Bioorganic & Medicinal Chemistry Letters 23 (2013) 4319–4323

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Effects of the methoxy group in the side chain of debromoaplysiatoxin on its tumor-promoting and anti-proliferative activities

Ryo C. Yanagita [a,b], Hiroaki Kamachi [a], Masayuki Kikumori [a], Harukuni Tokuda [c], Nobutaka Suzuki [c], Kiyotake Suenaga [d], Hiroshi Nagai [e], Kazuhiro Irie [a,*]

[a] Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[b] Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan
[c] Department of Complementary and Alternative Medicine, Clinical R&D, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan
[d] Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan
[e] Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan

ARTICLE INFO

Article history:
Received 1 May 2013
Revised 25 May 2013
Accepted 30 May 2013
Available online 7 June 2013

Keywords:
Aplysiatoxin
Debromoaplysiatoxin
Protein kinase C
Tumor promotion
Cancer
Anti-proliferative activity

ABSTRACT

Debromoaplysiatoxin (DAT) is a tumor promoter isolated from sea hare and exhibits anti-proliferative activity against several cancer cell lines. To clarify key residues that are responsible for its tumor-promoting activity, we focused on the chiral methoxy group in the side chain, whose role had not yet been discussed or examined before. Demethoxy-DAT (**8**) was derived from DAT and we evaluated its tumor-promoting activity, anti-proliferative activity, and ability to bind to protein kinase C (PKC) isozymes. Compound **8** showed somewhat weaker tumor-promoting activity than that of DAT both in vitro and in vivo, but showed higher anti-proliferative activity against several cancer cell lines. Although the affinity to novel PKC isozymes of **8** was comparable to that of DAT, the affinity to conventional PKC isozymes decreased slightly. These results suggest that the methoxy group of DAT is one of the key residues critical for tumor-promoting activity but not for anti-proliferative activity. Since the methoxy group has little influence on the molecular hydrophobicity, this is the first report showing that structural factors other than hydrophobicity in the side chain of DAT affected its biological activities.

Protein kinase C (PKC) is a family of serine/threonine protein kinases consisting of at least 10 isozymes and is involved in many cellular events such as proliferation, differentiation, and apoptosis.[1] The PKC family is subdivided into three groups based on their structures and cofactor requirements: conventional PKC (cPKC: α, βI, βII, and γ), novel PKC (nPKC: δ, ε, η, and θ), and atypical PKC (aPKC: ζ and λ/ι) isozymes.[1] Tumor promoters such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA), ingenol esters, teleocidins, and aplysiatoxins (Fig. 1) bind to the tandem C1 domains (C1A and C1B) in the regulatory domain of cPKCs and nPKCs, and strongly activate them.[1]

Since over-expression and/or down-regulation of cPKCs and nPKCs are observed in many cancers,[2] PKC isozymes are expected to be potential therapeutic targets in cancer.[3] Recent studies also indicated that the modulation of PKC isozymes could be a possible strategy in the treatment of Alzheimer's disease[4] and acquired immunodeficiency syndrome (AIDS).[5] Since PKC modulators could be promising reagents for the treatment of cancer, clinical trials of TPA-type PKC activators for certain types of cancer have been conducted so far,[6,7] for example, phase I trials of TPA for patients with hematological malignancies, but serious adverse events were observed in that case.[7] Recently, ingenol 3-angelate (ingenol mebutate), isolated from *Euphorbia peplus*, was approved by the US Food and Drug Administration (FDA) for the treatment of actinic keratosis, a precancerous skin lesion;[8] however, the acute inflammatory response by the ingenol ester might be a limiting factor in uses other than topical application.

Bryostatin 1 (bryo-1), isolated from bryozoan *Bugula neritina*, is a scarce PKC activator[9] displaying little tumor-promoting activity in vivo[10] and a promising anticancer activity that has led to clinical trials for cancer under the condition of bryo-1 alone or in combination with other chemotherapeutic agents.[10] In spite of its potential as a new medicinal lead, the isolation yield of bryo-1 from natural sources is quite low (0.00014%),[11] and there is no reliable method to obtain bryo-1 in large quantities sufficient for further research and medicinal use. Recently, Wender's and Keck's groups have been developed truncated analogues of bryo-1, which provided the promising ways to address the problem of supply.[12,13]

Aplysiatoxin (ATX) and debromoaplysiatoxin (DAT) are TPA-type tumor promoters[14] isolated from the sea hare *Stylocheilus longicauda*[15] and marine cyanobacteria belonging to genera

* Corresponding author. Tel.: +81 75 753 6281; fax: +81 75 753 6284.
E-mail address: irie@kais.kyoto-u.ac.jp (K. Irie).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.05.096

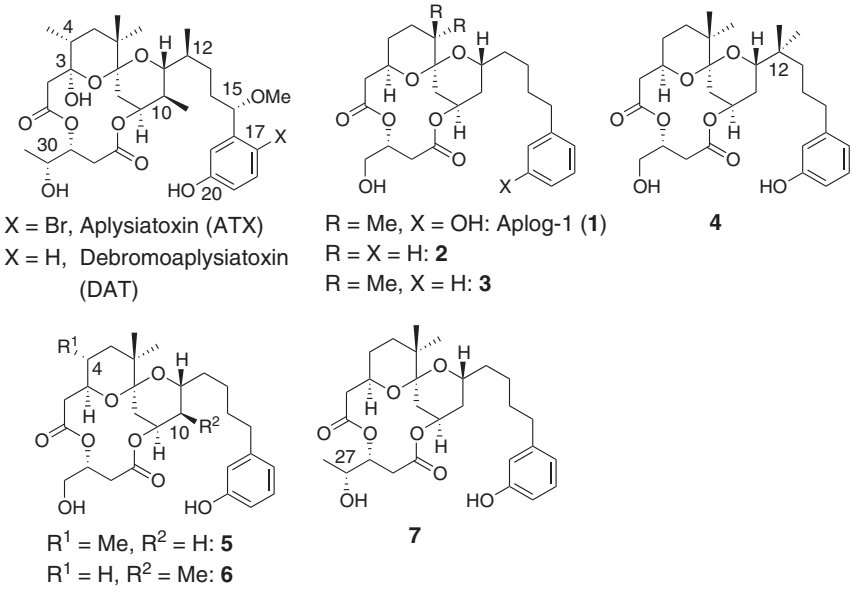


Figure 1. Structure of aplysiatoxin (ATX), debromoaplysiatoxin (DAT), and synthetic analogues of DAT.[17–23]

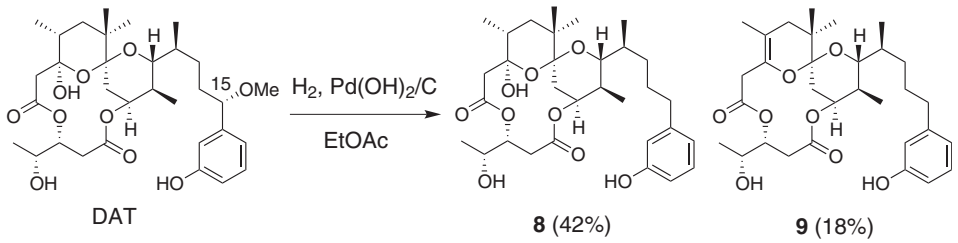
Lyngbya, *Schizothrix*, and *Planktothrix* (*Oscillatoria*).[16] Among naturally-occurring potent tumor promoters, DAT has relatively low lipophilicity and weak tumor-promoting activity. In order to address the supply problem of bryo-1, we have recently developed simplified, synthetically accessible, and less lipophilic analogs of DAT, named 'aplogs' (**1**–**7**, Fig. 1).[17–23] Like bryo-1, aplog-1 (**1**) inhibited the growth of several human cancer cell lines and showed little tumor-promoting activity in vitro and in vivo.[17,21] In particular, the ability of 10-methyl-aplog-1 (**6**) to bind to nPKC isozymes was comparable to that of DAT, whereas **6** did not show any tumor-promoting activity in vivo.[20]

To develop more potent and safer aplogs, we need to know the key structural components indispensable for the tumor-promoting activity of ATX and DAT. In aplog-1 (**1**), a hydroxyl group at position 3, methyl groups at positions 4, 10, 12, and 30, a methoxy group at position 15, and a bromine atom at position 17 were removed from ATX. Kishi and colleagues reported that 3-deoxy-DAT and DAT are equipotent as PKC activators,[24] and Shimomura and colleagues reported that removal of the bromine atom diminished the tumor-promoting activity.[12,25] Our previous studies have shown that re-installation of each methyl group at positions 4,[20] 10,[20] 12,[19] or 27[23] did not restore the tumor-promoting activity; however, the role of the chiral methoxy group of aplysiatoxins remains unclear. In this Letter, we report the preparation and biological activities of demethoxy-DAT (**8**) along with its dehydration product (**9**).

Compound **8** was prepared from DAT, which was isolated from the extracts of cyanobacteria collected in Ishigaki Island (Okinawa) and a red alga *Gracilaria coronopifolia*, which was parasitized by cyanobacteria, collected in Hawaii[26] by a sequence of column chromatography. The spectroscopic data (^{1}H and ^{13}C 1D NMR spectra, and optical rotation in Supplementary data) were in good agreement with those reported in the literature.[26,27] Catalytic hydrogenation of DAT afforded **8** (42%) along with the dehydrated derivative **9** (18%) in a single step (Scheme 1). A hemiacetal group at position 3 of ATX is easily dehydrated under acidic conditions,[28] indicating that **9** would be generated from **8** during biological evaluations. Thus, the biological activities of **9** as well as **8** were also evaluated.

We first assessed the in vitro tumor-promoting activity of **8** and **9** by the Epstein–Barr virus early antigen (EBV-EA) induction test using Raji cells (EBV genome-carrying lymphoblastoid cells) as described previously.[29] Tumor promoters such as TPA activate the latent EBV genome and induce EA. Figure 2 shows the EBV-EA inducing ability of DAT, **8**, and **9**. DAT strongly induced EA at 10^{-8}, 10^{-7}, and 10^{-6} M (21.1%, 22.9%, and 24.4%, respectively), while the EA-inducing ability of **8** (14.3%, 17.9%, and 18.6%, respectively) was significantly lower than that of DAT. EA-inducing ability of **9** was slightly but significantly lower than that of **8**.

Evans et al. reported that, in the EBV-EA induction test, non-tumor-promoting diterpene esters such as sapintoxin A were virtually equipotent to tumor-promoting compounds;[30] therefore, the tumor-promoting activity of **8** in vivo was further evaluated by the two-stage carcinogenesis test on mouse skin (Fig. 3). The skin on the back of male ICR mice was treated with a single dose of 390 nmol of 7,12-dimethylbenz[*a*]anthracene (DMBA) and from 1 week later, with 1.7 or 3.4 nmol of DAT or **8** twice a week. In a control experiment using TPA (1.7 nmol twice a week), the first tumor appeared in week 6 and the proportion of tumor-bearing mice reached 100% at week 11 (Fig. 3A). The number of papillomas per



Scheme 1. Synthesis of **8** and **9**.