

**RECURSO DE REPOSICIÓN CONTRA LA RESOLUCIÓN NO. 72827 DEL 01 DE DICIEMBRE DE 2014 DESFIJADO EL EDICTO EL 09 DE ENERO DE 2015**

Señores

**Superintendencia de Industria y Comercio**  
Dirección de Nuevas Creaciones

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



**No. 13-261736- -00008-0000**

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E. S. D.

**Ref: Expediente No. 13-261736**

**Solicitud de registro de patente de invención denominada  
“ANÁLOGOS DE ÁCIDO EPOXIEICOSATRIENÓICO”**

**Solicitante: THE MEDICAL COLLEGE OF WISCONSIN /  
THE BOARD OF REGENTS OF THE UNIVERSITY OF  
TEXAS SYSTEM.**

**Luz Helena Adarve**, identificada como aparece al pie de mi firma, y obrando en mi condición de apoderada de **THE MEDICAL COLLEGE OF WISCONSIN**, domiciliada en 8701 Watertown Plank Road, Milwaukee, WI 53226, Estados Unidos de América, y de **THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM**, domiciliada en 201 W. 7th St., Austin, TX 78701, Estados Unidos de América, atentamente manifiesto a usted que interpongo recurso de reposición contra la Resolución **No. 72827**, dictada por el señor Superintendente de Industria y Comercio, mediante la cual se niega la patentabilidad de la totalidad de las reivindicaciones de la solicitud de patente de la referencia.

A continuación me permito fundamentar el recurso en el siguiente hecho:

- Mediante Resolución **No. 72827**, se niega la patentabilidad de las reivindicaciones 1 a 5 de la presente invención basándose en la alegada falta nivel inventivo de las mismas.

En vista de lo que establece la Resolución Impugnada, se puede apreciar que las reivindicaciones 1 a 5 fueron negadas debido a que no describen características que les proporcionen nivel inventivo frente a los documentos **Falk JR. et al. J Med Chem. Vol. 27;52(16):5069-75 (D1)** y **Parrish et al. Cell Biol Toxicol. Vol. 25(3):217-25 (D2)**.

Más específicamente, el Examinador menciona lo siguiente:

*“Los documentos D1 y D2 sí revelan compuestos derivados de ácido epoxieicosatrienoico para el tratamiento de la hipertensión, tal como los compuestos propuestos en la presente solicitud, y como ya se indicó en el análisis, el grupo farmacofórico, causante de la actividad de dichos compuestos, ya había sido revelado en el documento D1, por lo cual, era obvio para la persona del oficio normalmente tomar las enseñanzas de dichos documentos D1 y D2 para lograr la solución al problema técnico planteado en la presente solicitud. Además, la solución al problema técnico no fue demostrada para todos los compuestos reclamados, sino solamente para tres compuestos, así que se reitera que, no se puede asegurar que todos los compuestos reclamados van a tener los mismos efectos mejorados, de modo que el efecto logrado no es inesperado.”*

Por esta razón, mediante el presente escrito se pretende ilustrar de una manera clara las razones por las cuales la presente solicitud contiene características diferenciadoras con respecto al estado de la técnica presentado, y enfocar la atención de ese Despacho al hecho de que se **cumple** con las exigencias técnicas y legales para gozar del privilegio de patente solicitado.

A continuación se presenta la siguiente argumentación que justifica lo previamente enunciado:

**I. En relación a la objeción por Nivel Inventivo:**

- 1. Los compuestos en D1 no enseñan o sugieren en ninguna forma a los compuestos reivindicados.**
- a. Como se indica en la opinión escrita de la búsqueda internacional (ISA), con fecha 25 de julio de 2012 (se adjunta copia), *"D1 reporta sustitutos de ácido 14,15- epoxyeicosa - 5,8, 11- trienoico y su uso en el tratamiento de la hipertensión. Los compuestos en D1 difieren de los de la aplicación en su estructura. Por tanto, el contenido de las reivindicaciones 1-15 se considera como novedoso sobre D1 por la ISA "* (traducción de: pág. 4, último párrafo). Además, la ISA llega a la conclusión de que *"Los compuestos de D1 difieren en la naturaleza de los radicales ambos unidos a los átomos de nitrógeno de urea. Como tales análogos no son de ninguna manera*

***sugiere en D1, la solución propuesta por el solicitante se considera como inventiva***" (énfasis añadido) (traducción de: pág. 5, párrafo 3).

- b. Como señaló el ISA, D1 está dirigida a sustitutos 14,15-EET y su uso en el tratamiento de la hipertensión. Las 14,15-EETs descritos en D1 son en gran parte libre de ácidos carboxílicos, excepto para el éster de metilo se encuentra en el compuesto # 26. En contraste, los compuestos reivindicados incluyen en gran medida bioisómeros. Nada en D1 o sugiere esta diferencia fundamental en la estructura.
- c. Los 14,15-EETs descritos en D1 tienen características funcionales muy diferentes a las que presentan los compuestos reivindicados debido a las diferencias en las estructuras. Algunas se enuncian a continuación:
  - Solubilidades.
  - Absorción desde el intestino (traducción del inglés: *gut*) (estómago).
  - Vida media plasmática (relacionado con la duración de la acción).
  - Físico-química (incluyendo acidez).
  - La absorción, la distribución y el comportamiento metabólico.

Nada en D1 enseña o sugiere las diferentes propiedades o las diferentes estructuras de los compuestos reivindicados.

## **2. Los compuestos en D2 no enseñan o sugieren en ninguna forma a los compuestos reivindicados.**

- a. En el ISA señalado anteriormente se confirma que "*D2 reporta el uso del n-butyl éster del 12- (3-adamantan-1-il-ureiido) ácido- dodecanoico (nbAUDA) para atenuar la nefrotoxicidad inducida por cisplatino. Los compuestos de la presente solicitud no se dan a conocer en D2. El contenido de las reivindicaciones 1-15, por tanto es novedoso sobre D2*" (traducción de: pág. 5, párrafo 1).

Además, la ISA llega a la conclusión de que "*Los compuestos de la aplicación difieren del n-butyl éster del ácido 12- (3-adamantan-1-il-ureiido) ácido dodecanoico descrito en D2 en la naturaleza de los dos radicales unidos a el nitrógeno de la urea. Como tales análogos no son sugeridos de ninguna manera en D2, la solución propuesta por el solicitante es considerado como inventiva*" (énfasis añadido) (traducción de: pág. 5, párrafo 4).

- b. Como señaló el ISA, D2 está dirigida al uso de nbAUDA para aliviar la nefrotoxicidad inducida por cisplatino. El nbAUDA descrito en D2 contiene un adamantano, que es un hidrocarburo policíclico muy voluminoso en un lado de la isómero epóxido (urea). Los compuestos reivindicados, por el contrario, tienen todas cadenas lineales en ambos lados. **Nada en D2 enseña o sugiere esta diferencia fundamental en la estructura.**
- c. El nbAUDA descrito en D2 no tiene carbonos Sp<sup>2</sup> o Sp- hibridados en la cadena, excluyendo el isómero epóxido (urea). En contraste, todos los compuestos reivindicados tienen carbonos Sp<sup>2</sup> o Sp- hibridados a excepción de los compuestos 30-32, que tienen un grupo heterocíclico extendido que sustituye a la unidad de olefina que se encuentra en los otros compuestos. **Nada en D2 o sugiere esta diferencia fundamental en la estructura.**
- d. El nbAUDA descrito en D2 no contiene ningún heteroátomo (N, S, O) en la cadena principal de carbono. En contraste, muchos de los compuestos reivindicados sí contienen heteroátomos en la cadena principal de carbono. **Nada en D2 o sugiere esta diferencia fundamental en la estructura.**
- e. El nbAUDA descrito en D2 depende de un mecanismo de la biosíntesis de funcionamiento EET para ser eficaz. En contraste, los compuestos reivindicados son eficaces incluso si la biosíntesis de EET no está funcionando. **Nada en D2 o sugiere esta diferencia crítica.**
- f. D2 no deja claro si la disminución en el daño renal se debe a un aumento en los niveles de 14,15-EET. AUDA es un inhibidor hidrolasa epóxido soluble y aumenta la relación (ratio) de epóxido a diol. Esto es cierto para todos los epóxidos de ácidos grasos y podría ser cierto para otros epóxidos ácido no graso. Todo lo que se puede concluir con certeza en D2 es que la inhibición de la enzima SEH por AUDA tuvo un efecto protector renal. **Nada en D2 enseña o sugiere los compuestos reivindicados, teniendo dichas estructuras tan diferentes a las reivindicadas.**
3. **Nada en D1 o D2, ya sea solo o en combinación, enseña o sugiere los compuestos reivindicados. Este rechazo debe ser en base a los argumentos anteriores.**

**II. En relación a la objeción de ausencia de efecto inesperado basado en falta de pruebas novedosas contenidas en la descripción:**

**1. El Examinador expone lo siguiente:**

*El efecto técnico observado es que los compuestos de la presente solicitud se emplean como agonistas de los ácidos epoxieicosatrienoicos (EET) efectos contra hipertensión y enfermedad cardiovascular y neural relacionada; la descripción enseña efectos encontrados de 3análogos de EET en cuanto a mejora en la presión sanguínea, frecuencia cardíaca, en el tratamiento de la nefrotoxicidad de cisplatina, disminución en la disfunción renal inducida por cisplatina, disminución de la tensión oxidativa renal inducida por cisplatina, respuesta inflamatoria y tensión del retículo endoplasmático. No obstante, estos ensayos no se realizaron en todos los compuestos que se reivindican, por lo cual no se puede asegurar que todos los compuestos reclamados van a tener los mismos efectos mejorados, de modo que el efecto logrado no es inesperado (énfasis añadido).*

2. Los datos proporcionados en la solicitud presentada, son representativos de los efectos de todos los compuestos reivindicados. Específicamente, los resultados que se describen en los Ejemplos son representativos de todos los compuestos. Además, la descripción de la solicitud presentada, deja claro para un experto en la técnica cómo hacer y usar todos los compuestos reivindicados a fin de lograr los mismos efectos mejorados que se muestran en los ejemplos.
3. Por ejemplo, el lenguaje en el Ejemplo 6 establece claramente que los compuestos descritos en el Ejemplo 6 son representativos de los compuestos reivindicados y que todos los compuestos reivindicados tendrán los mismos efectos mejorados como se muestra. Además, adjunto sírvase encontrar una publicación reciente de los inventores que muestran los diversos compuestos reivindicados en uso. En concreto, la Tabla 1 muestra la máxima relajación y ED50 de la relajación de la arteria coronaria bovina para los compuestos. Lo anterior indica que cada compuesto tiene la actividad esperada.

4. Para mayor claridad se presenta una traducción al Español de la tabla mencionada:

Tabla 1. Vaso-relajación De Arteria Coronaria Bovina Precontraída E Inhibición In Vitro De Hidrolasa Epóxido Soluble Recombinante Humana<sup>a,b</sup>

| Comp | Análogo | Relajación Vascular sEHi |                                |                          | Comp | Análogo | Relajación Vascular sEHi |                                |                          |
|------|---------|--------------------------|--------------------------------|--------------------------|------|---------|--------------------------|--------------------------------|--------------------------|
|      |         | %<br>(10 $\mu$ M)        | EC <sub>50</sub><br>( $\mu$ M) | IC <sub>50</sub><br>(nM) |      |         | %<br>(10 $\mu$ M)        | EC <sub>50</sub><br>( $\mu$ M) | IC <sub>50</sub><br>(nM) |
| 1    |         | 86                       | 2.9                            | >500                     | 18   |         | 74                       | 1.0                            | >500                     |
| 2    |         | 72                       | 5.1                            | 255                      | 19   |         | 119                      | 0.18                           | 11                       |
| 3    |         | 104                      | 1.5                            | >500                     | 20   |         | 110                      | 1.1                            | 32                       |
| 4    |         | 95                       | 1.9                            | >500                     | 21   |         | 96                       | 1.7                            | >500                     |
| 5    |         | 92                       | 2.75                           | >500                     | 22   |         | 89                       | 1.1                            | 65                       |
| 6    |         | 91                       | 1.6                            | 392                      | 23   |         | 109                      | 0.34                           | 10                       |
| 7    |         | 73                       | 6.0                            | 41                       | 24   |         | 109                      | 0.32                           | >500                     |
| 8    |         | 61                       | 6.7                            | 71                       | 25   |         | 116                      | 0.36                           | >500                     |
| 9    |         | 75                       | 3.4                            | 32                       | 26   |         | 93                       | 3.3                            | 31                       |
| 10   |         | 71                       | >10                            | >500                     | 27   |         | 54                       | 2.4                            | 231                      |
| 11   |         | 63                       | 7.6                            | 57                       | 28   |         | 47                       | >10                            | 57                       |
| 12   |         | 48                       | >10                            | 6                        | 29   |         | 73                       | 3.3                            | 282                      |
| 13   |         | 78                       | 5.0                            | 11                       | 30   |         | 96                       | 1.3                            | >500                     |
| 14   |         | 85                       | 3.7                            | 22                       | 31   |         | 73                       | 0.9                            | >500                     |
| 15   |         | 53                       | 9.8                            | 32                       | 32   |         | 96                       | 2.4                            | >500                     |
| 16   |         | 92                       | 3.5                            | 96                       |      |         |                          |                                |                          |
| 17   |         | 92                       | 3.1                            | 23                       |      |         |                          |                                |                          |

<sup>a</sup>A 10  $\mu$ M, 14, 15 – EET induce 85% de vaso

– relajación y su ED<sub>50</sub> es 2.2  $\mu$ M. Para sEH humano recombinante, el IC<sub>50</sub> para ácido 12

– (3 – adamantan – 1il

– ureido)dodecanoico (AUDA) es 3nM. <sup>b</sup> Determinaciones Biossary (n) = 35.

En vista de lo presentado anteriormente, se puede concluir que no se puede deducir a partir de los documentos D1 y D2 de manera evidente las características técnicas esenciales reveladas por la presente solicitud, y por ende la misma posee novedad y nivel inventivo sobre el estado de la técnica presentado por el Examinador en el oficio mencionado anteriormente. En consecuencia no deben serle negados los derechos de patentabilidad a la presente invención.

### Conclusión

En consecuencia, atentamente me permito solicitar a ese Despacho, se sirva **tener en cuenta los argumentos aquí expuestos** y con base en ellos, revocar la decisión contenida en la Resolución impugnada No. **72827** y conceder el privilegio de patente solicitado para las reivindicaciones 1-5 de la presente invención, toda vez que **ya se ha demostrado que las misma cumplen con los requisitos de claridad, novedad, nivel inventivo y aplicación industrial** que exige la normativa Andina en materia de patentes.

### Anexos

- Capítulo reivindicatorio de la solicitud.
- Copia de la opinión escrita de la búsqueda internacional (ISA) de la solicitud de patente.
- Publicación reciente de los inventores que muestran los diversos compuestos reivindicados en uso. 14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates: Carboxylate Modifications. Journal of Medicinal Chemistry. Autores: John R. Falck,\*, Sreenivasulu Reddy Koduru, Seetaram Mohapatra, Rajkumar Manne, Raju Atcha, Vijaya L. Manthathi, Jorge H. Capdevila, Sarah Christian,§ John D. Imig,§ and William B. Campbell§. Acceso desde: 13 Agosto de 2015.

Atentamente,



Luz Helena Adarve Gómez

C.C. 41.575.435 de Bogotá

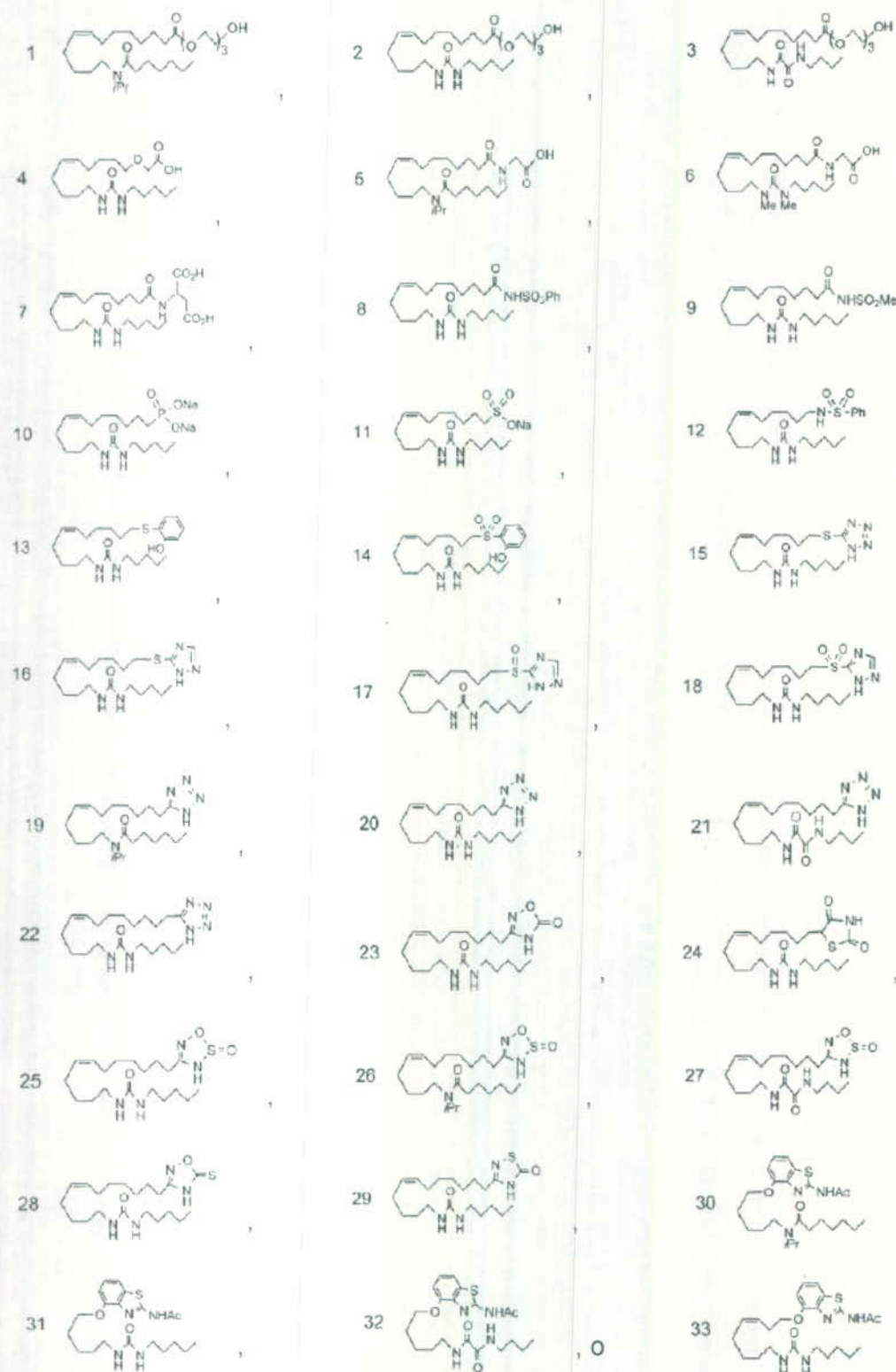
T. P. 14.884 del CSJ

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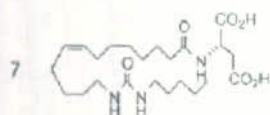
ABM/ P-759

## CAPÍTULO REIVINDICATORIO

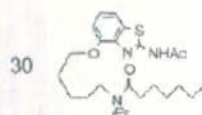
1. Un compuesto seleccionado del grupo que consiste de



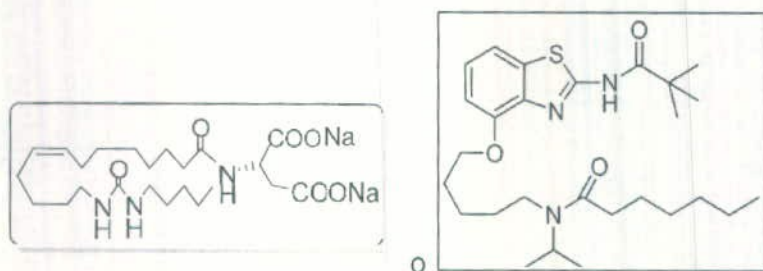
2. El compuesto de la reivindicación 1, en donde la estructura es



3. El compuesto de la reivindicación 1, en donde la estructura es



4. Un compuesto seleccionado del grupo que consiste de



5. Una composición que comprende un compuesto de las reivindicaciones 1 o 4 y un portador farmacéuticamente aceptable.

## PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY  
(PCT Rule 43bis.1)

Date of mailing  
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference  
see form PCT/ISA/220

**FOR FURTHER ACTION**  
See paragraph 2 below

International application No.  
PCT/US2012/032090

International filing date (day/month/year)  
04.04.2012

Priority date (day/month/year)  
06.04.2011

International Patent Classification (IPC) or both national classification and IPC  
INV. C07C233/46 C07C233/56 C07C275/20 A61P9/12 A61K31/17 A61K31/16

Applicant  
MCW RESEARCH FOUNDATION, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



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Date of completion of  
this opinion

see form  
PCT/ISA/210

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**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/US2012/032090

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**Box No. I Basis of the opinion**

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1. With regard to the **language**, this opinion has been established on the basis of:
  - ☒ the international application in the language in which it was filed
  - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
  - a. (means)
    - ☐ on paper
    - ☐ in electronic form
  - b. (time)
    - ☐ in the international application as filed
    - ☐ together with the international application in electronic form
    - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

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**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

|                               |             |             |
|-------------------------------|-------------|-------------|
| Novelty (N)                   | Yes: Claims | <u>1-15</u> |
|                               | No: Claims  |             |
| Inventive step (IS)           | Yes: Claims | <u>1-15</u> |
|                               | No: Claims  |             |
| Industrial applicability (IA) | Yes: Claims | <u>1-15</u> |
|                               | No: Claims  |             |

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/US2012/032090

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**Box No. VII Certain defects in the international application**

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The following defects in the form or contents of the international application have been noted:

see separate sheet

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**Box No. VIII Certain observations on the international application**

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The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)

International application No.

PCT/US2012/032090

Re Item V

Reference is made to the following documents:

- D1 FALCK J R ET AL: "14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates Containing Epoxide Bioisosteres: Influence upon Vascular Relaxation and Soluble Epoxide Hydrolase Inhibition", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 52, no. 16, 27 August 2009 (2009-08-27), pages 5069-5075, XP002548924, ISSN: 0022-2623, DOI: 10.1021/JM900634W [retrieved on 2009-08-04]
- D2 PARRISH, A.R. ET AL: "Attenuation of cisplatin nephrotoxicity by inhibition of soluble epoxide hydrolase", CELL. BIOL. TOXICOL., vol. 25, 2007, pages 217-225,

The patentability of claims 5, 8-10 and 13 is dependent upon their formulation. The EPO does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Patentability, in particular novelty and inventive step, of claims 5, 8-10 and 13 has been assessed on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

The present application concerns epoxyeicosatrienoic acid analogues and their use in the treatment of hypertension and nephrotoxicity.

D1 reports 14,15- epoxyeicosa-5,8,11- trienoic acid surrogates and their use in the treatment of hypertension. The compounds of D1 differ from the one of the application in their structure. The content of claims 1-15 is therefore regarded as novel.

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2012/032090

D2 reports the use of the n-butyl ester of 12-(3-adamantan-1-yl-ureiido)-dodecanoic acid to attenuate cisplatin-induced nephrotoxicity. The compounds of the present application are not disclosed in D2. The content of claims 1-15 is therefore novel over D2.

D1 is regarded as closest prior art for the assessment of inventive step of claims 1-7.

The difference between D1 and the present application has been highlighted above. The effect caused by this difference is unknown. The remaining objective problem underlying the content of claims 1-7 is therefore identified in the provision of alternative epoxyeicosatrienoic acid analogues to use in the treatment of hypertension. The compounds of D1 differ in the nature of the both radicals attached to the urea nitrogens. As such analogues are not in any way suggested in D1, the solution proposed by the applicant is regarded as inventive.

D2 is regarded as closest prior art for the assessment of inventive step of claims 1-5 and 8-15.

The difference between D2 and the present application has been highlighted above. The effect caused by this difference is unknown. The remaining objective problem underlying the content of claims 1-5 and 8-15 is therefore identified in the provision of alternative epoxyeicosatrienoic acid analogues to use in the treatment of nephrotoxicity. The compounds of the application differ from the n-butyl ester of 12-(3-adamantan-1-yl-ureiido)-dodecanoic acid disclosed in D2 in the nature of both radicals attached to the urea nitrogen. As such analogues are not in any way suggested in D2, the solution proposed by the applicant is regarded as inventive.

**Re Item VII**

The vague and imprecise statements in the description in paragraphs 1 and 297 imply that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them.

Contrary to the requirements of Rule 5.1 (a) (ii) PCT, the relevant background art disclosed in D1 and D2 is not mentioned in the description, nor are these documents identified therein.

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)

International application No.

PCT/US2012/032090

Re Item VIII

Although claims 1 and 4 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.

## PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

**PCT**

To:

Rabe, Ann E.  
 QUARLES & BRADY LLP  
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 ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF  
 THE INTERNATIONAL SEARCH REPORT AND  
 THE WRITTEN OPINION OF THE INTERNATIONAL  
 SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

|                                                       |                                                                         |
|-------------------------------------------------------|-------------------------------------------------------------------------|
|                                                       | Date of mailing<br>(day/month/year) 25 July 2012 (25-07-2012)           |
| Applicant's or agent's file reference<br>650053.00227 | <b>FOR FURTHER ACTION</b> See paragraphs 1 and 4 below                  |
| International application No.<br>PCT/US2012/032090    | International filing date<br>(day/month/year) 4 April 2012 (04-04-2012) |
| Applicant<br>MCW RESEARCH FOUNDATION, INC.            |                                                                         |

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

**Filing of amendments and statement under Article 19:**

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

**When?** The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

**Where?** Directly to the International Bureau of WIPO, 34 chemin des Colombettes  
 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

**For more detailed instructions, see PCT Applicant's Guide, International Phase, paragraphs 9.004 - 9.011.**

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

**4. Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see [www.wipo.int/pct/en/texts/time\\_limits.html](http://www.wipo.int/pct/en/texts/time_limits.html) and the *PCT Applicant's Guide, National Chapters*.

|                                                                                                                                                                                                                                                                                    |                                                                        |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Name and mailing address of the International Searching Authority<br> European Patent Office, P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk<br>Tel. (+31-70) 340-2040<br>Fax: (+31-70) 340-3016 | Authorized officer<br>MONTALVILLO, Almudena<br>Tel: +31 (0)70 340-4823 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2012/032090

| <p>A. CLASSIFICATION OF SUBJECT MATTER</p> <p>INV. C07C233/46 C07C233/56 C07C275/20 A61P9/12 A61K31/17</p> <p>A61K31/16</p> <p>ADD.</p> <p>According to International Patent Classification (IPC) or to both national classification and IPC</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                             |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------------|-----------------------|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols)</p> <p>C07C A61K A61P</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)</p> <p>EP0-Internal</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                             |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| <p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>A</td> <td> <p>FALCK J R ET AL:</p> <p>"14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates Containing Epoxide Bioisosteres: Influence upon Vascular Relaxation and Soluble Epoxide Hydrolase Inhibition",</p> <p>JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US,</p> <p>vol. 52, no. 16,</p> <p>27 August 2009 (2009-08-27), pages 5069-5075, XP002548924,</p> <p>ISSN: 0022-2623, DOI: 10.1021/JM900634W [retrieved on 2009-08-04]</p> <p>the whole document</p> <p>-----</p> <p>-/--</p> </td> <td>1-15</td> </tr> </tbody> </table>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                             | Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | A | <p>FALCK J R ET AL:</p> <p>"14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates Containing Epoxide Bioisosteres: Influence upon Vascular Relaxation and Soluble Epoxide Hydrolase Inhibition",</p> <p>JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US,</p> <p>vol. 52, no. 16,</p> <p>27 August 2009 (2009-08-27), pages 5069-5075, XP002548924,</p> <p>ISSN: 0022-2623, DOI: 10.1021/JM900634W [retrieved on 2009-08-04]</p> <p>the whole document</p> <p>-----</p> <p>-/--</p> | 1-15 |
| Category*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                       | Relevant to claim No.                                                       |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <p>FALCK J R ET AL:</p> <p>"14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates Containing Epoxide Bioisosteres: Influence upon Vascular Relaxation and Soluble Epoxide Hydrolase Inhibition",</p> <p>JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US,</p> <p>vol. 52, no. 16,</p> <p>27 August 2009 (2009-08-27), pages 5069-5075, XP002548924,</p> <p>ISSN: 0022-2623, DOI: 10.1021/JM900634W [retrieved on 2009-08-04]</p> <p>the whole document</p> <p>-----</p> <p>-/--</p> | 1-15                                                                        |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| <p><input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                             |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                             |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| <p>Date of the actual completion of the international search</p> <p>10 July 2012</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <p>Date of mailing of the international search report</p> <p>25/07/2012</p> |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |
| <p>Name and mailing address of the ISA/<br/>European Patent Office, P.B. 5818 Patentlaan 2<br/>NL - 2280 HV Rijswijk<br/>Tel. (+31-70) 340-2040,<br/>Fax: (+31-70) 340-3016</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <p>Authorized officer</p> <p>Tabanella, Stefania</p>                        |           |                                                                                    |                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |      |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/032090

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                             | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | <p>PARRISH, A.R. ET AL: "Attenuation of cisplatin nephrotoxicity by inhibition of soluble epoxide hydrolase",<br/>CELL. BIOL. TOXICOL.,<br/>vol. 25, 2007, pages 217-225, XP002679542,<br/>the whole document</p> <p>-----</p> | 1-15                  |

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

|                                                       |                                                                                                  |                                                         |
|-------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| Applicant's or agent's file reference<br>650053.00227 | <b>FOR FURTHER ACTION</b><br>see Form PCT/ISA/220<br>as well as, where applicable, item 5 below. |                                                         |
| International application No.<br>PCT/US2012/032090    | International filing date (day/month/year)<br>04/04/2012                                         | (Earliest) Priority Date (day/month/year)<br>06/04/2011 |
| Applicant<br>MCW RESEARCH FOUNDATION, INC.            |                                                                                                  |                                                         |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

## 1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed  
☐ a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☐ **Unity of invention is lacking** (see Box No. III)

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant  
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant  
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. 1  
☒ as suggested by the applicant  
☐ as selected by this Authority, because the applicant failed to suggest a figure  
☐ as selected by this Authority, because this figure better characterizes the invention  
b. ☐ none of the figures is to be published with the abstract

## 14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates: Carboxylate Modifications

John R. Falck,<sup>\*,†</sup> Sreenivasulu Reddy Koduru,<sup>†</sup> Seetaram Mohapatra,<sup>†</sup> Rajkumar Manne,<sup>†</sup> Raju Atcha,<sup>†</sup> Vijaya L. Manthathi,<sup>†</sup> Jorge H. Capdevila,<sup>‡</sup> Sarah Christian,<sup>§</sup> John D. Imig,<sup>§</sup> and William B. Campbell<sup>§</sup>

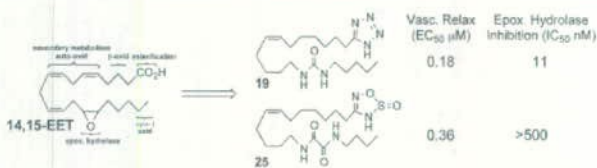
<sup>†</sup>Departments of Biochemistry and Pharmacology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390, United States

<sup>‡</sup>Departments of Medicine and Biochemistry, Vanderbilt University Medical School, Nashville, Tennessee 37232, United States

<sup>§</sup>Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States

### Supporting Information

**ABSTRACT:** The cytochrome P450 eicosanoid 14,15-epoxyeicosa-5,8,11-trienoic acid (14,15-EET) is a powerful endogenous autacoid that has been ascribed an impressive array of physiologic functions including regulation of blood pressure. Because 14,15-EET is chemically and metabolically labile, structurally related surrogates containing epoxide bioisosteres were introduced and have become useful in vitro pharmacologic tools but are not suitable for in vivo applications. A new generation of EET mimics incorporating modifications to the carboxylate were prepared and evaluated for vasorelaxation and inhibition of soluble epoxide hydrolase (sEH). Tetrazole **19** (ED<sub>50</sub> 0.18  $\mu$ M) and oxadiazole-5-thione **25** (ED<sub>50</sub> 0.36  $\mu$ M) were 12- and 6-fold more potent, respectively, than 14,15-EET as vasorelaxants; on the other hand, their ability to block sEH differed substantially, i.e., 11 vs >500 nM. These data will expedite the development of potent and specific in vivo drug candidates.



## INTRODUCTION

An imposing body of studies,<sup>1</sup> spanning more than three decades, has cogently elucidated the involvement of epoxyeicosatrienoic acids (EETs) in a wide array of critical physiological functions, inter alia, blood pressure regulation,<sup>2</sup> nociception,<sup>3</sup> adipogenesis,<sup>4</sup> anti-inflammatory activity,<sup>5</sup> organ regeneration,<sup>6</sup> insulin potentiation,<sup>7</sup> podocyte integrity,<sup>8</sup> and cellular responses to bacterial infection.<sup>9</sup> The most prominent regioisomer,<sup>10</sup> 14,15-epoxyeicosa-5(Z),8(Z),11(Z)-trienoic acid (14,15-EET), along with other members of this autacoid family, arise via metabolism of arachidonic acid by cytochromes P450, especially members of the 2C<sup>11</sup> and 2J<sup>12</sup> families. The ratio of EET isomers and their stereocomposition are CYP P450 isoform-dependent<sup>13</sup> and, thus, species- and tissue-specific.<sup>14</sup>

Numerous chemical and metabolic factors conspire to complicate investigations into the roles of 14,15-EET and restrain its potential therapeutic utility (Figure 1).<sup>15</sup> Its susceptibility toward aerial oxidation or auto-oxidation, i.e., a nonenzymatic, free radical process involving the 1,4-dienyl

substructures characteristic of polyunsaturated fatty acids, necessitates handling and/or storage under strict conditions that minimize exposure to oxygen and trace transition metals.<sup>16</sup> A precis of inactivating enzymatic processes includes esterification,<sup>17</sup> metabolism by other pathways of the arachidonate cascade,<sup>18</sup> conjugation,<sup>19</sup>  $\beta$ -oxidation,<sup>20</sup> chain elongation,<sup>21</sup> and hydrolysis of the epoxide.<sup>22</sup> In biological milieu, hydration of the epoxide by soluble epoxide hydrolase (sEH) is a major determinant in maintaining the steady state levels of 14,15-EET,<sup>23</sup> whose half-life has been estimated at no more than seconds to minutes.<sup>24</sup> The pioneering studies of Hammock<sup>25</sup> and others<sup>26</sup> confirmed inhibition of sEH can elevate EET levels both in vitro and in vivo, thus offering an indirect means for pharmacologic intervention in EET-mediated processes. However, this strategy might prove less efficacious than an agonist replacement therapy whenever endogenous EET biosynthesis is compromised, for instance, as a consequence of disease, inflammation, radiotherapy, aging, and/or exposure to xenobiotics and drugs that inhibit cytochromes P450.<sup>27</sup>

## RESULTS AND DISCUSSION

To address some of the stability limitations of natural EETs, our laboratories previously prepared several iterations of EET surrogates with improved stability.<sup>28,29</sup> Advanced versions were

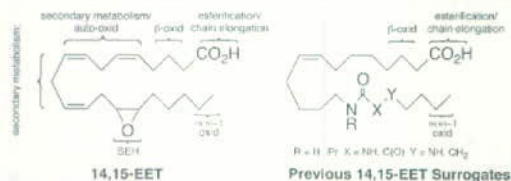


Figure 1. Major routes of metabolism/degradation (oxid = oxidation).

Table 1. Vasorelaxation of Precontracted Bovine Coronary Artery and in Vitro Inhibition of Recombinant Human Soluble Epoxide Hydrolase<sup>a,b</sup>

| Compd | Analog | Vascular Relax. |                  | sEH <sup>i</sup> | Compd | Analog | Vascular Relax. |                  | sEH <sup>i</sup> |
|-------|--------|-----------------|------------------|------------------|-------|--------|-----------------|------------------|------------------|
|       |        | %               | EC <sub>50</sub> | IC <sub>50</sub> |       |        | %               | EC <sub>50</sub> | IC <sub>50</sub> |
|       |        | (10 $\mu$ M)    | ( $\mu$ M)       | (nM)             |       |        | (10 $\mu$ M)    | ( $\mu$ M)       | (nM)             |
| 1     |        | 86              | 2.9              | >500             | 18    |        | 74              | 1.0              | >500             |
| 2     |        | 72              | 5.1              | 255              | 19    |        | 119             | 0.18             | 11               |
| 3     |        | 104             | 1.5              | >500             | 20    |        | 110             | 1.1              | 32               |
| 4     |        | 95              | 1.9              | >500             | 21    |        | 96              | 1.7              | >500             |
| 5     |        | 92              | 2.75             | >500             | 22    |        | 89              | 1.1              | 65               |
| 6     |        | 91              | 1.6              | 392              | 23    |        | 109             | 0.34             | 10               |
| 7     |        | 73              | 6.0              | 41               | 24    |        | 109             | 0.32             | >500             |
| 8     |        | 61              | 6.7              | 71               | 25    |        | 116             | 0.36             | >500             |
| 9     |        | 75              | 3.4              | 32               | 26    |        | 93              | 3.3              | 31               |
| 10    |        | 71              | >10              | >500             | 27    |        | 54              | 2.4              | 231              |
| 11    |        | 63              | 7.6              | 57               | 28    |        | 47              | >10              | 57               |
| 12    |        | 48              | >10              | 6                | 29    |        | 73              | 3.3              | 282              |
| 13    |        | 76              | 5.0              | 11               | 30    |        | 96              | 1.3              | >500             |
| 14    |        | 85              | 3.7              | 22               | 31    |        | 73              | 0.9              | >500             |
| 15    |        | 53              | 9.8              | 32               | 32    |        | 96              | 2.4              | >500             |
| 16    |        | 92              | 3.5              | 96               |       |        |                 |                  |                  |
| 17    |        | 92              | 3.1              | 23               |       |        |                 |                  |                  |

<sup>a</sup>At 10  $\mu$ M, 14,15-EET induces 85% of maximum vasorelaxation and its ED<sub>50</sub> is 2.2  $\mu$ M. For recombinant human sEH, the IC<sub>50</sub> for 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) is 3 nM. <sup>b</sup>Bioassay determinations ( $n$ ) = 3–5.

evaluated for relaxation of precontracted bovine coronary artery rings and for in vitro inhibition of recombinant human sEH.<sup>15</sup> Depending upon the bioisostere and its position along the carbon chain, varying levels of vascular relaxation and/or sEH inhibition were observed. Generally, oxamides and *N*-*i*Pr-amides displayed useful 14,15-EET agonist activities but were modest to poor sEH inhibitors. Unsubstituted ureas proved to be both potent 14,15-EET agonists and sEH inhibitors. The in vitro success of this generation of analogues prompted us to consider further structural iterations.<sup>30</sup>

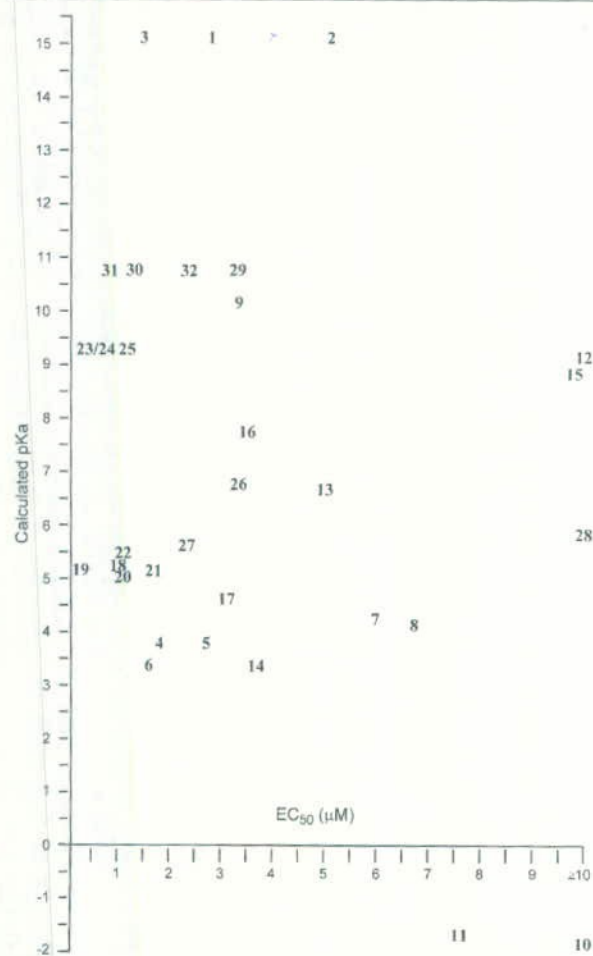
The surrogates described herein modify the free carboxylic acid of the previous generation of 14,15-EET mimics while, for the most part, retaining some key structural features of the pharmacophore identified earlier (i.e., *cis*- $\Delta^{8,9}$ -olefin and an epoxide bioisostere). They were evaluated as described before in precontracted bovine coronary artery rings for (i) % vasorelaxation at 10  $\mu$ M relative to a 14,15-EET control, (ii) EC<sub>50</sub> for vasorelaxation, and (iii) IC<sub>50</sub> for sEH inhibition (Table 1).<sup>15</sup> Interestingly, the simple expedient of conjugating the carboxylate with a short poly(ethylene oxide) (PEG) unit improved both % vasorelaxation and the EC<sub>50</sub> somewhat

compared with the parent free acids<sup>31</sup> regardless the type of epoxide bioisostere, viz., *N*-*i*Pr-amide **1**, urea **2**, and oxamide **3**; on the other hand, the soluble epoxide hydrolase (sEH) inhibitory activity of urea **2** was significantly compromised versus the parent free acid ( $EC_{50}$  7.5  $\mu$ M,  $IC_{50}$  46 nM). Linkage of the carboxylate to the nitrogen of glycine (**4** and **5**) or aspartic acid (**6**) resulted in a modest boost to the vasoactivities while the sEH  $IC_{50}$  of **6** dropped by an order of magnitude compared to the parent free acid. Conversion to the *N*-phenyl and *N*-methylsulfonimides **7** and **8**, respectively, left the biological activities virtually unchanged in all categories while the simplified phenylsulfonamide **9** led to an improvement in the  $EC_{50}$  by a factor of  $\sim 2$  and smaller improvements in the other parameters.

Given the generally lackluster behavior of the esters and amides, our focus changed to replacement of the carboxylate with a variety of heterocyclic bioisosteres identified from literature sources.<sup>32</sup> For phosphonate **10** and sulfonate **11**, the increase in polarity did not improve potency as seen with the PEG esters. Interestingly, sulfonate **11** retained its ability to suppress sEH, in contrast to phosphonate **10** and PEG ester **2**. Vasorelaxation by *S*-alkylthiocatechol **12** was poor, but its  $IC_{50}$  for sEH was pushed down into the single digits.<sup>33</sup> The sulfone variant **13** regained some vasopotency, probably due to an increase in the acidity of the phenol but not sufficiently to be viable. Replacement of the phenol moiety in **12** with a tetrazole ring<sup>34</sup> produced **14** and was encouraging, but this trend was not continued in triazole **15**. Sequential oxidation of the sulfur to sulfoxamide **16** and sulfonamide **17**, as seen for **12**  $\rightarrow$  **13**, incrementally improved the  $EC_{50}$ . The  $ED_{50}$  jumped in the tetrazole bioisostere series **18**–**21**, achieving a submicromolar value for urea **19** (12-fold better than 14,15-EET), while the % vasorelaxation versus 14,15-EET peaked. Factoring in a very respectable low nanomolar  $IC_{50}$ , **19** is the best dual activity analogue in the study and a leading candidate for further development. Notably, all three parameters began to erode, albeit minimally, in the one-carbon homologue **20** and further in oxamide **21**. The performance of another five-membered heterocycle,<sup>35</sup> oxadiazol-5-one **22**, while acceptable, was not comparable to urea **19**. The closely related oxathiadiazole-2-oxides<sup>35</sup> **23** and **24**, on the other hand, demonstrated good vasorelaxant activities; sEH inhibition potency was consistent with our previous observations that ureas  $\gg$  *N*-*i*Pr-amides. The isomeric thioxo-1,2,4-oxadiazol<sup>35</sup> **25** registered a 6-fold improvement in  $ED_{50}$  over 14,15-EET yet was a comparatively poor sEH inhibitor and, thus, is a good option whenever an EET agonist with minimal impact on sEH function is required. The corresponding urea thioxo-oxadiazol,<sup>36</sup> **26**, could not sustain the vasopotency; its  $ED_{50}$  sagged by almost an order of magnitude versus **25** while at the same time its sEH  $IC_{50}$  improved by more than an order of magnitude. A final variant on this heterocyclic theme, 5-thioxothiadiazol<sup>36</sup> **27**, fell short of expectations on all levels and further study of this particular heterocycle was terminated. Despite its success in the glitazone series of antidiabetic drugs,<sup>37</sup> the 2,4-thiazolidinedione modification proved disappointing when incorporated into **28**. Given the larger size of the *N*-(4-hydroxy-2-benzothiazolyl)acetamide bioisostere,<sup>38</sup> the carbon chain utilized in most of the analogues (Figure 1) was trimmed by four carbons before attachment via the phenolic oxygen to generate **29**. The undistinguished results caused us to re-evaluate our assumptions. Following inspection of molecular models, the carbon chain was shortened even further and the

olefin was deleted, leading to *N*-*i*Pr-amide **30**, urea **31**, and oxamide **32**, whose total chain length more closely resembled that of 14,15-EET. As hoped, the  $EC_{50}$  values increased relative to **29**, although the % vasorelaxation was variable. All were comparatively poor sEH inhibitors, including unexpectedly urea **31**.

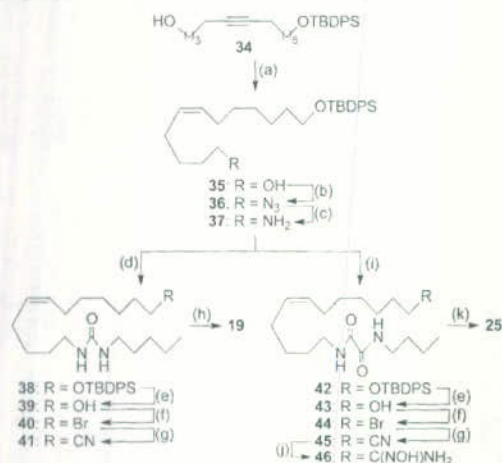
Contrary to expectations, there was no evident correlation between the  $pK_a$  of the EET analogues and the  $ED_{50}$  for vasorelaxation (Figure 2), suggesting factors other than ionic interactions are involved in binding the carboxylate at the putative EET binding site.



**Figure 2.** Plot of calculated  $pK_a$  vs  $ED_{50}$  ( $\mu$ M) of analogues in Table 1. The protonated form of analogues **6**, **11**, and **12** was used for the calculation.

**Chemistry.** The syntheses of tetrazole **19** and thioxo-1,2,4-oxadiazol **25** are summarized in Scheme 1 and illustrate the approach used to prepare the other analogues. Semihydrogenation of the known<sup>39</sup> acetylene **34** using P-2 nickel and  $H_2$  led to *cis*-olefin **35** that was subjected to azidation using diphenylphosphoryl azide (DPPA) under Mitsunobu conditions. Staudinger reduction of the product, azide **36**, led to primary amine **37** that was reacted without purification with *n*-pentyl isocyanate to furnish urea **38**. An uneventful series of functional group interchanges proceeding through alcohol **39**, bromide **40**, and ending with nitrile **41** set the stage for the zinc

**Scheme 1. Synthesis of Representative 14,15-EET Analogues<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (a) P2-Ni/(H<sub>2</sub>NCH<sub>2</sub>)<sub>2</sub>, H<sub>2</sub> (1 atm), EtOH, rt, 1 h (96%); (b) DIAD/Ph<sub>3</sub>P/Ph<sub>3</sub>P(O)N<sub>3</sub>, THF, -20 to 23 °C, 4 h (72%); (c) Ph<sub>3</sub>P, H<sub>2</sub>O/THF, rt, 12 h (76%); (d) C<sub>6</sub>H<sub>11</sub>NCO, THF, rt, 3 h (76%); (e) nBu<sub>4</sub>NF, THF, rt, 12 h (82–89%); (f) CBr<sub>4</sub>/Ph<sub>3</sub>P, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h (83–84%); (g) KCN, DMSO, rt, 12 h (78–81%); (h) NaN<sub>3</sub>/ZnBr<sub>2</sub>, iPrOH/H<sub>2</sub>O (1:3), 110 °C, 18 h (76%); (i) HO-(CO)<sub>2</sub>NH(CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>, EDCI, DMF, rt, 12 h (73%); (j) H<sub>2</sub>NOH/Na<sub>2</sub>CO<sub>3</sub>, MeOH/H<sub>2</sub>O (4:1), 60 °C, 18 h (62%); (k) Im<sub>2</sub>C(S), THF, rt, 45 min (63%).

bromide mediated annulation<sup>40</sup> with sodium azide that delivered tetrazole 19.

Condensation of 37 with 2-(*n*-butylamino)-2-oxoacetic acid<sup>15</sup> gave rise to oxamide 42. Following the same sequence of transformations as described above, 42 was converted into nitrile 45. Condensation of hydroxylamine 46, obtained by addition<sup>41</sup> of hydroxylamine to 45, with 1,1'-(thiocarbonyl)-diimidazole yielded thioxo-1,2,4-oxadiazol 25 as a crystalline solid.

## EXPERIMENTAL SECTION

**General Methods and Materials.** Final compounds were judged ≥95% pure by HPLC using a Zorbax Eclipse C18 column (250 mm × 4.6 mm; Agilent) connected to an Agilent 1200 API/LC-MS using acetonitrile/water combinations as eluent unless otherwise noted. Nuclear magnetic resonance (NMR) spectra were recorded on Varian 300, 400, or 500 spectrometers at operating frequencies of 300/400/500 MHz (<sup>1</sup>H) or 75/100/125 MHz (<sup>13</sup>C) in CDCl<sub>3</sub> with TMS as internal standard, unless otherwise stated. <sup>1</sup>H NMR data are reported as follows: chemical shift (ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, app q = apparent quartet, qn = quintet, app qn = apparent quintet, m = multiplet), and coupling constant (Hz). High resolution mass spectra (HRMS) were obtained at UT-Arlington using a Shimadzu IT-TOF mass spectrometer or at the Medical College of Wisconsin by Prof. Kasem Nithipatikom. Infrared (IR) spectra were obtained using a PerkinElmer Spectrum 1000 Fourier transform spectrometer. Melting points were measured using an OptiMelt from Stanford Research Systems and are uncorrected. Analytical thin layer chromatography (TLC) used EMD Chemicals TLC silica gel 60 F254 plates (0.040–0.063 mm) with visualization by UV light and/or KMnO<sub>4</sub> or phosphomolybdic acid (PMA) solution followed by heating. All oxygen and/or moisture sensitive reactions were performed under an argon atmosphere using oven-dried glassware and anhydrous solvents. Extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtered prior to removal of all volatiles under reduced pressure. Chromatographic purifications utilized preparative

TLC or flash chromatography using prepacked SiO<sub>2</sub> columns on a CombiFlash R<sub>4</sub>200 chromatograph (Teledyne Isco). Unless otherwise noted, yields refer to isolated, purified material with spectral data consistent with assigned structures or, if known, were in agreement with published data. Reagents were purchased at the highest commercial quality available and used without further purification, unless otherwise noted. Anhydrous solvents were dried using a Glass Contours solvent system by passage through columns of activated packing material under argon immediately prior to use.

**1-(12-(1*H*-Tetrazol-5-yl)dodec-5(*Z*)-en-1-yl)-3-pentylurea (19).** A mixture of 1-(12-cyanododec-5(*Z*)-enyl)-3-*n*-pentylurea (41) (500 mg, 1.55 mmol), sodium azide (100 mg, 1.55 mmol), and zinc bromide (335 mg, 1.48 mmol) was heated at 110 °C in 2-propanol/H<sub>2</sub>O (1:3, 8 mL) while stirring vigorously in a sealed tube.<sup>40</sup> After 18 h, the mixture was cooled to room temperature and the pH was adjusted to 1 using aq HCl (3 N, 4 mL). Ethyl acetate (10 mL) was added, and the stirring was continued until no solid was present. The organic layer was isolated and the aqueous layer extracted with EtOAc (2 × 25 mL). The combined organic fractions were washed with water (3 × 25 mL), dried, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the tetrazole 19 (431 mg, 76%) as a colorless solid, mp 205.6–205.8 °C. TLC: 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R*<sub>f</sub> ~ 0.30. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ 5.40–5.30 (m, 2H), 3.06–3.11 (m, 4H), 2.93 (t, *J* = 8.0 Hz, 2H), 1.98–2.10 (m, 4H), 1.70–1.82 (m, 2H), 1.24–1.50 (m, 16H), 0.90 (t, *J* = 7.6 Hz, 3H). <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ 160.16, 156.81, 129.77, 129.47, 39.81, 39.68, 29.88, 29.80, 29.35, 28.99, 28.69, 28.55, 27.48, 26.85, 26.81, 26.68, 22.96, 22.31, 13.22. HRMS calcd for C<sub>19</sub>H<sub>37</sub>N<sub>6</sub>O [M + 1]<sup>+</sup> 365.3029, found 365.3030.

**N1-Butyl-N2-(12-(2-oxido-3*H*-1,2,3,5-oxathiadiazol-4-yl)-dodec-5(*Z*)-en-1-yl)oxalamide (25).** A mixture of N<sup>1</sup>-(13-amino-13-(hydroxymino)tridec-5(*Z*)-enyl)-N<sup>2</sup>-*n*-butyloxalamide (46) (100 mg, 0.27 mmol) and 1,1'-(thiocarbonyl)diimidazole (57 mg, 0.32 mmol) in dry THF (5 mL) was stirred at room temperature for 45 min. The mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with water and dried, and the solvent was evaporated in vacuo. The residue was dissolved in acetonitrile (5 mL) to which was then added DBU (61 mg, 0.40 mmol). After stirring at room temperature for 1 h, the mixture was diluted with water (10 mL), adjusted pH ~ 4 with 1 N HCl, and extracted with ethyl acetate (3 × 10 mL) to give thioxo-1,2,4-oxadiazol 25 (71 mg, 63%) as a white solid, mp 110.6–110.8 °C. TLC: MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:9), *R*<sub>f</sub> ~ 0.55. <sup>1</sup>H NMR (400 MHz) δ 8.90 (br s, NH, 1H), 7.52 (br s, NH, 2H), 5.28–5.40 (m, 2H), 3.20–3.40 (m, 4H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.98–2.10 (m, 4H), 1.21–1.70 (m, 16H), 0.92 (t, *J* = 7.3 Hz, 3H). <sup>13</sup>C NMR (100 MHz) δ 160.12, 160.08, 153.31, 130.62, 129.46, 39.93, 39.85, 31.35, 29.33, 28.94, 28.89, 28.68, 27.02, 26.84, 26.69, 23.96, 20.23, 13.90. HRMS calcd for C<sub>19</sub>H<sub>35</sub>N<sub>4</sub>O<sub>4</sub>S [M + 1]<sup>+</sup> 415.2379, found 415.2372.

**12-(*tert*-Butyldiphenylsilyloxy)dodec-5(*Z*)-en-1-ol (35).** NaBH<sub>4</sub> (82 mg, 2.28 mmol) was added in portions with vigorous stirring to a room temperature solution of Ni(OAc)<sub>2</sub>·4H<sub>2</sub>O (567 mg, 2.28 mmol) in absolute ethanol (20 mL) under a hydrogen atmosphere (1 atm). After 15 min, freshly distilled ethylenediamine (0.30 mL, 4.56 mmol) was added to the black suspension, followed after a further 15 min by a solution of 12-(*tert*-butyldiphenylsilyloxy)-dodec-5-yn-1-ol<sup>39</sup> (34) (4.0 g, 9.16 mmol) in absolute EtOH (10 mL). After 1 h, the reaction mixture was diluted with Et<sub>2</sub>O (20 mL) and passed through a small bed of silica gel. The bed was rinsed with another portion of Et<sub>2</sub>O (5 mL). The combined ethereal filtrates were concentrated under reduced pressure to afford alcohol 35 (3.85 g, 96%) as a colorless oil sufficiently pure to be used directly in the next step. TLC: EtOAc/hexanes (3:7), *R*<sub>f</sub> ~ 0.46. <sup>1</sup>H NMR (300 MHz) δ 7.64–7.68 (m, 4H), 7.34–7.42 (m, 6H), 5.42–5.28 (m, 2H), 3.63 (t, *J* = 6.4 Hz, 4H), 2.08–1.96 (m, 4H), 1.50–1.60 (m, 4H), 1.40–1.24 (m, 10H), 1.04 (s, 9H). <sup>13</sup>C NMR (100 MHz) δ 135.81, 134.40, 130.61, 129.71, 129.60, 127.80, 64.21, 63.14, 32.78, 32.60, 29.98, 29.27, 27.42, 27.14, 27.10, 26.08, 25.92, 19.48. HRMS calcd for C<sub>28</sub>H<sub>43</sub>O<sub>3</sub>Si [M + 1]<sup>+</sup> 439.3032, found 439.3027.

**1-tert-Butyldiphenylsilyloxy-12-azidododec-7(Z)-ene (36).** Diisopropyl azodicarboxylate (DIAD; 1.46 mL, 7.35 mmol) was added dropwise to a  $-20^{\circ}\text{C}$  solution of  $\text{PPh}_3$  (2.10 g, 8.0 mmol) in dry THF (45 mL) under an argon atmosphere. After 10 min, a solution of 12-(tert-butyldiphenylsilyloxy)dodec-5(Z)-en-1-ol (35) (3.20 g, 7.35 mmol) in dry THF (10 mL) was added dropwise. After 30 min, the mixture was warmed to  $0^{\circ}\text{C}$  and diphenylphosphoryl azide (1.58 mL, 7.35 mmol) was added dropwise. After stirring 4 h at rt, the reaction mixture was quenched with water (150 mL) and extracted with EtOAc ( $2 \times 100$  mL). The combined organic extracts were washed with brine (100 mL), dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated under reduced pressure. The residue was purified by  $\text{SiO}_2$  column chromatography eluting with 4% EtOAc/hexane to afford azide 36 (2.45 g, 72%). TLC: EtOAc/hexanes (1:9),  $R_f \sim 0.55$ .  $^1\text{H}$  NMR (400 MHz)  $\delta$  7.64–7.68 (m, 4H), 7.34–7.42 (m, 6H), 5.28–5.42 (m, 2H), 3.70 (t,  $J = 5.8$  Hz, 2H), 3.27 (t,  $J = 6.3$  Hz, 2H), 1.96–2.10 (m, 4H), 1.24–1.64 (m, 12H), 1.04 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  135.84, 134.41, 130.93, 129.75, 129.12, 127.83, 64.22, 51.62, 32.81, 29.93, 29.30, 28.68, 27.46, 27.14, 27.02, 26.90, 25.96, 19.49. IR (neat) 2930, 2783, 2331, 2097, 1106  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{28}\text{H}_{42}\text{N}_3\text{OSi}$  [ $M + 1$ ] $^+$  464.3097, found 464.3099.

**1-tert-Butyldiphenylsilyloxy-12-aminododec-7(Z)-ene (37).** Triphenylphosphine (1.18 g, 4.50 mmol) was added to a stirring solution of 1-tert-butyldiphenylsilyloxy-12-azidododec-7(Z)-ene (36) (1.90 g, 4.10 mmol) in THF (12 mL) containing 10 drops of deionized water. After 12 h, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL), dried, and concentrated in vacuo to give amine 37 (1.36 g, 76%) as a viscous, colorless oil that was used directly in the next reaction without further purification. TLC: MeOH/ $\text{CH}_2\text{Cl}_2$  (1:4),  $R_f \sim 0.25$ .  $^1\text{H}$  NMR (400 MHz)  $\delta$  7.62–7.68 (m, 4H), 7.32–7.40 (m, 6H), 5.30–5.40 (m, 2H), 3.63 (t,  $J = 5.2$  Hz, 2H), 2.62 (t,  $J = 4.8$  Hz, 2H), 1.92–2.06 (m, 4H), 1.40–1.58 (m, 4H), 1.20–1.40 (m, 8H), 1.03 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  135.79, 134.37, 130.42, 129.70, 127.78, 64.19, 42.28, 33.44, 32.77, 29.93, 29.28, 27.40, 27.21, 27.10, 25.92, 19.44. HRMS calcd for  $\text{C}_{28}\text{H}_{44}\text{NOSi}$  [ $M + 1$ ] $^+$  438.3192, found 438.3186.

**1-(12-(tert-Butyldiphenylsilyloxy)dodec-5(Z)-enyl)-3-n-pentylurea (38).** A solution of 1-tert-butyldiphenylsilyloxy-12-aminododec-7(Z)-ene (37) (1.32 g, 3.0 mmol) in THF (5 mL) was added dropwise to a stirring solution of *n*-pentyl isocyanate (0.386 mL, 3.0 mmol) in THF (10 mL). After 3 h stirring at room temperature, all volatiles were removed under reduced pressure and the residue was purified by  $\text{SiO}_2$  column chromatography eluting with 20% EtOAc/hexane to afford urea 38 (1.26 g, 76%) as a viscous oil. TLC: EtOAc/hexanes (2:3),  $R_f \sim 0.40$ .  $^1\text{H}$  NMR (300 MHz)  $\delta$  7.60–7.70 (m, 4H), 7.35–7.42 (m, 6H), 5.28–5.42 (m, 2H), 5.16 (br s,  $-\text{NH}$ , 2H), 3.65 (t,  $J = 6.5$  Hz, 2H), 3.08–3.20 (m, 4H), 1.96–2.08 (m, 4H), 1.22–1.60 (m, 18H), 1.02 (s, 9H), 0.89 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  159.23, 135.80, 134.24, 130.52, 129.74, 129.49, 127.82, 64.22, 40.62, 40.54, 32.80, 30.33, 29.95, 29.37, 29.32, 27.46, 27.34, 27.18, 27.11, 25.97, 22.71, 19.46, 14.29. HRMS calcd for  $\text{C}_{34}\text{H}_{50}\text{N}_2\text{O}_2\text{Si}$  [ $M + 1$ ] $^+$  551.4033, found 551.4032.

**1-(12-Hydroxydodec-5(Z)-enyl)-3-n-pentylurea (39).** A mixture of 1-(12-(tert-butyldiphenylsilyloxy)dodec-5(Z)-enyl)-3-n-pentylurea (38) (1.12 g, 2.0 mmol) and tetra-*n*-butylammonium fluoride (2.20 mL of 1 M soln in THF, 2.2 mmol) in dry THF (10 mL) was stirred at room temperature under an argon atmosphere for 12 h and then evaporated to dryness in vacuo. The residue was dissolved in EtOAc (50 mL) and washed with water (30 mL), brine (30 mL), dried, and evaporated in vacuo. Purification of the residue via  $\text{SiO}_2$  column chromatography gave alcohol 39 (0.56 g, 89%) as a colorless solid, mp  $63.7$ – $63.8^{\circ}\text{C}$ . TLC: EtOAc/hexanes (7:3),  $R_f \sim 0.30$ .  $^1\text{H}$  NMR (300 MHz)  $\delta$  5.25–5.42 (m, 2H), 4.48 (br s,  $-\text{NH}$ , 2H), 3.64 (d,  $J = 6.5$  Hz, 2H), 3.08–3.20 (m, 4H), 1.96–2.14 (m, 4H), 1.22–1.60 (m, 18H), 0.88 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (125 MHz)  $\delta$  159.26, 130.23, 129.62, 63.72, 40.33, 40.29, 32.92, 30.30, 30.26, 29.74, 29.35, 29.13, 27.26, 27.20, 27.13, 25.82, 22.69, 14.27. HRMS calcd for  $\text{C}_{18}\text{H}_{37}\text{N}_2\text{O}_2$  [ $M + 1$ ] $^+$  313.2855, found 313.2857.

**1-(12-Bromododec-5(Z)-enyl)-3-n-pentylurea (40).**  $\text{CBr}_4$  (0.55 g, 1.66 mmol) and  $\text{PPh}_3$  (0.43 g, 1.66 mmol) were added to a  $0^{\circ}\text{C}$

solution of 1-(12-hydroxydodec-5(Z)-enyl)-3-n-pentylurea (39) (0.43 g, 1.38 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL). After 2 h at room temperature, the reaction mixture was concentrated in vacuo and the residue was purified via  $\text{SiO}_2$  column chromatography to give 1-(12-bromododec-5(Z)-enyl)-3-n-pentylurea (40) (0.43 g, 83%) as a solid, mp  $46.7$ – $46.8^{\circ}\text{C}$ . TLC: EtOAc/hexanes (2:3),  $R_f \sim 0.60$ .  $^1\text{H}$  NMR (300 MHz)  $\delta$  5.22–5.42 (m, 2H), 4.40 (br s, 2H), 3.42 (t,  $J = 9.3$  Hz, 2H), 3.10–3.20 (m, 4H), 1.98–2.10 (m, 4H), 1.80–1.90 (m, 2H), 1.25–1.55 (m, 16H), 0.92 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  159.51, 130.14, 129.69, 40.48, 40.39, 34.20, 32.96, 30.34, 29.67, 29.36, 28.58, 28.25, 27.31, 27.27, 27.17, 22.68, 14.26. HRMS calcd for  $\text{C}_{18}\text{H}_{35}\text{BrN}_2\text{O}$  [ $M + 1$ ] $^+$  375.2011, found 375.2014.

**1-(12-Cyanododec-5(Z)-enyl)-3-n-pentylurea (41).** A mixture of potassium cyanide (0.23 g, 3.54 mmol) and 1-(12-bromododec-5(Z)-enyl)-3-n-pentylurea (40) (0.90 g, 2.40 mmol) was stirred in DMSO (5 mL) at room temperature. After 12 h, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate ( $2 \times 50$  mL). The combined organic extracts were washed with water ( $2 \times 25$  mL), brine (25 mL), dried ( $\text{Na}_2\text{SO}_4$ ), and passed through a silica gel column to give nitrile 41 (0.62 g, 81%) as a colorless solid, mp  $56$ – $57^{\circ}\text{C}$ . TLC: EtOAc/hexanes (2:3),  $R_f \sim 0.45$ .  $^1\text{H}$  NMR (300 MHz)  $\delta$  5.29–5.40 (m, 2H), 4.27 (br s,  $-\text{NH}$ , 2H), 3.10–3.20 (m, 4H), 2.34 (t,  $J = 7.0$  Hz, 2H), 1.98–2.08 (m, 4H), 1.24–1.70 (m, 18H), 0.89 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (125 MHz)  $\delta$  159.41, 129.94, 129.86, 120.14, 40.45, 40.35, 30.30, 29.50, 29.33, 28.70, 28.51, 27.26, 27.16, 25.47, 22.66, 17.28, 14.24. IR (neat) 2930, 2281, 2184, 2042, 1936, 1613, 1197, 1042  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{19}\text{H}_{36}\text{N}_2\text{O}$  [ $M + 1$ ] $^+$  322.2858, found 322.2867.

***N*<sup>1</sup>-*n*-Butyl-*N*<sup>2</sup>-(12-(tert-butyldiphenylsilyloxy)dodec-5(Z)-enyl)oxalamide (42).** A mixture of 2-(*n*-butylamino)-2-oxoacetic acid<sup>15</sup> (0.40 g, 2.70 mmol), 1-tert-butyldiphenylsilyloxy-12-aminododec-7(Z)-ene (37) (1.20 g, 2.70 mmol), 1-hydroxybenzotriazole (HOBt; 0.44 g, 3.30 mmol), and [1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride] (EDCI; 0.63 g, 3.30 mmol) in dry DMF (5 mL) was stirred at room temperature overnight. The reaction mixture was quenched with water (30 mL) and extracted into ethyl acetate ( $3 \times 20$  mL). The combined organic extracts were washed with water ( $2 \times 10$  mL) and brine (10 mL), dried, and concentrated in vacuo. The residue was purified by  $\text{SiO}_2$  column chromatography to give *N*<sup>1</sup>-*n*-butyl-*N*<sup>2</sup>-(12-(tert-butyldiphenylsilyloxy)dodec-5(Z)-enyl)oxalamide (42) (1.10 g, 73%). TLC: EtOAc/hexanes (2:3),  $R_f \sim 0.55$ .  $^1\text{H}$  NMR (400 MHz)  $\delta$  8.05 (br s,  $-\text{NH}$ , 2H), 7.66–7.74 (m, 4H), 7.32–7.42 (m, 6H), 5.30–5.42 (m, 2H), 3.67 (t,  $J = 3.9$  Hz, 2H), 3.31 (q,  $J = 5.2$  Hz, 4H), 1.96–2.10 (m, 4H), 1.50–1.64 (m, 6H), 1.22–1.44 (m, 10H), 1.06 (s, 9H), 0.92 (t,  $J = 7.8$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  160.33, 135.80, 134.35, 130.73, 129.74, 129.20, 127.83, 64.17, 39.89, 39.69, 32.79, 31.48, 29.94, 29.29, 29.07, 27.46, 27.23, 27.14, 27.0, 25.96, 20.29, 19.46, 13.96. HRMS calcd for  $\text{C}_{34}\text{H}_{53}\text{N}_3\text{O}_3\text{Si}$  [ $M + 1$ ] $^+$  565.3826, found 565.3824.

***N*<sup>1</sup>-*n*-Butyl-*N*<sup>2</sup>-(12-hydroxydodec-5(Z)-enyl)oxalamide (43).** *N*<sup>1</sup>-*n*-Butyl-*N*<sup>2</sup>-(12-(tert-butyldiphenylsilyloxy)dodec-5(Z)-enyl)oxalamide (42) (1.20 g, 2.12 mmol) was desilylated as described above for 39 to give *N*<sup>1</sup>-*n*-butyl-*N*<sup>2</sup>-(12-hydroxydodec-5(Z)-enyl)oxalamide (43) (0.568 g, 82%) as a colorless solid, mp  $102.8$ – $102.9^{\circ}\text{C}$ . TLC: EtOAc/hexanes (7:3),  $R_f \sim 0.55$ .  $^1\text{H}$  NMR (400 MHz)  $\delta$  7.69 (br s, 2H), 5.20–5.35 (m, 2H), 3.56 (t,  $J = 4.2$  Hz, 2H), 3.26 (q,  $J = 5.6$  Hz, 4H), 2.17 (br s, 1H), 1.95–2.02 (m, 4H), 1.44–1.56 (m, 6H), 1.20–1.40 (m, 10H), 0.87 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  160.15, 130.66, 129.21, 62.98, 39.80, 39.63, 32.93, 31.39, 29.77, 29.18, 28.95, 27.26, 27.0, 26.88, 25.80, 20.18, 13.85. HRMS calcd for  $\text{C}_{18}\text{H}_{35}\text{N}_2\text{O}_3$  [ $M + 1$ ] $^+$  327.2648, found 327.2648.

***N*<sup>1</sup>-(12-Bromododec-5(Z)-enyl)-*N*<sup>2</sup>-*n*-butyloxalamide (44).** *N*<sup>1</sup>-*n*-Butyl-*N*<sup>2</sup>-(12-hydroxydodec-5(Z)-enyl)oxalamide (43) (330 mg, 1.0 mmol) was brominated as described above for 40 to give *N*<sup>1</sup>-(12-bromododec-5(Z)-enyl)-*N*<sup>2</sup>-*n*-butyloxalamide (44) (330 mg, 84%) as a white solid, mp  $46.0$ – $46.3^{\circ}\text{C}$ . TLC: EtOAc/hexanes (3:2),  $R_f \sim 0.55$ .  $^1\text{H}$  NMR (400 MHz)  $\delta$  7.79 (br s,  $-\text{NH}$ , 1H), 7.77 (br s,  $-\text{NH}$ , 1H), 5.20–5.32 (m, 2H), 3.32 (t,  $J = 6.4$  Hz, 2H), 3.22 (q,  $J = 7.2$  Hz, 4H), 1.90–2.00 (m, 4H), 1.72–1.82 (m, 2H), 1.42–1.56 (m, 4H), 1.20–1.40 (m, 10H), 0.85 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz)  $\delta$

160.17, 160.15, 130.40, 129.34, 39.77, 39.59, 34.12, 32.93, 31.40, 29.62, 29.0, 28.54, 27.25, 27.24, 27.0, 26.91, 20.18, 13.85. HRMS calcd for  $C_{18}H_{34}BrN_2O_2$   $[M + 1]^+$  389.1804, found 389.1809.

***N*<sup>1</sup>-*n*-Butyl-*N*<sup>2</sup>-(12-cyanododec-5(*Z*)-enyl)oxalamide (45).** *N*<sup>1</sup>-(12-Bromododec-5(*Z*)-enyl)-*N*<sup>2</sup>-*n*-butyloxalamide (44) (250 mg, 0.642 mmol) was treated with potassium cyanide as described above for 41 to give *N*<sup>1</sup>-*n*-butyl-*N*<sup>2</sup>-(12-cyanododec-5(*Z*)-enyl)oxalamide (45) (168 mg, 78%) as a colorless solid, mp 83.0–83.3 °C. TLC: EtOAc/hexanes (3:2),  $R_f \sim 0.35$ . <sup>1</sup>H NMR (400 MHz)  $\delta$  7.45 (br s, –NH, 2H), 5.30–5.40 (m, 2H), 3.34 (q,  $J = 8.6$  Hz, 4H), 2.32 (t,  $J = 7.6$  Hz, 2H), 1.98–2.08 (m, 4H), 1.30–1.68 (m, 16H), 0.92 (t,  $J = 7.2$  Hz, 3H). <sup>13</sup>C NMR (100 MHz)  $\delta$  160.03 (2C), 130.03, 129.08, 120.10, 39.88, 39.42, 31.22, 29.40, 28.82, 28.60, 28.42, 27.07, 27.06, 26.82, 25.54, 20.06, 17.01, 13.80. HRMS calcd for  $C_{19}H_{34}N_2O_2$   $[M + 1]^+$  336.2651, found 336.2650.

***N*<sup>1</sup>-(13-Amino-13-(hydroxyimino)tridec-5(*Z*)-enyl)-*N*<sup>2</sup>-*n*-butyloxalamide (46).** To a suspension of *N*<sup>1</sup>-*n*-butyl-*N*<sup>2</sup>-(12-cyanododec-5(*Z*)-enyl)oxalamide (45) (420 mg, 1.29 mmol) in MeOH/H<sub>2</sub>O (4:1; 12 mL) was added H<sub>2</sub>NOH·HCl (228 mg, 3.28 mmol) and Na<sub>2</sub>CO<sub>3</sub> (344 mg, 3.25 mmol).<sup>41</sup> The reaction mixture was heated at 60 °C for 18 h then cooled to room temperature, and all volatiles were removed in vacuo. The residue was diluted with water (30 mL) and extracted into ethyl acetate (2 × 25 mL). The combined organic extracts were washed with water (2 × 10 mL) and brine (10 mL), dried, and purified via silica gel column chromatography to give *N*<sup>1</sup>-(13-amino-13-(hydroxyimino)tridec-5(*Z*)-enyl)-*N*<sup>2</sup>-*n*-butyloxalamide (46) (287 mg, 62%) as a colorless solid, 116.3–116.4 °C. TLC: MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:4),  $R_f \sim 0.20$ . <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  5.28–5.40 (m, 2H), 3.24 (t,  $J = 6.4$  Hz, 4H), 1.98–2.00 (m, 6H), 1.50–1.60 (m, 6H), 1.26–1.40 (m, 10H), 0.92 (t,  $J = 7.3$  Hz, 3H). <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz)  $\delta$  160.55 (2C), 156.31, 130.05, 129.18, 39.23, 39.09, 31.18, 30.63, 29.51, 28.83, 28.69, 27.10, 26.87, 26.59, 19.88, 12.88. HRMS calcd for  $C_{19}H_{37}N_4O_3$   $[M + 1]^+$  369.2866, found 369.2864.

**Bioassays.** The influence of eicosanoids and analogues on coronary vascular tone was measured by the induced changes in isometric tension of bovine coronary artery rings precontracted with the thromboxane-mimetic, U46619, as previously described.<sup>42,43</sup> Synthetic 14,15-EET was used as a control. All assays were conducted in triplicate or greater and are means  $\pm$  10% SD of the reported value.

Recombinant human sEH was produced in a baculovirus expression system<sup>44</sup> and was purified by affinity chromatography.<sup>45</sup> Inhibition potencies (IC<sub>50</sub>s) were determined using a fluorescent-based assay.<sup>46</sup> Human sEH ( $\sim 1$  nM) was incubated with inhibitors ( $0.4 < [I]_{\text{final}} < 100000$  nM) for 5 min in 25 mM bis-tris/HCl buffer (200 mL, pH 7.0) at 30 °C before the substrate, cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyl-oxyran-2-yl)methyl carbonate (CMNPC;  $[S]_{\text{final}} = 5$  mM), was added. Activity was assessed by measuring the appearance of the fluorescent 6-methoxynaphthaldehyde product ( $\lambda_{\text{em}} = 330$  nm,  $\lambda_{\text{ex}} = 465$  nm) at 30 °C during a 10 min incubation (Spectramax M2, Molecular Device, Inc., Sunnyvale, CA).<sup>46</sup> IC<sub>50</sub>s refer to the concentrations of inhibitor that reduced activity by 50% and are the averages of three replicates.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental procedures and copies of the <sup>1</sup>H/<sup>13</sup>C NMR spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare the following competing financial interest(s): JRF, JDI, and WBC authored patents describing the synthesis and clinical uses of the analogues described herein

and assigned all rights to the Medical College of Wisconsin and the University of Texas Southwestern Medical Center.

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## ■ ABBREVIATIONS USED

14,15-EET, *cis*-14,15-epoxyeicosa-5(*Z*),8(*Z*),11(*Z*)-trienoic acid; sEH, soluble epoxide hydrolase

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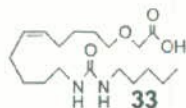
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