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Synthesis and biological evaluation of novel cytotoxic phospholipids for prostate cancer☆

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Abstract—We describe herein synthesis, SAR, and biological evaluation of a novel series of cytotoxic serine amide phosphates (SAPs) for prostate cancer. These compounds were tested for their cytotoxicity in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU), and in CHO and RH7777 cells (negative controls). Comparison of anticancer effects of these compounds with a standard chemotherapeutic agent 5-fluorouracil shows that they are very effective in killing prostate cancer cells with low micromolar cytotoxicity and provide us a new lead for the development of drugs for prostate cancer.

Prostate cancer is the most common malignancy affecting men and is the second-leading cause of cancer deaths in the US.[1] The risk of developing prostate cancer is associated with age,[2] ethnicity,[2] family history,[3,4] diet,[5] and other factors. Treatment for prostate cancer depends on the stage at which cancer is found and the age and health status of the patient. Clinically, localized disease is potentially curable[6] with standard surgery and/or radiation therapy. Standard treatment involves either removal of the entire prostate gland (radical prostatectomy) or radiation therapy aimed at the pelvic area. However, almost half of the men initially diagnosed with local disease are found to have tumors, which have advanced to the periprostatic area or beyond at the time of surgery. A variety of chemotherapeutic agents[7] are used alone or in combination with radiotherapy to treat the advanced disease. None of the conventional approaches to cancer therapy have proven to be highly successful for prostate cancer.

One promising drug development strategy for prostate cancer involves identifying and testing agents that interfere with growth factors and other molecules involved in the cancer cell's signaling pathways. Lysophosphatidic acid[8,9] (**1**, LPA) is a natural glycerophospholipid that possesses a range of biological actions and is the best characterized member of the phospholipid growth factors (PLGFs) family. LPA elicits its effects via multiple subtypes of membrane spanning G protein-coupled receptors (GPCR) that include the EDG and PSP family of receptors. The most prominent effects of LPA include stimulation of cell proliferation[10] and tumor cell invasion.[11] LPA stimulates phospholipase D activity and cell proliferation in PC-3 cell lines.[12] One of the LPA receptors, LPA_3, has been detected in prostate tissue.[13] Ovarian cancer cells produce and respond to LPA.[14] Moreover, vascular endothelial growth factor released from ovarian cancer cells in response to LPA has been reported to induce angiogenesis in endothelial cells.[15] We found that one of the compounds (**2**) prepared in our laboratory inhibits LPA induced chloride currents in frog oocytes.[16] Thus, we thought that it would be interesting to determine the actions of a small library of these agents in several prostate cancer cell lines. Preliminary studies[17] have shown that compounds **2** and **3** are effective in killing prostate cancer cells (Fig. 1). Encouraged by these results and due to important biological effects of LPA and its implications in the pathophysiology of a variety of cancers we decided to

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optimize the activity of SAPs for in vitro anticancer activity against prostate tumor cell lines. We report in this paper the synthesis, SAR, and in vitro cytotoxic activity of SAP derivatives against five human prostate cancer cell lines and two nontumor cell lines to determine their selectivity (Fig. 1).

The general synthesis of SAPs and serine amide alcohols (SAAs) is shown in Scheme 1. Commercially available *N*-Boc-serine (*R* or *S* form) was allowed to react with an appropriate amine in the presence of EDC/HOBt to form amide **5**. Amide **5** was treated with TFA to give serine amide alcohol (**6**). Phosphorylation of amide **5** and concurrent removal of protecting groups under hydrogenolysis conditions using Pd/C in ethanol gave **2**. During the progress of this work a report [18] appeared for the synthesis of **2b**. However, according to our knowledge these type of compounds (SAPs) have never been examined for prostate cancer therapy. We synthesized unsaturated analogues **9** and **11** by similar procedures as shown in Scheme 2. Serine diamide phosphates (SDAPs) and other amine derivatives were synthesized starting from *O*-benzyl *N*-Boc-serine following the earlier procedures (Scheme 3). LAH mediated reduction of compound **5e** gave long chain *N*-alkyl amino alcohols **17** and **18** (Scheme 4). Compound **20**, which has an ethanolamine amide backbone rather than the serine amide backbone was synthesized according to the reported procedure.[19]

All compounds were characterized by ^{1}H and ^{13}C NMR, mass spectroscopy and, in certain cases, elemental analysis.[20]

We examined the cytotoxicity of synthesized compounds in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU) and in two negative control cell lines (CHO and RH7777) using the sulforhodamine B (SRB) assay.[21] Cells were exposed to a wide range of concentrations (0–100 μM) of the particular compound for 96 h in 96-well plates. Cells were fixed with 10% trichloroacetic acid, washed five times with water. The plates were air dried overnight and fixed cells were stained with SRB solution. The cellular protein-bound SRB was measured at 540 nm using a plate reader. Cell numbers at the end of the treatment were measured. IC_{50} (i.e., concentration that inhibited cell growth by 50% of untreated control) values were obtained by nonlinear regression analysis using WinNonlin. For comparative purposes and to understand the degree of

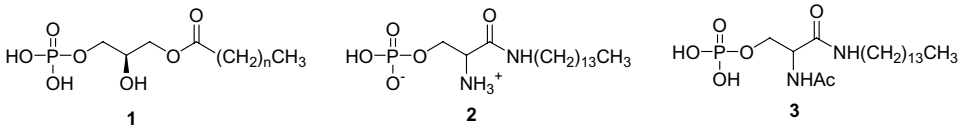
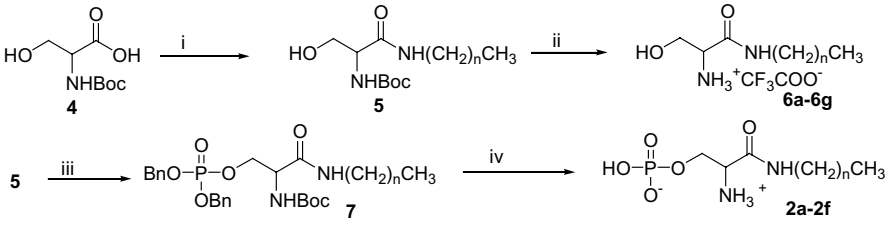
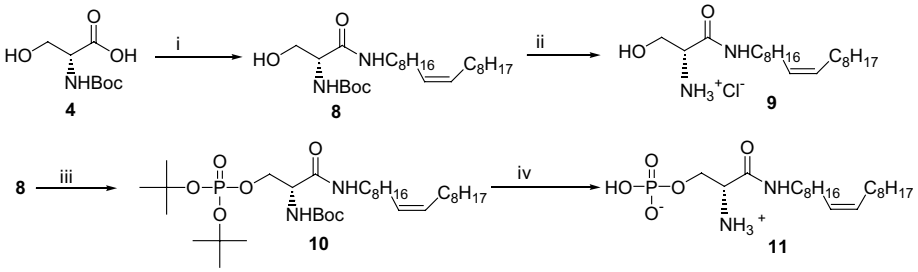


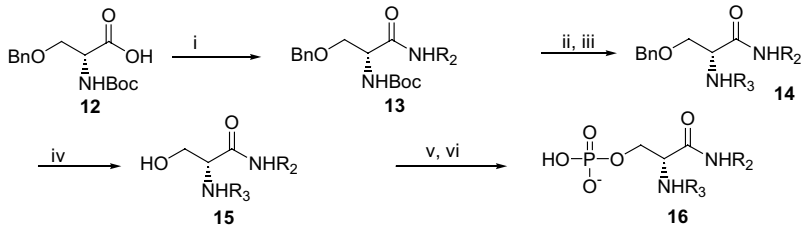
Figure 1. Structures of LPA and SAPs.



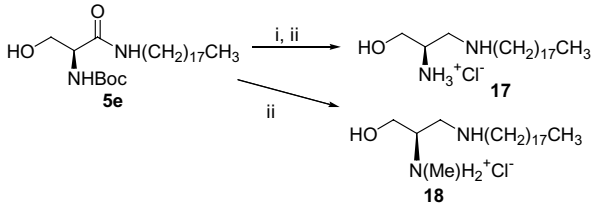
Scheme 1. Reagents and conditions: (i) $CH_3(CH_2)_nNH_2$, EDC, HOBt, CH_2Cl_2, rt, 5 h; (ii) TFA, CH_2Cl_2, rt, 0.5 h; (iii) tetrazole, dibenzyl diisopropylphosphoramidite, CH_2Cl_2, rt, 0.5 h, H_2O_2, rt, 0.5 h; (iv) H_2, 10% Pd/C, EtOH, rt, 3 h.



Scheme 2. Reagents and conditions: (i) $C_8H_{17}(CH{:}CH)C_8H_{16}NH_2$, EDC, HOBt, CH_2Cl_2, rt, 5 h; (ii) 2 M HCl/Et_2O, rt, overnight; (iii) tetrazole, di-*tert*-butyl diisopropylphosphoramidite, CH_2Cl_2, rt, 0.5 h, H_2O_2, rt, 0.5 h; (iv) TFA, CH_2Cl_2, rt, 0.5 h.



Scheme 3. Reagents and conditions: (i) R_2NH_2, EDC, HOBt, CH_2Cl_2, rt, 5 h; (ii) TFA, CH_2Cl_2, rt, 0.5 h; (iii) TEA, R_3SO_2Cl or R_3NCO or R_3COCl; (iv) H_2, 10% Pd/C, EtOH, rt, 3 h; (v) tetrazole, dibenzyl diisopropylphosphoramidite, CH_2Cl_2, rt, 0.5 h, H_2O_2, rt, 0.5 h; (vi) H_2, 10% Pd/C, EtOH, rt, 3 h.



Scheme 4. Reagents and conditions: (i) TFA, CH_2Cl_2, rt, 0.5 h (ii) (a) LAH, Et_2O, reflux, 7 h; (b) HCl.

cytotoxicity we tested 5-fluorouracil against all five prostate cancer cell lines. The results are summarized in Table 1.

From the cytotoxicity data it is clear that most of the compounds tested showed good anticancer activity against all five prostate cancer cell lines. It is noteworthy, to mention that serine amide alcohols (**6b**,**e**,**f**) without a phosphate group are as effective as SAPs. A direct relationship was observed between length of the alkyl chain and cytotoxicity of the tested compounds. Accordingly, all of these compounds showed an alkyl chain length dependent cytotoxicity. Compounds with shorter alkyl chains (**2a**, **6b**, **15d**, **16d**) are less cytotoxic than analogues with longer alkyl chains (see Table 1). Compound **2f** emerged as one of the most potent SAPs tested so far with an IC_{50} of 1.8 μM against PPC-1 cell line. However, serine amide alcohols are more potent than corresponding SAPs when the alkyl chain length is below 18C, but no significant difference in the cytotoxicity is observed between serine amide alcohols and SAPs with alkyl chain more than 18C.

IC_{50} values for enantiomers of serine amide alcohols (**6c**,**d**) and SAPs (**2b**,**c**) are approximately equivalent, which suggests that chirality is not important for the antiproliferative activity of these compounds in prostate cancer. Introduction of a double bond in the alkyl chain lowered the potency of both serine amide alcohol **9** and SAP **11**.

To understand the importance of the amine functionality we derivatized the amine group to the corresponding Set B amide, sulfonamide and urea derivatives. Serine diamide phosphate **16d** with a shorter alkyl chains failed to demonstrate cytotoxicity at concentration below 100 μM in four prostate cancer cell lines except TSU prostate cell line. The inhibitory activity of sulfonamide derivatives **15b** and **16b** and urea derivative **15c** in all five prostate cancer cell lines showed a general decreasing trend suggesting that derivatization of C2 amine group is not tolerable for their ability to kill prostate cancer cells.

To further investigate the extent of structural tolerance permitted in the serine amide backbone region, we replaced the serine amide group with simple ethanolamine amide by synthesizing compounds **19** and **20**. However, these ethanolamine amide analogues were less potent and particularly compound **19** did not show any activity against DU-145, PC-3, and LNCaP prostate cancer cell lines.

When the amide group in serine amide alcohols was reduced to produce long chain *N*-alkyl amino alcohols **17** and **18**, these analogues retained cytotoxicity and were very effective in killing prostate cancer cell lines with low micromolar cytotoxicity. To determine the selectivity, several of synthesized compounds were also examined for their cytotoxicity in CHO and RH7777 cells as negative controls. Many of the potent compounds showed similar cytotoxicity and were nonselective in their action against prostate cancer cell lines and nontumor negative control cells.

In summary we have shown that SAPs and SAAs, derivatives of LPA, represent a novel class of cytotoxic phospholipids for prostate cancer. We have designed and synthesized a number of SAP derivatives and evaluated for their inhibitory activity toward the growth of human prostate cancer cell lines. Several of these, such as **2f**, **17**, and **18** are potent inhibitors of prostate tumor cell proliferation at low micromolar cytotoxicity. Despite their high cytotoxicity, the same compounds were non selective against nontumor CHO cells and receptor-negative RH7777 cells. This initial report suggests that further optimization is necessary to increase the selectivity. Efforts are in progress in our laboratory to enhance potency and selectivity of this class of compounds for the treatment of prostate cancer.

Acknowledgements

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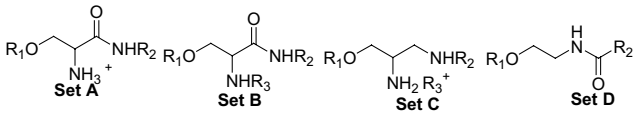
Table 1. Anti-proliferative effects of synthesized compounds in prostate cancer and negative control cell lines



Set	Compound (chirality)	IC_{50} (μM)									
		R_1	R_2	R_3	CHO[a]	RH7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU[b]
A	**2a** (2*R*)	PO_3H	$C_{10}H_{21}$	—	ND	ND	50.2	36.0	44.7	22.1	31.5
	2b (2*R*)	PO_3H	$C_{14}H_{29}$	—	ND	ND	20.6	>50	10.1	>10	>10
	2c (2*S*)	PO_3H	$C_{14}H_{29}$	—	ND	ND	32.0	>50	19.7	>10	>10
	2d (2*R*)	PO_3H	$C_{18}H_{37}$	—	ND	ND	11.7	19.1	7.2	5.6	4.8
	2e (2*R*)	PO_3H	$C_{19}H_{39}$	—	3.7	ND	5.7	15.3	5.8	1.8	5.0
	2f (2*S*)	PO_3H	$C_{20}H_{41}$	—	7.8	ND	10.8	>20	3.6	1.8	11.1
	6a (2*S*)	H	C_8H_{17}	—	>100	ND	>100	>100	>100	>100	>100
	6b (2*R*)	H	$C_{10}H_{21}$	—	ND	ND	52.2	35.0	31.0	15.9	26.0
	6c (2*R*)	H	$C_{14}H_{29}$	—	ND	ND	8.2	10.2	8.1	6.3	7.5
	6d (2*S*)	H	$C_{14}H_{29}$	—	ND	ND	6.9	10.3	10.0	6.2	9.2
	6e (2*R*)	H	$C_{18}H_{37}$	—	2.5	2.6	5.4	5.2	3.8	2.2	4.4
	6f (2*R*)	H	$C_{19}H_{39}$	—	2.4	3.2	5.1	5.3	5.3	1.8	3.9
	6g (2*S*)	H	$C_{20}H_{41}$	—	4.1	ND	7.0	6.6	3.9	2.6	6.6
	9 (2*S*)	H	C_8H_{17}–CH:CH–C_8H_{16}	—	5.2	6.8	6.9	5.9	6.6	5.1	5.5
	11 (2*S*)	PO_3H	C_8H_{17}–CH:CH–C_8H_{16}	—	11.9	28.6	16.0	39.2	12.2	21.1	12.4
B	**14a** (2*S*)	OBn	$C_{18}H_{37}$	H	3.0	9.9	11.2	6.2	10.9	2.9	6.8
	14b (2*S*)	OBn	$C_{18}H_{37}$	SO_2Me	>50	>50	>50	47.3	Not active	16.7	>50
	14c (2*S*)	OBn	$C_{18}H_{37}$	CO NH Ph (3,5-difluoro)	18.5	>20	20	>20	>20	>20	15.9
	14d (2*S*)	OBn	C_8H_{17}	COC_7H_{15}	9.2	12.9	22.9	31.3	35.0	4.0	10.0
	15b (2*S*)	H	$C_{18}H_{37}$	SO_2Me	12.9	9.2	23.1	13.6	16.0	10.2	20.5
	15c (2*S*)	H	$C_{18}H_{37}$	CO NH Ph (3,5-difluoro)	20	>20	20	>20	>20	>20	15.3
	15d (2*S*)	H	C_8H_{17}	COC_7H_{15}	>100	ND	>100	81.5	>100	81.2	93.8
	16b (2*S*)	PO_3H	$C_{18}H_{37}$	SO_2Me	>50	50	43.2	>50	15.1	17.8	35.7
	16d (2*S*)	PO_3H	C_8H_{17}	COC_7H_{15}	>100	>100	>100	>100	Not active	>100	79.0
C	**17** (2*R*)	H	$C_{18}H_{37}$	H	2.2	2.9	4.1	2.6	5.1	1.9	2.2
	18 (2*R*)	H	$C_{18}H_{37}$	Me	1.7	2.5	3.2	2.4	3.3	1.6	1.1
D	**19**	H	C_8H_{17}–CH:CH–C_8H_{16}	—	>20	>20	Not active	Not active	Not active	>20	Not active
	20	PO_3H	C_8H_{17}–CH:CH–C_8H_{16}	—	>50	>50	>50	>50	Not active	50	>50
	5-FU	—	—	—	—	—	11.9	12.0	4.9	6.4	3.6

[a] Control cell lines.
[b] Prostate cancer cell lines.

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20. All synthesized compounds were purified by column chromatography and purity was confirmed by elemental analysis and HPLC. Characteristic data for some compounds is given below.
    Compound **2e**: ^{1}H NMR (300 MHz, CF_3CO_2D) δ 0.77 (br s, 3H), 1.20 (br s, 32H), 1.53 (br s, 2H), 3.32–3.33 (m, 2H), 4.50 (br s, 1H), 4.67–4.72 (m, 2H); ^{13}C NMR (300 MHz, CF_3CO_2D) δ 11.92, 21.61, 25.78, 27.65, 28.13, 28.46, 28.49, 28.62, 28.67, 28.74, 31.0, 40.71, 54.27, 63.54, 164.99; MS (ESI) *m/z* 449 [M−H]. Anal. Calcd for $C_{22}H_{47}N_2O_5P\cdot 0.5EtOH$: C, 58.64; H, 10.51; N, 6.22. Found: C, 58.33; H, 10.64; N, 5.91.
    Compound **16b**: ^{1}H NMR (300 MHz, CF_3CO_2D) δ 0.79 (t, J = 6.6 Hz, 3H), 1.22 (br s, 30H), 1.56 (m, 2H), 3.20 (s, 3H), 3.35 (t, J = 6.9 Hz, 2H), 4.50–4.70 (br m, 3H); ^{13}C NMR (300 MHz, CF_3CO_2D) δ 11.77, 21.50, 25.69, 27.63, 28.05, 28.35, 28.42, 28.51, 28.56, 28.63, 30.97, 39.70, 40.55, 56.04, 67.72, 66.73, 169.85; MS (ESI) *m/z* 513.2 [M−H].
    Compound **18**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, J = 6.9 Hz, 3H), 1.23 (br s, 32H), 2.66 (br s, 3H), 2.92 (m, 2H), 3.2 (m, 2H), 3.52 (br s, 1H), 3.62–3.82 (m, 2H), 9.11 (br s, 1H), 9.35 (m, 2H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 13.85, 21.99, 25.26, 25.87, 28.44, 28.6, 28.73, 28.87, 28.9, 28.95, 30.0, 31.2, 44.39, 47.33, 47.45, 56.16, 57.05; MS (ESI) *m/z* 357.5 [M+H].
    Compound **20**: The spectroscopic properties of this compound were inconsistent with the assigned structure reported in the literature.[19]
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