Bioorganic & Medicinal Chemistry Letters 25 (2015) 5389–5392

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Bastadins, brominated-tyrosine derivatives, suppress accumulation of cholesterol ester in macrophages

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ARTICLE INFO

Article history:
Received 30 March 2015
Revised 22 August 2015
Accepted 10 September 2015
Available online 10 September 2015

Keywords:
Bastadin
Brominated-tyrosine derivatives
Cholesterol ester
Atherosclerosis

ABSTRACT

The formation of foam cells in macrophages has been suggested to play an essential role in the progression of early atherosclerotic lesions in vivo and, thus, its suppression is considered to be one of the major approaches for the treatment of atherosclerosis. We isolated eight brominated-tyrosine derivatives, bastadins, from the EtOH extract of the marine sponge *Ianthella vasta* as inhibitors of the formation of foam cells induced by acetylated low-density lipoproteins in human monocyte-derived macrophages. Bastadin 6 was the strongest inhibitor of foam cell formation due to its suppression of acyl-coenzyme A:cholesterol acyltransferase.

Foam cells derived from macrophages form large clusters in the subendothelial spaces of early atherosclerotic lesions and produce various endogenous signaling molecules, for example, cytokines, growth factors, and proteases, which play important roles in the development and progression of atherosclerotic lesions.[1] Free cholesterol is incorporated into macrophages through scavenger receptors as a constituent of chemically modified low-density lipoproteins (LDL) and is then released from LDL in its free form in cells. Since free cholesterol is toxic to cells, it is esterified to cholesterol esters (CE) by acyl-coenzyme A:cholesterol acyltransferase (ACAT) in the rough endoplasmic reticulum.[2] Through these processes, macrophages are converted to foam cells that contain the accumulated CE. A number of anti-atherosclerotic approaches that prevent the formation of foam cells, that is, the inhibition of LDL oxidation,[3] scavenger receptor expression,[4] and ACAT activity,[5] have been investigated to date. We previously isolated pheophytin *b*,[6] manzamine A,[7] and esculeoside A[8] as inhibitors of foam cell formation from a Chinese cabbage, marine sponge, and tomato, respectively. Among these, manzamine A and esculeoside A suppressed hyperlipidemia and atherosclerosis in atherogenic mice by inhibiting ACAT activity. A further search for drug candidates for the treatment of atherosclerosis revealed that an extract of the marine sponge *Ianthella vasta*, collected in Indonesia, inhibited the formation of foam cells in human monocyte-derived macrophages (HMDMs). We herein described the isolation of eight known bastadins from the sponge and their inhibitory effects on the formation of foam cells in HMDMs.

The EtOAc-soluble fraction of the EtOH extract of the marine sponge *Ianthella vasta*[9] inhibited the formation of foam cells in HMDMs[7] (65% inhibition at 100 μg/mL). Bioassay-guided purification from the EtOAc-soluble fraction by repeated column chromatography followed by ODS HPLC[10] afforded bastadins 4[11] (**1**), 5[11,13] (**2**), 6[11] (**3**), 7[11] (**4**), 9[12] (**5**), 12[14] (**6**), and 16[15] (**7**) as well as hemibastadin 1[16] (**8**) (Fig. 1).

The inhibitory activities of **1**–**8** against foam cell formation at concentrations of 5 μM were measured (Fig. 2).[17] Of these, **3**, which contains six bromine atoms, showed 96% inhibition at 5 μM and was the strongest inhibitor, while **1**, **2**, **4**, **5**, **6**, **7**, and **8** showed 36%, 58%, 39%, 80%, 80%, 88%, and 5% inhibition, respectively, at 5 μM without cytotoxicity. Thus, we proposed that the dimeric bastadins **1**–**7** were active with respect to inhibition, while the monomeric hemibastadin 1 (**8**) was inactive.

In order to clarify the mechanisms underlying the inhibitory effects of bastadins on the accumulation of CE in HMDMs, we examined the effects of bastadins on ACAT activity. Two human ACAT isozymes (hACAT-1 and hACAT-2) have been identified to date.[18] In the present study, we used Chinese hamster ovary

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http://dx.doi.org/10.1016/j.bmcl.2015.09.024
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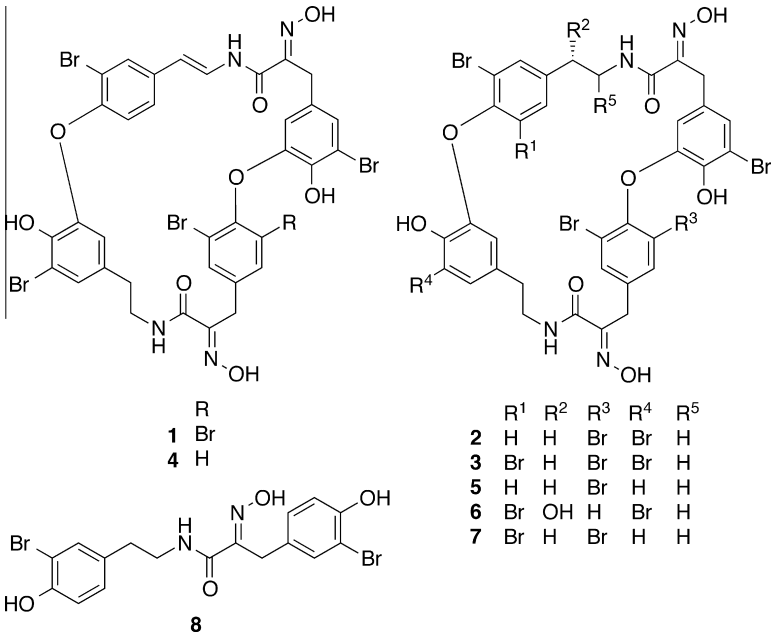

Figure 1. Structures of bastadins (**1**–**8**).

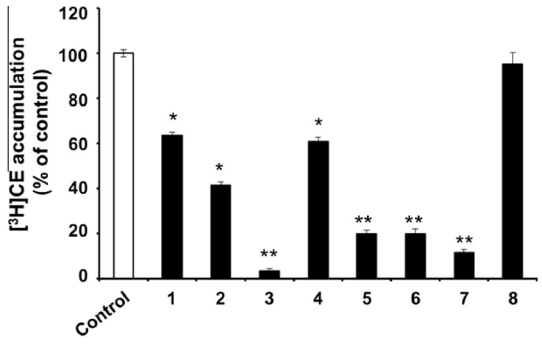

Figure 2. Inhibitory effects of **1**–**8** on CE accumulation in HMDMs induced by acetylated LDL (AcLDL). HMDMs were incubated with 50 μg/mL AcLDL and 0.1 mM [^{3}H]oleate conjugated with BSA in the absence or presence of **1**–**8** (5 μM). After an incubation for 24 h, cholesteryl-[^{3}H]oleate was separated from the cellular lipids of HMDMs by TLC and its radioactivity was measured with a radioscanner. Experiments were carried out in triplicate and the error bars represent the standard deviation. Asterisks show significant differences at $^*P$ <0.01 and $^{**}P$ <0.001.

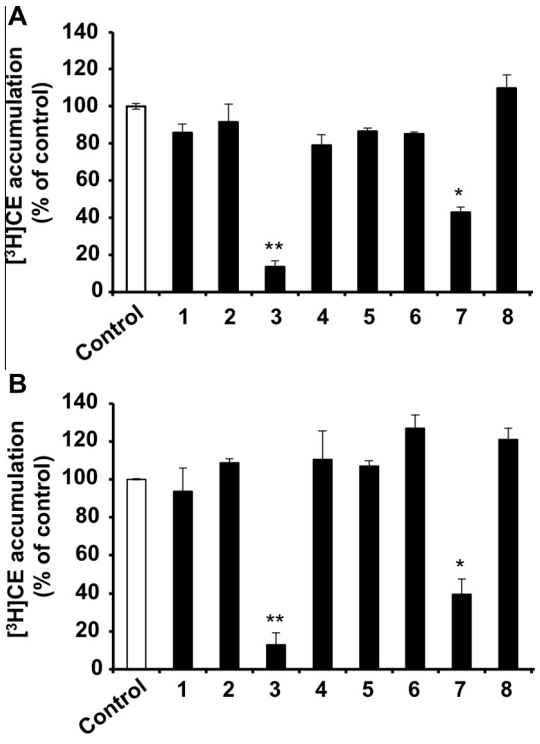

Figure 3. Inhibitory effects of **1**–**8** on CE accumulation in CHO cells overexpressing human ACAT-1 or ACAT-2. hACAT-1 CHO (A) and hACAT-2 CHO (B) cells were incubated with the medium containing 10% fetal calf serum in the presence of 0.1 mM [^{3}H]oleate-conjugated BSA and **1**–**8** (5 μM), and the accumulation of [^{3}H]CE in cells was measured, as described in Figure 2. Experiments were carried out in triplicate and the error bars represent the standard deviation. Asterisks show significant differences at $^*P$ <0.01 and $^{**}P$ <0.001.

(CHO) cells overexpressing human ACAT-1 (hACAT-1 CHO) and human ACAT-2 (hACAT-2 CHO). When the above two CHO cell lines were incubated with [^{3}H]oleate for 24 h, an increase in CE was detected and, among the eight compounds isolated, **3** was identified as the strongest inhibitor of the accumulation of CE in both hACAT-1 (Fig. 3A, 86% inhibition) and hACAT-2 CHO cells (Fig. 3B, 87% inhibition) without cytotoxicity.[17] The second strongest inhibitor was **7** (57% and 61% inhibition for hACAT-1 and hACAT-2 CHO cells, respectively) without cytotoxicity. However, the inhibitory effects of the remaining six compounds were negligible. These results suggested that **3** and **7** both inhibited the esterification of cholesterol by inhibiting the activity and/or expression of ACAT. Therefore, we investigated the effects of **3** on ACAT activity in HMDMs. Microsomes prepared from HMDMs were used as an enzyme preparation and were incubated with 250 μmol/L [^{14}C]oleoyl-CoA for 15 min in the presence (5 μM) or absence of **3**. ACAT activity, which was based on the formation of cholesteryl [^{14}C]oleate, was inhibited by **3** (Fig. 4A).[17] On the other hand, the protein levels of ACAT-1 and ACAT-2 in HMDMs remained unchanged in the presence of **3** (5 μM) for 24 h (Fig. 4B),[17] which indicated that **3** did not affect the expression of ACAT. Furthermore, **3** (5 μM) did not influence the protein levels of three scavenger receptors,[19] that is, the class A scavenger receptor[20] (SR-A), class B scavenger receptor type-I[21] (SR-BI), and class B