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Review

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Plant-Based Modulators of Endocannabinoid Signaling

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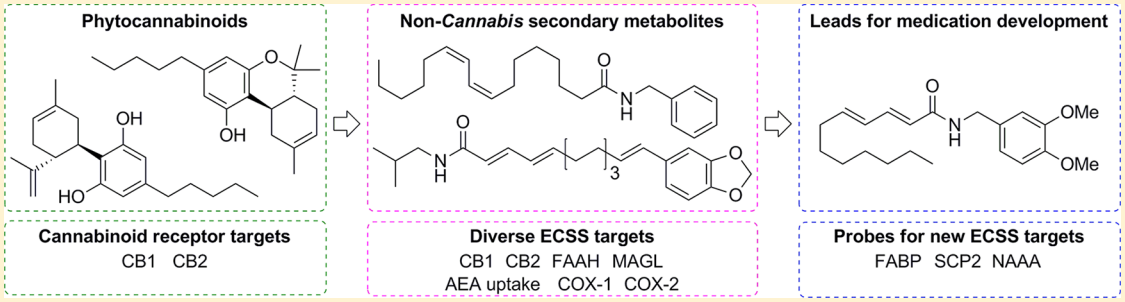

ABSTRACT: Extracts from *Cannabis* species have aided the discovery of the endocannabinoid signaling system (ECSS) and phytocannabinoids that possess broad therapeutic potential. Whereas the reinforcing effects of *C. sativa* are largely attributed to CB1 receptor agonism by Δ^9-tetrahydrocannabinol (Δ^9-THC), the observed medicinal effects of *Cannabis* arise from the combined actions of various compounds. In addition to compounds bearing a classical cannabinoid structure, naturally occurring fatty acid amides and esters resembling anandamide and 2-arachidonoyl glycerol isolated from non-*Cannabis* species are also valuable tools for studying ECSS function. This review highlights the potential of plant-based secondary metabolites from *Cannabis* and unrelated species as ECSS modulators.

INTRODUCTION

The recreational, spiritual, and medicinal use of *Cannabis sativa* L. (Cannabaceae) has been documented in Chinese and Indian texts since before 2000 B.C., along the way accumulating around 5000 years of anecdotal and documented evidence of its psychoactive effects.[1,2] The discovery of tetrahydrocannabinol (THC) by Mechoulam and colleagues[3] paved the way for subsequent discoveries of endocannabinoids (eCBs)[4,5] and the cannabinoid receptors.[6,7] Since the legalization of *Cannabis*-containing products by the states of Colorado and Washington in 2012,[8] 22 of the 50 United States have decriminalized possession of small amounts of *Cannabis*, and many others have passed legislation that facilitates the medical use of *Cannabis* products.[9]

Whereas the psychoactive effects may be sought after by recreational and spiritual users, secondary metabolites produced by *Cannabis sativa* L. and *Cannabis indica* Lam. (Cannabaceae) possess diverse therapeutic and medicinal potential. As such, the development of *Cannabis*-based medicinal products hinges on the ability to remove the consciousness-altering principles from the remainder of the medicinal plant. In *C. sativa*, this active principle is Δ^9-THC (**1a**), a terpene and polyketide biosynthesis product with partial agonist effects at the cannabinoid receptors CB1 and CB2.[10] Δ^9-THC is one of over 60 phytocannabinoids present in *Cannabis* spp.[11] and is structurally related to various other polyketide/terpene biosynthesis products (**1**−**6**, Figure 1). The pharmacologic and behavioral activity of Δ^9-THC is well-documented: Δ^9-THC produces anxiolytic and orexant effects while altering the level of consciousness.[12] The reinforcing effects of Δ^9-THC can lead to a dependence and withdrawal

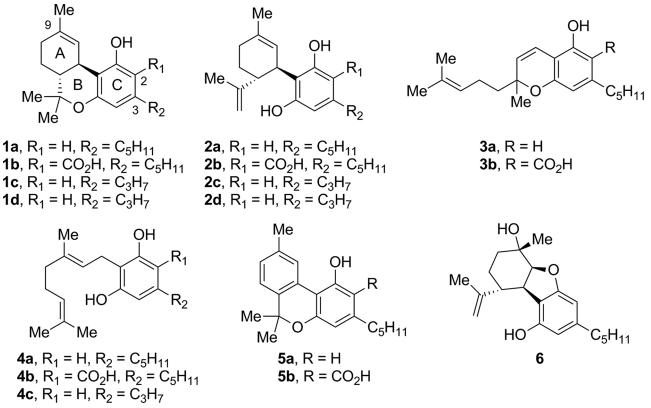

Figure 1. Major phytocannabinoids in *Cannabis* spp.: Δ^9-Tetrahydrocannabinol (Δ^9-THC, **1a**), Δ^9-THC-2-carboxylic acid (Δ^9-THCA, **1b**), tetrahydrocannabivarin (THCV, **1c**), THCV-2-carboxylic acid (THCVA, **1d**), cannabidiol (CBD, **2a**), CBD-2-carboxylic acid (CBDA, **2b**), cannabidivarin (CBDV, 2c), CBDV-2-carboxylic acid (CBDVA, **2d**), cannabichromene (CBC, **3a**), CBC-2-carboxylic acid (CBCA, **3b**), cannabigerol (CBG, **4a**), CBG-2-carboxylic acid (CBGA, **4b**), cannabigivarin (CBGV, **4c**), cannabinol (CBN, **5a**), CBN-2-carboxylic acid (CBNA, **5b**), cannabielsoin (CBE, **6**).

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syndrome in heavy users.[13] The pharmacologic profiles of several other closely related plant constituents are similarly well-documented and reveal vastly different therapeutic potential. For example, cannabidiol (CBD, **2a**) is a ring-opened analogue of Δ^9-THC that has antiseizure and anxiolytic effects without also exhibiting the altered level of consciousness and reinforcing effects of Δ^9-THC.[14,15] Indeed, Epidiolex is an oromucosal spray formulation of CBD and was the first agent extracted from *C. sativa* to be approved by the United States Food and Drug Administration (FDA) in June 2018.[16] Epidiolex is approved to treat several severe forms of childhood epilepsy that are refractory to other antiepileptic drugs.[17,18] Different from Δ^9-THC, CBD has no efficacy at CB1 or CB2 receptors and has no dependence liability; this contributes, in part, to the confusing regulatory state of CBD-containing products in the United States.[19] In fact, the therapeutic mechanism of action of CBD is proving to be quite complex and likely includes many pharmacologic targets in the eCB signaling system (ECSS) and beyond.[20,21] Thus, ECSS modulators represent an important class of agents that indirectly modulate eCB signaling.

Realizing the full pharmacologic potential of the ECSS requires discovery and development of selective probes for ECSS targets. Just as secondary metabolites from *Cannabis* helped characterize cannabinoid receptors, natural ECSS modulators from diverse plant sources have led to the development of key pharmacologic probes. Among them, Peruvian ginseng *Lepidium meyenii* Walp. (Brassicaceae), purple coneflower *Echinacea purpurea* (L.) Moench (Asteraceae), and *Piper nigrum* L. (Piperaceae) produce potent compounds that inhibit key ECSS proteins. Furthermore, anti-inflammatory flavones and other flavonoids produced by *Camelia sinensis* (L.) Kuntze (Theaceae) may also modulate the ECSS through various mechanisms. *Cannabis* spp. essential oils contain terpenoid constituents that may also contribute to the pharmacologic effects of *Cannabis* and ECSS-modulating medicinal plants.

The purpose of this review is to highlight the therapeutic potential of ECSS modulation from plant-based sources. The main focus here is on the actions of phytocannabinoids and related terpenes and polyketides on receptors, enzymes, and trafficking proteins within the ECSS; the activity of Δ^9-THC and CBD have been described in great detail elsewhere and will not be discussed here.[12,20,22]

PHARMACOLOGIC TARGETS INVOLVED IN ECB SIGNALING

The ECSS is broadly distributed throughout the central (CNS) and peripheral (PNS) nervous systems (Figure 2) and is a regulator of stress and anxiety, pain, and metabolic function.[23−27] As a regulator of GABAergic and dopaminergic neurotransmission in the mesolimbic system, the ECSS also impacts the reinforcing effects of drugs of abuse.[28,29] eCBs are headgroup-modified fatty acid analogues, with the most well-characterized being anandamide (arachindonic acid *N*-ethanolamide, AEA, **7**), 2-arachidonoyl glycerol (2-AG, **8**), and palmitoylethanolamide (PEA, **9**). Ethanolamide eCBs AEA and PEA are generated by the enzyme *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), which hydrolyzes the precursor *O*-phosphate into the corresponding primary alcohol.[30] The enzyme diacylglycerol lipase-α (DAGLα) converts esterified fatty acid glycerol esters to 2-AG.[31] The actions of NAPE-PLD and DAGLα take place

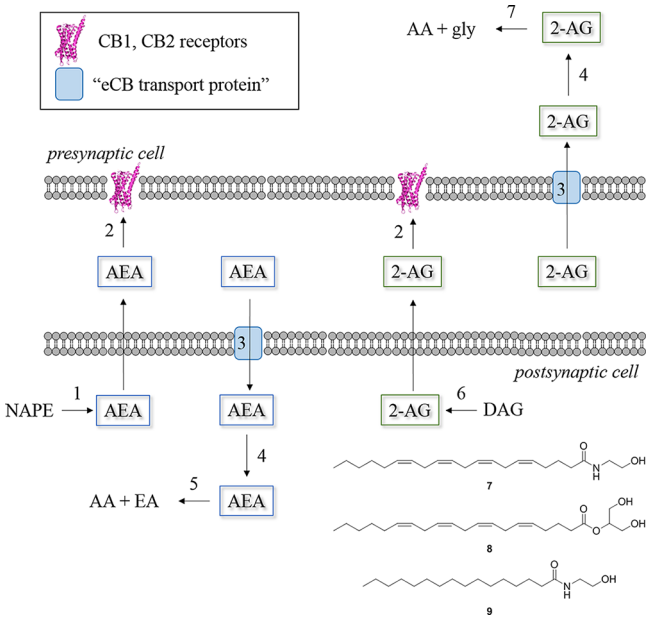

Figure 2. General schematic showing proteins involved in neuronal eCB synthesis, degradation, and transport. AEA: NAPE-PLD converts NAPE into AEA (1), which is then transported in a retrograde fashion where the eCB may activate presynaptic CB1 and CB2 receptors (2). Putative eCB transport proteins (3) actively transport AEA across the postsynaptic membrane. Intracellular carrier proteins, including FABP7 and possibly SCP-2 (4), transport AEA to FAAH, which hydrolyzes AEA into arachidonic acid (AA) and ethanolamine (EA) (5). 2-AG: DAGLα catalyzes the hydrolysis of DAG to 2-AG (6). Following activation of CB1 and CB2 receptors (2), 2-AG may be transported across the presynaptic membrane (3) and carried via carrier proteins (4) to MAGL. Hydrolysis of 2-AG by MAGL (7) produces AA and glycerol (gly).

on postsynaptic plasma membranes. eCBs travel across the synapse in a retrograde fashion, where they stimulate inhibitory presynaptic receptors.

Two heavily characterized cannabinoid receptors are CB1 and CB2. These receptors are inhibitory G protein-coupled receptors (GPCRs) expressed on presynaptic membranes. Agonist binding causes a conformational change in the receptor that recruits $G\alpha_i$ proteins and other intracellular signaling partners such as β-arrestins.[10] These receptors are expressed throughout the CNS and PNS, with CB1 expression largest in the CNS and CB2 expression on autoimmune targets and microglial cells.[32−34] The actions of phytocannabinoids as ion channel modulators are reviewed elsewhere.[35−37]

Termination of eCB signaling is afforded primarily by three enzymes, namely, monoacylglycerol lipase (MAGL),[38] fatty acid amide hydrolase (FAAH),[39] and *N*-acylethanolamine acid amide hydrolase (NAAA).[40] These enzymes hydrolyze the fatty acid headgroup to reveal the free fatty acid, which is inactive at ECSS receptors. Other enzymes terminate eCB signaling via biotransformation of the lipophilic fatty acid tail. Like other polyunsaturated fatty acids, the arachidonate tails of **7**−**9** are subject to oxidative biotransformation by cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LO).[41]

Cellular trafficking and transport mechanisms contribute to the observed pharmacologic effects of eCBs. The presence of a membrane-bound transporter or transporters that facilitate eCB transport remains a subject of much debate.[42−45] As amphiphilic molecules with poor aqueous solubility, eCBs are

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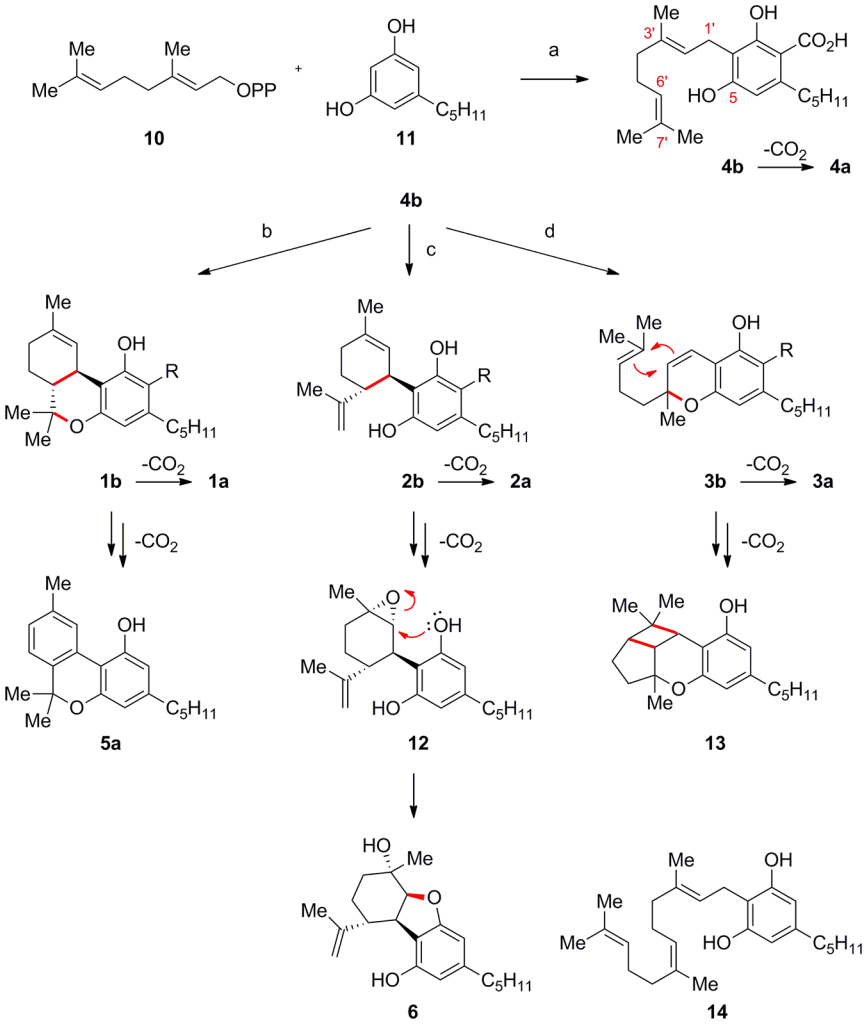
Scheme 1. Biosynthetic Pathways for Common Phytocannabinoids[a]



[a]Enzymatic conditions: (a) CBGA synthase (CBGAS). (b) THCA synthase (THCAS). (c) CBDA synthase (CBDAS). (d) CBCA synthase (CBCAS).

targeted by various classes of lipid-binding proteins. The fatty acid binding protein (FABP) family consists of seven isoforms expressed in various tissues, including cardiac cells, hepatocytes, and the brain.[46−49] Much evidence indicates that FABP7, a brain-specific isoform, regulates eCB transport and influences CB1 signaling.[50−52] Interestingly, Schroeder and colleagues recently reported that genetic knockout of FABP1, a liver-specific binding protein, also influences brain eCB function in male but not female mice.[53−55] Another recently characterized eCB-binding protein is sterol carrier protein-2 (SCP-2). SCP-2 is expressed in brain homogenates and synaptosomes.[56,57] In 2014, SCP-2 was characterized as a binding protein for eCBs,[58] and a subsequent study in 2017 showed that SCP-2 binds AEA and 2-AG with sub-μM potency (K_i^{AEA} = 0.68 ± 0.05 μM, $K_i^{2\text{-AG}}$ = 0.37 ± 0.02 μM).[59] Blocking eCB transport proteins augments eCB concentration at the receptor level and enhances receptor signaling.[60]

BIOSYNTHESIS OF PHYTOCANNABINOIDS AND RELATED SECONDARY METABOLITES

Phytocannabinoids are generated by *C. sativa* via a common biosynthetic pathway shown in Scheme 1.[61,62] Geranyl diphosphate (GPP, **10**) and olivetolic acid (**11**) are combined in the presence of CBGA synthase (CBGAS), also known as geranylpyrophosphate:olivetolate geranyltransferase (GOT), to produce CBGA (**4b**).[63] CBGA is a precursor for other phytocannabinoid classes that differ structurally in their connectivity. For example, the enzyme THCA synthase (THCAS) catalyzes the formation of a C−C bond between the 1′- and 6′-positions and a C−O bond between C-7′ and the 5-phenol,[64] whereas CBDA synthase (CBDAS) only catalyzes the C-1′−C-6′ connection.[65,66] CBCA synthase (CBCAS) catalyzes the connection of the 5-phenol with the C-3′-position that affords the six-membered chromene pyran ring.[67] In each case, the 2-carboxylic acid is subject to nonenzymatic degradation to the corresponding decarboxylated products. Whereas monoterpenoid conjugates are most abundant in *Cannabis* spp., Appendino and colleagues reported a sesquiterpene derivative (sesquicannabigerol, **14**) that has 5-fold higher CB2 potency compared to CBG.[68]

Oxidation of the A ring of **1b**, **2b**, and **3b** results in new molecular scaffolds. For example, CBN (**5a**) is a product of nonenzymatic oxidative aromatization of Δ^9-THC. Oxidation of CBD affords the chemically unstable CBD-9,10-epoxide (CBD-E, **12**), which is converted to cannabielsoin (CBE, **6**) on intramolecular nucleophilic attack by the 1-phenol.[69] CBE

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has also been isolated from guinea-pig microsomal enzymes as a metabolite of CBD.[70] Cannabicyclol (CBL, **13**) is formed as a result of UV-mediated cyclization of CBC.[71] Further oxidation of the A ring of **13** results in the bibenzofurans **15** and **16** (Figure 3).[72] The related cannabioxepane (CBX, **17**) was recently isolated from *C. sativa* var. Carmagnola; in line with similar oxidized products, **17** was found to have no activity at CB1, CB2, or TRPA1 receptors.[73] Other A-ring oxidation products have been isolated from a high-potency strain of *C. sativa* by ElSohly and colleagues that demonstrate CB1 and/or CB2 activity in vitro and in vivo (**18** and **19**).[74] Cannabimovone (**20**) and anhydrocannabimovone (**21**) are examples of A-ring-contracted forms of CBD.[75] Compound **21** was found to produce a similar pharmacologic profile as Δ^9-THC, whereas **20** was only active at ionotropic receptors. The absolute configuration of anhydrocannabimovone was recently updated to **21** based on total synthesis.[76] Similar oxidized CBD- and CBE-like compounds are generated in non-cannabinoid species that produce phytocannabinoids. Two examples include ferruginenes B and C isolated from *Rhododendron* spp. (**22** and **23**).[77]

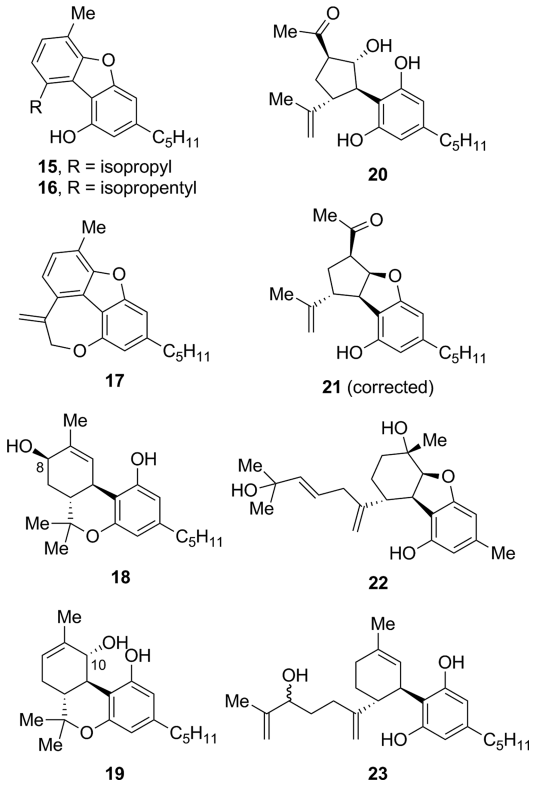

Figure 3. A-ring-oxidized phytocannabinoids isolated from *C. sativa* (**15**–**21**) and *Rhododendron* spp. (**22** and **23**).

The same conceptual biosynthetic scheme affords related cannabinoid classes modified at the 3-position. Biosynthesis of the 3-*n*-propyl congeners THCVA (**1c**) and CBDVA (**2c**) arises from the common intermediate CBGVA (**4c**) likely via similar enzymatic mechanisms in *Cannabis* spp. Other plants are capable of generating 3-arylalkyl phytocannabinoid derivatives (Figure 4). Examination of an acetone extract of the South African spiritual and medicinal plant *Heliochrysum umbraculigerum* Less (Asteraceae) revealed over a dozen resorcinol and phloroglucinol metabolites that include the cannabino-bibenzyl helicannabigerol (**24**), a weak CB2-preferring ligand (K_i = 0.828 ± 0.094 μM).[78] Amorfrutins A (**25**) and B (**26**) and the cannabino-stilbene amorphastilbol (**27**) have been isolated from various sources.[79−81] Amorfrutin B is a PPARγ ligand that has potent antidiabetic activity.[82−84] Cannabino-stilbenes (machaeriol A, **28**, and machaeridiol A, **30**)[85] and cannabino-benzofurans (machaeriol B, **29**, and machaeridiol B, **31**)[86] have been isolated from *Machaerium multiform* Spruce (Fabaceae), a Peruvian liana. It should be noted that the machaeriols and machaeridiols have the

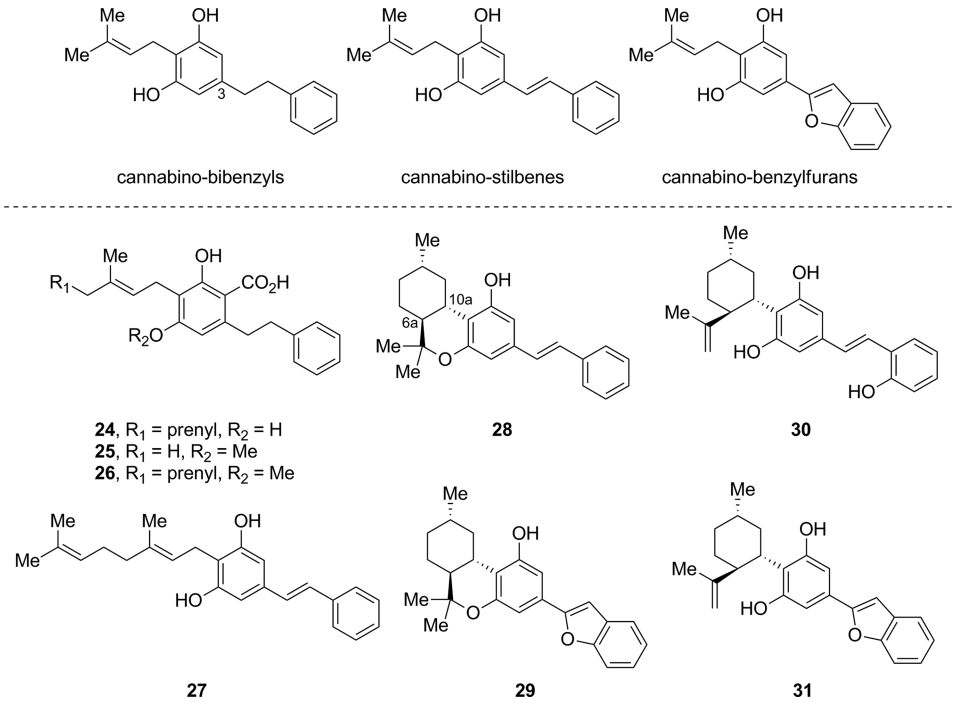

Figure 4. Phytocannabinoid analogues with modified 3-position arylalkyl groups.

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opposite C6a/C10a stereochemistry compared to the corresponding THC and CBD analogues in the *C. sativa* plant. It is unclear whether or not these arylalkyl cannabinoids modulate ECS signaling.

ECSS-MODULATING SECONDARY METABOLITES FROM *CANNABIS* SPP.

The ECS modulating activity of selected phytocannabinoids **1**−**5** isolated from *Cannabis* spp. is shown in Table 1. These data indicate that each of the nonpsychoactive phytocannabinoids possesses its own unique pharmacodynamic profile that may differently modulate the behavioral effects of Δ^9-THC. Metabolites **1b** and **4a** bind CB1 and CB2 receptors with K_i values in the range 10−60 nM, the most potent of the nonpsychoactive phytocannabinoids.[87,88] It is considered that Δ^9-THCA (**1b**) may be a peripherally restricted CB1 receptor agonist.[89] Compounds with this pharmacologic profile may be useful as analgesics lacking psychoactive effects.[90−92] Not all phytocannabinoids are CB1 and CB2 receptor agonists: for example, THCV (**1c**) behaves as a competitive antagonist of structurally diverse cannabinoid receptor agonists.[87] A comprehensive study in 2011 examined the effect of phytocannabinoids on various ECSS targets.[93] The most potent inhibitors of DAGLα found were the CBD analogues CBDA (**2b**) and CBDV (**2c**). CBDVA (**2d**) was less active (IC_{50} = 35 μM, E_{max} = 65%) and CBD (**2a**) was inactive in this study. This suggests the active site of DAGLα is highly sensitive to structural modifications. Few phytocannabinoids are potent inhibitors of the eCB degrative enzymes: MAGL, FAAH, and NAAA. THCA is the most potent and efficacious inhibitor of MAGL, albeit at concentrations likely outside of physiological relevance (IC_{50} 46 μM, E_{max} 86%). No phytocannabinoid inhibited FAAH at concentrations below 50 μM, and CBDA was the most potent NAAA inhibitor (IC_{50} 23 μM, E_{max} 62.5%). Several phytocannabinoids emerged as inhibitors of AEA uptake: CBC, CBG, CBD, and CBDV. These phytocannabinoids may serve as leads for developing more potent AEA uptake inhibitors and probes to elucidate the elusive pharmacologic target or targets of this class.

The essential oils of medicinal plants from the family Cannabaceae are rich in monoterpenes and sesquiterpenes. Some ranges of major constituents of *Cannabis* and *Humulus* essential oils are shown in Table 2;[94−98] as the total terpenoid content of essential oils can vary greatly between plant strains, cultivation conditions, and isolation method, these data are provided to be representative of the recent literature and not exact values or ranges. Ranging from 12% to 67% of the essential oil fraction, myrcene (**32**, Figure 5) is a principal component of *C. sativa*, *C. indica*, and *H. lupulus*. α-Pinene (**33**), terpinolene (**34**), and α-humulene (**39**) are produced in relatively higher concentrations in *C. sativa* compared to *C. indica*. Linalool (**35**), menthol (**36**), and eucalyptol (**37**) are found in higher levels in *C. indica* compared to *C. sativa* essential oil. Higher levels of the sesquiterpene β-caryophyllene (**38**) are found in *Cannabis* spp. compared to *H. lupulus*, whereas α-humulene (**39**) predominates in *H. lupulus* compared to *Cannabis*. Of these essential oil constituents, (*E*)-β-caryophyllene has CB2-selective agonist activity (EC_{50} = 1.9 ± 0.3 μM), whereas the related sesquiterpene α-humulene was found to be inactive.[99] Essential oil terpenoids may contribute to the observed pharmacologic effects of *Cannabis* and related medicinal plants. Their activity as ECSS modulators remains understudied; it would be of value to

Table 1. ECSS-Modulating Activities of Nonpsychoactive Phytocannabinoids Biosynthesized by *Cannabis* spp.[a]

		CB1	CB2	DAGLα[b]		MAGL[b]		FAAH[b]		NAAA[b]		AEA uptake[b]	
ID	compd	K_i	K_i	IC_{50}	E_{max}	IC_{50}	E_{max}	IC_{50}	E_{max}	IC_{50}	E_{max}	IC_{50}	E_{max}[c]
1b	THCA	0.024 ± 0.004[d]	0.056 ± 0.008[d]	27.3 ± 1.6	86.2 ± 6.9[f]	46.0 ± 1.2	86.0 ± 4.1[e]	>50	38.8 ± 3.1[f]	>50	21.1 ± 1.8[f]	>25	34.4 ± 2.8
1c	THCV	0.0754 (0.0534−0.1063)[g]	0.063 (0.053−0.075)[g]	>50	8.8 ± 0.7[f]	>50	18.2 ± 5.4[f]	>50	26.8 ± 3.4[e]	>100	18.2 ± 9.1[e]	>25	46.9 ± 2.0
1d	THCVA	N.D.	N.D.	>50	71.6 ± 5.1[f]	>50	16.9 ± 9.6[f]	>100	27.0 ± 3.6[e]	>100	20.5 ± 3.5[e]	>25	47.2 ± 2.1
2a	CBD	1.459 ± 0.159[d]	0.372 ± 0.058[d]	>100	0	>100	0	53.2 ± 11.3	68.6 ± 4.1[f]	>100	47.2 ± 2.6[e]	25.3 ± 1.8	49.9 ± 1.3
2b	CBDA	N.D.	N.D.	19.4 ± 2.7	90.2 ± 2.6[f]	>50	18.5 ± 2.1[f]	>50	42.2 ± 4.0[f]	23.0 ± 1.3	62.5 ± 1.1[f]	>25	36.3 ± 3.3
2c	CBDV	14.71 ± 5.734[d]	0.574 ± 0.146[d]	16.6 ± 4.1	83.3 ± 1.8[f]	>100	2.5 ± 0.1[e]	>50	36.2 ± 3.1[e]	72.3 ± 18.4	54.5 ± 6.1[e]	21.3 ± 1.8	52.7 ± 3.0
2d	CBDVA	N.D.	N.D.	35.0 ± 5.6	61.0 ± 0.7[f]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3a	CBC	0.714 ± 0.318[d]	0.257 ± 0.094[d]	>100	39.5 ± 2.9[e]	50.1 ± 12.1	64.5 ± 3.6[e]	>100	46.5 ± 1.3[e]	>100	40.6 ± 2.1[e]	12.3 ± 2.7	83.2 ± 3.5
4a	CBG	0.897 ± 0.596[d]	0.153 ± 0.043[d]	>100	44.8 ± 4.1[e]	95.7 ± 32.4	54.2 ± 3.8[e]	>100	44.9 ± 1.4[e]	>100	19.4 ± 2.1[e]	11.3 ± 4.2	85.4 ± 0.8
4b	CBGA	N.D.	N.D.	30.5 ± 1.4	67.6 ± 0.3[e]	>50	17.2 ± 1.8[e]	>50	46.2 ± 4.9[f]	>50	33.2 ± 2.9[f]	>25	43.6 ± 5.0
5c	CBGV	N.D.	N.D.	>50	18.9 ± 6.9[f]	>50	4.9 ± 1.9[f]	>50	38.7 ± 3.8[f]	>50	38.4 ± 4.0[f]	>25	31.2 ± 4.0
5a	CBN	0.013 ± 0.009[d]	0.016 ± 0.011[d]	>50	26.9 ± 3.8[f]	>50	31.5 ± 3.3[f]	>50	29.1 ± 3.3[f]	>50	31.4 ± 2.9[f]	~25	50.0 ± 31.8

[a]Data presented in μM. [b]Ref 93. [c]At 25 μM. [d]Ref 88. [e]At 100 μM. [f]At 50 μM. [g]Ref 87.

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Table 2. Some Representative Ranges for Essential Oil Monoterpenes and Sesquiterpenes in *Cannabis* and *Humulus*

compd	name	ranges (in %)					
		a	*b*	*c*	*d,e*	*d,f*	*g*
32	myrcene	12−30	33−67	14.2			22.9
33	α-pinene	10−17	1−2	16.4			7.7
34	terpinolene	3−10	0.1−1.1	9.6			12
35	linalool	0.2−0.5	2−5			20.8−22.1	0.3
36	menthol					7.2−9.4	
37	eucalyptol					9.7−12.1	
38	(*E*)-β-caryophyllene	10−14	1.3−5.5	23.8	40.6−50	21.1−25.1	18.7
39	α-humulene	4−66	0.3−2	8.3	9.5−16		6.2

[a]Ref 94. [b]Ref 95. [c]Ref 96. [d]Ref 97. [e]*C. sativa*. [f]*C. indica*. [g]Ref 98.

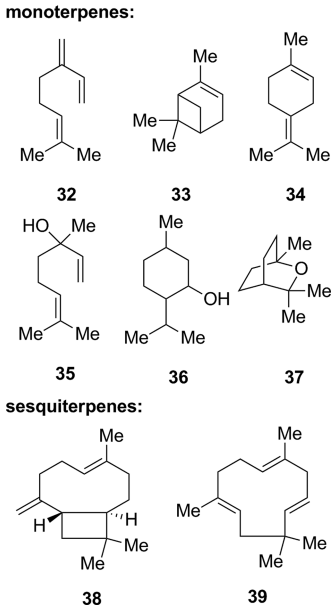

Figure 5. Essential oil constituents of plants from the family Cannabaceae. Monoterpenes: myrcene (**32**), α-pinene (**33**), terpinolene (**34**), linalool (**35**), menthol (**36**), eucalyptol (**37**). Sesquiterpenes: (*E*)-β-caryophyllene (**38**), α-humulene (**39**).

understand whether their role in the "entourage effect"[20] of *Cannabis* is related to ECSS modulation or some other mechanism.

ECSS-MODULATING SECONDARY METABOLITES FROM NON-*CANNABIS* MEDICINAL PLANTS

Plants from the genus *Echinacea* (Asteraceae), including *E. purpurea* and *E. angustifolia*, have a long history of medicinal use in Europe and the United States. Most common uses for these sunflowers include treatments for inflammatory diseases and the common cold and for pain relief.[100] Like other members of the Asteraceae family, *Echinacea* spp. produce fatty acid polyketide amide secondary metabolites called alkamides. The first report of ECSS modulatory activity of *Echinacea* alkamides came in 2004 when Gertsch and colleagues showed inhibition of lipopolysaccharide (LPS)-stimulated TNFα by a standardized *Echinacea* tincture via a CB2-mediated mechanism.[101] This and subsequent investigations revealed a series of *N-iso*-butyl C_{12} fatty acid amides with potent CB2-binding affinity (**40**−**49**, Figure 6, Table 3).[102−104] Bauer and colleagues also reported C_{11}−C_{15} fatty acid alkamides from the roots of *E. angustifolia* that showed modest CB1 and CB2 receptor binding affinities.[102] Some members of this class inhibit FAAH in vitro,[105] and others are reported to be COX-2 inhibitors.[106] Optimization of **41** led to the development of a potent eCB uptake inhibitor (WOBE437) that produced anxiolytic, anti-inflammatory, and antinociceptive effects in mouse models, highlighting the potential for natural ECSS modulators to produce important therapeutic tools.[107,108]

Roots of Peruvian ginseng, *Lepidium meyenii* Walp. (Brassicaceae), are used traditionally to treat male infertility and menopausal symptoms and are locally known as maca.[109] More recently, spray-dried extracts of black and red *L. meyenii* were found to be safe and well tolerated and improved quality of life scores in volunteers living in high and low altitudes.[110] Although phylogenically unrelated to the Cannabaceae, *L. meyenii* produces long-chain, unsaturated fatty acid amides called macamides that resemble the eCBs AEA and PEA and the alkylamides of *Echinacea* spp. (Table 4).[111−114] All macamides contain an *N*-benzylamide headgroup and unbranched fatty acid tails. Fatty acid groups can contain one, two, or three degrees of unsaturation, and several are oxidized as ketones. Two studies determined the ability of macamides **50**−**56** to bind ECSS targets. Gertsch and colleagues identified **53** as a sub-micromolar affinity CB1 ligand with approximately 9-fold preference for binding CB1 over CB2 receptors.[112] This is in contrast to the *Echinacea* alkylamides, which preferentially bind CB2 receptors. Compound **53** was also the most potent inhibitor of FAAH and AEA uptake. Macamides **52**, **54**, and **56** were neuroprotective in a Mn^{2+} toxicity model that was reversed by CB1 antagonist AM251.[114] The authors suggest PPARγ as a potential target for these macamides. The data collected in Table 4 can be used to determine structure−activity relationships (SAR) for this series of alkylamides at diverse ECSS targets.

Other alkamides isolated from various plants are shown in Figure 7. A series of alkamides isolated from the Mediterranian daisy, *Otanthus maritimus* L. (Asteraceae), were tested for binding affinity at cannabinoid (CB1, CB2) and opioid (mu, delta) receptors (Table 5).[115] Compounds **57**−**61** were found to be moderate-potency, CB2-preferring ligands, with compound **61** showing sub-μM potency at CB1 and CB2. None of the compounds tested were more potent at opioid receptors compared to cannabinoid receptors, although binding affinity values were similar for all targets. Alkamides **62**−**64** isolated from *Heliopsis helanthoides* var. *scabra* contain an α,β,γ,δ-unsaturated amide that affords conformational rigidity to the C_{18} tail.[112] These compounds are potent AEA uptake inhibitors (IC_{50} < 5 μM), and compound **64** is a moderate-affinity CB1 ligand (K_i 0.31 μM). The *E*- vs *Z*-

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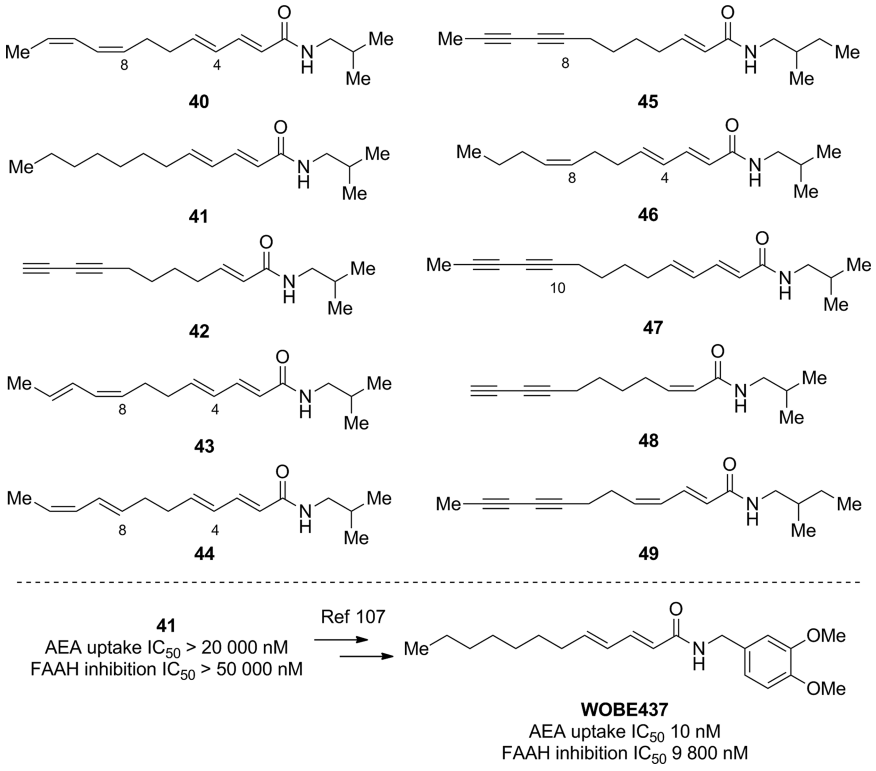

Figure 6. *Echinacea* alkamides with ECSS modulatory activity. Optimization of **41** led to WOBE437.[107]

Table 3. Selected ECSS Activities of *Echinacea* Alkamides

	CB1	CB2		
compd	K_i ± SD (95% C.I.)[a]	K_i ± SD (95% C.I.)[a]	ref	COX-2 inhibition,[106] [b]
40	6210 ± 800	57 ± 14	103	no
41	1940 ± 370	60 ± 13	103	no
42	>40 000	>40 000	103	no
43	N.D.	9044 ± 2985	104	no
44	N.D.	4535 ± 711	104	N.D.[c]
45	6731 (4700–9600)	7204 (4600–11 400)	102	yes
46	10,994 (6900–17 300)	8379 (5500–12 700)	102	no
47	24,644 (18 900–32 100)	1867 (1000–3400)	102	N.D.
48	N.D.	N.D.	N.A.[d]	yes
49	N.D.	N.D.	N.A.	yes

[a]In nM. [b]Yes/no = significant or nonsignificant inhibition of COX-2-mediated formation of PGE_2 at 10 μM. [c]N.D. = not determined. [d]N.A. = not applicable.

stereochemical designation of the C-9/C-10 and C-12/C-13 double bonds of **64** could not be determined.

A high-throughput screen of ethyl acetate extracts of over 400 medicinal plants identified two members of the pepper species, *Piper nigrum* L. (Piperaceae) and *P. longum* L., as inhibitors of AEA uptake.[116] Bioactivity-guided fractionation revealed several polyunsaturated fatty acid amides (**65**–**68**) that are ECSS modulators (Figure 8). The most potent AEA uptake inhibitor, guineensine (**67**), was found to be a highly selective inhibitor of AEA uptake over inhibition of FAAH and MAGL, with weak affinity for CB2 receptors, no affinity for CB1 receptors, and moderate inhibition of COX-1. Guineensine produced dose-dependent cannabimimetic behavioral effects in the tetrad test when administered via the intraperitoneal (i.p.) route in mice (locomotor suppression, antinociception, hypothermia, catalepsy) that were reversed by the CB1 antagonist rimonabant. A subsequent report that included a CEREP screen of 45 receptors, transporters, and ion channels indicated potential off-target binding to 5-$HT_{2A,2B}$ receptors, sigma receptors, and the dopamine transport protein (DAT).[117]

The green tea catechins epigallocatechin-3-*O*-gallate (EGCG, **69**), epigallocatechin (EGC, **70**), and epicatechin-3-*O*-gallate (ECG, **71**) produced by *C. sinensis* have diverse pharmacologic activity[118−122] that includes modest ECSS modulatory activity (Table 6). Korte et al. reported moderate, nonselective CB1 and CB2 binding affinity for **69**–**71** that was approximately 1000-fold lower than for a reference standard (CP-55,940).[123] The most abundant secondary metabolite in *C. sinensis*, EGCG, has been shown to influence COX expression and function under therapeutically relevant conditions.[124−127] In light of the fact that AEA and 2-AG are substrates for oxidation by COX enzymes, EGCG may indirectly alter ECSS function by modulating arachidonate metabolism. EGCG and other green tea phenolics have the potential to interfere with lipoxygenase metabolism. At 30 μM, phenols **55**–**57** inhibited LOX activity by 30–75% in two human tumor tissues that resulted in nonselective reduction in the production of 5-, 12-, and 15-oxidized metabolites.[128] An X-ray crystal structure of soybean LOX-3 (2.1 Å, PDB:[129] 1JNQ) was reported that suggests EGCG inhibits LOX-3 through a molecular mechanism involving a modified EGC.[130] Another proposed mechanism of action of EGCG and related green tea catechins involves inhibition of fatty acid synthase (FAS), which is up-regulated in several cancer cell lines.[131,132] This is potentially relevant to ECSS function, as Kunos and colleagues demonstrated that treatment of mice with CB1

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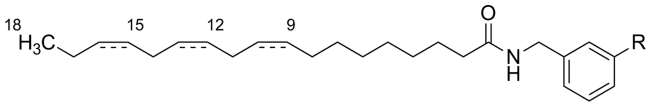
Table 4. ECSS Modulatory Activities of a Series of Macamides Present in *Lepidium meyenii* Roots



			K_i (μM)		FAAH		AEA uptake		
compd	Δ	R	CB1	CB2	IC_{50} (μM)	efficacy (%)	IC_{50} (μM)	efficacy (%)	ref
50		H			>500	N/A			113
51	Δ^9	H			16.7 ± 2.9	76.1			113
52	Δ^9	OCH_3			11.0 ± 2.3	76.4			113
53	$\Delta^{9,12}$	H	0.48 (0.33−0.67)	4.11 (3.11−5.66)	4.07 (2.95−5.62)	83	0.67 (0.47−0.97)	73	112
					10.8 ± 1.3	83.2			113
54	$\Delta^{9,12}$	OCH_3			10.3 ± 1.3	87.5			113
55	$\Delta^{9,12,15}$	H	8.88 (3.79−10.19)	43.96 (24.16 to >50)	11.48 (6.81−18.78)	85	84.36 (72.44 to >100)	53	112
					41.8 ± 2.4	79.7			113
56	$\Delta^{9,12,15}$	OCH_3	8.67 (4.66−13.75)	>50	19.05 (13.80−28.18)	85	>100	44	112
					13.7 ± 1.3	82.8			113

[a]Data shown as K_i or IC_{50} (95% C.I.) or as IC_{50} ± SD.

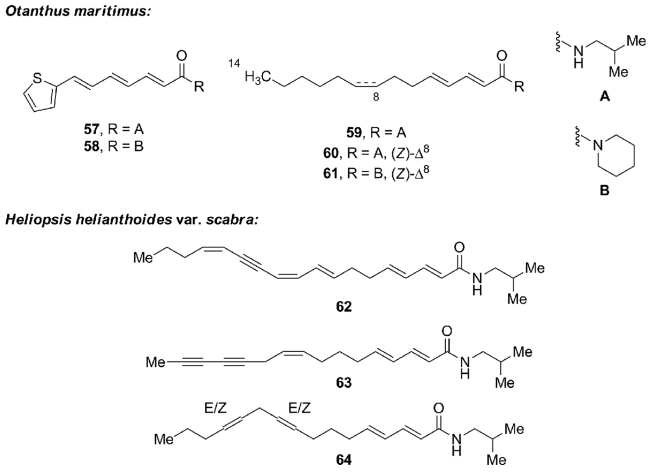


Figure 7. Polyunsaturated fatty acid amides with ECSS modulatory activity isolated from *O. maritimus* and *H. helianthoides* var. *scabra*.

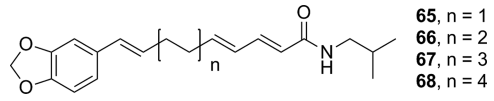
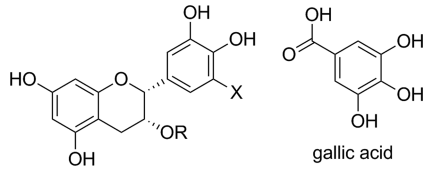


Figure 8. ECSS-modulating fatty acid amides from *Piper* spp. The following data were collected for the most potent AEA uptake inhibitor, guineensine (**67**):[116] AEA uptake (EC_{50} = 290 nM, efficacy = 65% inhibition), FAAH inhibition (IC_{50} = 46.8 μM, 59% inhibition at 100 μM), MAGL (K_i > 100 μM), CB1 receptor (K_i > 20 μM), CB2 receptor (K_i = 38.3 μM), FABP5 (K_i > 100 μM).

Table 6. ECSS Modulatory Activity of Catechins from *Camelia sinensis*



compound	X	R	CB1 K_i (95% CI)[a]	CB2 K_i (95% CI)[a]
69, EGCG	OH	H	33.6 (17.2−65.7)	42.8 (17.9−101.0)
70, EGC	OH	gallic acid	35.7 (3.5−368.20	361.3 (0.5−24 000)
71, ECG	H	gallic acid	47.3 (16.7−133.8)	95.6 (23.0−397.4)

[a]Data in μM.

receptor agonists caused increased expression of FAS in hepatocytes and in the hypothalamus.[133] Whether EGCG inhibits FAS in these contexts may reveal more therapeutic potential for green tea catechins to modulate obesity and metabolic disorders. Although the potential impact of EGCG on ECSS signaling following oral administration is limited due to poor oral bioavailability, future research into novel dosage formulations may help to deliver EGCG-based therapeutics to the clinic. A formulation of a purified botanical extract (sinecatechins, Veregen) is available for topical use.

Table 5. ECSS Modulatory Activity of 57−64

compd	CB1 K_i ± SD (95% C.I.)[a]	CB2 K_i ± SD (95% C.I.)[a]	FAAH IC_{50} (95% C.I.)[a]	efficacy (%)	AEA uptake (95% C.I.)[a]	efficacy (%)	ref
57	8.8 ± 2.2	8.25 ± 1.4	N.D.	N.D.	N.D.	N.D.	115
58	4.1 ± 0.8	1.24 ± 0.3	N.D.	N.D.	N.D.	N.D.	115
59	1.4 ± 0.1	1.17 ± 0.28	N.D.	N.D.	N.D.	N.D.	115
60	1.2 ± 0.3	0.55 ± 0.07	N.D.	N.D.	N.D.	N.D.	115
61	0.8 ± 0.1	0.16 ± 0.04	N.D.	N.D.	N.D.	N.D.	115
62	8.60 (2.39−10.92)	9.18 (5.41−9.84)	17.78 (13.18−25.70)	84	2.45 (1.28−4.70)	75	112
63	>20	22.55 (12.68−25.30)	12.30 (8.68−16.95)	79	4.33 (2.69−6.97)	75	112
64	0.31 (0.18−0.59)	1.21 (0.90−1.71)	19.95 (12.59−33.11)	80	2.15 (0.87−5.31)	51	112

[a]Data in μM.

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CONCLUSIONS

The vast therapeutic potential of the ECSS highlights the need for discovering new, selective small-molecule probes. Natural products have been critical in advancing our understanding of ECSS structure and function, and the evidence compiled here indicates the potential to discover future ECSS modulators from medicinal plants. For example, there is little information concerning the ECSS-modulating ability of 3-position-modified resorcinol derivatives such as those included in Figure 4. In light of the well-understood SAR surrounding classical and nonclassical cannabinoids, it may be possible to discover agents selective for eCB transporters or metabolic enzymes over CB1 and CB2 receptors. The true test for plant-based ECSS modulators will be translation into in vivo systems. As lipophilic lead molecules with many rotatable bonds, the polyketide ECSS modulators described here possess physicochemical properties that may prove problematic for oral and parenteral drug development. The need to develop useful pharmacologic probes and therapeutic agents targeting ECSS proteins has never been greater.

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Notes

The author declares no competing financial interest.

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DEDICATION

§Dedicated to Dr. Barbara N. Timmerman, University of Kansas, for her pioneering work on bioactive natural products.

REFERENCES

(1) Farnsworth, N. R. *J. Am. Pharm. Assoc.* **1969**, *9*, 410−414.
(2) Russo, E. B. *Chem. Biodiversity* **2007**, *4*, 1614−1648.
(3) Mechoulam, R.; Shvo, Y. *Tetrahedron* **1963**, *19*, 2073−2078.
(4) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946−1949.
(5) Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.* **1995**, *50*, 83−90.
(6) Devane, W. A.; Dysarz, F. A., 3rd; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605−613.
(7) Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61−65.
(8) Yates, D.; Speer, J. *Int. J. Drug Policy* **2018**, *59*, 63−66.
(9) Marijuana Overview. National Conference of State Legislatures. Updated 10/4/2018. http://www.ncsl.org/research/civil-and-criminal-justice/marijuana-overview.aspx (accessed October 12, 2018).
(10) Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2002**, *54*, 161−202.
(11) Izzo, A. A.; Borrelli, F.; Capasso, R.; Di Marzo, V.; Mechoulam, R. *Trends Pharmacol. Sci.* **2009**, *30*, 515−527.
(12) Russo, E. B.; Marcu, J. *Adv. Pharmacol.* **2017**, *80*, 67−134.
(13) Ramesh, D.; Schlosburg, J. E.; Wiebelhaus, J. M.; Lichtman, A. H. *ILAR J.* **2011**, *52*, 295−308.
(14) Grotenhermen, F.; Russo, E.; Zuardi, A. W. *Cannabis Cannabinoid Res.* **2017**, *2*, 1−4.
(15) Schoedel, K. A.; Szeto, I.; Setnik, B.; Sellers, E. M.; Levy-Cooperman, N.; Mills, C.; Etges, T.; Sommerville, K. *Epilepsy Behav* **2018**, *88*, 162−171.
(16) Kaufman, M. B. *Pharmacy Therapeutics* **2018**, *43*, 528−530.
(17) Devinsky, O.; Patel, A. D.; VanLandingham, K. E. N. *N. Engl. J. Med.* **2018**, *379*, 795.
(18) Devinsky, O.; Patel, A. D.; Cross, J. H.; Villanueva, V.; Wirrell, E. C.; Privitera, M.; Greenwood, S. M.; Roberts, C.; Checketts, D.; VanLandingham, K. E.; Zuberi, S. M. *N. Engl. J. Med.* **2018**, *378*, 1888−1897.
(19) Corroon, J.; Kight, R. *Cannabis Cannabinoid Res.* **2018**, *3*, 190−194.
(20) Russo, E. B. *Br. J. Pharmacol.* **2011**, *163*, 1344−1364.
(21) Laprairie, R. B.; Bagher, A. M.; Kelly, M. E.; Denovan-Wright, E. M. *Br. J. Pharmacol.* **2015**, *172*, 4790−4805.
(22) Hanus, L. O.; Meyer, S. M.; Munoz, E.; Taglialatela-Scafati, O.; Appendino, G. *Nat. Prod. Rep.* **2016**, *33*, 1357−1392.
(23) Hillard, C. J. *Int. Rev. Neurobiol.* **2015**, *125*, 1−47.
(24) Hillard, C. J. *Neuropsychopharmacology* **2018**, *43*, 155−172.
(25) Hillard, C. J.; Beatka, M.; Sarvaideo, J. *Compr. Physiol.* **2016**, *7*, 1−15.
(26) Pascual, D.; Sanchez-Robles, E. M.; Garcia, M. M.; Goicoechea, C. *Biochem. Pharmacol.* **2018**, *157*, 33−42.
(27) Lu, H. C.; Mackie, K. *Biol. Psychiatry* **2016**, *79*, 516−525.
(28) Hillard, C. *Life Sci.* **2005**, *77*, 1531−1542.
(29) Manzanares, J.; Cabanero, D.; Puente, N.; Garcia-Gutierrez, M. S.; Grandes, P.; Maldonado, R. *Biochem. Pharmacol.* **2018**, *157*, 108−121.
(30) Okamoto, Y.; Morishita, J.; Tsuboi, K.; Tonai, T.; Ueda, N. *J. Biol. Chem.* **2004**, *279*, 5298−5305.
(31) Tanimura, A.; Yamazaki, M.; Hashimotodani, Y.; Uchigashima, M.; Kawata, S.; Abe, M.; Kita, Y.; Hashimoto, K.; Shimizu, T.; Watanabe, M.; Sakimura, K.; Kano, M. *Neuron* **2010**, *65*, 320−327.
(32) Tsou, K.; Brown, S.; Sanudo-Pena, M. C.; Mackie, K.; Walker, J. M. *Neuroscience* **1998**, *83*, 393−411.
(33) Cabral, G. A.; Raborn, E. S.; Griffin, L.; Dennis, J.; Marciano-Cabral, F. *Br. J. Pharmacol.* **2008**, *153*, 240−251.
(34) Atwood, B. K.; Mackie, K. *Br. J. Pharmacol.* **2010**, *160*, 467−479.
(35) Di Marzo, V.; De Petrocellis, L. *Curr. Med. Chem.* **2010**, *17*, 1430−1449.
(36) Janero, D. R.; Makriyannis, A. *ACS Chem. Neurosci.* **2014**, *5*, 1097−1106.
(37) De Petrocellis, L.; Nabissi, M.; Santoni, G.; Ligresti, A. *Adv. Pharmacol.* **2017**, *80*, 249−289.
(38) Dinh, T. P.; Carpenter, D.; Leslie, F. M.; Freund, T. F.; Katona, I.; Sensi, S. L.; Kathuria, S.; Piomelli, D. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 10819−10824.
(39) Egertova, M.; Cravatt, B. F.; Elphick, M. R. *Neuroscience* **2003**, *119*, 481−496.
(40) Sun, Y. X.; Tsuboi, K.; Zhao, L. Y.; Okamoto, Y.; Lambert, D. M.; Ueda, N. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2005**, *1736*, 211−220.
(41) Piomelli, D.; Sasso, O. *Nat. Neurosci.* **2014**, *17*, 164−174.
(42) Chicca, A.; Marazzi, J.; Nicolussi, S.; Gertsch, J. *J. Biol. Chem.* **2012**, *287*, 34660−34682.
(43) Nicolussi, S.; Gertsch, J. *Vitam. Horm.* **2015**, *98*, 441−485.
(44) Glaser, S. T.; Abumrad, N. A.; Fatade, F.; Kaczocha, M.; Studholme, K. M.; Deutsch, D. G. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 4269−4274.
(45) Hillard, C. J.; Jarrahian, A. *Br. J. Pharmacol.* **2003**, *140*, 802−808.
(46) Owada, Y.; Yoshimoto, T.; Kondo, H. *J. Chem. Neuroanat.* **1996**, *12*, 113−122.

DOI: 10.1021/acs.jnatprod.8b00874
J. Nat. Prod. XXXX, XXX, XXX−XXX

(47) Furuhashi, M.; Tuncman, G.; Gorgun, C. Z.; Makowski, L.; Atsumi, G.; Vaillancourt, E.; Kono, K.; Babaev, V. R.; Fazio, S.; Linton, M. F.; Sulsky, R.; Robl, J. A.; Parker, R. A.; Hotamisligil, G. S. *Nature* **2007**, *447*, 959−965.
(48) Furuhashi, M.; Hotamisligil, G. S. *Nat. Rev. Drug Discovery* **2008**, *7*, 489−503.
(49) Myers-Payne, S. C.; Hubbell, T.; Pu, L.; Schnutgen, F.; Borchers, T.; Wood, W. G.; Spener, F.; Schroeder, F. *J. Neurochem.* **1996**, *66*, 1648−1656.
(50) Kaczocha, M.; Glaser, S. T.; Deutsch, D. G. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6375−6380.
(51) Howlett, A. C.; Reggio, P. H.; Childers, S. R.; Hampson, R. E.; Ulloa, N. M.; Deutsch, D. G. *Br. J. Pharmacol.* **2011**, *163*, 1329−1343.
(52) Sanson, B.; Wang, T.; Sun, J.; Wang, L.; Kaczocha, M.; Ojima, I.; Deutsch, D.; Li, H. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2014**, *70*, 290−298.
(53) Martin, G. G.; Chung, S.; Landrock, D.; Landrock, K. K.; Huang, H.; Dangott, L. J.; Peng, X.; Kaczocha, M.; Seeger, D. R.; Murphy, E. J.; Golovko, M. Y.; Kier, A. B.; Schroeder, F. *J. Neurochem.* **2016**, *138*, 407−422.
(54) Martin, G. G.; Landrock, D.; Chung, S.; Dangott, L. J.; Seeger, D. R.; Murphy, E. J.; Golovko, M. Y.; Kier, A. B.; Schroeder, F. *J. Neurochem.* **2017**, *140*, 294−306.
(55) Martin, G. G.; Chung, S.; Landrock, D.; Landrock, K. K.; Dangott, L. J.; Peng, X.; Kaczocha, M.; Murphy, E. J.; Kier, A. B.; Schroeder, F. *Lipids* **2016**, *51*, 1007−1020.
(56) Myers-Payne, S. C.; Fontaine, R. N.; Loeffler, A.; Pu, L.; Rao, A. M.; Kier, A. B.; Wood, W. G.; Schroeder, F. *J. Neurochem.* **1996**, *66*, 313−320.
(57) Avdulov, N. A.; Chochina, S. V.; Igbavboa, U.; Warden, C. S.; Schroeder, F.; Wood, W. G. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **1999**, *1437*, 37−45.
(58) Liedhegner, E. S.; Vogt, C. D.; Sem, D. S.; Cunningham, C. W.; Hillard, C. *Mol. Neurobiol.* **2014**, *50*, 149−158.
(59) Hillard, C. J.; Huang, H.; Vogt, C. D.; Rodrigues, B. E.; Neumann, T. S.; Sem, D. S.; Schroeder, F.; Cunningham, C. W. *Methods Enzymol.* **2017**, *593*, 99−121.
(60) Deutsch, D. G. *Front. Pharmacol.* **2016**, *7*, 370.
(61) Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed.; Wiley: Chichester, UK, 2009.
(62) Shoyama, Y.; Yagi, M.; Nishioka, I.; Yamauchi, T. *Phytochemistry* **1975**, *14*, 2189−2192.
(63) Fellermeier, M.; Zenk, M. H. *FEBS Lett.* **1998**, *427*, 283−285.
(64) Taura, F.; Morimoto, S.; Shoyama, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1995**, *117*, 9766−9767.
(65) Taura, F.; Sirikantaramas, S.; Shoyama, Y.; Yoshikai, K.; Shoyama, Y.; Morimoto, S. *FEBS Lett.* **2007**, *581*, 2929−2934.
(66) Taura, F.; Morimoto, S.; Shoyama, Y. *J. Biol. Chem.* **1996**, *271*, 17411−17416.
(67) Morimoto, S.; Komatsu, K.; Taura, F.; Shoyama, Y. *J. Nat. Prod.* **1997**, *60*, 854−857.
(68) Pollastro, F.; Taglialatela-Scafati, O.; Allara, M.; Munoz, E.; Di Marzo, V.; De Petrocellis, L.; Appendino, G. *J. Nat. Prod.* **2011**, *74*, 2019−2022.
(69) Shani, A.; Mechoulam, R. *Tetrahedron* **1974**, *30*, 2437−2466.
(70) Yamamoto, I.; Gohda, H.; Narimatsu, S.; Yoshimura, H. *J. Pharmacobio-Dyn.* **1988**, *11*, 833−838.
(71) Begley, M. J.; Clarke, D. G.; Crombie, L.; Whiting, D. A. *J. Chem. Soc. D* **1970**, *0*, 1547−1548.
(72) Friedrich-Fiechtl, J.; Spiteller, G. *Tetrahedron* **1975**, *31*, 479−487.
(73) Pagani, A.; Scala, F.; Chaianese, G.; Grassi, G.; Appendino, G.; Taglialatela-Scafati, O. *Tetrahedron* **2011**, *67*, 3369−3373.
(74) Radwan, M. M.; ElSohly, M. A.; El-Alfy, A. T.; Ahmed, S. A.; Slade, D.; Husni, A. S.; Manly, S. P.; Wilson, L.; Seale, S.; Cutler, S. J.; Ross, S. A. *J. Nat. Prod.* **2015**, *78*, 1271−1276.
(75) Taglialatela-Scafati, O.; Pagani, A.; Scala, F.; de Petrocellis, L.; di Marzo, V.; Grassi, G.; Appendino, G. *Eur. J. Org. Chem.* **2010**, *2010*, 2067−2072.
(76) Carreras, J.; Kirillova, M. S.; Echavarren, A. M. *Angew. Chem., Int. Ed.* **2016**, *55*, 7121−7125.
(77) Seephonkai, P.; Popescu, R.; Zehl, M.; Krupitza, G.; Urban, E.; Kopp, B. *J. Nat. Prod.* **2011**, *74*, 712−717.
(78) Pollastro, F.; De Petrocellis, L.; Schiano-Moriello, A.; Chianese, G.; Heyman, H.; Appendino, G.; Taglialatela-Scafati, O. *Fitoterapia* **2017**, *123*, 13−17.
(79) Bohlmann, F.; Hoffmann, E. *Phytochemistry* **1979**, *18*, 1371−1374.
(80) Asakawa, Y.; Toyota, M.; Takemoto, T. *Phytochemistry* **1978**, *17*, 2005−2010.
(81) Mitscher, L. A.; Park, Y. H.; Alshamma, A.; Hudson, P. B.; Haas, T. *Phytochemistry* **1981**, *20*, 781−785.
(82) Weidner, C.; de Groot, J. C.; Prasad, A.; Freiwald, A.; Quedenau, C.; Kliem, M.; Witzke, A.; Kodelja, V.; Han, C. T.; Giegold, S.; Baumann, M.; Klebl, B.; Siems, K.; Muller-Kuhrt, L.; Schurmann, A.; Schuler, R.; Pfeiffer, A. F.; Schroeder, F. C.; Bussow, K.; Sauer, S. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 7257−7262.
(83) Weidner, C.; Wowro, S. J.; Freiwald, A.; Kawamoto, K.; Witzke, A.; Kliem, M.; Siems, K.; Muller-Kuhrt, L.; Schroeder, F. C.; Sauer, S. *Diabetologia* **2013**, *56*, 1802−1812.
(84) Fuhr, L.; Rousseau, M.; Plauth, A.; Schroeder, F. C.; Sauer, S. *J. Nat. Prod.* **2015**, *78*, 1160−1164.
(85) Muhammad, I.; Li, X. C.; Dunbar, D. C.; ElSohly, M. A.; Khan, I. A. *J. Nat. Prod.* **2001**, *64*, 1322−1325.
(86) Muhammad, I.; Li, X. C.; Jacob, M. R.; Tekwani, B. L.; Dunbar, D. C.; Ferreira, D. *J. Nat. Prod.* **2003**, *66*, 804−809.
(87) Thomas, A.; Stevenson, L. A.; Wease, K. N.; Price, M. R.; Baillie, G.; Ross, R. A.; Pertwee, R. G. *Br. J. Pharmacol.* **2005**, *146*, 917−926.
(88) Rosenthaler, S.; Pohn, B.; Kolmanz, C.; Huu, C. N.; Krewenka, C.; Huber, A.; Kranner, B.; Rausch, W. D.; Moldzio, R. *Neurotoxicol. Teratol.* **2014**, *46*, 49−56.
(89) Moreno-Sanz, G. *Cannabis Cannabinoid Res.* **2016**, *1*, 124−130.
(90) Zhang, H.; Lund, D. M.; Ciccone, H. A.; Staatz, W. D.; Ibrahim, M. M.; Largent-Milnes, T. M.; Seltzman, H. H.; Spigelman, I.; Vanderah, T. W. *Pain* **2018**, *159*, 1814−1823.
(91) Yu, X. H.; Cao, C. Q.; Martino, G.; Puma, C.; Morinville, A.; St-Onge, S.; Lessard, E.; Perkins, M. N.; Laird, J. M. *Pain* **2010**, *151*, 337−344.
(92) Pertwee, R. G. *Philos. Trans. R. Soc., B* **2012**, *367*, 3353−3363.
(93) de Petrocellis, L.; Ligresti, A.; Schiano-Moriello, A.; Allara, M.; Bisogno, T.; Petrosino, S.; Stott, C. G.; Di Marzo, V. *Br. J. Pharmacol.* **2011**, *163*, 1479−1494.
(94) Nissen, L.; Zatta, A.; Stefanini, I.; Grandi, S.; Sgorbati, B.; Biavati, B.; Monti, A. *Fitoterapia* **2010**, *81*, 413−419.
(95) Ross, S. A.; ElSohly, M. A. *J. Nat. Prod.* **1996**, *59*, 49−51.
(96) Benelli, G.; Pavela, R.; Petrelli, R.; Cappellacci, L.; Santini, G.; Fiorini, D.; Sut, S.; Dall'Acqua, S.; Canale, A.; Maggi, F. *Ind. Crops Prod.* **2018**, *122*, 308−315.
(97) Naz, S.; Hanif, M. A.; Bhatti, H. N.; Ansari, T. M. *J. Essent. Oil-Bear. Plants* **2017**, *20*, 175−184.
(98) Gulluni, N.; Re, T.; Loiacono, I.; Lanzo, G.; Gori, L.; Macchi, C.; Epifani, F.; Bragazzi, N.; Firenzuoli, F. *Evid. Based Complement. Alternat. Med.* **2018**, *2018*, 1709182.
(99) Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J. Z.; Xie, X. Q.; Altmann, K. H.; Karsak, M.; Zimmer, A. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 9099−9104.
(100) Rondanelli, M.; Miccono, A.; Lamburghini, S.; Avanzato, I.; Riva, A.; Allegrini, P.; Faliva, M. A.; Peroni, G.; Nichetti, M.; Perna, S. *Evid. Based Complement. Alternat. Med.* **2018**, *2018*, 5813095.
(101) Gertsch, J.; Schoop, R.; Kuenzle, U.; Suter, A. *FEBS Lett.* **2004**, *577*, 563−569.
(102) Woelkart, K.; Xu, W.; Pei, Y.; Makriyannis, A.; Picone, R. P.; Bauer, R. *Planta Med.* **2005**, *71*, 701−705.
(103) 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.

DOI: 10.1021/acs.jnatprod.8b00874
J. Nat. Prod. XXXX, XXX, XXX−XXX

(104) Matovic, N.; Matthias, A.; Gertsch, J.; Raduner, S.; Bone, K. M.; Lehmann, R. P.; Devoss, J. *Org. Biomol. Chem.* **2007**, *5*, 169−174.
(105) Gertsch, J. *Planta Med.* **2008**, *74*, 638−650.
(106) Hinz, B.; Woelkart, K.; Bauer, R. *Biochem. Biophys. Res. Commun.* **2007**, *360*, 441−446.
(107) Chicca, A.; Nicolussi, S.; Bartholomaus, R.; Blunder, M.; Aparisi Rey, A.; Petrucci, V.; Reynoso-Moreno, I. D. C.; Viveros-Paredes, J. M.; Dalghi Gens, M.; Lutz, B.; Schioth, H. B.; Soeberdt, M.; Abels, C.; Charles, R. P.; Altmann, K. H.; Gertsch, J. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E5006−E5015.
(108) Nicolussi, S.; Chicca, A.; Rau, M.; Rihs, S.; Soeberdt, M.; Abels, C.; Gertsch, J. *Biochem. Pharmacol.* **2014**, *92*, 669−689.
(109) Valerio, L. G., Jr.; Gonzales, G. F. *Toxicol. Rev.* **2005**, *24*, 11−35.
(110) Gonzales-Arimborgo, C.; Yupanqui, I.; Montero, E.; Alarcon-Yaquetto, D. E.; Zevallos-Concha, A.; Caballero, L.; Gasco, M.; Zhao, J.; Khan, I. A.; Gonzales, G. F. *Pharmaceuticals* **2016**, *9*, No. E49.
(111) Zhao, J.; Muhammad, I.; Dunbar, D. C.; Mustafa, J.; Khan, I. A. *J. Agric. Food Chem.* **2005**, *53*, 690−693.
(112) Hajdu, Z.; Nicolussi, S.; Rau, M.; Lorantfy, L.; Forgo, P.; Hohmann, J.; Csupor, D.; Gertsch, J. *J. Nat. Prod.* **2014**, *77*, 1663−1669.
(113) Wu, H.; Kelley, C. J.; Pino-Figueroa, A.; Vu, H. D.; Maher, T. J. *Bioorg. Med. Chem.* **2013**, *21*, 5188−5197.
(114) Gugnani, K. S.; Vu, N.; Rondon-Ortiz, A. N.; Bohlke, M.; Maher, T. J.; Pino-Figueroa, A. *Toxicol. Appl. Pharmacol.* **2018**, *340*, 67−76.
(115) Ruiu, S.; Anzani, N.; Orru, A.; Floris, C.; Caboni, P.; Maccioni, E.; Distinto, S.; Alcaro, S.; Cottiglia, F. *Bioorg. Med. Chem.* **2013**, *21*, 7074−7082.
(116) Nicolussi, S.; Viveros-Paredes, J. M.; Gachet, M. S.; Rau, M.; Flores-Soto, M. E.; Blunder, M.; Gertsch, J. *Pharmacol. Res.* **2014**, *80*, 52−65.
(117) Reynoso-Moreno, I.; Najar-Guerrero, I.; Escareno, N.; Flores-Soto, M. E.; Gertsch, J.; Viveros-Paredes, J. M. *J. Agric. Food Chem.* **2017**, *65*, 9435−9442.
(118) Valcic, S.; Timmermann, B. N.; Alberts, D. S.; Wachter, G. A.; Krutzsch, M.; Wymer, J.; Guillen, J. M. *Anti-Cancer Drugs* **1996**, *7*, 461−468.
(119) Valcic, S.; Burr, J. A.; Timmermann, B. N.; Liebler, D. C. *Chem. Res. Toxicol.* **2000**, *13*, 801−810.
(120) Valcic, S.; Muders, A.; Jacobsen, N. E.; Liebler, D. C.; Timmermann, B. N. *Chem. Res. Toxicol.* **1999**, *12*, 382−386.
(121) Roth, M.; Timmermann, B. N.; Hagenbuch, B. *Drug Metab. Dispos.* **2011**, *39*, 920−926.
(122) Li, G.; Lin, W.; Araya, J. J.; Chen, T.; Timmermann, B. N.; Guo, G. L. *Toxicol. Appl. Pharmacol.* **2012**, *258*, 268−74.
(123) Korte, G.; Dreiseitel, A.; Schreier, P.; Oehme, A.; Locher, S.; Geiger, S.; Heilmann, J.; Sand, P. G. *Phytomedicine* **2010**, *17*, 19−22.
(124) Kim, S. Y.; Ahn, B. H.; Min, K. J.; Lee, Y. H.; Joe, E. H.; Min, D. S. *J. Biol. Chem.* **2004**, *279*, 38125−38133.
(125) Peng, G.; Dixon, D. A.; Muga, S. J.; Smith, T. J.; Wargovich, M. *Mol. Carcinog.* **2006**, *45*, 309−319.
(126) Shimizu, M.; Deguchi, A.; Joe, A. K.; McKoy, J. F.; Moriwaki, H.; Weinstein, I. B. *J. Exp. Ther. Oncol.* **2005**, *5*, 69−78.
(127) Lee, D. H.; Kim, Y. J.; Kim, H. H.; Cho, H. J.; Ryu, J. H.; Rhee, M. H.; Park, H. *Biomol. Ther.* **2013**, *21*, 54−59.
(128) Hong, J.; Smith, T. J.; Ho, C. T.; August, D. A.; Yang, C. S. *Biochem. Pharmacol.* **2001**, *62*, 1175−1183.
(129) Protein Data Bank, www.pdb.org.
(130) Skrzypczak-Jankun, E.; Zhou, K.; Jankun, J. *Int. J. Mol. Med.* **2003**, *12*, 415−420.
(131) Brusselmans, K.; De Schrijver, E.; Heyns, W.; Verhoeven, G.; Swinnen, J. V. *Int. J. Cancer* **2003**, *106*, 856−862.
(132) Yeh, C. W.; Chen, W. J.; Chiang, C. T.; Lin-Shiau, S. Y.; Lin, J. K. *Pharmacogenomics J.* **2003**, *3*, 267−276.
(133) Osei-Hyiaman, D.; DePetrillo, M.; Pacher, P.; Liu, J.; Radaeva, S.; Batkai, S.; Harvey-White, J.; Mackie, K.; Offertaler, L.; Wang, L.; Kunos, G. *J. Clin. Invest.* **2005**, *115*, 1298−1305.

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J. Nat. Prod. XXXX, XXX, XXX−XXX