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Synthesis and Biological Activities of the Simplified Analogs of Natural PKC Ligands, Bryostatin-1 and Aplysiatoxin

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ABSTRACT: Protein kinase C (PKC) isozymes play central roles in the signal transduction on the cell surface and could serve as promising therapeutic targets of intractable diseases like cancer, Alzheimer’s disease, and acquired immune deficiency syndrome (AIDS). Although natural PKC ligands like phorbol esters, ingenol esters, and teleocidins have the potential to become therapeutic leads, most of them are potent tumor promoters in mouse skin. By contrast, bryostatin-1 (bryo-1) isolated from marine bryozoan is a potent PKC activator with little tumor-promoting activity. Numerous investigations suggested bryo-1 to be a promising therapeutic candidate for the above intractable diseases. However, there is a supply problem of bryo-1 both from natural sources and by organic synthesis. Recent approaches on the synthesis of bryo-1 have focused on its simplification, without decreasing the ability to activate PKC isozymes, to develop new medicinal leads. Another approach is to use the skeleton of natural PKC ligands to develop bryo-1 surrogates. We have recently identified 10-methyl-aplog-1 (26), a simplified analog of tumor-promoting aplysiatoxin (ATX), as a possible therapeutic lead for cancer. This review summarizes recent investigations on the simplification of natural PKC ligands, bryo-1 and ATX, to develop potential medicinal leads.

Keywords: anti-proliferative, aplysiatoxin, bryostatin-1, debromoaplysiatoxin, phorbol ester, protein kinase C, tumor promoter
Introduction

Natural products as secondary metabolites have been considered to be possible lead compounds for treatment of human diseases. However, natural products themselves cannot be used as drugs in general because they have adverse effects in addition to the desirable effects; furthermore, they cannot be obtained in sufficient amounts from natural sources and their total synthesis normally requires multiple steps. It is reported that over 50% of the approved drugs from 1981 to 2010 were synthetic small compounds and that “natural product medicines” such as daptomycin, artemisinin, trabectedin, and paclitaxel (taxol) were rare cases (ca. 6%).[1] It is obvious that nature does not make compounds for human health care. Moreover, recent investigations have revealed the multiligandable nature of natural products.[1b] Many natural products should be regarded as “a bunch of keys” that have many cellular targets. To develop medicinal leads from natural products, it is indispensable to identify the structural motif (a special key) that is responsible for desirable effects. Function-oriented synthesis of natural products or the synthesis of suitably truncated ligands is one of the promising approaches to obtain new medicinal leads.[2]

The development of eribulin mesylate (Halaven)[3] is one of the successful examples. Halicondrin B[4] is a marine natural product with potent anticancer activities isolated from the marine sponge Halichondria okadai. After synthesizing over 200 derivatives of halicondrin B (conventional structure–activity studies), eribulin was found to be effective against breast cancer. This was an extremely hard work, and thus more rational approaches are necessary to uncover the essential structure of natural products that possesses the same desirable effects. If their main targets were identified, structural analysis of a ligand–target complex based on X-ray crystallography and/or NMR studies would shorten the procedure.

This review focuses on the development of simplified analogs of two natural products, bryostatin-1 (bryo-1)[5] and aplysia toxin (ATX)[6], as potential medicinal leads for intractable diseases. The main targets of these compounds are identified as protein kinase C (PKC) isozymes,[7] a key enzyme family involved in cell surface signal transduction. PKC isozymes are attractive targets in the treatment of cancer,[8] Alzheimer’s disease (AD),[9]
acquired immune deficiency syndrome (AIDS),\textsuperscript{[10]} vascular complications of diabetes,\textsuperscript{[11]} and neuropathic pain.\textsuperscript{[12]}

**Protein kinase C and C1 domain receptors**

Protein kinase C (PKC) is a family of serine/threonine kinases involved in cellular signal transduction via a second messenger, 1,2-diacyl-sn-glycerol (DG).\textsuperscript{[7]} Tumor-promoting phorbol esters, teleocidins, and alysiatoxins activate PKC isozymes by binding to the C1 domains in the regulatory region.\textsuperscript{[7a,13]} PKC isozymes activated by tumor promoters and DG are conventional PKCs (α, βI, βII, and γ) and novel PKCs (δ, ε, η, and θ) (Fig. 1a).\textsuperscript{[7b]} The former are also regulated by calcium ions, but the latter are insensitive to calcium despite the fact that they have a C2-like domain at the N-terminus. All of these PKC isozymes have two C1 domains (C1A, C1B),\textsuperscript{[14]} to which natural PKC ligands and DG bind in the presence of phosphatidylserine. They are inactive under physiological conditions because the C-terminal kinase domain is occupied by a pseudosubstrate sequence of the N-terminus. However, the binding of tumor promoters or DG to the C1 domains of the PKC isozymes induces their translocation from the cytoplasm to the plasma membrane, where they become activated (Fig. 1b). Nuclear translocation also occurs when the ligands are hydrophilic, as exemplified in phorbol esters with short acyl chains and bryo-1.\textsuperscript{[15]}

Since each C1 domain consists of a cysteine-rich sequence of 50 amino acid residues,\textsuperscript{[14]} we synthesized all C1 peptides by solid-phase peptide synthesis to determine the \( K_d \) values for \[^{3}H\] phorbol 12,13-dibutyrate (PDBu).\textsuperscript{[16]} Both C1 peptides of conventional PKCs, except for PKCα-C1B, showed potent binding to PDBu comparable to that for the whole PKCs. On the other hand, only the C1B peptides of novel PKCs bound strongly to PDBu. It is noteworthy that PDBu bound to at least one of the C1 domains of all conventional and novel PKCs with \( K_d \) values of 0.5-1.5 nM (Table 1).\textsuperscript{[16d]} The \( K_i \) values for the C1 peptides of indolactam-V,\textsuperscript{[17]} a biosynthetic precursor of tumor-promoting teleocidins,\textsuperscript{[17d,e]} obtained by the inhibition of \[^{3}H\]PDBu binding were almost equal to those of the corresponding whole PKC isozymes obtained by the well-established procedure of Blumberg’s group\textsuperscript{[18]} (Table
Thus, the C1 peptide library gives a unique opportunity for finding compounds with PKC isozyme and/or C1-domain selectivity.

However, PKC isozymes are not sole receptors for tumor-promoting phorbol esters and DG (Fig. 2). Recent investigations revealed that protein kinase D (PKD), diacylglycerol kinase (DGK) β and γ, chimaerins (α1, α2, β1, and β2), Ras guanyl nucleotide-releasing protein (RasGRP) 1, 3, and 4, and Unc13s (Munc-13-1, -2, and -3), bound potently to PDBu and DG. These proteins have at least one C1 homology domain present in PKC isozymes. By contrast, there are several proteins with C1 homology domains that do not bind to PDBu and DG. They include atypical PKCs (ζ and ι), DGKs other than DGKβ and γ, RasGRP2, c-Raf (a kinase suppressor of Ras), and Vav1 (a guanine nucleotide exchange factor).

Since PKCs and some of the other C1-domain-containing proteins are involved in diverse biological events like proliferation, differentiation, apoptosis, angiogenesis, and so on, they are attractive targets for cancer and other diseases such as AD and AIDS. Natural PKC ligands like phorbol esters might serve as their therapeutic leads. In fact, 12-O-tetradecanoylphorbol 13-acetate (TPA) and ingenol 3-angelate were subjected to clinical trials for some cancers. However, they might elicit tumor-promoting activity and severe inflammation as off-target activities. Since these compounds exhibit pleiotropic effects like cell differentiation, cell adhesion, and virus induction other than tumor-promoting and inflammatory activities, they should be regarded as “a bunch of keys” or “a master key”. Suitable derivatization of phorbol esters, indolactam compounds, or DG might separate desirable activities from off-target activities. Wender’s highly potent prostratin analogs, Kozikowski’s benzolactam compounds, and Marquez’s DAG-lactones could be successful examples (Fig. 3). Indolactam-V, the basic ring-structure of teleocidins, is a weak tumor promoter, but showed anti-proliferative effects against several cancer cell lines (unpublished results). However, such attempts to separate desirable activity from off-target activity like tumor-promoting activity seems to be quite difficult with phorbol esters and teleocidins since there is a good correlation between their tumor-promoting activity and ability to activate PKC isozymes.
Bryostatin-1, a unique PKC activator with anti-proliferative activity

Bryostatin-1 (bryo-1) is a mysterious PKC activator with little tumor-promoting activity and antagonizes the effects of TPA in several cell lines.\cite{28} Major differences between bryo-1 and tumor promoters like TPA and teleocidin B-4 are the hydrophobicity/hydrophilicity of the molecules. TPA and teleocidin B-4 are highly hydrophobic molecules (calculated \( \log P > 6.0 \)) while bryo-1 is hydrophilic (\( \log P = 2.9 \)).\cite{29} The hydrophobicity of phorbol or teleocidin derivatives correlated well with their binding potency to PKC isozymes as well as their tumor-promoting activity.\cite{15a,27} However, the hydrophilic compound bryo-1 binds strongly to PKC isozymes with a potency similar to phorbol and ingenol esters.\cite{28b,30} Although the reason why bryo-1 behaves like this remains unknown, its larger molecular size might be related to this behavior.

Bryo-1 has attracted much attention as a therapeutic agent for cancer treatment due to its unique character as a potent PKC activator without tumor-promoting activity and its abilities to induce apoptosis, to reverse multidrug resistance, and to modulate the immune system. Hitherto, the anticancer activity of bryo-1 for solid or blood cancers including epithelial ovarian cancer, advanced pancreatic carcinoma, myeloid, advanced esophageal cancer, and so on, has been investigated in at least 43 phase I and II clinical trials.\cite{8,31} Since most of these trials with the independent application of bryo-1 were disappointing,\cite{31} its combination with some anticancer drugs like paclitaxel,\cite{32} cisplatin,\cite{33} or vincristine\cite{34} has also been carried out. Among them, bryo-1 in combination with vincristine was found to be effective in selected patients with aggressive non-Hodgkin’s lymphoma (NHL).\cite{34} Moreover, the results of bryo-1 and fludarabine in the treatment of progressive chronic lymphocytic leukemia (CLL) or refractory indolent NHL were encouraging.\cite{35} A major adverse effect of bryo-1 is myalgia, the mechanism of which is unclear.\cite{36} While bryo-1 had promising effects for the treatment strategy against AD and HIV infection, clinical trials for these diseases have not yet been conducted.

Overall, bryo-1 has a broad therapeutic potential. However, it is a very rare natural
product. Only 18 g of bryo-1 was obtained from 14 tons of marine bryozoan.\textsuperscript{37} Since it has been used in numerous clinical trials, it is almost out of stock. It is quite difficult to purchase bryo-1 even for mechanistic studies as 10 µg cost $250 (Sigma, USA). Bryo-1 congeners (e.g., bryo-7 and bryo-10)\textsuperscript{38} could be used instead of bryo-1 (Fig. 4). In fact, a recent investigation on bryo-7 revealed that it could become a surrogate of bryo-1.\textsuperscript{39} Although over 20 congeners of bryo-1 have been isolated, their abundance is quite low.\textsuperscript{38} In our recent attempt to isolate bryostatin congeners, we only obtained 3 mg of bryo-10 from 6 kg of Japanese bryozoan as a major product.\textsuperscript{40}

Synthetic studies on bryo-1 and its natural congeners have been extensively carried out. Initial studies on the total synthesis of bryo-7, bryo-2, and bryo-3 required about 75-90 steps in total;\textsuperscript{41} however, in recent studies on bryo-7, bryo-16, bryo-1, and bryo-9, the number of steps required was shortened to about 35-70.\textsuperscript{42} However, amounts sufficient for clinical studies cannot be supplied at present. Genetic engineering is not practical since bryo-1 is a molecule too large to be synthesized using \textit{E. coli}.\textsuperscript{43}

**Development of simplified analogs of bryostatin-1 with potent anti-proliferative activity against several cancer cell lines**

To overcome the supply problem, the development of simplified analogs of bryostatin-1 (bryo-1) with equally potent anti-proliferative activity could be an effective strategy. Wender noticed this in the early 1980’s and designed a simplified PKC analog based on the pharmacophoric model of phorbol esters.\textsuperscript{44} In 1998, the first simplified analog of bryo-1 (1, bryolog) was synthesized by Wender and co-workers.\textsuperscript{45} They thought that the bottom half of bryo-1 containing the C-ring is strictly recognized by PKC isozymes,\textsuperscript{44b} but that its A- and B-rings play only a role to fix the molecule in a suitable conformation. The B-ring was thus replaced with a dioxane for acetal-driven macrocyclization. The designed analog 1 showed potent binding to a PKC isozyme mixture with a $K_i$ value of 3.4 nM, which is comparable to that of bryo-1 (1.35 nM).

Since then, Wender’s group has synthesized over 100 analogs of bryo-1 to
investigate the structure–activity relationship in PKC binding. Deletion of the asymmetric methyl group at position 26 significantly enhanced the PKC binding with a $K_i$ value of 0.25 nM (2, picolog).

More importantly, such simplification enhanced the binding selectivity to novel PKC isozymes. Especially, the C7-OH and C8 dimethyl analog (3) showed PKCδ-selective membrane translocation in CHO-K1 cells over PKCβI, whereas bryo-1 induced the membrane translocation of both isozymes. Although the A-ring itself proved to be unnecessary for PKC binding, substitution of the six-membered B-ring with a five-membered ring changed the compound into one selective for novel PKC isozymes similar as observed for 3. Modification of the B-ring has been accomplished through a Prins-driven macrocyclization strategy to yield 4. The molecular basis for the change of the PKC isozyme selectivity remains elusive.

The anti-proliferative activities of these analogs against several cancer cell lines were examined at the National Cancer Institute. The corresponding data for 1 and 2 are summarized in Table 2 along with those of bryo-1 as a control. The data clearly suggest that these analogs are superior to bryo-1 in the anti-proliferative activities against several cancer cell lines. Accordingly, they might become bryo-1 surrogates in future clinical trials. However, their off-target activity such as tumor-promoting activity and inflammation has not yet been reported.

Recently, Keck and colleagues have synthesized several analogs of bryo-1 simplified at the A- and B-rings (5 and 6). They examined the effects of A- and B-ring modifications on the proliferation and attachment of U-937 human leukemia cells. Unexpectedly, the behavior of 5 in U-937 cells was similar to that of TPA. By contrast, 6 which lacks only the carbomethoxy group at the B-ring of bryo-1 displayed the same properties as bryo-1 in the assay using U-937 cells; unlike TPA, both 6 and bryo-1 did not inhibit proliferation of U-937 cells. These results indicate that the A-ring of bryo-1 plays a significant role in its unique biological activities. So far, however, the in vivo tumor-promoting activity of bryo-1 has only been reported in one study. In the reported work, in which SENCAR mice were used, the amount of bryo-1 for topical application was
equal to that of TPA (1.7 nmol/mouse). Further experiments with a high dose of bryo-1 and its analogs (at least 5-fold excess of TPA) seem to be necessary.

Although both TPA and bryo-1 are potent PKC activators, the former is a potent tumor promoter while the latter is not.[28a] Moreover, bryo-1 antagonizes many cellular responses induced by TPA.[28c] Bryo-1 blocked the TPA-induced differentiation of a human colon cancer cell line and the human promyelocytic leukemia HL-60 cell line.[50] The mechanistic difference between TPA and bryo-1 in U-937 human myeloid leukemia cells has recently been investigated.[51] The enhanced m-RNAs (top 20) were almost similar to each other. However, the response to bryo-1 was transient while that to TPA was continuous. Another difference is that TPA, but not bryo-1, induces a down-regulation of PKCδ,[52] which plays a tumor suppressor role. In primary mouse keratinocytes, bryo-1 at low concentrations (<1 nM) down-regulated PKCδ to a similar extent as TPA; however, at high concentrations (>100 nM) bryo-1 did not down-regulate PKCδ but protected it from TPA.[52a] This might be ascribable to the cellular membrane translocation of PKCδ by TPA and to the nuclear membrane translocation by bryo-1.[15] However, TPA as well as bryo-1 did not down-regulate PKCδ in K562 cells (a human immortalised myelogenous leukemia line), whereas bryo-1, but not TPA, down-regulated PKCe and RasGRP3.[49d] The pattern of cellular response to TPA and bryo-1 might therefore vary depending on the conditions and/or cell lines. Since bryo-1 is regarded as a bunch of keys, its simplified analogs would be powerful tools to reveal some stages of the mechanism of bryo-1. For example, Wender’s analogs[48] with selectivity against novel PKC isoforms could be useful.

**Development and biological activities of the simplified analogs of debromoaplysiatoxin**

**Analog design:** Even the synthesis of Wender’s simplified analogs of bryo-1 requires at least about 30 steps, of which some are technically difficult.[46,48] The development of synthetically more accessible compounds with bryo-1 like activities is thus necessary. After scrutiny of natural PKC ligands, we decided to use the skeleton of aplysiatoxin (ATX),[6] which is
isolated from the digestive gland of the sea hare *Stylocheilus longicauda*, to develop a bryo-1 surrogate. The main reason to adopt the ATX structure is that it is regarded as a conformationally fixed analog of endogenous DG, though the atom sequence of one ester carbonyl moiety is reversed (Fig. 5). In addition, ATX and bryo-1 share the common structural feature of a macrolactone. The most interesting structural character of ATX in its biological activities is the role of the bromine atom in the side chain. The tumor-promoting activity of debromoaplysia toxin (DAT) is significantly weaker than that of ATX, while the binding potency to PKC isozymes is quite similar. This characteristic was not observed in phorbol esters and teleocidins, where hydrophobicity plays a critical role in their tumor-promoting activity and binding potency to PKC isozymes.

Unlike bryo-1, ATX is a potent tumor promoter. The simplification should thus be done so as to reduce the tumor-promoting activity without decreasing the binding potency to PKC isozymes, if possible, to PKCδ, which plays a tumor suppressor role, because the activation levels of PKCα, β, and ε are elevated in tumor tissues. First, we designed three simplified analogs (7-9). Four chiral methyl groups and a methoxy group as well as a bromine atom were removed to decrease the hydrophobicity and synthetic difficulty. Moreover, the labile hydroxyl group at position 3 was replaced with a hydrogen atom to increase its stability. Compound 7, named aplog-1, has geminal methyl groups at the spiroketal moiety and a phenolic hydroxyl group at the benzene ring. Compound 8 lacks the phenolic hydroxyl group, and 9 lacks both. The calculated log P values (partition coefficients between n-octanol and water) of TPA, teleocidin B-4, and ATX exceed 5.0, while those of these analogs are less than 3.0, which are values comparable to the measured log P value of bryo-1 (2.9).

We thought that the dimethyl groups of the spiroketal at position 6 of ATX could be more important compared to other methyl groups at positions 4, 10, 12, or 30 to retain the binding ability to PKC isozymes based on the pharmacophoric model of PKC activators. Polar groups like oxygen functionality at positions 12 and 13 of phorbol esters remarkably reduced the PKC binding potency; phorbol or phorbol 13-acetate could only weakly bind to
PKC isozymes. The positions 12 and 13 of phorbol esters corresponds roughly to the positions 6 and 11 of ATX on the pharmacophore model.\[44,56\] Since one of the oxygen atom at the spiroketal moiety directs outer side of the macrolactone ring, it would be indispensable to protect the oxygen atom by the hydrophobic dimethyl groups at position 6 to form the complex with each PKC isozyme and phosphatidylyserine. On the basis of this consideration, 7 was designed as a first simplified analog of ATX with substantial affinity to PKC isozymes without tumor-promoting activity.

**Synthesis of aplog-1 and its congeners:** The total synthesis of ATX first reported by Kishi and co-workers comprised over 53 steps.\[57\] Subsequently, the synthesis of its related compounds was carried out by the groups of Katsuki\[58\] and Yamamura.\[59\] The synthetic difficulty is the instability of the hydroxyl group at position 3 and the hydrogenolysis-sensitive methoxy group at the side chain. Because of this difficulty, the synthesis by Ireland et al. did not accomplish the total synthesis of ATX.\[60\] Since aplog-1 (7) is a highly simplified analog of ATX, the synthetic route was quite different from those of the four groups mentioned above.

As shown in Fig. 6, \textit{m}-hydroxycinnamic acid was converted into the aldehyde 11 in 8 steps. Asymmetric Keck’s allylation\[61\] of 11 provided 12, which was followed by Smith’s iodocarbonate cyclization reaction,\[62\] methanolysis, and protection, to yield the epoxide 13. Coupling of the epoxide moiety in 13 with the dithiane unit 14 following the protocol of Ide and Nakata,\[63\] and subsequent conversions provided the aldehyde 15. Keck’s allylation of 15 produced then the homoallylic alcohol 16, which was hydrolyzed to form the desired spiroketal 17 in 49\% yield. The undesired spiroketal 18 was partially converted into 17 by treatment with pyridinium \textit{p}-toluenesulfonate (PPTS). Although 17 seems to be more stable than 18 due to intramolecular hydrogen bonding, 18 might be stabilized by a double anomer effect. Condensation of the spiroketal 17 with the carboxylic acid 19 using Yamaguchi’s method\[64\] yielded 20, the \textit{p}-methoxybenzyl (MPM) group of which was substituted with the triethylsilyl (TES) group. Oxidative cleavage of the double bond, followed by Yamaguchi’s
lactonization and deprotection, gave Aplog-1 (7). The total yield of 7 over 27 steps was 1.5%. Aplog-2 (9), which does not contain the dimethyl group at the spiroketal moiety and the hydroxyl group in the benzene ring, was similarly synthesized. DM-aplog-2 (8) was obtained from 7 by catalytic hydrogenation.

The log P value of 7 was estimated experimentally by high-performance liquid chromatography (HPLC), as recommended by OECD. The retention time of 7 on a reverse-phase column could be correlated to the log P values using appropriate reference compounds with known log P values. The log P value of 7 (3.3) was almost equal to that of bryo-1 (2.9) reported by Bignami et al.

**Biological activities of aplog-1 (7) and its congeners:** The binding of aplog-1 (7) and its derivatives (8 and 9) to the C1B domain of PKCδ, whose activation might be related to the unique biological activities of bryo-1, are shown in Table 3. Compound 7 showed potent binding to the C1B domain with a $K_i$ value of 7.4 nM. The binding affinity of aplog-2 (9) without the dimethyl and phenolic hydroxyl groups was 20 times weaker than that of 7. By contrast, DM-aplog-2 (8) showed a binding affinity similar to that of 7, thus indicating that the dimethyl group at the spiroketal moiety plays a significant role in the PKC binding.

The most critical point in developing a bryo-1 surrogate from ATX is to make structural modifications that result in a decrease in the tumor-promoting activity. The skin of the back of ICR mice was first treated with a single dose of 100 µg of 7,12-dimethylbenz[a]anthracene (DMBA), a strong carcinogen, and one week later with 8.5 nmol of 7. Whereas an amount as low as 1.7 nmol (one fifth) of TPA or DAT resulted in significant tumor-promoting effects as reported previously, 7 did not show any tumor-promoting effects and inflammation at this higher dosage (unpublished results).

To evaluate the anti-proliferative activities of 7-9, a panel of 39 human cancer cell lines established by Yamori and co-workers was employed. The average of the log GI$_{50}$ values (where GI$_{50}$ denotes the concentration required for inhibiting cell growth by 50% compared to an untreated control) for all 39 human cancer cell lines were expressed as a
MG-MID (mean-graph midpoint). The cell lines with log GI\textsubscript{50} values less than −5.00 are listed in Table 4. To our delight, 7 exhibited a significant activity comparable to that of bryo-1 against several cancer cell lines. Compound 9 without the dimethyl and hydroxyl groups showed an activity that was one order of magnitude lower. The activities of 7 and 8 were similar. The affinity for PKCδ and anti-proliferative activity correlated well. After publication of these data, 7 was featured in “this week in therapeutics” of Science–Business eXchange.\textsuperscript{[68]}

As mentioned above, PKCδ is considered to be one of the major targets of bryo-1.\textsuperscript{[15b]} We found that 7 exhibited an activation profile for PKCδ similar to that of bryo-1.\textsuperscript{[55a]} Upon the treatment of PKCδ with 7 or bryo-1, PKCδ translocated from the cytosol to the nuclear membrane and perinuclear region in CHO-K1 (Chinese hamster ovary) cells, while TPA induced mainly translocation to the plasma membrane. To examine the correlation between PKCδ binding and anti-proliferative activity, we prepared three derivatives of aplog-1 (21-23) that differ in the ability to bind PKCδ.\textsuperscript{[69]} These are 27-(R)-methyl-aplog-1 (21), which has an absolute configuration at position 27 that is the same as that of DAT at position 30, the 27-epimer of 21 (22), and 27-O-methyl-aplog-1 (23) (Fig. 5). As expected from previous structure-activity studies on DAT,\textsuperscript{[56b]} 21 bound to and activated PKCδ with a potency similar to 7, while 22 and 23 were completely inactive.

The anti-proliferative activities of 21-23 were evaluated using the aforementioned panel of 39 human cancer cell lines. The cell lines with log GI\textsubscript{50} values of aplog-1 (7) less than −5.50 are listed in the upper half of Table 5.\textsuperscript{[69]} Compound 21 as well as 7 showed significant anti-proliferative activities. By contrast, 22 and 23 exhibited weak activities in these cell lines. These data suggest that aplog-1 and its derivatives (8, 9, and 21) might inhibit the growth of cells of these cancer cell lines through a PKCδ-dependent mechanism. As a reference, several aplog-insensitive cell lines (log GI\textsubscript{50} < −5.00) are also listed in the lower half of Table 5. They might constitute cell lines with a lower degree of PKCδ expression or a higher degree of expression of other PKC isozymes. The involvement of PKCδ in the anti-proliferative activities of the aplog-sensitive cancer cell lines might be demonstrated by knockout of each PKC isozyme using siRNA. However, targets other than PKC isozymes
might also exist that are responsible for the activities of aplogs, as 22 and 23 had a weak bjt substantial anti-proliferative activity.

**Structure-activity studies on the phenol side chain of aplog-1 (7):** The next step is to optimize the anti-proliferative activity of 7 without increasing off-target activities such as tumor-promoting activity and inflammation. The simplest way for this purpose is to modify the phenolic side chain of 7 that is deduced to be involved in non-specific hydrophobic interactions with phospholipids when bound to PKC isozymes.\[^{56}\] As shown in Fig. 7,\[^{66}\] the change in the anti-proliferative activity (–MG-MID) was dominated by the change in the log $P$ values of the aplog-1 derivatives, which were determined by the HPLC as mentioned above. The optimal range of log $P$ proved to be 4.0-4.5, which corresponds to that of the mono-halogenated derivative of 7. However, the increase in MG-MID of 21-bromo-aplog-1 (−5.20) and 21-iodo-aplog-1 (−5.19) in comparison with 7 (−4.98) was only about 0.2. This indicates that a suitable modification at the macrolactone ring of 7 is indispensable for developing highly potent analogs. It is also noteworthy that 21-bromo-aplog-1 did not show any tumor-promoting activity in a two-stage carcinogenesis experiment using ICR mice even when applied at a five-fold excess compared with DAT and TPA.\[^{66}\]

DAT has an asymmetric methoxy group at the side chain. To investigate the role of this functional group in the biological activities, demethoxy-DAT (24) was derived from DAT by catalytic hydrogenation.\[^{70}\] The two-stage carcinogenesis test in mouse skin suggested that 24 is a significantly weaker tumor promoter than DAT. By contrast, 24 showed a stronger anti-proliferative activity compared with DAT against several aplog-sensitive cancer cell lines, as shown in Tables 4 and 6. These data suggest that the methoxy group at the side chain could increase the tumor-promoting activity.

**Structure-activity studies on the spiroketal moiety of aplog-1 (7):** Since the hydrophobicity around the spiroketal moiety of 7 plays a critical role in PKCδ binding and anti-proliferative activities as exemplified in aplog-2 (9), methyl groups were installed
systematically into position 4, 10, or 12 of 7. These derivatives were synthesized by the method employed in the synthesis of 7 with suitable modifications.\[71\] Interestingly, 10-methyl-aplog-1 (26) bound to the C1B domain of PKCδ with a $K_i$ value of 0.46 nM, 7-20 times stronger than 4-methyl-aplog-1 (25, 3.3 nM), 12,12-dimethyl-aplog-1 (27, 9.1 nM), and aplog-1 (7, 7.1 nM). The anti-proliferative activities of these analogs against “aplog-sensitive” cell lines are shown in Table 6. Compound 26 exhibited 5-20 times stronger inhibitory effects on the growth of aplog-sensitive cell lines than 7, 25, and 27. The MG-MID of 26 (–5.24) was nearly equal to that of the tumor-promoting DAT (–5.22).

Since the hydrophobicity is a critical factor for increasing the tumor-promoting activity, the tumor-promoting activity of 26 and 27 was examined in vivo by the method mentioned above. The skin of the back of ICR mice was treated with a single dose of 100 µg of DMBA and, one week later, with 8.5 nmol (5-fold excess of TPA and DAT) of 26 or 27. These aplogs did not show any tumor-promoting effects under these conditions.\[71b,72\] In a control experiment, 1.7 nmol of TPA led to a significant tumor-promoting effect. Moreover, 27 was shown to be a suppressor of TPA as reported for bryo-1 (Fig. 8).\[72\]

The anti-proliferative activity of 26 against several aplog-sensitive cell lines was even higher than that of DAT, as shown in Table 4, whereas 26 showed little tumor-promoting activity unlike DAT. These results suggest that DAT should be regarded as “a master key” for pleiotropic effects like tumor promotion in addition to anti-proliferative activity, while 26 should be regarded as “a special key” for anti-proliferative activity. Compound 26 could therefore become a possible medicinal lead for cancer treatment.

The mechanism underlying the unique biological activities of aplogs: The activation of PKC isozymes could be related to the anti-proliferative and tumor-promoting activity of aplogs. Recent investigations revealed that each PKC isozyme mediates unique cellular functions and phosphorylates unique protein substrates.\[7\] However, their roles in tumor promotion, inflammation, or cell proliferation are complicated and controversial. For example, PKCα is found to be important for inflammation and proliferation, but appears to
suppress tumor promotion, and the DMBA/TPA protocol did not induce squamous cell carcinoma in PKCα knockout mice. Another study contradictorily reported that a low dose of TPA promoted the papilloma in PKCα-overexpressing mice but not in wild-type mice. PKCε transgenic mice exhibited enhanced carcinoma formation but reduced tumor promotion. By contrast, PKCδ transgenic mice were resistant to skin tumor promotion, and PKCη knockout mice were more susceptible to tumor promotion. Taken together, although PKC isozymes (α, δ, ε, and η) might play a tumor suppressor role, PKCα and ε appear to enhance tumor progression, and in some cases PKCα could enhance tumor promotion.

Using our synthetic PKC C1 peptide receptors, we found that aplog-1 (7) and 10-Me-aplog-1 (26) differed from the tumor promoters DAT, indolactam-V, and PDBu in their affinity for PKC isozymes (Fig. 9). The C1A peptides were used as conventional PKC surrogates, whereas the C1B peptides were used as novel PKC surrogates because these peptides are main binding sites of PDBu. Tumor promoters like DAT, indolactam-V, and PDBu bound significantly to both conventional and novel PKC isozymes. By contrast, the anti-proliferative aplogs bound selectively to novel PKC isozymes, that is, PKCδ, η, and θ. It is interesting that Wender’s simplified analogs of bryo-1 also showed novel PKC isozyme selectivity. Bryo-1 itself was selective for novel PKCs in our assay system using PKC C1 peptides, whereas the data of Kazanietz et al. using whole PKC isozymes are slightly different from our results. However, the relative binding affinity against conventional PKCα and β was lower for bryo-1 compared with PDBu. These results suggest that the unique biological activities of aplogs might be ascribable in part to the ability to bind PKCδ and η, although weak binding to conventional PKC isozymes might also be important.

A number of studies revealed the involvement of PKCδ in apoptosis signaling in a variety of cell types. PKCδ plays important roles in both receptor-mediated and DNA-damage-induced cell death. For example, in U-937 (a human leukemia cell) and MCF-7 (a breast cancer cell), TPA induced translocation of PKCδ to mitochondria and subsequent release of cytochrome c. Several lines of evidence support the notion that the
cleavage of PKCδ by caspase and nuclear translocation of the fragment is required for the apoptosis event. In LNCaP (an androgen-sensitive human prostate adenocarcinoma cell), PKCδ mediated TPA-induced apoptosis without its cleavage by caspase-3. PKCδ is also involved in cell cycle arrest in G1/S and G2/M phases. Therefore, activation of PKCδ by aplogs might contribute to its anti-proliferative effect in certain cell types (Fig. 10). However, PKCδ provides survival signaling in some cases. Further investigations on the regulation mechanism of PKCδ and other isozymes are required for the development of safer PKC ligands for anti-cancer therapy.

**Summary and Outlook**

Natural PKC ligands like phorbol esters have the potential to become therapeutic leads for intractable diseases, such as cancer, Alzheimer’s disease, and AIDS, but most of them are potent tumor promoters. Bryostatin-1 (bryo-1) isolated from marine bryozoan is a fascinating PKC activator without tumor-promoting activity. Although bryo-1 is a promising therapeutic candidate for these intractable diseases, amounts sufficient for clinical trials could not be supplied both by isolation and organic synthesis. Hence, the development of simplified analogs of bryo-1 could be a highly effective strategy.

As an alternative way to address the supply problem, we developed simple, easily produced aplysiatoxin analogs as possible anti-cancer leads. Although debromoaplysiatoxin (DAT) is a tumor promoter, 10-methyl-aplog-1 (26) did not show any tumor promotion *in vivo* even when applied in 5-fold excess compared with DAT. Interestingly, the anti-proliferative effects on 39 human cancer cell lines (MG-MID) were quite similar to each other, and the anti-proliferative activity of 26 against several cancer cell lines was even higher than that of DAT. These data indicate that the removal of three methyl and one methoxy groups along with a hemiacetal hydroxyl group reduced only the tumor-promoting activity. Such simplification would be quite important for finding new medicinal leads using complex skeletons of bioactive natural products. DAT can be regarded as a master key, at least for tumor promotion and anti-proliferative activity, while 26 can be
regarded as a special key for receptors related to anti-proliferative activity.

The next step is to unveil the molecular mechanism underlying the unique biological characteristics of aplogs. Both the anti-proliferative and the tumor-promoting activities of PKC ligands have been mostly attributed to its modulating effect on the PKC isozymes. However, even pro-apoptotic PKCδ isozymes also mediate survival signaling, and other PKC isozymes have dual roles in many cases. Therefore, a careful interpretation of the anti-cancer mechanism of PKC ligands is required. Recent studies also found that several C1 domain-containing proteins such as Ras GRPs and Munc13s are involved in apoptosis signaling.\cite{79} Moreover, the existence and involvement of unidentified targets of PKC ligands like cytosolic-nuclear tumor promoter-specific binding protein (CN-TPBP)\cite{80} should be considered. For this purpose, aplogs could become new molecular probes to identify the receptors involved in the anti-proliferative activity other than PKC isozymes. Such studies are underway in our laboratory.

**Acknowledgements**

Our data presented in this review were obtained in collaboration with Dr. Yu Nakagawa and several graduate students (Mr. Keisuke Tanaka, Hiroaki Kamachi, Masayuki Kikumori, and Yusuke Hanaki) of the Department of Food Science and Biotechnology of the Graduate School of Agriculture at Kyoto University. We thank Associate Professor Harukuni Tokuda of the Department of Complementary and Alternative Medicine, Clinical R&D, of the Graduate School of Medical Science at Kanazawa University for conducting the two-stage carcinogenesis experiments, and Professor Hiroshi Nagai of the Department of Ocean Sciences at Tokyo University of Marine Science and Technology and Associate Professor Kiyotake Suenaga of the Faculty of Science and Technology at Keio University for supplying aplysiatoxin and debromoaplysiatoxin. We also thank the Screening Committee for Anticancer Drugs supported by a Grant-in-aid for Scientific Research on Innovative areas “Scientific Support Programs for Cancer Research” from The Ministry of Education, Culture, Sports, Science and Technology, Japan. This research was partly supported by a Grant-in-aid
for Scientific Research on Innovative Areas ‘Chemical Biology of Natural Products’ (Grant No. 23102011 to K.I. and R.C.Y.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
REFERENCES


Figure Captions

Fig. 1. (a) Structure of protein kinase C (PKC) isozymes. Natural PKC ligands like phorbol esters as well as 1,2-diacyl-sn-glycerol (DG) bind to the C1 domains of these PKC isozymes. (b) Activation of a novel PKC isozyme by natural PKC ligands. Tumor promoters or endogenous DG produced by phospholipase C (PLC) bind to the C1A and/or C1B domains to induce the plasma membrane translocation. Nuclear membrane translocation also occurs depending on the structure and/or hydrophobicity of the ligands. C1, protein kinase C conserved region 1; C2, protein kinase C conserved region 2; IP<sub>3</sub>, inositol-1,4,5-triphosphate; PIP<sub>2</sub>, phosphatidylinositol-4,5-diphosphate; PS, phosphatidylserine.

Fig. 2. Structure of 1,2-diacyl-sn-glycerol (DG) receptors with C1 homology domains other than PKC isozymes. EF, EF hand; MHD1, 2, Munc13 homology domain 1, 2; PH, pleckstrin homology domain; Ras-GEF, guanine nucleotide exchange factor for Ras-like small GTPases; REM, Ras exchange motif; Rho-GAP, GTPase-activator protein for Rho-like GTPases; SH2, Src homology 2 domain.

Fig. 3. Structure of PKC activators: TPA, ingenol 3-angelate, teleocidin B-4, indolactam-V, prostratin, benzolactam-V8s, and DAG-lactones.

Fig. 4. Structure of bryo-1, bryo-7, bryo-10, and simplified analogs of bryo-1 synthesized by Wender (1-4) and Keck (5 and 6)

Fig. 5. Structure of ATX, DAT, and their simplified analogs (7-9, 21-27).

Fig. 6. A synthetic route for aplog-1 (7).<sup>55a</sup>

Fig. 7. Correlation between the anti-proliferative activity and log P values. “R” signifies the
macrolactone moiety and alkyl side chain of aplog-1 (7).

Fig. 8. Tumor-promoting activity of TPA, 10-methyl-aplog-1 (26), and 12,12-dimethyl-aplog-1 (27). The back of each male 6-week-old ICR mice was shaved with surgical clippers. From a week after initiation by a single application of 390 nmol of DMBA in 0.1 mL acetone, 8.5 nmol of 26 or 27 in 0.1 mL of acetone was applied twice a week from week 1 to week 20. The control group was treated with 390 nmol of DMBA and 1.7 nmol of TPA. Anti-tumor-promoting activity of 27 (8.5 nmol) against TPA (1.7 nmol) is also shown. Ten male ICR mice were tested in each group.

Fig. 9. Values of $K_i$ for the inhibition of $[^3H]$PDBu binding by aplogs (7 and 26) and tumor promoters (DAT and indolactam-V).

Fig. 10. Possible roles of PKC isozymes for the anti-proliferative activity of aplogs.
Table 1. $K_d$ and $K_i$ values of the PKC C1 peptides for PDBu and indolactam-V$^{[16d,19]}$

<table>
<thead>
<tr>
<th>PKC isozymes</th>
<th>PDBu ($K_d$: nM)</th>
<th>Indolactam-V ($K_i$: nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKCα (whole)</td>
<td>0.46</td>
<td>20</td>
</tr>
<tr>
<td>PKCα (whole)$^a$</td>
<td>0.15</td>
<td>11</td>
</tr>
<tr>
<td>α-C1A (72-mer)</td>
<td>1.1</td>
<td>21</td>
</tr>
<tr>
<td>α-C1B (72-mer)</td>
<td>5.3</td>
<td>4,000</td>
</tr>
<tr>
<td>PKCβ (whole)</td>
<td>0.54</td>
<td>31</td>
</tr>
<tr>
<td>PKCβ (whole)$^a$</td>
<td>0.14</td>
<td>6.1</td>
</tr>
<tr>
<td>β-C1A (72-mer)</td>
<td>1.3</td>
<td>19</td>
</tr>
<tr>
<td>β-C1B (51-mer)</td>
<td>1.3</td>
<td>140</td>
</tr>
<tr>
<td>PKCγ (whole)</td>
<td>1.8</td>
<td>91</td>
</tr>
<tr>
<td>PKCγ (whole)$^a$</td>
<td>0.37</td>
<td>19</td>
</tr>
<tr>
<td>γ-C1A (52-mer)</td>
<td>1.5</td>
<td>140</td>
</tr>
<tr>
<td>γ-C1B (51-mer)</td>
<td>1.2</td>
<td>210</td>
</tr>
<tr>
<td>PKCδ (whole)</td>
<td>0.76</td>
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</tr>
<tr>
<td>PKCδ (whole)$^a$</td>
<td>0.71</td>
<td>8.2</td>
</tr>
<tr>
<td>δ-C1A (52-mer)</td>
<td>52</td>
<td>1,900</td>
</tr>
<tr>
<td>δ-C1B (51-mer)</td>
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<td>11</td>
</tr>
<tr>
<td>PKCε (whole)</td>
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<td>6.6</td>
</tr>
<tr>
<td>PKCε (whole)$^a$</td>
<td>0.63</td>
<td>22</td>
</tr>
<tr>
<td>ε-C1A (53-mer)</td>
<td>5.6</td>
<td>4,100</td>
</tr>
<tr>
<td>ε-C1B (51-mer)</td>
<td>0.81</td>
<td>7.7</td>
</tr>
<tr>
<td>PKCη (whole)</td>
<td>0.95</td>
<td>4.8</td>
</tr>
<tr>
<td>PKCη (whole)$^a$</td>
<td>0.58</td>
<td>15.6</td>
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<td>η-C1A (53-mer)</td>
<td>4.3</td>
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<tr>
<td>η-C1B (51-mer)</td>
<td>0.45</td>
<td>5.5</td>
</tr>
<tr>
<td>PKCθ (whole)</td>
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<td>ND</td>
</tr>
<tr>
<td>θ-C1A (52-mer)</td>
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<tr>
<td>θ-C1B (51-mer)</td>
<td>0.72</td>
<td>8.7</td>
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$^a$ The data in the absence of calcium reported by Kazanietz et al.$^{[19a]}$
Table 2. Growth inhibition of Wender’s simplified analogs (1 and 2) of bryo-1 against several cancer cell lines along with that of bryo-1 \[46\]

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
<th>1</th>
<th>2</th>
<th>Bryo-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>MDA-MB-231</td>
<td>–5.01</td>
<td>–5.69</td>
<td>–5.20</td>
</tr>
<tr>
<td>CNS</td>
<td>SF-295</td>
<td>–6.56</td>
<td>–6.05</td>
<td>–5.20</td>
</tr>
<tr>
<td>Colon</td>
<td>HCC-2998</td>
<td>–3.50</td>
<td>–7.54</td>
<td>–5.30</td>
</tr>
<tr>
<td>Lung</td>
<td>NCI-H460</td>
<td>–7.47</td>
<td>&lt;–8.00</td>
<td>–5.60</td>
</tr>
<tr>
<td></td>
<td>A549</td>
<td>–6.49</td>
<td>–6.62</td>
<td>–5.20</td>
</tr>
<tr>
<td>Melanoma</td>
<td>SK-MEL5</td>
<td>–5.05</td>
<td>–6.43</td>
<td>–5.70</td>
</tr>
<tr>
<td>Ovarian</td>
<td>OVACAR-5</td>
<td>&gt;–4.00</td>
<td>–5.28</td>
<td>&gt;–5.00</td>
</tr>
<tr>
<td>Prostate</td>
<td>PC-3</td>
<td>–5.55</td>
<td>–5.60</td>
<td>–5.30</td>
</tr>
</tbody>
</table>
**Table 3.**  \(K_i\) values for the inhibition of binding of \[^3\text{H} \text{PDBu}\) by aplog-1 (7), 8, 9, aplysiatoxin (ATX), debromoaplysiatoxin (DAT), and bryo-1

<table>
<thead>
<tr>
<th>PKC(\delta) and C1 peptides</th>
<th>Aplog-1 (7)</th>
<th>8</th>
<th>9</th>
<th>ATX</th>
<th>DAT</th>
<th>Bryo-1</th>
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<tbody>
<tr>
<td>PKC(\delta)</td>
<td>15</td>
<td>400</td>
<td>NT(^a)</td>
<td>3.0</td>
<td>NT(^a)</td>
<td>0.84</td>
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<tr>
<td>PKC(\delta)-C1A</td>
<td>140</td>
<td>6,800</td>
<td>130</td>
<td>12</td>
<td>9.7</td>
<td>5.3</td>
</tr>
<tr>
<td>PKC(\delta)-C1B</td>
<td>7.4</td>
<td>170</td>
<td>9.8</td>
<td>0.41</td>
<td>0.20</td>
<td>0.60</td>
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</table>

[a] Not tested.
Table 4. Growth inhibition of aplog-1 (7), 8, 9, and debromoaplysiatoxin (DAT) against aplog-sensitive cancer cell lines along with that of bryo-1

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
<th>Log GI&lt;sub&gt;50&lt;/sub&gt; (M)</th>
<th>Aplog-1 (7)</th>
<th>8</th>
<th>9</th>
<th>DAT</th>
<th>Bryo-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>HBC-4</td>
<td>–6.33</td>
<td>–5.32</td>
<td>–6.20</td>
<td>–6.47</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231</td>
<td>–5.61</td>
<td>–4.55</td>
<td>–5.67</td>
<td>–6.03</td>
<td>–5.20</td>
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<tr>
<td>Colon</td>
<td>HCC2998</td>
<td>–5.43</td>
<td>–4.57</td>
<td>–5.53</td>
<td>–6.09</td>
<td>–5.30</td>
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</tr>
<tr>
<td>Lung</td>
<td>NCI-H460</td>
<td>–5.60</td>
<td>–4.70</td>
<td>–5.83</td>
<td>–6.46</td>
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<td>–5.94</td>
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<tr>
<td>Melanoma</td>
<td>LOX-IMVI</td>
<td>–5.74</td>
<td>–4.66</td>
<td>–5.17</td>
<td>–5.69</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>St-4</td>
<td>–5.55</td>
<td>–5.04</td>
<td>–6.05</td>
<td>–6.44</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MKN45</td>
<td>–5.33</td>
<td>–4.74</td>
<td>–6.09</td>
<td>–4.98</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>MG-MID of cell lines</td>
<td>39 cancer</td>
<td>–4.98</td>
<td>–4.27</td>
<td>–5.09</td>
<td>–5.22</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

[a] Not tested.
Table 5. Growth inhibition of aplog-1 (7), 27-(R)-methyl-aplog-1 (21), whose absolute configuration at position 27 is the same as that of DAT at position 30, the 27-epimer of 21 (22), and 27-O-methyl-aplog-1 (23) against several cancer cell lines.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
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<th>21</th>
<th>22</th>
<th>23</th>
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</thead>
<tbody>
<tr>
<td>Breast</td>
<td>HBC-4</td>
<td>-6.33</td>
<td>-6.15</td>
<td>-4.78</td>
<td>-4.71</td>
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<tr>
<td>Lung</td>
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<td>-5.66</td>
<td>-4.78</td>
<td>-4.70</td>
</tr>
<tr>
<td>Melanoma</td>
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<td>-5.74</td>
<td>-5.18</td>
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<td>-4.80</td>
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<tr>
<td>Stomach</td>
<td>St-4</td>
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<tr>
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<td>Stomach</td>
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<td>-4.76</td>
<td>-4.80</td>
</tr>
<tr>
<td>Prostate</td>
<td>DU-145</td>
<td>-4.85</td>
<td>-4.80</td>
<td>-4.73</td>
<td>-4.67</td>
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Table 6. Growth inhibition of demethoxy-DAT (24), 4-methyl-aplog-1 (25), 10-methyl-aplog-1 (26), and 12,12-dimethyl-aplog-1 (27) against aplog-sensitive cancer cell lines

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
<th>Log GI_{50} (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Breast</td>
<td>HBC-4</td>
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</tr>
<tr>
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<td>MDA-MB-231</td>
<td>−7.31</td>
</tr>
<tr>
<td>CNS</td>
<td>SF-295</td>
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<td>MG-MID of cell lines</td>
<td>39 cancer</td>
<td>−5.61</td>
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</tbody>
</table>
Biographical sketch of the corresponding author

Kazuhiro Irie, Ph. D.

Dr. Kazuhiro Irie is a Full Professor at the Division of Food Science and Biotechnology at Kyoto University. He received his B. Agr. (1982), M. Agr. (1984), and D. Agr. (1988) from Kyoto University. He was appointed as an instructor at the Department of Food Science and Technology in Kyoto University in 1984. In 1992, he joined to the group of Professor Paul A. Wender at the Department of Chemistry at Stanford University as a visiting scholar for one year. He then became an Associate Professor at Kyoto University in 1999. Since 2007, he has been a Full Professor of the Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University. His research interests are in the area of organic chemistry related to life sciences, with an emphasis on tumor promotion and Alzheimer’s disease.
Figure 1.
Figure 2.
Figure 3.
Figure 4.

R¹ = OAc, R² = \( \text{CH}_3 \): Bryo-1
R¹ = R² = OAc: Bryo-7
R¹ = OPiv, R² = H: Bryo-10
Figure 5.
Figure 6.
\[
-MG-MID = -0.25 \log P^2 + 2.2 \log P + 0.50 \\
(t = 0.95)
\]
Figure 8.
Figure 9.
Figure 10.

Aplog-1

PKCδ

PKCα

PKCε

stronger binding

weaker binding

Cell cycle arrest
Apoptosis

Cell survival
Metastasis