**EDITORIAL**

# Antitumor Alkylphospholipid Analogs: A Promising and Growing Family of Synthetic Cell Membrane-Targeting Molecules for Cancer Treatment

Faustino Mollinedo*

*Instituto de Biología Molecular y Celular del Cáncer, Centro de Investigación del Cáncer, CSIC-Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain*

Antitumor alkylphospholipid analogs (APLs), also known collectively as antitumor lipids (ATLs), were originally synthesized as synthetic ether-linked metabolically stable analogs of lysophospholipids in the late 1960's as immune modulators [1, 2], but later on they were shown to destroy tumor cells in the late 1970's [1, 2]. The mechanisms underlying the antitumor activity of APLs remained an enigma until the 1990's, when it was found that the APL edelfosine was a potent inducer of apoptosis in tumor cells, whereas normal cells were not affected [3-5]. This selectivity was mainly due to a preferential uptake of the ether lipid edelfosine by cancer cells [5-10], by mechanisms that remain to be fully elucidated. In contrast to most of the antitumor drugs of the time, it was found that APLs did not target DNA, but acted at the cell membrane. These findings made this ether lipid unique compared to the other anticancer drugs of the time, and rekindled a great interest in these APLs as promising and selective antitumor drugs.

At the beginning of the synthesis of APLs, the ester linkages at C1 and C2 of the glycerol backbone of lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosphocholine) were replaced for ether bonds to yield analogs resistant to the action of acyltransferases or lysophospholipases. Among these synthesized ether lipid analogs of lysophosphatidylcholine, edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine, initially known as ET-18-$OCH_3$) (Fig. **1**) resulted the most effective antitumor drug, which became, and still is, the gold standard for assessing the antitumor activities of new APLs in *in vitro* and *in vivo* trials. The first synthesis of edelfosine was reported in 1969, and it is interesting to mention that the chemical structure of this synthetic compound surprisingly turned out to be very close to that of the physiological molecule known as platelet-activating factor (PAF) (Fig. **1**), a major biologically active phospholipid inflammatory mediator whose structure was identified in 1979, that is a decade after the synthesis of edelfosine. The only difference between PAF and edelfosine lies at C2, namely an acetoxy group bound through an ester bond to the glycerol backbone in PAF, whereas edelfosine contains a methoxy group bound through an ether linkage to C2 (Fig. **1**). From the chemical point of view, APLs were primarily distributed into two main classes: a) the alkyl ether phospholipids (AEPs), also named as antitumor ether lipids (AELs) or alkyl-lysophospholipid analogs (ALPs), which contained ether bonds in the phospholipid glycerol backbone, as exemplified by edelfosine (Fig. **1**); b) the alkylphosphocholines (APCs), which lacked the glycerol backbone and were formed by a simple long-chain alcohol esterified to a phosphobase, as exemplified by hexadecylphosphocholine (HPC; miltefosine) that resulted to be the minimal structural requirement of APLs to exert antitumor activity [11] (Fig. **1**). So far, miltefosine, under the trademark name of Miltex®, is the only APL that has been approved to enter the clinic as antitumor drug, being used for the topical treatment of metastatic skin lesions in breast cancer [12]. Thus, a major difference between the above two classes of APLs lies in the lack of a glycerol backbone in APCs. The above two major APL categories are considered as the first and second generation of APLs, respectively. Three new drugs have been synthesized as variants of miltefosine, considered as the third-generation APLs, and have been named as: perifosine (octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate, D-21266) (Fig. **1**), where the choline moiety of miltefosine is replaced by a heterocyclic piperidine group; erucylphosphocholine (Fig. **1**), that contains a longer hydrocarbon chain than miltefosine with a *cis* double bond; and its closely related congener erufosine (erucylphosphohomocholine, erucylphospho-N,N,N-trimethylpropylammonium, ErPC3) (Veenman *et al.*, this issue) (Fig. **1**). These third-generation APLs, especially perifosine, show promise as putative anticancer drugs, and are currently in preclinical and clinical trials (Fensterle *et al.*, this issue). More recently, a novel group of APLs has been synthesized that contain a sugar moiety, known as glycosylated antitumor ether lipids (GAELs) and considered as fourth-generation APLs, that display significant antitumor activities in the concentration range of the gold standard edelfosine. Two major classes of GAELs can be distinguished: a) glycosydated phospholipids (Semini *et al.*, this issue), that contain carbohydrates or carbohydrate-related molecules at the *sn*-2 position of the glycerol backbone, inositol-C2-platelet-activating factor (Ino-C2-PAF) (1-*O*-octadecyl-2-*O*-(2-(myo-inositolyl-ethyl)-*sn*-glycero-3-(*R*/*S*)-phosphatidylcholine) (Fig. **1**) exhibiting the highest antitumor efficacy; b) non-phosphorous GAELs (Arthur and Bittman, this issue), that have a sugar moiety in place of the phosphobase present in ALPs, 1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(2'-amino-2'-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (ET-16-$OCH_3$-Gln) (Fig. **1**) being the most active compound of this new family to date. ALPs and APCs kill cancer cells by inducing apoptosis [1, 13], whereas GAELs appear to kill cells through apoptosis-independent mechanisms [14, 15]. All of these molecules, which are distinguished by their low rates of metabolism *in vitro* and *in vivo*, are dealt with in the present thematic issue in order to provide a comprehensive overview of the state of the art on this promising family of compounds. The eleven articles included in this thematic issue provide up-to-date research on the field of APLs, involving basic, translational and clinical work. The distinct original and review articles, comprised in this thematic issue, address numerous conceptual and experimental considerations on how these molecules target and affect membrane-related events that eventually lead to their antitumor effects. Elucidation of the mechanisms of action of membrane-targeted APLs has served to identify new ways to regulate cancer cell death as well as to provide novel targets and approaches for cancer therapy. In this regard, lipid raft membrane domains, endoplasmic reticulum and mitochondria have turned out as major subcellular structures in the mechanism of action of APLs (Gajate and Mollinedo, this issue) as well as in the modulation of cell death. Membrane-related signaling and metabolic processes are critical events in APL action and in the regulation of cell demise. The studies shown here indicate that a remarkable and singular

*Address correspondence to this author at the Instituto de Biología Molecular y Celular del Cáncer, Centro de Investigación del Cáncer, CSIC - Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain; Tel: (+34) 923 294806; Fax: (+34) 923 294795;
E-mail: fmollin@usal.es

1875-5992/14 $58.00+.00 

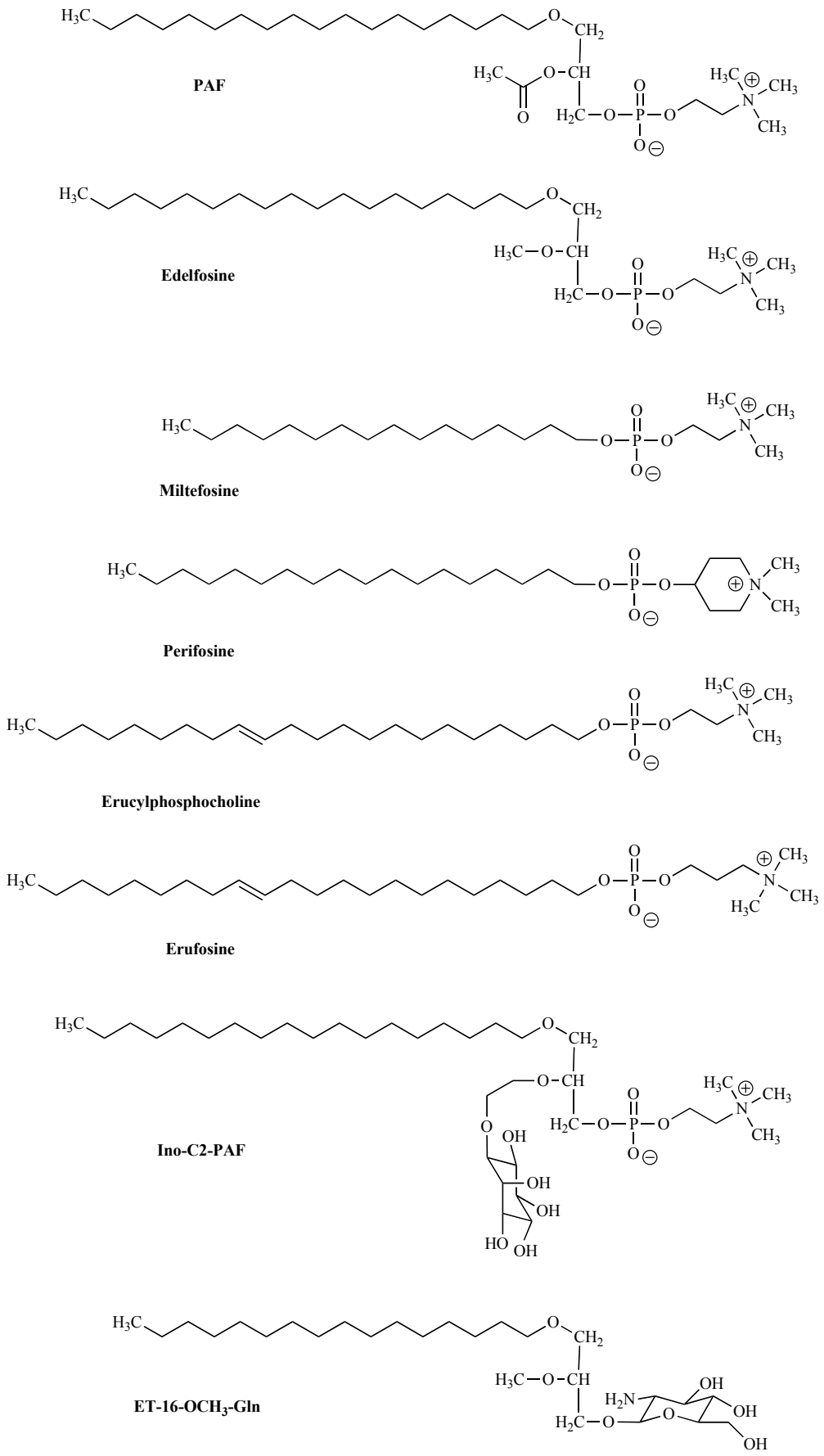


**Fig. (1).** Chemical structures of PAF, edelfosine, miltefosine, perifosine, erucylphosphocholine, erufosine, Ino-C2-PAF, and ET-16-$OCH_3$-Gln.

action of APLs lies in their effects on phospholipid and cholesterol metabolism (Marco *et al.*, this issue) as well as on cholesterol-rich lipid raft membrane domains, as evidenced both in cancer cells (Gajate and Mollinedo, this issue) and model membranes (Dynarowicz-Lątka and Hąc-Wydro, this issue), thus leading to alterations in the membrane structure and composition that eventually affect signaling processes and the cell fate. Ongoing studies on the singular action of these fascinating compounds are unveiling new ways to regulate cell death as well as to identify new targets for cancer therapy. The studies on edelfosine and lipid rafts led for the first time to the involvement of lipid rafts in the regulation of apoptosis signaling, and highlighted the role of lipid rafts in cancer chemotherapy, thus supporting the potential of lipid rafts as novel and attractive targets in cancer therapy [6, 8, 9, 16-20]. This membrane-targeted action of APLs not only affects cell death signaling [6, 8, 9, 18-21], but it also alters survival, adhesion and migration signaling (Chometon *et al*., this issue). The notion of the raft-targeted action of APLs established in the last thirteen years, favoring cell death signaling and inhibiting survival pathways [6, 8, 9, 16-20, 22], further supports the potential use of APLs in combination therapy. On these grounds, because of the rather unique mechanism of action of APLs, these compounds are appealing candidates to combine with other therapies, including radiotherapy (Verheij *et al.*, this issue).

New fluorescent APL analogs have been valuable tools to analyze the subcellular localization of APLs and have highlighted the role of endoplasmic reticulum and mitochondria in the action of APLs [23-27], but also have shown that the cytotoxicity exerted by these antitumor drugs largely depends on specific constituents of the cancer cells rather than only in the subcellular drug localization (Samadder *et al.*, this issue). Studies in yeast also suggest that the cytotoxic effect of edelfosine does not only depend on the accumulation of edelfosine in certain subcellular structures [28]. Interestingly, the effects of APLs on cancer cell invasion might also account, at least in part, for their antitumor action (Van slambrouck and Steelant, this issue). Thus, the interest for APLs lies not only for their potential role as antitumor drugs, but for their use as tools to study general biological processes, such as apoptosis/survival regulation and tumor cell invasion.

This thematic issue gives an up-to-date overview on most relevant aspects regarding APLs as anticancer drugs, and provides some novel insights into the potential chemotherapeutic role of these molecules as anticancer drugs as well as into the corresponding underlying processes.

## ACKNOWLEDGEMENTS

F.M.'s team is supported by grants from Ministerio de Economia y Competitividad of Spain (SAF2011-30518), Red Temática de Investigación Cooperativa en Cáncer, Instituto de Salud Carlos III, cofunded by the Fondo Europeo de Desarrollo Regional of the European Union (RD12/0036/0065), European Community's Seventh Framework Programme FP7-2007-2013 (Grant HEALTH-F2-2011-256986, PANACREAS), and Junta de Castilla y León (CSI221A12-2).

## REFERENCES

[1] Gajate, C.; Mollinedo F. Biological activities, mechanisms of action and biomedical prospect of the antitumor ether phospholipid ET-18-$OCH_3$ (edelfosine), a proapoptotic agent in tumor cells. *Curr. Drug Metab.*, **2002**, *3*, 491-525.

[2] Munder, P.G.; Westphal, O. Antitumoral and other biomedical activities of synthetic ether lysophospholipids. *Chem. Immunol.*, **1990**, *49*, 206-235.

[3] Mollinedo, F.; Martinez-Dalmau, R.; Modolell, M. Early and selective induction of apoptosis in human leukemic cells by the alkyl-lysophospholipid ET-18-$OCH_3$. *Biochem. Biophys. Res. Commun.*, **1993**, *192*, 603-609.

[4] Diomede, L.; Colotta, F.; Piovani, B.; Re, F.; Modest, E.J.; Salmona, M. Induction of apoptosis in human leukemic cells by the ether lipid 1-octadecyl-2-methyl-*rac*-glycero-3-phosphocholine. A possible basis for its selective action. *Int. J. Cancer*, **1993**, *53*, 124-130.

[5] Mollinedo, F.; Fernandez-Luna, J.L.; Gajate, C.; Martin-Martin, B.; Benito, A.; Martinez-Dalmau, R.; Modolell, M. Selective induction of apoptosis in cancer cells by the ether lipid ET-18-$OCH_3$ (Edelfosine): Molecular structure requirements, cellular uptake, and protection by Bcl-2 and Bcl-$X_L$. *Cancer Res.*, **1997**, *57*, 1320-1328.

[6] Gajate, C.; Del Canto-Janez, E.; Acuna, A.U.; Amat-Guerri, F.; Geijo, E.; Santos-Beneit, A.M.; Veldman, R.J.; Mollinedo, F. Intracellular triggering of Fas aggregation and recruitment of apoptotic molecules into Fas-enriched rafts in selective tumor cell apoptosis. *J. Exp. Med.*, **2004**, *200*, 353-365.

[7] Gajate, C.; Fonteriz, R.I.; Cabaner, C.; Alvarez-Noves, G.; Alvarez-Rodriguez, Y.; Modolell, M.; Mollinedo, F. Intracellular triggering of Fas, independently of FasL, as a new mechanism of antitumor ether lipid-induced apoptosis. *Int. J. Cancer*, **2000**, *85*, 674-682.

[8] Gajate, C.; Mollinedo, F. Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downstream signaling molecules into lipid rafts. *Blood,* **2007**, *109*, 711-719.

[9] Mollinedo, F.; De la Iglesia-Vicente, J.; Gajate, C.; De Mendoza, E-H.A.; Villa-Pulgarin, J.A.; Campanero, M.A.; Blanco-Prieto, M.J. Lipid raft-targeted therapy in multiple myeloma. *Oncogene,* **2010**, *29*, 3748-3757.

[10] Mollinedo, F.; De la Iglesia-Vicente, J.; Gajate, C.; De Mendoza, E-H.A.; Villa-Pulgarin, J.A.; De Frias, M.; Roue, G.; Gil, J.; Colomer, D.; Campanero, M.A.; Blanco-Prieto, M.J. *In vitro* and *in vivo* selective antitumor activity of Edelfosine against mantle cell lymphoma and chronic lymphocytic leukemia involving lipid rafts. *Clin. Cancer Res.*, **2010**, *16*, 2046-2054.

[11] Eibl, H.; Unger, C. Hexadecylphosphocholine: A new and selective antitumor drug. *Cancer Treat. Rev.*, **1990**, *17*, 233-242.

[12] Terwogt, J.M.; Mandjes, I.A.; Sindermann, H.; Beijnen, J.H.; Ten Bokkel Huinink, W.W. Phase II trial of topically applied miltefosine solution in patients with skin-metastasized breast cancer. *Br. J. Cancer*, **1999**, *79*, 1158-1161.

[13] Mollinedo, F.; Gajate, C.; Martin-Santamaria, S.; Gago, F. ET-18-$OCH_3$ (edelfosine): a selective antitumour lipid targeting apoptosis through intracellular activation of Fas/CD95 death receptor. *Curr. Med. Chem.*, **2004**, *11*, 3163-3184.

[14] Samadder, P.; Bittman, R.; Byun, HS.; Arthur, G. A glycosylated antitumor ether lipid kills cells *via* paraptosis-like cell death. *Biochem. Cell Biol.*, **2009**, *87*, 401-414.

[15] Jahreiss, L.; Renna, M.; Bittman, R.; Arthur, G.; Rubinsztein, D.C. 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(2'-acetamido-2'-deoxy-beta-D-glucopyranosyl)-*sn*-glycerol (Gln) induces cell death with more autophagosomes which is autophagy-independent. *Autophagy*, **2009**, *5*, 835-846.

[16] Van der Luit, A.H.; Budde, M.; Ruurs, P.; Verheij, M.; Van Blitterswijk, W.J. Alkyl-lysophospholipid accumulates in lipid rafts and induces apoptosis *via* raft-dependent endocytosis and inhibition of phosphatidylcholine synthesis. *J. Biol. Chem.*, **2002**, *277*, 39541-39547.

[17] Van der Luit, A.H.; Vink, S.R.; Klarenbeek, J.B.; Perrissoud, D.; Solary, E.; Verheij, M.; Van Blitterswijk, W.J. A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells. *Mol. Cancer Ther.*, **2007**, *6*, 2337-2345.

[18] Gajate, C.; Mollinedo, F. The antitumor ether lipid ET-18-$OCH_3$ induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells. *Blood*, **2001**, *98*, 3860-3863.

[19] Mollinedo, F.; Gajate, C. Fas/CD95 death receptor and lipid rafts: New targets for apoptosis-directed cancer therapy. *Drug Resist. Updat.*, **2006**, *9*, 51-73.

[20] Mollinedo, F.; Gajate, C. Lipid rafts and clusters of apoptotic signaling molecule-enriched rafts in cancer therapy. *Future Oncol.*, **2010**, *6*, 811-821.

[21] Mollinedo, F.; Gajate, C. Lipid rafts, death receptors and CASMERs: new insights for cancer therapy. *Future Oncol.*, **2010**, *6*, 491-494.

[22] Reis-Sobreiro, M.; Roue, G.; Moros, A.; Gajate, C.; De la Iglesia-Vicente, J.; Colomer, D.; Mollinedo, F. Lipid raft-mediated Akt signaling as a therapeutic target in mantle cell lymphoma. *Blood Cancer J.*, **2013**, *3*, e118.

[23] Gajate, C.; Matos-da-Silva, M.; Dakir, EL.; Fonteriz, RI.; Alvarez, J.; Mollinedo, F. Antitumor alkyl-lysophospholipid analog edelfosine induces apoptosis in pancreatic cancer by targeting endoplasmic reticulum. *Oncogene,* **2012**, *31*, 2627-2639.

[24] Nieto-Miguel, T.; Fonteriz, R.I.; Vay, L.; Gajate, C.; Lopez-Hernandez, S.; Mollinedo, F. Endoplasmic reticulum stress in the proapoptotic action of edelfosine in solid tumor cells. *Cancer Res.*, **2007**, *67*, 10368-10378.

[25] Nieto-Miguel, T.; Gajate, C.; Mollinedo, F. Differential targets and subcellular localization of antitumor alkyl-lysophospholipid in leukemic *versus* solid tumor cells. *J. Biol. Chem.*, **2006**, *281*, 14833-14840.

[26] Mollinedo, F.; Fernandez, M.; Hornillos, V.; Delgado, J.; Amat-Guerri, F.; Acuna, A.U.; Nieto-Miguel, T.; Villa-Pulgarin, J.A.; Gonzalez-Garcia, C.; Cena, V.; Gajate, C. Involvement of lipid rafts in the localization and dysfunction effect of the antitumor ether phospholipid edelfosine in mitochondria. *Cell Death Dis.*, **2011**, *2*, e158.

[27] Kuerschner, L.; Richter, D.; Hannibal-Bach, H.K.; Gaebler, A.; Shevchenko, A.; Ejsing, C.S.; Thiele, C. Exogenous ether lipids predominantly target mitochondria. *PLoS ONE,* **2012**, *7*, e31342.

[28] Cuesta-Marban, A.; Botet, J.; Czyz, O.; Cacharro, L.M.; Gajate, C.; Hornillos, V.; Delgado, J.; Zhang, H.; Amat-Guerri, F.; Acuna, A.U.; McMaster, C.R.; Revuelta, J.L.; Zaremberg, V.; Mollinedo, F. Drug uptake, lipid rafts, and vesicle trafficking modulate resistance to an anticancer lysophosphatidylcholine analogue in yeast. *J. Biol. Chem.*, **2013**, *288*, 8405-8418.