

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/263442354>

Identification of Endocannabinoid System-Modulating N-Alkylamides from *Heliopsis helianthoides* var. *scabra* and *Lepidium meyenii*

ARTICLE in JOURNAL OF NATURAL PRODUCTS · JUNE 2014

Impact Factor: 3.8 · DOI: 10.1021/np5000292g

READS

194

8 AUTHORS, INCLUDING:



Zsanett Hajdu

RotaChrom Technologies

6 PUBLICATIONS 39 CITATIONS

SEE PROFILE



Simon Nicolussi

Universität Bern

17 PUBLICATIONS 65 CITATIONS

SEE PROFILE



Judit Hohmann

University of Szeged

234 PUBLICATIONS 2,452 CITATIONS

SEE PROFILE



Jürg Gertsch

Universität Bern

144 PUBLICATIONS 2,581 CITATIONS

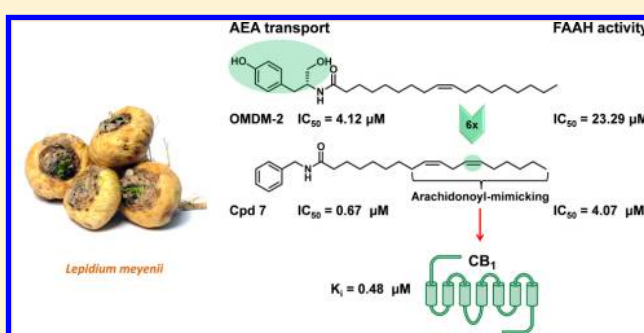
SEE PROFILE

Identification of Endocannabinoid System-Modulating *N*-Alkylamides from *Heliopsis helianthoides* var. *scabra* and *Lepidium meyenii*Zsanett Hajdu,[†] Simon Nicolussi,[‡] Mark Rau,[‡] László Lorántfy,[†] Peter Forgo,[†] Judit Hohmann,[†] Dezső Csupor,^{*,†} and Jürg Gertsch^{*,†}[†]Department of Pharmacology, University of Szeged, H-6720 Szeged, Hungary[‡]Institute of Biochemistry and Molecular Medicine, NCCR TransCure, University of Bern, CH-3012 Bern, Switzerland

S Supporting Information

ABSTRACT: The discovery of the interaction of plant-derived *N*-alkylamides (NAAs) and the mammalian endocannabinoid system (ECS) and the existence of a plant endogenous *N*-acyl ethanolamine signaling system have led to the re-evaluation of this group of compounds. Herein, the isolation of seven NAAs and the assessment of their effects on major protein targets in the ECS network are reported. Four NAAs, octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaene-12-ynoic acid isobutylamide (**1**), octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaene-12-ynoic acid 2'-methylbutylamide (**2**), hexadeca-2*E*,4*E*,9*Z*-triene-12,14-diynoic acid isobutylamide (**3**), and hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2'-methylbutylamide (**4**), were identified from *Heliopsis helianthoides* var. *scabra*. Compounds **2**–**4** are new natural products, while **1** was isolated for the first time from this species. The

previously described macamides, *N*-(3-methoxybenzyl)-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**5**), *N*-benzyl-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**6**), and *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**7**), were isolated from *Lepidium meyenii* (Maca). *N*-Methylbutylamide **4** and *N*-benzylamide **7** showed submicromolar and selective binding affinities for the cannabinoid CB₁ receptor (*K_i* values of 0.31 and 0.48 μM, respectively). Notably, compound **7** also exhibited weak fatty acid amide hydrolase (FAAH) inhibition (IC₅₀ = 4 μM) and a potent inhibition of anandamide cellular uptake (IC₅₀ = 0.67 μM) that was stronger than the inhibition obtained with the controls OMDM-2 and UCM707. The pronounced ECS polypharmacology of compound **7** highlights the potential involvement of the arachidonoyl-mimicking 9*Z*,12*Z* double-bond system in the linoleoyl group for the overall cannabimimetic action of NAAs. This study provides additional strong evidence of the endocannabinoid substrate mimicking of plant-derived NAAs and uncovers a direct and indirect cannabimimetic action of the Peruvian Maca root.



Plant-derived *N*-alkylamides (NAAs) or alkamides are lipophilic substances present in some plants, where they exert growth regulatory functions similar to those of *N*-acyl ethanolamines (NAEs) via yet poorly characterized mechanisms involving a plant fatty acid amide hydrolase (FAAH) and jasmonic acid biosynthesis.^{1–3} With the discovery of the functional interaction of *N*-alkylamides with the endocannabinoid system (ECS),^{4–9} a new pharmacological mode of action of medicinal and food plants containing NAEs has been uncovered. In mammalian tissues, different NAAs from medicinal plants exert analgesic, anti-inflammatory, and immunomodulatory effects, potentially also via the ECS.^{10,11} For instance, NAAs seem to play a major role in the bioactivity of the widely used medicinal *Echinacea* species.^{4–6,11} While the interaction of *Echinacea* NAAs with cannabinoid receptors has been studied in more detail, ECS interacting NAAs from other plants have been described only recently.^{8,9} The assumption that specific NAAs interfere with different proteins of the ECS because of their structural similarity to endocannabinoids, such

as anandamide [*N*-arachidonylethanolamide (AEA) (Figure 1)], is intuitive, but comparisons, in particular in the alkyl chain double bonds, are hampered by the lack of structure–activity relationship (SAR) data. Chemically diverse plant NAAs showing different saturation patterns and double-bond configurations therefore provide a source for further analysis of the ECS binding interactions of this class of natural products. Moreover, insights from such studies may inspire the generation of scaffolds for the development of novel chemical probes or may explain the traditional use of certain plants.

NAAs are present in several Asteraceae species, especially in the genera *Achillea*, *Acmella*, *Echinacea*, *Heliopsis*, and *Spilanthes*, as well as in some species of the plant families Brassicaceae, Piperaceae, Rutaceae, and Solanaceae.¹² To date, more than 70 NAAs have been identified in the Heliantheae tribe (Asteraceae),¹³ but only a few have been systematically tested

Received: April 2, 2014

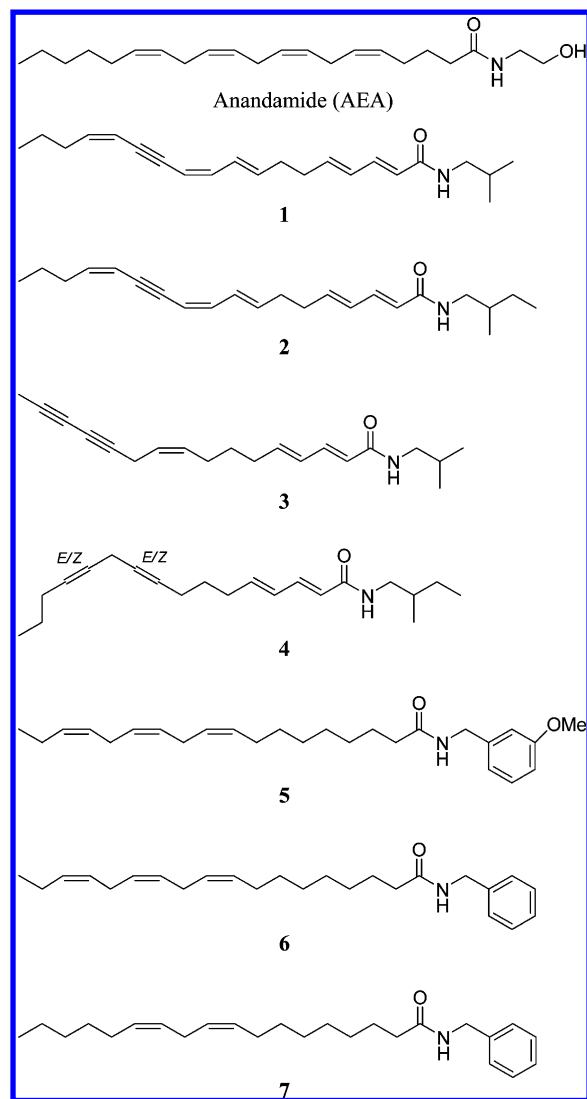


Figure 1. Molecular structures of anandamide (AEA) and isolated N-alkylamides 1–7.

on the different known targets of the ECS. *Lepidium meyenii* Walp. (Brassicaceae) is a South American plant, also known as Maca in Peru where it is extensively cultivated. Its hypocotyl is consumed as a common vegetable and also employed traditionally for medicinal purposes, including general invigoration and as a purported fertility enhancer for people and domesticated animals.^{14–17} At present, 19 macamides have been described from this species.¹⁸ It has been shown that the macamide N-3-methoxybenzyl linoleamide weakly inhibits the major AEA-degrading enzyme FAAH and may thus exert indirect cannabimimetic neuroprotective effects.¹⁹ This was recently confirmed in a study in which synthesized macamides and analogues were indeed shown to act as FAAH inhibitors, although no further ECS targets were tested.¹⁸

Members of the genus *Heliopsis*, including *Heliopsis helianthoides*, have been used by the North-American Indians as medicinal plants. *Heliopsis longipes* has been used primarily as a pain remedy. The analgesic effect has been attributed to the alkylamide content of this species.^{21,22} Besides two insecticidal N-alkylamides, scabrin and heliopsin,^{23,24} identified from *Heliopsis scabra* [syn. *H. helianthoides* var. *scabra* (Dunal)

Fernald²⁵], the NAA composition of this taxon has not been elucidated in detail.

The aim of this study was the isolation and structural elucidation of NAAs from *H. helianthoides* var. *scabra* and *L. meyenii* and to test them on different targets within the ECS. An interesting preliminary SAR of C₁₈ alkyl chain-bearing macamides and the synthetic reference AEA transport inhibitor OMDM-2 was uncovered. Moreover, two NAAs from *H. helianthoides* var. *scabra* (4) and *L. meyenii* (7) with a noteworthy submicromolar binding affinity for CB₁ cannabinoid receptors are reported. Macamide 7 showed a significant direct interaction with CB₁ receptors, revealing new opportunities for further pharmacological exploration of this Peruvian medicinal plant.

RESULTS AND DISCUSSION

Multistep chromatographic purification of *H. helianthoides* var. *scabra* and *L. meyenii* resulted in the isolation of seven pure compounds in total (Figure 1). The structures of the compounds were established by mass spectrometry and advanced one- and two-dimensional (2D) nuclear magnetic resonance (NMR) methods, including ¹H NMR, JMOD, ¹H–¹H COSY, HMQC, and HMBC techniques, and by comparison with literature data.^{18,23,26}

Compound 1 was isolated as a colorless oil. It was shown by APCIMS to exhibit a quasi-molecular ion peak at *m/z* 326 [*M* + *H*]⁺, which resulted in a fragment ion at *m/z* 253 in the MS² spectrum due to the loss of isobutylamine. In accordance with this, the ¹H NMR spectrum of 1 confirmed an *N*-isobutylamide structure [δ_{H} 3.15 t, 1.80 sept., 0.91 d (6H)] and additionally included signals proving the octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaene-12-ynoic acid isobutylamide structure.²⁶ This compound was isolated previously from another species of the genus (*Heliopsis buphthalmoides*),^{26,27} but its ¹³C NMR data are reported here for the first time.

Compound 2 was obtained as a colorless oil with a light tingling effect. Its APCIMS spectrum contained a quasi-molecular peak at *m/z* 340 [*M* + *H*]⁺, which afforded fragment ions at *m/z* 253 [(*M* + *H*) – C₅H₁₃N]⁺, 235 [(*M* + *H*) – C₅H₁₃N – H₂O]⁺, 225 [(*M* + *H*) – C₅H₁₃N – CO]⁺, and 211 [(*M* + *H*) – C₅H₁₃N – CH₂CO]⁺ revealing a methylbutylamide structural portion. Analysis of the ¹H NMR spectrum confirmed that compounds 1 and 2 are homologues because they exhibited the same spectroscopic features with regard to the acid part of the molecule. The ¹H NMR spectra were different only in the signals of a 2-methylbutylamide [δ_{H} 3.28 m (H-2'a), 3.15 m (H-2'b), 1.57 m (H-3'), 1.40 m (H-4'a), 1.17 m (H-4'b), and 0.91 (H-5', H-6')] in compound 2 instead of isobutylamide. Therefore, the new compound 2 was identified as octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaene-12-ynoic acid 2'-methylbutylamide.

Compound 3 was isolated as a colorless oil, and its HRESIMS spectrum displayed a quasi-molecular ion peak at *m/z* 298.2094 [*M* + *H*]⁺, indicating a molecular formula of C₂₀H₂₇NO. The ¹H and ¹³C NMR spectra exhibited resonances for an isobutylamide [δ_{H} 3.17 t (H-2'), 1.81 sept. (H-3'), and 0.93 d (H-4', 5'); δ_{C} 46.9 (C-2'), 28.6 (C-3'), and 20.1 (C-4', 5')] and a polyunsaturated C₁₆ acid portion. From the HSQC spectrum, the chemical shifts of the protonated carbons were assigned, and the proton–proton connectivities were then studied. The ¹H–¹H COSY spectrum defined the following structural fragment with correlated protons: –CH=CH–CH=CH–CH₂–CH₂–CH₂–CH=CH–CH₂– [δ_{H} 5.78 d,

Table 1. Summary of the Effects of the Isolated NAAs on ECS Targets^a

compound	AEA uptake IC ₅₀ (μM) (95% CI)	efficacy (%)	FAAH IC ₅₀ (μM) (95% CI)	efficacy (%)	MAGL IC ₅₀ (μM)	CB ₁ K _i (μM) (95% CI)	CB ₂ K _i (μM) (95% CI)
1	2.45 (1.28–4.70)	75	17.78 (13.18–25.70)	84	>100	8.60 (2.39–10.92)	9.18 (5.41–9.84)
3	4.33 (2.69–6.97)	75	12.30 (8.68–16.95)	79	>100	>20	22.55 (12.68–25.30)
4	2.15 (0.87–5.31)	51	19.95 (12.59–33.11)	80	>100	0.31 (0.18–0.59)	1.21 (0.90–1.71)
5	>100	44	19.05 (13.80–28.18)	85	>100	8.67 (4.66–13.75)	>50
6	84.36 (72.44 to >100)	53	11.48 (6.81–18.78)	85	>100	8.88 (3.79–10.19)	43.96 (24.16 to >50)
7	0.67 (0.47–0.97)	73	4.07 (2.95–5.62)	83	>100	0.48 (0.33–0.67)	4.11 (3.11–5.66)
OMDM-2	4.12 (2.01–12.19)	72	23.29 (10.72–48.98)	82	nd ^b	nd ^b	nd ^b
UCM707	1.46 (1.18–1.80)	67	7.24 (6.03–13.18)	79	nd ^b	nd ^b	nd ^b

^aData are mean values of at least three independent experiments and the 95% confidence intervals (CI) are listed. OMDM-2 and UCM707 were used as positive controls for AEA uptake inhibition. ^bNot determined.

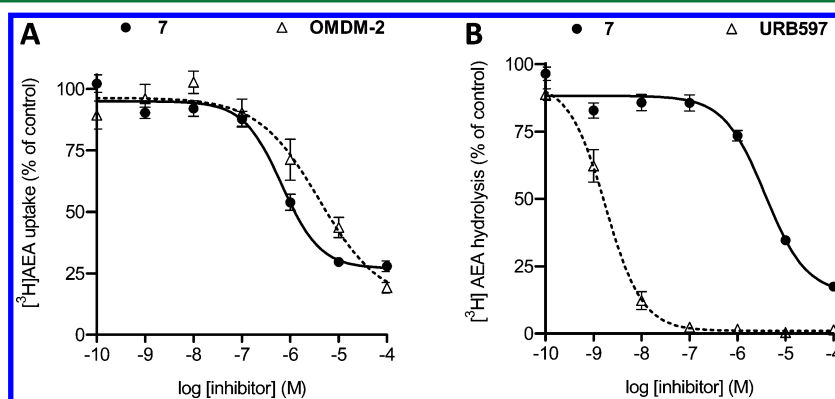


Figure 2. Effects of compound 7 on the cellular uptake of anandamide (AEA) into U937 cells (A) and degradation by FAAH (B). OMDM-2 was used as positive control for AEA uptake and URB597 as the positive control for FAAH inhibition. Data are means \pm the standard error of the mean (SEM).

7.19 dd, 6.15 dd, 6.03 dt, 2.16, 1.52 dq, 2.04 q, 5.48 dt, 5.40 dt and 3.01 d (H-2–H-11)]. This structural unit, a tertiary methyl (δ_{H} 1.95 s), the carbonyl carbon (δ_{C} 166.3), and acetylene carbons (δ_{C} 75.2, 64.9, 60.9, and 59.7) were connected by analysis of the long-range C–H correlations observed in the HMBC spectrum. The two- and three-bond correlations between the carbonyl carbon (δ_{C} 166.3) and proton signals at δ_{H} 5.78 and 7.19 (H-2 and H-3) and between the methyl group (δ_{H} 1.95 s) and δ_{C} 64.9 and 60.9 (C-14 and C-15) demonstrated the presence of a hexadeca-2,4,9-trien-12,14-diynoic acid isobutylamide structure. The geometry of the C-2/C-3 and C-4/C-5 double bonds was concluded to be *E* from the coupling constants ($J = 15.5$ and 15.1 Hz, respectively) and *Z* for the C-9/C-10 olefinic unit with regard to a coupling constant J of 10.3 Hz. On the basis of these data, the structure hexadeca-2*E*,4*E*,9*Z*-trien-12,14-diynoic acid isobutylamide (3) was elucidated for this compound.

Compound 4, a colorless oil, was found to have a molecular formula of $\text{C}_{21}\text{H}_{35}\text{NO}$ as confirmed by the quasi-molecular ion peak at m/z 317.2717 in the HRESIMS spectrum. In accordance with this, the ^1H and ^{13}C NMR spectra showed signals for a 2-methylbutylamide [δ_{H} 3.28 m (H-2'a), 3.15 m (H-2'b), 1.57 m (H-3'), 1.40 m (H-4'a), 1.17 m (H-4'b), and 0.93 t (H-5', H-6'); δ_{C} 45.2 (C-2'), 35.0 (C-3'), 27.0 (C-4'), 11.3 (C-5'), and 17.2 (C-6')]. Additionally, the NMR spectra exhibited resonances for a C_{16} unsaturated fatty acid residue containing one methyl, six methylenes, eight methines, and a carbonyl group. Analysis of the proton–proton connectivities in the ^1H – ^1H COSY spectrum led to the identification of a hexadeca-2,4,9,12-tetraenoate chain. The geometry of the C-2/C-3 and C-4/C-5 olefins was established as *E* on the basis of a

$J_{2,3}$ of 15.0 Hz and a $J_{4,5}$ of 15.3 Hz. Unfortunately, the *E* or *Z* geometry of the C-9/C-10 and C-12/C-13 double bond could not be determined because of the overlapping proton signals. The HMBC spectrum provided information about the connection of the acyl and amine part of the molecule from the long-range correlation between C-1 (δ_{C} 166.1) and H-2' (δ_{H} 3.28 and 3.15 m) and corroborated the hexadeca-2,4,9,12-tetraenoate structure. This evidence was used to propose the structure of compound 4 as hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2'-methylbutylamide.

Therefore, four NAAs (1–4), including the new compounds 2–4, were isolated from the roots of *H. helianthoides* var. *scabra*. Two alkamides (1 and 2) thus identified are based on pentaene-12-ynoic acid. 3 contains a trien-12,14-diynoic unit, while 4 is based on a tetraenoic acid functionality. In the tribe Heliantheae, olefinic alkamides have been detected only in six genera, including *Acmella*, *Echinacea*, *Heliopsis*, *Salmea*, *Sanvitalia*, and *Wedelia*, and even in these genera, not all the species examined to date have yielded alkamides.²⁸ Hence, compound 4 represents a chemotaxonomically important constituent within the alkamides of *H. helianthoides* var. *scabra*.

From *L. meyenii*, the previously described²⁹ major macamides 5–7 were isolated with the use of centrifugal partition chromatography combined with HPLC. This method allows for a quick and effective separation of these chemically rather instable compounds and offers the possibility of isolating NAAs for biological investigations.

Next, the isolated NAAs were investigated on different targets of the ECS, including cannabinoid CB₁ and CB₂ receptor binding, inhibition of FAAH, AEA transport, and monoacylglycerol lipase (MAGL), which is the major 2-

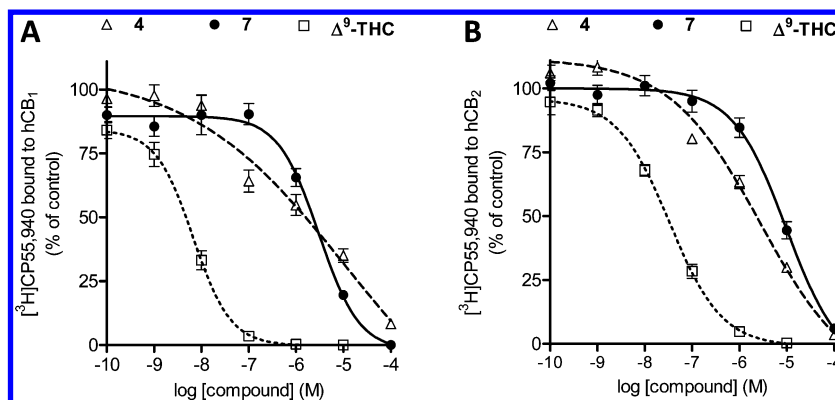


Figure 3. Binding affinity of compounds 4 and 7 for cannabinoid receptor type 1 (CB₁) (A) and cannabinoid receptor type 2 (CB₂). (B) Concentration-dependent displacement of $[^3\text{H}]$ CP55,940 from CHO-transfected CB₁ and CB₂ membranes. Δ^9 -THC was used as the positive control. Data are means \pm SEM.

arachidonoylglycerol (2-AG)-degrading enzyme. The data obtained are summarized in Table 1. While no NAA had any significant effect on MAGL, as expected, macamide 7 showed weak micromolar inhibition of FAAH.¹⁹ As shown in Figure 2, 7 also showed a significant submicromolar inhibition of AEA uptake, which was even more potent than the inhibition measured with the reference inhibitors OMDM-2 and UCM707 (Table 1). Because AEA cellular uptake inhibition by 7 was more potent than FAAH inhibition, this compound shows a certain selectivity toward the first process, similar to the recently described potent AEA cellular uptake inhibitor guineensine, which does not inhibit FAAH.²⁰ Experimental issues cannot be excluded to explain the difference in potency of compound 7 in inhibiting FAAH versus AEA cellular uptake, including the amount of serum albumin used (0.1% BSA in the FAAH assay but no BSA in the AEA uptake assay) or the potential cellular accumulation of the inhibitor via active transport. Notably, 7 also showed a significant binding affinity for CB receptors, with an unexpected 9-fold selectivity toward CB₁ over CB₂ receptors (Table 1 and Figure 3). The NAAs from *H. helianthoides* var. *scabra* were not active on FAAH or AEA uptake, but 4 showed a potent binding interaction with the CB₁ receptor ($K_i = 0.31 \mu\text{M}$) (Table 1 and Figure 3).

Previous studies with NAAs have allowed the generation of some SAR information with respect to CB receptor binding.^{30,31} However, the binding interactions of NAAs with cannabinoid receptors CB₁ or CB₂ do not reflect a straightforward SAR because headgroup modifications and variation in alkyl chain length and double-bond configurations all appear to play a role.^{5,6,9} In the study presented here, the potent new CB₁ receptor ligand 4 is described, comprising a methylbutylamide headgroup combined with the typical 2*E*,4*E* double bonds in the alkyl chain,⁶ with additional double bonds at carbons 9 and 12. This NAA shows an approximately 4-fold selectivity for CB₁ receptors over CB₂ receptors. Moderate CB₁ receptor interactions have been described with NAAs all containing the 2*E* double bond,^{5,9} but there is no clear SAR visible. On the basis of the findings with the macamides, it is possible to identify further crucial molecular elements for CB₁ binding and FAAH and AEA transport inhibition (Table 1 and Figure 4). Macamide 7 seems to fulfill the criteria for endocannabinoid substrate mimicking as this is the only NAA known so far to interact with several ECS targets at low micromolar or even submicromolar concentrations. It is tempting to speculate that the 9*Z*,12*Z*-octadecadiene alkyl

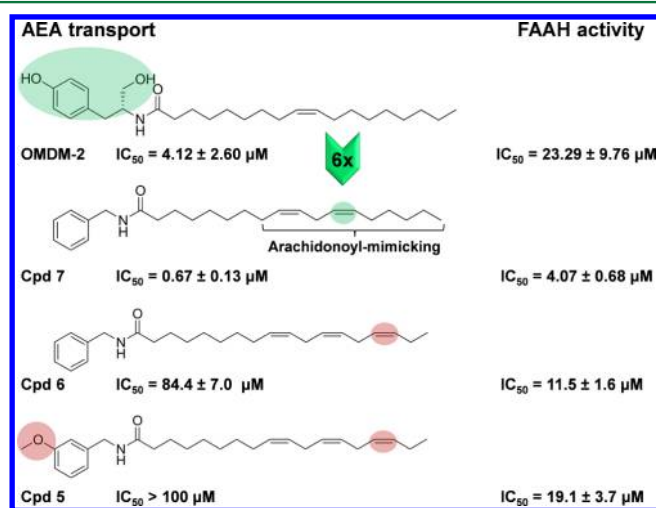


Figure 4. Preliminary SAR of macamides with respect to AEA cellular uptake and FAAH inhibition. While OMDM-2 seems to have an optimal 4-hydroxyphenyl headgroup,³² macamide 7 has an optimal 9*Z*,12*Z*-octadecadiene alkyl chain possibly mimicking the arachidonoyl tail of AEA. Activity-enhancing elements are colored green and activity-decreasing modifications red. Notably, 7 is the only macamide in this series to also show binding interactions with CB receptors (see Table 1). IC₅₀ values \pm the standard deviation are shown.

chain present in 7 mimics the end of the arachidonoyl chain present in endocannabinoids (Figure 4). Thus, it will be interesting to develop this new insight further by studying NAAs with ideal headgroups containing the 9*Z*,12*Z*-octadecadiene alkyl chain. Because OMDM-2 has been described as relatively specific inhibitor of AEA cellular uptake in RBL-2H3 cells,³² the superior potency of compound 7 shown in this study is noteworthy. In their original study, Ortar et al. showed that arvanil (*N*-vanillylarachidonamide) bearing an arachidonoyl chain is more potent on AEA cellular uptake inhibition and CB₁ receptor binding than olvanil (*N*-vanillyloleoylamide) harboring an oleoyl chain,³² which is in agreement with the assumption that the position of the double bonds in the alkyl chain critically mediates potency. In comparison to the macamide 7, the additional 15*Z* double bonds present in compounds 5 and 6 are detrimental to potency (Table 1 and Figure 4). Interestingly, the *N*-benzylamide headgroup present in 7 seems to be ideal as substituted *N*-phenylamides have weaker potency.³² With respect to the combined significant

direct and indirect ECS effects of macamide 7, future studies will have to establish whether this natural product is a functional agonist or antagonist at CB receptors. Moreover, it would be interesting to test whether the cannabimimetic action expected from this compound could relate to the ethnopharmacological use of Maca. Given the fact that CB₁ receptors are expressed in sperm and that the ECS appears to play a role in fertility and sperm quality,³³ the fertility enhancing effects of Maca widely reported in the ethnopharmacological literature^{14–17} should be studied in light of the data presented here. It has already been shown that Maca supplementation improves bovine sperm quality,³⁴ and it would be interesting to test the hypothesis that these effects are mediated, at least in part, by ECS-targeting macamides, such as compound 7. Overall, this study provides additional evidence of the structural and functional similarity between NAAs and endocannabinoids, potentially interlinking NAA chemodiversity, the use of NAA-containing medicinal plants, and botanical dietary supplements with the ECS as a potentially major site of action.

■ EXPERIMENTAL SECTION

General Experimental Procedures. For vacuum liquid chromatography (VLC), SiO₂ (silica gel 60 GF₂₅₄, 15 μ m, Merck, Darmstadt, Germany) and reverse-phase SiO₂ (LiChroprep RP-18, 40–63 μ m, Merck) were applied. Separations were monitored by TLC (aluminum sheets coated with silica gel 60 F₂₅₄, 0.25 mm, Merck 5554 and silica gel 60 RP-18 F₂₅₄, Merck) plates. The chromatograms were visualized at 254 and 366 nm, and by spraying with concentrated H₂SO₄, followed by heating at 110 °C. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi apparatus (Büchi Labortechnik AG, Flawil, Switzerland) using a 40 mm \times 75 mm RP18ec column (Büchi, 40–63 μ m). Preparative thin-layer chromatography was conducted on silica gel 60 F₂₅₄ (0.25 mm, Merck), and centrifugal planar chromatography (CLC) was performed with a Chromatotron instrument (model 8924, Harrison Research, Palo Alto, CA) on manually coated SiO₂ plates (silica gel 60 GF₂₅₄, Merck 7730).

HPLC experiments were conducted on a Young-Lin 9100 series HPLC system (Young-Lin, Anyang-si, Korea), equipped with an UV detector and online degasser using an RP-C₁₈ column (YMC-Pack ODS-A, 250 mm \times 4.6 mm, 5 μ m, 120 Å, YMC, Dinslaken, Germany) at 30 °C and on a Waters 600 system (Waters Corp., Milford, MA), equipped with an UV detector and online degasser using a reverse-phase C₁₈ column (LiChroCART 5 μ m, 100 Å, 250 mm \times 4 mm, Merck) at 25 °C. Centrifugal partition chromatography (CPC) was conducted on an Armen SCPC apparatus (Armen Instrument Sas, Saint-Avé, France) equipped with a gradient pump, a 10 mL sample loop, an ASC/DSC valve, a 250 mL column, a UV detector, and an automatic fraction collector. The system was controlled by Armen Glider software. NMR spectra were recorded in CDCl₃ on a Bruker Avance DRX 500 spectrometer (Bruker, Fallanden, Switzerland) at 500 MHz (¹H) or 125 MHz (¹³C). 2D data were acquired and processed with standard Bruker software. For ¹H–¹H COSY, HSQC, and HMBC experiments, gradient-enhanced versions were used. Mass spectrometric measurements were taken in the positive ionization mode with an API 2000 MS/MS instrument equipped with an atmospheric-pressure chemical ionization (APCI) interface (AB Sciex Instruments, Foster City, CA). The source temperature was 400 °C (*H. helianthoides* var. *scabra* samples) and 450 °C (*L. meyenii* samples). Acquisition and evaluation of data were performed with Analyst version 1.5.2.

Plant Material. The roots of *H. helianthoides* var. *scabra* (Dunal) Fernald “Asahi” were obtained from a nursery (Hegede Flower Nursery Ltd., Kecskemét, Hungary) in the flowering period in September 2009. A voucher specimen (No. 819) has been preserved in the Herbarium of the Department of Pharmacognosy of the University

of Szeged. The plant material was washed, cleaned, and processed in a fresh form.

The yellow-colored dry hypocotyl powder of *L. meyenii* Walp. originated from Peru and was purchased from Raw Organic Maca Powder, EverTrust Ltd. (U.K., batch no. M-010177-11-220312). A representative sample (No. 823) is available at the Department of Pharmacognosy of the University of Szeged.

Extraction and Isolation. The fresh roots of *H. helianthoides* var. *scabra* (9 kg) were extracted with MeOH (90 L) at room temperature. After evaporation, the MeOH extract was subjected to solvent partitioning to obtain CHCl₃- and H₂O-soluble fractions. The alkylamide-containing CHCl₃ phase was concentrated *in vacuo*, yielding 80 g of material, which was subjected to silica gel VLC (2 \times 250 g), with an *n*-hexane/EtOAc gradient system (10:0, 9:1, 8:2, 7:3, 1:1, and 0:10). In total, 78 fractions were collected and combined into 12 main fractions (I–XII) after TLC monitoring. Alkylamide-containing fractions (VIII–IX, 9 g) were selected for further purification, which was conducted by RP-VLC, using MeOH/H₂O (8:2, 85:15, 9:1, and 10:0) and then MeOH/EtOAc (9:1, 6:4, and 10:0) gradients. From the 75 fractions, selected ones (fractions 35–40, 1.15 g, and fractions 41–46, 668 mg) were further purified by medium-pressure liquid chromatography with an *n*-hexane/EtOAc gradient system (1:0, 9:1, 8:2, 7:3, 6:4, 1:1, and 0:1), with a flow rate of 60 mL/min.

Fractions 35–40 were separated into 45 subfractions, and of these, fractions 33–40 were chromatographed via CLC on a 2 mm silica gel plate (*n*-hexane/Me₂CO, 9:1, 8:2, 7:3, and 0:1; flow rate of 5 mL/min) and then purified twice on a 1 mm CLC plate with a flow rate of 3 mL/min, affording compound 1 (15 mg). Fractions 41–45 were purified via CLC (1 mm plate, benzene/CH₂Cl₂/Et₂O, 1:1:1; flow rate of 3 mL/min) and then by preparative TLC using two solvent systems (benzene/CH₂Cl₂/Et₂O, 1:1:1; *n*-hexane/Me₂CO, 8:2), resulting in the purification of compound 3 (3 mg).

Fractions 41–46 were separated into 78 subfractions, and fractions 20–36 were further chromatographed by CLC with an *n*-hexane/Me₂CO gradient system (9:1, 8:2, and 1:0). The material was subjected to separation on a 1 mm silica gel CLC plate, at a flow rate of 3 mL/min. Three main fractions were collected (1–3), of which fractions 1 and 3 were purified further by preparative TLC (benzene/CH₂Cl₂/Et₂O, 1:1:1), affording pure compounds 2 (4 mg) and 4 (3 mg).

Dried *L. meyenii* hypocotyl powder (1.2 kg) was extracted twice with *n*-hexane (30 °C, ultrasonic bath, 2 \times 15 min, 2 \times 3000 mL) and then centrifuged. The supernatant was concentrated *in vacuo*, yielding 9 g of material, which was subjected in four parts to CPC using a two-phase solvent system consisting of *n*-hexane, EtOAc, MeOH, and H₂O (9:1:9:1) (2200 rpm, flow rate of 12 mL/min, 60 min) in the ascending mode. Nine main fractions (I–IX) were obtained, from which fractions V (370 mg) and VI (235 mg) were purified again via CPC (MeCN/H₂O, 1:1; descending mode; 2200 rpm; flow rate of 12 mL/min; 60 min), and then the alkylamide-containing fractions and fraction VII (80 mg) were subjected to HPLC (Young-Lin, semipreparative RP-C₁₈ column, MeCN/H₂O, 9:1). Three almost pure compounds were afforded, and the final purification was conducted via RP-HPLC (Waters, MeCN/H₂O, 95:5), which resulted in the isolation of compounds 5 (1 mg), 6 (8 mg), and 7 (92 mg).

Octadeca-2E,4E,8E,10Z,14Z-pentaene-12-ynoic acid isobutylamide (1): colorless oil; ¹H NMR (CDCl₃, 500 MHz) δ 5.85 (1H, d, *J* = 15.0 Hz, H-2), 7.18 (1H, dd, *J* = 15.0, 10.8 Hz, H-3), 6.15 (1H, dd, *J* = 15.1, 10.8 Hz, H-4), 6.07 (1H, m, H-5), 2.29 (4H, m, H-6, H-7), 5.93 (1H, dt, *J* = 10.8, 3.2 Hz, H-8), 6.63 (1H, dd, *J* = 15.1, 10.8 Hz, H-9), 6.33 (1H, t, *J* = 10.8 Hz, H-10), 5.50 (1H, dd, *J* = 10.6, 1.6 Hz, H-11), 5.66 (1H, d, *J* = 10.5 Hz, H-14), 6.08 (1H, m, H-15), 2.33 (2H, q, *J* = 7.4 Hz, H-16), 1.47 (2H, m, H-17), 0.95 (3H, t, *J* = 6.5 Hz, H-18), 3.15 (1H, t, *J* = 6.5 Hz, H-2'), 1.80 (1H, sept, *J* = 6.7 Hz, H-3'), 0.91 (6H, d, *J* = 6.7 Hz, H-4', H-5'); ¹³C NMR (CDCl₃, 125 MHz) δ 166.4 (C-1), 143.5, 141.1, 140.6, 139.0, 136.7, 128.8, 128.4, 122.4, 109.3, 107.7 (C-2–C-5, C-8–C-11, C-14, C-15), 92.4, 90.8 (C-12, C-13), 46.9 (C-1'), 32.4, 32.3, 32.2, 32.1 (C-6, C-7, C-16, C-17), 28.5 (C-1'), 22.0 (C-2'), 20.0 (C-3', C-4'), 13.8 (C-18); APCIMS

(positive) m/z 326 $[M + H]^+$, 253 $[(M + H) - C_4H_{11}N]^+$, 225 $[(M + H) - C_4H_{11}N - CO]^+$, 211 $[(M + H) - C_4H_{11}N - CH_2CO]^+$, 185, 181, 171, 158, 143, 131, 117.

Octadeca-2E,4E,8E,10Z,14Z-pentaene-12-ynoic acid 2'-methylbutylamide (2): colorless oil; 1H NMR ($CDCl_3$, 500 MHz) δ 5.78 (1H, d, $J = 15.0$ Hz, H-2), 7.19 (1H, dd, $J = 15.0, 10.9$ Hz, H-3), 6.15 (1H, dd, $J = 15.0, 10.4$ Hz, H-4), 6.06 (1H, m, H-5), 2.27 (4H, m, H-6, H-7), 5.93 (1H, m, H-8), 6.64 (1H, dd, $J = 15.0, 10.9$ Hz, H-9), 6.33 (1H, t, $J = 10.9$ Hz, H-10), 5.51 (1H, brd, $J = 10.4$ Hz, H-11), 5.66 (1H, d, $J = 10.4$ Hz, H-14), 6.06 (1H, m, H-15), 2.33 (2H, q, $J = 7.3$ Hz, H-16), 1.47 (2H, m, H-17), 0.96 (3H, t, $J = 7.4$ Hz, H-18), 3.28 (1H, m, H-2'a), 3.15 (1H, m, H-2'b), 1.57 (1H, m, H-3'), 1.40 (1H, m, H-4'a), 1.17 (1H, m, H-4'b), 0.91 (3H, t, $J = 7.2$ Hz, H-5'), 0.91 (3H, d, $J = 6.8$ Hz, H-6'); APCIMS (positive) m/z 340 $[M + H]^+$, 253 $[(M + H) - C_5H_{13}N]^+$, 235 $[(M + H) - C_5H_{13}N - H_2O]^+$, 225 $[(M + H) - C_5H_{13}N - CO]^+$, 211 $[(M + H) - C_5H_{13}N - CH_2CO]^+$, 185, 181, 159; HRESIMS m/z 340.2559 $[M + H]^+$ (calcd for $C_{23}H_{34}NO$, 340.2562).

Hexadeca-2E,4E,9Z-triene-12,14-diynoic acid isobutylamide (3): colorless oil; 1H NMR ($CDCl_3$, 500 MHz) δ 5.78 (1H, d, $J = 15.5$ Hz, H-2), 7.19 (1H, dd, $J = 15.5, 10.8$ Hz, H-3), 6.15 (1H, dd, $J = 15.1, 10.8$ Hz, H-4), 6.03 (1H, dt, $J = 15.1, 7.0$ Hz, H-5), 2.16 (2H, $J = 7.0$ Hz, H-6), 1.52 (2H, dq, $J = 7.3, 7.3$ Hz, H-7), 2.04 (2H, q, $J = 7.1$ Hz, H-8), 5.48 (1H, dt, $J = 10.3, 7.2$ Hz, H-9), 5.40 (1H, dt, $J = 10.3, 6.8$ Hz, H-10), 3.01 (2H, d, $J = 7.3$ Hz, H-11), 1.95 (3H, s, H-16), 5.53 (1H, brs, NH), 3.17 (2H, t, $J = 6.5$ Hz, H-2'), 1.81 (1H, sept, $J = 6.7$ Hz, H-3'), 0.93 (6H, d, $J = 6.7$ Hz, H-4', H-5'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3 (C-1), 122.1 (C-2), 141.0 (C-3), 128.9 (C-4), 142.1 (C-5), 32.1 (C-6), 28.0 (C-7), 26.4 (C-8), 132.3 (C-9), 122.4 (C-10), 17.8 (C-11), 75.2, 64.9, 60.9, 59.7 (C-12–C-15), 4.5 (C-16), 46.9 (C-2'), 28.6 (C-3'), 20.1 (C-4', 5'); HRESIMS m/z 298.2094 $[M + H]^+$ (calcd for $C_{20}H_{28}NO$, 298.2092).

Hexadeca-2E,4E,9,12-tetraenoic acid 2'-methylbutylamide (4): colorless oil; 1H NMR ($CDCl_3$, 500 MHz) δ 5.75 (1H, d, $J = 15.0$ Hz, H-2), 7.19 (1H, dd, $J = 15.0, 10.6$ Hz, H-3), 6.14 (1H, dd, $J = 15.3, 10.6$ Hz, H-4), 6.05 (1H, dt, $J = 15.3, 6.8$ Hz, H-5), 2.17 (2H, $J = 6.8$ Hz, H-6), 1.50 (2H, m, H-7), 2.06 (2H, m, H-8), 5.46 (4H, m, H-9, H-10, H-12, H-13), 3.02 (1H, brd, $J = 5.1$ Hz, H-11a), 3.09 (1H, d, $J = 5.7$ Hz, H-11b), 2.06 (2H, m, H-14), 1.41 (2H, m, H-15), 0.92 (3H, $J = 7.0$ Hz, H-16), 3.28 (1H, m, H-2'a), 3.15 (1H, m, H-2'b), 1.57 (1H, m, H-3'), 1.41 (1H, m, H-4'a), 1.17 (1H, m, H-4'b), 0.93 (3H, t, $J = 7.2$ Hz, H-5'), 0.93 (3H, d, $J = 6.8$ Hz, H-6'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.1 (C-1), 122.0 (C-2), 141.0 (C-3), 128.6 (C-4), 142.4 (C-5), 32.3 (C-6), 28.4 (C-7), 26.5 (C-8), 2×124.9 and 2×130.9 (C-9, C-10, C-12, C-13), 17.8 (C-11), 26.5 (C-14), 22.5 (C-15), 13.6 (C-16), 45.2 (C-2'), 35.0 (C-3'), 27.0 (C-4'), 11.3 (C-5'), 17.2 (C-6'); HRESIMS m/z 318.2717 $[M + H]^+$ (calcd for $C_{21}H_{36}NO$, 318.2718).

[3H]AEA Uptake. Arachidonoyl ethanolamide (anandamide or AEA) uptake inhibition was assessed according to the method of Nicolussi et al.,²⁰ with minor modifications. Samples with 0.5×10^6 U937 cells in RPMI (37 °C) were dispensed in a volume of 250 μ L into Aquasil silanized glass vials (Chromacol 1.1-MTV) in a 96-well format. All compounds and vehicle controls (DMSO) were added in volumes of 5 μ L. The commercially available control inhibitors OMDM-2 and UCM707 were always run as controls at a final concentration of 10 μ M. For both controls, IC_{50} values were generated for comparison. After preincubation for 15 min, 0.5 nM [3H]AEA (60 Ci/mmol) and 99.5 nM nonlabeled AEA, resulting in a final AEA concentration of 100 nM, were incubated for a period of 15 min. AEA uptake was stopped by filtration over UniFilter-GF/C filters (PerkinElmer) presoaked with PBS and 0.25% BSA. The cells were washed three times with 1% fatty acid free BSA in PBS buffer, which removed residual AEA. The plates were dried and sealed after the addition of 45 μ L of MicroScint 20 scintillation cocktail (PerkinElmer). Radioactivity measurements were performed on a PerkinElmer Wallac Trilux MicroBeta 1450 instrument for 2 min. Relative IC_{50} values, efficacies, and 95% confidence intervals (CIs) were calculated using GraphPad Prism. All data are reported as means

\pm the standard error of the mean (SEM) of at least three independent experiments conducted in triplicate.

FAAH and MAGL Activity. The enzyme activity assays were conducted exactly as previously described.²⁰ Hydrolysis of [3H]AEA (60 Ci/mmol) by FAAH and that of [3H]-2-OG by MAGL were performed in U937 cellular homogenate (0.18 mg of protein per sample) and mouse brain homogenate (0.2 mg of protein per sample), respectively. Protein quantification was conducted using the BCA assay (Thermo Scientific). The homogenate samples were dissolved in 10 mM Tris-HCl, 1 mM EDTA, and 0.1% fatty acid free BSA at pH 8 in a volume of 490 μ L. Then, a 10 μ L aliquot of the compound or vehicle control (DMSO) was added followed by a 15 min incubation period. AEA (0.5 nM [3H]AEA and 99.5 nM unlabeled AEA) or 1 nM [3H]-2-OG was added as a substrate for FAAH or MAGL, respectively. Both substrates were incubated for 15 min (37 °C) in the respective homogenates. The hydrolysis reaction was terminated via the addition of 1 mL of an ice-cold $CHCl_3$ /MeOH mixture (1:1) and subsequent vortexing. Centrifugation at 10000g and 4 °C for 10 min resulted in phase separation. The upper aqueous phase was measured by liquid scintillation counting on a Tri-Carb 2100 TR instrument after addition of 3.5 mL of Ultima Gold scintillation liquid (PerkinElmer). Absolute IC_{50} values, efficacies, and 95% CIs were calculated using GraphPad Prism. All data are reported as means \pm SEM of three independent experiments conducted in triplicate.

Membrane Preparation. Membrane preparations were made following the method of Nicolussi et al.²⁰ In brief, CHO-K1 cells transfected with hCB_1 or hCB_2 were cultured in flasks up to 90% confluence. Cells were scraped after being washed twice with PBS, transferred into a falcon tube, and homogenized by Polytron PT1300D for 5 min at 30K rpm. A short centrifugation time (1400g for 5 min) was used to remove debris. The supernatant underwent ultracentrifugation (64000g for 45 min at 4 °C) to enrich the membrane fraction in the pellet. Each pellet was resuspended, and the protein content was determined with a BCA assay (Thermo Scientific).

Cannabinoid (CB) Receptor Binding. CB_1 and CB_2 receptor binding was assessed in a radioligand displacement assay described in detail by Nicolussi et al.²⁰ Briefly, 20 μ g of protein of CHO-K1 hCB_1 or hCB_2 membrane preparation samples was resuspended in 500 μ L of binding buffer [50 mM Tris-HCl, 2.5 mM EDTA, 5 mM $MgCl_2$, and 0.5% fatty acid free BSA (pH 7.4)] in silanized glass vials. [3H]CP55,940 (168 Ci/mmol) (PerkinElmer) was added, reaching a final concentration of 0.5 nM followed by the incubation of compounds at indicated concentrations in DMSO or vehicle (5 μ L). Equilibration of membrane binding for 2 h at 37 °C was followed by filtration through a 0.1% PEI presoaked UniFilter-96 GF/B plate (PerkinElmer) and 12 washes with 167 μ L of ice-cold buffer. After addition of 45 μ L of MicroScint 20 scintillation cocktail, the plates were sealed and counted in a PerkinElmer 1450 Microbeta TRILUX liquid scintillation counter. WIN55,212-2 was used to assess unspecific binding at 10 μ M and subtracted from all the measurements. A K_d of 0.124 nM with CP55,940 for hCB_1 and a K_d of 0.589 nM for hCB_2 , as determined previously,²⁰ were used to calculate the K_i values with the Cheng–Prusoff equation [$K_i = IC_{50}/(1 + [CP55,940]/K_d)$]. GraphPad Prism was used to express IC_{50} as the 50% displacement of the radioligand. Data are reported as means of three independent experiments.

■ ASSOCIATED CONTENT

● Supporting Information

Additional NMR and JMOD spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Authors

*Telephone: +36 62 545559. Fax: +36 62 545751. E-mail: csupor.dezso@pharmacognosy.hu.

*Telephone: +41 31 6314124. Fax: +41 31 6313737. E-mail: gertsch@ibmm.unibe.ch.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the European Union and the State of Hungary, cofinanced by the European Social Fund in the framework of the TÁMOP 4.2.4.A/2-11-1-2012-0001 “National Excellence Program”. This work was also supported by New Hungary Development Plan Projects TÁMOP-4.2.2.A-11/1/KONV-2012-0035 and TÁMOP-4.1.1.C-12/1/KONV-2012-0014, the Swiss National Science Foundation (SNSF) NCCR TransCure, and the University of Bern. Financial support from the Hungarian Scientific Research Fund (OTKA K109846) is gratefully acknowledged. We thank N. Jedlinski for mass spectrometric measurements.

REFERENCES

- (1) Blancaflor, E. B.; Kilaru, A.; Keereetaweep, J.; Khan, B. R.; Faure, L.; Chapman, K. D. *Plant J.* **2014**, in press.
- (2) Ramírez-Chávez, E.; López-Bucio, J.; Herrera-Estrella, L.; Molina-Torres, J. *Plant Physiol.* **2004**, *134*, 1058–1068.
- (3) Méndez-Bravo, A.; Calderón-Vázquez, C.; Ibarra-Laclette, E.; Raya-González, J.; Ramírez-Chávez, E.; Molina-Torres, J.; Guevara-García, A. A.; López-Bucio, J.; Herrera-Estrella, L. *PLoS One* **2011**, *6*, e27251.
- (4) Gertsch, J.; Schoop, R.; Kuenzle, U.; Suter, A. *FEBS Lett.* **2004**, *577*, S63–S69.
- (5) Woelkart, K.; Xu, W.; Pei, Y.; Makriyannis, A.; Picone, R. P.; Bauer, R. *Planta Med.* **2005**, *71*, 701–705.
- (6) Raduner, S.; Majewska, A.; Chen, J. Z.; Xie, X. Q.; Hamon, J.; Faller, B.; Altmann, K. H.; Gertsch, J. *J. Biol. Chem.* **2006**, *281*, 14192–14206.
- (7) Gertsch, J. *Planta Med.* **2008**, *74*, 638–650.
- (8) Ruiti, S.; Anzani, N.; Orrù, A.; Floris, C.; Caboni, P.; Maccioni, E.; Distinto, S.; Alcaro, S.; Cottiglia, F. *Bioorg. Med. Chem.* **2013**, *21*, 7074–7082.
- (9) Dossou, K. S.; Devkota, K. P.; Morton, C.; Egan, J. M.; Lu, G.; Beutler, J. A.; Moaddel, R. *J. Nat. Prod.* **2013**, *76*, 2060–2064.
- (10) Chicca, A.; Raduner, S.; Pellati, F.; Strompen, T.; Altmann, K. H.; Schoop, R.; Gertsch, J. *Int. Immunopharmacol.* **2009**, *9*, 850–858.
- (11) Woelkart, K.; Bauer, R. *Planta Med.* **2007**, *73*, 615–623.
- (12) Rios, M. Y. In *Drug Discovery Research in Pharmacognosy*; Vallisuta, O.; Olimat, S. M., Eds.; InTech: Rijeka, Croatia, 2012; Chapter 6, pp 107–144.
- (13) Boonen, J.; Bronselaer, A.; Nielandt, J.; Veryser, L.; De Tré, G.; De Spiegeleer, B. *J. Ethnopharmacol.* **2012**, *142*, S63–S90.
- (14) Gonzales, G. F. *J. Evidence-Based Complementary Altern. Med.* **2012**, 193496.
- (15) Zhao, J.; Muhammad, I.; Dunbar, D. C.; Mustafa, J.; Khan, I. A. *J. Agric. Food Chem.* **2005**, *53*, 690–693.
- (16) Wang, Y.; Wang, Y.; McNeil, B.; Harvey, L. M. *Food Res. Int.* **2007**, *40*, 783–792.
- (17) Quirós, C. F.; Cardenas, R. A. In *Andean Roots and Tubers: Ahipa, Arracacha, Maca and Yacon*; Hermann, M., Heller, J., Eds.; International Plant Genetic Resources Institute: Rome, 1997; pp 173–198.
- (18) Wu, H.; Kelley, C. J.; Pino-Figueroa, A.; Vu, H. D.; Maher, T. J. *Bioorg. Med. Chem.* **2013**, *21*, S188–S197.
- (19) Almukadi, H.; Wu, H.; Böhlke, M.; Kelley, C. J.; Maher, T. J.; Pino-Figueroa, A. *Mol. Neurobiol.* **2013**, *48*, 333–339.
- (20) Nicolussi, S.; Viveros-Paredes, J. M.; Gachet, M. S.; Rau, M.; Flores-Soto, M. E.; Blunder, M.; Gertsch, J. *Pharmacol. Res.* **2014**, *80*, S2–S5.
- (21) Rios, M. Y.; Aguilar-Guadarrama, B.; Del Carmen Gutiérrez, M. *J. Ethnopharmacol.* **2007**, *110*, 364–367.
- (22) Hernández, I.; Márquez, L.; Martínez, I.; Dieguez, R.; Delporte, C.; Prieto, S.; Molina-Torres, J.; Garrido, G. *J. Ethnopharmacol.* **2009**, *124*, 649–652.
- (23) Jacobson, M. *J. Am. Chem. Soc.* **1951**, *73*, 100–103.
- (24) Jacobson, M. *J. Am. Chem. Soc.* **1957**, *79*, 356–358.
- (25) <http://tropicos.org/Name/2726551>.
- (26) Bohlmann, F.; Gerke, T.; Ahmed, M.; King, M. R.; Robinson, H. *Liebigs Ann. Chem.* **1983**, *7*, 1202–1206.
- (27) Jakupovic, J.; Schuster, A.; Bohlmann, F.; King, R. M.; Robinson, H. *Planta Med.* **1986**, *52*, 18–20.
- (28) Molinartorres, J.; Salgado-Garciglia, R.; Ramirez-Chavez, E.; Del Rio, R. E. *Biochem. Syst. Ecol.* **1996**, *24*, 43–47.
- (29) McCollom, M. M.; Villinski, J. R.; McPhail, K. L.; Craker, L. E.; Gafner, S. *Phytochem. Anal.* **2005**, *16*, 463–469.
- (30) Gertsch, J.; Raduner, S.; Altmann, K. H. *J. Recept. Signal Transduction Res.* **2006**, *26*, 709–730.
- (31) Matovic, N.; Matthias, A.; Gertsch, J.; Raduner, S.; Bone, K. M.; Lehmann, R. P.; Devoss, J. J. *Org. Biomol. Chem.* **2007**, *5*, 169–174.
- (32) Ortá, G.; Ligresti, A.; De Petrocellis, L.; Morera, E.; Di Marzo, V. *Biochem. Pharmacol.* **2003**, *65*, 1473–1481.
- (33) Battista, N.; Mecceriello, R.; Cobellis, G.; Fasano, S.; Di Tommaso, M.; Pirazzi, V.; Konje, J. C.; Pierantoni, R.; Maccarone, M. *Mol. Cell. Endocrinol.* **2012**, *355*, 1–14.
- (34) Clement, C.; Kneubühler, J.; Urwyler, A.; Witschi, U.; Kreuzer, M. *Theriogenology* **2010**, *74*, 173–183.