomes were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compound, testosterone, was run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37 °C for 3 minutes. The reaction was initiated by the addition of cofactor, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 8-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 8a and 8b.

Reaction Composition

Liver Microsomes	0.5 mg/mL
NADPH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 µL

Table 8a. PEA stability observed in human, rat, mouse, and dog liver microsomes.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-6	Human	100	82	58	66	68	>60 (97)	<0.0231 (0.0143)
	Rat	100	68	67	62	53	>60 (63)	<0.0231 (0.0219)
	Mouse	100	84	74	73	61	>60 (84)	<0.0231 (0.0164)
	Dog	100	103	100	91	77	>60	<0.0231
I-2	Human	100	10	1.6	<1.0	<1.0	3.1	0.451
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
	Dog	100	5.4	<1.0	<1.0	<1.0	2.4	0.581
I-9	Human	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
	Rat	100	1.9	<1.0	<1.0	<1.0	1.8	0.791
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
I-3	Human	100	83	79	74	53	>60 (69)	<0.0231 (0.0200)
	Rat	100	81	88	72	68	>60 (112)	<0.0231 (0.0124)
	Mouse	100	82	83	60	70	>60 (100)	<0.0231 (0.0138)
	Dog	100	94	85	85	74	>60	<0.0231
I-5	Human	100	90	82	79	62	>60 (90)	<0.0231 (0.0154)
	Rat	100	77	70	69	57	>60 (75)	<0.0231 (0.0186)
	Mouse	100	72	57	56	55	59	0.0236
	Dog	100	84	75	75	67	>60 (104)	<0.0231 (0.0133)
I-11	Human	100	3.1	<1.0	<1.0	<1.0	2.0	0.696
	Rat	100	3.3	<1.0	<1.0	<1.0	2.0	0.681
	Mouse	100	3.2	<1.0	<1.0	<1.0	2.0	0.687

	Dog	100	3.6	<1.0	<1.0	<1.0	2.1	0.665
I-7	Human	100	4.6	1.3	<1.0	<1.0	2.3	0.612
	Rat	100	45	17	11	2.1	8.5	0.164
	Mouse	100	1.7	1.2	<1.0	<1.0	1.7	0.814
	Dog	100	1.6	1.4	1.3	<1.0	1.7	0.823

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Similarly, if the calculated half-life is less than the shortest time point, the half-life is expressed as < that time point and the calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	27	0.0505	≤ 41
	Rat	1.8	0.760	≤ 15
	Mouse	4.9	0.285	≤ 15
	Dog	33	0.0415	≤ 40

Table 8b. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-6	Human	PEA	0	0	0	0	0
	Rat		0.14	0.064	0.017	0.0098	0.0012
	Mouse		0.022	0.055	0.039	0.041	0.027
	Dog		0.030	0.087	0.091	0.080	0.084
I-2	Human		0.15	0.23	0.19	0.15	0.071
	Rat		0.40	0.11	0.028	0.0083	0
	Mouse		0.48	0.34	0.19	0.12	0.023
	Dog		0.23	0.48	0.38	0.29	0.17
I-9	Human		0.47	0.39	0.22	0.16	0.058
	Rat		0.22	0.066	0.017	0	0
	Mouse		0.52	0.29	0.18	0.11	0.036
	Dog		0.39	0.42	0.32	0.26	0.18
I-3	Human		0	0.026	0.017	0.019	0.027
	Rat		0.032	0.039	0.018	0.0067	0
	Mouse		0.0079	0.025	0.025	0.042	0.025
	Dog		0.00074	0.023	0.037	0.042	0.053
I-5	Human		0.014	0.020	0.025	0.022	0.016
	Rat		0.034	0.033	0.015	0.015	0.0027
	Mouse		0.048	0.080	0.052	0.048	0.024
	Dog		0.025	0.081	0.090	0.087	0.063
I-11	Human		0.046	0.29	0.20	0.16	0.057
	Rat		0.023	0.048	0.031	0.0086	0
	Mouse		0.22	0.32	0.18	0.12	0.030
	Dog		0.032	0.32	0.26	0.20	0.097
I-7	Human		0.22	0.39	0.28	0.22	0.080
	Rat		0.054	0.14	0.087	0.045	0.0085
	Mouse		0.28	0.29	0.18	0.11	0.034
	Dog		0.59	0.50	0.47	0.38	0.20

Liver S9 Stability

[365] Mixed gender human (Lot# 1210091), male Sprague-Dawley rat (Lot# 1410265), male CD-1 mouse (Lot# 1510255), and male Beagle dog (Lot# 1210278) liver S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin (7-HC), were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. The reaction was initiated by the addition of cofactor cocktail (see below), and the mixture was then incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples

were immediately combined with 300 µL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 µL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 8-1*. Test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 8c and 8d.

Reaction Composition

Liver S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 µM

Table 8c. PEA stability observed in human, rat, mouse, and dog liver S9.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-6	Human	100	80	102	95	77	>60	<0.0116
	Rat	100	64	82	93	89	>60	<0.0116
	Mouse	100	87	87	99	75	>60	<0.0116
	Dog	100	101	106	102	110	>60	<0.0116
I-2	Human	100	6.0	1.3	<1.0	<1.0	2.5	0.279
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Dog	100	5.1	2.0	<1.0	<1.0	2.4	0.293
I-9	Human	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Rat	100	13	2.1	<1.0	<1.0	3.4	0.207
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
I-3	Human	100	104	89	95	66	>60 (104)	<0.0116 (0.00665)
	Rat	100	66	69	71	52	>60 (72)	<0.0116 (0.00967)
	Mouse	100	82	61	78	61	>60 (87)	<0.0116 (0.00797)
	Dog	100	104	89	80	86	>60	<0.0116
I-5	Human	100	80	81	70	72	>60	<0.0116
	Rat	100	98	82	75	67	>60 (91)	<0.0116 (0.00764)
	Mouse	100	66	50	59	50	53	0.0132
	Dog	100	72	79	74	85	>60	<0.0116
I-11	Human	100	2.8	<1.0	<1.0	<1.0	1.9	0.359
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Dog	100	4.6	1.6	<1.0	<1.0	2.3	0.306
I-7	Human	100	1.9	<1.0	<1.0	<1.0	1.8	0.396
	Rat	100	43	22	11	1.4	8.8	0.0786
	Mouse	100	1.6	<1.0	<1.0	<1.0	1.7	0.415
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Similarly, if the calculated half-life is less than the shortest time point, the half-life is expressed as < that time point and the calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	16	0.0447	≤34
	Rat	4.7	0.148	≤15
	Mouse	8.7	0.0800	≤37
	Dog	14	0.0485	≤42
7-hydroxycoumarin	Human	10	0.0668	≤18
	Rat	4.6	0.152	≤15
	Mouse	4.1	0.171	≤15
	Dog	1.4	0.502	≤15

Table 8d. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-6	Human	PEA	0.014	0.037	0.022	0.022	0.016
	Rat		0.020	0.035	0.021	0.021	0.013
	Mouse		0.030	0.041	0.033	0.031	0.015
	Dog		0.016	0.044	0.041	0.049	0.037
I-2	Human		0.20	0.23	0.18	0.096	0.033
	Rat		0.50	0.31	0.17	0.11	0.023
	Mouse		0.51	0.26	0.19	0.12	0.022
	Dog		0.23	0.38	0.35	0.21	0.11
I-9	Human		0.35	0.30	0.18	0.088	0.028
	Rat		0.20	0.31	0.18	0.11	0.029
	Mouse		0.52	0.23	0.13	0.077	0.022
	Dog		0.32	0.29	0.21	0.18	0.078
I-3	Human		0.00032	0.0058	0.017	0.015	0.0096
	Rat		0.015	0.045	0.033	0.017	0.013
	Mouse		0.013	0.031	0.022	0.023	0.025
	Dog		0.010	0.030	0.035	0.031	0.029
I-5	Human		0.024	0.036	0.036	0.025	0.018
	Rat		0.020	0.025	0.037	0.026	0.013
	Mouse		0.046	0.061	0.043	0.029	0.018
	Dog		0.024	0.044	0.036	0.041	0.038
I-11	Human		0.047	0.25	0.18	0.14	0.033
	Rat		0	0.14	0.091	0.059	0.0062
	Mouse		0.18	0.24	0.13	0.083	0.015
	Dog		0.069	0.51	0.34	0.29	0.13
I-7	Human		0.20	0.32	0.17	0.13	0.042
	Rat		0.045	0.15	0.16	0.12	0.039
	Mouse		0.22	0.22	0.14	0.084	0.020
	Dog		0.21	0.16	0.15	0.12	0.049

Intestinal S9 Fraction Stability

[366] Mixed-gender human (Lot# 1410073), male Sprague-Dawley rat (Lot# 1510303), male CD-1 mouse (Lot# 1510194), and male Beagle dog (Lot# 1510226) intestinal S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin, were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. The reaction was initiated by the addition of cofactor cocktail, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 8-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 8e and 8f.

Reaction Composition

Intestinal S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate, pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 8e. PEA stability observed in human, rat, mouse, and dog intestinal S9 fraction.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-6	Human	100	109	103	82	70	>60 (102)	<0.0116 (0.00682)
	Rat	100	78	92	79	59	>60 (91)	<0.0116 (0.00765)
	Mouse	100	62	69	61	53	>60 (66)	<0.0116 (0.0105)
	Dog	100	54	53	45	38	34	0.0201
I-2	Human	100	73	41	28	9.6	17	0.0415
	Rat	100	35	<1.0	<1.0	<1.0	5.6	0.123
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Dog	100	36	12	4.2	<1.0	6.7	0.104
I-9	Human	100	28	5.7	1.2	<1.0	5.3	0.131
	Rat	100	45	26	15	3.6	10	0.0695
	Mouse	100	56	33	21	6.5	13	0.0537
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
I-3	Human	100	76	86	83	60	>60 (98)	<0.0116 (0.00706)
	Rat	100	53	46	42	31	26	0.0268
	Mouse	100	68	59	49	33	33	0.0211
	Dog	100	46	46	34	18	19	0.0368
I-5	Human	100	82	49	38	45	32	0.0216
	Rat	100	57	48	46	44	38	0.0182
	Mouse	100	68	67	66	49	60	0.0116
	Dog	100	50	48	36	36	26	0.0263
I-11	Human	100	4.1	1.2	<1.0	<1.0	2.2	0.318
	Rat	100	1.4	<1.0	<1.0	<1.0	1.6	0.425
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Dog	100	1.8	<1.0	<1.0	<1.0	1.7	0.404
I-7	Human	100	46	22	9.8	3.8	9.0	0.0772
	Rat	100	60	37	31	13	16	0.0431
	Mouse	100	59	35	27	5.7	14	0.0485
	Dog	100	1.1	<1.0	<1.0	<1.0	1.5	0.452

[a] When the calculated half-life is longer than the duration of the experiment, the half-life is expressed as > the longest incubation time. Similarly, if the calculated half-life is less than the shortest time point, the half-life is expressed as < that time point and the calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)
Testosterone	Human	7.8	0.0889
	Rat	>60 (106)	<0.0116 (0.00652)
	Mouse	>60 (75)	<0.0116 (0.00919)
	Dog	>60 (96)	<0.0116 (0.00722)
7-hydroxycoumarin	Human	13	0.0545
	Rat	27	0.0257
	Mouse	5.0	0.139
	Dog	8.6	0.0803

Table 8f. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-6	Human	PEA	0.0089	0.023	0.043	0.056	0.072
	Rat		0.069	0.088	0.094	0.10	0.11
	Mouse		0.12	0.14	0.17	0.15	0.17
	Dog		0.26	0.42	0.38	0.35	0.36
I-2	Human		0.011	0.057	0.075	0.067	0.11
	Rat		0.69	0.63	0.60	0.52	0.52
	Mouse		0.52	0.50	0.52	0.45	0.45
	Dog		0.11	0.40	0.50	0.53	0.54
I-9	Human		0.048	0.18	0.23	0.22	0.18
	Rat		0.034	0.22	0.31	0.36	0.39
	Mouse		0.0024	0.12	0.15	0.19	0.23
	Dog		0.26	0.48	0.47	0.46	0.45
I-3	Human		0	0.0027	0.025	0.034	0.048
	Rat		0	0.024	0.037	0.047	0.073
	Mouse		0	0.026	0.024	0.026	0.043
	Dog		0.015	0.23	0.28	0.29	0.50
I-5	Human		0	0.050	0.063	0.074	0.10
	Rat		0.029	0.062	0.099	0.11	0.18
	Mouse		0.050	0.11	0.076	0.093	0.14
	Dog		0.13	0.26	0.25	0.25	0.28
I-11	Human		0.018	0.13	0.20	0.21	0.18
	Rat		0	0.13	0.24	0.35	0.38
	Mouse		0	0.021	0.059	0.12	0.084
	Dog		0.032	0.23	0.38	0.74	0.88
I-7	Human		0.090	0.46	0.62	0.63	0.48
	Rat		0.042	0.24	0.35	0.47	0.60
	Mouse		0.033	0.24	0.29	0.39	0.38
	Dog		0.38	0.36	0.55	0.51	0.50

Plasma Stability

[367] Studies were carried out in mixed-gender human plasma (Lot# GLP530-5), male Sprague-Dawley rat (Lot# RAT297944, RAT313140), male CD-1 mouse (Lot# MSE237700), and male Beagle dog (Lot# BGL87670, BGL82614), collected on sodium heparin. Plasma was adjusted to pH 7.4 prior to initiating the experiments. DMSO stocks were first prepared for the test articles. Aliquots of the DMSO solutions were dosed into 700 µL of plasma, which had been pre-warmed to 37 °C, at a final test article concentration of 1 µM. Aliquots (100 µL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 µL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 8-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 8g and 8h.

Table 8g. PEA stability observed in human, rat, mouse, and dog plasma.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-6	Human	100	79	102	74	53	>120 (147)
	Rat	100	93	119	100	29	110
	Mouse	100	83	89	39	23	56
	Dog	100	55	42	34	22	36
I-2	Human	100	96	91	84	51	>120 (135)
	Rat	100	58	59	46	18	49
	Mouse	100	72	71	56	21	62
	Dog	100	95	71	54	24	61
I-9	Human	100	6.4	1.4	<1.0	<1.0	3.8
	Rat	100	2.5	<1.0	<1.0	<1.0	2.8
	Mouse	100	49	17	2.3	<1.0	13
	Dog	100	8.7	12	1.2	<1.0	4.7
I-3	Human	100	109	84	65	77	>120 (211)
	Rat	100	94	79	76	79	>120
	Mouse	100	23	24	9.1	4.7	9.4
	Dog	100	90	90	95	99	>120
I-5	Human	100	110	81	82	69	>120 (195)
	Rat	100	85	90	87	83	>120
	Mouse	100	109	86	73	75	>120 (213)
	Dog	100	87	98	87	83	>120
I-11	Human	100	67	47	34	10	34
	Rat	100	9.0	23	<1.0	<1.0	5.6
	Mouse	100	2.9	2.1	1.9	<1.0	3.0
	Dog	100	61	40	18	5.4	23
I-7	Human	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0

Table 8h. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-6	Human	PEA	0	0	0	0	0
	Rat		0	0	0.020	0.017	0.056
	Mouse		0	0	0.015	0.059	0.11
	Dog		0	0	0	0	0
I-2	Human		0.00043	0.035	0.064	0.10	0.21
	Rat		0.052	0.22	0.28	0.28	0.38
	Mouse		0.019	0.068	0.095	0.14	0.23
	Dog		0.0061	0.12	0.18	0.21	0.26
I-9	Human		0.049	0.41	0.37	0.49	0.35
	Rat		0.77	1.2	1.3	1.2	1.1
	Mouse		0.51	0.60	0.62	0.64	0.89
	Dog		0.13	0.58	0.61	0.62	0.55
I-3	Human		0	0	0	0	0.0071
	Rat		0.0029	0.0043	0.013	0.020	0.033
	Mouse		0.015	0.10	0.25	0.36	0.42
	Dog		0	0	0	0	0
I-5	Human		0.0017	0.034	0.055	0.083	0.096
	Rat		0.021	0.11	0.14	0.18	0.17
	Mouse		0.074	0.19	0.19	0.18	0.29
	Dog		0	0.040	0.047	0.070	0.072
I-11	Human		0.011	0.13	0.26	0.38	0.53
	Rat		0.0022	0.016	0.017	0.025	0.025
	Mouse		0.19	0.38	0.56	0.60	0.76
	Dog		0.012	0.20	0.35	0.52	0.55
I-7	Human		0.11	0.54	0.53	0.49	0.53
	Rat		0.29	0.59	0.54	0.56	0.60
	Mouse		0.87	0.75	0.81	0.81	0.91
	Dog		0.17	0.52	0.55	0.53	0.51

Simulated Intestinal Fluid Stability

[368] Studies were carried out in simulated intestinal fluid in the presence of various enzymes. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Aliquots of this solution were taken and the pH was adjusted to 6.8. Individual enzymes were then spiked into aliquots for each experiment. A DMSO stock was first prepared for the test article. Aliquots of the DMSO solution were dosed into 700 μL of matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then

centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 8-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 8i and 8j.

Table 8i. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-6	SIF + Pancreatin	100	27	11	3.1	1.7	8.3
	SIF + Elastase	100	95	74	64	69	>120 (185)
	SIF + Carboxypeptidase A	100	77	46	60	56	>120 (134)
	SIF + Carboxypeptidase B	100	87	93	54	19	62
	SIF + Chymotrypsin	100	106	99	51	55	101
	SIF + Trypsin	100	116	87	83	123	>120
I-2	SIF + Pancreatin	100	93	91	98	60	>120 (205)
	SIF + Elastase	100	61	42	30	28	37
	SIF + Carboxypeptidase A	100	79	70	56	43	92
	SIF + Carboxypeptidase B	100	75	54	40	17	43
	SIF + Chymotrypsin	100	78	70	60	58	>120 (144)
	SIF + Trypsin	100	82	72	75	46	>120 (121)
I-9	SIF + Pancreatin	100	35	34	20	13	17
	SIF + Elastase	100	49	22	10	6.3	15
	SIF + Carboxypeptidase A	100	43	15	10	5.5	12
	SIF + Carboxypeptidase B	100	44	14	6.1	4.7	12
	SIF + Chymotrypsin	100	45	24	11	7.2	14
	SIF + Trypsin	100	67	49	22	12	29
I-3	SIF + Pancreatin	100	<1.0	<1.0	<1.0	<1.0	<1.0
	SIF + Elastase	100	94	61	44	29	55
	SIF + Carboxypeptidase A	100	76	66	36	20	46

	SIF + Carboxypeptidase B	100	86	77	41	15	49
	SIF + Chymotrypsin	100	102	84	61	53	107
	SIF + Trypsin	100	67	60	55	43	93
I-5	SIF + Pancreatin	100	13	<1.0	<1.0	<1.0	5.1
	SIF + Elastase	100	103	70	48	35	65
	SIF + Carboxypeptidase A	100	84	63	39	15	44
	SIF + Carboxypeptidase B	100	94	62	43	14	45
	SIF + Chymotrypsin	100	75	59	37	23	47
	SIF + Trypsin	100	81	67	44	26	56
I-11	SIF + Pancreatin	100	<1.0	<1.0	<1.0	<1.0	<1.0
	SIF + Elastase	100	70	50	33	17	37
	SIF + Carboxypeptidase A	100	73	78	42	18	53
	SIF + Carboxypeptidase B	100	68	50	33	9.6	34
	SIF + Chymotrypsin	100	84	63	42	21	50
	SIF + Trypsin	100	77	62	45	23	53
I-7	SIF + Pancreatin	100	40	19	6.5	2.7	12
	SIF + Elastase	100	44	14	3.8	3.5	12
	SIF + Carboxypeptidase A	100	30	10	4.1	3.9	8.8
	SIF + Carboxypeptidase B	100	33	14	5.7	2.2	10
	SIF + Chymotrypsin	100	42	23	13	15	14
	SIF + Trypsin	100	66	44	38	33	50

Table 8j. Measured Concentrations of Drug.

Test Article	Treatment	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-6	SIF + Pancreatin	PEA	-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-2	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-9	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0

	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-3	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-5	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-11	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-7	SIF + Pancreatin		-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0

PEA was found to be endogenous in pancreatin and thus was not quantified in the assay samples.

Appendix 8-1

Liquid Chromatography

Column: Waters ACQUITY UPLC BEH C18 30 x 2.1 mm, 1.7µm
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.8 mL/minute
Gradient Program:

Time (min)	%A	%B
0.00	50	50
0.75	1	99
1.25	1	99
1.30	50	50
1.50	50	50

Total Run Time: 1.5 minutes
Autosampler: 5 µL injection volume
Wash1: Water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2: 0.1% formic acid in water

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 1.5 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-6	+562.6/282.4	133	10	29	19	5500	500	7	30	50	50
I-2	+370.3/282.4	107	10	21	18	5500	500	7	30	50	50
I-3	+726.6/282.4	129	10	38	18	5500	500	7	30	50	50
I-5	+584.5/282.4	135	10	24	20	5500	500	7	30	50	50
I-11	+614.6/282.4	123	10	35	20	5500	500	7	30	50	50
I-7	+724.5/282.4	120	10	40	19	5500	500	7	30	50	50
PEA	+300.3/62.1	123	10	29	10	5500	500	7	30	50	50

Liquid Chromatography

Column: Thermo BDS Hypersil C18 30 x 2.0 mm, 3 µm, with guard column
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 350 µL/minute
Gradient Program:

Time (min)	%A	%B
0.00	50	50
.080	25	75
1.50	0	100
2.00	0	100
2.10	50	50
3.00	50	50

Total Run Time: 3.0 minutes
Autosampler: 10 µL injection volume
Autosampler Wash: water/methanol/2-propanol:1/1/1; with 0.2% formic acid

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 3.0 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-9	+562.6/282.4	107	10	29	19	5500	500	7	20	20	30
PEA	+370.3/282.4	123	10	29	10	5500	500	7	20	20	30

Example 9: Determination of the Bioavailability of Palmitoylethanolamide (PEA) Following Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats.

[369] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs in male Sprague-Dawley rats.

[370] Oral bioavailability of palmitoylethanolamide (PEA) was evaluated in male Sprague-Dawley rats following oral dosing of a PEA pro-drug, **I-13**. **I-13** was dosed orally (PO) at 24.3 mg/kg, which is equivalent to a 10 mg/kg dose of PEA. Blood samples were collected up to 8 hours post-dose, and plasma concentrations of PEA were determined by LC-MS/MS. Pharmacokinetic parameters, with the exception of C_{max} and t_{max}, were not determined due to a

lack of quantifiable data points. Following PO dosing of **I-13** (in 20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H20), nearly all rat plasma samples were below the limit of quantitation. Maximum plasma concentrations (average of 2.70 ± 0.0681 ng/mL) were observed between 2 and 8 hours post dosing. No AUCs or bioavailability values were determined.

Preparation for Dosing Formulations

[371] Pro-drugs were dosed so that a total dose of 10 mg/kg of PEA was administered. Prodrugs were formulated in a vehicle comprised of 10% Solutol HS15, 10% n-methyl pyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400) and 70% water. Formulations were prepared fresh on the day of dosing.

Animal Dosing

[372] Pharmacokinetics of PEA were evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study.

[373] Blood samples (~300 µL) were collected from the rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 µL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 µL) was then transferred to a chilled, labeled polypropylene tube containing 15 µL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 9a.

Table 9a: Study Design.

Test Article	Dosing Route	Total Animals n=	Dose (mg/kg of pro-drug)*	Dosing Solution Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sample Time Points
I-13	PO	3	24.3	3	8.1	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours

*All doses are based on mg/kg of the pro-drugs, and deliver 10 mg/kg of active drug, PEA.

[374] An LC-MS/MS method for the determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[375] Pharmacokinetic parameters, with the exception of C_{max} and t_{max}, were not determined due to a lack of quantifiable data points. Maximum plasma concentration (C_{max}) and time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from the data. Samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

Results

[376] No adverse reactions were observed following oral administration of PEA pro-drug in male Sprague-Dawley rats in this study.

[377] Dosing solutions were not analyzed by LC-MS/MS. Nominal dosing level was used in all calculations. Individual and average plasma concentrations for PEA are shown in Table 9b. Data are expressed as ng/mL of the free drug. Samples that were below the limit of quantitation were not used in the calculation of averages. Plasma concentrations versus time data are plotted in FIG. 6. Endogenous levels of PEA found in all rats were below limit of quantitation; and therefore, measured concentrations of PEA in plasma samples were not corrected.

Table 9b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-13** (in 20% (Solutol HS15:NMP 1:1) 10% PEG400, 70% H_2O) at 24.3 mg/kg in Male Sprague-Dawley Rats.

Oral (24.3 mg/kg **I-13** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	61	62	63	Mean	SD
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	BLOQ	BLOQ	BLOQ	ND	ND
0.25	BLOQ	BLOQ	BLOQ	ND	ND
0.50	BLOQ	BLOQ	BLOQ	ND	ND
1.0	BLOQ	BLOQ	BLOQ	ND	ND
2.0	BLOQ	2.75	2.62	2.69	ND
4.0	BLOQ	BLOQ	BLOQ	ND	ND
8.0	2.72	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.239	0.241	0.228	0.236	0.007
Volume Dosed (mL)	1.94	1.95	1.85	1.91	0.06
C_{max} (ng/mL)	2.72	2.75	2.62	2.70	0.0681
t_{max} (hr)	8.00	2.00	2.00	4.00	3.46
$t_{1/2}$(hr)	ND[1]	ND[1]	ND[1]	ND	ND
MRT_{last} (hr)	ND[1]	ND[1]	ND[1]	ND	ND
AUC_{last} (hr ng/mL)	ND[1]	ND[1]	ND[1]	ND	ND
AUC_{∞} (hr ng/mL)	ND[1]	ND[1]	ND[1]	ND	ND

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below limit of quantitation (2.5 ng/mL); [1]not determined due to a lack of quantifiable data points.

Example 10: PEA Stability in Human, Rat, Mouse and Dog Liver Microsomes, Human, Rat, Mouse and Dog Liver S9 Fraction, Human, Rat, Mouse and Dog Intestinal S9 Fraction, Human, Rat, Mouse and Dog Plasma, and Simulated Intestinal Fluid.

[378] The present Example describes PEA stability observed in 1) human, rat, mouse, and dog liver microsomes; 2) human, rat, mouse, dog liver S9 fraction; human, rat, mouse, and dog intestinal S9 fraction; 4) human, rat, mouse, and dog plasma; and 5) simulated intestinal fluid containing various enzymes.

Liver Microsomal Stability

[379] Mixed-gender human (Lot# 1010420), male Sprague-Dawley rat (Lot# 1510115), male CD-1 mouse (Lot# 1510043), and male Beagle dog (Lot# 0810143) liver microsomes were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compound, testosterone, were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37 °C for 5 minutes. Reaction was initiated by the addition of cofactor, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both the dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 10-1*. Test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 10a and 10b.

Reaction Composition

Liver Microsomes	0.5 mg/mL
NADPH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 10a. PEA stability observed in human, rat, mouse, and dog liver microsomes.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-13	Human	100	79	73	60	42	47	0.0296
	Rat	100	91	72	60	38	41	0.0336
	Mouse	100	69	75	54	44	48	0.0291
	Dog	100	78	76	56	32	38	0.0362

[a] When the calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than the shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	21	0.0667	≤ 41
	Rat	1.4	1.01	≤ 15
	Mouse	7.3	0.190	≤ 15
	Dog	33	0.0419	≤ 40

Table 10b. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-13	Human	PEA	0	0	0	0	0
	Rat		0	0	0.013	0.013	0
	Mouse		0	0	0	0	0
	Dog		0	0	0	0	0

Liver S9 Stability

[380] Mixed gender human (Lot# 1210091), male Sprague-Dawley rat (Lot# 1410265), male CD-1 mouse (Lot# 1510255), and male Beagle dog (Lot# 1310285) liver S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was

added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin (7-HC), were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 5 minutes. Reaction was initiated by the addition of cofactor cocktail (see below), and mixture was then incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 10-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 10c and 10d.

Reaction Composition

Liver S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 10c. PEA stability observed in human, rat, mouse, and dog liver S9.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-13	Human	100	93	87	78	45	58	0.0119
	Rat	100	76	74	59	45	50	0.0138
	Mouse	100	89	90	79	78	>60	<0.0116
	Dog	100	70	61	61	37	41	0.0169

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	28	0.0247	≤34
	Rat	2.7	0.260	≤15
	Mouse	7.1	0.0976	≤37
	Dog	14	0.0493	≤42
7-hydroxycourmarin	Human	15	0.0472	≤18
	Rat	1.9	0.362	≤15
	Mouse	3.8	0.182	≤15
	Dog	1.4	0.512	≤15

Table 10d. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-13	Human	PEA	0	0	0	0	0
	Rat		0	0	0	0	0
	Mouse		0	0	0	0	0
	Dog		0.015	0.025	0.020	0.024	0.021

Intestinal S9 Fraction Stability

[381] Mixed-gender human (Lot# 1410073), male Sprague-Dawley rat (Lot# 1010042), male CD-1 mouse (Lot# 1510194), and male Beagle dog (Lot# 1510226) intestinal S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin, were run simultaneously with test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 5 minutes. Reaction was initiated by the addition of cofactor cocktail, and mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were

withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 µL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 µL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 10-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 10e and 10f.

Reaction Composition

Intestinal S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate, pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 µM

Table 10e. PEA stability observed in human, rat, mouse, and dog intestinal S9 fraction.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-13	Human	100	86	86	86	59	>60 (92)	<0.0116 (0.00757)
	Rat	100	80	82	81	55	>60 (81)	<0.0116 (0.00859)
	Mouse	100	97	71	81	57	>60 (73)	<0.0116 (0.00951)
	Dog	100	67	63	46	49	45	0.0153

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on CL_{int} = k/P, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)
Testosterone	Human	14	0.0509
	Rat	>60	<0.0116
	Mouse	>60	<0.0116
	Dog	>60	<0.0116
7-hydroxycourmarin	Human	9.9	0.0698
	Rat	22	0.0320
	Mouse	4.3	0.160
	Dog	8.7	0.0799

Table 10f. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-13	Human	PEA	0	0.010	0.014	0.018	0.031
	Rat		0	0.020	0.048	0.064	0.092
	Mouse		0.012	0.050	0.073	0.096	0.089
	Dog		0.058	0.095	0.10	0.089	0.17

Plasma Stability

[382] Studies were carried out in mixed-gender human plasma (Lot# AS1650-2), male Sprague-Dawley rat (Lot# RAT297944), male CD-1 mouse (Lot# MSE237700), and male Beagle dog (Lot# BGL91384), collected on sodium heparin. Plasma was adjusted to pH 7.4 prior to initiating the experiments. DMSO stocks were first prepared for the test articles. Aliquots of the DMSO solutions were dosed into 700 μL of plasma, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 10-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 10g and 10h.

Table 10g. PEA stability observed in human, rat, mouse, and dog plasma.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-13	Human	100	104	115	95	40	120
	Rat	100	69	76	36	5.1	42
	Mouse	100	82	71	49	35	70
	Dog	100	90	72	54	23	61

Table 10h. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-13	Human	PEA	0	0	0	0	0
	Rat		0	0	0	0.022	0.053
	Mouse		0	0.016	0.037	0.068	0.090
	Dog		0	0	0	0	0

Simulated Intestinal Fluid Stability

[383] Studies were carried out in simulated intestinal fluid in the presence of various enzymes. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Aliquots of this solution were taken and the pH was adjusted to 6.8. Individual enzymes were then spiked into aliquots for each experiment. A DMSO stock was first prepared for the test article. Aliquots of the DMSO solution were dosed into 700 μL of matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 10-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 10i and 10j.

Table 10i. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-13	SIF + Pancreatin	100	1.3	3.7	3.4	<1.0	<15 (2.5)
	SIF + Elastase	100	41	16	5.6	5.0	12
	SIF + Carboxypeptidase A	100	77	53	27	37	47
	SIF + Carboxypeptidase B	100	65	31	14	8.7	20
	SIF + Chymotrypsin	100	82	67	45	15	49
	SIF + Trypsin	100	78	70	55	<1.0	47

Table 10j. Measured Concentrations of Drug.

Test Article	Treatment	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-13	SIF + Pancreatin	PEA	-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0

[384] PEA was found to be endogenous in pancreatin and thus could not be quantified in the assay samples.

Appendix 10-1

Liquid Chromatography

Column: Waters ACQUITY UPLC BEH C18 30 x 2.1 mm, 1.7µm
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.8 mL/minute
Gradient Program:

Time (min)	%A	%B
0.00	50	50
0.75	1	99
1.50	1	99
1.55	50	50
2.00	50	50

Total Run Time: 2.0 minutes
Autosampler: 10 µL injection volume
Wash1: water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2: 0.1% formic acid in water

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 2.0 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-13	+998.9/282.2	141	10	68	28	5500	500	7	30	50	50
	+1015.9/282.2	42	10	53	7	5500	500	7	30	50	50
	+1015.9/998.9	63	10	20	17	5500	500	7	30	50	50
PEA	+300.3/62.1	123	10	29	10	5500	500	7	30	50	50

Example 11: PEA Stability in Simulated Intestinal Fluid.

Simulated Intestinal Fluid Stability

[385] Studies were carried out in simulated intestinal fluid containing pancreatin. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Pancreatin was then added to the solution and the pH was adjusted to 6.8. A DMSO stock was first prepared for the test articles. Aliquots of the DMSO solution were dosed into 300

µL of matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 µM. An individual tube was dosed for each time point. At each time point (0, 15, 30, 60, and 120 minutes), 900 µL of ice-cold acetonitrile containing 1.0% formic acid was added to an individual tube. Starting time for each tube was staggered such that all timepoints would finish at the same time. After the conclusion of the experiment, tubes were mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization. Analytical conditions are outlined in *Appendix 11-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 11a and 11b.

Table 11a. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-15	SIF + Pancreatin	100	<2.2	<2.2	<2.2	<2.2	ND
I-14	SIF + Pancreatin	100	25	<2.2	<2.2	<2.2	7.6

Table 11b. Measured Concentrations of Drug.

Test Article	Treatment	Concentration (µM)				
		0 min	15 min	30 min	60 min	120 min
I-15	SIF + Pancreatin	0.18	0	0	0	0
I-14	SIF + Pancreatin	0.19	0.047	0	0	0

Appendix 11-1

Liquid Chromatography

Column: Waters ACQUITY UPLC BEH C18 x2.1 mm, 1.7 µm
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.7 mL/minute
Gradient Program:

Time (min)	%A	%B
0.00	50	50
0.75	1	99
1.00	1	99
1.05	50	50
1.50	50	50

Total Run Time: 1.5 minutes
Autosampler: 1 µL Injection Volume
Wash1: water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2: 0.1% formic acid in water

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 1.5 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-15	+614.5/282.6	112	10	40	7	5500	500	7	30	50	50
I-14	+614.5/282.8	124	10	30	8	5500	500	7	30	50	50

Example 12: Determination of the Bioavailability of Palmitoylethanolamide (PEA) Following Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats.

[386] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs in male Sprague-Dawley rats.

[387] Oral bioavailability of palmitoylethanolamide (PEA) was evaluated in male Sprague-Dawley rats following oral dosing of a PEA pro-drug, **I-12**. **I-12** was dosed orally (PO) at 35.2 mg/kg in two different formulations, which is equivalent to a 10 mg/kg dose of PEA. Blood samples were collected up to 8 hours post-dose, and plasma concentrations of PEA were determined by LC-MS/MS. Following PO dosing of Group 1 of **I-12** (in 20% (Solutol

HS15:NMP 1:1) 10% PEG400; 70% H_2O) with analysis of PEA, maximum plasma concentrations (average of 12.8 ± 1.68 ng/mL) were observed at 1 hour post dosing. Average half-life could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for PEA based on the dose-normalized AUC_{last} was 2.23 ± 1.08 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for PEA (Group 1) was 3.60 ± 1.73%. Following PO dosing of Group 2 of **I-12** (in 0.5% methyl cellulose in 20% (Solutol HS15:NMP 1:1); 10% PEG400: 70% H2O) with analysis of PEA, maximum plasma concentrations (average of 16.1 ± 3.62 ng/mL) were observed at 1 hour post dosing. Average half-life after PO dosing could not be determined; however, half-life for one rat was 4.34 hours. Average exposure for PEA based on the dose-normalized AUC_{last} was 3.43 ± 1.03 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for PEA (Group 2) was 5.52 ± 1.66%.

Preparation of Dosing Formulations

[388] Pro-drugs were dosed so that a total dose of 10 mg/kg of PEA was administered. Pro-drugs were formulated in a vehicle comprised of 10% Solutol HS15, 10% n-methyl pyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400) and 70% water for Group 1 or in in a vehicle comprised of 0.5% methyl cellulose in 10% Solutol HS15, 10% NMP, 10% PEG400 and 70% water (Group 2). Formulations were prepared fresh on the day of dosing.

Animal Dosing

[389] Pharmacokinetics of PEA were evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study. Blood samples (~300 µL) were collected from rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 µL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 µL) was then transferred to a chilled, labeled polypropylene tube containing 15 µL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 12a.

Table 12a: Study Design.

Dose group	Test Article	Dosing Route	Total Animals n=	Dose (mg/kg of pro-drug)*	Dosing Solution Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sample Time Points
1	I-12	PO	3	35.2	5.03	7	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours
2		PO	3	35.2	5.03	7	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours

*All doses are based on mg/kg of the pro-drugs, and deliver 10 mg/kg of active drug, PEA.

[390] An LC-MS/MS method for the determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[391] Pharmacokinetic parameters were calculated from the time course of the plasma concentration. Maximum plasma concentration (C_{max}) and time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from the data. Area under the time concentration curve (AUC) was calculated using the linear trapezoidal rule with calculation to the last quantifiable data point, and with extrapolation to infinity if applicable. At least three quantifiable data points were required to determine the AUC. Plasma half-life ($t_{1/2}$) was calculated from 0.693/slope of the terminal elimination phase. Mean residence time, MRT, was calculated by dividing area under the moment curve (AUMC) by the AUC. Bioavailability was determined by dividing the individual dose-normalized PO AUC_{last} values by the average IV AUC_{last} value (IV data from Example 5). Samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

Results

[392] No adverse reactions were observed following the oral administration of PEA pro-drug in male Sprague-Dawley rats in this study.

[393] Dosing solutions were not analyzed by LC-MS/MS. Nominal dosing level was used in all calculations. Individual and average plasma concentrations for PEA and are shown in Tables 12b and 12c. Data are expressed as ng/mL of the free drug. Samples that were below the limit of quantitation were not used in the calculation of averages. Plasma concentrations versus time data are plotted in FIGs. 7A through 7D. Endogenous levels of PEA found in all rats were below the limit of quantitation; and therefore, measured concentrations of PEA in plasma samples were not corrected.

Table 12b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-12** (in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in Male Sprague-Dawley Rats (Group 1).

Oral (35.2 mg/kg **I-12** equals 10 mg/kg PEA)

Time (hr)	**Rat #**				
	13	**14**	**15**	**Mean**	**SD**
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	BLOQ	BLOQ	BLOQ	ND	ND
0.25	3.84	5.64	3.69	4.39	1.09
0.50	7.08	10.2	9.06	8.78	1.58
1.0	11.9	11.7	14.7	12.8	1.68
2.0	6.14	6.11	10.1	7.45	2.30
4.0	BLOQ	BLOQ	4.40	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.291	0.279	0.277	0.282	0.008
Volume Dosed (mL)	2.04	1.95	1.94	1.98	0.06
C_{max} (ng/mL)	11.9	11.7	14.7	12.8	1.68
t_{max} (hr)	1.00	1.00	1.00	1.00	0.00
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.07	1.02	1.75	1.28	0.409
AUC_{last} (hr ng/mL)	15.5	16.8	34.7	22.3	10.8
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	1.55	1.68	3.47	2.23	1.08
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	2.49	2.71	5.60	3.60	1.73

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dosenormalized by dividing the parameter by the nominal dose in mg/kg; [2]Bioavailability determined by dividing the individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable data points trailing the C_{max}.

Table 12c. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-12** (in 0.5% Methyl Cellulose in 20% (Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 35.2 mg/kg in Male Sprague-Dawley Rats (Group 2).

Oral (35.2 mg/kg **I-12** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	16	17	18	Mean	SD
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	BLOQ	BLOQ	BLOQ	ND	ND
0.25	5.79	3.55	2.98	4.11	1.49
0.50	8.27	9.21	7.72	8.40	0.753
1.0	20.1	13.0	15.3	16.1	3.62
2.0	6.78	2.71	**6.56**	5.35	2.29
4.0	3.96	BLOQ	**5.02**	4.49	ND
8.0	BLOQ	3.17	**2.54**	2.86	ND
Animal Weight (kg)	0.283	0.282	0.275	0.280	0.004
Volume Dosed (mL)	1.98	1.97	1.93	1.96	0.03
C_{max} (ng/mL)	20.1	13.0	15.3	16.1	3.62
t_{max} (hr)	1.00	1.00	1.00	1.00	0.00
$t_{1/2}$(hr)	ND[3]	ND[3]	4.34	ND	ND
MRT_{last} (hr)	1.58	2.90	2.97	2.48	0.780
AUC_{last} (hr ng/mL)	33.5	24.3	45.0	34.3	10.3
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[4]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	3.35	2.43	4.50	3.43	1.03
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[4]	ND	ND
Bioavailability (%)[2]	5.40	3.92	7.24	5.52	1.66

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]not determined due to lack of quantifiable data points trailing the C_{max}; [4]not determined because the AUC_{∞} was a greater than 25% extrapolation above the AUC_{last}.

Example 13: PEA Stability in Human, Rat, Mouse and Dog Liver Microsomes, Human, Rat, Mouse and Dog Liver S9 Fraction, Human, Rat, Mouse and Dog Intestinal S9 Fraction, Human, Rat, Mouse and Dog Plasma, and Simulated Intestinal Fluid.

[394] The present Example describes PEA stability observed in 1) human, rat, mouse, and dog liver microsomes; 2) human, rat, mouse, dog liver S9 fraction; human, rat, mouse, and dog intestinal S9 fraction; 4) human, rat, mouse, and dog plasma; and 5) simulated intestinal fluid containing various enzymes.

Liver Microsomal Stability

[395] Mixed-gender human (Lot# 1010420), male Sprague-Dawley rat (Lot# 1510115), male CD-1 mouse (Lot# 1610148), and male Beagle dog (Lot# 0810143) liver microsomes were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compound, testosterone, was run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 13-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 13a and 13b.

Reaction Composition

Liver Microsomes	0.5 mg/mL
NADPH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 µL

Table 13a. PEA stability observed in human, rat, mouse, and dog liver microsomes.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-12	Human	100	105	96	91	73	>60	<0.0231
	Rat	100	85	77	62	52	58	0.0240
	Mouse	100	83	88	68	61	>60 (82)	<0.0231 (0.0169)
	Dog	100	93	79	78	60	>60 (80)	<0.0231 (0.0173)

[a] When the calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than the shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	21	0.0667	≤ 41
	Rat	1.4	1.01	≤ 15
	Mouse	3.1	0.444	≤ 15
	Dog	33	0.0419	≤ 40

Table 13b. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (µM)				
			0 min	10 min	20 min	30 min	60 min
I-12	Human	PEA	0	0	0	0	0
	Rat		0.022	0.022	0.015	0.011	0.0048
	Mouse		0.037	0.038	0.030	0.022	0.011
	Dog		0.019	0.032	0.036	0.035	0.033

Liver S9 Stability

[396] Mixed gender human (Lot# 1210091), male Sprague-Dawley rat (Lot# 1410265), male CD-1 mouse (Lot# 1510255), and male Beagle dog (Lot# 1310285) liver S9 fraction were

provided. The reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin (7-HC), were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor cocktail (see below), and the mixture was then incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 13-1*. Test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 13c and 13d.

Reaction Composition

Liver S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 13c. PEA stability observed in human, rat, mouse, and dog liver S9.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-12	Human	100	89	73	68	43	49	0.0141
	Rat	100	82	85	75	61	>60 (90)	<0.0116 (0.00769)
	Mouse	100	75	66	58	50	53	0.0130
	Dog	100	69	53	51	47	43	0.0161

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	28	0.0247	≤34
	Rat	2.7	0.260	≤15
	Mouse	9.0	0.0770	≤37
	Dog	14	0.0493	≤42
7-hydroxycourmarin	Human	15	0.0472	≤18
	Rat	1.9	0.362	≤15
	Mouse	2.2	0.313	≤15
	Dog	1.4	0.512	≤15

Table 13d. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-12	Human	PEA	0.016	0.042	0.047	0.040	0.035
	Rat		0	0.018	0.017	0.020	0
	Mouse		0.037	0.049	0.041	0.033	0.021
	Dog		0.026	0.047	0.053	0.051	0.033

Intestinal S9 Fraction Stability

[397] Mixed-gender human (Lot# 1410073), male Sprague-Dawley rat (Lot# 1010042), male CD-1 mouse (Lot# 1510194), and male Beagle dog (Lot# 1510226) intestinal S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin, were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a

shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor cocktail, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 µL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 µL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 µL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in Appendix *13-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 13e and 13f.

Reaction Composition

Intestinal S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate, pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 µM

Table 13e. PEA stability observed in human, rat, mouse, and dog intestinal S9 fraction.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-12	Human	100	98	82	79	65	>60 (89)	<0.0116 (0.00782)
	Rat	100	92	88	74	53	>60 (67)	<0.0116 (0.0103)
	Mouse	100	80	58	64	43	46	0.0151
	Dog	100	61	44	48	47	41	0.0169

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on CL_{int} = k/P, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)
Testosterone	Human	14	0.0509
	Rat	>60	<0.0116
	Mouse	>60	<0.0116
	Dog	>60	<0.0116
7-hydroxycourmarin	Human	9.9	0.0698
	Rat	22	0.0320
	Mouse	4.3	0.160
	Dog	8.7	0.0799

Table 13f. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-12	Human	PEA	0.0089	0.023	0.024	0.032	0.043
	Rat		0	0.039	0.056	0.074	0.10
	Mouse		0.022	0.084	0.11	0.15	0.16
	Dog		0.088	0.19	0.22	0.23	0.26

Plasma Stability

[398] Studies were carried out in mixed-gender human plasma (Lot# AS1650-2), male Sprague-Dawley rat (Lot# RAT297944), male CD-1 mouse (Lot# MSE237700), and male Beagle dog (Lot# BGL91384), collected on sodium heparin. Plasma was adjusted to pH 7.4 prior to initiating the experiments. DMSO stocks were first prepared for test articles. Aliquots of the DMSO solutions were dosed into 700 μL of plasma, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in Appendix *13-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent

remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 13g and 13h.

Table 13g. PEA stability observed in human, rat, mouse, and dog plasma.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-12	Human	100	114	123	87	55	>120 (137)
	Rat	100	85	71	89	61	>120 (216)
	Mouse	100	104	107	74	42	102
	Dog	100	85	99	88	48	>120 (146)

Table 13h. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-12	Human	PEA	0	0.016	0.026	0.025	0.042
	Rat		0.031	0.059	0.073	0.099	0.13
	Mouse		0.046	0.073	0.094	0.098	0.10
	Dog		0	0.023	0.027	0.039	0.048

Simulated Intestinal Fluid Stability

[399] Studies were carried out in simulated intestinal fluid in the presence of various enzymes. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Aliquots of this solution were taken and the pH was adjusted to 6.8. Individual enzymes were then spiked into aliquots for each experiment. A DMSO stock was first prepared for test article. Aliquots of the DMSO solution were dosed into 700 μL of matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of experiment. After final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in Appendix *13-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine

the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 13i and 13j.

Table 13i. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-12	SIF + Pancreatin	100	24	14	4.7	<1.0	<15 (8.2)
	SIF + Elastase	100	101	88	63	33	79
	SIF + Carboxypeptidase A	100	74	71	25	12	38
	SIF + Carboxypeptidase B	100	71	48	24	3.4	28
	SIF + Chymotrypsin	100	86	64	31	4.1	37
	SIF + Trypsin	100	77	55	64	37	86

Table 13j. Measured Concentrations of Drug.

Test Article	Treatment	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-12	SIF + Pancreatin	PEA	-	-	-	-	-
	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0

[400] PEA was found to be endogenous in pancreatin and thus could not be quantified in the assay samples.

Appendix 13-1

Liquid Chromatography

Column:	Waters ACQUITY UPLC BEH C18 30 x 2.1 mm, 1.7 µm
M.P. Buffer:	25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A):	90% water, 10% buffer
Organic Reservoir (B):	90% acetonitrile, 10% buffer
Flow Rate:	0.8 mL/minute
Gradient Program:	

Time (min)	%A	%B
0.00	50	50
0.75	1	99
1.50	1	99
1.55	50	50
1.75	50	50

Total Run Time:	1.75 minutes
Autosampler:	10 µL Injection Volume
Wash1:	water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2:	0.1% formic acid in water

Mass Spectrometer

Instrument:	PE SCIEX API 4000
Interface:	Turbo Ionspray
Mode:	Multiple reaction monitoring
Method:	1.75 minute duration
Settings:	

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-12	+1042.9/282.4	175	10	49	18	5500	500	7	30	50	50
	+1059.9/282.4	60	10	62	18	5500	500	7	30	50	50
	+1059.9/1043.0	60	10	29	37	5500	500	7	30	50	50
PEA	+300.3/62.1	123	10	29	10	5500	500	7	30	50	50

Example 14: PEA Stability in Simulated Intestinal Fluid.

Simulated Intestinal Fluid Stability

[401] Studies were carried out in simulated intestinal fluid containing pancreatin. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Pancreatin was then added to the solution and the pH was adjusted to 6.8. A DMSO stock was first prepared for test articles. Aliquots of DMSO solution were dosed into 300 µL of

matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. An individual tube was dosed for each time point. At each time point (0, 15, 30, 60, and 120 minutes), 900 μL of ice-cold acetonitrile containing 1.0% formic acid was added to an individual tube. The starting time for each tube was staggered such that all time points would finish at the same time. After the conclusion of the experiment, tubes were mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization. Analytical conditions are outlined in *Appendix 14-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 14a and 14b.

Table 14a. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-8	SIF + Pancreatin	100	17	6.8	<1.3	<1.3	6.2
I-16	SIF + Pancreatin	100	<4.5	<4.5	<4.5	<4.5	ND

Table 14b. Measured Concentrations of Drug.

Test Article	Treatment	Concentration (μM)				
		0 min	15 min	30 min	60 min	120 min
I-8	SIF + Pancreatin	0.94	0.16	0.064	0	0
I-16	SIF + Pancreatin	0.088	0	0	0	0

Appendix 14-1

Column: Waters ACQUITY UPLC BEH C18 30 x 2.1 mm, 1.7 µm
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.7 mL/minute
Gradient Program:

Time (min)	%A	%B
0.0	50	50
.75	1	99
1.00	1	99
1.05	50	50
1.50	50	50

Total Run Time: 1.5 minutes
Autosampler: 1 µL Injection Volume
Wash1: water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2: 0.1% formic acid in water

Mass Spectrometer

Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 1.5 minute duration
Settings:

Test Article	Q1/Q3	DP	EP	CE	CXP	IS	TEM	CAD	CUR	GS1	GS2
I-8	+474.3/282.2	112	10	28	18	5500	500	7	30	50	50
I-16	+614.5/282.6	123	10	37	18	5500	500	7	30	50	50

Example 15: Determination of the Bioavailability of Palmitoylethanolamide (PEA) Following Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats.

[402] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs in male Sprague-Dawley rats.

[403] Oral bioavailability of palmitoylethanolamide (PEA) was evaluated in male Sprague-Dawley rats following oral administration of PEA pro-drugs, **I-15** and **I-14**. Each test article was dosed orally (PO) at 20.7 mg/kg, which was equivalent to a 10 mg/kg dose of PEA. Blood samples were collected up to 8 hours post-dose, and plasma concentrations of PEA were determined by LC-MS/MS. Following PO dosing of **I-15** (in 20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H20), average C_{max} of 11.7 ± 2.34 ng/mL was observed between 15 minutes and 1

hour post dose. Average exposure for **I-15** (Group 1) based on the dose-normalized AUC_{last} was 2.13 ± 1.05 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-15** (Group 1) was 3.42 ± 1.69%. Following PO dosing of **I-14** (in 20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H20), average C_{max} of 16.9 ± 1.47 ng/mL was observed at 30 minutes post dose in all rats. Average exposure for **I-14** (Group 2) based on dose-normalized AUC_{last} was 2.72 ± 0.854 hr*kg*ng/mL/mg. Based on the IV data from Example 5, average oral bioavailability for **I-14** (Group 2) was 4.39 ± 1.37%.

Preparation of Dosing Formulations

[404] Pro-drugs were dosed so that a total dose of 10 mg/kg of PEA was administered. Prodrugs were formulated in a vehicle comprised of 10% Solutol HS15, 10% n-methyl pyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400) and 70% water.

Animal Dosing

[405] Pharmacokinetics of PEA was evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study. Blood samples (~300 µL) were collected from the rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 µL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 µL) was then transferred to a chilled, labeled polypropylene tube containing 15 µL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 15a.

Table 15a: Study Design.

Dose group	Test Article	Dosing Route	Total Animals n=	Dose (mg/kg of pro-drug)*	Dosing Solution Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sample Time Points
1	**I-15**	PO	3	20.7	3	6.9	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours
2	**I-14**	PO	3	20.7	3	6.9	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours

*All doses are based on mg/kg of the pro-drugs, and deliver 10 mg/kg of active drug, PEA.

[406] An LC-MS/MS method for the determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[407] Pharmacokinetic parameters were calculated from the time course of the plasma concentration. Maximum plasma concentration (C_{max}) and the time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from data. Area under the time concentration curve (AUC) was calculated using the linear trapezoidal rule with calculation to the last quantifiable data point, and with extrapolation to infinity if applicable. At least three quantifiable data points were required to determine the AUC. Plasma half-life ($t_{1/2}$) was calculated from 0.693/slope of the terminal elimination phase. Mean residence time, MRT, was calculated by dividing area under the moment curve (AUMC) by AUC. Bioavailability was determined by dividing individual dose-normalized PO AUC_{last} values by the average IV AUC_{last} value (IV data from Example 5). Samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

Results

[408] No adverse reactions were observed following the oral administration of PEA pro-drug in male Sprague-Dawley rats in this study. Dosing solutions were not analyzed by LC-MS/MS. Nominal dosing level was used in all calculations. Individual and average plasma concentrations for PEA and are shown in Table 15b and Table 15c. Data are expressed as ng/mL of the free drug. Samples that were below the limit of quantitation were not used in the calculation of

averages. Plasma concentrations versus time data are plotted in FIGs. 15A through 15D. Endogenous levels of PEA found in all rats were below the limit of quantitation; and therefore, measured concentrations of PEA in plasma samples were not corrected.

Table 15b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-15** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in Male Sprague-Dawley Rats (Group 1).

Oral (20.7 mg/kg **I-15** equals 10 mg/kg PEA)

Time (hr)	Rat #				
	305	**306**	**307**	**Mean**	**SD**
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	BLOQ	3.92	BLOQ	3.92	ND
0.25	8.62	9.44	9.00	9.02	0.410
0.50	11.4	12.6	8.92	11.0	1.88
1.0	13.4	6.95	4.60	8.32	4.56
2.0	9.93	10.7	4.36	8.33	3.46
4.0	BLOQ	3.41	BLOQ	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.250	0.235	0.247	0.244	0.008
Volume Dosed (mL)	1.73	1.62	1.70	1.68	0.06
C_{max} (ng/mL)	13.4	12.6	9.00	11.7	2.34
t_{max} (hr)	1.0	0.50	0.25	0.58	0.38
$t_{1/2}$(hr)	ND[3]	ND[4]	ND[4]	ND	ND
MRT_{last} (hr)	1.07	1.69	0.917	1.23	0.409
AUC_{last} (hr ng/mL)	21.1	31.9	10.9	21.3	10.5
AUC_{∞} (hr ng/mL)	ND[3]	ND[4]	ND[4]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	2.11	3.19	1.09	2.13	1.05
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[4]	ND[4]	ND	ND
Bioavailability (%)[2]	3.40	5.13	1.75	3.42	1.69

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}. [4]Not determined because the line defining the terminal elimination phase had an $r^2 > 0.85$.

Table 15b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-14** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in Male Sprague-Dawley Rats (Group 1).

Oral (20.7 mg/kg **I-14** (equals 10 mg/kg PEA)

Time (hr)	Rat #				
	308	**309**	**310**	**Mean**	**SD**
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	4.46	BLOQ	4.54	4.50	ND
0.25	11.3	4.91	14.1	10.1	4.71
0.50	15.8	16.4	18.6	16.9	1.47
1.0	14.8	13.7	**11.0**	13.2	1.96
2.0	9.02	5.99	**9.41**	8.14	1.87
4.0	BLOQ	BLOQ	**3.97**	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.239	0.248	0.249	0.245	0.006
Volume Dosed (mL)	1.65	1.71	1.72	1.69	0.04
C_{max} (ng/mL)	15.8	16.4	18.6	16.9	1.47
t_{max} (hr)	0.50	0.50	0.50	0.50	0.00
$t_{1/2}$(hr)	ND[3]	ND[3]	1.96	ND	ND
MRT_{last} (hr)	0.970	0.959	1.54	1.16	0.331
AUC_{last} (hr ng/mL)	24.4	20.4	36.8	27.2	8.54
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	48.1	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	2.44	2.04	3.68	2.72	0.854
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	4.81	ND	ND
Bioavailability (%)[2]	3.94	3.29	5.93	4.39	1.37

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Example 16: Determination of the Bioavailability of Palmitoylethanolamide (PEA) Following Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats.

[409] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs **I-8** and **I-16** in male Sprague-Dawley rats according to the methods described in, e.g., Example 15. Individual and average plasma concentrations for PEA and are shown in Table 16a and Table 16b. Plasma concentrations versus time data are plotted in FIGs. 9A through 9D.

Table 16a. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 16 mg/kg in Male Sprague-Dawley Rats (Group 1).

Oral (16 mg/kg **I-8** equals 10 mg/kg PEA)

Time (hr)	**Rat #**				
	317	**318**	**319**	**Mean**	**SD**
0	BLOQ	BLOQ	BLOQ	ND	ND
0.083	3.06	3.99	4.73	3.93	0.837
0.25	11.2	8.54	11.4	10.4	1.60
0.50	52.5	33.1	42.9	42.8	9.70
1.0	65.1	41.4	65.0	57.2	13.7
2.0	29.2	26.9	21.8	26.0	3.79
4.0	8.08	6.40	7.22	7.23	0.840
8.0	BLOQ	2.50	BLOQ	ND	ND
Animal Weight (kg)	0.275	0.269	0.271	0.272	0.003
Volume Dosed (mL)	1.46	1.43	1.44	1.44	0.02
C_{max} (ng/mL)	65.1	41.4	65.0	57.2	13.7
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	1.86	ND[3]	ND	ND
MRT_{last} (hr)	1.56	2.23	1.49	1.76	0.411
AUC_{last} (hr ng/mL)	114	105	103	107	6.17
AUC_{∞} (hr ng/mL)	ND[3]	112	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	11.4	10.5	10.3	10.7	0.617
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	11.2	ND[3]	ND	ND
Bioavailability (%)[2]	18.4	16.9	16.5	17.3	0.994

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last

observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Table 16b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 16 mg/kg in Male Sprague-Dawley Rats (Group 1).

Oral (16 mg/kg **I-16** equals 10 mg/kg PEA)

Time (hr)	**Rat #**				
	320	**321**	**322**	**Mean**	**SD**
0	BLOQ	BLOQ	BLOQ	ND	ND
0.083	2.79	2.93	BLOQ	2.86	ND
0.25	8.32	5.06	4.65	6.01	2.01
0.50	43.4	23.0	18.5	28.3	13.3
1.0	55.0	34.3	40.2	43.2	10.7
2.0	27.8	14.0	12.8	18.2	8.34
4.0	6.81	8.79	5.15	6.92	1.82
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.279	0.269	0.278	0.275	0.006
Volume Dosed (mL)	1.93	1.86	1.92	1.90	0.04
C_{max} (ng/mL)	55.0	34.3	40.2	43.2	10.7
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.58	1.72	1.54	1.62	0.0980
AUC_{last} (hr ng/mL)	101	62.5	61.7	75.0	22.4
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	10.1	6.25	6.17	7.50	2.24
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	16.2	10.1	9.93	12.1	3.60

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Example 17: PEA Stability in Human, Rat, Mouse and Dog Liver Microsomes, Human, Rat, Mouse and Dog Liver S9 Fraction, Human, Rat, Mouse and Dog Intestinal S9 Fraction, Human, Rat, Mouse and Dog Plasma, and Simulated Intestinal Fluid.

[410] The present Example describes PEA stability observed in 1) human, rat, mouse, and dog liver microsomes; 2) human, rat, mouse, dog liver S9 fraction; human, rat, mouse, and dog intestinal S9 fraction; 4) human, rat, mouse, and dog plasma; and 5) simulated intestinal fluid containing various enzymes.

Liver Microsomal Stability

[411] Mixed-gender human (Lot# 1010420), male Sprague-Dawley rat (Lot# 1510115), male CD-1 mouse (Lot# 1610148), and male Beagle dog (Lot# 1110044) liver microsomes were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 µM. Control compound, testosterone, was run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor) was equilibrated in a shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 µL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 µL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 µL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 17-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 17a and 17b.

Reaction Composition

Liver Microsomes	0.5 mg/mL
NADPH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM

Magnesium Chloride	5 mM
Test Article	1 μL

Table 17a. PEA stability observed in human, rat, mouse, and dog liver microsomes.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-8	Human	100	2.8	<1.0	<1.0	<1.0	<10 (2.0)	>0.139 (0.712)
	Rat	100	4.3	1.2	<1.0	<1.0	<10 (2.2)	>0.139 (0.624)
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0	>1.38
I-16	Human	100	8.0	3.5	1.9	<1.0	<10 (2.8)	>0.139 (0.493)
	Rat	100	9.7	4.2	3.1	<1.0	<10 (3.1)	>0.139 (0.452)
	Mouse	100	7.6	3.0	2.1	1.8	<10 (2.8)	>0.139 (0.503)
	Dog	100	4.4	1.6	1.4	1.5	<10 (2.2)	>0.139 (0.618)

[a] When the calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than the shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.

[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	9.2	0.151	≤ 41
	Rat	1.5	0.911	≤ 15
	Mouse	2.1	0.674	≤ 15
	Dog	23	0.0593	≤ 40

Table 17b. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-8	Human	PEA	0.063	0.34	0.25	0.21	0.070
	Rat		0.033	0.041	0.019	0	0
	Mouse		0.45	0.30	0.15	0.086	0
	Dog		0.33	0.54	0.46	0.44	0.20
I-16	Human		0	0.22	0.15	0.14	0.069
	Rat		0.025	0.033	0.015	0	0
	Mouse		0.29	0.27	0.12	0.089	0.0093
	Dog		0.053	0.59	0.48	0.43	0.20

Liver S9 Stability

[412] Mixed gender human (Lot# 0910396), male Sprague-Dawley rat (Lot# 1410265), male CD-1 mouse (Lot# 1310026), and male Beagle dog (Lot# 1310285) liver S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin (7-HC), were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor cocktail (see below), and the mixture was then incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and the expected drug (PEA). Analytical conditions are outlined in *Appendix 17-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 17c and 17d.

Reaction Composition

Liver S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 17c. PEA stability observed in human, rat, mouse, and dog liver S9.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-8	Human	100	1.4	<1.0	<1.0	<1.0	<10 (1.6)	>0.0693 (0.431)
	Rat	100	17	2.8	<1.0	<1.0	<10 (3.9)	>0.0693 (0.177)
	Mouse	100	4.6	1.0	<1.0	<1.0	<10 (2.3)	>0.0693 (0.305)
	Dog	100	32	11	3.5	<1.0	<10 (6.1)	>0.0693 (0.113)
I-16	Human	100	4.9	2.1	<1.0	<1.0	<10 (2.3)	>0.0693 (0.299)
	Rat	100	3.0	<1.0	<1.0	<1.0	<10 (2.0)	>0.0693 (0.352)
	Mouse	100	3.7	<1.0	<1.0	<1.0	<10 (2.1)	>0.0693 (0.330)
	Dog	100	2.1	<1.0	<1.0	<1.0	<10 (1.8)	>0.0693 (0.385)

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)	Acceptable Range ($t_{1/2}$, min)
Testosterone	Human	15	0.0463	≤34
	Rat	2.7	0.260	≤15
	Mouse	17	0.0417	≤37
	Dog	25	0.0272	≤42
7-hydroxycourmarin	Human	11	0.0628	≤18
	Rat	2.5	0.283	≤15
	Mouse	4.3	0.162	≤15
	Dog	2.1	0.334	≤15

Table 17d. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-8	Human	PEA	0.19	0.57	0.47	0.41	0.28
	Rat		0.069	0.17	0.092	0.061	0.013
	Mouse		0.29	0.50	0.44	0.38	0.37
	Dog		0.13	0.33	0.34	0.33	0.20
I-16	Human		0.10	0.46	0.42	0.39	0.27
	Rat		0.050	0.13	0.13	0.058	0.020
	Mouse		0.33	0.78	0.68	0.59	0.43
	Dog		0.053	0.26	0.33	0.29	0.23

Intestinal S9 Fraction Stability

[413] Mixed-gender human (Lot# 1410073), male Sprague-Dawley rat (Lot# 1510303), male CD-1 mouse (Lot# 1510194), and male Beagle dog (Lot# 1510226) intestinal S9 fraction were provided. Reaction mixture, minus cofactors, was prepared as described below. Test article was added into the reaction mixture at a final concentration of 1 μM. Control compounds, testosterone and 7-hydroxycoumarin, were run simultaneously with the test article in a separate reaction. An aliquot of the reaction mixture (without cofactor cocktail) was equilibrated in a shaking water bath at 37 °C for 3 minutes. Reaction was initiated by the addition of cofactor cocktail, and the mixture was incubated in a shaking water bath at 37 °C. Aliquots (100 μL) were withdrawn at 0, 10, 20, 30, and 60 minutes. Test article samples were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Control samples were immediately combined with 400 μL of ice-cold 50/50 acetonitrile (ACN)/dH_2O containing 0.1% formic acid and internal standard to terminate the reaction. Samples were then mixed and centrifuged to precipitate proteins. Calibration standards were prepared in matched matrix.

Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 17-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent remaining at each time point. Halflives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 17e and 17f.

Reaction Composition

Intestinal S9 Fraction	1.0 mg/mL
NADPH (cofactor)	1 mM
UDPGA (cofactor)	1 mM
PAPS (cofactor)	1 mM
GSH (cofactor)	1 mM
Potassium Phosphate, pH 7.4	100 mM
Magnesium Chloride	5 mM
Test Article	1 μM

Table 17e. PEA stability observed in human, rat, mouse, and dog intestinal S9 fraction.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)	CL_{int}[b] (mL/min/mg protein)
		0 min	10 min	20 min	30 min	60 min		
I-8	Human	100	31	7.0	1.8	<1.0	<10 (5.7)	>0.0693 (0.122)
	Rat	100	58	37	22	6.2	14	0.0503
	Mouse	100	65	45	31	12	18	0.0389
	Dog	100	<1.0	<1.0	<1.0	<1.0	<10 (1.5)	>0.0693 (0.473)
I-16	Human	100	3.2	<1.0	<1.0	<1.0	<10 (2.0)	>0.0693 (0.344)
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0	>0.691
	Mouse	100	2.0	<1.0	<1.0	<1.0	<10 (1.8)	>0.0693 (0.389)
	Dog	100	1.7	<1.0	<1.0	<1.0	<10 (1.7)	>0.0693 (0.409)

[a] When calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is elimination rate constant and P is protein concentration in the incubation.

Control Compound	Species	Half-life (min)	CL_{int} (ml/min/mg protein)
Testosterone	Human	4.8	0.144
	Rat	>60 (102)	<0.0116 (0.00680)
	Mouse	>60 (90)	<0.0116 (0.00767)
	Dog	>60	<0.0116
7-hydroxycourmarin	Human	13	0.0522
	Rat	29	0.0242
	Mouse	5.0	0.138
	Dog	9.0	0.0770

Table 17f. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	10 min	20 min	30 min	60 min
I-8	Human	PEA	0.021	0.32	0.39	0.38	0.38
	Rat		0.022	0.22	0.34	0.42	0.45
	Mouse		0.012	0.12	0.19	0.22	0.26
	Dog		0.33	0.71	0.81	0.67	0.81
I-16	Human		0	0.17	0.29	0.33	0.32
	Rat		0	0.20	0.28	0.34	0.38
	Mouse		0	0.10	0.17	0.18	0.26
	Dog		0.042	0.50	0.57	0.54	0.68

Plasma Stability

[414] Studies were carried out in mixed-gender human plasma (Lot# AS1650-2), male Sprague-Dawley rat (Lot# RAT320835), male CD-1 mouse (Lot# MSE260693), and male Beagle dog (Lot# BGL91384), on sodium heparin. Plasma was adjusted to pH 7.4 prior to initiating the experiments. DMSO stocks were first prepared for the test articles. Aliquots of the DMSO solutions were dosed into 700 μL of plasma, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 17-1*. Test article concentration at each time point was compared to test article concentration at time 0 to determine the percent

remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 17g and 17h.

Table 17g. PEA stability observed in human, rat, mouse, and dog plasma.

Test Article	Species	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-8	Human	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Rat	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Mouse	100	<1.0	<1.0	<1.0	<1.0	<1.0
	Dog	100	<1.0	<1.0	<1.0	<1.0	<1.0
I-16	Human	100	78	51	24	5.8	31
	Rat	100	20	9.3	4.1	1.6	<15 (6.9)
	Mouse	100	10	4.0	1.1	<1.0	<15 (4.7)
	Dog	100	91	65	39	13	44

Table 17h. Measured Concentrations of Drug.

Dosed Test Article	Species	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-8	Human	PEA	0.27	1.25	1.30	1.16	1.50
	Rat		0.21	0.62	0.64	0.55	0.59
	Mouse		0.97	0.93	0.92	1.05	1.30
	Dog		0.33	0.72	0.72	0.73	0.69
I-16	Human		0	0.19	0.38	0.53	0.67
	Rat		0	0.23	0.24	0.24	0.30
	Mouse		0.12	0.53	0.55	0.68	0.79
	Dog		0	0.12	0.18	0.34	0.44

Simulated Intestinal Fluid Stability

[415] Studies were carried out in simulated intestinal fluid in the presence of various enzymes. Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 1.0 L of water. Aliquots of this solution were taken and the pH was adjusted to 6.8. Individual enzymes were then spiked into aliquots for each experiment. A DMSO stock was first prepared for the test article. Aliquots of the DMSO solution were dosed into 700 μL of matrix, which had been pre-warmed to 37 °C, at a final test article concentration of 1 μM. Aliquots (100 μL) were taken at each time point (0, 15, 30, 60, and 120 minutes) and were immediately combined with 300 μL of ice-cold acetonitrile containing 1% formic acid. Samples were stored at 4 °C until the end of the experiment. After the final time point was sampled, the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Calibration standards were prepared in matched matrix. Samples and standards were assayed by LC-MS/MS using electrospray ionization for both dosed

prodrug and expected drug (PEA). Analytical conditions are outlined in *Appendix 17-1*. Test article concentration at each time point was compared to the test article concentration at time 0 to determine the percent remaining at each time point. Half-lives were calculated using GraphPad software, fitting to a single-phase exponential decay equation. Results are shown in Tables 17i and 17j.

Table 17i. PEA stability observed in simulated intestinal fluid (SIF).

Test Article	Treatment	% Remaining of Initial (n=1)					Half-life[a] (min)
		0 min	15 min	30 min	60 min	120 min	
I-8	SIF + Elastase	100	38	17	16	17	<15 (12)
	SIF + Carboxypeptidase A	100	33	8.7	<1.0	<1.0	<15 (9.2)
	SIF + Carboxypeptidase B	100	30	7.1	<1.0	<1.0	<15 (8.5)
	SIF + Chymotrypsin	100	36	8.7	<1.0	<1.0	<15 (9.6)
	SIF + Trypsin	100	52	31	28	25	25
I-16	SIF + Elastase	100	72	50	27	7.1	31
	SIF + Carboxypeptidase A	100	88	74	60	32	75
	SIF + Carboxypeptidase B	100	94	87	68	37	89
	SIF + Chymotrypsin	100	103	90	68	35	84
	SIF + Trypsin	100	89	82	62	37	85

[a] When the calculated half-life is longer than the duration of the experiment, half-life is expressed as > the longest incubation time. Similarly, if calculated half-life is less than the shortest time point, half-life is expressed as < that time point and calculated half-life is also listed in parentheses.

Table 17j. Measured Concentrations of Drug.

Test Article	Treatment	Analyte	Concentration (μM)				
			0 min	15 min	30 min	60 min	120 min
I-8	SIF + Elastase	PEA	0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0
I-16	SIF + Elastase		0	0	0	0	0
	SIF + Carboxypeptidase A		0	0	0	0	0
	SIF + Carboxypeptidase B		0	0	0	0	0
	SIF + Chymotrypsin		0	0	0	0	0
	SIF + Trypsin		0	0	0	0	0

Appendix 17-1

Liquid Chromatography

Column: Waters ACQUITY UPLC BEH Phenyl 30 x 2.1 mm, 1.7μm
M.P. Buffer: 25 mM ammonium formate buffer, ,pH 3.5
Aqueous Reservoir (A): 90% water, 10% buffer
Organic Reservoir (B): 90% acetonitrile, 10% buffer
Flow Rate: 0.7 mL/minute
Gradient Program:

Time (min)	**%A**	**%B**
0.0	50	50
2.00	15	85
2.05	50	50
2.50	50	50

Total Run Time: 2.5 minutes
Autosampler: 3 μL injection volume
Wash1: water/methanol/2-propanol:1/1/1; with 0.2% formic acid
Wash2: 0.1% formic acid in water

Mass Spectrometer
Instrument: PE SCIEX API 4000
Interface: Turbo Ionspray
Mode: Multiple reaction monitoring
Method: 2.5 minute duration
Settings:

Test Article	+/–	Q1	Q3	DP	EP	CE	CXP	IS
I-8	+	474.3	282.2	112	10	28	18	5500
I-16	+	614.5	282.6	123	10	37	18	5500
PEA	+	300.3	62.0	100	10	32	10	5500

Table A. Summary of Half Life and Oral Bioavailability Data.

Compound	Half life (mins)	Bioavailability
PEA	n/a	0.56 %
I-11	<1	4.5 %
I-15	<1	3.42 %
I-14	7.5	4.39 %
I-16	<1	12.1 %
I-8	6.2	17.3 %

Example 18: Determination of the Bioavailability of Palmitoylethanolamide (PEA) Following Oral Administration of PEA-Prodrug in Male Sprague-Dawley Rats.

[416] The present Example describes oral bioavailability of PEA following administration of PEA prodrugs in male Sprague-Dawley rats.

[417] Ora the oral bioavailability of palmitoylethanolamide (PEA) was evaluated in male Sprague-Dawley rats following oral dosing of PEA pro-drugs, **I-8** and **I-16**. **I-8** was dosed orally (PO) at 4, 8 and 16 mg/kg, and **I-16** was dosed orally (PO) at 5.2, 10.35 and 20.7 mg/kg in a formulation consisting of 20% (Solutol HS15:NMP 1:1), 10% PEG400, and 70% water. Each prodrug dosed was equivalent to 2.5, 5, or 10 mg/kg dose of PEA. Blood samples were collected up to 8 hours post-dose, and plasma concentrations of PEA were determined by LC-MS/MS. Bioavailability was calculated using IV data from Example 5.

[418] Following PO dosing of **I-8** at 4 mg/kg (2.5 mg/kg PEA equivalent) maximum plasma concentrations (average of 25.3 ± 6.67 ng/mL) were observed at 1 hour post dosing. Halflife could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for **I-8** based on the dose-normalized AUC_{last} was 13.5 ± 4.65 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 21.7 ± 7.48%.

[419] Following PO dosing of **I-8** at 8 mg/kg (5 mg/kg PEA equivalent) maximum plasma concentrations (average of 46.9 ± 13.6 ng/mL) were observed at 1 hour post dosing. Halflife could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for **I-8** based on the dose-normalized AUC_{last} was 14.8 ± 1.19 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 23.9 ± 1.92%.

[420] Following PO dosing of **I-8** at 16 mg/kg (10 mg/kg PEA equivalent) maximum plasma concentrations (average of 102 ± 31.8 ng/mL) were observed at 1 hour post dosing. Half-life could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average

exposure for **I-8** based on the dose-normalized AUC_{last} was 16.8 ± 3.80 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 27.1 ± 6.13%.

[421] Following PO dosing of **I-16** at 5.2 mg/kg (2.5 mg/kg PEA equivalent) maximum plasma concentrations (average of 25.3 ± 23.6 ng/mL) were observed between 30 minutes and 1 hour post dosing. Half-life could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for **I-16** based on the dose-normalized AUC_{last} was 9.08 ± 6.08 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 14.6 ±11.1%.

[422] Following PO dosing of **I-16** at 10.35 mg/kg (5 mg/kg PEA equivalent) maximum plasma concentrations (average of 43.9 ± 7.33 ng/mL) were observed at 1 hour post dosing. Halflife could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for **I-16** based on the dose-normalized AUC_{last} was 10.6 ± 0.544 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 17.0 ± 0.876%.

[423] Following PO dosing of **I-16** at 20.7 mg/kg (10 mg/kg PEA equivalent) maximum plasma concentrations (average of 68.3 ± 11.4 ng/mL) were observed at 1 hour post dosing. Halflife could not be determined due to a lack of quantifiable data points trailing the C_{max}. Average exposure for **I-16** based on the dose-normalized AUC_{last} was 11.2 ± 1.01 hr*kg*ng/mL/mg. Average oral bioavailability for PEA in this group was 18.0 ± 1.63%.

[424] Following each dose of **I-8**, there was a dose proportional increase in C_{max} for PEA. Average PEA C_{max} values after **I-8** dosing were 25.3, 46.9, and 102 ng/mL following the 4, 8, and 16 mg/kg doses, respectively. Average dose normalized AUC_{last} values (13.5, 14.8, and 16.8 hr*kg*ng/mL/mg) and bioavailability (21.7, 23.9, and 27.1%) were also after similar the 4, 8, and 16 mg/kg **I-8** doses, respectively.

[425] Following each dose of **I-16**, there was a dose proportional increase in C_{max} for PEA. Average PEA C_{max} values after **I-16** dosing were 25.3, 43.9, 68.3 ng/mL following the 5.2, 10.35, and 20.7 mg/kg doses, respectively. Average dose normalized AUC_{last} values (9.08, 10.6, 11.2 hr*kg*ng/mL/mg) and bioavailability (14.6, 17.0, 18.0%) were also similar after the 5.2, 10.35, and 20.7 mg/kg **I-16** doses, respectively.

Preparation of Dosing Formulations

[426] Pro-drugs were dosed so that a total dose of 2.5, 5, 10 mg/kg of PEA was administered. Each prodrug was formulated in a vehicle comprised of 10% Solutol HS15, 10% n-methyl pyrrolidone (NMP), 10% polyethylene glycol 400 (PEG400) and 70% water. Formulations were prepared fresh on the day of dosing.

Animal Dosing

[427] Pharmacokinetics of PEA were evaluated in fasted male Sprague-Dawley rats. Rats were housed one per cage. Each rat was fitted with a jugular vein cannula (JVC) for blood collection. Each study group was dosing in triplicate. Rats were fasted for a minimum of twelve hours prior to dosing. Food was returned at four hours post dosing. Animals had free access to water throughout the study. Blood samples (~300 µL) were collected from the rats via a JVC and placed into chilled polypropylene tubes containing sodium heparin as an anticoagulant, and 30 µL of 0.5 M citric acid. Samples were maintained chilled throughout processing. Blood samples were centrifuged at 4 °C and 3,000 g for 5 minutes. Plasma (~150 µL) was then transferred to a chilled, labeled polypropylene tube containing 15 µL of 10% formic acid, placed on dry ice, and stored in a freezer maintained at -60 °C to -80 °C. Blood sampling times are shown in Table 18a.

Table 18a: Study Design.

Group #	Test Article	Dosing Route	Total Animals n=	Dose (mg/kg of pro-drug)*	Dosing Solution Conc. (mg/mL)	Dosing Volume (mL/kg)	Vehicle	Blood Sample Time Points
1	I-8	PO	3	4	2	2	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours
2		PO	3	8	2	4		
3		PO	3	16	3	5.3		
4	I-16	PO	3	5.2	2	2.6	20% (Solutol HS15:NMP 1:1) 10% PEG400; 70% H_2O	Pre-dose, 5, 15, 30 min, 1, 2, 4, 8 hours
5		PO	3	10.35	3	3.45		
6		PO	3	20.7	3	6.9		

*All doses are based on mg/kg of the pro-drugs, and deliver 10 mg/kg of active drug, PEA.

[428] An LC-MS/MS method for the determination of PEA and PEA-prodrug is described above (see e.g., Example 3).

[429] Pharmacokinetic parameters were calculated from the time course of the plasma concentration. Maximum plasma concentration (C_{max}) and the time to reach maximum plasma drug concentration (T_{max}) after oral dosing were observed from the data. Area under the time concentration curve (AUC) was calculated using the linear trapezoidal rule with calculation to the last quantifiable data point, and with extrapolation to infinity if applicable. At least three

quantifiable data points were required to determine the AUC. Mean residence time (MRT) was calculated by dividing the area under the moment curve (AUMC) by the AUC. Bioavailability was determined by dividing the individual dose-normalized PO AUC_{last} values by the average IV AUC_{last} value (IV data from Example 5). Samples below the limit of quantitation were treated as zero for pharmacokinetic data analysis.

Results

[430] No adverse reactions were observed following the oral administration of PEA pro-drugs in male Sprague-Dawley rats. Dosing solutions were not analyzed by LC-MS/MS. Nominal dosing level was used in calculations. Concentrations are expressed as mg/mL of the free base.

[431] Individual and average plasma concentrations for PEA and are shown in Table 18b through Table 18g. Data are expressed as ng/mL of the free drug. Samples that were below the limit of quantitation were not used in the calculation of averages. Plasma concentration versus time data are plotted in FIGs. 10A through 10L. Endogenous levels of PEA were found in the majority of all the rats. Measured concentrations of PEA in plasma samples were corrected by subtracting the concentration of PEA measured in the pre-dose samples. Corrected values are reported in tables below and were used to determine pharmacokinetic parameters. Corrected values that were negative are reported as not determined (ND).

Table 18b. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 4 mg/kg in Male Sprague-Dawley Rats.

Oral (4 mg/kg **I-8** equals 2.5 mg/kg PEA)

Time (hr)	Rat #				
	446	447	448	Mean	SD
0 (pre-dose)	BLOQ	BLOQ	BLOQ	ND	ND
0.083	6.36	BLOQ	BLOQ	ND	ND
0.25	4.60	2.71	BLOQ	3.66	ND
0.50	9.80	16.9	14.3	13.7	3.59
1.0	18.1	31.3	26.4	25.3	6.67
2.0	6.19	6.38	10.4	7.66	2.38
4.0	BLOQ	BLOQ	4.57	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.294	0.287	0.294	0.292	0.004
Volume Dosed (mL)	0.59	0.57	0.59	0.58	0.01
C_{max} (ng/mL)	18.1	31.3	26.4	25.3	6.67
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	0.991	0.988	1.59	1.19	0.345
AUC_{last} (hr ng/mL)	22.1	33.6	45.3	33.7	11.6
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	8.84	13.4	18.1	13.5	4.65
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	14.2	21.6	29.2	21.7	7.48

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Table 18c. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 8 mg/kg in Male Sprague-Dawley Rats.

Oral (8 mg/kg **I-8** equals 5 mg/kg PEA)

Time (hr)	Rat #				
	449	**450**	**451**	**Mean**	**SD**
0 (pre-dose)	ND	ND	ND	ND	ND
0.083	3.16	1.41	0.920	1.83	1.18
0.25	9.62	1.31	2.86	4.60	4.42
0.50	46.2	30.0	13.7	30.0	16.3
1.0	61.8	43.7	35.1	46.9	13.7
2.0	9.52	18.4	22.0	16.6	6.41
4.0	0.0300	1.13	3.37	1.51	1.70
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.302	0.301	0.287	0.297	0.008
Volume Dosed (mL)	1.12	1.20	1.15	1.19	0.03
C_{max} (ng/mL)	61.8	43.7	35.1	46.9	13.6
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.05	1.34	1.58	1.32	0.268
AUC_{last} (hr ng/mL)	80.4	73.2	68.5	74.0	5.97
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	16.1	14.6	13.7	14.8	1.19
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	25.9	23.6	22.1	23.9	1.92

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Table 18d. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-8** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 16 mg/kg in Male Sprague-Dawley Rats.

Oral (16 mg/kg **I-8** equals 5 mg/kg PEA)

Time (hr)	Rat #				
	452	**453**	**454**	**Mean**	**SD**
0 (pre-dose)	ND	ND	BLOQ	ND	ND
0.083	ND	2.60	5.10	3.85	ND
0.25	5.75	BLOQ	13.4	9.58	ND
0.50	70.2	45.0	80.6	65.3	18.3
1.0	95.8	74.4	137	102	31.8
2.0	28.6	38.0	46.0	37.5	8.72
4.0	6.97	13.3	6.56	8.94	3.78
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.288	0.289	0.292	0.290	0.002
Volume Dosed (mL)	1.53	1.53	1.55	1.54	0.01
C_{max} (ng/mL)	95.8	74.4	137	102	31.8
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.33	1.62	1.33	1.43	0.162
AUC_{last} (hr ng/mL)	149	143	212	168	38.0
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	14.9	14.3	21.2	16.8	3.80
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	24.0	23.1	34.1	27.1	6.13

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Table 18e. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 5.2 mg/kg in Male Sprague-Dawley Rats.

Oral (5.2 mg/kg **I-16** equals 2.5 mg/kg PEA)

Time (hr)	Rat #				
	455	**456**	**457**	**Mean**	**SD**
0 (pre-dose)	ND	ND	ND	ND	ND
0.083	ND	ND	ND	ND	ND
0.25	ND	ND	2.57	ND	ND
0.50	2.83	6.02	52.2	20.3	27.6
1.0	15.1	8.52	25.5	16.4	8.53
2.0	5.93	3.51	6.25	5.23	1.50
4.0	ND	ND	ND	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.284	0.283	0.285	0.284	0.001
Volume Dosed (mL)	0.74	0.74	0.74	0.74	0.00
C_{max} (ng/mL)	15.1	8.52	52.2	25.3	23.6
t_{max} (hr)	1.0	1.0	0.50	0.83	0.29
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.16	1.06	0.833	1.02	0.167
AUC_{last} (hr ng/mL)	15.4	10.4	42.4	22.7	17.2
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	6.14	4.16	16.9	9.08	6.88
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	9.89	6.70	27.3	14.6	11.1

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

Table 18f. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 10.35 mg/kg in Male Sprague-Dawley Rats.

Oral (10.35 mg/kg **I-16** equals 2.5 mg/kg PEA)

Time (hr)	Rat #				
	458	**459**	**460**	**Mean**	**SD**
0 (pre-dose)	ND	ND	ND	ND	ND
0.083	ND	ND	ND	ND	ND
0.25	0.400	ND	0.960	0.680	ND
0.50	30.0	11.4	24.4	21.9	9.52
1.0	47.2	35.5	49.0	43.9	7.31
2.0	5.98	12.5	16.1	11.5	5.13
4.0	ND	5.02	ND	ND	ND
8.0	BLOQ	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.288	0.298	0.284	0.290	0.007
Volume Dosed (mL)	0.99	1.03	0.98	1.00	0.03
C_{max} (ng/mL)	47.2	35.5	49.0	43.9	7.33
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	0.946	1.58	1.06	1.20	0.337
AUC_{last} (hr ng/mL)	49.7	54.7	54.2	52.8	2.72
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	9.94	10.9	10.8	10.6	0.544
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	16.0	17.6	17.4	17.0	0.876

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

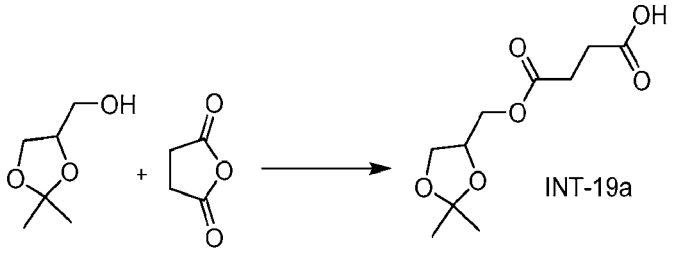
Table 18g. Individual and Average Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters for PEA after Oral Administration of **I-16** in 20% Solutol HS15:NMP (1:1), 10% PEG400, 70% H_2O) at 20.7 mg/kg in Male Sprague-Dawley Rats.

Oral (20.7 mg/kg **I-16** equals 2.5 mg/kg PEA)

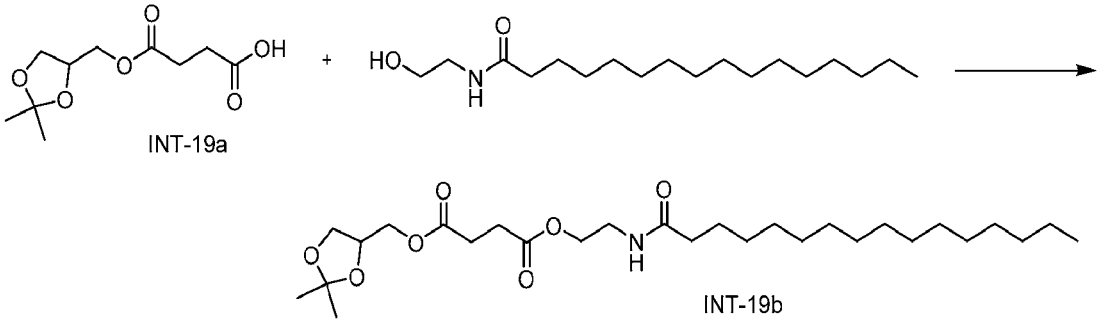
Time (hr)	Rat #				
	461	**462**	**463**	**Mean**	**SD**
0 (pre-dose)	ND	ND	ND	ND	ND
0.083	ND	1.53	1.96	1.75	ND
0.25	4.19	10.8	9.44	8.14	3.49
0.50	31.1	30.7	30.8	30.9	0.225
1.0	64.0	81.2	59.6	68.3	11.4
2.0	33.4	20.5	24.5	26.2	6.62
4.0	12.6	2.68	8.74	8.02	5.02
8.0	ND	BLOQ	BLOQ	ND	ND
Animal Weight (kg)	0.280	0.284	0.285	0.283	0.003
Volume Dosed (mL)	1.93	1.96	1.97	1.95	0.02
C_{max} (ng/mL)	64.0	8.2	59.6	68.3	11.4
t_{max} (hr)	1.0	1.0	1.0	1.0	0.0
$t_{1/2}$(hr)	ND[3]	ND[3]	ND[3]	ND	ND
MRT_{last} (hr)	1.66	1.29	1.53	1.49	0.190
AUC_{last} (hr ng/mL)	123	108	104	112	10.1
AUC_{∞} (hr ng/mL)	ND[3]	ND[3]	ND[3]	ND	ND
Dose-normalized Values[1]					
AUC_{last} (hr kg ng/mL/mg)	12.3	10.8	10.4	11.2	1.01
AUC_{∞} (hr kg ng/mL/mg)	ND[3]	ND[3]	ND[3]	ND	ND
Bioavailability (%)[2]	19.8	17.4	16.7	18.0	1.63

C_{max}: maximum plasma concentration; t_{max}: time of maximum plasma concentration; $t_{1/2}$: half-life, data points used for half-life determination are in bold; MRT_{last}: mean residence time, calculated to the last observable time point; AUC_{last}: area under the curve, calculated to the last observable time point; AUC_{∞}: area under the curve, extrapolated to infinity; ND: not determined; BLOQ: below the limit of quantitation (2.5 ng/mL); [1]Dose-normalized by dividing the parameter by nominal dose in mg/kg; [2]Bioavailability determined by dividing individual dose-normalized oral AUC_{last} values by the average IV AUC_{last} value 62.1 hr*ng/mL from Example 5; [3]Not determined due to lack of quantifiable data points trailing the C_{max}.

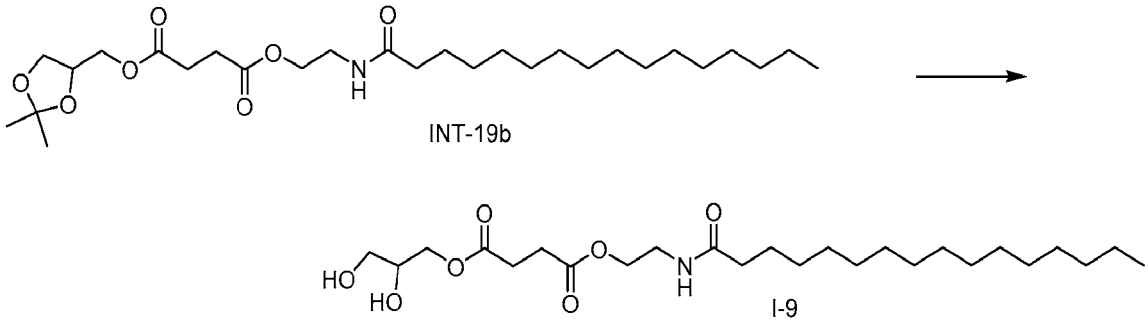
Example 19: Synthesis of compound **I-9**



[432] A mixture of solketal (21 gm, 0.16 mol), succinic anhydride (15.9 gm, 0.16 mol) and pyridine (500 mL) was heated to reflux for 16 hrs. Conversion was monitored by NMR. Pyridine was removed under high vacuum. Approximately 15-20% pyridine was still remaining. Mixture was taken to next step as is without further purification.



[433] A solution of INT-19a (62.13 mg, 0.27 mol) and PEA (61.46 mg, 0.27 mol) in DCM (1 lire) was cooled to 0 °C. To this solution was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (152.5 mg, 0.8 mol) followed by 4-dimethylaminopyridine (DMAP) (9.8 mg, 0.08 mol) in portions. Reaction mixture was warmed to room temperature and stirred for 24 hrs. Reaction mixture was washed with water and brine and extracted with DCM. Organic layers were separated and dried over anhydrous sodium sulfate, filtered and concentrated. Crude material was purified by column chromatography with hexanes and ethyl acetate to obtain 88 mg of pure INT-19b as white solid.



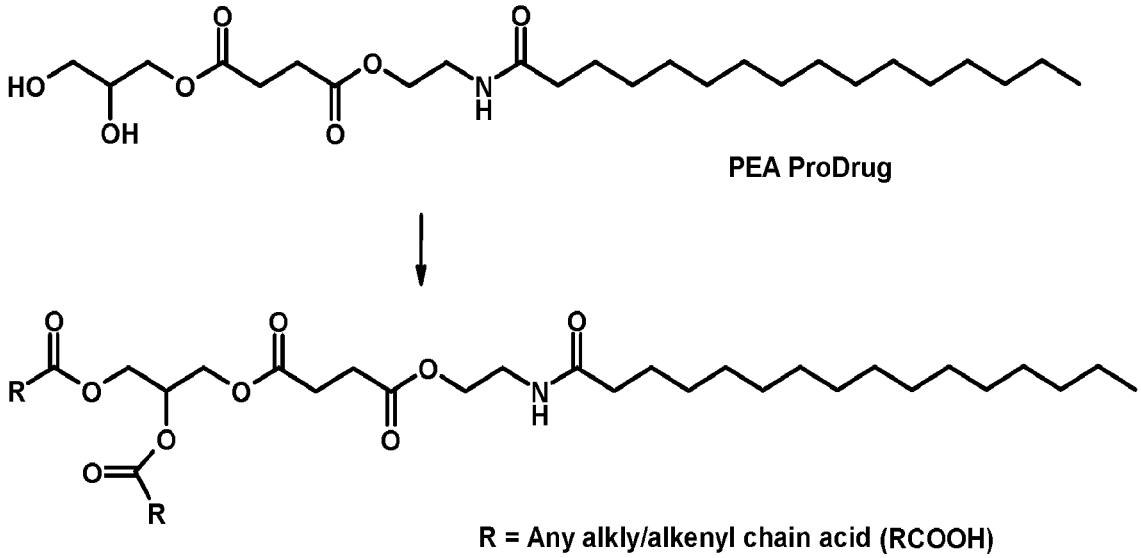
[434] INT-19b (88 mg) was dissolved in methanol (4 Liter) and cooled to 5 °C. To this solution was added Dowex H^+ resin (45 mg) and stirred at 5 °C for 8 hrs. Then resin was filtered

off with a pad of celite. Filtrate was concentrated to obtain off white solid. Solids were recrystallized with ethyl acetate to give 73.6 mg of pure **I-9**.

Example 20: General Synthesis of Diester PEA Prodrugs.

[435] Compounds of the present invention may be synthesized according to Scheme 20.

Scheme 20.

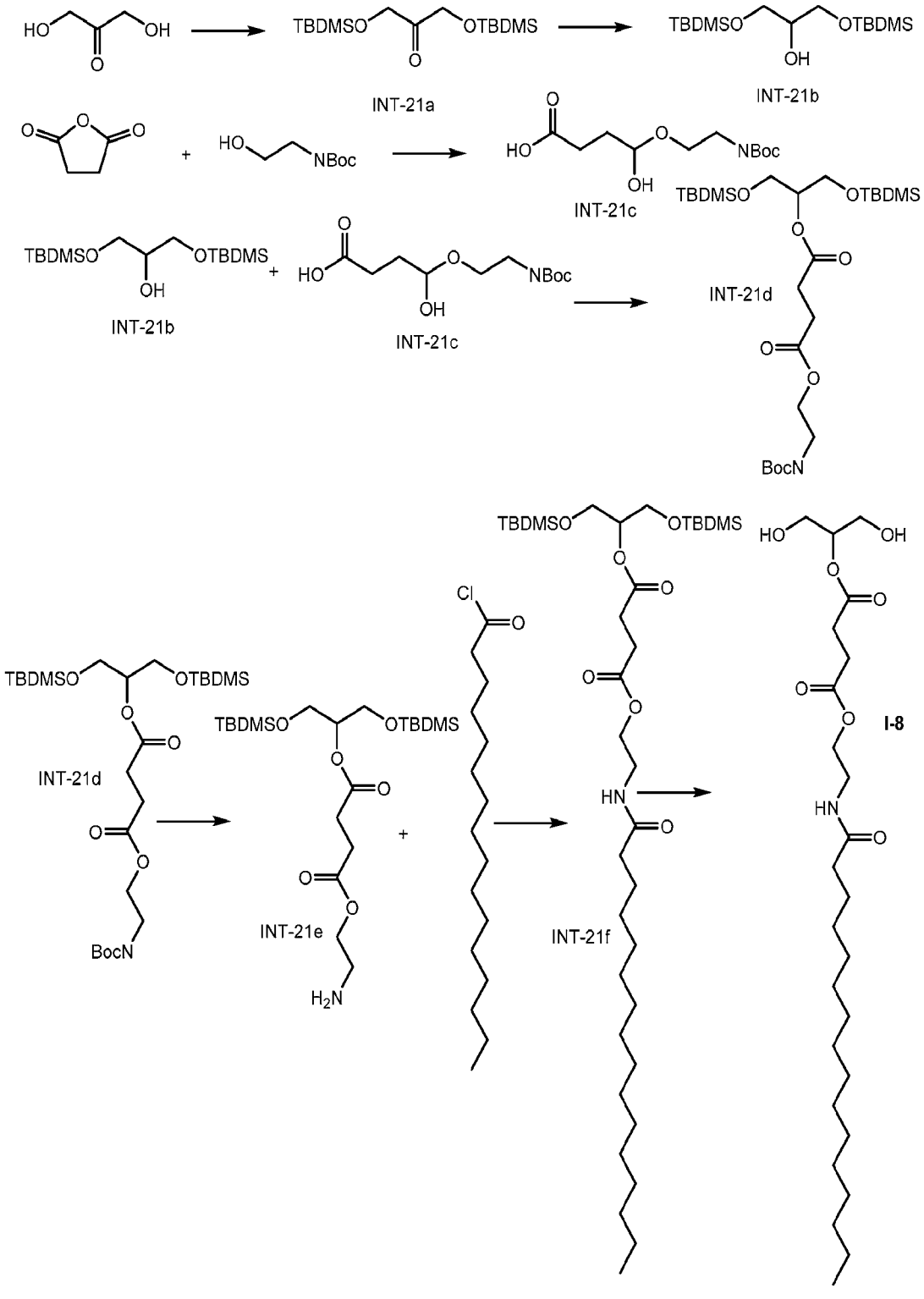


[436] A solution of PEA prodrug (1 eq), RCOOH (2.2-3.0 eq) in DCM (10 vol) was cooled to 0 ^{o}C. To this solution was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (3 eq) followed by addition of 4-dimethylaminopyridine (DMAP) (0.3 eq) in portions. Progress of the reaction was monitored by TLC/NMR. After conversion, the reaction mixture is diluted with DCM, washed with water, sat.aq sodium bicarbonate and brine and extracted with DCM. The organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The crude residue was purified by column chromatography with increasing gradient of ethylacetate in hexanes.

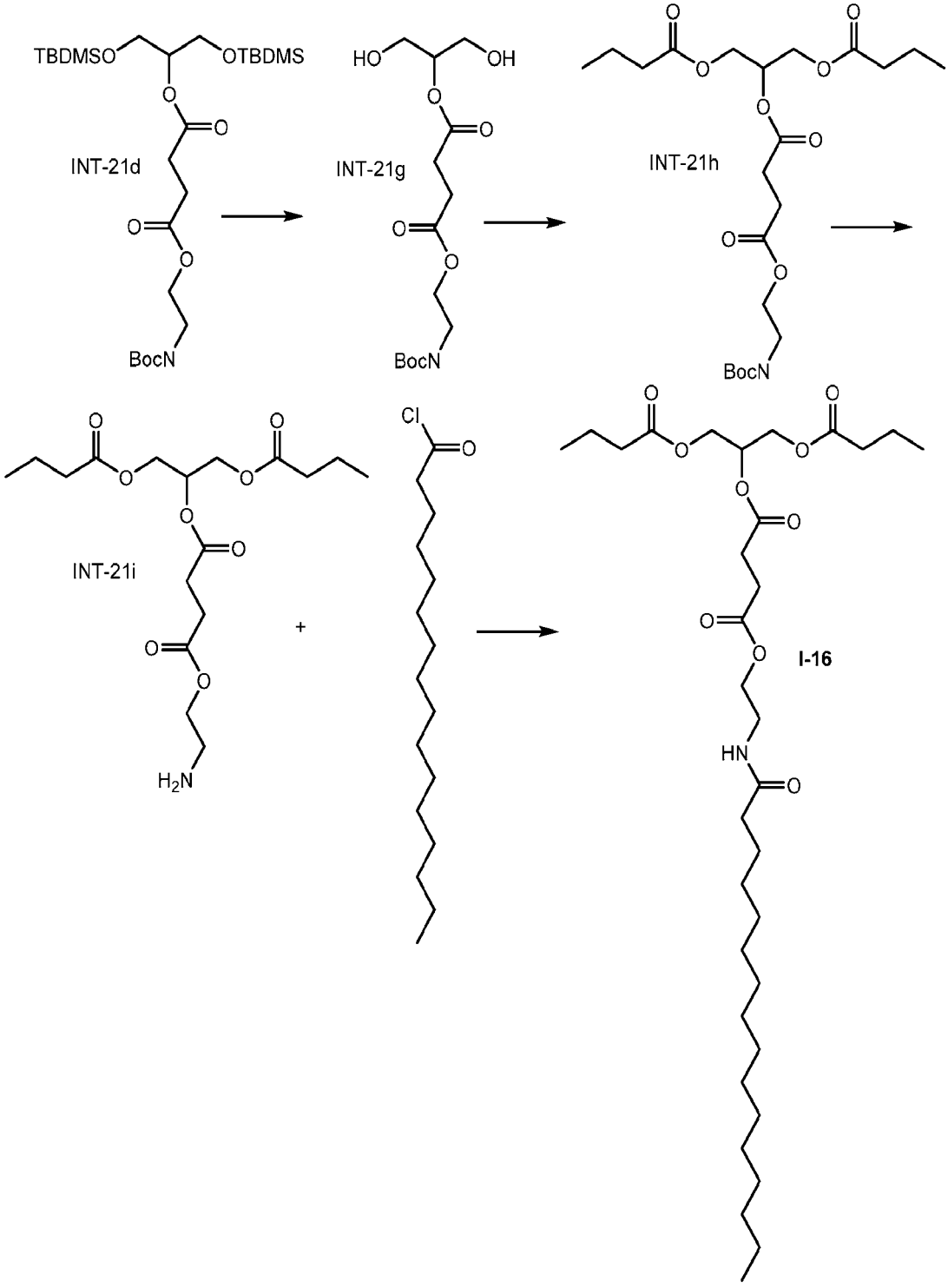
Example 21: Synthesis of compounds **I-8** and **I-16**

[437] Compounds **I-8** and **I-16** may be synthesized according to Schemes 21a and 21b.

Scheme 21a.


HO
OH
O
TBDMSO
OTBDMS
INT-21a
INT-21b
NBoc
INT-21c
INT-21d
BocN
INT-21e
H_2N
INT-21f
Cl
HN
I-8

Scheme 21b.



Example 22: Assessing the analgesic effects of I-16 in the Carrageenan-induced inflammatory model using thermal hyperalgesia testing.

[438] Sixty male Sprague Dawley rats were used in this study. Baseline thermal hyperalgesia thresholds were determined on day -1; animals were divided into 6 groups based on baseline thermal hyperalgesia thresholds. On day 0, animals received an oral dose of vehicle or I-16.

Approximately 30 minutes after dosing the animals received an intra-plantar injection of 2% carrageenan solution. The animals were assessed for thermal hyperalgesia approximately 4 and approximately 24 hours after carrageenan injection.

[439] Thermal hyperalgesia was assessed at baseline (prior to dosing with either vehicle or I-16), 4 hours,and 24 hours post-carrageenan injection. Oral administration of 10.25 mg/kg (equivalent to 5 mg/kg equivalents of PEA) I-16 did not significantly reduce the thermal hyperalgesia induced by carrageenan injection into the hind paw at any time point. Oral administration of 20.50 mg/kg (equivalent to 15 mg/kg of PEA) I-16 significantly reduced thermal hyperalgesia at the 4-hour time point, but did not significantly reduce thermal hyperalgesia at the 24-hour time point (FIG. 11).

[440] Mean ± SEM ipsilateral paw withdrawal latencies following carrageenan injection in vehicle and I-16 treated animals during the pharmacological assessment period (day 0). All animals received a mixture of 10% solutol, 10% n-methyl pyrrolidone, 10% PEG 400, and 70% water (10 mL/kg) or I-16 (10.25 or 20.50 mg/kg) via oral gavage (n = 10).

[441] These results indicate that administration of I-16 significantly reduces the degree of thermal hyperalgesia associated with inflammatory pain. Administration of I-16 produced a dose- and time- dependent reduction of thermal hyperalgesia with administration of 10.25 mg/kg I-16 producing no significant effect, and administration of 20.50 mg/kg I-16 significantly reducing thermal hyperalgesia at the 4-hour time point.

Example 23: Evaluation of Analgesic Effects in Rat Chronic Constriction Injury (CCI) model.

[442] Two test compounds (I-16 and Gabapentin) were formulated in 15% Solutol® HS15/ 15% Polyethylene glycol (PEG) 400/ 70% water for injection (WFI) for oral (PO) administrations for 17 consecutive days (qdx17). A dosing volume of 10 mL/kg was applied.

Methods:

[443] Male Sprague Dawley rats weighing 180 ± 20 g were used. Under pentobarbital (50 mg/kg, 5 ml/kg, IP) anesthesia, the left sciatic nerve was exposed at mid-thigh level. Four chromic gut ligatures, about 1 mm apart, were loosely tied around the nerve. The animals were then housed socially in cages with soft bedding for at least 10 days before testing for mechanical allodynia and thermal hyperalgesia.

Mechanical allodynia

[444] The rats were placed under inverted Plexiglas cages on a wire mesh rack and allowed to acclimate for 20 to 30 minutes. Allodynia was evaluated by the Chaplin up/down method using von Frey filaments to the plantar surface of the left hind paw. All rats were assessed for mechanical allodynia for pre-surgical allodynia threshold on Day -3 (pre-surgery baseline). For gabapentin group, the rats were pre-selected for experimentation only if the pain threshold on Day 13 after nerve ligation (pre-treatment) is reduced by 10 grams of force relative to the response of the individual paw before nerve ligation (pre-ligation), namely, with clear presence of allodynia. On Day 14, the mechanical allodynia test was performed at 1 hour after administrations of I-16, vehicle, or gabapentin.

Thermal hyperalgesia

[445] Thermal hyperalgesia was measured by the IITC Model-336G (IITC Inc., USA) apparatus. Each rat was placed within a plastic box atop a glass floor for 20 to 30 minutes. A light beam under the floor was aimed at the plantar surface of the left hind paw. The time was measured automatically when the paw was withdrawn away from the thermal stimulus. A cut-off latency of 23 sec was imposed. The latency to withdrawal is obtained for each rat and defined as the heat pain threshold. All rats were assessed for thermal hyperalgsia for pre-surgical threshold on Day -3 (pre-surgery baseline). For gabapentin group, the rats were pre-selected for experimentation only if the pain threshold on Day 13 after nerve ligation (pre-treatment) is reduced by 15 seconds. On Day 14, the thermal hyperalgesia test was performed at 1.5 hour after administrations of I-16, vehicle, or gabapentin.

[446] Group differences were compared to the vehicle control group. Differences were considered significant at $P<0.05$.

[447] The formulations and dosing protocols are provided in Table 23 and visualized in FIG. 12A:

Table 23

Group	Test Article	Route	Conc. mg/mL	Dosage mL/kg	Dosage mg/kg	Rats (Male)
1	Vehicle[a]	PO	NA	10	NA, qd x 17 (Days -2~14)	8[b]
2	Gabapentin	PO	10	10	100, qd x 1	8[c]
3	I-16	PO	3.1	10	31, qd x 17 (Days -2~14)	8[b]

a Vehicle: 15% solutol® HS15, 15% PEG400 & 70% WFI.
Dose preparation instructions:
first mix the TA in solutol, then add PEG400 and vortex, then add water and vortex to ensure a clear solution.
b The rats were randomized on Day -3.
c The rats were randomized on Day 13. Each group was underwent mechanical allodynia and thermal hyperalgesia testing on Day 14.

[448] The results of an assay measuring the mechanical allodynia at post treatment (1-hr) and (1.5-hr) is reported in FIG. 12B.

Equivalents

[449] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

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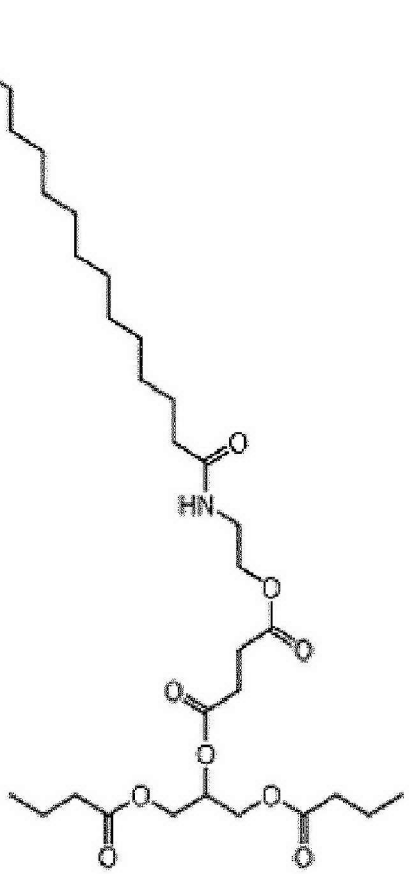
CLAIMS:

1. A compound which is:



.

2. The compound of claim 1, wherein the compound is in a salt form.

3. The compound of claim 2, wherein the salt form is a pharmaceutically acceptable salt form.

4. A pharmaceutical composition comprising the compound of any one of claims 1 to 3 and a pharmaceutically acceptable excipient.

5. The pharmaceutical composition of claim 4, which contains additional therapeutic agents.

6. The pharmaceutical composition of claim 4 or claim 5, which is formulated for oral delivery.

7. The pharmaceutical composition of claim 6, which is formulated as a solid formulation.

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8. The pharmaceutical composition according to claim 7, wherein the solid formulation is a capsule.

9. The pharmaceutical composition according to claim 8, wherein the capsule encloses a liquid.

10. The pharmaceutical composition according to any one of claims 4 to 9, for use in therapy.

11. A method of treatment of pain, the method comprising administering to a subject in need thereof the compound according to any one of claims 1 to 3 or the pharmaceutical composition according to any one of claims 4 to 9.

12. A method of treatment of inflammatory pain, the method comprising administering to a subject in need thereof the compound according to any one of claims 1 to 3 or the pharmaceutical composition according to any one of claims 4 to 9.

13. A method of treatment of neuropathic pain, the method comprising administering to a subject in need thereof the compound according to any one of claims 1 to 3 or the pharmaceutical composition according to any one of claims 4 to 9.

14. Use of the compound according to any one of claims 1 to 3 in the manufacture of a medicament for the treatment of pain.

15. Use of the compound according to any one of claims 1 to 3 in the manufacture of a medicament for the treatment of inflammatory pain.

16. Use of the compound according to any one of claims 1 to 3 in the manufacture of a medicament for the treatment of neuropathic pain.

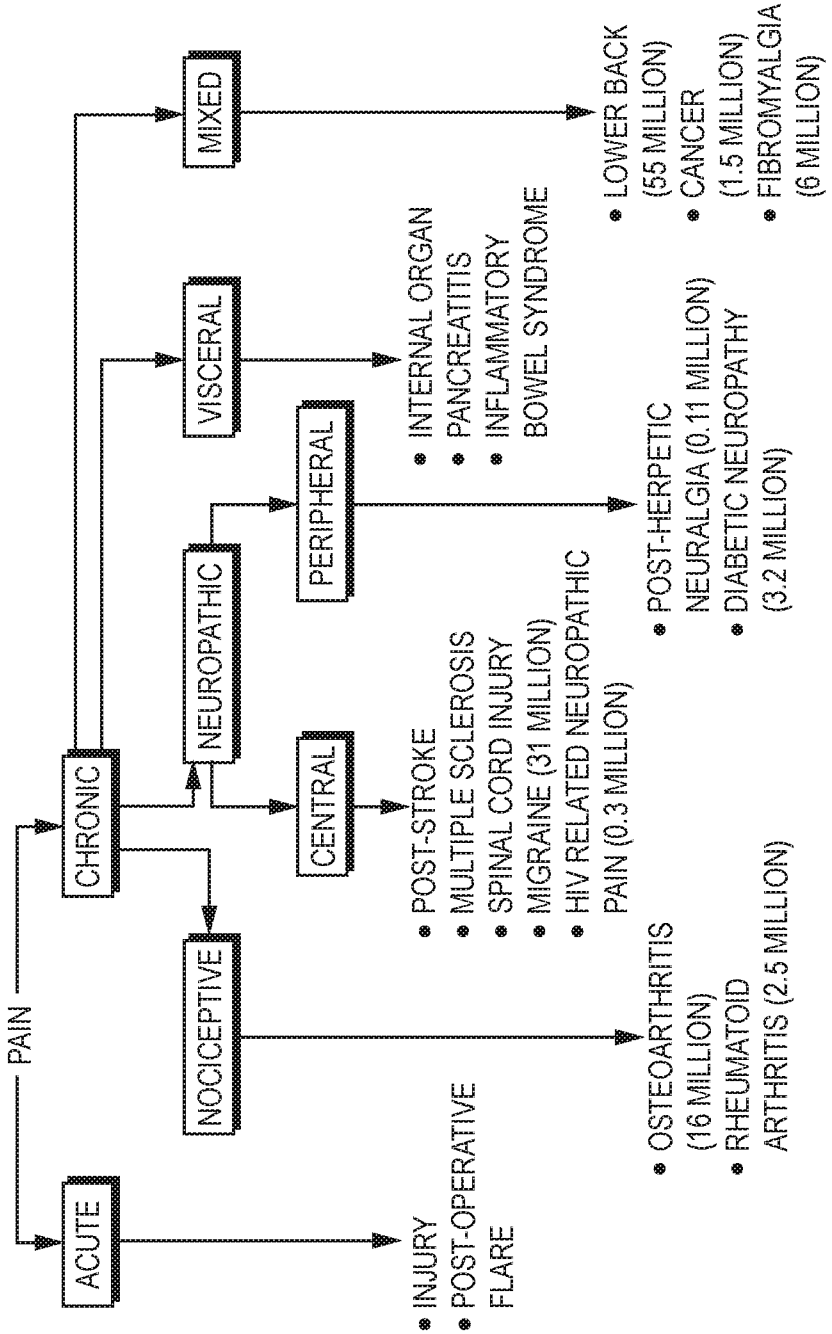

PAIN
ACUTE
CHRONIC
NOCICEPTIVE
NEUROPATHIC
CENTRAL
PERIPHERAL
VISCERAL
MIXED
• INJURY
• POST-OPERATIVE FLARE
• POST-STROKE
• MULTIPLE SCLEROSIS
• SPINAL CORD INJURY
• MIGRAINE (31 MILLION)
• HIV RELATED NEUROPATHIC PAIN (0.3 MILLION)
• OSTEOARTHRITIS (16 MILLION)
• RHEUMATOID ARTHRITIS (2.5 MILLION)
• POST-HERPETIC NEURALGIA (0.11 MILLION)
• DIABETIC NEUROPATHY (3.2 MILLION)
• INTERNAL ORGAN
• PANCREATITIS
• INFLAMMATORY BOWEL SYNDROME
• LOWER BACK (55 MILLION)
• CANCER (1.5 MILLION)
• FIBROMYALGIA (6 MILLION)

FIG. 1

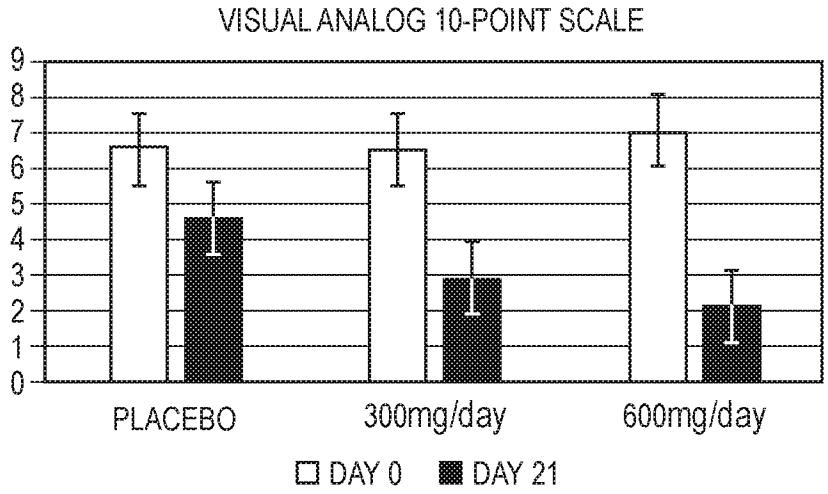

VISUAL ANALOG 10-POINT SCALE
9
8
7
6
5
4
3
2
1
0
PLACEBO
300mg/day
600mg/day
DAY 0
DAY 21

FIG. 2A

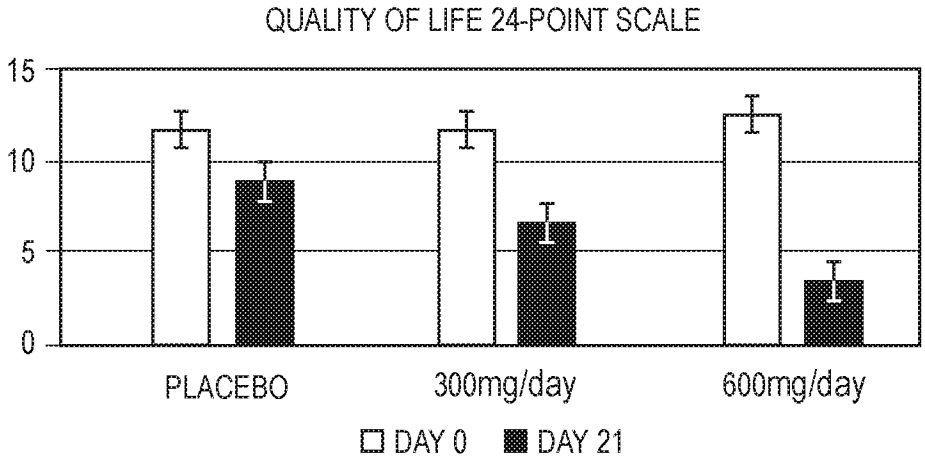
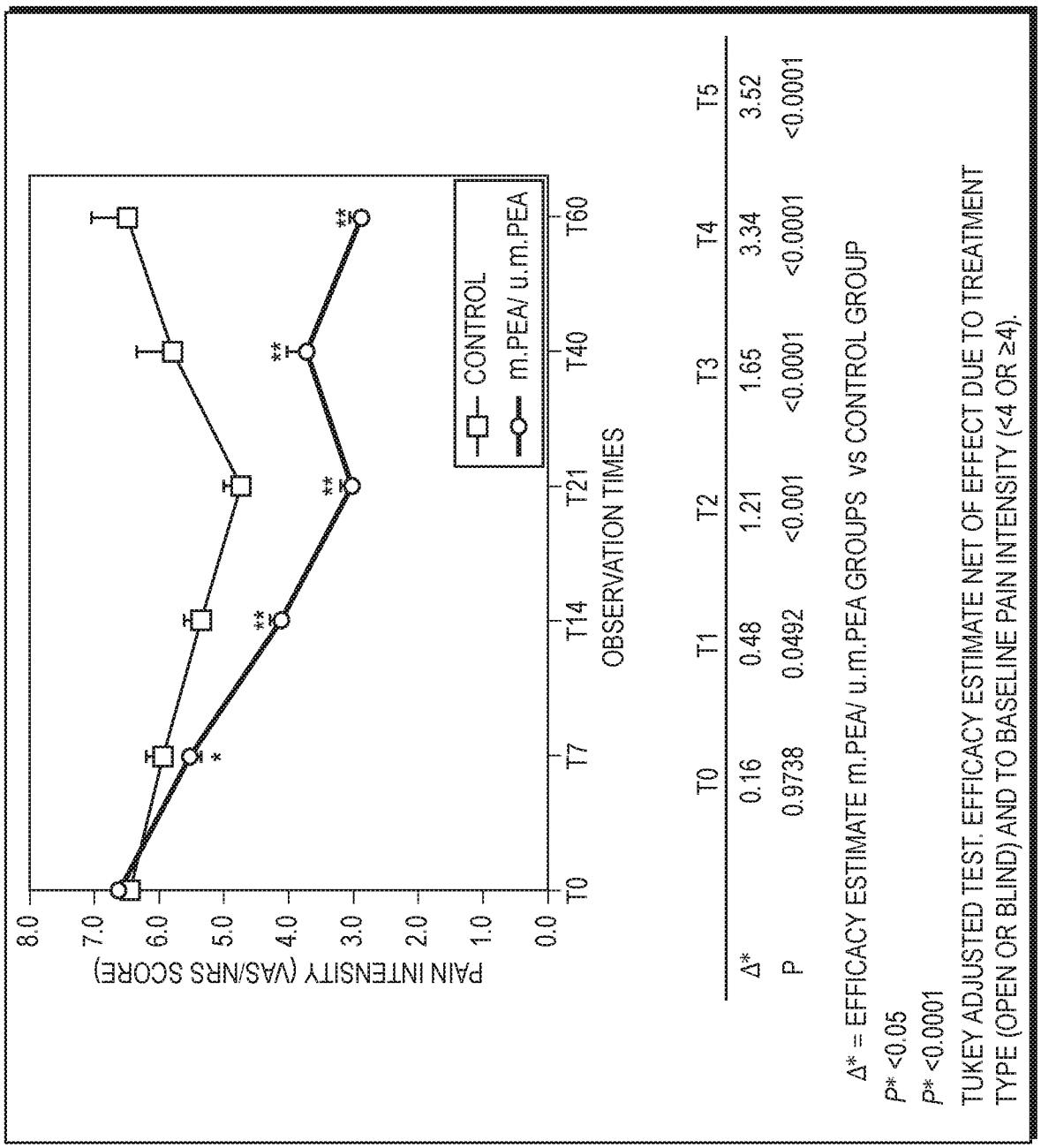

QUALITY OF LIFE 24-POINT SCALE
15
10
5
0
PLACEBO
300mg/day
600mg/day
DAY 0
DAY 21

FIG. 2B



	T0	T1	T2	T3	T4	T5
Δ*	0.16	0.48	1.21	1.65	3.34	3.52
P	0.9738	0.0492	<0.001	<0.0001	<0.0001	<0.0001

Δ* = EFFICACY ESTIMATE m.PEA/ u.m.PEA GROUPS vs CONTROL GROUP

*P** <0.05

*P** <0.0001

TUKEY ADJUSTED TEST. EFFICACY ESTIMATE NET OF EFFECT DUE TO TREATMENT TYPE (OPEN OR BLIND) AND TO BASELINE PAIN INTENSITY (<4 OR ≥4).

FIG. 2C

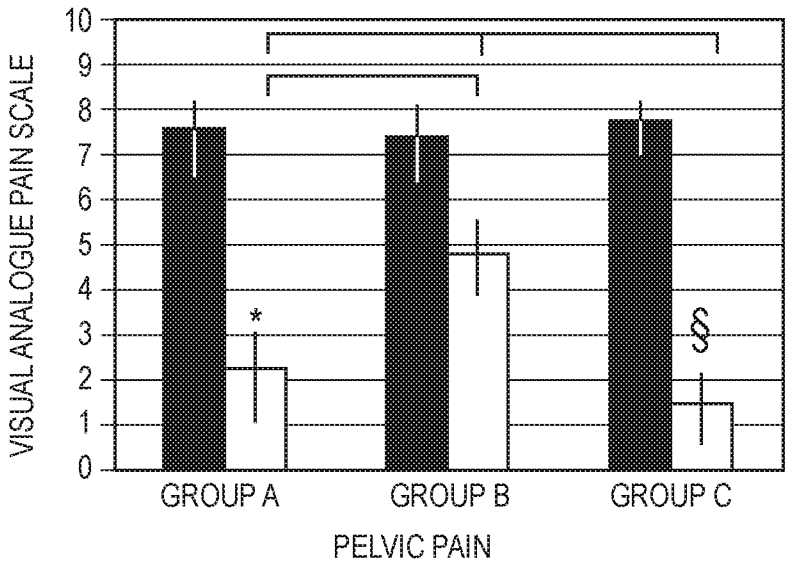


FIG. 2D

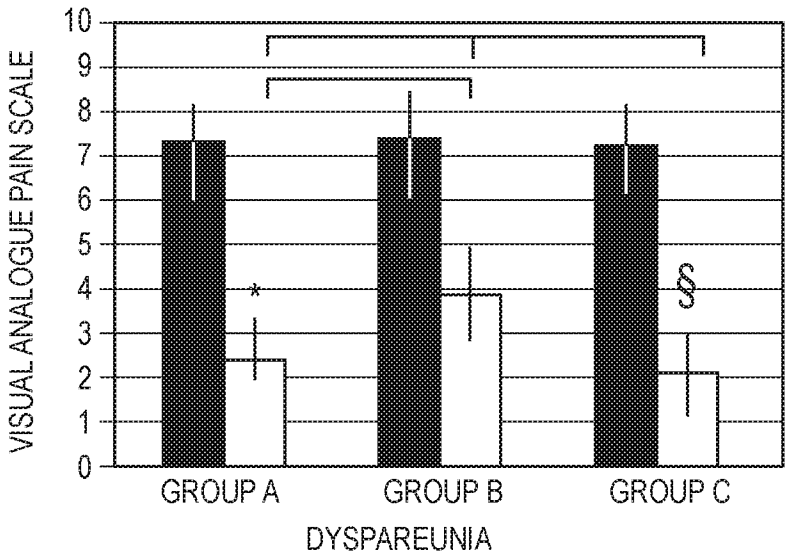


FIG. 2E

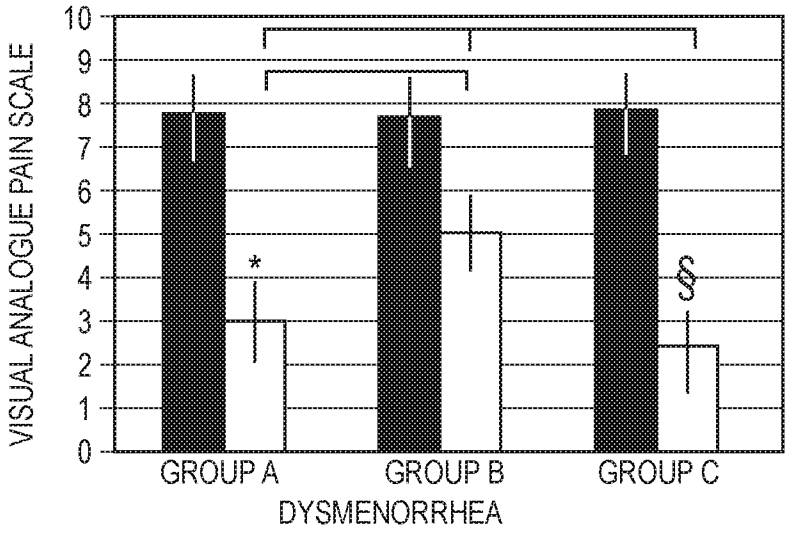


FIG. 2F

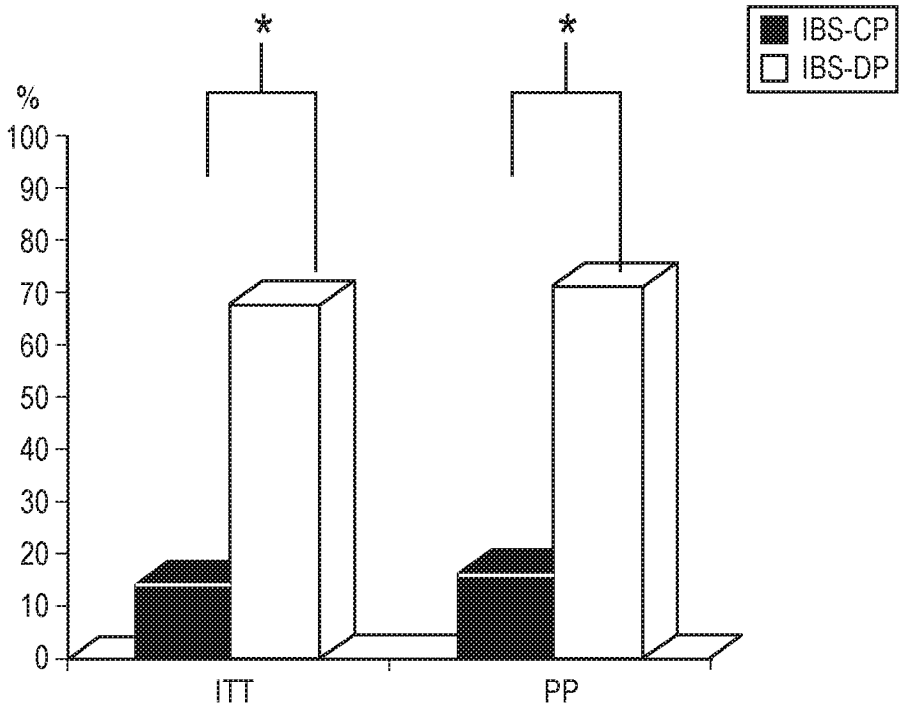

IBS-CP
IBS-DP
%
100
90
80
70
60
50
40
30
20
10
0
*
*
ITT
PP

FIG. 2G

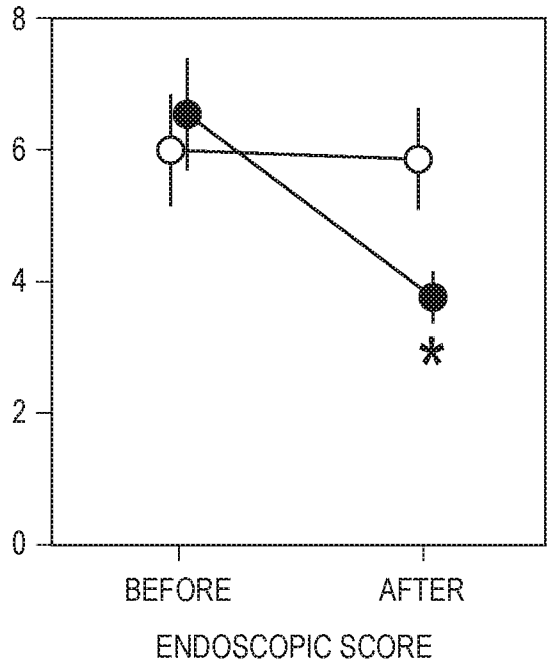

8
6
4
2
0
*
BEFORE
AFTER
ENDOSCOPIC SCORE

FIG. 2H

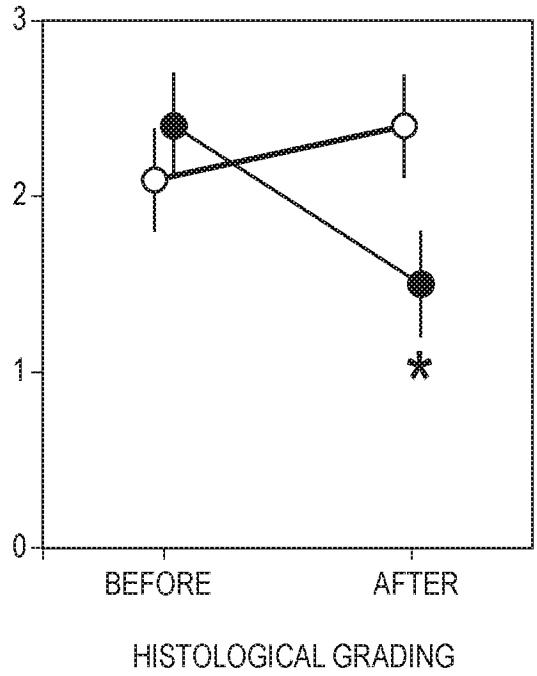

3
2
1
0
*
BEFORE
AFTER
HISTOLOGICAL GRADING

FIG. 2I

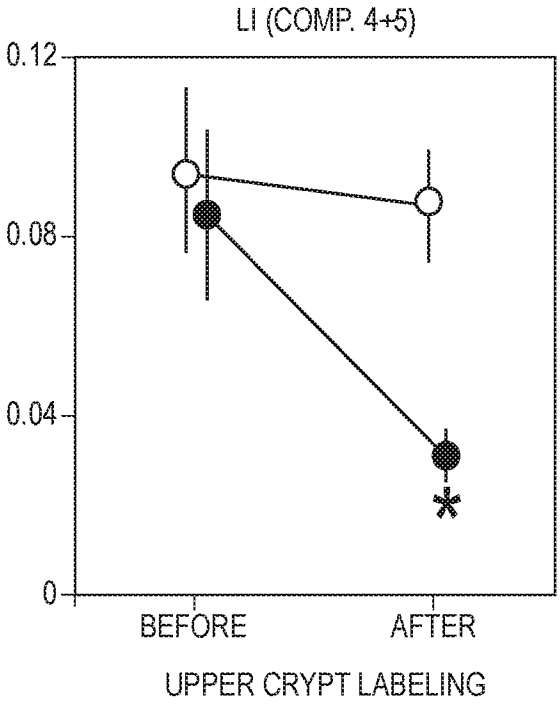

LI (COMP. 4+5)
0.12
0.08
0.04
0
*
BEFORE
AFTER
UPPER CRYPT LABELING

FIG. 2J

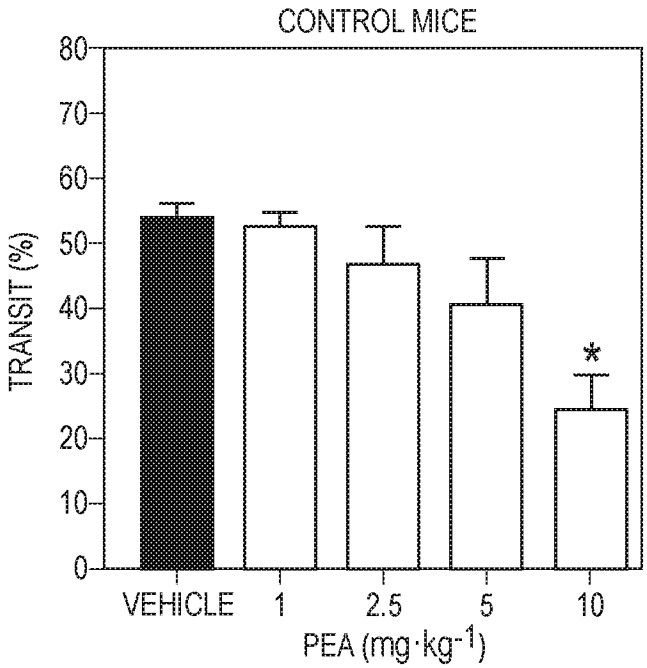

CONTROL MICE
TRANSIT (%)
80
70
60
50
40
30
20
10
0
*
VEHICLE
1
2.5
5
10
PEA (mg·kg-1)

FIG. 2K

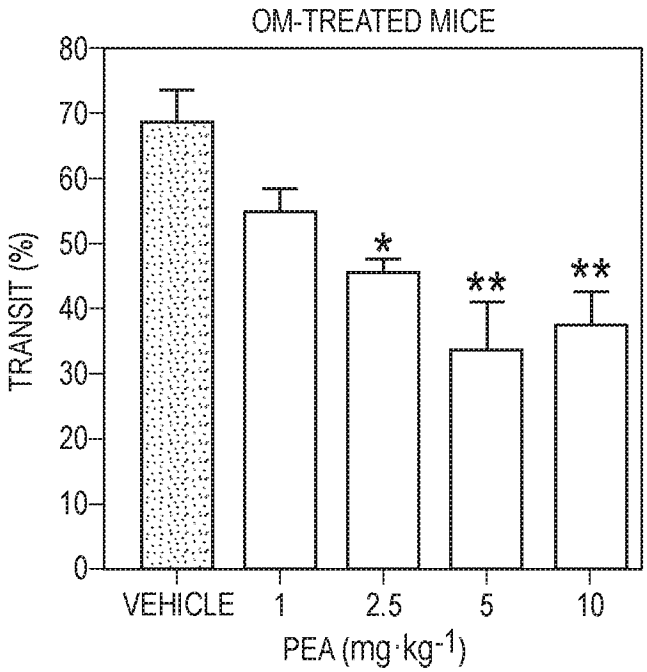

OM-TREATED MICE
TRANSIT (%)
80
70
60
50
40
30
20
10
0
*
**
**
VEHICLE
1
2.5
5
10
PEA (mg·kg-1)

FIG. 2L

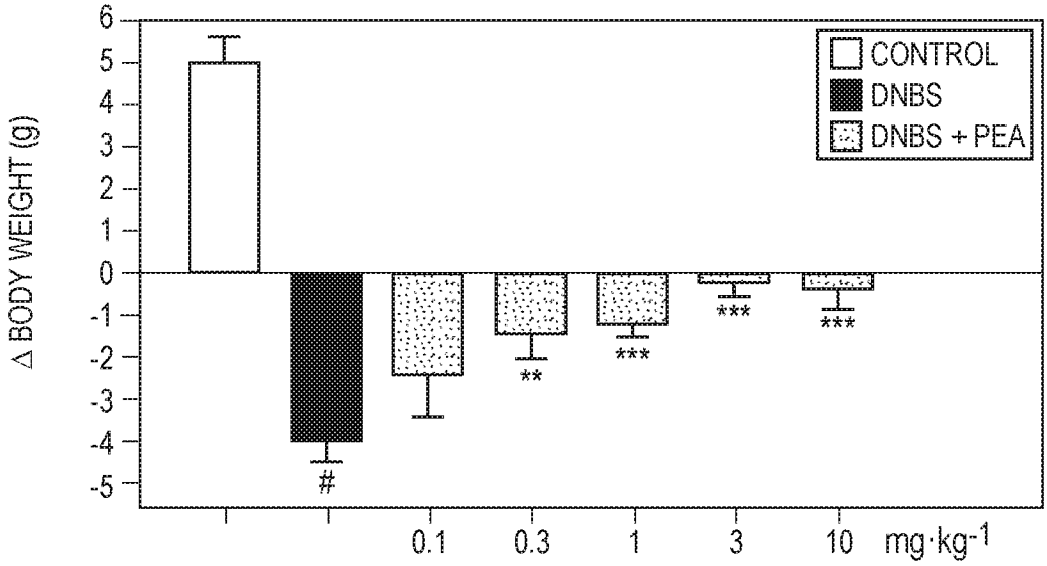


FIG. 2M

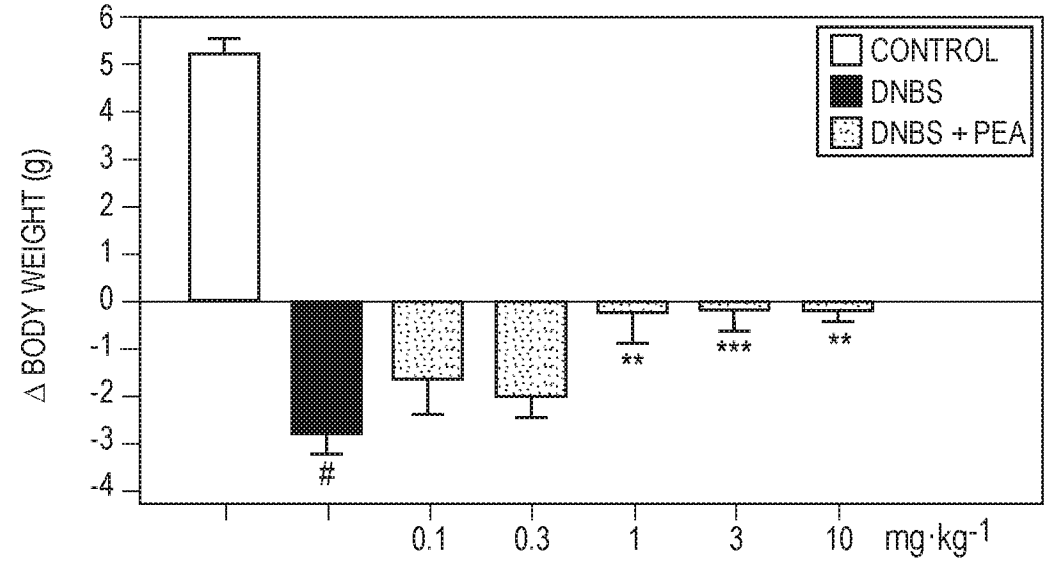


FIG. 2N

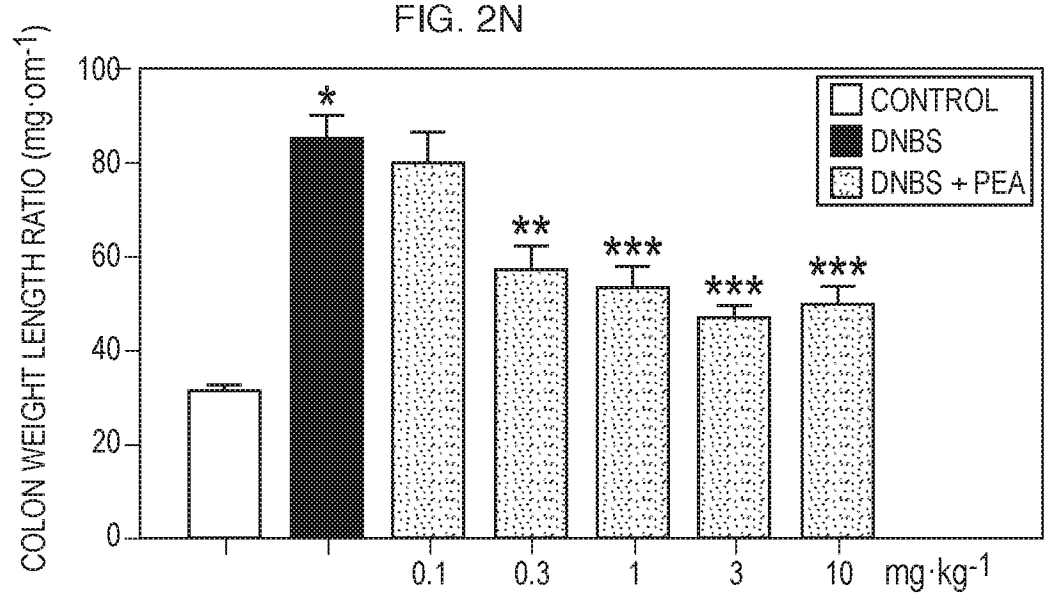


FIG. 2O

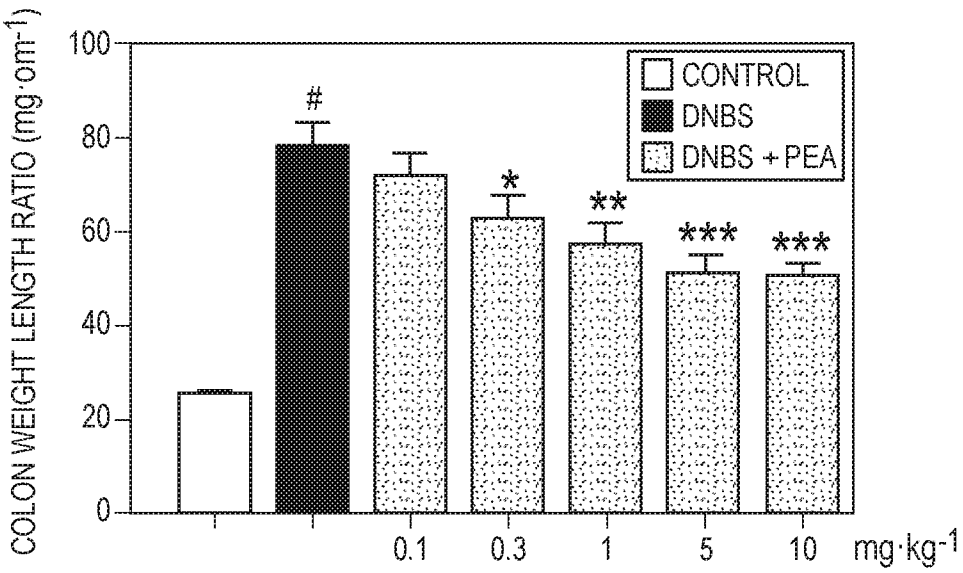

COLON WEIGHT LENGTH RATIO (mg·om-1)
100
80
60
40
20
0
CONTROL
DNBS
DNBS + PEA
#
*
**
***
***
0.1
0.3
1
5
10
mg·kg-1

FIG. 2P.

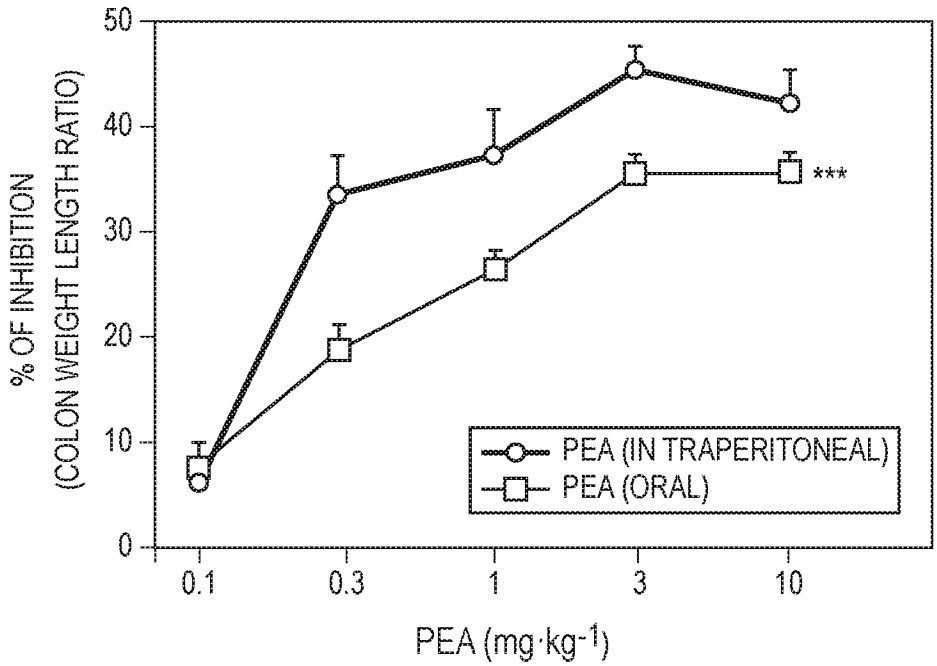

% OF INHIBITION
(COLON WEIGHT LENGTH RATIO)
50
40
30
20
10
0
***
PEA (IN TRAPERITONEAL)
PEA (ORAL)
0.1
0.3
1
3
10
PEA (mg·kg-1)

FIG. 2Q.

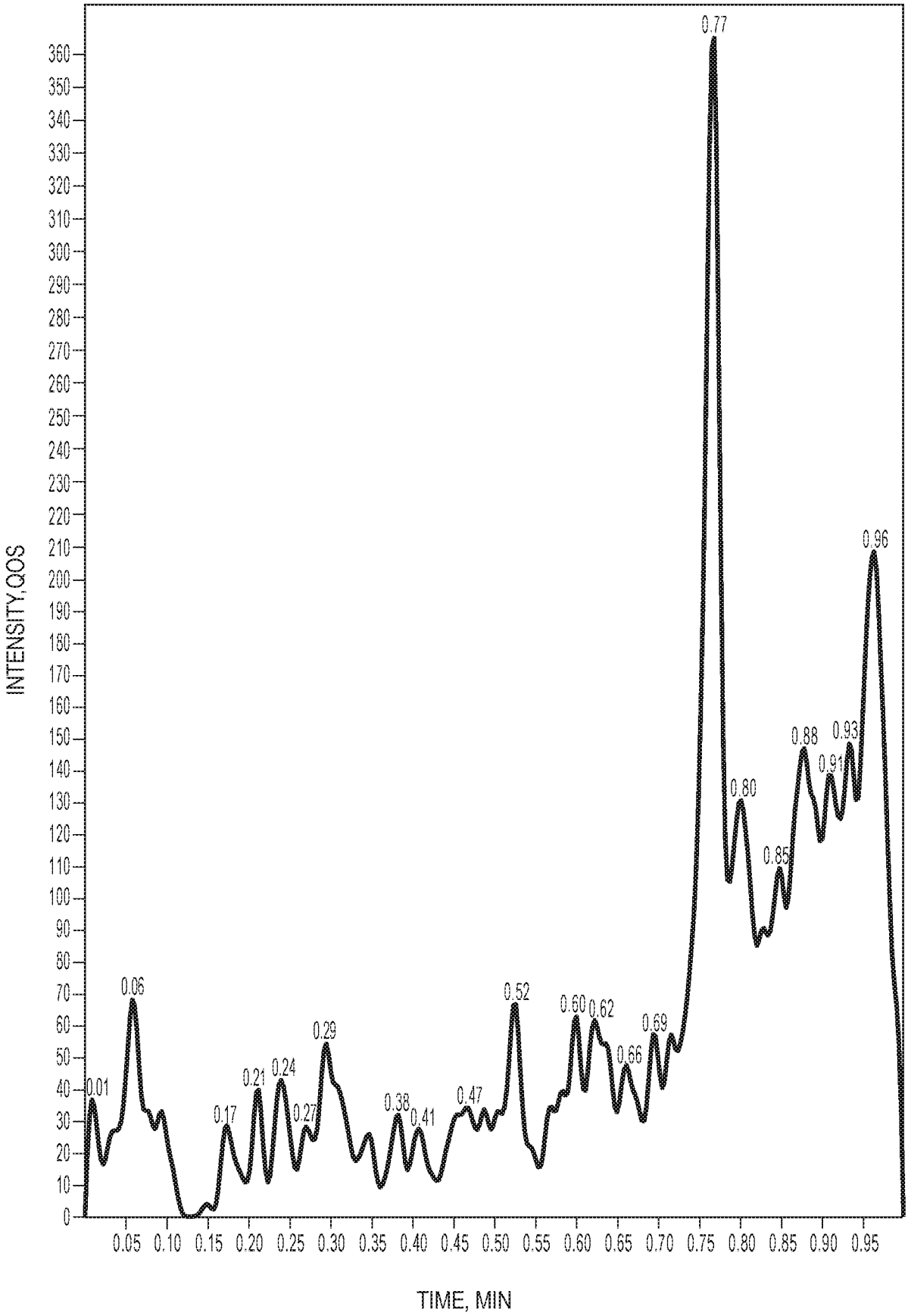

INTENSITY,QOS
TIME, MIN
0.01
0.06
0.17
0.21
0.24
0.27
0.29
0.38
0.41
0.47
0.52
0.60
0.62
0.66
0.69
0.77
0.80
0.85
0.88
0.91
0.93
0.96

FIG. 3A

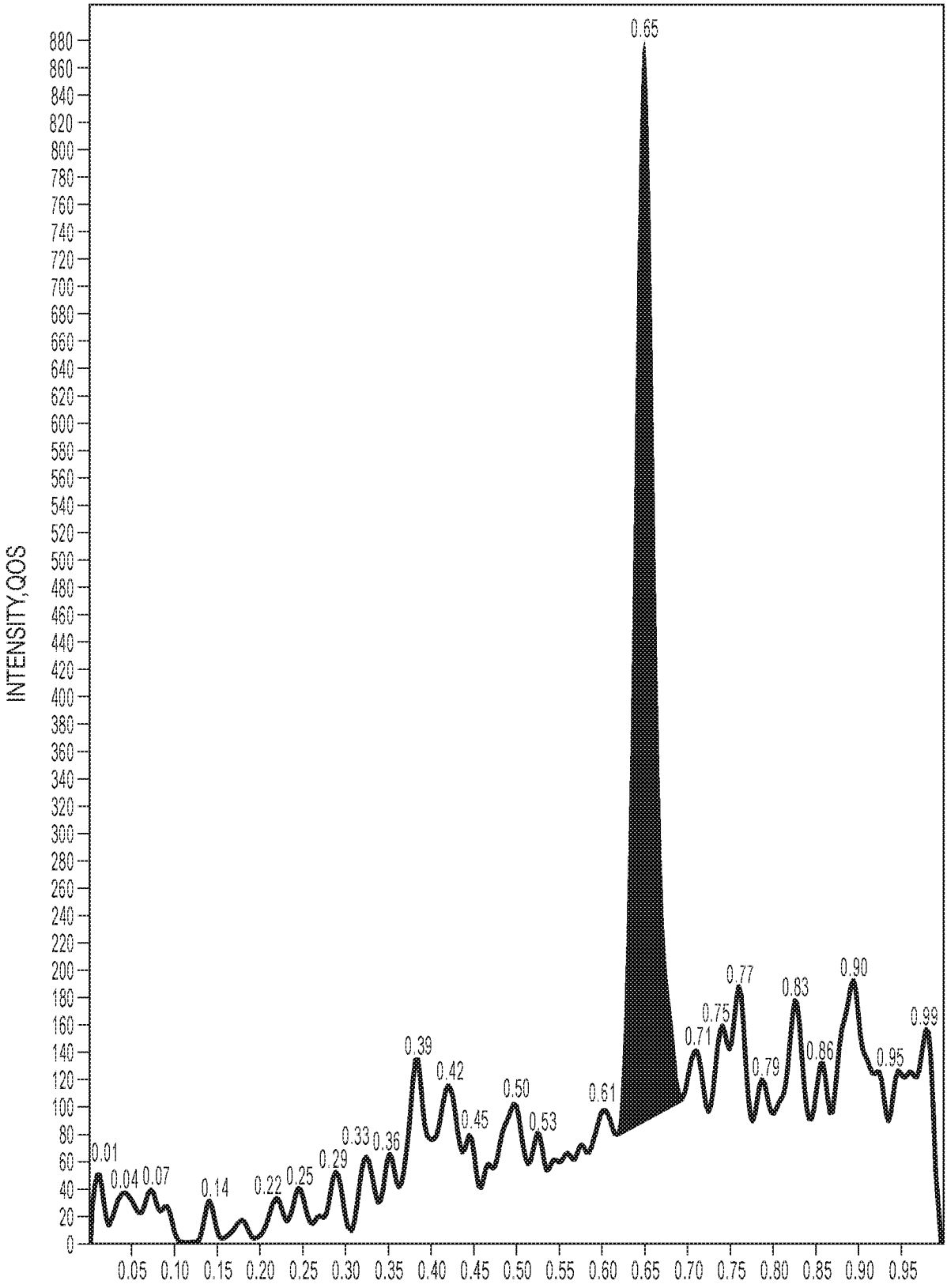

INTENSITY,QOS
0.01
0.04
0.07
0.14
0.22
0.25
0.29
0.33
0.36
0.39
0.42
0.45
0.50
0.53
0.61
0.65
0.71
0.75
0.77
0.79
0.83
0.86
0.90
0.95
0.99

FIG. 3B

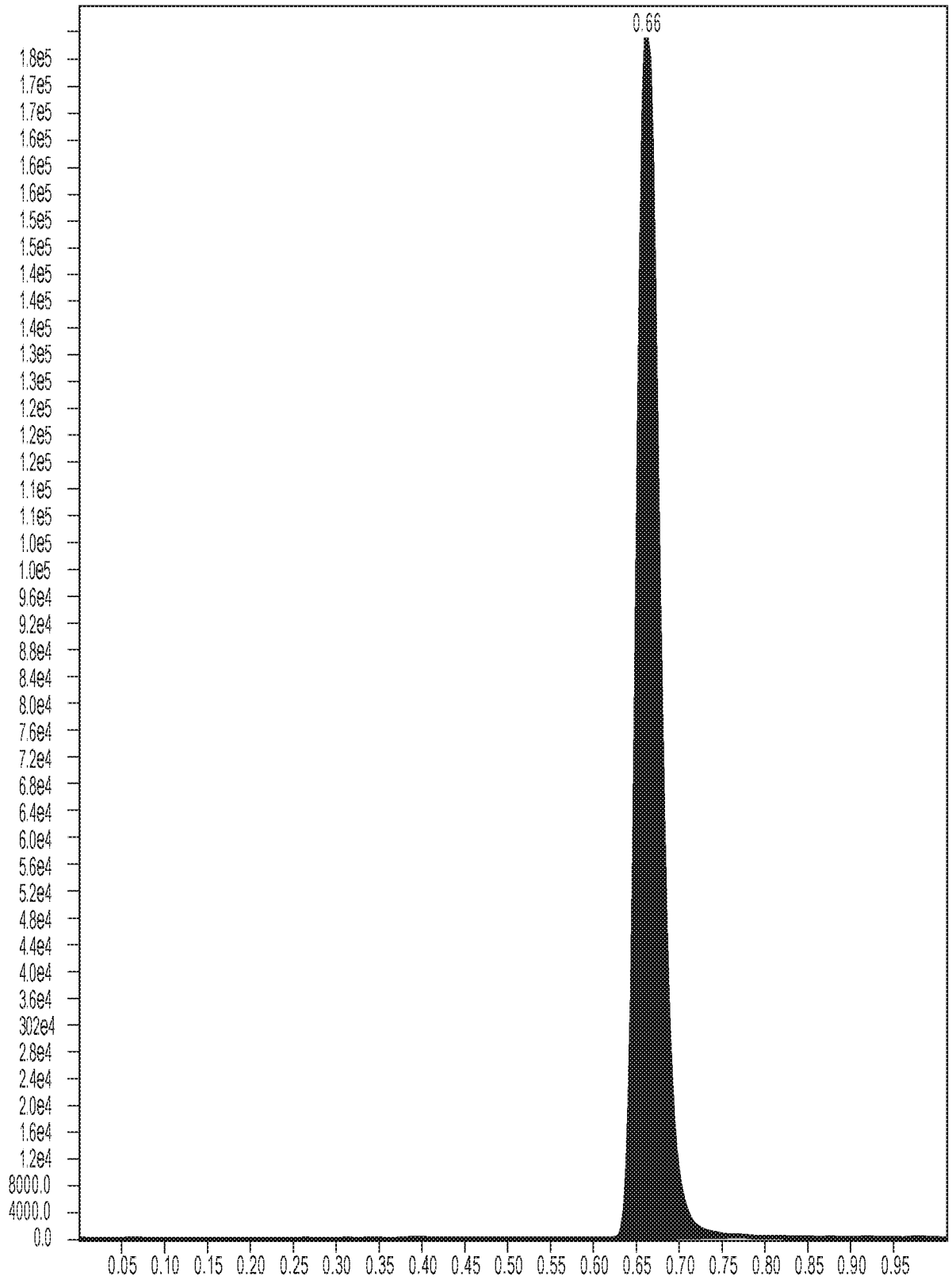

0.66
1.8e5
1.7e5
1.7e5
1.6e5
1.6e5
1.6e5
1.5e5
1.5e5
1.4e5
1.4e5
1.4e5
1.3e5
1.3e5
1.2e5
1.2e5
1.2e5
1.1e5
1.1e5
1.0e5
1.0e5
9.6e4
9.2e4
8.8e4
8.4e4
8.0e4
7.6e4
7.2e4
6.8e4
6.4e4
6.0e4
5.6e4
5.2e4
4.8e4
4.4e4
4.0e4
3.6e4
302e4
2.8e4
2.4e4
2.0e4
1.6e4
1.2e4
8000.0
4000.0
0.0
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95

FIG. 3C

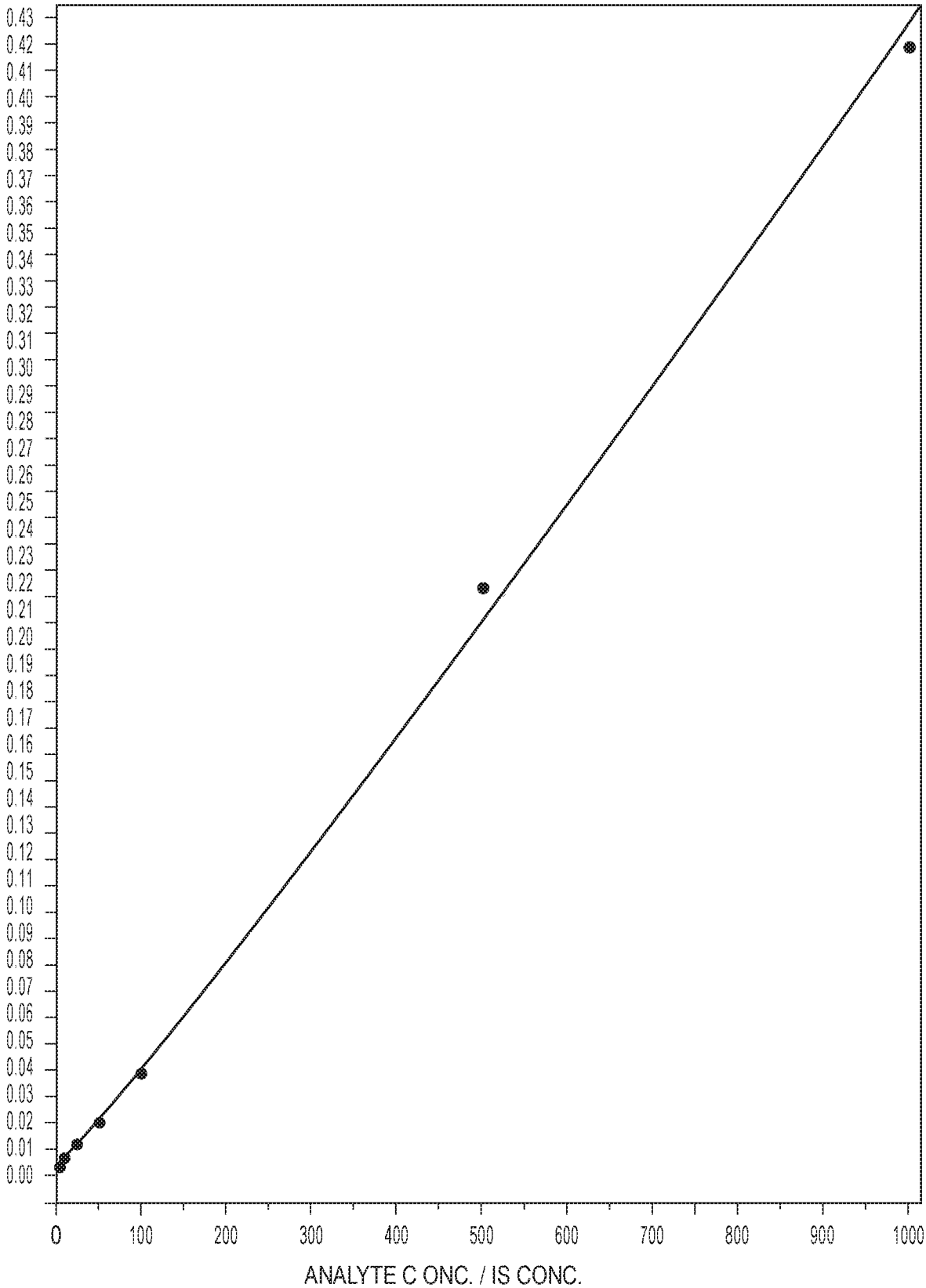

0.43
0.42
0.41
0.40
0.39
0.38
0.37
0.36
0.35
0.34
0.33
0.32
0.31
0.30
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
100
200
300
400
500
600
700
800
900
1000
ANALYTE C ONC. / IS CONC.

FIG. 3D

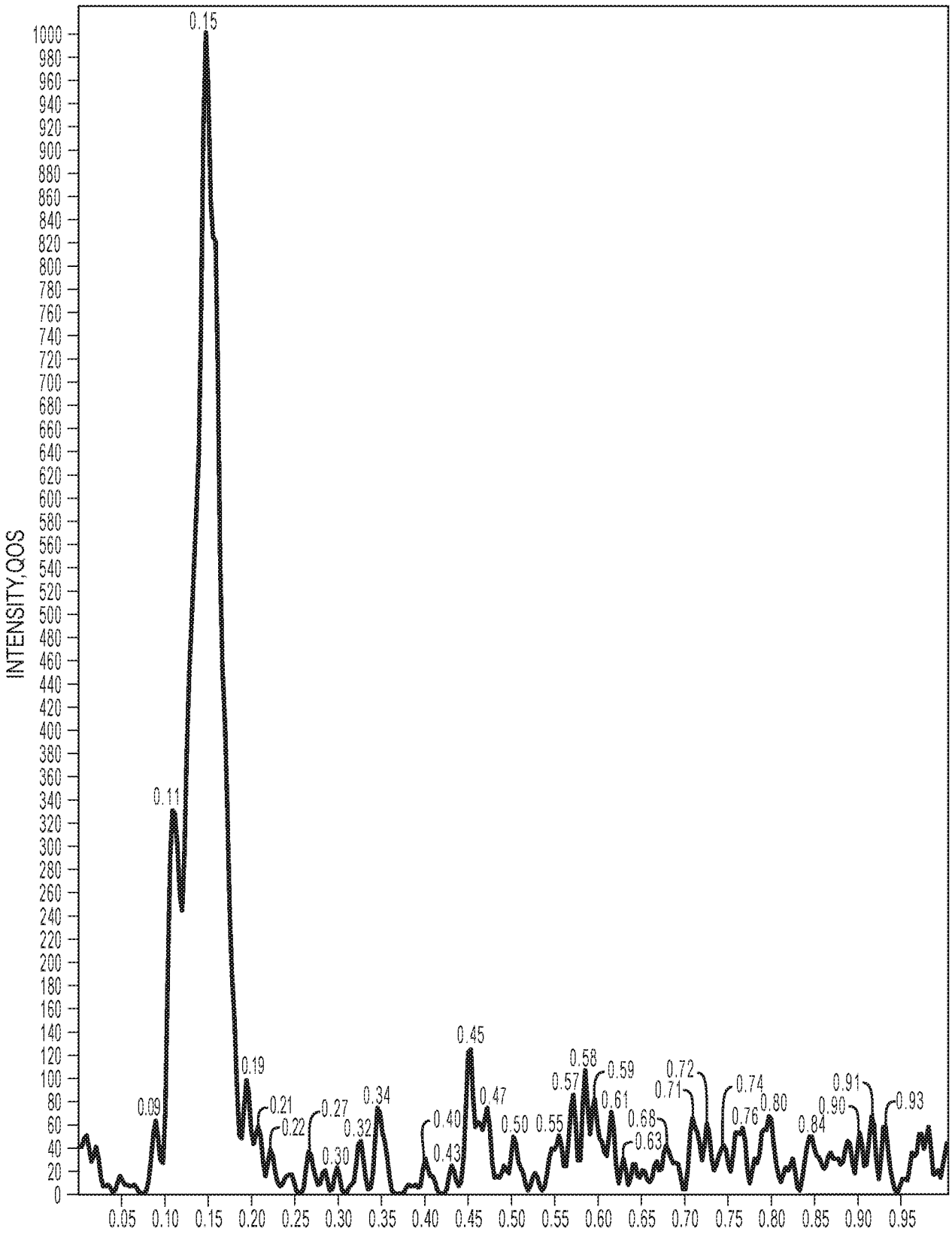

INTENSITY,QOS
0.15
0.11
0.09
0.19
0.21
0.22
0.27
0.30
0.32
0.34
0.40
0.43
0.45
0.47
0.50
0.55
0.57
0.58
0.59
0.61
0.63
0.68
0.71
0.72
0.74
0.76
0.80
0.84
0.90
0.91
0.93

FIG. 3E

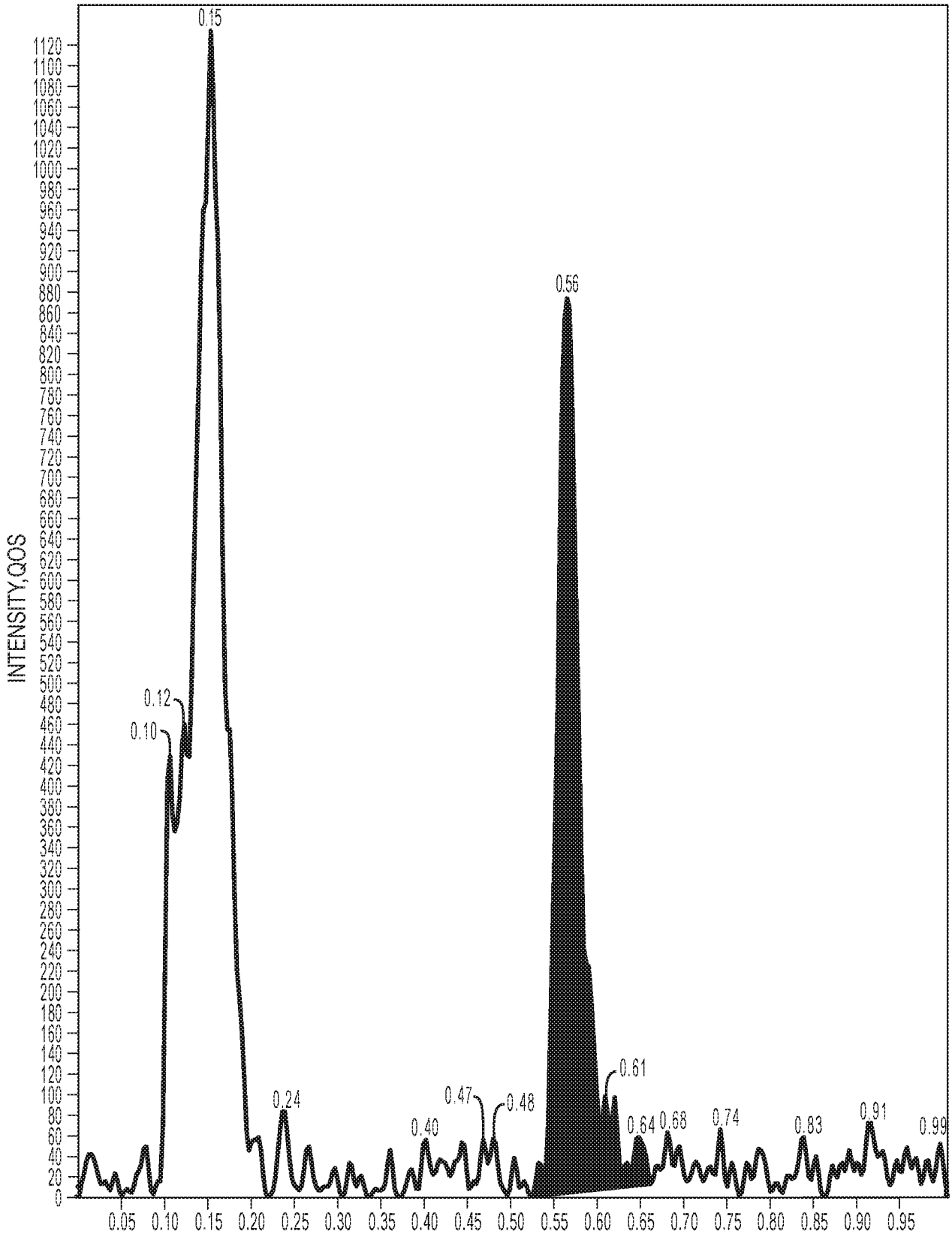

INTENSITY,QOS
0.15
0.56
0.12
0.10
0.24
0.40
0.47
0.48
0.61
0.64
0.68
0.74
0.83
0.91
0.99

FIG. 3F

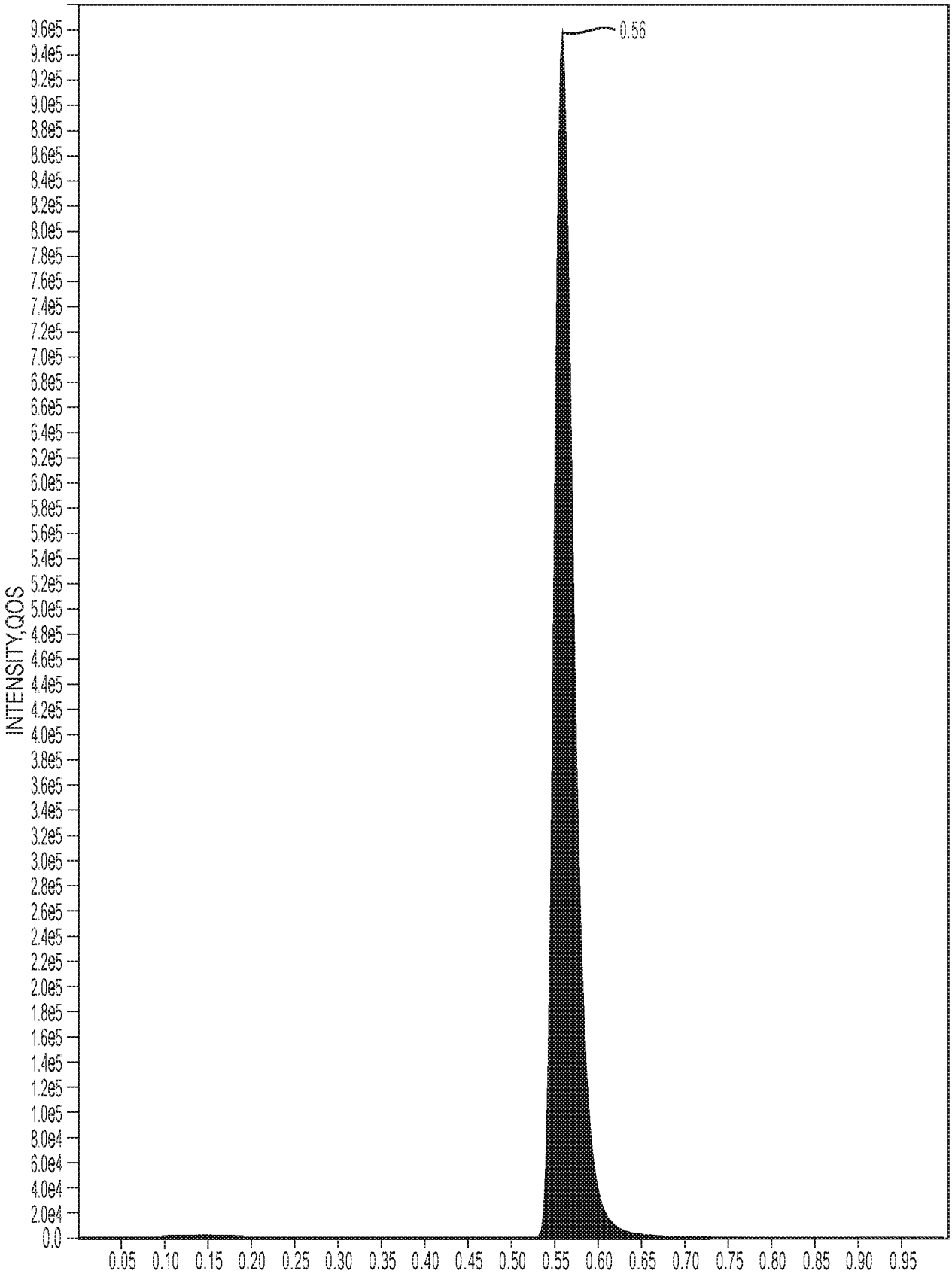

0.56
INTENSITY,QOS
9.6e5
9.4e5
9.2e5
9.0e5
8.8e5
8.6e5
8.4e5
8.2e5
8.0e5
7.8e5
7.6e5
7.4e5
7.2e5
7.0e5
6.8e5
6.6e5
6.4e5
6.2e5
6.0e5
5.8e5
5.6e5
5.4e5
5.2e5
5.0e5
4.8e5
4.6e5
4.4e5
4.2e5
4.0e5
3.8e5
3.6e5
3.4e5
3.2e5
3.0e5
2.8e5
2.6e5
2.4e5
2.2e5
2.0e5
1.8e5
1.6e5
1.4e5
1.2e5
1.0e5
8.0e4
6.0e4
4.0e4
2.0e4
0.0
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95

FIG. 3G

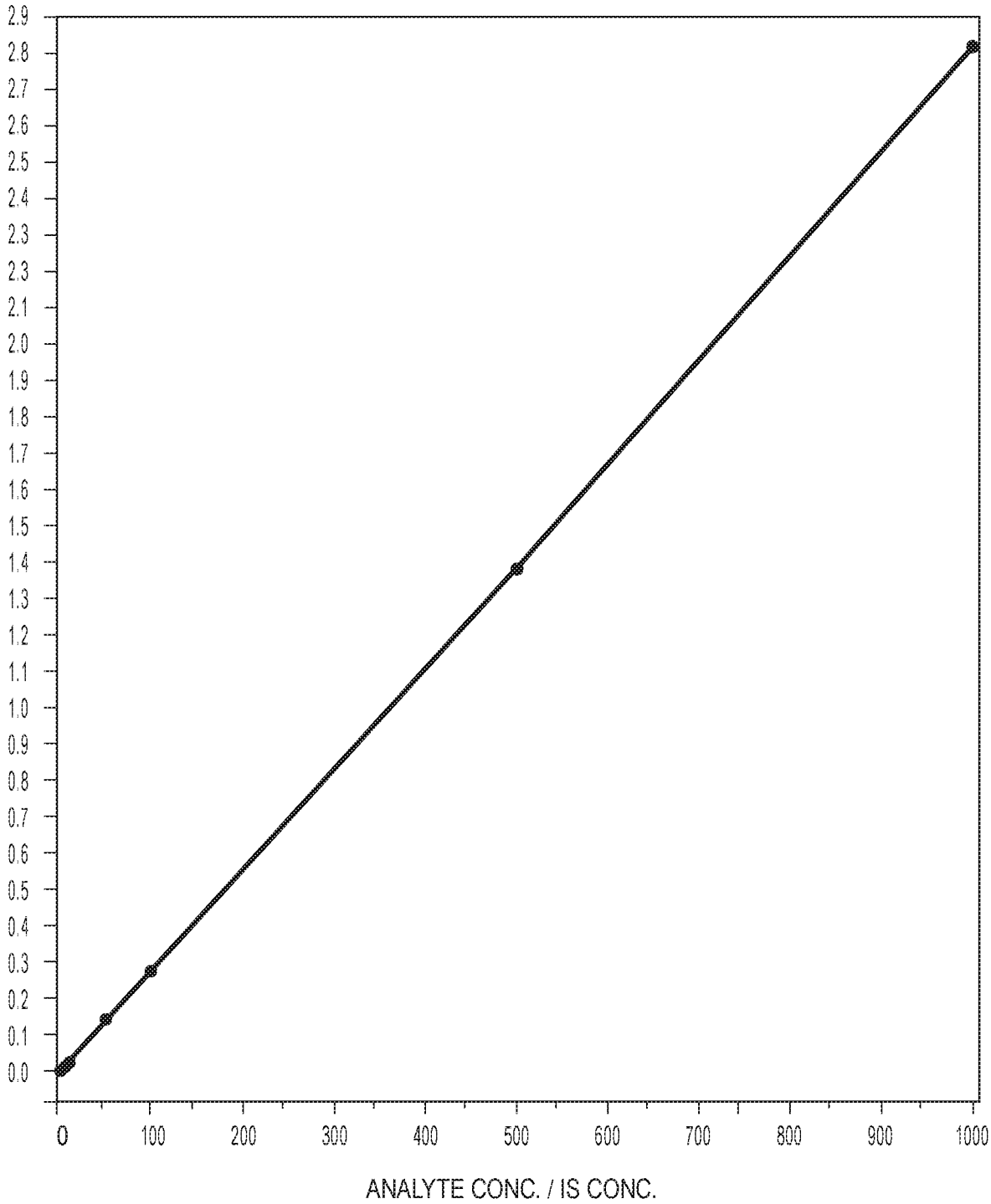

2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.3
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
100
200
300
400
500
600
700
800
900
1000
ANALYTE CONC. / IS CONC.

FIG. 3H

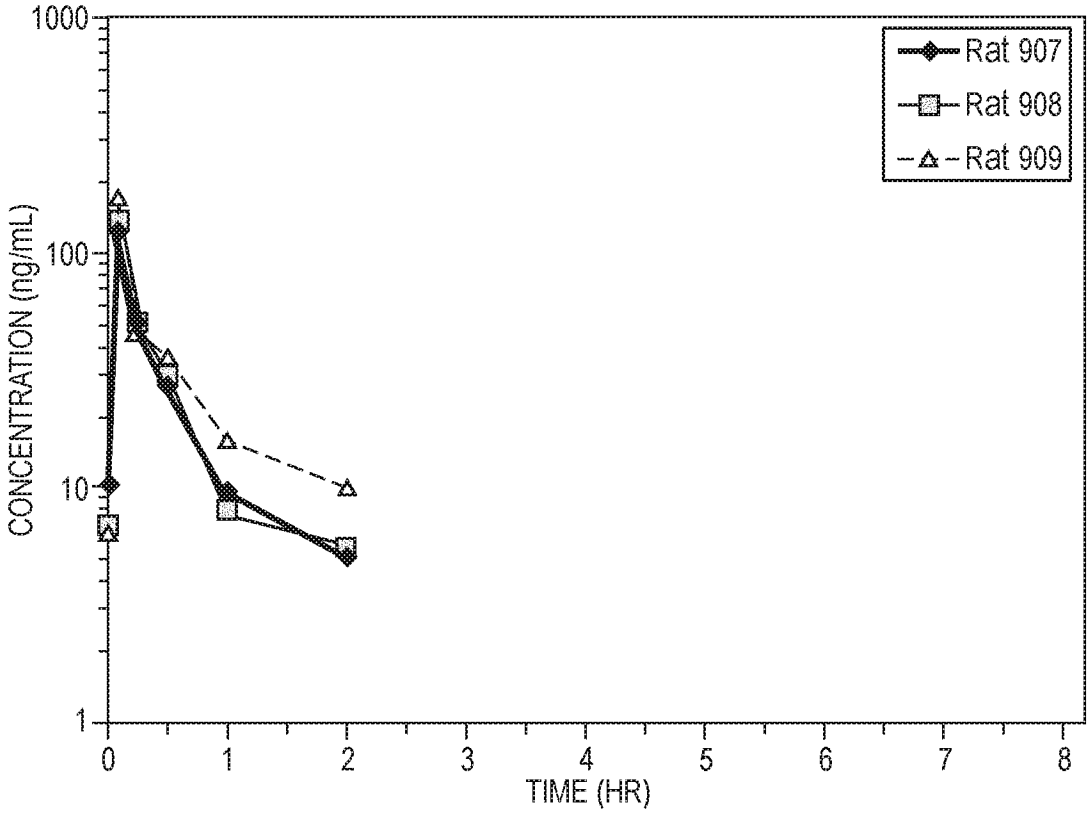

1000
100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
Rat 907
Rat 908
Rat 909

FIG. 4A

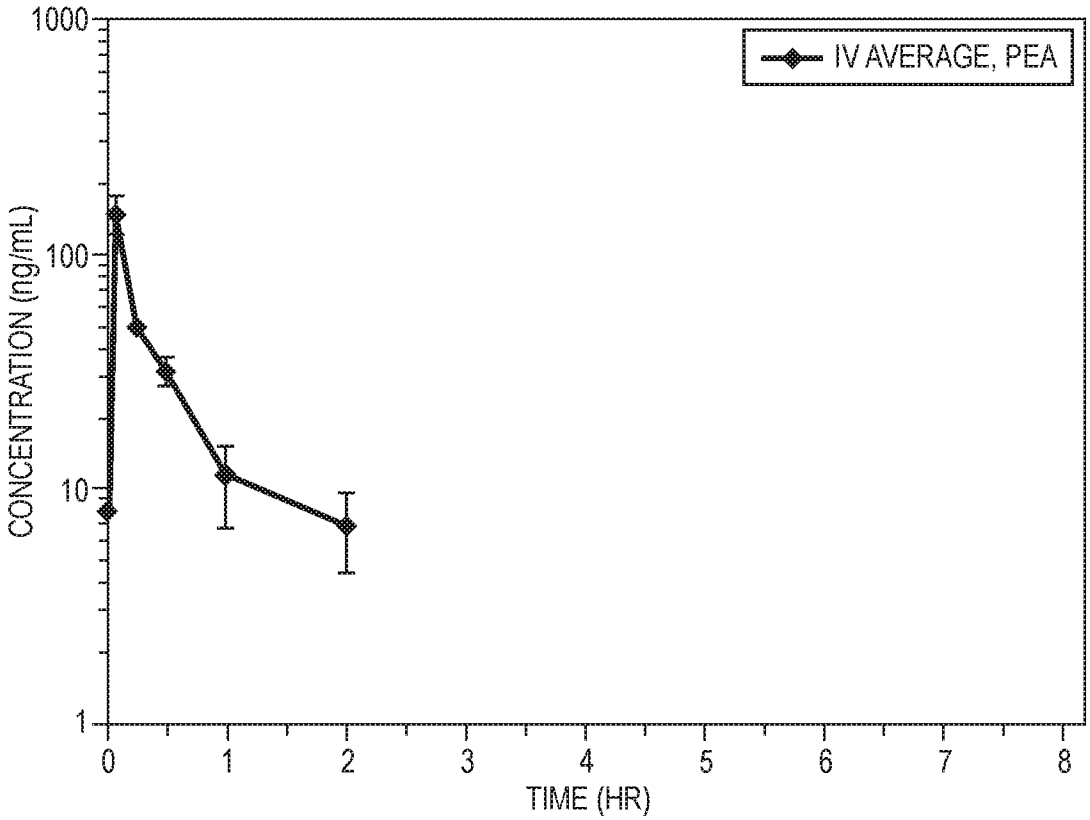

1000
100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
IV AVERAGE, PEA

FIG. 4B

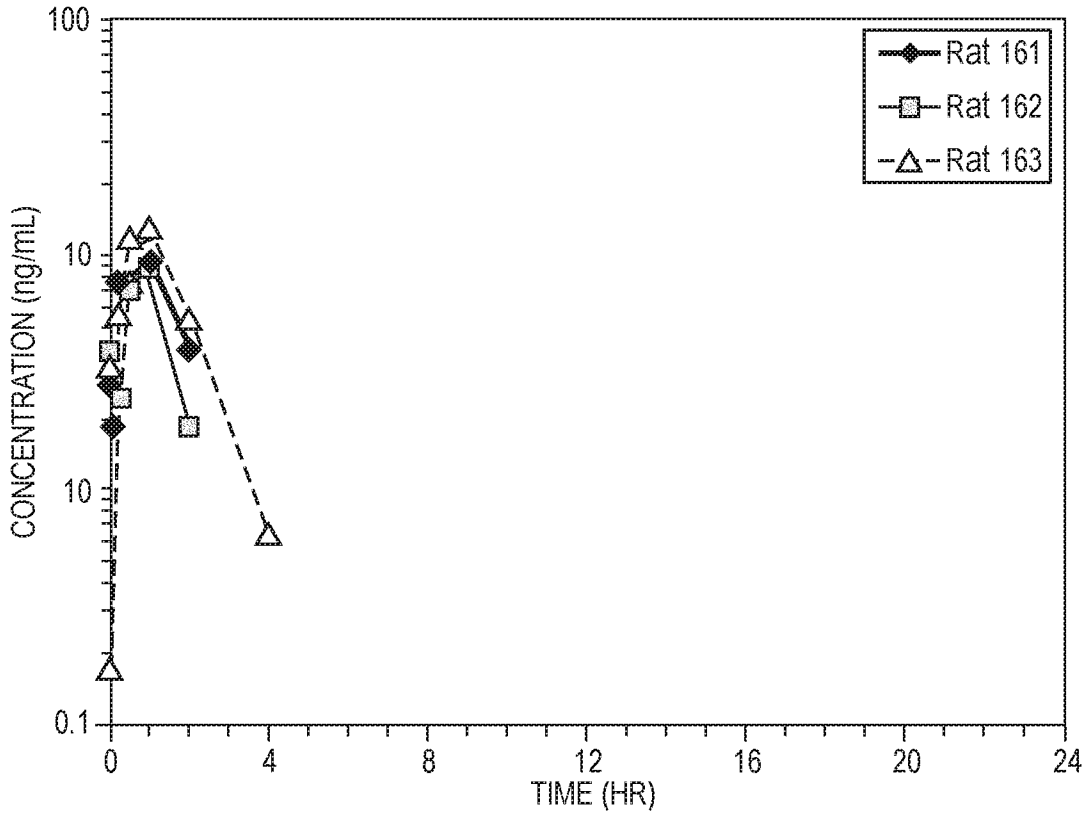

100
10
10
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 161
Rat 162
Rat 163

FIG. 5A

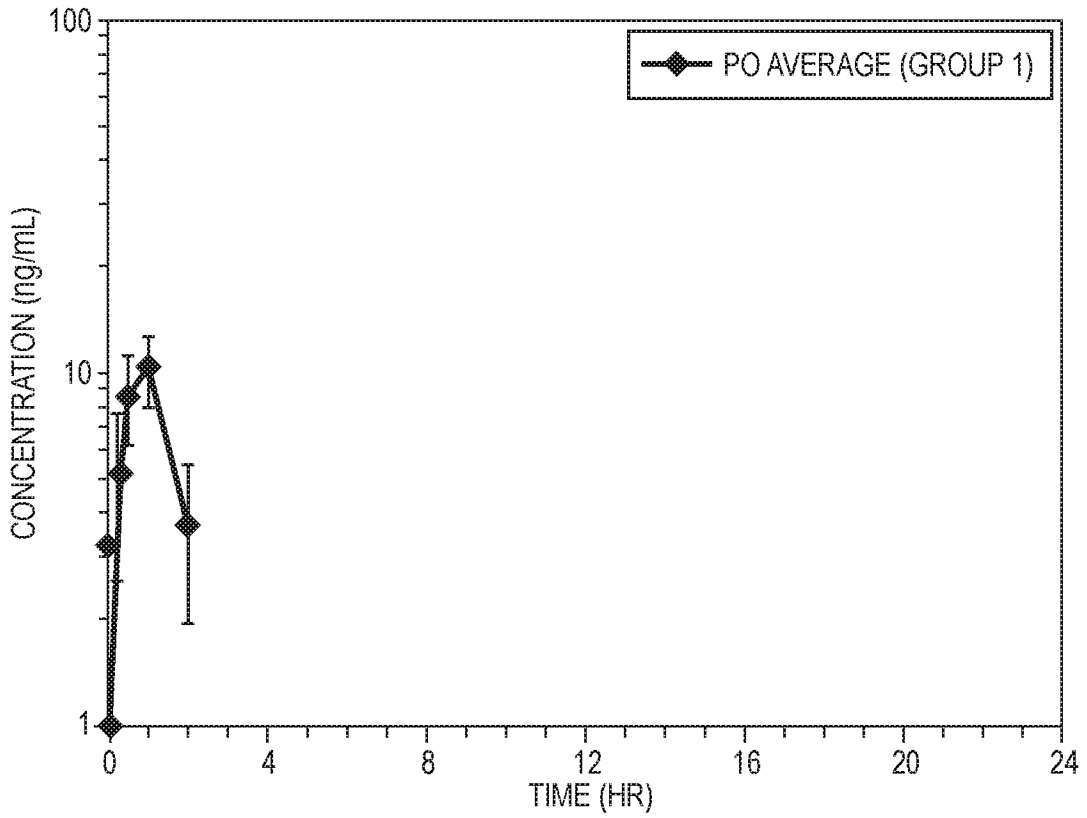

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 1)

FIG. 5B

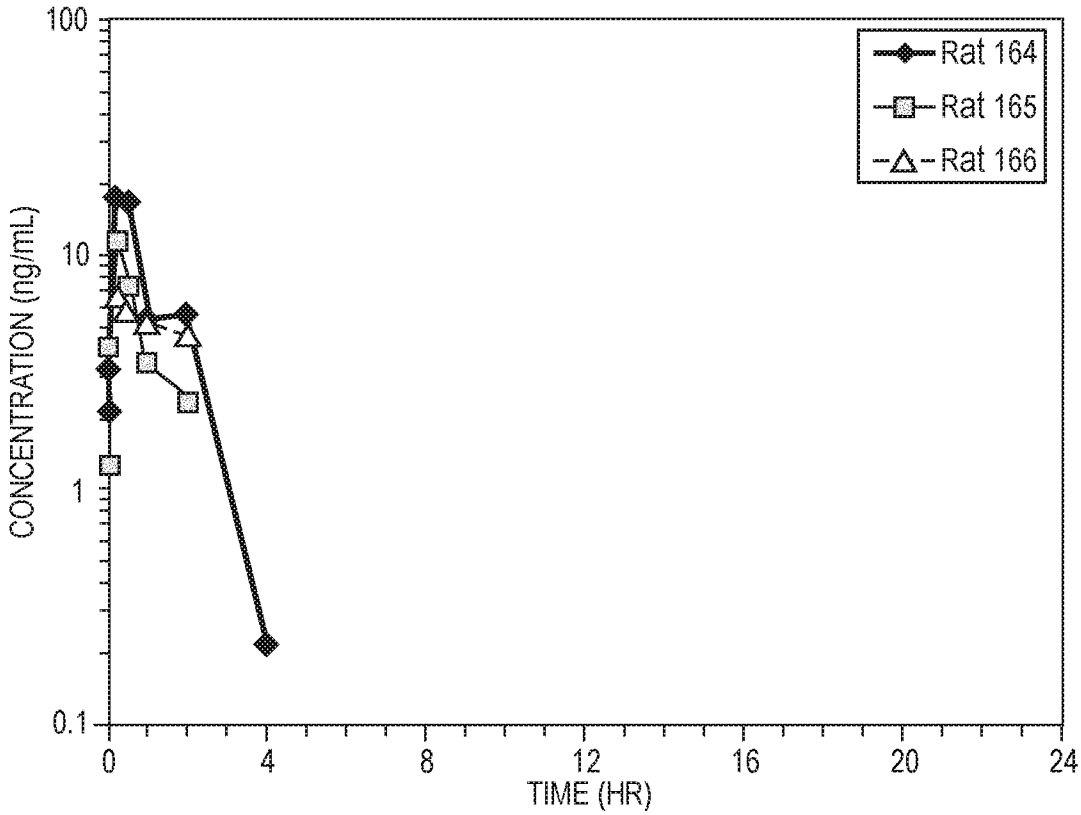

100
10
1
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 164
Rat 165
Rat 166

FIG. 5C

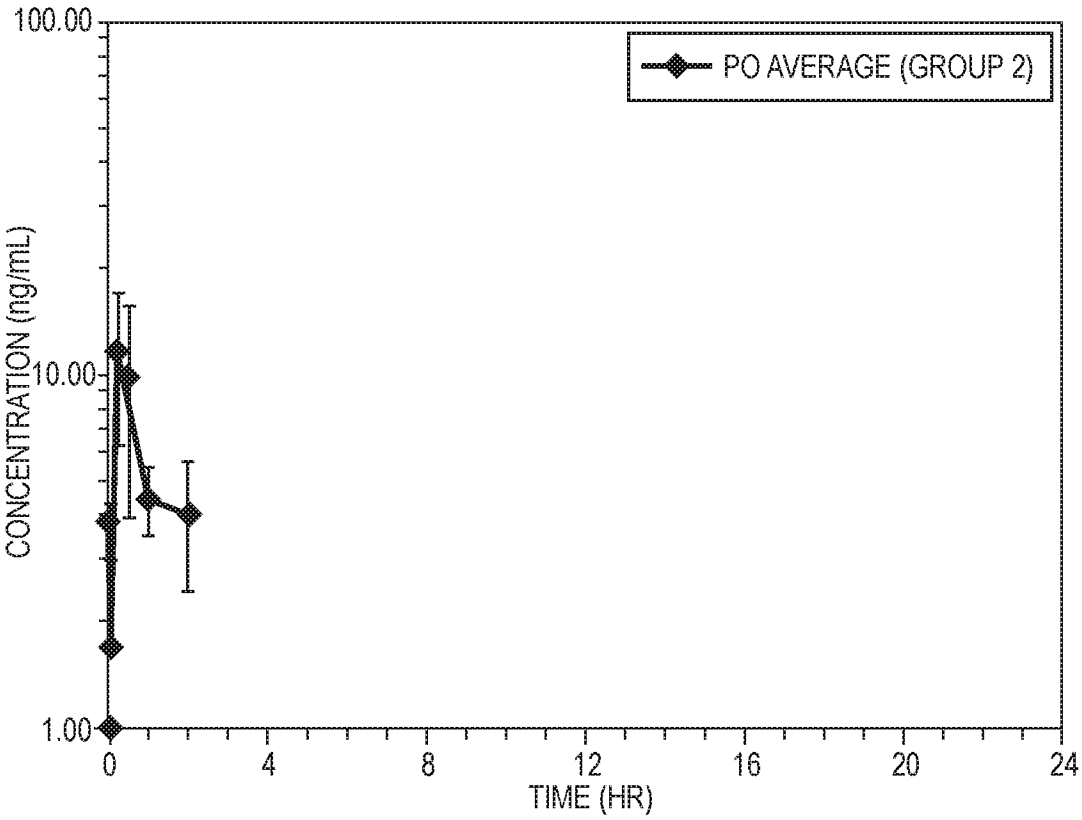

100.00
10.00
1.00
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 2)

FIG. 5D

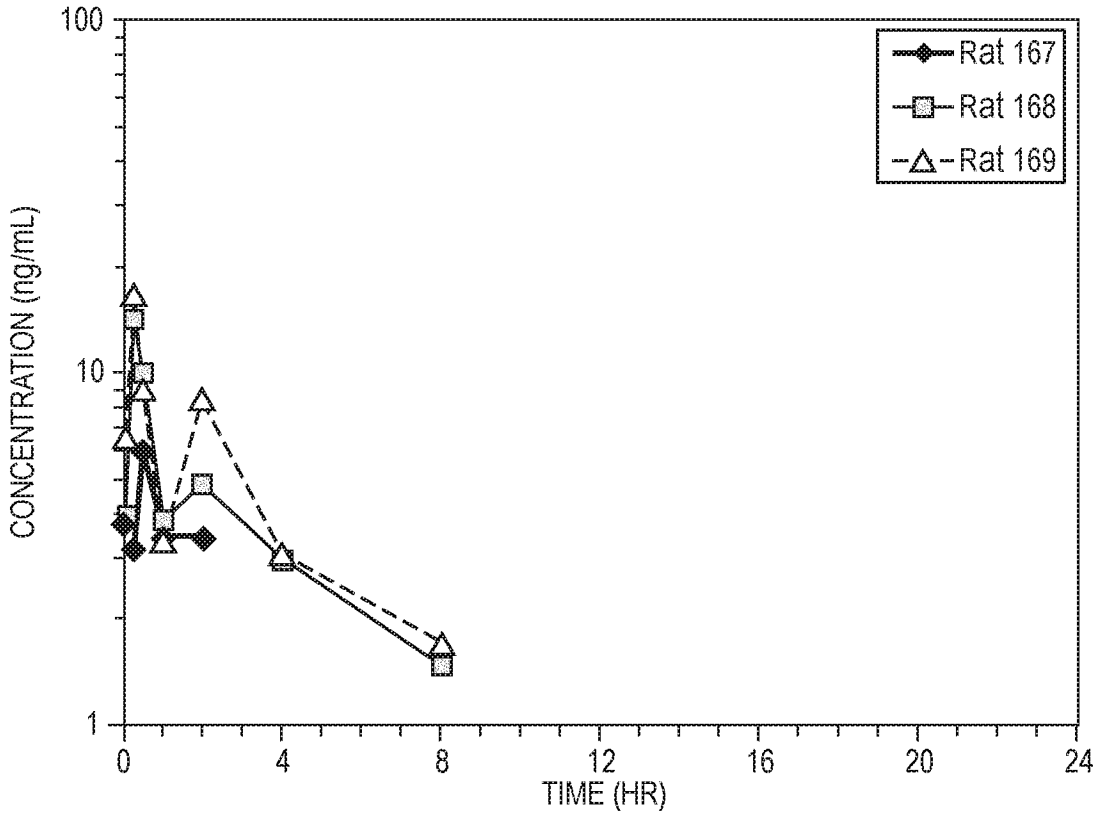

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 167
Rat 168
Rat 169

FIG. 5E

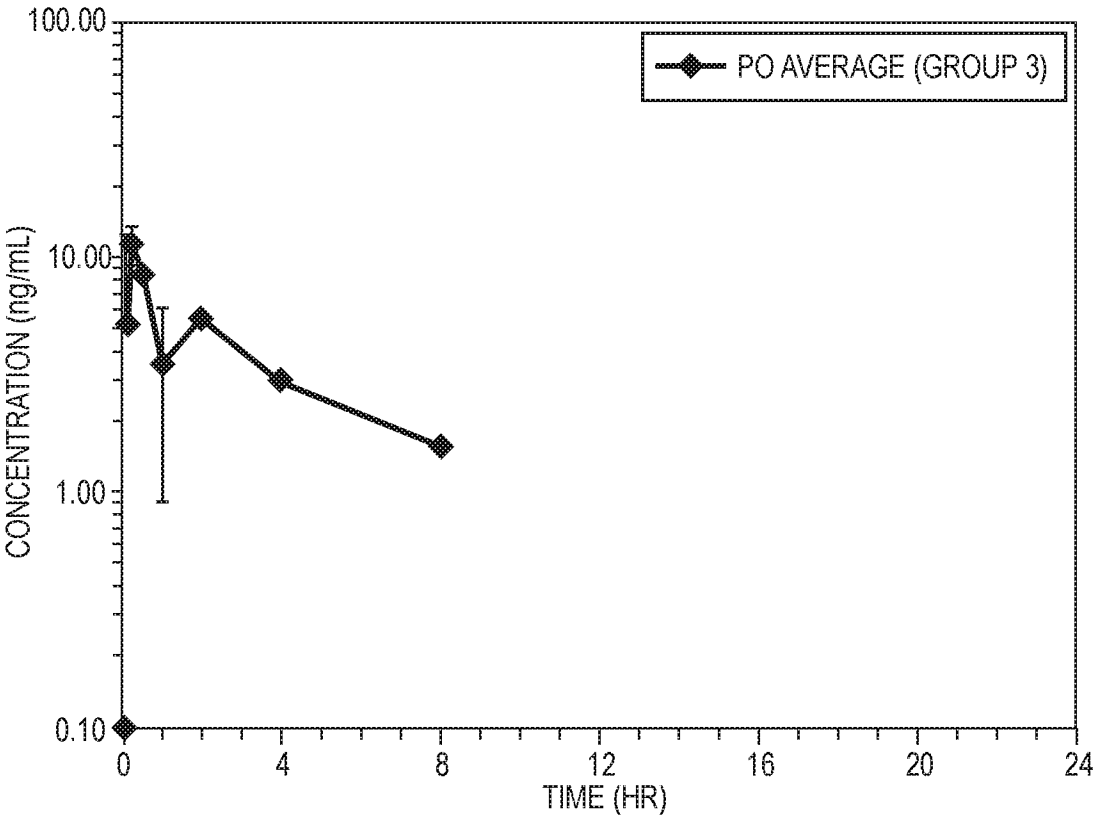

100.00
10.00
1.00
0.10
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 3)

FIG. 5F

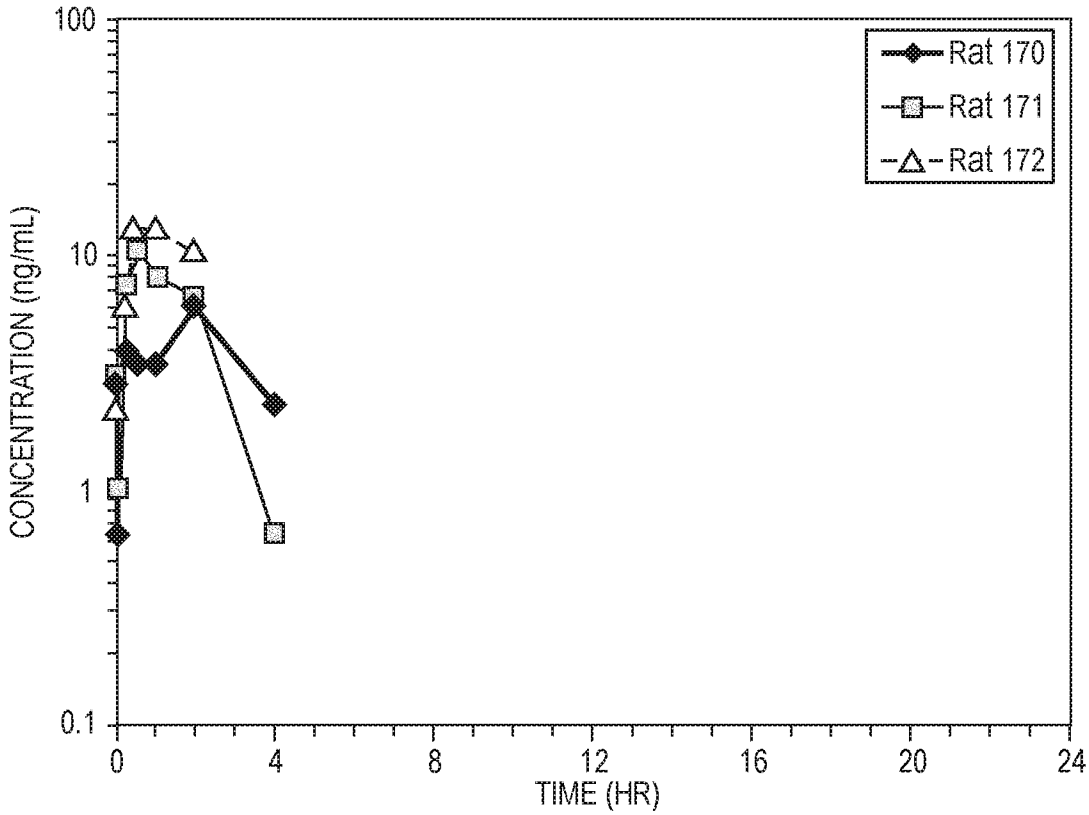

100
10
1
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 170
Rat 171
Rat 172

FIG. 5G

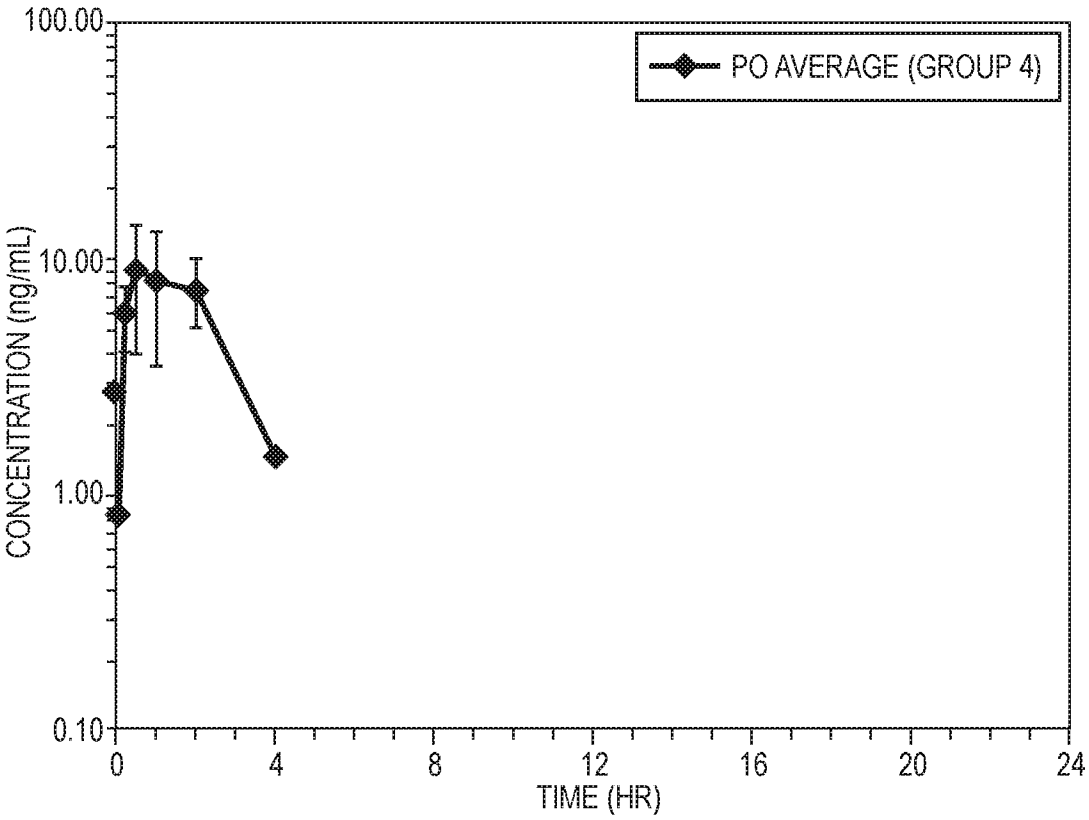

100.00
10.00
1.00
0.10
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 4)

FIG. 5H

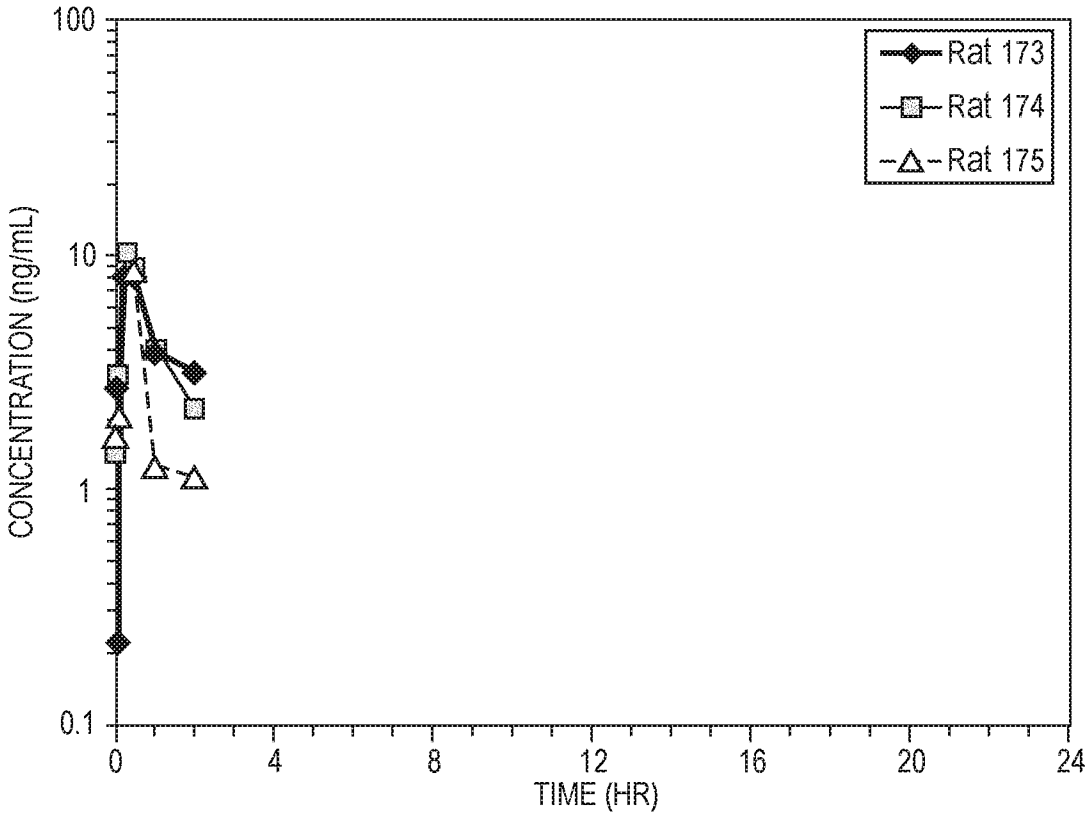

100
10
1
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 173
Rat 174
Rat 175

FIG. 5I

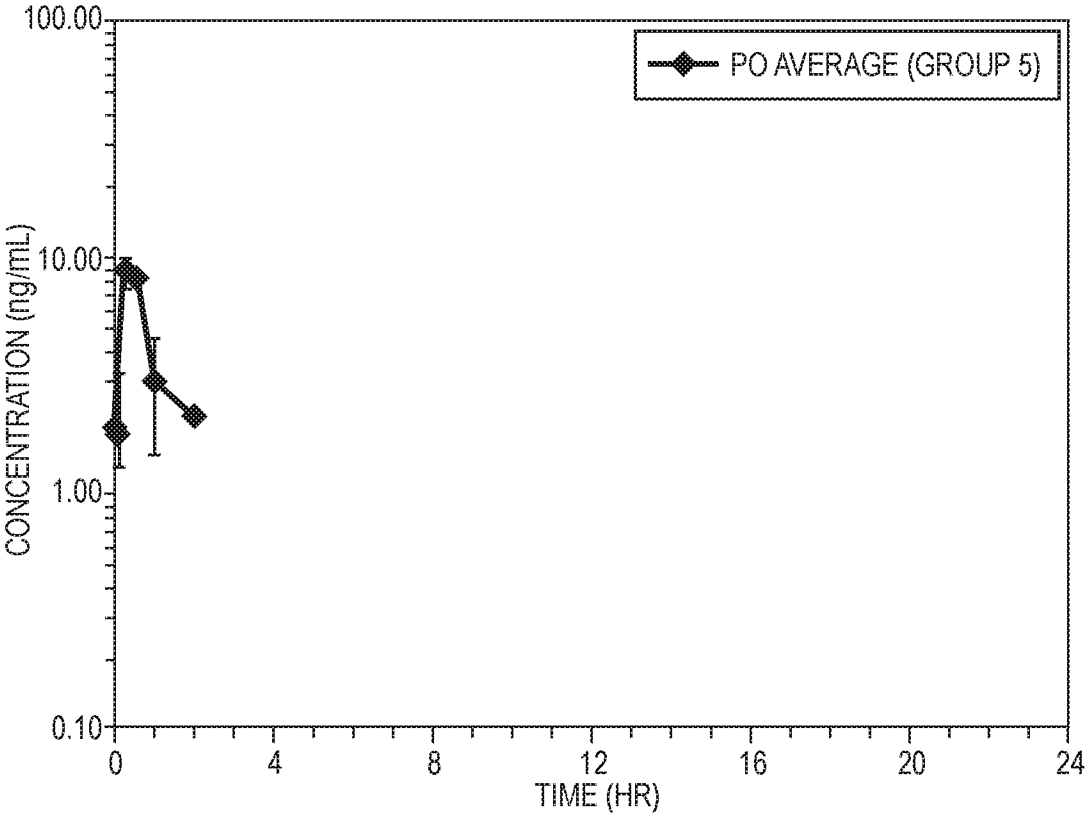

100.00
10.00
1.00
0.10
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 5)

FIG. 5J

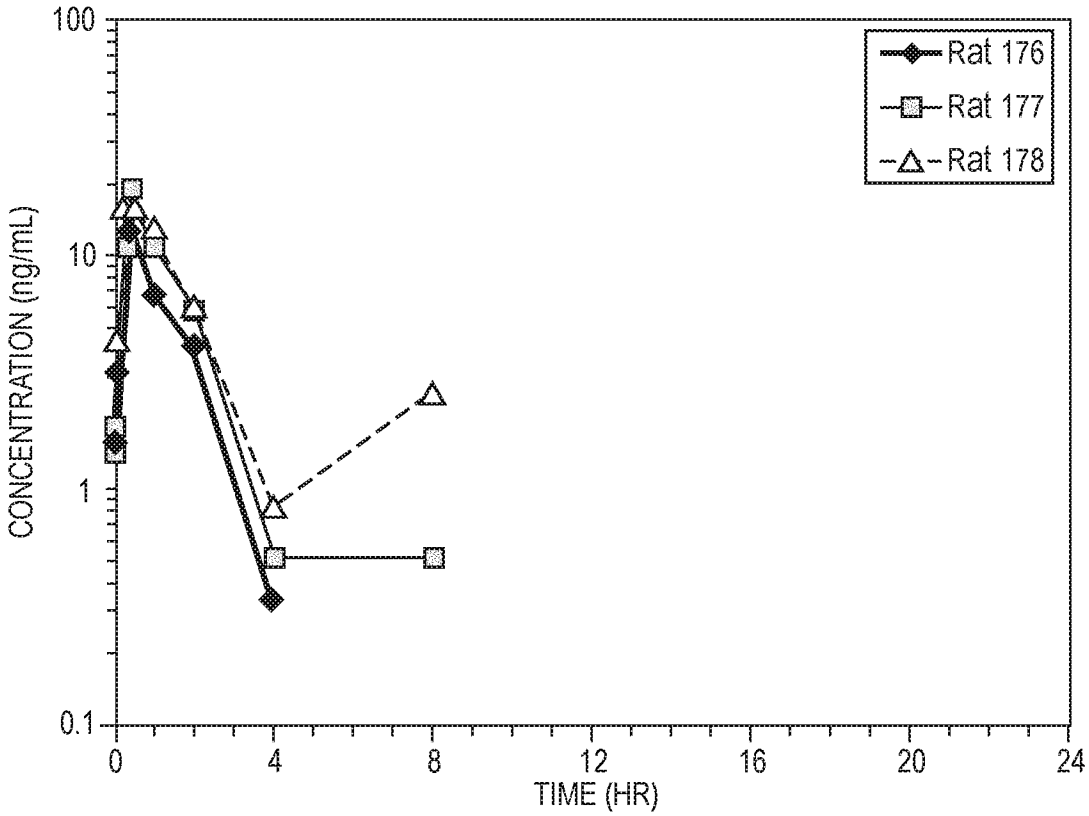

100
10
1
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 176
Rat 177
Rat 178

FIG. 5K

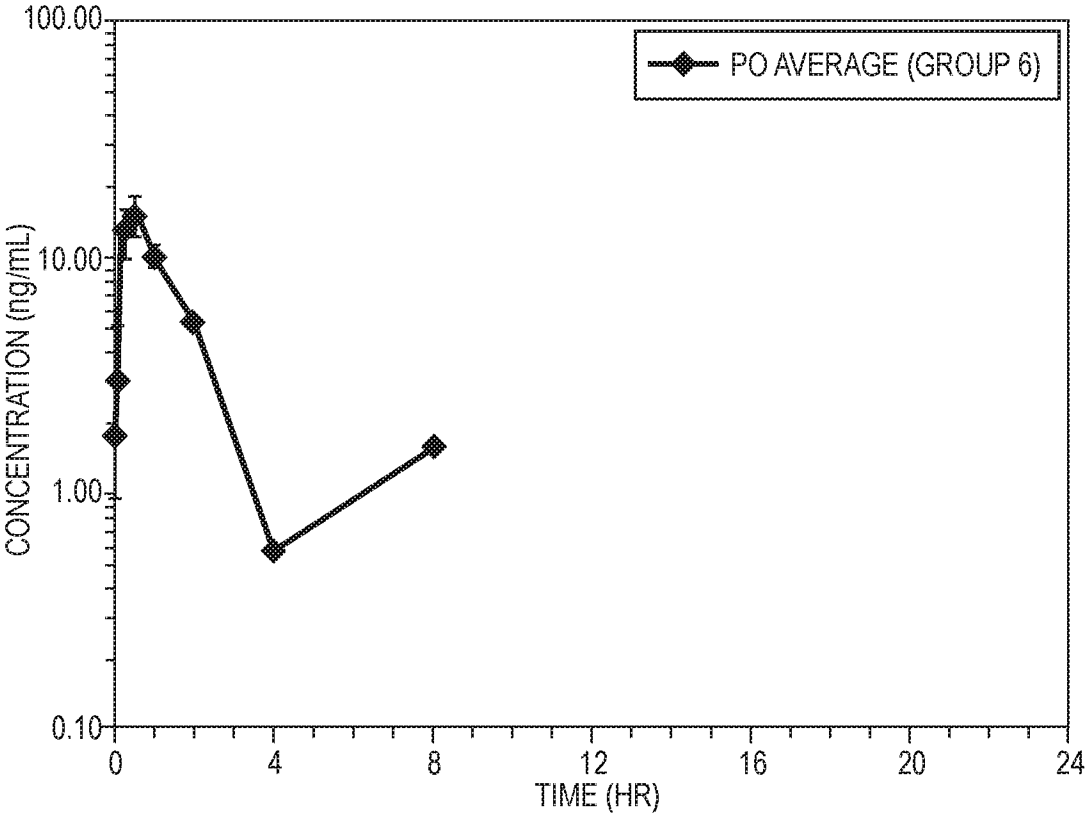

100.00
10.00
1.00
0.10
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 6)

FIG. 5L

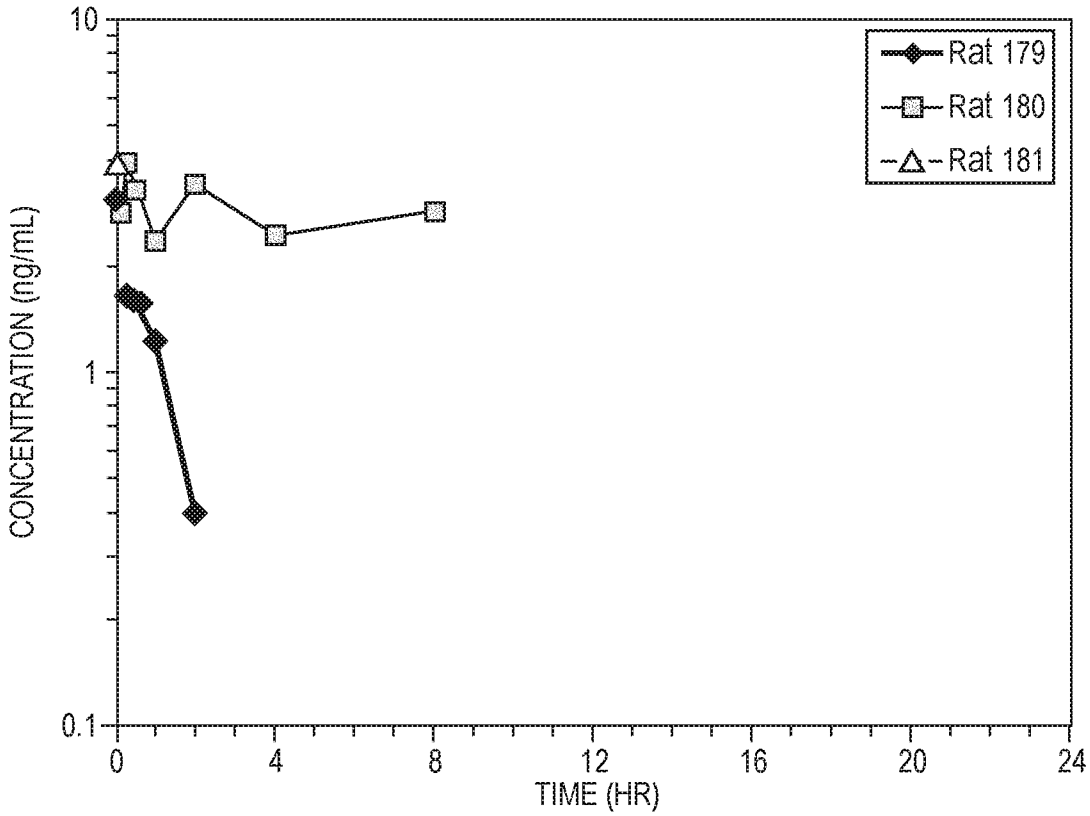

10
1
0.1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 179
Rat 180
Rat 181

FIG. 5M

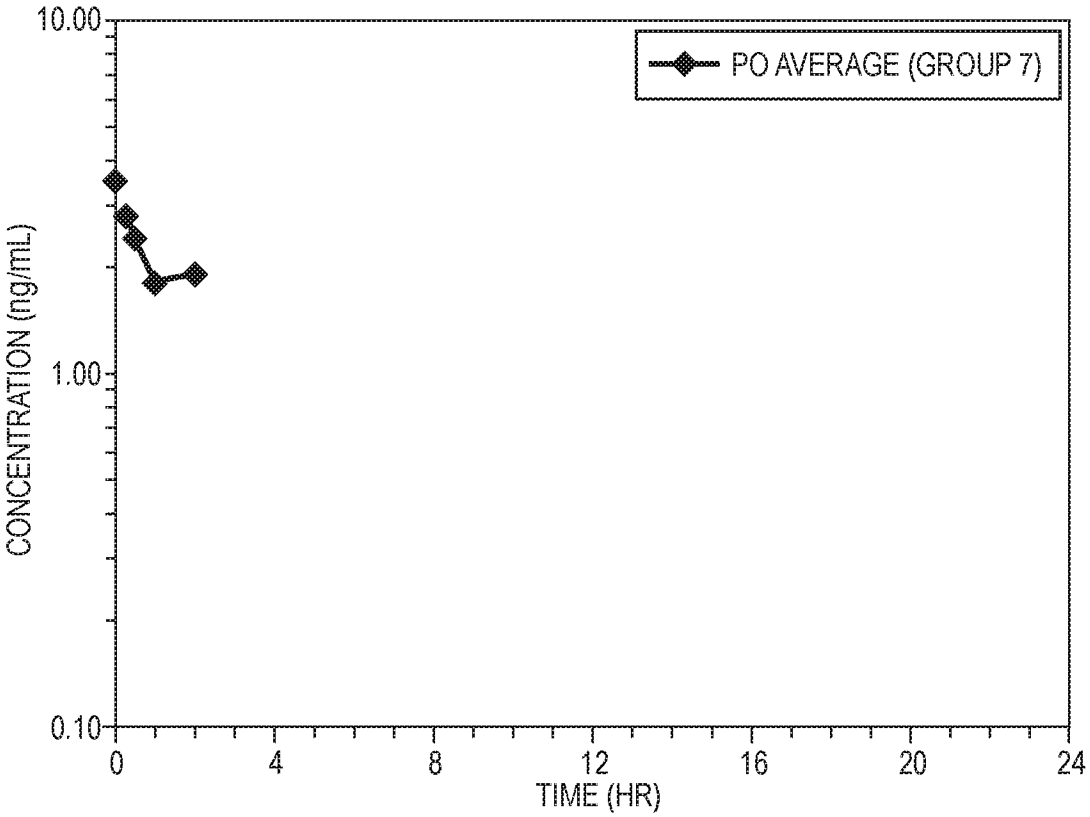

10.00
1.00
0.10
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
PO AVERAGE (GROUP 7)

FIG. 5N

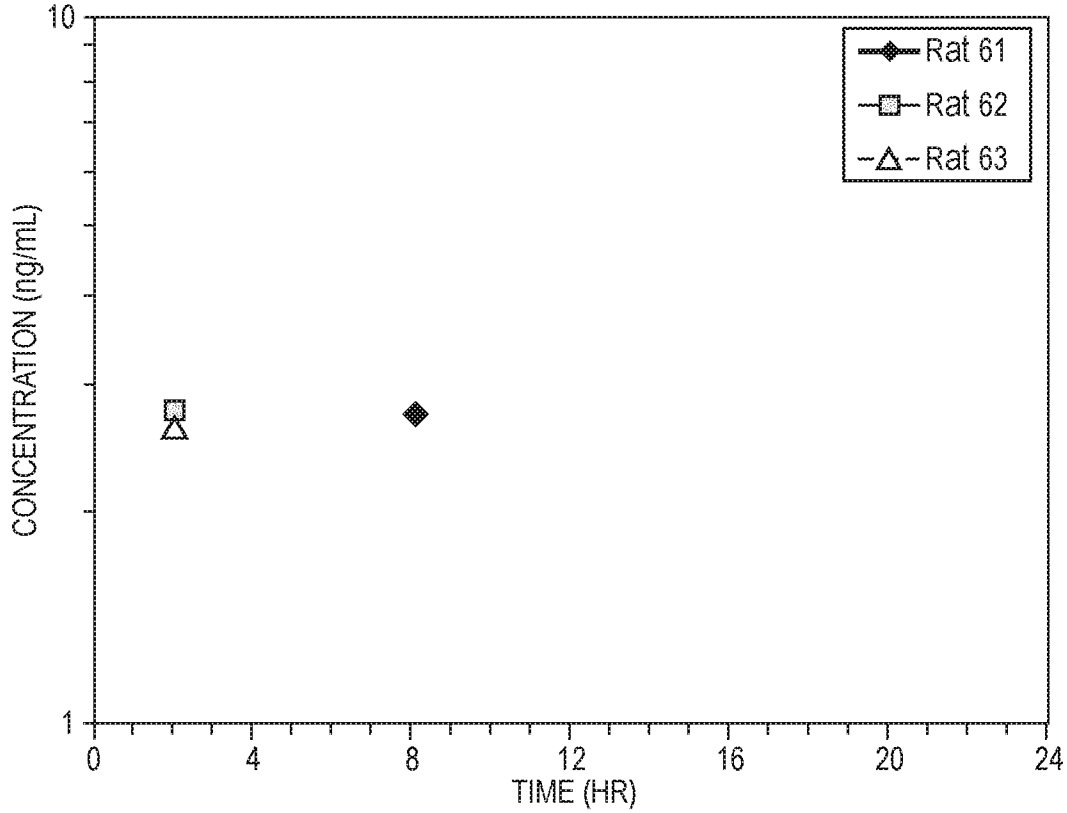

10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 61
Rat 62
Rat 63

FIG. 6

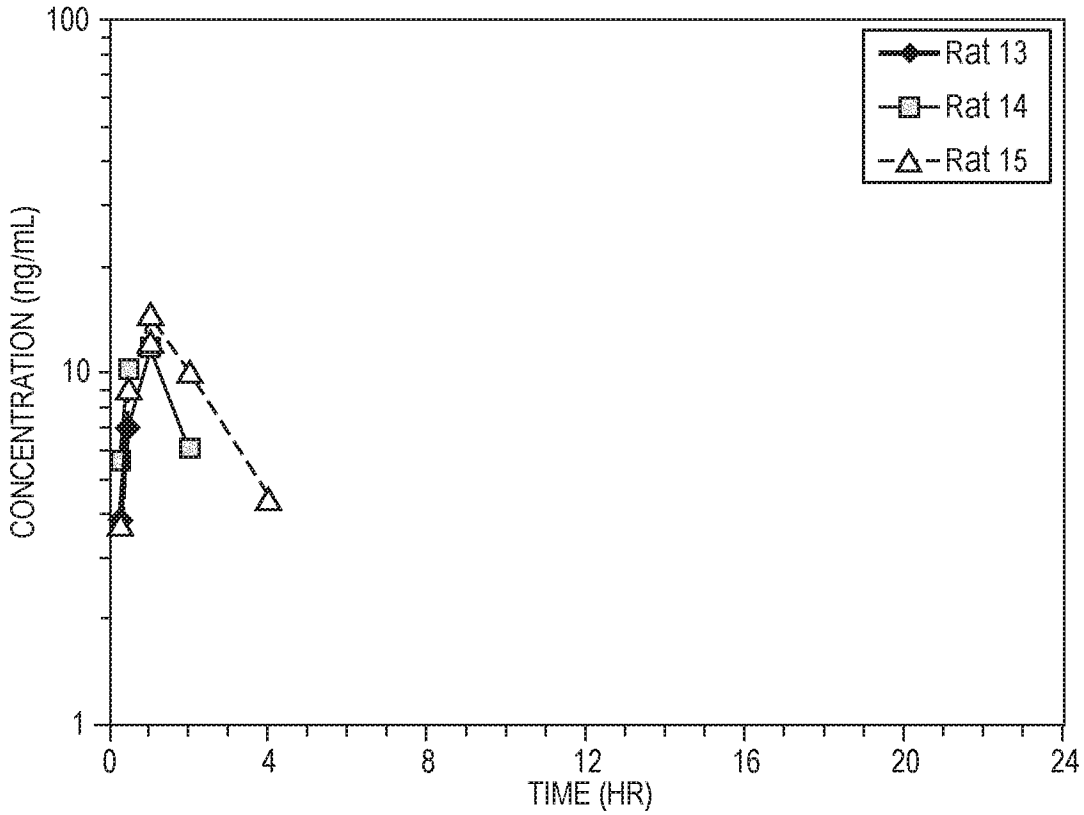

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 13
Rat 14
Rat 15

FIG. 7A

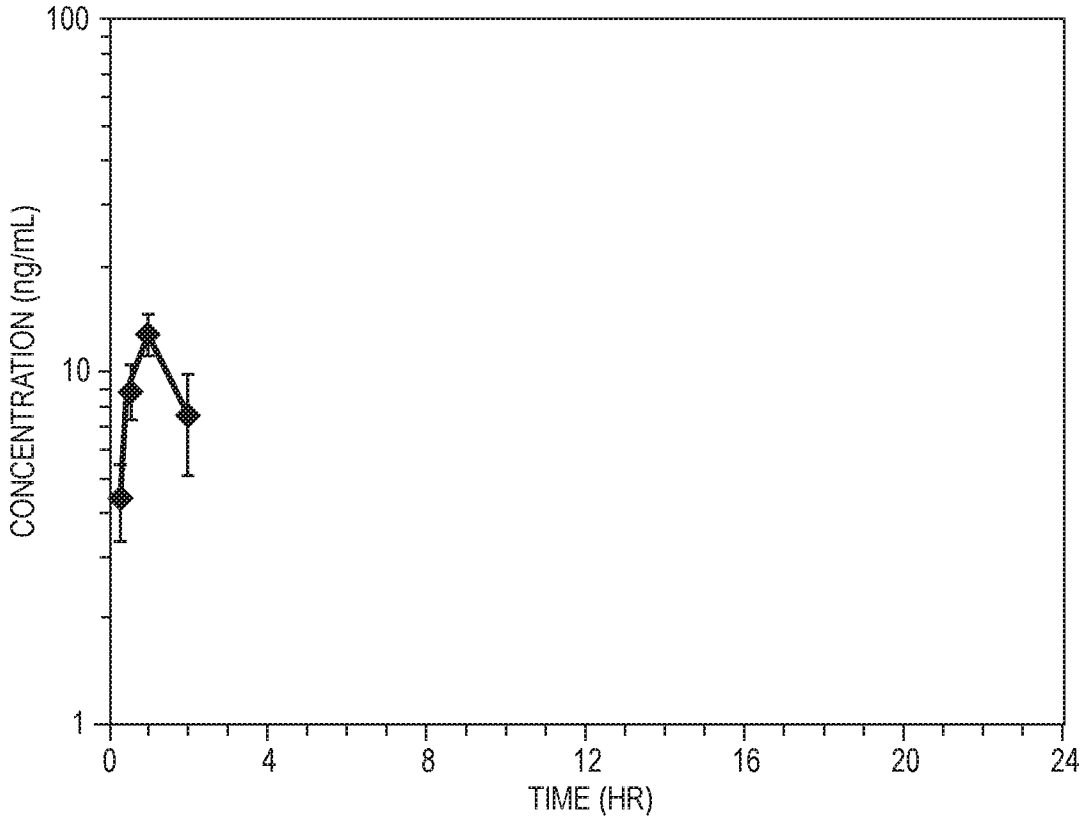

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)

FIG. 7B

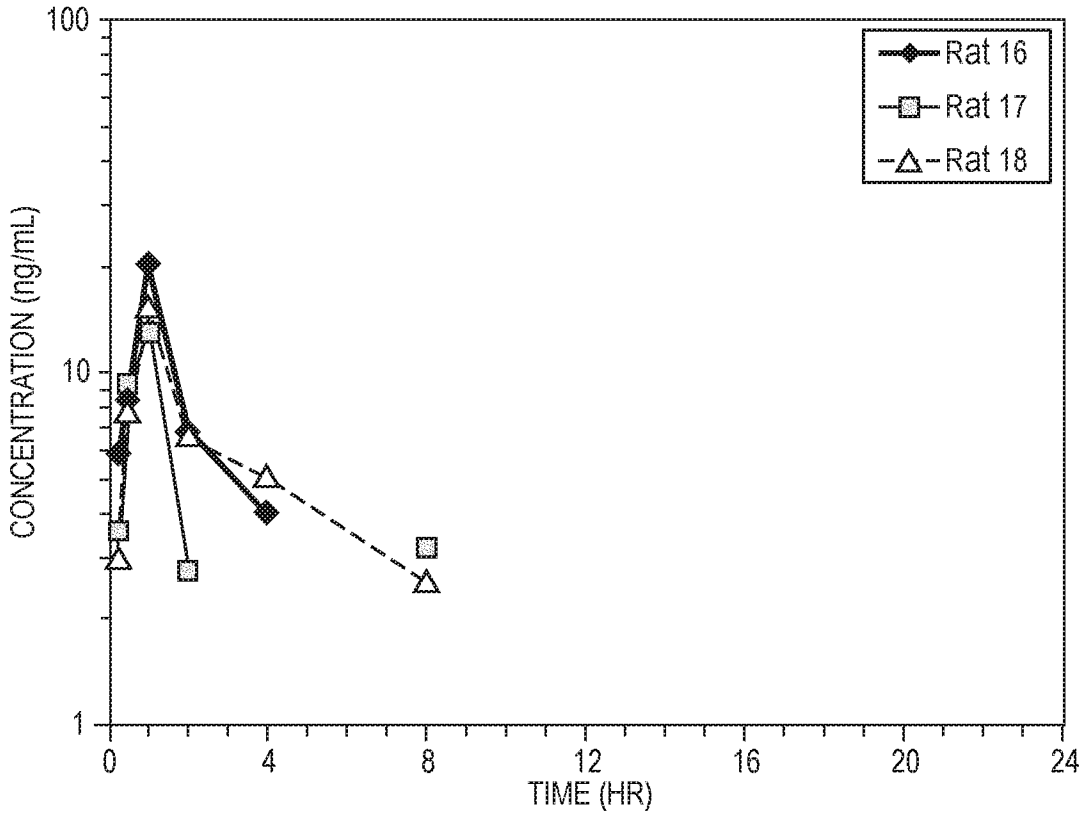

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)
Rat 16
Rat 17
Rat 18

FIG. 7C

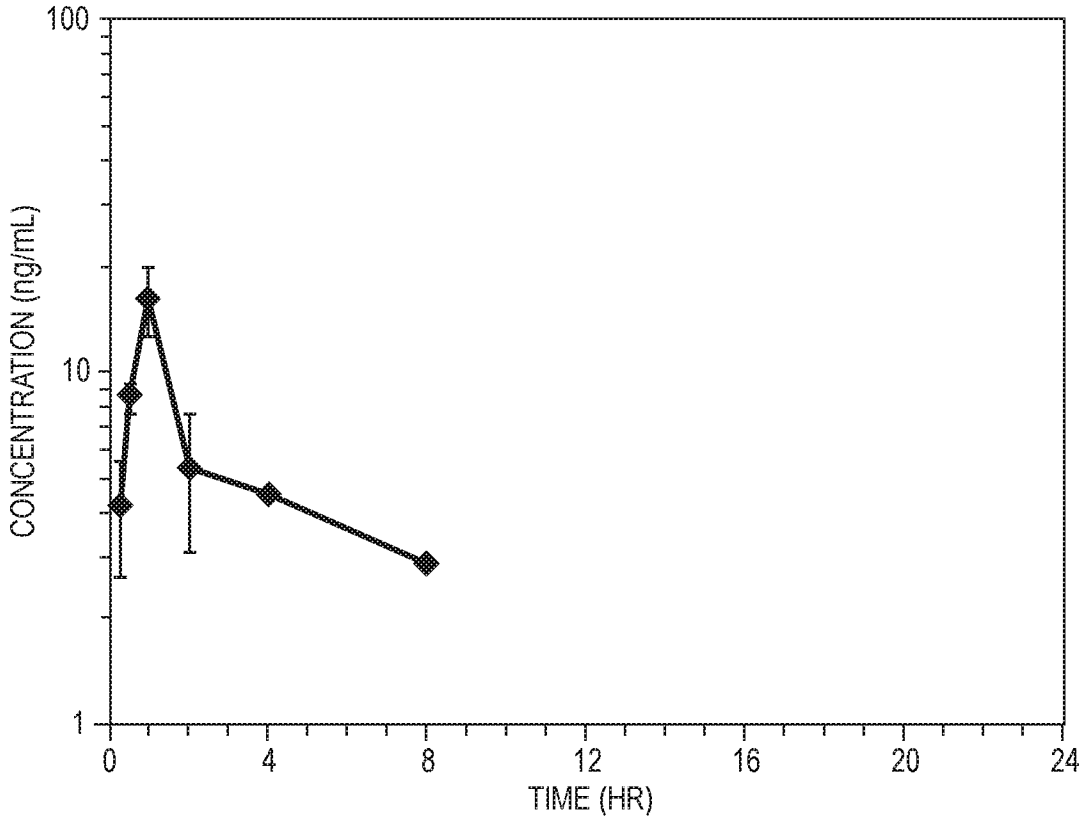

100
10
1
CONCENTRATION (ng/mL)
0
4
8
12
16
20
24
TIME (HR)

FIG. 7D

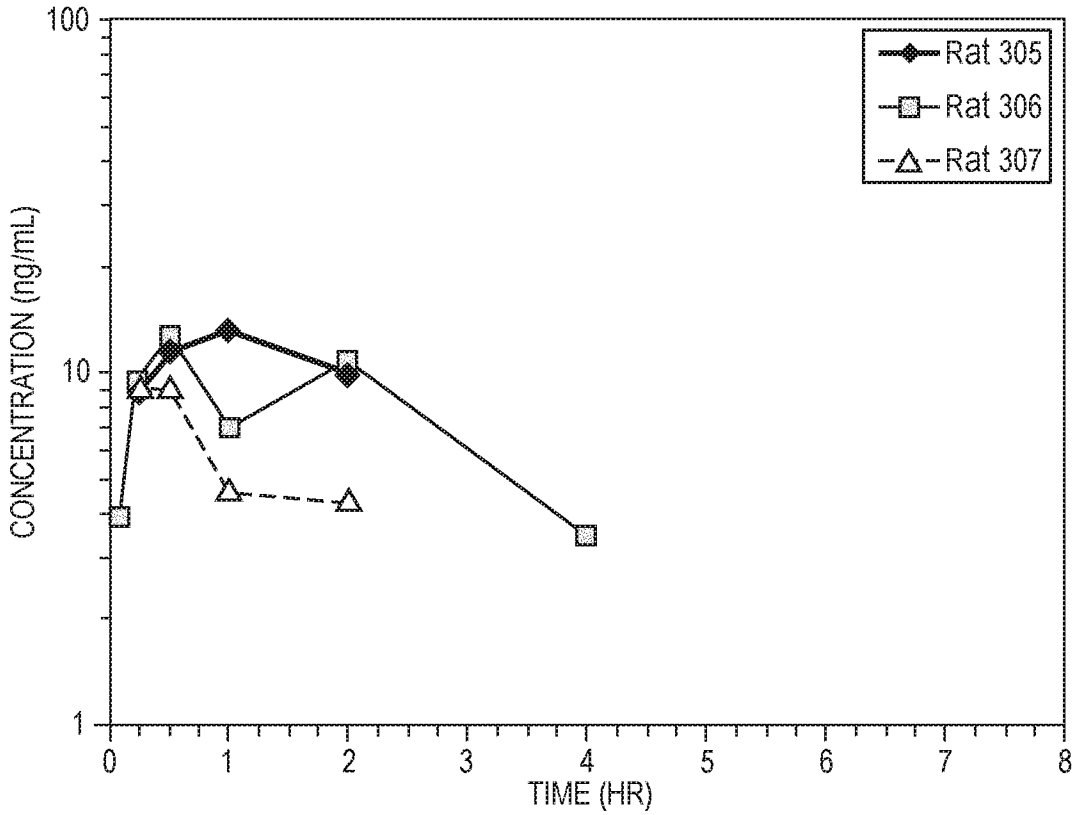

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
Rat 305
Rat 306
Rat 307

FIG. 8A

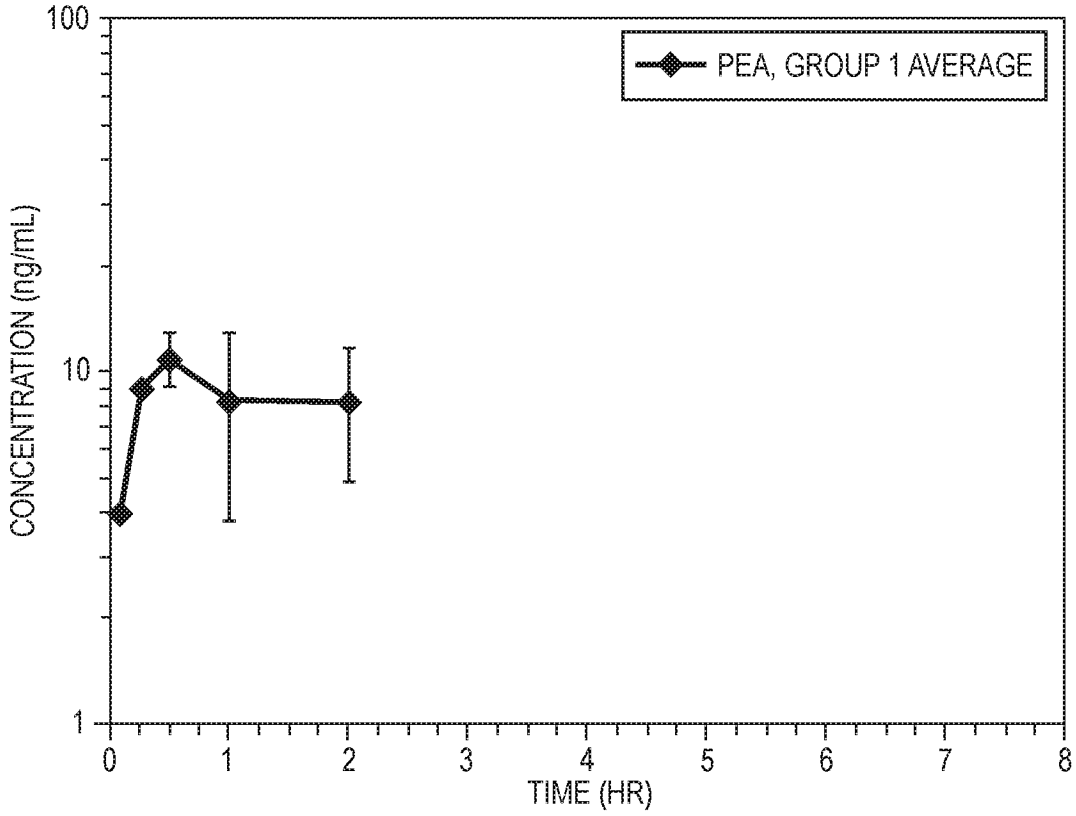

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
PEA, GROUP 1 AVERAGE

FIG. 8B

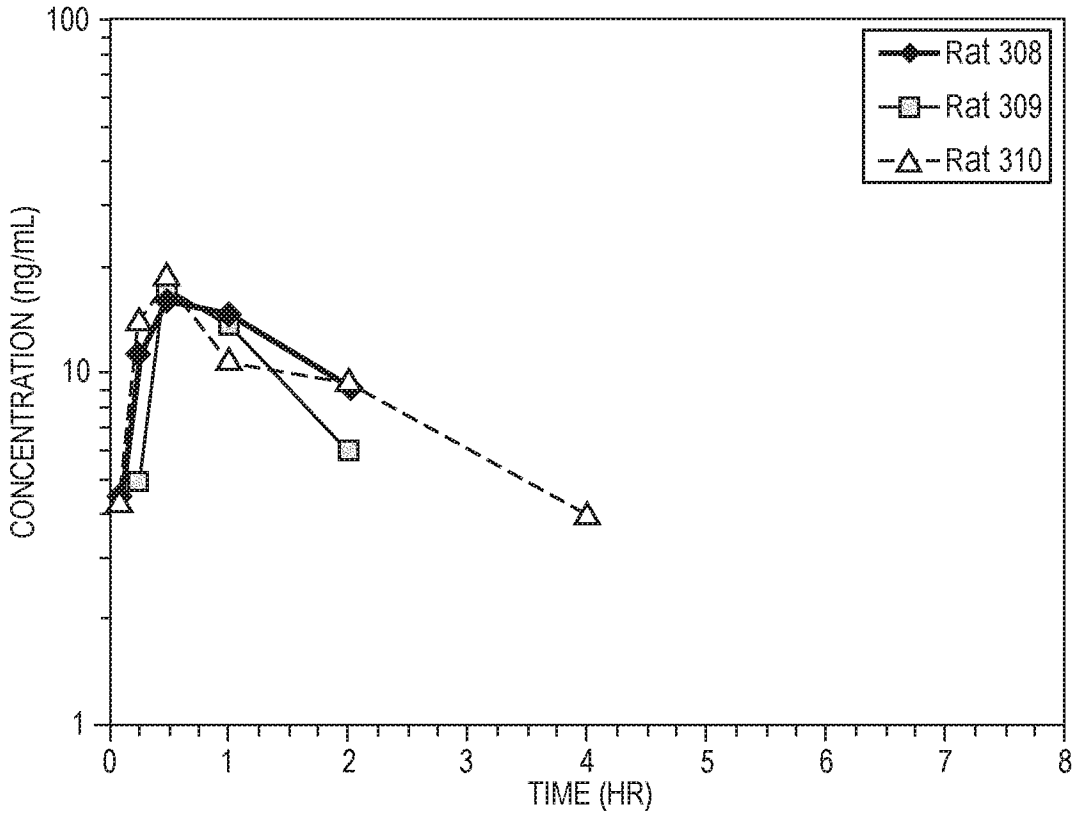

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
Rat 308
Rat 309
Rat 310

FIG. 8C

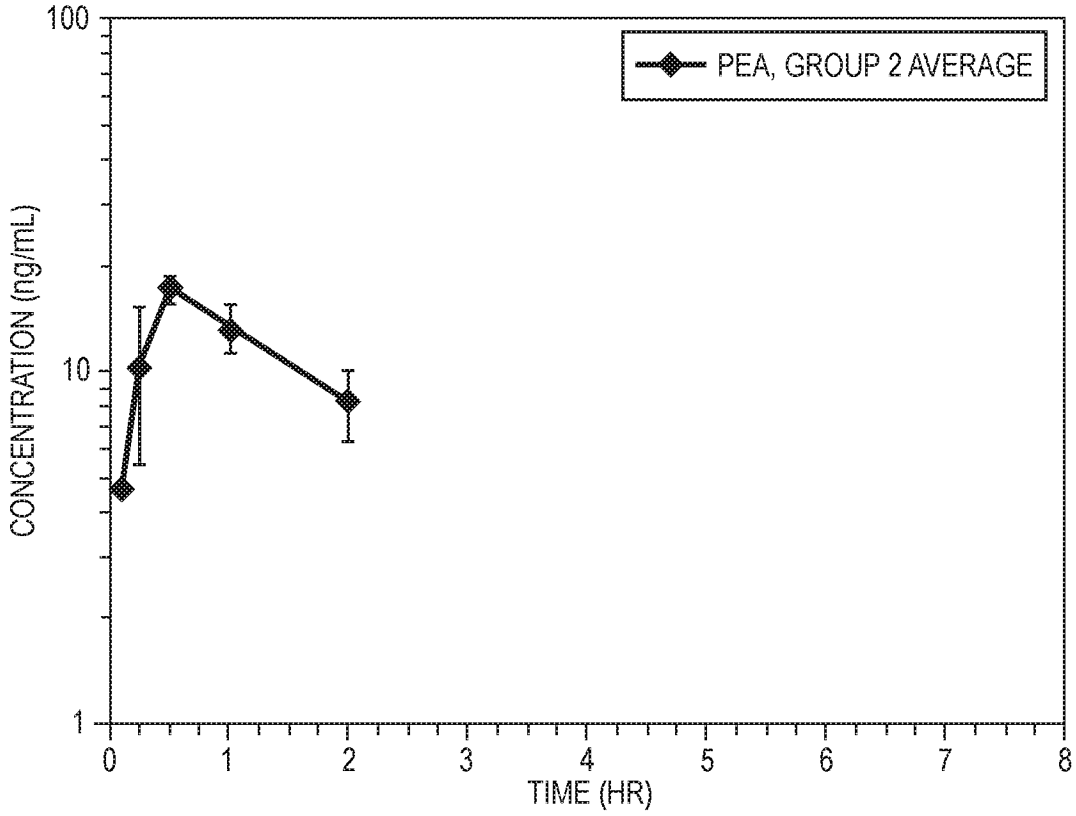

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
PEA, GROUP 2 AVERAGE

FIG. 8D

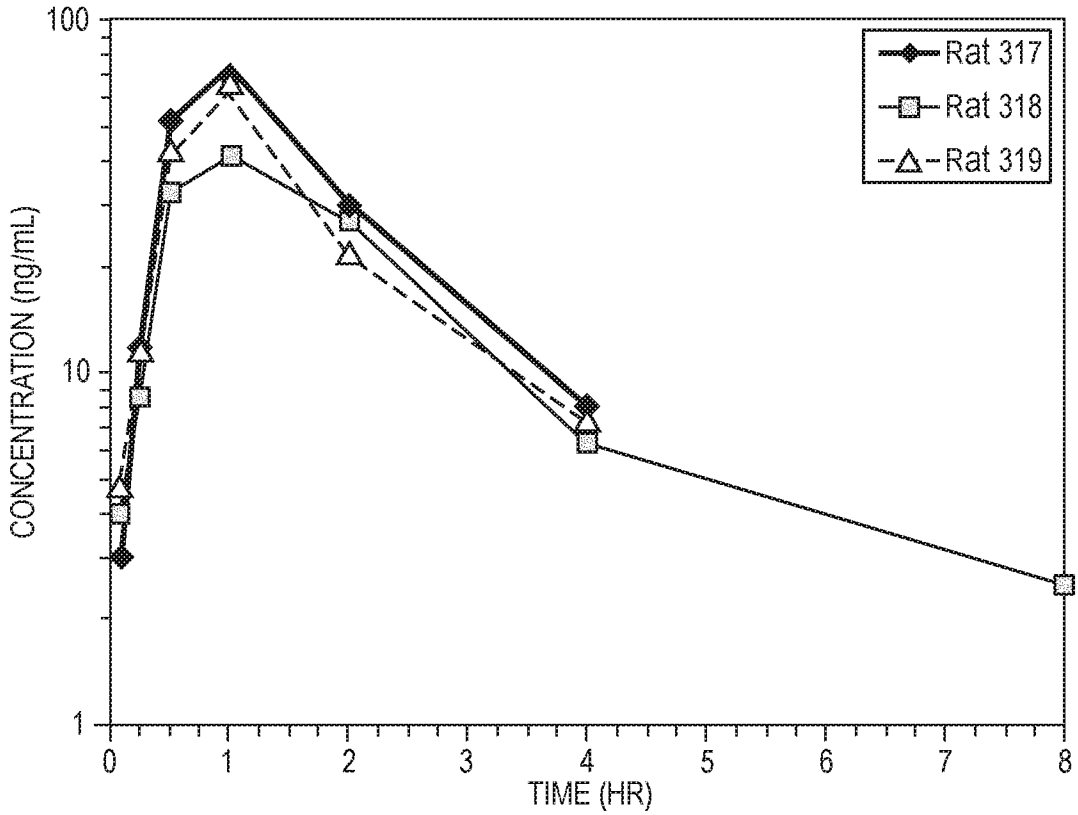

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
Rat 317
Rat 318
Rat 319

FIG. 9A

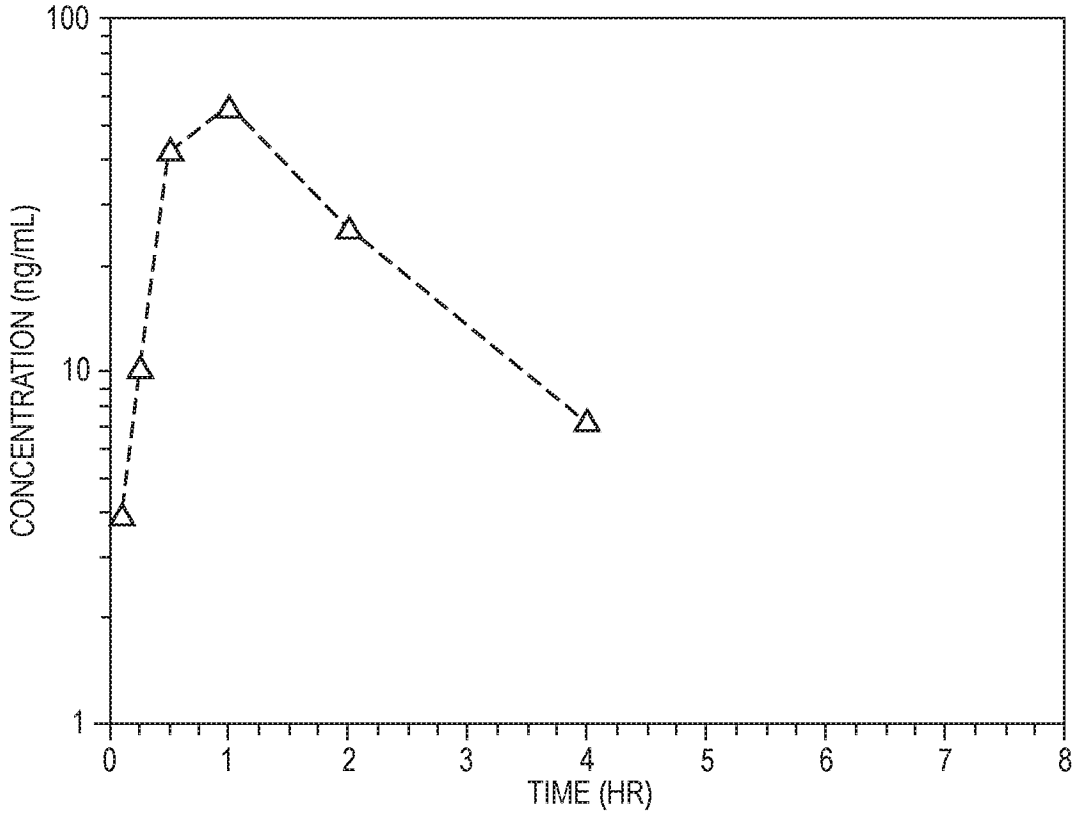

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)

FIG. 9B

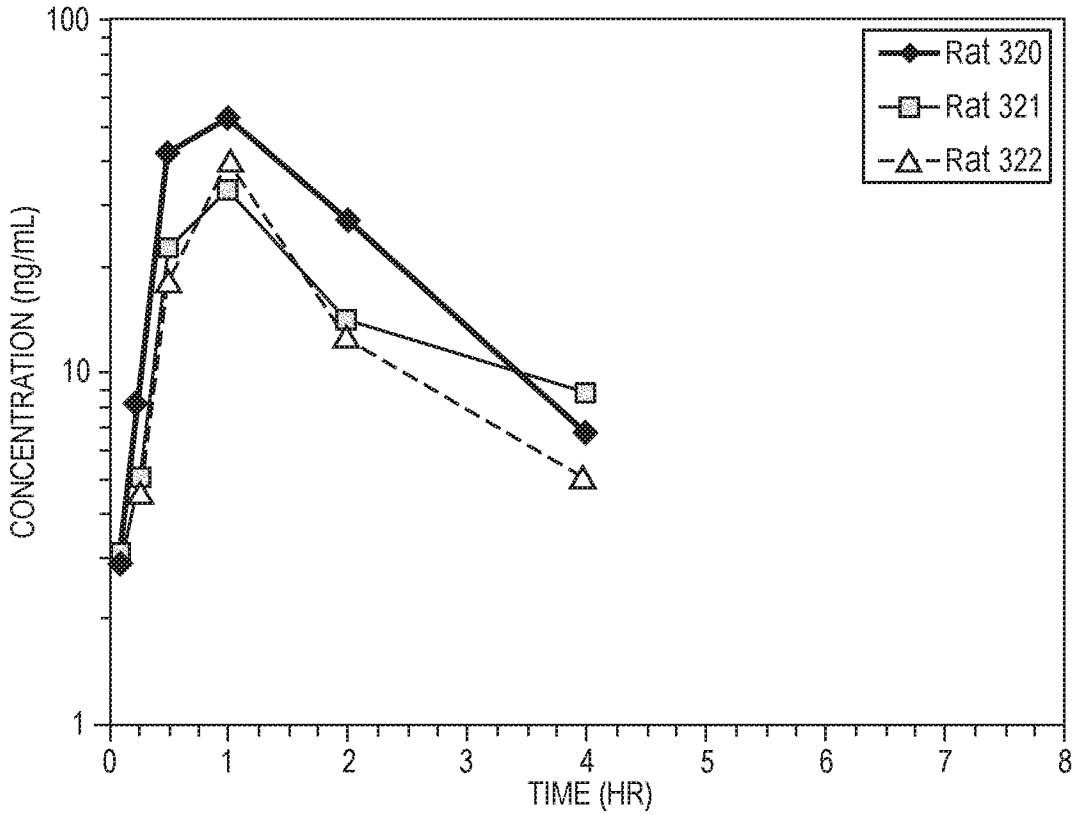

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)
Rat 320
Rat 321
Rat 322

FIG. 9C

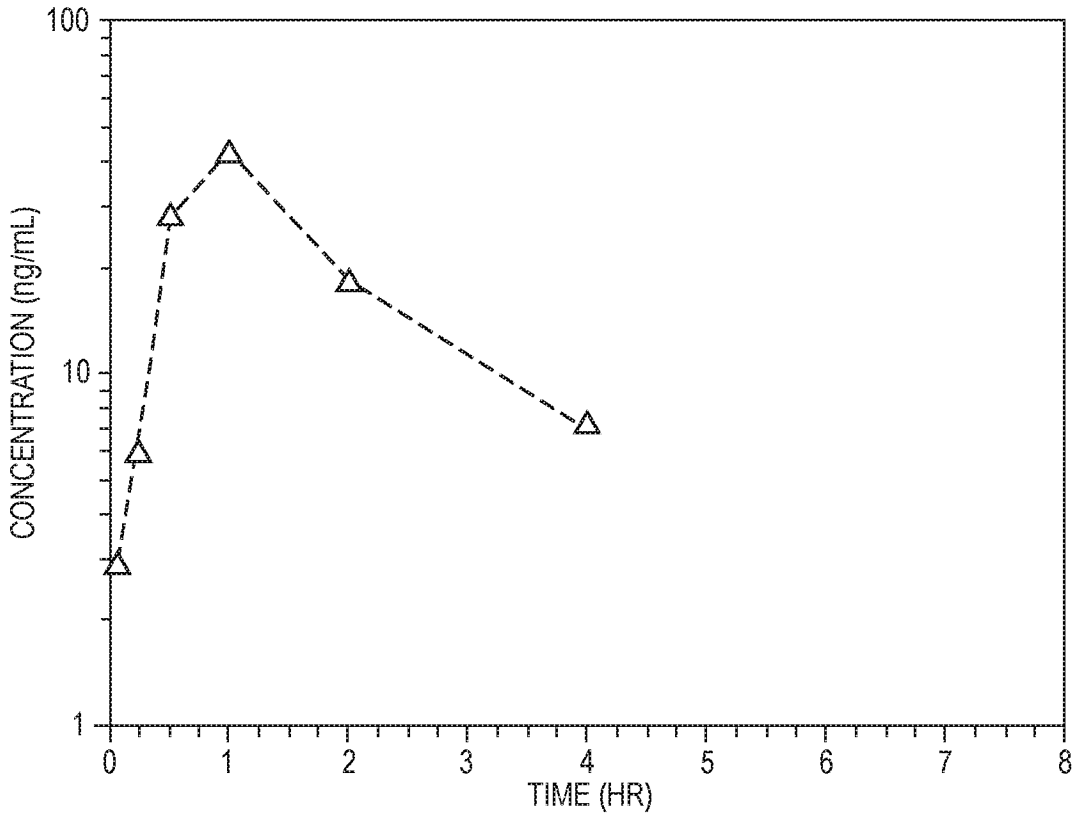

100
10
1
CONCENTRATION (ng/mL)
0
1
2
3
4
5
6
7
8
TIME (HR)

FIG. 9D

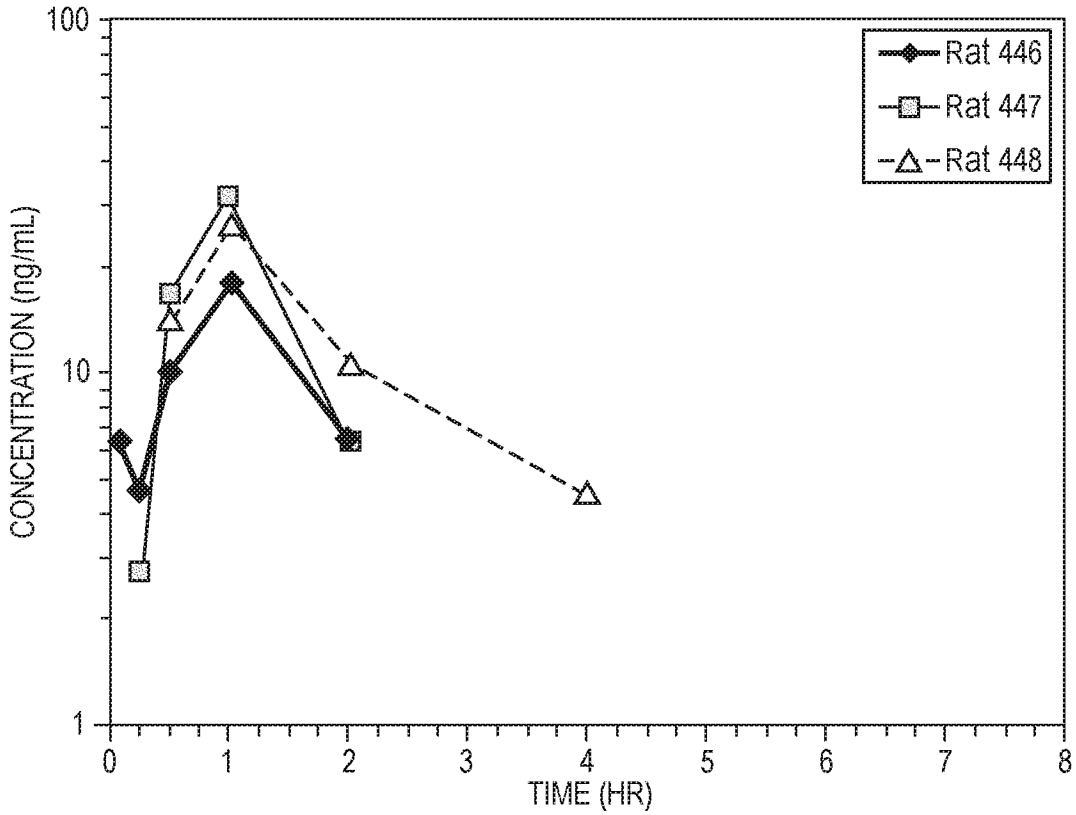


FIG. 10A

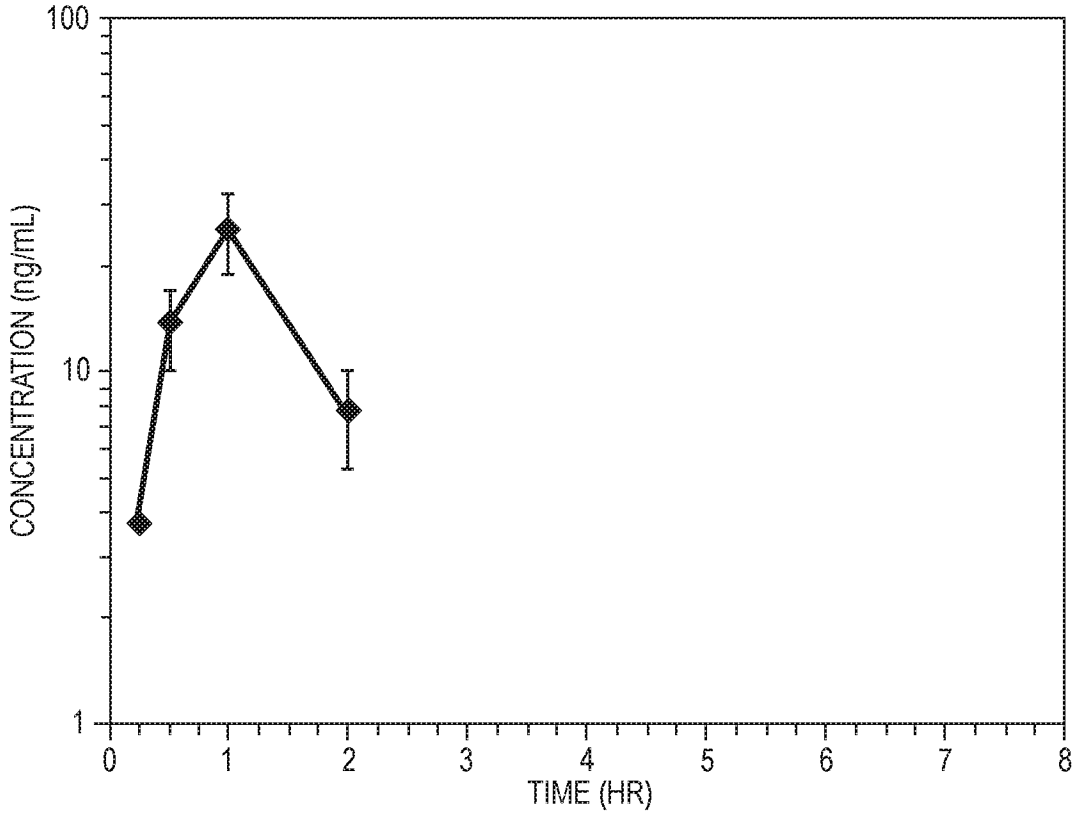


FIG. 10B

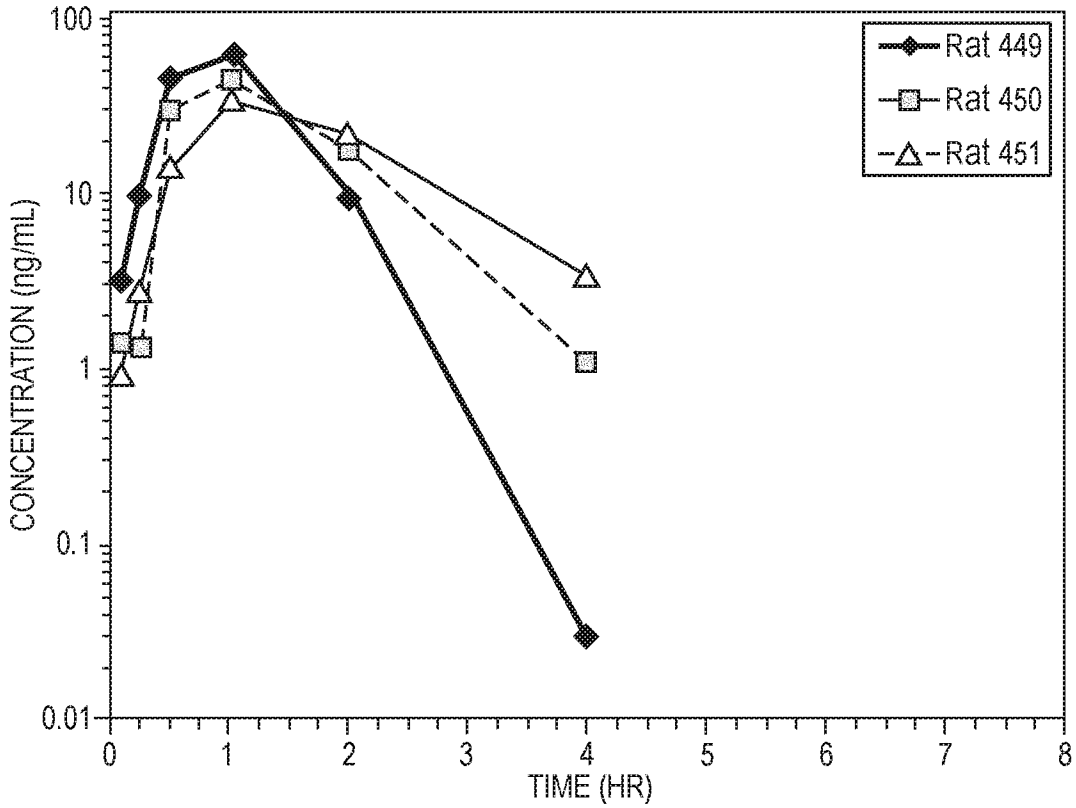


FIG. 10C

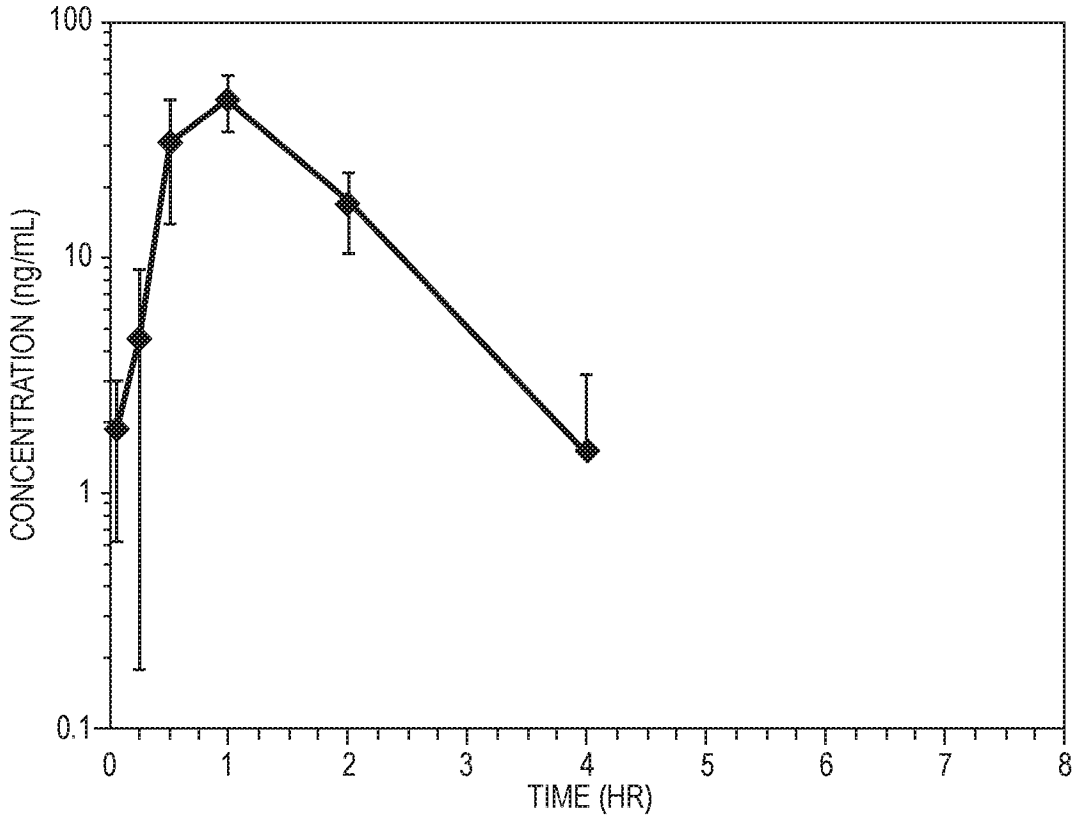


FIG. 10D

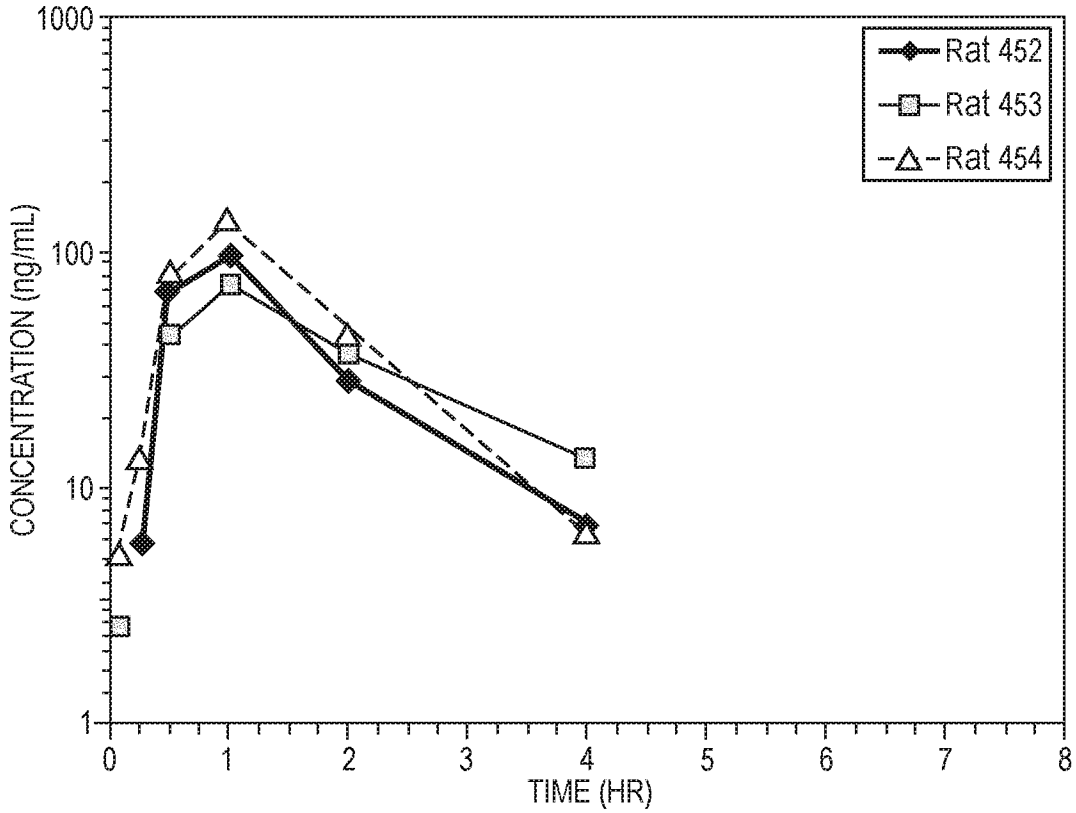


FIG. 10E

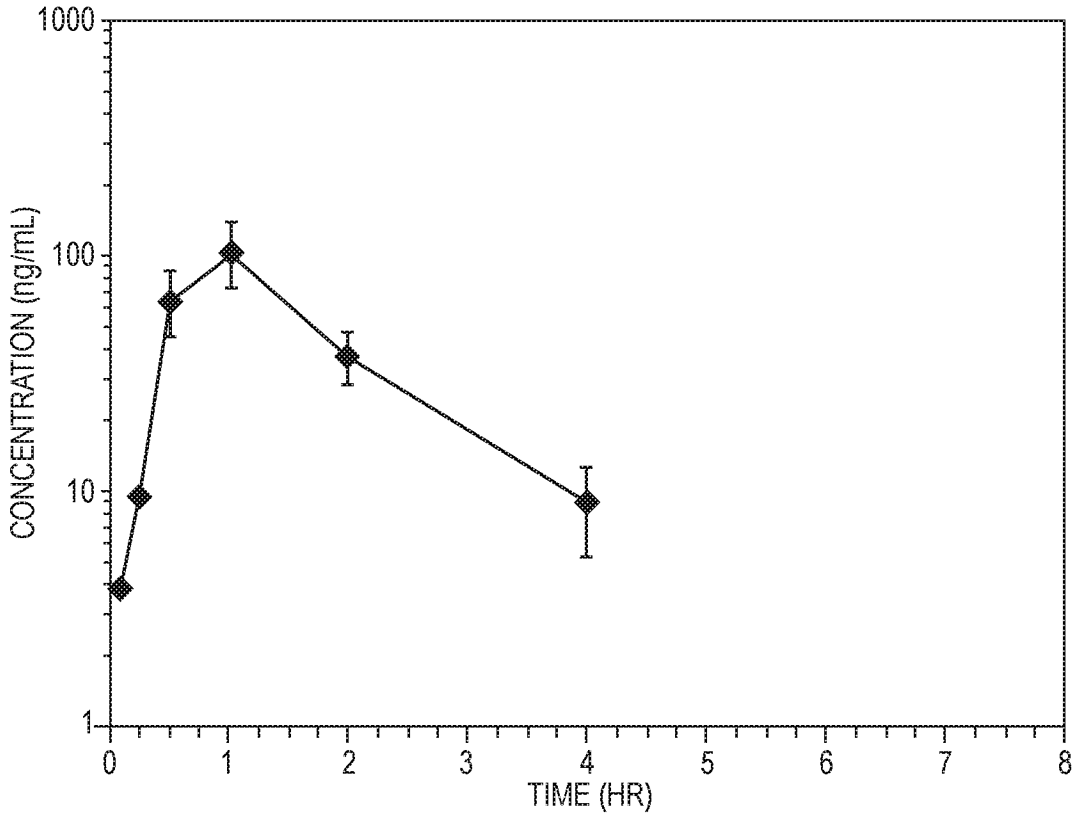


FIG. 10F

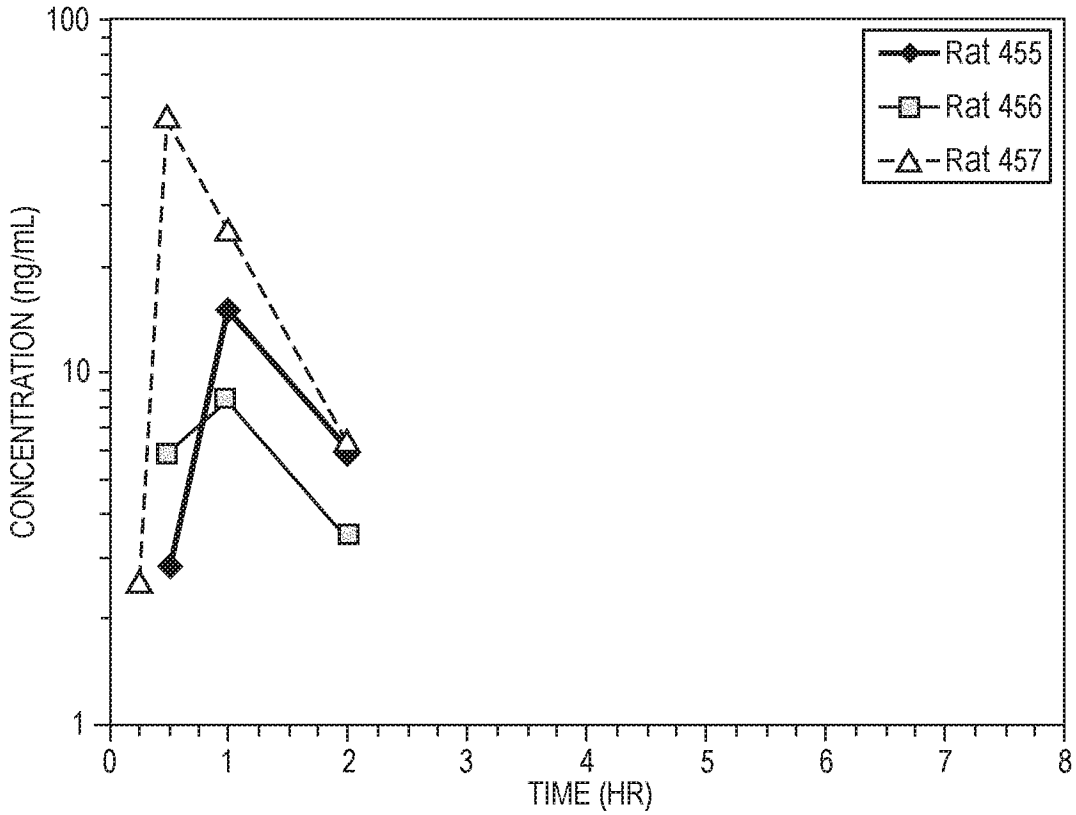


FIG. 10G

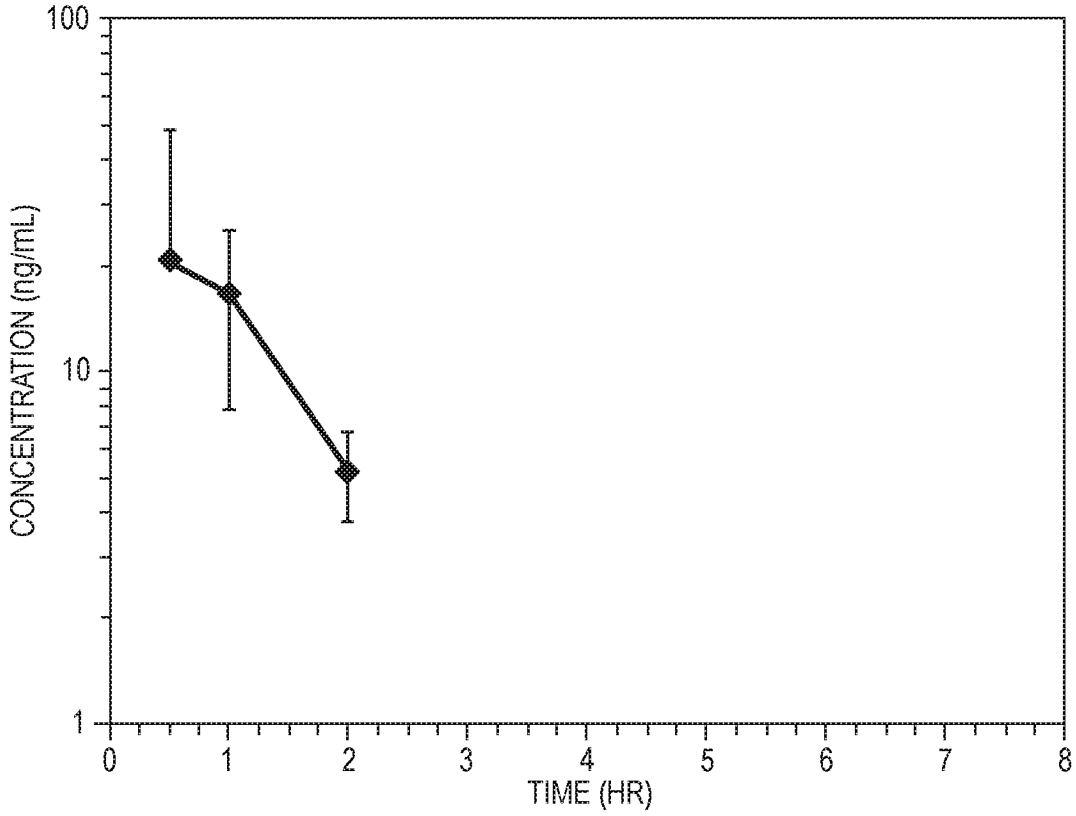


FIG. 10H

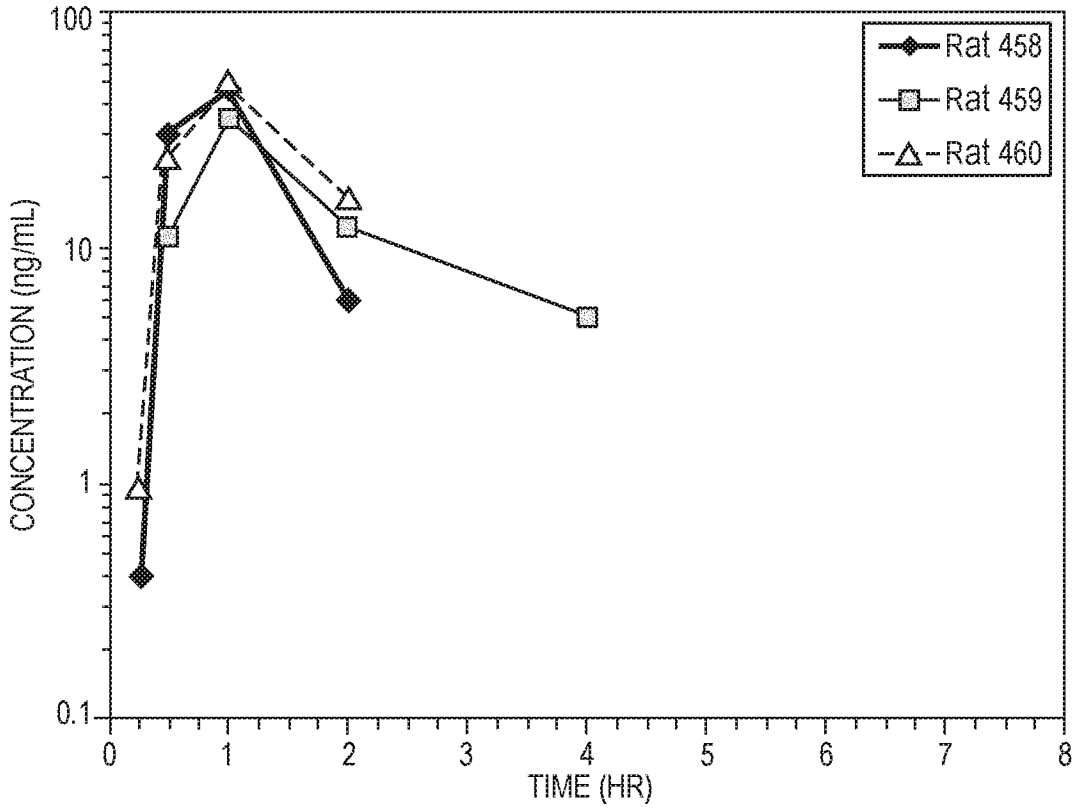


FIG. 10I

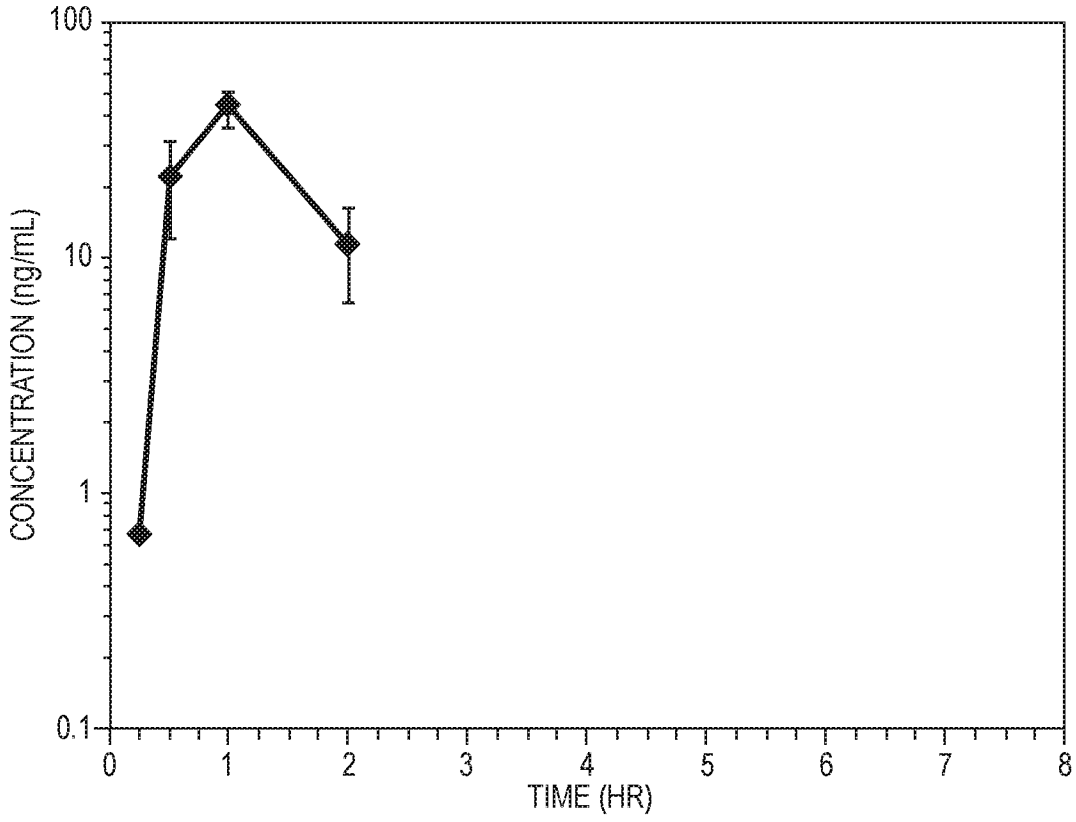


FIG. 10J

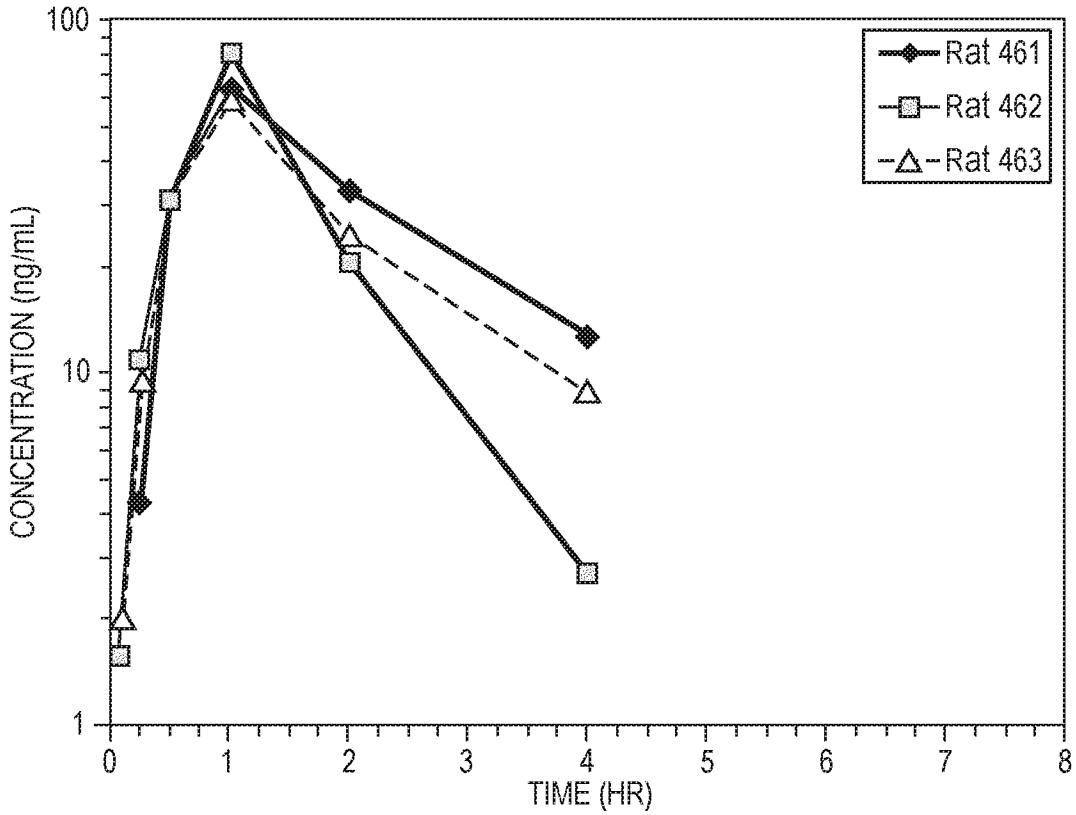


FIG. 10K

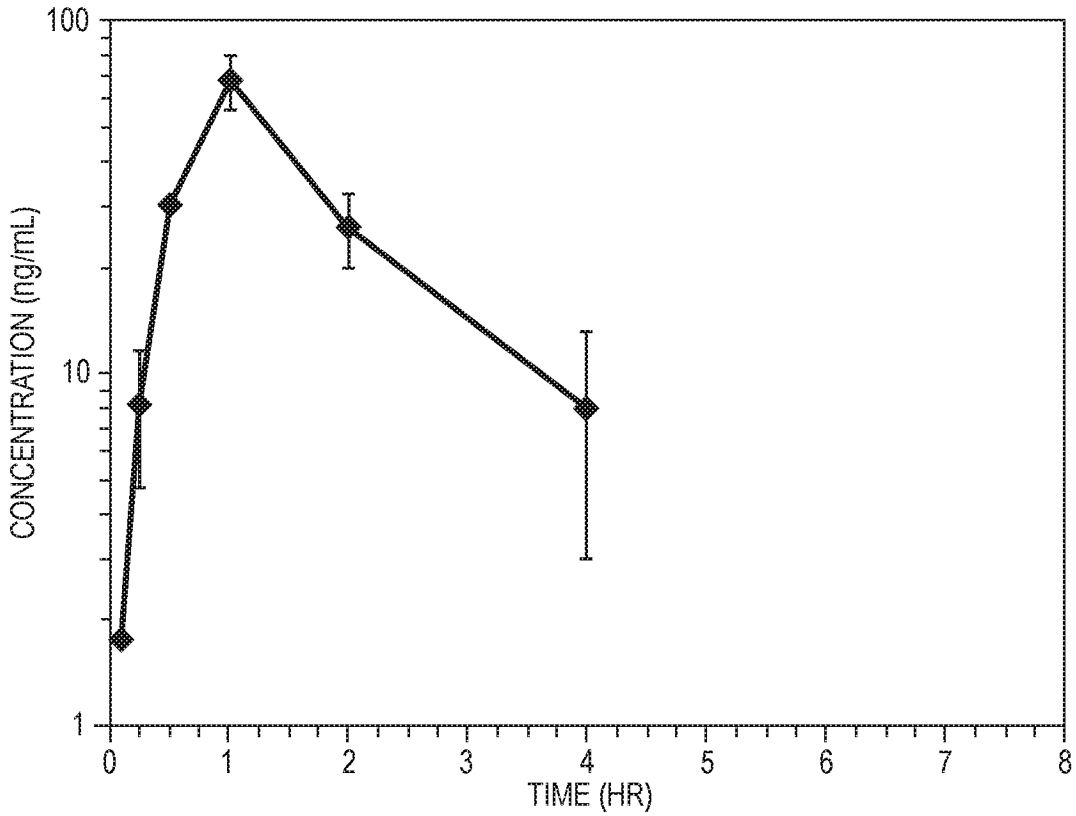


FIG. 10L

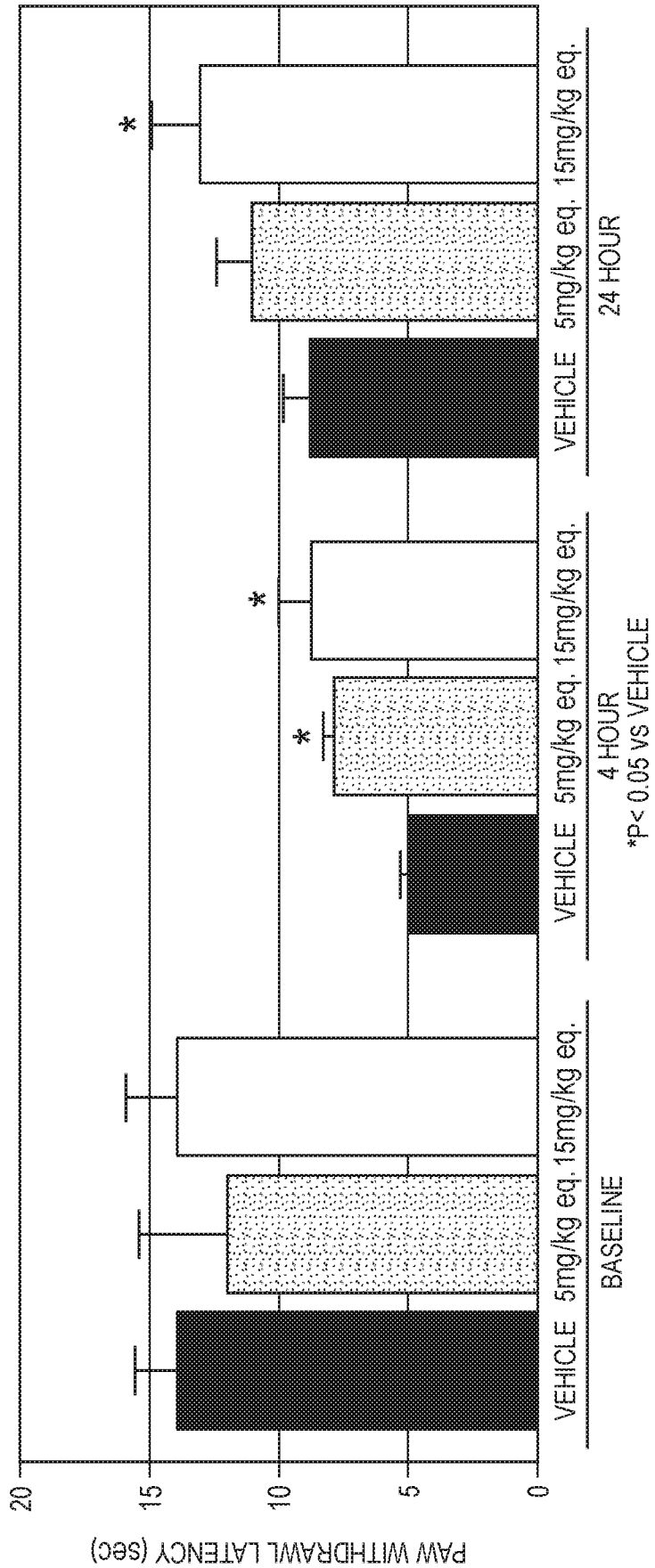

PAW WITHDRAWL LATENCY (sec)
20
15
10
5
0
*
*
*
VEHICLE
5mg/kg eq.
15mg/kg eq.
BASELINE
VEHICLE
5mg/kg eq.
15mg/kg eq.
4 HOUR
VEHICLE
5mg/kg eq.
15mg/kg eq.
24 HOUR
*P< 0.05 vs VEHICLE

FIG. 11

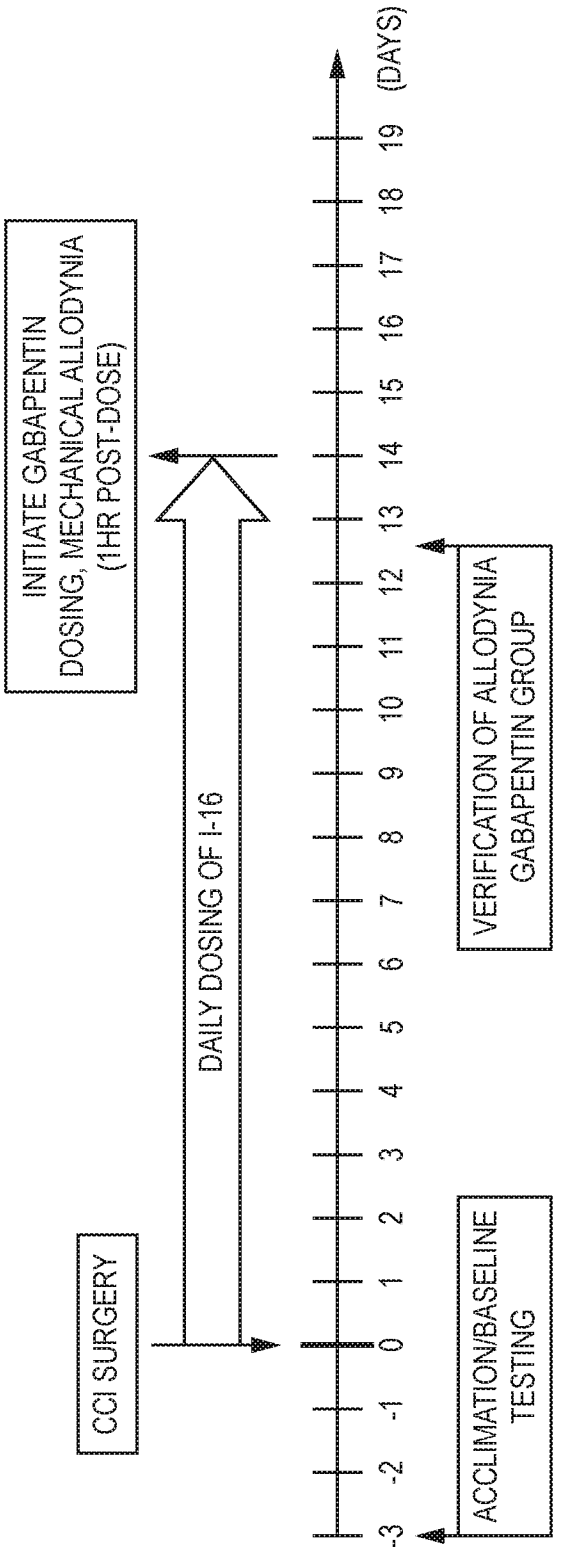


FIG. 12A

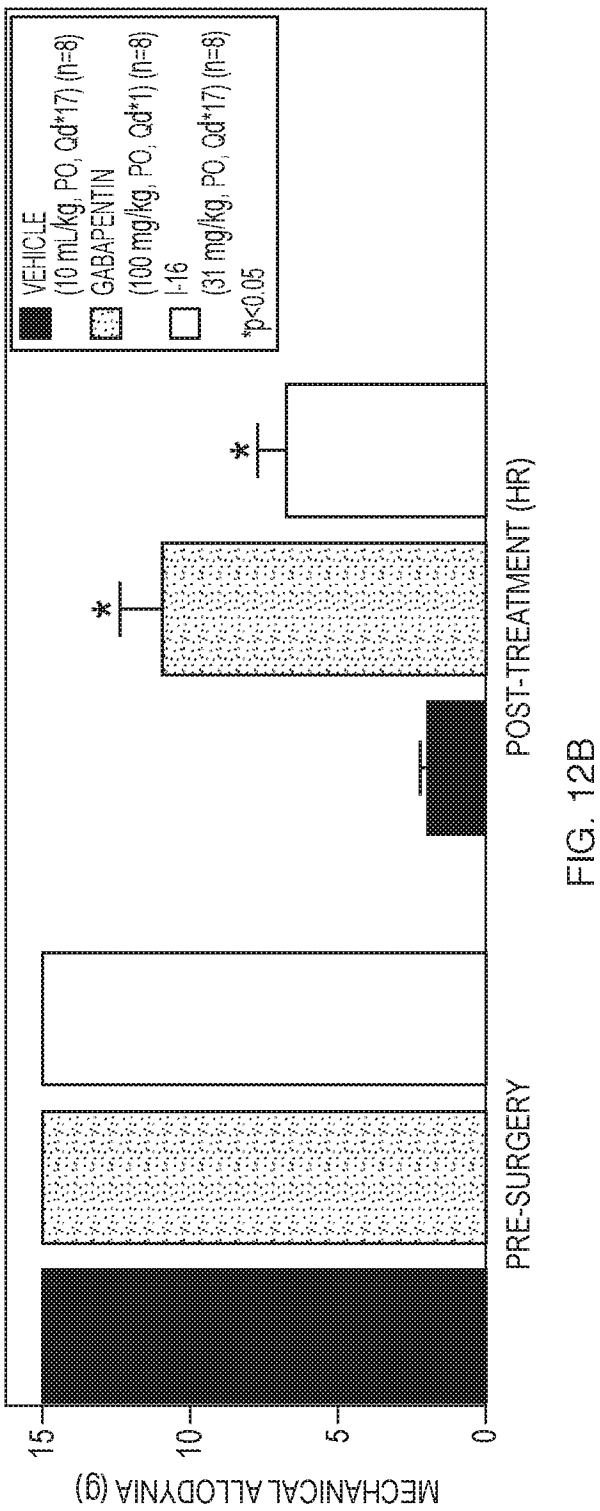


FIG. 12B