Design, Synthesis and Biological Evaluation of Novel Arachidonic Acid Derivatives as Highly Potent and Selective Endocannabinoid Transporter Inhibitors

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Abstract: In the present work, we have designed and synthesized a series of arachidonic acid derivatives of general structure **I** which have been characterized as highly potent and selective inhibitors of anandamide transporter ($IC_{50} = 24-0.8$ μM, K_i > 1000−5000 nM for CB_1 and CB_2 cannabinoid receptors and vanilloid VR_1 receptor). Among them, *N*-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide deserves special attention as being the most potent endocannabinoid transporter inhibitor ($IC_{50} = 0.8$ μM) described to date.

Introduction. The different elements of the endogenous cannabinoid system (ECS) constitute a variety of pharmacological targets for the broad group of compounds generally termed as cannabinoids. Included among these elements are two types of G-protein-coupled membrane receptors (the central CB_1[1] and the peripheral CB_2[2]), the endogenous ligands anandamide,[3] 2-arachidonoylglycerol,[4] and the recently reported 2-arachidonyl glyceryl ether,[5] as well as a mechanism for the termination of biological activity of these compounds composed of a carrier-mediated transport system (ANT)[6−8] and a hydrolyzing enzyme, named fatty acid amide hydrolase (FAAH).[9] Cannabinoid agonists include both exogenous active molecules as well as endocannabinoids. Exogenous agonists are usually classified as classical cannabinoids (*Cannabis sativa* derived compounds as, for example, Δ^9-THC and their analogues), nonclassical cannabinoids (which lack the characteristic tricyclic structure of classical ones, as, for instance, CP55940), and aminoalkylindoles (WIN552122 being the most representative), whereas endogenous cannabinoids belong to the eicosanoid class. Among the antagonists, diarylpyrazoles merit special mention as being the most widely used compounds.[10]

The ECS seems to be involved in the regulation of a wide variety of central and peripheral processes[11] such as antinociception, brain development, retrograde neuronal communication, memory, appetite, psychomotor control, cardiovascular and immune regulation, and cellular proliferation, among others. This broad spectrum of action makes the ECS an important therapeutic target for the treatment of diverse pathologies,[12,13] including asthma, pain, multiple sclerosis, malignant gliomas, and neurodegenerative diseases.

Recent findings suggest that in mammalian cells an increased level of endocannabinoids can be obtained by inhibiting its uptake and/or degradation, raising the possibility of producing local cannabimimetic effects without directly activating cannabinoid receptors with classic agonists and therefore avoiding their associated undesirable side effects. On this basis, such synthetic inhibitors may be of potential therapeutic value for the treatment of disorders characterized by a low endocannabinoid activity and where direct agonists have proven to be effective, yet produce other undesirable effects.[14] In particular, the therapeutic utility of such uptake inhibitors has been considered for the treatment of diverse pathologies as Huntington's chorea[13] or multiple sclerosis.[15] However, the number of compounds with a demonstrated capacity to increase the endocannabinoid tonus by blocking the mechanism of termination of their biological activity is limited.[16−20] Among them, the *N*-(4-hydroxyphenyl)arachidonamide (AM404) is a unique compound that has been extensively studied due to its ability to inhibit the transporter in a potent and selective way,[7] although recent results have revealed an agonist activity of AM404 at vanilloid VR_1 receptors.[20−22] Additionally, the lack of molecular characterization of the transporter and the scarce structure−affinity relationship (SAFIR) studies reported to date[16,17] make studies regarding the structural features of the carrier involved in the recognition and translocation of substrates an important area of research aimed at providing new potential therapeutic agents for the treatment of hypofunctionalities of the ECS. The intracellular fate of these uptake inhibitors is an additional point to be taken into consideration due to the possibility of producing toxic metabolites through enzymatic hydrolysis. For example, it has been described that some aminophenols, which could be produced as possible metabolites of AM404 hydrolysis, generate methemoglobin both in vivo and in vitro.[23] Thus, there remains a need for the design of selective inhibitors.

In this line, we have designed and synthesized a series of arachidonic acid derivatives of general structure **I** which have proved to be highly potent and selective inhibitors of anandamide reuptake[24] (Table 1). In these derivatives we have analyzed the effect of the replacement of ethanolamine moiety of anandamide with a fragment containing a five-membered ring with one heteroatom. Thus, we have evaluated the influence of several factors such as aromaticity, position of the arachidonic chain, as well as the nature of the heteroatom. Also, we have compared amides with its isosteric corresponding esters in order to assess the ability of the carrier to uptake both types of endocannabinoids. In particular, compound **9** deserves special attention as being the most active derivative with an IC_{50} value of 0.8 μM, which makes it the most potent uptake inhibitor described to date.

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Table 1. Inhibition of ANT and Binding Data of Compounds **I**

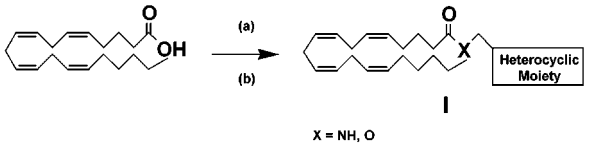
Compound	HM	X	ANT IC_{50} (μM)[a]	Receptor Affinity $K_i CB_1$ (nM)[b]	$K_i CB_2$ (nM)[c]	$K_i VR_1$ (nM)[e]
1		O	24±14	>1000	>1000	>5000
2		O	14±2	>1000	>1000	*f*
3		O	18±7	>1000	>1000	>5000
4		O	*d*	>1000	>1000	-
5		O	Inactive	>1000	>1000	>5000
6		O	3±2	>1000	>1000	>5000
7		NH	5.0±0.7	124±1	70±5	>5000
8		NH	5±2	>1000	>1000	*f*
9		NH	0.8±0.4	>1000	67±6	>5000
10		NH	8±2	>1000	>1000	>5000
11		NH	5.7±0.6	>1000	>1000	>5000
AM404			4±2	>1000	>1000	>5000
WIN552122			-	4.5±0.4	3.9±0.8	-
CP55940			-	1.3±0.4	0.15±0.05	-
Anandamide			-	285±14	>1000	169±33
RTX			-	-	-	0.040±0.001

[a] Inhibition of anandamide transport was determined using human lymphoma U937 cells and [^{3}H]anandamide. [b] Affinity of compounds for the CB_1 receptor was evaluated using rat cerebellum membranes and [^{3}H]WIN552122. [c] Affinity of compounds for the CB_2 receptor was assayed using [^{3}H]CP55940 in HEK293EBNA human CB_2 receptor transfected cells. K_i and IC_{50} values were obtained from two or three independent experiments, respectively, carried out in triplicate and are expressed as the mean ± standard error. [d] This compound presented a strange profile which did not fit the sigmoid dose−response, characteristic of the rest of the compounds. [e] Affinity of compounds for the VR_1 receptor was evaluated using rat spinal cord membranes and [^{3}H]RTX. [f] These compounds exhibited a partial capacity to displace [^{3}H]RTX (K_i < 5000 nM).

The present study explores the structural features involved in the recognition of substrates for the endocannabinoid transporter in a SAFIR study focused on the influence exerted by the heterocyclic moiety on the affinity for the carrier.

Results and Discussion. The synthesis of the amides and esters of general structure **I** listed in Table 1 is detailed in Scheme 1. In general, these compounds were prepared from arachidonic acid by treatment of the acyl chloride[25] with the appropriate amine or alcohol. The noncommercial amines were prepared from the corresponding aldehydes by catalytic reductive amination in 28% ammonium hydroxide solution using Raney nickel as catalyst.[26]

Scheme 1. Synthesis of Compounds of General Structure **I**[a]



[a] Reagents: (a) oxalyl chloride, DMF, CH_2Cl_2, rt; (b) HX−CH_2−HM, HM = heterocyclic moiety.

All new compounds were assessed for their ability to inhibit [^{3}H]anandamide uptake in human lymphoma U937 cells, for their affinity for CB_1 and CB_2 cannabinoid receptors in radioligand binding assays using [^{3}H]-WIN552122 in rat cerebellum membranes and [^{3}H]-CP55940 in HEK293EBNA human CB_2 receptor transfected cells, respectively, as well as for their affinity for the vanilloid VR_1 receptor in radioligand binding assays using [^{3}H]Rexiniferatoxin ([^{3}H]RTX) in rat spinal cord membranes (Table 1). For comparison purposes and as reference values, we have also included results obtained for AM404, anandamide, RTX, and for the two prototypical cannabinoid ligands WIN552122 and CP55940.

Most of compounds **I** display an excellent ability to inhibit anandamide reuptake with IC_{50} values in the low micromolar range as well as selectivity for the transporter, as deduced from their affinity constants for both CB_1 and CB_2 receptors (K_i > 1000 nM) as well as for VR_1 receptors (K_i > 5000 nM). Well-characterized cannabinoid[10] or vanilloid ligands such as WIN552122 and CP55940 or RTX, respectively, exhibit K_i values in the low nanomolar or even in the picomolar range, as shown in Table 1.

In particular, special attention should be paid to compounds **6** and **9** which exhibit the best IC_{50} values (3 and 0.8 μM, respectively).

The compounds described here allow us to ascertain the effect of the heterocyclic moiety on the transporter affinity as well as affinity for cannabinoid receptors. With respect to the ANT, the most important points can be summarized as follows:

(i) The position of the arachidonic chain attached to the C-2 or the C-3 of the heterocyclic ring seems to exert a different influence depending on the heterocycle. Thus, in case of the furan ring it does not seem to play a relevant role in affinity for the transporter when esters are considered (IC_{50} (**1**) = 24 μM; IC_{50} (**2**) = 14 μM), whereas in amides (compound **8** vs **9**) this change improves the affinity more than 6 times. Furthermore, this substitution pattern has a crucial influence in the affinity of thienyl derivatives (compound **5** vs **6**) as well as in the behavior of tetrahydrofuryl derivatives (compound **3** vs **4**).

(ii) Regarding esters, when the arachidonic chain is attached to the 3-position of the heterocyclic ring, replacement of the furan with a thiophene ring improves more than 4 times the affinity for the transporter (IC_{50} (**2**) = 14 μM; IC_{50} (**6**) = 3 μM), whereas when the arachidonic chain is at the C-2 position, the same isosteric change implies a complete loss of affinity (compound **1** vs **5**). However, in the case of amides, the inhibitory potency is comparable regardless of the

heteroatom of the heterocycle, as deduced from IC_{50} values for compounds **7**, **8**, and **11**.

(iii) With respect to the aromaticity of the heterocyclic moiety, when the arachidonic chain is attached to the C-2 position, the equipotent IC_{50} values observed for compounds **1** vs **3** and **8** vs **10** indicate that the presence of an aromatic fragment is not essential for affinity. However, when the arachidonic chain is attached to the C-3 position (compound **2** vs **4)**, aromaticity becomes an important factor in the inhibitory trend observed, which does not fit the sigmoid dose−response typical of the rest of compounds. This rare behavior may reveal the existence of different mechanisms of interaction or recognition between the transporter and its substrates or involve some other explanation, such as an additional interaction with the new specific 2-arachidonoylglycerol transporter recently described and which is not inhibited by AM404.[27]

(iv) In general, carboxamide and carboxylate groups are able to compete with [^{3}H]anandamide for transport. Comparison between furan esters and amides suggests that the presence of the NH group enhances the affinity for the carrier, as shown from the IC_{50} values for analogues **3** vs **10** (about 2-fold), **1** vs **8** (5-fold), and especially **2** vs **9** (nearly 20-fold). Regarding the thienyl derivative **5**, the substitution of the ester for the amide bond allows recovery of its inhibitory ability, leading to **11** ($IC_{50} = 5.7\ \mu M$).

(v) With respect to cannabinoid receptors, most of compounds analyzed here turned out to have little affinity for both CB_1 and CB_2 ($K_i > 1000$ nM). However, it should be pointed out that the presence of *N*-methyl pyrrole fragment dramatically increases affinity for both cannabinoid receptors as deduced from the K_i values obtained for **7**.

(vi) Regarding the interaction of compounds with vanilloid VR_1 receptors, the data obtained here show that most of the compounds synthesized are essentially unable to bind to VR_1 ($K_i > 5000$ nM), especially when comparing these data with the K_i values of high affinity VR_1 ligands such as RTX ($K_d = 25$ pM,[28] which is in accordance with the value determined in our experiment). The fact that AM404 appears to be devoid of affinity for VR_1 receptors seems to be in apparent contradiction with previously published results[19−21] which describe this inhibitor as a potent agonist at VR_1 receptors. However, it should be taken into account that these studies reflect a measure of agonist activity by evaluating the Ca^{2+} influx within the cell and that this action is determined using concentrations of AM404 or of the other compounds tested as potential agonists in the micromolar range (3−10 μM).[20−22] Another important point that deserves consideration is the difference existing between the model most widely used (cells transfected with and overexpressing hVR1)[20−22] and the one described here (rat spinal cord membranes). Finally, it is also possible that an alternative mechanism could explain these different observations since neither the interaction between both systems (VR_1 and ANT) nor the physiological functions, endogenous ligands, and transduction mechanisms of vanilloid receptor have been completely elucidated. Thus, perhaps compounds with low affinity for the VR_1 receptors (such as AM404) provoke a slight Ca^{2+} influx within the cell. Then, this slight increase of Ca^{2+} could trigger the release of a potent ligand of VR_1 (as anandamide, for example) which would be responsible for the activation of all VR_1 receptors and therefore for the great increase in the intracellular levels of Ca^{2+}, which are measured in agonist assays.

The capacity of analogues **2** and **8** to displace [^{3}H]-RTX at its binding sites and also to inhibit the ANT further support the existence of a partially common pharmacophore between these two targets.[20]

From all the above considerations, it should be stressed that the small variations in the heterocyclic moiety reported here have allowed us to obtain different selectivities and affinities in the various test systems. Also, affinity values of compound **7** at CB_1 and CB_2 cannabinoid receptors and the affinity value of compound **9** at CB_2 seem to suggest that the receptors and the transporter share some of the requirements involved in recognition and binding of substrates but also that they indicate the existence of significant differences which could be used to develop future ligands with enhanced affinity, efficacy, and selectivity for the various proteins that constitute the ECS. Furthermore, the fact that compound **9**, the most potent derivative in the transporter, seems to bind with moderate affinity only to CB_2 raises the possibility of selectively activating this receptor and simultaneously inhibiting the metabolism of endocannabinoids, with all the interesting and attractive therapeutic applications that this implies in search of new agents capable of increasing the physiological levels of anandamide but devoid of non desired psychoactive effects of CB_1 ligands.

With all these points taken into consideration, further research is still required to elucidate the nature of the interaction of the new compounds with biological targets such as the FAAH and VR_1 to determine the extent of their common ligand recognition properties, and this is currently under investigation in our laboratory.

In conclusion, in the present work we have synthesized new arachidonic acid derivatives which act as potent inhibitors of anandamide reuptake with IC_{50} values similar or even better than previously reported inhibitors (AM404). Particularly, special attention should be paid to compound **9** whose excellent IC_{50} value ($IC_{50} = 0.8\ \mu M$) makes it a valuable candidate for future pharmacological studies.

Results of these studies may help to develop insights in this emerging area of interest in order to further explore the molecular features involved in the recognition and/or translocation of substrates. Ultimately, new agents may be identified (such as compound **9**) which could be useful not only as tools to improve our understanding of the ECS but also as novel therapeutic approaches for the treatment and cure of a broad range of pathological dysfunctions.

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Supporting Information Available: Experimental procedures and NMR data are available free of charge via the Internet at http://pubs.acs.org.

References

(1) Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a Cannabinoid Receptor and Functional Expression of the Cloned cDNA. *Nature* **1990**, *346*, 561−564.

(2) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* **1993**, *365*, 61−65.

(3) Devane, W. A.; Hanuš, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor. *Science* **1992**, *258*, 1946−1949.

(4) Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol: a Possible Endogenous Cannabinoid Receptor Ligand in Brain. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89−97.

(5) Hanuš, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. 2-Arachidonyl Glyceryl Ether, an Endogenous Agonist of the Cannabinoid CB_1 Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662−3665.

(6) Hillard, C. J.; Edgemond, W. S.; Jarrahian, A.; Campbell, W. B. Accumulation of *N*-Arachidonoylehanolamine (Anandamide) into Cerebellar Granule Cells Occurs via Facilitated Diffusion. *J. Neurochem.* **1997**, *69*, 631−638.

(7) Beltramo, M.; Stella, N.; Calignano, A.; Lin, S. Y.; Makriyannis, A.; Piomelli, D. Functional Role of High-Affinity Anandamide Transport, as Revealed by Selective Inhibition. *Science* **1997**, *277*, 1094−1097.

(8) Hillard, C. J.; Jarrahian, A. The Movement of *N*-arachidonoylethanolamine (Anandamide) across Cellular Membranes. *Chem. Phys. Lipids* **2000**, *108*, 123−134.

(9) Ueda, N.; Puffenbarger, R. A.; Yamamoto, S.; Deutsch, D. G. The Fatty Acid Amide Hydrolase (FAAH). *Chem. Phys. Lipids* **2000**, *108*, 107−121.

(10) Pertwee, R. G. Cannabinoid Receptor Ligands: Clinical and Neuropharmacological Considerations, Relevant to Future Drug Discovery and Development. *Expert Opin. Invest. Drugs* **2000**, *9*, 1−19.

(11) (a) Calignano, A.; La Rana, G.; Giuffrida, A.; Piomelli, D. Control of Pain Initiation by Endogenous Cannabinoids. *Nature* **1998**, *394*, 277−281. (b) Walker, J. M.; Hohmann, A. G.; Martin, W. J.; Strangman, N. M.; Huang, S. M.; Tsou, K. The Neurobiology of Cannabinoid Analgesia. *Life Sci.* **1999**, *65*, 665−673. (c) Fernández-Ruiz, J.; Berrendero, F.; Hernández, M. L.; Ramos, J. A. The Endogenous Cannabinoid System and Brain Development. *Trends Neurosci.* **2000**, *23*, 14−20. (d) Wilson, R. I.; Nicoll, R. A.; Endogenous Cannabinoids Mediate Retrograde Signaling at Hippocampal Synapses. *Nature* **2001**, *410*, 588−592. (e) Hampson, R. E.; Deadwyler, S. A. Cannabinoids, Hippocampal Function and Memory. *Life Sci.* **1999**, *65*, 715−723. (f) Di Marzo, V.; Goparaju, S. K.; Wang, L.; Liu, J.; Bátkai, S.; Járai, Z.; Fezza, F.; Miura, G. I.; Palmiter, R. D.; Sugiura, T.; Kunos, G. Leptin-Regulated Endocannabinoids Are Involved in Maintaining Food Intake. *Nature* **2001**, *410*, 822−825. (g) Giuffrida, A.; Piomelli, D. The Endocannabinoid System: a Physiological Perspective on its Role in Psychomotor Control. *Chem. Phys. Lipids* **2000**, *108*, 151−158. (h) De Petrocellis, L.; Melck, D.; Bisogno, T.; Di Marzo, V. Endocannabinoids and Fatty Acid Amides in Cancer, Inflammation and Related Disorders. *Chem. Phys. Lipids* **2000**, *108*, 191−209.

(12) (a) Calignano, A.; Kátona, I.; Désarnaud, F.; Giuffrida, A.; La Rana, G.; Mackie, K.; Freund, T. F.; Piomelli, D. Bidirectional Control of Airway Responsiveness by Endogenous Cannabinoids. *Nature* **2000**, *408*, 96−101. (b) Baker, D.; Pryce, G.; Croxford, J. L.; Brown, P.; Pertwee, R. G.; Huffman, J. W.; Layward, L. Cannabinoids Control Spasticity and Tremor in a Multiple Sclerosis Model. *Nature* **2000**, *404*, 84−87. (c) Galve-Roperh, I.; Sánchez, C.; Cortés, M. L.; Gómez del Pulgar, T.; Izquierdo, M.; Guzmán, M. Antitumoral Action of Cannabinoids: Involvement of Sustained Ceramide Accumulation and Extracellular Signal-Regulated Kinase Activation. *Nat. Med.* **2000**, *6*, 313−319. (d) Pertwee, R. G. Pharmacology of Cannabinoid Receptor Ligands. *Curr. Med. Chem.* **1999**, *6*, 635−664.

(13) Lastres-Becker, I.; Hansen, H. H.; Berrendero, F.; de Miguel, R.; Pérez-Rosado, A.; Manzanares, J.; Ramos, J. A.; Fernández-Ruiz, J. J. Loss of Cannabinoid CB_1 Receptors and Alleviation of Motor Hyperactivity and Neurochemical Deficits by Endocannabinoid Uptake Inhibition in a Rat Model of Huntington's Disease. *Neuroscience* In press.

(14) Piomelli, D.; Giuffrida, A.; Calignano, A.; Rodríguez de Fonseca, F. The Endocannabinoid System as a Target for Therapeutic Drugs. *Trends Pharmacol. Sci.* **2000**, *21*, 218−224.

(15) Baker, D.; Pryce, G.; Croxford, J. L.; Brown, P.; Pertwee, R. G.; Makriyannis, A.; Khanolkar, A.; Layward, L.; Fezza, F.; Bisogno, T.; Di Marzo, V. Endocannabinoids Control Spasticity in a Multiple Sclerosis Model. *FASEB J.* **2001**, *15*, 300−302.

(16) Piomelli, D.; Beltramo, M.; Glasnapp, S.; Lin, S. Y.; Goutopoulos, A.; Xie, X.-Q.; Makriyannis, A. Structural Determinants for Recognition and Translocation by the Anandamide Transporter. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5802−5807.

(17) Jarrahian, A.; Manna, S.; Edgemond, W. S.; Campbell, W. B.; Hillard, C. J. Structure−Activity Relationships Among *N*-Arachidonylehanolamine (Anandamide) Head Group Analogues for the Anandamide Transporter. *J. Neurochem.* **2000**, *74*, 2597−2606.

(18) Muthian, S.; Nithipatikom, K.; Campbell, W. B.; Hillard, C. J. Synthesis and Characterization of a Fluorescent Substrate for the *N*-Arachidonoylethanolamine (Anandamide) Transmembrane Carrier. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 289−295.

(19) Bisogno, T.; Melck, D.; Bobrov, M. Y.; Gretskaya, N. M.; Bezuglov, V. V.; De Petrocellis, L.; Di Marzo, V. *N*-acyldopamines: Novel Synthetic CB_1 Cannabinoid-Receptor Ligands and Inhibitors of Anandamide Inactivation with Cannabimimetic Activity in vitro and in vivo. *Biochem. J.* **2000**, *351*, 817−824.

(20) De Petrocellis, L.; Bisogno, T.; Davis, J. B.; Pertwee, R. G.; Di Marzo, V. Overlap between the Ligand Recognition Properties of the Anandamide Transporter and the VR1 Vanilloid Receptor: Inhibitors of Anandamide Uptake with Negligible Capsaicin-Like Activity. *FEBS Lett.* **2000**, *483*, 52−56.

(21) Zygmunt, P. M.; Chuang, H.-h.; Movahed, P.; Julius, D.; Högestätt, E. D. The Anandamide Transport Inhibitor AM404 Activates Vanilloid Receptors. *Eur. J. Pharmacol.* **2000**, *396*, 39−42.

(22) Smart, D.; Jerman, J. C. Anandamide: an Endogenous Activator of the Vanilloid Receptor. *Trends Pharmacol. Sci.* **2000**, *21*, 134.

(23) Klaassen, C. D. *Casarett & Doull's Toxicology The Basic Science of Poisons*, 5th ed.; McGraw-Hill Companies, Inc.: New York, 1996; p 345.

(24) López-Rodríguez, M. L.; Viso, A.; Ortega-Gutiérrez, S.; Lastres-Becker, I.; González, S.; Fernández-Ruiz, J. J.; Ramos, J. A. PCT/ES01/00305, 2001.

(25) Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Wright, S. W. Rationally Designed, Potent Competitive Inhibitors of Leukotriene Biosynthesis. *J. Am. Chem. Soc.* **1984**, *106*, 1503−1504.

(26) Hudlický, M. *Reductions in Organic Chemistry*, 2nd ed.; American Chemical Society: Washington, DC, 1996; pp 187−190.

(27) Maccarrone, M.; Bari, M.; Menichelli, A.; Giuliani, E.; Del Principe, D.; Finazzi-Agrò, A. Human Platelets Bind and Degrade 2-Arachidonoylglycerol, Which Activates These Cells Through a Cannabinoid Receptor. *Eur. J. Biochem.* **2001**, *268*, 819−825.

(28) Szallasi, A.; Bíró, T.; Szabó, T.; Modarres, S.; Petersen, M.; Klusch, A.; Blumberg, P. M.; Krause, J. E.; Sterner, O. A Nonpungent Triprenyl Phenol of Fungal Origin, Scutigeral, Stimulates Rat Dorsal Root Ganglion Neurons Via Interaction at Vanilloid Receptors. *Br. J. Pharmacol.* **1999**, *126*, 1351−1358.

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