

P-2018/16104

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIRECCIÓN DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. **NC2018/0011299**

Solicitud de patente a nombre de **RECEPTOR LIFE SCIENCES INC.**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, obrando como apoderada de la sociedad RECEPTOR LIFE SCIENCES INC., domiciliada en Seattle, Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. **200536** notificado electrónicamente el 30 de abril de 2020, relacionado con los requerimientos de fondo de la solicitud, y para el cual solicité prórroga de 30 días el 31 de julio de 2020, atentamente me permito presentar la siguiente información:

1. Título

Respecto al requerimiento del señor examinador solicitando una modificación al título de la presente solicitud, se solicita amablemente que dicho título sea reemplazado por:

"COMPOSICIONES DE BASE VEGETAL QUE PUEDEN COMPRENDER UNO O MÁS AMINOÁCIDOS GRASOS N-ACILADOS, AGENTES QUE POTENCIAN LA ABSORCIÓN, Y/O OTROS DIVERSOS PORTADORES FAVORABLES, TALES COMO TENSIOACTIVOS, DETERGENTES, AZONAS, PIRROLIDONAS, GLICOLES, Y SALES BILIARES EN UNA FORMULACIÓN ORAL"

2. Modificaciones a las Reivindicaciones

Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio, el cual consta de doce (12) reivindicaciones y reemplaza completamente el capítulo presentado anteriormente. Las modificaciones realizadas se presentan a continuación:

2.1 Se han eliminado las antiguas reivindicaciones 1 a 5 con el fin de dar claridad y definir el objeto de la invención. Ahora el pliego reivindicatorio se encuentra dirigido a la formulación oral con el fin de superar las objeciones del examinador.

2.2 La antigua reivindicación 6 y sus reivindicaciones dependientes (nuevas reivindicaciones 1 a 12) se han modificado para definir los componentes de la formulación oral reclamada de conformidad con las reivindicaciones concedidas en la solicitud US 10, 588,974, la cual se adjunta a este memorial. Adicionalmente, se han incluido las proporciones de los componentes en la formulación oral reclamada de acuerdo con lo divulgado en la descripción y las reivindicaciones previamente presentadas.

2.3 Se ha modificado la antigua reivindicación 20 (nueva reivindicación 12) eliminando todas las especies excepto la especie Cannabis de acuerdo con lo divulgado en la descripción, y así superar las objeciones del examinador.

2.4 Se ha eliminado las antiguas reivindicaciones 9, 10 y 12 de acuerdo con las modificaciones realizadas a la nueva reivindicación 1.

2.5 Las reivindicaciones restantes, así como sus dependencias, fueron reenumeradas.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con

el ánimo de superar las objeciones de claridad y patentabilidad planteadas por el examinador en su concepto técnico.

3. Pago de tasas

En relación con el número de reivindicaciones, atentamente nos permitimos decir que no se adjunta tasa por concepto de reivindicaciones adicionales, toda vez que el nuevo capítulo reivindicatorio tiene menor número de reivindicaciones a las presentadas anteriormente, y en esencia es el mismo objeto, pero definido con mayor claridad.

4. Claridad y concisión de las Reivindicaciones

El solicitante desea manifestar que mediante el nuevo capítulo reivindicatorio quedan subsanadas la totalidad de las objeciones, ya que la nueva reivindicación proporciona claridad y concisión a la invención reclamada, cuenta con pleno soporte en la descripción de la solicitud en estudio, y además sigue las recomendaciones realizadas por el examinador en su concepto técnico. En particular, frente a cada objeción, me permito señalar lo siguiente:

4.1 El examinador indica que *"La reivindicación 1 no es clara, ya que en ella aparentemente se conjugan características de composición, el uso de un ingrediente activo, un resultados a alcanzar y una caracterización por funcionalidades o ventajas, y por tanto, salvo en el primer caso (composición), no son aceptables como características técnicas ya que no definen específicamente el objeto de la invención y además contradicen el preámbulo de la misma cuando supuestamente se pretende reivindicar un "método", pero éste no contiene características técnicas que así lo identifiquen. Debido a la ambigüedad de la reivindicación 1, se tomarán únicamente las características técnicas esenciales para poder analizar el estado del arte y se sugiere replantear el capítulo reivindicatorio y dirigir las reivindicaciones de conformidad con los ensayos sustentados en la descripción (...) La anterior objeción aplica también para las reivindicaciones 2 a 5. Se sugiere realizar las correcciones y modificaciones necesarias (...) Adicionalmente, la reivindicación 1 no es concisa cuando señala el término "ingrediente activo", con el cual se podrían presentar*

múltiples opciones de distintas formulaciones orales que no encuentran sustento en el capítulo descriptivo. Se sugiere realizar las modificaciones necesarias de conformidad con el sustento de la descripción". En este respecto y como se indicó previamente, se han eliminado las reivindicaciones 1 a 5 con el fin de dar claridad y definir el objeto de la invención. Ahora el pliego reivindicatorio se encuentra dirigido a la formulación oral con el fin de superar las objeciones del examinador.

4.2 El examinador indica en el Oficio: *"La reivindicación 6 no es concisa cuando emplea los siguientes términos: "materia vegetal" y "catión monovalente, un catión metálico divalente o un catión orgánico", con los cuales se podrían presentar múltiples opciones de distintas composiciones que no encuentran sustento en el capítulo descriptivo. Se sugiere realizar las modificaciones necesarias de conformidad con el sustento de la descripción. Las reivindicaciones 6 a 8, 10 a 19 no son claras, porque aparentemente se pretende reivindicar una composición farmacéutica, pero luego de revisar las características técnicas esenciales, se observa que no cumple con la caracterización para este tipo de productos. Cualquier composición farmacéutica se debe caracterizar por sus componentes particulares y claramente establecidos y las proporciones de cada uno de los mismos en una forma farmacéutica conocida. Se sugiere realizar las aclaraciones necesarias. (...)"*. En este respecto y como se indicó previamente, la antigua reivindicación 6 y sus reivindicaciones dependientes (nuevas reivindicaciones 1 a 12) se han modificado para definir los componentes de la formulación oral reclamada de conformidad con las reivindicaciones concedidas en la solicitud US 10, 588,974, la cual se adjunta a este memorial. Adicionalmente, se han incluido las proporciones de los componentes en la formulación oral reclamada de acuerdo con lo divulgado en la descripción y las reivindicaciones previamente presentadas. Por tanto, se superan las objeciones realizadas.

4.3 El examinador indica "La reivindicación 20 no es clara cuando menciona: "donde la materia vegetal con una solubilidad en agua menor que 0,1 mg/ml se deriva de *Calophyllum brasiliense*, *Calophyllum caledonicum*, *Calophyllum inophyllum*, *Calophyllum soulattri*, *Uncaria tomentosa*, *Thymus vulgaris*, *Matricaria recutita*, *Salix alba*, *Calendula officinalis*, *Usnea barbata*, *Ligusticum porterii-osha*, *Gaultheria procumbens*, *Camellia sinensis*, *Vaccinium myrtillus*,

Melissa officinalis, Allium sativum, Camellia sinensis, Krameria triandra, Punica granatum, Viburnum plicatum, Nicotiana tabacum, Duboisia hopwoodii, Asclepias syriaca, Curcuma longa, Cannabis sativa, Cannabis indica, Cannabis ruderalis y Acer spp, o un extracto de estas”, ya que al revisar los ejemplos de la solicitud, sólo se desarrollaron composiciones con Cannabis, por lo que no tienen sustento para que involucren las demás especies. Adicionalmente, en el caso de las especies Calophyllum brasiliense, Uncaria tomentosa, Usnea barbata, Krameria triandra (Sin. Krameria lappacea) y Nicotiana tabacum, éstas son nativas de Colombia y si eventualmente se hubiese desarrollado una invención a partir éstas, deberían aportar el contrato de acceso a recursos genéticos y productos derivados, tal como lo establece la normatividad vigente”. En este respecto y como se indicó previamente, se ha modificado la antigua reivindicación 20 (nueva reivindicación 12) eliminando todas las especies excepto la especie Cannabis de acuerdo con lo divulgado en la descripción, y así superar las objeciones del examinador. Por tanto, se solicita amablemente retirar la objeción.

En consecuencia, nos permitimos indicar que el presente capítulo reivindicatorio, que incluye doce (12) reivindicaciones, se ajusta a las disposiciones del artículo 30 de la Decisión 486/00.

5. Descripción

En relación con lo indicado por el examinador *“Tal como lo establece la decisión 486 de 2000, artículo 28, la descripción de la invención debe ser clara y completa, de manera que una persona versada en la materia pueda ejecutarla. Lo anterior no se cumple en el capítulo descriptivo, cuando se menciona que se pueden desarrollar composiciones de base vegetal que comprenden “Calophyllum brasiliense, Calophyllum caledonicum, Calophyllum inophyllum, Calophyllum soulattri, Uncaria tomentosa, Thymus vulgaris, Matricaria recutita, Salix alba, Calendula officinalis, Usnea barbata, Ligusticum porterii-oshai, Gaultheria procumbens, Camellia sinensis, Vaccinium myrtillus, Melissa officinalis, Allium sativum, Camellia sinensis, Krameria triandra, Punica granatum, Viburnum plicatum, Nicotiana tabacum, Duboisia hopwoodii, Asclepias syriaca, Curcuma longa, Acer spp, o un extracto de estas”, los cuales*

en ninguna parte del capítulo descriptivo se encuentran sustentados ni ejemplificados siquiera. Tal como se mencionó anteriormente, al revisar los ejemplos de la solicitud, sólo se desarrollaron composiciones con Cannabis, por lo que no tienen sustento para que involucren las demás especies y en el caso de las especies Calophyllum brasiliense, Uncaria tomentosa, Usnea barbata, Krameria triandra (Sin. Krameria lappacea) y Nicotiana tabacum, son nativas de Colombia y si se hubiese desarrollado una invención a partir éstas, deberían aportar el contrato de acceso a recursos genéticos y productos derivados, tal como lo establece la normatividad vigente. Se sugiere eliminar cualquier referencia, tanto en la descripción como en las reivindicaciones, correspondiente a materiales vegetales distintos a Cannabis”.

En este respecto, el solicitante desea manifestar respetuosamente su desacuerdo, ya que aunque las especies mencionadas anteriormente están incluidas en la descripción, la presente invención define claramente que se ha desarrollado con derivados de Cannabis como se menciona en el párrafo [0017] “... En modalidades particulares, las composiciones de base vegetal incluyen la planta de cannabis, o un extracto de esta”, párrafo [0037] “En modalidades particulares, las composiciones de base vegetal incluyen materia vegetal derivada de la planta de cannabis (...)”, párrafo [0136] “... Una composición de base vegetal de cualquiera de las modalidades 1-3, donde la materia vegetal se deriva del cannabis (...)” y los Ejemplos. Adicionalmente y como se indicó previamente, la antigua reivindicación 20 (nueva reivindicación 12) se ha modificado para que solo recite las especies de cannabis. Por lo tanto, se solicita amablemente sea retirada dicha objeción.

6. Argumentos de la Oposición

El examinador ha señalado los argumentos del Oponente en contra de la presente invención. No obstante, el solicitante desea llamar la atención del examinador sobre los argumentos presentados en respuesta a dicha Oposición, ya que como se evidencia en el Oficio que se contesta, los argumentos presentados en respuesta a esta Oposición no fueron considerados por el examinador, por lo cual, serán incluidos como parte de los argumentos contra las objeciones del examinador en esta respuesta. Adicionalmente, se adjunta a

este memorial la respuesta presentada en contra de la Oposición para su consideración.

7. Nivel Inventivo

El examinador establece que las reivindicaciones 1 a 20 carecen de nivel inventivo a la luz de las enseñanzas descritas en los documentos US 2015/057341 (D1) y US2008/194676 (D2).

A este respecto, me permito decir que el artículo 18 de la Decisión 486 establece que *"Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica"*.

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

De acuerdo con lo anterior, la solicitante desea manifestar respetuosamente su desacuerdo, ya que teniendo en cuenta las modificaciones presentadas en el nuevo capítulo reivindicatorio, se superan las objeciones realizadas, por cuanto **dichas características no se encuentran divulgadas individualmente en los documentos citados por el examinador.**

Después de revisar los documentos citados, ninguno de ellos revela las características del nuevo capítulo reivindicatorio como se demuestra a continuación.

El documento D1 no anticipa el presente pliego reivindicatorio porque no enseña un aminoácido graso *N*-acilado o una sal del mismo o *N*-[8-(2-hidroxibenzoil)amino]caprilato (SNAC) como agente de mejora de la absorción.

El documento D2 solo describe el uso de SNAC como agente de entrega para cromolina. D2 describe a la cromolina como "altamente insoluble en lípidos y principalmente ionizado" (Párrafo [0009]). Debido a que la cromolina es un lípido insoluble e ionizada, es soluble en agua y polar, y por lo tanto, no es "cannabinoide con una solubilidad en agua menor que 0.1 mg/ml".

Adicionalmente, el solicitante no está de acuerdo con la apreciación del examinador por las siguientes razones:

La Dra. Andrea Leone-Bay, una de las inventoras de la presente solicitud, determinó en la década de 1990 que el N-[8-(2-hidroxibenzoil)amino] caprilato (SNAC) podría utilizarse para mejorar la permeabilidad intestinal de materiales como la heparina y la cromolina. Ella es una de las principales expertas del mundo en relación con el SNAC, sus propiedades y sus efectos fisiológicos (Adjunto se presenta el documento de Declaración Firmada por la Dra. Andrea Leone-Bay para su referencia).

Los hallazgos de la Dra. Leone-Bay descritos en la presente solicitud, junto con el co-inventor Gregory Wesner, fueron altamente imprevistos, es decir, que los aminoácidos grasos N-acilados, como el SNAC, podrían proporcionar una mayor biodisponibilidad de cannabinoide con una solubilidad en agua menor que 0.1mg/ml.

La Dra. Leone-Bay fue una de las inventoras de la clase de aminoácidos grasos N-acilados descrita en la patente US 5,650,386 y, específicamente ella propuso la estructura química original del N-[8-(2-hidroxibenzoil) amino] caprilato (SNAC) y un método para sintetizarlo.

Los aminoácidos grasos N-acilados fueron diseñados específicamente para proporcionar un mecanismo que permita la entrega oral de macromoléculas que se administraban previamente mediante inyección. El objetivo era mejorar tanto las opciones terapéuticas como el cumplimiento del paciente. Las macromoléculas inicialmente seleccionadas para una entrega mejorada incluían proteínas (por ejemplo, hormona de crecimiento humana recombinante),

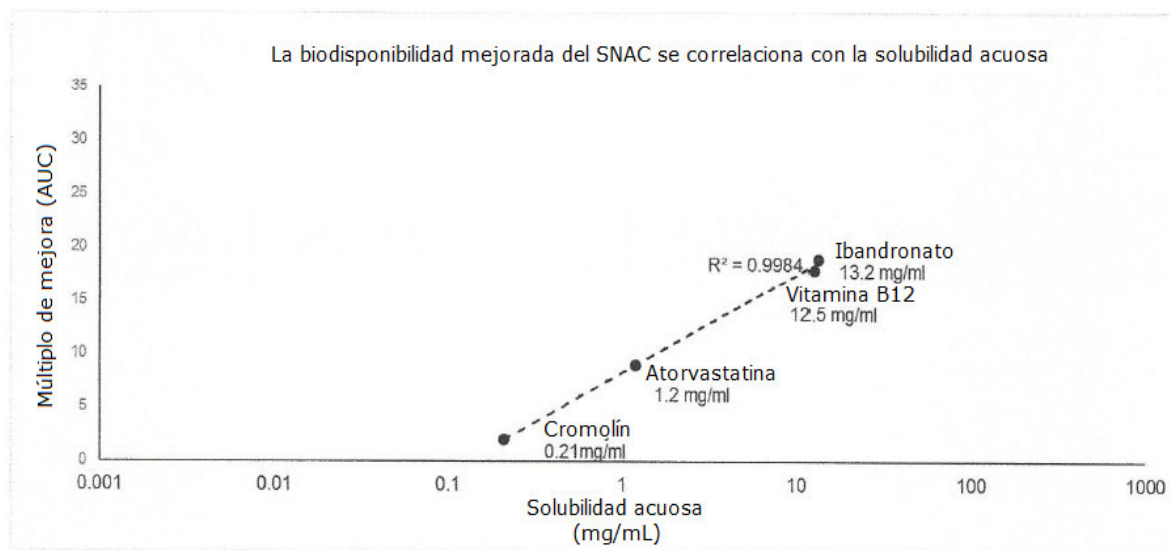
péptidos (por ejemplo, hormona paratiroidea) y oligosacáridos (por ejemplo, heparina).

Los aminoácidos grasos *N*-acilados fueron diseñados para ser solubles en agua porque los medicamentos descritos anteriormente son solubles en agua. La idea detrás del diseño era que el tracto gastrointestinal (GI) es un ambiente acuoso, por lo que un potenciador de la absorción soluble en agua (por ejemplo, SNAC) junto con un fármaco soluble en agua optimizaría el transporte a través de la membrana GI y, a su vez, optimizaría la absorción.

El SNAC funcionó bien con proteínas solubles en agua, péptidos y fármacos oligosacáridos. Véase, por ejemplo, Baughman *et al.*, "Oral delivery of anticoagulant doses of heparin: a randomized, double-blind, controlled study in humans" *Circulation* 1998; 98:1610-1615 (Apéndice B); Kidron, *et al.*, "A novel per-oral insulin formulation: proof-of-concept study in non-diabetic subjects" *Diabetic Medicine*, 2003; 21: 354-357 (Apéndice C); y Kremer *et al.*, "Pharmacokinetics, pharmacodynamics and tolerability of oral cromolyn sodium/SNAC capsules in healthy and allergic male subjects" 2003; *BrJ Clin Pharmacol* 56, 467-468 (Apéndice D). Continuando con este éxito, el SNAC también impulsa el único tratamiento con péptidos orales para la diabetes recientemente aprobado por la FDA (septiembre de 2019), Rybelsus.

El uso del SNAC también se ha extendido a otras clases de fármacos, incluidas moléculas pequeñas, y existe una gran cantidad de patentes y literatura científica sobre el SNAC desde la invención del compuesto en 1994. A medida que se evaluaron más compuestos, se observó una tendencia en donde la capacidad del SNAC para facilitar la absorción del fármaco se correlacionaba con la solubilidad en agua del compuesto. Esto no fue sorprendente dado el trabajo de diseño del compuesto emprendido inicialmente.

Al presentar la presente Solicitud, se utilizaron puntos de datos publicados de la extensa literatura sobre aminoácidos grasos *N*-acilados y SNAC para resaltar y demostrar la tendencia de solubilidad en agua bien entendida por aquellos familiarizados con esta clase de compuestos. El siguiente gráfico muestra el múltiplo de mejora de la biodisponibilidad del SNAC en función de la solubilidad en agua para Cromolín, véase col. 27, tabla 5 de U.S. 8513300B2 (Apéndice E); Atorvastatina, véase pág. 13, line 10, tabla 2 de WO2009100245A1 (Apéndice F); vitamina B12, véase col. 5, tabla 1 de U.S. 8022048B2 (Apéndice G); e Ibandronato, página 239, tabla 5 de Bittner *et al.*, "Phase I clinical study to select a novel oral formulation for ibandronate containing the excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC)" 2012; *Pharmazie* 67, 233-241 (Apéndice H). Estos puntos de datos proporcionan la siguiente línea de tendencia para la eficiencia de entrega del SNAC basada en la solubilidad en agua de los fármacos entregados:



Con base en la línea de tendencia anterior, los inventores representan cómo un experto en la materia predeciría la eficacia de los aminoácidos grasos *N*-acilados para aumentar la entrega de varios ingredientes activos con solubilidades en agua variables y en la presente solicitud, los inventores explican por qué los

efectos del SNAC con materia vegetal con una solubilidad en agua menor que 0.1 mg/ml fueron tan inesperados. En particular, la Figura 1B de la presente solicitud (presentada a continuación) muestra lo que un experto en la técnica **hubiera** esperado basándose en moléculas que se habían probado con SNAC antes de la presente invención. Antes del trabajo que conducía a la presente solicitud, una mejora de la absorción basada en SNAC correlacionada con la solubilidad acuosa de una molécula. La Figura 1B traza además la solubilidad acuosa del THC (0,0028 mg/ml) a la línea de tendencia logarítmica y el efecto predicho de SNAC basado en el mismo. Según este conocimiento, un experto en la materia habría predicho que el SNAC **no** proporcionaría un incremento en la biodisponibilidad de cannabinoides con una solubilidad en agua menor que 0,1 mg/ml.

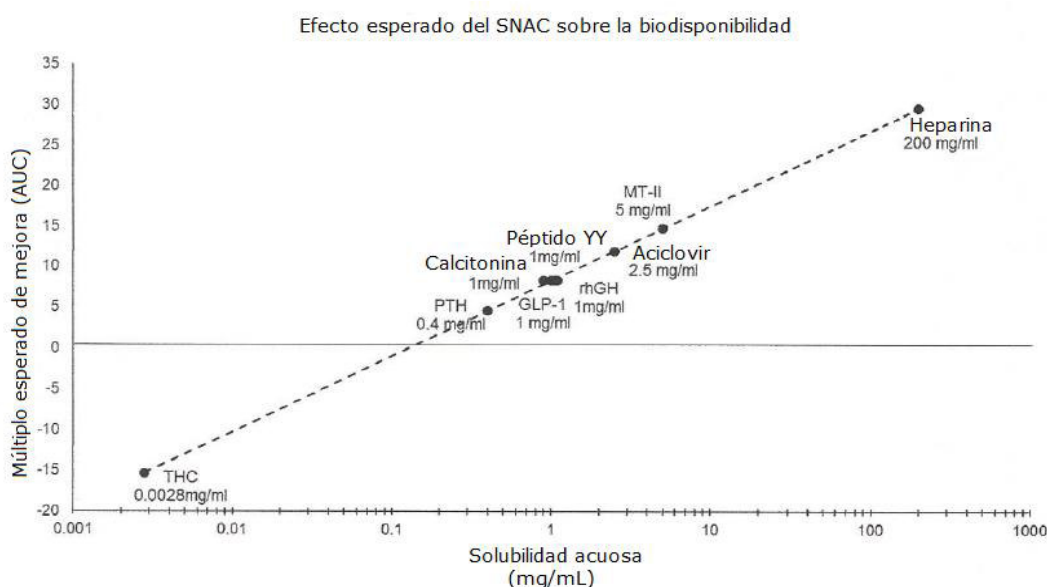


Figura 1B de la Presente Solicitud

En consecuencia, en la fecha de presentación efectiva de la presente solicitud, no había motivación para crear una formulación oral de un aminoácido graso *N*-acilado y un cannabinoide con solubilidad en agua menor que 0.1 mg/ml, ni había una expectativa razonable de éxito de que la combinación de un

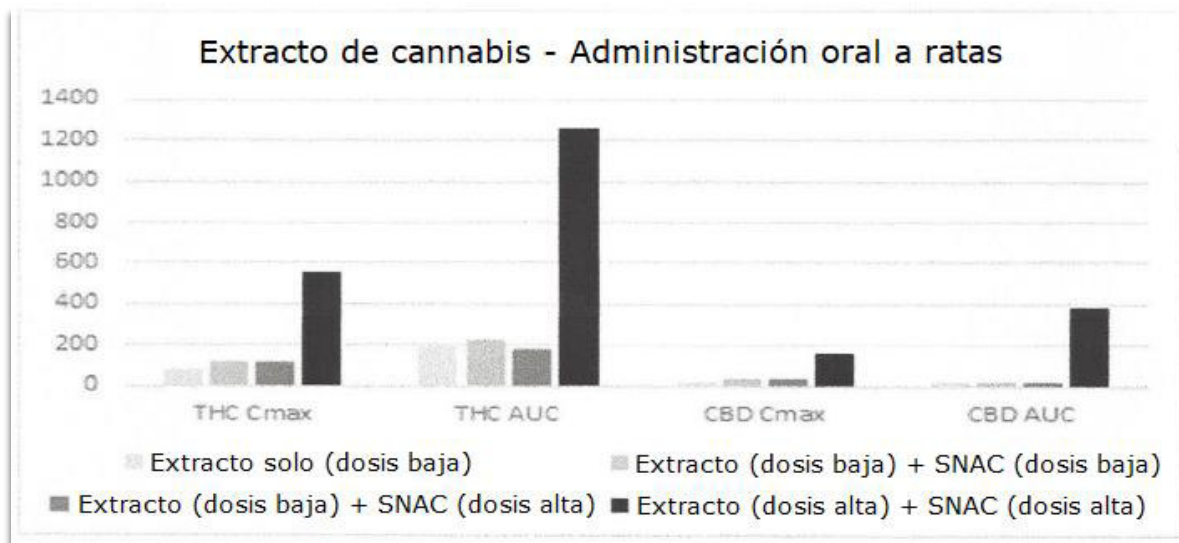
aminoácido graso *N*-acilado, como SNAC, con un cannabinoide con una solubilidad en agua menor que 0,1 mg/ml proporcionaría una formulación con una mayor biodisponibilidad.

Como lo demuestra esta solicitud, la Dra. Leone-Bay y su compañero inventor, Gregory Wesner, lograron un gran avance en la administración de moléculas insolubles en agua cuando combinaron un aminoácido graso *N*-acilado con un cannabinoide con una solubilidad en agua menor que 0,1 mg/ml, como el THC. Contrario a los resultados esperados de que SNAC no mejoraría la biodisponibilidad del THC, los inventores pudieron mejorar significativamente la biodisponibilidad del THC combinando THC con SNAC (ver, por ejemplo. Figuras 5A, 5B, y 6A-6F).

En 2016, Receptor Life Sciences comenzó a abordar los desafíos de entrega asociados con las terapias con cannabinoides utilizando un enfoque científico y clínico sólido con el objetivo de cambiar la medicina cannabinoide para que sea segura y efectiva para los pacientes. Con base en el amplio conocimiento de la clase de aminoácidos grasos *N*-acilados descrita en la Patente US 5,650,386 de la Dra. Leone-Bay, Receptor Life Sciences eligió administrar el SNAC con THC para observar sus efectos en un estudio en ratas. Los fundamentos de los inventores para llevar a cabo este estudio fue obtener información sobre los potenciadores de la absorción, en general, e informar la elección y el análisis de posibles compuestos potenciadores de la absorción en el futuro. Los inventores no esperaban ver un aumento en la biodisponibilidad con el SNAC.

En el primer estudio descrito en Receptor Life Sciences, las ratas Sprague Dawley en ayunas (6/grupo de dosis) recibieron dosis orales únicas (por sonda oral) de SNAC solo, cannabis solo o varias combinaciones de cannabis/SNAC. Se recogió sangre en serie en los siguientes momentos: predosis, 5, 15, 30, 45 minutos y 1, 2 y 4 horas. Sorprendentemente, los inventores observaron que el SNAC mejoró en gran medida la biodisponibilidad de THC y CBD. A dosis de SNAC de 250 mg/kg o 500 mg/kg, la $C_{m\acute{a}x}$ de THC y CBD aumentó 1.4 veces y 2.8 veces, respectivamente, sobre el grupo de cannabis solo. Las $AUC_{0-Tfinal}$ fueron comparables. El aumento de las dosis de cannabis y SNAC en 2 veces dio como resultado aumentos de la $C_{m\acute{a}x}$ de THC y CBD de 6.3 veces y 14.2 veces,

respectivamente, sobre el grupo de cannabis solo. El $AUC_{0-T_{final}}$ para THC y CBD aumentó en 6.9 veces y 22.1 veces, respectivamente, sobre el grupo de cannabis solo. En general, estos datos muestran que el SNAC mejora la absorción de cannabis cuando se administra a ratas por sonda oral, un resultado que no se habría previsto cuando se realizó el trabajo.

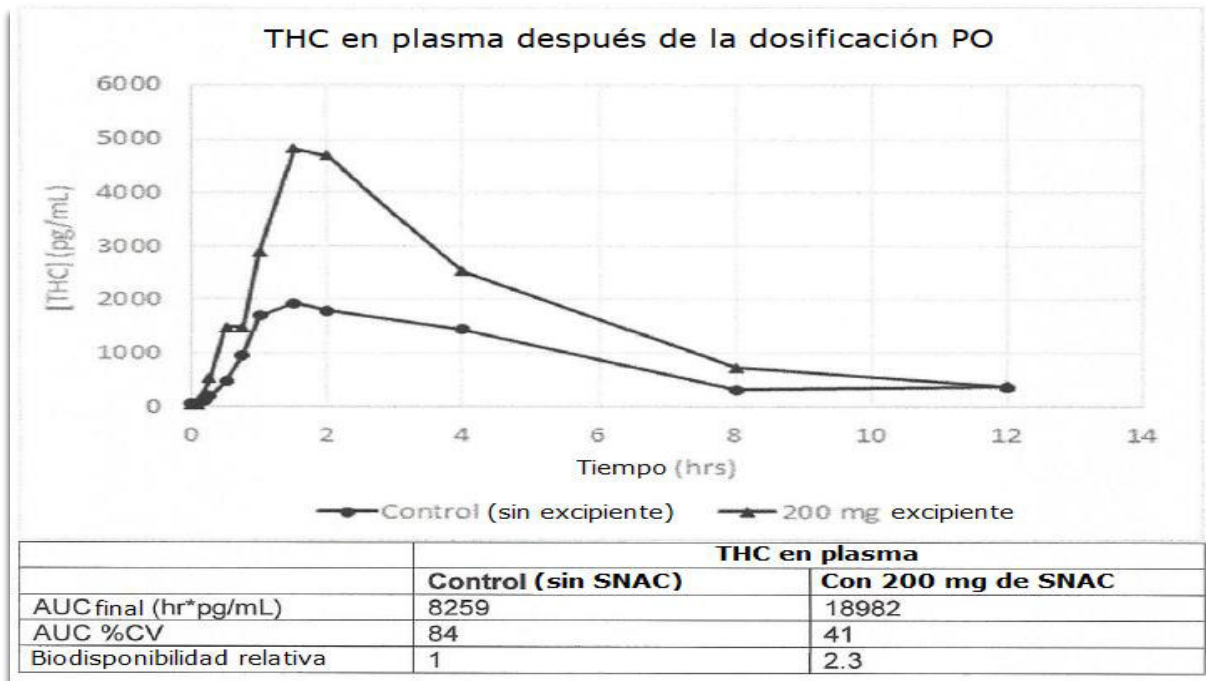


Dosis THC/CBD (mg/kg)	Dosis SNAC (mg/kg)	THC				CBD			
		T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0-T_{final}}$ (hr*ng/mL)	En comparación con cannabis solo C_{max} (AUC)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0-T_{final}}$ (hr*ng/mL)	En comparación con cannabis solo C_{max} (AUC)
12.5/12.5	0	1.00	79.13	200.3	NA	1.00	11.23	17.29	NA
12.5/12.5	250	1.00	112.57	218.45	1.4 (1.1)	0.50	32.9	16.11	2.9 (0.93)
12.5/12.5	500	1.00	111.5	170.64	1.4 (0.85)	1.00	31.7	13.17	2.8 (0.79)
25/25	500	2.00	546.17	1256.49	6.9 (6.3)	0.25	159.3	382.14	14.2 (22.1)

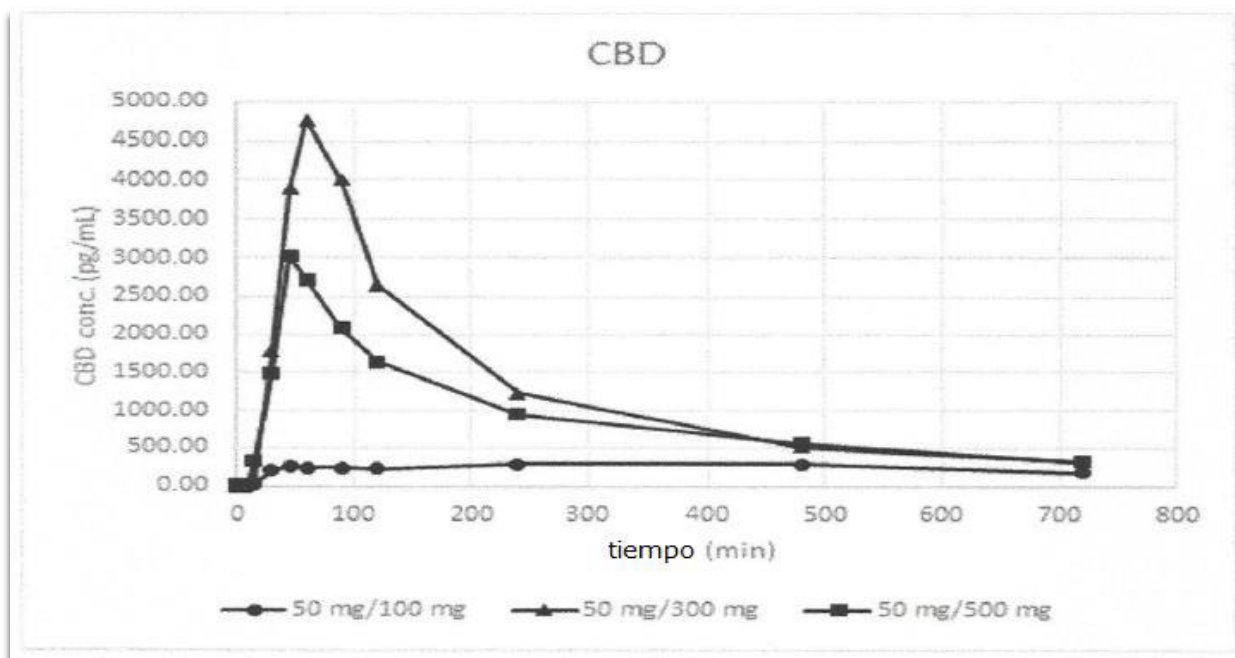
Con base en los datos no clínicos descritos anteriormente, Receptor Life Sciences realizó dos estudios clínicos de prueba de concepto. En el primer estudio, se evaluó SNAC con THC. En el segundo estudio, se evaluó SNAC con CBD. En ambos estudios, SNAC mejoró la biodisponibilidad, redujo la variabilidad y disminuyó el tiempo de inicio de la absorción en humanos.

En el estudio farmacocinético de SNAC/THC, a voluntarios sanos se les administró una solución de SNAC/THC o THC solo. Se recogieron muestras de sangre en serie y se analizaron mediante un ensayo sensible y específico para

el contenido de THC. Los datos mostraron claramente que el SNAC mejora la biodisponibilidad de THC en 2.3 veces en comparación con el control sin SNAC.



En el estudio farmacocinético de SNAC/CDB, a voluntarios sanos se les administró una cápsula que contenía una cantidad fija de CBD (50 mg) y cantidades variables de SNAC (100 mg, 300 mg, 500 mg). Se recogieron muestras de sangre en serie y se analizaron mediante un ensayo sensible y específico para el contenido de CBD. Los datos mostraron claramente que el SNAC mejoró la biodisponibilidad del CBD de manera saturable y dependiente de la dosis. La biodisponibilidad del CBD fue 4 veces mayor con 500 mg de SNAC y 6 veces mayor con 300 mg de SNAC.



Estos hallazgos con compuestos insolubles en agua fueron inesperados con base en lo que se sabía sobre la clase de aminoácidos grasos *N*-acilados y el SNAC en el momento en que se realizó el trabajo y no se habrían predicho con una expectativa razonable de éxito.

Por lo tanto, ni D1 ni D2, solos o en combinación, podrían sugerir la formulación oral de la presente invención con propiedades de almacenamiento mejoradas que, por lo tanto, presenten una actividad inventiva.

De esta forma, teniendo en cuenta que los documentos D1 y D2 **no mencionan, describen o sugieren**, en forma individual o en combinación, todas las características técnicas reclamadas como se describe en la presente invención, **dichas reivindicaciones son novedosas, e inventivas sobre el estado del arte.**

En este sentido, y siguiendo el Manual Andino de Patentes, la presente invención tal y como se encuentra definida en las reivindicaciones 1 a 12, tiene diferencias con el estado del arte e implicó un nivel inventivo durante su desarrollo, por lo cual debe ser considerada como novedosa e inventiva según las disposiciones de los artículos 16 y 18 de la Decisión 486.

8. Conclusión

Con las modificaciones introducidas y los argumentos presentados puede concluirse que:

- i) La presente solicitud es clara, concisa y está debidamente soportada en la descripción, cumpliendo con los requerimientos del Artículo 30 de la Decisión 486; y
- ii) Las reivindicaciones actuales son patentables sobre D1 y D2, cumpliendo así con los requerimientos del Artículo 18 de la Decisión 486.

Por tanto, atentamente me permito solicitar a ese Despacho otorgar el privilegio de patente solicitado a la solicitud en estudio, toda vez que cumple a cabalidad con los requerimientos de la Decisión 486/00.

En consecuencia, solicitamos a ese Despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

9. Anexos

- Nuevo capítulo reivindicatorio conformado por 12 reivindicaciones.
- Declaración firmada por la Dra. Andrea Leone-Bay y su traducción al Español.
- Apéndices de la Declaración.
- Solicitud US 10,588,974
- Respuesta a la Oposición presentada el 19 de mayo de 2019.

Señora Directora,



CAROLINA DAZA MONTALVO

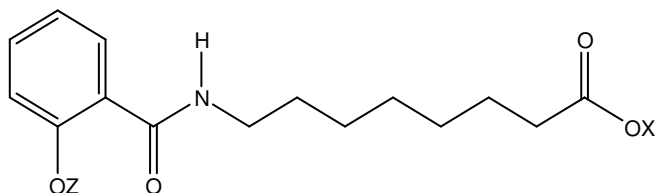
C.C. 66.783.790 de Palmira Valle

TP-141563 CSJ

notificaciones@clarkemodet.com.co

REIVINDICACIONES MODIFICADAS

1. Una formulación oral que comprende entre 1 y 99 % (p/p) de un cannabinoide con una solubilidad en agua menor que 0,1 mg/ml y un aminoácido graso *N*-acilado o una sal de este que comprende la fórmula:



donde X y Z son independientemente H, un catión monovalente que comprende sodio o potasio, un catión metálico divalente que comprende calcio o magnesio, o un catión orgánico que comprende amonio o tetrametilamonio, donde el aminoácido graso *N*-acilado o una sal de este está en una cantidad de 100-200 mg.

2. La formulación oral de la reivindicación 1, donde X es H, un catión monovalente que comprende sodio o potasio, un catión metálico divalente que comprende calcio o magnesio, o un catión orgánico que comprende amonio o tetrametilamonio.

3. La formulación oral de la reivindicación 61, donde Z es H, un catión monovalente que comprende sodio o potasio, o un catión divalente que comprende calcio o magnesio.

4. La formulación oral de la reivindicación 1, donde X es H y Z es H.

5. La formulación oral de la reivindicación 1, donde X es H y Z es sodio.

6. La formulación oral de la reivindicación 1, donde X es sodio y Z es sodio.

7. La formulación oral de la reivindicación 1, que comprende Δ 9-tetrahidrocannabinol (THC) y cannabidiol (CBD), cannabigerol (CBG), cannabicromeno (CBC), cannabinol (CBN), cannabinodiol (CBDL), cannabicitrol (CBL), cannabivarina (CBV), tetrahidrocannabivarina (THCV), cannabidivarina (CBDV), cannabicromevarina (CBCV), cannabigerovarina (CBGV), éter monometílico de cannabigerol (CBGM), ácido cannabinerólico, ácido cannabidiólico (CBDA), variante de propilo de cannabinol (CBNV), cannabitriol (CBO), ácido tetrahidrocannabinólico (THCA), ácido tetrahidrocannabivarínico (THCVA) y/o mezclas de estos.

8. La formulación oral de la reivindicación 1, en donde el cannabinoide comprende Δ 9-tetrahidrocannabinol (THC) y/o cannabidiol (CBD).

9. La formulación oral de la reivindicación 8, en donde el Δ 9-tetrahidrocannabinol (THC) y/o cannabidiol (CBD) se extrae de una planta de *Cannabis sativa*, una planta de *Cannabis ruderalis*, o una planta de *Cannabis indica*.

10. La formulación oral de la reivindicación 1, en donde el aminoácido graso *N*-acilado o una sal de este comprende *N*-[8-(2-hidroxibenzoil)amino]caprilato.

11. La formulación oral de la reivindicación 1, en donde la formulación oral además comprende compuestos flavonoides, terpenos o terpenoides.

12. La formulación oral de la reivindicación 1, en donde el cannabinoide con una solubilidad en agua menor que 0,1 mg/ml se deriva de *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis*, o un extracto de estas.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial Number	16/094,802
Confirmation Number	3639
Filing Date	October 18, 2018
Inventors	Andrea Leone-Bay and Gregory Wesner
Applicant	RECEPTOR HOLDINGS, INC.
Group Art Unit	1612
Examiner	Benjamin J. Packard
Attorney Docket Number	R115-0003US
Customer No.	29150
Title	FAST-ACTING PLANT-BASED MEDICINAL COMPOUNDS AND NUTRITIONAL SUPPLEMENTS

DECLARATION OF ANDREA LEONE-BAY, PH.D. UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Andrea Leone-Bay, Ph.D., declare and state as follows:

1. I am an internationally recognized expert in drug delivery. I am currently the Chief Scientific Officer of and a Founder of Receptor Life Sciences, Inc. which was founded in 2016. I was the Vice President of Pharmaceutical Development at MannKind Corporation from 2006-2015 and before I assumed this role, I was the Senior Directory of Chemistry at MannKind from 2003-2006. Before joining MannKind, I was the Senior Director of Chemistry and Formulations at Emisphere Technologies from 1993-2002.

2. I am an inventor on 110 issued U.S. patents and the author or co-author of 48 peer-reviewed journal articles. I have been an invited speaker at dozens of drug delivery conferences both in the U.S. and internationally.

3. I received a B.A. in Chemistry, magna cum laude, from Mount Holyoke College in 1975 and a Ph.D. in Organic Chemistry from Texas A&M University in 1979. I was a Lecturer in Chemistry at the University of Maryland from 1979-1980 and an NIH postdoctoral fellow at The Ohio State University in natural product synthesis from 1980-1982. My Curriculum Vitae is attached as Appendix A.

4. I have reviewed the non-final Office Action dated October 3, 2019 ("OA") for U.S. Patent Application Serial No. 16/094,802 ("the Application") and the pending claims. It is my understanding that claims 56-75 are rejected under 35 U.S.C. § 103 as obvious over U.S.

Publication No. 2015/0057341 ("Perry") in view of U.S. Patent No. 5,650,386 ("Leone-Bay"). OA, page 3.

5. My positions and experience in the fields noted in ¶¶ 1-3 above, as well as my status as an inventor of the Application and the cited art "Leone-Bay", allow me to speak as one of skill in the relevant art including the scope and interpretation of Leone-Bay and whether its disclosures could be combined with Perry to arrive at the currently claimed invention with a reasonable expectation of success.

6. A reasonable expectation of success of combining Leone-Bay with Perry would require one to expect that the *N*-acylated fatty amino acids that Leone-Bay describes function regardless of the characteristics of the active agent to be delivered. As will be explained in more detail below, however, this is not the case. Before the current Application, the efficacy of the *N*-acylated fatty amino acids described in Leone-Bay were understood to correlate with the water-solubility of the compound to be delivered.

7. I invented the *N*-acylated fatty amino acid class described in Leone-Bay with Koc-Kan Ho and Jeffrey Bruce. I particularly led the scientific design aspects of this project and proposed the original chemical structure of *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) and a method to synthesize it.

8. The *N*-acylated fatty amino acids were specifically designed to provide a mechanism to allow oral delivery of macromolecules that were previously administered through injection. The goal was to improve both therapeutic options and patient compliance. The macromolecules initially selected for improved delivery included proteins (e.g. recombinant human growth hormone), peptides (e.g. parathyroid hormone), and oligosaccharides (e.g., heparin).

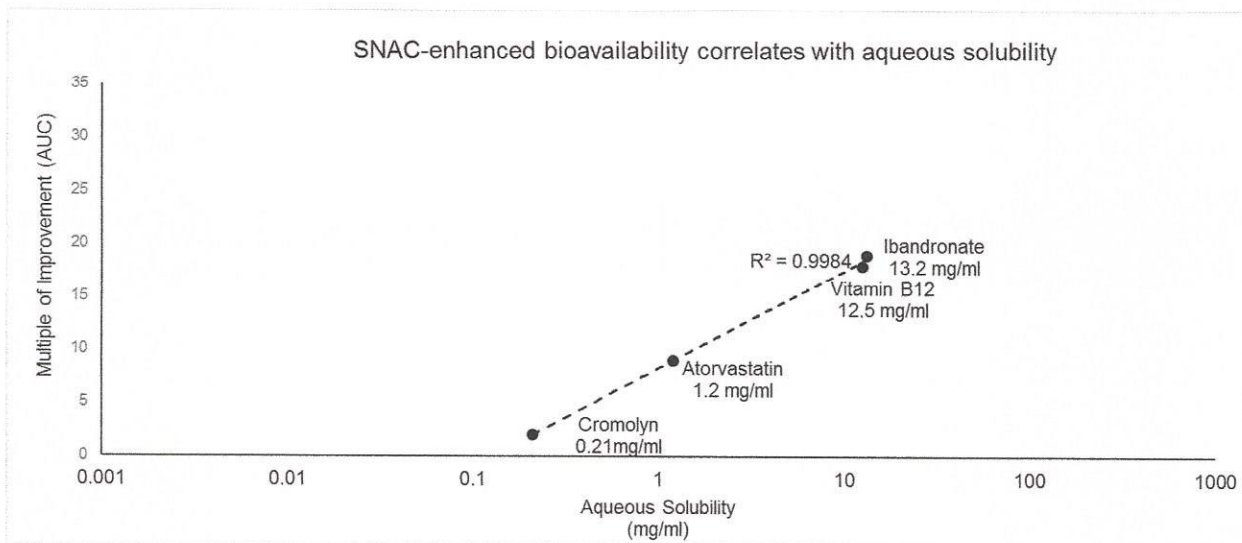
9. I designed the *N*-acylated fatty amino acids described in Leone-Bay to be water soluble because the drugs described above are water soluble. The thought behind the design was that the gastrointestinal (GI) tract is an aqueous environment so a water-soluble absorption enhancer (e.g., SNAC) coupled with a water-soluble drug would optimize transport through the GI membrane and, in turn, optimize absorption.

10. SNAC worked well with water-soluble protein, peptide, and oligosaccharide drugs. See, for example, Baughman *et al.*, "Oral delivery of anticoagulant doses of heparin: a randomized, double-blind, controlled study in humans" *Circulation* 1998; 98:1610-1615 (Appendix B); Kidron, *et al.*, "A novel per-oral insulin formulation: proof-of-concept study in non-diabetic subjects" *Diabetic Medicine*, 2003; 21: 354-357 (Appendix C); and Kremer *et al.*, "Pharmacokinetics, pharmacodynamics and tolerability of oral cromolyn sodium/SNAC capsules in healthy and allergic male subjects" 2003; *Br J Clin Pharmacol* 56, 467-468 (Appendix D). Continuing with this

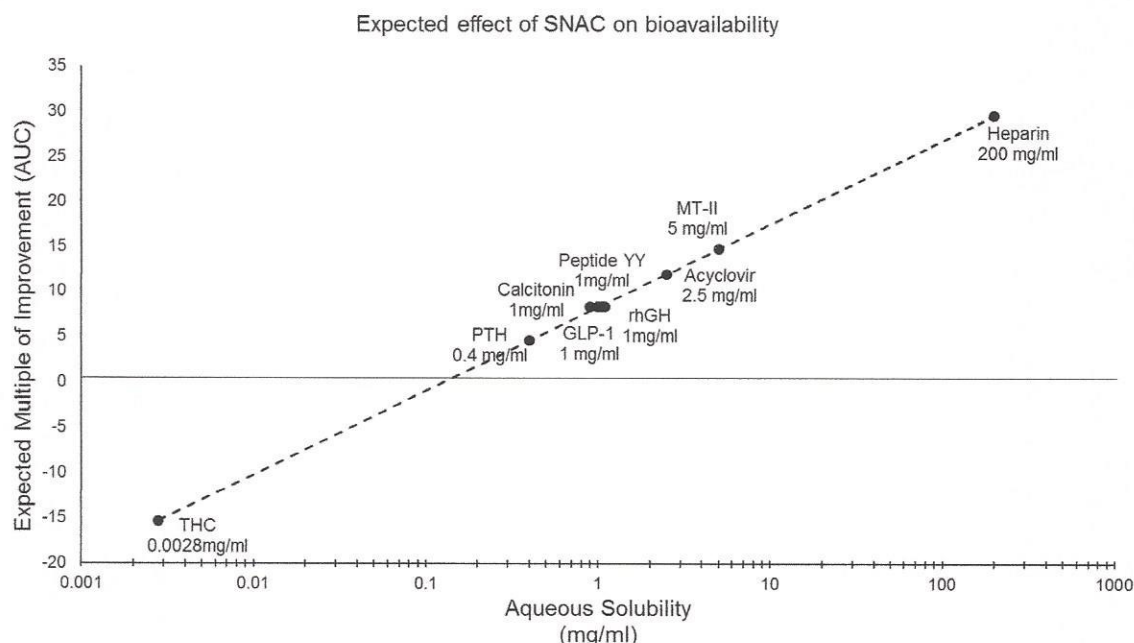
success, SNAC additionally drives the recently FDA-approved (September 2019) and only oral peptide treatment for diabetes, Rybelsus.

11. The use of SNAC has also been extended to other drug classes including small molecules, and there is a large amount of patent and scientific literature regarding SNAC that I have followed since inventing the compound in 1994. As more compounds were evaluated, a trend was observed wherein SNAC's ability to facilitate drug absorption correlated with the water-solubility of the compound. This was not surprising given the compound design work we had initially undertaken.

12. When filing the present Application, published data points from the extensive SNAC and *N*-acylated fatty amino acid literature were used to highlight and demonstrate the water-solubility trend well understood by those familiar with this class of compounds. The graph below plots SNAC's multiple of improvement of bioavailability as a function of water solubility for Cromolyn, see col. 27, table 5 of U.S. 8513300B2 (Appendix E); Atorvastatin, see pg. 13, In. 10, table 2 of WO2009100245A1 (Appendix F); vitamin B12, see col. 5, table 1 of U.S. 8022048B2 (Appendix G); and Ibandronate, page 239, table 5 of Bittner *et al.*, "Phase I clinical study to select a novel oral formulation for ibandronate containing the excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC)" 2012; *Pharmazie* 67, 233-241 (Appendix H). These data points provide the following trend line for SNAC's delivery efficiency based on the water-solubility of the delivered drugs:



13. Based on the trend line above, we depicted how one of ordinary skill in the art would predict the efficacy of *N*-acylated fatty amino acids to increase the delivery of various active ingredients with varying water solubilities and generated the following extended trend line:

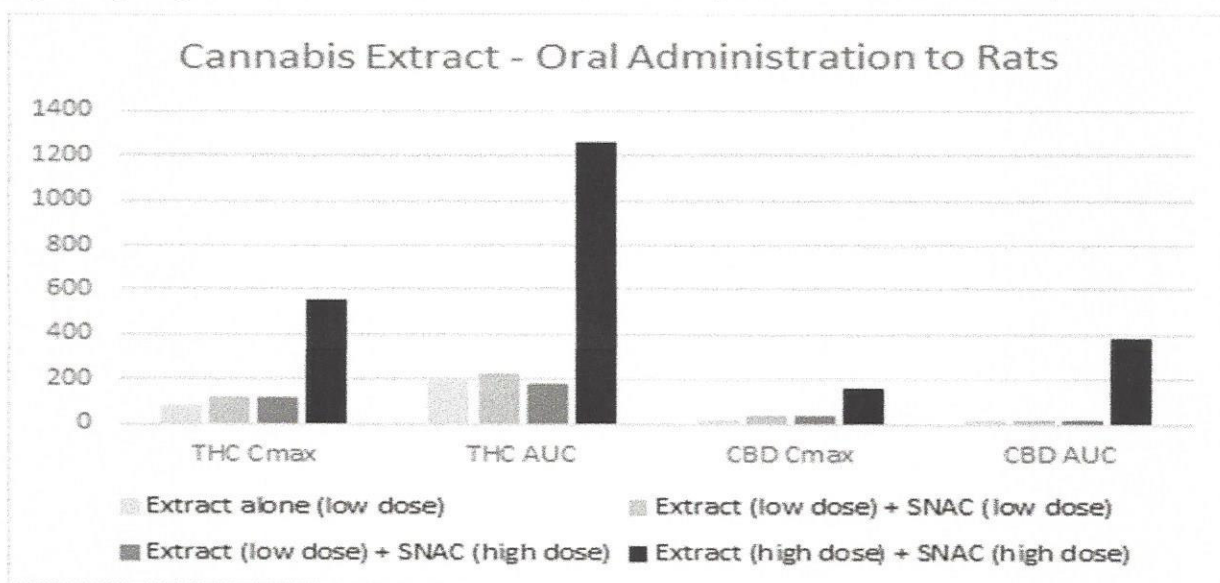


14. In fact, up to the present Application, the predictions illustrated in the graph above were in line with my experience with the *N*-acylated fatty amino acids class of compounds. This experience, coupled with data reported in the literature, further support that one of skill in the art would not have expected to see any improvement in absorption for water-insoluble molecules, such as THC and CBD.

15. In 2016, Receptor Life Sciences began to address the delivery challenges associated with cannabinoid therapies using a robust scientific and clinical approach with the goal of changing cannabinoid medicine to make it safe and effective for patients. Based on my extensive knowledge of the *N*-acylated fatty amino acid class described in Leone-Bay, Receptor Life Sciences chose to administer SNAC with THC to observe its effects in a rat study. Our rationale for conducting this study was to obtain information about absorption enhancers, generally, and inform the choice and analysis of potential absorption-enhancing compounds going forward. We did not expect to see an increase in bioavailability with SNAC.

16. In the described first study at Receptor Life Sciences, fasted Sprague Dawley rats (6/dose group) received single oral doses (by oral gavage) of SNAC alone, cannabis alone or various combinations of cannabis/SNAC. Blood was collected serially at the following times: pre-dose, 5, 15, 30, 45 minutes and 1, 2 and 4 hours. Surprisingly, we observed that SNAC greatly improved the bioavailability of both THC and CBD. At SNAC doses of 250 mg/kg or 500 mg/kg, THC and

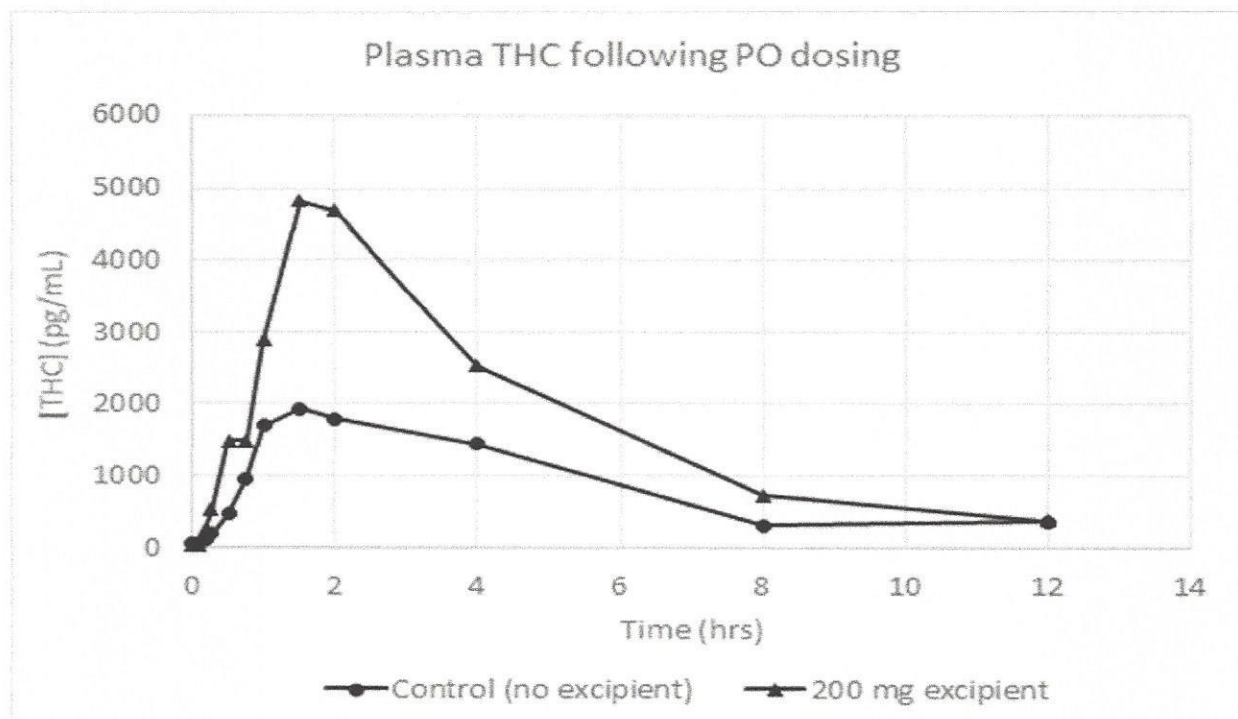
CBD C_{max} were increased 1.4-fold and 2.8-fold, respectively, over the cannabis alone group. $AUC_{0-Tlast}$ were comparable. Increasing both the cannabis and SNAC doses by 2-fold resulted in THC and CBD C_{max} increases of 6.3-fold and 14.2-fold, respectively, over the cannabis alone group. $AUC_{0-Tlast}$ for THC and CBD increased by 6.9-fold and 22.1-fold, respectively, over the cannabis alone group. Overall, these data show that SNAC enhances cannabis absorption when administered to rats by oral gavage, a result that would not have been anticipated when the work was performed.



Dose THC/CBD (mg/kg)	Dose SNAC (mg/kg)	THC				CBD			
		T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0-Tlast}$ (hr*ng/mL)	Compared to cannabis alone C_{max} (AUC)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0-Tlast}$ (hr*ng/mL)	Compared to cannabis alone C_{max} (AUC)
12.5/12.5	0	1.00	79.13	200.3	NA	1.00	11.23	17.29	NA
12.5/12.5	250	1.00	112.57	218.45	1.4 (1.1)	0.50	32.9	16.11	2.9 (0.93)
12.5/12.5	500	1.00	111.5	170.64	1.4 (0.85)	1.00	31.7	13.17	2.8 (0.79)
25/25	500	2.00	546.17	1256.49	6.9 (6.3)	0.25	159.3	382.14	14.2 (22.1)

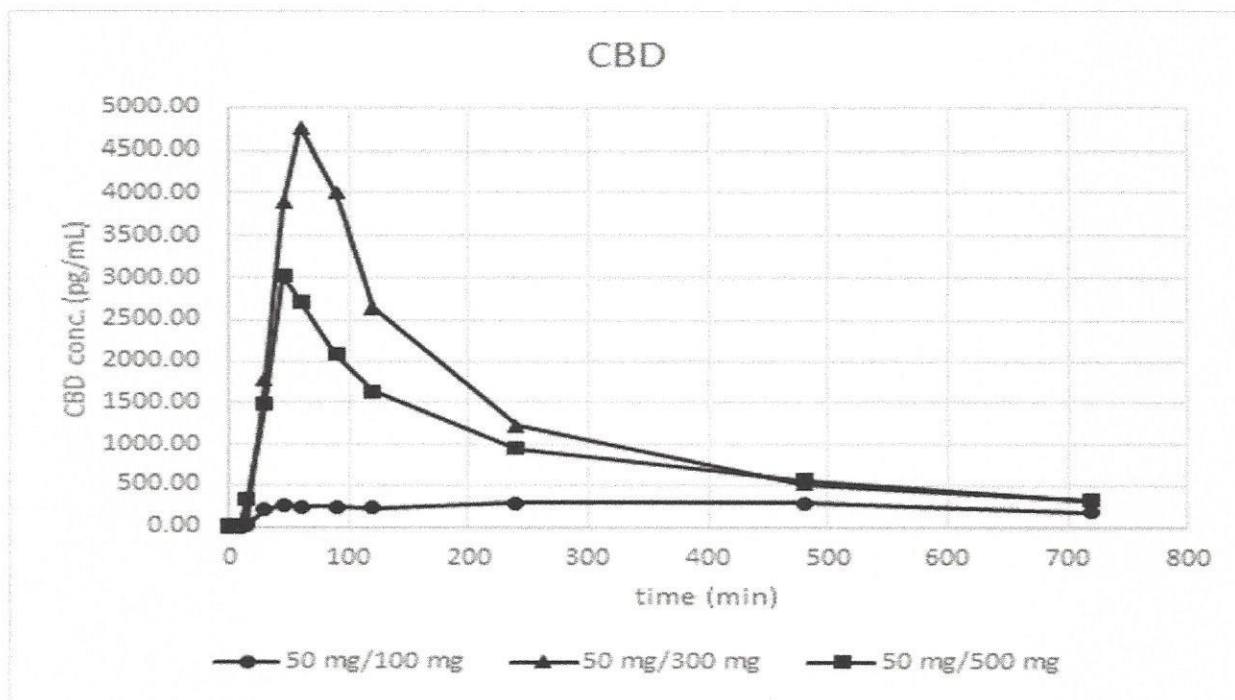
17. Based on the nonclinical data described above, Receptor Life Sciences conducted two proof-of-concept clinical studies. In the first study SNAC was evaluated with THC. In the second study, SNAC was evaluated with CBD. In both studies, SNAC improved bioavailability, reduced variability and decreased time to onset of absorption in humans.

18. In the SNAC/THC pharmacokinetic study, healthy volunteers were administered a solution of either SNAC/THC or THC alone. Blood samples were collected serially and analyzed by a sensitive and specific assay for THC content. The data clearly showed that SNAC improves THC bioavailability by 2.3-fold compared to the non-SNAC control.



	Plasma THC	
	Control (no SNAC)	With 200 mg SNAC
AUC last (hr*pg/mL)	8259	18982
AUC %CV	84	41
Relative Bioavailability	1	2.3

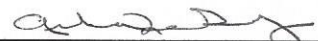
19. In the SNAC/CBD pharmacokinetic study, healthy volunteers were administered a capsule containing a fixed amount of CBD (50 mg) and varying amounts of SNAC (100 mg, 300 mg, 500 mg). Blood samples were collected serially and analyzed by a sensitive and specific assay for CBD content. The data clearly showed that SNAC improved CBD bioavailability in a dose-dependent and saturable manner. CBD bioavailability was 4-fold higher with 500 mg SNAC and 6-fold higher with 300 mg SNAC.



20. These findings with water-insoluble compounds were unexpected based on what was known about the *N*-acylated fatty amino acid class and SNAC at the time the work was performed and would not have been predicted with a reasonable expectation of success.

21. In summary, it is my opinion that one of ordinary skill in the art would not have had a reasonable expectation of success in achieving the benefits of the claimed invention based on the combination of Perry with Leone-Bay.

22. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.



Andrea Leone-Bay, Ph.D.

Date 01/02/20

EN LA OFICINA DE PATENTES Y MARCAS DE ESTADOS UNIDOS

Número de serie	16/094,802
Número de confirmación	3639
Fecha de presentación	Octubre 18, 2018
Inventores	Andrea Leone-Bay y Gregory Wesner
Solicitante	RECEPTOR HOLDINGS, INC.
Unidad de arte grupal	1612
Examinador	Benjamin J. Packard
Número de expediente del abogado	R115-0003US
No. de cliente	29150
Título	COMPUESTOS MEDICINALES Y SUPLEMENTOS NUTRICIONALES BASADOS EN PLANTAS DE ACCIÓN RÁPIDA

DECLARACIÓN DE ANDREA LEONE-BAY, PH.D. BAJO LA SECCIÓN 1.132 DEL TÍTULO 37 DEL CÓDIGO DE REGULACIONES FEDERALES

Comisionado de Patentes
Apartado de correos 1450
Alexandria, VA 22313-1450

Yo, Andrea Leone-Bay, Ph.D., declaro y afirmo lo siguiente:

1. Soy una experta reconocida internacionalmente en entrega de fármacos. Actualmente soy la Directora Científica y una Fundadora de Receptor Life Sciences, Inc., que fue fundada en 2016. Fui Vicepresidenta de Desarrollo Farmacéutico en MannKind Corporation de 2006 a 2015 y antes de asumir este cargo, era la Directora Senior de Química en MannKind de 2003 a 2006. Antes de unirme a MannKind, fui la Directora Senior de Química y Formulaciones en Emisphere Technologies de 1993 a 2002.

2. Soy inventora de 110 patentes de EE. UU. emitidas y autora o coautora de 48 artículos de revistas revisados por pares. He sido una ponente invitada en docenas de conferencias sobre entrega de fármacos tanto en los EE. UU. como a nivel internacional.

3. Recibí una licenciatura en Química, magna cum laude, de Mount Holyoke College en 1975 y un Ph.D. en Química Orgánica de la Universidad de Texas A&M en 1979. Fui profesora de Química en la Universidad de Maryland de 1979 a 1980 y becaria postdoctoral de los Institutos Nacionales de Salud (NIH, por sus siglas en inglés) en la Universidad Estatal de Ohio en síntesis de productos naturales de 1980 a 1982. Mi Currículum Vitae se adjunta como Apéndice A.

4. He revisado la Acción Oficial no final con fecha del 3 de octubre de 2019 ("AO") para la Solicitud de Patente de EE. UU. No. de Serie 16/094,802 ("la Solicitud") y las reivindicaciones pendientes. Tengo entendido que las reivindicaciones 56 a 75 son rechazadas bajo la Sección 103 del Título 35 del Código de EE. UU. como obvias sobre la Publicación de EE. UU. No. 2015/0057341 ("Perry") en vista de la patente de EE. UU. No. 5,650,386 ("Leone-Bay"). AO, página 3.

5. Mis posiciones y experiencia en los campos mencionados en los puntos 1 a 3 anteriores, así como mi estado de inventora de la Solicitud y el documento del estado del arte citado "Leone-Bay", me permiten hablar como una persona experta en el arte relevante que incluye el alcance y la interpretación de Leone-Bay y si sus revelaciones podrían combinarse con Perry para llegar a la invención actualmente reivindicada con una expectativa razonable de éxito.

6. Una expectativa razonable de éxito de combinar Leone-Bay con Perry requeriría que uno esperara que los aminoácidos grasos *N*-acilados que describe Leone-Bay funcionen independientemente de las características del agente activo a entregar. Como se explicará con más detalle a continuación, sin embargo, este no es el caso. Antes de la presente Solicitud, se entendía que la eficacia de los aminoácidos grasos *N*-acilados descritos en Leone-Bay se correlacionaba con la solubilidad en agua del compuesto a entregar.

7. Yo inventé la clase de aminoácidos grasos *N*-acilados descrita en Leone-Bay con Koc-Kan Ho y Jeffrey Bruce. Yo particularmente dirigí los aspectos de

diseño científico de este proyecto y propuse la estructura química original del *N*-[8-(2-hidroxibenzoil) amino] caprilato (SNAC) y un método para sintetizarlo.

8. Los aminoácidos grasos *N*-acilados fueron diseñados específicamente para proporcionar un mecanismo que permita la entrega oral de macromoléculas que se administraban previamente mediante inyección. El objetivo era mejorar tanto las opciones terapéuticas como el cumplimiento del paciente. Las macromoléculas inicialmente seleccionadas para una entrega mejorada incluían proteínas (por ejemplo, hormona de crecimiento humana recombinante), péptidos (por ejemplo, hormona paratiroidea) y oligosacáridos (por ejemplo, heparina).

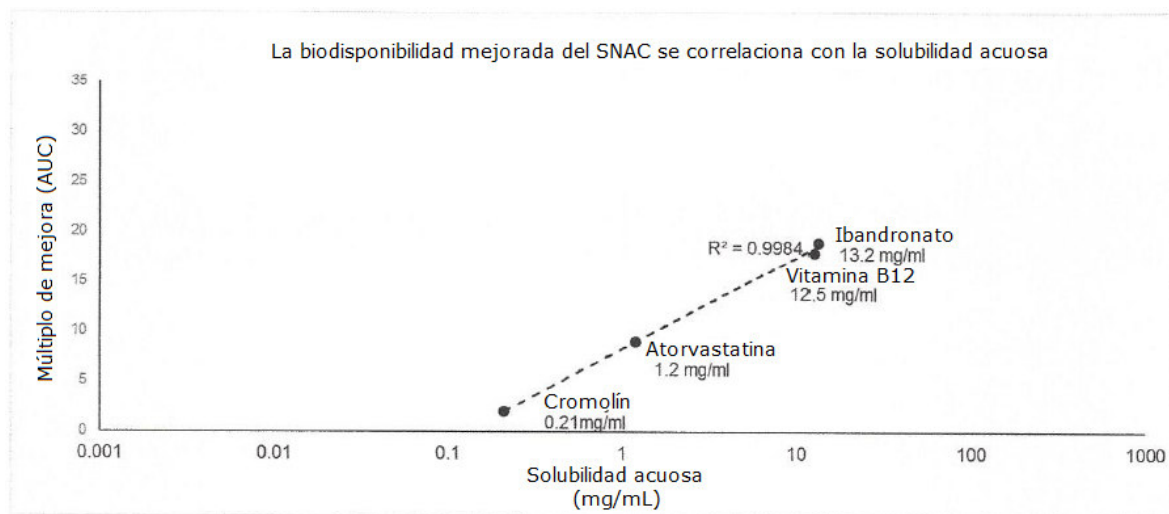
9. Yo diseñé los aminoácidos grasos *N*-acilados descritos en Leone-Bay para que fueran solubles en agua porque los medicamentos descritos anteriormente son solubles en agua. La idea detrás del diseño era que el tracto gastrointestinal (GI) es un ambiente acuoso, por lo que un potenciador de la absorción soluble en agua (por ejemplo, SNAC) junto con un fármaco soluble en agua optimizaría el transporte a través de la membrana GI y, a su vez, optimizaría la absorción.

10. El SNAC funcionó bien con proteínas solubles en agua, péptidos y fármacos oligosacáridos. Véase, por ejemplo, Baughman *et al.*, "Oral delivery of anticoagulant doses of heparin: a randomized, double-blind, controlled study in humans" *Circulation* 1998; 98:1610-1615 (Apéndice B); Kidron, *et al.*, "A novel per-oral insulin formulation: proof-of-concept study in non-diabetic subjects" *Diabetic Medicine*, 2003; 21: 354-357 (Apéndice C); y Kremer *et al.*, "Pharmacokinetics, pharmacodynamics and tolerability of oral cromolyn sodium/SNAC capsules in healthy and allergic male subjects" 2003; *Br J Clin Pharmacol* 56, 467-468 (Apéndice D). Continuando con este éxito, el SNAC también impulsa el único tratamiento con péptidos orales para la diabetes recientemente aprobado por la FDA (septiembre de 2019), Rybelsus.

11. El uso del SNAC también se ha extendido a otras clases de fármacos, incluidas moléculas pequeñas, y existe una gran cantidad de patentes y literatura científica sobre el SNAC que he seguido desde la invención del compuesto en 1994. A medida que se evaluaron más compuestos, se observó una tendencia en donde la capacidad del SNAC para facilitar la absorción del

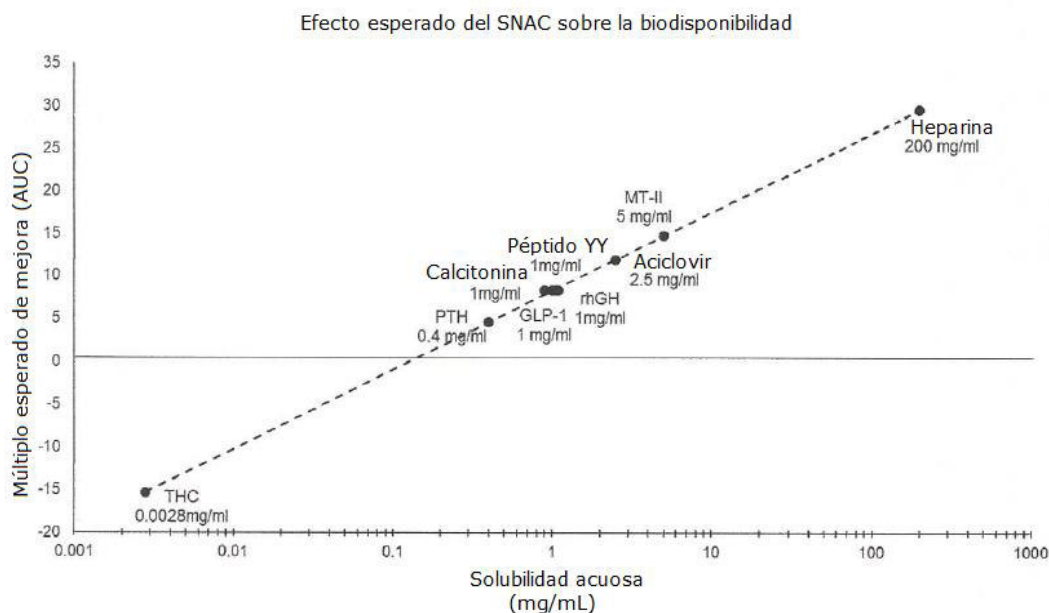
fármaco se correlacionaba con la solubilidad en agua del compuesto. Esto no fue sorprendente dado el trabajo de diseño del compuesto que habíamos emprendido inicialmente.

12. Al presentar la presente Solicitud, se utilizaron puntos de datos publicados de la extensa literatura sobre aminoácidos grasos *N*-acilados y SNAC para resaltar y demostrar la tendencia de solubilidad en agua bien entendida por aquellos familiarizados con esta clase de compuestos. El siguiente gráfico muestra el múltiplo de mejora de la biodisponibilidad del SNAC en función de la solubilidad en agua para Cromolín, véase col. 27, tabla 5 de U.S. 8513300B2 (Apéndice E); Atorvastatina, véase pág. 13, line 10, tabla 2 de WO2009100245A1 (Apéndice F); vitamina B12, véase col. 5, tabla 1 de U.S. 8022048B2 (Apéndice G); e Ibandronato, página 239, tabla 5 de Bittner *et al.*, "Phase I clinical study to select a novel oral formulation for ibandronate containing the excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC)" 2012; *Pharmazie* 67, 233-241 (Apéndice H). Estos puntos de datos proporcionan la siguiente línea de tendencia para la eficiencia de entrega del SNAC basada en la solubilidad en agua de los fármacos entregados:



13. Con base en la línea de tendencia anterior, representamos cómo un experto en la materia predeciría la eficacia de los aminoácidos grasos *N*-acilados

para aumentar la entrega de varios ingredientes activos con solubilidades en agua variables y generamos la siguiente línea de tendencia extendida:

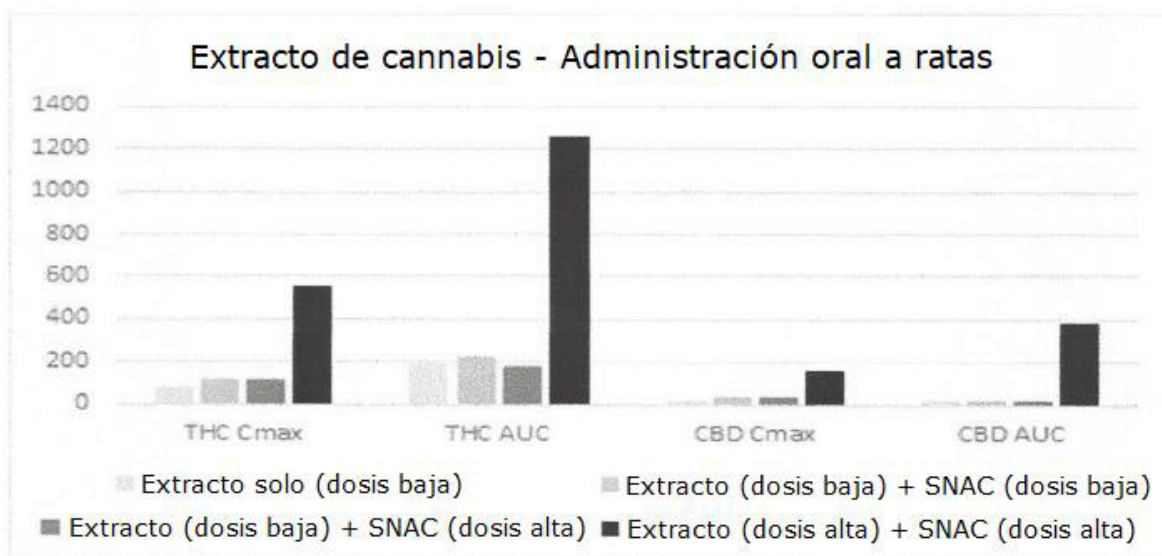


14. De hecho, hasta la presente Solicitud, las predicciones ilustradas en el gráfico anterior estaban en línea con mi experiencia con la clase de compuestos de aminoácidos grasos *N*-acilados. Esta experiencia, junto con los datos reportados en la literatura, respalda aún más que un experto en la materia no habría esperado ver ninguna mejora en la absorción de moléculas insolubles en agua, como THC y CBD.

15. En 2016, Receptor Life Sciences comenzó a abordar los desafíos de entrega asociados con las terapias con cannabinoides utilizando un enfoque científico y clínico sólido con el objetivo de cambiar la medicina cannabinoide para que sea segura y efectiva para los pacientes. Con base en mi amplio conocimiento de la clase de aminoácidos grasos *N*-acilados descrita en Leone-Bay, Receptor Life Sciences eligió administrar el SNAC con THC para observar sus efectos en un estudio en ratas. Nuestro fundamento para llevar a cabo este estudio fue obtener información sobre los potenciadores de la absorción, en general, e informar la elección y el análisis de posibles compuestos

potenciadores de la absorción en el futuro. No esperábamos ver un aumento en la biodisponibilidad con el SNAC.

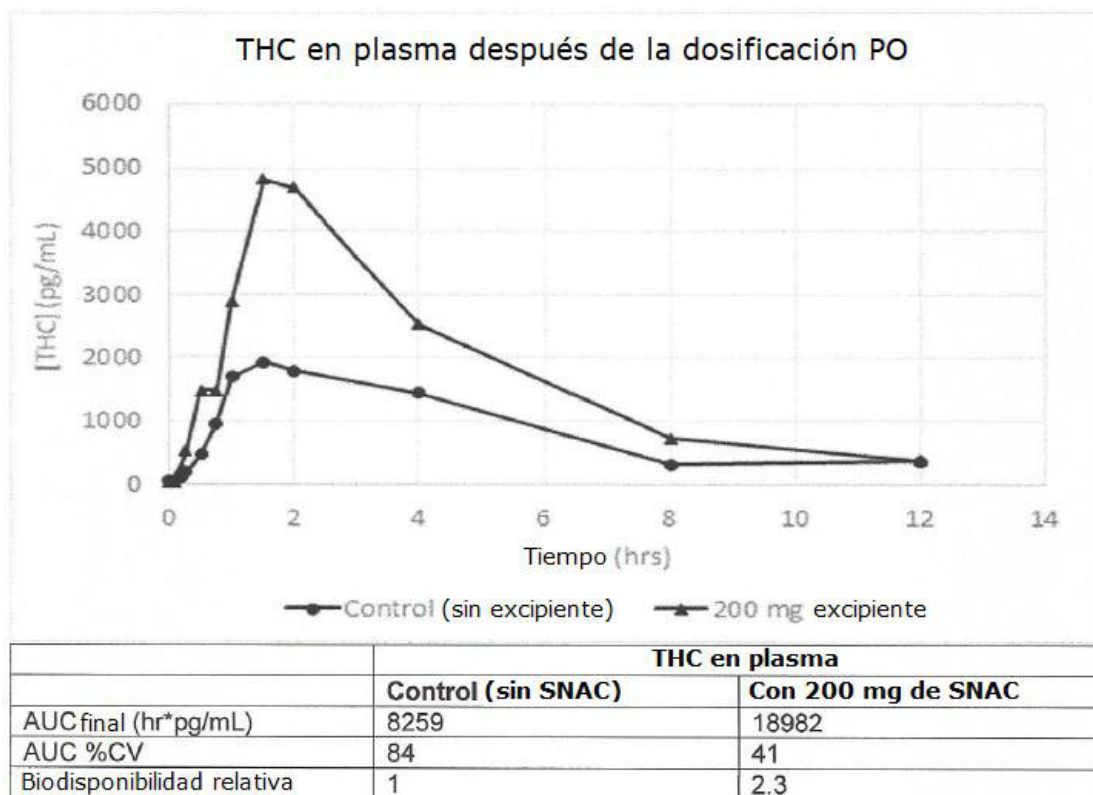
16. En el primer estudio descrito en Receptor Life Sciences, las ratas Sprague Dawley en ayunas (6/grupo de dosis) recibieron dosis orales únicas (por sonda oral) de SNAC solo, cannabis solo o varias combinaciones de cannabis/SNAC. Se recogió sangre en serie en los siguientes momentos: predosis, 5, 15, 30, 45 minutos y 1, 2 y 4 horas. Sorprendentemente, observamos que el SNAC mejoró en gran medida la biodisponibilidad de THC y CBD. A dosis de SNAC de 250 mg/kg o 500 mg/kg, la $C_{\text{máx}}$ de THC y CBD aumentó 1.4 veces y 2.8 veces, respectivamente, sobre el grupo de cannabis solo. Las $AUC_{0-T\text{final}}$ fueron comparables. El aumento de las dosis de cannabis y SNAC en 2 veces dio como resultado aumentos de la $C_{\text{máx}}$ de THC y CBD de 6.3 veces y 14.2 veces, respectivamente, sobre el grupo de cannabis solo. El $AUC_{0-T\text{final}}$ para THC y CBD aumentó en 6.9 veces y 22.1 veces, respectivamente, sobre el grupo de cannabis solo. En general, estos datos muestran que el SNAC mejora la absorción de cannabis cuando se administra a ratas por sonda oral, un resultado que no se habría previsto cuando se realizó el trabajo.



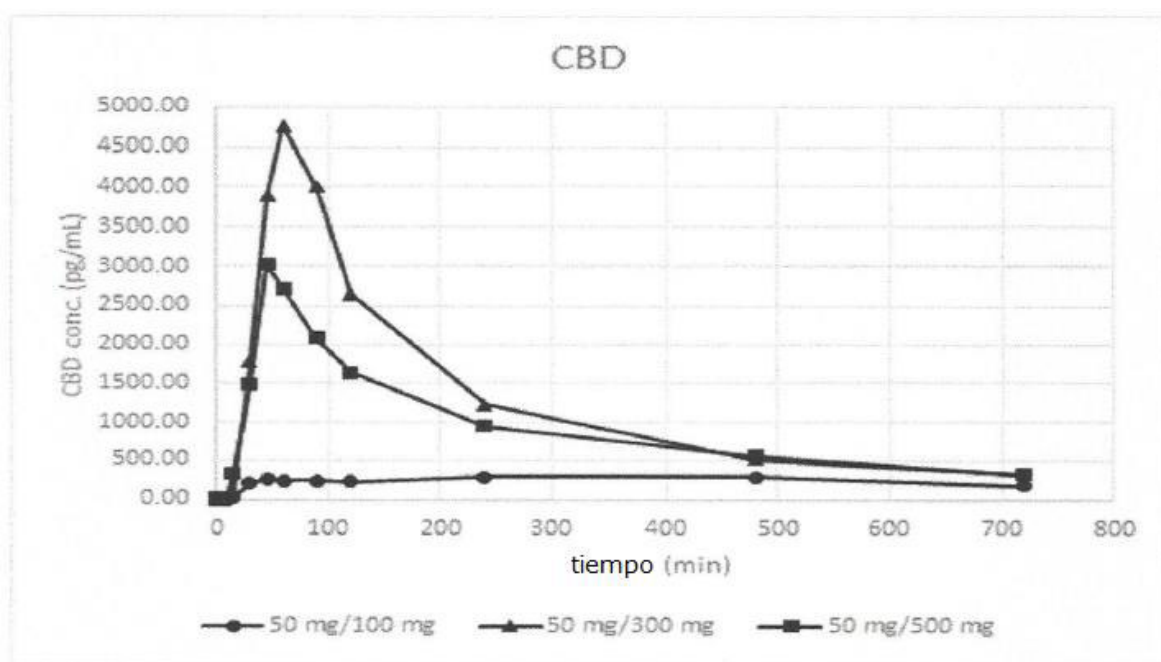
Dosis THC/CBD (mg/kg)	Dosis SNAC (mg/kg)	THC				CBD			
		T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-Tfinal} (hr*ng/mL)	En comparación con cannabis solo C _{max} (AUC)	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-Tfinal} (hr*ng/mL)	En comparación con cannabis solo C _{max} (AUC)
12.5/12.5	0	1.00	79.13	200.3	NA	1.00	11.23	17.29	NA
12.5/12.5	250	1.00	112.57	218.45	1.4 (1.1)	0.50	32.9	16.11	2.9 (0.93)
12.5/12.5	500	1.00	111.5	170.64	1.4 (0.85)	1.00	31.7	13.17	2.8 (0.79)
25/25	500	2.00	546.17	1256.49	6.9 (6.3)	0.25	159.3	382.14	14.2 (22.1)

17. Con base en los datos no clínicos descritos anteriormente, Receptor Life Sciences realizó dos estudios clínicos de prueba de concepto. En el primer estudio, se evaluó SNAC con THC. En el segundo estudio, se evaluó SNAC con CBD. En ambos estudios, SNAC mejoró la biodisponibilidad, redujo la variabilidad y disminuyó el tiempo de inicio de la absorción en humanos.

18. En el estudio farmacocinético de SNAC/THC, a voluntarios sanos se les administró una solución de SNAC/THC o THC solo. Se recogieron muestras de sangre en serie y se analizaron mediante un ensayo sensible y específico para el contenido de THC. Los datos mostraron claramente que el SNAC mejora la biodisponibilidad de THC en 2.3 veces en comparación con el control sin SNAC.



19. En el estudio farmacocinético de SNAC/CBD, a voluntarios sanos se les administró una cápsula que contenía una cantidad fija de CBD (50 mg) y cantidades variables de SNAC (100 mg, 300 mg, 500 mg). Se recogieron muestras de sangre en serie y se analizaron mediante un ensayo sensible y específico para el contenido de CBD. Los datos mostraron claramente que el SNAC mejoró la biodisponibilidad del CBD de manera saturable y dependiente de la dosis. La biodisponibilidad del CBD fue 4 veces mayor con 500 mg de SNAC y 6 veces mayor con 300 mg de SNAC.



20. Estos hallazgos con compuestos insolubles en agua fueron inesperados con base en lo que se sabía sobre la clase de aminoácidos grasos N-acilados y el SNAC en el momento en que se realizó el trabajo y no se habrían predicho con una expectativa razonable de éxito.

21. En resumen, opino que un experto en la materia no habría tenido una expectativa razonable de éxito en lograr los beneficios de la invención reivindicada basándose en la combinación de Perry con Leone-Bay.

22. Declaro además que todas las declaraciones hechas aquí de mi propio conocimiento son verdaderas y que todas las declaraciones hechas sobre

información y creencias se consideran verdaderas; y, además, estas declaraciones se hacen con el conocimiento de que las declaraciones falsas intencionadas y similares se castigan con multa o prisión, o ambas, de conformidad con la Sección 1001 del Título 18 del Código de los Estados Unidos.

Firma ilegible

Andrea Leone-Bay, Ph.D.

Fecha 01/02/20

APPENDIX A

Andrea Leone-Bay, Ph.D.

Summary

Accomplished, enthusiastic, and effective scientific leader with strong interdisciplinary strategic planning and portfolio development expertise. Significant experience successfully evaluating, initiating, managing and directing development projects (IND to NDA) in a multi-disciplinary environment, in start-ups, early stage and mature business environments. Very strong verbal and written communication skills with comprehensive knowledge of formulations, chemistry, drug development and drug delivery for oral, injected and inhaled products. Extensive list of publications and patents. Comprehensive understanding of Good Manufacturing Practices (GMP) and regulatory guidelines. Published over 40 articles in peer-reviewed journals and inventor on over 90 issued US patents. Have had the privilege of making major contributions to the development programs for two commercial products.

Experience

Receptor Life Sciences (10/15 to present; privately held start-up)

Chief Scientific Officer

Responsible for strategic scientific direction, portfolio planning, and new product development.

MannKind Corporation (4/03 to 9/15)

Vice President, Pharmaceutical Research & Development (7/06 to 9/15)

Responsible for discovery through late stage pharmaceutical product planning and development. Led scientific team evaluating potential pipeline products followed by planning and implementation of selected programs. Provided strategy, process, and tactical leadership to facilitate progression of the company's pharmaceutical research and development efforts in pipeline development. Represented R&D to corporate management ensuring business needs were met in a timely and cost-effective manner.

- Built, directed and implemented pharmaceutical R&D strategies to develop product pipeline of protein, peptide and small molecule drugs over a variety of clinical indications
- Scientific leader, in collaboration with medical/regulatory and business development, in establishing a product portfolio
- Provided expert knowledge of chemistry, formulations, drug development, and drug delivery to evaluate, develop and implement R&D projects
- Established and maintained pharmaceutical research and development infrastructure focused on science, technology and quality internally and with appropriately managed outsourcing
- Facilitated new business opportunities through external scientific presentations
- Managed external collaborations with pharmaceutical and academic partners
- Key member of the Scientific Advisory Board
- Represented MannKind science and technology externally
- Led formulation and chemical development for inhaled insulin formulation/device combination product (Afrezza® Inhaled Insulin)
- Led formulation development for injected insulin formulation for use in insulin pump
- Led formulation development for small molecule and peptide products, inhaled dry powders and injected solution products
- Led medical writing team for NDA submission
- Key member of Afrezza inhaled insulin FDA Advisory Committee team
- NDA submission support and technical transfer for API and clinical supply manufacture
- Built, led and developed a department of 28 staff members in two locations, east/west coast US
- Accountable for on-time, on-budget delivery for drug product development
- Planned and administered budgets; full responsibility for \$15M annual budget
- Interfaced across the organization to ensure integration and alignment of activities
- Key member of management team for strategic business direction

Sr. Director, Chemistry and Formulations (4/03 to 7/06)

Responsible for the management of formation development, new formulations technologies, chemical development for inhalation drug delivery and medicinal chemistry for small molecules.

- Directed pharmaceutical development for dry powder insulin product including technical/process transfer to manufacturing
- Led CMC team for the early development of a dry powder inhalation product
- Provided and implemented IND strategies and filings
- Directed drug delivery platform expansion and characterization program
- Built, lead and develop a department of 15 staff members
- Planned and administered budgets; full responsibility for \$8M annual budget
- Directed the design and synthesis of small molecule NCE for oncology programs
- Directed the chemical development, scale-up, and manufacture of bulk drug substances and drug products

Consultant (5/02 to 3/03)

Consultant to executive management team of two small biopharmaceutical companies.

- Reviewed IND for a Phase II oncology drug and prepared a comprehensive preclinical plan (CMC and Pharm/Tox requirements, budget, resources, etc.) to NDA filing.
- Directed the resolution of issues surrounding drug substance analysis, stability, and manufacture.
- Designed and initiated a toxicology program.
- Directed the scale up and outsourcing of a small molecule API.

Emisphere Technologies, Inc. (3/93 to 5/02)

Sr. Director of Project Management (4/00 to 5/02). Responsible for the management of drug development programs from preclinical development through Phase III. Major accomplishments:

- Established/grew the Project Management function from 2 to 8 staff members
- Directed/facilitated clinical operations for a international, multicentre Phase III clinical program (oral heparin)
- Directed oral heparin (Phase II/Phase III), oral cromolyn (preclinical), oral PTH (preclinical to Phase I), and oral insulin (preclinical to Phase I) programs
- Planned and administered budgets
- Directed/facilitated preclinical programs (pharmacology, pharmacokinetics, ADME, process chemistry, toxicology, formulation development, IDB, clinical protocol, and regulatory application preparation, etc.) to bring 5 new chemical entities to first human dose studies in both the US and Europe
- Acted as the primary liaison between Emisphere and Lilly for the development of oral PTH
- Provided strategy and directed the filing of 3 US INDs for new chemical entities

Sr. Director of Chemistry (9/97 to 4/00) and Acting Director of Program Management (10/99 to 4/00). Responsible for the direction of 10 chemists in the area of oral macromolecule delivery and 3 project managers in the area of drug development. Major accomplishments:

- Directed the discovery of 2 new chemical entities that entered clinical studies (one of these progressed to Phase III and the other progressed to Phase II)
- Facilitated the interactions between the research and development departments
- Directed the oral heparin pharmacology program
- Directed process transfer to external manufacturing organizations
- Acted as the primary technical liaison between Emisphere and Elan for the development of oral heparin
- Acted as the primary technical liaison between Emisphere and Lilly for the discovery of oral PTH and oral rhGH delivery agents
- Coordinated/directed the filing of 1 US IND (currently open) for a new chemical entity

Director of Organic Chemistry (3/93 to 9/97). Responsible for the direction of 10 chemists in the area of small molecule medicinal chemistry. Major accomplishments:

- Established/grew the Medicinal Chemistry function from 3 to 10 staff members
- Directed the design and synthesis of small molecule oral delivery agents
- Directed the discovery of 3 new chemical entities that entered clinical studies
- Evolved Emisphere's oral delivery agent technology from multicomponent systems to single new chemical entities
- Acted at the primary technical liaison to patent counsel for the preparation and filing of over 2 dozen new chemical entity patent applications
- Directed the discovery of an oral calcitonin delivery agent that is currently in Phase II

American Cyanamid (3/88 to 2/93)

Senior Research Chemist (3/88 to 2/93). Organic and polymer syntheses and process development with responsibility for the direction of three chemists. Major accomplishments:

- Developed processes for the manufacture of pharmaceutical intermediates and APIs
- Developed polymers for use in the water treating industry

Stauffer Chemical Company (9/82 to 2/88)

Senior Research Chemist (10/87 to 2/88), Principal Research Chemist (10/84 to 10/87) and Research Chemist (9/82 to 10/84) at Stauffer Chemical Company. Organic synthesis and process development of compounds of agricultural and pharmaceutical interest. Major accomplishments:

- Designed and synthesized two novel herbicides that extended patent life of commercial products
- Developed novel synthetic methods for existing pesticide products

Education

- NIH postdoctoral fellow and postdoctoral fellow at The Ohio State University, Columbus, Ohio, in natural product synthesis. Advisor - Leo A. Paquette.
- Lecturer (Organic Chemistry) at the University of Maryland, College Park, Maryland.
- Ph.D. in Organic Chemistry Texas A&M University, College Station, Texas. Dissertation title "The Photochemistry of *N*-Vinyl-2-Pyridones and their Acyclic Analogs". Advisor - Patrick S. Mariano.
- B.A. magna cum laude, Mount Holyoke College, South Hadley, Massachusetts. Thesis title "Photodecarbonylation of Cyclic Ketones". Advisor - Mary H. Delton.

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- Diabetes Summit (4/14 Boston, MA)
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- Protein Protein Interactions Summit (10/14 Boston, MA)

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Journal Reviewer

Therapeutic Delivery, Journal of Organic Chemistry, Pharmaceutical Research, Canadian Journal of Physiology, Journal of Medicinal Chemistry, Journal of Pharmacy and Pharmacology

References

Available upon request

APPENDIX B

Oral Delivery of Anticoagulant Doses of Heparin

A Randomized, Double-Blind, Controlled Study in Humans

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James Kisicki, MD; Francesca Catella-Lawson, MD; Garret A. FitzGerald, MD

Background—Parenteral heparin is the anticoagulant of choice in hospitalized patients. Continued anticoagulation is achieved by subcutaneous administration of low-molecular-weight heparin or with an orally active anticoagulant such as warfarin. An oral heparin formulation would avoid the inconvenience of subcutaneous injection and the unfavorable drug interactions and adverse events associated with warfarin. A candidate delivery agent, sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), was evaluated with escalating oral heparin doses in a randomized, double-blind, controlled clinical study for safety, tolerability, and effects on indexes of anticoagulation.

Methods and Results—Increases in activated partial thromboplastin time (aPTT), anti-factors IIa and Xa, and tissue factor pathway inhibitor (TFPI) concentrations were detected when normal volunteers were dosed with 10.5 g SNAC/20 000 IU heparin by gavage in some subjects. For the entire group, 30 000 IU SNAC and heparin elevated TFPI from 74.9 ± 7.6 to 254.2 ± 12.3 mg/mL ($P < 0.001$) 1 hour after dosing ($P < 0.001$). Similar changes occurred in anti-factor IIa and anti-factor Xa. aPTT rose from 28 ± 0.5 to 42.2 ± 6.3 seconds 2 hours after dosing ($P < 0.01$). No significant changes in vital signs, physical examination, ECGs, or clinical laboratory values were observed. Neither 30 000 IU heparin alone nor 10.5 g SNAC alone altered the hemostatic parameters. Emesis was associated with 10.5 g SNAC. A taste-masked preparation of SNAC 2.25 g was administered orally with heparin 30 000 to 150 000 IU. Both aPTT and anti-factor Xa increased with escalating doses of heparin. This preparation was well tolerated.

Conclusions—Heparin, administered orally in combination with the delivery agent SNAC, produces significant elevations in 4 indexes of anticoagulant effect in healthy human volunteers. These results establish the feasibility of oral delivery of anticoagulant doses of heparin in humans and may have broader implications for the absorption of macromolecules. (*Circulation*. 1998;98:1610-1615.)

Key Words: heparin ■ anticoagulants ■ oral administration

Heparin, a naturally occurring glycosaminoglycan, is a potent inhibitor of blood coagulation, primarily through formation of a protease inhibitory complex with antithrombin (AT) III.¹⁻³ Parenteral administration of heparin has been shown to prevent venous thrombosis and pulmonary embolism in patients undergoing surgery.^{1,4,5} It has also been shown to prevent peripheral arterial embolism and reduce the incidence of myocardial infarction and death in patients with unstable angina.^{6,7} Heparin has been suggested to improve outcome in acute ischemic stroke.⁸ Heparin is composed of glucosamine and either L-iduronic acid or D-glucuronic acid in chains of variable length, having a molecular weight range of 5000 to 30 000. It is sulfated and highly acidic.⁴⁻⁸ Fractionated, low-molecular-weight heparins have also established clinical efficacy.³⁻⁵ Clinical prevention of thrombosis with heparin is attained at doses that modify tests of anticoagulant function. These include the activated partial thromboplastin time (aPTT) and tissue factor pathway inhibitor

(TFPI). Clinical benefit has been associated with heparin-induced prolongation of aPTT by 1.5- to 2.5-fold.^{1,2,4-8} Less information is available that relates alterations in TFPI to clinical outcome.

Heparin is not absorbed in the gastrointestinal tract, presumably because of its size and ionic repulsion from negatively charged epithelial tissue.⁹ As such, it has poor oral bioavailability and is administered parenterally, either by continuous or intermittent infusion or by deep subcutaneous injection. Thus, parenteral heparin is usually replaced by the orally active anticoagulant warfarin for long-term outpatient therapy. However, the specific adverse effects of warfarin and pharmacokinetic interactions involving this highly protein-bound drug may limit its practical utility.¹⁰ Low-molecular-weight heparins may be self-administered by patients after hospital discharge. This approach does not require intensive monitoring and has established clinical efficacy, at least over short periods.⁷ Given this observation, continuation

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of heparin therapy with an oral dosage form might prove to be a clinically attractive alternative. Although thrombin generation may persist during parenteral heparin administration,¹¹ abrupt cessation of therapy may result in a rebound increase in thrombin generation and reactivation of clinical syndromes of vascular occlusion.¹²

Several recent attempts to develop effective nonparenteral heparin formulations have been reported but have met with limited success.¹³⁻¹⁹ We have synthesized delivery agents based on *N*-acylated α -amino acids²⁰ and *n*-acylated non- α -amino acids²¹ and demonstrated in animal models that they promote oral absorption of macromolecules. *N*-[8-(2-hydroxybenzoyl) amino]caprylate (SNAC)-mediated gastrointestinal absorption of heparin occurs in a passive transcellular process,²² without causing apparent damage to intestinal epithelium.²³ Transport is thought to be facilitated by the formation of a noncovalent complex between SNAC and heparin.²⁴ Investigations, both *in vitro* and *in vivo*, revealed that the *n*-acylated non- α -amino acid SNAC has no pharmacological activity.²¹ However, it increased aPTT in primates dosed orally with heparin. Hypoglycemia, nausea, and emesis were observed at high doses (1800 mg/kg) of SNAC with an unformulated solution in cynomolgus macaques.²⁵

We now report that SNAC facilitated the gastrointestinal absorption of heparin in healthy volunteers, at tolerated doses, with no change in glucose or insulin levels. Furthermore, 4 indexes of anticoagulant effect were prolonged into the range associated with clinical utility by the SNAC-heparin solution. These observations suggest the potential for development of an orally available heparin preparation for clinical investigation.

Methods

Study Design

Three clinical studies were performed at the Clinical Research Unit of Harris Laboratories. All studies were approved by the Institutional Review Board. In the first study, healthy male ($n=27$) and surgically sterile female ($n=3$) volunteers were evaluated for inclusion in the study after providing written informed consent. All had unremarkable histories and physical examinations. No abnormalities were detected on 12-lead ECG, clinical chemistry, hematology, and urinalysis (including drug screen). Subjects were selected and randomly assigned to receive ascending doses (1.4 to 10.5 g) of SNAC by gavage (group 1), 10 000 IU heparin with ascending doses (1.4 to 10.5 g) of SNAC (group 2), or 20 000 or 30 000 IU heparin with a fixed dose (10.5 g) of SNAC (group 3). Subjects randomized to a heparin treatment (group 2 or 3) were screened with 4000 IU heparin (heparin sodium injection, USP, 1000 IU/mL SC, Schein Pharmaceuticals) for an atypical heparin response (aPTT increase >2 times baseline) or untoward reaction. In the second study, 4 volunteers (3 men, 1 surgically sterile woman) were administered 30 000 IU heparin alone under identical conditions. Again, indexes of hemostatic function were monitored.

In the third study, the oral administration of a taste-masked SNAC-heparin solution was evaluated. SNAC and heparin were formulated into a sugar syrup containing other sweeteners and flavors. The syrup was administered orally from unit dose vials at a dose volume of 30 mL. In this study, a fixed, lower dose (2.25 g) of SNAC was used with escalating doses (30 000, 60 000, 90 000, and 150 000 IU) of heparin. Before oral dosing, 14 volunteers (12 men) were administered heparin 10 000 IU SC. The aPTT was measured before and 0.33, 0.67, 1, 1.5, 2, and 4 hours after dosing. Volunteers were excluded from the study if the aPTT was prolonged >2.5 times control.

Subjects satisfying all inclusion and exclusion criteria for the studies were admitted to the clinical research unit the evening before dosing. All subjects were provided with standardized meals and snacks throughout the study periods. Subjects were not allowed food or beverages containing xanthine-related agents during the 12-hour period before dosing. Alcohol intake was prohibited during the 24-hour predosing period and throughout the study. Vital signs and oral temperature were recorded at screening; immediately before each dose; and 1, 2, 4, 8, 12, and 24 hours after each dose. Glucose and insulin were measured 5 minutes before dosing and at 1 and 2 hours after dosing for each study period.

For the first 2 studies, dosing solutions were compounded on each study day, according to a predetermined randomization scheme provided by the research pharmacist. Individual dosing solutions were labeled by the pharmacy with the subject's initials, study number, and study day and date. The test solutions were composed of SNAC (Emisphere Technologies) and/or heparin sodium USP (Scientific Protein Laboratories, 166.9 USP U/mg) in 25% vol/vol aqueous propylene glycol. The vehicle control was 25% vol/vol aqueous propylene glycol. In these studies, all oral doses were administered by gavage through a 16F, Levin-type seamless stomach tube, which had been shown to be compatible with the dosing solution. Delivery into the stomach was designed to standardize dosing to the site of absorption. After an overnight fast, the subjects were administered 70 mL dosing solution via the stomach tube. In the third study, a formulated, taste-masked, oral SNAC-heparin solution was administered. Both the study physician and the subject were blinded as to which preparation was being administered. Similarly, the analyses were performed without knowledge of patient name, sex, or treatment.

Prothrombin time (PT), aPTT, AT III, and triglycerides were determined before dosing and at 2 and 4 hours after dosing in groups 2 and 3 of study 1. Arachidonic acid-induced platelet aggregation was measured before and 2 hours after dosing as previously described.²⁶ Blood samples were drawn 30, 15, and 5 minutes before dosing and 0.33, 0.67, 1, 1.5, 2, 4, and 8 hours after dosing. Samples taken 30 minutes before and 1, 2, and 4 hours after dosing were analyzed for TFPI. Supplementary blood and urine samples for safety determination, including hematology (complete blood count, platelets), PT, aPTT, and urinalysis (dipstick), were collected 24 hours after dosing. In the third study, aPTT was measured before and 0.5, 1, 2, 4, and 24 hours after dosing. Anti-factor Xa was determined before and 0.08, 0.17, 0.33, 0.5, 0.67, 1.0, 1.33, 1.67, 2.0, 4.0, 6.0, and 8.0 hours after dosing.

Analytical Procedures

Anti-factors IIa and Xa and TFPI were assayed in the Center for Experimental Therapeutics, University of Pennsylvania School of Medicine, except for study 3, in which anti-factor Xa was assayed by Bioanalytical Services, Elan Pharmaceutical Technologies. Insulin and AT III were assayed in the Clinical Laboratory, University of Nebraska Medical Center. All other laboratory work was performed in the Clinical Laboratory at Harris Laboratories.

Anti-factor Xa activity was determined with a chromogenic substrate by use of a kit from Diagnostica. The assay has a coefficient of variation of $<4\%$ at a limit of detection (LOD) of 0.1 IU/mL. Values reported <0.1 IU/mL were generated from a standard curve by use of lower concentration standards. Anti-factor IIa activity was determined with a kit from Kabi Diagnostics. The coefficient of variation is $<5\%$ at an LOD of 0.05 IU/mL. TFPI concentrations were determined by use of an ELISA (American Diagnostic, Inc). The LOD for this assay is 0.5 ng/mL. The intra-assay coefficient of variation is $<5\%$. A subset of samples subjected to a previous freeze/thaw cycle was also assayed for TFPI. These data, however, were considered of qualitative value only.

Data Management

All clinical data were entered into an Oracle database. All computations and statistical analyses were carried out with the statistical software package SAS, version 6.09 or greater. aPTT, anti-factors IIa and Xa, and TFPI values were analyzed by ANOVA, with subse-

TABLE 1. Subject Distribution and Gastrointestinal Side Effects by Treatment Group in Study 1

Group	Period	Dose Level (No. of Subjects)	Nausea	Emesis
1	1	1.4 g SNAC (n=7)	0	0
		Vehicle control (n=3)	0	0
	2	3.5 g SNAC (n=7)	3	0
		Vehicle control (n=3)	0	0
	3	7.0 g SNAC (n=6)	4	0
		Vehicle control (n=3)	0	0
	4	10.5 g SNAC (n=6)	3	0
		Vehicle control (n=3)	0	0
2	1	1.4 g SNAC and 1000 IU heparin (n=6)	2	0
		1000 IU heparin (n=2)	0	0
	2	3.5 g SNAC and 1000 IU heparin (n=6)	5	1
		1000 IU heparin (n=1)	0	0
	3	7.0 g SNAC and 1000 IU heparin (n=5)	2	1
		1000 IU heparin (n=2)	1	0
	4	10.5 g SNAC and 10 000 IU heparin (n=5)	2	3
		1000 IU heparin (n=2)	0	0
3	1	10.5 g SNAC and 2000 IU heparin (n=6)	1	4
		10.5 g SNAC (n=3)	2	2
	2	10.5 g SNAC and 3000 IU heparin (n=6)	3	2
		10.5 g SNAC (n=1)	1	1

quent pairwise comparison as appropriate. Data are reported as mean $\pm$ SEM. Differences were considered statistically significant at $P < 0.05$.

Results

Subjects in the first study were randomized to treatment groups as listed in Table 1; dropouts and no-shows were not replaced. There were no changes between preenrollment and discharge physical examinations in all subjects. All ECG results and vital sign measurements were considered unremarkable throughout the study.

Neither heparin nor SNAC induced significant changes in serum chemistry, glucose, insulin, hematology, or urinalysis. There were no trends observed for any dosing group. The PT, AT III levels, and triglycerides remained normal throughout the study.

Measures of anticoagulant effect were unaltered by SNAC alone (group 1) or by escalating doses of SNAC with 10 000 IU heparin. Specifically, TFPI did not change significantly from levels before (69.7 ± 12.8 ng/mL) to 2 hours (79.9 ± 8.8 ng/mL) and 4 hours (78.7 ± 14.2 ng/mL) after 10.5 g SNAC alone. Similarly, anti-factor IIa and Xa levels were undetectable before or after 10.5 g SNAC. Administration of 20 000 and 30 000 IU heparin with the 10.5 g SNAC altered the indexes of anticoagulant effect. aPTT increased significantly ($P < 0.01$) from a control level of 28.0 ± 0.5 to 42.2 ± 6.3 seconds 2 hours after dosing (Figure 1A) in subjects receiving 30 000 IU heparin. Anti-factor IIa activity was detected in 4 of 6 volunteers after the lower dose and in all but 1 of the 6

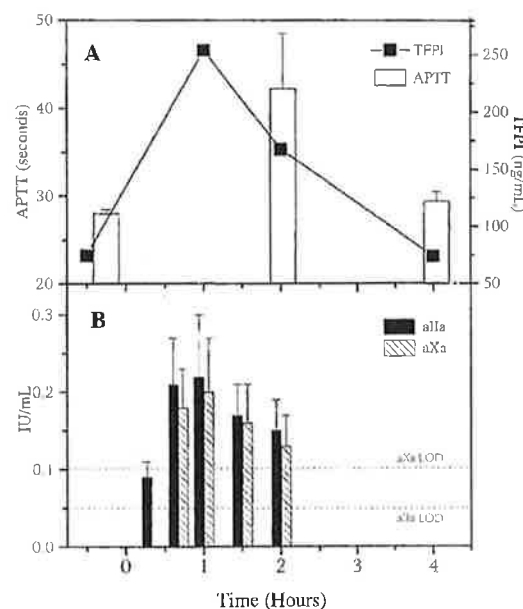


Figure 1. A, aPTT values (seconds) and TFPI concentrations (ng/mL) after oral administration of 10.5 g SNAC and 30 000 IU heparin in human volunteers. B, Anti-factor IIa and Xa concentrations (IU/mL) after oral dosing of 10.5 g SNAC and 30 000 IU heparin in normal volunteers. Stippled lines represent the LOD for anti-factor Xa (aXa) and anti-factor IIa (aIIa), respectively.

subjects receiving 30 000 IU heparin. An anti-factor Xa response was measured in 2 of 6 subjects at 20 000 IU heparin and 4 of the 5 volunteers at 30 000 IU heparin, reaching a peak concentration of 0.20 ± 0.05 IU/mL 1 hour after dosing. Anti-factor IIa was significantly ($P < 0.05$) elevated at 0.67, 1, and 1.5 hours after dosing, reaching a peak concentration of 0.22 ± 0.08 IU/mL 1 hour after 30 000 IU heparin (Figure 1B). TFPI rose from 90.0 ± 8.0 to 224.2 ± 66.7 ng/mL 1 hour after dosing with 20 000 IU heparin ($P < 0.003$) and from 74.9 ± 7.6 to 254.2 ± 12.3 ng/mL 1 hour after the 30 000 IU heparin dose ($P < 0.001$). Following this observation, the -0.5 , 0.33 , 0.67 , and 1.5 -hour samples remaining from the anti-factor IIa analysis were submitted for TFPI assay. This involved a second freeze/thaw cycle, so the data were viewed qualitatively. These data suggest 1.5- to 2-fold increases in TFPI as early as 0.33 hours after dosing in those subjects in whom a significant increase in anti-factor IIa was detected 1 and 1.5 hours after dosing with 30 000 IU heparin.

In the second study, TFPI levels were unaltered in any of the 4 subjects when 30 000 IU heparin was administered orally via nasogastric tube but without SNAC (Table 2). Consistent with these observations, neither anti-factor IIa nor anti-factor Xa was detectable at any time point in any individual.

In the third study, 4 of 12 subjects were excluded from further dosing for safety reasons, because their aPTT response to heparin 10 000 IU SC exceeded baseline values by a factor of ≥ 2.5 -fold. The remaining 7 subjects (6 men) received ascending doses of oral SNAC-heparin. One patient was excluded because of an aPTT elevation > 2.5 -fold greater

TABLE 2. Individual Responses of TFPI to Combination of Heparin Plus Carrier and Either Alone

	Time, h			
	-0.5	1.0	2.0	4.0
Group A, 30 000 IU heparin plus 10.5 g SNAC	75.2	268.3	155.9	65.1
	90.3	287.3	234.6	76.7
	66.6	215.7	96.0	66.9
	91.1	240.1	99.7	63.9
	50.8	259.8	251.8	97.4
Mean±SD	74.8±17.0	254.2±27.4	167.6±73.2	74.0±14.0
Group B, 30 000 IU heparin alone	60.4	61.1	57.7	68.0
	60.7	62.5	67.5	64.3
	66.2	69.4	58.0	59.1
	60.2	55.8	63.1	56.3
Mean±SD	61.9±2.9	62.2±5.6	61.6±4.7	61.9±5.2
Group C				
10.5 g SNAC in vehicle	82.5		98.3	94.1
	77.1		78.3	76.6
	60.6		61.9	73.7
	88.2		79.9	96.3
	45.5		72.3	51.4
	61.3		75.0	65.1
	82.1		88.9	90.1
Vehicle only	73.7		74.3	79.1
	69.5		90.0	86.3
	69.3		79.4	86.3
Mean±SD	69.7±12.8		79.9±8.8	78.8±14.2

No samples were collected at the 1-hour time point after the carrier or its vehicle. TFPI responses are given in ng/mL.

after the third oral dose. The group as a whole exhibited dose-dependent prolongations of mean aPTT and anti-factor Xa (Figure 2). Thus, despite exclusion of the volunteers likely to exhibit a more sensitive response to heparin, mean aPTT was prolonged $172\pm22\%$ above baseline values 30 minutes

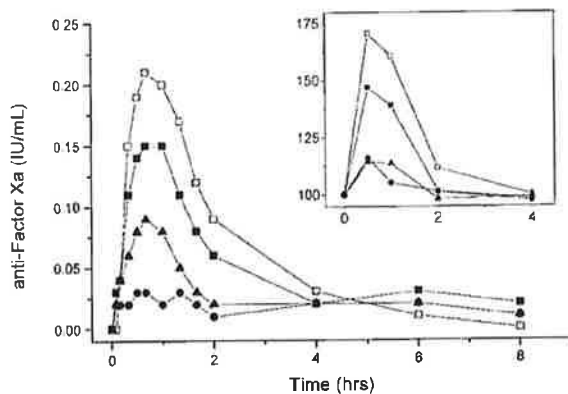


Figure 2. Mean dose-dependent prolongation of anti-factor Xa activity and aPTT as percent at baseline (inset) in response to taste-masked syrup containing SNAC 2.25 g and rising doses of heparin ingested per os. ● indicates 30 000 IU heparin; ▲, 60 000 IU heparin; ■, 90 000 IU heparin; and □, 150 000 IU heparin.

after dosing with 2.25 g SNAC and 150 000 IU heparin. One subject (included in the mean data) failed to exhibit a prolongation in aPTT at any oral dose of SNAC-heparin. After administration of heparin 10 000 IU SC, the aPTT in this individual was 27 seconds at baseline and 33, 34, 34, 37, and 27 seconds at 0.5, 1.0, 2.0, 4.0, and 24.0 hours after dosing. After oral SNAC-heparin, a detectable prolongation of anti-factor Xa was measured in this subject only at 0.5 (0.07 IU/mL) and 1 (0.06 IU/mL) hour after dosing with 2.25 g SNAC and 150 000 IU heparin.

There were no clinically important adverse events in 111 dosings in the 34 subjects. Nausea was reported after 29 of the 111 dosing events. Excluding the vehicle and heparin control groups (16 doses), nausea appeared to be unrelated to SNAC or heparin dosages. Emesis occurred after 14 of the 111 doses and was associated with higher SNAC dose levels only in study 1 (Table 1). Emesis was self described as mild (easily tolerated) in 13 of these individuals. One experienced emesis that interfered with usual activity. Less frequent side effects in study 1 were headache, diarrhea, abdominal pain, dyspepsia, and pharyngitis. Gastrointestinal side effects were not noted in the third study. Neither alterations in blood glucose or serum insulin nor bleeding complications were observed in any of the studies.

Discussion

This report describes the first controlled evidence for the oral absorption of an anticoagulant dose of heparin in humans. Our initial observations were obtained after drug delivery by gavage under double-blind conditions and involved 4 indexes of anticoagulant effect. The results indicate that SNAC does promote oral absorption of heparin in humans. The evidence that links the anticoagulant effect of heparin to prevention of thrombosis holds most strongly for aPTT. The changes in aPTT observed after the single oral dose of heparin administered by gavage were modest. However, TFPI may also reflect the effects of heparin in vivo.²⁷ The maximal effects on TFPI that we observed after oral heparin were an increase of 2.5- to 3-fold. Such increases are within the range of those reported after intravenous dosing of 7500 U heparin (1.5- to 6.5-fold)²⁷ and similar to that observed after 5000 anti-factor Xa units of a low-molecular-weight heparin.²⁷ However, interindividual differences in the anticoagulant response to conventional heparins are well recognized.^{27,28} The peak TFPI concentration we observed was at 1 hour after dosing. This is similar to the response reported by Alban et al²⁸ in healthy male and female human volunteers. Peak values for anti-factor IIa and Xa values were also observed 1 hour after dosing. Thus, the TFPI and anti-factor IIa and Xa concentrations suggest that the timing of the aPTT samples in the first study may have led to an underestimation of the maximum response to oral heparin on this index of anticoagulant effect. Similarly, plasma levels of heparin were not estimated by protamine titration.²⁹ Such an approach may minimize variability in aPTT responses attributable to differences in test reagents.³⁰

Preclinical data from cynomolgus macaques suggested that SNAC at high doses may lower fasting blood sugar.²² There was no evidence, as assessed by glucose and insulin, of a hypoglycemic response to SNAC in any individual at any dosing level. However, administration of a large volume (70 mL) of this unformulated solution to fasting volunteers in the initial study did produce digestive symptoms, most commonly nausea. These accounted for more than half the reported side effects. Twelve of the 30 subjects who received SNAC in combination with an anticoagulant dose of heparin in the first study reported gastrointestinal discomfort. Of the 30 subjects, 13 experienced mild emesis and 1 reported moderate emesis at this SNAC dose level. This last event was considered dose limiting. Although tolerated, SNAC has a bitter taste as an unformulated solution.

To address the issue of gastrointestinal intolerance, we developed a taste-masked preparation. Furthermore, we reduced the dose of SNAC to 2.25 g while evaluating the pharmacodynamic efficacy of increasing doses of heparin when the preparation was administered per os rather than by gavage. Given the interindividual variability in response to heparin, we wished to bias our experience against including those individuals most sensitive to heparin, for safety reasons. Thus, we excluded 5 of the 12 volunteers whose aPTT was prolonged by ≥ 2.5 times in response to 10 000 IU heparin SC from exposure to SNAC-heparin. Despite this, heparin dose dependently prolonged both indexes of anticoagulation. For example, 0.5 hours after dosing with 150 000 IU SNAC and

2.25 g heparin, aPTT had been prolonged from 30.3 ± 2.4 to 51.7 ± 8.6 seconds. Furthermore, with the individual who failed to prolong the aPTT with any dose of SNAC-heparin excluded, aPTT was elevated from 30.7 ± 2.3 to 55.3 ± 7.4 seconds. It is unclear why this 1 subject failed to respond to SNAC-heparin.

This study establishes the feasibility of oral heparin delivery in humans. Both unfractionated and low-molecular-weight heparins have been demonstrated to be efficacious in the prevention of thrombotic venous and arterial disease.^{1-7,31} Furthermore, nonanticoagulant properties of heparin may also reduce cardiovascular risk.³² Long-term therapy with subcutaneous administration of low-molecular-weight heparins is being evaluated currently in comparison with long-term oral warfarin therapy in the prevention of postoperative venous thrombosis.⁴ Indeed, long-term subcutaneous administration of specific ATs may prove even more efficacious than low-molecular-weight heparins.³³ Although further modifications of drug delivery are desirable for clinical application, the present observations raise the possibility of extended oral dosing with heparins or specific ATs.^{34,35} They may also have a broader relevance to the absorption of macromolecules in general.

Acknowledgments

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APPENDIX C

A novel per-oral insulin formulation: proof of concept study in non-diabetic subjects

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Abstract

Aims The aim of our study was to examine the absorption of insulin from the gastrointestinal (GI) tract, using a novel oral formulation—adding a delivery agent SNAC (sodium N-[8-(2-hydroxybenzoyl)amino] caprylate) in combination with insulin.

Methods Capsules containing insulin and SNAC, in various combinations, were administered orally, as a single dose, to 12 non-diabetic subjects and four control subjects (receiving SNAC or insulin only) in order to assess its biological effect and safety. Plasma glucose levels, insulin and C-peptide concentrations, as well as SNAC levels, were determined, at timed intervals up to 4 h.

Results In all cases, a glucose-lowering effect was demonstrated, preceded by an increase in plasma insulin levels. The nadir of plasma glucose levels appeared after 30–50 min, following the ingestion of the mixture. The plasma insulin levels were found to parallel the blood SNAC levels. Plasma C-peptide levels were suppressed by the lowered glucose levels achieved concurrent with the increasing amount of exogenous insulin absorbed, indicating that the secretion of endogenous hormone was partially abolished. There were no biological effects regarding blood glucose levels upon administration of SNAC or insulin when given alone. No adverse effects were detected during the trial or several weeks after the trial.

Conclusions Insulin in combination with a novel delivery agent, SNAC, given orally, is absorbed through the GI tract in a biologically active form. This was demonstrated by a glucose lowering effect of the mixture as well as a suppression of an endogenous insulin secretion.

Diabet. Med. 21, 354–357 (2004)

Keywords C-peptide, gastrointestinal absorption, oral insulin

Abbreviations GI, gastrointestinal; IRB, investigational review board; SNAC, (sodium N-[8-(2-hydroxybenzoyl) amino] caprylate)

Introduction

Since its discovery in 1922, insulin has become essential therapy for patients with Type 1 and many with Type 2 diabetes [1]. Over the years, improvements in the structure of insulin and/or its delivery have had a great impact on therapy. Today the most common and preferred insulin is synthesized by

recombinant DNA technology [2]. Many synthetic insulin analogues are available with improved pharmacokinetic profiles [3–5].

Because insulin is a peptide, it is not absorbed to any extent from the gastrointestinal (GI) tract, presumably due to its molecular size and enzymatic degradation. Two obstacles had to be overcome in order to enable the hormone to penetrate into the blood stream—dermal and mucosal barriers and the degradation of the molecule by proteolytic enzymes in the GI tract [6]. The rectal and oral routes have a potential theoretical advantage over other routes of administration as these routes

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allow the absorption of the drug through the liver, closely mimicking the physiological pathway [7]. Transdermal, nasal, pulmonary and oral preparations are still in developmental stages, and none are as yet commercially available [8].

For some years, our group has published research results pertaining to rectal and oral administration of insulin and heparin [7,9,10]. We have demonstrated the capacity of different enhancers to alter the mucosal barrier and promote the transfer of peptides and polysaccharides from the intestinal lumen into the blood circulation.

In this report, we present initial data on utilizing a novel formulation, which enables the absorption of insulin following oral ingestion in non-diabetic volunteers. We used an Emisphere delivery agent, which was different from the traditional permeation enhancers. This new delivery agent reversibly and non-covalently binds to the peptide itself, changing it to a more lipophilic form, that enables gastrointestinal absorption of the peptide while retaining its biological effect.

Patients and methods

Subjects

The study included 16 healthy, non-smoking, male volunteers. Mean age was 26 years (range 22–33), and mean BMI was 25 (range 22–27). Subjects were enrolled after giving written informed consent for participation in the study, which was approved by the Investigational Review Board (IRB) of the Hospital. Twelve subjects received SNAC and insulin in various doses. Of the four control subjects, two received 2100 mg SNAC alone and two received 350 U insulin alone.

Experimental design

One week prior to the study, volunteers had a complete physical examination, blood count and blood chemistry. The study took place in the morning hours, after an overnight fast. A catheter was inserted into the ante-cubital arm vein.

Blood was withdrawn at baseline twice, 5–10 min apart, and at timed intervals (every 5–15 min) after the administration of the test drug (with a glass of water), for the determination of plasma glucose, insulin, the delivery agent SNAC and C-peptide. Blood was sampled every 5 min during the first hour, and after that, every 15 min until blood glucose reached basal levels.

Materials and methods

The delivery agent SNAC was supplied by Emisphere Technologies Inc., Tarrytown, NY, USA and was stored desiccated at room temperature (22°–24°C) until use.

Recombinant Crystal Human Zinc Insulin was shipped directly by Eli Lilly and Company (Indianapolis, IN, USA), and was stored at –20°C. Standard capsules were made of gelatin, size 000. Each capsule contained 700 mg SNAC. Nine of the 12 subjects received three capsules, resulting in a total dose of 2100 mg SNAC. The three remaining subjects received four

capsules for a total of 2800 mg SNAC. Insulin was given in a dose escalation format, each capsule contained 50, 100, 135 or 150 units of insulin. The control group received a total dose of either 2100 mg of SNAC or 350 units of insulin. The capsules were prepared by a pharmacist at Hadassah Medical Organization 1 day prior to the administration.

During the trial, blood glucose levels were measured in real time by Glucometer Elite (Bayer Corporation, Elkhart, Indianapolis, IN, USA). At the end of the trial, plasma glucose concentrations were measured in all the samples collected using an Enzymatic UV test (Roche Diagnostics Indianapolis, IN, USA). Plasma insulin and C-peptide concentrations were determined using radio-immunoassay kits produced by Linco Research, Inc., St Charles, MO, USA. SNAC levels were measured using HPLC pump, model LC-410 with PE Nelson 900 A/D, Perkin Elmer Corporation, Shelton, CT and RP-HPLC column, Luna C18 IB-Sil 5 µ 254 × 4.60 mm, with 5-µm mini filter, Phenomenex, Torrance, CA, USA.

Results

Young, fasting, non-diabetic volunteers ingested a mixture of SNAC and insulin. A decrease in blood glucose levels is shown in Fig. 1, which depicts a representative pattern of plasma insulin and glucose values achieved in one volunteer during the trial; every volunteer demonstrated the same pattern. The increase in insulin levels appeared 20–40 min after the ingestion of the capsules and preceded the drop in glucose levels. The nadir of the glucose concentrations ranged between 15 and 55% of the initial values, proportional to the total insulin dose given. The maximal decrements attained are shown in a dose–response curve model (Fig. 2). This figure represents the results of nine volunteers, who received a fixed dose of 2100 mg SNAC with increasing doses of insulin. In order to examine the possibility of a greater response, by increasing the dose of the delivery agent, three volunteers received a higher dose SNAC (2800 mg) with 400 U insulin. This resulted in an attenuated response, most likely due to a loss of solubility due to the excess carrier (results not shown).

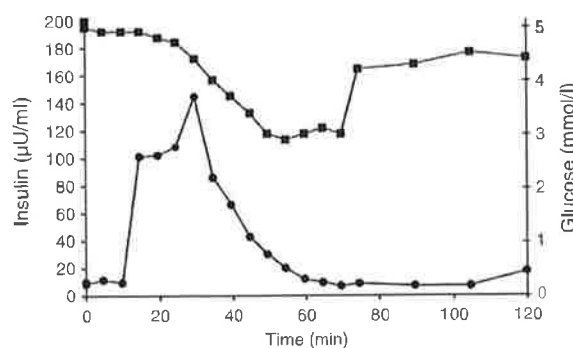


Figure 1 A representative pattern of the timed response of plasma insulin (●) and glucose (■) levels following the ingestion of 400 units of insulin together with 2100 mg of SNAC.

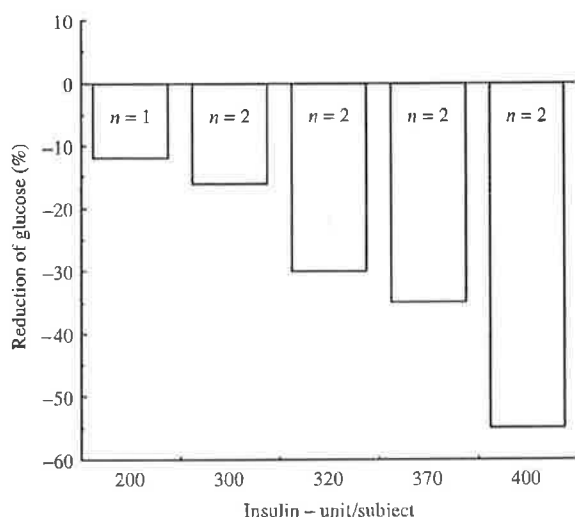


Figure 2 Per cent decrease of plasma glucose levels in relation to oral dosing of 200–400 units insulin with 2100 mg SNAC.

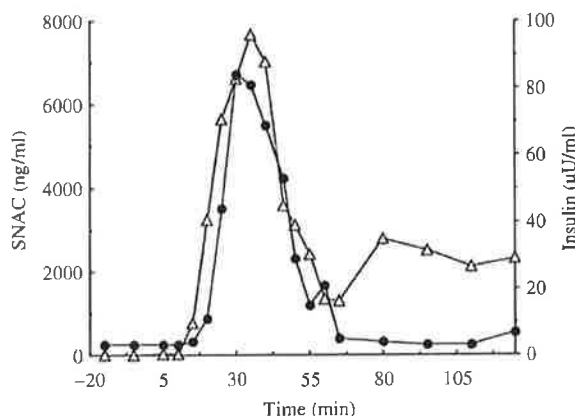


Figure 3 A representative pattern of plasma insulin levels (●) and plasma SNAC levels (Δ) following the ingestion of 400 units of insulin and 2100 mg of SNAC.

Figure 3 shows representative blood levels of SNAC and insulin in one subject (similar pattern was shown with other volunteers). It is apparent that the absorption of both ingredients occurred concurrently.

SNAC (in a single dose of 2100 mg) or insulin (in a single dose of 350 units), each given alone to the four control subjects, did not change the blood glucose concentrations level.

To evaluate the extent of enteral absorption of insulin, and the associated hypoglycaemia, we measured the plasma C-peptide concentration. The absorption of insulin brought about a decrease in glucose levels (as depicted in Fig. 2) and an average drop of 30% in the C-peptide levels, indicating a decrease in endogenous insulin secretion.

Discussion

In this preliminary dose escalation study, we demonstrated that insulin absorption through the gastrointestinal tract in humans is feasible. The delivery agent may interact selectively with a drug and does not facilitate the indiscriminate transport of other substances near the cell membrane [11,12]. This interaction is fully reversible. The concomitant appearance in the plasma of the delivery agent and insulin, indicates the necessary presence of both the carrier and the hormone at the absorption site in the gut. Comprehensive toxicological screening regarding the use of SNAC in animals indicated that it is free of adverse effects [13]. Moreover, clinical follow-up on our volunteers did not reveal any adverse effects, even after 8 weeks.

In each dose given to the volunteers, a glucose-lowering response was apparent, and the lowest level was accomplished 30–50 min after the ingestion of the test drug. However, the short lag-time between ingestion of the capsules and the insulin peak attained, suggests that the hormone was absorbed in the upper part of the gastrointestinal tract.

The delivery efficiency pertaining to insulin given orally indicates that insulin absorption is still low. With the Emisphere formulation, the efficiency of absorption appears to be at least 5–10%, but needs to be further assessed. Enteral absorption carries the insulin into the body through the liver. It is believed that portal transport of insulin is metabolically superior than when given peripherally [14,15].

In conclusion, fasting non-diabetic volunteers given increasing doses of insulin (200–400 units) orally with the delivery agent SNAC (2100 mg), show a decrease in glucose levels in a dose-response fashion with an increase in insulin levels that preceded the glucose lowering effect of the mixture. A hypoglycaemic-mediated suppression of C-peptide secretion was also noted. In this preliminary study, the use of oral insulin appears efficient.

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APPENDIX D

talizations, co-morbidity, use of diuretics, ACE inhibitors, calcium-channel blockers, NSAIDs, sex hormones, and other lipid-lowering drug therapies. Statin use was defined as a minimum use of six months.

In the study base, 3129 cases were identified and matched to 16,976 controls. Statin use (79.6% simvastatin) was associated with a risk reduction of cancer of 20% (adjusted odds ratio (OR) 0.80; 95% CI: 0.66–0.96). Statins were protective when used longer than

4 years (adjusted OR 0.64; 95% CI: 0.44–0.93) or when more than 1350 Defined Daily Doses were taken (adjusted OR 0.60; 95% CI: 0.40–0.91). Patients that had their statin therapy stopped returned to baseline risks within six months.

This observational study provides evidence for a protective effect of statins against cancer.

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Population pharmacokinetics of efavirenz in an unselected cohort of HIV-1-infected individuals

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Introduction: Efavirenz (EFV) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI), which shows good inhibitory activity against HIV-1. EFV is always used as part of combination regimens to ensure adequate suppression of viral replication and thus to prevent resistance. Several studies in HIV-infected patients showed that treatment failure and side effects were associated with low and high EFV plasma levels, respectively.

Several patient characteristics (e.g. demographics, co-morbidity) may have large impact on the pharmacokinetics of EFV. Knowledge of these factors is very useful in the treatment of HIV-infected patients. In order to establish the pharmacokinetics and factors involved in the variability of therapy, a population pharmacokinetic analysis was performed in an unselected cohort of HIV-1-infected individuals.

Methods: Included patients were ambulatory HIV-1-infected patients from the outpatient clinic of the Academic Medical Centre and the Slotervaart Hospital, Amsterdam, The Netherlands. Data were retrospectively collected from September 1998 to January

2002. The pharmacokinetics of EFV were described with a two-compartment model with first-order elimination from the central compartment. A chain of transition compartments between the absorption and the central compartment described the absorption process. The rate constants (K_{12}) between these compartments were set equal to each other. NONMEM (Version V1.1) was used throughout. Population pharmacokinetic parameters (clearance (CL/F), volume of distribution (V/F) and transition rate constant (K_{12})) were estimated. Interindividual (IIV) and interoccasion variability (IOV) in the pharmacokinetic parameters were estimated. Residual variability was modelled with a combined additive and proportional error model.

Results: From 173 outpatients a total number of 315 EFV plasma concentrations at a single timepoint and full pharmacokinetic curves of 40 patients were available resulting in a database of 1009 EFV plasma concentrations. A chain of three transition compartments proved to be the best model to describe the absorption process, which shows a slow onset of absorption. CL/F of EFV was 11.4 L/h with an IIV and IOV of 43% and 39%, respectively. V/F was 181 L (IIV = 58%) and the K_{12} was 3.05 h⁻¹ (IIV = 42%).

Conclusions and further perspectives: The pharmacokinetics of EFV were adequately described with the developed population pharmacokinetic model. In future studies the influence of patient characteristics on the pharmacokinetics of EFV will be determined. Furthermore, this model will be validated and a Bayesian TDM strategy based on this model will be developed and validated.

Pharmacokinetics, pharmacodynamics and tolerability of oral cromolyn sodium/SNAC capsules in healthy and allergic male subjects

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Introduction: cromolyn (cromoglycate) sodium is used by inhalation as a prophylactic agent in the treatment of mild to moderate asthma and as a nasal inhaler to treat seasonal allergic rhinitis. Cromolyn, when given orally, is poorly absorbed from the gastrointestinal tract. SNAC is an acetylated amino acid molecule that facilitates the oral absorption of (macro)molecules. In a sheep model of allergic asthma, orally delivered cromolyn/SNAC was successful in mitigating the early and late phase responses of the allergic reaction.

Aims: To evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of cromolyn when given as capsules twice daily for 10 days in combination with SNAC.

Methods: Double-blind, randomised, placebo-controlled, multiple dose study with oral administration of cromolyn/SNAC twice daily for 10 days in two groups of 24 male subjects, Group 1: 12 healthy subjects (8 subjects cromolyn 80 mg/SNAC 600 mg, 2 subjects cromolyn 80 mg/placebo, 2 subjects placebo/placebo). Group 2: 12 allergic subjects (8 cromolyn 80 mg/SNAC 600 mg, 2 subjects cromolyn 80 mg/placebo, 2 subjects placebo/placebo). Plasma samples were collected for PK assessments on cromolyn and SNAC. The following PD parameters were assessed: wheal and flare reactions after intracutaneous codeine/histamine (Group 1) and after intracutaneous allergen/histamine (Group 2); VAS scores and peak nasal inspiratory flow (PNIF) after intranasal allergen provocation (Group 2).

Results: Cromolyn PK parameters are summarized in Table 1. Cromolyn given orally in combination with SNAC was rapidly absorbed with peak concentrations at approx. 20 min in fasting volunteers (allergic and healthy). When cromolyn was administered without SNAC, the oral absorption was negligible. The plasma-concentrations of cromolyn and SNAC peaked almost concomitantly and were almost back to zero after 4 hours. Cromolyn and SNAC PK parameters were similar on days 1 and 10, with considerable between-subject but limited within-subject variability.

Table 1 Cromolyn PK parameters; mean (SD).

Treatment	Day	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)
80 mg cromolyn/ 600 mg SNAC n = 16	1	63.0 (41.2)	0.34 (0.07)	40.3 (26.4)	39.7 (26.3)
	10	71.3 (46.4)	0.35 (0.10)	48.8 (26.1)	—
80 mg cromolyn/ placebo n = 3 (*n = 2)	1	1.1 (0.3)	1.83 (0.29)	— (0.1)*	4.7
	10	2.3 (0.7)	3.17 (2.47)	—	—

Accumulation of linezolid in critically-ill patients with complete renal failure is prevented using continuous venovenous hemofiltration (CVVH)

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Introduction: Linezolid, a new oxazolidinone antibacterial agent, is being increasingly used in critically ill patients. An optimal dosing regime of linezolid in these patients receiving continuous renal replacement therapy is not available. Two patients with acute renal failure were evaluated to obtain specific pharmacokinetic characteristics.

Methods: Two patients, a 42-year-old man with gut perforation (SOFA-score 16) and a 55-year-old man with necrotising colon (SOFA-score 12), were treated with linezolid 600 mg(IV) every 12 hours. Both patients were receiving CVVH (Baxter PSIIF-1200 hemofilter, predilution (2 L/h), ultrafiltrate-flow: 150 ml/min), during the initial doses and were without CVVH during the following doses. Both blood (1st and 7th dosage interval) and ultrafiltrate samples (1st) were collected. The pharmacy has developed an HPLC-assay to determine and quantify linezolid in serum and dialysate. Using a non-compartment model MW-Pharm Kin-fit software calculated the pharmacokinetic variables.

Results: Figure 1 shows the serum concentration-time curves of the 2 patients with (day 1) and without CVVH (day 4). The pharmacokinetic characteristics compared to population-data of patients with normal renal function (Perry and Jarvis, 2001) are summarised in Table 1.

Conclusion: In critically ill patients with complete renal failure accumulation of linezolid occurs. During CVVH normal to high clearance of linezolid occurs.

However, clearance of linezolid via CVVH appears to be limited. The differences between the total clearance with or

There was no relationship found between SNAC and cromolyn plasma AUCs. For the PD parameters no apparent differences were found between treatments, and no changes from baseline within treatments.

Conclusions: Simultaneous administration of SNAC clearly enhanced the oral absorption of cromolyn, with considerable between-subject variability but limited within-subject variability. No effects could be observed on PD parameters assessed in this study. SNAC and cromolyn, either alone or in combination were well tolerated after oral administration in healthy and allergic human subjects.

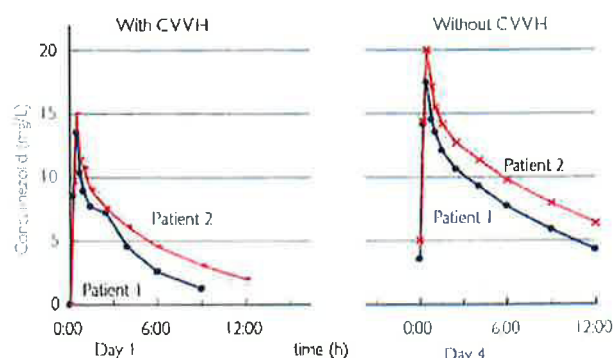


Figure 1

Table 1

	V_d (L)	$T_{1/2}$ (h)	Cl_{tot} (ml/min)	Cl_{nr} (ml/min)	Cl_{CVVH} (ml/min)	Rec_{CVVH} (mg (%))	S.C.
Pt 1 _{CVVH}	47.9	2.6	214.8	189.8	25	60.2 (10%)	0.81
Pt 1	43.0	7.1	70.3	70.3	—	—	—
Pt 2 _{CVVH}	55.8	4.9	130.3	105.3	25	93.9 (16%)	0.69
Pt 2	39.2	9.6	47.5	47.5	—	—	—
Pt _{ref} (SD)	45 (5)	4.4 (2.4)	138 (39)	90 (25)	—	—	—

V_d = volume of distribution; $T_{1/2}$ = elimination half-life; Rec_{CVVH} = CVVH-recovery; S.C. = sieving-coefficient; Cl_{tot} = total-, Cl_{nr} = nonrenal- and Cl_{CVVH} = CVVH-clearance.

without CVVH cannot be explained by CVVH clearance only. The accumulation might also be the result of an equilibrium with the major, rate-limiting metabolite of linezolid, PNU-142586 (Slatter *et al.*, 2001). Due to its physiochemical properties this metabolite can only be eliminated by renal or CVVH clearance.

Perry CM, Jarvis B. *Drugs* 2001; **61**: 525–551.

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APPENDIX E



US008513300B2

(12) **United States Patent**
Abbas et al.

(10) **Patent No.:** **US 8,513,300 B2**
(45) **Date of Patent:** **Aug. 20, 2013**

(54) **FORMULATIONS FOR ORAL
ADMINISTRATION OF CROMOLYN SODIUM**

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patent is extended or adjusted under 35
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(21) Appl. No.: **12/103,821**

(22) Filed: **Apr. 16, 2008**

(65) **Prior Publication Data**
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Related U.S. Application Data

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application No. PCT/US02/38247 on Nov. 29, 2002,
now abandoned.

(60) Provisional application No. 60/334,395, filed on Nov.
29, 2001, provisional application No. 60/384,916,
filed on May 24, 2002.

(51) **Int. Cl.**
A01N 43/16 (2006.01)
A61K 31/35 (2006.01)

(52) **U.S. Cl.**
USPC **514/451**; 514/183; 514/449; 424/400

(58) **Field of Classification Search**
USPC 514/183
See application file for complete search history.

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Primary Examiner — Jeffrey S. Lundgren

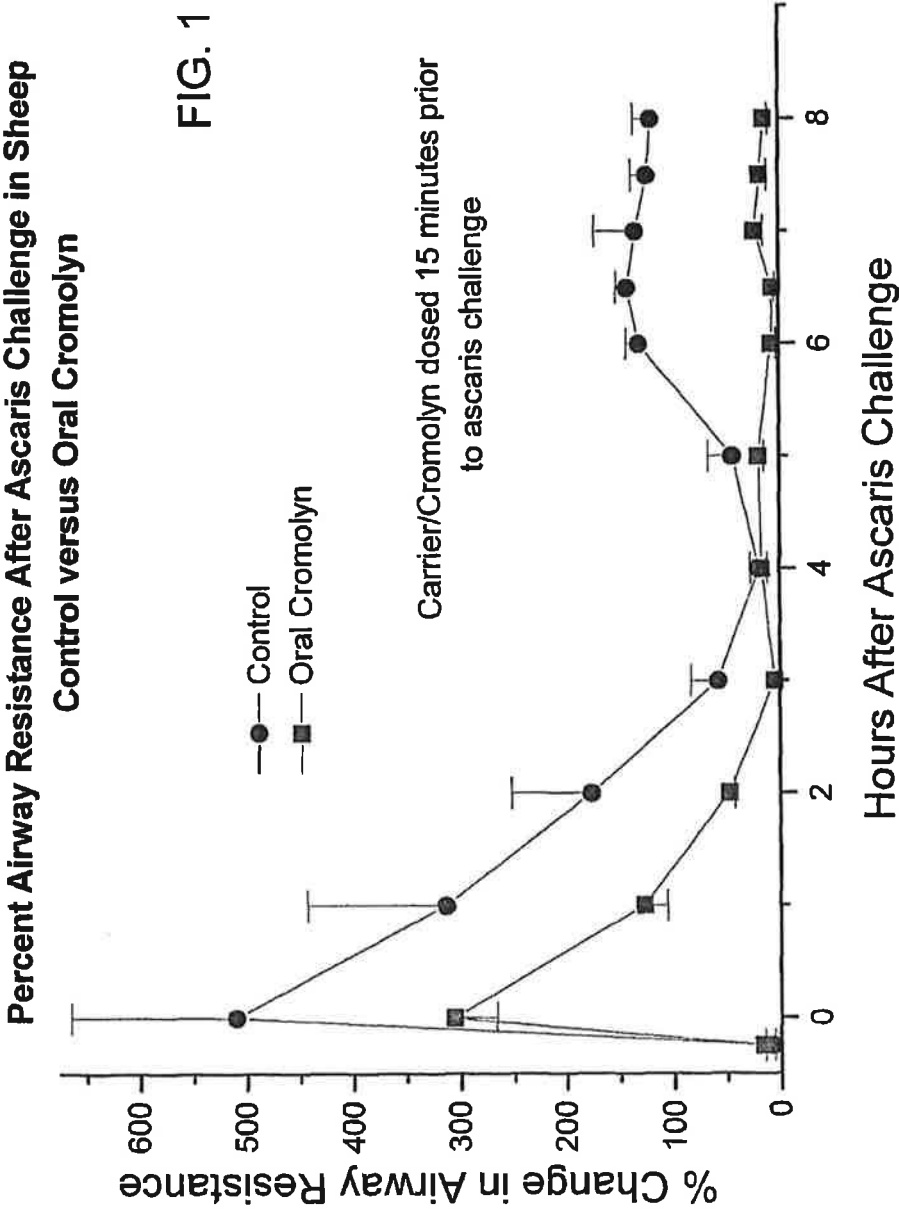
Assistant Examiner — Meghan Finn

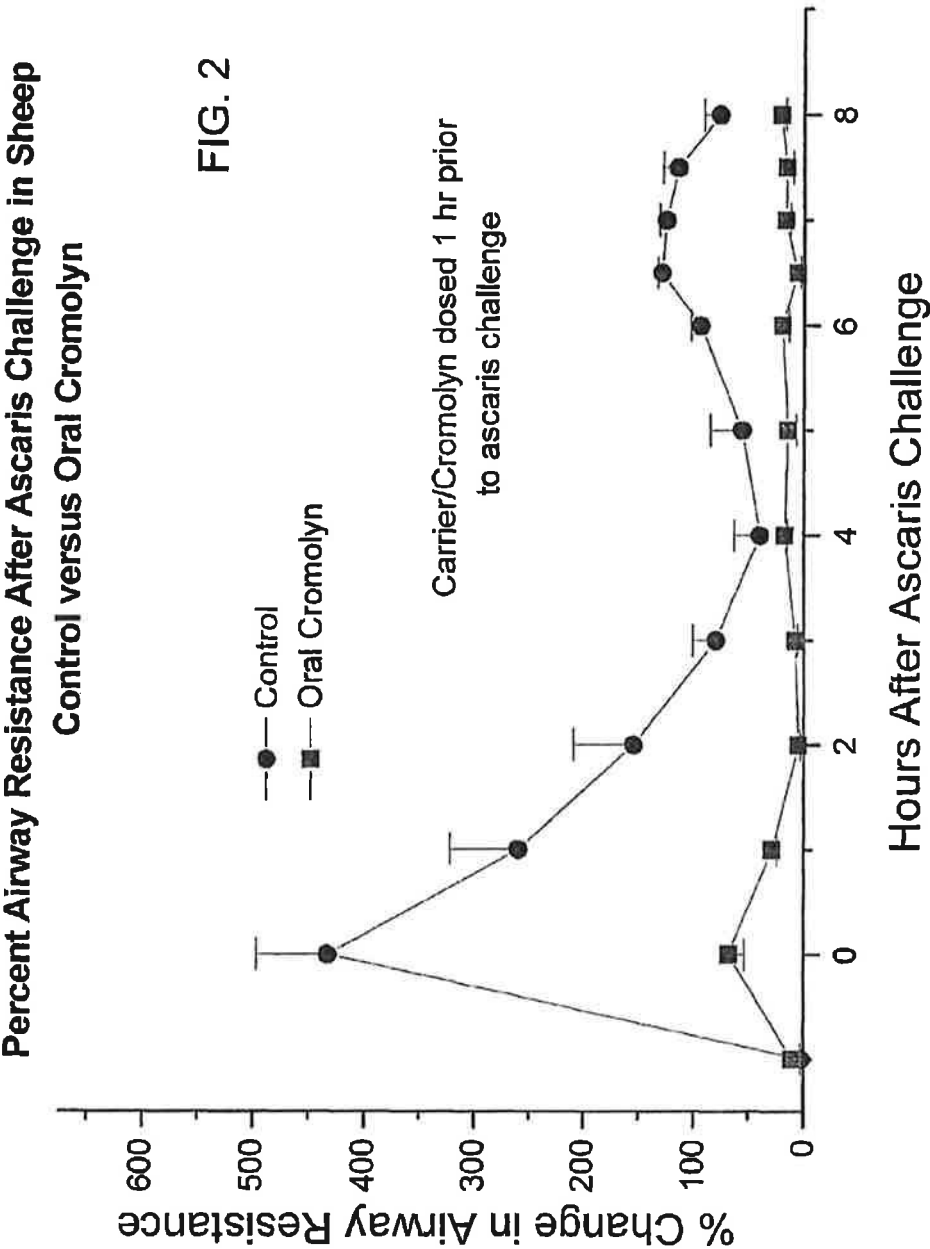
(74) Attorney, Agent, or Firm — Blank Rome LLP

(57) **ABSTRACT**

An oral dosage form comprises cromolyn sodium (sodium or disodium cromoglycate), and an acylated amino acid delivery agent.

28 Claims, 12 Drawing Sheets





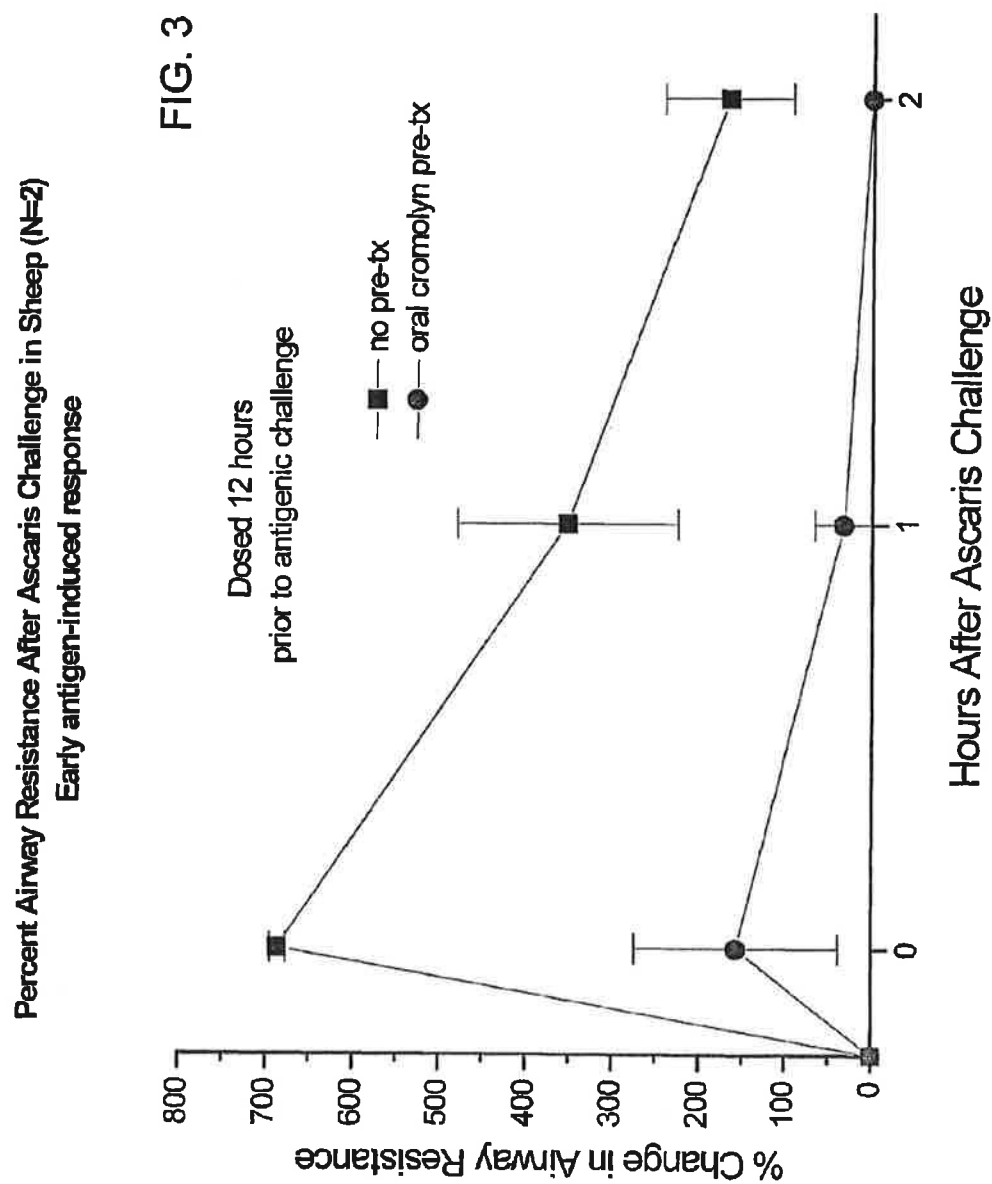


Figure 4: Treatment Group 1 (40 mg Cromolyn/1200 mg SNAC) Cromolyn Concentrations (ng/mL) in Human Plasma

Sample ID	Time, h	Subject									
		S001	S002	S003	S004	S005	S006	S007	S008	S009	S010
P0	0.000	bql	bql	bql	bql	bql	bql	bql	bql	bql	bql
P1	0.083	bql	bql	bql	bql	bql	0.566	bql	bql	bql	bql
P2	0.167	1.54	6.00	0.818	0.614	9.33	7.16	bql	bql	bql	bql
P3	0.250	8.73	26.87	16.01	7.44	28.48	29.32	2.93	7.55	bql	bql
P4	0.333	13.16	41.79	24.00	13.66	32.31	32.13	5.66	15.72	bql	0.325
P5	0.500	8.77	31.66	16.71	9.81	21.26	21.33	5.15	10.87	bql	0.696
P6	0.750	5.31	22.12	11.86	5.50	11.35	10.62	4.10	6.21	1.10	0.930
P7	1.00	4.07	14.62	6.78	3.12	8.59	6.18	2.92	3.76	0.963	0.836
P8	1.25	2.48	9.99	4.98	1.99	5.81	4.44	1.72	2.58	1.31	1.46
P9	1.50	2.01	5.94	3.71	1.18	3.89	2.55	2.36	1.75	1.16	2.64
P10	2.00	1.02	3.02	1.50	0.879	1.83	1.43	1.82	0.928	1.30	2.94
P11	2.50	0.723	2.15	0.863	0.453	0.661	0.948	0.884	0.771	1.26	1.99
P12	3.00	0.430	1.47	0.407	bql	0.584	0.636	0.659	0.570	1.71	1.24
P13	4.00	bql	0.624	bql	bql	0.392	0.538	0.593	0.490	0.692	0.500
P14	6.00	bql	bql	0.554	1.13	bql	0.484	bql	0.463	1.01	0.535
P15	8.00	bql	bql	0.343	0.616	0.356	0.682	0.451	bql	0.724	0.946

bql: < 0.3125 ng/mL

NC: Not Calculated

Run Numbers: 011108-S06, 011108-S07, and 011109-S09

Figure 5: Treatment Group 2 (80 mg Cromolyn/1200 mg SNAC) Cromolyn Concentrations (ng/mL) in Human Plasma

Sample ID	Time, h	Subject									
		S001	S002	S003	S004	S005	S006	S007	S008	S009	S010
P0	0.000	bql	bql	bql	bql	bql	bql	bql	bql	bql	bql
P1	0.083	bql	bql	bql	bql	bql	bql	bql	bql	bql	bql
P2	0.167	1.34	1.10	1.06	bql	9.26	8.10	bql	bql	bql	bql
P3	0.250	9.22	47.82	31.62	15.62	57.58	46.96	12.42	12.62	bql	bql
P4	0.333	19.52	75.16	40.16	32.40	80.12	46.72	35.24	19.74	bql	bql
P5	0.500	12.08	49.84	37.22	26.40	52.62	29.02	31.10	13.92	bql	bql
P6	0.750	6.36	31.76	21.56	15.54	28.84	14.54	18.42	8.26	bql	bql
P7	1.00	4.72	22.44	14.10	12.68	16.74	9.00	10.78	4.12	bql	bql
P8	1.25	2.92	14.78	11.38	9.98	10.50	6.26	6.70	3.26	bql	bql
P9	1.50	2.04	9.40	7.90	5.98	7.08	5.14	4.66	2.30	bql	bql
P10	2.00	1.06	5.76	5.06	3.16	3.88	3.68	2.42	1.24	bql	bql
P11	2.50	0.632	3.46	2.28	1.67	2.12	1.47	1.09	0.746	bql	bql
P12	3.00	bql	2.76	2.04	0.686	1.59	1.18	0.904	0.890	bql	bql
P13	4.00	bql	1.51	0.866	bql	0.802	0.710	0.616	0.450	bql	bql
P14	6.00	0.862	0.782	2.22	1.44	0.514	0.812	0.596	0.570	bql	bql
P15	8.00	bql	bql	3.24	1.21	0.716	0.658	bql	bql	bql	bql

bql: < 0.3125 ng/mL

NC: Not Calculated

Run Numbers: 011106-S04 and 011107-S05-r

Figure 6: Treatment Group 3 (80 mg Cromolyn/600 mg SNAC) Cromolyn Concentrations (ng/mL) in Human Plasma

Sample ID	Time, h	Subject							
		S001	S002	S003	S004	S005	S006	S007	S008
P0	0	bql	bql	bql	bql	bql	bql	bql	bql
P1	0.083	bql	bql	bql	bql	bql	bql	bql	bql
P2	0.167	bql	bql	30.22	0.700	16.34	8.40	2.14	1.26
P3	0.25	16.60	26.62	64.76	11.90	29.44	40.76	16.20	41.56
P4	0.333	27.30	53.32	100.9	36.50	30.10	52.56	24.66	40.28
P5	0.5	21.50	40.10	62.46	29.60	16.82	25.66	19.16	26.60
P6	0.75	11.32	19.76	29.02	15.26	10.22	14.80	10.76	16.76
P7	1	9.34	14.78	15.20	12.94	7.06	8.30	13.16	9.92
P8	1.25	6.58	14.08	21.23	10.13	5.71	5.18	6.06	5.11
P9	1.50	4.45	9.25	11.61	7.24	4.10	4.10	3.89	3.68
P10	2.00	2.46	5.07	5.98	5.11	2.32	1.99	2.06	1.97
P11	2.50	1.25	3.00	3.12	4.13	1.38	1.05	0.990	1.38
P12	3.00	1.01	2.07	1.77	3.05	0.954	1.15	0.903	0.917
P13	4.00	0.406	1.03	1.93	1.22	0.672	0.701	0.538	0.719
P14	6.00	0.839	0.567	1.86	1.23	0.495	0.423	0.600	0.546
P15	8.00	0.399	bql	1.01	0.906	1.06	0.359	0.445	0.437

bql: <0.3125 ng/mL

NC: Not Calculated

Run Numbers: 011103-S03 and 011111-S10

Figure 7: Treatment Group 4 (120 mg Cromolyn/200 mg SNAC) Cromolyn Concentrations (ng/mL) in Human Plasma

Sample ID	Time, h	Subject							
		S001*	S002	S003	S004	S005*	S006*	S007	S008
P0	0.000	bql	bql	bql	bql	bql	bql	bql	bql
P1	0.083	0.374	bql	1.02	bql	3.67	0.333	bql	bql
P2	0.167	2.90	1.00	1.90	0.551	7.28	bql	1.90	3.72
P3	0.250	11.11	82.26	1.73	10.46	41.48	3.50	5.10	12.61
P4	0.333	29.59	85.90	2.18	46.05	45.16	26.54	5.57	13.22
P5	0.500	36.07	55.25	2.74	54.53	29.30	13.46	5.73	7.85
P6	0.750	28.46	27.32	3.38	31.78	14.05	8.50	4.02	5.46
P7	1.00	19.53	15.75	3.14	17.70	10.93	5.72	3.82	3.01
P8	1.25	13.74	11.39	2.86	12.77	7.35	5.55	3.14	1.87
P9	1.50	10.05	8.11	2.66	10.86	5.30	4.24	2.15	1.48
P10	2.00	7.00	9.90	2.80	8.01	3.02	2.50	1.38	0.941
P11	2.50	5.90	4.25	3.08	7.18	1.60	1.49	1.15	0.694
P12	3.00	3.67	6.21	2.47	5.54	1.30	1.48	0.760	0.606
P13	4.00	2.87	6.11	2.78	2.71	0.943	0.928	0.963	0.861
P14	6.00	2.25	1.76	2.84	2.05	0.524	0.485	0.686	1.44
P15	8.00	1.25	0.461	2.18	1.26	0.849	0.522	0.895	0.912

bql: < 0.3125 ng/mL

NC: Not Calculated

Run Numbers: 011109-S08 and 011109-S09

* The 0 and 0.083 h samples were switched during initial sample processing and confirmed by reassay

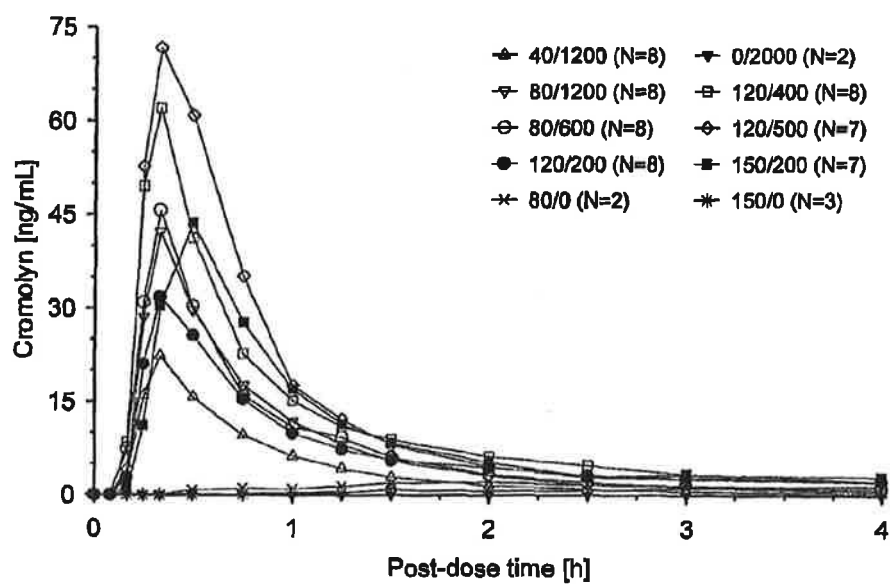


FIG. 8

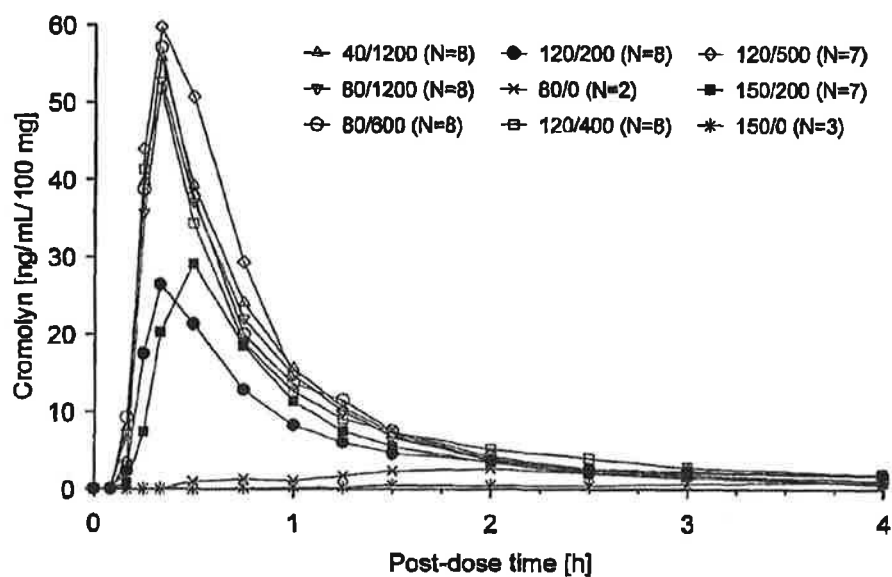


FIG. 9

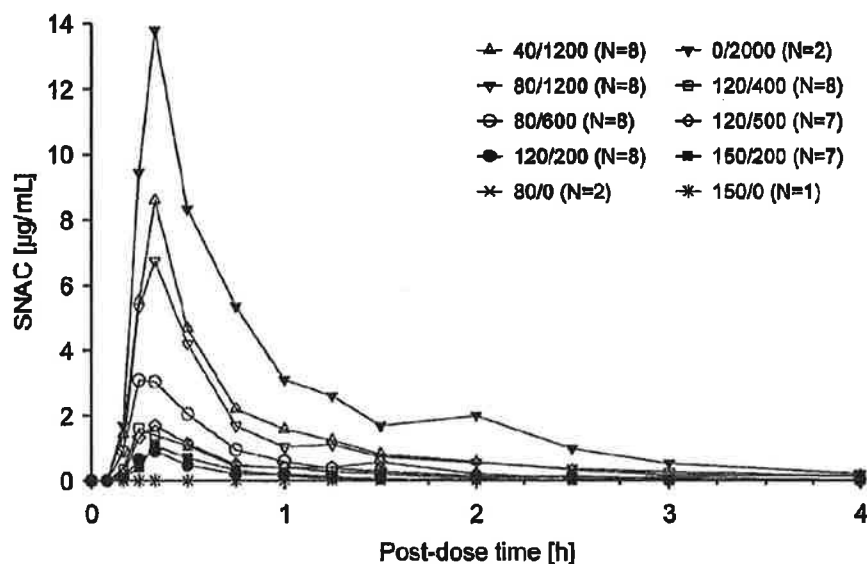


FIG. 10

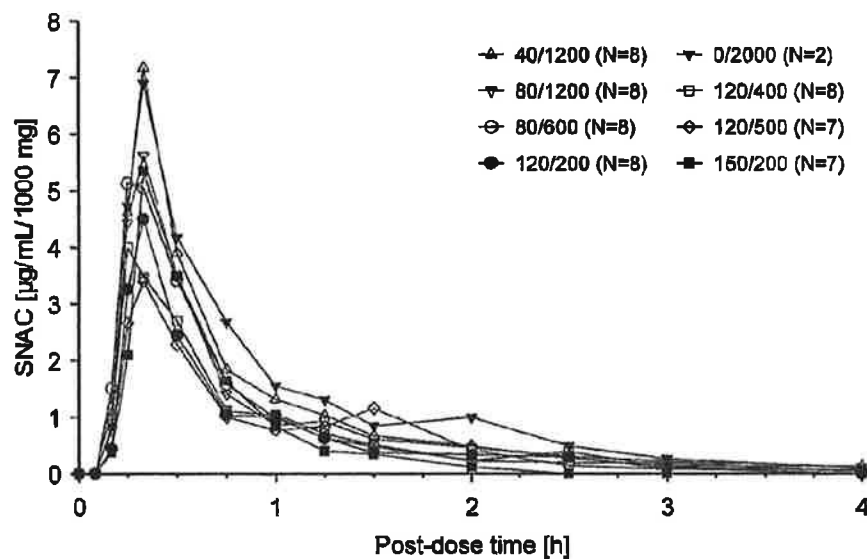


FIG. 11

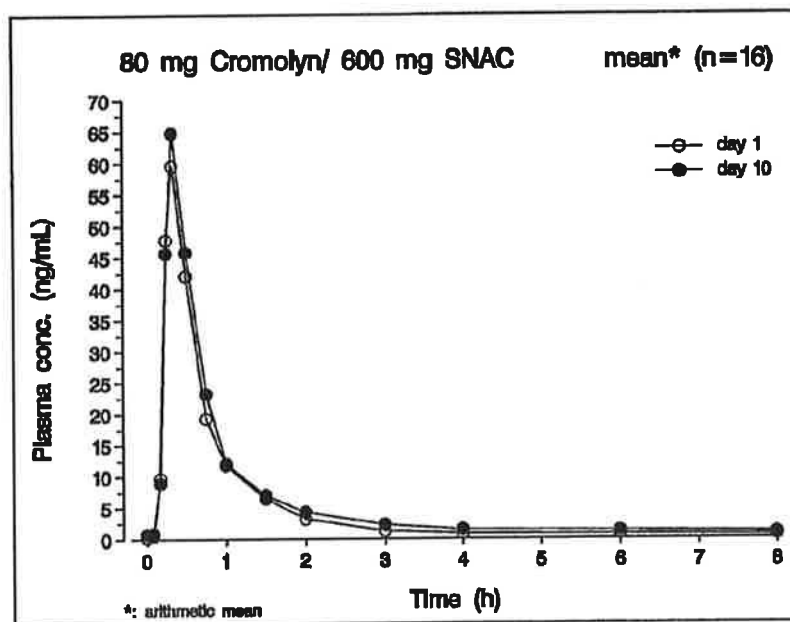


FIG. 12

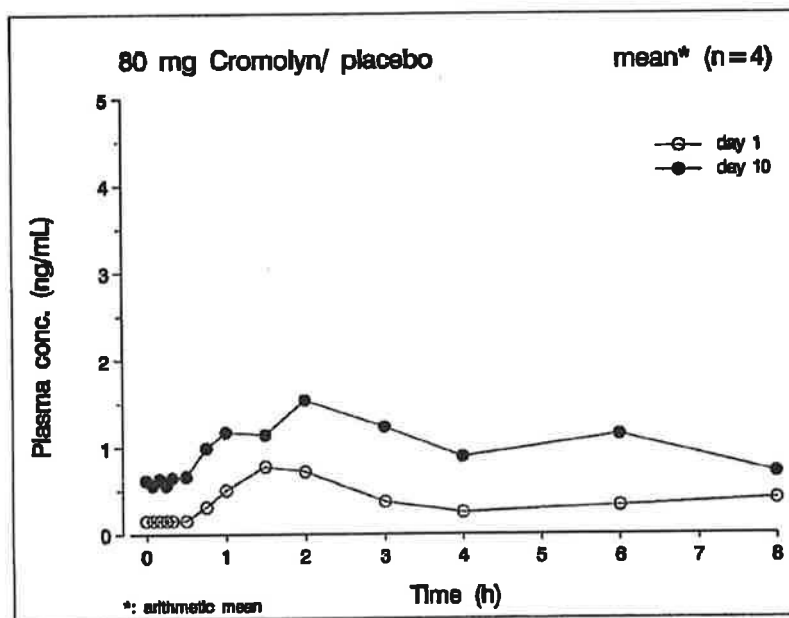


FIG. 13

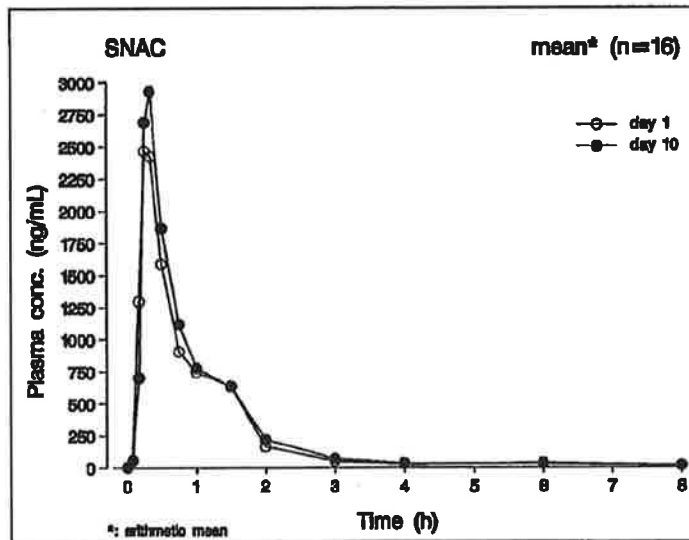


FIG. 14

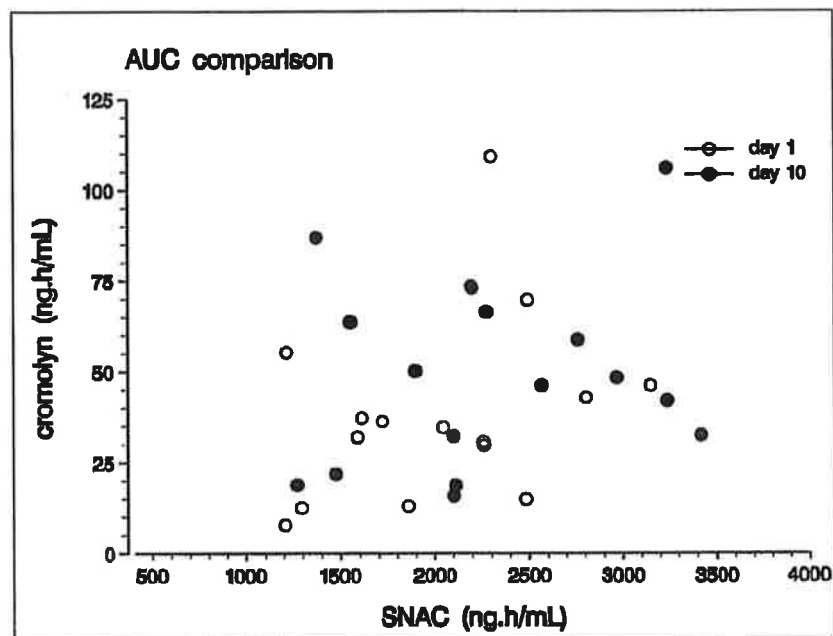


FIG. 15

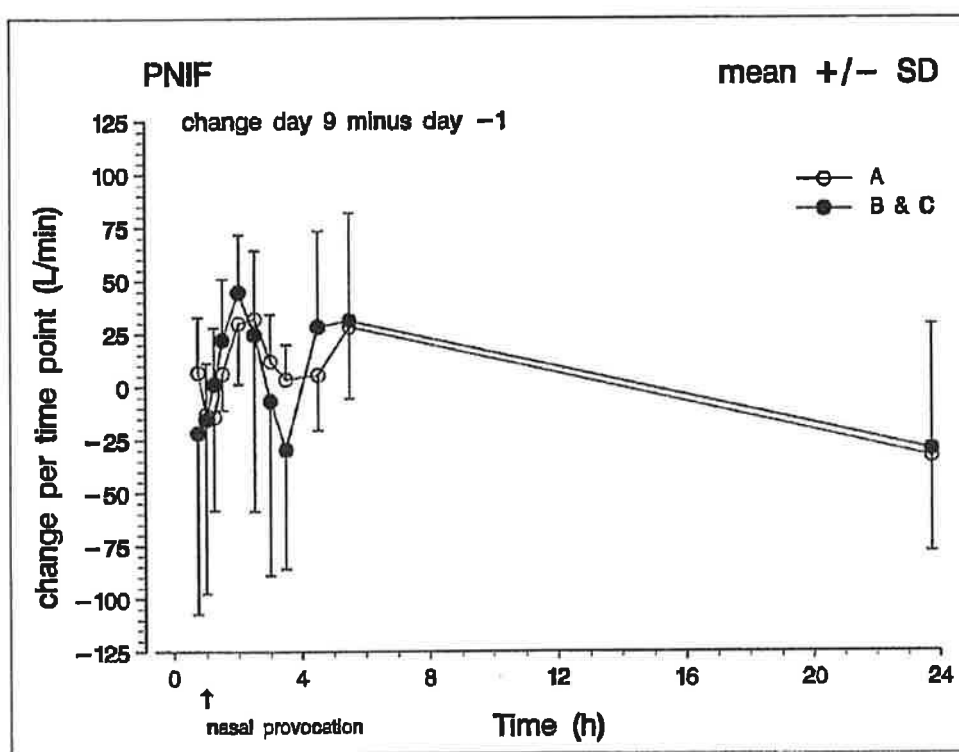


FIG. 16

1

FORMULATIONS FOR ORAL ADMINISTRATION OF CROMOLYN SODIUM

CROSS REFERENCE TO RELATED APPLICATION

This application is a divisional of U.S. patent application Ser. No. 10/497,286, filed May 28, 2004 now abandoned as a national phase application under 35 U.S.C. §371 of International Patent Application No. PCT/US02/38247, filed Nov. 29, 2002, and claimed the benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 60/334,395, filed Nov. 29, 2001, and No. 60/384,916, filed May 24, 2002.

TECHNICAL FIELD OF THE INVENTION

This invention relates to delivery of therapeutic polar organic compounds in a bioavailable and bioactive form. This invention further relates to oral administration of polar organic compounds as active agents as part of a therapeutic regimen. This invention further relates to the oral administration of cromolyn in a bioavailable and bioactive form for the treatment of asthma, allergies and viral infections.

This invention is directed to formulations of a delivery agent and cromolyn for oral administration. This invention further relates to formulations of a delivery agent and cromolyn for oral administration that releases cromolyn which is both bioavailable and bioactive for the treatment of asthma, allergies and viral infections.

This invention relates to providing methods for the granulation and encapsulation of a formulation comprising a delivery agent and cromolyn for oral administration. This invention further relates to providing methods for the granulation and encapsulation of a formulation comprising an absorption agent molecule and cromolyn for oral administration that releases cromolyn which is both bioavailable and bioactive for the treatment of allergies and asthma.

BACKGROUND OF THE INVENTION

Conventional means for delivering active agents are often severely limited by biological, chemical, and physical barriers. Typically, these barriers are imposed by the environment through which delivery occurs, the environment of the target for delivery, or the target itself.

Biologically or chemically active agents are particularly vulnerable to such barriers. For example in the delivery to humans and animals of pharmacological and therapeutic agents, barriers are imposed by the body. Examples of physical barriers are the skin and various organ membranes that must be traversed before reaching a target. Chemical barriers include, but are not limited to, pH variations, lipid bilayers, and degrading enzymes. These barriers are of particular significance in the design of oral delivery systems. Oral delivery of many biologically or chemically active agents would be the route of choice for administration to humans and animals if not for biological, chemical, and physical barriers such as varying pH in the gastro-intestinal (GI) tract, powerful digestive enzymes, and active agent impermeable gastro-intestinal membranes. Among the numerous agents which are not typically amenable to oral administration are biologically or chemically polar organic compounds, such as cromolyn, antibiotics and other organic substances. These agents are rapidly rendered partially or totally ineffective, pass through partially or totally unabsorbed, or are partially or totally destroyed in the gastro-intestinal tract by acid hydrolysis, enzymes, or the like.

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Earlier methods for orally administering vulnerable pharmacological agents, such as polar organic compounds and biological macromolecules have relied on the co-administration of adjuvants (e.g., resorcinols and non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to artificially increase the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and aprotinin) to inhibit enzymatic degradation.

The oral administration of therapeutic agents that require parenteral administration would greatly improve patient compliance and reduce the costs and risks associated with injections. Absorption of drugs via the gastrointestinal (GI) tract, however, is hampered by natural barriers including acid-induced hydrolysis in the stomach, enzymatic degradation throughout the GI tract, poor solubility in the intestinal environment, and lack of permeation through the epithelial cells. The latter is particularly problematic because it can exclude the passage of compounds across the tissue based on size, charge and/or lipophilicity. A common strategy to overcome this obstacle is the use of general penetration enhancers and/or nonspecific protease inhibitors. These systems, however, are highly inefficient and have been shown to cause transient to long-lasting membrane damage. Such membrane effects have the added disadvantage of allowing nonselective transport of toxic materials.

In current practice, cromolyn sodium is used by inhalation as a prophylactic agent in the treatment of mild to moderate asthma, as a nasal inhaler to treat seasonal bronchial asthma and as an ophthalmic solution to treat allergic or vernal conjunctivitis. Cromolyn is a mast cell stabilizer and as such attacks the initial step in the cascade that results in the manifestation of allergy including asthma and allergic rhinitis. The mechanism of action of cromolyn is through inhibiting the release of histamine and leukotrienes from the mast cells. Cromolyn has a high safety profile but exhibits poor absorption from the gastrointestinal tract. The clinical practicality and patient compliance of this drug is reduced because it is available mainly as an inhalation agent, and several weeks of therapy may be required before improvement is apparent. Cromolyn, when given orally, is poorly absorbed from the gastrointestinal tract because it is highly lipid-insoluble and mainly ionized.

Cromolyn is available only as an inhalation agent, except for an oral concentrate commercially available in the United States under the trade name Gastrocrom® from Celltech Pharmaceuticals, Inc. Gastrocrom® is provided in 5 ml ampoules containing 100 mg cromolyn sodium, USP, in purified water. Gastrocrom® is indicated in the management of patients with mastocytosis, and use of this product is said to be associated with improvement in diarrhea, flushing, headaches, vomiting, urticaria, abdominal pain, nausea and itching in some patients. No more than 1% of an administered dose of Gastrocrom® is absorbed by humans after oral administration (Physician's Desk Reference® 2001, page 1840-1841).

Many investigators believe that if cromolyn were to be available in an oral form and with good absorption from the gastrointestinal tract, it would become a valuable addition with broad application in the treatment of allergic asthma and rhinitis.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide polar organic compounds in an orally bioavailable and bioactive form.

It is an object of the present invention to provide cromolyn in a bioavailable and bioactive form.

It is also an object of the present invention to provide bioavailable cromolyn as an active agent as part of a therapeutic regimen.

It is another object of the present invention to provide formulations of a delivery agent and cromolyn for oral administration.

It is still another object of the present invention to provide formulations of a delivery agent and cromolyn for oral administration that provides cromolyn in a form which is both bioavailable and bioactive for the treatment of allergies and asthma.

It is yet another object of the present invention to provide methods for the granulation and encapsulation of a formulation comprising a delivery agent and cromolyn for oral administration.

It is a further object of the present invention to provide cromolyn sodium in an oral dosage form for the treatment of any inflammatory process involving the release of cytokines and/or histamine from mast cells.

It is still a further object of the present invention to provide an oral dosage form of cromolyn sodium suitable for the treatment of inflammatory diseases.

It is yet a further object of the present invention to provide an oral dosage form of cromolyn sodium suitable for the prophylactic treatment of allergies including allergic rhinitis, asthma, rheumatoid arthritis, etc.

SUMMARY OF THE INVENTION

The following terms will be used throughout the application as defined below:

Effective amount of drug—an amount of the drug (e.g., cromolyn sodium) that is effective to treat or prevent a condition in a living organism to whom it is administered over some period of time, e.g., provides a therapeutic effect during a desired dosing interval.

Effective amount of delivery agent—an amount of the delivery agent that promotes the absorption of a desired amount of the drug from the gastrointestinal tract.

Organic solvents—any solvent of non-aqueous origin, including liquid polymers and mixtures thereof. Organic solvents suitable for the present invention include: acetone, methyl alcohol, methyl isobutyl ketone, chloroform, 1-propanol, isopropanol, 2-propanol, acetonitrile, 1-butanol, 2-butanol, ethyl alcohol, cyclohexane, dioxane, ethyl acetate, dimethylformamide, dichloroethane, hexane, isooctane, methylene chloride, tert-butyl alcohol, toluene, carbon tetrachloride, or combinations thereof.

Patient—refers to any animal that is to be treated with the formulations and compositions by the methods herein disclosed.

Peptide—a polypeptide of small to intermediate molecular weight, usually 2 or more amino acid residues and frequently but not necessarily representing a fragment of a larger protein.

Protein—a complex high polymer containing carbon, hydrogen, oxygen, nitrogen and usually sulfur and composed of chains of amino acids connected by peptide linkages. Proteins in this application refer to glycoproteins, antibodies, non-enzyme proteins, enzymes, hormones and peptides. The molecular weight range for proteins includes peptides of 1000 Daltons to glycoproteins of 600 to 1000 kiloDaltons.

Prophylactically effective amount—an amount of a formulation or composition which is effective to prevent or reduce the incidence or severity of a condition in a living organism to whom it is administered over some period of time.

Reconstitution—dissolution of formulations or compositions in an appropriate buffer or pharmaceutical formulation.

Shelf stability—the loss of specific activity that results in decreased activity over time incubated under specified conditions.

Unit-Dose Forms—refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. It is contemplated for purposes of the present invention that dosage forms of the present invention comprising therapeutically effective amounts of cromolyn may include one or more unit doses (e.g., tablets, capsules) to achieve the therapeutic effect.

The term “therapeutically effective” as is used herein refers to the release of drug from the dosage form and the absorption of the drug to an extent at which blood (e.g., plasma) concentrations (levels) of the drug (e.g., cromolyn) are maintained within the therapeutic range (above the minimum effective concentration or “MEC”) but below toxic levels over the dosing interval.

The term “ C_{max} ” as is used herein means the highest plasma concentration of the drug attained within the dosing interval.

The term “ T_{max} ” as is used herein means the time period that elapses after administration of the dosage form at which the plasma concentration of the drug attains the C_{max} within the dosing interval.

The term “ K_{el} ” as is used herein means the terminal elimination rate constant calculated by linear regression of the terminal linear portion of the log concentration vs. time curve.

The term “ λ_z ” as is used herein means the terminal elimination rate constant.

The term “AUC” as used herein means area under the plasma concentration-time curve, as calculated by the trapezoidal rule over the complete dosing interval, e.g., 24-hour interval.

The term “ $AUC_{(0-t)}$ ” as used herein means the area under the plasma concentration-time curve using linear trapezoidal summation from time zero to time t post-dose, where t is the time of the last measurable concentration (C_t).

The term “ $AUC_{(0-\infty)}$ ” as used herein means the area under the plasma concentration-time curve from time 0 to infinity, $AUC_{(0-\infty)} = AUC_{(0-t)} + C_t/K_{el}$.

The term “single dose” means that the human patient has received a single dose of the drug formulation and the drug plasma concentration has not achieved steady state.

The term “multiple dose” means that the human patient has received at least two doses of the drug formulation in accordance with the dosing interval for that formulation.

Unless specifically designated as “single dose” or at “steady-state” the pharmacokinetic parameters disclosed and claimed herein encompass both single dose and steady-state conditions.

The term “mean”, when preceding a pharmacokinetic value (e.g., mean T_{max}) represents the arithmetic mean value of the pharmacokinetic value unless otherwise specified.

The term “CL/F” as used herein means the apparent total body clearance calculated as $Dose/AUC_{(0-\infty)}$.

The term “MRT” as used herein means the mean residence time calculated as the ratio of the Area Under the first moment of the plasma concentration-time curve (AUMC) and the area under the plasma concentration-time curve, $(AUMC)/AUC_{(0-\infty)}$.

The term “ $T_{1/2}$ ” as used herein means the terminal half-life calculated as $\ln(2)/K_{el}$.

The term “ $T_{1/2}(\lambda_z)$ ” as used herein means the terminal elimination half life of the drug.

The term " V_d/F " as used herein means the apparent volume of distribution calculated as $(CL/F)/K_{el}$.

The term "% Extr," as used herein means the percentage of $AUC_{0-\infty}$ extrapolated to infinity.

The term " γ -GTP" as used herein means Gamma-Glutamyl Transpeptidase.

The term "PNIF" as used herein means the Peak Nasal Inspiratory Flow.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. In addition, reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

In accordance with the above and other objects, the present invention is related in part to oral dosage forms comprising a polar organic compound, particularly cromolyn sodium, and a delivery agent or salts thereof in an effective amount to provide good absorption of cromolyn from the gastrointestinal tract.

More particularly, the present invention is related in part to an oral dosage form comprising cromolyn sodium (sodium or disodium cromoglycate) and a delivery agent in an amount effective to provide an absorption of cromolyn from the gastrointestinal tract of greater than about 2% of the dose of cromolyn contained in the dosage form, by weight, said formulation containing an amount of said cromolyn sodium necessary to render a therapeutic effect.

The invention is further related to an oral dosage form comprising cromolyn sodium and a pharmaceutically acceptable delivery agent in an effective amount to provide therapeutically effective blood plasma levels of cromolyn upon oral administration, said dosage form providing a T_{max} of cromolyn in less than about 1 hour, and in certain embodiments preferably at from about 0.2 to about 0.5 hours after oral administration.

The invention is further related to an oral dosage form comprising cromolyn sodium and a pharmaceutically acceptable delivery agent in an effective amount to provide therapeutically effective blood plasma levels of cromolyn upon oral administration, the dosage form providing a C_{max} of cromolyn from about 10 ng/ml to about 1700 ng/ml. In one embodiment of the present invention, the dosage form provides a C_{max} of cromolyn from about 22.3 ng/ml to about 200 ng/ml. In another embodiment of the present invention, the dosage form provides a C_{max} of cromolyn from about 20 ng/ml to about 100 ng/ml. In still another embodiment of the present invention, the dosage form provides a C_{max} of cromolyn from about 25 ng/ml to about 80 ng/ml. In yet another embodiment of the present invention, the dosage form provides a C_{max} of cromolyn from about 40 ng/ml to about 60 ng/ml.

The invention is further related to an oral dosage form comprising cromolyn sodium and a pharmaceutically acceptable absorption enhancing delivery agent in an effective amount to provide a T_{max} of cromolyn after oral administration of the dosage form at from about 0.2 to about 0.5 hours after oral administration, and a C_{max} of cromolyn from about 10 ng/ml to about 1700 ng/ml, and in certain embodiments from about 4 ng/ml to about 250 ng/ml.

In certain embodiments of the invention, the oral dosage form described herein provides a mean plasma level of cromolyn from about 4 ng/ml to about 250 ng/ml during a dosage interval. In other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 10 ng/ml to about 60 ng/ml during a dosage interval. In yet other embodi-

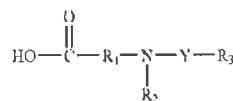
ments, the oral dosage form provides a mean plasma level of cromolyn from about 30 ng/ml to about 60 ng/ml during a dosage interval.

In certain embodiments of the invention, the oral dosage form described herein provides a mean C_{max} from about 10 ng/ml to about 250 ng/ml.

In certain embodiments of the invention, the oral dosage form contains from about 40 mg to about 1 gram cromolyn sodium (either in one unit dose or in multiple unit doses, e.g., tablets or capsules), in certain embodiments the oral dosage form contains from about 40 mg to about 800 mg cromolyn sodium, and in other embodiments the oral dosage form contains from 240 mg cromolyn sodium; and further contains from about 100 mg to about 2500 mg delivery agent (either in one unit dose or in multiple unit doses, e.g., tablets or capsules), and in certain embodiments contains from about 200 mg to about 1200 mg delivery agent. In certain preferred embodiments, the delivery agent is included in the dosage form in an amount from about 200 to about 800 mg. In yet other embodiments, the delivery agent is included in the dosage form in an amount of about 500 mg to about 700 mg.

Alternatively, and in accordance with the methods of the invention, the delivery agent in that same amount may be separately administered to the animal in need of treatment.

In certain embodiments of the invention, the delivery agent used in the oral dosage form and methods described herein has the formula or salts thereof:

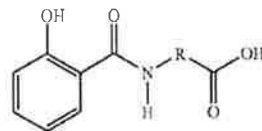


wherein Y is carbonyl (CO) or SO_2 ; R_1 is C_3 - C_{24} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkyne, cycloalkyl, or aromatic; R_2 is hydrogen, C_1 - C_4 alkyl, or C_2 - C_4 alkenyl; and R_3 is C_1 - C_7 alkyl, C_3 - C_{10} cycloalkyl, aryl, thienyl, pyrrolo, or pyridyl, where R_3 is optionally substituted by one or more C_1 - C_5 alkyl group, C_2 - C_4 alkenyl group, halogen preferably For Cl, OH, SO_2 , COOH , or SO_3H .

In a preferred embodiment, R_1 is C_3 - C_{12} alkyl, more preferably C_3 - C_9 alkyl, more preferably C_5 - C_7 alkyl, more preferably C_7 alkyl.

In a preferred embodiment, R_3 is 2OH substituted phenyl or preferably 2OH substituted phenyl further substituted with a halogen such as F or Cl.

In other preferred embodiments, the delivery agent used in the oral dosage form and methods described herein has the formula or salts thereof:



wherein R is C_3 - C_{24} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkyne, cycloalkyl, or aromatic.

In certain preferred embodiments of the invention, the delivery agent is Sodium N-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) or salts thereof.

In certain embodiments, the cromolyn sodium and the delivery agent are simultaneously administered. In other embodiments, the cromolyn sodium and the delivery agent are sequentially administered.

In certain preferred embodiments, the delivery agent is in an amount effective to provide an absorption of cromolyn from the gastrointestinal tract of greater than about 3.5% of the dose of cromolyn contained in the dosage form.

Also contemplated is a method for preparing these compositions which comprises mixing at least one biologically active agent with at least one delivery agent as described above and, optionally, a dosing vehicle (e.g., pharmaceutical excipients).

In an alternative embodiment, these non-toxic delivery agents are orally administered to animals as part of a delivery system by blending or mixing the delivery agent(s) with the biologically active agent prior to administration. Also contemplated by the present invention are dosage unit forms that include these compositions.

According to the invention, modified amino acids are prepared by reacting single amino acids or mixture of two or more kinds of amino acids with an acylating or sulfonating agent which reacts with free amino moieties present in the amino acids to form amides or sulfonamides, respectively. The modified amino acids are then recovered from the mixture.

The modified amino acids are non-toxic and can be orally administered to mammals as a drug delivery system by simply mixing the modified amino acids with an active agent prior to administration. Alternatively, the drug and the delivery agent may be sequentially administered.

In certain embodiments, the invention provides an oral dosage form comprising a dose of cromolyn sodium and a delivery agent that upon oral administration provides a systemic absorption of the cromolyn sodium in an amount effective to inhibit the release of histamine and/or cytokines from mast cells. The delivery agent could comprise sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) in a ratio from about 1:30 to about 3:4 (weight/weight).

In other embodiments, the invention provides an oral dosage form comprising cromolyn sodium (sodium or disodium cromoglycate), and an acylated amino acid delivery agent in an amount effective to provide an absorption of cromolyn from the gastrointestinal tract of greater than about 2% of the dose of cromolyn contained in the dosage form, by weight, said formulation containing an amount of said cromolyn sodium necessary to render a systemic therapeutic effect.

In certain embodiments, the oral dosage form is solid. The oral dosage form could alternatively be in the form of a tablet, capsule or oral suspension. In certain embodiments, the oral dosage form is suitable for being administered once a day, twice a day, three times a day or four times a day.

In certain embodiments, the oral dosage form comprises a dose of cromolyn sodium of from about 40 mg to about 240 mg. In certain other embodiments, the oral dosage form comprises a dose of cromolyn sodium of from about 50 mg to about 100 mg.

In certain embodiments, the oral dosage form comprises a dose of the delivery agent of from about 100 mg to about 1200 mg. In certain other embodiments, the oral dosage form comprises a dose of the delivery agent of from about 200 mg to about 800 mg.

In certain embodiments, the oral dosage form provides a T_{max} for cromolyn sodium at about 0.1 to about 1.5 hours after oral administration. In certain other embodiments, the oral dosage form provides a T_{max} for cromolyn sodium at about 0.2 to about 0.5 hours after administration.

In certain embodiments, the oral dosage form provides an AUC for cromolyn sodium of between about 15 and about 60

ng·h/mL. In certain other embodiments, the oral dosage form provides an AUC for cromolyn sodium of between about 30 and about 40 ng·h/mL.

In certain embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 4 ng/mL to about 1700 ng/mL during a dosage interval. In certain other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 10 ng/mL to about 60 ng/mL during a dosage interval. In certain other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 30 ng/mL to about 60 ng/mL during a dosage interval.

In certain embodiments, the oral dosage form provides a cromolyn sodium C_{max} from about 22 ng/mL to about 1700 ng/mL. In certain other embodiments, the oral dosage form provides a cromolyn sodium C_{max} from about 22 to about 77 ng/mL. In certain other embodiments, the oral dosage form provides a cromolyn sodium C_{max} from about 40 to about 65 ng/mL.

In certain embodiments, the oral dosage form provides a cromolyn sodium C_{max} from about 22 to about 77 ng/mL within about 0.2 to about 1 hour after oral administration.

In certain embodiments, the invention provides for the use of cromolyn sodium in the manufacture of a medicament for the oral treatment of inflammatory diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells. In certain other embodiments, the invention provides cromolyn sodium for use in oral therapy for treatment of inflammatory diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells.

In certain other embodiments, the invention provides for the use of cromolyn sodium in the manufacture of a medicament comprising cromolyn sodium and a delivery agent, said delivery agent comprising Sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), in ratios from about 1:30 to about 3:4 (weight/weight), for the oral treatment of inflammatory diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells.

In certain embodiments, the invention provides a method for treating inflammatory diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, comprising orally administering cromolyn sodium in an amount from about 40 mg to about 1 g together with a delivery agent in an amount from about 100 mg to about 2500 mg or in a ratio from about 1:30 (weight/weight) to about 3:4 (weight/weight).

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, comprising orally administering a therapeutically effective amount of cromolyn sodium together with a delivery agent in a ratio from about 1:30 (mg/mg) to about 3:4 (weight/weight) on a prophylactic basis.

In certain other embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein the dose of cromolyn sodium and drug delivery agent is selected from the group consisting of about 40 mg cromolyn sodium/about 1200 mg delivery agent, about 80 mg cromolyn sodium/about 1200 mg delivery agent, about 80 mg cromolyn sodium/about 600 mg delivery agent, about 80 mg cromolyn sodium/about 1200 mg delivery agent, about 120 mg cromolyn sodium/about 200 mg delivery agent, about 120 mg cromolyn sodium/about 400 mg delivery agent, 120 mg cromolyn sodium/about 500 mg delivery agent and about 150 mg cromolyn sodium/about 200 mg delivery agent.

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein the T_{max} for cromolyn sodium occurs at a time less than about 1 hour after oral administration. In certain other embodiments, the T_{max} for cromolyn sodium occurs at about 0.1 to about 1.5 hours after oral administration. In certain other embodiments, the T_{max} for cromolyn sodium occurs at about 0.2 to about 0.5 hours after oral administration.

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein an AUC for cromolyn sodium of between about 16 and about 400 ng·h/mL is attained. In certain other embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein an AUC for cromolyn sodium of between about 25 and about 40 ng·h/mL is attained. In certain other embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein an AUC for cromolyn sodium of between about 30 and about 35 ng·h/mL is attained.

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein a cromolyn sodium C_{max} that is from about 20 to about 250 ng/mL is attained. In certain other embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein a cromolyn sodium C_{max} that is from about 22 to about 77 ng/mL is attained. In certain other embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein a cromolyn sodium C_{max} that is from about 40 to about 65 ng/mL is attained.

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein a cromolyn sodium C_{max} that is from about 22.3 to about 76.7 ng/mL within about 0.2 to about 1 hour after oral administration is attained.

In certain embodiments, the invention provides a method for treating allergic rhinitis, asthma, rheumatoid arthritis, or autoimmune diseases caused or exacerbated by the release of histamine and/or cytokines from mast cells, wherein cromolyn sodium and an acylated amino acid delivery agent are sequentially administered.

In certain embodiments, the invention provides an oral dosage form comprising cromolyn sodium and a pharmaceutically acceptable absorption enhancing delivery agent in an effective amount to provide therapeutically effective blood plasma levels of cromolyn upon oral administration, said dosage form providing a T_{max} of cromolyn at from about 0.2 to about 0.5 hours after oral administration.

In certain embodiments, the invention provides an oral dosage form comprising cromolyn sodium and a pharmaceutically acceptable bioavailability enhancing delivery agent in an effective amount to provide therapeutically effective blood

plasma levels of cromolyn upon oral administration, said dosage form providing a C_{max} of cromolyn from about 4 ng/mL to about 250 ng/mL. In certain other embodiments, the delivery agent is included in said dosage form in an amount from about 200 to about 800 mg. In certain other embodiments, the delivery agent is included in said dosage form in an amount of about 500 mg to about 700 mg.

In certain embodiments, the invention provides a method of providing a therapeutically effective orally administrable dose of cromolyn sodium, comprising combining from about 40 mg to about 1 g of unmodified cromolyn with from about 100 mg to about 2500 mg of a pharmaceutically acceptable delivery agent which facilitates absorption of said cromolyn from the gastrointestinal tract of human patients, and orally administering said unit dose to a human patient to provide a therapeutic effect.

In certain embodiments, the invention provides a method of treating asthma, allergic rhinitis, autoimmune diseases and rheumatoid arthritis comprising: orally administering to a patient in need of treatment a dosage form comprising a dose of cromolyn sodium together with a delivery agent which facilitates the absorption of the cromolyn sodium from the gastrointestinal tract to provide a therapeutically effective systemic dose of cromolyn sodium.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the percent airway resistance after ascaris challenge in sheep given oral cromolyn or control compositions 15 minutes prior to ascaris challenge;

FIG. 2 depicts the percent airway resistance after ascaris challenge in sheep given oral cromolyn or control compositions 60 minutes prior to ascaris challenge;

FIG. 3 depicts the percent airway resistance after ascaris challenge in sheep given oral cromolyn or control compositions 12 hours prior to ascaris challenge;

FIG. 4 depicts the plasma levels attained over time of cromolyn for individual patients given treatment period 1 in Example 4;

FIG. 5 depicts the plasma levels of cromolyn for individual patients given treatment period 2 in Example 4;

FIG. 6 depicts the plasma levels of cromolyn for individual patients given treatment period 3 in Example 4;

FIG. 7 depicts the plasma levels of cromolyn for individual patients given treatment period 4 in Example 4;

FIG. 8 depicts plasma cromolyn concentrations vs. time profiles after single oral administration of different combinations of cromolyn sodium and SNAC dosages;

FIG. 9 depicts plasma cromolyn concentrations vs. time profiles normalized to a 100-mg dose of cromolyn sodium after single oral administration of different combinations of cromolyn sodium and SNAC dosages;

FIG. 10 depicts plasma SNAC concentrations vs. time profiles after single oral administration of different combinations of cromolyn sodium and SNAC dosages;

FIG. 11 depicts plasma SNAC concentrations vs. time profiles normalized to a 1000-mg dose of SNAC after single oral administration of different combinations of cromolyn sodium and SNAC dosages;

FIG. 12 depicts mean plasma concentration versus time profiles of cromolyn for Day 1 and Day 10 after an oral administration of a combination of cromolyn sodium and SNAC dosages;

FIG. 13 depicts mean plasma concentration versus time profiles of cromolyn for Day 1 and Day 10 after an oral administration of a combination of cromolyn sodium and placebo;

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FIG. 14 depicts mean plasma concentration versus time profiles for SNAC for Day 1 and Day 10 after administration of a combination of cromolyn sodium and SNAC; and

FIG. 15 depicts a correlation of the AUC values for plasma concentrations of SNAC and cromolyn.

FIG. 16 shows the PNIF results as mean changes from baseline per time point (values on Day 9 minus the values on Day 1).

DETAILED DESCRIPTION

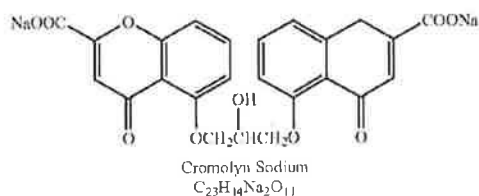
The specific compositions of the present invention include an active agent and a modified amino acid. These compositions may be used to deliver various active agents through various biological, chemical, and physical barriers and are particularly suited for delivering active agents which are subject to environmental degradation. The compositions of the subject invention are particularly useful for delivering or administering biologically or chemically active agents to any animals such as birds; mammals, such as primates and particularly humans; and insects.

Other advantages of the present invention include the use of easy to prepare, inexpensive raw materials. The compositions and the formulation methods of the present invention are cost effective, simple to perform, and amenable to industrial scale up for commercial production.

Active agents suitable for use in the present invention include biologically active agents and chemically active agents, including, but not limited to pharmacological agents and therapeutic agents, particularly polar organic compounds and particularly compounds which by themselves do not pass (or which pass only a fraction of the administered dose) through the gastro-intestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastro-intestinal tract and are rendered less active or inactive; or any combination thereof.

In a preferred embodiment of the present invention, pharmaceutical compositions comprising cromolyn and a delivery agent molecule are provided. The term "cromolyn" is defined for purposes of the present invention as any pharmaceutically acceptable form of the drug. Pharmaceutical compositions containing cromolyn sodium, an antiallergic, are useful for mammals suffering from respiratory afflictions, such as asthma, hay fever or allergies.

Cromolyn is a synthetic compound, and it is commercially available, originally produced in the search for an improved bronchodilator. Although cromolyn has no bronchodilator activity, it was found to inhibit antigen-induced bronchospasm. Currently, its major use is as a prophylactic agent in the treatment of mild to moderate asthma. It also is used as a nasal inhaler to treat seasonal allergic rhinitis, as an ophthalmic solution to treat allergic or vernal conjunctivitis, and orally to treat systemic mastocytosis and ulcerative colitis. The chemical structure for cromolyn sodium is as follows:



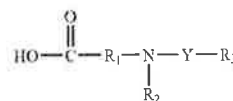
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Cromolyn, or 5,5'-[(2-hydroxy-1,3-propanediyl)bis-(oxy)]4-oxo-4H-1-benzopyran-2-carboxylic acid has a molecular weight of 468.38 with the empirical formula of $C_{23}H_{16}O_{11}$. Cromolyn is also known as cromolyn sodium or disodium chromoglycate, has a melting point of 241-241° C. and is freely soluble in water. See Merck Index, Eleventh Edition, compound number 2594, page 406, the text of which is hereby incorporated by reference.

Modified Amino Acid Delivery Agent Compounds

The present invention provides compounds and compositions that are useful in the oral delivery of therapeutic compounds. Further, the present invention utilizes compounds having the following formula, or salts thereof, or mixtures thereof in the granulation and encapsulation of cromolyn. Such formulations serve to make cromolyn bioavailable and bioactive when orally administered.

In certain embodiments, the delivery agent compound has the formula and salts thereof:

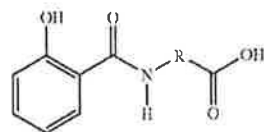


wherein Y is carbonyl (CO) or SO_2 ; R_1 is C_3 - C_{24} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkyne, cycloalkyl, or aromatic; R_2 is hydrogen, C_1 - C_4 alkyl, or C_3 - C_4 alkenyl; and R_3 is C_1 - C_7 alkyl, C_3 - C_{10} cycloalkyl, aryl, thienyl, pyrrolo, or pyridyl, where R_3 is optionally substituted by one or more C_1 - C_5 alkyl group, C_2 - C_4 alkenyl group, F, Cl, OH, SO_2 , COOH, or SO_3H .

In a preferred embodiment, R_1 is C_3 - C_{12} alkyl, more preferably C_3 - C_9 alkyl, more preferably C_5 - C_7 alkyl, more preferably C_7 alkyl.

In a preferred embodiment, Y is carbonyl (CO).

In certain preferred embodiments, the delivery agent compound has the formula and salts thereof:



wherein R is C_3 - C_{24} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkyne, cycloalkyl, or aromatic.

In certain preferred embodiments of the invention, the delivery agent is Sodium N-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) or salts thereof. These compounds, and methods of preparation of the same, are described in U.S. Pat. No. 5,650,386 (Leone-Bay, et. al.), which is hereby incorporated by reference in its entirety.

The terms modified amino acid, modified poly amino acid and modified peptide are meant to include amino acids that have been modified, or poly amino acids and peptides in which at least one amino acid has been modified, by acylating or sulfonating at least one free amine group with an acylating or sulfonating agent which reacts with at least one of the free amine groups present.

Amino acids, poly amino acids and peptides, in modified form, may be used to deliver active agents including, but not limited to, biologically or chemically active agents such as, for example, pharmacological and therapeutic agents. An

amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids.

Poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, e.g., an ester, anhydride or an anhydride linkage. Peptides are two or more amino acids joined by a peptide bond. Peptides can vary in length from dipeptides with two amino acids to poly peptides with several hundred amino acids. See Chambers Biological Dictionary, editor Peter M. B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. Special mention is made of di-peptides, tri-peptides, tetra-peptides and penta-peptides. Modified amino acids are typically prepared by modifying the amino acid or an ester thereof.

Many of these compounds may be prepared, e.g., by acylation or sulfonation with agents having the formula



wherein R^4 is the appropriate radical to yield the modification indicated in the final product, Y is a carbonyl (CO) or SO_2 , and X is a leaving group. Typical leaving groups include, but are not limited to, halogens such as, for example, chlorine, bromine and iodine. Additionally, the corresponding anhydrides are modifying agents.

Many other compounds suitable for the present invention can be readily prepared from amino acids by methods within the skill of those in the art based upon the disclosure of U.S. Pat. No. 5,650,386 to A. Leone-Bay et al., the disclosure of which is hereby incorporated by reference in its entirety. For example, compounds derived from aminobutyric acid and compounds derived from aminocaproic acid may be suitable for the present invention. For example, the modified amino acid compounds above may be prepared by reacting the single amino acid with the appropriate modifying agent which reacts with free amino moiety present in the amino acids to form amides. Protecting groups may be used to avoid unwanted side reactions, as would be known to those skilled in the art.

The amino acid can be dissolved in aqueous alkaline solution of a metal hydroxide, e.g., sodium or potassium hydroxide, and heated at a temperature ranging between about 5 EC and about 70 EC, preferably between about 10 EC and about 40 EC, for a period ranging between about 1 hour and about 4 hours, preferably about 2.5 hours. The amount of alkali employed per equivalent of NH_2 groups in the amino acid generally ranges between about 1.25 and about 3 mmole, preferably between about 1.5 and about 2.25 mmole per equivalent of NH_2 . The pH of the solution generally ranges between about 8 and about 13, preferably ranging between about 10 and about 12.

Thereafter, the appropriate amino modifying agent can be added to the amino acid solution while stirring. The temperature of the mixture may be maintained at a temperature generally ranging between about 5 EC and about 70 EC, preferably between about 10 EC and about 40 EC, for a period ranging between about 1 and about 4 hours. The amount of amino modifying agent employed in relation to the quantity of amino acid is based on the moles of total free NH_2 in the amino acid. In general, the amino modifying agent is employed in an amount ranging between about 0.5 and about 2.5 mole equivalents, preferably between about 0.75 and about 1.25 equivalents, per molar equivalent of total NH_2 group in the amino acid.

The reaction is quenched by adjusting the pH of the mixture with a suitable acid, e.g., concentrated hydrochloric acid, until the pH reaches between about 2 and about 3. The mix-

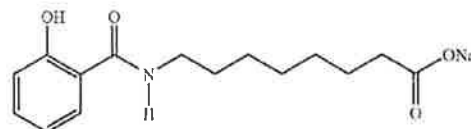
ture separates on standing at room temperature to form a transparent upper layer and a white or off-white precipitate. The upper layer is discarded, and the modified amino acid is collected from the lower layer by filtration or decantation. The crude modified amino acid is then dissolved in water at a pH ranging between about 9 and about 13, preferably between about 11 and about 13. Insoluble materials are removed by filtration and the filtrate is dried in vacuo. The yield of modified amino acid generally ranges between about 30 and about 60%, and usually about 45%.

If desired, amino acid esters, such as, for example, benzyl, methyl or ethyl esters of amino acid compounds, may be used to prepare the modified amino acids of the invention. The amino acid ester, dissolved in a suitable organic solvent, such as dimethylformamide, pyridine or tetrahydrofuran, is reacted with the appropriate amino modifying agent at a temperature ranging between about 5 EC and about 70 EC, preferably about 25 EC, for a period ranging between about 7 and about 24 hours. The amount of amino modifying agent used relative to the amino acid ester is the same as described above for amino acids. This reaction may be carried out with or without a base such as, for example, triethylamine or diisopropylethylamine.

Thereafter, the reaction solvent is removed under negative pressure and the ester functionality is removed by hydrolyzing the modified amino acid ester with a suitable alkaline solution, e.g., 1N sodium hydroxide, at a temperature ranging between about 50 EC and about 80 EC, preferably about 70 EC, for a period of time sufficient to hydrolyze off the ester group and form the modified amino acid having a free carboxyl group. The hydrolysis mixture is then cooled to room temperature and acidified, e.g., aqueous 25% hydrochloric acid solution, to a pH ranging between about 2 and about 2.5. The modified amino acid precipitates out of solution and is recovered by conventional means such as filtration or decantation. Benzyl esters may be removed by hydrogenation in an organic solvent using a transition metal catalyst.

The modified amino acid may be purified by recrystallization or by fractionation on solid column supports. Suitable recrystallization solvent systems include acetonitrile, methanol and tetrahydrofuran. Fractionation may be performed on a suitable solid column supports such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase column supports using trifluoroacetic acid/acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water as the mobile phase. When anion exchange chromatography is performed, preferably a subsequent 0-500 mM sodium chloride gradient is employed.

In certain preferred embodiments, the delivery agent compound is Sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), having the chemical structure:



This delivery agent, SNAC, may be synthesized by methods known in the art, such as described in Examples 1, and 8-11 in International Publication Number WO 00/46182 by Gschneidner et al., (2000), which is hereby incorporated by reference.

The delivery agent compounds may be in the form of the carboxylic acid or salts thereof. Suitable salts include, but are

not limited to, organic and inorganic salts, for example alkali-metal salts, such as sodium, potassium and lithium; alkaline-earth metal salts, such as magnesium, calcium or barium; ammonium salts; basic amino acids, such as lysine or arginine; and organic amines, such as dimethylamine or pyridine. Preferably, the salts are sodium salts. The salts may be mono- or multi-valent salts, such as monosodium salts and disodium salts. The salts may also be solvates, including ethanol solvates, and hydrates.

In certain embodiments of the invention, the oral dosage form described herein provides a mean plasma level of cromolyn from about 4 ng/ml to about 250 ng/ml during a dosage interval. In other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 10 ng/ml to about 200 ng/ml during a dosage interval. In yet other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 30 ng/ml to about 70 ng/ml during a dosage interval. In yet other embodiments, the oral dosage form provides a mean plasma level of cromolyn from about 40 ng/ml to about 60 ng/ml during a dosage interval.

The delivery agent may be used directly by simply mixing one or more such agents with the active agent prior to administration. The delivery agent and active agent may be mixed in dry powder form or wet granulated together. Other pharmaceutically acceptable excipients may be added to this mixture. The mixture may then be tableted or placed into capsules containing a unit dose of the active agent and the delivery agent. Alternatively, the delivery agent/active agent mixture may be prepared as an oral solution or suspension. The delivery agent and active agent need not be mixed together prior to administration. In other words, in certain embodiments, the unit dose of active agent (with or without other pharmaceutically acceptable excipients) is orally administered without the delivery agents of this invention, and the delivery agent is separately orally administered (with or without other pharmaceutically acceptable excipients) before, after or simultaneously with the active agent. In yet another alternative embodiment, the delivery agent may be used to form microspheres containing the active agent.

The administration mixtures may be prepared, e.g., by mixing an aqueous solution of the delivery agent with an aqueous solution of the active ingredient, just prior to administration. Alternatively, the delivery agent and the biologically or chemically active ingredient can be admixed during the manufacturing process. The solutions may optionally contain additives such as phosphate buffer salts, citric acid, acetic acid, gelatin and gum acacia.

Stabilizing additives may be incorporated into the delivery agent solution. With some drugs, the presence of such additives promotes the stability and dispersibility of the agent in solution. The stabilizing additives may be employed at a concentration ranging between about 0.1 and 5% (W/V), preferably about 0.5% (W/V). Suitable, but non-limiting, examples of stabilizing additives include gum acacia, gelatin, methyl cellulose, polyethylene glycol, carboxylic acids and salts thereof, and polylysine. The preferred stabilizing additives are gum acacia, gelatin and methyl cellulose.

The amount of active agent, e.g., cromolyn sodium, is an amount effective to accomplish the purpose of the particular active agent. The amount in the composition typically is a pharmacologically or biologically effective amount. However, the amount can be less than a pharmacologically or biologically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of delivery agent/biologically or chemically active agent compositions or may contain a divided pharmacologically or bio-

logically effective amount. The total effective amounts can then be administered in cumulative units containing, in total, pharmacologically or biologically or chemically active amounts of biologically or pharmacologically active agent.

The total amount of active agent, and particularly cromolyn sodium, to be used can be determined by those skilled in the art. However, it has surprisingly been found that with some biologically or chemically active agents, the use of the presently disclosed delivery agents provides extremely efficient delivery.

The amount of delivery agent in the present composition is a delivery effective amount and can be determined for any particular delivery agent/active agent combination by methods known to those skilled in the art.

The oral dosage forms of the present invention, containing a mixture of the active agent, e.g., cromolyn sodium, and the delivery agent, e.g., SNAC, or separately containing the active agent and the delivery agent, may include additional materials known to those skilled in the art as pharmaceutical excipients. Any excipient or ingredient, including pharmaceutical ingredients or excipients. Such pharmaceutical excipients include, for example, the following: Acidifying agents (acetic acid, glacial acetic acid, citric acid, fumaric acid, hydrochloric acid, diluted hydrochloric acid, malic acid, nitric acid, phosphoric acid, diluted phosphoric acid, sulfuric acid, tartaric acid); Aerosol propellants (butane, dichlorodifluoromethane, dichlorotetrafluoroethane, isobutane, propane, trichloromonofluoromethane); Air displacements (carbon dioxide, nitrogen); Alcohol denaturants (denatonium benzoate, methyl isobutyl ketone, sucrose octacetate); Alkalinizing agents (strong ammonia solution, ammonium carbonate, diethanolamine, diisopropanolamine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, triethylamine); Anticaking agents (see glidant); Antifoaming agents (dimethicone, simethicone); Antimicrobial preservatives (benzalkonium chloride, benzalkonium chloride solution, benzethonium chloride, benzoic acid, benzyl alcohol, butylparaben, methylparaben, methylparaben sodium, phenol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric nitrate, potassium benzoate, potassium sorbate, propylparaben, propylparaben sodium, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimerosal, thymol); Antioxidants (ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, tocopherols excipient); Buffering agents (acetic acid, ammonium carbonate, ammonium phosphate, boric acid, citric acid, lactic acid, phosphoric acid, potassium citrate, potassium metaphosphate, potassium phosphate monobasic, sodium acetate, sodium citrate, sodium lactate solution, dibasic sodium phosphate, monobasic sodium phosphate); Capsule lubricants (see tablet and capsule lubricant); Chelating agents (edetate disodium, ethylenediaminetetraacetic acid and salts, edetic acid); Coating agents (sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methacrylic acid copolymer, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, zein); Colorants (caramel, red, yellow, black or blends, ferric oxide); Complexing agents (ethylenediaminetetraacetic acid and salts (EDTA), edetic acid, gentisic acid ethanolamide, oxyquinoline sulfate); Desiccants (calcium chloride, calcium sulfate, silicon dioxide);

Emulsifying and/or solubilizing agents (acacia, cholesterol, diethanolamine (adjunct), glyceryl monostearate, lanolin alcohols, lecithin, mono- and di-glycerides, monoethanolamine (adjunct), oleic acid (adjunct), oleyl alcohol (stabilizer), poloxamer, polyoxyethylene 50 stearate, polyoxyl 35 castor oil, polyoxyl 40 hydrogenated castor oil, polyoxyl 10 oleyl ether, polyoxyl 20 cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, propylene glycol diacetate, propylene glycol monostearate, sodium lauryl sulfate, sodium stearate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, stearic acid, trolamine, emulsifying wax); Filtering aids (powdered cellulose, purified siliceous earth); Flavors and perfumes (anethole, benzaldehyde, ethyl vanillin, menthol, methyl salicylate, monosodium glutamate, orange flower oil, peppermint, peppermint oil, peppermint spirit, rose oil, stronger rose water, thymol, tolu balsam tincture, vanilla, vanilla tincture, vanillin); Glidants and/or anticaking agents (calcium silicate, magnesium silicate, colloidal silicon dioxide, talc); Humectants (glycerin, hexylene glycol, propylene glycol, sorbitol); Plasticizers (castor oil, diacetylated monoglycerides, diethyl phthalate, glycerin, mono- and di-acetylated monoglycerides, polyethylene glycol, propylene glycol, triacetin, triethyl citrate); Polymers (e.g., cellulose acetate, alkyl celluloses, hydroxyalkylcelluloses, acrylic polymers and copolymers); Solvents (acetone, alcohol, diluted alcohol, amylene hydrate, benzyl benzoate, butyl alcohol, carbon tetrachloride, chloroform, corn oil, cottonseed oil, ethyl acetate, glycerin, hexylene glycol, isopropyl alcohol, methyl alcohol, methylene chloride, methyl isobutyl ketone, mineral oil, peanut oil, polyethylene glycol, propylene carbonate, propylene glycol, sesame oil, water for injection, sterile water for injection, sterile water for irrigation, purified water); Sorbents (powdered cellulose, charcoal, purified siliceous earth); Carbon dioxide sorbents (barium hydroxide lime, soda lime); Stiffening agents (hydrogenated castor oil, cetostearyl alcohol, cetyl alcohol, cetyl esters wax, hard fat, paraffin, polyethylene excipient, stearyl alcohol, emulsifying wax, white wax, yellow wax); Suspending and/or viscosity-increasing agents (acacia, agar, alginic acid, aluminum monostearate, bentonite, purified bentonite, magma bentonite, carbomer 934p, carboxymethylcellulose calcium, carboxymethylcellulose sodium, carboxymethylcellulose sodium 12, carrageenan, microcrystalline and carboxymethylcellulose sodium cellulose, dextrin, gelatin, guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium aluminum silicate, methylcellulose, pectin, polyethylene oxide, polyvinyl alcohol, povidone, propylene glycol alginate, silicon dioxide, colloidal silicon dioxide, sodium alginate, tragacanth, xanthan gum); Sweetening agents (aspartame, dextrates, dextrose, excipient dextrose, fructose, mannitol, saccharin, calcium saccharin, sodium saccharin, sorbitol, solution sorbitol, sucrose, compressible sugar, confectioner's sugar, syrup); Tablet binders (acacia, alginic acid, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methylcellulose, polyethylene oxide, povidone, pregelatinized starch, syrup); Tablet and/or capsule diluents (calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextrates, dextrin, dextrose excipient, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, compressible sugar, confectioner's sugar); Table disintegrants (alginic acid, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrifin potassium, sodium starch glycolate, starch, pregelatinized starch); Tablet

and/or capsule lubricants (calcium stearate, glyceryl behenate, magnesium stearate, light mineral oil, polyethylene glycol, sodium stearyl fumarate, stearic acid, purified stearic acid, talc, hydrogenated vegetable oil, zinc stearate); Tonicity agent (dextrose, glycerin, mannitol, potassium chloride, sodium chloride); Vehicle: flavored and/or sweetened (aromatic elixir, compound benzaldehyde elixir, iso-alcoholic elixir, peppermint water, sorbitol solution, syrup, tolu balsam syrup); Vehicle: oleaginous (almond oil, corn oil, cottonseed oil, ethyl oleate, isopropyl myristate, isopropyl palmitate, mineral oil, light mineral oil, myristyl alcohol, octyldodecanol, olive oil, peanut oil, persic oil, scame oil, soybean oil, squalane); Vehicle: solid carrier (sugar spheres); Vehicle: sterile (Bacteriostatic water for injection, bacteriostatic sodium chloride injection); Viscosity-increasing (see suspending agent); Water repelling agent (cyclomethicone, dimethicone, simethicone); and Wetting and/or solubilizing agent (benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, docusate sodium, nonoxynol 9, nonoxynol 10, octoxynol 9, poloxamer, polyoxyl 35 castor oil, polyoxyl 40, hydrogenated castor oil, polyoxyl 50 stearate, polyoxyl 10 oleyl ether, polyoxyl 20, cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, sodium lauryl sulfate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, tyloxapol). This list is not meant to be exclusive, but instead merely representative of the classes of excipients and the particular excipients that may be used in oral dosage forms of the present invention.

Clinical Applications

In preferred embodiments in which the active agent is cromolyn sodium, the pivotal roles mast cell degranulation and release of mediators play in the genesis of allergic reactions, and the potential role of cromolyn of modifying the immune response at the top of the inflammatory cascade, are the rationale to utilize this drug in an oral form. The conditions for which cromolyn is most suited are mild to moderate asthma and allergic rhinitis.

Asthma is a serious, chronic and potentially life-threatening condition affecting an estimated 14 to 15 million people in the United States. More than 5000 people die of asthma annually, and almost all of these deaths are preventable. Prevalence rates have increased 29% in the last decade, hospitalizations by 300% and asthma death rates by 31%. Asthma causes more than 100 million days annually of restricted activity due to symptoms and 7 million outpatient visits per year for asthma-related symptoms. The costs of asthma care are enormous, approaching \$6 billion per year.

Allergic rhinitis is the fifth most common chronic illness in the U.S. and, according to the Joint Task Force on the Diagnosis and Treatment of Rhinitis, allergic rhinitis affects 20 to 40 million people in the U.S. annually, including 10% to 30% of adults and up to 40% of children. The disease occurs either during the seasons of airborne pollens and molds or throughout the year, triggered by indoor allergens such as dust mites, house pets, cockroaches and molds. Allergic rhinitis usually starts in childhood, on average at about the age of 10 years. Like that of asthma, its prevalence is rising, especially in urban areas, for obscure reasons. The severity of allergic rhinitis ranges from mild to seriously debilitating and has a large impact on the quality of life of affected patients. Although many assume that allergic rhinitis is more of a nuisance than a clinically significant health problem, it is responsible for \$6 billion annually in health care costs in the United States, and it frequently causes absence or poor performance at work and school.

Allergic rhinitis and asthma are atopic diseases implying IgE-mediated diseases. Individuals with atopy have a hereditary predisposition to produce IgE antibodies against common environmental allergens and often have one or more atopic diseases. On inhaling aeroallergens derived from pollen, house-dust mites, and cat dander the individual without atopy will mount a low-grade immunologic response; they produce allergen-specific IgG1 and IgG4 antibodies, and in vitro their T cells respond to the allergen with a moderate degree of proliferation and the production of interferon- γ by type 1 helper T (Th1) cells. Persons with atopy, by contrast, have an exaggerated response characterized by the production of allergen-specific IgE antibodies; they have elevated serum levels of IgE antibodies and positive reactions to extracts of common aeroallergens on skin-prick tests. T cells from their blood respond to allergens in vitro by inducing cytokines produced by type 2 helper T (Th2) cells (i.e., interleukin-4, 5, and 13), rather than cytokines produced by Th1 cells (interferon- γ and interleukin-2). The immunopathological hallmark of allergic disease is the infiltration of affected tissue by Th2 cells.

The development of specific allergic diseases may be related to alterations in the target organ. For example, the cofactors required for an asthma attack may include respiratory virus infections and exposure to allergens, tobacco smoke and air pollutants. These factors, alone or in combination, may alter immunoregulatory mechanisms at mucosal surfaces in ways that promote a Th2-mediated allergic inflammatory response. Acute allergic reactions result from the release of preformed granule-associated mediators, membrane-derived lipids, cytokines and chemokines when an allergen interacts with IgE that is bound to mast cells or basophils by the chain of the high-affinity IgE receptor.

The most important inducers of the production of IgE are interleukin-4 and interleukin-13. These cytokines initiate transcription of the gene for the epsilon class of the constant region (C) of the immunoglobulin heavy chain. The production of IgE also requires two transcription factors, nuclear factor B and STAT-6; the former pathway involves the costimulatory molecules CD40 and the CD40 ligand (CD 154), and the latter is activated when interleukin-4 binds to the high-affinity chain of the interleukin-4 receptor.

In a person with atopy, exposure of the airway to a single dose of allergen produces an immediate hypersensitivity reaction manifesting itself as sneezing and runny nose, or wheezing within minutes. Depending on the amount of the allergen, these immediate hypersensitivity reactions are followed by a late-phase reaction, which reaches a peak six to nine hours after exposure to the allergen and then slowly resolves.

Immediate hypersensitivity is the basis of acute allergic reactions. It is caused by molecules released by mast cells when an allergen interacts with membrane-bound IgE. The complex of allergen, IgE and Fc ϵ R1 on the surface of the mast cell triggers a noncytotoxic, energy-dependent release of preformed, granule-associated histamine and tryptase and the membrane-derived lipid mediators leukotrienes, prostaglandins, and platelet-activating factor. (Tryptase is a four-chain neutral protease that activates the protease-activated receptors on endothelial and epithelial cells). The leukotrienes produced by mast cells are the three cysteinyl leukotrienes C₄, D₄ and E₄, which cause the contraction of smooth muscles, vasodilatation, increased vascular permeability and the hypersecretion of mucus when they bind to specific receptors. The activation of the specific receptors initiates a cascade of events, including the up-regulation of adhesion molecules

that selectively attract eosinophils and basophils. The mast-cell mediators have a critical role in anaphylaxis, rhinoconjunctivitis and urticaria.

In the late-phase asthmatic and nasal reaction, eosinophils and neutrophils accumulate, and then CD4⁺ T cells (in the cutaneous late phase response also basophils infiltrate the site). Depending on the target organ, late-phase reactions can be provoked by the activation of mast cells or T cells. Late-phase reactions can be induced in patients with atopic asthma in the absence of immediate hypersensitivity involving mast cells.

Pharmacokinetics:

Systemic bioavailability of oral cromolyn (administered without the delivery agents of the present invention) is approximately 1%. Nevertheless, cromolyn is administered orally to treat systemic mastocytosis and inflammatory bowel disease. Minimal systemic absorption occurs after either intranasal or ophthalmic use. Roughly 5-10% of an inhaled dose reaches the lungs. The amount reaching the lungs after inhalation is affected by the degree of bronchoconstriction present. Cromolyn does not traverse cell membrane walls well because it is highly lipid-insoluble and mainly ionized. Several weeks of therapy may be required before improvement is apparent. Little of the drug crosses the placenta or distributes into breast milk. Roughly 98% of the dose is eliminated unchanged in the feces.

Mechanism of Action:

Mucosal inflammation is characterized by early and late phases. The early phase results from IgE-mediated mast cell degranulation. Cromolyn works at the surface of the mast cell to inhibit its degranulation. This, in turn, prevents the release of histamine and slow-reacting substance of anaphylaxis (SRS-A), mediators of type I allergic reactions. Cromolyn also may reduce the release of inflammatory leukotrienes. It has been postulated that cromolyn produces these effects by inhibiting calcium influx, but its exact mechanism of action is unclear. Cromolyn does not interfere with the binding of IgE to the mast cell or with the binding of antigen to IgE. Because cromolyn is not a bronchodilator, an antihistamine or a vasoconstrictor, its beneficial effects in the treatment of asthma are largely prophylactic. Cromolyn can reduce hyperreactivity of the bronchi, inhibiting asthmatic responses to antigenic challenge (e.g., cold air, allergens, environmental pollutants) or to exercise.

The late phase bronchospastic response of asthma is characterized by interstitial edema, mucous glycoprotein release, and eosinophil infiltration of the airways. Leukotrienes attract cellular infiltrates producing epithelial injury, abnormalities in neural mechanisms, increases in airway smooth muscle responsiveness and airway obstruction. Cromolyn may reduce the release of inflammatory leukotrienes. It has been postulated that cromolyn produces these effects by inhibiting calcium influx, but its exact mechanism of action is unclear. An exaggerated bronchoconstrictor response, airway hyperresponsiveness, can be induced by a variety of causes including cold air, allergens, environmental pollutants or exercise. Cromolyn can reduce hyperreactivity of the bronchi, inhibiting asthmatic responses to antigenic challenge.

The guidelines for the diagnosis and management of asthma depend on the severity of the disease and patient age, factors which determine which therapy to initiate. Both adults and children with chronic mild asthma can effectively prevent asthma attacks with cromolyn. Cromolyn is first line therapy for prophylaxis because it is well tolerated, displaying only minor adverse reactions. For the chronic treatment of moder-

ate asthma, cromolyn continues to be the respiratory anti-inflammatory agent of choice, with inhaled or oral corticosteroids as an acceptable option.

As the severity of the disease progresses, therapy becomes more intense. Inhaled corticosteroids are first line agents for both adults and children, with or without cromolyn or other agents. If not effectively controlling symptoms, short burst of oral corticosteroids or chronic alternate day therapy should be considered. If symptoms are severe enough in children, systemic corticosteroid therapy may be considered; risk-benefit should be weighed in this therapeutic decision. The use of intravenous corticosteroids is limited to the treatment of acute exacerbations of asthma in patients in the emergency room or in hospitalized patients.

As discussed above, cromolyn sodium inhibits antigen-triggered release of mediators from sensitized mast cells. Cromolyn sodium stabilizes the mast cell membrane and is believed to function in part by being bound to the mast cell membranes.

In contrast to previously available low dosages of cromolyn sodium (i.e., Gastrocrom®), which is available in 100 mg ampoules and which upon oral administration of which no more than 1% of the administered dose is absorbed, the oral formulations of the present invention provide a greatly enhanced oral absorption of cromolyn sodium and can achieve this increased absorption at a more rapid rate. For this reason, the oral doses of cromolyn sodium plus delivery agent as described herein are contemplated for use in a wide range of disease states which may be mediated or exacerbated by a proliferation or infiltration of mast cells and degranulation of these mast cells, thereby causing an inflammatory condition. Plus, it is contemplated that the formulations of the present invention may be used as anti-inflammatory formulations to prevent the release of mediators from sensitized mast cells. Inflammatory conditions contemplated for treatment by the formulations of the present invention include any systemic inflammatory condition known to those skilled in the art, including but not limited to autoimmune diseases, rheumatoid arthritis, and cardiovascular disease. Many other disease states which are caused by or exacerbated by inflammatory conditions caused by the release of mediators from sensitized mast cells may also be treated utilizing the oral formulations of the present invention.

By virtue of the fact that the oral dosage forms of the present invention provide an increased absorption of cromolyn sodium as compared to any other treatments to date, the cromolyn sodium may be delivered to the systemic circulation of the effected patients in a higher level than previously possible. In view of the fact that cromolyn sodium binds to mast cells and functions in this manner, the duration of action of the cromolyn sodium may be dependent upon the amount absorbed. Therefore, it is contemplated that the oral dosage forms of the present invention provide a longer acting product, and may be administrable at greater intervals (i.e. once a day) and still achieve the desired therapeutic effect.

Furthermore, it is contemplated that, in contrast to generally accepted treatment regimens, the systemic administration of cromolyn sodium may be more efficacious in certain disease states to treat a localized inflammatory condition (e.g., asthma) than a localized treatment of cromolyn sodium form asthma (e.g., via a metered dose inhaler (MDI)). This is because, in disease states such as asthma, the most diseased airways are restricted to the greatest extent and therefore have the least airflow, while the least diseased airways have the greatest airflow. Therefore, local administration of cromolyn sodium via MDI will likely result in the greatest amount of drug flowing to the areas that least require treatment. In con-

trast, via the systemic administration of cromolyn sodium via the oral formulations of the present invention, the localized area (e.g., lungs) can be treated equally across the entire area, thereby insuring that sufficient levels of cromolyn sodium reach the most restricted passage ways of the lung.

In further embodiments of the present invention, it is contemplated that the oral dosage forms of the invention may be used to treat any inflammatory condition wherein inflammatory process is related at least in part to the release of cytokines or histamine from mast cells.

It is further contemplated that the oral dosage forms of the present invention may be used to treat alert allergies, including but not limited to allergic rhinitis. For seasonal allergies, it is contemplated that the oral dosage forms of the present invention are administered on a regular basis beginning early in the season and are continued prophylactically throughout the allergy season. For very severe allergies, it is contemplated that the administration of the oral dosage forms of the present invention may occur on a once-every-four-hours basis. However, it is contemplated that the oral dosage forms of the invention can be administered for treatment of allergy on a once-a-day basis in many situations, as well.

It is further contemplated that the oral dosage of the present invention can be utilized for the treatment of allergic conjunctivitis. In such embodiments, it is contemplated that a sufficient plasma concentration of cromolyn sodium is obtained via the oral administration to reach the affected area and provide an effective treatment.

In certain preferred embodiments, the oral formulations of the present invention include cromolyn sodium in unmodified form in an amount from about 40 mg to about 1 g, and more preferably in an amount from about 80 mg to 100 mg, together with a drug delivery agent (e.g. SNAC) in an amount from about 200 mg to about 2500 mg.

As demonstrated by the appended examples, the oral dosage forms of the present invention in certain preferred embodiments provide a rapid absorption of cromolyn sodium systemically in a significantly greater percentage than previously reported. In certain preferred embodiments, this rapid absorption is characterized by the time to maximum plasma concentration T_{max} within about 1 hour after oral administration of the dosage form, and in certain embodiments occurring at a time point at from about 0.2 to about 0.5 hours after oral administration of the oral dosage forms of the present invention.

The oral dosage forms of the present invention containing cromolyn sodium plus the delivery agent are described by virtue of the C_{max} and AUC of the cromolyn sodium following oral administration of the dose. Although these pharmacokinetic parameters are characteristic of certain embodiments of the oral dosage forms of the present invention, it will be appreciated by those skilled in the art that the measured plasma concentration of cromolyn sodium may not be directly tied to efficacy or activity of the oral dosage form, as there have been no studies reported to date concerning the absolute relationship in humans between plasma level and activity or disease states contemplated treatment in the present invention.

Due to the greater levels of systemic absorption afforded to cromolyn sodium by the oral dosage forms of the present invention, it is contemplated that the duration of action may be dependent on the total amount of drug absorbed from the gastrointestinal tract. Therefore, it is contemplated that the oral dosage forms of the present invention may provide the prolonged activity of cromolyn sodium in comparison to other cromolyn sodium treatments used to date. However, it is also contemplated that, in certain circumstances, e.g., in the

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treatment of severe inflammatory conditions, the oral dosage forms of the present invention may be administered as often as every 4 hours to human patients. It is further contemplated that the oral dosage forms of the present invention may be administered on a less frequent basis, e.g., 4 times a day, 3 times a day, twice a day, and once a day.

In certain preferred embodiments of the present invention, the oral dosage form is a solid dosage form which contains the cromolyn sodium in an effective amount together with the drug delivery agents of the present invention in a requisite ratio to provide suitably enhanced absorptivity of the cromolyn sodium from the gastrointestinal tract upon oral administration. In embodiments of the present invention wherein a relatively large amount of cromolyn sodium is administered together with the requisite amount of drug delivery agent, or wherein a large amount of drug delivery is required to provide the requisite absorptivity, it is further contemplated that the dose may be divided into multiple capsules or tablets and administered to the patient at the same time. In this manner, even large amounts of drug/delivery agent combinations can be administered orally to the patient without patient experiences problems swallowing the dose. In yet other embodiments of the invention contemplated herein, the cromolyn sodium/drug delivery agent combination may be prepared in the form of a dry powder which is suspended in a liquid for oral administration prior to use, thereby negating the necessity of administering multiple unit doses of the dosage forms of the present invention in order to attain the desired dosage level of drug.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods, compositions, reagents, cells, similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are described herein. All publications mentioned herein are incorporated herein, including all figures, graphs, equations, illustrations, and drawings, to describe and disclose specific information for which the reference was cited in connection with.

The publications discussed above are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than as limitations on the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In order that this invention may be better understood, the following examples are set forth to illustrate various aspects of the present invention. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention or the scope of the claims in any manner.

Example 1

Male Sprague-Dawley rats weighing 225-275 grams were fasted for 24 hours. Rats were anesthetized by intramuscular injection with ketamine and thorazine. Experimental groups were dosed by oral gavage or by intravenous injection. Blood samples were collected serially via the tail artery. Serum obtained from whole blood was acidified and cromolyn

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extracted with ethyl acetate. The ethyl acetate was added to phosphate buffer or back extraction of cromolyn. Pharmacokinetic parameters were determined from extracted serum by HPLC. For intravenous administered cromolyn (1.5 mg/kg), the area under the serum concentration-time curve (AUC), peak serum concentration (C_{max}) and time of peak serum concentration (T_{max}) were 20.4 $\mu\text{g}\cdot\text{min}/\text{ml}$, 1.3 $\mu\text{g}/\text{ml}$ and 0 minutes, respectively. When a delivery agent and cromolyn (30 mg/kg) are co-administered orally, the AUC, C_{max} and T_{max} were 18.3 $\mu\text{g}\cdot\text{min}/\text{ml}$, 0.65 $\mu\text{g}/\text{ml}$ and 15 minutes, respectively. No detectable serum cromolyn concentrations were observed in the control groups of delivery agent alone and cromolyn alone. An absolute oral bioavailability of 4.5% was achieved in rats. Results for the administration of cromolyn to rats are in Table 1.

TABLE 1

Administration of Cromolyn to Rats							
Dose		Pharmacokinetic Parameters					
Delivery Agent (mg/kg)	Cromolyn (mg/kg)	Route of Administration	n	Avg. C_{max} ($\mu\text{g}/\text{ml}$)	T_{max} (min)	Avg. AUC ($\mu\text{g}\cdot\text{min}/\text{ml}$)	
200	1.5	IV	5	1.3	0	20.4	
	30	P.O.	5	0.04	15	1.03	
	30	P.O.	5	0.10	30	9.03	
		P.O.	5	0.65	120	18.3	

Example 2

Male and female Cynomolgus monkeys were fasted for 24 hours. Monkeys were gavaged with a solution of delivery agent and cromolyn. Serum obtained from whole blood was acidified and cromolyn extracted with ethyl acetate. The ethyl acetate was added to phosphate buffer for back extraction of cromolyn. Pharmacokinetic parameters were determined from extracted serum by HPLC. The AUC, C_{max} and T_{max} from orally dosing capsules containing the delivery agent and cromolyn (25 mg/kg) were 48.8 $\mu\text{g}\cdot\text{min}/\text{ml}$, 0.30 $\mu\text{g}/\text{ml}$ and 130 minutes, respectively.

Example 3

In order to obtain an in vivo evaluation of the oral administration of cromolyn to sheep, a preparation of delivery agent and cromolyn was prepared and administered to fasted sheep by oral gavage, 15 minutes prior to challenge with ascaris. A control formulation without cromolyn was also administered to an additional group. The biological effect of the cromolyn was assessed using changes in airway resistance of the sheep after challenge with ascaris.

In one study, the oral doses of cromolyn and delivery agent were administered 15 minutes before ascaris challenge. The results are depicted in FIG. 5, which is a graph depicting the percent airway resistance after ascaris challenge in the sheep over time. As can be seen from FIG. 5, in this study, the oral cromolyn reduced the change in airway resistance by 200%. In animals not receiving cromolyn, the change in airway resistance was plus 500% after ascaris challenge and in animals receiving cromolyn the change in airway resistance was 300%. Even given within 15 minutes of ascaris challenge the allergic symptoms were substantially alleviated.

In a second study, the oral doses of cromolyn and delivery agent were administered 60 minutes before ascaris challenge. The results are depicted in FIG. 6, which is a graph depicting

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the percent airway resistance after ascaris challenge in the sheep over time. As can be seen from the results depicted in FIG. 6, in this study the oral cromolyn reduced the change in airway resistance between about 350 to 400%. In animals not receiving cromolyn, the change in airway resistance was plus 440% after ascaris challenge and in animals receiving cromolyn the change in airway resistance was approximately 60%. Therefore, if the cromolyn formulations of the present invention are given about 1 hour prior to ascaris challenge the allergic symptoms were substantially eliminated.

In a third study, an oral dose of 100 mg/kg cromolyn and 300 mg/kg delivery agent were administered to fasted sheep by oral gavage, 12 hours prior to challenge with ascaris. A control formulation without cromolyn was also administered to an additional group. The biological effect of the cromolyn was assessed using changes in airway resistance of the sheep after challenge with ascaris. The results are tabulated in Table 2 below and are depicted in FIG. 8, which is a graph depicting the percent airway resistance after ascaris challenge in the sheep over time.

TABLE 2

Administration of Cromolyn to Sheep, 12 hours prior to ascaris challenge: Compound: CR-1 & Cromolyn Dose: 300 mg/kg CR-1 & 100 mg/kg Cromolyn given oral 12 hrs. before Ag. challenge <i>Ascaris Acute Study</i>							
Sheep#	Baseline	P-Ascaris		+1 hr.		+2 hr.	
	RL	RL	%	RL	%	RL	%
Control Trial:							
2067	0.98	7.77	693%	3.55	262%	2.10	114%
2080	0.94	7.33	680%	5.10	442%	3.00	219%
Means:	0.96	7.55	687%	4.33	352%	2.55	167%
S.D.:	0.03	0.31	9%	1.10	127%	0.64	74%
S.E.:	0.02	0.22	6%	0.78	90%	0.45	53%
Drug Trial:							
2067	0.95	1.64	73%	1.04	9%	0.97	2%
2080	0.98	3.33	240%	1.54	57%	0.98	0%
Means:	0.97	2.49	157%	1.29	33%	0.98	1%
S.D.:	0.02	1.20	118%	0.35	34%	0.01	1%
S.E.:	0.01	0.85	84%	0.25	24%	0.01	1%

In animals not receiving cromolyn, the change in airway resistance was plus 687% after ascaris challenge and in animals receiving cromolyn the change in airway resistance was approximately 157%. The mean protection with this carrier vs. the peak control response is 77%. Therefore, if the cromolyn formulations of the present invention are given about 12 hours prior to ascaris challenge the allergic symptoms were substantially eliminated.

Example 4

Cromolyn sodium was orally administered to healthy human subjects in a study designed to evaluate the safety, tolerability, oral absorption and pharmacokinetics data following various doses of cromolyn orally administered in combination with the delivery agent SNAC as capsules. The oral administration was accomplished using capsules containing the specified dose of cromolyn sodium and sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) synthesized, e.g., as described in Examples 1 and 8-11 in International Publication Number WO 00/46182 by Gschneidner et

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al., (2000). This study was a randomized, open label, oral, single escalating dose administration study in healthy, fasted volunteers.

The volunteers were all males between 18 and 50 years of age, in good health, with a body weight resulting in a Body Mass Index (BMI) between 18.0 and 28.0 kg/m² inclusive, calculated as the body mass/(Height)². All laboratory values (electrocardiogram, hematology, serum chemistries, and urinalysis) obtained during screening were within normal ranges.

The study was carried out in two parts. In the first clinical part, ten subjects received four escalating single doses of Cromolyn/SNAC, and two subjects received two other treatments (80 mg of cromolyn alone as a first treatment, and 2000 mg of SNAC alone as a second treatment). The dosing regimen is indicated in Table 3 below. Eight subjects (subjects nos. 11 to 18) were included in the second clinical part, and dosed as indicated in Table 4 below. Safety and pharmacokinetic measures were collected and evaluated following each dose. If the observed cromolyn concentration was higher than the desired concentration (i.e., about 10-20 ng/ml range), the next dose was modified.

TABLE 3

Dosing regimen in subjects nos. 1-10		
Treatment Period	Cromolyn/SNAC (mg/mg) (subjects nos. 1 to 8)	Cromolyn/SNAC alone (mg/mg) (subjects nos. 9 and 10)
Period 1	40/1200	80/0
Period 2	80/1200	0/2000
Period 3	80/600	ND
Period 4	120/200	ND
ND: no more dosing		

TABLE 4

Dosing regimen in additional subjects nos. 11 to 18		
Treatment Period	Cromolyn/SNAC (mg/mg) (7 subjects)	Cromolyn/SNAC (mg/mg) (one subject)
Period 1	120/400	150/0
Period 2	120/500	150/0
Period 3	150/200	150/0

The initial cromolyn dose of 40 mg was selected based on preclinical studies and cromolyn therapeutic concentration levels. The clinical therapeutic concentration of cromolyn is about 0.01 µg/ml. It is stated in the Physician's Desk Reference that the therapeutic levels of cromolyn was achieved after inhalation dose of 20 mg with an cromolyn absolute bioavailability of about 8%. Preclinical study with cromolyn/SNAC in rats demonstrated that the cromolyn absolute bioavailability of about 4%. Assuming similar absolute bioavailability in rats and humans, the initial dose was selected to be 40 mg cromolyn combined with 1200 mg SNAC. The escalation of the SNAC dose from 1200 mg to 2000 mg was selected based upon the results of the nonclinical studies and a clinical safety and tolerability study.

On day one of each study treatment period, medication (capsules) was administered following an 8-hour overnight fast. The capsules were swallowed with up to 120 mL of water to the subjects in an upright position. After each dosing, safety, and pharmacokinetic measures were taken, and cromolyn concentrations were evaluated. The sponsor and the

investigator together decided the administration of the next dose after reviewing safety measurements and cromolyn plasma concentration of the current dose. If the observed cromolyn concentration was higher than the therapeutic concentration (i.e., about 10-20 ng/mL range), the next dose was reduced or the current dose was repeated.

Cromolyn/SNAC capsule formulations containing varying cromolyn/SNAC ratios were prepared extemporaneously at the study site. The process could be summarized as follows: First, sieve SNAC powder through a screen, or delump it using a pestle/mortar. Then, weigh the exact dose of SNAC powder (delumped) and add to a mortar or to a suitable container, and weigh the exact dose of cromolyn and add to SNAC pre-weighed in the container. Carefully mix cromolyn/SNAC powders and fill all the powder in the capsule shells provided. For the subjects receiving cromolyn alone, the appropriate amount for cromolyn was blended with an inactive excipient, METHIOCEL E15 PREMIUM LV, and packaged in four hard gelatin capsules. Each capsule contained about 2100 mg solids (SNAC and Cromolyn sodium USP). Capsules were prepared at the following dosages (mg cromolyn/mg SNAC): 0/2000, 40/1200, 80/0, 80/600, 80/1200, 120/200, 120/400, 120/500, 150/0 and 150/200.

Following administration, blood samples (5 mL in tri-sodium citrate tube) were drawn immediately before dosing (0), and at 5, 10, 15, 20, 30, and 45 minutes and 1, 1.25, 1.5, 2, 2.5, 3, 4, 6 and 8 hours postdose (16 samples per treatment) for cromolyn and SNAC measurements in all treatment groups.

The effect of oral administration of either SNAC alone or cromolyn alone in human subjects was evaluated. The average pharmacokinetic parameters derived for cromolyn in plasma after single administration of the different combination treatments of cromolyn sodium and SNAC dosages are summarized in Table 5 below.

The plasma levels of cromolyn for individual subjects given treatment number 1 are depicted in FIG. 4. The plasma levels of cromolyn for individual subjects given treatment number 2 are depicted in FIG. 5. The plasma levels of cromolyn for individual subjects given treatment number 3 are depicted in FIG. 6. The plasma levels of cromolyn for individual subjects given treatment number 4 are depicted in FIG. 7.

Profiles of average plasma cromolyn concentration versus time after single oral administration of different combinations of cromolyn sodium and SNAC dosages expressed as cromolyn/SNAC (mg/mg) are illustrated in FIG. 8 (original values) and FIG. 9 (after normalization to a 100 mg dose of cromolyn). In these figures, values are arithmetic means.

The cromolyn absorbed through oral administration of capsules containing SNAC and cromolyn is bioavailable and bioactive. As summarized in Table 4, cromolyn that has been granulated with SNAC and encapsulated achieves a maximal plasma level in human subjects in less than 30 minutes. As shown in Table 5, mean plasma levels ranging from about 22.3 ng/ml to about 76.7 ng/ml are achieved using this oral formulation.

No cromolyn was administered to subjects with a dosage of 2000 mg of SNAC. Regarding the peak (C_{max}) and extent ($AUC_{(0-t)}$) of exposure to cromolyn, the other treatments could be classified in three groups:

When patients were administered cromolyn without SNAC (i.e., 80/0 and 150/0), the plasma cromolyn concentration was very low with normalized C_{max} below 3 ng/mL/100 mg and $AUC_{(0-t)}$ not exceeding 10 ng·h/mL/100 mg. The peak cromolyn concentration occurred between 2 to 8 hours after administration and plasma concentrations never reached the therapeutic level (i.e., at least 10 ng/mL). When cromolyn is administered without the delivery agent of the present invention, it is not significantly absorbed or bioavailable. See FIGS. 8 and 9.

When patients were administered cromolyn with 200 mg of SNAC (i.e., 120/200 and 150/200), the plasma cromolyn concentration increased rapidly with normalized C_{max} around 30 ng/mL/100 mg and $AUC_{(0-t)}$ around 30 ng·h/mL/100 mg. In all subjects, the peak concentration was observed within 45 minutes after administration. The therapeutic cromolyn plasma level was not reached in subjects nos. 3 and 7 after administration of 120 mg of cromolyn, but was reached for at least 30 minutes in all subjects after administration of 150 mg.

When patients were administered cromolyn with higher SNAC doses (400 to 1200 mg), cromolyn absorption was still higher with normalized C_{max} between 53 and 64 ng/mL/100 mg and $AUC_{(0-t)}$ between 39 and 50 ng·h/mL/100 mg. In all subjects, the peak concentration was observed within 30 minutes after administration. The therapeutic cromolyn plasma level was not reached in subject no. 7 after administration of 40 mg of cromolyn sodium combined with 1200 mg of SNAC, and in subject no. 14 administered with 120 mg of cromolyn sodium and 400 mg of SNAC. High cromolyn plasma concentrations were found in subject no. 13 after administration of 120 mg of cromolyn combined with 400 and 500 mg of SNAC (C_{max} reached respectively 172 and 229 ng/mL).

The plasma levels of cromolyn achieved with these formulations were significantly higher than those seen with pulmonary delivery of cromolyn at therapeutic doses.

TABLE 5

Cromolyn/SNAC [mg/mg]	C_{max} [ng/mL]	T_{max} [h]	$AUC_{(0-t)}$ [ng·h/mL]	$MRT_{(0-t)}$ [h]	C_{max}/D^* [ng/mL]	$AUC_{(0-t)}/D^*$ [ng·h/mL]
40/1200 (N = 8)	22.3 ± 12.3	0.33-0.33	16.5 ± 7.9	1.31 ± 0.57	55.8 ± 30.7	41.2 ± 19.8
80/1200 (N = 8)	42.3 ± 21.3	0.25-0.33	31.4 ± 15.7	1.22 ± 0.44	52.9 ± 26.6	39.2 ± 19.6
80/600 (N = 8)	45.9 ± 24.7	0.25-0.37	33.4 ± 14.2	1.41 ± 0.23	57.3 ± 30.9	41.8 ± 17.8
120/200 (N = 8)	33.8 ± 28.0	0.33-0.75	34.9 ± 22.4	2.23 ± 0.83	28.2 ± 23.3	29.1 ± 18.7
80/0 (N = 2)	2.33 ± 0.87	2.00-3.00	7.97 ± 0.24	3.65 ± 0.37	2.91 ± 1.09	9.96 ± 0.30
120/400 (N = 8)	65.5 ± 52.5	0.25-0.50	52.7 ± 37.1	1.94 ± 0.64	54.5 ± 43.7	43.9 ± 30.9
120/500 (N = 7)	76.7 ± 75.5	0.33-0.50	59.7 ± 60.9	1.64 ± 0.40	64.0 ± 62.9	49.8 ± 50.7
150/200 (N = 7)	51.1 ± 29.3	0.33-0.52	48.5 ± 25.1	2.14 ± 0.46	34.1 ± 19.5	32.3 ± 16.7
150/0 (N = 3)	2.06 ± 0.85	4.00-8.00	8.53 ± 2.94	5.06 ± 0.57	1.37 ± 0.56	5.69 ± 1.96

*Normalized to a 100-mg dose of cromolyn sodium

Values are mean ± SD, except range for T_{max}

Further evidence of the bioavailability of oral cromolyn formulated according to the present invention is demonstrated by the area under the plasma concentration-time curve (AUC) of cromolyn after oral administration. As shown in Table 5, the AUC ranged from 16.5 to 59.7 ng·hr/mL.

Profiles for average SNAC plasma concentration versus time after single oral administration of different combinations of cromolyn sodium and SNAC dosages expressed as cromolyn/SNAC (mg/mg) are illustrated in FIG. 10 (original values) and FIG. 11 (after normalization to a 1000 mg dose of SNAC) below.

Average pharmacokinetic parameters derived for SNAC in plasma after single oral administration of the different combinations of cromolyn sodium and SNAC dosages are summarized in Table 6 below.

TABLE 6

Cromolyn/SNAC [mg/mg]	C_{max} [μg/mL]	T_{max} [h]	$AUC_{(0-t)}$ [μg·h/mL]	$MRT_{(0-t)}$ [h]	C_{max}/D^* [μg/mL]	$AUC_{(0-t)}/D^*$ [μg·h/mL]
40/1200 (N = 8)	8.64 ± 5.54	0.33-2.00	5.16 ± 1.44	1.26 ± 0.77	7.20 ± 4.62	4.30 ± 1.20
80/1200 (N = 8)	6.91 ± 2.88	0.25-0.50	4.56 ± 1.50	1.28 ± 0.42	5.76 ± 2.40	3.80 ± 1.25
80/600 (N = 8)	3.73 ± 1.59	0.25-0.75	1.98 ± 0.69	0.94 ± 0.23	6.22 ± 2.66	3.30 ± 1.15
120/200 (N = 8)	0.93 ± 0.87	0.25-0.33	0.52 ± 0.24	1.00 ± 0.45	4.65 ± 4.35	2.58 ± 1.18
0/2000 (N = 2)	13.8 ± 9.2	0.33-0.33	10.1 ± 2.9	1.18 ± 0.12	6.89 ± 4.61	5.05 ± 1.41
120/400 (N = 8)	1.86 ± 1.01	0.25-1.00	1.12 ± 0.29	1.07 ± 0.36	4.66 ± 2.53	2.79 ± 0.72
120/500 (N = 7)	2.05 ± 1.13	0.33-1.50	1.34 ± 0.45	1.10 ± 0.21	4.09 ± 2.25	2.67 ± 0.91
150/200 (N = 7)	1.25 ± 0.83	0.33-0.52	0.51 ± 0.15	0.72 ± 0.21	6.23 ± 4.13	2.57 ± 0.75

*Normalized to a 1000-mg dose of SNAC

Values are mean ± SD, except range for T_{max}

SNAC, when administered alone as described above, did not display any significant effect on human subjects.

No SNAC was found in control subjects administered with cromolyn only (80/0: two subjects, 150/0: one subject).

The differences observed among SNAC C_{max} normalized to a 1000-mg dose of SNAC (means between 4.09 and 7.20 μg/mL) were not relevant when compared to the large observed inter-subject variability (CV between 42 and 94%). By contrast, $AUC_{(0-t)}$ normalized to a 1000-mg dose of SNAC exhibited a lower inter-subject variability (CV between 26 and 46%) and increased with the SNAC dose, in particular for SNAC dosages above 500 mg.

Due to the design of the study, high SNAC dosages (>500 mg) were associated with low doses of cromolyn (≤80 mg), whereas lower SNAC dosages were associated with at least 120 mg of cromolyn. An explorative statistical analysis showed that the increase observed for dose-normalized $AUC_{(0-t)}$ was due rather to the increase of SNAC dosages ($p=0.025$) than to an influence of cromolyn ($p=0.11$).

Pharmacokinetics

When 80 or 150 mg of cromolyn were administered without SNAC, absorption of cromolyn was very low, and plasma concentrations never reached the therapeutic level (i.e., at least 10 ng/mL). However, co-administration with SNAC enhanced the oral absorption of cromolyn. When 120 or 150 mg of cromolyn were administered in combination with 200 mg of SNAC, the dose-normalized C_{max} and $AUC_{(0-t)}$ reached 30 ng/mL/100 mg and 30 ng·h/mL/100 mg, respectively.

When 40 to 120 mg of cromolyn were administered in combination with 400 to 1200 mg of SNAC, the oral absorption of cromolyn was still higher with dose-normalized C_{max} and $AUC_{(0-t)}$ reaching respectively 53.64 ng/mL/100 mg and 39.50 ng·h/mL/100 mg. However, no marked increase of dose-normalized parameters of cromolyn was observed when increasing the SNAC dose from 400 to 1200 mg.

After administration of 80 mg of cromolyn combined with 600 mg of SNAC, all subjects reached the therapeutic level

(i.e., at least 10 ng/mL) for at least 30 minutes. The therapeutic level was also reached in all subjects with 80/1200, 120/500 and 150/200 (mg/mg) cromolyn/SNAC combinations, but for less than 30 minutes in some subjects. With each of the other treatments, a few subjects failed to reach the therapeutic level.

SNAC absorption was not influenced by co-administration of cromolyn sodium. In view of its large inter-individual variability (CV between 42 and 94%), the SNAC peak absorption did not markedly diverge from dose-proportionality, with dose-normalized C_{max} ranging from 4.09 to 7.20 μg/mL/1000 mg. For doses between 200 and 500 mg, the SNAC extent of absorption was also dose-proportional (dose-normalized $AUC_{(0-t)}$ between 2.58 and 2.79 μg·h/mL/1000 mg) but increased more than dose-proportionally for higher

doses (from 3.30 to 5.05 μg·h/mL/1000 mg when SNAC doses were increased from 600 to 2000 mg).

This example shows that administration of SNAC with cromolyn clearly enhances the oral absorption of cromolyn. The best combination is 80 mg cromolyn administered with 600 mg SNAC, allowing all subjects to reach the 10-ng/mL therapeutic level for at least 30 minutes. All tested cromolyn sodium/SNAC combinations were well tolerated and did not produce any clinically relevant modifications of the clinical status of the subjects.

Example 5

In the previous example, the single dose study demonstrated that cromolyn was rapidly absorbed into the systemic circulations, reached maximum plasma concentrations at about 20 minutes post-dose and was rapidly cleared from the body within 6-8 hours with a mean elimination half-life of 1-2 hours. In addition, the cromolyn plasma concentration level was not influenced when the SNAC dose was reduced from 1200 to 600 mg. Based on the results of the single dose safety, tolerability and pharmacokinetics study shown in the previous example, wherein rapid clearance of cromolyn from the body indicated no accumulation of cromolyn would occur after multiple dosing of cromolyn, 80 mg cromolyn plus 600 mg SNAC twice a day dose was selected for a multiple dose study in healthy and allergic patients.

The purpose of this study was to evaluate the safety, tolerability, oral absorption, pharmacokinetics and pharmacodynamics following multiple dosing of oral cromolyn when given in combination with SNAC in healthy and allergic subjects. In order to be able to distinguish between cromolyn and other not-drug related issues, the study was placebo controlled. In order to evaluate the absorption of cromolyn, cromolyn was given with and without SNAC, a regimen that was expected to improve absorption of cromolyn. In order to

evaluate the pharmacodynamics, it was important to compare the effect of cromolyn between healthy and allergic subjects (target population).

Accordingly, a double-blind, randomized, placebo-controlled, multiple dose study was conducted to evaluate the safety, tolerability, oral absorption, and pharmacokinetics of cromolyn when given as capsules twice daily for ten days in combination with SNAC. Additionally, the study was conducted to evaluate the effect of oral cromolyn on the wheal and flare reaction after intracutaneous injection of codeine and histamine in healthy male subjects. Thirdly, the study was conducted to evaluate the effect of cromolyn on the wheal and flare reaction after intracutaneous injection with an allergen in male subjects with allergies.

The volunteers were all males between 18 and 45 years of age, in good health, with a weight within 15% of normal range based upon Metropolitan Life Insurance Co. tables. All laboratory values (electrocardiogram, hematology, serum chemistries and urinalysis) obtained during screening were within normal ranges.

Two groups of human males were defined: group 1 consisted of 12 healthy male volunteers, and group 2 had 12 allergic male volunteers. Eight volunteers from each of Group 1 and 2 received 80 mg Cromolyn/600 mg SNAC; two volunteers from each group received 80 mg of Cromolyn/0 mg SNAC as a control and two volunteers from each group received controls of capsules with 0 mg Cromolyn/0 mg SNAC. The dosing regimen is illustrated in Table 7 below.

Cromolyn/SNAC capsule, Cromolyn control capsule, and placebo capsule formulations were prepared extemporaneously at the study site. The composition of the capsules varied, depending on the treatment administered. Each capsule containing the cromolyn/SNAC combination contained about 340 mg solids (SNAC and cromolyn sodium USP).

TABLE 7

Dosing regimen		
Number of subjects	Group 1 Per Dose, mg-Cromolyn/mg-SNAC	Group 2 Per Dose, mg-Cromolyn/mg-SNAC
8	80/600 (2 capsules)	80/600 (2 capsules)
2	80/placebo (2 capsules)	80/placebo (2 capsules)
2	Placebo (2 capsules)	Placebo (2 capsules)

Each subject received oral dosing of the study medication twice a day for nine days. On the last day (i.e., day 10), only the morning dose was administered. The capsules were administered with up to 200 mL of water to the subjects in an upright position. The first dose of each day was administered following an overnight fast, and the second dose of each day was administered at least 1 hour before a meal or 4 hours after a meal. No food was allowed for at least 4 hours before the oral dosing.

Blood Samples for pharmacokinetics of cromolyn and SNAC (4.5 mLx2, one tube for cromolyn and one tube for SNAC, in chilled tri-sodium citrate tubes) were drawn in both treatment groups 15 minutes before the morning dose and at 20 minutes after the morning dose on days 2, 3, 4, 5, 6, 7, 8 and 9, and 15 minutes before the morning dose and at 5, 10, 15, 20,

30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, and 8 hours after the morning dose on days 1 and 10.

From the literature there are indications that nasal and lung mast cells are more sensitive to cromolyn. Therefore, for the allergic subjects it was proposed to determine the effect of cromolyn/SNAC on symptoms generated by mast cell degranulation in the nasal mucosa induced by intranasal provocation with allergen. Healthy subjects received codeine/histamine to induce wheal and flare reaction, and allergic subjects received allergen/histamine to induce wheal and flare reaction.

Wheal and flare reaction was read 15 min. after the injections of codeine/histamine or allergen/histamine, traced by pen onto transparent cellophane and the length and width of the reaction was measured. Wheal and flare reaction was measured for Group 1 (healthy subjects) after intracutaneous injection of codeine/histamine before the morning dose on day 1, at 45 minutes after the morning dose on days 1, 2, 4, 6, 8 and 10, and at 4 and 8 hours after the morning dose on days 2, 4 and 10. Wheal and flare reaction was measured for Group 2 (subjects with allergies) after injection with allergen/histamine before the morning dose on day 1, at 60 minutes after the morning dose on days 4 and 10, and at 8 hours after the morning dose on day 4.

The allergic subjects in group 2 were also tested for response to allergen (pollen, trees, house dust mite, cats or dogs) using a nasal provocation test in which the allergen challenge vehicle was administered intranasally. The procedure was initiated by spraying dilution fluid (as was used for the allergen challenge; sodium hydrogen carbonate 2.5 mg, phenol 5 mg, sodium chloride 5 mg, HSA 0.3 mg in 1 ml water) into each nostril, followed 15 minutes later by spraying each nostril with one challenge puff, each 0.089 ml in volume of allergen at a concentration of 10,000 BU/mL. This resulted in a total dose of 1780 BU, 890 BU per nostril, which is considered a mild provocation.

The following assessments for nasal tolerability were made following allergen challenge: VAS scores and PNIF measurement (each time in triplicate). The nasal provocation test was performed at day -1 (baseline) and 60 min post dose on day 9. Nasal tolerability was assessed immediately before administration with vehicle and 15 min post vehicle challenge (-pre allergen challenge), and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5 and 23 hours post-challenge.

The criteria for evaluating pharmacokinetics were C_{max} , T_{max} , AUC_{0-12} , $AUC_{0-\infty}$, K_{el} , $t_{1/2}$, MRT, CL/F, V_d/F of Cromolyn and SNAC. Pharmacodynamics were measured for Group 1 and 2 by measuring the length and width of the wheal and flare reaction, and permanent records were generated by marking on transparent tapes and/or by photographs. For Group 2 only, pharmacodynamics were also measured by VAS scores and PNIF measurements before and after nasal provocation test.

Pharmacokinetics

Summary statistics for the pharmacokinetic parameters derived from plasma cromolyn for Day 1 and Day 10 are presented in Table 8 below. The mean plasma concentration versus time profile of cromolyn for Day 1 and Day 10 for 80 mg Cromolyn/600 mg SNAC is shown in FIG. 12, and the mean plasma concentration versus time profile of cromolyn for Day 1 and Day 10 for 80 mg Cromolyn/placebo is shown in FIG. 13.

TABLE 8

Treatment	day	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12} (ng · h/mL) mean (SD)	AUC_{last} (ng · h/mL)	AUC_{inf} (ng · h/mL)
80 mg Cromolyn/ 600 mg SNAC N = 16	1	63.0 (41.2)	0.34 (0.07)	40.3 (26.4)	39.7 (26.3)	40.3 (26.4)
	10	71.3 (46.4)	0.35 (0.10)	48.8 (26.1)	—	—
80 mg Cromolyn/ placebo N = 3	1	1.1 (0.3)	1.83 (0.29)	—	4.7 (0.1)*	—
	10	2.3 (0.7)	3.17 (2.47)	—	—	—

*N = 2

As shown, cromolyn given orally in combination with SNAC was rapidly absorbed, with peak concentrations seen at approximately 20 minutes after drug administration in fasting male volunteers (allergic and healthy). Due to the short plasma half-life of the two compounds (cromolyn ± 45 min, SNAC ± 25 min), there was no accumulation during multiple dose treatment. In fact, plasma concentrations declined to negligible levels within 4 hour after each drug administration. The plasma-concentrations were almost back to zero after 4 hours, and there was no difference between AUC_{0-12} and AUC_{inf} .

For subjects treated with Cromolyn combined with SNAC (N=16), the mean C_{max} was somewhat higher on Day 10 than on Day 1. However, the between subject variability was large, which is also reflected in the large SD. When comparing the results on day 10 versus day 1, the within-subject variability appears to be less pronounced.

When cromolyn was administered without SNAC, the oral absorption was negligible (<2.3 ng/mL) and occurred. When cromolyn was given in combination with 600 mg SNAC, mean plasma concentrations of up to 65 ng/mL were reached.

A summary of the descriptive statistics for pharmacokinetic parameters derived from plasma SNAC concentrations for Day 1 and Day 10 are presented in Table 9 below. Mean plasma concentrations-time data for SNAC for Day 1 and Day 10 after administration of 80 mg cromolyn/600 mg SNAC are shown in FIG. 14.

TABLE 9

Summary statistics of plasma SNAC pharmacokinetic parameters						
Treatment	day	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12} (ng · h/mL) mean (SD)	AUC_{last} (ng · h/mL)	AUC_{inf} (ng · h/mL)
80 mg Cromolyn/600 mg SNAC N = 16	1	2861 (2021)	0.50 (0.37)	2030 (567)	2013 (563)	2032 (568)
	10	3416 (1473)	0.41 (0.32)	2285 (692)	—	—

The mean profiles for SNAC look rather similar for Day 1 and Day 10. For subjects treated with SNAC (N=16), the mean C_{max} for SNAC was somewhat higher on Day 10 than on Day 1. The variation of SNAC concentration-time profiles was rather large between subjects. However, there was only limited within-subject variability (Day 10 versus Day 1).

T_{max} was reached in about 30 minutes. The AUC was similar on Day 1 and Day 10.

After analysis of cromolyn and SNAC plasma concentrations, a large within and between subject variability in plasma SNAC and cromolyn concentrations was found. The correlation of individual SNAC versus cromolyn concentrations, shown in FIG. 15, shows that the relation for relative bioavailability between SNAC and cromolyn was weak, i.e. the large

between variability of individual cromolyn concentrations was not due to the variability of SNAC concentration for the same subject. There was no direct relationship found between SNAC and cromolyn plasma concentrations on Day 1 and Day 10.

Pharmacodynamics

FIG. 16 shows the PNIF results as mean changes from baseline per time point (values on Day 9 minus the values on Day -1). For all subjects, a baseline wheal and flare reaction was found after challenge with codeine (healthy subjects) or with allergen (allergic subjects). However, the wheal and flare test for healthy subjects (codeine/histamine) and allergic subjects (allergen/histamine) did not show any treatment effect. Overall, both healthy and allergic subjects showed no clear change from baseline (pre-dose) for the wheal and flare skin reactions.

There were no apparent differences in mean change from baseline for PNIF scores (values on Day 9 minus the values on Day -1) between the treatments, obtained from the allergic subjects only. There was a smaller decrease of PNIF scores, for all treatments, after provocation on Day 9 than for Day -1 (shown as positive change from baseline in FIG. 16). There was also a large variation between subjects, and no difference was found between Cromolyn/SNAC and Cromolyn alone or placebo.

All subjects showed a change in VAS score after nasal provocation. However, the VAS scores were not influenced by

any of the treatments. The nose and throat were less sore after dosing with Cromolyn/SNAC. For the rest of the scales, no obvious differences in VAS scores were found before and after dosing with Cromolyn/SNAC.

CONCLUSIONS

It is clear that SNAC enhances the absorption of cromolyn when the two compounds are orally administered in combination. When cromolyn was administered alone, i.e., without SNAC, the oral absorption was practically negligible. Consequently, twice daily administration may not have been enough to achieve continuous exposure to cromolyn during the combined cromolyn/SNAC treatment, and this could

explain why no clear effect of treatment could be observed for the pharmacodynamic parameters.

Between individuals, there was a large variation in the cromolyn and SNAC exposures in terms of AUC values. However, within individuals the variation was clearly less pronounced. The large plasma cromolyn concentration variability on the Day 2 to Day 9, measured at 20 min after dosing, is probably due to the large variability of T_{max} between subjects.

There was no correlation found between the SNAC and Cromolyn plasma concentrations. The cromolyn relative bioavailability (AUC) seemed to vary independently of the plasma SNAC concentrations, i.e., the large variability for cromolyn in plasma cannot be explained by the variability in plasma SNAC concentrations.

For the wheal and flare reactions, the VAS scores and PNIF scores it can be firmly concluded that the challenge was indeed strong enough, since a clear effect was generated after each challenge that was administered, independent of group or treatment. The wheal and flare skin reactions were independent of treatment administered and did not clearly change from baseline (pre-dose). Apparently, oral cromolyn had no significant effects on skin mast cell degranulation when stimulated with codeine. However, the results show no apparent differences in mean change from baseline for PNIF scores (values on Day 9 minus the values on Day -1) between the treatments, obtained from the allergic subjects only.

For most VAS scores, the variability between subjects was too large to draw a general conclusion for this group. From the results of the nasal provocation test it was not possible to draw conclusions on the effect of Cromolyn/SNAC administration.

In this study, the wheal and flare reactions and the VAS scores were measured relatively soon after the challenge. This might be an explanation for the absence of clinically significant differences after cromolyn administration. An alternative explanation for the minimal effects of cromolyn on the pharmacodynamic parameters, despite significantly increased plasma levels compared to dosing without SNAC, might be that the achieved plasma levels were still too low to produce a local response to oral dosing with cromolyn. After inhalation of cromolyn, the local bioavailability of cromolyn is possibly much higher than the local bioavailability of cromolyn after oral dosing combined with SNAC.

As demonstrated by the data in the Examples and Figures, the use of compositions of the subject invention shows significant advantages for the delivery of biologically active agents.

While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic constructions can be altered to provide other embodiments which utilize the processes and compositions of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than by the specific embodiments which have been presented hereinbefore by way of example.

What is claimed is:

1. A method for treating a patient in need of treatment of an inflammatory disease, comprising administering to said patient an oral dosage form comprising:

(a) cromolyn sodium; and

(b) a delivery agent sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) that upon oral administration provides a systemic absorption of the cromolyn sodium in a physiologically effective amount, wherein the physiologically effective amount is enough to inhibit the release of histamine or cytokines or histamine and cytokines from mast cells,

wherein the ratio of cromolyn sodium to the delivery agent is from 1:7.5 to 1:30 (weight/weight).

2. The method of claim 1 wherein the patient is a human.

3. The method of claim 1 wherein cromolyn sodium is in an amount from about 40 mg to about 1 g together with a delivery agent in an amount from about 100 mg to about 2500 mg.

4. The method of claim 1 wherein the inflammatory disease is allergic rhinitis, asthma, rheumatoid arthritis or an autoimmune disease.

5. The method of claim 1, wherein the T_{max} for cromolyn sodium occurs at a time less than about 1 hour after oral administration.

6. The method of claim 1, wherein an AUC for cromolyn sodium of between about 16 and about 60 ng·h/mL is attained.

7. The method of claim 1, wherein a cromolyn sodium C_{max} that is from about 10 to about 250 ng/mL is attained.

8. The method of claim 1 wherein the physiologically effective amount is enough to provide an absorption of cromolyn from the gastrointestinal tract of greater than about 3.5%, by weight, of the dose of cromolyn contained in the dosage form.

9. The method of claim 1 wherein the physiologically effective amount is enough to provide an absorption of cromolyn from the gastrointestinal tract of greater than about 2%, by weight, of the dose of cromolyn contained in the dosage form.

10. The method of claim 1, wherein the oral dosage form is in the form of a tablet, capsule or oral suspension.

11. The method of claim 1, wherein the oral dosage form is solid.

12. The method of claim 1, wherein the dose of cromolyn sodium is from about 40 mg to about 1 g.

13. The method of claim 12, wherein the dose of cromolyn sodium is from about 40 mg to about 240 mg.

14. The method of claim 12, wherein the dose of cromolyn sodium is from about 50 mg to about 150 mg.

15. The method of claim 1, wherein the dose of the delivery agent is from about 100 mg to about 1200 mg.

16. The method of claim 15, wherein the dose of the delivery agent is from about 200 mg to about 800 mg.

17. The method of claim 1, wherein said administration provides a T_{max} for cromolyn sodium at about 0.1 to about 1.5 hours after oral administration.

18. The method of claim 17, wherein said administration provides a T_{max} for cromolyn sodium at less than about 1 hours after oral administration.

19. The method of claim 17, wherein said administration provides a T_{max} for cromolyn sodium at about 0.2 to about 0.5 hours after administration.

20. The method of claim 1, wherein said administration provides an AUC for cromolyn sodium of between about 15 and about 60 ng·h/mL.

21. The method of claim 20, wherein said administration provides an AUC for cromolyn sodium of between about 30 and about 40 ng·h/mL.

22. The method of claim 1, wherein said administration provides a cromolyn sodium C_{max} from about 10 ng/mL to about 250 ng/mL.

23. The method of claim 22, wherein said administration provides a cromolyn sodium C_{max} from about 20 to about 100 ng/mL.

24. The method of claim 22, wherein said administration provides a cromolyn sodium C_{max} from about 40 to about 65 ng/mL.

25. The method of claim 22, wherein said administration provides a cromolyn sodium C_{max} from about 20 to about 80 ng/mL within about 0.2 to about 1 hour after oral administration.

26. The method of claim 1, wherein the dose of cromolyn sodium is about 80 mg and the dose of delivery agent is about 600 mg. 5

27. The method of claim 1, wherein the ratio of cromolyn sodium to the delivery agent is 1:7.5 (weight/weight).

28. The method of claim 1, wherein the dose of cromolyn sodium and delivery agent is selected from the group consisting of about 40 mg cromolyn sodium and about 1200 mg delivery agent, about 80 mg cromolyn sodium and about 1200 mg delivery agent, and about 80 mg cromolyn sodium and about 600 mg delivery agent. 10 15

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APPENDIX F



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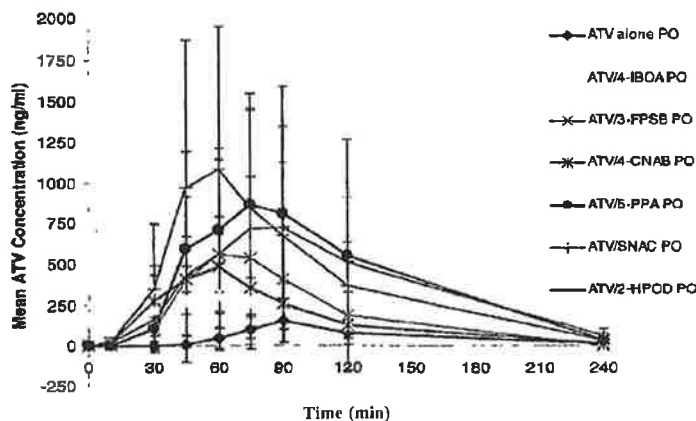
Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.1 7(H))
- of inventorship (Rule 4A 7(iv))

[Continued on next page]

(54) Title: LOW DOSE HMG-COA REDUCTASE INHIBITOR WITH REDUCED SIDE EFFECTS

Figure 2



(57) Abstract: The present invention relates to pharmaceutical formulations for lowering cholesterol in a human subject comprising (a) a HMG-CoA reductase inhibitor, (b) at least one of SNAC, SNAD, 5CNAC, 4CNAB, 4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHIA, 3-IIPSB, 4-IBOA and 3-FPSB (c) optionally CoEnzyme Q and (d) optionally, a pharmaceutically acceptable excipient.

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Low Dose HMG-CoA Reductase Inhibitor With Reduced Side Effects

FIELD OF THE INVENTION

[0001] The present invention relates to pharmaceutical formulations containing a
5 HMG-CoA reductase inhibitor, (b) at least one of SNAC, SNAD, 5CNAC, 4CNAB, 4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB.

BACKGROUND OF THE INVENTION

[0002] HMG-CoA reductase inhibitors, such as atorvastatin, have been used as
pharmaceutical agents to lower cholesterol levels in people with or at risk for cardiovascular
10 disease. These inhibitors lower cholesterol by inhibiting the enzyme HMG-CoA reductase which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver stimulates LDL receptors, resulting in an increased clearance of low-density lipoprotein (LDL) from the bloodstream and a decrease in blood cholesterol levels.

15 [0003] One of the most popular HMG-CoA reductase inhibitors is atorvastatin which is currently marketed by Pfizer under the trade name Lipitor®, in tablets (10, 20, 40 or 80 mg) for oral administration. Tablets are white, elliptical, and film coated. In some countries it may also be known as: Sortis, Torvast, Totalip, Tulip, Xarator, Atorpic, or Lipimar. It is also packaged in combination with other drugs, such as is the case with Pfizer's Caduet. With
20 2005 sales of US\$ 12.2 billion under the brand name Lipitor®, it is the largest selling drug in the world (see Jerry Avorn, M.D., "Torcetrapib and Atorvastatin— Should Marketing Drive the Research Agenda?" *New England Journal of Medicine* Volume 352:2573-2576 June 23, 2005).

[0004] However, atorvastatin, like other HMG-CoA reductase inhibitors, causes
25 many adverse drug reactions, including muscle pain, myalgia, muscle cramps, mild transient gastrointestinal symptoms, elevated hepatic transaminase concentrations, headache, insomnia, and/or dizziness. Myopathy and rhabdomyolysis can also occur in some patients. Such risk increases in patients with renal impairment, serious concurrent illness; and/or concomitant use of drugs which inhibit CYP3A4 (see Nissen S. et al., "Effect of very high-
30 intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial," *JAMA* 295 (13): 1556-65, 2006). So far, various efforts to improve the safety profile of

HMG-CoA reductase inhibitors, such as atorvastatin, have failed. With millions of patients taking HMG-CoA reductase inhibitors every day, there is a need to reduce the side effects of these HMG-CoA reductase inhibitors.

SUMMARY OF THE INVENTION

- 5 [0005] The present invention provides a pharmaceutical composition for lowering cholesterol in a human subject comprising (a) a HMG-CoA reductase inhibitor, (b) at least one of SNAC, SNAD, 5-CNAC, 4-CNAB, 4-MOAC, 4-HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB (c) optionally CoEnzyme Q and (d) optionally, a pharmaceutically acceptable excipient.
- 10 [0006] The present invention also provides a solid pharmaceutical dosage form for lowering cholesterol level in a human subject comprising (a) from about 0.1 mg to about 200 mg of HMG-CoA reductase inhibitor, and (b) from about 25 mg to about 600 mg of at least one of SNAC, SNAD, 5-CNAC, 4-CNAB, 4-MOAC, 4-HPO₅, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB.
- 15 [0007] The present invention further provides a method of lowering cholesterol in a human subject, comprising the step of orally administering an effective amount of the pharmaceutical formulation which comprises (a) a HMG-CoA reductase inhibitor, (b) at least one of SNAC, SNAD, 5-CNAC, 4-CNAB, 4-MOAC, 4-HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB (c) optionally CoEnzyme Q and (d) optionally, a
- 20 pharmaceutically acceptable excipient.
- [0008] The contents of the patents and publications cited herein and the contents of documents cited in these patents and publications are hereby incorporated herein by reference to the extent permitted.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Fig. 1 is a graph showing the Mean serum ATV (atorvastatin) concentration Value in ng/ml over Time in minutes at a dose of 10 mg/kg.

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[0010] Fig. 2 is a graph showing the Mean serum ATV (atorvastatin) concentration Value in ng/ml over Time in minutes at a dose of 40 mg/kg.

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DETAILED DESCRIPTION OF THE INVENTION

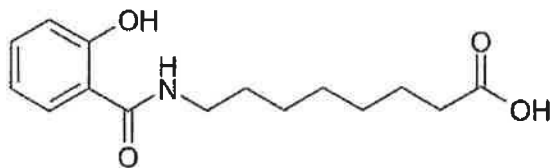
[0011] The phrase "pharmaceutically acceptable" refers to compounds or compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal.

[0012] The term "bioavailability" refers to the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes systematically available.

[0013] As used herein, the term "HMG-CoA Reductase Inhibitor" means statin, a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease. They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Commonly used HMG-CoA Reductase inhibitors include, but are not limited to

[0014] The term "CoEnzyme Q" means a class of vitamin-like substance that are present in most human cells and are responsible for the production of the body's energy. One of the most common CoEnzyme Q is CoEnzyme Q10, wherein Q refers to the quinone chemical group and 10 refers to the number of isoprenyl chemical subunits.

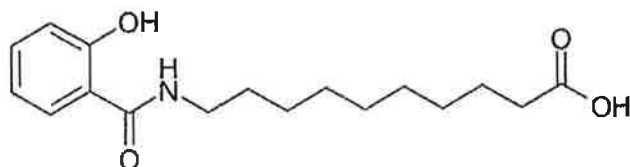
[0014] The term "SNAC" as used herein refers to N-(8-[2-hydroxybenzoyl]-amino) caprylic acid and pharmaceutically acceptable salts thereof, including its monosodium and disodium salt as well as SNAC free acid. The term "SNAC free acid" refers to N-(8-[2-hydroxybenzoyl]- amino) caprylic acid which has the following structure:



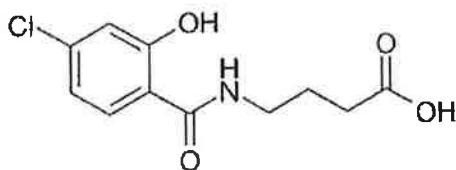
Unless otherwise noted, the term "SNAC" refers to all forms of SNAC, including all amorphous and polymorphic forms of SNAC, such as SNAC trihydrate and those described in U.S. Serial Nos. 60/619,418 and 60/569,476, both of which are hereby incorporated by reference. The term "SNAC trihydrate" as used herein refers to a crystalline form of SNAC in which three molecules of water are associated with each molecule of SNAC. SNAC can

be prepared by the procedures described in U.S. Patent No. 5,650,386 and International Publication Nos. WO00/46182 and WOOO/59863.

[0014] The term "SNAD" as used herein refers to N-(10-[2-hydroxybenzoyl]-amino) decanoic acid and pharmaceutically acceptable salts thereof, including its monosodium salt and disodium salts as well as SNAD free acid. Unless otherwise noted, the term "SNAD" refers to all forms of SNAD, including all amorphous and polymorphic forms of SNAD. The term "SNAD free acid" refers to N-(10-[2-hydroxybenzoyl]-amino) decanoic acid which corresponds to the following structure:

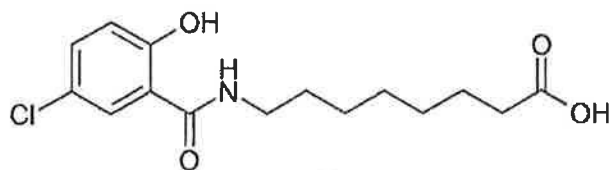


[0015] The term "4-CNAB" as used herein refers to 4-[(4-chloro-2-hydroxy-benzoyl)amino]butanoic acid (also known as 4-[(2-hydroxy-4-chlorobenzoyl)amino]butanoate) and pharmaceutically acceptable salts thereof, including its sodium salt (e.g., monosodium salt and disodium salt) as well as 4-CNAB free acid. Unless otherwise noted, the term "4-CNAB" refers to all forms of 4-CNAB, including all amorphous and polymorphic forms of 4-CNAB. The term "sodium 4-CNAB" and "mono-sodium 4-CNAB" refer to monosodium 4-[(2-hydroxy-4-chlorobenzoyl)amino]butanoate, including anhydrous, monohydrate, and isopropanol solvates thereof and amorphous and polymorphic forms thereof (including those described in International Publication No. WO 03/057650 which is hereby incorporated by reference), unless otherwise indicated. The term "4-CNAB free acid" refers to 4-[(4-chloro-2-hydroxy-benzoyl)amino]butanoic acid which has the following structure:



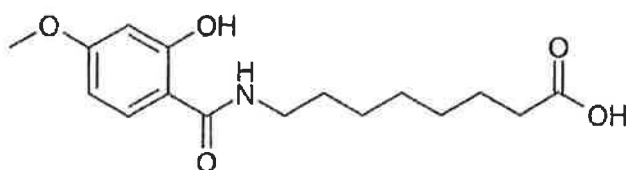
[0016] The term "5-CNAC" as used herein refers to N-(8-[5-chloro-2-hydroxybenzoyl]-amino)octanoic acid and pharmaceutically acceptable salts thereof, including its monosodium and disodium salts. Unless otherwise noted, the term "5-CNAC"

refers to all forms of 5-CNAC, including all amorphous and polymorphic forms of 5-CNAC. The term "5-CNAC free acid" refers to N-(8-[5-chloro-2-hydroxybenzoyl]-amino) octanoic acid which corresponds to the following structure:



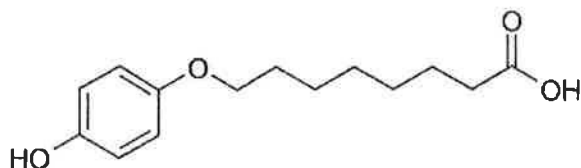
5 [0017] The term "4-MOAC" as used herein refers to N-(8-[4-methoxy-chloro-2-hydroxybenzoyl]-amino) octanoic acid and pharmaceutically acceptable salts thereof, including its sodium salt and disodium salt as well as 4-MOAC free acid. The term "4-MOAC free acid" refers to N-(8-[4-methoxy-chloro-2-hydroxybenzoyl]-amino) octanoic acid which has the following structure:

10



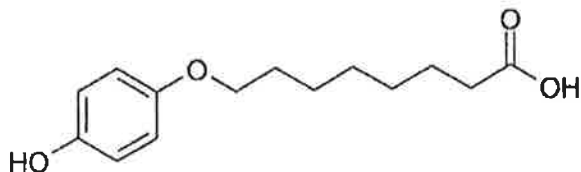
[0018] The term "4-HPO" as used herein refers to 8-(4-hydroxyphenoxy) octanoic acid and pharmaceutically acceptable salts thereof, including its sodium salt and disodium salt as well as 4-HPO free acid. The term "4-HPO free acid" refers to 8-(4-hydroxyphenoxy) octanoic acid which has the following structure:

15



[0018] The term "4-HPO" as used herein refers to 8-(4-hydroxyphenoxy) octanoic acid and pharmaceutically acceptable salts thereof, including its monosodium and disodium salts. Unless otherwise noted, the term "4-HPO" refers to all forms of 4-HPO, including all amorphous and polymorphic forms of 4-HPO. The term "4-HPO free acid" refers to 8-(4-hydroxyphenoxy) octanoic acid which corresponds to the following structure

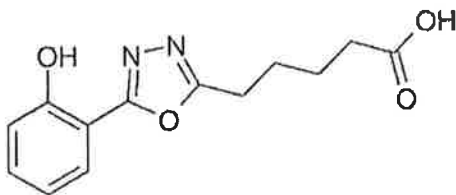
20



[0019] The term "5-PPA" as used herein refers to 5-phenylpentanoic acid and
 5 pharmaceutically acceptable salts thereof, including its monosodium salt. Unless otherwise
 noted, the term "5-PPA" refers to all forms of 5-PPA, including all amorphous and
 polymorphic forms. The term "5-PPA free acid" refers to 5-phenylpentanoic acid which
 corresponds to the following structure:

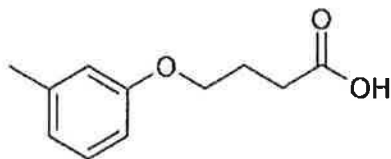


10 [0020] The term "2-PHOD" as used herein refers to 2-(5-pentanoic acid)-5-(2-
 hydroxyphenyl)-1,3,4-oxadiazole and pharmaceutically acceptable salts thereof, including its
 monosodium and disodium salts. Unless otherwise noted, the term "2-PHOD" refers to all
 forms of 2-PHOD, including all amorphous and polymorphic forms of 2-PHOD. The term
 "2-PHOD free acid" refers to 2-(5-pentanoic acid)-5-(2-hydroxyphenyl)-1,3,4-oxadiazole
 15 which corresponds to the following structure:

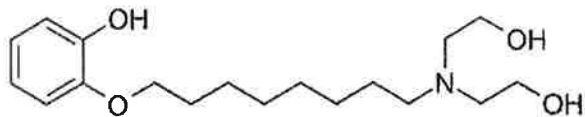


[0021] The term "3-TBA" as used herein refers to 4-m-tolyloxybutyric acid and
 pharmaceutically acceptable salts thereof, including its monosodium salt. Unless otherwise
 20 noted, the term "3-TBA" refers to all forms of 3-TBA, including all amorphous and

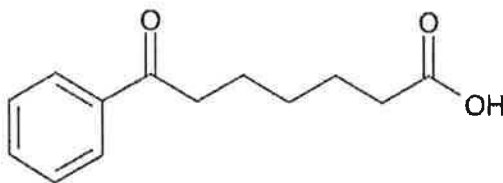
polymorphic forms of 3-TBA. The term "3-TBA free acid" refers to 4-m-tolyloxybutyric acid which corresponds to the following structure:



5 [0022] The term "2-HPOD" as used herein refers to 8-(2-hydroxyphenoxy)octyldiethanolamine and pharmaceutically acceptable salts thereof, including its hydrochloride, hydrobromide, mesylate, acetate, trifluoroacetate, propionate, fumarate, tartrate, citrate, phosphate, succinate, bisulfate, or besylate salts. Unless otherwise noted, the term "2-HPOD" refers to all forms of 2-HPOD, including all amorphous and
10 polymorphic forms of 2-HPOD. The term "2-HPOD free base" refers to 8-(2-hydroxyphenoxy)octyldiethanolamine which corresponds to the following structure:



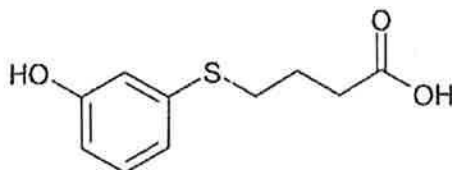
[0023] The term "7-OPHA" as used herein refers to 7-oxo-7-phenylheptanoic acid and pharmaceutically acceptable salts thereof, including its monosodium salt. Unless
15 otherwise noted, the term "7-OPHA" refers to all forms of 7-OPHA, including all amorphous and polymorphic forms of 7-OPHA. The term "7-OPHA free acid" refers to 7-oxo-7-phenylheptanoic which corresponds to the following structure:



20

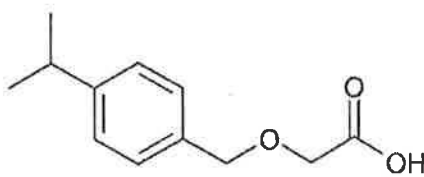
[0024] The term "3-HPSB" as used herein refers to 4-(3-hydroxyphenylsulfanyl)butyric acid and pharmaceutically acceptable salts thereof, including

its monosodium and disodium salts. Unless otherwise noted, the term "3-HPSB" refers to all forms of 3-HPSB, including all amorphous and polymorphic forms of 3-HPSB. The term "3-HPSB free acid" refers to 4-(3-hydroxyphenylsulfanyl)butyric acid which corresponds to the following structure:

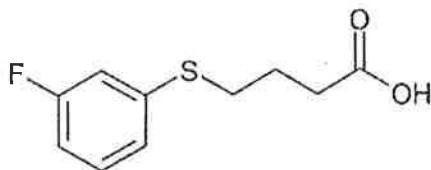


5

[0025] The term "4-IBOA" as used herein refers to (4-isopropylbenzyloxy)acetic acid and pharmaceutically acceptable salts thereof, including its monosodium salt. Unless otherwise noted, the term "4-IBOA" refers to all forms of 4-IBOA, including all amorphous and polymorphic forms of 4-IBOA. The term "4-IBOA free acid" refers to (4-isopropylbenzyloxy)acetic acid which corresponds to the following structure:



[0026] The term "3-FPSB" as used herein refers to 4-(3-fluorophenylsulfanyl)butyric acid and pharmaceutically acceptable salts thereof, including its monosodium salt. Unless otherwise noted, the term "3-FPSB" refers to all forms of 3-FPSB, including all amorphous and polymorphic forms of 3-FPSB. The term "3-FPSB free acid" refers to 4-(3-fluorophenylsulfanyl)butyric acid which corresponds to the following structure:



20

[0027] Pharmaceutical composition may be in the form of tablets, capsules (including hard and soft gelatin capsules), and particles, such as powders and sachets. An oral tablet is a

preferred dosage form of the present invention. Solid pharmaceutical dosage forms may be prepared by mixing the solid form of the earlier with the solid form of the HMG-CoA reductase inhibitor.

[0028] The pharmaceutical composition can include any one or combination of
5 excipients, diluents, disintegrants, lubricants, fillers, plasticizers, colorants, flavorants, taste-
masking agents, sugars, sweeteners, salts, and dosing vehicles, including, but not limited to,
water, 1,2-propane diol, ethanol, olive oil, or any combination thereof.

[0029] The amount of HMG-CoA reductase inhibitors, such as atorvastatin, included
10 in the pharmaceutical composition is an amount effective to accomplish the purpose of the
atorvastatin for the target indication of lowering cholesterol level in mammals. The amount
of HMG-CoA reductase inhibitors, such as atorvastatin in the pharmaceutical composition
typically is a pharmacologically, biologically, therapeutically, or chemically effective
amount.

[0030] One embodiment of the present invention provides a pharmaceutical
15 composition containing a HMG-CoA reductase inhibitor selected from lovastatin,
simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin and their pharmaceutically
acceptable salt, ester and lactone forms thereof. Preferably atorvastatin, its pharmaceutically
acceptable salt, ester and lactone forms thereof. More preferably a potassium salt, sodium
salt, or calcium salt of atorvastatin. Such pharmaceutical composition further comprises
20 CoEnzyme Q, preferably CoEnzyme Q10. A preferred carrier is SNAC, SNAD, 5CNAC,
4CNAB, 4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA or 3-FPSB.
A more preferred carrier is SNAC, 4-CNAB and 5-CNAC. Such pharmaceutical composition
reduces the side effects of administering HMG-CoA reductase inhibitors to human subjects.

[0031] The pharmaceutical composition can be in tablet or other forms. Preferably
25 sodium, potassium or calcium salt of atorvastatin is used in the pharmaceutical composition
in an amount from about 0.1 mg to about 200 mg, preferably from about 0.2 mg to about 150
mg, more preferably from about 0.4 mg to about 100 mg and the most preferably from about
1 mg to about 50 mg. The amount of at least one of SNAC, SNAD, 5CNAC, 4CNAB,
4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB is from
30 about 20 mg to about 700 mg, preferably from about 40 mg to about 600 mg, more preferably

from about 60 mg to about 400 mg and the most preferably from about 100 mg to about 200 mg.

[0023] The pharmaceutical composition of the present invention can be administered to treat and/or prevent any disorder for which HMG-CoA reductase inhibitors, such as atorvastatin are known to be capable of treating and/or preventing. Typically, an effective amount of the pharmaceutical composition is administered to treat and/or prevent the desired disorder. Such disorders include, but are not limited to, high cholesterol. The pharmaceutical compositions are useful for administering HMG-CoA reductase inhibitors, such as atorvastatin to mammals including, but not limited to, horses, rodents, cows, pigs, dogs, cats, primates, and particularly humans.

[0024] In a preferred embodiment of the present invention, the pharmaceutical composition reduces cholesterol level in a mammal using substantially reduced dosage of HMG-CoA reductase inhibitors, such as atorvastatin while reducing the hazardous effects of administering HMG-CoA reductase inhibitors alone, such as reduced level of myalgia, muscle cramps and liver damage.

[0025] In another embodiment of the present invention, the combination of atorvastatin with SNAC increasing the absolute bioavailability of atorvastatin 9-fold as compared with administration of atorvastatin alone.

[0026] In still another embodiment of the present invention, the combination of atorvastatin with 5-PPA increases the absolute bioavailability of atorvastatin 8-fold as compared with administration of atorvastatin alone.

[0027] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviations, per practice in the art. Alternatively, "about" with respect to the formulations can mean a range of up to 10%, preferably up to 5%.

[0028] The following examples are given as specific illustrations of the invention. It should be understood, however, that the invention is not limited to the specific details set forth in the examples. All parts and percentages in the examples, as well as in the remainder of the specification, are by weight unless otherwise specified.

Example 1

[0029] The study was conducted in fasted male Sprague-Dawley rats (n=5 for each group as seen in Fig. 1). Atorvastatin was designated ATV. ATV was administered alone to rats orally or intravenously (IV) as controls. For oral dosing, single dose of two mini-tablets were administered at time 0, in the following manner: (a) each carrier (200 mg/kg) in combination with ATV (10 mg/kg) was administered orally to rats; (b) ATV (10 mg/kg) alone was administered orally to rats; and (c) Lipitor (10mg/kg) alone was administered orally to rats. For IV dosing, a bolus shot of ATV alone (2mg/kg) was administered to a group of rats. Blood samples were collected by rail clipping pre-dosing (0 minute), and 5, 15, 30, 40, 50, 60, 120 and 240 minutes after dosing. The testing results are shown in Table 1.

Table 1. Increased Absorption of ATV (at 10 mg/kg) In The Presence Of Carriers

<u>Group</u>	<u>C_{max}</u> <u>(ng/ml)</u>	<u>T_{max}</u> <u>(min)</u>	<u>AU Clast (min*ng/ml)</u>
ATV alone (10 mg/kg) PO	25.6	54.0	985
ATV(10 mg/kg) PO with 5-PPA	137.8	66.0	6852
ATV(10mg/kg)PO with 4-CNAB	73.8	54.0	4669
ATV(10 mg/kg) PO with 3-TBA	31.2	52.0	1997
ATV/alone (2mg/kg) IV	1529	3.0	31872
ATV(10 mg/kg) PO with 2-PHOD	81.5	48.0	4942
ATV(10 mg/kg) PO with 3-HPSB	89.6	45.0	7435
ATV(10 mg/kg) PO with 7-OPHA	87.9	58.0	5027
Lipitor (10 mg/kg) PO	10.1	42.5	162

5

Example 2

[0030] The process of example 1 was repeated except that each carrier (200 mg/kg) in combination with ATV (40 mg/kg) was administered orally to rats. The testing results are shown in Table 2.

10

Table 2. Increased Absorption of ATV (at 40 mg/kg) In The Presence Of Carriers

<u>Group</u>	<u>C_{max} (ng/ml)</u>	<u>T_{max} (min)</u>	<u>AUC_{last} (min*ng/ml)</u>
ATV alone (40 mg/kg) PO	157	90	9577
ATV(40 mg/kg) PO with SNAC	1168	63	86709
ATV(40 mg/kg) PO with 5-PPA	1006	105	79572
ATV(40 mg/kg) PO with 2-HPOD	876	72	74796
ATV(40 mg/kg) PO with 3-FPSB	603	66	38546
ATV(40 mg/kg) PO with 4-CNAB	526	54	35434
ATV(40 mg/kg) PO with 4-IBOA	355	57	29455

[0031] The principles, preferred embodiments, and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since these are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art, without departing from the spirit of the invention.

20

WHAT IS CLAIMED IS:

5

1. A pharmaceutical composition for lowering cholesterol in a human subject comprising (a) a HMG-CoA reductase inhibitor, (b) at least one of SNAC, SNAD, 5-CNAC, 4-CNAB, 4-MOAC, 4-HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB (c) optionally CoEnzyme Q and (d) optionally, a pharmaceutically acceptable
10 excipient.

2. The pharmaceutical composition of claim 1, wherein said HMG-CoA reductase inhibitor is selected from lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin and their pharmaceutically acceptable salt, ester and lactone forms
15 thereof.

3. The pharmaceutical composition of claim 1, further comprising CoEnzyme Q.

4. The pharmaceutical composition of claim 3, wherein said CoEnzyme Q is
20 CoEnzyme Q10.

5. The pharmaceutical composition of claim 1, wherein said HMG-CoA reductase inhibitor is atorvastatin, its pharmaceutically acceptable salt, ester and lactone forms thereof.
25

6. The pharmaceutical composition of claim 5, wherein said atorvastatin is a potassium salt, sodium salt, or calcium salt.

7. The pharmaceutical composition of claim 5, comprising (a) atorvastatin and
30 (b) SNAC.

8. The pharmaceutical composition of claim 1, comprising (a) atorvastatin and (b) SNAD, 5CNAC, 4CNAB, 4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA or 3-FPSB.

5 9. The pharmaceutical composition of claim 1, wherein the side effects of said HMG-CoA reductase inhibitor is reduced.

10 10. A solid pharmaceutical dosage form for lowering cholesterol level in a human subject comprising (a) from about 0.1 mg to about 200 mg of HMG-CoA reductase inhibitor, and (b) from about 25 mg to about 600 mg of at least one of SNAC, SNAD, 5CNAC, 4CNAB, 4MOAC, 4HPO, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-IBOA and 3-FPSB.

15 11. The solid pharmaceutical dosage form of claim 10, wherein said HMG-CoA reductase inhibitor is selected from lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin and their pharmaceutically acceptable salt, ester and lactone forms thereof.

20 12. The solid pharmaceutical dosage form of claim 10, wherein said HMG-CoA reductase inhibitor is atorvastatin, its pharmaceutically acceptable salt, ester and lactone forms thereof.

25 13. The solid pharmaceutical dosage form of claim 12, wherein said atorvastatin is a potassium salt, sodium salt, or calcium salt.

14. The solid pharmaceutical dosage form of claim 10, further comprising from about 10 mg to about 300 mg of CoEnzyme Q.

30 15. The solid pharmaceutical dosage form of claim 14, wherein said CoEnzyme Q is CoEnzyme Q10.

16. The solid pharmaceutical dosage form of claim 10, comprising from about 25 mg to about 600 mg of SNAC.

17. The solid pharmaceutical dosage form of claim 10, wherein the side effects of said HMG-CoA reductase inhibitor is reduced.

18. A method of lowering cholesterol in a human subject, comprising the step of orally administering an effective amount of the pharmaceutical formulation of claim 1 to said human subject.

10

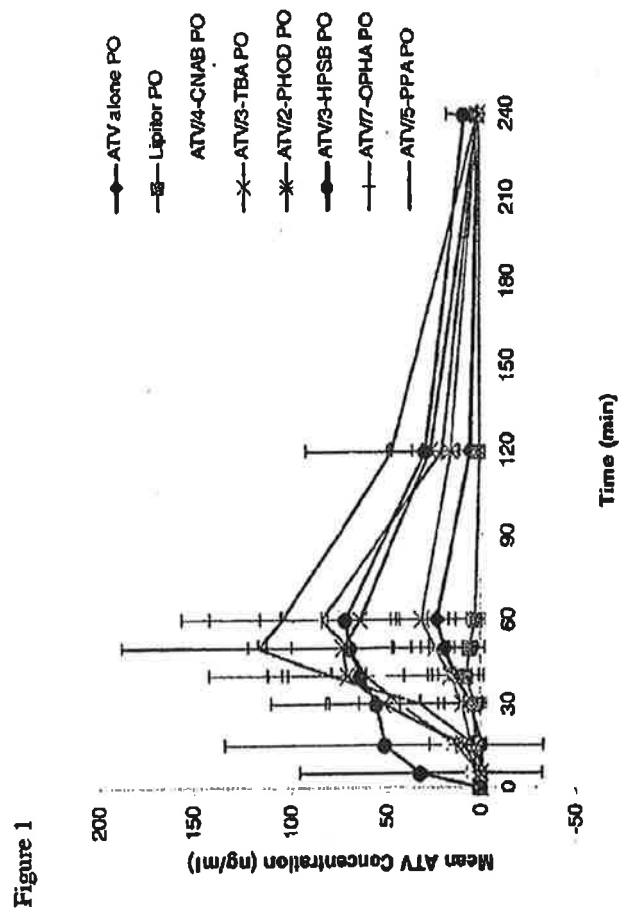
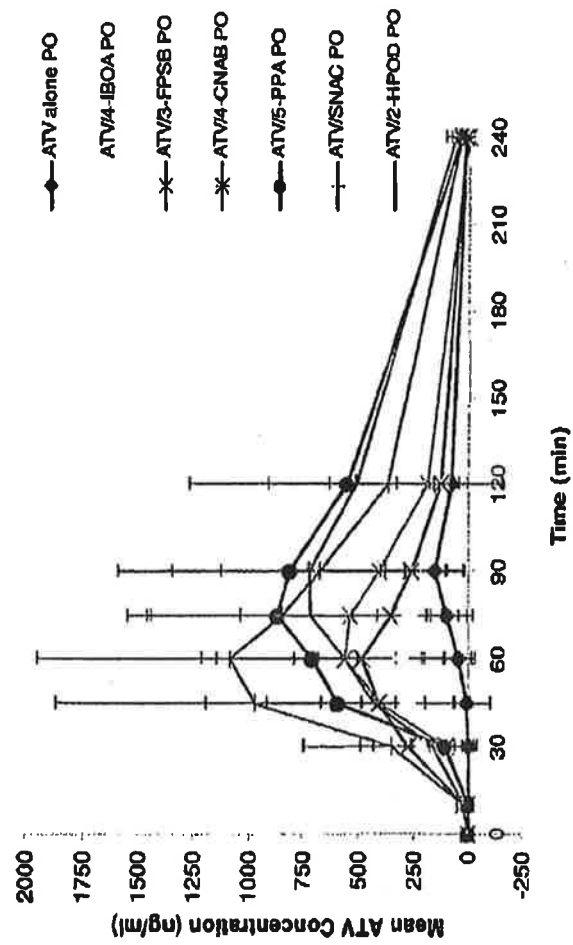


Figure 2



INTERNATIONAL SEARCH REPORT

International application No

PCT/JS 09/33255

A CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61 K 9/22; C07D 207/00 (2009.01)

USPC - 548/537; 424/468

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

B FIELDS SEARCHEDMinimum documentation searched (classification system followed by classification symbols)
USPC 548/537, 424/468Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 514/423, 424/408 (see search terms below)

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

PubWEST (USPT, PGPB, EPAB, JPAB), Google Scholar

Search Terms HMG-CoA reductase inhibitor, cholesterol, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, SNAC, SNAD, 5-CNAC, 4-CNAB, 4-MOAC, 4-HOP, 5-PPA, 3-TBA, 2-HPOD, 7-OPHA, 3-HPSB, 4-BOA, 3-FPSB

C DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X		1-2, 5-13 and 16-17
Y	US 2005/0186267 A1 (THOMPSON et al) 25 August 2005 (25 08 2005) para [0198], [0233]	3-4, 14-15 and 18
Y	US 2005/0147620 A1 (BOZICEVIC) 07 July 2005 (07 07 2005) abstract, para [0027]	3-4, 14-15 and 18

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

* Special categories of cited documents	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 April 2009 (06 04 2009)

Date of mailing of the international search report

17 APR 2009

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Authorized officer

Lee W Young

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PCT OSP- 571-272-7774

APPENDIX G



US008022048B2

(12) **United States Patent**
Castelli et al.(10) **Patent No.:** **US 8,022,048 B2**
(45) **Date of Patent:** **Sep. 20, 2011**(54) **METHOD OF TREATING VITAMIN B12 DEFICIENCY**(75) Inventors: **Cristina Castelli**, New York, NY (US);
Laura Kragie, Chevy Chase, MD (US)(73) Assignee: **Emisphere Technologies, Inc.**, Cedar Knolls, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 339 days.

(21) Appl. No.: **12/262,677**(22) Filed: **Oct. 31, 2008**
(Under 37 CFR 1.47)(65) **Prior Publication Data**

US 2010/0016255 A1 Jan. 21, 2010

Related U.S. Application Data

(60) Provisional application No. 60/984,898, filed on Nov. 2, 2007, provisional application No. 61/020,108, filed on Jan. 9, 2008, provisional application No. 61/083,566, filed on Jul. 25, 2008.

(51) **Int. Cl.**
A61K 31/70 (2006.01)(52) **U.S. Cl.** **514/52**(58) **Field of Classification Search** None
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

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(57) **ABSTRACT**A novel method and composition for treating vitamin B₁₂ deficiency mammals that fail to respond to oral vitamin B₁₂ therapy.**29 Claims, 1 Drawing Sheet**

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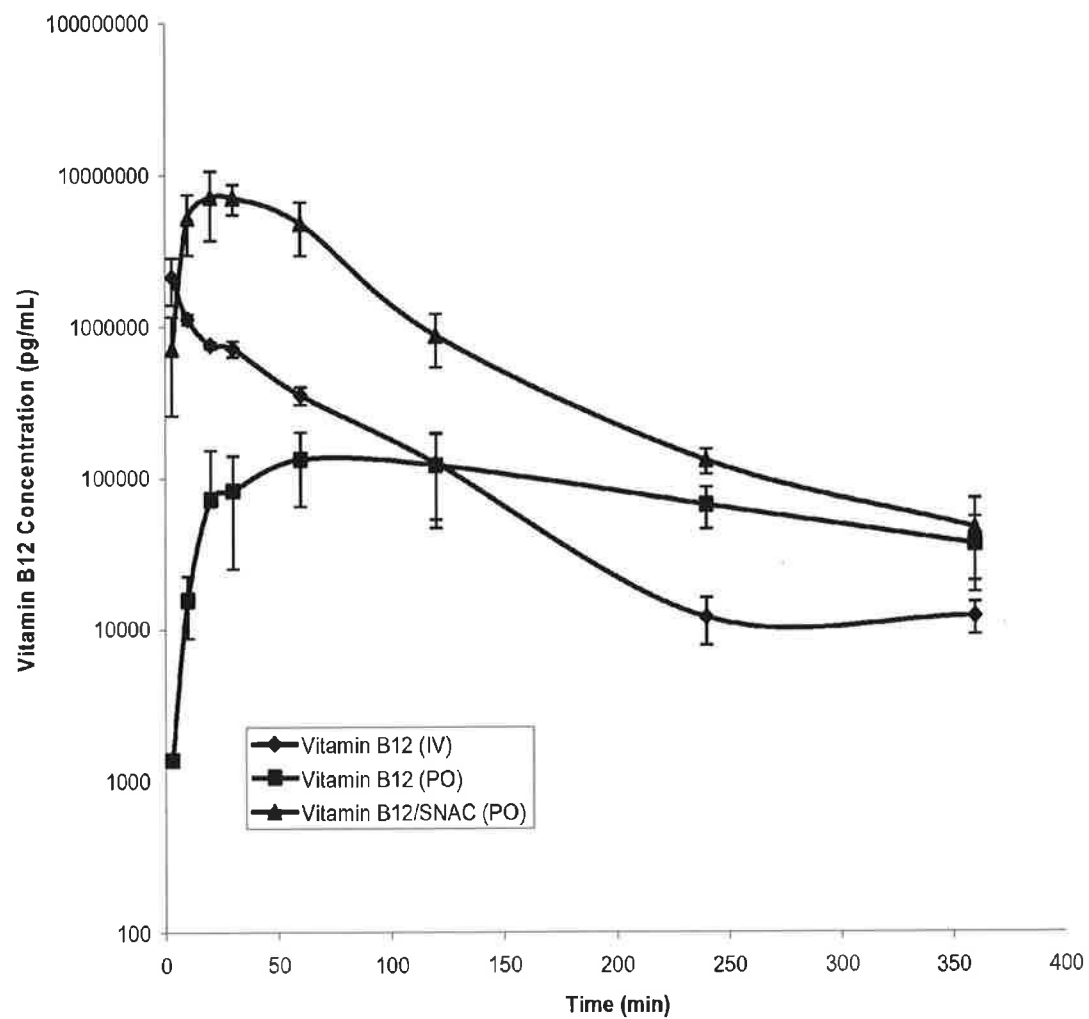
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Figure 1



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METHOD OF TREATING VITAMIN B₁₂ DEFICIENCY

CROSS-REFERENCE TO PRIOR APPLICATIONS

This is a Non Provisional U.S. Application of three provisional applications, claiming the benefit of U.S. Provisional Application No. 60/984,898, filed Nov. 2, 2007; U.S. Provisional Application No. 61/020,108 filed on Jan. 9, 2008; and US Provisional Application No. 61/083,566 filed on Jul. 25, 2008.

FIELD OF THE INVENTION

The present invention relates generally to methods of treating vitamin B₁₂ deficiency and pharmaceutical compositions for such treatment.

BACKGROUND OF THE INVENTION

Vitamin B₁₂ is important for the normal functioning of the brain and nervous system and for the formation of blood. It is involved in the metabolism of every cell of the body, especially affecting the DNA synthesis and regulation but also fatty acid synthesis and energy production. Its effects are still not completely known.

Cyanocobalamin is the most stable and widely used form of vitamin B₁₂. It is bound to plasma proteins and stored in the liver. Vitamin B₁₂ is excreted in the bile and undergoes some enterohepatic recycling. Absorbed vitamin B₁₂ is transported via specific B₁₂ binding proteins, transcobalamin I and II, to the various tissues. The liver is the main organ for vitamin B₁₂ storage.

Vitamin B₁₂ deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. Oral tablets containing vitamin B₁₂ have been developed to treat vitamin B₁₂ deficiency. However, many patients with vitamin B₁₂ deficiency do not respond to oral vitamin B₁₂ treatment. There is a need to develop a treatment for these patients.

BRIEF SUMMARY OF THE INVENTION

One aspect of the invention is directed to a method for treating vitamin B₁₂ deficiency in a subject, comprising the steps of (a) preparing a pharmaceutical composition for oral administration containing (1) vitamin B₁₂ and (2) at least one substance selected from the group consisting of N-[8-(2-hydroxybenzoyl)amino]caprylic acid and its pharmaceutically acceptable salts; and (b) administering the pharmaceutical composition to the subject to effectively treat said vitamin B₁₂ deficiency.

Another aspect of the invention is directed to a pharmaceutical composition for treating vitamin B₁₂ deficiency in a subject, comprising (1) vitamin B₁₂ and (2) at least one substance selected from the group consisting of N-[8-(2-hydroxybenzoyl)amino]caprylic acid and its pharmaceutically acceptable salts; wherein said subject had failed to respond to existing oral vitamin B₁₂ treatment.

The contents of the patents and publications cited herein and the contents of these documents cited in these patents and publications are hereby incorporated herein by reference to the extent permitted.

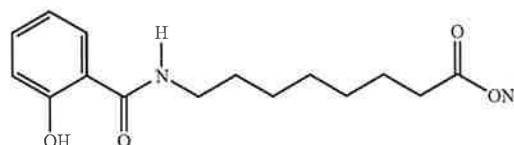
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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of serum vitamin B₁₂ concentration as a function of time.

DETAILED DESCRIPTION

As used herein, the term "SNAC" means Sodium-N-salicyloyl-8-aminocaprylate, Monosodium 8-(N-salicyloylamino) octanoate, N-(salicyloyl)-8-aminooctanoic acid monosodium salt, monosodium N-[8-(2-phenoxybenzoyl) amino]octanoate, E414 monosodium salt or sodium 8-[(2-hydroxybenzoyl)amino]octanoate. It has the structure



"N-[8-(2-hydroxybenzoyl) amino]caprylic acid" has an empirical formula C₁₅H₂₁NO₄.

The term "Vitamin B₁₂" means any member of a group of cobalt-containing compounds known as cobalamins which include, but is not limited to cyanocobalamin, hydroxocobalamin, methylcobalamin, and 5-deoxyadenosylcobalamin.

The term "treatment" or "treating" means any treatment of a disease or disorder in a mammal, including: preventing or protecting against the disease or disorder, that is, causing the clinical symptoms not to develop; inhibiting the disease or disorder, that is, arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder, that is, causing the regression of clinical symptoms. The term "mammal" include human subjects.

The terms "carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent" are as defined in the Handbook of Pharmaceutical Excipients (fourth edition) by Raymond C. Rowe, Paul J. Sheskey and Paul J. Weller, the content of which is herein incorporated by reference.

The term "intrinsic factor protein" means is a glycoprotein produced by the parietal cells of the stomach. It is necessary for the absorption of vitamin B₁₂ later on in the terminal ileum.

In a preferred embodiment, the treatment is directed to subjects that had failed to respond to existing oral vitamin B₁₂ treatment. Preferably, tablets are used for the treatment. Such tablets contain from about 0.01 mg to about 25 mg of vitamin B₁₂ and from about 1 mg to about 600 mg of SNAC each, preferably from about 0.02 mg to about 25 mg of vitamin B₁₂ and more preferably from about 0.1 mg to about 20 mg of vitamin B₁₂ and the most preferably from about 0.5 mg to 10 mg of vitamin B₁₂ and from about 10 mg to about 200 mg of SNAC in each tablet.

The preferred weight ratio of vitamin B₁₂ and SNAC in the tablet is from about 2:1 to about 1:700, more preferably from about 1:2 to about 1:600 or from about 1:3 to about 1:20 and the most preferably from about 1:4 to about 1:10.

In a preferred embodiment, the pharmaceutical composition is in the form of tablets. Preferably, each tablet contains from about 0.01 mg to about 25 mg of vitamin B₁₂ and from about 50 mg to about 600 mg of SNAC. More preferably, each tablet contains from about 0.02 mg to about 20 mg of vitamin B₁₂. More preferably, each tablet contains from about 0.1 mg to about 10 mg of vitamin B₁₂. The most preferably, each

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tablet contains about 15 to 20 mg of vitamin B₁₂ and about 50 to 100 mg of SNAC, or about 0.1 to 1.5 mg of vitamin B₁₂ and about 25 to 150 mg of SNAC.

In another preferred embodiment, the tablet further contains at least one of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent or combinations thereof.

In another preferred embodiment, the tablet optionally contains from about 1 to 25 mg of Capmul PG-8 and optionally contains from about 0.5 to 10 mg of providone. Preferably, Capmul PG-8 is in an amount from about 2 to 20 mg and Providone is in an amount from about 1 to 8 mg. Preferably, Capmul PG-8 is in an amount from about 5 to 15 mg and the Providone is in an amount from about 1.5 to 5 mg. More preferably, Capmul PG-8 is in an amount from about 5 to 10 mg and Providone is in an amount from about 1.5 to 5 mg.

Without intending to be bound by any particular theory of operation, it is believed that gastrointestinal absorption of vitamin B₁₂ depends on the presence of sufficient intrinsic factor protein, secreted from gastric parietal cells. The average diet supplies about 10 mcg/day of vitamin B₁₂ in a protein-bound form that is available for absorption after normal digestion. Vitamin B₁₂ is bound to intrinsic factor during transit through the stomach; separation occurs in the terminal ileum, and vitamin B₁₂ enters the mucosal cell for absorption via a receptor mediated process. It is then transported by the transcobalamin binding proteins. A small amount (approximately 1% of the total amount ingested) is absorbed by simple diffusion, but this mechanism is adequate only with very large doses. It is also believed that SNAC will allow B₁₂ to bypass its usual receptor mediated process.

The following examples are given as specific illustrations of the invention. It should be understood, however, that the invention is not limited to the specific details set forth in the examples. All parts and percentages in the examples, as well as in the remainder of the specification, are by weight unless otherwise specified.

Further, any range of numbers recited in the specification or paragraphs hereinafter describing or claiming various aspects of the invention, such as that representing a particular set of properties, units of measure, conditions, physical states or percentages, is intended to literally incorporate expressly herein by reference or otherwise, any number falling within such range, including any subset of numbers or ranges subsumed within any range so recited. The term "about" when used as a modifier for, or in conjunction with, a variable, is intended to convey that the numbers and ranges disclosed herein are flexible and that practice of the present invention by those skilled in the art using concentrations, amounts, contents, carbon numbers, and properties that are outside of the range or different from a single value, will achieve the desired result, namely, effective treatment of a subject with vitamin B₁₂ deficiency which failed to respond to existing oral vitamin B₁₂ tablets as well as pharmaceutical compositions for such treatment.

Example 1

Preparation of N-[8-(2-hydroxybenzoyl)amino]caprylic Acid and SNAC

The preparation method for N-[8-(2-hydroxybenzoyl)amino]caprylic acid and SNAC involves the following steps: The starting material is salicylamide, which is converted to form Carsalam. The second step involves the alkylation of Carsalam. The penultimate step is a hydrolysis to cleave the

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ethyl protection group at the end of the alkyl chain and spring open the heterocyclic ring forming the free acid of SNAC. In the final step, the sodium salt of the SNAC free acid is formed by reaction with a 1% excess stoichiometric amount of sodium hydroxide base. Upon cooling the precipitated product is isolated by centrifugation and vacuum dried prior to packaging. The in-process controls for the synthetic scheme are given in Table I.

TABLE I

In-process controls for SNAC Manufacturing Process.				
Step	Reaction	Desired Product	Specification	In-Process Control
1	Carsalam	Carsalam	<10% salicylamide	HPLC
2	Alkylation	Alkylated Carsalam	<8% Carsalam	HPLC
3	Hydrolysis	SNAC Free acid	<0.5%	LOD
4	Sodium Salt	SNAC Sodium salt	95-105%	HPLC

Example 2

Preparation of Vitamin B₁₂ Tablets

The tablet die and punches are checked to ensure that they are clean and that their surfaces are dusted with magnesium stearate powder. Vitamin B₁₂, SNAC, carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent are screened through a #35 sieve and transferred into a sealed containers. 50 mg of Vitamin B₁₂ is weighed and mixed thoroughly with 11 grams of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent and/or solubilizing agent. 100 vitamin B₁₂ tablets are made, with each tablet containing 0.5 mg of Vitamin B₁₂ and 110 mg of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent and/or solubilizing agent. These tablets are used as a control.

Example 3

Preparation of Vitamin B₁₂ and SNAC Tablets

50 mg of Vitamin B₁₂, 1 gram of SNAC are weighed and thoroughly mixed with 10 grams of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent and/or solubilizing agent. 100 vitamin B₁₂ tablets are made, with each tablet containing 0.5 mg of Vitamin B₁₂. 10 mg of SNAC and 100 mg of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent and/or solubilizing agent. The process is repeated to make tablet batches containing 1.0 mg, 0.8 mg, 0.6 mg, 0.4 mg and 0.2 of Vitamin B₁₂, respectively. These tablets have the following specifications for release of SNAC component:

Tests	Specification	Analytical Method
Appearance	White to light-tan powder with pink hue	AM001
Identification		
Test for Sodium FTIR	Confirms presence of Sodium Conforms to reference standard	USP <191> USP <197K>

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-continued

Tests	Specification	Analytical Method
Melting Range/ Temperature	193-203° C. with a range not to exceed 5° C.	USP <741>
Water Content	NMT 3.0%	USP <921> Method I
Heavy Metals	<20 ppm	USP <231> Method II
Sodium Content	6.9 to 8.4%	AM017
Residual Solvents		
Ethanol	Less than 4000 ppm	AM008
Heptane	Less than 500 ppm	AM008
Assay as SNAC	90.0-110.0% w/w	AM016
Sodium salt (As Is)		

Example 4

Preparation of Tablets for Testing on Rats

Tablets with four types of different ingredients were made as follows: (1) 8.8 mg of vitamin B₁₂, 35 mg of SNAC were weighed, thoroughly mixed and made into a tablet for dosing on rat; (2) 8.8 mg of vitamin B₁₂, 35 mg of SNAC and 5 mg of Capmul PG-8 were weighed, thoroughly mixed and made into a tablet; (3) 8.8 mg of vitamin B₁₂, 35 mg of SNAC and 0.9 mg of Providone were weighed, thoroughly mixed and made into a tablet. Each of the four processes was repeated to produce more tablets.

Example 5

Dosing Sprague-Dawley Rats

Male Sprague-Dawley rats (325-350 g) were dosed with vitamin B₁₂ intravenously (0.5 mg/kg) alone, or orally with the tablets made in Example 4 at a dose of 50 mg/kg vitamin B₁₂ alone or in combination with SNAC at 200 mg/kg. Blood samples were collected at 0, 3, 10, 20, 30, 60, 120, 240 and 360 minutes post dosing. Plasma samples were analyzed for B12 by RIA. The model independent PK metrics obtained following B12-SNAC combination were compared to those obtained following B12 alone. The testing results are shown in Table 1.

TABLE 1

Comparative Testing Results for Vitamin B ₁₂ Absorption							
Group	Cmax		Tmax		AUC		Mean Bio-availability %
	(ug/mL)		(min)		(ug * min/mL)		
(N = 5)	Mean	S.D	Mean	S.D	Mean	S.D	
0.5 mg/kg Vitamin B ₁₂ (IV)	2.15	0.64	4.4	3.13	65.84	11	
50 mg/kg Vitamin B ₁₂ alone (PO)	0.14	0.07	52	17.9	28.72	13	0.42
50 mg/kg Vitamin B ₁₂ + 200 mg/kg SNAC (PO)	7.99	2.41	24	5.48	522.37	179	7.93

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Example 6

Preparation of Tablets for Testing on Human Subjects

Tablets were made from Cyanocobalamin, SNAC, Kollidon 90F, Anhydrous Emcompress USP/EP and Magnesium Stearate, NF/BP/EP/JP. Each tablet contains the followings:

Ingredients	mg/tablet
Cyanocobalamin, USP (Intrgranular)	5.00
SNAC (Intrgranular)	100.00
Kollidon 90F, NF/EP/JP	2.00
(Providone K90; Intrgranular)	
Anhydrous Emcompress USP/EP (Diabasic Calcium Phosphate, Anhydrous; Intrgranular)	70.00
Anhydrous Emcompress USP/EP (Diabasic Calcium Phosphate, Anhydrous; Extrgranular)	21.00
Magnesium Stearate, NF/BP/EP/JP (extrgranular)	2.00
Total Weight	200.0

Example 7

Dosing Human Subjects

Sixteen healthy male subjects were randomized to receive one of the following treatments:

- (1) Treatment B: a single oral dose of cyanocobalamin/SNAC (5 mg cyanocobalamin/100 mg SNAC) administered in the fasted state as a tablet. (6 subjects);
- (2) Treatment C: a single oral dose of cyanocobalamin alone (5 mg cyanocobalamin, VitaLabs, commercial) administered in the fasted state as a tablet. (6 subjects).
- (3) Treatment D: a single intravenous dose of cyanocobalamin (1 mg cyanocobalamin) administered in the fasted state. (4 subjects). Each subject received a 1 mL intravenous injection of a 1 mg/mL (1000 µg/mL) solution resulting in a total dose of 1 mg cyanocobalamin.

The subjects were fasted overnight prior to dosing and had no liquids (including water) consumption for at least one hour before and after dosing. The oral forms of cyanocobalamin/SNAC tablets were administered in a single dose as tablets with 50 mL of plain water. Twenty-five blood samples were drawn for cyanocobalamin analyses at the following time points: within 30 minutes pre-dose and at Minutes 2, 5, 10, 20, 30, 40, 50, and at Hours 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20 and 24 post-dose.

Pharmacokinetic metrics was obtained following a model independent pharmacokinetic analysis of individual cyanocobalamin concentrations. Descriptive statistics was used to summarize the results.

Following 1 tablet of 5 mg B12/100 mg SNAC mean B12 peak concentration is 12847±6613 µg/mL and occur within 1 hour post dose (mean tmax of 0.50±0.21 hours). Mean AUClast (0-24) value is 54618±16392 hr*pg/mL. The percent coefficient of variation (% CV) is 51.5% for Cmax and 30.0% for AUC.

Following a single oral dose of cyanocobalamin alone (5 mg cyanocobalamin, VitaLabs, commercial) mean B12 peak concentration is 1239±450 µg/mL and occur between 3 to 10 hours post-dose (mean tmax of 6.8±3.2 hours). Mean AUClast (0-24) value is 23131±8343 hr*pg/mL. The percent coefficient of variation (% CV) is 36.3% for Cmax and 36.1% for AUC.

Following a single intravenous dose of cyanocobalamin (1 mg cyanocobalamin) administered in the fasted state (4 subjects). Mean B12 peak concentration is 221287 ± 80248 pg/mL and mean AUClast (0-24) value is 215391 ± 44602 hr*pg/mL. The percent coefficient of variation (% CV) is 36.3% for Cmax and 20.7% for AUC.

The mean bioavailability of 1 tablet of 5 mg vitamin B12 alone, 1 tablet of 5 mg vitamin B12/100 mg SNAC, and 2 tablets of 5 mg vitamin B12/100 mg SNAC are $2.15 \pm 0.77\%$, 5.07 ± 1.52 , and $5.92 \pm 3.05\%$, respectively. (Note: 2 tablets of 5 mg vitamin B12/100 mg SNAC were dosed previously in a pilot arm are designated Treatment A).

The mean tmax of 1 tablet of 5 mg vitamin B12 alone, 1 tablet of 5 mg vitamin B12/100 mg SNAC, and 2 tablets of 5 mg vitamin B12/100 mg SNAC are 6.8 ± 3.2 hours, 0.50 ± 0.21 hours, and 0.54 ± 0.32 hours, respectively.

No adverse events were observed during the given treatments. All formulations appear to be safe and well tolerated.

It was found surprisingly that the extent of B12 absorption, measured as Cmax and AUC, was significantly enhanced by the administration of the cyanocobalamin/SNAC combination. Vitamin B12 bioavailability was ~240% greater for the 1 tablet of 5 mg B12/100 mg SNAC compared to 5 mg B12 commercial formulation. Mean peak B12 concentrations following B12 commercial oral formulation occurred significantly later compared to that following the B12/SNAC combinations likely due to a different site of absorption between the two oral formulations. This is consistent with literature data describing intestinal absorption of B12 occurring in the distal section of the gastrointestinal tract in the absence of the carrier.

The principles, preferred embodiments, and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since these are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art, without departing from the spirit of the invention.

What is claimed is:

1. A method for treating vitamin B₁₂ deficiency in a subject in need thereof, comprising orally administering to the subject a pharmaceutical composition comprising (1) vitamin B₁₂ and (2) a compound selected from N-[8-(2-hydroxybenzoyl)amino]caprylic acid and pharmaceutically acceptable salts thereof, wherein the pharmaceutical composition comprises an effective amount of the compound such that, upon oral administration of the pharmaceutical composition, the T_{max} of the vitamin B₁₂ is decreased.

2. The method of claim 1, wherein said subject has failed to respond to existing oral vitamin B₁₂ treatment.

3. The method of claim 1, wherein said pharmaceutical composition is a tablet.

4. The method of claim 3, wherein said tablet comprises from about 0.01 mg to about 25 mg of vitamin B₁₂ and from about 1 mg to about 600 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate.

5. The method of claim 3, wherein said tablet comprises from about 0.02 mg to about 25 mg of vitamin B₁₂.

6. The method of claim 3, wherein said tablet comprises from about 0.1 mg to about 20 mg of vitamin B₁₂.

7. The method of claim 3, wherein said tablet comprises about 0.5 mg of vitamin B₁₂ and about 17.5 mg of monosodium N-[8-(2-hydroxybenzoyl) amino]caprylate.

8. The method of claim 1, wherein said pharmaceutical composition has a weight ratio of vitamin B₁₂ to monosodium N-[8-(2-hydroxybenzoyl) amino]caprylate of from about 2:1 to about 1:700.

9. The method of claim 8, wherein said weight ratio is from about 1:2 to about 1:600.

10. The method of claim 8, wherein said weight ratio is from about 1:3 to about 1:20.

11. The method of claim 8, wherein said weight ratio is about 1:4.

12. The method of claim 8, wherein said weight ratio is from about 1:500 to about 1:700.

13. The method of claim 3, wherein said pharmaceutical composition further comprises at least one of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent or combinations thereof.

14. An oral pharmaceutical composition comprising (1) vitamin B₁₂ and (2) an effective amount of a compound selected from N-[8-(2-hydroxybenzoyl)amino]caprylic acid and pharmaceutically acceptable salts thereof such that, upon oral administration of the pharmaceutical composition, the T_{max} of the vitamin B₁₂ is decreased.

15. The pharmaceutical composition of claim 14, wherein said pharmaceutical composition is in the form of a tablet.

16. The pharmaceutical composition of claim 15, wherein said tablet comprises from about 0.01 mg to about 25 mg of vitamin B₁₂ and from about 50 mg to about 600 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate.

17. The pharmaceutical composition of claim 15, wherein said tablet comprises from about 0.02 mg to about 20 mg of vitamin B₁₂.

18. The pharmaceutical composition of claim 15, wherein said tablet comprises from about 0.1 mg to about 10 mg of vitamin B₁₂.

19. The pharmaceutical composition of claim 15, wherein said tablet comprises about 1 to 15 mg of vitamin B₁₂ and about 50 to 200 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate.

20. The pharmaceutical composition of claim 14, further comprising at least one of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent or combinations thereof.

21. An oral pharmaceutical composition comprising 0.5 to 10 mg of vitamin B₁₂ and from 10 to 200 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate, wherein the pharmaceutical composition comprises an effective amount of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate such that, upon oral administration of the pharmaceutical composition, the T_{max} of the vitamin B₁₂ is decreased.

22. The oral pharmaceutical composition of claim 21, wherein the pharmaceutical composition is a tablet.

23. A method of treating vitamin B₁₂ deficiency in a subject in need thereof, the method comprising orally administering to the subject a pharmaceutical composition comprising 0.5 to 10 mg of vitamin B₁₂ and from 10 to 200 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate, wherein the pharmaceutical composition comprises an effective amount of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate such that, upon oral administration of the pharmaceutical composition, the T_{max} of the vitamin B₁₂ is decreased.

24. The method of claim 23, wherein the subject is a human.

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25. The method of claim 1, wherein, upon oral administration, the pharmaceutical composition exhibits a mean T_{max} for the vitamin B₁₂ of 0.50 hours.

26. The pharmaceutical composition of claim 14, wherein the pharmaceutical composition exhibits a mean T_{max} for the vitamin B₁₂ of 0.50 hours. 5

27. The pharmaceutical composition of claim 21, wherein, upon oral administration, the pharmaceutical composition exhibits a mean T_{max} for the vitamin B₁₂ of 0.50 hours.

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28. The method of claim 23, wherein the pharmaceutical composition exhibits a mean T_{max} for the vitamin B₁₂ of 0.50 hours.

29. The oral pharmaceutical composition of claim 14, wherein the oral pharmaceutical composition comprises about 0.1 to 1.5 mg of vitamin B₁₂ and from 25 to 100 mg of monosodium N-[8-(2-hydroxybenzoyl)amino]caprylate.

* * * * *

APPENDIX H

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Phase I clinical study to select a novel oral formulation for ibandronate containing the excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC)

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The aim of this study was to select a novel oral formulation for ibandronate (IBN, CAS number: 138926-19). In four cohorts of 28, 21, 19 and 29 healthy volunteers, the impact of the carrier molecule sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, CAS number: 203787-91-1) on the bioavailability of IBN was investigated. Within each cohort different oral formulations with one dose of ibandronate (30 mg) and three different ratios of IBN:SNAC (1:5, 1:10 and 1:20) were compared to the approved oral IBN tablet formulations (150 and 50 mg IBN) in a 4-way cross-over design and a one week washout between the administrations. The highest mean IBN exposure was achieved with a capsule formulation containing drug-coated beadlets and an IBN:SNAC ratio of 1:5. AUC_{last} and C_{max} of IBN were approximately 1.3- and 2.2-fold higher compared to the reference treatment (150 mg IBN without SNAC). Increasing the post-dose fasting duration from 15 to 30 min resulted in a more than 2-fold increase in AUC_{last}, while superimposable IBN serum concentration-time profiles were achieved after a 30 and 60 min fast. The tolerability of the IBN/SNAC treatments in all cohorts was similar to that in the IBN reference groups and most adverse events (AEs) were of mild to moderate intensity.

1. Introduction

When combined with another charged molecule (e.g. heparin), the pharmaceutical excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, CAS number: 203787-91-1) has been shown to facilitate the transport of that molecule across the gastrointestinal tract (Leone-Bay et al. 1998). In the current investigation, SNAC has been co-formulated with ibandronate (IBN, CAS number: 138926-19), a potent, nitrogen-containing bisphosphonate that has been shown to induce significant increases in bone mineral density (BMD) at the lumbar spine and the hip and decrease the risk of osteoporotic fractures in postmenopausal women (Delmas et al. 2002; Chesnut et al. 2004; Miller et al. 2005; Reginster et al. 2006) and is approved for the treatment and prevention of postmenopausal osteoporosis and for the treatment of metastatic bone disease. Ibandronate exhibits a low and highly variable oral bioavailability (~0.6%). As its oral absorption is reduced by concomitant food intake (Barrett et al. 2004), the bisphosphonate must be taken under fasting conditions and fasting must continue for at least 60 min (Bonviva® 150 mg tablets) or 30 min (Bondronat® 50 mg tablets) post-dose to ensure adequate absorption and efficacy. These food intake restrictions may result in inconvenience for some patients, which might affect long-term compliance. Co-formulation of SNAC with IBN is expected to result in a faster absorption of IBN from the gastrointestinal tract, which might allow for a reduction of the post-dose fasting duration.

The primary objective of the study was to select an IBN/SNAC formulation type as well as the appropriate IBN:SNAC ratio required to increase the oral bioavailability of the bisphosphonate. A parallel cohort design (cohorts I to III) was selected to compare different formulation types to permit a full assessment of safety, i.e. maximizing the number of subjects exposed to better characterize the safety profile of the IBN/SNAC combination. Within each cohort, a cross-over design was chosen to compare the impact of the IBN:SNAC ratio on the absorption of IBN for a formulation type. The selected formulation type and IBN:SNAC ratio from cohorts I to III was further evaluated with respect to the impact of the post-dose fasting duration on the exposure to IBN in a sequential cohort IV.

2. Investigations and results

2.1. Demographics

The majority of subjects enrolled were male. The percentage of females was 21%, 10%, 32% and 45% in cohorts I, II, III and IV, respectively. More than 90% of subjects were Caucasian. The age of the subjects varied between 18 and 60 years with mean values of 31.6, 24.2, 24.7 and 36.5 years in cohorts I to IV. Body weight, height and body mass index were comparable between the cohorts, ranging from 52 to 89.5 kg, 156 to 193 cm, and 18.2 to 33.3 kg/m², respectively.

2.2. Safety

The majority of the adverse events were mild to moderate in intensity. No adverse event was of serious intensity. There was no increase in the incidence of adverse events with increasing doses of SNAC. The most common adverse events across all four cohorts were musculoskeletal and connective tissue disorders (mainly back pain), gastrointestinal disorders (mainly nausea), and nervous system disorders (mainly headache). There were no clinically significant changes in vital signs and ECG parameters.

2.3. IBN and SNAC dissolution from formulation

As shown in Fig. 1, tablet formulations 1 and 2 showed comparable sigmoidal dissolution profiles for SNAC and IBN, suggesting co-dissolution of the ingredients. A lag-time of approximately 30 min was observed prior to IBN release from the formulation. The capsules containing drug-coated beadlets (formulation 3) or the spray-granulate (formulation 4) did exhibit an early release of ibandronate, with a slight lag in release of SNAC. The spray granulate did show a first order release pattern for IBN and a zero-order release profile for SNAC, while the beadlet formulation resulted in a zero-order release for both IBN and SNAC, an indication for co-dissolution.

2.4. NIR Chemical Imaging

For formulations 3 and 4, spectra extracted from each sample image were projected onto the PLS model and score images for IBN, SNAC and PVC were calculated. Fig. 2 shows the SNAC images in formulation 2 (drug-coated beadlets) and formulation 4 (spray-granulate) along with the histogram plots of the score values and statistical parameters. The pixel score value corresponds to the concentration of SNAC in the sample matrix. The darker pixels symbolize higher localized concentration of SNAC. Results demonstrated that SNAC is more uniformly distributed around IBN in the beadlet formulation than in the granulate. In addition, the lower standard deviation and smaller kurtosis value in the beadlets sample image indicate lower variability, thus a higher degree of homogeneous SNAC distribution within the sample matrix.

Results obtained from PLS score images of SNAC were further verified by the RGB images constructed using the individual score images of IBN, SNAC, and PVP, as shown in Fig. 3. RGB images revealed that SNAC is more homogeneously mixed with IBN in the beadlet formulation, whereas evident agglomeration of SNAC and IBN was observed in the granule sample. In the beadlet formulation, IBN particles alone were difficult to be spatially resolved under the 20 μm /pixel objective of the image system, which may be an indication for a uniform and close contact between SNAC and IBN, prohibiting self-aggregation of IBN. Tables 1–4

2.5. Pharmacokinetics

The pharmacokinetics of IBN were studied in each cohort. Mean serum concentration-time plots of IBN by treatment for each cohort are shown in Fig. 4. Mean ($\pm$ SD) PK parameters by treatment for each cohort are summarized in Tables 1–4. Estimated ratios for AUClast and Cmax with respect to treatment by cohort are shown in Table 5.

In cohorts I and II where two different types of SNAC containing tablet formulations (formulations 1 and 2) containing 30 mg IBN each were compared to the approved 150 mg IBN tablet formulation, the exposure to IBN expressed as AUClast was the highest with reference formulation. In cohort I, this higher

exposure with the reference formulation was statistically significant compared to all IBN/SNAC ratios, while in cohort II the advantage with the reference formulation was statistically significant compared to the IBN/SNAC ratios of 1:5 and 1:20 only. In both cohorts, AUClast decreased in the following order: reference 150 mg formulation > IBN/SNAC 1:10 ratio > IBN/SNAC 1:5 ratio > IBN/SNAC 1:20 ratio. The mean AUClast for the 150 mg reference formulation was approximately 2.2- and 1.6-fold higher than for the IBN/SNAC formulation with the 1:10 ratio in cohorts I and II, respectively.

The highest overall exposure to IBN was achieved with the SNAC containing formulation type 3 tested in cohort III (drug-coated beadlets). AUClast of IBN decreased in the following order: IBN/SNAC 1:5 ratio > IBN/SNAC 1:10 ratio > IBN/SNAC 1:20 ratio > reference 150 mg formulation. AUClast and Cmax of IBN with an IBN/SNAC 1:5 ratio were approximately 1.3- and 2.2-fold higher than with the reference 150 mg treatment. This difference was statistically significant for Cmax only.

The coated beadlets formulation with an IBN/SNAC ratio of 1:5 was selected for a food effect assessment in cohort IV. Compared to a post-dose fasting duration of 15 min as studied in cohort III, an increase of the fasting time to 30 and 60 min did result in a 2.3- and 2.5-fold increase in AUClast and a 1.7- and 1.8-fold increase in Cmax of IBN, respectively. IBN serum concentration-time profiles after a 30 and 60 min fast were superimposable.

As a protocol amendment, in cohort IV, an additional formulation type 4 (granulate formulation administered in hard gelatin capsules, IBN/SNAC ratio 1:5, post-dose fast of 60 min) was investigated. Results demonstrated that the mean AUClast and Cmax after a 60 min fast with the granulate formulation were approximately 4.6- and 4.2-fold lower, respectively, as compared to the beadlet formulation with a 30 or 60 min post-dose fast. This difference was statistically significant.

Median Tmax with all SNAC containing formulations was higher compared to Tmax of the reference formulations (average 0.33 h vs. 0.667 h).

3. Discussion

Absorption of a substance following ingestion is determined largely by its physicochemical properties such as molecular weight, solubility pKa and lipophilicity (Song et al. 2004). Thus, the poor absorption of bisphosphonates is proposed to be due to their polarity, being negatively charged at physiological pH. This polarity hinders transcellular transport through epithelial membranes, and paracellular transport is thought the likely route of absorption (Lin 1996). As for other bisphosphonates, it is expected that IBN absorption occurs high in the gastro-intestinal tract (Brayden et al. 1997; Mitchell et al. 1998). This assumption is supported by the rapid absorption within 1 hour, as well as by the influence of gastric acidity and the presence of food on the extent of absorption (Barrett et al. 2004). Consequently, an early release from the formulation together with a protection from degradation in the gastric environment is necessary for increasing the absorption of IBN.

The carrier molecule SNAC, when combined with a target substance, has been shown to form a weak, non-covalent complex that enables absorption of the target substance while preserving its chemical integrity (Brayden et al. 1997; Wu and Robinson 1999a, b; Leone-Bay et al. 2001; Malkov et al. 2002). SNAC and related carriers enable absorption primarily via the transcellular pathway, without compromising the integrity of the intestinal epithelium (Ding et al. 2004).

This clinical investigation was undertaken to select the formulation type that results in the highest systemic exposure to IBN

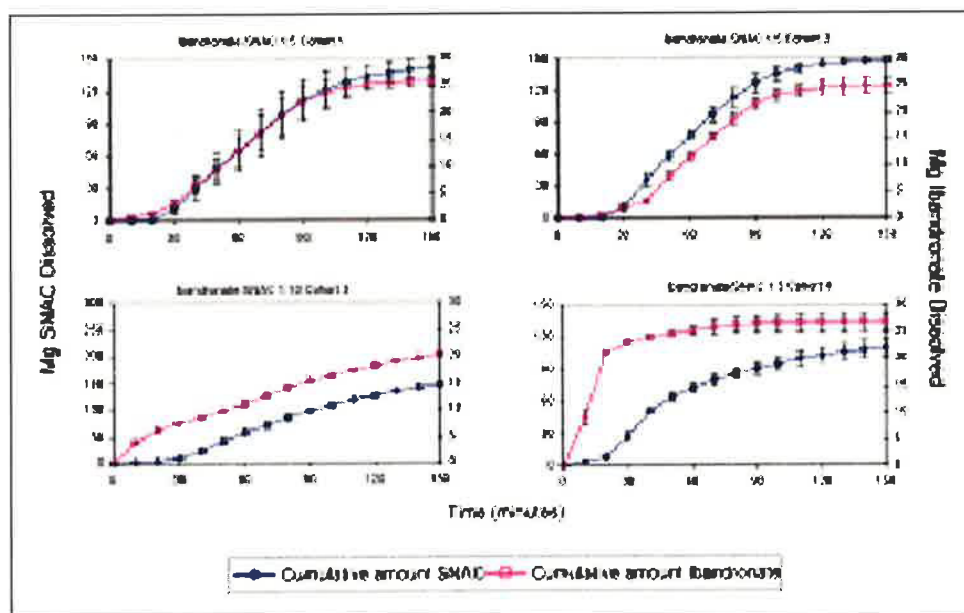


Fig. 1: Dissolution profiles of ibandronate and SNAC from different formulations

in the presence of SNAC. The underlying assumption was that intimate contact between the bisphosphonate and the carrier at the site of absorption would be required. This hypothesis is based on a study in beagle dogs. In a cross-over study which used 4 dogs, heparin and SNAC were either co-formulated or administered alone but concomitantly in two different soft gel capsules. The results showed that heparin absorption occurred

only when heparin was formulated (or processed) along with SNAC (Emisphere unpublished data).

In the current trial, the drug-coated beadlet formulation (formulation 3) tested in cohort III resulted in a markedly greater extent of IBN detectable in the systemic circulation compared to all other formulations investigated. This observation might be explained considering the dissolution profiles of SNAC and IBN.

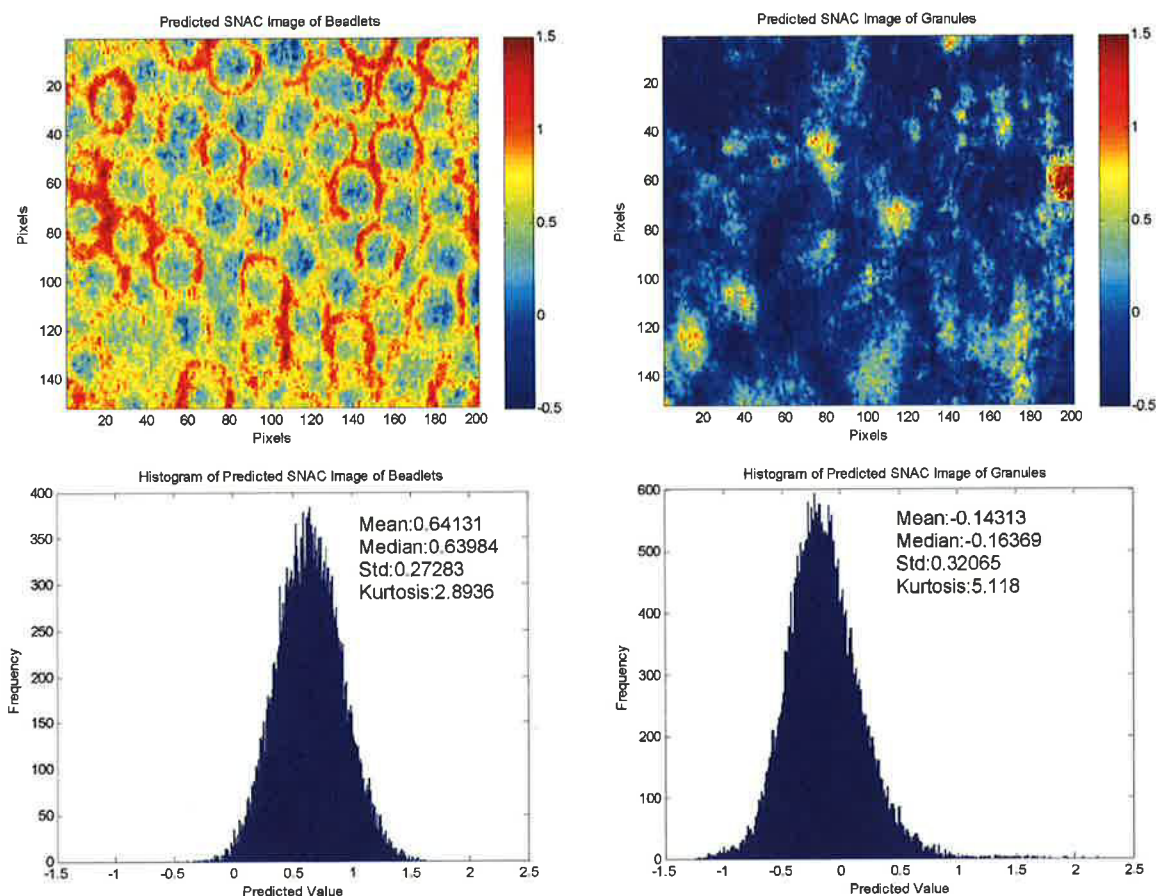


Fig. 2: Example of score images of SNAC generated from one sample image using the developed discriminant PLS model

Table 1: Summary of pharmacokinetic parameters (mean $\pm$ S.D.) of IBN in serum by treatment in cohort I

Treatment ^a	A N = 19	B N = 19	C N = 19	D N = 19
C_{max} (ng/mL)	68.7 $\pm$ 36.6	82.1 $\pm$ 35.9	56.8 $\pm$ 27.3	116 $\pm$ 88.3
Median	69.9	75.0	54.3	89.0
Range	(8.69–131)	(17.8–165)	(2.72–115)	(28.6–378)
CV (%)	53.3	43.7	48.1	76.2
T_{max} (h)	0.356 $\pm$ 0.0531	0.325 $\pm$ 0.0386	0.456 $\pm$ 0.310	0.875 $\pm$ 0.224
Median	0.333	0.333	0.333	0.983
Range	(0.333–0.500)	(0.167–0.350)	(0.167–1.50)	(0.333–1.02)
CV (%)	14.9	11.9	68.0	25.6
AUC_{last} (h*ng/mL)	96.7 $\pm$ 54.2	112 $\pm$ 44.8	86.8 $\pm$ 41.6	243 $\pm$ 125
Median	89.0	97.5	86.5	204
Range	(16.1–207)	(31.6–194)	(8.67–175)	(81.8–571)
CV (%)	56.1	40.1	47.9	51.4
$t_{1/2}$ (h)	14.6 $\pm$ 7.00	13.2 $\pm$ 4.22	13.1 $\pm$ 5.48	10.5 $\pm$ 3.70
Median	13.9	13.2	12.7	10.5
Range	(3.22–30.6)	(6.74–24.0)	(3.37–24.7)	(3.46–19.0)
CV (%)	47.9	32.0	41.8	35.3

^a A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN. A, B and C: film-coated tablets; D: reference

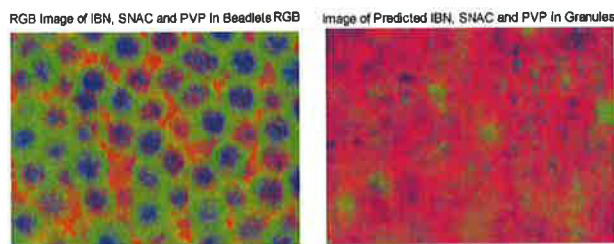


Fig. 3: Example of RGB images of IBN (red), SNAC (green), and PVP (blue), in beadlet and granule formulations

With the tablet formulations 1 and 2, IBN dissolution starts about 30 min post-dose only. Given that food was given already 15 min post-dose, the amount of IBN available for absorption was limited. By contrast, both for the beadlets and granulate formulations, IBN dissolution started immediately upon exposure to the test fluid. While with the granulate formulation IBN is almost quantitatively released prior to the start of marked SNAC dissolution at about 30 min post-dose, with the beadlets formulation IBN and

SNAC dissolve slowly and with a parallel release pattern. Thus the presence of SNAC may have protected in parts complexation of IBN once food was administered at 15 min post-dose. NIR imaging that was conducted to gain further insight into the different absorption behavior from the beadlets and granulate formulations showed a more uniform and intimate interaction between SNAC and IBN in the beadlet formulation. Such close contact between IBN and SNAC seems to have facilitated the formation of an IBN/SNAC complex to improve the IBN permeability *in vivo*, which potentially led to the enhanced bioavailability. The cross-cohort food effect assessment comparing a 15, 30 and 60 min post-dose fasting duration demonstrated that at 15 min after drug administration absorption from the gastrointestinal tract is not yet complete. This is shown by the more than 2-fold higher AUC when applying a 30 and 60 min fast compared to the 15 min fast. A reduction of the 60 min post-dose fasting duration to 15 min for the novel SNAC formulation is therefore not recommended. Patients who would not comply with the fasting restrictions and eat later than 15 min after drug administration, would have markedly higher IBN plasma levels

Table 2: Summary of pharmacokinetic parameters (mean $\pm$ S.D.) of IBN in serum by treatment in cohort II

Treatment ^a	A N = 17	B N = 17	C N = 17	D N = 17
C_{max} (ng/mL)	61.6 $\pm$ 51.7	102 $\pm$ 45.0	53.2 $\pm$ 23.4	73.3 $\pm$ 46.4
Median	66.0	104	50.5	69.0
Range	(1.80–187)	(19.5–187)	(23.2–107)	(18.5–173)
CV (%)	83.9	44.1	44.0	63.3
T_{max} (hr)	0.338 $\pm$ 0.104	0.355 $\pm$ 0.0889	0.393 $\pm$ 0.0815	0.938 $\pm$ 0.684
Median	0.333	0.333	0.333	0.667
Range	(0.167–0.50)	(0.333–0.700)	(0.333–0.50)	(0.167–3.00)
CV (%)	30.8	25.1	20.7	72.9
AUC_{last} (hr*ng/mL)	74.2 $\pm$ 55.6	120 $\pm$ 43.9	76.7 $\pm$ 44.5	187 $\pm$ 106
Median	68.9	118	69.2	150
Range	(2.07–199)	(28.5–204)	(25.0–210)	(54.4–441)
CV (%)	74.9	36.7	58.1	56.8
$t_{1/2}$ (hr)	12.0 $\pm$ 6.52	13.9 $\pm$ 3.76	12.5 $\pm$ 6.97	13.5 $\pm$ 6.65
Median	12.3	14.1	10.7	12.4
Range	(1.78–27.8)	(3.83–19.8)	(3.86–30.1)	(4.17–35.3)
CV (%)	54.3	27.0	55.8	49.3

^a A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN. A, B and C: film-coated tablets; D: reference

Table 3: Summary of pharmacokinetic parameters (mean $\pm$ S.D.) of IBN in serum by treatment in cohort III

Treatment ^a	A N = 19	B N = 19	C N = 19	D N = 19
$C_{\max}$ (ng/mL)	259 $\pm$ 127	197 $\pm$ 142	147 $\pm$ 59.4	116 $\pm$ 149
Median	277	158	146	76.4
Range	(42.5–500)	(12.2–571)	(52.2–263)	(27.9–627)
CV (%)	49.0	72.1	40.3	128
$T_{\max}$ (hr)	0.308 $\pm$ 0.0919	0.360 $\pm$ 0.121	0.363 $\pm$ 0.112	0.661 $\pm$ 0.264
Median	0.25	0.333	0.333	0.667
Range	(0.25–0.583)	(0.250–0.667)	(0.250–0.700)	(0.250–0.983)
CV (%)	29.8	33.7	30.9	39.9
AUC_{last} (hr*ng/mL)	287 $\pm$ 150	227 $\pm$ 149	178 $\pm$ 59.0	223 $\pm$ 203
Median	236	192	182	152
Range	(40.0–681)	(23.3–486)	(82.8–332)	(60.6–825)
CV (%)	52.4	65.5	33.2	91.2
$t_{1/2}$ (hr)	9.12 $\pm$ 2.26	10.7 $\pm$ 5.36	9.68 $\pm$ 3.06	7.77 $\pm$ 3.31
Median	9.31	9.00	10.2	8.58
Range	(3.51–12.2)	(4.82–26.1)	(3.51–14.9)	(3.50–13.8)
CV (%)	24.8	50.1	31.6	42.6

^a): A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN. A, B and C: Beadlets in capsules; D: reference

than those adhering to the dosing instructions. The finding that the IBN plasma concentration-time profiles are superimposable when applying a 30 or 60 min post-dose fast demonstrates that absorption is complete at 30 min post-dose, and there is no need to wait more than 30 min prior to food intake with the new IBN/SNAC formulation.

In summary, this study has shown that when combined with the carrier molecule SNAC, the systemic exposure to IBN could be markedly increased. With the SNAC formulation, an oral IBN dose of 30 mg resulted in a more than 2-fold greater exposure compared to the approved 150 mg tablet formulation. A prerequisite for the improved absorption from the gastro-intestinal tract is the use of an optimal formulation type, namely drug-coated beadlets presenting the homogenous IBN/SNAC mixture at their surface, this way allowing a fast release and subsequent absorption already in the upper part of the gastro-intestinal tract. The exact IBN dose that results in comparable AUC to the approved tablet formulation will be determined in a subsequent trial in healthy post-menopausal females.

4. Experimental

4.1. Subjects

Ninety-seven healthy male and female subjects were enrolled in this study. Subjects were in good general health as assessed by a physician on the basis of a medical history, general physical examination, vital signs, ECG and laboratory tests. Subjects were excluded in case of a history of clinically significant gastro-intestinal, cardiovascular, musculoskeletal, endocrine, hematological, psychiatric, hepatic, broncho-pulmonary, neurological, renal or allergic disease including aspirin-sensitive asthma, abnormalities of the oesophagus which could delay oesophageal emptying such as stricture or achalasia, active upper gastrointestinal disease such as dysphasia, gastritis, duodenitis or ulcers. Subjects who participated in a clinical study with an investigational drug within 6 weeks or 6 times the elimination half-life (whichever was longer) of first drug administration or who donated/lost more than 400 mL of blood in the 3 month period prior to first drug administration were not allowed to enter the trial. Each subject gave written informed consent. The protocol was approved by the Upper South B Regional Ethics Committee, Ministry of Health, Christchurch, New Zealand. The study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki.

Table 4: Summary of pharmacokinetic parameters (mean $\pm$ S.D.) of IBN in serum by treatment in cohort IV

Treatment ^a	A N = 19	B N = 19	C N = 19	D N = 19
$C_{\max}$ (ng/mL)	364 $\pm$ 123	340 $\pm$ 123	86.7 $\pm$ 59.5	20.9 $\pm$ 15.8
Median	366	349	75.4	17.0
Range	(157–571)	(136–619)	(13.2–226)	(8.14–76.0)
CV (%)	33.8	36.1	68.6	75.4
$T_{\max}$ (hr)	0.356 $\pm$ 0.134	0.345 $\pm$ 0.128	0.446 $\pm$ 0.224	0.611 $\pm$ 0.0971
Median	0.333	0.333	0.333	0.667
Range	(0.167–0.667)	(0.167–0.667)	(0.250–1.02)	(0.333–0.683)
CV (%)	37.6	37.0	50.1	15.9
AUC_{last} (hr*ng/mL)	561 $\pm$ 245	518 $\pm$ 293	123 $\pm$ 66.0	40.4 $\pm$ 26.2
Median	542	447	129	31.6
Range	(152–1140)	(157–1240)	(26.5–271)	(19.1–132)
CV (%)	43.7	56.7	53.5	64.8
$t_{1/2}$ (hr)	8.98 $\pm$ 1.78	9.20 $\pm$ 1.33	9.41 $\pm$ 3.02	15.5 $\pm$ 7.82
Median	8.87	9.46	9.03	13.0
Range	(6.37–14.0)	(7.37–11.6)	(3.36–15.4)	(7.44–36.6)
CV (%)	19.9	14.4	32.1	50.4

^a): A: 30/150 mg IBN/SNAC, cohort III formulation (drug-coated beadlets), 60 min fast; B: 30/150 mg IBN/SNAC cohort III formulation, 30 min fast; C: 30/150 mg IBN/SNAC, granulate formulation; D: 50 mg IBN

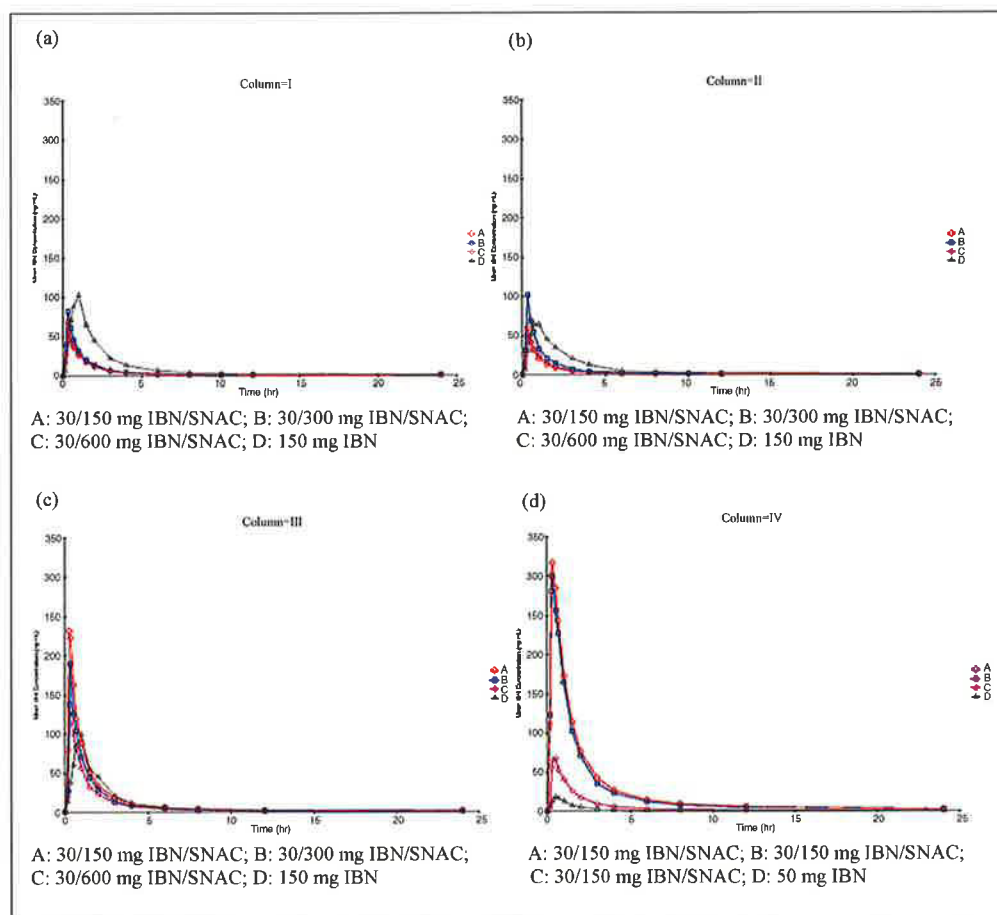


Fig. 4: Mean serum concentration-time profiles of IBN by treatment in (a) cohort I, (b) cohort II, (c) cohort III, (d) cohort IV

4.2. Study design

This was a single centre, open-label, randomized, four-cohort study. Within each cohort, formulations were investigated in a four-period crossover design. Subjects underwent a thorough screening medical examination between day -28 and day -2 of the study. Three different types of oral formulations (1 [film coated tablet], 2 [film coated tablet], and 3 [drug-coated beadlets in a hard gelatin capsule]) with 30 mg of ibandronate were studied in cohorts I to III. For each type of formulation, three different ratios of IBN:SNAC (1:5 [30/150 mg], 1:10 [30/300 mg] and 1:20 [30/600 mg]) were tested. The reference formulation in cohorts I to III was the 150 mg IBN tablet approved for the osteoporosis indication (Table 6).

Based on the results from cohorts I to III, the cohort III formulation (drug-coated beadlets in a hard gelatin capsule) was further evaluated, in cohort IV, to assess the impact of the timing of food intake on the absorption of IBN. The protocol was amended to include a modified hard gelatin capsule formulation (granulate) with 30 mg ibandronate at a ratio of IBN:SNAC of 1:5 (i.e., 30/150 mg, formulation 4) into cohort IV. To also generate data that allow a direct comparison to the 50 mg IBN tablet approved for the oncology indication, this tablet was used as the reference treatment D in cohort IV. In cohorts I, II and III subjects received a standardized breakfast 15 min after drug intake for the SNAC containing formulations in treatment arms A, B and C. For the reference treatment arm D, a standardized breakfast was given 60 min after drug intake as per clinical practice. For the food effect assessment in cohort IV, a standardized breakfast was given 30 min (treatments B and D) or 60 min (treatments A and C) after drug intake. Subjects received 500 mg supplemental calcium once-daily throughout the course of the study. Table 6 details the treatments and dosing conditions in the different cohorts.

4.3. Blood sampling and determination of IBN concentrations

On Day -1, a 45 mL venous blood sample was collected to create calibration curves for the enzyme-linked immunosorbent assay (Ibandronate ELISA, Microcoat GmbH, Bernried, Germany) for each individual. On Day 1 of each treatment period, 5 mL venous blood samples were collected in 5 mL plain glass Vacutainer tubes at pre-dose and at 10, 15, 20, 30, 40, 60 min, 1.5,

2, 3, 4, 6, 8, 12 and 24 h post-dose. Serum obtained from these blood samples were assayed for levels of IBN. The calibration range was 25–1600 pg/mL (including “0” standard). The lower limit of quantification for this assay was 50 pg/mL. Quality control serum samples at three different concentrations (150, 300 and 600 pg/mL) were analyzed with each assay batch (approx. 8% quality controls referenced to the number of unknown samples). Results from study samples were only accepted if not more than 33% of the quality control samples within each assay batch had an inaccuracy greater than 15%.

4.4. Pharmacokinetics

Pharmacokinetic parameters were estimated using non-compartmental methods according to the Roche internal “Guideline for calculation and analyses of non compartmental pharmacokinetic parameters” issued April 2005 and using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA).

Primary pharmacokinetic parameters for IBN were C_{max} (maximum plasma concentration) and AUC_{last} (area under the plasma concentration-time curve from time zero to the time of the last measurable plasma concentration). Secondary pharmacokinetic parameters were t_{max} (time of maximum plasma concentration) and $t_{1/2}$ (elimination half-life).

4.5. Statistical methods

The ANOVA model:

$$y_{ijklm} = \mu' + \alpha_i + s_{(j)} + \tau_k + \pi_l + \epsilon_{ijklm}$$

$$(i = f, m; j = 1, \dots, N; k = A, B, C, D; l = 1, 2, 3, 4)$$

was applied to the logarithmically transformed variables AUC_{last} and C_{max} , where μ' denotes the general mean of the transformed variable, α_i the gender effect, s_j the effect of subject j (nested in gender), τ_k the direct effect of treatment k , π_l the effect of period l , ϵ_{ijklm} the random deviation and N the number of subjects included into the analysis.

Table 5: Estimated ratios for AUClast and Cmax with respect to treatment by cohort

Cohort	Treatment Ratio	AUClast Estimate	AUClast 90% confidence interval	
			Lower limit	Upper limit
I	A/D	0.260	0.177	0.381
	B/D	0.455	0.308	0.673
	C/D	0.342	0.231	0.505
II	A/D	0.212	0.093	0.485
	B/D	0.646	0.415	1.007
	C/D	0.528	0.344	0.808
III	A/D	1.372	0.993	1.896
	B/D	0.889	0.580	1.363
	C/D	0.898	0.690	1.168
IV	A/D	14.232	10.833	18.698
	B/D	12.614	9.601	16.572
	C/D	2.915	2.219	3.829
Cohort	Treatment Ratio	Cmax Estimate	Cmax 90% confidence interval	
			Lower limit	Upper limit
I	A/D	0.386	0.247	0.603
	B/D	0.872	0.594	1.280
	C/D	0.550	0.318	0.950
II	A/D	0.340	0.131	0.881
	B/D	1.409	0.829	2.394
	C/D	1.056	0.669	1.666
III	A/D	2.629	1.830	3.776
	B/D	1.571	0.959	2.573
	C/D	1.506	1.080	2.101
IV	A/D	19.467	15.076	25.138
	B/D	18.176	14.155	23.338
	C/D	3.788	2.612	5.494

The *estimated population relative bioavailability* of the test treatment k was defined as $\mu_k/\mu_D = \exp(\tau_k - \tau_D)$, where μ_k (k = A, B, C, D) denotes the population (geometric) means of treatment k for the untransformed variables.

The primary parameters for assessing the relative bioavailability were the area under the concentration-time curve AUC_{last} and C_{max} . The relative

bioavailability was defined as the ratio $\frac{AUC_k}{AUC_D}$, where the subscript k stands for a test formulation and D for the reference formulation (Bonviva® 150 mg in cohorts I to III and Bondronat® 50 mg in cohort IV). The ratios $\frac{C_{max,k}}{C_{max,D}}$ were treated in the same way.

Other pharmacokinetic parameters were regarded as secondary.

Table 6: Doses and dosing conditions in cohorts I to IV

Cohort	Formulation	Treatment	Fasting duration after drug intake
Cohort I	Film coated tablet (1)	A: 30 mg IBN:150 mg SNAC	15 min
	Film coated tablet (1)	B: 30 mg IBN:300 mg SNAC	15 min
	Film coated tablet (1)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort II	Film coated tablet (2)	A: 30 mg IBN:150 mg SNAC	15 min
	Film coated tablet (2)	B: 30 mg IBN:300 mg SNAC	15 min
	Film coated tablet (2)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort III	Beadlets in capsules (3)	A: 30 mg IBN:150 mg SNAC	15 min
	Beadlets in capsules (3)	B: 30 mg IBN:300 mg SNAC	15 min
	Beadlets in capsules (3)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort IV	Beadlets in capsules (3)	A: 30 mg IBN:150 mg SNAC	60 min
	Beadlets in capsules (3)	B: 30 mg IBN:150 mg SNAC	30 min
	Granulate in capsules (4)	C: 30 mg IBN:150 mg SNAC	60 min
	Reference	D: 50 mg IBN	30 min

Table 7: IBN/SNAC formulations

Formulation	Technology	Excipients
1: Film-coated tablets (cohort I)	Aqueous spray granulation in fluid bed dryer; Water sprayed onto powder bed of intragranular materials (including IBN and SNAC)	SNAC, lactose monohydrate, croscarmellose sodium, povidone, sodium stearyl fumarate, hypromellose, macrogol, titanium dioxide
2: Film-coated tablets (cohort II)	Aqueous spray granulation in fluid bed dryer; IBN dissolved in water prior to spray granulating the remaining intragranular materials	SNAC, lactose monohydrate, croscarmellose sodium, povidone, sodium stearyl fumarate, hypromellose, macrogol, titanium dioxide
3: Drug-coated beadlets in hard gelatin capsules (cohorts III and IV)	Spray coating of IBN and SNAC solution in water onto the beadlets in fluid bed dryer	SNAC, microcrystalline cellulose (as beadlets), povidone, talc, calcium silicate; gelatin, titanium dioxide
4: Granules in hard gelatin capsules (cohort IV)	Aqueous spray granulation in fluid bed dryer; Water sprayed onto powder bed of intragranular materials (including IBN and SNAC)	SNAC, croscarmellose sodium, povidone, sodium stearyl fumarate, calcium silicate

4.6. Formulation

IBN was supplied for this study as either oblong, white to off-white film-coated tablets or white hard gelatin capsules. Labeled strength was based on the free acid (e.g., a 30 mg film-coated tablet contains 30 mg ibandronic acid in the form of 33.76 mg ibandronate monosodium salt monohydrate). In addition to the drug substance, each tablet did contain SNAC (sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate) as an excipient (IBN:SNAC ratios of 1:5, 1:10, and 1:20). All formulation principles are summarized in Table 7.

4.7. Dissolution testing

Dissolution testing was performed on each formulation using the flow-through cell apparatus (USP Dissolution Apparatus IV) in media and flow conditions simulating fasted state conditions. In a sequential manner, the media, which was pumped through the cell at 6 mL/min, was changed over from simulated gastric fluid (SGF) to maleate buffer pH 6.5 after 20 min to simulate the transition of the dosage form from the stomach to the small intestine. Collected samples were analyzed for dissolved IBN by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and for SNAC by high-performance liquid chromatography (HPLC).

4.8. Chemical imaging characterization of the formulation

Near infrared (NIR) chemical imaging was employed to examine distribution of IBN and SNAC in the different formulations. NIR chemical images were collected for each sample using the Sapphire™ NIR chemical imaging system (Malvern Instruments Inc., Columbia, MD) at a wavelength range of 1850 to 2200 nm. The image spatial resolution was 20 µm/pixel with the field of view of 5.1 × 6.4 mm. To eliminate the spectra variation due to physical interferences and to enhance spectra characteristics, all sample spectra were pre-processed using standard normal variate and Savitsky-Golay first derivative (7,2,1).

A discriminant partial least square (PLS) model was developed using pure component spectra of IBN, SNAC, and another main excipient, polyvinylpyrrolidone (PVP). The statistical parameters of each SNAC PLS score image were calculated as a measure of the degree of SNAC distribution in the formulation. Red-green-blue (RGB) images simultaneously mapping IBN, SNAC and PVP, respectively, were generated to get additional information on the distribution of the three components in the capsule formulations tested in cohorts III (drug-coated beadlets) and IV (granulate). Principle component analysis (PCA) was performed on the sample spectra to determine whether the physical and chemical properties of beadlet and granule formulations are intrinsically distinctive.

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(54) **FAST-ACTING PLANT-BASED MEDICINAL COMPOUNDS AND NUTRITIONAL SUPPLEMENTS**

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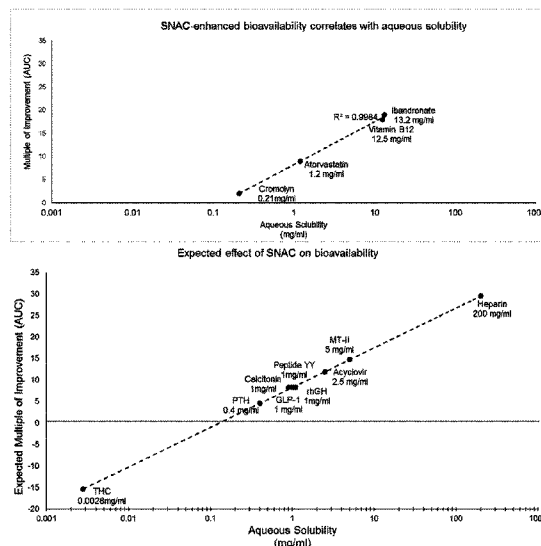
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(57) **ABSTRACT**

Plant-based medicinal compounds or nutritional supple-
ments in various carrier combinations are described. The
carriers can include N-acylated fatty amino acids, penetra-
tion enhancers, and/or various other beneficial carriers. The
plant-based composition/carrier combinations can create
administration benefits.

25 Claims, 15 Drawing Sheets



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FIG. 1A

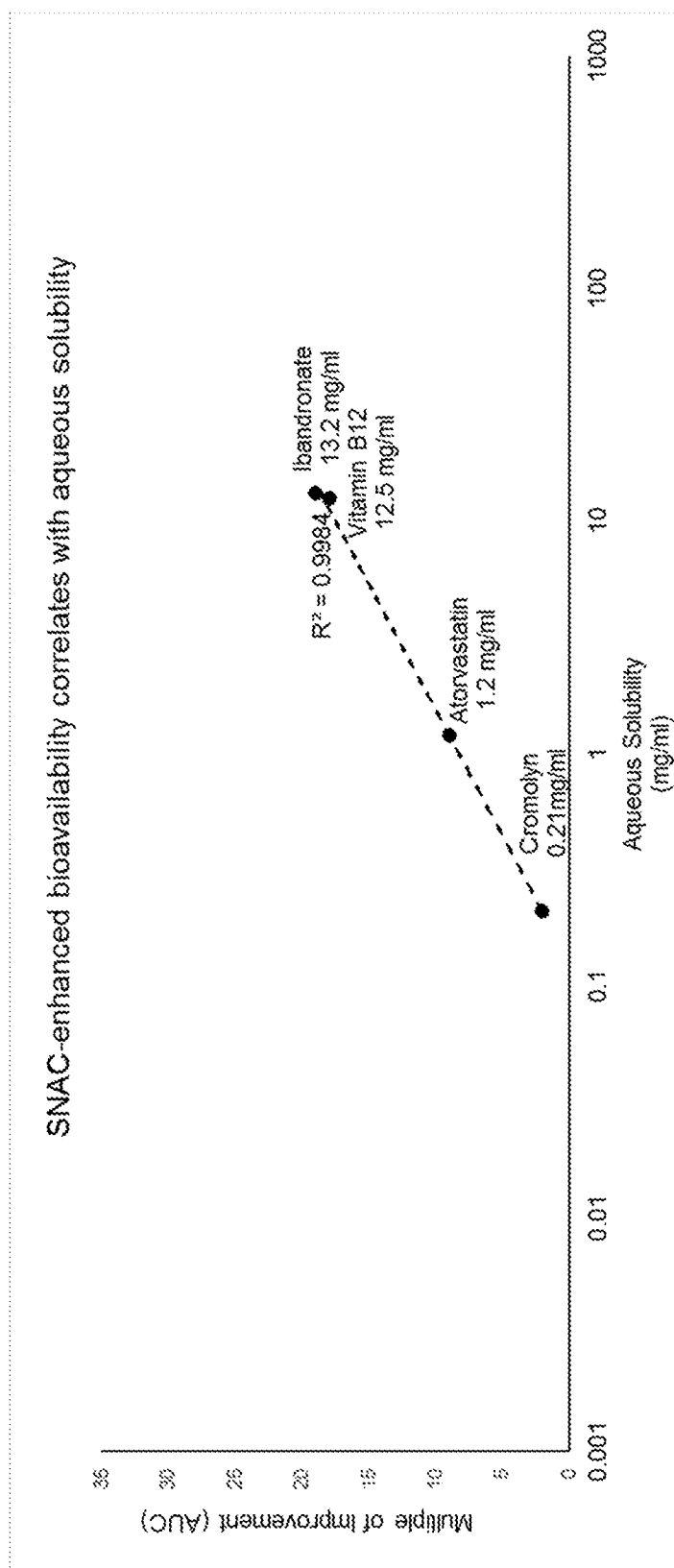


FIG. 1B

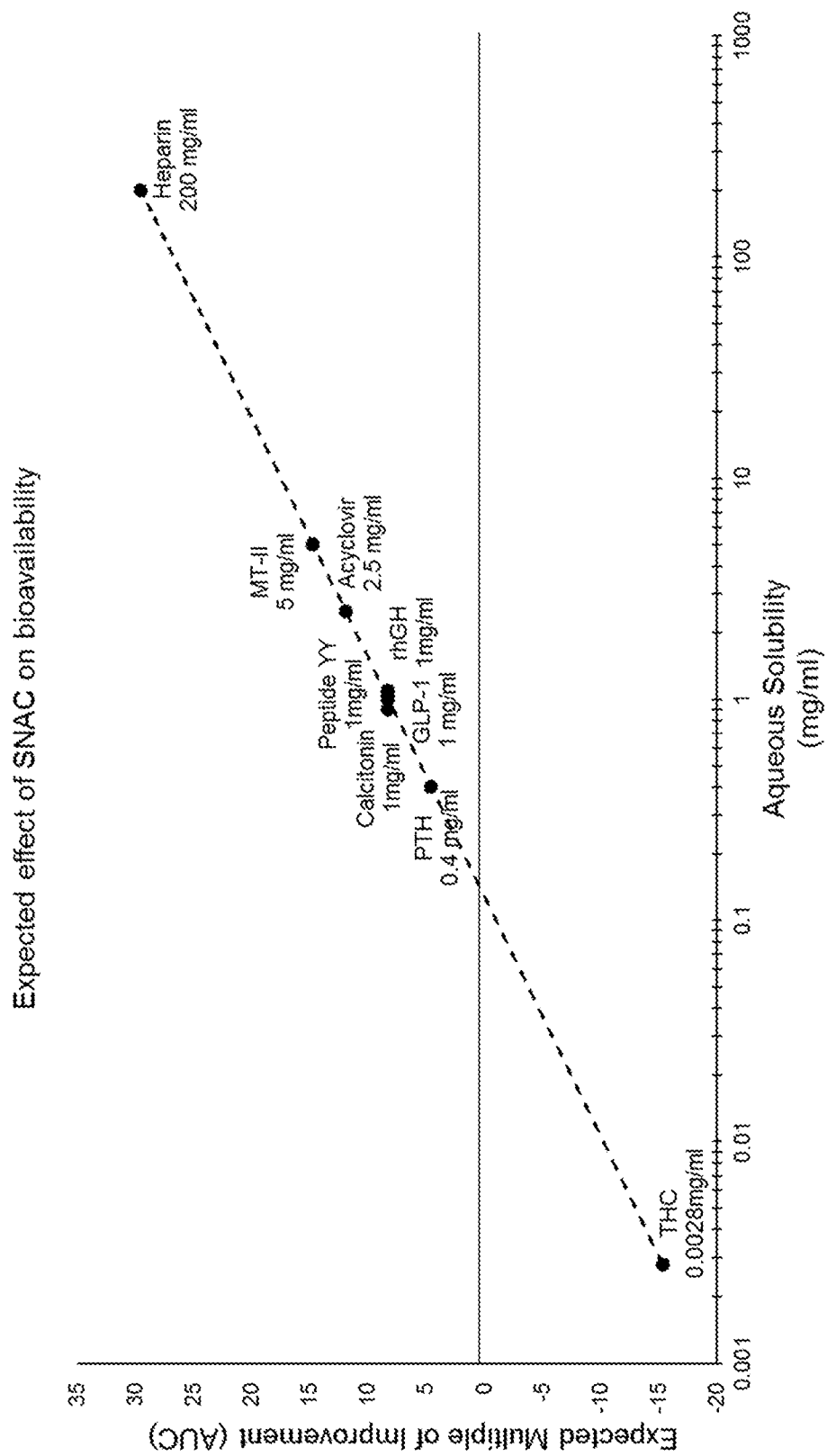


FIG. 2

Name	Structure	Name	Structure
CBC	Chemical structure of CBC (Cannabichrome): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 3-methyl-3-pentenyl group.	CBN	Chemical structure of CBN (Cannabinol): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.
CBCV	Chemical structure of CBCV (Cannabichromene varietal): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 3-methyl-3-pentenyl group.	CBNV	Chemical structure of CBNV (Cannabinol varietal): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.
CBD	Chemical structure of CBD (Cannabidiol): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.	CBO	Chemical structure of CBO (Cannabiole): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.
CBDA	Chemical structure of CBDA (Cannabidiolic acid): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.	THC	Chemical structure of THC (Tetrahydrocannabinol): A tricyclic cannabinoid with a chromene core. It features a methyl group at C-1, a hydroxyl group at C-2, and a pentyl group at C-3. The C-4 position is substituted with a 1-methyl-1-propenyl group.

FIG. 2 cont'd

CBDV	Chemical structure of CBDV (Cannabidiol varin). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond and a hydroxyl group.	THCA	Chemical structure of THCA (Tetrahydrocannabinolic acid). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond, a hydroxyl group, and a carboxylic acid group.
CBG	Chemical structure of CBG (Cannabigerol). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond and a hydroxyl group.	THCV	Chemical structure of THCV (Tetrahydrocannabivarin). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond, a hydroxyl group, and a carboxylic acid group.
CBGV	Chemical structure of CBGV (Cannabigerol varin). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond and a hydroxyl group.	THCVA	Chemical structure of THCVA (Tetrahydrocannabivarinic acid). It features a cyclohexene ring with a methyl group and a propyl group, and a side chain containing a double bond, a hydroxyl group, and a carboxylic acid group.
CBL	Chemical structure of CBL (Cannabicyclol). It features a bicyclic system with a methyl group and a propyl group, and a side chain containing a double bond and a hydroxyl group.		

FIG. 3

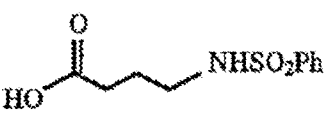
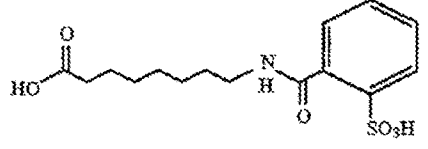
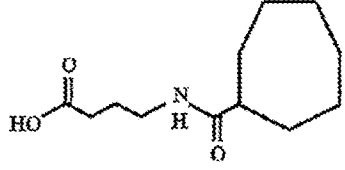
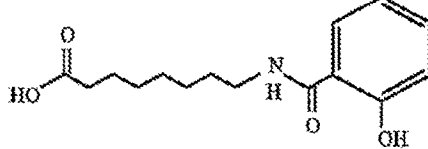
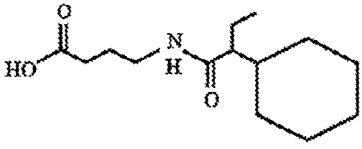
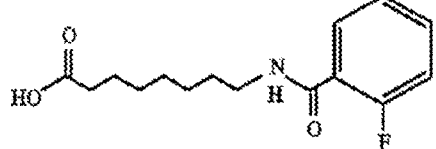
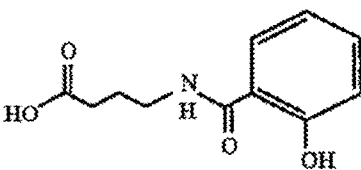
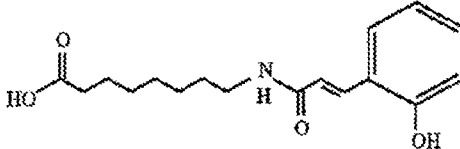
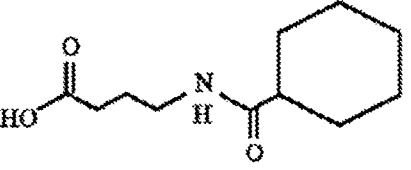
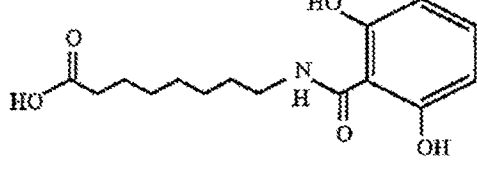
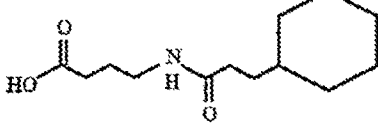
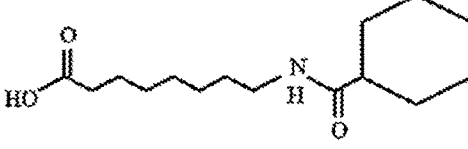
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III		XX	
IV		XXI	
V		XXII	
VI		XXIII	

FIG. 3 cont'd

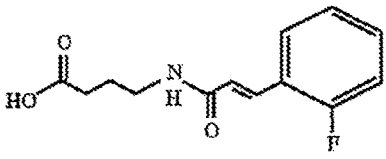
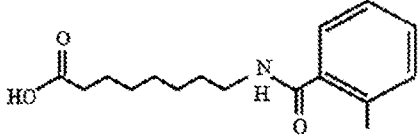
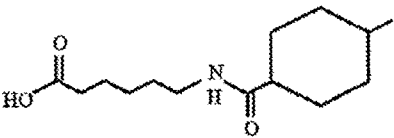
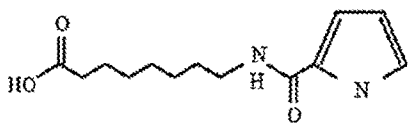
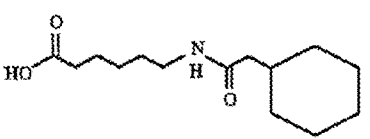
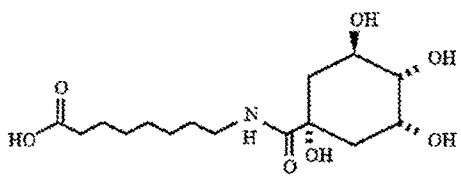
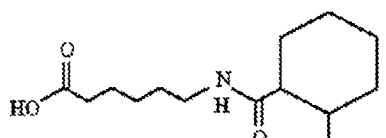
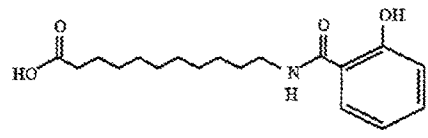
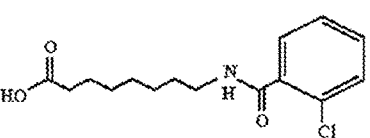
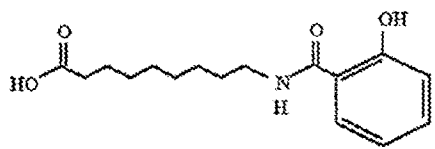
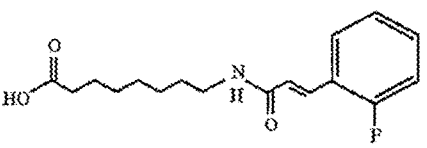
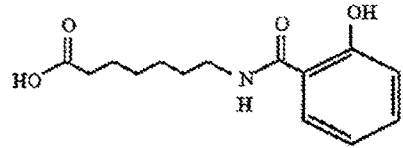
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XII		XXIX	

FIG. 3 cont'd

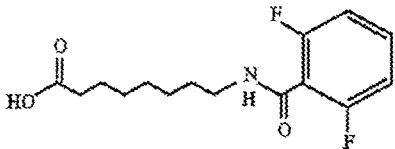
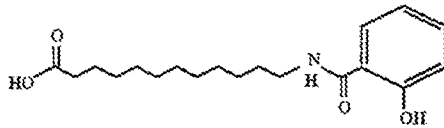
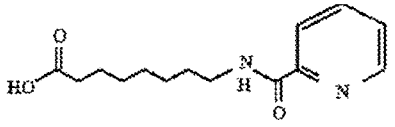
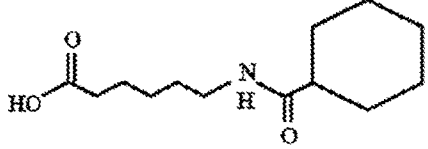
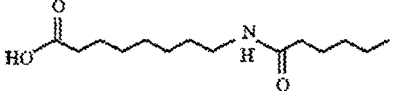
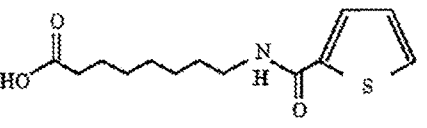
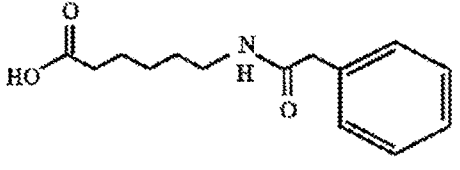
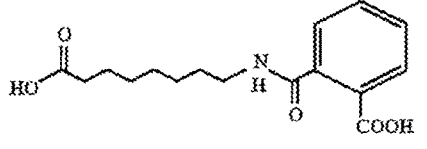
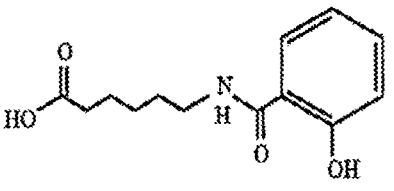
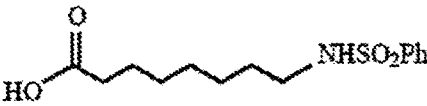
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FIG. 4

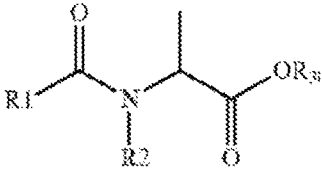
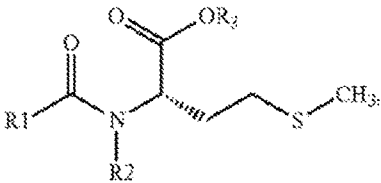
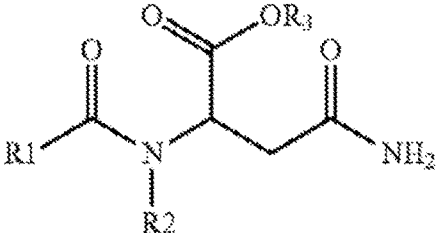
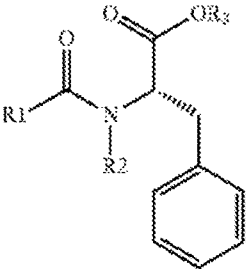
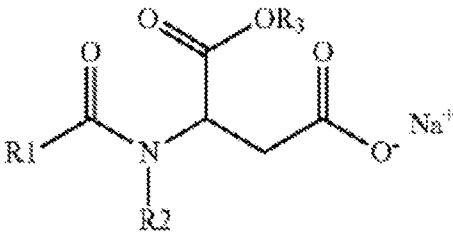
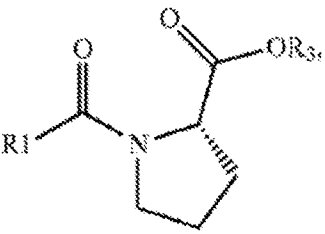
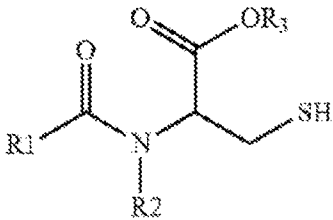
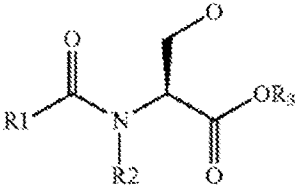
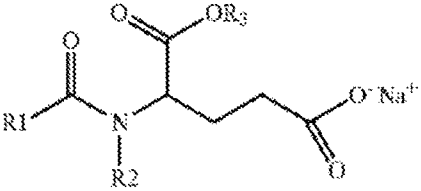
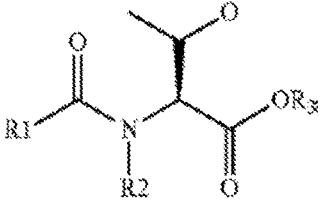
#	Structure	#	Structure
a		j	
b		k	
c		l	
d		m	
e		n	

FIG. 4 cont'd

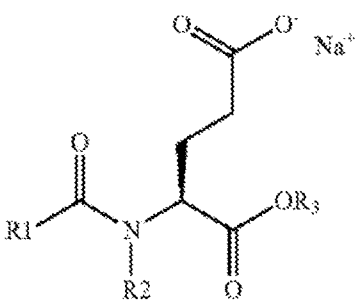
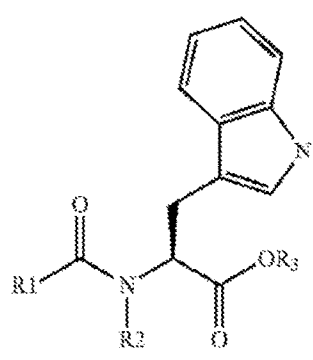
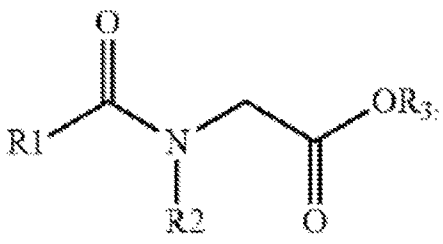
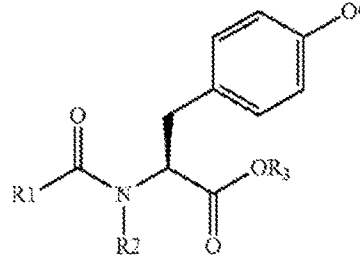
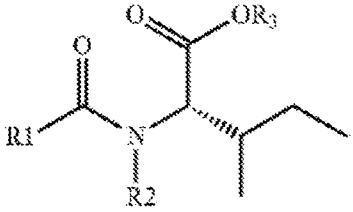
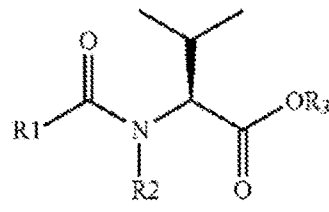
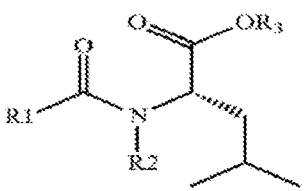
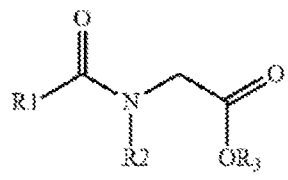
f		o	
g		p	
h		q	
i		r	

FIG. 5A

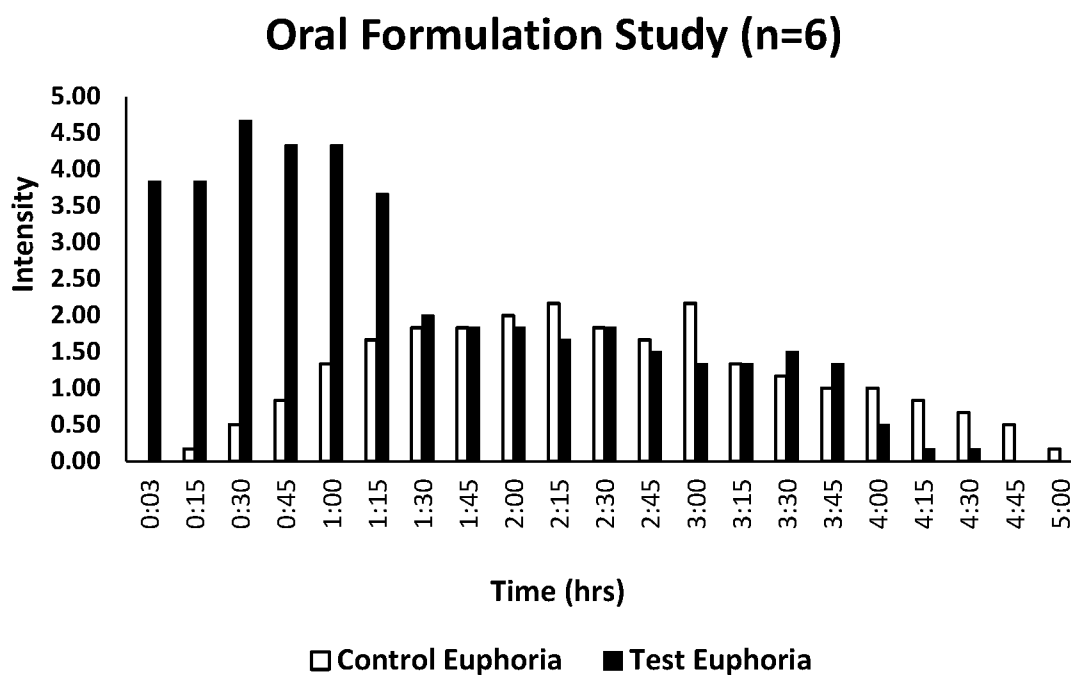


FIG. 5B

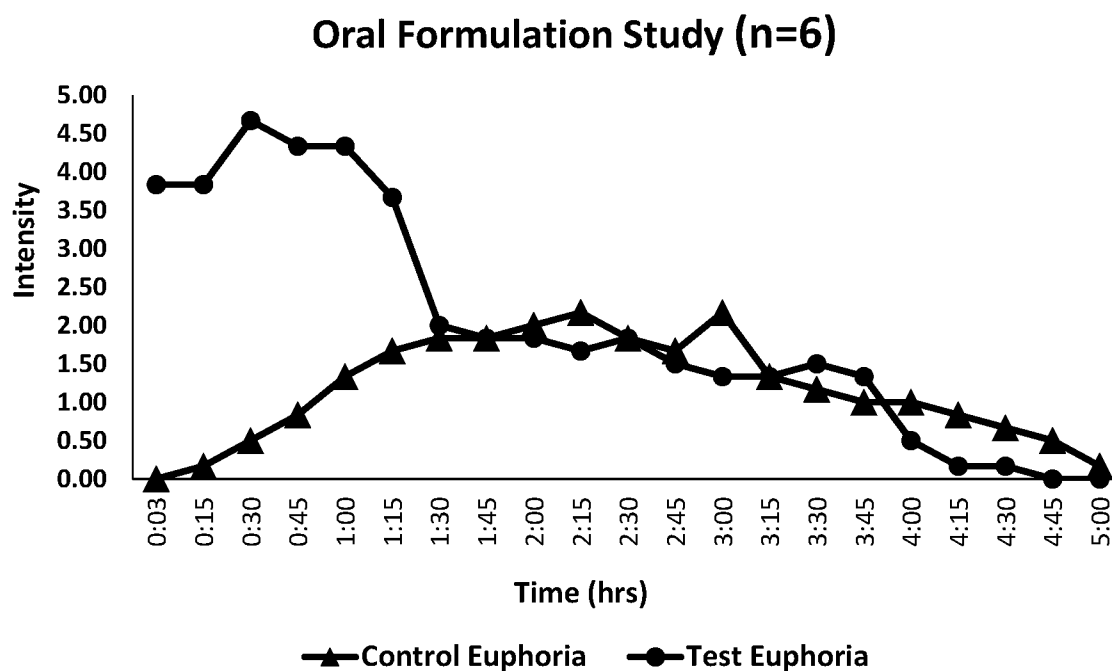


FIG. 6A

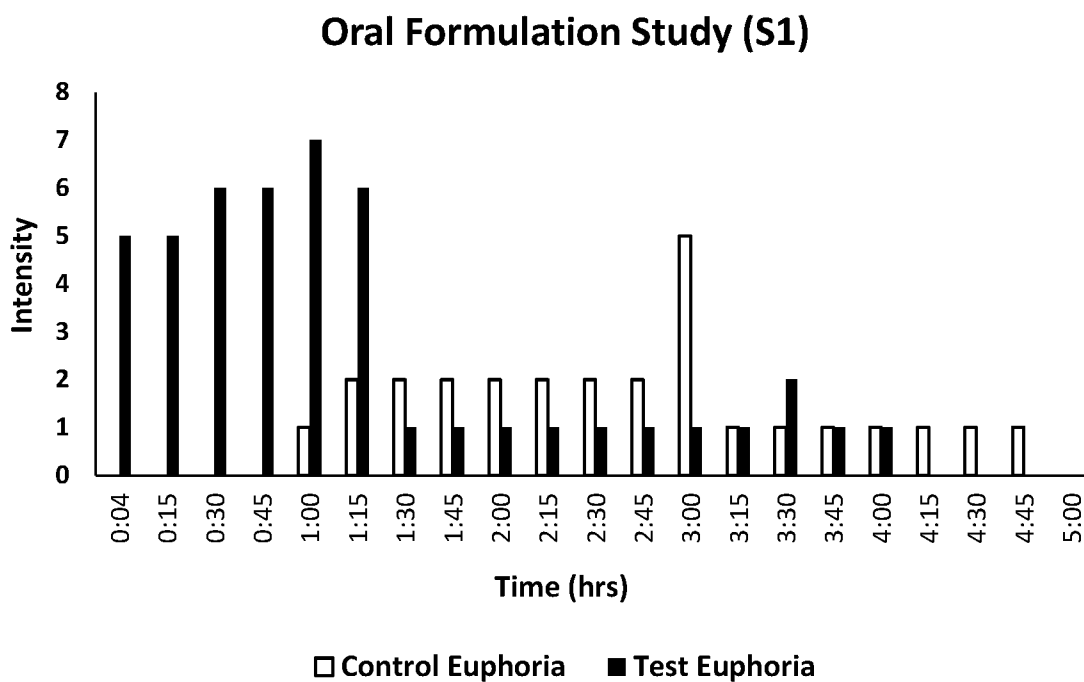


FIG. 6B

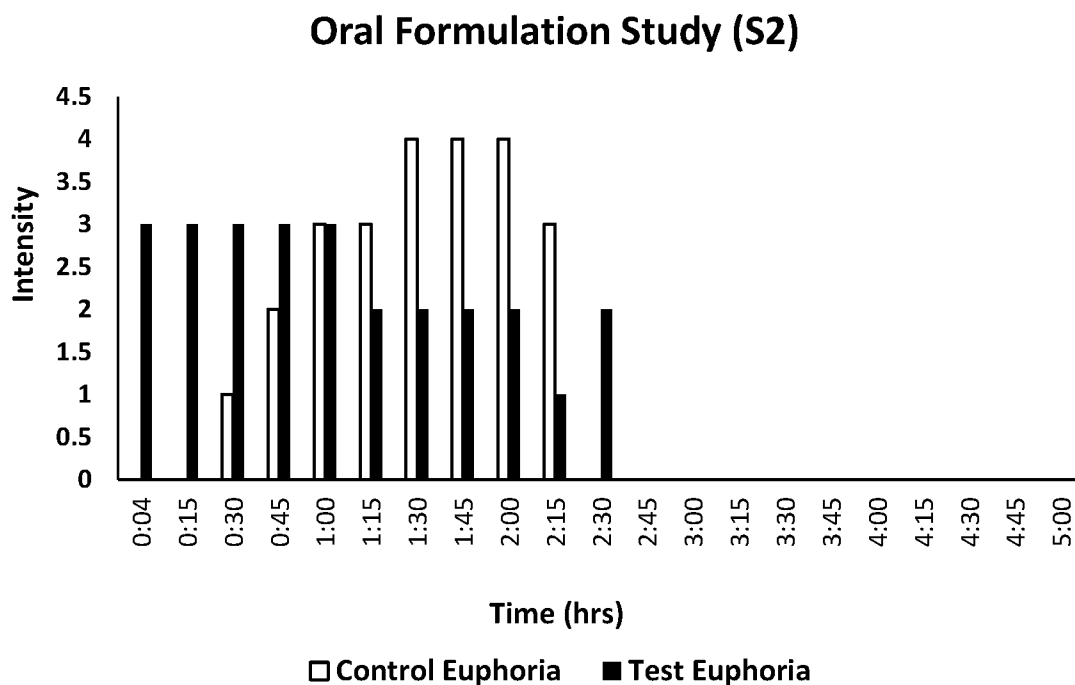


FIG. 6C

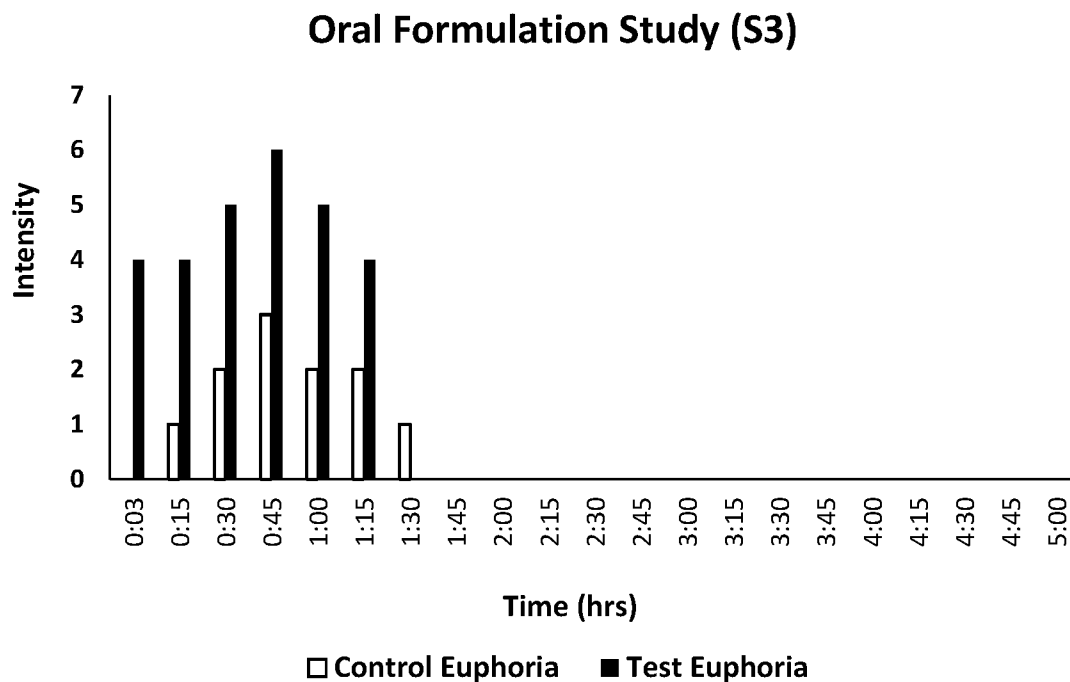


FIG. 6D

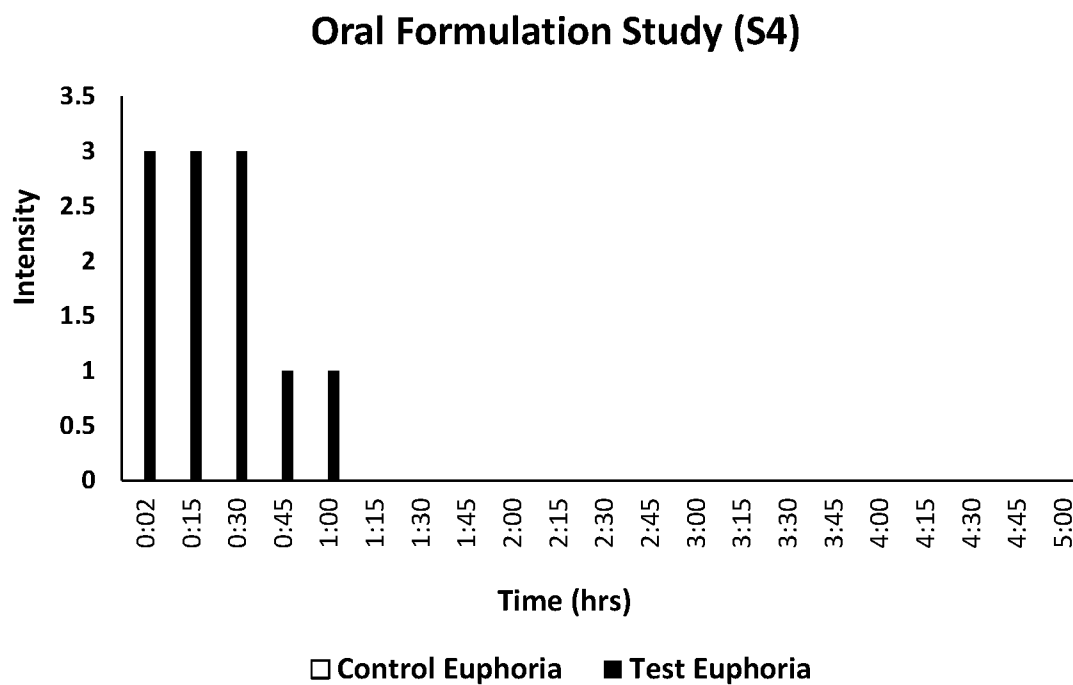


FIG. 6E

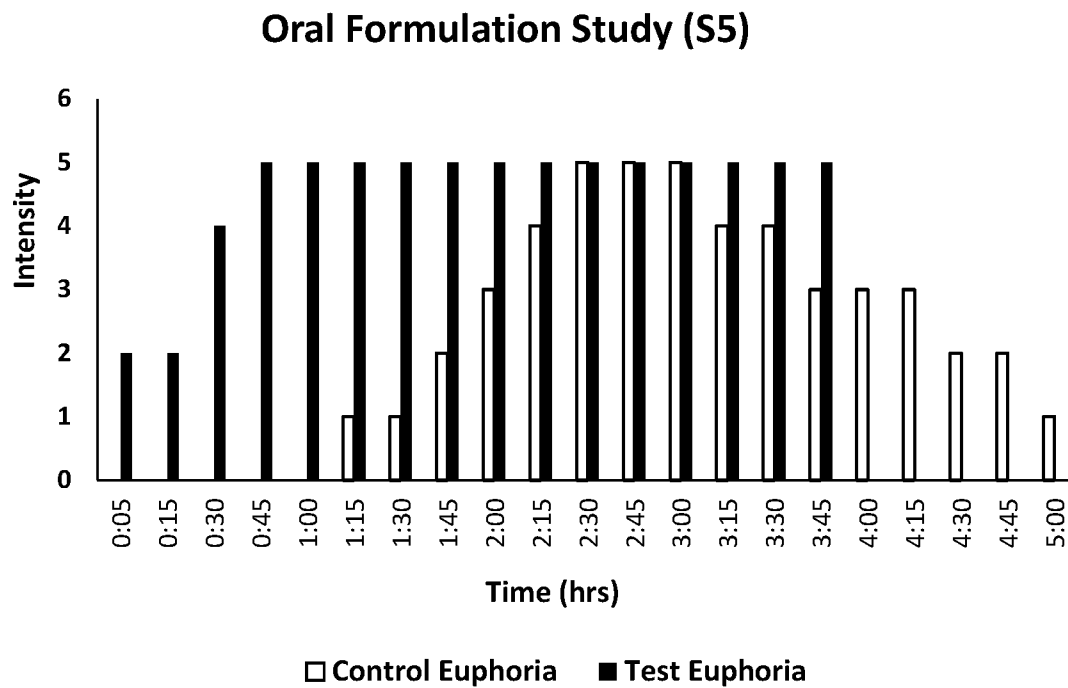


FIG. 6F

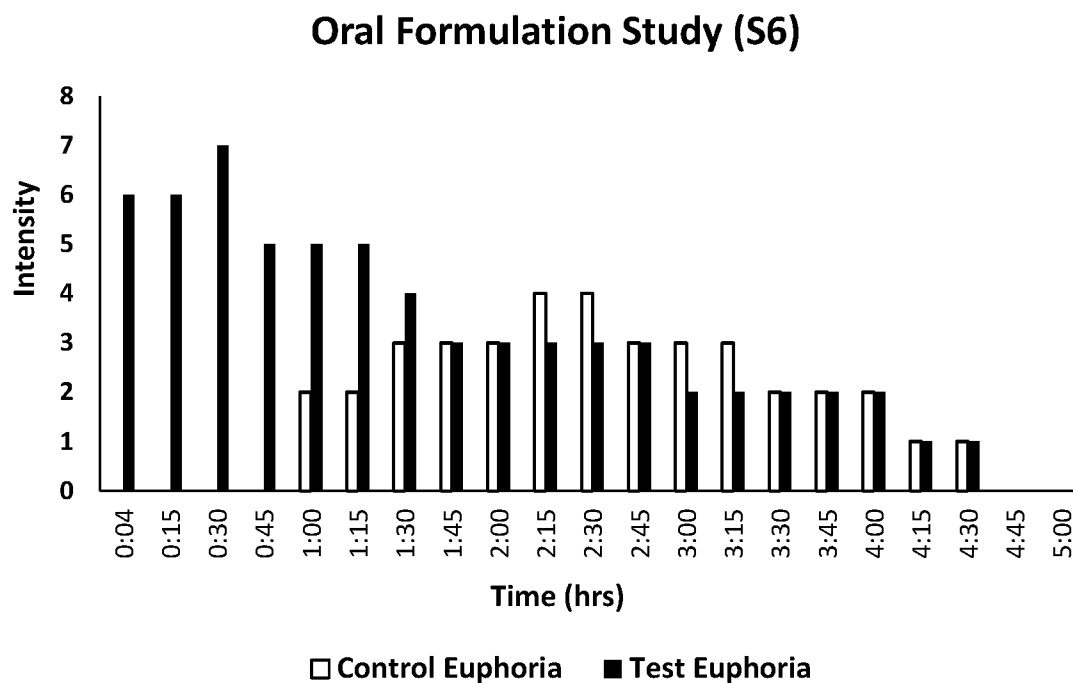


FIG. 7

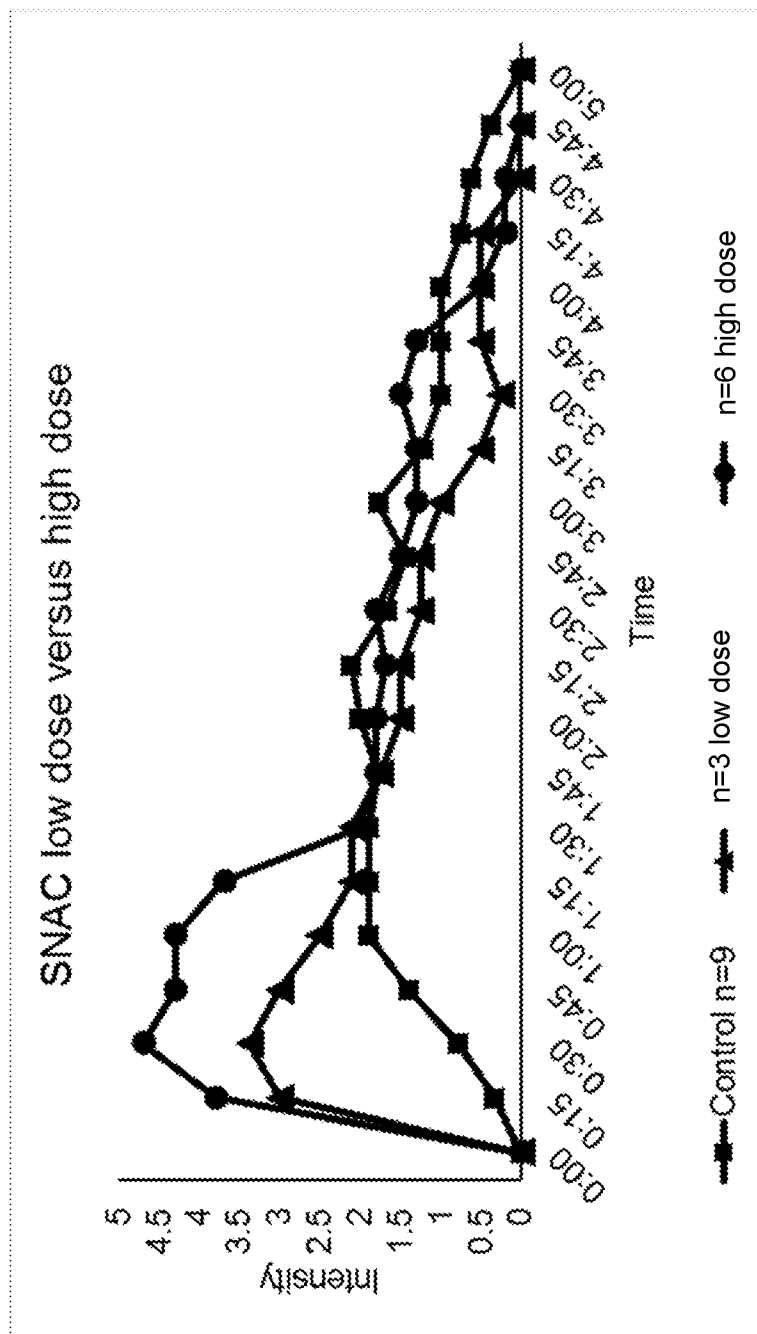
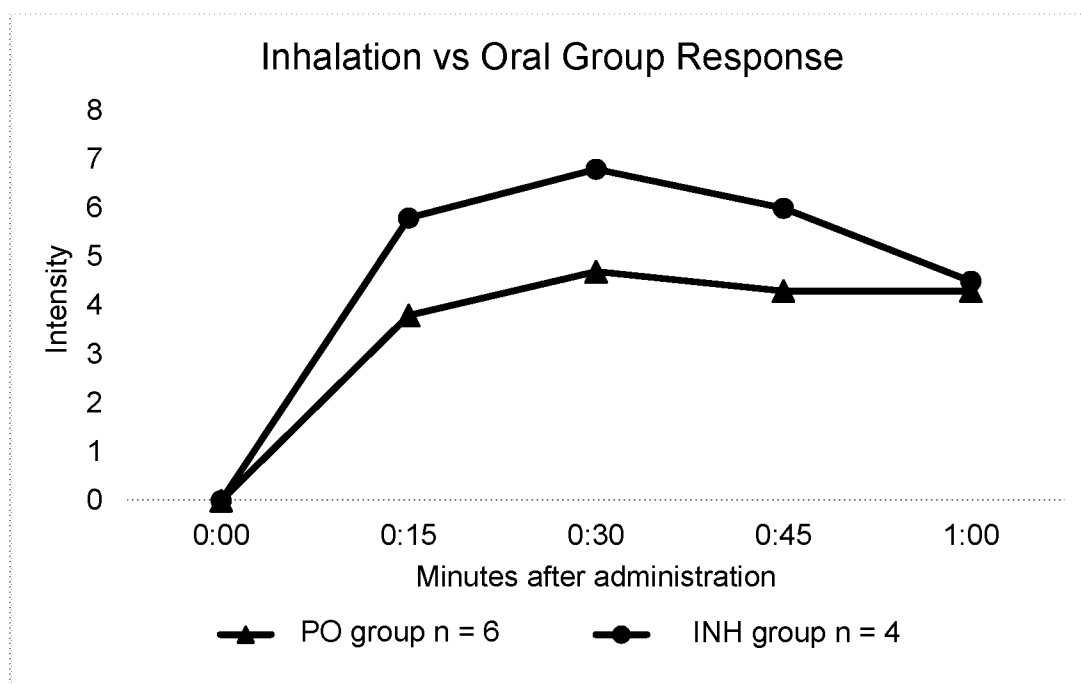
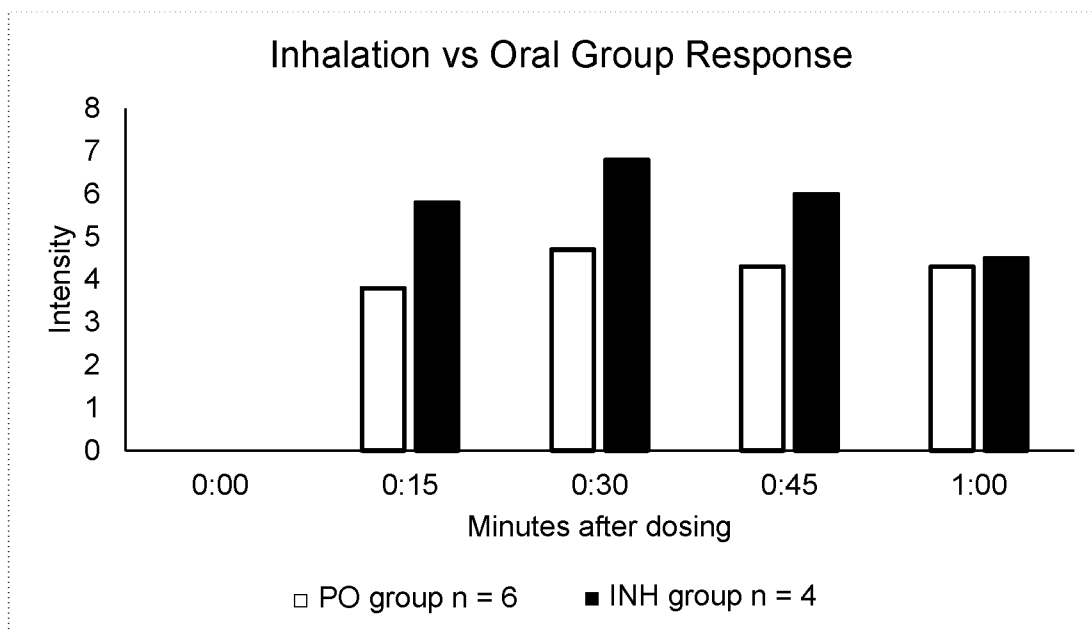


FIG. 8



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FAST-ACTING PLANT-BASED MEDICINAL COMPOUNDS AND NUTRITIONAL SUPPLEMENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This Application is a US National Phase Patent Application based on PCT/US2017/028953, filed Apr. 21, 2017, which claims priority to U.S. Provisional Patent Application No. 62/326,490 filed Apr. 22, 2016 and to U.S. Provisional Patent Application No. 62/429,544 filed Dec. 2, 2016, the entire contents of each of which are incorporated by reference herein.

FIELD OF THE DISCLOSURE

The current disclosure provides fast-acting plant-based medicinal compounds or nutritional supplements in various carrier combinations. The carriers can include N-acylated fatty amino acids, penetration enhancers, and/or various other beneficial carriers. The plant-based composition/carrier combinations can create administration benefits following oral administration.

BACKGROUND OF THE DISCLOSURE

Historically, the plant world has been the most important source of medicinal agents for the treatment of human and animal disease, and for use as preventative agents in maintaining good health. However, for at least the last 150 years, Western medicine has been dominated by synthetic chemical agents.

It is now being increasingly recognized, however, that many plants and plant extracts are highly effective agents for the prevention and treatment of disease. A single plant can possess a large number of pharmaceutically active agents, and extracts obtained therefrom can exert their activities on a variety of physiologic processes, increasing the range of the desired therapeutic effect.

As one example, U.S. Publication No. 2015/0050373 describes use of plants from the *Calophyllum* genus to treat metabolic disorders. *Calophyllum* is a flowering plant genus of around 180-200 species of tropical evergreen trees. The *Calophyllum* genus includes four subcategories: *Calophyllum brasiliense*, *Calophyllum caldonicum*, *Calophyllum inophyllum* and *Calophyllum soulattri*. *Calophyllum inophyllum*, is a medium to large sized evergreen tree that averages 25-65 feet in height. Different medicinal uses of this plant have been reported in the literature, for example, decoction of the bark of this plant treats internal hemorrhages. The oil extracted from *Calophyllum inophyllum* seeds is used to treat rheumatoid arthritis or joint disorders; itching; eczema; pimples appearing on head; eye diseases; and kidney failure.

U.S. Publication No. 2014/0193345 describes use of the plants *Uncaria tomentosa*, *Thymus vulgaris*, *Matricaria recutita*, *Salix alba*, *Calendula officinalis*, *Usnea barbata*, *Ligusticum porteri-oshia*, *Gaultheria procumbens*, *Camellia sinensis*, *Vaccinium myrtillus*, *Melissa officinalis*, *Allium sativum*, *Camellia sinensis* and *Krameria triandra* to treat mucosal lesions.

U.S. Publication No. 2010/0068297 describes use of the plants *Punica granatum*, *Viburnum plicatum*, *Camellia sinensis*, and *Acer* spp. as antimicrobials.

Numerous medical uses have also been identified for the *cannabis* plant. For example, delta-9-tetrahydrocannabinol

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(THC, also referred to as Dronabinol), an extract of the *cannabis* plant has been formulated in sesame oil for oral delivery. THC exhibits complex effects on the central nervous system (CNS), including central sympathomimetic activity. THC has been shown to have a marked appetite stimulant effect and has been used in the treatment of AIDS-related anorexia. THC demonstrates effects on appetite, mood, cognition, memory and perception. Furthermore, the drug has anti-emetic properties and is used to control nausea and vomiting associated with cancer chemotherapy. These effects appear to be dose related.

THC's efficacy in pain treatment has been described in Pharm. J. 259, 104, 1997 and in Pharm. Sci. 3, 546, 1997. Nabilone, a synthetic cannabinoid has also been reported to be an anti-emetic and anxiolytic, and also useful for treating pain of various etiologies such as multiple sclerosis (MS), peripheral neuropathy and spinal injuries (Lancet, 1995, 345, 579, Pharm. J. 259, 104, 1997; Baker & Pryce, Expert Opin Investig Drugs. 2003 April; 12(4):561-7). THC has also been reported to be useful in the treatment of AIDS (J. Pain. Symptom Manage. 1995, 10, 89-97) when given orally.

Another cannabinoid with well-documented health benefits is cannabidiol (CBD). In contrast to THC, CBD does not exert psychoactive effects. CBD is reported to have antidepressant (Zanelati T, et al. Journal of Pharmacology. 2010. 159(1):122-8;), anti-anxiety (Resstel B M, et al. Br J Pharmacol. 2009. 156(1):181-188), anti-inflammatory (Vuolo F, et al. Mediators of Inflammation. 2015. 538670), and neuroprotective effects (Campos A C, et al. Pharmacol Res. 2016. 112:119-127).

Additional uses for the *cannabis* plant include treatment of addiction (De Vries, et al., Psychopharmacology (Berl). 2003 July; 168(1-2):164-9); ADHD (O'Connell and Ché, Harm Reduction Journal. 2007; 4:16); alcoholism (Basavarajappa & Hungund, Alcohol. 2005 January-February; 40(1):15-24); Alzheimer's disease (Eubanks et al., Mol Pharm. 2006 November-December; 3(6):773-7); amyotrophic lateral sclerosis (ALS) (Raman et al., Amyotroph Lateral Scler Other Motor Neuron Disord. 2004 March; 5(1):33-9); anxiety (The British Journal of Psychiatry February 2001, 178 (2) 107-115); asthma (Tashkin et al., American Review of Respiratory Disease, 1975; 112, 377); autoimmune diseases (Lyman et al., J Neuroimmunol. 1989 June; 23(1):73-81); bacterial infections (Nissen et al., Fito-terapia. 2010 July; 81(5):413-9); bone loss (Bab et al., Ann Med. 2009; 41(8):560-7); brain injury/stroke (Shohami et al., Br J Pharmacol. 2011 August; 163(7):1402-10); cancer (Guindon & Hohmann, Br J Pharmacol. 2011 August; 163 (7):1447-63); heart disease (Walsh et al., Br J Pharmacol. 2010 July; 160(5):1234-42); Huntington's disease (Lastres-Becker et al., J Neurochem. 2003 March; 84(5):1097-109); inflammation (AAPS J. 2009 March; 11(1): 109-119); Parkinson's disease (Sieradzan et al., Neurology. 2001 Dec. 11; 57(11):2108-11); and psoriasis (Trends Pharmacol Sci. 2009 August; 30(8): 411-420).

Additional documented uses for the *cannabis* plant include treating acquired hypothyroidism, acute gastritis, agoraphobia, ankyloses, arthritis, Asperger's syndrome, atherosclerosis, autism, bipolar disorder, blood disorders, cachexia, carpal tunnel syndrome, cerebral palsy, cervical disk disease, cervicobrachial syndrome, chronic fatigue syndrome, chronic pain, cluster headache, conjunctivitis, Crohn's disease, cystic fibrosis, depression, dermatitis, diabetes, dystonia, eating disorders, eczema, epilepsy, fever, fibromyalgia, flu, fungal infection, gastrointestinal disorders, glaucoma, glioma, Grave's disease, hepatitis, herpes,

hypertension, impotence, incontinence, infant mortality, inflammatory bowel disease (IBD), insomnia, liver fibrosis, mad cow disease, menopause, migraine headaches, motion sickness, MRSA, muscular dystrophy, nail patella syndrome, neuroinflammation, nicotine addiction, obesity, obsessive compulsive disorder (OCD), pancreatitis, panic disorder, periodontal disease, phantom limb pain, poison ivy allergy, premenstrual syndrome (PMS), proximal myotonic myopathy, post-traumatic stress disorder (PTSD), Raynaud's disease, restless leg syndrome, schizophrenia, scleroderma, septic shock, shingles herpes zoster), sickle cell disease, seizures, sleep apnea, sleep disorders, stress, stuttering, temporomandibular joint disorder (TMJ), tension headaches, tinnitus, Tourette's syndrome, traumatic memories, wasting syndrome, and withdrawal.

Despite the numerous benefits associated with plant-based compounds and nutritional supplements, when administered in oral form, their onset of action can be slow, which can detract from their usefulness in some instances. For example, after oral administration, THC has an onset of action of fifteen minutes at the very earliest to 1.5 hours and a peak effect at 2-4 hours. The duration of action for psychoactive effects is 4-6 hours, but the appetite stimulant effect may continue for 24 hours or longer after administration. THC is almost completely absorbed (90-95%) after single oral doses. However, due to a combined effect of first pass hepatic metabolism and poor aqueous solubility (THC water solubility is 2.8 mg/L), only 10-20% of the administered dose reaches the systemic circulation. Therefore, oral consumption of *cannabis* is characterized by low bioavailability of cannabinoids, and slow onset of action. Thus, as this one example provides, there is room for improvement in the oral administration of plant-based compounds and nutritional supplements.

SUMMARY OF THE DISCLOSURE

The current disclosure provides fast-acting plant-based medicinal compounds and nutritional supplements (collectively, plant-based compositions) formulated for oral delivery. By providing fast-acting delivery, physiological benefits are observed earlier increasing the usefulness of these compounds.

The disclosed fast-acting plant-based compositions can create various administration benefits in providing therapeutically effective amounts in a variety of conditions. Exemplary administration benefits include increased absorption, increased bioavailability, faster onset of action, higher peak concentrations, faster time to peak concentrations, shorter duration of action, increased subjective therapeutic efficacy, and increased objective therapeutic efficacy.

The fast-acting nature of the plant-based compositions is created by including one or more N-acylated fatty amino acids, absorption enhancing agents, and/or various other beneficial carriers, such as surfactants, detergents, azones, pyrrolidones, glycols and bile salts in an oral formulation. In particular embodiments, N-acylated fatty amino acids can be linear, branched, cyclic, bicyclic, or aromatic including, for example, 1-50 carbon atoms in an oral formulation. That use of N-acylated fatty amino acids could provide a fast-acting benefit with a plant-based composition was unexpected given particular aspects of plant-based components described further herein. For example, the ability of N-acylated fatty amino acids to increase absorption of compounds is proportional to the water-solubility of a compound. Many

plant-based compounds are not water-soluble and would not have been expected to be affected by the presence of an N-acylated fatty amino acid.

In particular embodiments, the plant-based compositions include *Calophyllum brasiliense*, *Calophyllum caledonicum*, *Calophyllum inophyllum*, *Calophyllum soulattri*, *Uncaria tomentosa*, *Thymus vulgaris*, *Matricaria recutita*, *Salix alba*, *Calendula officinalis*, *Usnea barbata*, *Ligusticum porterii-oshia*, *Gaultheria procumbens*, *Camellia sinensis*, *Vaccinium myrtillus*, *Melissa officinalis*, *Allium sativum*, *Camellia sinensis*, *Krameria triandra*, *Punica granatum*, *Viburnum plicatum*, *Nicotiana tabacum*, *Duboisia hopwoodii*, *Asclepias syriaca*, *Curcuma longa*, *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis*, and *Acer* spp., or an extract thereof. In particular embodiments, the plant-based compositions include the *cannabis* plant, or an extract thereof.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A and 1B show an established correlation between water-solubility and the ability of SNAC to improve a molecule's absorption. FIG. 1A shows the multiple of improvement from SNAC plotted for cromolyn, vitamin B12, atorvastatin, and ibandronate, along with the aqueous solubility of each molecule. The plotted data shows a striking fit to a logarithmic trendline ($R^2=0.998$), indicating a logarithmic relationship between the aqueous solubility of each and the extent to which SNAC improves absorption. FIG. 1B plots the aqueous solubility of heparin, acyclovir, rhGH, PTH, MT-II, GLP-1, calcitonin, yy peptide, and THC according to the logarithmic trendline derived from FIG. 1A.

FIG. 2 provides exemplary cannabinoid structures.

FIG. 3 provides modified amino acids of compounds I-XXXV.

FIG. 4 provides fatty acid amino acids of formula (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), (p), (q), and (r), wherein R1 is an alkyl group including 5 to 19 carbon atoms, R2 is H (i.e. hydrogen) or CH3 (i.e. methyl group), and R3 is H; or a salt or the free acid form thereof.

FIGS. 5A and 5B provide the average results of the study comparing onset and duration of action of orally administered *cannabis*/N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, "test") formulation and *cannabis* (without SNAC, "control") formulation.

FIGS. 6A-6F provide the results for each individual participant in the study comparing onset and duration of action of orally administered *cannabis*/N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, "test") formulation and *cannabis* (without SNAC, "control") formulation.

FIG. 7 shows a comparison of intensity, duration and onset of action of orally administered *cannabis* formulations with a high SNAC dose (200 mg, "high dose"), a low SNAC dose (100 mg, "low dose") and no SNAC ("control").

FIG. 8 shows intensity, duration and onset of action of *cannabis* formulated with SNAC administered orally ("PO") compared to *cannabis* administered by inhalation ("INH").

DETAILED DESCRIPTION

Despite the numerous benefits associated with plant-based compounds and nutritional supplements, when administered in oral form, their onset of action can be slow, which can detract from their usefulness in some instances. For example, after oral administration, THC has an onset of action of fifteen minutes at the very earliest to 1.5 hours and a peak effect at 2-4 hours. The duration of action for

psychoactive effects is 4-6 hours, but the appetite stimulant effect may continue for 24 hours or longer after administration. THC is almost completely absorbed (90-95%) after single oral doses. However, due to a combined effect of first pass hepatic metabolism and poor aqueous solubility (THC water solubility is 2.8 mg/L) only 10-20% of the administered dose reaches the systemic circulation. Therefore, oral consumption of *cannabis* is characterized by low bioavailability of cannabinoids, and slow onset of action. Thus, as this one example provides, there is room for improvement in the oral administration of plant-based compounds and nutritional supplements.

The current disclosure provides fast-acting plant-based medicinal compounds and nutritional supplements (collectively, plant-based compositions) formulated for oral delivery. By providing fast-acting delivery, physiological benefits are observed earlier increasing the usefulness of these compounds.

The disclosed fast-acting plant-based compositions can create various administration benefits in providing therapeutically effective amounts in a variety of conditions. Exemplary administration benefits include increased absorption, increased bioavailability, faster onset of action, higher peak concentrations, faster time to peak concentrations, shorter duration of action, increased subjective therapeutic efficacy, and increased objective therapeutic efficacy.

The fast-acting nature of the plant-based compositions is created by including one or more N-acylated fatty amino acids, absorption enhancing agents, and/or various other beneficial carriers, such as surfactants, detergents, azones, pyrrolidones, glycols and bile salts in an oral formulation. In particular embodiments, N-acylated fatty amino acids can be linear, branched, cyclic, bicyclic, or aromatic including, for example, 1-50 carbon atoms in an oral formulation. That use of N-acylated fatty amino acids could provide a fast-acting benefit with a plant-based composition was unexpected given particular aspects of plant-based components described further herein. For example, the ability of N-acylated fatty amino acids to increase absorption of compounds is proportional to the water-solubility of a compound. Many plant-based compounds are not water-soluble and would not have been expected to be affected by the presence of an N-acylated fatty amino acid.

Molecules that have been shown to have improved absorption when co-administered with an N-acylated fatty amino acid (e.g., SNAC) include water-soluble molecules such as cromolyn, vitamin B12, atorvastatin, ibandronate, heparin, acyclovir, recombinant human growth hormone (rhGH), parathyroid hormone 1-34 (PTH 1-34), α -melanotropin (MT-II), GLP-1, calcitonin, and peptide yy.

FIG. 1A shows an established correlation between water-solubility and the ability of SNAC to improve a molecule's absorption. For cromolyn, vitamin B12, atorvastatin, and ibandronate, published results include area under the curve (AUC), which is calculated from a time-course of plasma levels. To quantify the effect of co-administration with SNAC, a multiple of improvement can be calculated by dividing the AUC for a molecule with SNAC by the AUC for the molecule without SNAC. FIG. 1A shows the multiple of improvement from SNAC plotted for cromolyn, vitamin B12, atorvastatin, and ibandronate, along with the aqueous solubility of each molecule. The plotted data shows a striking fit to a logarithmic trendline ($R^2=0.998$), indicating a logarithmic relationship between the aqueous solubility of each and the extent to which SNAC improves its absorption.

Heparin, acyclovir, rhGH, PTH, MT-II, GLP-1, calcitonin, and yy peptide are other molecules have been shown

to have SNAC-improved absorption, as demonstrated by Cmax (maximum drug plasma level) and/or Tmax (the time taken to reach maximum drug plasma level). As shown in FIG. 1B, each of these molecules has an aqueous solubility of more than 0.15 mg/ml, and therefore, the model accurately predicts that SNAC can improve their absorption. This result demonstrates that a SNAC-based absorption improvement correlates with a molecule's aqueous solubility. FIG. 1B further plots the aqueous solubility of THC (0.0028 mg/ml) to the logarithmic trendline and SNAC's predicted effect based on the same. Based at least on the foregoing, the results described herein were unexpected and would not have been reasonably expected by those of ordinary skill in the art.

Aspects of the disclosure are now described in more detail.

The current disclosure provides fast-acting plant-based compositions including vegetable matter and a carrier as an oral formulation. Plant-based compositions refer to plant-based medicinal compounds and plant-based nutritional supplements. Plant-based medicinal compounds provide therapeutically-effective amounts to treat a condition, such as those described in the Background of the Disclosure. Plant-based nutritional supplements claim a benefit related to a classical nutrient deficiency; describes how the supplement is intended to affect the structure or function of the human body; characterizes a documented mechanism by which the supplement acts to maintain such structure or function; and/or describes general well-being associated with consumption of the product. In particular embodiments, a nutritional supplement may not claim to diagnose, mitigate, treat, cure, or prevent a specific disease or class of diseases.

Plant-based compositions include vegetable matter. Vegetable matter is matter produced by a plant and includes any whole plant or plant part (e.g., bark, wood, leaves, stems, roots, flowers, fruits, seeds, or parts thereof) and/or exudates or extracts thereof. In particular embodiments, plant-based compositions include botanical products. Botanical products can include plant materials, algae, macroscopic fungi, and/or combinations thereof. In particular embodiments, plant-based compositions include a mixture of various types of vegetable matter. Plant-based compositions can also include materials derived from vegetable matter including resins, oils, dried flowers, kief, tinctures, infusions, etc. In particular embodiments, the vegetable matter has little or no water solubility. In particular embodiments, plant-based compositions do not include synthetic, semi-synthetic, or chemically-modified drugs.

In particular embodiments, the plant-based compositions include vegetable matter derived from *Calophyllum brasiliense*, *Calophyllum caledonicum*, *Calophyllum inophyllum*, *Calophyllum soulattri*, *Uncaria tomentosa*, *Thymus vulgaris*, *Matricaria recutita*, *Salix alba*, *Calendula officinalis*, *Usnea barbata*, *Ligusticum porterii-osha*, *Gaultheria procumbens*, *Camellia sinensis*, *Vaccinium myrtillus*, *Melissa officinalis*, *Allium sativum*, *Camellia sinensis*, *Krameria triandra*, *Punica granatum*, *Viburnum plicatum*, *Nicotiana tabacum*, *Duboisia hopwoodii*, *Asclepias syriaca*, *Curcuma longa*, *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis* and/or *Acer* spp. or an extract thereof.

In particular embodiments, the plant-based compositions include vegetable matter derived from the *cannabis* plant. The *cannabis* plant refers to a flowering plant including the species (or sub-species) *Cannabis sativa*, *Cannabis ruderalis*, and *Cannabis indica*.

Particular extracts of the *cannabis* plant include cannabinoids. Cannabinoids are a group of cyclic molecules from *cannabis* plants that activate cannabinoid receptors (i.e., CB1 and CB2) in cells. There are at least 85 different cannabinoids that can be isolated from *cannabis*. Many cannabinoids produced by *cannabis* plants, such as Δ^9 -Tetrahydrocannabinol (THC) and cannabidiol (CBD), have very low or no solubility in water. The most notable cannabinoids are THC and CBD. Additional examples include cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabiniol (CBDL), cannabicyclol (CBL), cannabivarin (CBV), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), cannabigerol monomethyl ether (CBGM), cannabinerolic acid, cannabidiolic acid (CBDA), Cannabinol propyl variant (CBNV), cannabitrilol (CBO), tetrahydrocannabinolic acid (THCA), and tetrahydrocannabivarinic acid (THCVA). See, for example, FIG. 2. Extracts of the *cannabis* plant similarly include flavonoid compounds, terpenes, terpenoid, and synthetic, semisynthetic or highly purified versions of any such constituent.

Components of plant-based compositions can be produced by, e.g., pulverization, decoction, expression, and extraction of a starting plant product. The term "extract" can include all of the many types of preparations containing some or all of the active ingredients found in the relevant plants. Extracts may be produced by cold extraction techniques using a variety of different extraction solvents including water, fatty solvents (such as olive oil), and alcoholic solvents (e.g. 70% ethanol). Cold extraction techniques are typically applied to softer parts of the plant such as leaves and flowers, or in cases wherein the desired active components of the plant are heat labile. Alternatively, the aforementioned solvents may be used to produce extracts of the desired plants by a hot extraction technique, wherein said solvents are heated to a high temperature, the precise value of said temperature being dependent on the properties of the chosen solvent, and maintained at that temperature throughout the extraction process. Hot extraction techniques are more commonly applied to the harder, tougher parts of the plant, such as bark, woody branches and larger roots. In some cases, sequential extractions can be performed in more than one solvent, and at different temperatures. The plant extract may be used in a concentrated form. Alternatively, the extract may be diluted as appropriate to its intended use.

WO2004/026857 provides a method for preparing a purified *cannabis* extract, wherein the cannabinoids are purified to at least 99% wt % THC (Δ^9 -tetrahydrocannabinol). In this method a crude ethanolic extract of *Cannabis* plant material is passed through a column of activated charcoal and evaporated by means of rotary evaporation. The resulting THC enriched extract is subsequently passed through a column packed with Sephadex LH20 and eluted with chloroform/dichloromethane. The solvent used is removed by means of rotary evaporation. In order to further increase the purity of the THC enriched extract, the extract is dissolved in methanol and subsequently in pentane and subjected to rotary evaporation twice.

US2015/0126754 describes a) providing a crude solvent extract of *Cannabis* plant material; b) subjecting the crude extract to thin film evaporation to obtain a refined extract; c) chromatographically fractionating the refined extract to produce one or more high purity fractions having a THC content higher than a preset value and one or more low purity fractions having a THC content lower than the preset value, wherein the preset value is in the range of 95-99% by weight of dry matter; d) subjecting the one or more high purity

fractions to another thin film evaporation; and e) collecting a THC isolate containing at least 97% THC by weight of dry matter; and wherein in step b) and/or in step d) the thin film evaporation is carried out by using wiped film evaporation. This method offers the advantage that it yields a high purity THC extract in good yield and without using solvents that pose a health risk. The method further offers the advantage that it is highly reproducible in that it produces THC-isolate with a specific cannabinoid profile. More particularly, the method yields a THC isolate that contains at least 97.0-99.5% THC and 0.4-2.0% of other cannabinoids, including at least 0.3% Cannabinol and Cannabidiol (all percentages by weight of dry matter).

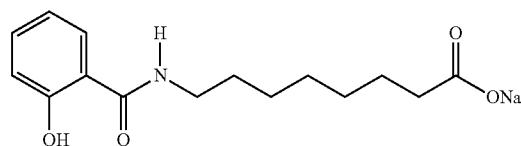
Additional procedures for producing plant extracts (including hot extraction, cold extraction and other techniques) are described in publications including "Medicinal plants: a field guide to the medicinal plants of the Land of Israel (in Hebrew), author: N. Krispil, Har Gilo, Israel, 1986" and "Making plant medicine, author: R. Cech, pub. by Horizon Herbs, 2000".

In particular embodiments, plant components of plant-based compositions (e.g., plant extracts) may be sterilized, for example by autoclaving, and then allowed to cool and stored at an appropriate temperature (e.g., -20° C.). In particular embodiments, further purification to a molecular weight cut-off (e.g., below 10,000 Da) can be carried out, for example, by membrane ultrafiltration before storage.

In particular embodiments, plant-based compositions include carriers such as modified amino acids, a surfactant, a detergent, an azone, a pyrrolidone, a glycol, or a bile salt. An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring, non-naturally occurring and synthetic amino acids. Poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, e.g. an ester, anhydride, or an anhydride linkage. Peptides are two or more amino acids joined by a peptide bond. Peptides can vary in length from dipeptides with two amino acids to poly peptides with several hundred amino acids. See Chambers Biological Dictionary, editor Peter M. B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. Di-peptides, tri-peptides, tetra-peptides, and penta-peptides can also be used.

Carriers which are modified amino acids include acylated fatty acid amino acids (FA-aa) or a salt thereof, which are typically prepared by modifying the amino acid or an ester thereof by acylation or sulfonation. Acylated fatty acid amino acids include N-acylated FA-aa or an amino acid acylated at its alpha amino group with a fatty acid.

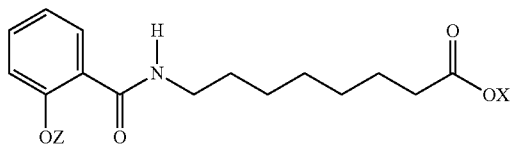
Exemplary N-acylated fatty amino acid salts include sodium N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC). Other names for SNAC include Sodium-N-salicyloyl-8-aminocaprylate, Monosodium 8-(N-salicyloylamino) octanoate, N-(salicyloyl)-8-aminooctanoic acid monosodium salt, monosodium N-{8-(2-hydroxybenzoyl) amino}octanoate, or sodium 8-[(2-hydroxybenzoyl)amino] octanoate. SNAC has the structure:



Salts of SNAC may also be used as a carrier.

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Other forms of SNAC include:



wherein X and Z are independently H, a monovalent cation, a divalent metal cation, or an organic cation. Examples of monovalent cations include sodium and potassium. Examples of divalent cations include calcium and magnesium. Examples of organic cations include ammonium and tetramethylammonium.

Exemplary modified amino acids, such as N-acylated FA-aas, are provided as compounds I-XXXV (see FIG. 3). Salts of these compounds and other N-acylated FA-aa can also be used as carriers.

Many of the compounds can be readily prepared from amino acids by methods within the skill of those in the art based upon the present disclosure. For example, compounds I-VII are derived from aminobutyric acid. Compounds VIII-X and XXXI-XXXIV are derived from aminocaproic acid. Compounds XI-XXVI and XXXV are derived from aminocaprylic acid. For example, the modified amino acid compounds above may be prepared by reacting the single amino acid with the appropriate modifying agent which reacts with free amino moiety present in the amino acids to form amides. Protecting groups may be used to avoid unwanted side reactions as would be known to those skilled in the art.

The amino acid can be dissolved in aqueous alkaline solution of a metal hydroxide, e.g., sodium or potassium hydroxide, and heated at a temperature ranging between 5° C. and 70° C., preferably between 10° C. and 40° C., for a period ranging between 1 hour and 4 hours, preferably 2.5 hours. The amount of alkali employed per equivalent of NH₂ groups in the amino acid generally ranges between 1.25 and 3 mmole, preferably between 1.5 and 2.25 mmole per equivalent of NH₂. The pH of the solution generally ranges between 8 and 13, preferably ranging between 10 and 12.

Thereafter, the appropriate amino acid modifying agent is added to the amino acid solution while stirring. The temperature of the mixture is maintained at a temperature generally ranging between 5° C. and 70° C., preferably between 10° C. and 40° C., for a period ranging between 1 and 4 hours. The amount of amino acid modifying agent employed in relation to the quantity of amino acid is based on the moles of total free NH₂ in the amino acid. In general, the amino acid modifying agent is employed in an amount ranging between 0.5 and 2.5 mole equivalents, preferably between 0.75 and 1.25 equivalents, per molar equivalent of total NH₂ group in the amino acid.

The reaction is quenched by adjusting the pH of the mixture with a suitable acid, e.g., concentrated hydrochloric acid, until the pH reaches between 2 and 3. The mixture separates on standing at room temperature to form a transparent upper layer and a white or off-white precipitate. The upper layer is discarded, and the modified amino acid is collected from the lower layer by filtration or decantation. The crude modified amino acid is then dissolved in water at a pH ranging between 9 and 13, preferably between 11 and 13. Insoluble materials are removed by filtration and the filtrate is dried in vacuo. The yield of modified amino acid generally ranges between 30 and 60%, and usually 45%.

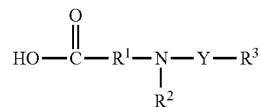
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If desired, amino acid esters, such as, for example benzyl, methyl, or ethyl esters of amino acid compounds, may be used to prepare the modified amino acids. The amino acid ester, dissolved in a suitable organic solvent such as dimethylformamide, pyridine, or tetrahydrofuran can be reacted with the appropriate amino acid modifying agent at a temperature ranging between 5° C. and 70° C., preferably 25° C., for a period ranging between 7 and 24 hours. The amount of amino acid modifying agent used relative to the amino acid ester is the same as described above for amino acids. This reaction may be carried out with or without a base such as, for example, triethylamine or diisopropylethylamine.

Thereafter, the reaction solvent is removed under negative pressure and the ester functionality is removed by hydrolyzing the modified amino acid ester with a suitable alkaline solution, e.g. 1N sodium hydroxide, at a temperature ranging between 50° C. and 80° C., preferably 70° C., for a period of time sufficient to hydrolyze off the ester group and form the modified amino acid having a free carboxyl group. The hydrolysis mixture is then cooled to room temperature and acidified, e.g. aqueous 25% hydrochloric acid solution, to a pH ranging between 2 and 2.5. The modified amino acid precipitates out of solution and is recovered by conventional means such as filtration or decantation. Benzyl esters may be removed by hydrogenation in an organic solvent using a transition metal catalyst.

The modified amino acid may be purified by recrystallization or by fractionation on solid column supports. Suitable recrystallization solvent systems include acetonitrile, methanol and tetrahydrofuran. Fractionation may be performed on a suitable solid column supports such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase column supports using trifluoroacetic acid/acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water as the mobile phase. When anion exchange chromatography is performed, preferably a subsequent 0-500 mM sodium chloride gradient is employed.

In particular embodiments, modified amino acids having the formula



wherein Y is



or SO₂;

R¹ is C₃-C₂₄ alkylene, C₂-C₂₀ alkenylene, C₂-C₂₀ alkynylene, cycloalkylene, or an aromatic, such as arylene;

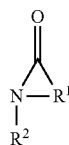
R² is hydrogen, C₁-C₄ alkyl, or C₂-C₄ alkenyl; and

R³ is C₁-C₇ alkyl, C₃-C₁₀ cycloalkyl, aryl, thienyl, pyrrolo, or pyridyl; and

R³ is optionally substituted by one or more C₁-C₅ alkyl group, C₂-C₄ alkenyl group, F, Cl, OH, OR¹, SO₂, COOH, COOR¹ or, SO₃H;

may be prepared by reacting in water and the presence of a base a lactam having the formula

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with a compound having the formula R^3-Y-X , wherein Y, R^1 , R^2 , and R^3 are as above and X is a leaving group. A lactam as shown in the above formula can be prepared, for example by the method described in Olah et al., Synthesis, 537-538 (1979).

In particular embodiments, modified amino acids also include an amino acid acylated at its alpha amino group with a fatty acid, which can be represented by the general formula A-X, wherein A is the alpha-amino acid residue and X is a fatty acid attached by acylation to A's alpha-amino group. The amino acids include cationic and non-cationic amino acids. In particular embodiments the term "non-cationic amino acid" refers to an amino acid selected from the group consisting of non-polar hydrophobic amino acids, polar non-charged amino acids, and polar acidic amino acids. In particular embodiments the term "non-cationic amino acid" as used herein refers to amino acids selected from the group consisting of Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Phenylalanine (Phe), Tryptophane (Trp), Methionine (Met), Proline (Pro), Sarcosine, Glycine (Gly), Serine (Ser), Threonine (Thr), Cysteine (Cys), Tyrosine (Tyr), Asparagine (Asn), and Glutamine (Gln), Aspartic acid (Asp), and Glutamic acid

In particular embodiments, the acylated FA-aa includes an alpha amino acid residue of a non-polar hydrophobic amino acid. In particular embodiments, the acylated FA-aa may be represented by the general formula A-X, wherein A is the amino acid residue of a non-polar hydrophobic amino acid and X is a fatty acid attached by acylation to A's alpha-amino group. In particular embodiments the term "non-polar hydrophobic amino acid" as used herein refers to categorisation of amino acids used by the person skilled in the art. In particular embodiments the term "non-polar hydrophobic amino acid" refers to an amino acid selected from the group consisting of Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Phenylalanine (Phe), Tryptophane (Trp), Methionine (Met), Proline (Pro) and Sarcosine.

In particular embodiments, the acylated FA-aa includes the amino acid residue of a polar non-charged amino acid. In particular embodiments the acylated FA-aa may be represented by the general formula A-X, wherein A is the amino acid residue of a polar non-charged amino acid and X is a fatty acid attached by acylation to A's alpha-amino group. In particular embodiments the term "polar non-charged amino acid" as used herein refers to categorisation of amino acids used by the person skilled in the art. In particular embodiments the term "polar non-charged amino acid" refers to an amino acid selected from the group consisting of Glycine (Gly), Serine (Ser), Threonine (Thr), Cysteine (Cys), Tyrosine (Tyr), Asparagine (Asn), and Glutamine (Gln).

In particular embodiments, the acylated FA-aa includes the amino acid residue of a polar acidic amino acid. In particular embodiments, the acylated FA-aa may be represented by the general formula A-X, wherein A is the amino acid residue of a polar acidic amino acid and X is a fatty acid attached by acylation to A's alpha-amino group. In particular embodiments, the term "polar acidic amino acid" as used herein refers to categorisation of amino acids used by the person skilled in the art. In particular embodiments, the term

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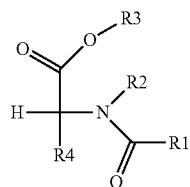
"polar acidic amino acid" refers to an amino acid selected from the group consisting of Aspartic acid (Asp) and Glutamic acid (Glu).

In particular embodiments, the amino acid residue of the acylated FA-aa includes the amino acid residue of an amino acid that is not encoded by the genetic code. Modifications of amino acids by acylation may be readily performed using acylation agents known in the art that react with the free alpha-amino group of the amino acid.

In particular embodiments, the alpha-amino acids or the alpha-amino acid residues herein are in the L-form unless otherwise stated.

In particular embodiments, the amino acid residue is in the free acid form and/or a salt thereof, such as a sodium (Na^+) salt thereof.

Exemplary embodiments of acylated FA-aas may be represented by the general Fa-aa formula 1:



wherein R_1 is an alkyl or aryl group including 5 to 19 carbon atoms; R_2 is H (i.e. hydrogen), CH_3 (i.e. methyl group), or covalently attached to R_4 via a $(CH_2)_3$ group; R_3 is H or absent; and R_4 is an amino acid side chain or covalently attached to R_2 via a $(CH_2)_3$ group; or a salt thereof.

The FA-aa can be acylated with a fatty acid including a substituted or unsubstituted alkyl group consisting of 5 to 19 carbon atoms. In particular embodiments, the alkyl group consists of 5 to 17 carbon atoms. In particular embodiments, the alkyl group consists of 5-15 carbon atoms. In particular embodiments the alkyl group consists of 5-13 carbon atoms. In particular embodiments the alkyl group consists of 6 carbon atoms.

In particular embodiments, the acylated FA-aa is soluble at intestinal pH values, particularly in the range pH 5.5 to 8.0, such as in the range pH 6.5 to 7.0. In particular embodiments, the acylated FA-aa is soluble below pH 9.0.

In particular embodiments, the acylated FA-aa has a solubility of at least 5 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 10 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 20 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 30 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 40 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 50 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 60 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 70 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 80 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 90 mg/mL. In particular embodiments, the acylated FA-aa has a solubility of at least 100 mg/mL. In particular embodiments, solubility of the acylated FA-aa is determined in an aqueous solution at a pH value 1 unit above or below pK_a of the FA-aa at 37° C. In particular embodiments, solubility of the acylated FA-aa is determined in an aqueous solution at pH 8 at 37° C. In particular embodiments, solubility of the acylated

FA-aa is determined in an aqueous solution at a pH value 1 unit above or below pl of the FA-aa at 37° C. In particular embodiments, solubility of the acylated FA-aa is determined in an aqueous solution at a pH value 1 units above or below pl of the FA-aa at 37° C., wherein said FA-aa two or more ionisable groups with opposite charges. In particular embodiments, solubility of the FA-aa is determined in an aqueous 50 mM sodium phosphate buffer, pH 8.0 at 37° C.

In particular embodiments the acylated FA-aa is selected from the group consisting of formula (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), (p), (q), and (r), wherein R1 is an alkyl group including 5 to 19 carbon atoms, R2 is H (i.e. hydrogen) or CH₃ (i.e. methyl group), and R3 is H; or a salt or the free acid form thereof. Formulas (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), (p), (q), and (r) are provided in FIG. 4.

In particular embodiments, the acylated FA-aa can be selected from one or more of sodium N-dodecanoyl alaninate, N-dodecanoyl-L-alanine, sodium N-dodecanoyl isoleucinate, N-dodecanoyl-L-isoleucine, sodium N-dodecanoyl leucinate, N-dodecanoyl-L-leucine, sodium N-dodecanoyl methioninate, N-dodecanoyl-L-methionine, sodium N-dodecanoyl phenylalaninate, N-dodecanoyl-L-phenylalanine, sodium N-dodecanoyl proline, N-dodecanoyl-L-proline, sodium N-dodecanoyl tryptophanate, N-dodecanoyl-L-tryptophane, sodium N-dodecanoyl valinate, N-dodecanoyl-L-valine, sodium N-dodecanoyl sarcosinate, N-dodecanoyl-L-sarcosine, sodium N-oleoyl sarcosinate, sodium N-decyl leucine, sodium N-decanoyl alaninate, N-decanoyl-L-alanine, sodium N-decanoyl leucinate, N-decanoyl-L-leucine, sodium N-decanoyl phenylalaninate, N-decanoyl-L-phenylalanine, sodium N-decanoyl valinate, N-decanoyl-L-valine, sodium N-decanoyl isoleucinate, N-decanoyl-L-isoleucine, sodium N-decanoyl methioninate, N-decanoyl-L-methionine, sodium N-decanoyl proline, N-decanoyl-L-proline, sodium N-decanoyl threoninate, N-decanoyl-L-threonine, sodium N-decanoyl tryptophanate, N-decanoyl-L-tryptophane, sodium N-decanoyl sarcosinate, N-decanoyl-L-Sarcosine, N-dodecanoyl asparaginate, N-dodecanoyl-L-asparagine, sodium N-dodecanoyl aspartic acid, N-dodecanoyl-L-aspartic acid, sodium N-dodecanoyl cysteinate, N-dodecanoyl-L-cysteine, sodium N-dodecanoyl glutamate, N-dodecanoyl-L-glutamine, sodium N-dodecanoyl glycinate, N-dodecanoyl-L-glycine, sodium N-dodecanoyl serinate, N-dodecanoyl-L-serine, sodium N-dodecanoyl threoninate, N-dodecanoyl-L-threonine, sodium N-dodecanoyl tyrosinate, N-dodecanoyl-L-tyrosine, sodium N-decanoyl asparaginate, N-decanoyl-L-asparagine, sodium N-decanoyl aspartic acid, N-decanoyl-L-aspartic acid, sodium N-decanoyl cysteinate, N-decanoyl-L-cysteine, sodium N-decanoyl glutamate, N-decanoyl-L-glutamine, sodium N-decanoyl glutamate, N-decanoyl-L-glutamic acid, Amisoft HS-11 P (sodium Stearoyl Glutamate, Amisoft MS-11 (sodium Myristoyl Glutamate), Amisoft LS-11 (sodium Dodecanoyl Glutamate), Amisoft CS-11 (sodium Cocoyl Glutamate), sodium N-cocoyl glutamate, Amisoft HS-11 P, Amisoft HS-11 P (sodium N-stearoyl glutamate), (sodium N-myristoyl glutamate)), (sodium N-dodecanoyl glutamate), and Amisoft HS-11 P.

The following acylated FA-aas are commercially available:

Brand Name	Chemical Name	Provider (per Apr. 14, 2011)
Hamposyl L-95	sodium N-dodecanoyl sarcosinate	Chattem Chemicals
Hamposyl O	sodium N-oleoyl sarcosinate	Chattem Chemicals
Hamposyl C	sodium N-cocoyl sarcosinate	Chattem Chemicals
Hamposyl L-30	sodium N-dodecanoyl sarcosinate	Chattem Chemicals
Amisoft HS-11 P	sodium N-stearoyl glutamate	Ajinomoto
Amisoft LS-11	sodium N-dodecanoyl glutamate	Ajinomoto
Amisoft CS-11	sodium N-cocoyl glutamate	Ajinomoto
Amisoft MS-11	sodium N-myristoyl glutamate	Ajinomoto
Amilite GCS-11	sodium N-cocoyl glycinate	Ajinomoto

In particular embodiments the terms “fatty acid N-acylated amino acid”, “fatty acid acylated amino acid”, or “acylated amino acid” are used interchangeably herein and refer to an amino acid that is acylated with a fatty acid at its alpha-amino group.

Particular embodiments utilize vegetable matter with low solubility, or very low solubility. Particular embodiments utilize vegetable matter that is essentially water insoluble. In particular embodiments, solubility in water is defined as low to zero by the United States pharmacopeia (USP 32) according to the amount of water necessary for the dissolution of one part of solute: Low solubility: 100 to 1000 parts of water necessary for dissolution of one part of solute; very low solubility: 1000 to 10 000 parts of water necessary; essentially water insoluble more than 10 000 parts of water necessary. At a basic pH, however, SNAC and other modified amino acids and FA-aas described herein are water soluble. Thus, the administration benefits, as described herein could not be reasonably predicted. In particular embodiments, very low solubility can refer to a solubility in water or an aqueous solution of less than 1 mg/ml, less than 0.1 mg/ml, or less than 0.01 mg/ml.

In particular embodiments, N-acylated fatty amino acids act as absorption enhancing agents, thereby creating an administration benefit. Absorption enhancing agents refer to compounds that promote gastrointestinal absorption. Absorption enhancing agents can improve drug absorption by improving the solubility of the drug in the gastrointestinal tract or by enhancing membrane penetration, as compared to a formulation that does not include the absorption enhancing agents. Additional examples of absorption enhancing agents include surfactants, detergents, azones, pyrrolidones, glycols or bile salts.

In particular embodiments, N-acylated fatty amino acids act as bioavailability enhancing agents. Bioavailability refers to the fraction of active ingredient that is actually absorbed by a subject and reaches the bloodstream. In particular embodiments, bioavailability enhancing agents increase the fraction of active ingredient in the bloodstream or result in detection of active ingredient in the bloodstream earlier in time, as compared to a formulation that does not include the bioavailability enhancing agent.

In particular embodiments, additional administration benefits created by absorption enhancing agents and/or bioavailability enhancing agents include faster onset of action, higher peak concentrations, faster time to peak concentrations, shorter duration of action, increased subjective therapeutic efficacy, and/or increased objective therapeutic efficacy as compared to a control plant-based composition or oral formulation based on the same, similar in all aspects but for inclusion of the absorption enhancing agents and/or bioavailability enhancing agents.

Embodiments utilizing absorption enhancing agents and/or bioavailability enhancing agents (e.g., and in particular

embodiments, N-acylated fatty amino acids) can be beneficial because many oral plant-based compositions designed to address various physiological conditions are inadequate because they are characterized by a delayed onset of action, and low bioavailability. Delayed onset of action presents challenges in clinical indications that require rapid therapeutic effect (e.g. pain and migraine); and low bioavailability requires patients to ingest significantly higher doses than would be required by alternative dosing forms (e.g. smoking, vaping). Particular embodiments disclosed herein provide plant-based composition oral formulations with improved bioavailability and shorter time to onset of therapeutic effect.

As stated, in particular embodiments, N-acylated fatty amino acids act as subjective therapy enhancing agents. Subjective therapy enhancement refers to a noticeable alleviation of a symptom, as perceived by a subject. In particular embodiments, subjective therapy enhancing agents increase the alleviation of a symptom or alleviate a symptom more quickly, as compared to a formulation that does not include the subjective therapy enhancing agent.

In particular embodiments, N-acylated fatty amino acids act as objective therapy enhancing agents. Objective therapy enhancement refers to alleviation of a clinical measure, such as a nutritional deficiency detected by a blood or saliva assay or a test of wellness, as administered by a physician. In particular embodiments, objective therapy enhancing agents increase the alleviation of an objective clinical measure or result in alleviation more quickly, as compared to a formulation that does not include the objective therapy enhancing agent.

Particular embodiments include *cannabis* and an absorption enhancing agent and/or bioavailability enhancing agent. These embodiments can allow more rapid *cannabis* absorption and higher bioavailability compared to *cannabis* ingested by currently available oral dosage forms.

In particular embodiments, carriers disclosed herein create administration benefits selected from: increased absorption, increased bioavailability, faster onset of action, higher peak concentrations, faster time to peak concentrations, shorter duration of action, increased subjective therapeutic efficacy, increased objective therapeutic efficacy, improved taste, and improved mouthfeel. Administration benefits related to increased absorption, increased bioavailability, faster onset of action, higher peak concentrations, faster time to peak concentrations, shorter duration of action can alleviate adverse conditions more rapidly (for example, alleviation of pain). "Mouthfeel" refers to non-taste-related aspects of the pleasantness experienced by a person while ingesting (e.g., chewing or swallowing) an oral dosage form. Aspects of mouthfeel include the hardness and brittleness of a composition, whether the composition is chewy, gritty, oily, creamy, watery, sticky, easily dissolved, astringent, effervescent, and the like, and the size, shape, and form of the composition (tablet, powder, gel, etc.).

Plant-based compositions can be manufactured for administration to a subject by adding vegetable matter, a carrier that provides an administration benefit, and one or more excipients, mixing, suspending, dissolving, blending, granulating, tableting, encapsulating, or performing other dosage-form-specific procedures, followed by packaging. For clarity, carriers contribute to providing an administration benefit. Excipients can, but need not, contribute to an administration benefit.

Particular embodiments include plant-based compositions prepared as oral formulations. Exemplary oral formulations include capsules, coated tablets, edibles, elixirs, emulsions,

gels, gelcaps, granules, gums, juices, liquids, oils, pastes, pellets, pills, powders, rapidly-dissolving tablets, sachets, semi-solids, sprays, solutions, suspensions, syrups, tablets, etc.

Exemplary excipient classes include binders, buffers, chelators, coating agents, colorants, complexation agents, diluents (i.e., fillers), disintegrants, emulsifiers, flavoring agents, glidants, lubricants, preservatives, releasing agents, surfactants, stabilizing agents, solubilizing agents, sweeteners, thickening agents, wetting agents, and vehicles.

Binders are substances used to cause adhesion of powder particles in granulations. Exemplary binders include acacia, compressible sugar, gelatin, sucrose and its derivatives, maltodextrin, cellulosic polymers, such as ethylcellulose, hydroxypropylcellulose, hydroxypropylmethyl cellulose, carboxymethylcellulose sodium and methylcellulose, acrylic polymers, such as insoluble acrylate ammoniomethacrylate copolymer, polyacrylate or polymethacrylic copolymer, povidones, copovidones, polyvinylalcohols, alginic acid, sodium alginate, starch, pregelatinized starch, guar gum, and polyethylene glycol.

Colorants may be included in the oral formulations to impart color to the formulation. Exemplary colorants include grape skin extract, beet red powder, beta carotene, annatto, carmine, turmeric, and paprika. Additional colorants include FD&C Red No. 3, FD&C Red No. 20, FD&C Yellow No. 6, FD&C Blue No. 2, D&C Green No. 5, FD&C Orange No. 5, D&C Red No. 8, caramel, and ferric oxide.

Diluents can enhance the granulation of oral formulations. Exemplary diluents include microcrystalline cellulose, sucrose, dicalcium phosphate, starches, lactose and polyols of less than 13 carbon atoms, such as mannitol, xylitol, sorbitol, maltitol and pharmaceutically acceptable amino acids, such as glycine.

Disintegrants also may be included in the oral formulations in order to facilitate dissolution. Disintegrants, including permeabilising and wicking agents, are capable of drawing water or saliva up into the oral formulations which promotes dissolution from the inside as well as the outside of the oral formulations. Such disintegrants, permeabilising and/or wicking agents that may be used include starches, such as corn starch, potato starch, pre-gelatinized and modified starches thereof, cellulosic agents, such as Ac-di-sol, montmorillonite clays, cross-linked PVP, sweeteners, bentonite, microcrystalline cellulose, croscarmellose sodium, alginates, sodium starch glycolate, gums, such as agar, guar, locust bean, karaya, pectin, Arabic, xanthan and tragacanth, silica with a high affinity for aqueous solvents, such as colloidal silica, precipitated silica, maltodextrins, beta-cyclodextrins, polymers, such as carbopol, and cellulosic agents, such as hydroxymethylcellulose, hydroxypropylcellulose and hydroxypropylmethylcellulose. Dissolution of the oral formulations may be facilitated by including relatively small particles sizes of the ingredients used.

Exemplary dispersing or suspending agents include acacia, alginate, dextran, fragalanth, gelatin, hydrogenated edible fats, methylcellulose, polyvinylpyrrolidone, sodium carboxymethyl cellulose, sorbitol syrup, and synthetic natural gums.

Exemplary emulsifiers include acacia and lecithin.

Flavorants are natural or artificial compounds used to impart a pleasant flavor and often odor to oral formulations. Exemplary flavorants include, natural and synthetic flavor oils, flavoring aromatics, extracts from plants, leaves, flowers, and fruits and combinations thereof. Such flavorants include anise oil, cinnamon oil, vanilla, vanillin, cocoa, chocolate, natural chocolate flavor, menthol, grape, pepper-

mint oil, oil of wintergreen, clove oil, bay oil, anise oil, eucalyptus, thyme oil, cedar leave oil, oil of nutmeg, oil of sage, oil of bitter almonds, cassia oil; citrus oils, such as lemon, orange, lime and grapefruit oils; and fruit essences, including apple, pear, peach, berry, wildberry, date, blueberry, kiwi, strawberry, raspberry, cherry, plum, pineapple, and apricot. In particular embodiments, flavorants that may be used include natural berry extracts and natural mixed berry flavor, as well as citric and malic acid.

Glidants improve the flow of powder blends during manufacturing and minimize oral formulation weight variation. Exemplary glidants include silicon dioxide, colloidal or fumed silica, magnesium stearate, calcium stearate, stearic acid, cornstarch, and talc.

Lubricants are substances used in oral formulations that reduce friction during composition compression. Exemplary lubricants include stearic acid, calcium stearate, magnesium stearate, zinc stearate, talc, mineral and vegetable oils, benzoic acid, poly(ethylene glycol), glyceryl behenate, stearyl fumarate, and sodium lauryl sulfate.

Exemplary preservatives include methyl p-hydroxybenzoates, propyl p-hydroxybenzoates, and sorbic acid.

Exemplary sweeteners include aspartame, dextrose, fructose, high fructose corn syrup, maltodextrin, monoammonium glycyrrhizinate, neohesperidin dihydrochalcone, potassium acesulfame, saccharin sodium, stevia, sucralose, and sucrose.

Particular embodiments include swallowable compositions. Swallowable compositions are those that do not readily dissolve when placed in the mouth and may be swallowed whole without chewing or discomfort. U.S. Pat. Nos. 5,215,754 and 4,374,082 describe methods for preparing swallowable compositions. In particular embodiments, swallowable compositions may have a shape containing no sharp edges and a smooth, uniform and substantially bubble free outer coating.

To prepare swallowable compositions, each of the ingredients may be combined in intimate admixture with a suitable carrier according to conventional compounding techniques. In particular embodiments of the swallowable compositions, the surface of the compositions may be coated with a polymeric film. Such a film coating has several beneficial effects. First, it reduces the adhesion of the compositions to the inner surface of the mouth, thereby increasing the subject's ability to swallow the compositions. Second, the film may aid in masking the unpleasant taste of certain ingredients. Third, the film coating may protect the compositions from atmospheric degradation. Polymeric films that may be used in preparing the swallowable compositions include vinyl polymers such as polyvinylpyrrolidone, polyvinyl alcohol and acetate, celluloses such as methyl and ethyl cellulose, hydroxyethyl cellulose and hydroxylpropyl methylcellulose, acrylates and methacrylates, copolymers such as the vinyl-maleic acid and styrene-maleic acid types, and natural gums and resins such as zein, gelatin, shellac and acacia.

In particular embodiments, the oral formulations may include chewable compositions. Chewable compositions are those that have a palatable taste and mouthfeel, are relatively soft and quickly break into smaller pieces and begin to dissolve after chewing such that they are swallowed substantially as a solution.

U.S. Pat. No. 6,495,177 describes methods to prepare chewable compositions with improved mouthfeel. U.S. Pat. No. 5,965,162, describes kits and methods for preparing comestible units which disintegrate quickly in the mouth, especially when chewed.

In order to create chewable compositions, certain ingredients should be included to achieve the attributes just described. For example, chewable compositions should include ingredients that create pleasant flavor and mouthfeel and promote relative softness and dissolvability in the mouth. The following discussion describes ingredients that may help to achieve these characteristics.

Sugars such as white sugar, corn syrup, sorbitol (solution), maltitol (syrup), oligosaccharide, isomaltoligosaccharide, sucrose, fructose, lactose, glucose, lycasin, xylitol, lactitol, erythritol, mannitol, isomaltose, dextrose, polydextrose, dextrin, compressible cellulose, compressible honey, compressible molasses and mixtures thereof may be added to improve mouthfeel and palatability. Fondant or gums such as gelatin, agar, arabic gum, guar gum, and carrageenan may be added to improve the chewiness of the compositions. Fatty materials that may be used include vegetable oils (including palm oil, palm hydrogenated oil, corn germ hydrogenated oil, castor hydrogenated oil, cotton-seed oil, olive oil, peanut oil, palm olein oil, and palm stearin oil), animal oils (including refined oil and refined lard whose melting point ranges from 30° to 42° C.), Cacao fat, margarine, butter, and shortening.

Alkyl polysiloxanes (commercially available polymers sold in a variety of molecular weight ranges and with a variety of different substitution patterns) also may be used to enhance the texture, the mouthfeel, or both of chewable compositions. By "enhance the texture" it is meant that the alkyl polysiloxane improves one or more of the stiffness, the brittleness, and the chewiness of the chewable composition, relative to the same preparation lacking the alkyl polysiloxane. By "enhance the mouthfeel" it is meant that the alkyl polysiloxane reduces the gritty texture of the chewable composition once it has liquefied in the mouth, relative to the same preparation lacking the alkyl polysiloxane.

Alkyl polysiloxanes generally include a silicon and oxygen-containing polymeric backbone with one or more alkyl groups pending from the silicon atoms of the backbone. Depending upon their grade, they can further include silica gel. Alkyl polysiloxanes are generally viscous oils. Exemplary alkyl polysiloxanes that can be used in swallowable, chewable or dissolvable compositions include monoalkyl or dialkyl polysiloxanes, wherein the alkyl group is independently selected at each occurrence from a C₁-C₆-alkyl group optionally substituted with a phenyl group. A specific alkyl polysiloxane that may be used is dimethyl polysiloxane (generally referred to as simethicone). More specifically, a granular simethicone preparation designated simethicone GS may be used. Simethicone GS is a preparation which contains 30% simethicone USP.

Simethicone USP contains not less than 90.5% by weight (CH₃)₃-Si{OSi(CH₃)₂}CH₃ in admixture with 4.0% to 7.0% by weight SiO₂.

To prevent the stickiness that can appear in some chewable compositions and to facilitate conversion of the active ingredients to emulsion or suspension upon taking, the compositions may further include emulsifiers such as glycerin fatty acid ester, sorbitan monostearate, sucrose fatty acid ester, lecithin and mixtures thereof. In particular embodiments, one or more of such emulsifiers may be present in an amount of 0.01% to 5.0%, by weight of the administered formulations. If the level of emulsifier is lower or higher, in particular embodiments, an emulsification cannot be realized, or wax value will rise.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or

they may be presented as a dry product for reconstitution with water or other suitable vehicles before use.

In addition to those described above, any appropriate fillers and excipients may be utilized in preparing the swallowable, chewable and/or dissolvable compositions or any other oral formulation described herein so long as they are consistent with the described objectives.

Oral formulations also include edibles. Edibles refer to any product that can be consumed as a food or a drink. In some cases, edibles are made by infusion of plant extracts into a foodstuff. Examples of edible foods appropriate for use include candy, a candy bar, bread, a brownie, cake, cheese, chocolate, cocoa, a cookie, gummy candy, a lollipop, a mint, a pastry, peanut butter, popcorn, a protein bar, rice cakes, yogurt, etc. While technically not edible, gums can also be used. Examples of edible drinks include beer, juice, flavored milk, flavored water, liquor, milk, punch, a shake, soda, tea, and water. In particular embodiments, edibles are made by combining a plant extract with ingredients used to make an edible. Examples include butters and oils. Exemplary oils include coconut oil, grape seed oil, olive oil, palm oil, papaya seed oil, peanut oil, sesame oil, sprouted wheat oil, wheat germ oil, or any combination thereof.

Oral formulations can be individually wrapped or packaged as multiple units in one or more packages, cans, vials, blister packs, or bottles of any size. Doses are sized to provide therapeutically effective amounts.

In particular embodiments, the oral formulations include vegetable matter (e.g., plant parts or extracts) of at least 0.1% w/v or w/w of the oral formulation; at least 1% w/v or w/w of oral formulation; at least 10% w/v or w/w of oral formulation; at least 20% w/v or w/w of oral formulation; at least 30% w/v or w/w of oral formulation; at least 40% w/v or w/w of oral formulation; at least 50% w/v or w/w of oral formulation; at least 60% w/v or w/w of oral formulation; at least 70% w/v or w/w of oral formulation; at least 80% w/v or w/w of oral formulation; at least 90% w/v or w/w of oral formulation; at least 95% w/v or w/w of oral formulation; or at least 99% w/v or w/w of oral formulation.

In particular embodiments, the oral formulations include carrier of at least 0.1% w/v or w/w of the oral formulation; at least 1% w/v or w/w of oral formulation; at least 10% w/v or w/w of oral formulation; at least 20% w/v or w/w of oral formulation; at least 30% w/v or w/w of oral formulation; at least 40% w/v or w/w of oral formulation; at least 50% w/v or w/w of oral formulation; at least 60% w/v or w/w of oral formulation; at least 70% w/v or w/w of oral formulation; at least 80% w/v or w/w of oral formulation; at least 90% w/v or w/w of oral formulation; at least 95% w/v or w/w of oral formulation; or at least 99% w/v or w/w of oral formulation.

In particular embodiments, the oral formulations include excipient of at least 0.1% w/v or w/w of the oral formulation; at least 1% w/v or w/w of oral formulation; at least 10% w/v or w/w of oral formulation; at least 20% w/v or w/w of oral formulation; at least 30% w/v or w/w of oral formulation; at least 40% w/v or w/w of oral formulation; at least 50% w/v or w/w of oral formulation; at least 60% w/v or w/w of oral formulation; at least 70% w/v or w/w of oral formulation; at least 80% w/v or w/w of oral formulation; at least 90% w/v or w/w of oral formulation; at least 95% w/v or w/w of oral formulation; or at least 99% w/v or w/w of oral formulation.

In particular embodiments, 10 g of dried plant extract may be used in 150 ml of water. This may give an effective concentration of between 1 and 99% (w/w) plant extract, between 2 and 80% (w/w) plant extract, and between 5 and 50% (w/w) plant extract.

Excipients are commercially available from companies such as Aldrich Chemical Co., FMC Corp, Bayer, BASF, Alexi Fres, Witco, Mallinckrodt, Rhodia, ISP, and others.

Additional information can be found in WADE & WALLER, HANDBOOK OF PHARMACEUTICAL EXCIPIENTS (2nd ed. 1994) and Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, formulations can be prepared to meet sterility, pyrogenicity, general safety, and purity standards as required by U.S. FDA and/or other relevant foreign regulatory agencies.

Plant-based compositions disclosed herein can be used to treat subjects (humans, veterinary animals (dogs, cats, reptiles, birds, etc.), livestock (horses, cattle, goats, pigs, chickens, etc.), and research animals (monkeys, rats, mice, fish, etc.)). Treating subjects includes providing therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments, and/or therapeutic treatments.

An "effective amount" is the amount of a plant-based composition necessary to result in a desired physiological change in a subject. Effective amounts are often administered for research purposes. Representative effective amounts disclosed herein can reduce pain perception in an animal model (neuropathic pain, acute pain, visceral pain), stimulate appetite in an animal model, reduce seizures (e.g., epileptic seizures) in an animal model, reverse bone loss in an animal model, relieve migraine (vasoconstrict cranial blood vessels) in an animal model, treat addiction in an animal model, reduce anxiety in an animal model, and/or reduce symptoms of asthma in an animal model.

A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a disease or nutritional deficiency, or displays only early signs or symptoms of a disease or nutritional deficiency, such that treatment is administered for the purpose of diminishing, preventing, or decreasing the risk of developing the disease or nutritional deficiency further. Thus, a prophylactic treatment functions as a preventative treatment against the development of diseases or nutritional deficiencies.

As one example of a prophylactic treatment, an oral formulation disclosed herein can be administered to a subject who is at risk of developing a migraine headache. An effective prophylactic treatment of a migraine headache occurs when the number of migraines per month experienced by a subject is reduced by at least 10% or in particular embodiments, by 25%.

As another example of a prophylactic treatment, an oral formulation disclosed herein can be administered to a subject who is at risk of having an epileptic seizure. An effective prophylactic treatment of epileptic seizures occurs when the number of seizures per month is reduced by at least 10% or in particular embodiments, by 25%.

As another example of a prophylactic treatment, an oral formulation disclosed herein can be administered to a subject who is at risk of suffering from neuropathic pain. An effective prophylactic treatment of neuropathic pain occurs when the occurrence of the neuropathic pain is reduced by at least 10%, or in particular embodiments, by 25% as measured by a standard subjective or objective pain assessment.

As another example of a prophylactic treatment, an oral formulation disclosed herein can be administered to a subject who is at risk of developing breakthrough pain. An effective prophylactic treatment of breakthrough pain occurs when the occurrence of breakthrough pain is reduced by

10%, and in particular embodiments, by 25% by a standard subjective or objective pain assessment.

As another example of a prophylactic treatment, an oral formulation disclosed herein can be administered to a subject who is at risk of developing chemotherapy induced nausea and vomiting (CINV). An effective prophylactic treatment of CINV occurs when CINV is reduced by 10%, and in particular embodiments, by 25% measured by a standard subjective or objective CINV assessment.

As an example of a prophylactic treatment of a nutritional deficiency, an oral formulation disclosed herein can be administered to a subject who is at risk of developing rickets from insufficient vitamin C, anemia from insufficient dietary iron, and/or bone loss from insufficient calcium. An effective prophylactic treatment of these conditions occurs when the conditions are avoided or delayed due to nutritional supplementation with an oral formulation disclosed herein.

A "therapeutic treatment" includes a treatment administered to a subject who has a disease or nutritional deficiency and is administered to the subject for the purpose of curing or reducing the severity of the disease or nutritional deficiency.

As one example of a therapeutic treatment, an oral formulation disclosed herein can be administered to a subject who has a migraine headache. An effective therapeutic treatment of the migraine headache occurs when the severity of the headache is reduced or relieved completely and/or the headache resolves more quickly measured by a standard subjective or objective headache assessment.

Another example of a therapeutic treatment includes administration of an oral formulation disclosed herein to a subject experiencing CINV. A therapeutic treatment of CINV occurs when the vomiting is reduced or ceases (or ceases more quickly) and the nausea is relieved measured by a standard subjective or objective CINV assessment.

Another example of a therapeutic treatment, includes administration of an oral formulation disclosed to a subject who has osteoporosis. An effective therapeutic treatment of osteoporosis occurs when bone density has increased by 10% and in particular embodiments, by 25%.

Another example of a therapeutic treatment includes administration of an oral formulation disclosed herein to a subject who has anxiety. An effective therapeutic treatment of anxiety occurs when the severity of the anxiety is reduced or relieved completely and/or more quickly measured by a standard subjective or objective anxiety assessment.

Another example of a therapeutic treatment includes administration of an oral formulation disclosed herein to a subject who has multiple sclerosis. An effective therapeutic treatment of multiple sclerosis occurs when the score in a standard walk test improves by 10% and in particular embodiments, by 25%.

As one example of a therapeutic treatment of a nutritional deficiency, an oral formulation disclosed herein can be administered to a subject who has rickets from insufficient vitamin C, anemia from insufficient dietary iron, and/or bone loss from insufficient calcium. An effective therapeutic treatment of these conditions occurs when the conditions are reduced or resolved due to nutritional supplementation with an oral formulation disclosed herein.

Therapeutic treatments can be distinguished from effective amounts based on the presence or absence of a research component to the administration. As will be understood by one of ordinary skill in the art, however, in human clinical trials effective amounts, prophylactic treatments and therapeutic treatments can overlap.

For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies. Such information can be used to more accurately determine useful doses in subjects of interest.

The actual dose amount administered to a particular subject can be determined by the subject, a physician, veterinarian, or researcher taking into account parameters such as physical, physiological and psychological factors including target, body weight, condition, previous or concurrent therapeutic interventions, and/or idiopathy of the subject.

Useful doses can range from 0.1 to 5 µg/kg or from 0.5 to 1 µg/kg. In other non-limiting examples, a dose can include 1 µg/kg, 5 µg/kg, 10 µg/kg, 15 µg/kg, 20 µg/kg, 25 µg/kg, 30 µg/kg, 35 µg/kg, 40 µg/kg, 45 µg/kg, 50 µg/kg, 55 µg/kg, 60 µg/kg, 65 µg/kg, 70 µg/kg, 75 µg/kg, 80 µg/kg, 85 µg/kg, 90 µg/kg, 95 µg/kg, 100 µg/kg, 150 µg/kg, 200 µg/kg, 250 µg/kg, 350 µg/kg, 400 µg/kg, 450 µg/kg, 500 µg/kg, 550 µg/kg, 600 µg/kg, 650 µg/kg, 700 µg/kg, 750 µg/kg, 800 µg/kg, 850 µg/kg, 900 µg/kg, 950 µg/kg, 1000 µg/kg, 0.1 to 5 mg/kg or from 0.5 to 1 mg/kg. In other non-limiting examples, a dose can include 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg, 65 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 85 mg/kg, 90 mg/kg, 95 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 550 mg/kg, 600 mg/kg, 650 mg/kg, 700 mg/kg, 750 mg/kg, 800 mg/kg, 850 mg/kg, 900 mg/kg, 950 mg/kg, 1000 mg/kg or more.

Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., hourly, every 2 hours, every 3 hours, every 4 hours, every 6 hours, every 9 hours, every 12 hours, every 18 hours, daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, or monthly).

One or more active agent(s) can be administered simultaneously or within a selected time window, such as within 10 minutes, 1 hour, 3 hour, 10 hour, 15 hour, 24 hour, or 48 hour time windows or when the complementary active agent(s) is within a clinically-relevant therapeutic window.

The Exemplary Embodiments and Examples below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

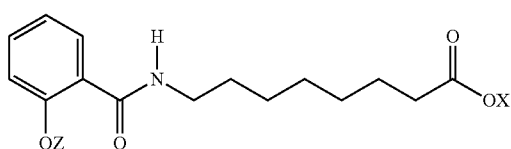
EXEMPLARY EMBODIMENTS

1. A plant-based composition including vegetable matter and an N-acylated fatty amino acid or a salt thereof.
2. A plant-based composition of embodiment 1 including a botanical product.
3. A plant-based composition of embodiment 1 or 2 wherein the vegetable matter is derived from *Calophyllum brasiliense*, *Calophyllum caledonicum*, *Calophyllum inophyllum*, *Calophyllum soulattri*, *Uncaria tomentosa*, *Thymus vulgaris*, *Matricaria recutita*, *Salix alba*, *Calendula officinalis*, *Usnea barbata*, *Ligusticum porteri*, *osha*, *Gaultheria procumbens*, *Camellia sinensis*, *Vaccinium myrtillus*, *Melissa officinalis*, *Allium sativum*, *Camellia sinensis*, *Krameria triandra*, *Punica granatum*, *Viburnum plicatum*, *Nicotiana tabacum*, *Duboisia hopwoodii*, *Asclepias*

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syriaca, *Curcuma longa*, *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis* and/or *Acer* spp, or an extract thereof.

4. A plant-based composition of any of embodiments 1-3 wherein the vegetable matter is derived from *cannabis*.
5. A plant-based composition of any of embodiments 1-4 wherein the vegetable matter is derived from *Cannabis sativa*, *Cannabis ruderalis*, or *Cannabis indica*.
6. A plant-based composition of any of embodiments 1-5 including a *cannabis* extract.
7. A plant-based composition of any of embodiments 1-6 including cannabinoids.
8. A plant-based composition of any of embodiments 1-7 including Δ^9 -Tetrahydrocannabinol (THC) and cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabinodiol (CBDL), cannabicyclol (CBL), cannabivarin (CBV), tetrahydrocannabinavarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovaridin (CBGV), cannabigerol monomethyl ether (CBGM), cannabimerolic acid, cannabidiolic acid (CBDA), Cannabinol propyl variant (CBNV), cannabitriol (CBO), tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarinic acid (THCVA), and/or mixtures thereof.
9. A plant-based composition of any of embodiments 1-8 including flavonoid compounds, terpenes, or terpenoids.
10. A plant-based composition of any of embodiments 1-9 wherein the N-acylated fatty amino acid includes one or more of Compounds I-XXXV (FIG. 3), or Compounds a-r (FIG. 4).
11. A plant-based composition of any of embodiments 1-10 wherein the N-acylated fatty amino acid includes mono-sodium-N-salicyloyl-8-aminocaprylate, disodium-N-salicyloyl-8-aminocaprylate, and N-(salicyloyl)-8-aminocaprylic acid.
12. A plant-based composition of any of embodiments 1-11 wherein the N-acylated fatty amino acid or a salt thereof includes



wherein X and Z are independently H, a monovalent cation, a divalent metal cation, or an organic cation.

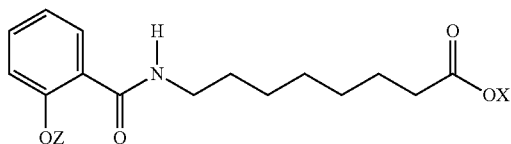
13. A plant-based composition of embodiment 12, wherein the monovalent cation includes sodium or potassium.
14. A plant-based composition of embodiment 12, wherein the metal cation includes calcium or magnesium.
15. A plant-based composition of embodiment 12, wherein the organic cation includes ammonium or tetramethylammonium.
16. The plant-based composition of embodiment 12, wherein X is H.
17. The plant-based composition of embodiment 12, wherein X is a monovalent cation including sodium or potassium.
18. The plant-based composition of embodiment 12, wherein X is a divalent metal cation including calcium or magnesium.
19. The plant-based composition of embodiment 12, wherein X is an organic cation including ammonium or tetramethylammonium.

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20. The plant-based composition of embodiment 12, wherein Z is H.

21. The plant-based composition of embodiment 12 wherein Z is a monovalent cation comprising sodium or potassium.
22. The plant-based composition of embodiment 12 wherein Z is a divalent cation comprising calcium or magnesium.
23. The plant-based composition of embodiment 12, wherein X is H and Z is H.
24. The plant-based composition of embodiment 12, wherein X is H and Z is sodium.
25. The plant-based composition of embodiment 12, wherein X is sodium and Z is sodium.
26. A plant-based composition of any of embodiments 1-25 wherein the N-acylated fatty amino acid provides an administration benefit.
27. A plant-based composition of embodiment 26 wherein the administration benefit is a dose-dependent administration benefit.
28. A plant-based composition of embodiment 27 wherein the dose-dependent administration benefit is at a dose of 100-200 mg.
29. A plant-based composition of embodiment 26 wherein the administration benefit includes one or more of increased absorption of a measured component of vegetable matter, increased bioavailability of a measured component of vegetable matter, faster onset of action of a measured component of vegetable matter, higher peak concentrations of a measured component of vegetable matter, faster time to peak concentrations of a measured component of vegetable matter, shorter duration of action, increased subjective therapeutic efficacy, increased objective therapeutic efficacy, improved taste, and improved mouthfeel as compared to a control composition without the N-acylated fatty amino acid.
30. A plant-based composition of any of embodiments 1-29 wherein the plant-based composition is a medicinal composition.
31. A plant-based composition of any of embodiments 1-30 wherein the plant-based composition is a nutritional supplement.
32. A plant-based composition of any of embodiments 1-31 including a surfactant, detergent, azone, pyrrolidone, glycol or bile salt.
33. A plant-based composition of any of embodiments 1-32 including a therapeutically effective amount of vegetable matter.
34. A plant-based composition of embodiment 33 wherein the therapeutically effective amount treats a symptom of acquired hypothyroidism, acute gastritis, addiction, ADHD, agoraphobia, AIDS, AIDS-related anorexia, alcoholism, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), ankyloses, anxiety, arthritis, Asperger's syndrome, asthma, atherosclerosis, autism, auto-immune diseases, bacterial infections, bipolar disorder, bone loss, blood disorders, brain injury/stroke, cachexia, cancer, carpal tunnel syndrome, cerebral palsy, cervical disk disease, cervicobrachial syndrome, chronic fatigue syndrome, chronic pain, cluster headache, conjunctivitis, Crohn's disease, cystic fibrosis, depression, dermatitis, diabetes, dystonia, eating disorders, eczema, epilepsy, fever, fibromyalgia, flu, fungal infection, gastrointestinal disorders, glaucoma, glioma, Grave's disease, heart disease hepatitis, herpes, Huntington's disease, hypertension, impotence, incontinence, infant mortality, inflammation, inflammatory bowel disease (IBD), insomnia, liver fibrosis, mad cow disease, menopause, metabolic disorders,

- migraine headaches, motion sickness, MRSA, multiple sclerosis (MS), muscular dystrophy, mucosal lesions, nail patella syndrome, nausea and vomiting associated with cancer chemotherapy, neuroinflammation, nicotine addiction, obesity, obsessive compulsive disorder (OCD), pain, pancreatitis, panic disorder, Parkinson's disease, periodontal disease, peripheral neuropathy, phantom limb pain, poison ivy allergy, premenstrual syndrome (PMS), proximal myotonic myopathy, post-traumatic stress disorder (PTSD), psoriasis, Raynaud's disease, restless leg syndrome, schizophrenia, scleroderma, septic shock, shingles herpes zoster), sickle cell disease, seizures, sleep apnea, sleep disorders, spinal injuries, stress, stuttering, temporomandibular joint disorder (TMJ), tension headaches, tinnitus, Tourette's syndrome, traumatic memories, wasting syndrome, and withdrawal.
35. A plant-based composition of any of embodiments 1-34 including vitamins or minerals.
 36. A plant-based composition of any of embodiments 1-34 including vitamins and minerals.
 37. A plant-based composition of embodiments 35 or 36 wherein the vitamins are selected from one or more of Vitamin A, Vitamin B1, Vitamin B6, Vitamin B12, Vitamin C, Vitamin D, Vitamin E, or Vitamin K.
 38. A plant-based composition of any of embodiments 35-36 wherein the minerals are selected from one or more of calcium, chromium, iodine, iron, magnesium, selenium or zinc.
 39. An oral formulation including a plant-based composition of any of embodiments 1-38.
 40. An oral formulation of embodiment 39 wherein the oral formulation is swallowable or chewable.
 41. An oral formulation of embodiment 39 or 40 wherein the oral formulation is liquid or solid.
 42. An oral formulation of any of embodiments 39-41 wherein the oral formulation is a solution, suspension, or spray.
 43. An oral formulation of any of embodiments 39-41 wherein the oral formulation is a tablet, capsule or sachet.
 44. An oral formulation of any of embodiments 39-43 wherein the oral formulation is flavored.
 45. A method of preparing an oral formulation of *cannabis* having a faster onset of action, wherein the method comprises adding an absorption enhancer to the oral formulation of *cannabis* and wherein the oral formulation of *cannabis* has a faster onset of action than an oral formulation of *cannabis* without an absorption enhancer.
 46. The method of embodiment 45, wherein the absorption enhancer is an N-acylated fatty amino acid or a salt thereof.
 47. The method of embodiment 45 or 46, wherein the N-acylated fatty amino acid or a salt thereof comprises



wherein X and Z are independently H, a monovalent cation, a divalent metal cation, or an organic cation.

48. The method of any one of embodiments 45-47, wherein the N-acylated fatty amino acid is selected from mono-

sodium-N-salicyloyl-8-aminocaprylate, disodium-N-salicyloyl-8-aminocaprylate, and N-(salicyloyl)-8-aminocaprylic acid.

49. A method of treating a subject in need thereof including administering a therapeutically effective amount of a composition of any of embodiments 1-39 or a formulation of any of embodiments 40-45 to the subject thereby treating the subject in need thereof.
50. A method of embodiment 50 wherein the therapeutically effective amount provides an effective amount, a prophylactic treatment, and/or a therapeutic treatment.
51. A method of reducing or eliminating one or more symptoms of a disease or disorder in a human subject, wherein said method includes delivering a therapeutically effective amount of a composition of any of embodiments 1-39 or oral formulation of any of embodiments 40-45 to the subject, thereby reducing or eliminating one or more symptoms of the disease or disorder, and wherein said disease or disorder is acquired hypothyroidism, acute gastritis, addiction, ADHD, agoraphobia, AIDS, AIDS-related anorexia, alcoholism, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), ankyloses, anxiety, arthritis, Asperger's syndrome, asthma, atherosclerosis, autism, auto-immune diseases, bacterial infections, bipolar disorder, bone loss, blood disorders, brain injury/stroke, cachexia, cancer, carpal tunnel syndrome, cerebral palsy, cervical disk disease, cervicobrachial syndrome, chronic fatigue syndrome, chronic pain, cluster headache, conjunctivitis, Crohn's disease, cystic fibrosis, depression, dermatitis, diabetes, dystonia, eating disorders, eczema, epilepsy, fever, fibromyalgia, flu, fungal infection, gastrointestinal disorders, glaucoma, glioma, Grave's disease, heart disease hepatitis, herpes, Huntington's disease, hypertension, impotence, incontinence, infant mortality, inflammation, inflammatory bowel disease (IBD), insomnia, liver fibrosis, mad cow disease, menopause, metabolic disorders, migraine headaches, motion sickness, MRSA, multiple sclerosis (MS), muscular dystrophy, mucosal lesions, nail patella syndrome, nausea and vomiting associated with cancer chemotherapy, neuroinflammation, nicotine addiction, obesity, obsessive compulsive disorder (OCD), osteoporosis, osteopenia, pain, pancreatitis, panic disorder, Parkinson's disease, periodontal disease, peripheral neuropathy, phantom limb pain, poison ivy allergy, premenstrual syndrome (PMS), proximal myotonic myopathy, post-traumatic stress disorder (PTSD), psoriasis, Raynaud's disease, restless leg syndrome, schizophrenia, scleroderma, septic shock, shingles herpes zoster), sickle cell disease, seizures, sleep apnea, sleep disorders, spinal injuries, stress, stuttering, temporomandibular joint disorder (TMJ), tension headaches, tinnitus, Tourette's syndrome, traumatic memories, wasting syndrome, or withdrawal syndrome.

EXAMPLES

Oral cannabinoid dosage form providing improved bioavailability and shortened time to onset of effect. Considering the wealth of medical conditions potentially benefiting from *cannabis* therapy, a significant unmet need exists for a faster-acting product that provides improved bioavailability in an oral format. Current oral *cannabis* products include edibles and traditional pharmaceutical dosage forms that are challenged by low bioavailability, and prolonged time to onset of action. The present disclosure addresses the short-

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comings of all of the currently available oral *cannabis* products to provide an improved time to onset of effect and improved bioavailability.

Example 1

Exemplary Formulations. Solution formulation. *Cannabis* and one or more N-acylated fatty amino acids are combined in an aqueous/organic solvent mixture. The resulting blend is stirred vigorously for an hour. If solution is incomplete, a surfactant can be added and stirring can be continued to prepare the final formulation.

Suspension formulation. *Cannabis* and one or more N-acylated fatty amino acids are combined in water, an aqueous/organic solvent mixture or an organic solvent mixture. The resulting blend can be stirred to effect suspension.

Solution formulation. *Cannabis* and one or more absorption enhancing agents are combined in an aqueous/organic solvent mixture. The resulting blend is stirred vigorously for an hour. If solution is incomplete, a surfactant can be added and stirring can be continued to prepare the final formulation.

Suspension formulation. *Cannabis* and one or more absorption enhancing agents are combined in water, an aqueous/organic solvent mixture or an organic solvent mixture. The resulting blend can be stirred to effect suspension.

Gelcap composition. A suspension formulation or solution formulation can be filled into a gelcap to contain up to 1 g of *cannabis*. The gelcap can be treated with an enteric coat or used without a coating.

Tablet/capsule composition. The solution formulation and the suspension formulation can be dried by evaporation, lyophilization, or spray drying. The resultant dry product can be combined with tableting excipients and compressed into tablets or caplets to contain up to 1 g of *cannabis*. Alternatively, the dry product can be filled into capsules.

Example 2

Onset and duration of action of orally administered *cannabis*/SNAC composition. This study was designed to assess the utility of SNAC in enabling a rapid-acting oral form of *cannabis*.

Selection of Participants. Six study participants were recruited to ingest *cannabis* compositions and record the onset, duration, and intensity of *cannabis*-induced euphoria and/or dysphoria. Study participants took part in two separate tests: 1) use of a control substance, which included liquid *cannabis* extract dissolved in aqueous ethanol, and 2) use of a test substance, which included the liquid *cannabis* extract dissolved in aqueous ethanol, as well as SNAC.

Formulations. The selected *cannabis* concentrate is commercially available and was provided to participants in an ethanol solution. The concentrate contains 8 mg THC per dose. It was selected because it contains a high percentage of THC, which provides a noticeable effect on user-reported "euphoria". Aqueous ethanol was used as solvent because it effectively dissolves *cannabis* extract, as well as SNAC.

Methods. For the Control experiment, each participant mixed the *cannabis* concentrate with 15 ml (one tablespoon) of aqueous ethanol, and immediately swallowed the mixture.

For the Test experiment, each participant mixed the *cannabis* concentrate with a pre-mixed solution of aqueous ethanol and 200 mg SNAC, and immediately swallowed the dissolved mixture.

For both the Control experiment and the Test experiment, each participant recorded the time of dose administration,

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the time of onset of euphoria and/or dysphoria, and the observed level of euphoria and/or dysphoria in fifteen minute intervals for five hours following administration of the *cannabis* dose. Euphoria and dysphoria were reported using a scale value, in a range from 1-10. Table 1 shows descriptions of euphoria and dysphoria levels for each scale value.

TABLE 1

Scale Values for Reporting Euphoria and Dysphoria	
Scale Value	Description
0	No observed effect
1-2	Mild observed effect; possibly psychological
3-4	Definite but mild effect
5-6	Definite substantial effect
7-8	Strong effect
9-10	Intense effect

Results. The results shown below are the average scale values obtained for all six participants (also shown in FIGS. 5A and 5B).

TABLE 2

Control Experiment (n = 6)			
Actual Time 12:00 PM	Time from Start 0:00	Observed "Euphoria" (0-10)	Observed "Dysphoria" (0-10)
	12:15 PM	0:15	0.17
	12:30 PM	0:30	0.50
	12:45 PM	0:45	0.83
	1:00 PM	1:00	1.33
	1:15 PM	1:15	1.67
	1:30 PM	1:30	1.83
	1:45 PM	1:45	1.83
	2:00 PM	2:00	2.00
	2:15 PM	2:15	2.17
	2:30 PM	2:30	1.83
	2:45 PM	2:45	1.67
	3:00 PM	3:00	2.17
	3:15 PM	3:15	1.33
	3:30 PM	3:30	1.17
	3:45 PM	3:45	1.00
	4:00 PM	4:00	1.00
	4:15 PM	4:15	0.83
	4:30 PM	4:30	0.67
	4:45 PM	4:45	0.50
	5:00 PM	5:00	0.17

TABLE 3

Test Experiment (n = 6)			
Actual Time 12:00 PM	Time from Start 0:00	Observed "Euphoria" (0-10)	Observed "Dysphoria" (0-10)
	12:03 PM	0:03	3.83
	12:15 PM	0:15	3.83
	12:30 PM	0:30	4.67
	12:45 PM	0:45	4.33
	1:00 PM	1:00	4.33
	1:15 PM	1:15	3.67
	1:30 PM	1:30	2.00
	1:45 PM	1:45	1.83
	2:00 PM	2:00	1.83
	2:15 PM	2:15	1.67
	2:30 PM	2:30	1.83
	2:45 PM	2:45	1.50
	3:00 PM	3:00	1.33
	3:15 PM	3:15	1.33

TABLE 3-continued

Test Experiment (n = 6)			
Actual Time	Time from Start	Observed "Euphoria" (0-10)	Observed "Dysphoria" (0-10)
12:00 PM	0:00		
3:30 PM	3:30	1.50	1.00
3:45 PM	3:45	1.33	0.00
4:00 PM	4:00	0.50	0.00
4:15 PM	4:15	0.17	0.00
4:30 PM	4:30	0.17	0.00
4:45 PM	4:45	0.00	0.00
5:00 PM	5:00	0.00	0.00

Onset: All six participants reported euphoria within five minutes of ingesting the *cannabis*/SNAC formulation (Test), with the time of onset ranging between two and five minutes. In contrast, the first time-point of euphoria reported by participants after ingestion of the *cannabis*-only formulation (Control) was fifteen minutes post-ingestion, with the time of onset ranging between fifteen minutes and one hour, fifteen minutes (see FIGS. 6A-6F for individual participant results). By fifteen minutes post-ingestion, the average reported euphoria scale value was 3.8 for the *cannabis*/SNAC formulation (Test). In contrast, fifteen minutes after ingestion of the *cannabis*-only formulation (Control), the average reported euphoria scale value was 0.17 (see FIGS. 5A-5B for averages at each time-point).

Intensity: The average peak euphoria scale value after ingestion of the *cannabis*/SNAC formulation (Test) was 4.7, which occurred thirty minutes post-ingestion. In contrast, the highest average euphoria scale value after ingestion of the *cannabis*-only formulation (Control) was 2.2, which was at the two hour, fifteen minute time-point (see FIGS. 5A and 5B). Therefore, ingestion of the *cannabis*/SNAC formulation led to a higher peak intensity of euphoria, which occurred an average of one hour and forty-five minutes faster than when the *cannabis*-only formulation was ingested. The intensity of observed dysphoria was minimal for both the Test and Control, with a peak average scale value of 0.83 for both experiments.

Duration: The results indicate that the addition of an absorption enhancer does not shorten the action of *cannabis*.

In summary, adding an absorption enhancer, such as SNAC, in an oral dosage formulation of *cannabis* provides faster onset of action and higher intensity of action at peak activity level of *cannabis*. Moreover, the absorption enhancer has no effect on the duration of action of *cannabis*.

Example 3

Onset and duration of action of orally administered *cannabis*/SNAC composition at a low SNAC dose. This study was designed to assess the utility of SNAC in enabling a rapid-acting oral form of *cannabis* at a low dose.

Selection of Participants. Three study participants were recruited to ingest *cannabis* compositions and record the onset, duration, and intensity of *cannabis*-induced euphoria and/or dysphoria. Study participants took part in two separate tests: 1) use of a control substance, which included liquid *cannabis* extract dissolved in aqueous ethanol, and 2) use of a test substance, which included the liquid *cannabis* extract dissolved in aqueous ethanol, as well as SNAC.

Formulations. The selected *cannabis* concentrate is commercially available and was provided to participants in an ethanol solution. The concentrate contains 8 mg THC per

dose. It was selected because it contains a high percentage of THC, which provides a noticeable effect on user-reported "euphoria". Aqueous ethanol was used as solvent because it effectively dissolves *cannabis* extract, as well as SNAC.

5 Methods. For the Control experiment, each participant mixed the *cannabis* concentrate with 15 ml (one tablespoon) of aqueous ethanol, and immediately swallowed the mixture.

For the Test experiment, each participant mixed the *cannabis* concentrate with a pre-mixed solution of aqueous ethanol and 100 mg SNAC, and immediately swallowed the dissolved mixture.

For both the Control experiment and the Test experiment, each participant recorded the time of dose administration, the time of onset of euphoria and/or dysphoria, and the observed level of euphoria and/or dysphoria in fifteen minute intervals for five hours following administration of the *cannabis* dose. Euphoria and dysphoria were reported using a scale value, in a range from 1-5. Table 1 shows descriptions of euphoria and dysphoria levels for each scale value.

Results. The results are combined with the data from Example 2 and are reported for all participants in FIG. 7.

Onset: All three participants reported euphoria within five minutes of ingesting the *cannabis*/SNAC formulation (Test), with the time of onset ranging between two and five minutes. In contrast, the first time-point of euphoria reported by participants after ingestion of the *cannabis*-only formulation (Control) was fifteen minutes post-ingestion, with the time of onset ranging between fifteen minutes and one hour, fifteen minutes. By fifteen minutes post-ingestion, the average reported euphoria scale value was 3.0 for the *cannabis*/SNAC formulation (Test). In contrast, fifteen minutes after ingestion of the *cannabis*-only formulation (Control), the average reported euphoria scale value was 0.25.

Intensity: The average peak euphoria scale value after ingestion of the *cannabis*/SNAC formulation (Test) was 3.4, which occurred thirty minutes post-ingestion. In contrast, the highest average euphoria scale value after ingestion of the *cannabis*-only formulation (Control) was 2.2, which was at the two hour, fifteen minute time-point. Compared to Example 2 where the SNAC dose was 200 mg, the participants in Example 3 ingested only 100 mg of SNAC combined with the same quantity of *cannabis* used in Example 2. This reduced quantity of SNAC resulted in a reduced *cannabis* effect demonstrating a clear dose-response relationship between observed *cannabis* effect (euphoria) and SNAC dose. Consistent with Example 2, ingestion of the *cannabis*/SNAC formulation led to a higher peak intensity of euphoria, which occurred an average of one hour and forty-five minutes faster than when the *cannabis*-only formulation was ingested.

Duration: The results indicate that the addition of an absorption enhancer does not shorten the action of *cannabis*.

In summary, adding an absorption enhancer, such as SNAC, in an oral dosage formulation of *cannabis* provides faster onset of action and higher intensity of action at peak activity level of *cannabis*. Moreover, the absorption enhancer has no effect on the duration of action of *cannabis*. The varying quantity of SNAC produces a clear dose-response relationship between observed *cannabis* effect (euphoria) and SNAC dose.

As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." As used herein, the transition term "comprise" or "comprises" means includes,

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but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. As used herein, a material effect would cause a statistically-significant reduction in an administration benefit when assessed in an experimental protocol disclosed herein.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limi-

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tations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

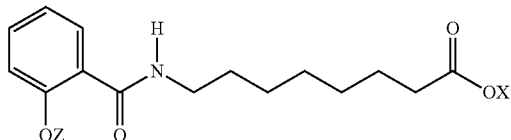
Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster’s Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

What is claimed is:

1. A method of providing increased bioavailability of a cannabinoid with an aqueous solubility of less than 0.1

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mg/ml in an oral formulation when administered to a subject wherein the method comprises adding an N-acylated fatty amino acid or a salt thereof comprising the formula:



to the oral formulation

wherein X and Z are independently H, a monovalent cation, a divalent metal cation, or an organic cation thereby providing increased bioavailability of the cannabinoid when administered to the subject as compared to the same oral formulation without the added N-acylated fatty amino acid or salt thereof.

2. The method of claim 1, wherein the N-acylated fatty amino acid or a salt thereof comprises N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC).

3. The method of claim 1, wherein X is H, a monovalent cation comprising sodium or potassium, a divalent metal cation comprising calcium or magnesium, or an organic cation comprising ammonium or tetramethylammonium.

4. The method of claim 1, wherein Z is H, a monovalent cation comprising sodium or potassium, or a divalent cation comprising calcium or magnesium.

5. The method of claim 1, wherein X is H and Z is H.

6. The method of claim 1, wherein X is H and Z is sodium.

7. The method of claim 1, wherein X is sodium and Z is sodium.

8. The method of claim 1, wherein the adding comprises adding 100-200 mg of the N-acylated fatty amino acid to the oral formulation.

9. The method of claim 1, wherein the cannabinoid comprises Tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabiniol (CBL), cannabivarin (CBV), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), cannabigerol monomethyl ether (CBGM), cannabimerolic acid, cannabidiolic acid (CBDA), Cannabinol propyl variant (CBNV), cannabitiol (CBO), tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarinic acid (THCVA) and/or mixtures thereof.

10. The method of claim 1, wherein the cannabinoid comprises THC and/or CBD.

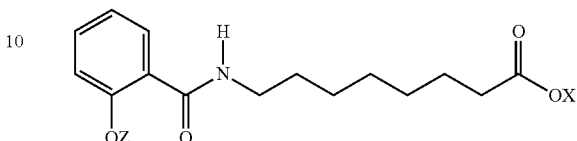
11. The method of claim 10, wherein the THC and/or CBD is extracted from a *Cannabis sativa* plant, a *Cannabis ruderalis* plant, or a *Cannabis indica* plant.

12. The method of claim 1, wherein the method also provides a faster onset of action of the cannabinoid within the subject when the oral formulation is administered to the subject as compared to the same oral formulation without the added N-acylated fatty amino acid or salt thereof.

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13. The method of claim 1, wherein the oral formulation further comprises flavonoid compounds, terpenes, or terpenoids.

14. An oral formulation comprising a cannabinoid with an aqueous solubility of less than 0.1 mg/ml and an N-acylated fatty amino acid or a salt thereof comprising the formula:



wherein X and Z are independently H, a monovalent cation, a divalent metal cation, or an organic cation.

15. The oral formulation of claim 14, wherein the N-acylated fatty amino acid or a salt thereof comprises N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC).

16. The oral formulation of claim 14, wherein X is H, a monovalent cation comprising sodium or potassium, a divalent metal cation comprising calcium or magnesium, or an organic cation comprising ammonium or tetramethylammonium.

17. The oral formulation of claim 14, wherein Z is H, a monovalent cation comprising sodium or potassium, or a divalent cation comprising calcium or magnesium.

18. The oral formulation of claim 14, wherein X is H and Z is H.

19. The oral formulation of claim 14, wherein X is H and Z is sodium.

20. The oral formulation of claim 14, wherein X is sodium and Z is sodium.

21. The oral formulation of claim 14, comprising 100-200 mg of the N-acylated fatty amino acid.

22. The oral formulation of claim 14, wherein the cannabinoid comprises Tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabiniol (CBL), cannabivarin (CBV), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), cannabigerol monomethyl ether (CBGM), cannabimerolic acid, cannabidiolic acid (CBDA), Cannabinol propyl variant (CBNV), cannabitiol (CBO), tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarinic acid (THCVA) and/or mixtures thereof.

23. The oral formulation of claim 14, wherein the cannabinoid comprises THC and/or CBD.

24. The oral formulation of claim 23, wherein the THC and/or CBD is extracted from a *Cannabis sativa* plant, a *Cannabis ruderalis* plant, or a *Cannabis indica* plant.

25. The oral formulation of claim 14, wherein the oral formulation further comprises flavonoid compounds, terpenes, or terpenoids.

* * * * *

P-2018/16104

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

Re: Expediente No. **NC2018/0011299**
Solicitud de patente a nombre de **RECEPTOR LIFE SCIENCES INC.**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la carrera 11 No. 86-53, obrando como apoderada de la sociedad **RECEPTOR LIFE SCIENCES INC.**, domiciliada en Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. **1356** notificado por comunicación electrónica el 21 de marzo de 2019, mediante el cual se nos comunica que la oposición presentada por LABORATORIOS LEGRAND S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación, nos permitimos manifestar:

1. MODIFICACIONES A LA SOLICITUD

Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio el cual consta de veinte (20) reivindicaciones.

En este nuevo capítulo reivindicatorio se han realizado las siguientes modificaciones:

1.1 Se modificó la reivindicación 1 con base en la antigua reivindicación 48 la cual hace referencia a un método para preparar a formulación oral. Además, se han incluido las características de la antigua reivindicación 2 con el fin de definir claramente la materia a proteger.

1.2 Además, se modificaron las reivindicaciones en correspondencia con la nueva reivindicación 1 dando claridad a la materia reclamada y definiendo las características esenciales de la presente invención con el fin de cumplir los requerimientos de la actual legislación.

1.3 Respecto a que el capítulo reivindicatorio previamente presentado carece de concisión, el solicitante respetuosamente desea manifestar su desacuerdo con la apreciación de la sociedad opositora que indica que *“La solicitud en mención reivindica independientemente 4 composiciones de base vegetal que poseen características similares, por lo tanto, se considera que la materia reivindicada carece de concisión”*, y teniendo en cuenta lo descrito en la Guía para Examen de Solicitudes de Patentes de Invención y Modelos de Utilidad de la SIC que establece respecto a la concisión que *“La finalidad de este requisito es evitar una excesiva complejidad para el examinador, a la hora de analizar las reivindicaciones, y evitar que terceros no puedan ver claramente cuál es el alcance de las reivindicaciones, por el excesivo número y complejidad de éstas”*¹, por lo cual esta objeción no aplica para la presente solicitud ya que el alcance de las reivindicaciones está claramente definido y es entendible para una persona versada en la materia.

1.4 La sociedad opositora indica que las reivindicaciones 28 a 34 previamente presentadas carecen de claridad porque no definen una composición en términos de sus ingredientes y proporciones. Amablemente se informa a este Despacho que las reivindicaciones 28 a 34 han sido eliminadas del nuevo capítulo reivindicatorio.

1.5 Adicionalmente, la sociedad opositora indica que las reivindicaciones 38 a 41 previamente presentadas carecen de claridad porque no indican el rango de cantidades en que pueden estar tales compuestos en la composición. Amablemente se informa a este Despacho que las reivindicaciones 38 a 41 han sido eliminadas del nuevo capítulo reivindicatorio.

¹ Guía para Examen de Solicitudes de Patentes de Invención y Modelo de Utilidad. Superintendencia de Industria y Comercio. 2012. Sección 2.6.6 (disponible en http://api.sic.gov.co/Documentos/Guia_Examen_Patentes.pdf).

1.6 La sociedad opositora indica que las reivindicaciones 42 a 47 previamente presentadas carecen de claridad porque no definen una composición en términos de sus ingredientes y proporciones. Amablemente se informa a este Despacho que las reivindicaciones 42 a 47 han sido eliminadas del nuevo capítulo reivindicatorio.

1.7 La sociedad opositora indica que las reivindicaciones 48 a 51 previamente presentadas carecen de claridad porque define inequívocamente el método de preparación de la composición reclamada. Amablemente se informa a este Despacho que las reivindicaciones 48 a 51 han sido eliminadas del nuevo capítulo reivindicatorio.

1.8 Además, la sociedad opositora indica que las reivindicaciones 37 y 52 a 54 se refieren a métodos de tratamiento terapéutico y profiláctico que corresponde a materia no patentable. Amablemente se informa a este Despacho que las reivindicaciones 37 y 52 a 54 han sido eliminadas del nuevo capítulo reivindicatorio.

1.9 Finalmente, se eliminaron las antiguas reivindicaciones 4, 12, 13, 28 a 36 y 38 a 40.

Conforme a lo anterior, es claro que las modificaciones realizadas al capítulo reivindicatorio no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de restringir la materia que se reclama, las cuales están enteramente sustentadas por la descripción.

En consecuencia, nos permitimos informar que las reivindicaciones 1 a 20 cumplen con las disposiciones del artículo 30 de la Decisión 486.

2. PAGO DE TASAS

En cuanto al número de reivindicaciones, atentamente nos permitimos decir que no se adjunta tasa por reivindicaciones adicionales, toda vez que el nuevo capítulo reivindicatorio tiene menor número de cláusulas que el originalmente presentado y en esencia es el mismo objeto, pero más limitado.

Por otra parte, nos permitimos adjuntar recibo por Tasa de modificación teniendo en cuenta las modificaciones realizadas al capítulo reivindicatorio.

3. PRETENSIONES DE LA OPOSICIÓN:

Nos oponemos a la pretensión del escrito de oposición por cuanto, la invención presentada por el solicitante es una invención patentable a la luz de la Decisión 486 y cumple con los requisitos de novedad, nivel inventivo y aplicación industrial. En consecuencia, se debe otorgar el privilegio de patente de invención y expedir el título correspondiente.

Como primera medida, respetuosamente nos permitimos decir ante ese Despacho que consideramos que el opositor no acredita su legítimo interés para presentar la oposición que nos ocupa, ya que no presenta ninguna evidencia con la cual confirme que se encuentra en preparativos para comercializar o bien se encuentre comercializando el producto de la presente invención.

Como segundo aspecto, atentamente nos permitimos manifestar que del análisis de la oposición presentada por LABORATORIOS LEGRAND S.A., no se desprende **NINGUN ARGUMENTO MOTIVADO Y FUNDAMENTADO** en contra de la materia objeto de la presente invención.

En este sentido, es deseo del solicitante presentar los siguientes argumentos en contra de las afirmaciones del oponente, que demuestran que la invención reclamada debe ser reconocida como patentable según los lineamientos de patentabilidad en Colombia.

4. OBSERVACIONES RESPECTO A LA FALTA DE NIVEL INVENTIVO

La parte opositora ha indicado que la presente solicitud carece de nivel inventivo a la luz de los documentos:

1. US 2015/057341 (D1)
2. US 2008/0194676 (D2)
3. US 2015/0181925 (D3)

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4. La publicación científica titulada ***“Investigation of the enhancing mechanism of sodium N-[8-(2-hydroxybenzoyl)amino]caprylate effect on the intestinal permeability of polar molecules utilizing a voltage clamp method”*** (D4)

Con respecto a los argumentos presentados por el oponente en contra de la presente solicitud, el Solicitante desea indicar que después de revisar los documentos citados, ninguno de ellos revela las características del nuevo capítulo reivindicatorio como se demuestra a continuación.

En primer lugar, el oponente indica que D1 anticipa la materia reclamada en la presente solicitud. Sin embargo, D1 no anticipa el presente pliego reivindicatorio porque no enseña un aminoácido graso *N*-acilado o una sal del mismo o *N*-[8-(2-hidroxibenzoil)amino]caprilato (SNAC) como agente de mejora de la absorción.

En segundo lugar, el oponente indica que D2 anticipa la materia reclamada en la presente solicitud. Sin embargo, D2 solo describe el uso de SNAC como agente de entrega para cromolina. D2 describe a la cromolina como “altamente insoluble en lípidos y principalmente ionizado” (Párrafo [0009]). Debido a que la cromolina es un lípido insoluble e ionizada, es soluble en agua y polar, y por lo tanto, no es “materia vegetal con una solubilidad en agua menor que 0.1 mg / ml”.

En tercer lugar, el oponente indica que D3 anticipa la materia reclamada en la presente solicitud. Al igual que D1, D3 no enseña el uso de un aminoácido graso *N*-acilado o una sal del mismo o SNAC como agente mejorador de la absorción. D3, por lo tanto, tampoco anticipa el nuevo pliego reivindicatorio.

En cuarto lugar, el oponente indica que D4 anticipa la materia reclamada en la presente solicitud. Sin embargo, D4 describe que “SNAC puede facilitar el transporte pasivo de moléculas con carga polar a través de la membrana de enterocitos epiteliales” (Resumen). En particular, D4 indica que SNAC puede mejorar la permeabilidad intestinal de rata de 6-carboxifluoresceína (6-CF) (Página 309). 6-CF es soluble en agua. *6-Carboxifluoresceína*, Sigma-Aldrich, disponible en <https://www.sigmaaldrich.com/catalog/product/sial/54115?lang=en®ion=US>.

www.clarkemodet.com

6-CF también se ioniza en solución. Por lo tanto, D4 tampoco anticipa las reivindicaciones dirigidas al uso de aminoácidos grasos *N*-acilados, como el SNAC, para proporcionar un inicio de acción más rápido y una mayor biodisponibilidad de “materia vegetal con una solubilidad en agua menor que 0,1 mg/ml”.

Además, el oponente indica que el objeto reclamado en la solicitud carece de nivel inventivo sobre una combinación de D2, D4 y D1. En este sentido, el solicitante no está de acuerdo con esta apreciación por las siguientes razones:

La Dra. Andrea Leone-Bay, una de las inventoras de la presente solicitud, determinó en la década de 1990 que el N-[8-(2-hidroxibenzoil)amino] caprilato (SNAC) podría utilizarse para mejorar la permeabilidad intestinal de materiales como la heparina y la cromolina. El documento D4, por ejemplo, cita múltiples artículos publicados por la Dra. Leone-Bay sobre el tema de SNAC. Ver, por ejemplo, Hess, page 307. Ella es una de las principales expertas del mundo en relación con el SNAC, sus propiedades y sus efectos fisiológicos.

Los hallazgos de la Dra. Leone-Bay descritos en la presente solicitud, junto con el co-inventor Gregory Wesner, fueron altamente imprevistos, es decir, que los aminoácidos grasos *N*-acilados, como el SNAC, podrían proporcionar un inicio de acción más rápido y una mayor biodisponibilidad de materia vegetal con una solubilidad en agua menor que 0.1mg/ml. Ninguna de las técnicas citadas aborda o describe el aumento de la acción o la biodisponibilidad de la materia vegetal con esta característica.

En la presente solicitud, los inventores explican por qué los efectos del SNAC con materia vegetal con una solubilidad en agua menor que 0.1 mg/ml fueron tan inesperados. En particular, la Figura 1B de la presente solicitud (presentada a continuación) muestra lo que un experto en la técnica **hubiera** esperado basándose en moléculas que se habían probado con SNAC antes de la presente invención. Antes del trabajo que conducía a la presente solicitud, había casi una relación lineal completa entre la eficacia del SNAC y la polaridad de la molécula administrada. Según este conocimiento, un experto en la materia habría predicho que el SNAC **no** proporcionaría un inicio de acción más rápido y **no** aumentaría la

biodisponibilidad de la materia vegetal con una solubilidad en agua menor que 0,1 mg/ml.

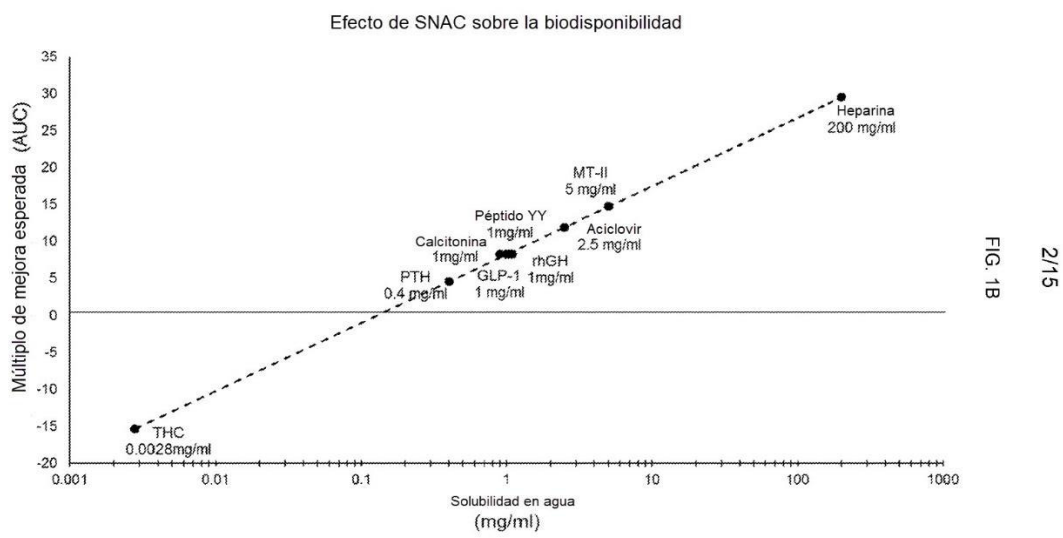


Figura 1B de la Presente Solicitud

En consecuencia, en la fecha de presentación efectiva de la presente solicitud, no se esperaba que la combinación de un aminoácido graso N-acilado, como el SNAC, proporcionaría un inicio de acción más rápido y una mayor biodisponibilidad de la molécula de materia vegetal con una solubilidad e agua menor que 0.1mg/ml.

Como lo demuestra esta solicitud, la Dra. Leone-Bay y su compañero inventor, Gregory Wesner, lograron un gran avance en la administración de fármacos basados en aminoácidos grasos N-acilados cuando combinaron dichos aminoácidos con materia vegetal con una solubilidad en agua menor que 0,1 mg/ml, como el THC. Al contrario de los resultados esperados de que SNAC no mejoraría la biodisponibilidad del THC, los inventores pudieron mejorar significativamente la biodisponibilidad del THC combinando THC con SNAC. La solicitud demuestra que *“SNAC, en una formulación de dosificación oral de cannabis proporciona un comienzo más rápido de la acción y una mayor intensidad de la acción en un nivel de actividad pico del cannabis”* (Solicitud PCT, Párrafo [0154], ver también Figuras 5A y 5B).

Según la Oposición presentada:

*“En vista de la baja solubilidad del THC (Solubilidad de 2,8 mg por litro), del CBD (insoluble en agua), o de una materia vegetal (Solubilidad menor que 0,1 mg/ml), una persona versada en la materia habría sido sugerida por **US2008194676 o por Hess** a emplear ... [SNAC], con el fin de mejorar la absorción de estos componentes”.*

En este respecto, el solicitante discrepa respetuosamente. D2 describe composiciones formuladas para administración oral que comprenden cromolina (un medicamento antiinflamatorio usado para tratar el asma) y SNAC. D4 describe composiciones que incluyen 6-CF y SNAC. Sin embargo, tanto la cromolina como el 6-CF son solubles en agua y principalmente ionizados en agua. Por lo tanto, D2 y D4 describen combinaciones convencionales de materiales solubles en agua con SNAC.

La Oposición presentada indica que *“la mera combinación de un compuesto polar con [SNAC] no es suficiente para demostrar nivel inventivo”*. Sin embargo, la nueva reivindicación 1 describe “materia vegetal con una solubilidad en agua menor que 0.1 mg/ml”, no un compuesto polar” Como se explicó anteriormente, una persona experta en la técnica reconocería que los compuestos polares tienen solubilidad en agua relativamente alta, no *“una solubilidad menor que 0.1 mg/ml”* como se describe en la nueva reivindicación 1.

Por al menos estas razones, ni D1 ni D2 ni D3 ni D4, solos o en combinación, enseñan o sugieren la presente invención. Por lo cual, las nuevas reivindicaciones son inventivas.

5. CONCLUSIÓN

Finalmente, a la luz de las modificaciones al pliego reivindicatorio y los argumentos presentados, es posible concluir lo siguiente:

5.1 La presente solicitud es clara, concisa y está debidamente soportada por la descripción, cumpliendo así con los requerimientos del Artículo 30 de la Decisión 486/00.

5.2 La presente solicitud es patentable sobre los documentos D1, D2, D3 y D4, y los argumentos indicados por LABORATORIO LEGRAND S.A., de acuerdo con los requerimientos de los Artículos 14, 16 y 18 de la Decisión 486/00.

5.3 La parte Opositora **NO APORTA NINGUN DOCUMENTO O PRUEBA QUE EVIDENCIE** la falta de nivel inventivo de la presente patente de invención. Por lo tanto, consideramos que dicha oposición es **infundada**, incumpliendo con lo establecido por el artículo 42 de la Decisión 486 del Tribunal Andino.

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio.

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional

6. ANEXOS

- Nuevo capítulo reivindicatorio conformado por 20 reivindicaciones.
- Recibo de pago correspondiente a la Tasa por modificación de las reivindicaciones.

Señor Director,


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