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by Henny Dien 10

Submission date: 13-Mar-2019 03:50PM (UTC+0700)
Submission ID: 1092549746
File name: 9._Desulfohaplosamate.pdf (261.76K)
Word count: 4906
Character count: 23642

Steroids 76 (2011) 998–1002

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Desulfohaplosamate, a new phosphate-containing steroid from *Dasychalina* sp., is a selective cannabinoid CB_2 receptor ligand

Giuseppina Chianese[a], Ernesto Fattorusso[a], Orazio Taglialatela-Scafati[a,*], Giorgio Bavestrello[b], Barbara Calcinai[b], Henny A. Dien[c], Alessia Ligresti[d], Vincenzo Di Marzo[d]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy
[b] Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[c] Faculty of Fishery and Marine Science, Sam Ratulangi University, Manado, Indonesia
[d] Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 22 March 2011
Accepted 25 March 2011
Available online 19 April 2011

Keywords:
Haplosamate
Sponges
Phosphated steroids
Cannabinoid receptors
CB_2 agonist

ABSTRACT

From the polar organic extract of the Indonesian sponge *Dasychalina* sp. we have isolated haplosamate A (**1**), a unique C_{28} sterol containing a sulfate group at C-3 and a methyl phosphate at C-15, along with its new desulfo analogue **2**, whose structure has been secured by detailed NMR investigation. Compounds **1** and **2**, as well as their semi-synthetic analogues **3**–**5**, have been evaluated for interaction with CB_1 and CB_2 receptors through a binding test. Desulfohaplosamate (**2**) showed a selective affinity for CB_2 receptors in the low μM range, while a semi-synthetic derivative with cleaved ring B showed a complete loss of affinity for both receptors, highlighting the importance of an intact steroid nucleus. To our knowledge, haplosamate derivatives represent the first CB receptor ligands belonging to the class of steroids.

1. Introduction

Phytocannabinoids, a class of meroterpenoids isolated from *Cannabis sativa* (hemp) and exemplified by Δ^9-THC, constitute a clear example of the profound impact that natural products can have on medicinal chemistry and pharmacology. Similarly to opioids, the investigation on these psychoactive natural products yielded to the discovery, about 25 years ago, of a specific receptor in the brain, named cannabinoid (CB) receptor CB_1 [1,2]. Soon after, the first endogenous ligand for this receptor was identified [3] and called anandamide, subsequently followed by a number of other endogenous CB_1 agonists belonging to the classes of fatty acid amides and esters.

Two CB receptors have been cloned and characterized to date: CB_1 and CB_2, both belonging to the superfamily of G-protein coupled receptors [4]. The existence of additional CB receptors different from CB_1 and CB_2 has long been pursued, since a number of cannabinoid-like effects persist in CB_1/CB_2 knockout mice [5], but it is still the object of an intense debate. Interaction with CB receptors has been unambiguously associated to a plethora of pharmacological effects, the most important being psychotropic, analgesic, immunomodulatory and motor effects (hypokinesia, antispasticity). While many of the psychoactive effects of Δ^9-THC appear to be mediated by CB_1 activation [6], CB_2 receptors are highly expressed in some cells of the immune system [7] and they are believed to play a role in the immune cell function. CB_2 receptors have also been suspected to be involved in neuroinflammation, atherosclerosis, and bone remodelling [8] and they are considered an attractive therapeutic target.

The research on CB receptors is a hot topic of modern pharmacology and medicinal chemistry and several classes of synthetic molecules have been proposed to act as specific agonists (or antagonists) of these receptors, e.g. pyrazole or imidazole derivatives as CB_2 agonists [9]. Conversely, a few natural products, with the obvious exception of meroterpenoids from *C. sativa*, have been tested as CB receptor ligands [10]. A recent example includes the polyine falcarinol (found in carrots, parsley and other plants) which exhibited micromolar binding affinity to both human CB receptors [11].

As part of our ongoing screening for biologically active secondary metabolites from Indonesian marine invertebrates [12–14], we had recently the opportunity to analyze a specimen of the sponge *Dasychalina* sp. (order Haposclerida, family Niphatidae). From the polar organic extract we have isolated consistent amounts of two steroidal derivatives, haplosamate A (**1**) and its new desulfo analogue **2**, whose structural identification is herein described. Compounds **1** and **2**, as well as their semi-synthetic analogues **3**–**5**,

* Corresponding author. Tel.: +39 081 678509; fax: +39 081 678552.
E-mail address: scatagli@unina.it (O. Taglialatela-Scafati).

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doi:10.1016/j.steroids.2011.03.013

have been evaluated for interaction with CB_1 and CB_2 receptors and in this paper we will discuss the obtained data.

2. Experimental

2.1. General experimental procedures

Optical rotations ($CHCl_3$) were measured at 589 nm on a P2000 Jasco polarimeter using a 10 cm microcell. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal (CD_3OD: δ_H 3.34, δ_C 49.0). 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-HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Through space ^1H connectivities were evidenced by using a ROESY experiment with a mixing time of 300 ms. Low- and high-resolution ESI-MS spectra were performed on LTQ Orbitrap XL (Thermo Scientific) mass spectrometer. Medium pressure liquid chromatography was performed on a Büchi apparatus using a silica gel (230–400 mesh) column; HPLC were achieved on a Knauer apparatus equipped with a refractive index detector and analytical LUNA (Phenomenex) RP18 (250× 4 mm) columns.

2.2. Collection, extraction and isolation

A specimen of the sponge *Dasychalina* sp. (order Haposclerida, family Niphatidae) was collected in January 2008 by hand in the area of the Bunaken Marine Park of Manado and kept frozen until extraction. A voucher sample (no. MAN08-07) has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II. The organism (210 g wet weight) was exhaustively extracted, in sequence, with methanol (MeOH) and dichloromethane (CH_2Cl_2) by soaking the diced sponge tissue. The extracts were combined and then partitioned between ethyl acetate (EtOAc) and H_2O. The polar material has been then partitioned between H_2O and butanol to obtain a polar organic phase (2.67 g), which was chromatographed on a silica gel column (230–400 mesh), using a gradient solvent system from EtOAc to MeOH. Fractions eluted with EtOAc/MeOH 7:3 to 1:1 were combined and further purified by reverse-phase HPLC (MeOH/H_2O 1:1) to obtain consistent amounts of haplosamate A (**1**, 480.5 mg) and the new desulfohaplosamate (**2**, 12.2 mg) in the pure state.

2.3. Desulfohaplosamate

Colorless powder. $[\alpha]_D = -3.8$ ($c = 0.03$ MeOH); ^1H and ^{13}C NMR (CD_3OD): Table 1. ESI-MS (negative ions) m/z 573 $[M-Na]^-$; HREIMS m/z 573.3200 $[M-Na]^-$ (calcd. for $C_{29}H_{50}O_9P$ 573.3198).

2.4. Oxidative cleavage of haplosamate A

To a solution of haplosamate A (43.4 mg, 0.062 mmol) in CH_2Cl_2 (1.2 mL) 50 μL of $NaHCO_3$ sat. solution and 28 mg (0.13 mmol mmol) of $NaIO_4$ were added. The mixture was left at room temp. for 3 h under vigorous stirring. Then, the reaction mixture was treated with dry Na_2SO_4 and filtered. The obtained filtrate was purified by RP18 HPLC (H_2O/MeOH 1:1) to give 27.0 mg (65% yield) of the dialdehyde **3**.

2.5. Dialdehyde 3

Colorless amorphous solid; $[\alpha]_D = -4.6$ ($c = 0.05$); ^1H NMR (CD_3OD, 500 MHz): δ 10.22 (H-7, bs), 9.81 (H-6, s), 4.60 (H-3, bs), 4.37 (H-15, m), 4.19 (H-4, bs), 3.68 (H-16, dd, $J = 10.5$, 2.0 Hz), 3.57 (OMe, d, $J = 10.5$ Hz), 3.36 (H-23, t, $J = 8.5$ Hz), 2.55 (H-8, t, $J = 11.0$ Hz), 2.56 (H-5, bs), 2.35 (H-9, m), 2.14 (H-2a, m), 2.09 (H-25, m), 1.84 (H-12a, overlapped), 1.80 (H-20, overlapped), 1.76 (H-2a, overlapped), 1.75 (H-14, overlapped), 1.73 (H-1a, overlapped), 1.63 (H-22a, overlapped), 1.60 (H-11a, overlapped), 1.55 (H-11b, overlapped), 1.41 (H-24, m), 1.30 (Me-19, s), 1.30 (H-[illegible] m), 1.27 (H-12b, overlapped), 0.96 (Me-18, s), 0.95 (Me-21, d, $J = 7.0$ Hz), 0.[illegible] (Me-26, d, $J = 7.0$ Hz), 0.86 (H-22b, m), 0.81 (Me-27, d, $J = 7.0$ Hz), 0.80 (Me-28, d, $J = 7.0$ Hz), 0.80 (H-17, overlapped). ^{13}C NMR (CD_3OD, 125 MHz): δ 205.2 (C-7), 204.8 (C-6), 90.8 (C-16), 82.3 (C-3), 81.4 (C-23), 77.5 (C-15), 70.8 (C-4), 62.0 (C-17), 55.2 (C-[illegible]), 54.0 (OMe), 51.9 (C-14), 44.2 (C-24), 43.5 (C-9), 43.4 (C-13), 40.5 (C-22), 40.1 (C-[illegible]), 37.8 (C-12), 34.3 (C-1), 33.5 (C-20), 33.2 (C-10), 27.2 (C-25), 22.8 (C-2), 22.5 (C-11), 21.8 (C-[illegible]), 20.5 (C-21), 18.5 (C-19), 16.8 (C-26), 15.4 (C-[illegible]), 11.0 (C-28). ESI-MS (negative ions) m/z 651 $[M-2Na+H]^-$, 673 $[M-Na]^-$; HR-ESI-MS m/z 673.2421 (calcd. for $C_{29}H_{47}NaO_{12}PS$ m/z 673.2429).

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR data of desulfohaplosamate (**2**) in CD_3OD.

Pos.	δ_C, mult.	δ_H, mult., J in, Hz
1a	30.3, CH_2	1.34[a]
1b		1.29[a]
2a	24.4, CH_2	2.04, m
2b		1.55[a]
3	69.9, CH	3.74, d, 2.5
4	78.5, CH	3.85, bs
5	44.9, CH	1.52[a]
6	78.2, CH	3.96, bs
7	80.0, CH	3.28, bd, 10.6
8	34.5, CH	2.29, ddd, 11.0, 10.6, 10.6
9	54.1, CH	0.86, m
10	36.8, C	
11a	20.0, CH_2	1.53[a]
11b		1.44[a]
12a	40.5, CH_2	1.84, m
12b		1.14, m
13	43.2, C	
14	58.2, CH	1.39, m
15	81.6, CH	4.72, m
16	91.8, CH	3.91, dd, 10.0,3,5
17	62.5, CH	0.73, m
18	15.5, CH_3	1.[illegible]9, s
19	17.5, CH_3	1.32, s
20	33.9, CH	1.82, m
21	20.5, CH_3	0.96, d, 7.0
22a	40.0, CH_2	1.65, m
22b		0.84, m
23	82.1, CH	3.45, ddd, 9.5, 8.5, 2.1
24	44.9, CH	1.[illegible]1[a]
25	27.8, CH	2.08, m
26	17.1, CH_3	0.81, d, 7.0
27	21.7, CH_3	0.89, d, 7.0
28	10.2, CH_3	0.76, d, 7.0
–OMe	52.8, CH_3	3.60, d, 11.0

[a] Overlapped with other signals.

2.6. Acetylation of haplosamate A

Haplosamate A (33.5 mg, 0.048 mmol) was dissolved in dry pyridine (0.5 mL) and treated with Ac_2O (0.5 mL). After standing overnight under stirring at room temp, the reaction was worked up by addition of a few drops methanol to destroy the excess Ac_2O, water (*ca.* 1 mL) and EtOAc (*ca.* 3 mL). The organic phase was washed sequentially with 2 N H_2SO_4, sat. $NaHCO_3$ and brine. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by HPLC (H_2O/MeOH 6:4) to afford 25.5 mg (75% yield) of the 7-monoacetate **4** and 8.5 mg (22% yield) of the 4,7-diacetate **5**.

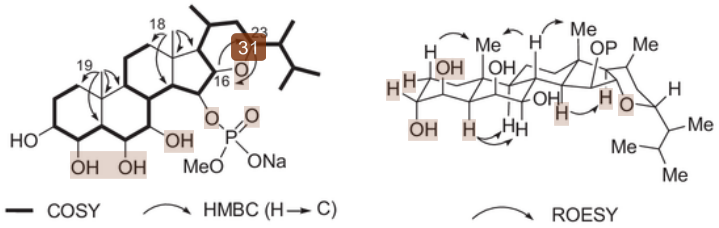


Fig. 1. COSY and key HMBC cross-peaks (left) (**1**) and ROESY correlations (right) of desulfohaplosamate (**2**).

2.7. Haplosamate A 7-acetate (**4**)

Colorless powder. $[\alpha]_D$ = 4 (c = 0.03); ^{1}H NMR (CD_3OD, 500 MHz): δ 4.64 (H-7, bd, J = 10.5), 4.43 (H-3, bs), 4.36 (H-15, m), 4.14 (H- s), 4.00 (H-16, dd, J = 10 2.0 Hz), 3.89 (H-6, bs), 3.64 (OMe, d, J = 10.5 Hz), 3.52 (H-23, t, J = 8.5 Hz), 2.70 (H-8, t, J = 11.0 Hz), 2.32 (7-OAc, s), 2.03 (H-2a, m), 2.00 (H-25, m), 1.81 (H-20, overlapped), 1.77 (H-12a, overlapped), 1.68 (H-5, bs), 1.55 (H-11a, overlapped), 1.53 (H-22a, overlapped), 1.51 (H-1a, m), 1.42 (H-24, overlapped), 1.40 (H-11b, overlapped), 1.30 (H-14, overlapped), 1.38 (Me-19, s), 1.33 (H-2a, overlapped), 1.27 (H-1b, m), 1.05 (Me-18, s), 1.01 (H-9, m), 0.99 (Me-21, d, J = 7.0 Hz), 0.89 (Me-26, d, J = 7.0 z), 0.88 (H-12b, overlapped), 0.86 (H-22b, m), 0.81 (Me-27, = 7.0 Hz), 0.75 (Me-28, d, J = 7.0 Hz), 0.70 (H-17, overlapped). ESI-MS (negative ions) m/z 695 $[M-2Na+H]^-$, 717 $[M-Na]^-$; HR-ESI-MS m/z 717.2702 (calcd. for $C_{31}H_{51}NaO_{13}PS$ m/z 717.2691).

2.8. Haplosamate A 4,7-diacetate (**5**)

Colorless powder. $[\alpha]_D$ = −10.6 (c = 0.08); ^{1}H NMR (CD_3OD, 500 MHz): δ 5.42 (H-4, bs), 4.6 -7, bd, J = 10.5 Hz), 4.40 (H-15, 4.36 (H-3, bs), 3.99 (H-16, dd, J = 1 2.0 Hz), 3.91 (H-6, bs), 3.67 (OMe, d, J = 10.5 Hz), 3.55 (H-23, t, J = 8.5 Hz), 2.70 (H-8, t, J = 11.0 Hz), 2.32 (7-OAc, s), 2.09 (4-OAc, s), 2.03 (H-2a, m), 2.00 (H-25, m), 1.81 (H-20, overlapped), 1.77 (H-12a, overlapped), 1.72 (H-5, bs), 1.55 (H-11a, overlapped), 1.53 (H-22a, overlapped), 1.51 (H-1a, m), 1.42 (H-24, overlapped), 1.40 (H-11b, overlapped), 1.35 (H-14, overlapped), 1.33 (H-2a, overlapped), 1.33 (M 9, s), 1.27 (H-1b, m), 1.05 (Me-18, s), 1.01 (H-9, m), 0.99 (Me-21, d, J = 7.0 Hz), 0.89 (Me-26, d, J = 7 Hz), 0.88 (H-12b, overlapped), 0.86 (H-22b, m), 0.81 (Me-27 = 7.0 Hz), 0.75 (Me-28, d, J = 7.0 Hz), 0.70 (H- verlapped). ESI-MS (negative ions) m/z 737 $[M-2Na+H]^-$, 759 $[M-Na]^-$; HR-ESI-MS m/z 759.2802 (calcd. for $C_{33}H_{53}NaO_{14}PS$ m/z 759.2797).

2.9. Cannabinoid CB_1 and CB_2 receptor binding assays

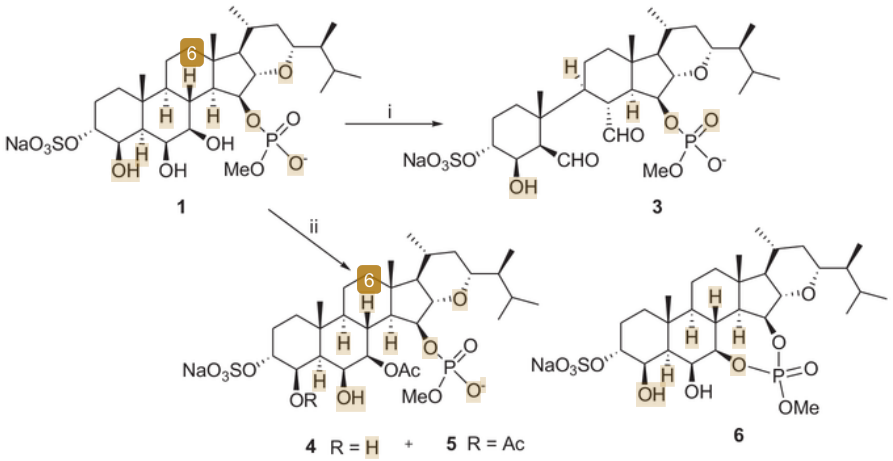
Membranes from HEK-293 cells transfected with the human recombinant CB_1 receptor (B_{max} = 2.5 pmol/mg protein) and human recombinant CB_2 receptor (B_{max} = 4.7 pmol/mg protein) were incubated th the radiolabelled high affinity ligand]-CP-55,940 (0.14 nM, Kd = 0.18 nM and 0.084 nM, Kd = 0.31 nM, for CB_1 and CB_2 receptors, respectively) and displaced with 10 μM WIN 55212-2 as the heterologous c petitor for non specific binding (K_i values 9.2 nM and 2.1 nM for CB_1 and CB_2 receptors, respectively). All compounds were tested following the procedure described by the manufacturer (PerkinElmer, Italia). Displacement curves were generated by incubating drugs with [^{3}H]-CP-55,940 for 90 min at 30 °C. K_i values were calculated by applying the Cheng-Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compound. Δ^9-THC was used as the reference compound. Data are means of 3 experiments.

3. Results and discussion

A specimen of the sponge *Dasychalina* sp. was collected by hand in the area of the Bunaken Marine Park of Manado and kept frozen until sequentially extracted with methanol (MeOH) and dichloromethane by soaking the diced sponge tissue (210 g wet weight). The extracts were concentrated, combined, and then partitioned between ethyl acetate (EtOAc) and water. The polar material has been then partitioned betwee ater and butanol to obtain a polar organic phase of 2.67 g. This was chromatographed on silica using a gradient solvent system from EtOAc to MeOH. Fractions eluted with EtOAc/MeOH 7:3 to 1:1 were combined and further purified by reverse-phase HPLC (MeOH/H_2O mixtures) obtain consistent amounts of haplosamate A (**1**, 480.5 mg, 0.2% based on wet weight) and the new desulfohaplosamate (**2**, 12.2 mg) in the pure form.

Haplosamate A is a unique C_{28} sterol containing seven oxygenated carbons and including a rare six-membered ether ring connecting C-16 and C-23, a sulfate group -3, and a methyl phosphate at C-15. Haplosamate A was first isolated from the sponge *Xestospongia* sp. [15] and then re-isolated (and structurall evised) from another Haposclerida sponge, *Cribrochalina* sp. [16]. Although steroidal sulfates are well represented in marine sponges, the group of natural sterols including both sulfate and phosphate groups is restricted to a ha ful of members [17].

Compound **2** showed a prominent ion peak at m/z 573 in the ESI-MS spectrum (negative ion mode) and high resol n measurements indicated the molecular formula $C_{29}H_{50}O_9P$. ^{1}H and ^{13}C NMR spectra of **2** (CD_3OD, Table 1) resembled parallel spectra of haplosamate A, but marked shifts were o erved in several resonances. In particular, the presence of the characteristic *O*-methyl doublet signal coupled to the phosphorous atom (δ_H 3.60, doublet, J = 11.0 Hz) clearly confirmed the presence of the methylphosphate functionality. All the proton resonances were associated to those of the directly attached carbon atoms through the 2D NMR HSQC experiment and then the proton multiplets were arranged in sequence through the COSY experiment, yielding to the single large spin system evidenced in bold in Fig. 1. HMBC cross-peaks exhibited by Me-18 an e-19 defined the tetracyclic steroidal skeleton, while the key HMBC correlations of H-16 (δ_H 3.91) with C-23 (δ_C 82.1) and H-23 with C-16 indicated the presence of a -membered ether ring. Carbon–phosphorous couplings were observed in the ^{13}C NMR spectrum of **2** for C-14 ($^3J_{C,P}$ = 8.5 Hz) and C-15 ($^2J_{C,P}$ = 7.0 Hz), thus placing the methylphosphate group at C-15, and completely defining the planar structure of **2**. The comparison of the pattern of ^{1}H–^{1}H coupling constants measured for **2** (Table 1) with those reported for haplosamate A, supported by the series of ROESY cross peaks shown in Fig. 1, indicated the relative configuration of **2**. Noteworthy, three of the four secondary hydroxyl groups are in axial orientation and they are all located on the "southern" part of rings A and B. The relatively low d shifted resonance of Me-19 (δ_H 1.32) is in agreement with the β orientation of both the hydroxyl groups at C-4 and C-6. Desulfohaplosamate (**2**) is only the fourth member of the haplosamate group



Scheme 1. Preparation of semisynthetic derivatives of haplosamate A. (i) $NaIO_4$; (ii) Ac_2O/Pyr.

of steroids, in addition to haplosamate A, haplosamate B (showing a second phosphate group at C-7) and a minor analogue showing ring A contraction [16].

Fusetani et al. reported that haplosamate A possessed a moderate inhibitory activity toward membrane type 1 matrix metalloproteinase, a key enzyme in tumor metastasis [16]. More recently, a seminal work by Andersen et al. [18] identified haplosamate A as CB_1 and CB_2 receptor agonists, on the basis of a functional cell-based bioassay (*Spodoptera frugiperda* cells expressing one or the other of the human cannabinoid receptors) and saturation transfer double-difference NMR experiments [19]. In the same study, the Authors also suggested that phosphate and sulfate groups are not essential for binding to cannabinoid receptors, tentatively identifying the "northern" less polar region of the molecule as responsible for this interaction. Since we have been interested on the chemistry of both natural [20] and synthetic [21] cannabinoids, the isolation of haplosamate compounds from *Dasychalina* sp. represented a unique opportunity to investigate the interaction of these steroidal derivatives with cannabinoid receptors through a binding test.

In order to increase the chemical diversity of the tested compounds, we took advantage of the relatively high amounts of haplosamate A obtained from the sponge to prepare a few simple semi-synthetic derivatives. Treatment of haplosamate A (**1**) with $NaIO_4$ gave in good yields (65%) the dialdehyde **3** (Scheme 1) originating from the regioselective oxidative cleavage of the C-6/C-7 bond. The ^1H NMR spectrum of compound **3** (CD_3OD) showed the appearance of two broad singlets at δ_H 9.81 (H-6) and 10.22 (H-7) and a marked downfield shift of the other ring B proton signals (H-5: from δ 1.50 to 2.56; H-8 from δ 2.26 to 3.15; H-9 from δ 0.90 to 2.35). Inspection of 2D NMR COSY, HSQC and HMBC experiments allowed the assignment of all the proton and carbon resonances as reported in Section 2. Acetylation of haplosamate A (**1**) in standard conditions (Ac_2O/pyr) yielded to the formation of the 7-acetate (**4**) and of the 4,7-diacetate (**5**) derivatives (Scheme 1). The acetylation positions in compounds **4** and **5** were unambiguously determined on the basis of the marked downfield shift of the relevant methine protons (e.g. H-7 is shifted from δ_H 3.34 to 4.64 in **4**) and of the $^3J_{H,C}$ HMBC cross peaks between each of these methines and the corresponding ester carbonyls. The easy esterification at C-7 can be rationalized on the basis of the equatorial position of the hydroxyl group; on the contrary, acetylation of the axial hydroxyl at C-4 was somewhat unexpected, due to the steric hindrance of Me-19. Noteworthy, Fusetani et al. reported that treatment of haplosmate A with benzoyl chloride not only failed to give esterification at C-4 but also at C-7, and the phosphotriester **6** (Scheme 1), formed by ester exchange, was the single reaction product [17]. This markedly different behaviour of the same compound upon treatment with benzoyl chloride and acetic anhydride appears intriguing.

The affinity of the natural haplosamates **1** and **2** and of the semisynthetic derivatives **3**–**5** for both human recombinant cannabinoid receptors CB_1 and CB_2 was evaluated (Table 2). A comparison of affinity data obtained for haplosamate A (**1**) and desulfohaplosamate (**2**) appears quite interesting, since these two compounds showed an opposite behaviour. Compound **1** exhibited significant affinity only for CB_1 receptors, its potency in the binding assay being in the mid μM range, significantly higher than previously reported using a different type of assay [18]. Instead, the desulfated analogue **2** showed a much higher affinity for CB_2 receptors, in the low μM range (K_i = 2.8 μM). The 7-monoacetylated derivative of haplosamate A showed an increase in the affinity for both CB receptors, compared to its parent compound, but the further acetylation at C-4 proved to be deleterious for affinity on both CB_1 and CB_2. Finally, the dialdehyde derivative **3** showed a complete loss of affinity for both receptors, highlighting the importance of an intact steroid nucleus and, in particular, of intact rings A and B. Δ^9-THC, used here as reference compound, was, as expected, about 1000-fold more potent than haplosamates in the same binding assays.

To our knowledge, haplosamate derivatives represent a new chemotype of cannabinoid receptor ligands. Natural products with cannabinomimetic activity, whose structures are not related to the meroterpenoids of hemp nor to the endocannabinoids (polyunsaturated fatty acid amides or esters) are extremely rare and haplosamates are the first CB ligands belonging to the class of steroids. The selectivity of desulfohaplosamate for CB_2 receptor is

Table 2
Effects of haplosamate derivatives **1**–**5** on cannabinoid receptors.

	K_i at hCB_1[a] (μM)	K_i at hCB_2[a] (μM)
Haplosamate A (**1**)	19.17	>50
Desulfohaplosamate (**2**)	19.47	2.82
Dialdehyde **3**	>50	>50
Haplosamate A-7-acetate (**4**)	9.74	8.89
Haplosamate A-4,7-diacetate (**5**)	19.98	11.85
Δ^9-Tetrahydrocannabinol	0.0016	0.0024

[a] K_i values are calculated by applying the Cheng-Prusoff equation.

particularly important and worthy of further investigation, given the involvement of this receptor in the immune response and inflammation [22] and the lack of abuse potential and strong central effects related to the use of CB_2 ligands.

Acknowledgments

This work was partially conduced during the Master Course "Tropical Marine Biodiversity and Natural Products" of Università Politecnica delle Marche. Mass and NMR spectra were recorded at "Centro di Ricerca Interdipartimentale di Analisi Strumentale" of the University of Naples "Federico II". The assistance of the staff is acknowledged. The authors also thank Mr. Marco Allarà for his valuable help in the binding assays.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.03.013.

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