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Short communication

Novel alkylphospholipid-DTC hybrids as promising agents against endocrine related cancers acting via modulation of Akt-pathway☆

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ABSTRACT

A new series of 2-(alkoxy(hydroxy)phosphoryloxy)ethyl dialkylcarbodithioate derivatives was synthesized and evaluated against endocrine related cancers, acting via modulation of Akt-pathway. Eighteen compounds were active at 7.24–100 μM against MDA-MB-231 or MCF-7 cell lines of breast cancer. Three compounds (**14**, **18** and **22**) were active against MCF-7 cells at IC_{50} significantly better than miltefosine and most of the compounds were less toxic towards non-cancer cell lines, HEK-293. On the other hand, twelve compounds exhibited cell growth inhibiting activity against prostate cancer cell lines, either PC-3 or DU-145 at 14.69–95.20 μM. While nine of these were active against both cell lines. The most promising compounds **14** and **18** were about two and five fold more active than miltefosine against DU-145 and MCF-7 cell lines respectively and significantly down regulated phospho-Akt. Possibly anti-cancer and pro-apoptotic activity was mostly due to blockade of Akt-pathway.

1. Introduction

Cancer is a group of diseases characterized by uncontrolled cell proliferation and spread of malignant cells. About 77% of all cancers are diagnosed in older persons with age 55 years or more. In US, the lifetime risk from cancer is about 1 in every 3 person [1]. The National Institute of Health (NIH) estimates that the overall costs of cancer in 2007 were US $ 226.8 billion: $103.8 for direct medical cost and $ 123.0 for indirect mortality costs [1]. The projections [2] for cancer survivors and cost of care during 2010–2020 reveal that there will be 18.1 million survivors in 2020 with the medical costs to be increased by 27% from 2010. The largest medical cost increase being in the two endocrine related cancers—prostate cancer in men (42%) and breast cancer in women (32%). These revelations have prompted the authors to design, synthesize and biologically evaluate chemical entities that may be useful in the management of both prostate and breast cancer.

Recently the concept of hybrid molecules has been attracting a lot of attention [3–6] as the presence of more than one active pharmacophore in a single molecule leads to an entirely novel class of compounds with better biological activity profile. It is supposed that a single chemical entity can modulate multiple targets simultaneously.

Alkylphosphocholines (APCs) [7] belong to a class of lipid molecules that include miltefosine (Fig. 1), perifosine (Fig. 1), erufosine (Fig. 1) and edelfosine (Fig. 1), which have known anti-tumor properties [8,9]. Unlike conventional chemotherapeutics, these compounds structurally resemble membrane lipid components, and thus, exhibit adequate drug delivery to target tumor tissues [9]. However, they can also target primarily the membrane-bound receptors associated with cancer-specific cellular phenotypes, such as, cellular proliferation and survival. More specifically, the possible mechanisms underlying the actions of APCs have been reported to be the involvement of PI3K/Akt (phosphatidylinositide 3-kinase/v-

Abbreviations: APCs, alkylphosphocholines; DDTC, diethyldithiocarbamate; HEK, human embryonic kidney; PI, propidium iodide; MMP, mitochondrial membrane potential; ATCC, American type of cell culture collection; DMEM, Dulbecco modified eagle medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PI3K, phosphatidylinositide 3-kinase.

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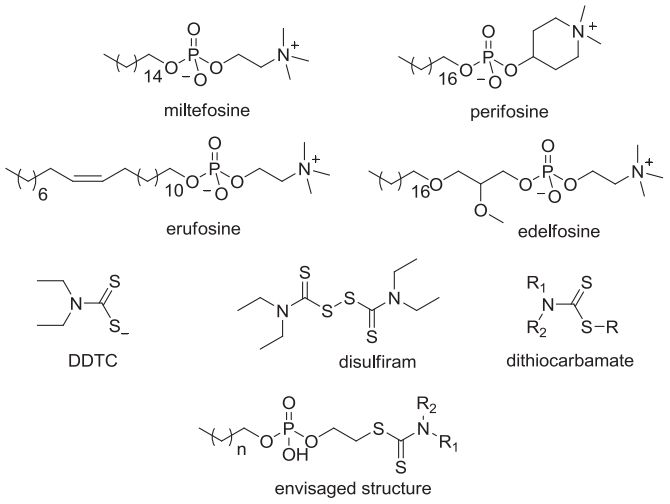


Fig. 1. Structures of known alkylphosphocholine, dithiocarbamate and envisaged structure.

Akt murine thymoma viral oncogene homolog) inhibition, their downstream signaling pathways, and the induction of apoptosis [7]. It is also known that APCs do not target the DNA, and they have cell selective effects of inhibiting the proliferation of cancer cells at low concentrations without affecting the normal cells [7]. Miltefosine (Fig. 1) shows a wide range of anti-tumor effects and as Milterx®, it has been approved for the topical treatment of skin metastases from breast cancer [7].

Likewise, dithiocarbamate is also a versatile pharmacophore exhibiting various biological activities due to its sulfur based metal chelating properties [10]. Diethyldithiocarbamate (DDTC; Fig. 1) is capable of binding accumulated copper and forming a new complex that could potently inhibit the proteasomal chemotrypsin-like activity, decrease expression of androgen receptor (AR), estrogen receptor (ER)-α and ER-β receptors proteins, and induce apoptosis in both prostate and breast cancer cells [10]. Pyrrolobenzodiazepine derivatives with DTC side chains inhibit the growth of prostate and breast cancer cell lines [11]. Tetraethylthiuram disulfide (Disulfiram, Fig. 1) and dithiocarbamate anion (Fig. 1) strongly inhibited the proliferation of cancer cells of a variety of cell types [12] by inhibiting the maturation of P-glycoprotein pump, an ATP-driven 170-kd efflux pump on the plasma membrane that pumps a variety of cytotoxic drugs out of the cell [13]. Another hybrid molecule of butenolide and dithiocarbamate have anti-tumor properties against several human cancer cell lines including the prostate and breast cancer cells [14].

Thus, it was thought worthwhile to incorporate dithiocarbamate moiety into alkylphospholipid scaffold in place of amine moiety (part of choline moeity) keeping the rest of the structure same. The hybrid structures (envisaged structure, Fig. 1) though not entirely alkylphosphocholine since it was modified in order to add the alkyldithiocarbamate moiety, were therefore addressed as alkylphospholipid. It was envisaged to arrive at a novel scaffold (Fig. 1) having anti-proliferative activity against both the breast and prostate cancer cell lines. Synthesis, biological activity and structure activity relationship (SAR) are being communicated.

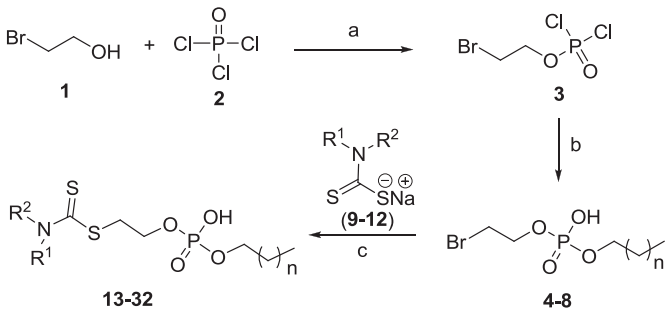
2. Chemistry

The 2-(alkoxy(hydroxy)phosphoryloxy)ethyl dialkylcarbodithioate derivatives (**13**–**32**) were synthesized according to Scheme 1. Bromoethanol (**1**) was treated with phosphorus oxychloride (**2**) to give 2-bromoethyl phosphorodichloridate (**3**). Subsequent reaction of **3** with suitable alcohol furnished 2-bromoethyl alkyl hydrogen phosphate (**4**–**8**), which after treatment with sodium salt of carbodithioic acid (**9**–**12**) provided 2-(alkoxy(hydroxy) phosphoryloxy)ethyl dialkylcarbodithioate (**13**–**32**).

3. Biological evaluation

3.1. Cell inhibitory activity in breast and prostate cancer cells

The above compounds **13**–**32** were evaluated against breast cancer cell lines, MCF-7 (estrogen responsive proliferative breast cancer model), MDA-MB-231 (estrogen independent aggressive breast cancer model); and prostate cancer cell lines, PC-3 (estrogen responsive both α and β), DU-145 (estrogen responsive β only) and non-cancer cell line, HEK-293 (human embryonic kidney) using MTT assay to assess cell inhibition [15,16]. Miltefosine, an alkylphosphocholine was used as standard drug. The results (Table 1) revealed that almost all the compounds except compounds **15** and **16** were inhibited both or either of MDA-MB-231 and MCF-7 breast cancer cell lines. Standard drug, miltefosine had IC_{50} 2.075 μM and 34.70 μM against MDA-MB-231 and MCF-7 cells respectively. Out of 20 compounds, three compounds (**14**, **18** and **22**) were active against MCF-7 cells with IC_{50} better than miltefosine. Most of the compounds were found to be less toxic towards non-cancer cell lines, HEK-293, except compound **21**, **31** and **32** which exhibited some degree of toxicity. Compound **18** exhibited potent cell growth inhibition against both MCF-7 and MD-MB-231 cells with IC_{50} of 7.24 ± 2.15 and 24.57 ± 5.69 μM respectively. It may be inferred that compound **18** is most promising compound with significant anti-proliferative activity against breast cancer cell lines. On the other hand twelve compounds (**13**–**21**, **23**, **27** and **31**) exhibited cell growth inhibiting activity against prostate cancer cell lines, either PC-3 or DU-145, at 14.69–95.20 μM while nine of these (**13**–**20** and **23**) were active against both the cell lines. The standard miltefosine had IC_{50} of 19.90 μM and 25.78 μM against PC-3 and DU-145 cell lines respectively. Compounds **14**, **15** and **19** exhibited better activity than miltefosine against DU-145 cell lines. Compound **14** and **15** were most promising as these two had lower IC_{50} values against both the prostate cancer cell lines. Sodium piperidine-1-carbodithioate was also evaluated against breast cancer cell lines, MCF-7, MDA-MB-231 and prostate cancer cell lines, PC-3, DU-145 to assess the effect of hybridization of alkylphospholipids and DTC. It has no effect against both the breast cancer cell lines and mildly inhibited the growth of prostate cancer cell lines, PC-3 and DU-145 at IC_{50} 25.5 ± 1.25 μM and 18.7 ± 0.84 μM respectively. These results



-NR^1R^2 = piperidine, pyrrolidine, azepane, N-methyl piperazine
n = 0, 4, 6, 8, 14

Scheme 1. Reagent and conditions; (a) CCl_4, reflux, 10 h; (b) (1) ROH, CCl_4, reflux, 10 h; (2) H_2O,rt, 6 h; (c) MeOH, reflux, 3 h.