Bioorganic & Medicinal Chemistry Letters 21 (2011) 1962–1964

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of indole alkaloids with cannabinoid CB1 receptor antagonistic activity

Mariko Kitajima [a], Masumi Iwai [a], Ruri Kikura-Hanajiri [b], Yukihiro Goda [b], Mitsuru Iida [c], Hisatoshi Yabushita [c], Hiromitsu Takayama [a,*]

[a] Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
[b] National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
[c] Otsuka Pharmaceutical Co., Ltd, 224-18 Hiraishi Ebisuno, Kawauchi-cho, Tokushima 771-0182, Japan

ARTICLE INFO

Article history:
Received 6 January 2011
Revised 8 February 2011
Accepted 9 February 2011
Available online 13 February 2011

Keywords:
Cannabinoid
CB1 receptor
Antagonist
Indole alkaloid
Iboga
Voacanga

ABSTRACT

Three indole alkaloids, voacamine (**1**), 3,6-oxidovoacangine (**2**), and a new alkaloid, 5-hydroxy-3,6-oxidovoacangine (**3**), isolated from *Voacanga africana* were found to exhibit potent cannabinoid CB1 receptor antagonistic activity. This is the first example of CB1 antagonists derived from natural alkaloids.

The endocannabinoid system is involved in a wide variety of psychological and physiological processes. The cannabinoid (CB) receptors are G-protein-coupled receptors (GPCRs) and two subtypes are known: CB1 and CB2.[1] The CB1 receptor is distributed throughout the body, mainly expressed in the brain, and involved in memory, cognitive process, pain, and appetite. It is also one of the targets of drug development for the treatment of obesity, metabolic syndrome, obesity-related cardiovascular disorder, substance abuse, and cognitive impairment. The therapeutic potential of CB1 receptor antagonists has been demonstrated.[1] In this Letter, we disclose three indole alkaloids possessing CB1 receptor antagonistic activity: voacamine (**1**), 3,6-oxidovoacangine (**2**), and a new alkaloid, 5-hydroxy-3,6-oxidovoacangine (**3**), isolated from *Voacanga africana* (Fig. 1).

The screening of plant extracts and isolated alkaloids for agonistic or antagonistic activity on the CB1 receptor was performed using the aequorin/GPCR cell-based Ca^{2+} functional assay.[2] CP55940 or rimonabant was used as the positive control for agonist or antagonist. As a result, we found that the MeOH extract of *V. africana* root bark showed potent activity. This prompted us to clarify the active principle in *V. africana*. Separation of the crude base that was prepared from *V. africana* root bark resulted in the isolation of three active indole alkaloids: one iboga-vobasine-type bis-indole alkaloid, voacamine (**1**),[3] and two iboga-type monomer alkaloids, 3,6-oxidovoacangine (**2**)[4] and 5-hydroxy-3,6-oxidovoacangine (**3**).[5] These compounds exhibited relatively potent CB1 receptor antagonistic activity with IC_{50} of 0.041, 0.199, and 0.141 μM, respectively, compared with that of rimonabant (IC_{50} 0.004 μM). These are a new class of small molecules possessing CB1 receptor antagonistic activity. Figure 2 shows that their effects were concentration-dependent. Interestingly, well-known co-existing alkaloids, such as voacangine (**4**), vobasine (**5**), and tabersonine (**6**), did not show the activity.

Among the three alkaloids, 5-hydroxy-3,6-oxidovoacangine (**3**) is a new alkaloid and its structure was deduced by spectroscopic analysis as follows. Compound **3** was found to have the molecular formula $C_{22}H_{26}N_2O_5$ from HREIMS [*m/z* 398.1844 (M^+)], which indicated that **3** has an extra oxygen atom compared to 3,6-oxidovoacangine (**2**). The UV spectrum exhibited a characteristic 5-methoxyindole chromophore. Its NMR spectra were similar to those of 3,6-oxidovoacangine (**2**) except for the existence of a low-field oxygenated methine group at δ_H 5.16 and δ_C 92.7 in the ^1H and ^{13}C NMR spectra, respectively, instead of a methylene group due to C-5. HMBC correlation of the proton at δ 5.16 to the carbon at δ 59.0 due to C-21, together with the above data, indicated that the hydroxyl group was attached to C-5 (Fig. 3). As a result of the rigid character of the skeleton, the relative configuration at C-3, C-6, C-14, C-16, and C-21 was restricted, that is, 3*S**, 6*R**, 14*R**, 16*S**, and 21*S**. The NOE correlation of H-5 to H-21 suggested that

* Corresponding author. Tel./fax: +81 (43) 290 2901.
E-mail address: htakayam@p.chiba-u.ac.jp (H. Takayama).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.036

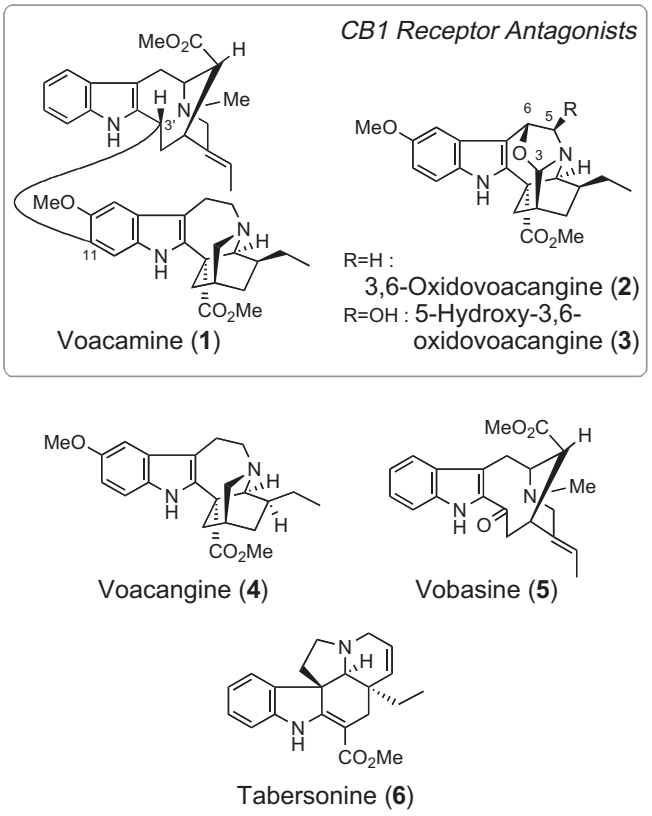

Figure 1. Structures of indole alkaloids **1**–**3** with CB1 receptor antagonistic activity and alkaloids **4**–**6** from *Voacanga africana*.

both protons were in *cis* relationship and the hydroxyl group at C-5 had R^* configuration. The absolute configuration at C-16 was deduced to be *S* from the negative Cotton effect that appeared at approximately 280 nm in the CD spectrum, which was the same as that of voacangine (**4**), whose absolute configuration is known.[6] Therefore, new alkaloid **3** was deduced to be 5-hydroxy-3,6-oxidovoacangine.

In conclusion, we have found three cannabinoid CB1 receptor antagonists, voacamine (**1**), 3,6-oxidovoacangine (**2**), and 5-hydroxy-3,6-oxidovoacangine (**3**), from *V. africana*. As far as we know, these are the first examples of natural alkaloids having CB1 receptor antagonistic activity. Further studies of the medicinal chemistry using these structurally and biologically unique alkaloids are under way in our laboratories.

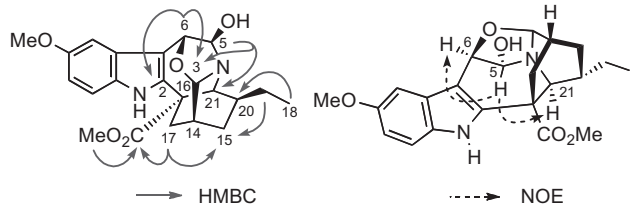

Figure 3. Selected HMBC and NOE correlations of 5-hydroxy-3,6-oxidovoacangine (**3**).

Acknowledgement

This work was supported by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

References and notes

1. For recent reviews on cannabinoid receptor agonists/antagonists, see: (a) Thomas, B. F. *Drug Dev. Res.* **2009**, *70*, 527; (b) Janero, D. R.; Makriyannis, A. *Expert Opin. Emerg. Drugs* **2009**, *14*, 43; (c) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205; (d) Muccioli, G.; Lambert, D. M. *Expert Opin. Ther. Patents* **2006**, *16*, 1405. and references cited therein.
2. Stables, J.; Green, A.; Marshall, F.; Fraser, N.; Knight, E.; Sautel, M.; Milligan, G.; Lee, M.; Rees, S. *Anal. Biochem.* **1997**, *252*, 115.
3. (a) Achenbach, H.; Schaller, E. *Chem. Ber.* **1976**, *109*, 3527; (b) Medeiros, W. L. B.; Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; Leal, K. Z.; Rodrigues-Filho, E.; Schripsema, J. *Magn. Reson. Chem.* **1999**, *37*, 676; (c) Agwada, J.; Patel, M. B.; Hesse, M.; Schmid, H. *Helv. Chim. Acta* **1972**, *55*, 1849.
4. (a) Ghorbel, N.; Damak, M.; Ahond, A.; Philogéne, E.; Poupat, C.; Potier, P.; Jacquemin, H. *J. Nat. Prod.* **1981**, *44*, 717; (b) Morfaux, A. M.; Mulamba, B.; Richard, B.; Delaude, C.; Massiot, G.; Le Men-Oliver, L. *Phytochemistry* **1982**, *21*, 1767.
5. The root bark of *Voacanga africana* (608 g dry weight) was extracted with MeOH to give a MeOH extract (70.12 g). The crude base (15.5 g) was prepared from the MeOH extract by a conventional method. The $CHCl_3$ extract was separated by silica gel open column chromatography with a MeOH/$CHCl_3$ gradient. The fraction eluted with 3% MeOH/$CHCl_3$ was purified by repeated chromatography to afford 5-hydroxy-3,6-oxidovoacangine (**3**, 1.4 mg). Compound **3**: UV (MeOH) λ_{max} nm (log ε) 285.0, 203.5; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.53 (1H, br s, NH), 7.14 (1H, d, *J* = 8.8 Hz, H-12), 7.02 (1H, d, *J* = 2.5 Hz, H-9), 6.80 (1H, dd, *J* = 8.8, 2.5 Hz, H-11), 5.27 (1H, s, H-6), 5.24 (1H, d, *J* = 3.8 Hz, H-3), 5.16 (1H, s, H-5), 3.88 (3H, s, CO_2Me), 3.84 (3H, s, 10-OMe), 3.69 (1H, br d, *J* = 2.7 Hz, H-21), 2.44 (1H, br d, *J* = 14.0 Hz, H-17), 2.30 (1H, m, H-14), 2.14 (1H, br ddd, *J* = 14.0, 3.4, 3.4 Hz, H-17), 1.92 (1H, m, H-15), 1.57 (1H, m, H-19), 1.42 (1H, m, H-19), 1.30 (1H, m, H-20), 1.08 (1H, m, H-15), 0.89 (3H, dd, *J* = 7.1, 7.1 Hz, H_3-18); ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 175.0 (CO_2Me), 154.7 (C-10), 139.0 (C-2), 128.8 (C-13), 127.1 (C-8), 112.7 (C-7), 112.6 (C-11), 111.7 (C-12), 99.5 (C-9), 93.4 (C-3), 92.7 (C-5), 74.9 (C-6), 59.0 (C-21), 55.9 (10-OMe), 53.0 (CO_2*Me*), 52.9 (C-16), 38.4 (C-20), 32.4 (C-17), 29.7 (C-15), 29.5 (C-14), 26.6 (C-19), 11.7 (C-18); EIMS

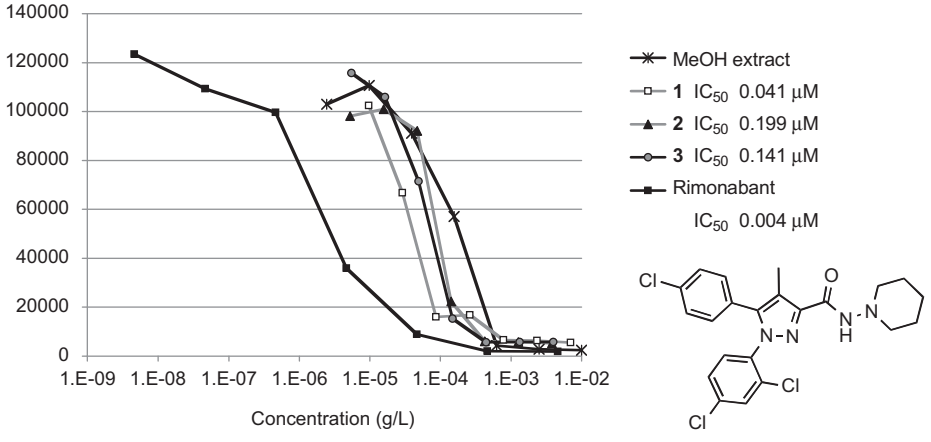

Figure 2. CB1 receptor antagonistic activity of MeOH extract of *Voacanga africana*, alkaloids **1**–**3**, and rimonabant.

m/z (%) 398 ([M]$^+$, 27), 75 (100); HREIMS *m/z* 398.1844 (calcd for $C_{22}H_{26}N_2O_5$ 398.1841); CD (MeOH, 24 °C, *c* 0.29 mmol/L) $\Delta\varepsilon$ (λ nm) 0 (356), −0.24 (320), 0 (306), −0.54 (281), 0 (265), +0.15 (255), 0 (251), −0.26 (241), 0 (231), +2.38 (217).

6. (a) Kutney, J. B.; Brown, R. T.; Piers, E. *Can. J. Chem.* **1966**, *44*, 637; (b) Bláha, K.; Koblicová, Z.; Trojánek, J. *Tetrahedron Lett.* **1972**, *27*, 2763.