Phospholipase Cγ as a target for the development of new anticancer agents from natural sources

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Summary
Phosphatidylinositol phospholipase C (PLC) is an attractive target for pharmacological intervention in a number of human diseases, particularly those related to abnormal cell proliferation. Several lines of evidence have suggested that an inhibitor of PLC, especially the γ-isofrom, would be a useful tool for development of anticancer agents. Recently, several new classes of PLCγ inhibitors have been isolated from natural sources. Active components from a number of medicinal plants have displayed inhibitory activity and include compounds of the biflavonoids, norlignans, triterpene esters, alkyl phenols, isocoumarin, prenylated flavonoids, prenylated isoflavonoids and retrochalcone classes. Furthermore, microbial secondary products have also shown activity and include a cage-like compound, salicylic acid derivatives, aminoglycosides, peptides, a macrolide, benzoaldehydes, a cyclic peptide, fatty acid derivatives and a macro lactam.

Of these inhibitors, it has been reported that alkyl phenols, triterpene esters, licochalcone A, norlignans and prenylated flavonoids exhibit cytotoxic activities. Interestingly, alkyl phenols and triterpene esters were less cytotoxic on a normal colon cell line (CCD-18-Co) as compared to the corresponding colon carcinoma cell line (HCT-15). These data suggest that PLCγ inhibitors may be candidates for a new class of anticancer agents that show less toxicity against normal tissues.

Introduction
Since aberrations in cell signaling pathways can result in hyperproliferative diseases and inflammatory conditions, interventions targeted at cell signaling have served as useful targets for chemotherapy and chemoprevention of these diseases (1). Among these targets, this review will focus on the γ-isofrom of phospholipase C (PLCγ) as a target for the development of new natural product anticancer agents.

Phosphoinositide specific-PLC (PI-PLC) plays a pivotal role in transmembrane signal transduction pathways. PLC generates the intracellular messengers inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) following hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2) (2). IP3 induces the release of Ca2+ from internal stores which produces a transient increase in cytoplasmic free Ca2+ concentration, while DAG is an activator of Ca2+ and phospholipid-dependent protein serine/threonine kinase, protein kinase C (PKC). The increase of Ca2+ concentration and the activation of PKC lead to a series of events that culminates in DNA synthesis, cell proliferation and cell differentiation (3, 4).

Each type of PLC isozyme contains several distinct subtypes including β1-4, γ1-2 and δ1-4 which have molecular weights of 150-154, 145-148 and 85-88 kDa, respectively (5). Two regions of high-sequence homology
PLC as a target for new anticancer agents

(40-60%), designated X and Y, constitute the PLC catalytic domain, with 170 and 260 amino acids, respectively, thought to be responsible for the recognition and hydrolysis of phosphoinositides (8). A pleckstrin homology (PH) domain in the NH2-terminal region mediates interaction with the membrane surface by binding to PIP2, and the C2 domain fixes the catalytic domain in a productive orientation on the membrane. In addition to these domains, the COOH terminal domain in PLCβ isoforms might contribute to the tethering of the enzyme to the membrane surface. In the PLCγ type, the area between the X and Y regions consists of 400 amino acid residue motifs with denoted Src homology (SH) (2 SH2 and 1 SH3) domains, while the PLCβ and δ types only possess 71 and 40 amino acids, respectively. These SH domains, first recognized as highly conserved regions in the products of oncogenes, abl and src (9), are sufficient for binding to activate growth factor receptors in PLCγ1, the regulatory subunit (p85) of PI3 kinase, members of the src family of protein tyrosine kinase, the Ras-GTPase-activating protein (GAP) and the adaptor proteins Grb2, Nck and Src (10). These domains may play a critical role in mitogenic signal transduction as a secondary or regulatory mediator (11).

The existence of multiple PLC isozymes reflects different activation pathways. In the PLCβ pathway, peptides such as angiostatin, bombesin, bradykinin and vasopressin act on a 7-spanning receptor coupled to a specific guanine nucleoside binding protein (G protein) which, in turn, activates a specific membrane bound PLCβ.

In the PLCγ pathway, PLCγ1 is activated through phosphorylation of specific tyrosine residues by receptor protein tyrosine kinases (PTK) and nonreceptor type PTKs in immune system receptors such as the membrane IgM receptor and the T cell antigen receptor (12, 13). Binding of growth factors (PDGF, EGF, NGF and FGF) to the PTK receptor induces a conformational change, enhancing PTK activity toward other substrates in the receptor (14, 15). Thus, the phosphotyrosine-induced activation of PLCγ1 via binding to the PTK receptor ultimately leads to DNA synthesis, proliferation and cell differentiation.

Although the crystal structure of a mammalian PLCδ1 has been elucidated (16) and its amino acid sequences found to be common to all PLC subfamilies, the mechanism(s) for the regulation of the PLCδ isotype are presently unknown.

As for the activation mechanism of PLC isoforms, aberrations in PLCβ and PLCγ cell signaling pathways resulted in inflammatory conditions and hyperproliferative diseases, respectively (1).

PLCγ and cancer

PLCgamma is an essential enzyme involved in cell proliferation whose activity is increased by a variety of mitogens. Several studies have reported that different PLC isoforms are responsible for the different regulatory mechanisms of PLC seen in normal and tumor cells.

The neu/HER2 protooncogene induces cellular transformation by tyrosine phosphorylation and activation of PLCγ (17). Overexpression of PLCγ1 by direct microinjection into NIH3T3 fibroblast cells induces DNA synthesis, growth and morphological transformation (18), while microinjection of PLCγ1 antibodies inhibits PLCγ1-, serum- and ras-induced mitogenesis (11, 19). Furthermore, microinjection of lipase-defective mutants, i.e., the SH223 domain (SH2-SH2-SH3) of PLCγ1, and catalytically inactive PLCγ1 into quiescent NIH3T3 fibroblasts also induces DNA synthesis, indicating that the noncatalytic region of PLCγ1 can induce mitogenesis (11, 20). In addition, overexpression of PLCγ1 from rat 3Y1 fibroblasts in culture and in mice led to malignant transformation (21).

Surprisingly, there are reports suggesting that the levels of PLCγ1 as measured by radioimmunoassay are increased as compared to corresponding normal tissues in various human tumor cells, such as melanoma grown as xenografts (22), colorectal cancer cells (23, 29), renal cell carcinoma (24), neoplastic keratinocytes (25, 26), glial tumor (27), malignant breast (28) and non-small cell lung carcinomas (24). PLCγ1 protein was significantly detectable in 70% of all human breast carcinomas compared to only 6% of nonmalignant breast tissues (28). On the other hand, protein levels of PLCγ1 were considerably higher in 15 of 17 colorectal cancer tissues as compared with their normal counterparts, while little difference was noted in the levels of PLCβ1 and PLCδ1 (29). In addition, while PLCγ1 overexpression was seen in 15 colorectal cancer tissues and correlated with either highly or moderately differentiated carcinoma, overexpression was not seen in 1 poorly differentiated and 1 mucinous carcinoma tissue (29). Western blot analysis of human glial tumors using antibodies to PLCs showed expression of PLCγ in all tumors, whereas PLCβ expression was seen in only some tumors (27).

As mentioned above, there is substantial evidence indicating that PLC fragments, especially the γ type, and their overexpression leads to human carcinogenesis. Therefore, specific inhibitors of PLCγ might contribute to growth inhibition of tumor cells and chemoprevention of cancer. Moreover, PLC inhibitors also appear to be much less toxic than classic chemotherapeutic agents due to the fact that they are endogenous cell signal interceptors (1). A previous review described PLC inhibitors known up to 1993 that showed antitumor or cytotoxic effects (7).

Nitro derivatives of aminochromene and coumarin were found to inhibit human melanoma PLC with IC50 values of 208 and 10 µM, respectively, and cell growth of melanoma was inhibited in culture at IC50 values of 10 and 2 µM, respectively (30). The ether lipids, ET-18-OCH3, predominantly inhibited fibroblast cytosolic PLCγ with an IC50 of 0.4 µM (31). The formation of inositol phosphates in intact Swiss 3T3 fibroblasts stimulated with PDGF or fluoroaluminate anion was also inhibited with an IC50 value of 10 µM, close to the cytotoxic concentration.
of ET-18-OCH₃ for this cell line (32). This compound which is a platelet-activating factor, is undergoing clinical trials as an anticancer drug (33). The antitumor and antitypanosomal drug suramin was also found to be an inhibitor of PLCγ with an IC₅₀ value of 63 μM (34). Stroidamine (U-73122) inhibited PLC with an IC₅₀ of 9-40 μM and was cytotoxic to Swiss fibroblasts at 10 μM (35). 3-F-phosphatidylinositol is an inhibitor of the Swiss 3T3 fibroblast PLCγ and mediated hydrolysis of PI with an IC₅₀ of 8 μM and inhibited the growth of HT-29 colon carcinoma cells with an IC₅₀ of 37 μM (7).

**PLCγ inhibitors from medicinal plants**

**Biflavonoid from Selaginella tamariscina**

Amentoflavone (1), a biflavonoid isolated as the first plant-derived PLCγ inhibitor from Selaginella tamariscina P. Beauv. (Selaginellaceae), inhibited PLCγ activity from bovine cerebellum with an IC₅₀ value of 29.0 μM and inhibited the formation of total inositol phosphates (IP₃) in PDGF-stimulated NIH3T3 (PLCγ overexpressing NIH3T3 fibroblasts) with an IC₅₀ value of 9.2 μM. However, it did not show inhibitory activity against PKC in vitro or against phorbol 12,13-dibutyrate (PDBu)-induced bleb formation in K562 cells (human leukemia). Accordingly, this compound (1) directly inhibited PLCγ leading to a reduction in the amount of intracellular IP₃ in PDGF-stimulated NIH3T3γ1 cells without directly affecting cellular PKC (36).

**Norlignans from Anemarrhena asphodeloides**

PLCγ inhibitory compounds isolated from the roots of Anemarrhena asphodeloides Bunge (Liliaceae) were characterized as 2 norlignans, cis-hinokiresinol (2) and monomethyl cis-hinokiresinol (3). These compounds showed weak PLCγ inhibitory activities with IC₅₀ values of 99.5 and 94.0 μM, respectively (37). The report suggested that the C-4” hydroxy group was a factor that may have affected activity, while the C-4’-hydroxy group has no effect in this regard. Of these compounds, hinokiresinol (2) showed selective and more potent cyto-

**Triterpene esters from Uncaria rhynchophylla**

Using bioactivity-guided fractionation and isolation, 8 PLCγ1 inhibitors from the hooks of Uncaria rhynchophyl-la (Miq.) Miq. Ex Havil. (Rubiaceae) were identified as triterpene esters, including 5 new natural products, uncarinic acids A-E (4-8) (39, 40). All of the compounds (4-11) exhibited dose-dependent inhibitory activity against PLCγ1 with IC₅₀ values ranging from 9.5-44.5 μM. The compounds also reduced PDGF-induced accumulation of toxicity against several human cancer cell lines overexpressing PLCγ1 than others, i.e., A-549 (lung; ED₅₀ = 12 μg/ml), HCT-15 (colon; ED₅₀ = 9.2 μg/ml), DLD1 (colon; ED₅₀ = 6.9 μg/ml), MCF-7 (breast; ED₅₀ = 15 μg/ml) as compared to SK-OV-3 (ovary; ED₅₀ = 34 μg/ml) and P-388 (leukemia; ED₅₀ = 26 μg/ml) (38).
A dihydroisocoumarin, (3R)-(−)-8-hydroxy-3-(6’-pentadecenyl)-3,4-dihydroisocoumarin (22) and a long chain ketone, 3-heptadecen-2-one (23) were also isolated as PLCγ1 inhibitors from the sarcotestas of G. biloba. These compounds exhibited IC₅₀ values of 9.7 and 25.6 µM, respectively, for PLCγ1 (42).

All 10 of these alkyl phenolic compounds inhibited the growth of human cancer cells such as HCT-15, MCF-7, A-549, HT-1197 and SK-OV-3. These compounds were less cytotoxic to the normal colon cell line (CCD-18-Co) than to its corresponding colon carcinoma cell line (unpublished results).

**Alkyl phenols and an isocoumarin from Ginkgo biloba**

The 10 phenolic compounds with saturated or unsaturated long chains isolated from the sarcotestas of Ginkgo biloba L. (Ginkgoaceae) (41) included the cardanols C₁₅:₁ (12), C₁₇:₁ (13), C₁₃:₀ (14) and C₁₅:₀ (15), the phenolic acids C₁₅:₁ (16), C₁₇:₁ (17) and C₁₅:₀ (18) and the bilobols C₁₅:₁ (19), C₁₇:₁ (20) and C₁₅:₀ (21). These compounds exhibited PLCγ1 inhibitory activities with IC₅₀ values ranging from 2.2-72.3 µM. The most active compound was phenolic acid C₁₇:₁ (17). A structure-activity relationship study revealed the importance of a long alkyl chain, a double bond in the alkyl chain, a phenolic OH and an aromatic COOH for effective PLCγ1 inhibitory activities. On the other hand, phenolic acids having an additional carboxyl group were more active than the corresponding cardanols, and bilobols possessing a supplementary hydroxyl group were less effective than the corresponding cardanols. The mode of PLCγ1 inhibition induced by cardanol C₁₅:₁ (12) was competitive, whereas inhibition by phenolic acid C₁₅:₁ (16) and bilobol C₁₅:₁ (19) was noncompetitive. Thus, these changes in activity caused by the additional carboxyl and hydroxyl groups seemed to result from the differences in reaction sites of phenolic acid and bilobol as compared to cardanol. These results indicated that the carboxyl and hydroxyl groups are essential for noncompetitive inhibition (41).

<table>
<thead>
<tr>
<th>RI C₅₀ (µM)</th>
<th>12 CH₂(CH₃)₆CH=CH(CH₂)₅CH₃ 12.9</th>
<th>13 CH₂(CH₃)₈CH=CH(CH₂)₅CH₃ 9.7</th>
<th>14 CH₂(CH₃)₁₀CH₃ 57.9</th>
<th>15 CH₂(CH₃)₁₂CH₃ 58.0</th>
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<tr>
<th>RI C₅₀ (µM)</th>
<th>16 CH₂(CH₃)₆CH=CH(CH₂)₅CH₃ 5.7</th>
<th>17 CH₂(CH₃)₈CH=CH(CH₂)₅CH₃ 2.2</th>
<th>18 CH₂(CH₃)₁₀CH₃ 16.3</th>
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A dihydroisocoumarin, (3R)-(−)-8-hydroxy-3-(6’-pentadecenyl)-3,4-dihydroisocoumarin (22) and a long chain ketone, 3-heptadecen-2-one (23) were also isolated as PLCγ1 inhibitors from the sarcotestas of G. biloba. These compounds exhibited IC₅₀ values of 9.7 and 25.6 µM, respectively, for PLCγ1 (42).

All 10 of these alkyl phenolic compounds inhibited the growth of human cancer cells such as HCT-15, MCF-7, A-549, HT-1197 and SK-OV-3. These compounds were less cytotoxic to the normal colon cell line (CCD-18-Co) than to its corresponding colon carcinoma (41). Several of the phenolic compounds, including C₁₅:₁, C₁₇:₁ and C₁₅:₀, have also been reported to have antitumor effects (43).

**Prenylated flavonoids from Sophora flavescens**

Eleven prenylated flavonoids from the roots of Sophora flavescens Aiton (Leguminosae) were reported to be cytotoxic compounds (44). With the exception of
Prenylated isoflavonoids from 
*Erythrina senegalensis*

Ariculatin (35) and 8-prenylluteone (36), 2 prenylated isoflavonoids isolated from the stem bark of *Erythrina senegalensis* DC. (Leguminosae), were found to inhibit PLCγ1 and PI turnover in NIH3T3γ1 cells (46). These compounds had similar inhibitory activity with an IC₅₀ value of 20 µM against PLCγ1 in vitro and inhibited the formation of inositol phosphate in PDGF-stimulated NIH3T3 cells. When compared to some common flavonoids, such as luteolin (flavone), quercetin (flavonol), hesperetin (flavanone) and genistein (isoflavone), the isoprenyl group at C-8 of 35 and 36 was shown to be related to the inhibitory activity against PLCγ1. These prenylated flavonoids showed moderate cytotoxicity (IC₅₀ = 9-20 µM) against kushenol H (27), sophoraflavone G (24), kurarinone (25), kushenol N (26), kushenol K (28), kushenol B (29), kushenol M (30), kosamol A (31), kushenol E (32), kushenol L (33) and kuraridin (34) also showed PLCγ1 inhibitory activities with IC₅₀ values ranging from 7.5-35 µM. The most active compound was kushenol B (29) which has a lavandulyl group at C-8 (R₃) and an isopentenyl group at C-6 (R₂). The presence of a hydroxyl group at C-3 resulted in a significant decrease in activity and the configuration of this hydroxyl is likely to be another factor influencing activity. In addition, dehydration of the C-4”"-C-5”" double bond of the lavandulyl side chain caused complete loss of activity. These data suggest that the lavandulyl side chain is important for high inhibitory activity and that the presence and configuration of a hydroxyl group at C-3 are related to inhibitory activity (45).

The prenylated flavonoids from *S. flavescens* exhibited moderate cytotoxicity against several human tumor cell lines, i.e., A-549, SK-OV-3, SK-MEL2 (skin), XF-498 (central nerve) and HCT-15, with ED₅₀ values of 5-14 µg/ml. The results were well in agreement with those reported for the inhibition of PLCγ1 (44).
Several human tumor cell lines in vitro, including PC-3 (prostate), NCI-H226 (lung) and CRL-1579 (melanoma) (46).

_Retrochalcone from Pogostemon cablin_

Licochalcone A (37), a characteristic retrochalcone with antitumor action, was isolated from the aerial parts of _Pogostemon cablin_ (Blanco) Benth. (Labiatae) using bioactivity-guided fractionation and isolation. It was found to be active against mouse leukemia cells (P-388; IC₅₀ = 3.6 µg/ml) (47, 48). This compound also showed inhibitory activity against PLCγ₁ (IC₅₀ = 30 µM) and exhibited selective cytotoxicity against human cancer cells overexpressing PLCγ₁, i.e., A-549 (IC₅₀ = 4.6 µg/ml), MCF-7 (IC₅₀ = 9.2 µg/ml), HCT-15 (IC₅₀ = 8.8 µg/ml), SK-OV-3 (IC₅₀ > 20 µg/ml) and Malme-3M (malignant melanoma; IC₅₀ > 20 µg/ml). In addition, it induced expression of NSE activity, a marker of macrophage (monocyte) formation (13.2 µM), but did not show activity in the NBT reduction assay, an indicator of granulocyte formation. Thus, licochalcone A appears to be an inducer of monocyte rather than granulocyte differentiation and may be useful as a cancer chemotherapeutic and chemopreventive agent (48).

**PLCγ inhibitors from microorganisms**

_Cage-like compound from C. hispidulum_

Hispidospermidine (38), a cage-like compound with a trimethylspermidine side chain isolated from the fungal culture broth of _Chaetoshaeronema hispidulum_, inhibited rat brain PLC with an IC₅₀ value of 16 µM but did not inhibit other signal transduction markers such as PKC and PLA₂ (49, 50). This compound also exhibited cytotoxic activity against HeLa (cervical cancer) cells (IC₅₀ = 36 µM) (49).

_Salicylic acid derivative from Caloporus dichrous_

Caloporoside (39) is a new salicylic acid derivative from fermentations of _Caloporous dichrous_ that selectively inhibits PLC. This compound exhibited marked selectivity towards PLC of pig brain (IC₅₀ = 18-31 µM) but not PLC from _Clostridium welchii_ and _Bacillus cereus_, phospholipase D, triacylglyceride lipase, PLA₂ and acetylcholine esterase (51). Caloporoside exhibited no significant cytotoxic effects against L-1210, HeLa 3S or Ehrlich ascitic tumor cells, but inhibited the incorporation of radioactive precursors into DNA, RNA and proteins in Ehrlich ascitic tumor cells (51).

_Aminoglycosides and peptides from bacteria_

Screening of over 150 bacteria yielded 5 compounds that were active PLC inhibitors with IC₅₀ values in the micromolar range (52). Two aminoglycosides, rhodomycin (40) and tobramycin (41) (IC₅₀ = 47.1 and 9.7 µM, respectively) appeared to act via a mechanism similar to neomycin which inhibits PLC by binding to PIP₂ (53). The other group of compounds that inhibited PLC were all peptides and included myrordin K (42), streptothricin B...
formation in HT-29 cells with IC_{50} values in the micromolar range and showed some selective cytotoxicity for SW-480 (colon), MCF-7 and A-375 (melanoma), but not for HL-60 (leukemia), A-549 and LNCaP (prostate). One of the peptides, myroridin K (IC_{50}, 6.7 µM), was reported to have antitumor activity when injected i.p. against Ehrlich ascites carcinoma and sarcoma 180 in mice (55).

Salicylic acid derivative from Pseudallescheria

Thielavin B (CRM-60109, 45), a salicylic acid derivative from Pseudallescheria sp. MT60109, showed direct PLCγ inhibitory activity with an IC_{50} value of 20 µM (56). The compound was previously isolated from Thielavia terricola as an inhibitor of prostaglandin biosynthesis (57). This compound inhibited the formation of IP_{3} in PDGF-stimulated NIH3T3 cells with an IC_{50} value of 20 µM; however, it did not show inhibitory activity against PKC in vitro and against TPA-induced bleb formation in K-562 cells.

Macrolide from Actinomycetes

Scopafungin (46), a 36-membered macrolide initially isolated as an antifungal agent, was purified as an inhibitor of PLCγ1 (IC_{50} = 30 µM) from the culture broth of a Streptomyces sp. No. 2511-5 (58).

Benzaldehydes from a fungal strain

Two benzaldehydes, anguillosporal (47) and CRM-51005 (48), were purified from the culture broth of a
methyl ester) are PLCγ1 inhibitors (IC50 = 80 and 50 µM, respectively) that were obtained from a culture broth of an unidentified Actinomycetes species (63). In comparison to other fatty acids (FA), the unsaturated fatty acids (UFAs) showed more potent activity than the saturated FAs, and the long chain monounsaturated FAs (MUFAs) appeared to be somewhat less effective. The highly polyunsaturated FAs (PUFAs) were relatively more effective than MUFAs. The mechanism of action of UFAs on PLCγ1 inhibition has been reported to involve a tau protein. Arachidonic acid interacts with one of the two pH domains of PLCγ1 and complexes with tau bound to the SH3 domain to enhance enzyme activity (64). In addition, UFAs directly activate PKC and free FAs are known to play a role as second messengers. UFAs are necessary for the full or sustained activation of PKC, and thereby, like Ca2+, DAG or IP3, play a role as regulatory molecules in signaling through the PKC pathway (63).

Macrolactam from Streptomyces

Fluvirucin B2 (52) is a macro lactam purified from the culture broth of Streptomyces sp. MJ677-72F5 that inhibited PI-PLC from A431 cells with an IC50 value of 1.6 µg/ml (65). This microbial metabolite did not inhibit PTK, protein tyrosine phosphatase, PKC or phosphatase phosphatase at a concentration of 100 µg/ml. However, it inhibited the formation of inositol phosphates in cultured A431 cells (IC50 = 9.4 µg/ml) and completely inhibited EGF-induced rapid rounding of A431 cells at a

Fatty acid derivatives from Actinomycetes

Two fatty acid esters, MT965-A (50, 14-methylpentadecanoic acid) and MT965-B (51, 16-methyllinoleic acid methyl ester) are PLCγ1 inhibitors (IC50 = 80 and 50 µM, respectively) that were obtained from a culture broth of an unidentified Actinomycetes species (63). In comparison to other fatty acids (FA), the unsaturated fatty acids (UFAs) showed more potent activity than the saturated FAs, and the long chain monounsaturated FAs (MUFAs) appeared to be somewhat less effective. The highly polyunsaturated FAs (PUFAs) were relatively more effective than MUFAs. The mechanism of action of UFAs on PLCγ1 inhibition has been reported to involve a tau protein. Arachidonic acid interacts with one of the two pH domains of PLCγ1 and complexes with tau bound to the SH3 domain to enhance enzyme activity (64). In addition, UFAs directly activate PKC and free FAs are known to play a role as second messengers. UFAs are necessary for the full or sustained activation of PKC, and thereby, like Ca2+, DAG or IP3, play a role as regulatory molecules in signaling through the PKC pathway (63).
concentration of 100 µg/ml. In addition, it inhibited the growth of A431 cells without inhibiting DNA, RNA or protein syntheses. Thus, fluvirucin B2 inhibited PI-PLC both in vitro and in situ (65).

Conclusions

PLCγ is an essential enzyme for cell proliferation and its aberration in the cell signaling pathway has resulted in abnormal hyperproliferative disease. Moreover, overexpression of this enzyme might lead to human carcinogenesis. Therefore, specific PLCγ inhibitors might contribute to inhibition of proliferation of tumor cells. Since 1993, several new structural classes of PLCγ1 inhibitors have been isolated from natural sources. The active components of some medicinal plants include alkyl phenols, amentoflavone, isocoumarin, licochalcone A, norlignans, prenylated flavonoids, prenylated isoflavonoids and triterpene esters. Furthermore, microbial secondary products with PLCγ1 inhibitory activity have also been identified and they include aminoglycosides, benzaldehydes, caloporoside, salicylic acid derivatives, cyclic peptide, fatty acid derivatives, fluvirucin B2, hispidospermidin, peptides and scopafungin.

The activity of many PLCγ1 inhibitors has been correlated to cytotoxic effects. Thus, alkyl phenols, caloporoside, ether lipids (ET-18-OCH3), licochalcone A, nitro derivatives of coumarin, norlignans, 3-F-phosphatidylinositol, prenylated flavonoids, prenylated isoflavonoids, stroidamine and triterpene esters inhibited growth of several cancer cells or exhibited cytotoxicity against cancer cells. In addition, 2 aminoglycosides (rhodomycin and tobramycin) inhibited colony formation in the HT-29 colon cancer cell line. Moreover, the antitumor drug suramin (tobramycin) inhibited colony formation in the HT-29 colon cells. In addition, 2 aminoglycosides (rhodomycin and eral cancer cells or exhibited cytotoxicity against cancer cells. The secondary products with PLCγ1 inhibitory activity also have been identified and they include aminoglycosides, benzaldehydes, caloporoside, salicylic acid derivatives, cyclic peptide, fatty acid derivatives, fluvirucin B2, hispidospermidin, peptides and scopafungin.

The activity of many PLCγ1 inhibitors has been correlated to cytotoxic effects. Thus, alkyl phenols, caloporoside, ether lipids (ET-18-OCH3), licochalcone A, nitro derivatives of coumarin, norlignans, 3-F-phosphatidylinositol, prenylated flavonoids, prenylated isoflavonoids, stroidamine and triterpene esters inhibited growth of several cancer cells or exhibited cytotoxicity against cancer cells. In addition, 2 aminoglycosides (rhodomycin and tobramycin) inhibited colony formation in the HT-29 colon cancer cell line. Moreover, the antitumor drug suramin was found to be an inhibitor of PLCγ1 and ET-18-OCH3, a platelet-activating factor, is selectively cytotoxic in cancer cells only and is undergoing phase III clinical trials as an anticancer drug. The inhibitory activity of aminoglycosides, peptides and prenylated flavonoids against PLCγ1 paralleled their cytotoxicity against cancer cells. The norlignans and licochalcone A exhibited selective antitumor activity against human cancer cells overexpressing PLCγ1, when compared with cell lines not overexpressing this enzyme. Interestingly, alkyl phenols and triterpene esters were less cytotoxic on a normal colon cell line (CCD-18-Co) as compared to corresponding colon carcinoma cells. In addition, licochalcone A is an inducer of monocyte differentiation.

The information presented in this review suggests that PLCγ1 inhibitors may be potential candidates for chemotherapeutic and chemopreventive anticancer agents, exhibiting less toxicity against normal tissues as compared to other compounds. However, the discovery of more potent PLCγ1 inhibitors and further cell signaling studies are necessary in order to develop new anticancer agents.

Acknowledgements

The authors are grateful to Dr. Jong Seog Ahn of Korea Research Institute of Bioscience and Biotechnology, Prof. Sung-Ho Ryu of Pohang Institute of Science and Technology and Prof. A. Douglas Kinghorn of the College of Pharmacy, University of Illinois at Chicago for assistance in preparing this manuscript. The studies described in the senior author's laboratory were supported by the Korean Science and Engineering Foundation (KOSEF) through the Research Center for New Drug Development (RCNDD) at Seoul National University.

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