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Neuroactive and Anti-inflammatory Frankincense Cembranes: A Structure–Activity Study

Federica Pollastro,[†] Samantha Golin,[†] Giuseppina Chianese,[‡] Masteria Yunovilsa Putra,[‡,#] Aniello Schiano Moriello,[§] Luciano De Petrocellis,[§] Victor García,[⊥] Eduardo Munoz,[⊥] Orazio Taglialatela-Scafati,[*,‡] and Giovanni Appendino[*,†]

[†]Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Largo Donegani 2, 28100 Novara, Italy

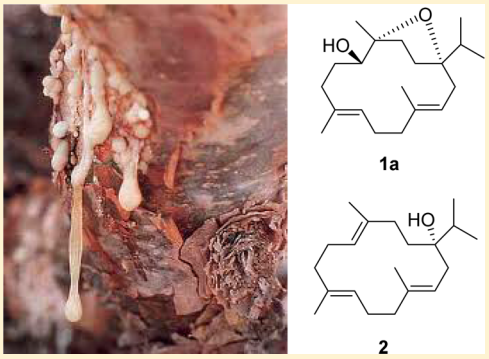
[‡]Dipartimento di Farmacia, Università di Napoli Federico II, Via Montesano 49, 80131 Napoli, Italy

[§]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, CNR, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy

[⊥]Maimonides Biomedical Research Institute of Córdoba, Reina Sofía University Hospital, Department of Cell Biology, Physiology and Immunology, University of Córdoba, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain

Supporting Information

ABSTRACT: An expeditious isolation method for the cembrane diterpene alcohols incensol (**1a**) and serratol (**2**) has been developed from respectively African and Indian frankincense. The two native alcohols and a series of semisynthetic derivatives of incensol were evaluated for transient receptor potential vanilloid 3 (TRPV3) activation and the inhibition of NF-κB, the putative molecular targets underlying the psychotropic and anti-inflammatory activities of incensol acetate (IA, **1b**). Serratol (**2**) was the most potent TRPV3 activator, outperforming by 2 orders of magnitude the reference agonist thymol and by 1 order of magnitude incensol acetate (**1b**). Acylation, epimerization, and oxidation did not significantly improve the affinity of incensol for TRPV3, while NF-κB inhibition, marginal for both natural alcohols, could be improved by esterification of incensol (**1a**) with lipophilic acids. Interestingly, incensol (**1a**) but not IA (**1b**) was a potent inhibitor of STAT3, raising the possibility that hydrolysis to incensol (**1a**) might be involved in the in vivo biological activity of IA (**1b**). Serratol was not amenable to chemical modification, but some marine cembranoids related to the frankincense diterpenoids showed a certain degree of TRPV3-activating properties, qualifying the aliphatic macrocyclic cembrane skeleton as a selective chemotype to explore the pharmacology of TRPV3, a thermo-TRP otherwise resistant to modulation by small molecules.



Cembranes are a biogenetically early class of diterpenoids for which their distribution in Nature encompasses marine invertebrates, lower and higher plants, and insects.[1] Accumulation of cembranes is, however, rare. Within marine organisms it is limited to soft corals and, to a lesser extent, to sponges, while some conifer resins, frankincense, and tobacco are the major sources of cembranes within terrestrial plants. The first member of the class was structurally elucidated in 1962,[2] but interest in plant cembranes has long been limited to the realm of perfumery, since these compounds are important constituents of the essential oil from frankincense and contribute, with a resinous and woody note, to its sensory properties.[3] More recently, incensol[4] acetate (IA, **1b**), a constituent of both African [*Boswellia papyrifera* Hochst., *B. carteri* Birdw. (Burseraceae)] and Arabian (*B. sacra* Flueck.) frankincense,[5] was identified as a potent activator of transient receptor potential vanilloid 3 (TRPV3),[6] a heat-sensitive ion channel highly expressed in keratinocytes and involved in skin heat sensitivity and thermoregulation.[6] TRPV3 is also expressed in the central nervous system (CNS),[7] and, using IA as a probe, its activation was associated with anxiolytic and antidepressant activity.[6] More recently, downregulation of the expression of TRPV3 in adipocytes was discovered to play a critical role in the accumulation of visceral fat.[8]

As an ester of an aliphatic macrocyclic alcohol, IA is a surprising agonist for TRPV3, for which the archetypal ligands are aromatic phenolics such as carvacrol and thymol from thyme, eugenol from clove,[7] and the phytocannabinoid cannabidiol and tetrahydrocannabivarin.[9] A few oxygenated monoterpenes, including camphor and eucalyptol have been reported to show a modest affinity for TRPV3,[10] but these compounds, as well as the phenolic TRPV3 agonists, are promiscuous in terms of affinity for thermo-TRPs, targeting also other members of this class of ion channels.[9,10] Conversely, IA did not show any significant affinity for other thermo-TRPs as well as for various receptors and ion channels of relevance for drug discovery.[6] Owing to its potency and selectivity, IA qualifies therefore as a unique ligand to investigate the function and the modulation of TRPV3, for which recent studies have

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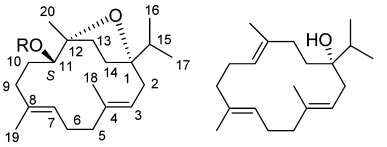
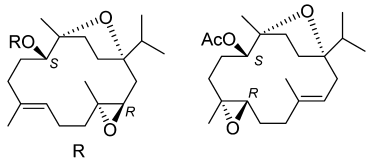
suggested has broad physiological implications, from human genetic diseases to inflammation, skin physiology, weight management, and brain function.[7] Interestingly, resurrection and functional testing of woolly mammoth tissues have shown that a mutation of TRPV3 was critical for their adaptation to cold, highlighting the evolutionary relevance of this thermosensor.[11]

Despite the interest for incensol acetate (**1b**) as a TRPV3 ligand, no structure−activity study has so far been carried out on this compound, and the activity of its closely related analogue serratol (**2**) from Indian frankincense (*B. serrata* Roxb. ex Colebr.)[12] is still unknown. Capitalizing on the development of an expeditious method for the isolation of incensol and serratol from commercial frankincense, we have explored the biological space around the cembrane chemotype of TRPV3 activators. Since IA shows in vivo neuroprotective properties[13] possibly associated with inhibition of NF-κB,[14] the interaction with this pro-inflammatory transcription factor was also evaluated.



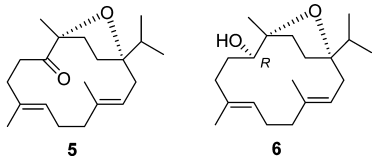
2

	R
1a	H
1b	Ac (-C(=O)CH_3)
1c	Non (-C(=O)-$(CH_2)_7$-CH_3)
1d	Bz (-C(=O)-C_6H_5)
1e	PhAc (-C(=O)-CH_2-C_6H_5)
1f	Cinn (*E* -C=O)-CH=CH-C_6H_5)



	R
3a	H
3b	Ac

4



5 **6**

RESULTS AND DISCUSSION

African frankincense contains a mixture of incensol (**1a**), a secondary alcohol, and its acetate (**1b**), while Indian frankincense contains the tertiary alcohol serratol (**2**).[15] The purification of the cembrane alcohols was easier than that of IA (**1b**), and therefore, it was found more convenient to synthesize IA (**1b**) from incensol rather than obtaining it by direct isolation. In practice, powdered, frozen African frankincense was extracted with petroleum ether, and, after filtration on neutral alumina to remove the acidic constituents and evaporation, the neutral extract was treated with $LiAlH_4$ in THF to reductively cleave the acetate group, which was otherwise sluggish to remove by hydrolysis. After acidic workup and extraction with EtOAc, the residue was purified by gravity column chromatography (CC) on silica gel, affording incensol (**1a**) in a yield of ca. 3.9% on the commercial gum resin. A similar protocol was used for serratol, omitting, however, the treatment with $LiAlH_4$.

Incensol was acylated to explore issues such as elongation of the alkyl chain (nonanoyl ester, **1c**), its aromatization (benzoyl ester, **1d**), and a combination of the two maneuvers (phenylacetate and cinnamate esters, **1e** and **1f**, respectively). The choice of these acids was inspired by previous studies on the acylative modification of polyol templates for the modulation of thermo-TRPs.[16] Serratol, a tertiary alcohol, proved resistant to acylation under a variety of protocols. Next, the epoxidation of the endocyclic double bonds of the natural polyols was investigated. Incensol afforded a crystalline monoepoxide by treatment with peracids[17] and was then acetylated to the acetate **3b**. Conversely, the epoxidation of incensol acetate was not chemoselective, affording a mixture of two regioisomeric epoxides (**3b** and **4**), separated by gravity CC and identified by comparison with the literature spectroscopic data.[17b] The reason for the different chemoselectivity associated with the epoxidation of the alcohol **1a** and its acetate **1b** is unclear and is seemingly associated with different conformational properties between the two compounds. The epoxidation of serratol, also under buffered conditions, gave a complex mixture from which no pure compound could be obtained. Finally, incensol (**1a**) was oxidized to the ketone **5** (incensone), for which reduction gave a mixture of incensol (**1a**) and epi-incensol (**6**).[17a]

The 12 cembranes **1a**,**f**−**6** were evaluated for their interaction with TRPV3. This ion channel shows 43% sequence homology with TRPV1 but has a distinct pharmacology.[7] Thus, while TRPV1 is activated by burning heat (>42 °C), TRPV3 has a lower thermal threshold (>33 °C), corresponding to a feeling of mild warmth,[18] and its activation should, in principle, be devoid of the painful side-effects typical of TRPV1 agonists. Furthermore, while activation of thermo-TRPs such as TRPA1, TRPV1, and TRPV4 is characterized by desensitization, the repeated thermal or chemical stimulation of TRPV3 is instead associated with sensitization, leading to an increase of activity and larger excitatory signals.[19] While there is no shortage of interest in the pharmacological modulation of TRPV3 for conditions such as inflammatory skin disorders, itch, pain sensation, hypothermia, obesity, and depression,[7] this ion channel has proved rather difficult to modulate with small molecules, and the ligands described so far are promiscuous, also targeting other thermo-TRPs [2-aminoethoxydiphenyl borate (2-APB), camphor, eugenol, thymol] as well as unrelated nonion channel targets (phytocannabinoids).[7] It was therefore important to explore the chemical space around IA (**1b**), the only selective TRPV3 agonist identified so far. TRPV3 activity was evaluated in rTRPV3-expressing HEK293 cells sensitized with the agonist 2-APB (100 μM). IA stimulated TRPV3-mediated calcium influx with high efficacy (43.4 ± 1.9% of the effect of 4 μM ionomycin) and potency (EC_{50} 1.4 ± 0.2 μM) (Table 1). The EC_{50} value observed in rTRPV3 was lower than that reported for mTRPV3 (16 μM),[6] suggesting a higher sensitivity to IA (**1b**) for the rat orthologue. Removal of the acetate (incensol, **1a**), and oxidation to incensone (**5**) had only a modest effect on potency, as evaluated by EC_{50}, while an increase of efficacy (65.1 ± 1.1% of 4 μM ionomycin) was surprisingly observed for incensol. Conversely, epimerization to epi-incensol (**6**) and epoxidation to the 3- and 7-epoxides (**3b** and **4**, respectively) caused a decrease in potency and efficacy, as did the replacement of the acetate by a cinnamate (**1f**). The decrease of activity was lower for the benzoate **1d** and the

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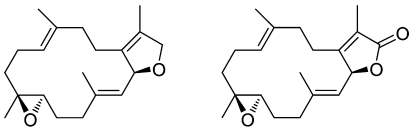
Table 1. Evaluation of the Cembranes 1a,f−8 on Calcium Influx in HEK293 Cells Transfected with rTRPV3 and Sensitized with 2-APB[a] and in NF-κB[b] and STAT3[c] Transcriptional Activities

compound	efficacy TRPV3 (% ionomycin 4 μM)	potency EC_{50} TRPV3 μM	IC_{50} TRPV3 μM (thymol 100 μM)	IC_{50} NF-κB (STAT3) (μM)
1a	65.1 ± 1.1	2.1 ± 0.2	2.1 ± 0.1	>50 (15.84 ± 2.88)
1b	43.4 ± 1.9	1.4 ± 0.2	2.2 ± 0.5	>50 (>50)
1c	35.4 ± 0.9	5.6 ± 0.8	24.1 ± 1.9	35.5 ± 1.1 (38.66 ± 3.99)
1d	10.3 ± 0.9	4.4 ± 1.1	>100	>50 (>50)
1e	25.6 ± 0.6	1.2 ± 0.2	21.7 ± 3.1	22.4 ± 2.4 (>50)
1f	14.1 ± 0.4	10.5 ± 1.5	94.4 ± 8.6	>50 (>50)
2	62.4 ± 1.0	0.17 ± 0.01	0.15 ± 0.01	>50 (>50)
3a	27.2 ± 1.0	25.4 ± 0.2	>100	>50 (>50)
3b	57.7 ± 2.3	8.8 ± 0.5	13.2 ± 1.2	>50 (>50)
4	31.6 ± 0.6	8.6 ± 0.4	21.1 ± 1.7	>50 (>50)
5	46.5 ± 0.6	2.4 ± 0.2	5.8 ± 0.6	>50 (>50)
6	38.0 ± 1.1	7.3 ± 0.3	12.3 ± 0.4	>50 (>50)
7	30.0 ± 2.4	29.2 ± 2.1	57.5 ± 4.9	>50 (>50)
8	21.3 ± 0.7	30.1 ± 3.8	>100	>50 (>50)
thymol	49.4 ± 0.9	29.0 ± 0.5		
SC415				7.9 ± 0.6

[a]TRPV3-epressing HEK293 cells were first sensitized with the structurally unrelated agonist 2-aminoethoxydiphenyl borate (100 μM). [b]NIH-3T3-KBF-Luc cells were preincubated with the compounds for 15 min, then stimulated with TNFα for 6 h, and luciferase activity was measured in the cell lysates. [c]HeLa-STAT3-Luc cells were preincubated with the compounds for 15 min, then stimulated with INFγ for 6 h, and luciferase activity was measured in the cell lysates.

nonanoate **1c**, while the phenylacetate **1e** showed similar potency, but decreased efficacy (25.6 ± 0.6% of 4 μM ionomycin), compared to IA (**1b**).

The two cembranes sarcophytoxide (**7**)[20] and sarcophine (**8**),[21] the major constituents of the Indonesian soft coral *Sarcophyton* sp. (order Alcyonacea, family Alcyoniidae), were also investigated for TRPV3-activation properties. Interestingly, these marine cembranes also showed significant TRPV3-activating properties, although at least 1 order of magnitude lower than IA (**1b**). In contrast, the tertiary cembrane alcohol serratol (**2**) was 1 order of magnitude more potent than IA and represents the first submicromolar TRPV3 agonist described to date. Serratol outperformed IA also in terms of efficacy, evaluated as maximal activation with 4 μM ionomycin (62.4 ± 1.0%), a value similar to the one measured for incensol (65.1 ± 1.1%).



7 **8**

The submicromolar affinity of serratol (**2**) for TRPV3 is surprising, since this compound has only one polar group and is otherwise hydrophobic. Strong intermolecular interactions are associated with the presence of polar groups, and the activity of **2** on TRPV3 is somewhat reminiscent of that of the hydrocarbon caryophyllene on the cannabinoid receptor CB_2.[22] The medium-sized hydrophobic ring of caryophyllene can adopt more than one conformation,[23] and an even higher number of geometries is possible for serratol, complicating the definition of a pharmacophoric model.[24]

IA (**1b**) has been shown to inhibit NF-κB activation, but the reported IC_{50} value in vitro (over 100 μM)[14] suggests a marginal activity, unlikely to significantly contribute to the neuroprotective activity observed in vivo.[13] Nevertheless, it was interesting to investigate the effect of cembranes on NF-κB activation, since incensol acetate could be rapidly deacetylated in vivo, and incensol itself could show higher potency. In any event, incensol and, with the exception of **1e** and **1c**, also its esters and analogues showed only marginal, if any, activity on TNFα-induced NF-κB activation (IC_{50} > 50 μM), casting doubts on the involvement of NF-κB in the in vivo activity of IA against neuroinflammation. On the other hand, incensol (**1a**), but not IA (**1b**), showed potent inhibitory activity on IL-6-induced STAT3 activation (IC_{50} = 15.8 ± 2.88 μM), and, given the relevance of STAT3 in neuro-inflammation,[25] it does not seem unrealistic to assume its involvements, secondary to enzymatic deacetylation, in the in vivo neuroprotective activity of IA (**1b**). The nonanoate and the phenylacetate esters of incensol (**1c** and **1e**, respectively) showed a significant inhibitory activity on NF-κB activation (Table 1), an interesting observation, since, unlike most NF-κB inhibitors, these compounds are devoid of electrophilicity and redox properties.[14] Furthermore, incensol nonanoate (**1c**) also inhibited STAT3 (IC_{50} = 38.7 ± 3.99 μM), while all the other analogues were inactive. Taken together, these observations suggest that the cembrane structural motif is an interesting platform for the noncovalent inhibition of transcriptions factors involved in inflammation, as exemplified by incensol (**1a**) and its nonanoate ester (**1c**), which could inhibit the activity of both NF-κB and STAT3.

The macrocyclic cembrane skeleton is also a privileged structure for targeting TRPV3 and evaluating the pharmacological potential of its modulation. Of special relevance is the identification of serratol (**2**) as a submicromolar agonist of TRPV3, although we were unable to derivatize this compound without obtaining complex mixtures requiring challenging chromatographic resolution. The in vivo evaluation of the neuroprotective activity of the phenolic monoterpene carvacrol to demonstrate the druggability of TRPV3 by small molecules gave unclear results due to the low potency of this agonist and its pleiotropic profile of activity.[25] Cembrane ligands such as IA (**1b**) and serratol (**2**) qualify as better probes because of their higher potency and, at least for IA and possibly also for serratol, selectivity within the TRP ion channel family and should be

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seriously considered for the in vivo investigation of the physiological role of TRPV3. Conversely, incensol (**1a**) seems an excellent candidate for neuroprotection, due to its pleiotropic mechanism of activity that includes, apart from the modulation of TRPV3, also the inhibition of the pro-inflammatory transcription factor STAT3.

The simple purification method developed in this investigation makes it possible to obtain incensol (**1a**) and serratol (**2**) from frankincense, an easily available biomass,[26] in amounts sufficient to sustain a systematic exploration of the biological space around the cembrane chemotype and for in vivo studies. Furthermore, the occurrence of a host of polar minor cembranoids in frankincense[27] will make it possible to extend this exploration also to areas of the pharmacophoric space unaccessible by chemical modification of the natural products but populated by naturally occurring analogues.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations ($CHCl_3$) were measured at 589 nm on a P2000 JASCO polarimeter. IR spectra were registered on an Avatar 370 FT-IR Techno-Nicolet apparatus. ^1H (500 and 400 MHz) and ^{13}C (125 and 100 MHz) NMR spectra were measured on Varian INOVA NMR spectrometers. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26, δ_C = 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment. One-bond heteronuclear $^1H-^{13}C$ connectivities were determined with the HSQC experiment. Two- and three-bond $^1H-^{13}C$ connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ = 9 Hz. Low- and high-resolution ESIMS were obtained on an LTQ OrbitrapXL (Thermo Scientific) mass spectrometer.

Silica gel 60 (70−230 mesh) used for gravity column chromatography was purchased from Macherey-Nagel. Reactions were monitored by TLC on Merck 60 F254 (0.25 mm) plates, visualized by staining with 5% H_2SO_4 in ethanol and heating. Organic phases were dried with Na_2SO_4 before evaporation. Chemical reagents and solvents were from Aldrich and were used without any further purification unless stated otherwise.

Plant Material. *Boswellia serrata* and *B. papyrifera* were purchased from Anbesa (Mestre, VE, Italy). The identity of the material was checked by TLC according to the method reported by Jauch and co-workers, with caryophyllene oxide, oxotirucallic acid, incensol, incensol acetate, and serratol as reference compounds.[15] The profile of occurrence/absence of these compounds matched that proposed for their differentiation, as did the odor of the extracts ("lemony" for *B. papyrifera*, "pine-like" for *B. serrata*).[15] A voucher specimen of the resin batch used in this investigation is kept at the laboratory in Novara for both *B. papyrifera* (2016-1) and *B. serrata* (2016-2).

Isolation of Incensol (1a). A sample (40.8 g) of *B. papyrifera* resin was cooled in a freezer at −18 °C for 3 h and then powered using a kitchen blender. The frozen powder was extracted twice with 400 mL of petroleum ether (ratio resin:solvent 1:10), affording, after filtration and evaporation, 13 g of a pale yellow oil with a lemon-like odor. The latter was dissolved in the minimal amount of petroleum ether and then vacuum-filtered over neutral alumina (40 g, ratio oil:stationary phase 1:3, elution volume: 300 mL) to remove acidic constituents. Removal of the solvent gave 4.19 g (10.3%) of an orange-odor oil, which was dissolved in dry THF (50 mL) and treated with an excess of freshly powdered $LiAlH_4$ (1.24 g, ca. 300 mg/g of crude purified fraction). After stirring under nitrogen for 12 h at room temperature, the reaction was worked up by the addition of EtOAc to quench the excess of $LiAlH_4$ and then with 2 N H_2SO_4 to hydrolyze the aluminate salts. Extraction with EtOAc afforded 3.9 g of an oil, which was purified by gravity CC on silica gel (180 mL) using petroleum ether−EtOAc (9:1) as eluant to afford 1.61 g (3.9%) of **1a** as a colorless and essentially odorless oil, identified by comparison with the published spectroscopic data.[17b]

Isolation of Serratol (2). A sample of *B. serrata* resin (10 g) was cooled in a freezer at −18 °C for 3 h and then powdered and extracted as described for *B. papyrifera*, affording a pale yellow oil with a pine odor (4.9 g). The latter was dissolved in the minimal amount of petroleum ether and then vacuum-filtered over neutral alumina (15 g, ratio oil:stationary phase 1:3, elution volume: 100 mL) to remove acidic constituents. Removal of the solvent gave 1.40 g (14%) of a colorless oil, which was purified by gravity CC on silica gel using petroleum ether−EtOAc (9:1) as eluant to afford 250 mg (2.5%) of **2** as a colorless and odorless oil, identified by comparison with the published spectroscopic data.[28] The purity of serratol obtained in this way was batch-dependent, and, in some cases, presumably because of the age of the resin sample used, a further purification by flash chromatography (petroleum ether−EtOAc, 95:5) was necessary to obtain a sample of suitable purity.

Isolation of Marine Cembranes. A specimen of *Sarcophyton* sp. was collected in January 2010 along the coasts of Bunaken Island in the Bunaken Marine Park of Manado (Indonesia). A frozen voucher sample (MAN-10-024) has been deposited at the Department of Pharmacy, University of Naples Federico II. After homogenization, the organism (98 g, wet weight) was exhaustively extracted, in sequence, with methanol and chloroform. The extracts were combined and then partitioned between ethyl acetate (EtOAc) and H_2O to obtain an organic phase (2.20 g), which was chromatographed on a silica gel column (230−400 mesh), using a gradient solvent system from hexane to EtOAc and then MeOH. Fractions eluted with *n*-hexane−EtOAc (9:1) afforded sarcophytoxide (**7**, 445 mg) as a pure compound, while fractions eluted with *n*-hexane−EtOAc (8:2) were combined and further subjected to HPLC (eluent: *n*-hexane−EtOAc, 96:4), affording sarcophyn (**8**, 15 mg). These two compounds were identified on the basis of the comparison between their physical and spectroscopic data ($[\alpha]_D$, ^1H and ^{13}C NMR) with those reported in the literature.[20,21]

Esterification of Incensol with Acyl Chlorides or Anhydrides. Synthesis of Incensol Acetate (1b) as a Representative. To a solution of incensol (**1a**, 100 mg, 0.33 mmol) in pyridine (2 mL) were added acetic anhydride (2 mL) and 4-dimethylaminopyridne (DMAP, 80 mg, 0.65 mmol, 2 molar equiv). After stirring for 5 h at room temperature, the reaction was quenched by dilution with water; 2 N H_2SO_4 was added and the reaction was extracted with EtOAc. The organic phase was dried, filtered, and evaporated, and the residue, a brown oil, was purified by gravity CC on silica gel using petroleum ether−EtOAc (98:2) as eluant to yield 58 mg (51%) of incensol acetate (**1b**).[17b] Incensol nonanoate (**1c**), incensol benzoate (**1d**), and incensol cinnamate (**1f**) were prepared in a similar way, in yields of 50%, 16%, and 89%, respectively.

*Incensol nonanoate (**1c**):* colorless oil; IR (KBr) ν_{max} 1738, 1457, 1373, 1164, 1105, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 5.17 (2H, overlapped, H-7, H-3), 4.87 (1H, d, J = 10.1 Hz, H-11), 2.30 (2H, overlapped, H_2-2′), 2.18 (2H, overlapped, H_2-6), 2.10 (2H, overlapped, H_2-5), 2.19 (2H, overlapped, H_2-2), 2.00 (2H, overlapped, H_2-9), 1.85 (1H, overlapped, H-15), 1.80−1.60 (4H, overlapped, H-13, H-14), 1.65 (2H, overlapped, H_2-10), 1.60 (2H, overlapped, H_2-3′), 1.59 (3H, s, H_3-19), 1.56 (3H, s, H_3-14), 1.24 (10H, overlapped, from H-4′ to 8′), 1.11 (3H, s, H_3-20), 0.88 (3H, d, J = 7.1 Hz, H_3-16), 0.86 (3H, d, J = 7.1 Hz, H-17) 0.85 (3H, overlapped, H_3-9′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 173.0 (C-1′), 135.2 (C-4), 133.3 (C-8), 125.2 (C-7), 121.1 (C-3), 89.1 (C-1), 83.0 (C-12), 76.7 (C-11), 38.5 (C-5), 35.8 (C-13), 34.9 (C-15), 34.1 (C-2′), 33.3 (C-9), 32.0 (C-2), 30.4 (C-15), 29.0−23.5 (C-3′ to 8′ not assigned), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7(C-19), 16.1 (C-18), 14.9 (C-9′); ESIMS (positive ion) m/z 469 $[M + Na]^+$; HRESIMS m/z 469.3660 (calcd for $C_{29}H_{50}NaO_3$ 469.3658).

*Incensol benzoate (**1d**):* colorless oil; IR (KBr) ν_{max} 1716, 1450, 1269, 1175, 1112, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.05 (2H, d, J = 7.0 Hz, H-3′, H-7′), 7.56 (1H, t, J = 7.6 Hz, H-5), 7.45 (2H, t, J = 7.6 Hz, H-4′, H-6′), 5.26 (1H, t, J = 6.1 Hz, H-7), 5.23 (1H, t, J = 6.1 Hz, H-3), 5.12 (1H, d, J = 10.1 Hz, H-11), 2.24 (2H, overlapped, H_2-6), 2.20 (2H, overlapped, H_2-2), 2.15 (2H, overlapped, H_2-5), 2.00 (2H, overlapped, H_2-9), 1.94 (1H, overlapped, H-15), 1.80−1.60 (4H, overlapped, H-13, H-14), 1.72 (2H, overlapped, H_2-

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10), 1.61 (3H, s, H_3-19), 1.61 (3H, s, H_3-14), 1.24 (3H, s, H_3-20), 0.93 (3H, d, J = 7.1 Hz, H_3-16), 0.92 (3H, d, J = 7.1 Hz, H_3-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 166.6 (C-1′), 135.3 (C-8), 133.3 (C-4), 132.7 (C-5′), 130.7 (C-2′), 129.6 (C-3′, C-7′), 128.3 (C-4′, C-6′), 125.4 (C-3), 121.1 (C-7), 89.3 (C-1), 83.2 (C-12), 76.7 (C-11), 38.5 (C-5), 35.8 (C-13), 34.9 (C-15), 33.3 (C-9), 32.0 (C-2), 30.4 (C-14), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7 (C-19), 16.1 (C-18); ESIMS (positive ion) m/z 433 $[M + Na]^+$; HRESIMS m/z 433.2725 (calcd for $C_{27}H_{38}NaO_3$ 433.2719).

*Incensol cinnamate (**1f**):* colorless oil; IR (KBr) ν_{max} 1712, 1639, 1449, 1300, 1163, 1001, 765 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.69 (1H, d, J = 16.0 Hz, H-3′), 7.53 (2H, overlapped, H-5′, H-9′), 7.38 (2H, overlapped, H-6′, H-8′), 7.36 (1H, overlapped, H-7′), 6.45 (1H, d, J = 16.0 Hz, H-2′), 5.23 (1H, t, J = 6.1 Hz, H-7), 5.20 (1H, t, J = 6.1 Hz, H-3), 5.01 (1H, d, J = 10.1 Hz, H-11), 2.21 (2H, overlapped, H_2-6), 2.13 (2H, overlapped, H_2-5), 2.19 (2H, overlapped, H_2-2), 2.00 (2H, overlapped, H_2-9), 1.96 (1H, overlapped, H-15), 1.80−1.60 (4H, overlapped, H-13, H-14), 1.68 (2H, overlapped, H_2-10), 1.61 (3H, s, H_3-19), 1.59 (3H, s, H_3-14), 1.18 (3H, s, H_3-20), 0.93 (3H, d, J = 7.1 Hz, H-16), 0.91 (3H, d, J = 7.1 Hz, H_3-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 167.2 (C-1′), 144.6 (C-3′), 135.3 (C-4), 134.5 (C-4′), 133.3 (C-8), 130.2 (C-7′), 129.0 (C-5′, C-9′), 128.0 (C-6′, C-8′), 125.4 (C-7), 121.1 (C-3), 118.6 (C-2′), 89.3 (C-1), 83.2 (C-12), 76.7 (C-11), 38.5 (C-5), 35.8 (C-13), 34.9 (C-15), 33.3 (C-9), 32.0 (C-2), 30.4 (C-14), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7 (C-19), 16.1 (C-18). ESIM (positive ion) m/z 459 $[M + Na]^+$; HRESIMS m/z 469.2871 (calcd for $C_{29}H_{40}NaO_3$ 469.2875).

Steglich Esterification of Incensol (1a). Synthesis of Incensol Phenylacetate (1e). To a solution of incensol (**1a**, 200 mg, 0.65 mmol) in EtOAc (10 mL) were added sequentially phenylacetic acid (533 mg, 3.9 mmol, 6 molar equiv), dicyclohexylcarbodiimide (809 mg, 3.9 mmol, 6 molar equiv), and DMAP (476 mg, 3.9 mmol, 6 molar equiv). After stirring at room temperature for 24 h, the solution was evaporated and the residue was taken up in toluene (10 mL) and cooled (−18 °C) for 1 h to precipitate dicyclohexylurea and the unreacted acid. After filtration on a bed of Celite and evaporation, the residue was purified by gravity CC on silica gel with petroleum ether−EtOAc (95:5) as eluent to afford 161 mg (58%) of incensol phenylacetate (**1e**).

*Incensol phenylacetate (**1e**):* colorless oil; IR (KBr) ν_{max} 1731, 1453, 1253, 1132, 1038, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.26−7.30 (5H, overlapped, from H-4′ to H-8′), 5.13 (1H, overlapped, H-7), 5.12 (1H, overlapped, H-3), 4.84 (1H, d, J = 10.1 Hz, H-11), 3.60 (2H, s, H_2-2′), 2.18 (2H, overlapped, H-6), 2.10 (2H, overlapped, H-5), 2.19 (2H, overlapped, H_2-2), 2.00 (2H, overlapped, H_2-9), 1.85 (1H, overlapped, H-15), 1.80−1.60 (4H, overlapped, H-13, H-14), 1.65 (2H, overlapped, H_2-10), 1.54 (3H, s, H_3-19), 1.53 (3H, s, H_3-14), 1.05 (3H, s, H_3-20), 0.88 (3H, d, J = 7.1 Hz, H_3-16), 0.86 (3H, d, J = 7.1 Hz, H_3-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 171.6 (C-1′), 135.2 (C-4), 134.4 (C-3′), 133.3 (C-8), 129.0 (C-4′, C-8′), 128.0 (C-5′, C-7′), 127.0 (C-6′), 125.2 (C-7), 121.1 (C-3), 89.1 (C-1), 83.0 (C-12), 76.7 (C-11), 42.1 (C-2′), 38.5 (C-5), 35.8 (C-13), 34.9 (C-15), 33.3 (C-9), 32.0 (C-2), 30.4 (C-15), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7 (C-19), 16.1 (C-18); ESIMS (positive ion) m/z 447 $[M + Na]^+$; HRESIMS m/z 447.2881 (calcd for $C_{28}H_{40}NaO_3$ 447.2875).

Epoxidation of Incensol (1a). To a cooled (0 °C) solution of incensol (**1a**, 100 mg, 0.33 mmol) in EtOAc (5 mL) were added sequentially NaOAc (62 mg) and *m*-CPBA (62 mg, 0.36 mmol, 1.1 molar equiv). The course of the reaction was followed by TLC [(petroleum ether−EtOAc (9:1) as eluant, R_f **1a** = 0.42; R_f **3a** = 0.28], and, after 10 min, the reaction was quenched by the addition of 5% $Na_2S_2O_3$, diluted with EtOAc, and washed with saturated $NaHCO_3$. Removal of the solvent left a yellowish oil, which was purified by gravity CC on silica gel with petroleum ether−EtOAc (9:1) as eluant to afford 200 mg (63%) of incensol oxide (**3a**) as white crystals.[17b]

Epoxidation of Incensol Acetate (1b). The reaction was carried out as described for incensol, following the reaction course by TLC [petroleum ether−EtOAc, 9:1, R_f **1b** = 0.66; R_f **3b** = 0.22; R_f **4** = 0.33) After workup, the residue was purified by gravity CC on silica gel with petroleum ether−EtOAc (9:1) as eluant to afford 25 mg (29%) of 7-epoxyincensol acetate (**4**)[17b] and 27 mg (29%) of 3-epoxyincensol acetate (**3b**).[17b]

Oxidation of Incensol. To a solution of incensol (**1a**, 200 mg, 0.65 mmol) in CH_2Cl_2 (5 mL) was added pyridinium chlorochromate (420 mg, 1.9 mmol, 3 molar equiv). After stirring at room temperature for 5 h, the solution was evaporated and the residue was taken up in ethyl ether (5 mL), filtered on a bed of Celite, and evaporated. The residue was purified by gravity CC on silica gel, using petroleum ether−EtOAc (95:5) as eluent, affording 85 mg (40%) of incensone (**5**).

*Incensone (**5**):* white powder; mp 72 °C; IR (KBr) ν_{max} 1718, 1444, 1366, 1039, 938, 890 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.00 (1H, overlapped, H-7), 4.98 (1H, overlapped, H-3), 3.16 (1H, ddd, J = 17.4, 11.2, 2.0 Hz, H-10a), 2.56 (1H, overlapped, H-13a), 2.51 (1H, overlapped, H-10b), 2.36 (1H, ddd, J = 17.4, 7.4, 2.0 Hz, H-9a), 2.21 (1H, dd, J = 15.7, 6.5 Hz, H-2a), 2.09 (2H, overlapped, H_2-5), 2.08 (1H, overlapped, H-9b), 2.03 (2H, overlapped, H_2-6), 1.97 (1H, overlapped, H-2b), 1.90 (1H, overlapped, H-13a), 1.84 (1H, overlapped, H-13b), 1.86 (1H, overlapped, H-14a), 1.66 (1H, overlapped, H-14b), 1.57 (3H, s, H_3-15), 1.49 (3H, s, H_3-14), 1.30 (3H, s, H_3-20), 0.96 (3H, d, J = 7.1 Hz, H_3-16), 0.91 (3H, d, J = 7.1 Hz, H_3-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 210.5 (C-11), 134.7 (C-4), 134.5 (C-8), 125.2 (C-7), 121.4 (C-3), 90.0 (C-1), 87.9 (C-12), 38.5 (C-5), 35.8 (C-13), 34.9 (C-15), 33.3 (C-9), 32.0 (C-2), 30.4 (C-15), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7 (C-19), 16.1 (C-18); ESIMS (positive ion) m/z 327 $[M + Na]^+$; HRESIMS m/z 327.2304 (calcd for $C_{20}H_{32}NaO_2$ 327.2300).

Reduction of Incensone (5). To a solution of incensone (**5**, 50 mg, 0.15 mmol) in dry THF (5 mL) was added an excess of freshly powdered $LiAlH_4$ (627 mg, 16.5 mmol). After stirring under nitrogen for 2 h at room temperature, the reaction was worked up by the addition of EtOAc to quench the excess of $LiAlH_4$, followed by 2 N H_2SO_4 to hydrolyze the aluminate salts. Removal of the solvent left a yellowish oil, which was purified by gravity CC on silica gel with petroleum ether−EtOAc (9:1) as eluant to afford 24 mg (53%) of 11-epi-incensol (**6**) and 13 mg (28%) of incensol (**1a**).

*11-Epi-incensol (**6**):* white powder; mp 69 °C; IR (KBr) ν_{max} 3653, 1469, 1447, 1072, 1039, 889 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.20 (1H, overlapped, H-7), 5.15 (1H, overlapped, H-3), 3.73 (1H, m, H-11), 2.25 (2H, overlapped, H_2-6), 2.15 (2H, overlapped, H_2-5), 2.19 (2H, overlapped, H_2-2), 2.00 (2H, overlapped, H_2-9), 1.99 (1H, overlapped, H-15), 1.65−1.78 (4H, overlapped, H-13, H-14), 1.73 (2H, overlapped, H_2-10), 1.58 (3H, s, H_3-19), 1.58 (3H, s, H_3-14), 1.13 (3H, s, H_3-20), 0.95 (3H, d, J = 7.1 Hz, H_3-16), 0.91 (3H, d, J = 7.1 Hz, H_3-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 137.6 (C-4), 137.3 (C-8), 122.8 (C-7), 122.8 (C-3), 89.5 (C-1), 86.1 (C-12), 77.2 (C-11), 40.5 (C-5), 35.7 (C-13), 34.3 (C-15), 33.2 (C-9), 31.0 (C-2), 30.4 (C-15), 28.0 (C-10), 24.8 (C-6), 22.2 (C-20), 18.1 (C-16), 18.0 (C-17), 17.7 (C-19), 15.5 (C-18); ESIMS (positive ion) m/z 329 $[M + Na]^+$; HRESIMS m/z 329.2477 (calcd for $C_{20}H_{34}NaO_2$ 329.2475).

TRPV3 Activity Experiments. HEK293 (human embryonic kidney) cells stably overexpressing recombinant rat TRPV3 were provided by Dr. Heather Bradshaw (Indiana University, USA). They were grown on 100 mm diameter Petri dishes as monolayers in minimum essential medium (MEM) supplemented with nonessential amino acids, 10% fetal bovine serum, and 2 mM glutamine and were maintained at 5% CO_2 at 37 °C. The effect of the substances on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by using Fluo-4 AM, a selective intracellular fluorescent probe for Ca^{2+}. On the day of the experiment, cells were loaded for 1 h at room temperature with the methyl ester Fluo-4 AM (4 μM in DMSO containing 0.02% Pluronic F-127, Invitrogen) in MEM without fetal bovine serum, then were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred (about 100 000 cells) to the quartz cuvette of the spectrofluorimeter (PerkinElmer LS 50B, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. TRPV3-expressing HEK293 cells were first sensitized with the structurally unrelated

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agonist 2-aminoethoxydiphenyl borate (100 μM). $[Ca^{2+}]_i$ was determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 516 nm). Curve fitting (sigmoidal dose−response variable slope) and parameter estimation were performed with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e., half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). The efficacy of the agonists was determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin (Invitrogen). The values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e., not transfected with any construct) HEK293 cells were taken as baseline and subtracted from the values obtained from transfected cells. Antagonist/desensitizing behavior was evaluated against thymol (100 μM), by adding the test compounds in a quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]_i$ elevation (IC_{50}), which was calculated again using GraphPad Prism software. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Dose−response curves were fitted by a sigmoidal regression with variable slope. All determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by the Bonferroni's test.

Luciferase Assays. The origin and characterization of the NIH-3T3-KBF-Luc and HeLa-STAT3-Luc cell lines have been previously described.[29] The cells were grown at 37 °C and 5% CO_2 in supplemented Dulbecco's modified Eagle medium containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin (50 U/mL), and streptomycin (50 μg/mL). For the anti-NF-κB activity, NIH-3T3-KBF-Luc cells were stimulated with TNFα (20 ng/mL) in the presence or the absence of the compounds for 6 h. For the anti-STAT3 activity, HeLa-STAT3-Luc cells were stimulated with INFγ (20 IU) in the presence or absence of the compounds for 6 h. After treatment, the cells were washed twice in phosphate buffer saline and lysed in 25 mM Tris-phosphate buffer (pH 7.8), 8 mM $MgCl_2$, 1 mM dithiothreitol, 1% Triton X-100, and 7% glycerol for 15 min at room temperature in a horizontal shaker. After centrifugation, luciferase activity in the supernatant was measured using an Autolumat LB 9510 (Berthold) in accordance with the instructions of the luciferase assay kit (Promega, Madison, WI, USA). SC-514, a selective IKK2 inhibitor, was used as a positive control, and IC_{50} calculations were performed using GraphPad Prism (Graph Pad Software). Sample population means were compared against control population means in an unpaired two-tailed Student's t test.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00141.

^{1}H and ^{13}C NMR spectra for all the new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +39-081-678509. Fax: +39-081678552. E-mail: scatagli@unina.it (O. Taglialatela-Scafati).

*Tel: +39-0321-375744. Fax: +39-0321375744. E-mail: giovanni.appendino@uniupo.it (G. Appendino).

Present Address

#Research Center for Oceanography, The Indonesian Institute of Sciences, Jl Pasir Putih 1, Ancol Timur, 14430 Jakarta Utara, Indonesia.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Weinheimer, A. J.; Chang, C. W. L.; Matson, J. A. *Progr. Chem. Org. Nat. Prod.* **1979**, *36*, 285−388. (b) Yang, B.; Zhou, X.-F.; Lin, X.-P.; Liu, J.; Peng, Y.; Yang, X.-W.; Liu, Y. *Curr. Org. Chem.* **2012**, *16*, 1512−1539.

(2) (a) Dauben, W. G.; Thiessen, W. E.; Resnick, P. R. *J. Am. Chem. Soc.* **1962**, *84*, 2015−2016. (b) Kobayashi, H.; Akiyoshi, S. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 1044−1045.

(3) Niebler, J.; Buettner, A. *Phytochemistry* **2015**, *109*, 66−75.

(4) Incensol was originally referred to as "incensole". Since the suffix of an alcohol is "ol" and the terminal "e" is often associated with nitrogen-containing compounds, we believe it would be better to drop this, also by analogy with the name of the related cembrane serratol and a host of other isoprenoid alcohols (phytol, bisabolol, menthol, etc.). The configuration of cembranes is difficult to represent because of the presence of re-entrant stereogenic carbons if their perimeter is drawn in the classic C_4-symmetric form. Since the meaning of wedges and broken lines at re-entrant angles should be reversed (β = below the plane and α = above the plane), for the sake of clarity we have also specified the absolute configuration of these carbons using the *R,S*-descriptors. Numbering was done starting from the carbon that bears the isopropyl group, assigning the locant 7 to a double-bond (or vestigial double-bond) carbon, as suggested in ref 1a.

(5) Corsano, S.; Nicoletti, R. *Tetrahedron* **1967**, *23*, 1977−1984.

(6) Moussaieff, A.; Rimmerman, N.; Bregman, T.; Straiker, A.; Felder, C. C.; Shoham, S.; Kashman, Y.; Huang, S. M.; Lee, H.; Shohami, E.; Mackie, K.; Caterina, M. J.; Walker, J. M.; Fride, E.; Mechoulam, R. *FASEB J.* **2008**, *22*, 3024−3034.

(7) Luo, J.; Hu, H. *Curr. Top. Membr.* **2014**, *74*, 325−364.

(8) Cheung, S. Y.; Huang, Y.; Kwan, H. Y.; Chung, H. Y.; Yao, X. *Endocrinology* **2015**, *156*, 2074−2086.

(9) De Petrocellis, L.; Orlando, P.; Schiano- Moriello, A.; Aviello, G.; Stott, C.; Izzo, A. A.; Di Marzo, V. *Acta Physiol.* **2012**, *204*, 255−266.

(10) Vogt-Eisele, A. K.; Weber, K.; Sherkheli, M. A.; Vielhaber, G.; Panten, J.; Gisselmann, G.; Hatt, H. *Br. J. Pharmacol.* **2007**, *151*, 530−540.

(11) Lynch, V. J.; Bedoya-Reina, O. C.; Ratan, A.; Sulak, M.; Drautz-Moses, D.; Perry, G. H.; Miller, W.; Schuster, S. C. *Cell Rep.* **2015**, *12*, 217−228.

(12) Klein, E.; Obermann, H. *Tetrahedron Lett.* **1978**, *4*, 349−352.

(13) Moussaieff, A.; Shein, N. A.; Tsenter, J.; Grigoriadis, S.; Simeonidou, C.; Alexander, G.; Alexandrovich, A. G.; Trembovler, V.; Ben-Neriah, Y.; Schmitz, M. L.; Fiebich, B. L.; Muñoz, E.; Mechoulam, R.; Shohami, E. *J. Cereb. Blood Flow Metab.* **2008**, *28*, 1341−1352.

(14) Moussaieff, A.; Shohami, E.; Kashman, Y.; Fride, E.; Schmitz, M. L.; Renner, F.; Fiebich, B. L.; Muñoz, E.; Ben-Neriah, Y.; Mechoulam, R. *Mol. Pharmacol.* **2007**, *72*, 1657−1664.

(15) Paul, M.; Bruning, G.; Bergmann, J.; Jauch, J. *Phytochem. Anal.* **2012**, *23*, 184−189.

(16) Appendino, G.; Cascio, M. G.; Bacchiega, S.; Morello, A. S.; Minassi, A.; Thomas, A.; Ross, R.; Pertwee, R.; De Petrocellis, L.; Di Marzo, V. *FEBS Lett.* **2006**, *580*, 568−574.

(17) (a) Nicoletti, R.; Forcellese, M. L. *Tetrahedron* **1968**, *24*, 6519−6525. (b) Hasegawa, T.; Kikuchi, A.; Saitoh, H.; Yamada, H. *J. Essent. Oil Res.* **2012**, *24*, 593−598.

DOI: 10.1021/acs.jnatprod.6b00141
J. Nat. Prod. 2016, 79, 1762−1768

(18) Moqrich, A.; Hwang, S. W.; Earley, T. J.; Petrus, M. J.; Murray, A. N.; Spencer, K. S. R.; Andahazy, M.; Story, G. M.; Patapoutian, A. *Science* **2005**, *307*, 1468−1472.
(19) Chung, M. K.; Lee, H.; Mizuno, A.; Suzuki, M.; Caterina, M. J. *J. Biol. Chem.* **2004**, *279*, 21569−21575.
(20) Bowden, B. F.; Coll, J. C.; Heaton, A.; Konig, G.; Bruck, M. A.; Cramer, R. E.; Klein, D. M.; Scheuer, P. J. *J. Nat. Prod.* **1987**, *50*, 650−659.
(21) Meng, Y.; Su, J.; Zeng, L. *Chin. J. Org. Chem.* **1997**, *17*, 542−546.
(22) 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.
(23) Chicca, A.; Caprioglio, D.; Minassi, A.; Petrucci, V.; Appendino, G.; Taglialatela-Scafati, O.; Gertsch, J. *ACS Chem. Biol.* **2014**, *9*, 1499−1507.
(24) Feketa, V. V.; Marrelli, S. P. *PLoS One* **2015**, *10*, e0141994.
(25) Liang, Z.; Wu, G.; Fan, C.; Xu, J.; Jiang, S.; Yan, X.; Di, S.; Ma, Z.; Hu, W.; Yang, Y. *Prog. Neurobiol.* **2016**, *137*, 1−16.
(26) Although in principle renewable, the collection of frankincense has been associated with a reduced regeneration in *B. papyrifera* (decreased flower and fruit production and increased percentage of mainly nonviable seeds), presumably because of competition for carbohydrates between the production of resin and that of fruits and seeds. Rijkers, T.; Woldeselassie, O.; Wessel, M.; Bongers, F. *J. Appl. Ecol.* **2006**, *43*, 1188−1195. Owing to a broader geographical distribution and the lack of perfumery relevance, the situation of *B. serrata* is much better in terms of conservation. In any case, *B. papyrifera* is not currently listed in the IUCN Red List of Threatened Species (http://www.iucnredlist.org, retrieved on February 12, 2016).
(27) Ren, J.; Wang, Y.-G.; Wang, A.-G.; Wu, L.-Q.; Zhang, H.-J.; Wang, W.-J.; Su, Y.-L.; Qin, H.-L. *J. Nat. Prod.* **2015**, *78*, 2322−2331.
(28) Schmidt, T. J.; Kaiser, M.; Brun, R. *Planta Med.* **2011**, *77*, 849−850.
(29) Del Prete, D.; Millán, E.; Pollastro, F.; Chianese, G.; Luciano, P.; Collado, J. A.; Munoz, E.; Appendino, G.; Taglialatela-Scafati, O. *J. Nat. Prod.* **2016**, *79*, 267−273.

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J. Nat. Prod. 2016, 79, 1762−1768